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Supporting Information

An Esterase-Activated Diketopyrrolopyrrole-Based Theranostic Prodrug for Precise Pyroptosis and Synergistic Chemo-Photodynamic Therapy of Pancreatic Cancer

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Experimental Procedures

1. Materials

The reagents for synthesis and in vitro analysis were purchased from commercial sources (Aladdin, Adamas-beta, Leyan.com) and used without further processing. The solvents used in spectrum analysis were of analytical grade. The solutions for analytical studies were prepared with deionized water treated using a Milli-Q System (Billerica, MA, USA).

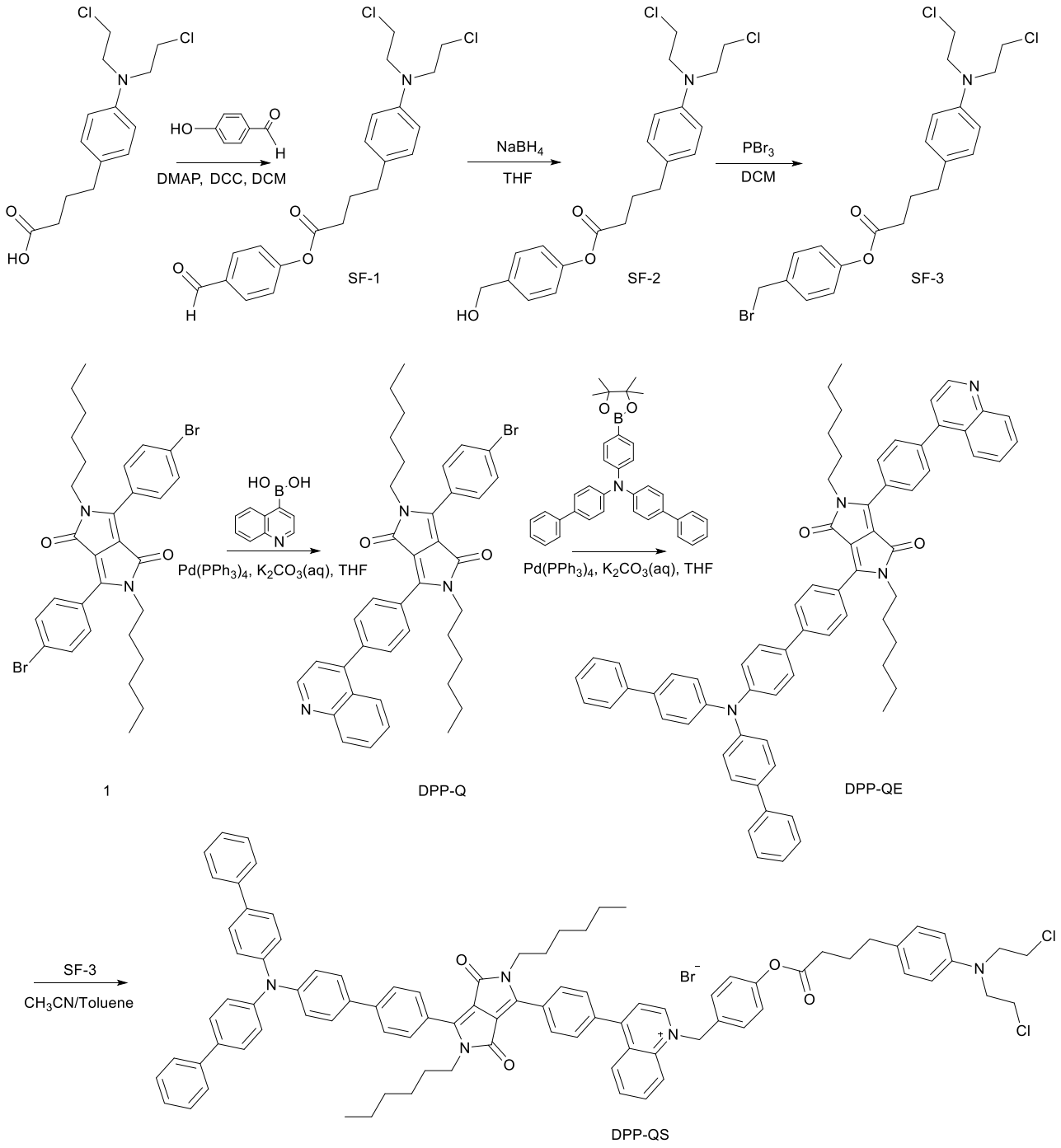
2. Characterization Techniques

^{1}H-NMR and ^{13}C-NMR were measured by Bruker AM-400 MHz NMR spectrometer 600MHz nuclear magnetic resonance spectrometer (Ascend). Chemical shifts were expressed in ppm (in dichloromethane-d_2(CD_2Cl_2), chloroform-d ($CDCl_3$) and DMSO-d_6; TMS as an internal standard) and coupling constants (J) in Hz. High resolution mass spectrometry (HRMS) data were obtained from GCT Premier and Xevo G2 TOF MS. UV-Vis absorption and fluorescence spectra were obtained from Varian Cary 500 spectrophotometer and FLS1000 Photoluminescence Spectrometer, respectively. Fluorescence quantum yields were measured using HAMAMATSU Quantaurus-QY C11347-11. Dynamic light scatting (DLS) experiments were performed on Zetasizer Nano ZS. CCK-8 results were recorded using Bio Tek Synergy H1. Confocal fluorescence images were obtained on Lecia TCS SP8 laser scanning confocal microscopy and processed with ImageJ software. SEM imaging was carried on a Field emission scanning electron microscope (FESEM, Nova NanoSEM 450). Hecho S5000 LED cold-light fountain provides the white light irradiation. LED lamp (530 nm) was used as excitation and the light intensity was 118 lm. The optical power density is measured by 1918-C Optical Power Meter. The immune reaction bands were detected by Tanon 5200 Chemiluminescent Imaging System. Imaging of living mouse cell was observed using PerkinElmer IVIS Lumina III with a Xe lamp as an excitation.

3. Cell culture

Human umbilical vascular endothelial (HUVEC) cells were provided by Feringa Nobel Prize Scientists Joint Research Center, East China University of Science and Technology. Human pancreatic cancer (MIA PaCa-2) cells and mouse embryonic fibroblasts (NIH/3T3) cells were provided by State Key Laboratory of Oncogenes and Related Genes Renji Hospital School of Biomedical Engineering Shanghai Jiao Tong University. DMEM High Glucose (DMEM), South America Fetal Bovine Serum (FBS), Penicillin-Streptomycin Solution 100X, Trypsin 0.25 % in PBS, Phosphate Buffered Saline (PBS) and CCK-8 solution were purchased from Yuli Biotechnology Co., LTD (Shanghai, China). Human interleukin-1β (IL-1β) detection kit and human interleukin-18 (IL-18) detection kit were purchased from Quanzhou Ruixin Biotechnology Co., LTD (Quanzhou, China). DiO (Cell membrane green fluorescent probe), DCFH-DA, Calcein AM/PI Live/Dead Viability/Cytotoxicity Assay Kit, Annexin V-FITC Apoptosis Detection Kit, DNA Damage Assay Kit by γ-H2AX Immunofluorescence, Enhanced ATP Assay Kit and LDH Release Assay Kit were purchased from Beyotime Biotechnology Co., LTD (Shanghai, China). BODIPY 505/515 was purchased from MCE (USA). Cleaved Caspase-1 rabbit antibody (D57A2), β-Actin (13E5) rabbit mAb and HRP-linked antibody (Anti-rabbit IgG) were purchased from Cell Signaling Technology (CA, USA). Anti-cleaved N-terminal GSDMD rabbit monoclonal antibody (ab215203) purchased from Abcam (Cambridge, UK).

4. Synthesis routes of DPP-QS



Scheme S1. Synthesis routes of **DPP-QS** in this work.

4.1 Synthesis of SF-1

Chlorambucil (1.52 g, 5 mmol), 4-dimethylaminopyridine (DMAP, 39 mg, 0.32 mmol), dicyclohexylcarbodiimide (DCC, 1.69 g, 8.2 mmol) were dissolved in ultra-dry dichloromethane (DCM,

10 mL) and stirred at room temperature for 30 min. p-Hydroxybenzaldehyde (649 mg, 5.3 mmol) dissolved in ultra-dry dichloromethane (10 mL) was added slowly by dropwise addition and stirred at room temperature for another 24 hours. When the reaction was complete based on TLC analysis, the mixture was filtered and the filtrate was concentrated under reduced pressure. The raw product was diluted with dichloromethane (35 mL), washed consecutively with aqueous acetic acid (35 mL, 1 M) and water (35 mL), dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography (silica gel, petroleum ether/ dichloromethane = (v/v) 2:1) to provide the compound **SF-1** (1.50 g, 73.5%) as a yellow syrup. ^{1}H NMR (400 MHz, CD_2Cl_2) δ: 9.97 (s, 1H), 7.93 – 7.88 (m, 2H), 7.29 – 7.22 (m, 2H), 7.14 – 7.08 (m, 2H), 6.71 – 6.65 (m, 2H), 3.73 – 3.62 (m, 8H), 2.61 (dt, J = 20.3, 7.5 Hz, 4H), 2.02 (m, J = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ: 191.23, 171.76, 155.84, 144.74, 134.40, 131.38, 130.85, 130.05, 122.79, 112.77, 53.89, 41.05, 34.20, 33.93, 26.96. HR EI-MS, m/z: $[M]^+$ calcd 407.1055, found: 407.1060.

4.2 Synthesis of SF-2

SF-1 (1.50 g, 3.68 mmol) were dissolved in ultra-dry tetrahydrofuran (THF, 10 mL), cooled down to 5 oC and stirred for 10 min. $NaBH_4$ (300 mg, 5.28 mmol) was added at below 5 ^{o}C and stirred for another 5 hours at room temperature. When the reaction was complete based on TLC analysis, the reaction was quenched by addition of aqueous NH_4Cl solution (10 mL, 1 M). The raw product was extracted with ethyl acetate (15 mL) and the organic layer was collected, dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography (silica gel, dichloromethane) to provide the compound **SF-2** (1.10 g, 73.1%) as a yellow syrup. ^{1}H NMR (400 MHz, CD_3Cl) δ: 7.37 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H), 7.04 (d, J = 8.5 Hz, 2H), 6.64 (d, J = 8.7 Hz, 2H), 4.67 (s, 2H), 3.74 – 3.67 (m, 4H), 3.66 – 3.59 (m, 4H), 2.65 (t, J = 7.5 Hz, 2H), 2.57 (t, J = 7.5 Hz, 2H), 2.03 (m, J = 7.5 Hz, 2H), 1.77 (s, 1H). 13C NMR (101 MHz, $CDCl_3$) δ: 172.18, 150.10, 144.43, 138.44, 130.31, 129.79, 128.08, 121.68, 112.19, 64.77,

53.60, 40.54, 33.95, 33.67, 26.73. HR EI-MS, m/z: $[M]^+$ calcd 409.1211, found: 409.1204.

4.3 Synthesis of SF-3

SF-2 (700 mg, 1.71 mmol) were dissolved in ultra-dry dichloromethane (DCM, 10 mL) and stirred at 5 ^oC for 10 min under argon protection. PBr_3 (0.2 mL) dissolved in ultra-dry dichloromethane (5 mL) was added slowly by dropwise addition and stirred at room temperature for another 12 hours. When the reaction was complete based on TLC analysis, the reaction was quenched by addition of saturated $NaHCO_3$ solution (10 mL). The raw product was extracted with dichloromethane (15 mL) and the organic layer was collected, dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography (silica gel, petroleum ether/ dichloromethane = (v/v) 2:1) to provide the compound **SF-3** (760 mg, 94.3%) as a colorless liquid. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.40 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H), 7.04 (t, J = 7.7 Hz, 2H), 6.64 (d, J = 8.6 Hz, 2H), 4.48 (s, 2H), 3.71 (t, J = 6.4 Hz, 4H), 3.62 (t, J = 6.4 Hz, 4H), 2.64 (t, J = 7.5 Hz, 2H), 2.57 (t, J = 7.4 Hz, 2H), 2.03 (m, J = 7.5 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 171.92, 150.60, 144.44, 135.28, 130.27, 130.23, 129.79, 121.96, 112.21, 53.61, 40.54, 33.94, 33.66, 32.75, 26.70. HR EI-MS, m/z: $[M]^+$ calcd 471.0367, found: 471.0367.

4.4 Synthesis of DPP-Q

Starting material 1 prepared by methods reported in the literature.[1,2] Compound 1 (1.47 g, 2.40 mmol), $Pd(PPh_3)_4$ (222 mg, 0.20 mmol) and K_2CO_3 aqueous solution (12 mL, 0.5 M) were dissolved in 15 mL THF. 4-Quinolineboronic acid (248 mg, 1.44 mmol) dissolved in 15 mL THF was added slowly by dropwise addition and stirred at 50 ^oC for another 6 hours under argon protection. When the reaction was complete based on TLC analysis, THF was removed by concentration under reduced pressure. The raw product was extracted with dichloromethane (30 mL) and water (30 mL) and the organic layer was collected, dried over Na2SO4 and concentrated in vacuum. The residue was purified by column chromatography

(silica gel, petroleum ether/ ethyl acetate = (v/v) 10:1 → 2:1) to provide the **DPP-Q** (600 mg, 37.7%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.99 (d, J = 4.4 Hz, 1H), 8.22 (d, J = 8.6 Hz, 1H), 8.02 – 7.94 (m, 3H), 7.80 – 7.67 (m, 7H), 7.55 (m, J = 8.3, 6.9, 1.3 Hz, 1H), 7.39 (d, J = 4.4 Hz, 1H), 3.80 (m, J = 30.6, 7.5 Hz, 4H), 1.64 (m, J = 22.4, 6.9 Hz, 4H), 1.25 (m, J = 12.8, 9.6, 5.7 Hz, 12H), 0.88 – 0.81 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 162.63, 162.58, 149.89, 148.60, 148.04, 147.43, 147.32, 140.81, 132.29, 130.14, 129.95, 129.67, 128.95, 128.29, 127.05, 126.99, 126.28, 125.84, 125.57, 121.26, 110.12, 110.08, 42.07, 42.00, 31.21, 29.47, 26.40, 22.48, 13.99, 13.96. HR EI-MS, m/z: $[M]^+$ calcd 661.2304, found: 661.2302.

4.5 Synthesis of DPP-QE

DPP-Q (663 mg, 1 mmol), $Pd(PPh_3)_4$ (69 mg, 0.06 mmol) and K_2CO_3 aqueous solution (5 mL, 0.5 M) were dissolved in 10 mL THF. Bis(biphenyl-4-yl)[4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)phenyl]amine (640 mg, 1.2 mmol) dissolved in 5 mL THF was added slowly by dropwise addition and stirred at 75 °C for another 12 hours under argon protection. When the reaction was complete based on TLC analysis, THF was removed by concentration under reduced pressure. The raw product was extracted with dichloromethane (30 mL) and water (30 mL) and the organic layer was collected, dried over Na_2SO_4 and concentrated in vacuum. The residue was purified by column chromatography (silica gel, dichloromethane / ethyl acetate = (v/v) 1:0 → 50:1) to provide the **DPP-QE** (720 mg, 73.5%) as a red solid. ^{1}H NMR (400 MHz, CD_2Cl_2) δ: 8.97 (d, J = 4.4 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 8.01 (m, J = 8.5, 4.3 Hz, 3H), 7.97 – 7.90 (m, 2H), 7.83 – 7.76 (m, 3H), 7.75 – 7.70 (m, 2H), 7.67 – 7.55 (m, 11H), 7.48 – 7.41 (m, 5H), 7.36 – 7.30 (m, 2H), 7.30 – 7.22 (m, 6H), 3.83 (m, J = 10.6, 7.5 Hz, 4H), 1.63 (h, J = 7.3, 6.7 Hz, 4H), 1.29 – 1.22 (m, 12H), 0.84 (m, J = 6.9, 1.8 Hz, 6H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ: 163.02, 162.82, 150.40, 149.20, 148.78, 148.14, 147.54, 147.40, 147.04, 143.50, 140.94, 140.83, 136.35, 134.09, 130.44, 130.36, 129.80, 129.77, 129.36, 129.22, 128.97, 128.33, 128.30, 127.41, 127.26, 127.08, 127.02, 126.69, 125.98, 125.23,

124.23, 121.76, 110.75, 110.19, 42.29, 42.18, 31.67, 31.65, 29.79, 26.78, 26.74, 22.91, 22.89, 14.19, 14.17. HR ESI-MS, m/z: $[M+H]^+$ calcd 979.4946, found: 979.4948.

4.6 Synthesis of DPP-QS

DPP-QE (196 mg, 0.2 mmol), **SF-3** (117 mg, 0.24 mmol) were dissolved in the mixture (toluene/acetonitrile = (v/v) 1:5, 12 mL) and stirred at 85 ^oC for another 12 hours under argon protection. When the reaction was complete based on TLC analysis (dichloromethane / methanol = (v/v) 50:1), the solution was removed by concentration under reduced pressure. The residue was successively added with 2 mL DCM and 10 mL petroleum ether, and the purple solid was filtered. After recrystallization twice, **DPP-QS** (60 mg, 20.7%) was obtained as a purple solid. ^1H NMR (400 MHz, CD_2Cl_2) δ: 10.69 (d, J = 6.1 Hz, 1H), 8.57 (d, J = 9.0 Hz, 1H), 8.18 (d, J = 8.5 Hz, 1H), 8.11 – 7.97 (m, 4H), 7.82 (d, J = 8.4 Hz, 2H), 7.76 (t, J = 7.7 Hz, 1H), 7.68 (d, J = 8.1 Hz, 4H), 7.56 – 7.46 (m, 12H), 7.34 (t, J = 7.7 Hz, 4H), 7.26 – 7.22 (m, 2H), 7.18 – 7.13 (m, 6H), 6.98 (d, J = 8.4 Hz, 4H), 6.71 (s, 2H), 6.62 – 6.48 (m, 2H), 3.80 – 3.69 (m, 4H), 3.68 – 3.43 (m, 8H), 2.50 (t, J = 7.5 Hz, 2H), 2.43 (t, J = 7.4 Hz, 2H), 1.87 (t, J = 7.5 Hz, 2H), 1.52 (t, J = 6.8 Hz, 4H), 1.19 – 1.12 (m, 12H), 0.78 – 0.72 (m, 6H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ: 172.20, 163.01, 162.58, 158.53, 151.72, 149.72, 148.14, 146.98, 146.01, 144.96, 143.65, 140.78, 138.84, 137.01, 136.36, 133.90, 131.39, 130.71, 130.61, 130.03, 129.93, 129.87, 129.73, 129.22, 128.58, 128.32, 128.29, 127.42, 127.00, 126.76, 125.27, 124.09, 122.98, 112.59, 111.47, 110.09, 64.61, 54.35, 41.20, 34.22, 33.89, 31.66, 31.64, 29.83, 29.72, 27.01, 26.76, 22.89, 14.20, 14.17. HR ESI-MS, m/z: $[M+H]^+$ calcd 1370.6051, found: 1370.6045.

5. Preparation of samples for UV absorption and fluorescence spectroscopy tests

Firstly, **DPP-QE** or **DPP-QS** were prepared by dissolving THF (AR) or dimethyl sulfoxide (DMSO, AR) to prepare a stock solution with a concentration of 1 mM. All ultraviolet-visible (UV-vis) absorption and

fluorescence spectroscopy tests were performed in ethanol/hexane or THF/PBS. To test the aggregation-induced emission properties of **DPP-QE**, different proportions of ethanol/hexane solutions were prepared to obtain 10 μM solutions with different hexane components (0 vol %, 10 vol %, 20 vol %, 30 vol %, 40 vol %, 50 vol %, 60 vol %, 70 vol %, 80 vol %, 90 vol %, 99 vol %). In extracellular ROS detection, **DPP-QE** or **DPP-QS** were dissolved in ethanol to form a solution with a concentration of 10 μM, and the final concentration of DPBF was 30 μM in the test.

6. *In vitro* monitoring esterase activity

DPP-QS was diluted into a mixture solvent of THF/PBS (v/v = 1/9, pH = 7.4) to give final concentration of 10 μM. Then the **DPP-QS** (10 μM) solution was incubated with esterase (0.01 $U \cdot mL^{-1}$) at 37 °C for different times, or **DPP-QS** (10 μM) was then incubated with different concentrations of esterase (0.001 $U \cdot mL^{-1}$ to 0.01 $U \cdot mL^{-1}$) for 60 min, and the fluorescence change was monitored in-situ. K_2CO_3, Mg_2SO_4, NaCl, NaClO, H_2O_2 (100 μM); Cys, GSH), HAS, BSA (50 μM); β-Gal, Trpsin, AchE, CE (0.1 $U \cdot mL^{-1}$), PLE (1 $U \cdot mL^{-1}$); CE (0.1 $U \cdot mL^{-1}$) with AEBSF (10 mM). λ_{ex} = 514 nm.

7. Calculation of LOD

The ratio of the corresponding maximum emission before and after the esterase reaction (I/I_0) was plotted as a function of esterase concentration for determine the limit of detection (LOD). LOD were calculated as 3δ/k (δ is the standard deviation of the maximum emission of eleven blank solutions, and k is the slope obtained after linear fitting the titration curves within certain ranges).

8. Validation of esterase activation mechanism

DPP-QS was diluted into a mixture solvent of THF/PBS (10 μm, v/v = 1/9, pH = 7.4), and then incubated with esterase (0.01 $U \cdot mL^{-1}$) for 60 min. An appropriate amount of reaction solution was diluted with methanol for HRMS analysis, and diluted with acetonitrile for HPLC analysis.

9. Cellular Studies

Cells of MIA PaCa-2, HUVEC and NIH/3T3 were cultured in DMEM-High glucose complete medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 g/mL streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. The cells were cultured in T25 cell culture flasks and grown to 80-90% confluence.

10. Confocal laser scanning microscopy (CLSM) imaging

The cell suspension (1 mL, 1×10^5) were plated in glass-bottomed petri dishes and allowed to adhere for 24 hours. To test the ability of **DPP-QS** to internalize in cells, MIA PaCa-2 cells were incubated with **DPP-QS** for 15 min, 30 min, 45 min, 60 min and 90 min, respectively. In order to study the specific activation of endogenous esterase on **DPP-QS**, MIA PaCa-2 cells were pretreated with AEBSF (1 mM, 2 mM, 4 mM, 8 mM) for 30 min before incubation with **DPP-QS**. To test the ability of **DPP-QS** to localize on cells, MIA PaCa-2, HUVEC and NIH/3T3 cells were incubated with **DPP-QS** (10 μM) for 15 min or 90 min, and stained with the membrane tracking agent DiO (5 μM) for 15 min or with BODIPY 505/515 (2 μM). Then the cells were washed three times with PBS and supplemented with fresh medium. CLSM images were acquired on a Leica Application Suite X scanning microscope using an oil immersion objective lens of 63× with the following parameters. Red channel for **DPP-QS**: λ_{ex} = 514 nm, λ_{em} = 575 - 750 nm; Green channel for DiO and BODIPY 505/515: λ_{ex} = 488 nm, λ_{em} =500 - 530 nm.

11. Cell Activity Assay

Cell suspensions (100 μL, 1×10^5) were inoculated in 96-well plates and incubated for 24 hours. Then, **DPP-QS** and chlorambucil were dissolved in fresh DEME to prepare solutions with different concentrations of 1.25 μM, 2.5 μM, 5 μM, 10 μM, and 15 μM. The above solutions replaced the old medium, and after 2 hours of incubation, for the light group, the cells were irradiated with white light (30 $mW \cdot cm^{-}$

^{2}) for 5 min or 10 min. After treatment for 24 hours, the cells were washed three times with PBS, and then incubated with CCK-8 solution (10 μM, 100 μL) at 37 °C for 1 hour, absorbance was measured at 450 nm using multifunctional microplate reader. The cell viability was determined using the following formula: Cells viability (%) = ($OD_{experiment} - OD_{blank}$) / ($OD_{experiment} - OD_{blank}$) × 100. where $OD_{experiment}$ is the OD value of **DPP-QS** or chlorambucil (1.25 μM ~ 15 μM) treated cells.

12. Live/dead cell co-staining method

Cell suspension (2 mL, 1×10^5) of MIA PaCa-2 was inoculated into 6-well plates and incubated for 24 hours. MIA PaCa-2 cells were incubated for another 24 hours with the following different conditions: Control, fresh medium; Chlorambucil, fresh medium containing 10 μM Chlorambucil; **DPP-QS**, fresh medium containing 10 μM **DPP-QS**; **DPP-QS**+L_1, fresh medium with 10 μM **DPP-QS** and followed by illumination (white light, 30 mW·cm^{-2})) for 5 min after 2 hours of incubation; **DPP-QS**+L_2, 10 μM **DPP-QS** in fresh medium and followed by illumination (white light, 30 mW·cm^{-2}) for 10 min after incubation for 2 hours. The Cells were stained with Calcein AM and PI according to the manufacturer's instructions. CLSM imaging was performed using the following parameters, Calcein AM: λ_{ex} = 494 nm, λ_{em} = 500 - 550 nm; PI: λ_{ex} = 535 nm, λ_{em} = 600 -700 nm.

13. Quantification of apoptosis/necrosis by flow cytometry

The cell sample processing method is as above. Cells were stained with Annexin V-FITC and PI according to the manufacturer's instructions. The treated cells were subjected to flow cytometry analysis, and the data were processed with FlowJo software.

14. Intracellular detection of ROS

To test the ROS generation ability of **DPP-QS** in cells, MIA PaCa-2 cells was incubated with **DPP-QS** for 2 hours. Then wash the cells three times with PBS and incubate with medium containing DCFH-DA (10

μM) for 30 minutes. Then the cells were washed three times with PBS and supplemented with fresh medium, followed by illumination (white light, 30 mW·cm^{-2}) for different time (0 min, 5 min, 10 min). CLSM imaging was performed using the following parameters, DCFH-DA: λ_{ex} = 488 nm, λ_{em} = 500 - 550 nm.

15. SEM imaging of Cell samples

Cell suspension (1 mL, 1 × 10^5) of MIA PaCa-2 was inoculated into 12-well plates fitted with silicon slice and incubated for 24 hours. The cells were cultured with the **DPP-QS** for 2 hours and followed by illumination (white light, 30 mW·cm^{-2}) for different time (0 min, 5 min, 10 min). After treatment, cells were rinsed with PBS and fixed with 2.5% glutaraldehyde at 37 °C for 20 min. Then the cells were dehydrated sequentially with 30%, 50%, 70%, 80%, 90%, 100% and 100% ethanol for 5 min, followed by de-ethanolisation with isoamyl acetate for 10 min and dried in a desiccator. The cells were observed under a field emission scanning electron microscope.

16. γ-H2AX Immunofluorescence Assay

Cell suspension (500 μL, 1 × 10^5) of MIA PaCa-2 was inoculated into 24-well plate and incubated for 24 hours. MIA PaCa-2 cells was incubated for another 24 hours with the following different conditions: control, fresh medium; 5 μM **DPP-QS**; 10 μM **DPP-QS**; 15 μM **DPP-QS**; 10 μM chlorambucil; 10 μM **DPP-QS**+L (white light, 30 mW·cm^{-2}, 10 min). After cells were washed with PBS, the cells were treated according to the DNA Damage Assay Kit. CLSM imaging was performed using the following parameters, DAPI: λ_{ex} = 405 nm, λ_{em} = 410 - 460 nm; γ-H2AX: λ_{ex} = 495 nm, λ_{em} = 510 - 550 nm.

17. LDH, ATP and cytokine release assays

Cell suspension (2 mL, 1 × 10^5) of MIA PaCa-2 was inoculated into 6-well plates and incubated for 24 hours. MIA PaCa-2 cells was incubated with the following different conditions: Control, fresh medium; Chlorambucil, fresh medium containing 10 μM Chlorambucil; **DPP-QS**, fresh medium containing 10 μM

DPP-QS; **DPP-QS**+L_1, fresh medium with 10 μM **DPP-QS** and followed by illumination (white light, 30 $mW \cdot cm^{-2}$) for 5 min after 2 hours of incubation; **DPP-QS**+L_2, 10 μM **DPP-QS** in fresh medium and followed by illumination (white light, 30 $mW \cdot cm^{-2}$) for 10 min. The cell supernatants were collected 3 hours after treatment, ATP levels were measured using an ATP assay kit and LDH levels were measured using an LDH kit according to the manufacturer's instructions. Meanwhile, the levels of IL-18 and IL-1β in the supernatants were quantified by ELISA method.

18.Western blotting analysis

The cell sample processing method is as above. Extract total protein using lysis buffer, separate the extracted protein by SDS-PAGE, and then transfer it to a polyvinylidene fluoride (PVDF) membrane (Bio Rad). The membranes were blocked with TBS solution containing bovine saline albumin (5%) and incubated with primary antibodies (1: 1000 dilution, cleaved caspase-1, cleaved N-terminal GSDMD, β-actin) at room 4 ºC overnight. The membranes were washed 5 times with 1x TBST buffer and incubated with HRP-conjugated secondary antibodies (1: 3000 dilution) for 2 hours. A chemiluminescence system was used to observe immune response bands.

19. Establishment of tumor model

Animal experiments were performed according to the protocol approved by the Animal Care and Use Renji Hospital, School of Medicine, Shanghai Jiaotong University (license no. RJ2023-131A). The BALB/c nude female mice were obtained from Jiangsu Jicui Pharmachem Biotechnology Co. 2×10^6 Mia PaCa-2 cells were suspended in 100 μL PBS and injected into the right back of 6-week-old BALB/c nude female mice.

20. *In vivo* imaging

The tumor grew to ~ 500 mm^3 before imaging. **DPP-QS** (0.1 mM, 100 μL) was injected into tumor-bearing mice by peritumoral injection for fluorescence imaging. Fluorescence images were acquired at different

time post-injection (0, 2, 4, 6, 8, 12, 24, 48 hours). A tumor-bearing mice was euthanized after 8 hours injection, and tumors and organs were collected and imaged.

21. *In vivo* therapy

When the tumor size reached approximately 100 mm^3, mice were randomly divided into five groups (n = 4/group): PBS (100 μL) only, PBS (100 μL) + L, **DPP-QS** (0.1 mM, 100 μL) only, chlorambucil (0.1 mM, 100 μL) only, and **DPP-QS** (0.1 mM, 100 μL) + L. Wherein, light group with white light (30 $mW \cdot cm^{-2}$, 8 min). The mice were given peritumoral injection every third day, and the weight and volume of the mice were measured and recorded at the same time. The volume of the tumor was determined according to the equation $V = ab^2 \times 1/2$, where a and b were separately the longest and shortest diameters of the tumors. After 12 days of treatment, all mice were euthanized, and tumors and major organs were collected and histopathological immunofluorescence analysis was performed. Next, the tumors and organs were washed with PBS three times, fixed with 10% neutral buffered formalin solution, embedded in paraffin, and cut into sections. H&E, TUNEL and Ki67 immunohistochemistry staining were used to histopathological analyze the tumor sections under a light microscope. Finally, H&E staining was also performed for heart, liver, spleen, lung, and kidney.

22. Statistical Analysis

Data were expressed as mean ± standard deviation. Student's t test was used to evaluate the statistical significance. P values < 0.05 were regarded statistically significant (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

Results and Discussion

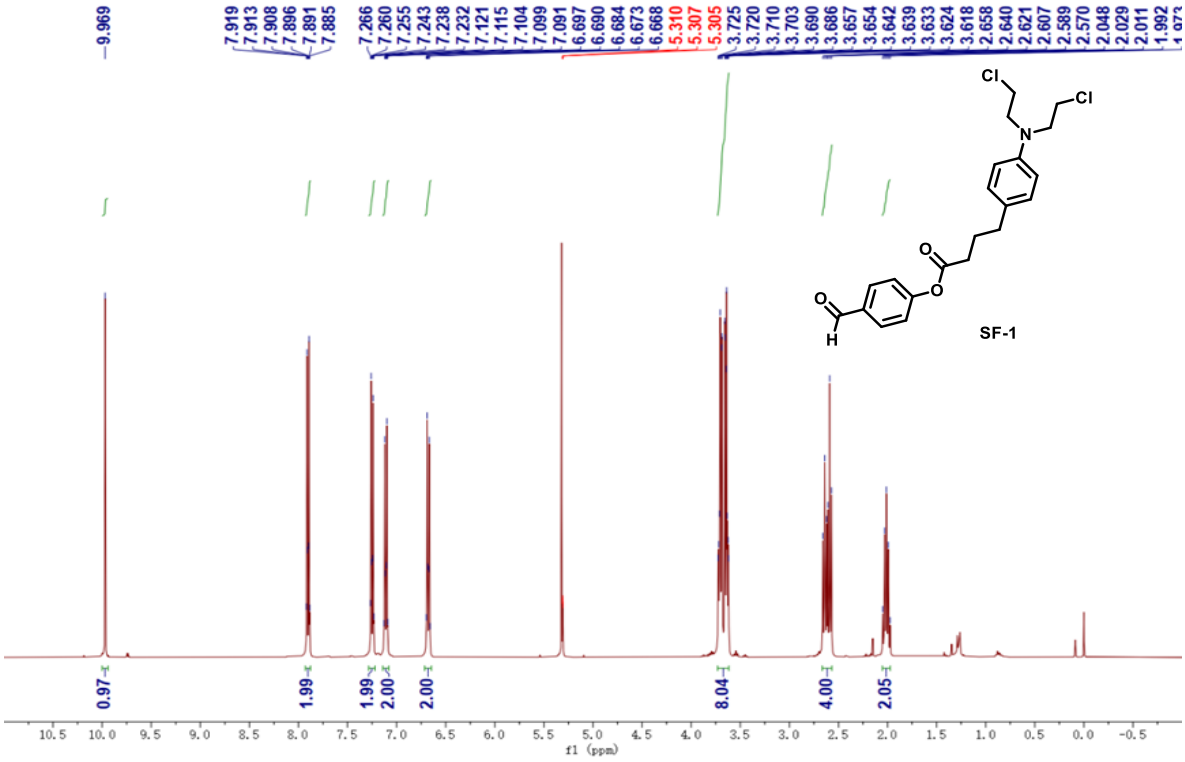


Fig. S1 ^{1}H NMR spectrum of **SF-1** (400 MHz, CD_2Cl_2).

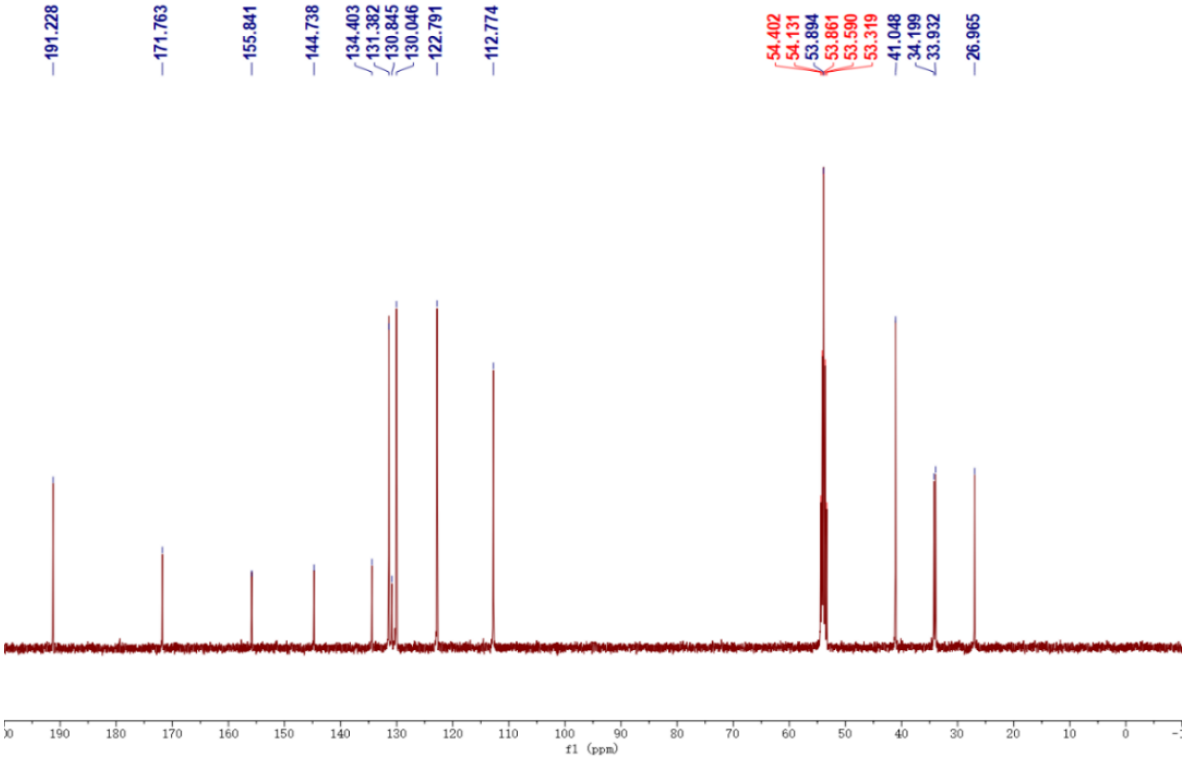


Fig. S2 ^{13}C NMR spectrum of **SF-1** (101 MHz, CD_2Cl_2).

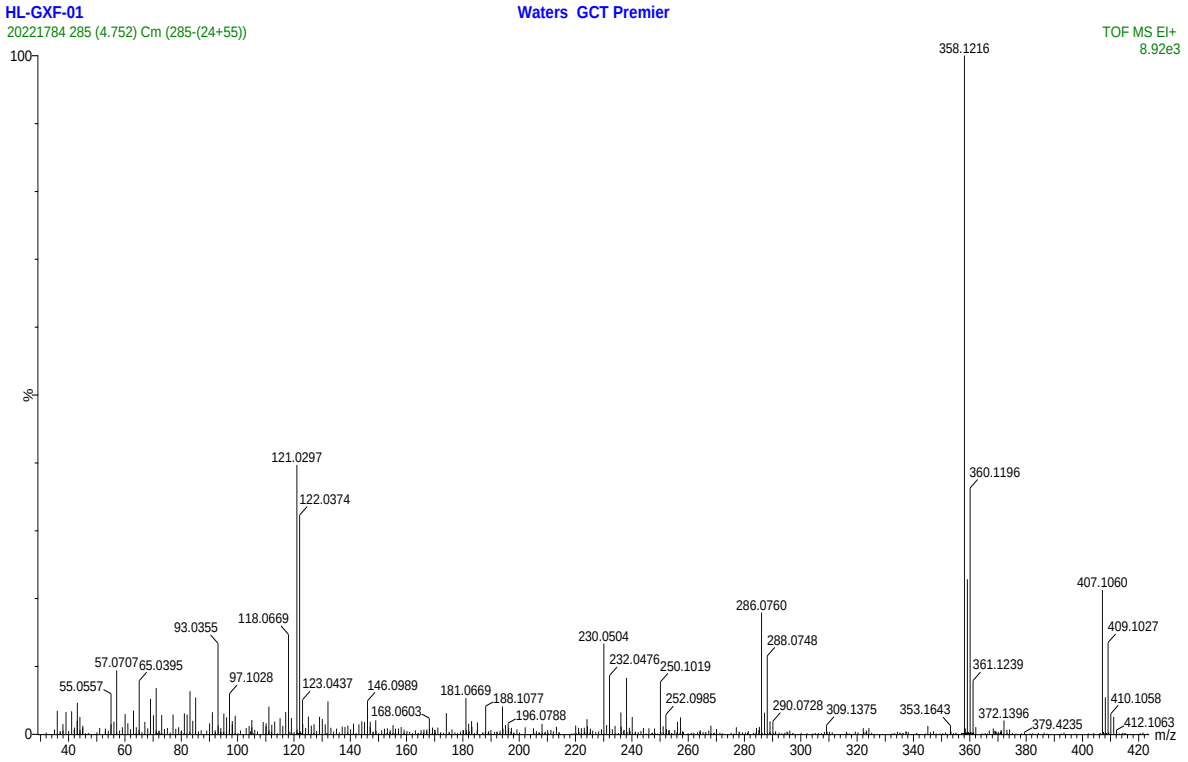


Fig. S3 Mass spectrum of **SF-1**.

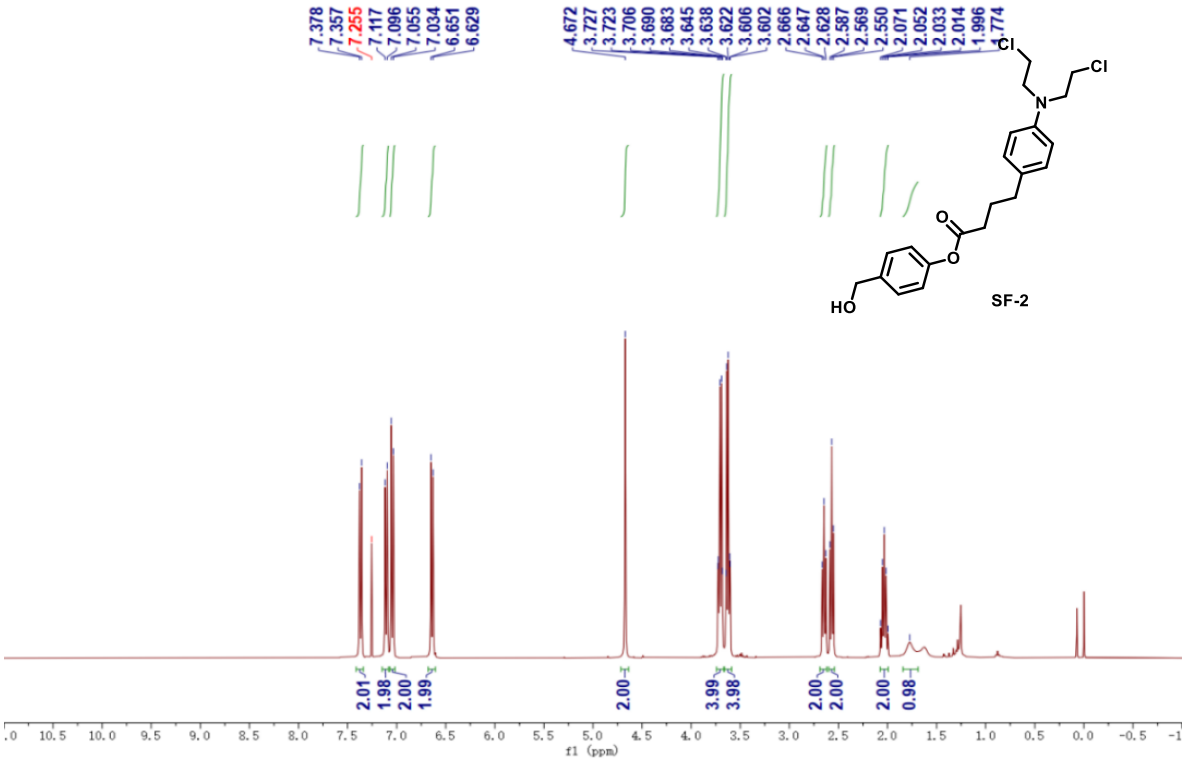


Fig. S4 ^{1}H NMR spectrum of **SF-2** (400 MHz, $CDCl_3$).

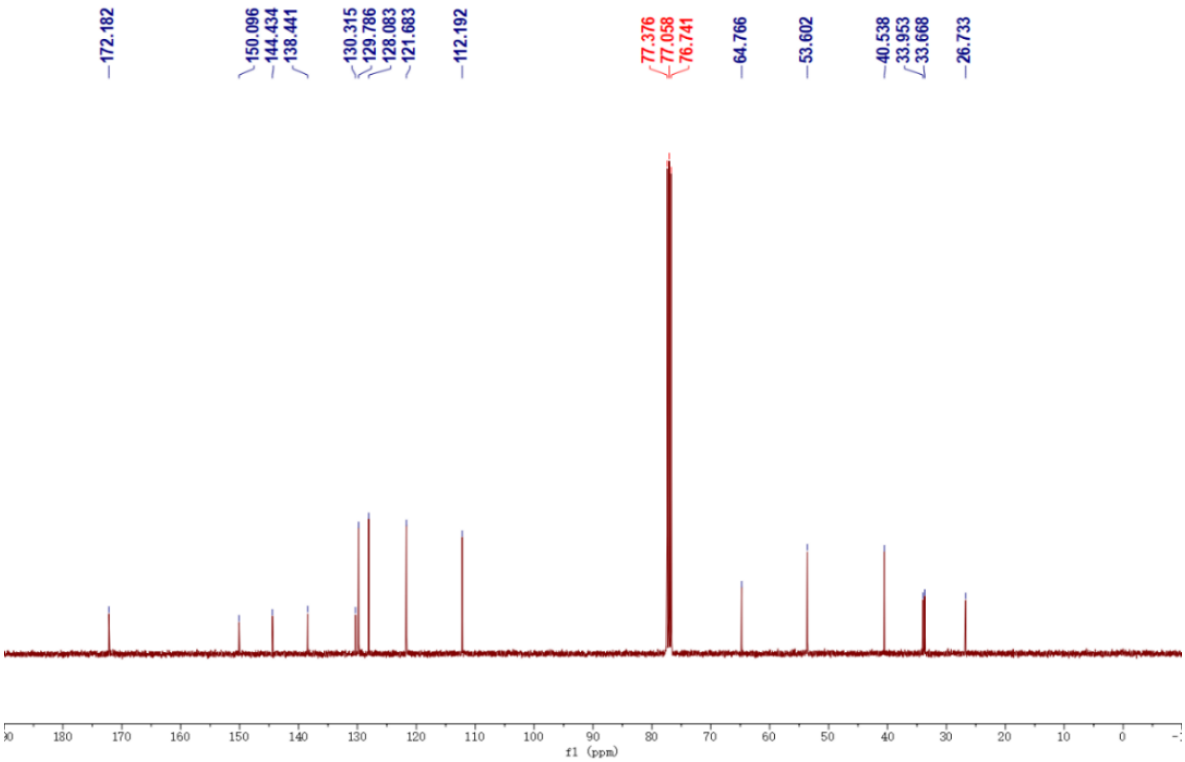


Fig. S5 ^{13}C NMR spectrum of **SF-2** (101 MHz, $CDCl_3$).

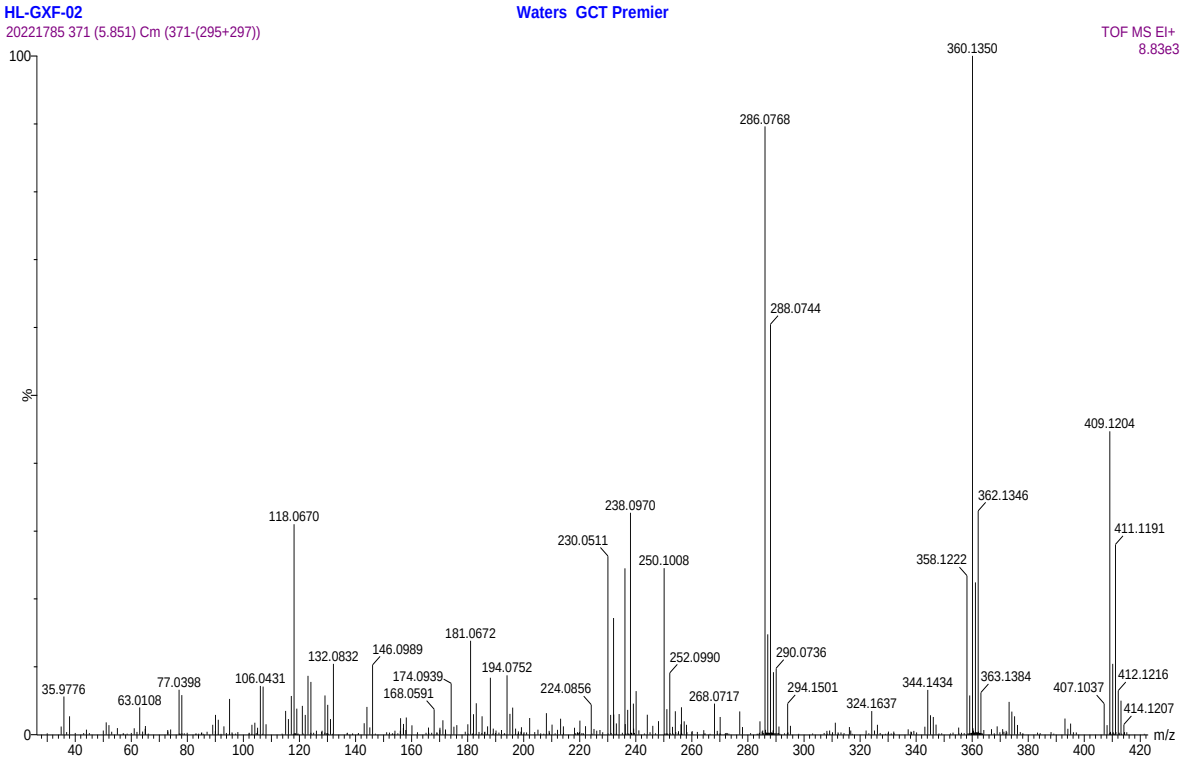


Fig. S6 Mass spectrum of **SF-2.**

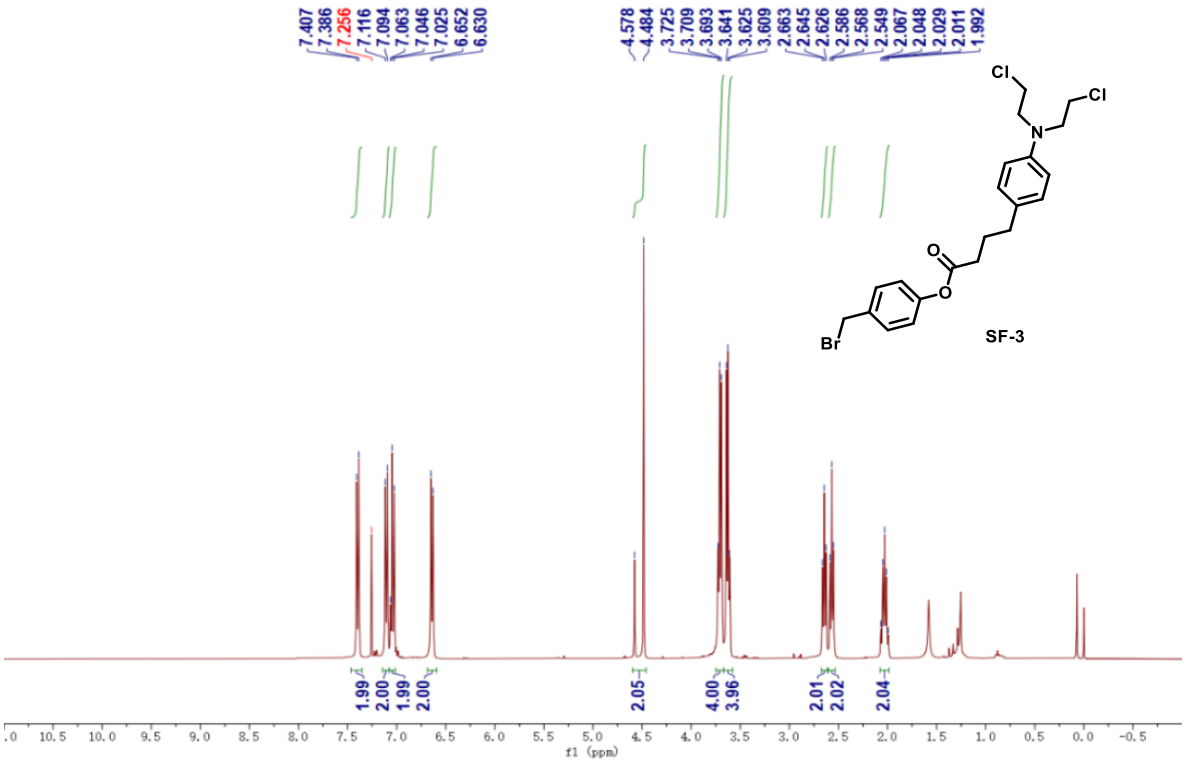


Fig. S7 ^{1}H NMR spectrum of **SF-3** (400 MHz, $CDCl_3$).

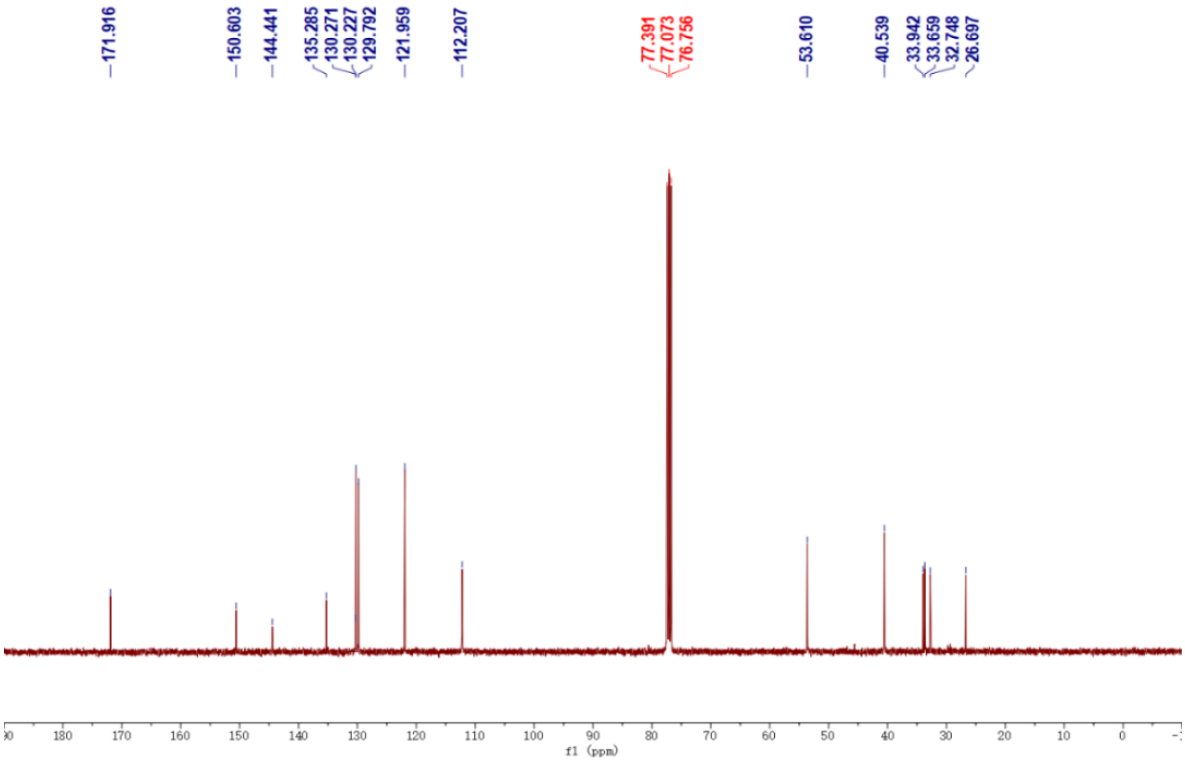


Fig. S8 ^{13}C NMR spectrum of **SF-3** (101 MHz, $CDCl_3$).

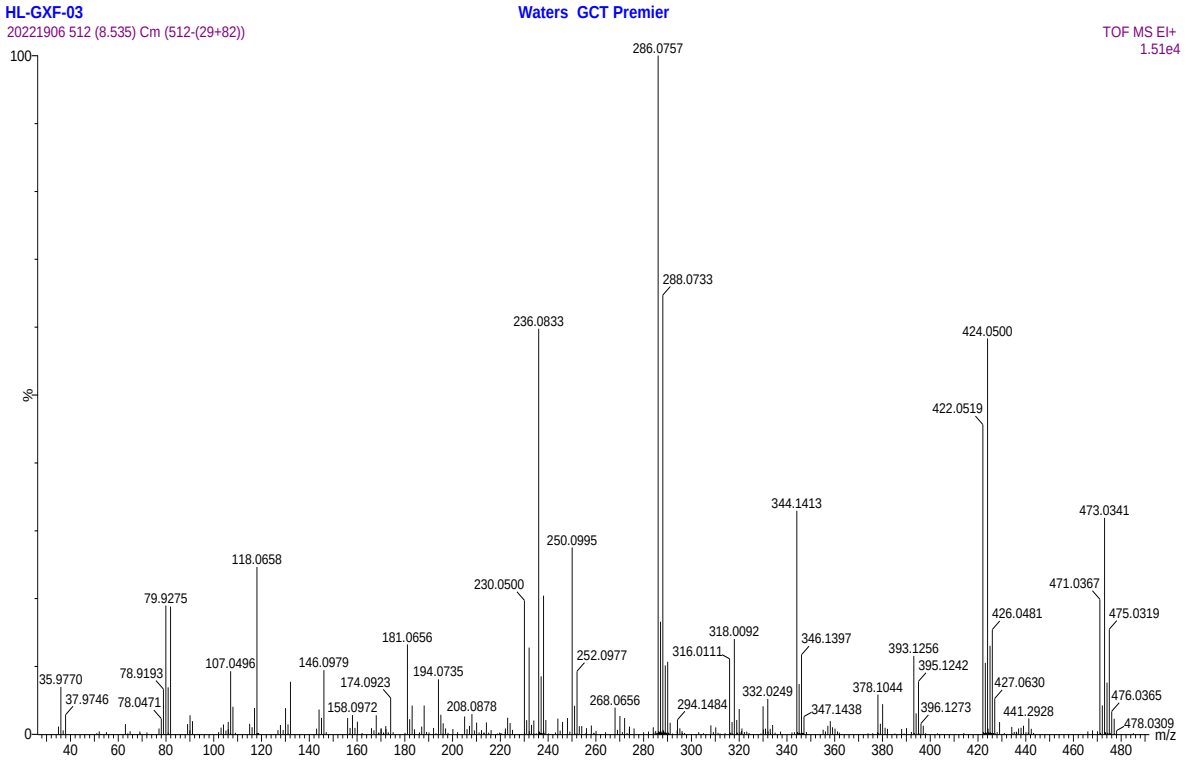


Fig. S9 Mass spectrum of **SF-3**.

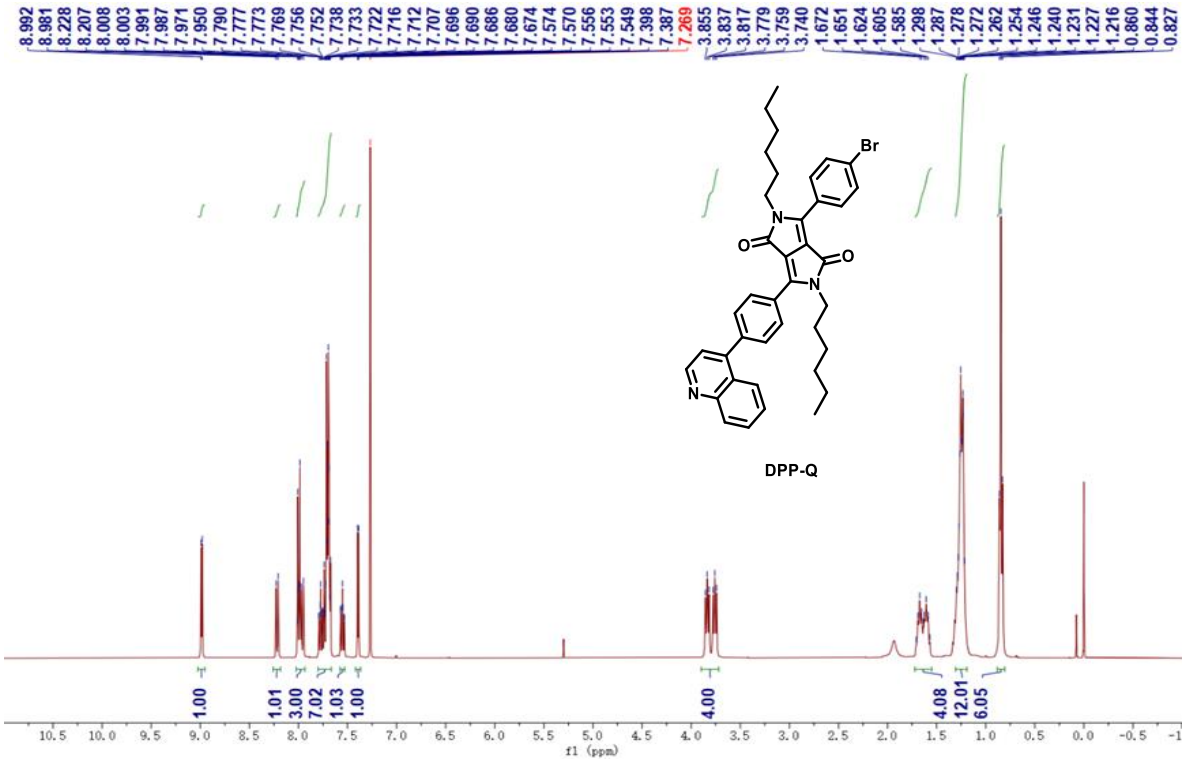


Fig. S10 ^{1}H NMR spectrum of **DPP-Q** (400 MHz, $CDCl_3$).

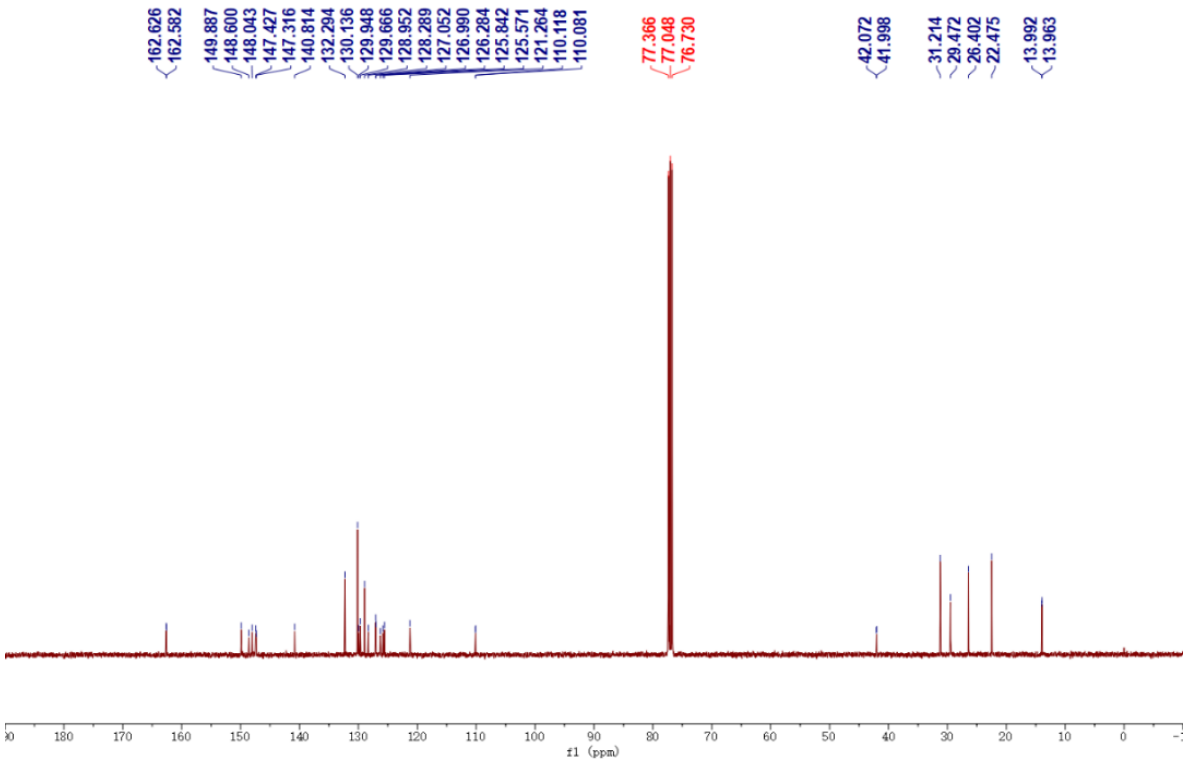


Fig. S11 ^{13}C NMR spectrum of **DPP-Q** (101 MHz, $CDCl_3$).

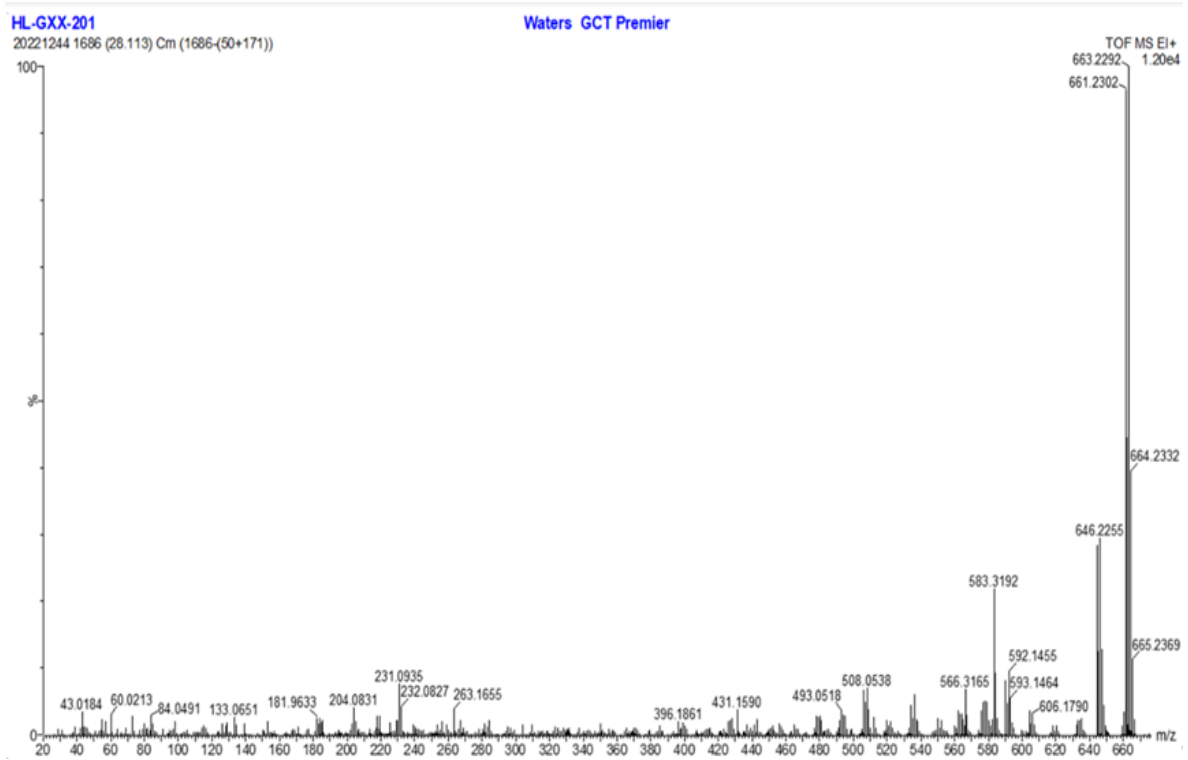


Fig. S12 Mass spectrum of **DPP-Q**.

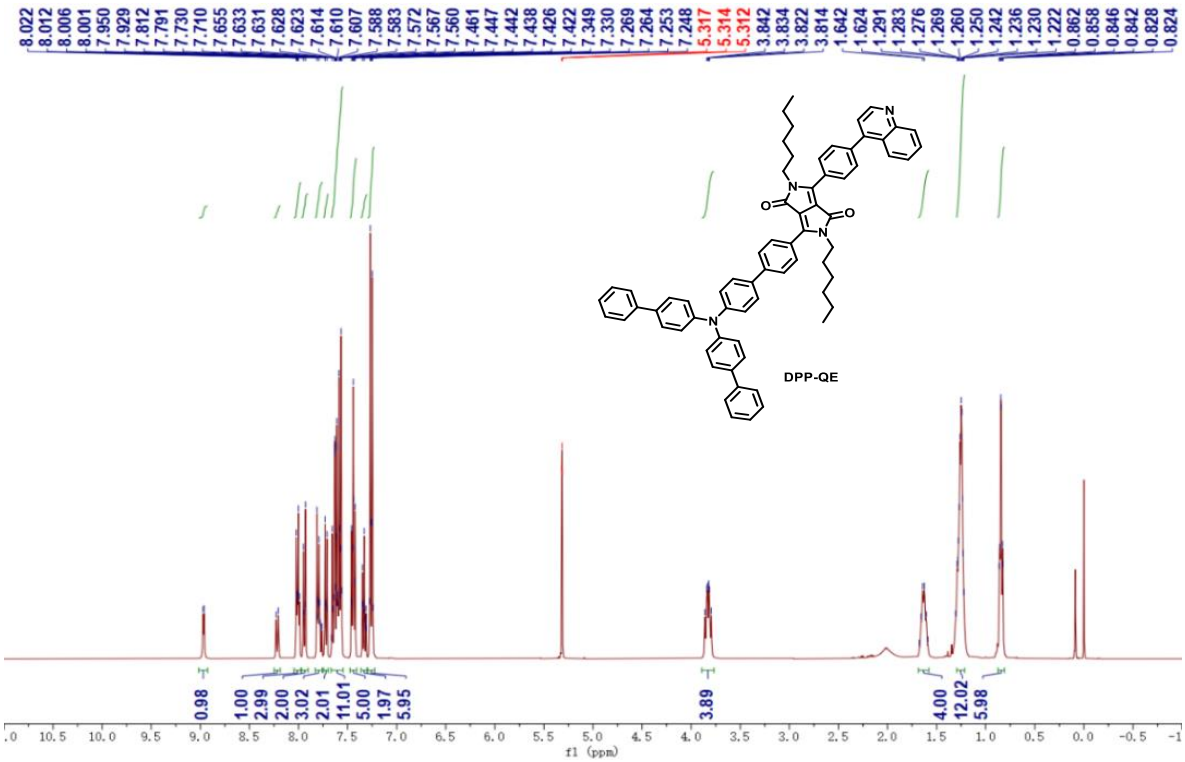


Fig. S13 ^{1}H NMR spectrum of **DPP-QE** (400 MHz, CD_2Cl_2).

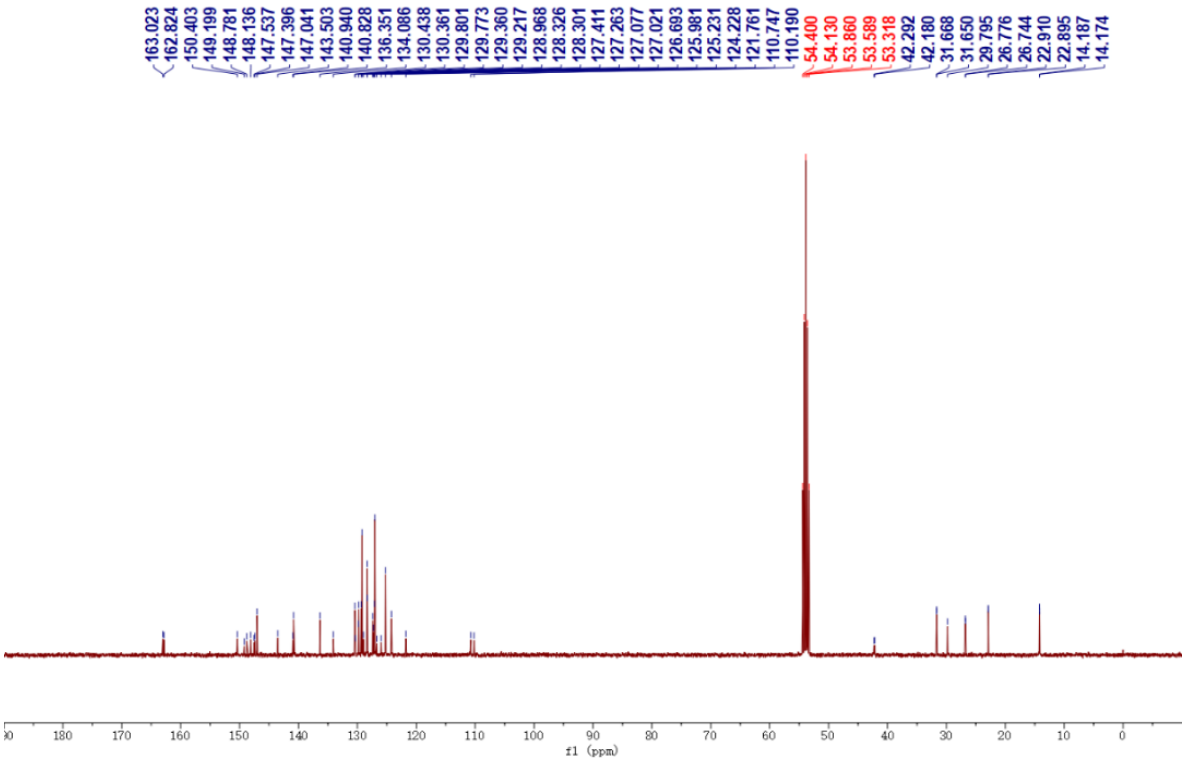


Fig. S14 ^{13}C NMR spectrum of **DPP-QE** (101 MHz, CD_2Cl_2).

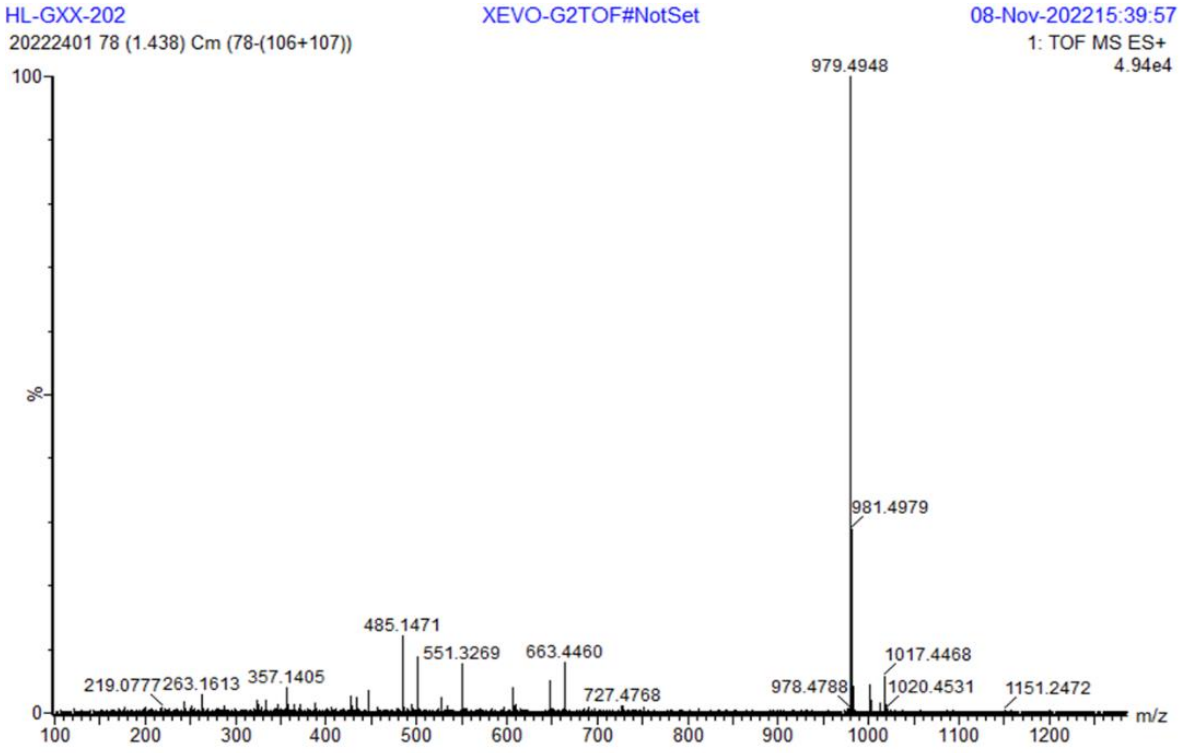


Fig. S15 Mass spectrum of **DPP-QE**.

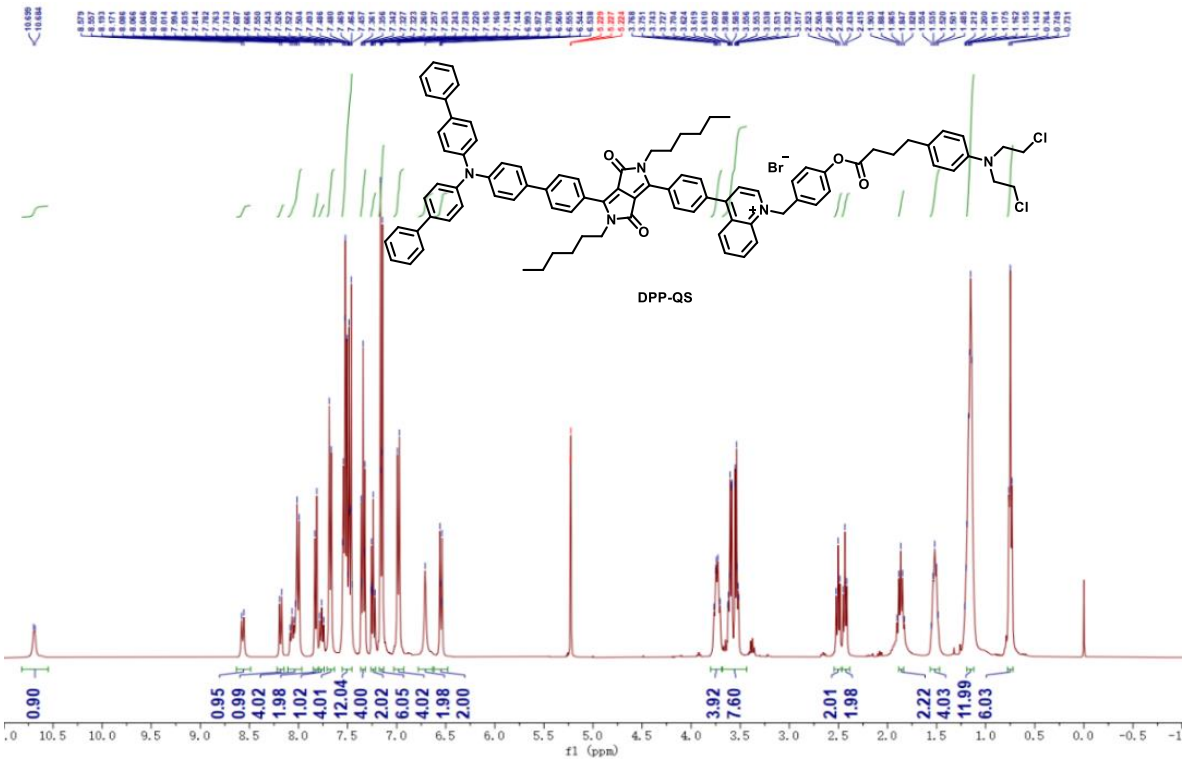


Fig. S16 ^{1}H NMR spectrum of **DPP-QS** (400 MHz, CD_2Cl_2).

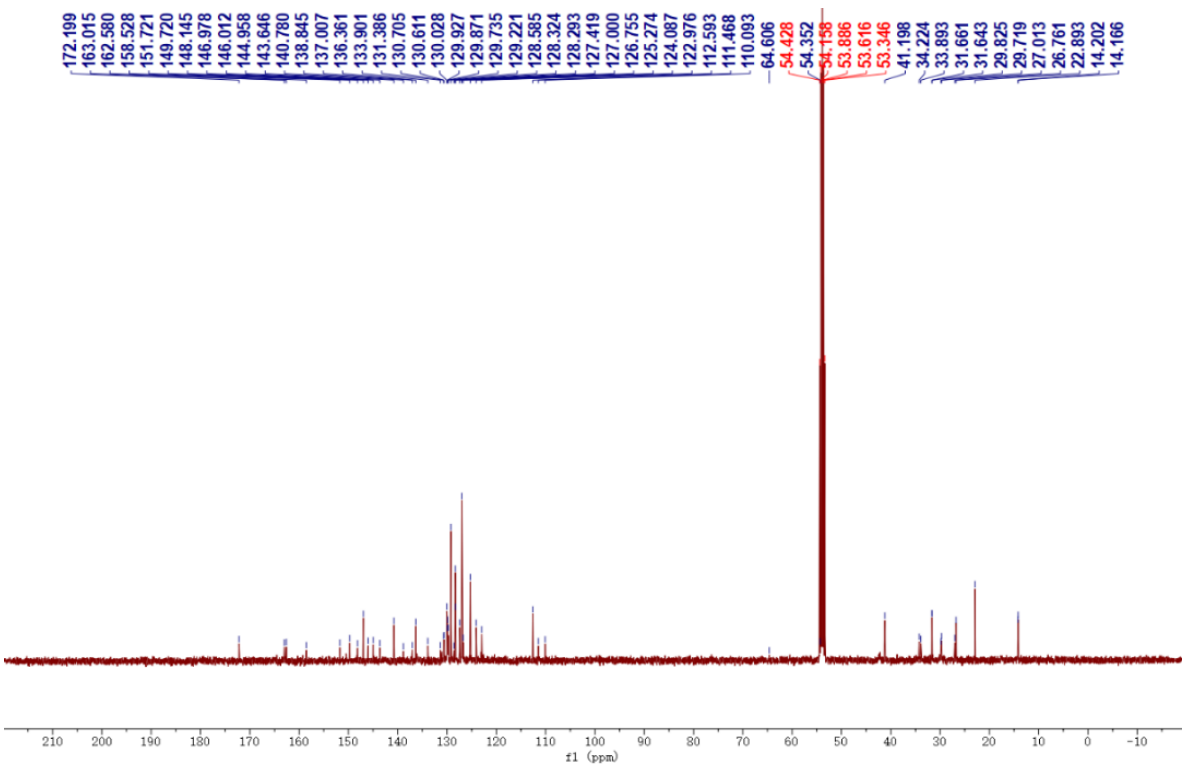


Fig. S17 ^{13}C NMR spectrum of **DPP-QS** (101 MHz, CD_2Cl_2).

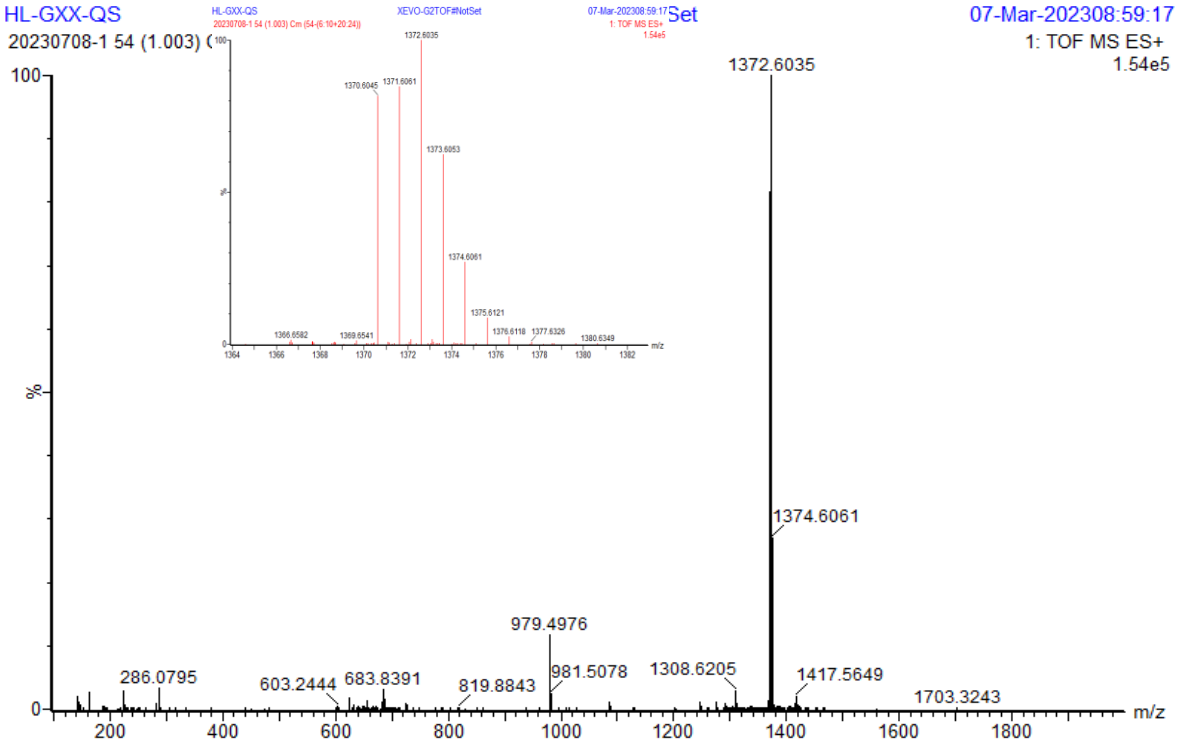


Fig. S18 Mass spectrum of **DPP-QS**.

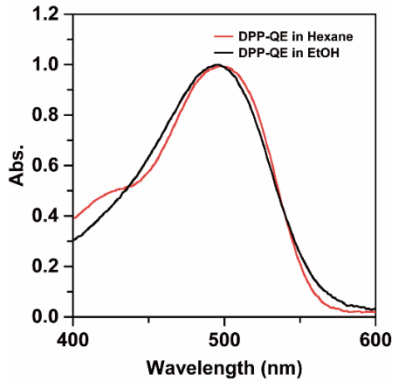


Fig. S19 Absorption spectra of **DPP-QS** and **DPP-QE** (10 μM) in Hexane or EtOH solution.

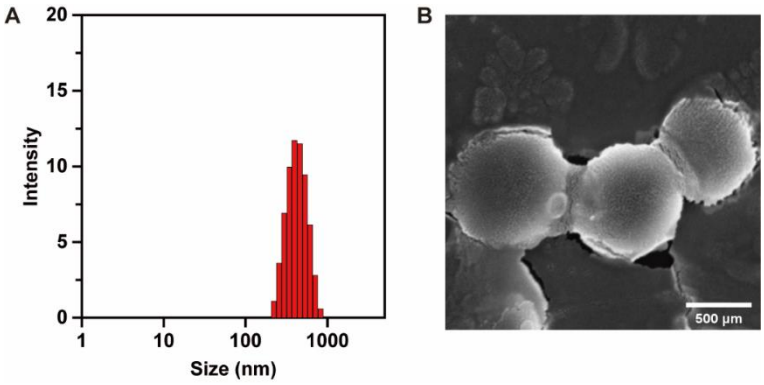


Fig. S20 (A) Particle size distribution and (B) SEM image of **DPP-QS** (10 μM) in EtOH/Hex (v/v = 1/99) solution.

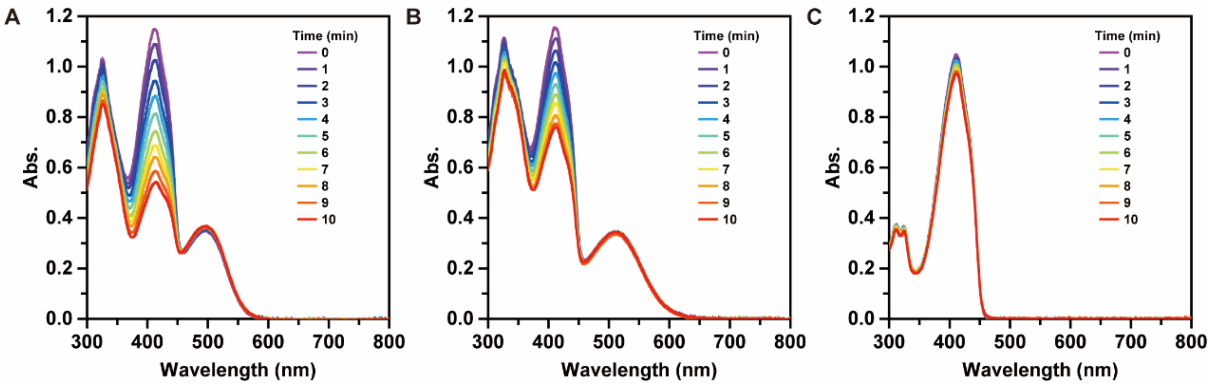


Fig. S21 Absorption spectra of DPBF (30 μM) in the presence of **DPP-QE** (A, 10 μM), **DPP-QS** (B, 10 μM) and Control (C, 0 μM) in EtOH under 530 nm laser irradiation with 30 mW·cm^{-2}.

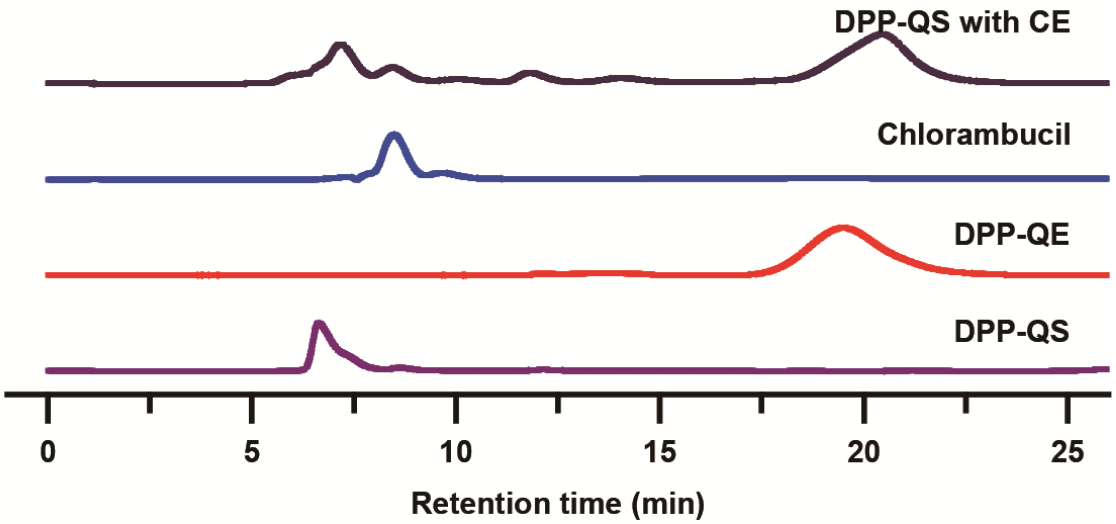


Fig. S22 HPLC analysis of **DPP-QS**, **DPP-QE**, chlorambucil, and **DPP-QS** with CE.

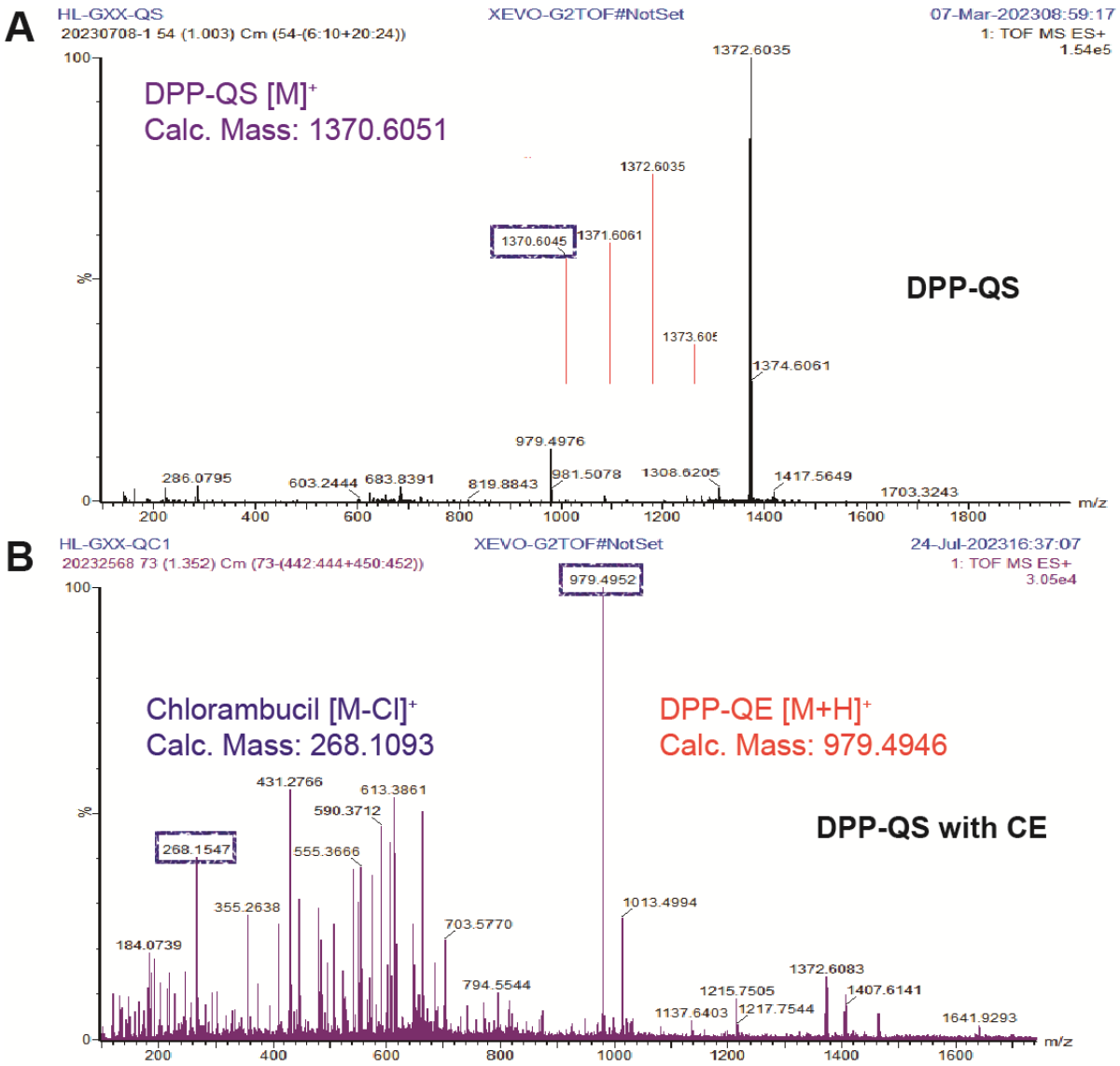


Fig. S23 HRMS analysis of **DPP-QS** (A) and **DPP-QS** with CE (B).

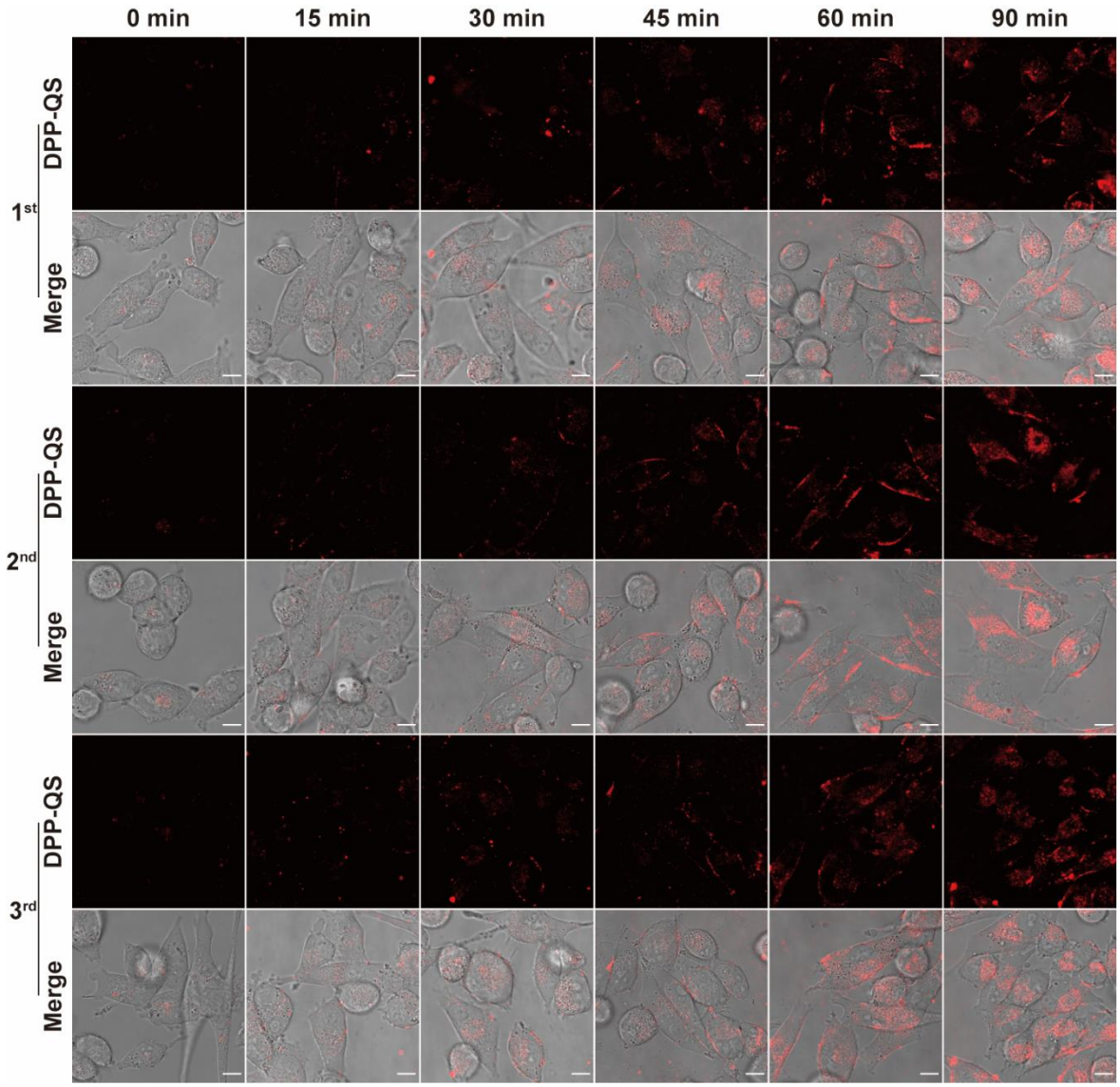


Fig. S24 Time-dependent CLSM images of Mia PaCa-2 cells stained with **DPP-QS** in three groups.

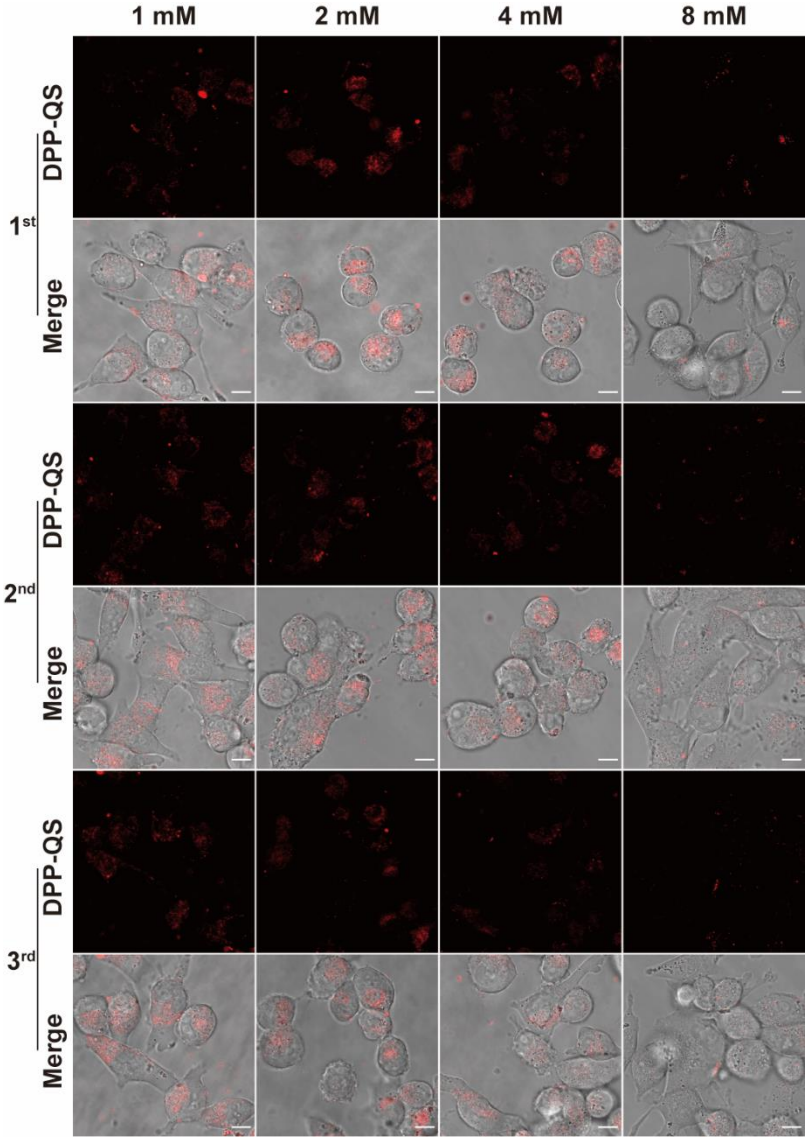


Fig. S25 AEBSF concentration inhibited CLSM images of Mia PaCa-2 cells stained with **DPP-QS** in three groups.

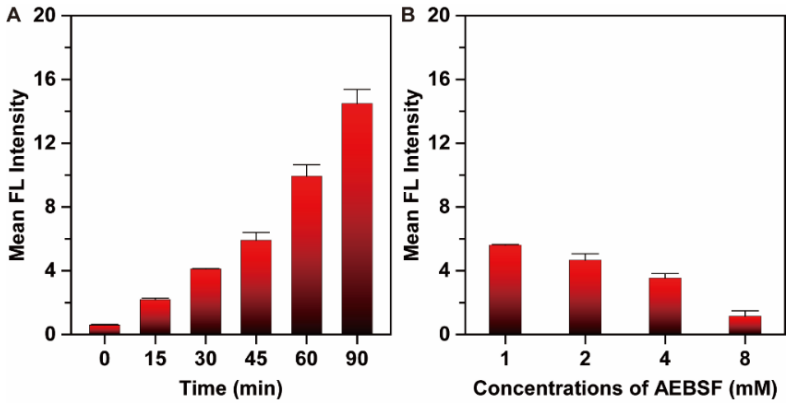


Fig. S26 (A) The Mean FL Intensity of Figure **S22**. (B) The Mean FL Intensity of Figure **S23**.

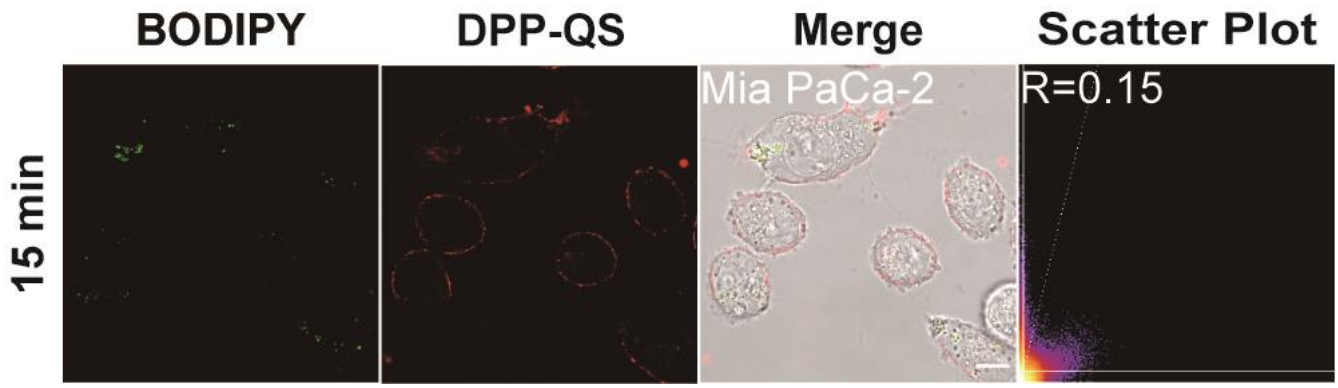


Fig. S27 Co-location of **DPP-QS** and BODIPY 505/515 in Mia PaCa-2 cells at 15 min. Scale bar: 10 μm.

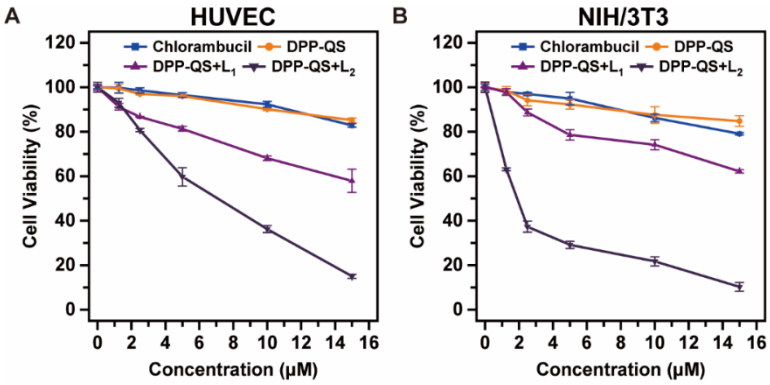


Fig. S28 Cell viability curves of NIH/3T3, HUVEC cells under different treatments. Data were expressed as mean ± standard error (n = 4).

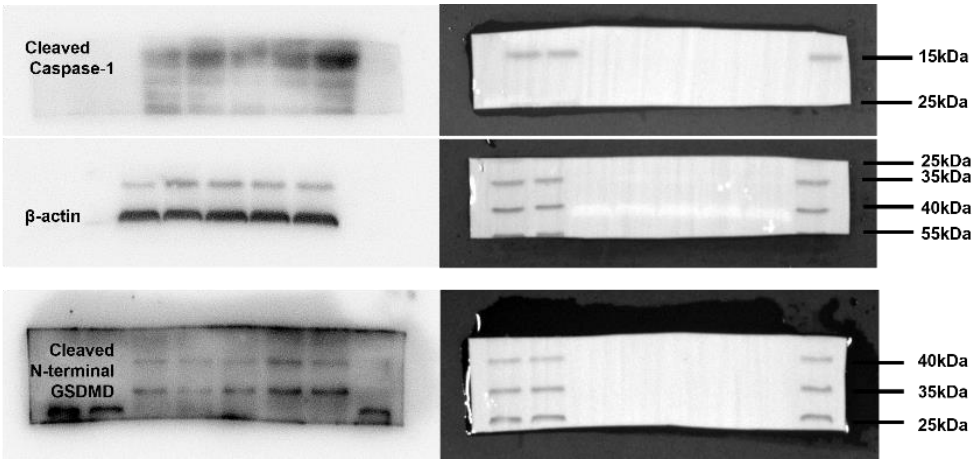


Fig. S29 The images of the full gel and blot.

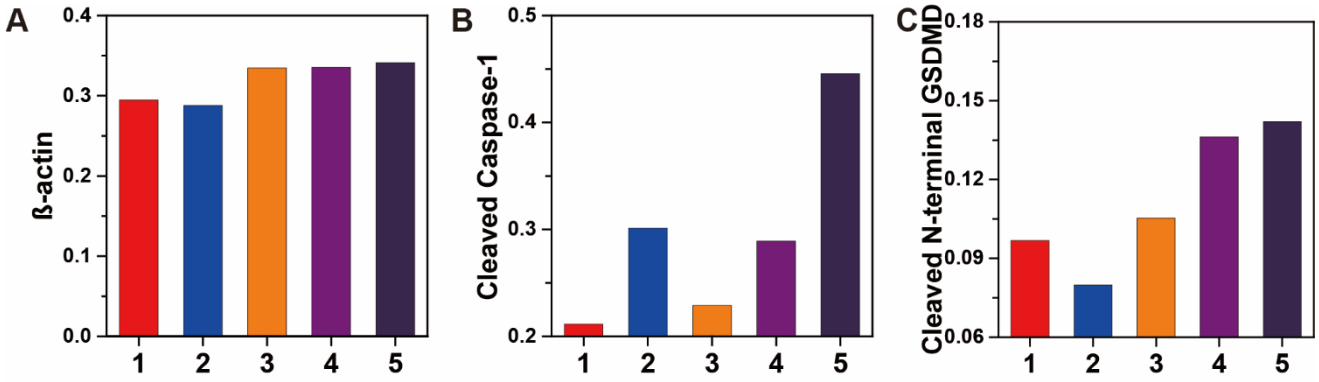


Fig. S30 Quantitative analysis results of Western blot. Group 1: control; Group 2: chlorambucil; Group 3: **DPP-QS**; Group 4: **DPP-QS**+L_1; Group 5: **DPP-QS**+L_2. L_1 refers to white light irradiation (30 mW·cm^{-2}) for 5 minutes, L_2 refers to white light irradiation (30 mW·cm^{-2}) for 10 minutes.

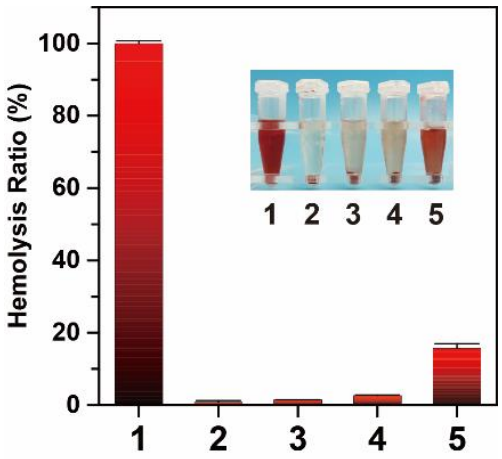


Fig. S31 Analysis of **DPP-QS** hemolysis rate. Group 1: Triton X-100; Group 2: DPP-QS, 25 μM; Group 3: DPP-QS, 50 μM; Group 4: DPP-QS, 100 μM; Group 5: DPP-QS, 200 μM. Data were expressed as mean ± standard error (n = 4).

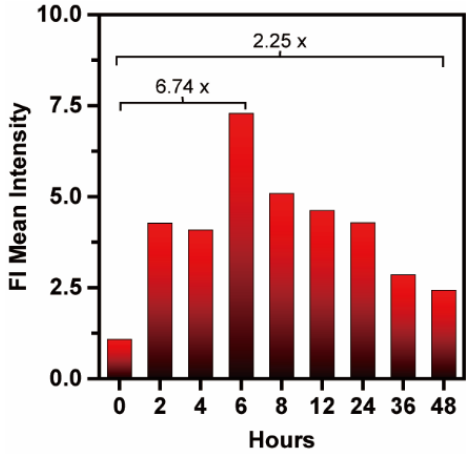


Fig. S32 Quantitative analysis of mean fluorescence intensity at the tumor site according to optical imaging at different times.

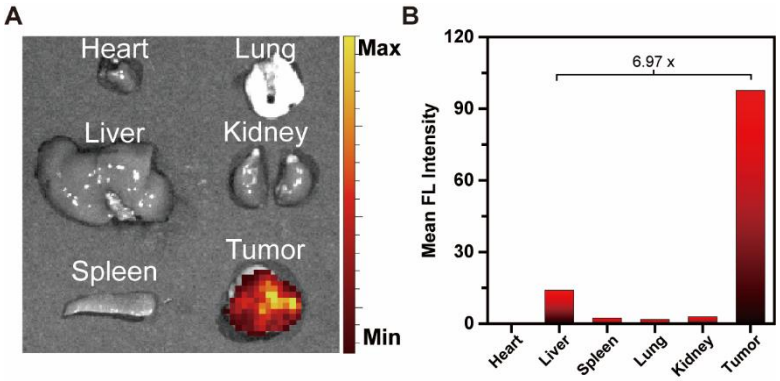


Fig. S33 (A) In vitro images of organs and tumor. (B) Quantitative analysis of mean fluorescence intensity of the organs at 8 hours.

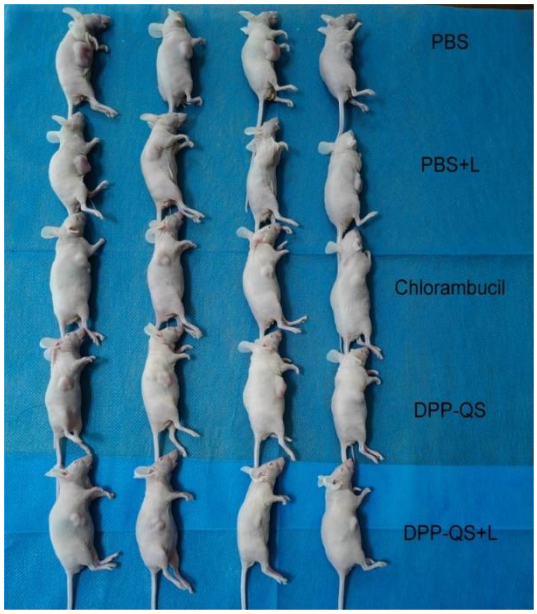


Fig. S34 Photos of mice at the end of treatment in different groups.

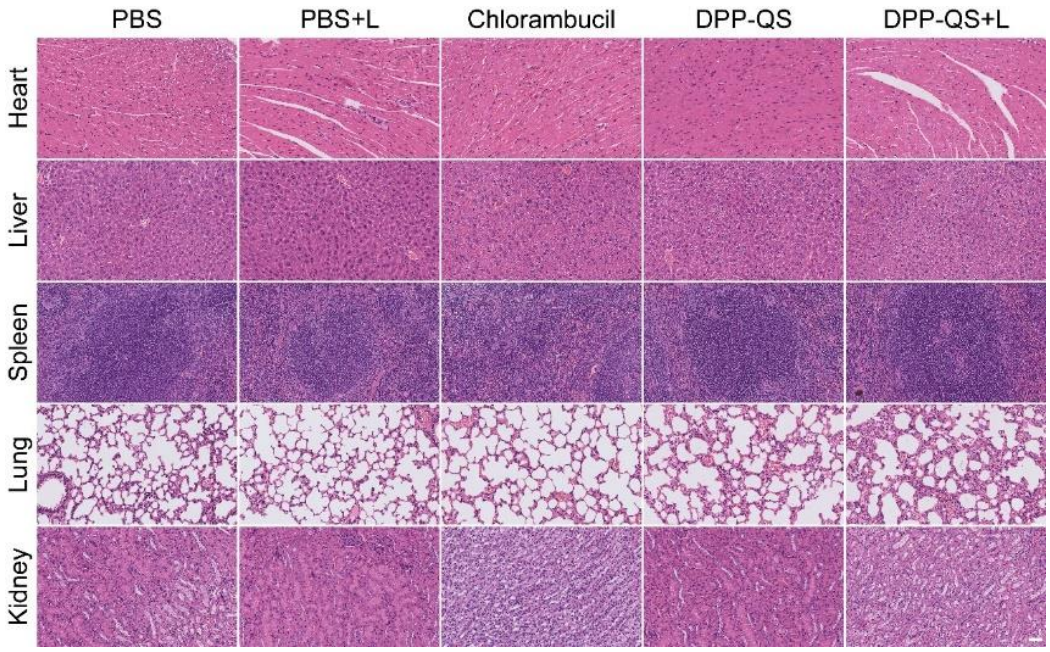


Fig. S35 H&E staining of major organs (heart, liver, spleen, lung, and kidney) after treatment period. Scale bars = 50 μm.

References

[1] Y. Gao, G. Feng, T. Jiang, C. Goh, L. Ng, B. Liu, B. Li, L. Yang, J. Hua, H. Tian, Biocompatible Nanoparticles Based on Diketo-Pyrrolo-Pyrrole (DPP) with Aggregation-Induced Red/NIR Emission for In Vivo Two-Photon Fluorescence Imaging, *Adv. Funct. Mater.*, 2015, **25**, 2857–2866.

[2] T. G. Hwang, G. R. Han, J. M. Lee, J. W. Lee, H. M. Kim, D. Hwang, S. K. Kim, J. P. Kim, Fluorescence Quenching of 4,4′-Dimethoxytriphenylamine-Substituted Diketopyrrolopyrrole via Intramolecular Photoinduced Electron Transfer, *J. Phys. Chem. C*, 2019, **123**, 24263–24274.