Supporting Information

for

Acceptor Planarized Type I Photosensitizer for Lipid Droplet-Targeted Two-Photon Photodynamic Therapy by Ferroptosis

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1. Experimental sections

1.1 Reagents and Apparatus

All commercial chemicals were used without further purification unless otherwise specified. 4,7-dibromo-2,1,3-benzothiadiazole, 7-bromo-2,1,3-benzothiadiazole-4-carboxaldehyde, 4-hydroxyphenylacetonitrile, [4-(diphenylamino)phenyl]boronic acid, 4-formylphenylboronic acid, (4-hydroxyphenyl)boronic acid, (4-bromophenyl)acetonitrile, sodium methoxide, tetrakis (triphenylphosphine)palladium(0), Rose Bengale (RB), 2’’,7’’-dichlorodihydrofluorescein diacetate (DCFH-DA), 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA), superoxide anion indicator (DHR123), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), K$_2$CO$_3$, 1,4-dioxane, MitoTracker Green, BODIPY 493/503, LysoTracker Green, phosphate-buffered saline (PBS) solution, Oxoid™ AnaeroGen™ 2.5 L Sachet and Oxoid™ Resazurin Anaerobic Indicator were obtained from commercial sources (Energy Chemistry, J&K, Innochem, Bioquest and Thermo Fisher Scientific). All organic solvents were purchase from Shanghai Titan Technology Co., LTD in analytical grade.

400 MHz $^1$H NMR spectra and 151 MHz $^{13}$C NMR spectra were acquired from a Bruker AV-400 spectrometer. High-resolution mass spectra-electrospray ionization (HRMS-ESI) were acquired from Thermo Scientific Q Exactive LC-MS/MS. UV–vis and fluorescence spectra were measured on Hitachi U-3900 absorbance spectrometer and Hitachi F-4500 fluorescence spectrometer, respectively. Two-photon spectra were measured using a Vitara-Legend Elite femtosecond titanium gemstone pulsed laser. The absorbance in cytotoxicity experiments was measured using a Spectra Max M5 enzyme-linked immunoassay. All cell imaging experiments were taken under Nikon A1 confocal microscope with two-photon femtosecond pulsed laser modules.

1.2 Synthesis

1.2.1 Synthesis of TBPCP
The photosensitizer TBPCP was synthesized by a specific synthetic route from 4,7-dibromo-2,1,3-benzothiadiazole (Scheme S1). Therein, as reported in previous literature, compound 1 [1] and compound 2 [2] were synthesized by Suzuki Reaction simply. Following, compound 2(300mg, 0.62 mmol) and 4-hydroxyphenylacetonitrile (83 mg, 0.62 mmol) were dissolved in anhydrous ethanol (15 mL), then CH$_3$ONa (50 mg) was added into the mixture after 5 minutes of stirring. After stirring at room temperature for 48 h, ethanol was removed under reduced pressure and the crude product was purified by silica gel chromatography with petroleum ether/ethyl acetate (v/v, 10:1) to afford red powder (247 mg, 67%). $^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.98 (s, 1H), 8.18 (d, $J = 7.2$ Hz, 2H), 8.05 (s, 3H), 7.98 (d, $J = 7.4$ Hz, 2H), 7.92 (s, 2H), 7.63 (d, $J = 7.4$ Hz, 2H), 7.36 (s, 4H), 7.11 (s, 8H), 6.91 (d, $J = 7.4$ Hz, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 158.71, 153.4, 153.3, 147.6, 146.9, 138.9, 138.2, 133.8, 132.3, 130.4, 130.2, 129.7, 129.4, 129.0, 128.7, 127.3, 124.6, 123.6, 122.2, 118.2, 116.0, 110.5. HRMS-ESI (m/z): calcd. for C$_{39}$H$_{25}$N$_4$OS [M-H]$^+$: 597.1749; found: 597.1757.

1.2.2 Synthesis of TBCP

The photosensitizer TBCP was synthesized by a simply synthetic route from 7-bromo-2,1,3-benzothiadiazole-4-carboxaldehyde. First of all, compound 3 was synthesized by Suzuki Reaction as reported literature [3]. Then Compound 3 (407 mg, 1 mmol), 4-hydroxyphenylacetonitrile (160 mg, 1.2 mmol) and t-BuOK (134 mg, 1.2 mmol) were successively added to anhydrous EtOH (20 mL) in a round bottomed flask in N$_2$ atmosphere, which was stirred at 80 °C for 12 h. After cooling to room temperature, the reactants are extracted and solvents was removed under reduced pressure, then the crude product was purified by silica gel chromatography with petroleum ether/ethyl acetate (v/v, 10:1) to afford dark red powder (210 mg, 40%). $^1$H NMR (600 MHz, DMSO-$d_6$) δ 10.09 (s, 1H), 8.47 (d, $J = 6.6$ Hz, 1H), 8.33 (s, 1H), 8.01 (d, $J = 6.8$ Hz, 3H), 7.66 (d, $J = 7.2$ Hz, 2H), 7.37 (s, 4H), 7.10 (d, $J = 21.1$ Hz, 8H), 6.94 (d, $J = 7.1$ Hz, 2H). $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 159.1, 154.1, 152.3, 147.9, 146.6, 133.8, 132.5, 130.3, 129.7, 129.5, 127.5, 126.8, 125.0, 124.7, 124.2,
13.7, 121.7, 117.8, 116.15, 112.29. HRMS-ESI (m/z): calcd. for C_{33}H_{22}N_{4}OSNa [M+Na]^+: 545.1412; found: 545.1407.

1.3 Characterizations of spectroscopic properties of photosensitizers

For absorption and fluorescence test, TBPCPP stocks in different solvents (1×10^{-3} M) were diluted to 10 μM separately and then tested with a quartz cuvette to measure fluorescence using the appropriate excitation wavelength.

The two-photon cross section was calculated according to published methods [4], and TBPCP and TBCP were dissolved in DMSO to obtain 1×10^{-3} M solution to be tested. Two-photon excited fluorescence (TPEF) spectra were measured using femtosecond laser pulse and Ti: sapphire system (700-1060 nm, 80 MHz, 140 fs) as the light source. Using a fiber optic spectrometer (Ocean Optics USB2000 CCD) as the detector, the fluorescence spectrum was recorded in a direction perpendicular to the laser beam. Rhodamine B in methanol as reference. The TPA cross-section (σ) values of the sample were calculated using the following equation:

\[ \delta_s = \frac{F_s}{F_r} \times \frac{\varphi_r}{\varphi_s} \times \frac{\eta_r}{\eta_s} \times \frac{C_r}{C_s} \delta_r \]

where the subscripts R and S stand for the reference and sample, respectively. F is the integral area of the fluorescence, ϕ is the fluorescence quantum yield, η is the overall fluorescence collection efficiency of the experimental apparatus and the default value is 1, and c is the concentration.

1.4 Detection of ROS in solution

1.4.1 Total ROS generation detection

Total ROS generation was measured using 2,7-dichlorofluorescein diacetate (DCFH-DA). Firstly, 5×10^{-2} mL DCFH-DA (1×10^{-2} M) in DMSO was activated by adding 2 mL NaOH (1×10^{-2} M) to form DCFH. After stirring for 30 minutes at room temperature, add 10 mL PBS (pH = 7.4) to neutralize the excess sodium hydroxide, and store the probe in the dark. Then, 200 μL DCFH (concentration: 50 μM) was mixed with the photosensitizer solution (1% DMSO in PBS, working concentration:
15 μM) to obtain 2 mL working solution and exposed to white light (400–700 nm, 50 mW/cm²). Meanwhile, the fluorescence intensities at ~525 nm were recorded immediately for every 10 seconds in the fluorescence spectrum. The control group was 2 mL PBS containing 200 μL DCFH. For DCFH-DA: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 508$–700 nm.

1.4.2 $^{1}$O$_{2}$ generation detection

9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) was used as the $^{1}$O$_{2}$ indicator agent. 200 μL of ABDA stock solution (1 mM in DMSO) was added to TBPCP and TBCP solutions (1% DMSO in PBS) to obtain 2 mL working solution respectively, then the mixed solution was exposed to white light (400–700 nm, 50 mW/cm²). The absorption of ABDA at 378 nm was recorded immediately for every 1 min in the absorbance spectrum to obtain the decay rate of the photosensitizers process. The control group was 2 mL PBS containing 200 μL ABDA stock solution.

1.4.3 Electron Spin Resonance (ESR)

ESR analysis was performed to monitor the generation of type I and type II ROS using 2,2,6,6-tetramethyl-4-piperidone hydrochloride (TEMP) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin trapping agents. For the detection of $^{1}$O$_{2}$, 10 μL of TEMP solution was added to 100 μL of the test solution (100 μM, DMSO), and then the solution was irradiated with 200 mW/cm² white light for 5 min and the spin spectra were monitored in the 3350–3650 G range. For $O_2^{-}$ detection, 10 μL of DMPO solution (0.2 M, DMSO) was added to 50 μL of the test solution (100 μM, DMSO), then the solution was irradiated with 200 mW/cm² white light for 5 min and the spin spectrum was monitored in the range 3350–3650 G. Controls were 2 mL of DMSO solution containing DMPO and TEMP, respectively, and background interferences were corrected with the samples before white light irradiation.

1.4.4 $O_2^{-}$ generation detection

Dihydrorhodamine 123 (DHR123) was used as indicator for detection of $O_2^{-}$ in
solution. When $O_2^-$ is generated in the detection system, DHR123 will be oxidized and emit strong fluorescence near 525 nm. DHR123 (work concentration: 10 μM) was mixed with the 2 mL photosensitizer solution (1% DMSO in PBS, working concentration: 15 μM) uniformly and exposed to white light (400–700 nm, 50 mW/cm²). Meanwhile, the fluorescence intensities at ~525 nm were recorded immediately for every 30 seconds in the fluorescence spectrum. The control group was 2 mL PBS containing 10 μM DHR123. Vitamin C (Vc) is a common free radical scavenger, for superoxide anion radical quenching experiment, 50 μM Vc was added to the above aqueous solution before light irradiation. As control, DHR123 aqueous solution without photosensitizers was subjected to irradiation. For DHR123: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 508$–700 nm.

1.5 Cell cultures and fluorescence imaging experiment

1.5.1 Cell cultures

HepG-2 cells were cultured in DMEM, supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin in a humidified incubator at 37 °C in 5% CO₂, then, the fresh culture medium was changed every 24 h.

1.5.2 Normoxic and hypoxic tumor model construction in vitro

Instead of normoxic environments, hypoxic tumor model in vitro is essential in this work. HepG-2 cells were cultured in DMEM, supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin in a humidified incubator at 37 °C in 5% CO₂ for 24 h. After that, cells were placed in sealed bags with anoxic bags (Oxoid™ AnaeroGen™ 2.5 L Sachet) and anaerobic indicators (Oxoid™ Resazurin Anaerobic Indicator) for another 12 h in incubator at 37 °C in 5% CO₂. When the anaerobic indicator changed from pink ($21\% O_2$) to colorless ($1\% O_2$), the hypoxic tumor model was established in vitro.

1.5.3 Cell Viability

Unless otherwise stated, TBPCP and TBCP ($1\times10^{-3}$ M) was prepared in DMSO and
stored at −20 °C as the stock solutions respectively.

For normoxic environment: HepG-2 cells were cultured in DMEM, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin in a humidified incubator at 37 °C in 5% CO2. Firstly, HepG-2 cells were planted in the 96-well plates with a density of 1~2×10^4 cells per well and incubated 24 h. Then, after washed with PBS buffer (pH = 7.4) three times, fresh DMEM solution with various concentrations TBPCP and TBCP (0-10 μM) were added to the wells. For dark cytotoxicity, the 96-wells plates were incubated for another 24 h at 37 °C in 5% CO2. For phototoxicity, after incubation for 2 h, the 96-well plates were exposed to white irradiation (400-700 nm, 200 mW/cm²) for 10 min and then incubated for another 24 h. After that, 20 μL MTT (5 mg/mL) solution was added to the previous DMEM and incubated for another 4 h at 37 °C in 5% CO2. Finally, the original medium was discarded and 100 μL DMSO was added to each well to obtain absorbance values for each well by using a microplate reader at 570 nm (Thermo Fisher Scientific Inc. USA).

For hypoxic environment: HepG-2 cells were planted in the 96-well plates with a density of 1~2×10^4 cells per well and then treated with the hypoxic tumor model in vitro following adherence. After washed with PBS buffer (pH = 7.4) three times, fresh DMEM solution with various concentrations TBPCP and TBCP (0-10 μM) were added to the wells and then the 96-well plates were placed in hypoxic condition. Subsequently, the dark cytotoxicity and phototoxic operation under hypoxic conditions was the same as that under normoxic conditions.

1.5.4 Cell imaging and co-localization of LD

HepG-2 cells were incubated with fresh DMEM medium containing 5 μM TBPCP and TBCP for 2 h, respectively. After washed three times with PBS buffer (pH = 7.4) to remove nonspecifically staining TBPCP and TBCP, one- and two-photon images were obtained by Nikon A1 and two-photon laser scanning confocal microscopy.

In co-localization experiment, HepG-2 cells were stained with 5 μM TBPCP and TBCP for 2 h, respectively. Then treated with 200 nM BODIPY 493/503, 200 nM
LysoTracker Green and 200 nM MitoTracker Green for 10 min, respectively. Finally, co-localization images were acquired by Nikon A1 after washed with PBS buffer (pH = 7.4) three times.

In all of the above imaging experiments, for TBPCP and TBCP, $\lambda_{ex} = 488$ nm, $\lambda_{em} = 560$–750 nm; for commercial dyes: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500$–530 nm.

1.6 Detection of intracellular ROS

1.6.1 Intracellular total ROS generation detection

HepG-2 cells were incubated with 5 μM TBPCP and TBCP for 2 h respectively followed by incubation 10 μM DCFH-DA for another 30 min. After that, cells were imaged by CLSM at excitation of femtosecond pulsed laser 940 nm with continuous irradiation in the designated area for 2 min, and fluorescence images were obtained. In addition to the hypoxic process, the operation is the same as the normoxic conditions. $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500$–530 nm.

1.6.2 Intracellular $^1$O$_2$ generation detection

After incubation with 5 μM TBCP and TBPCP for 2 h respectively, HepG-2 cells were fixed with ice methanol for 3 min and added with SOSG (5 μM) for 30 min. After that, cells the imaged by CLSM at excitation of femtosecond pulsed laser 940 nm with continuous irradiation in the designated area for 2 min, and fluorescence images were obtained. In addition to the hypoxic process, the operation is the same as the normoxic conditions. $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500$–530 nm.

1.6.3 Intracellular $\mathrm{O}_2^{•−}$ generation detection

HepG-2 cells were incubated with 5 μM TBCP TBPCP for 2 h respectively followed by incubation 10 μM DHE for another 30 min. After that, cells the imaged by CLSM at excitation of femtosecond pulsed laser 940 nm with continuous irradiation in the designated area for 2 min, and fluorescence images were obtained. In addition to the hypoxic process, the operation is the same as the normoxic conditions. $\lambda_{ex} = 488$ nm, $\lambda_{em} = 570$–620 nm.
1.7 Dead/live cell co-staining under two-photon laser irradiation

HepG-2 cells were cultured on 35 mm confocal dishes then exposed to different following treatments: 1) control group: HepG-2 cells in normoxic condition and hypoxic condition with two-photon irradiation (940 nm, 50 mW, 5 min). 2) experimental group: HepG-2 cells incubated with 5 μM TBPCP and TBPCP for 2 h respectively in normoxic condition and hypoxic condition, and then cells were irradiated with two-photon femtosecond pulsed laser (940 nm, 50 mW, 5 min). Subsequently, the cells of all groups were washed carefully with PBS and stained with Calcein-AM and PI solutions in DMEM medium for 30 min. Finally, the live/dead cells were viewed by CLSM. For Calcein-AM: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500–530$ nm.; for PI: $\lambda_{ex} = 561$ nm, $\lambda_{em} = 570–620$ nm.

1.8 GSH determination in HepG-2 cells

HepG-2 cells were cultured on 6-well plates and incubated until the cell density reached 70%–80%. Then exposed to different following treatments: 1) control group: HepG-2 cells were irradiated with white light (400–700 nm, 200 mW/cm$^2$) for 10 min. 2) HepG-2 cells incubated with 5 μM TBPCP and TBPCP for 2 h respectively. 3) HepG-2 cells incubated with 5 μM TBPCP and TBCP respectively and then cells were irradiated for 10 min with white light irradiation (400–700 nm, 200 mW/cm$^2$). After 12 h of incubation, GSH Assay Kit was used according to the manufacture instruction.

1.9 Detection of lipid peroxidation

Lipid peroxidation (LPO) was assessed by confocal imaging via C11- BODIPY 581/591 Lipid Peroxidation Sensor staining. HepG-2 cells were incubated on 35 mm glass-bottom culture dishes until the cell density reached 50%–60%, then exposed to different following treatments: 1) HepG-2 cells untreated. 2) HepG-2 cells incubated with 5 μM TBCP and TBPCP for 2 h respectively in normoxic and hypoxic condition without two-photon irradiation. 3) HepG-2 cells incubated with 5 μM TBCP and TBPCP for 2 h respectively in normoxic and hypoxic condition with two-photon irradiation (940 nm, 50 mW, 2 min). After that, cells were stained with C11 BODIPY
581/591 (10 μM) and nuclear-tracker Hochedest 33342 (5 μg/mL) for 30 min with fresh DMEM in the incubator. Subsequently, the cells were imaged with Nikon A1 immediately. For C11 BODIPY 581/591, $\lambda_{ex} = 561$ nm, $\lambda_{em} = 570–620$ nm; for oxidized C11 BODIPY 581/591, $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500–530$ nm.

1.10 Western Blot analysis

HepG-2 cells were cultured on 6-well plates and incubated until the cell density reached 70%-80%. Then cells were cultured with DMEM containing TBPCP and TBCP (5 μM and 10 μM) respectively. After 2 h of incubation at 37 °C in the dark, the cells were irradiated with white light (400–700 nm, 200 mW/cm$^2$) for 10 min. After 24 h of incubation, cells were lysed by RIPA lysis buffer with protease and phosphatase inhibitor in ice for 30 min. After measurement of protein concentration by BCA assay, SDS-PAGE Sample Loading Buffer (Biotime Biotechnology) was added and the samples were heated at 95 °C for 15 min, then stored at -20 °C. Then, equal amounts of protein were added to each lane of SDS-PAGE gel for electrophoresis to run for 2 h, and then transferred onto polyvinylidene fluoride (PVDF) membranes for 2 h and the PVDF membrane were blocked with skim milk (5% in TBST) for 1 h. After blocking by western blocking buffer and specific primary antibodies incubation at 4 °C overnight, membranes were incubated by peroxidase-labeled goatanti-rabbit HRP secondary antibodies for 1 h at room temperature. The immunoblots were visualized by FluorChem E (ProteinSimple Co, Ltd., USA) with BeyoECL Plus (Beyotime Biotechnology Co., Ltd., Shanghai).

1.11 Bio-TEM images

HepG-2 cells were cultured on 6-well plates and incubated until the cell density reached 70%-80%, then exposed to different following treatments: 1) HepG-2 cells were irradiated with white light (400–700 nm, 200 mW/cm$^2$) for 10 min. 2) HepG-2 cells incubated with 5 μM TBPCP and TBCP respectively and then cells were irradiated for 10 min with white light (400–700 nm, 200 mW/cm$^2$). After incubation for 24 h, cells were scraped off with a cell scraper and centrifuged. After PBS
washing 3 times, the electron microscope fixative (2.5% glutaraldehyde) was added. Transmission electron microscopy imaging post-fixed, dehydrated, embedded, cut, and mounted of the sample was done by the Institute of Biophysics, Chinese Academy of Sciences. The samples were viewed using a Tecnai Spirit microscope.

1.12 Generation and analysis of multicellular tumor spheroids (MCTS)

A number of 3000 diluted HepG-2 cells were transferred to 1% agarose-coated transparent 96-well plates with 200 μL of culture media. MCTS aggregates formed approximately 1000 μm in diameter after 3-4 days, and then were exchanged for fresh medium to continue incubation for 3 days before starting the experiment.

MCTS were treated with TBPCP and TBCP (10 μM) for 8 h in the dark. The culture medium was refreshed with DMEM and subjected to 940 nm two-photon irradiation (50 mW) for 5 min using a laser source equipped in Nikon A1 Confocal Microscope.

For live/dead MCTS assay. MCTS were treated with TBPCP and TBCP (10 μM) for 8 h in the dark. Moreover, the live/dead MCTS assay was tested with a stain similar to a monolayer of cells, but the cells are illuminated with two-photons (940 nm, 50 mW, 5 min). Subsequently, MCTS were stained with Calcein-AM ($\lambda_{ex} = 488$ nm, $\lambda_{em}$= 500–530 nm) and PI ($\lambda_{ex} = 561$ nm, $\lambda_{em}$ = 570–620 nm) solutions in fresh DMEM medium for 60 min. Images were obtained by using Nikon A1.
2. Supplemental Figures

Scheme S1 The synthetic route of TBPCP.

Scheme S2 The synthetic route of TBCP.
Figure S1 $^1$H NMR spectrum of TBPCP in DMSO-$d_6$.

Figure S2 $^{13}$C NMR spectrum of TBPCP in DMSO-$d_6$. 
Figure S3 The ESI-MS spectrum of TBPCP.

Figure S4 $^1$H NMR spectrum of TBCP in DMSO-$d_6$. 
Figure S5 $^{13}$C NMR spectrum of TBCP in DMSO-$d_6$.

Figure S6 The ESI-MS spectrum of TBCP.
**Figure S7** The normalized absorbance spectrum of (A) TBPCPP, (B) TBPCP, and (C) TBCP in different polar solvents. The normalized fluorescence spectrum of (D) TBPCPP, (E) TBPCP, and (F) TBCP in different polar solvents. Concentration: 10 μM.

**Figure S8** The singlet and triplet energy levels as well as ΔE_{ST} values calculated by the level of [B3LYP/6-311G(d)].

**Table S1** Photophysical data of TBPCP (10 μM) in different polarity solvents.

<table>
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<th>Solvent</th>
<th>Δf°</th>
<th>E(30)</th>
<th>λ_{abs}/nm</th>
<th>λ_{em}/nm</th>
<th>Φ_F/%</th>
<th>Stokes shift/nm</th>
<th>Δυ/cm⁻¹</th>
<th>ε/M⁻¹cm⁻¹</th>
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<td>THF</td>
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<td>6524</td>
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Table S2 Photophysical data of TBCP (10 μM) in different polarity solvents.

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<th>λ_F/nm</th>
<th>Φ_F%</th>
<th>Stokes shift/nm</th>
<th>Δυ/cm⁻¹</th>
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</table>

a: Polarity parameter: Δf = f (ε) − f (n²), f (ε) = (ε - 1) / (2ε + 1), f (n²) = (n² - 1) / (2n² + 1). Where ε and n are the dielectric constant and the refractive indices of the solvent at 25 °C, respectively. b: Absolute fluorescence quantum yield determined using a calibrated integrating sphere. c: Δυ = λ_a − λ_F.

Figure S9 Fluorescence spectra of (A) TBPCP and (B) TBCP in toluene/DMSO mixture solution system with various toluene fractions. (C) TBPCP and (D) TBCP in DMSO/H₂O mixture solution.
system with various water fractions Concentration: 10 μM.

**Figure S10** Characterization of two-photon properties of TBPCP and TBCP: normalized OPE and TPE fluorescence spectra of (A) TBPCP and (D) TBCP; two-photon excited fluorescence spectra of (B) TBPCP and (E) TBCP at different laser power; power dependence relationship of the fluorescence from (C)TBPCP and (F)TBCP under the 940 nm laser excitation. Concentration:1 mM.

**Figure S11** Photostability (A/A₀) of (A) TBPCP and (B) TBCP under white light irradiation (400–700 nm, 200 mW/cm²) for 1 h in DMSO/PBS (v/v=1:99).

**Figure S12** Fluorescence spectra of DCFH-DA in the presence of (A) blank, (B) TBPCP, and (C) TBCP under white light (50 mW/cm²) irradiation with different time in DMSO/PBS (v/v=1:99). Concentration: 15 μM (TBPCP and TBCP); 5 μM (DCFH-DA).
Figure S13 The absorption spectra of ABDA, the absorption peak area, and the decomposition rate constants of ABDA in the presence of (A) RB, (B) TBPCP and (C) TBCP under white light (50 mW/cm$^2$) irradiation with different time in DMSO/PBS (v/v=1:99). Concentration: 100 μM (ABDA).

Figure S14 Fluorescence spectra of DHR 123 in the presence of (A) blank, (B) TBPCP, and (C) TBCP under white light (50 mW/cm$^2$) irradiation with different time in DMSO/PBS (v/v=1:99). (D) Relative various of fluorescence spectra of DHR123 solutions containing TBPCP and TBCP at 525 nm upon white light irradiation or not. Concentration: 15 μM (TBPCP and TBCP); 10 μM (DHR123).
Figure S15 (A) Fluorescence spectra of the DHR123 probe for free radical ROS detection in the presence of (A) RB, (B) TBPCP, and (C) TBP with Vc under white light irradiation. Concentration: 15 μM (TBPCP, TBP and RB); 10 μM (DHR123); 50 μM (Vc).

Figure S16 Fluorescence spectra of DHE in the presence of (A) blank, (B) TBP under white light (50 mW/cm^2) irradiation with different time in DMSO/PBS (v/v=1:99). (C) Relative various of fluorescence spectra of DHE solutions containing TBP at 580 nm upon white light irradiation or not. Concentration: 15 μM (TBPCP); 5 μM (DHE).

Figure S17 (A) HepG-2 cells were stained with TBPCP (5 μM) and TBP (5 μM) for 2 h and co-located with Lyso-Tracker Green (LTG,200 nM). (B) HepG-2 cells were stained with TBPCP and TBP (5 μM) for 2 h and co-located with Mito-Tracker Green (MTG, 200 nM). For TBPCP and TBP, λ_ex = 488 nm; λ_em = 560–750 nm. For commercial dyes: λ_ex = 488 nm, λ_em = 500–530 nm. Scale bar:10 μm.
**Figure S18** Total ROS, $^{1}$O$_{2}$, and O$_{2}$$^{-}$ generation of TBPCP and TBCP (5 μM) in HepG-2 cells by using DCFH-DA, SOSG, and DHE as indicators in dark and two-photon irradiation (940 nm, 50mW, 2 min) under normoxic (21% O$_{2}$). Scale bar:10 μm. For DCFH-DA and SOSG: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500–530$ nm; for DHE: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 570–620$ nm; for Calcein AM: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500–530$ nm; for PI: $\lambda_{ex} = 561$ nm, $\lambda_{em} = 570–620$ nm.

**Figure S19** (A) Average fluorescence intensity of confocal images after incubation with different ROS probes in Figure S18 (normoxia); (B) Average fluorescence intensity of confocal images after incubation with different ROS probes in Figure 4A (hypoxia).
**Figure S20** Cellular dark toxicity of HepG-2 cells treated with different concentrations of (A) TBPCP and (B) TBCP (1-10 μM) under normoxic and hypoxic conditions.

<table>
<thead>
<tr>
<th>Normoxia (21% O₂)</th>
<th>Control</th>
<th>TBPCP</th>
<th>TBCP</th>
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<tr>
<td></td>
<td>dark</td>
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<td>light+Fer-1</td>
</tr>
<tr>
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</table>

**Figure S21** CLSM images of HepG-2 cells after incubation with TBPCP and TBCP (5 μM) under normoxic conditions and two-photon irradiation (940 nm, 50 mW, 2 min) followed by staining with C11-BODIPY 581/591, Hoechst 33342 and Fer-1. For C11-BODIPY 581/591: λ<sub>ex</sub> = 561 nm, λ<sub>em</sub> = 570-620 nm; For oxidized C11-BODIPY 581/591, λ<sub>ex</sub> = 488 nm, λ<sub>em</sub> = 500–530 nm. Scale bar: 10 μm.

**Figure S22** One- and two-photon fluorescence images of MCTS treated with TBPCP and TBCP (10 μM) for 8 h. For one-photon: λ<sub>ex</sub> = 488 nm, λ<sub>em</sub> = 560–750 nm; for two-photon: λ<sub>ex</sub> = 940 nm, λ<sub>em</sub> = 560–750 nm. Scale bar: 500 μm.
Figure S23 One-photon two-photon fluorescence images of MCTS stained with TBPCP (10 μM) for 8 h at different penetration depths along z-axis. For one-photon: $\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 560–750$ nm; for two-photon: $\lambda_{\text{ex}} = 940$ nm, $\lambda_{\text{em}} = 560–750$ nm. Scale bar: 500 μm.

Figure S24 Fluorescence images of MCTS treated with TBPCP (10 μM) for 8 h and then incubation with DCFH-DA for 30 min under dark or two-photon irradiation (940 nm, 50 mW, 5 min). For DCFH-DA: $\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 500–530$ nm. Scale bar: 500 μm.
Figure S25 Fluorescence images upon incubation of HepG-2 MCTS with TBPCP (10 μM) and the cell live (Calcein AM, green)/cell death (PI, red) stain upon two-photon irradiation (940 nm, 50 mW, 5 min). for Calcein AM: $\lambda_{ex} = 488$ nm, $\lambda_{em} = 500$–530 nm; for PI: $\lambda_{ex} = 561$ nm, $\lambda_{em} = 570$-620 nm. Scale bar: 500 μm.

3. Reference