New Cy5 photosensitizers for cancer phototherapy: 
low singlet-triplet gap provides high quantum yield of singlet oxygen

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# Content

1. Figure S1-12 and Table S1 .................................................................3

2. General Information ........................................................................11

3. Preparation of TCy5 and the Spectral Data .....................................12
   3.1 Preparation of quaternary ammonium salt and condensing agent ....12
   3.2 General procedure for the Synthesis of TCy5 ..............................15

4. Analysis test procedure ..................................................................18

5. $^1$H NMR, $^{13}$C NMR spectrogram and HRMS data .....................22

6. References ..................................................................................28
1. Figure S1-S12 and Table S1-S2.

**Figure S1.** Absorption (A) and fluorescence (B) of TCy5 compounds (4 μM) in ultrapure water. The slit of TCy5 is 5/5.

**Figure S2.** Time-correlated single-photon counting fluorescence intensity decay of TCy5 in air-saturated DCM. The solid lines are fitting curves. $\lambda_{ex} = 400$ nm, $\lambda_{probe} = 690$ nm (TCy5-H), 684 nm (TCy5-Ph-NO$_2$), 665 nm (TCy5-CHO) and 673 nm (TCy5-Btz).
Table S1. the fluorescence decay fitting curve parameters of dyes in DCM

| Compounds     | $\tau_1(A_1)$ | $\tau_2(A_2)$ |
|---------------|---------------|---------------|
| TCy5-H        | 2.0 ns (100 %)| -             |
| TCy5-Ph-NO$_2$| 1.2 ns (100 %)| -             |
| TCy5-CHO      | 1.2 ns (100 %)| -             |
| TCy5-Btz$^b$  | 1.3 ns (67 %) | 0.28 ns (33 %)|

$^a$Fluorescence lifetimes measured by TCSPC technique for TCy5 compounds, the percentages of fractional amplitudes exhibit in brackets. $^b$The fluorescence decay curves are fitted by the double exponential functions ($\tau_1$, $\tau_2$, $A_1$, $A_2$).

**Figure S3.** UV–vis absorption spectra of ABDA in TCy5 solutions (10 $\mu$M) irradiated for different durations with NIR light irradiation (20 mW cm$^{-2}$). In aqueous solutions, the absorbance of ABDA at 380 nm was recorded upon different irradiation time to obtain the decay rate of the photosensitizing process. A (TCy5-H, 660 nm), B (TCy5-Ph-OMe, 660 nm), C (TCy5-Ph-NO$_2$, 660 nm).

**Figure S4.** UV–vis absorption spectra of ABDA in (A) Rose Bengal (RB) (1 $\times$ 10$^{-5}$ M) (B) TCy5-CHO (1 $\times$ 10$^{-5}$ M), (C) TCy5-Btz (1 $\times$ 10$^{-5}$ M), irradiated for different durations with light irradiation (660 nm / 550 nm, 1 mW cm$^{-2}$). The linear fit of (D) RB, (E) TCy5-CHO, (F) TCy5-Btz based on absorbance of ABDA at 380 nm in aqueous solutions for different durations with light irradiation.
Figure S5. EPR signals of TEMP for $^{1}$O$_2$ characterization of TCy5-CHO, TCy5-Btz.

Figure S6. UV-vis spectra of (A) TCy5-CHO and (B) TCy5-Btz at absorption maximum against exposure time (10 mW cm$^{-2}$).
Figure S7. Cyclic voltammograms of TCy5-CHO, TCy5-Btz. Conditions: H₂O : EtOH = 3 : 4 containing 0.19 M KCl as the supporting electrolyte and Ag/AgCl as the reference electrode. Scan rates: 100 mV/s. c = 1.15 × 10⁻³ M, 20 °C.

Table S2. Electrochemical Redox Potentials of the Compounds

|         | E₁/₂(OX) (V vs Ag/AgCl) | E₁/₂(RED) (V vs Ag/AgCl) |
|---------|-------------------------|--------------------------|
| TCy5-CHO| +0.63, +0.88            | +0.44                    |
| TCy5-Btz| +0.75                   | +0.44                    |

*Not observed.

The electrochemical properties of TCy5-Btz, TCy5-CHO are studied. For TCy5-CHO, a reversible reduction waves at +0.63 V and +0.44 V are observed (vs Ag/AgCl). The irreversible oxidation waves, for TCy5-CHO and TCy5-Btz, are observed at +0.88 and +0.75 V, respectively. Compared to the oxidation potential of TCy5-CHO and TCy5-Btz, the TCy5-Btz is more easily oxidized by ¹O₂ matching with the sharp photobleaching *in vitro* experiment.
**Figure S8.** (A) Femtosecond transient absorption spectra of TCy5-H. (B) the kinetics at 507 nm of TCy5-H, the lifetimes associated with the components of monoexponential fitting are shown in the insets. $\lambda_{ex} = 380$ nm, in air-saturated DCM, $c$ (TCy5-H) = 50 $\mu$M. (C) Nanosecond transient absorption spectra, and (D) decay trace at 667 nm of TCy5-H. Excited with nanosecond laser at 355 nm, $c = 3.0 \times 10^{-5}$ M in deaerated DCM; at room temperature. Lifetime of the monoexponential fitting functions is given in the graph.
Figure S9. Phosphorescence spectrum of TCy5-CHO (10 μM) in 2-methyltetrahydrofuran. $\lambda_{ex} = 580$ nm, in liquid nitrogen.

Figure S10. Cellular uptake of TCy5 in MCF7 cells. Emissions are collected at 650-730 nm ($\lambda_{em} = 640$ nm).
Figure S11. Fluorescence imaging of MCF7 cells labeled with TCy5-CHO/TCy5-Btz and their colocalization with Lyso- and Golgi-tracker Green, respectively. The correlation coefficient is shown in the graph at the end of each line. $\lambda_{ex} = 488 \text{ nm}, \lambda_{em} = 500-580 \text{nm}, \lambda_{ex} = 640 \text{ nm}, \lambda_{em} = 650-730 \text{ nm}$. Scale bar = 30 $\mu$m.
Figure S12. Typical images of H&E-stained lung, heart, spleen, liver, and kidney from mice 3 days post different treatments of Balb/C mice: A) PBS, B) PBS + light (660 nm, 50 mW cm$^{-2}$), C) TCy5-Btz, D) TCy5-Btz + light (660 nm, 50 mW cm$^{-2}$), E) TCy5-CHO, F) TCy5-CHO + light (660 nm, 50 mW cm$^{-2}$).
2. General Information

In this report, the general chemicals including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Energy Chemical Co., and J&K Scientific Ltd., and all of the solvents were of analytic grade. Flash column chromatography was performed with silica gel (200–300 mesh) and dichloromethane/methanol was used as eluent. Singlet Oxygen Sensor Green (SOSG) was obtained from life technologies. Mitochondrial Membrane Potential Assay Kit with JC-1 and Calcein-AM/PI was obtained from Beijing Solarbio Life Sciences & Technology Co., Ltd. Lyso-, Golgi- and Mito-Tracker was purchased from Life Technologies Co. (USA). All the other solvents and reagents used in this study were of analytical grade. NMR spectra were detected by Bruker Avance III 500 spectrometer. Chemical shift (δ) was reported as ppm in DMSO-\textit{d}_6 with TMS as the internal standard. The mass spectrum was recorded on Agilent 6224 TOF LC/MS instruments. Absorption and emission spectra for six TCy5 were performed with a Lambda 35 UV-visible spectrophotometer (PerkinElmer) and a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018), respectively. Fluorescence quantum yield was obtained with the HAMAMATSU absolute fluorescence quantum yield spectrometer (Serial No. C11347). Nanosecond time-resolved transient absorption spectra were recorded on a LP980 laser flash photolysis spectrometer (Edinburgh Instruments Ltd.) in combination with a Nd:YAG laser (Surelite I-10, Continuum Electro-Optics, Inc.). Confocal laser scanning microscope (CLSM) images were performed on Olympus FV3000 confocal laser scanning microscope. For solution and in vitro tests, stock solution of TCy5 in DMSO (10 mM) was prepared. For in vivo test, TCy5 was dissolved in saline solution.
3. Preparation of TCy5 and the Spectral Data

3.1 Preparation of quaternary ammonium salt and condensing agent

Scheme S1. Preparation of compounds 2

Synthesis of compound 2: 3-benzyl-2-methylbenzo[d]thiazol-3-ium bromide
To a stirred solution of 2-Methylbenzothiazole (4 g, 26.8 mmol) in acetonitrile (10 mL) was added Benzyl bromide (3 g, 17.5 mmol) at room temperature. The reaction was heated to 60 °C for 24 hours. Subsequently, the reaction mixture was allowed to cool to room temperature. The precipitate was filtered and dried under vacuo to afford compound 2 as a pale green solid in 95% yield (5.5 g).

Scheme S2. Preparation of compounds 3-7

Synthesis of compound 3: N-((1E,3E)-3-(phenylimino)prop-1-en-1-yl)aniline
The title compound was prepared in accordance to the literature.[1] To a stirring mixture of 1,1,3,3-tetramethoxypropane (10 mL, 62 mol) and aniline (5.2 mL, 124 mol) in 100
mL ethanol was added to Conc. HCl (6 mL). The reaction mixture was stirred at ice-water bath for 1 hour followed by 3 hours at room temperature. The reaction mixture was then evaporated to remove EtOH, and then added water to the reaction residues, filtered, following washed with water and dry in vacuo to obtain 10 g (63 %) of product 3 as a yellow solid.

**Synthesis of compound 4: Bis(perchlorate)aminalacetal**

The title compound was prepared in accordance to the literature. The round-bottom flask was charged with solid bromoacetic acid (9 g, 65 mmol, 1 equiv). The solid was dissolved in dimethylformamide (30 mL). The reaction solution was cooled in an ice bath, then POCl₃ (29.8 g, 195 mmol, 3 equiv) was added to the stirring mixture dropwise. A colorless to yellowish slurry was obtained and the reaction mixture was stirred at 90 °C overnight. A suspension liquid was obtained following the reaction mixture was cooled to 0 °C. Ethanol (30 mL) was added dropwise within 30 minutes and the reaction mixture became clearer. Water (50 mL) was added dropwise within 20 minutes. Perchloric acid (70 % aq., 15 mL) was added dropwise and the reaction mixture was stirred for 3 hours at 0 °C. The yellow to orange solid was collected on a Büchner funnel, washed with ethanol, and dried overnight under reduced pressure. The desired product was obtained as a yellow solid (11.05 g, 29 mmol, 45 % yield). Which was used in the next step without further purification.

**Synthesis of compound 5: Triformylmethane**

The title compound was prepared in accordance to the literature. A round bottom flask was charged with solid bis(perchlorate) aminalacetal (4.1 g, 11 mmol, 1 equiv), and dissolved in methanol (8 mL). A slurry was obtained. Colorless, solid NaOH were added under stirring until a clear solution was obtained. Addition of NaOH solid was continued until significant amounts of a colorless precipitate were formed which were collected over a Büchner funnel, washed with methanol (8 mL), and transferred to round-bottom flask. CH₂Cl₂ (8 mL) and 6 M aq. HCl (6 mL) were added to this flask. After stirring for 5 min, the mixture became more homogeneous. Stirring was continued for 70 min. The reaction mixture was transferred to a separatory funnel and the aq. phase was three times extracted with CH₂Cl₂. The pooled organic layers were dried
over MgSO₄, filtered, and dried under reduced pressure. The title compound was obtained as a colorless powder (0.96 g, 9.6 mmol, 89% yield).

**Synthesis of compound 6: (E)-3-hydroxy-2-(4-methoxyphenyl) acrylaldehyde**

The title compound was prepared in accordance to the literature.[3] POCl₃ (2.1 mL, 22.5 mmol) was added dropwise to DMF (9 mL) at ice-water bath and stirred one hour. After that the mixture was stirred for three hours at room temperature. Subsequently, phenylacetic acid (7.5 mmol) was added to the mixture. The clear solution was heated to 90 °C keeping 4 hours, and then the reaction mixture cooled to room temperature. The crushed ice was added to the dark mixture followed by saturated NaClO₄ solution. The resulting white crystalline solid was filtered and carefully washed two times with saturated NaClO₄ solution to afford intermediate product, which was used in the next step without further purification. Intermediate product was added to 10 mL NaOH (0.4 g, 10 mmol) solution and the mixture was heated with stirring at 90 °C until the mixture was dissolved. The reaction mixture was cooled to room temperature and diluted with 10 mL water. The solution was acidified to pH 2 with 10% HCl solution resulting in the precipitation of the compound 6 (about 70 % yield).

**Synthesis of compound 7: (E)-3-hydroxy-2-(4-methoxyphenyl) acrylaldehyde**

Compound 7 was synthesized according to the synthetic procedure of compound 6 to give 60 % yield.
3.2 General procedure for the Synthesis of TCy5

Scheme S3. Preparation of TCy5

**Method A:**
A stirring mixture of condensating agent (0.5 mmol, 1 equiv), quaternary ammonium salt (1 mmol, 2 equiv) in ethyl alcohol (10 mL) was added a drop of pyridine. The resulting solution was heated to reflux and monitored by TLC analysis. After the yellow intermediate decreases obviously, the solvent was evaporated under reduced pressure to give a residue. The crude material was adsorbed onto silica gel and purified by column chromatography (dichloromethane/methyl alcohol = 20/1) to give the corresponding compound TCy5.

**Method B:**
Both of condensating agent (0.5 mmol, 1 equiv) and quaternary ammonium salt (1 mmol, 2 equiv) were dissolved in acetic acid (20 mL). The resulting solution was stirred...
in the room temperature and monitored by TLC analysis. After the yellow intermediate almost disappeared, the solvent was evaporated under reduced pressure to give a residue. Then, the residue was dissolved in 100 mL DCM, and organic layer was washed with saline and dried with anhydrous Na₂SO₄. Finally, the organic layer was evaporated to dryness and the crude material was adsorbed onto silica gel and purified by column chromatography (dichloromethane/methyl alcohol = 20/1) give the corresponding compound TCy5.

TCy5-H
The title compound was prepared according to Method B. The product was obtained as a purple powder with a metallic luster (146 mg, 49 % yield). ¹H NMR (500 MHz, DMSO-d₆): δ 8.03 (d, J = 8.0 Hz, 2H), 7.81 (t, J = 12.5 Hz, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.51 (t, J = 7.5 Hz, 2H), 7.44–7.34 (m, 6H), 7.64 (d, J = 7.0 Hz, 2H), 7.26 (d, J = 7.5 Hz, 4H), 6.63 (d, J = 12.5 Hz, 2H), 6.37 (t, J = 12.0 Hz, 1H), 5.66 (s, 4H) ppm; ¹³C NMR (125 MHz, DMSO-d₆): δ 164.25, 150.75, 141.47, 134.67, 128.96, 128.07, 127.96, 126.68, 125.27, 125.15, 123.17, 113.50, 101.00, 48.79, 40.00, 39.83, 39.6 6, 39.50, 39.33, 39.16, 39.00 ppm; HRMS(ESI): m/z calc. for [C₃₃H₂₇N₂S₂]⁺ 515.1610, found 515.1605 [M-Br]⁺.

TCy5-Ph-OMe
The title compound was prepared according to Method A. The product was obtained as a purple powder with a metallic luster (60 mg, 17 % yield). ¹H NMR (500 MHz, DMSO-d₆): δ 8.07 (d, J = 8.0 Hz, 2H), 7.92–7.80 (m, 4H), 7.55 (td, J = 7.5, 1.0 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.36–7.30 (m, 6H), 7.07–6.99 (m, 4H), 6.51 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 8.5 Hz, 2H), 5.88 (d, J = 13.5 Hz, 2H), 5.41 (s, 4H) ppm; ¹³C NMR (125 MHz, DMSO-d₆): δ 164.11, 158.46, 141.78, 134.53, 130.52, 128.91, 128.26, 128.21, 128.03, 126.79, 125.25, 125.23, 123.29, 114.26, 113.43, 99.52, 55.18, 49.15 ppm; HRMS(ESI): m/z calc. for [C₄₀H₃₃N₂OS₂]⁺ 621.2029, found 621.2033 [M-Br]⁺.

TCy5-Ph-NO₂
The title compound was prepared according to Method A. The product was obtained as a purple powder with a metallic luster (251 mg, 70 % yield). ¹H NMR (500 MHz,
DMSO-d$_6$: $\delta$ 8.22 (d, $J= 8.5$ Hz, 2H), 8.10 (d, $J= 8.0$ Hz, 2H), 7.95 (d, $J= 14.0$ Hz, 2H), 7.86 (d, $J= 8.5$ Hz, 2H), 7.57 (t, $J= 8.0$ Hz, 2H), 7.45 (t, $J= 7.5$ Hz, 2H), 7.35–7.24 (m, 6H), 6.20 (d, $J= 8.5$ Hz, 2H), 7.05–6.95 (m, 4H), 5.85 (d, $J= 13.5$ Hz, 2H), 5.48 (s, 4H) ppm; $^{13}$C NMR (125 MHz, DMSO-d$_6$): $\delta$ 165.51, 149.86, 146.96, 142.24, 135.13, 131.45, 129.39, 128.79, 128.53, 127.23, 125.98, 125.84, 124.60, 123.86, 114.21, 99.66, 74.36, 60.49, 58.49, 49.74 ppm; HRMS(ESI): m/z calc. for [C$_{39}$H$_{30}$N$_3$O$_2$S$_2$]$^+$ 636.1774, found 636.1779 [M-Br]$^+$.  

**TCy5-CHO**

The title compound was prepared according to Method A. The product was obtained as a purple powder with a metallic luster (143 mg, 46 % yield). $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ 9.75 (s, 1H), 8.20 (d, $J= 7.5$ Hz, 2H), 7.07 (d, $J= 14.5$ Hz, 2H), 7.94 (d, $J= 8.5$ Hz, 2H), 7.63 (t, $J= 8.0$ Hz, 4H), 7.54 (t, $J= 8.0$ Hz, 2H), 7.45–7.37 (m, 4H), 7.37–7.28 (m, 6H), 5.90 (s, 4H) ppm; $^{13}$C NMR (125 MHz, DMSO-d$_6$): $\delta$ 190.05, 169.72, 141.33, 134.31, 129.09, 128.56, 128.22, 126.88, 126.34, 125.98, 123.59, 117.68, 114.81, 49.84, 40.00, 39.83, 39.67, 39.50, 39.33, 39.17, 39.00 ppm; HRMS(ESI): m/z calc. for [C$_{34}$H$_{27}$N$_3$OS$_2$]$^+$ 543.1599, found 543.1564 [M-Br]$^+$.  

**TCy5-Btz**

The title compound was prepared according to Method A, and the ratio of reagent was 3:1. The product was obtained as a purple powder with a metallic luster (139 mg, 30 % yield). $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ 8.31 (d, $J= 8.0$ Hz, 3H), 8.11 (d, $J= 14.5$ Hz, 3H), 8.00 (d, $J= 8.0$ Hz, 3H), 7.70 (td, $J= 7.5$, 1.0 Hz, 3H), 7.62 (t, $J= 7.5$ Hz, 3H), 7.48 (d, $J= 14.5$ Hz, 3H), 7.44–7.40 (m, 6H), 7.38–7.32 (m, 9H), 6.09 (s, 6H) ppm; $^{13}$C NMR (125 MHz, DMSO-d$_6$): 169.36, 145.74, 141.32, 134.54, 129.19, 128.87, 128.33, 126.92, 126.61, 123.80, 117.26, 115.42, 104.27, 50.14, 39.99, 39.83, 39.66, 39.49, 39.33, 39.16, 38.99 ppm; HRMS(ESI): m/z calc. for [C$_{49}$H$_{39}$N$_3$S$_3$]$^{2+}$ 382.6148, found 382.6148 [M-2Br]$^{2+}$.  

TCy5-Btz
4. Analysis test procedure

Singlet oxygen (\(^1\)O\(_2\)) detection

The singlet oxygen generated by TCy5 was measured using \(^1\)O\(_2\) specificity capture agent, 9,10-anthracenedipropanoic acid (ABDA). Briefly, the absorbance of ABDA at 380 nm was adjusted to about 1.0 in UP water. Then, 10 \(\mu\)M TCy5 was added to this cuvette. After blending well, the cuvette was irradiated with 660 nm monochromatic light for various time, and absorption spectra were measured immediately.

Procedure for \(^1\)O\(_2\) Quantum Yield Measurement\(^4\)

In 3 mL of the ultrapure water, 10 \(\mu\)M of targeted molecule was incubated for 1 min, and the absorbance of 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) at 380 nm was adjusted to about 1.0 in water. UV–vis absorption spectra were recorded after the probe was exposed to a corresponding monochromatic light with a power density of 1 mW/cm\(^2\) for various time. The \(^1\)O\(_2\) quantum yield was measured using Rose Bengal (RB) as the reference photosensitizer and calculated using the following equation:

\[
\Phi_{probe} = \frac{K_{probe}A_{RB}}{\Phi_{RB}K_{RB}A_{probe}}
\]

where \(K_{probe}\) and \(K_{RB}\) were the decomposition rate constants of ABDA in the presence of the probe and RB, respectively. \(\Phi_{RB}\) was the \(^1\)O\(_2\) quantum yield of RB (\(\Phi_{RB} = 0.75\) in water). \(A_{RB}/A_{TCy5-CHO}\) and \(A_{RB}/A_{TCy5-Brz}\) at corresponding monochromatic light were 1.048, 1.795, respectively (Figure S6). The natural logarithm of the absorbance ratio (\(A_0/A\)) of ABDA at 380 nm was plotted against irradiation time and the slope is regarded as the decomposition rate.

Cyclic Voltammetry

Cyclic voltammetry curves were recorded by a CHI660D electrochemical workstation (CHI instruments, Inc., Shanghai, China) at a scan rate of 100 mV/s. Electrochemical measurements were performed at room temperature (RT) using 0.19 M potassium chloride (the mixture of deionized water and ethyl alcohol ratio 4:3 was used as the
solvent) as the supporting electrolyte, a glassy carbon electrode as the working electrode, and a glassy carbon electrode as the counter electrode. The reference electrode was Ag/AgCl (0.22V vs NHE).

**Laser flash photolysis**

Nanosecond time-resolved transient absorption spectra were recorded on a LP980 laser flash photolysis spectrometer (Edinburgh Instruments Ltd.) in combination with a Nd:YAG laser (Surelite I-10, Continuum Electro-Optics, Inc.). Samples in nitrogen atmosphere were excited by a 555 nm laser pulse (1 Hz, 100 mJ per pulse, fwhm ≈ 7 ns) at room temperature. The absorbances of TCy5-CHO and Cy5-CHO were lower than 0.5 OD at 588 nm in 1 cm path length quartz cuvettes.

**Time-correlated single photon counting (TCSPC)**

Time-resolved photoluminescence (TRPL) measurements were carried out by using a time-correlated single photon counting (TCSPC) spectrometer (PicoHarp 300, PicoQuant).[5] The second harmonic of a Ti-sapphire laser (Mai Tai DeepSee, Spectra-Physics, 150 fs, 80 MHz) at 400 nm was used as the excitation source. The instrument response function (IRF) of the detection was 30 ps and all the fluorescence lifetimes were achieved by deconvolution fitting.

**Computations details of B3LYP**

Density Functional Theory (DFT) Calculations: The DFT and TD-DFT calculations were performed using the Gaussian09 program to optimize the molecular geometries of S₀ state, S₁ state and T₁ state (the atomic standard orientations after geometry optimization of ground state and excited state have been given in supporting information). Becke’s three-parameter hybrid exchange functions with Lee–Yang–Parr gradient-corrected correlation functional (B3LYP functional) is the more reasonable choice to be in good accordance with the experimental results,[6] and used in both the DFT and TD-DFT methods in the sequential work. The 6-31G(d,p) basis set was chosen as the basis set throughout, which is an appropriate basis set for such organic compound.[7]

**Femtosecond Transient Absorption Spectra**

The femtosecond transient absorption spectra of compound TCy5-H and TCy5-CHO
were measured by a home-made ultrafast pump–probe setup. The pulse duration was 30 fs. The wavelength of the pump beam was chosen to be 380 nm. The used concentration of samples was 50 μM for both TCy5-H and TCy5-CHO in DCM at room temperature. Measurements were performed in 1 mm quartz cuvettes.

**Cell incubation**

MCF7 cells, HepG2 cells and COS-7 cells were purchased from the Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences and cultured with Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 10 % fetal bovine serum (Invitrogen) and 1 % antibiotics (penicillin/streptomycin, 100 U mL⁻¹) in an atmosphere of CO₂/air = 5%/95% at 37 ºC.

**Cytotoxicity assay**

The cytotoxicity in the presence or absence of light was evaluated by reducing MTT (3-(4,5)-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide) to formazan crystals with mitochondrial dehydrogenases. MCF7 cells, HepG2 cells and COS-7 cells were seeded in a 96-well plate with a concentration of 1 × 10⁵ cells per mL in 100 mL DMEM medium (containing 10 % FBS) incubated at 37 ºC for 24 h. For the photo-cytotoxicity evaluation, different concentrations of TCy5 from 0 to 2 μM in DMEM medium were added to the wells of cells, respectively. Then, the cells were further incubated for 1 h under normoxic conditions, respectively. Subsequently, the cells were subjected to 660 nm light (10 mW/cm², 10 min). Then the cells were further incubated for 24 h at 37 ºC. Next, MTT solution (5 mg/mL) was added in DMEM to each well following incubated the cells for 4 h, the solution in each well was removed out carefully, and added 100 μL DMSO to each well. The plate was then shaken for 10 min and the absorbance was determined on a microplate reader (Thermo Fisher Scientific) at 570 nm and 630 nm. The viability was expressed as a percent of the controlled one using the following equation:

\[
\text{Cell viability (\%)} = \frac{OD_{\text{dye570}} - OD_{\text{dye630}}}{OD_{\text{control570}} - OD_{\text{control630}}} \times 100\%
\]

For dark toxicity measurement of TCy5, the light irradiation was canceled and the other steps were same.
**PDT in the 4T1 tumor bearing mice model**

All procedures were in accordance with the Guide for the Care and Use of Laboratory Animal Resources and the National Research Council, and were approved by the Institutional Animal Care and Use Committee of the NIH. The 4–5 weeks old Balb/c mice were purchased from SPF experimental animal center of Dalian Medical University for transplanting 4T1 cells at a density of $5 \times 10^6$ under the subcutaneous of back. When the tumors volume reached 200 mm$^3$, the tumors mice were divided into 6 groups (PBS, PBS + Light, TCy5-CHO, TCy5-CHO + Light, TCy5-Btz, TCy5-Btz + Light, N = 5). The reagent (50 mM, 100 μL) was intratumorally injected into the tumor-bearing mice on day 0. After 2 h post-injection, tumor region was irradiated with 660 nm light at a power density of 50 mW cm$^{-2}$ for 15 min. The volume of tumors and the weight of mice were measured by a vernier caliper diebus tertius after treatments. The tumors volume was measured using a vernier caliper and calculated using the following equation:

$$V = \frac{a \times b^2}{2}$$

V represents the tumors volume, a and b represents the longer diameter and the shorter diameter, respectively

**In Vivo Biosafety Assay**

The in vivo biosafety assay was performed by using measurement mice weight and H&E slice histological analysis. After the 21th day treatment, all mice were sacrificed and the tumor tissues were collected. The main organs including heart, liver, spleen, lung, kidneys were harvested for histological analysis by means of hematoxylin-eosin (H&E) staining.
5. $^1$H NMR, $^{13}$C NMR spectrogram and HRMS data

TCy5-H
TCy5-Ph-OMe

[Chemical structure images]

Counts vs. Mass-to-Charge (m/z)

[Graphical representation of mass spectra]

[Additional data and comments related to chemical analysis]
TCy5-Ph-NO₂

[Chemical structure image]

ESI Scan (rt: 0.192 min) Frag=150.0V MAHE-0924.d Subtracted

Counts vs. Mass-to-Charge (m/z)
TCy5-CHO
6. References

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