Supplementary Information

A Lipid Droplet-Targeted Pt (II) Complex Enables Photoinduced Ferroptosis and Apoptosis against Cisplatin-Resistant Cells

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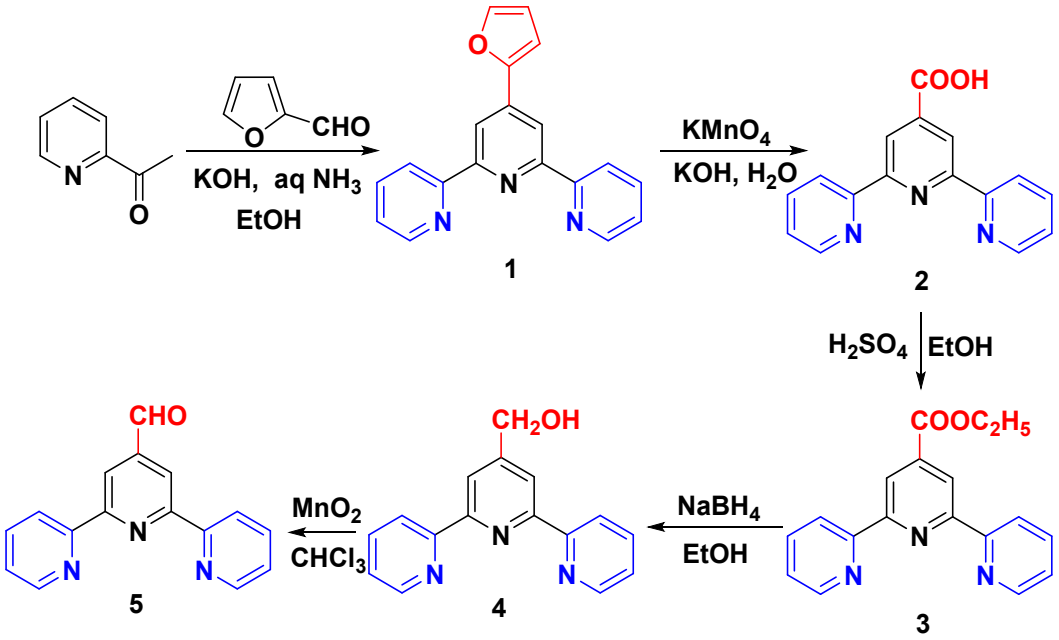
1. Experimental Details: Materials and Instruments

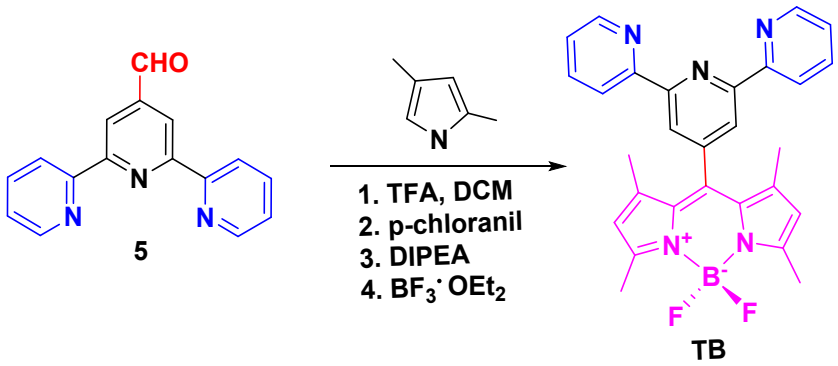
Apparatus included: ^{1}H-NMR and ^{13}C NMR spectra were measured on a Bruker Avance spectrometer (TMS as an internal standard in NMR). Finnigan LCQ-type mass spectrometer, Thermo Fisher, USA; UV-vis spectra were measured on a UV-5900 PC spectrophotometer, Shanghai Metash Instruments Co. Ltd; and HITACHI F-4600 Fluorescence Spectrophotometer, Hitachi Ltd, Japan; Leica TCS SP8 Confocal Microscope, Leica, Germany; Infinite 200 pro enzyme labeler; FACS Aria II flow cytometer (BD Biosciences, USA); Thermo Fisher cell culture incubator (37℃, CO_2 volume fraction 5%). Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Fetal bovine serum (FBS) was purchased from BaiDi Biotechnology Co., Ltd. (BDBIO). The reagents required for phosphate buffer solution (PBS) were purchased from Beyotime, and the triple lysis solution (10% sodium dodecyl sulfate, 5% isobutanol, 0.012 mol-L^{-1} HCI) and MTT reagent were purchased from Biosharp. Trypsin cell digest (0.25% trypsin), Annexin V-FITC/PI double staining apoptosis kit, and single-line oxygen test kit were purchased from Beyotime. Penicillin (100 units-mL^{-1}), RPMI-1640, MEM, and DMEM medium were purchased from Gibco. Human hepatocellular carcinoma cells HepG2, human non-small cell lung cancer cells A549, human lung adenocarcinoma cisplatin-resistant cells A549-DDP, human triple-negative breast cancer cells MDA-MB-231, human ovarian cancer cells A2780, human ovarian cancer cisplatin-resistant cells A2780-DDP, human breast cancer cells MCF-7 and human normal hepatocytes HL-7702 were supplied by the cell bank of Chinese Academy of Sciences.

2. Synthesis and characterization of TB and Pt-TB.

In this paper, compounds **1-5** were prepared according to the methodology reported in the literature [1,2].



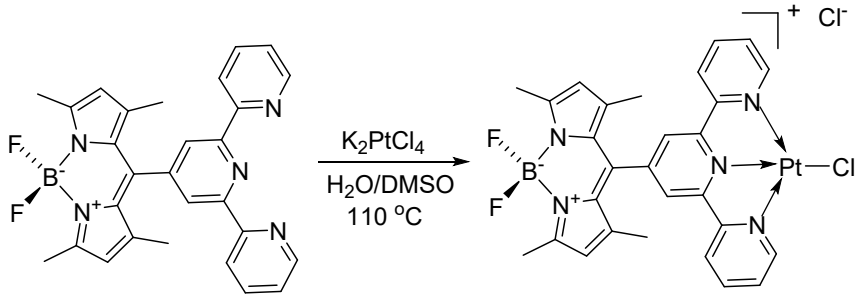
Scheme S1 Synthesis route of compounds **1-5**.



Scheme S2 Synthesis route for **TB**.

Synthesis of 4,4-difluoro-1,3,5,7-tetramethyl-8-(2,2':6',2''-Terpyridine)-4-bora-3a,4adiaza-s-indacene (TB). TB was prepared according to the methodology reported in the literature [3]: Under argon protection, 200 mL of argon deaerated CH_2Cl_2 was added to a round-bottomed flask, compound 5 (0.5 g, 1.91 mmol) and 2,4-dimethylpyrrole (0.473 mL, 4.59 mmol) were added, and TFA (0.22 mL, 2.87 mmol) was added dropwise with vigorous stirring, and then the reaction was stirred for 1 day, protected from light, and then tetrachlorobenzoquinone (0.47 g, 1.91 mmol), stirred for another 2 hours, added diisopropylethylamine (8.0 mL), after half an hour, then added boron trifluoride ethyl ether solution (8.0 mL), the reaction for 1 day away from light. The reaction solution was washed with water (3×200 mL), dried with anhydrous

Na_2SO_4, the solvent was evaporated, the residue was purified by column chromatography using dichloromethane: petroleum ether (V/V = 1:4) as the eluent to obtain product **TB** (0.385 g, 42% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.73 (d, J = 7.7 Hz, 4H, Py-*H*), 8.55 (s, 2H, Py-*H*), 7.94 (t, J = 7.4 Hz, 2H, Py-*H*), 7.43 - 7.37 (m, 2H, Py-*H*), 5.99 (s, 2H, -CH_2-), 2.58 (s, 6H, -CH_3), 1.53 (s, 6H, -CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.4(Py-*C*), 156.2(Py-*C*), 155.3(Py-*C*), 149.4(Py-*C*), 145.4(Pyr-*C*), 142.8(Py-*C*), 138.8(Py-*C*), 137.0(-*C*=C-), 130.6(Pyr-*C*), 124.3(Py-*C*), 121.6(Py-*C*), 121.2(Pyr-*C*), 120.6(Pyr-*C*), 15.2(-*C*H_3), 14.7(-*C*H_3). HR-MS (ESI, m/z): calcd. for $C_{28}H_{25}BF_2N_5$\{[M+H]$^+$\}: 480.2171, found 480.2148.



Scheme S3 Synthesis route for **Pt-TB**.

Synthesis of tripyridyl-modified BODIPY platinum complexes（Pt-TB）. Tripyridyl modified BODIPY platinum complex (**Pt-TB**) was synthesized according to the method reported [4]: K_2PtCl_4 (0.415 g, 1.0 mmol) was weighed and dissolved in 5 ml of distilled water, and **TB** (0.479 g, 1.0 mmol) was dissolved in 5 ml of DMSO, and then the aqueous potassium platinum chloride was added into the **TB** solution drop by drop, heated and stirred for 12 h, and then washed and recrystallized repeatedly with different organic solvents. Vacuum drying gives 0.450 g of the purer complex **Pt-TB** in 50% yield. The structure of this compound has been confirmed by ^{1}H NMR (Figures S1, S2) and ^{13}C NMR (Figures S4, S5), HR-MS (Figures S3, S7) characterization. The structural integrity of the BODIPY unit in the complex **Pt-TB** was confirmed by ^{11}B NMR spectra (Figure S6), and the purity of **Pt-TB** was also measured by HPLC (Figure S8). ^{1}H NMR (600 MHz, DMSO) δ 9.00 (d, *J* = 18.3 Hz, 4H, Py-*H*), 8.67 (d, *J* = 7.3

Hz, 2H, Py-*H*), 8.54 (t, *J* = 6.9 Hz, 2H, Py-*H*), 7.99 (s, 2H, Py-*H*), 6.33 (s, 2H, -CH_2-), 2.54 (s, 6H, -CH_3), 1.64 (s, 6H, -CH_3). ^{13}C NMR (101 MHz, DMSO) δ 158.7(Py-*C*), 157.4(Py-*C*), 155.9(Py-*C*), 151.7(Py-*C*), 143.5(Pyr-*C*), 143.2(Pyr-*C*) 136.2(Py-*C*) 129.9(Py-*C*) 129.7(Py-*C*), 127.0(-*C*=C-), 126.8(Pyr-*C*), 126.7(Pyr-*C*), 125.0(Py-*C*) 122.8(Py-*C*), 115.8(Pyr-*C*) 115.5(Pyr-*C*), 30.7(-*C*H_3), 16.1(-*C*H_3), 14.8(-*C*H_3). HR-MS (m/z): calcd for $C_{28}H_{24}BF_2N_5Pt$\{$[M]^+$\}: 709.1429, found 709.1426. The purity of **Pt-TB** measured at 365 nm and 532 nm channels was 95.7% and 95.4%, respectively.

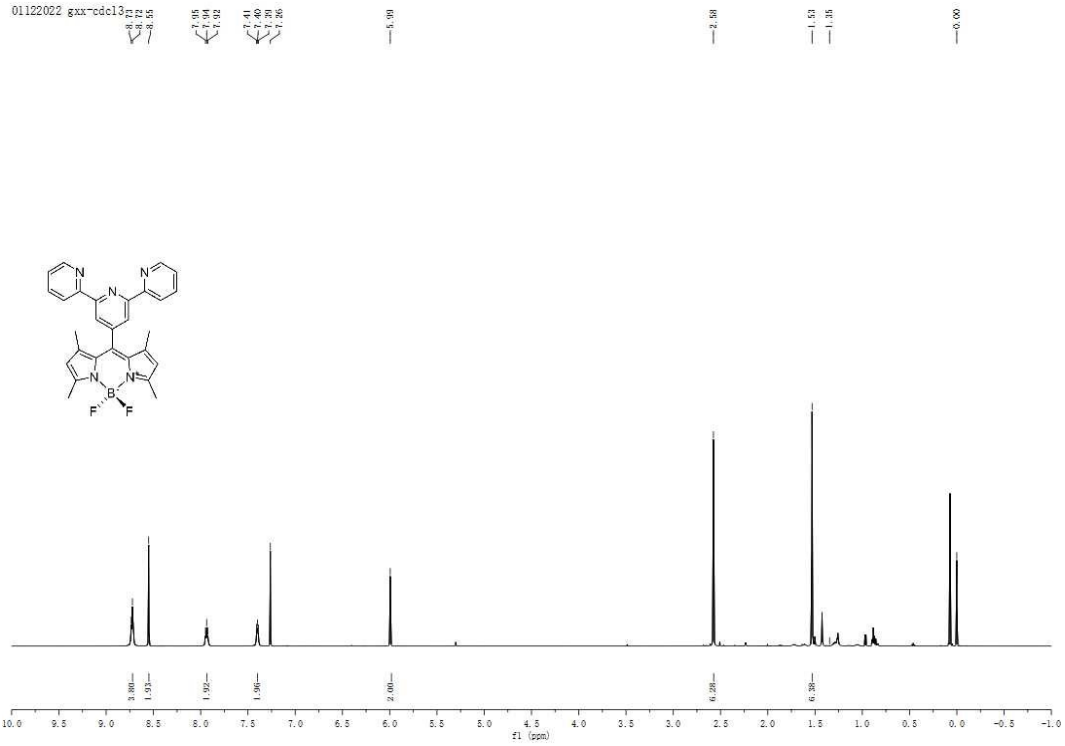


Fig. S1 ^{1}H NMR spectrum of **TB** in $CDCl_3$.

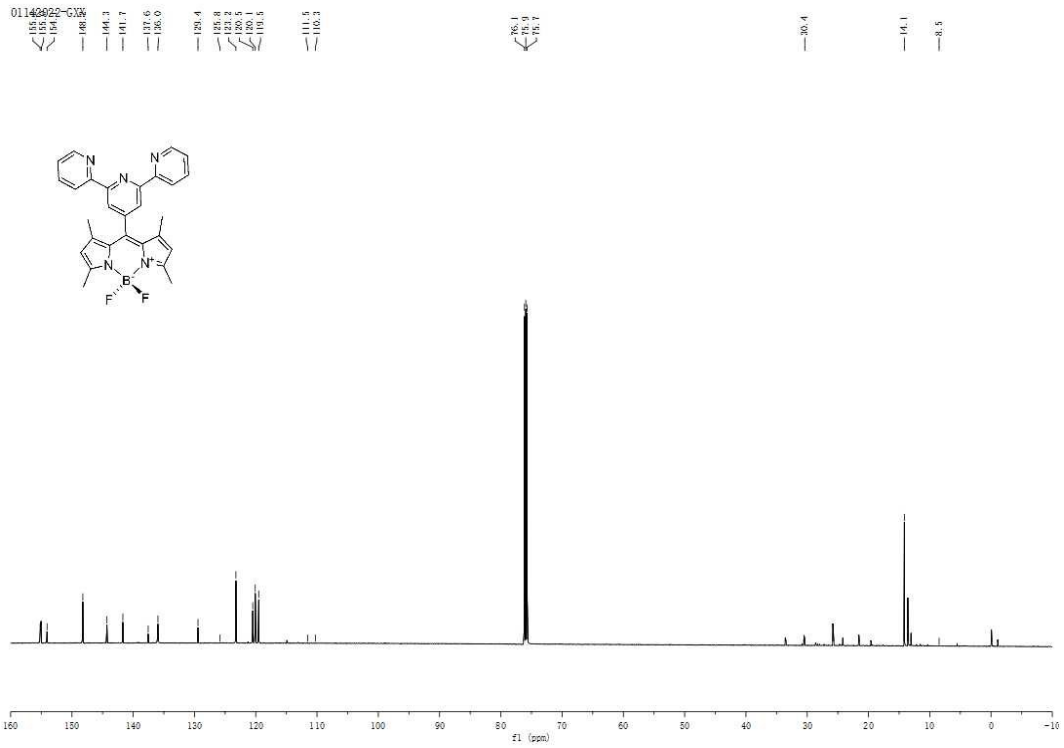


Fig. S2 ^{13}C NMR spectrum of **TB** in $CDCl_3$**.**

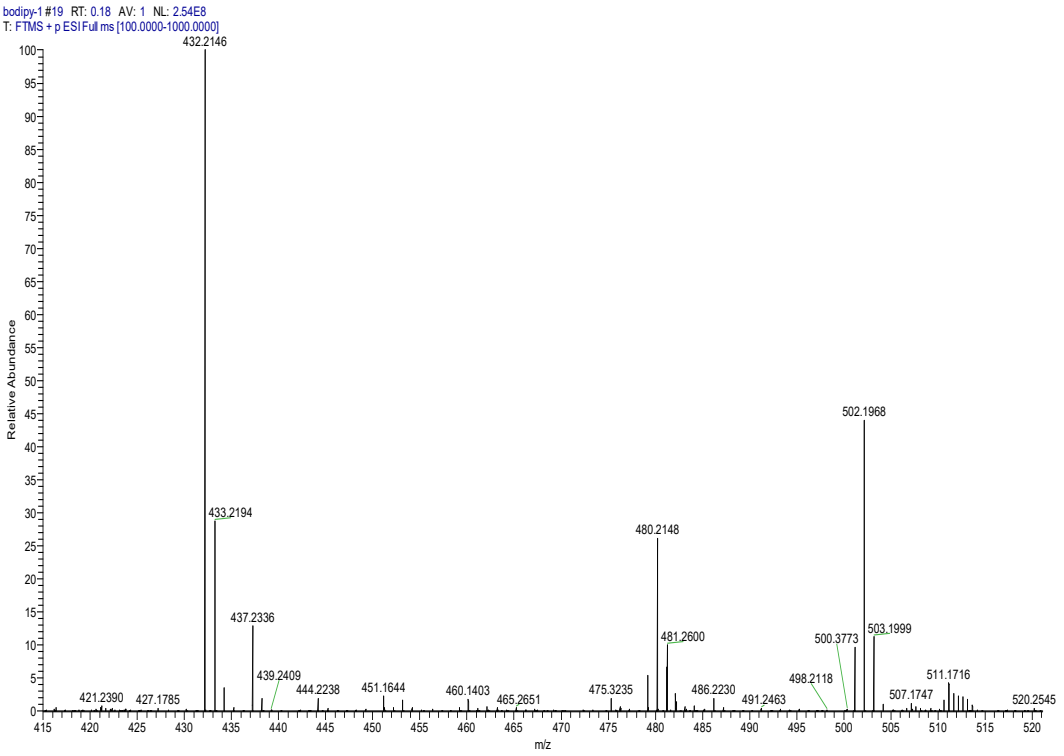


Fig. S3 HRMS of **TB.**

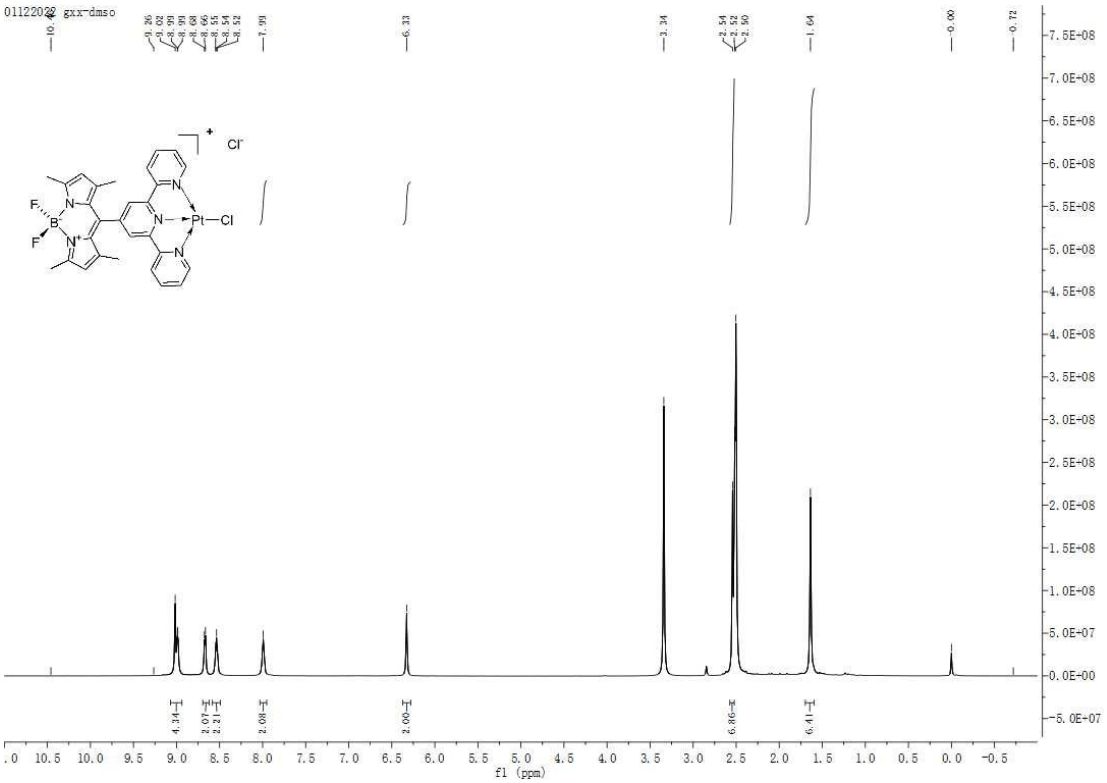


Fig. S4 ^{1}H NMR spectrum of **Pt-TB** in DMSO-d_6.

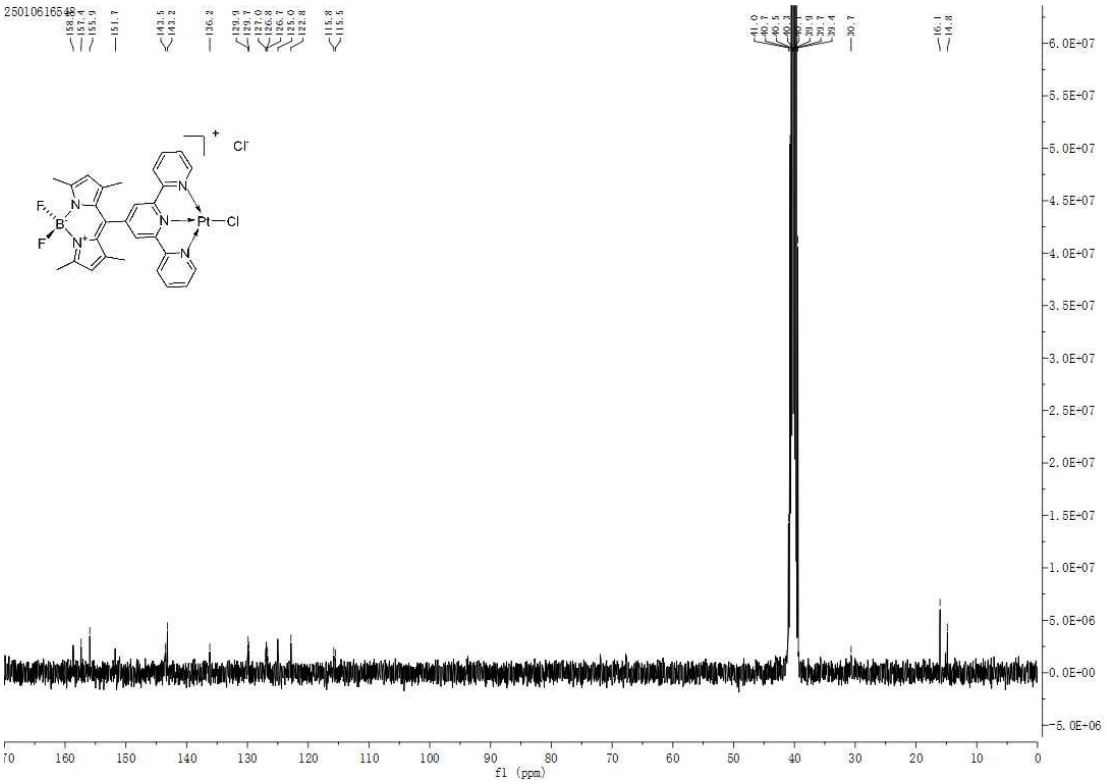


Fig. S5 ^{13}C NMR spectrum of **Pt-TB** in DMSO-d_6.

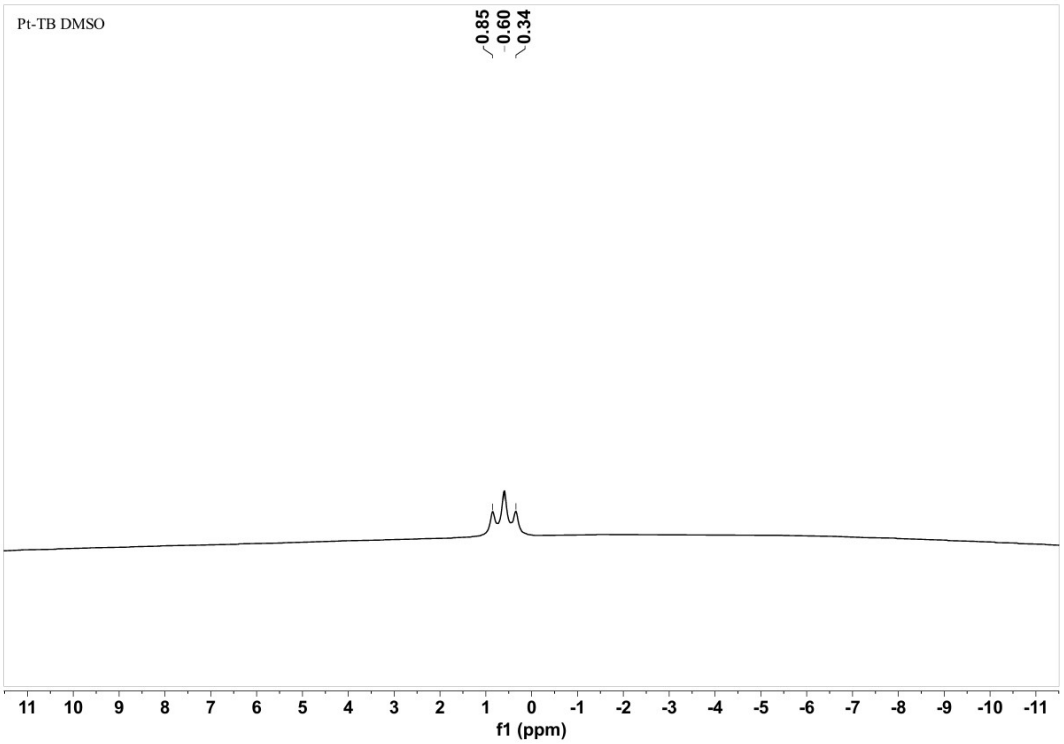


Fig. S6 ^{11}B NMR Spectrum of Pt-TB in DMSO-d_6 Solvent.

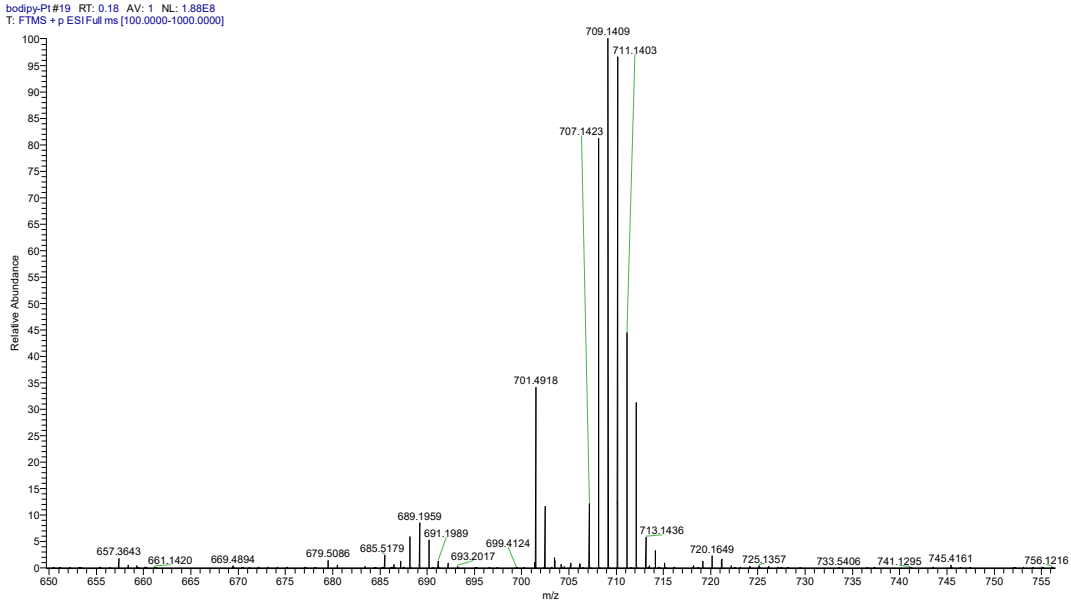


Fig. S7 HRMS of **Pt-TB.**

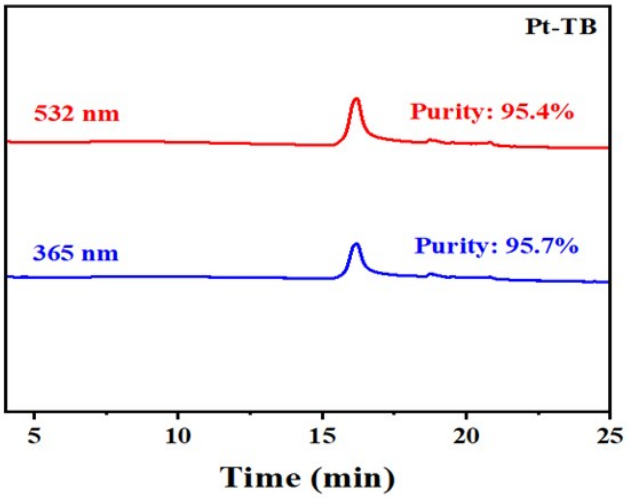


Fig. S8 HPLC analysis of Pt-TB (50 μM, 100 μL). Gradient: 20 % A for 2 min, then 95 % A for 22 min; A: CH_3OH, B: ddH_2O.

3. Photophysical properties of TB and Pt-TB

Determination of quantum yield of TB and Pt-TB and single-line oxygen yield. The formula for calculating the oxygen yield of a single line state is as follows:

$$\Phi_{\Delta sam} = \Phi_{std}\left(\frac{S_{sam}}{S_{stad}}\right)\left(\frac{F_{std}}{F_{sam}}\right)$$

Φ_Δ denotes the yield of single-linear oxygen; S is the slope of a straight line linearly fitted with the irradiation time as the x-axis and the UV absorption value of DPBF at 410 nm after the corresponding irradiation as the y-axis, and F stands for the absorption correction factor of the samples and the reference compounds, and $F = 1\text{-}10^{-OD}$, with the OD standing for the UV absorption value of the compounds at the irradiation wavelength (532 nm).

For the quantum yield calculation of ligand **TB** and complex **Pt-TB** in acetonitrile solution, rhodamine 6G was used as the reference material ($\Phi_f = 0.95$, H_2O), and its maximum excitation wavelength of 480 nm was used as the excitation wavelength, and its acetonitrile solution was subjected to UV absorption measurement and fluorescence spectrometry, respectively, and the obtained data were substituted as the following equations for the calculations:

$$\Phi_{fs} = \Phi_{fR^o}\frac{F_s A_R {n_S}^2}{F_R A_S {n_R}^2}$$

In the formula, F denotes the integral area of the fluorescence emission spectrum when excited by light at 480 nm, A denotes the absorbance at 480 nm, n is the refractive index of the sample solution, and the subscripts R and S represent the reference material and the sample, respectively, in which the quantum yield of the ligand **TB** was calculated to be 0.32 from the above formula. The absorbance of each sample should be guaranteed to be no greater than 0.05.

Excited-State Fluorescence and Phosphorescence Lifetime Detection. A transient fluorescence spectrometer was used to monitor the signal intensity at the maximum fluorescence/phosphorescence absorption peak of the photosensitizers **TB** and **Pt-TB** (10 μM) in degassed DMSO solvent over time upon excitation at 480 nm. The fluorescence and phosphorescence lifetimes were then calculated using the ExpDecy2 and ExpDecy3 formulas, respectively.

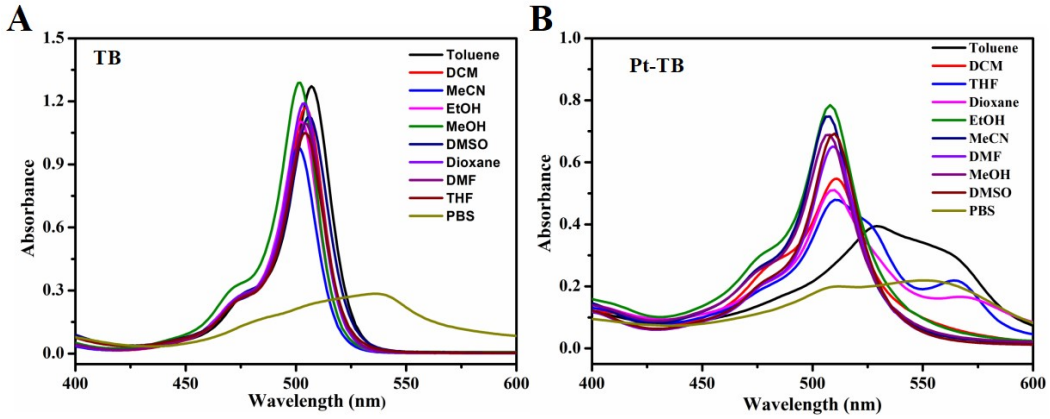


Fig. S9 UV-vis absorption spectra of **TB** (A) and **Pt-TB** (B**)** in different solvents.

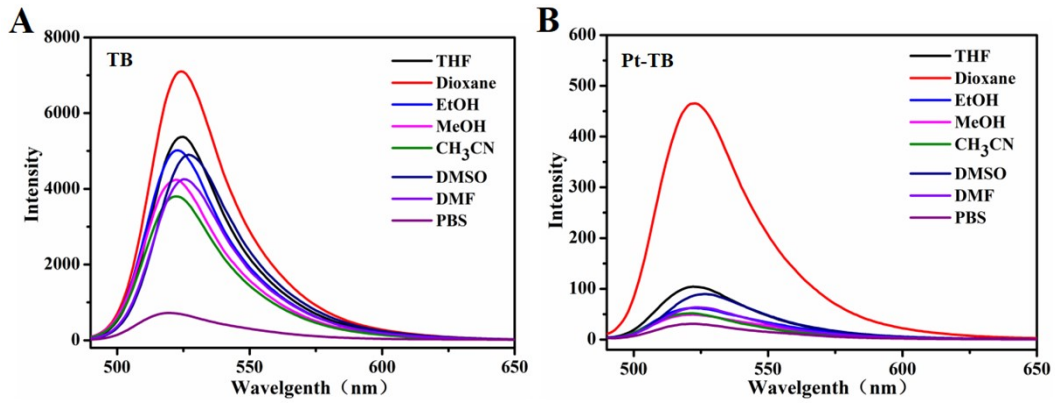


Fig. S10 Fluorescence emission spectra of **TB** (A) and **Pt-TB** (B**)** in different solvents. λ_{ex} (**TB**) = 470 nm; λ_{ex} (**Pt-TB**) = 490 nm.

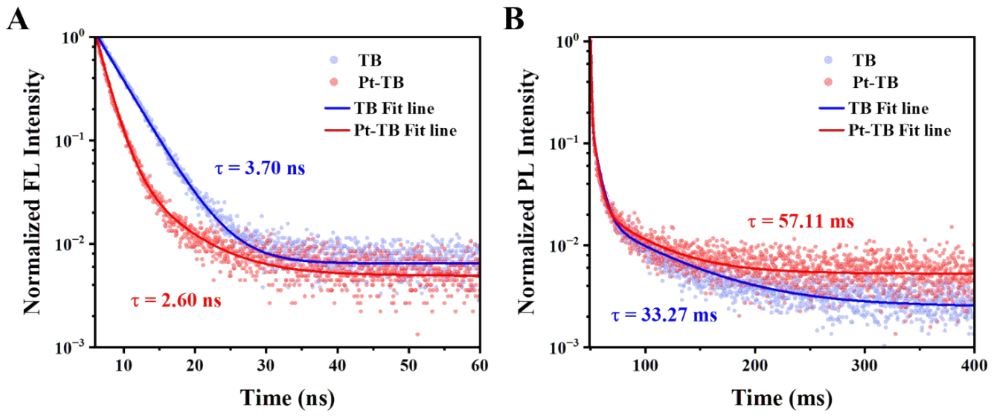


Fig. S11 Excited-state fluorescence (A) and phosphorescence (B) lifetimes of 10 μM **TB** and **Pt-TB** in degassed DMSO solvent (λ_{ex} = 480 nm).

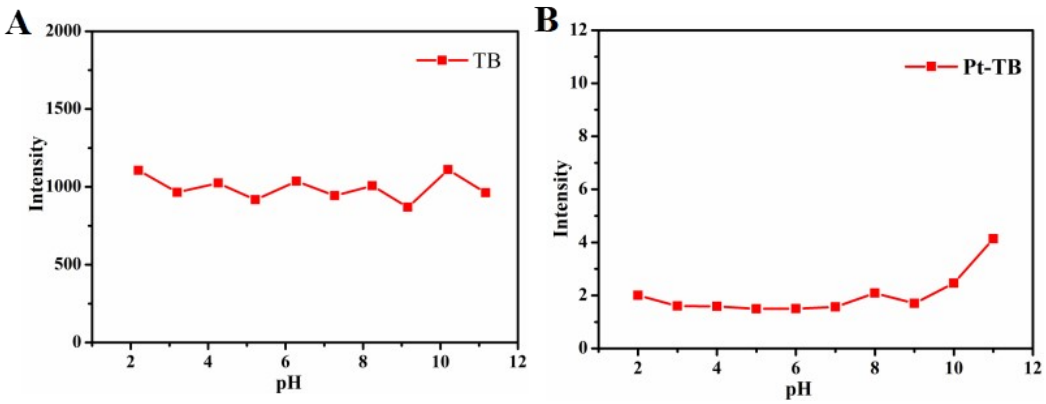


Fig. S12 Fluorescence emission spectra of **TB** (A) and **Pt-TB** (B) at pH 2-11. λ_{ex} (**TB**) = 470 nm; λ_{ex} (**Pt-TB**) = 490 nm.

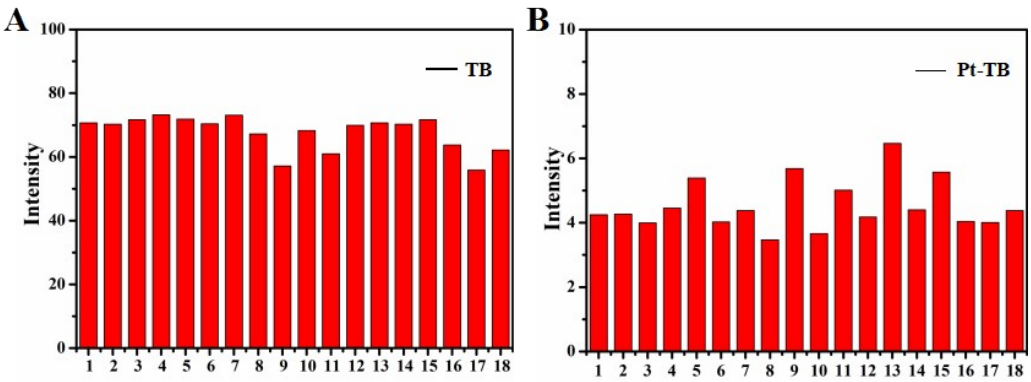


Fig. S13 Fluorescence emission spectra of **TB** (A), **Pt-TB** (B) in different amino acids and ions. λ_{ex} (**TB**) = 470 nm; λ_{ex} (**Pt-TB**) = 490 nm. (1: Zn^{2+}, 2: Ni^{2+}, 3: K^{+}, 4: I^{-}, 5: Mn^{2+}, 6: Co^{3+}, 7: F^{-}, 8: Na^{+}, 9: O_2^{-}, 10: SO_4^{2-}, 11: Al^{3+}, 12: Mg^{2+} ,13: Leu, 14: Lys, 15: Glu, 16: Val, 17: Gln, 18: Ser).

4. Biological experiments related to photodynamic therapy

Cell imaging. HepG2 cells used for cell imaging were cultured in a 37°C cell culture incubator containing 5% CO_2 and 95% air. Compounds were first dissolved in DMSO to 1 mM as a stock solution and then diluted to a working concentration (10 μM) with DMEM cell culture medium. For cellular imaging, we inoculated HepG2 cells at a density of 1×10^5 cells into confocal culture dishes for 24 h. Subsequently, HepG2 cells were co-incubated with 10 μM **TB** and **Pt-TB** for 30 min, respectively, and then the cells were washed with PBS buffer, and finally, the bioimaging experiments were performed. The cells were imaged with a confocal laser scanning microscope (Leica TCS SP8, Leica, Germany). Images were recorded with Leica Application Suite and processed using ImageJ 1.52a software (NIH, Bethesda, MD, USA).

Cell Viability. Seven human cancer cells and human normal hepatocytes HL-7702 in good condition were inoculated into 96-well plates at a density of 104 cells/well and cultured in an incubator at 37 °C overnight. Cells were grown to approximately 85% in 96-well plates prior to treatment. Compounds **TB** and **Pt-TB** are then added to the wells at the indicated concentrations. Prior to compound treatment, the cell culture medium is changed and an equal amount of the compound stock solution is diluted to obtain the final concentration. After 24 hours of incubation, replace with fresh DMEM/1640/MEM medium. After removing the MTT solution, 150 μL of DMSO was added to each well. Subsequently, after shaking the 96-well plate for 10 min, the optical density (OD) values were recorded using an enzyme meter (Infinite 200pro). Cell viability (%) = $(OD_1\text{-}OD_2)/(OD_3\text{-}OD_2) \times 100$ was then determined as follows, where OD_1 is the OD value of the treatment group, OD_2 is the OD value of the blank group and OD_3 is the OD value of the control group.

Apoptosis Test. Adjust the cell density culture and do the drug treatment, set the concentration as 0, 2, 4, 6, 8, 16 μmol-L^{-1}, wait for the drug action for 24 h, transfer the culture solution to the labeled centrifuge tube, PBS light wash and add 1 mL tryptic digest (EDTA-free) to digest the cells, wait for the end of digestion, remove the digest and add the original culture solution, the Blow and put into the corresponding centrifuge

tube as above, collect the cells by slow centrifugation, resuspend the cells (5×10^4~1×10^5 cells) with AnnexinV-FITC conjugate, add the AnnexinV-FITC staining solution and PI staining solution, and flick to mix. After incubation for 5~15 min at room temperature (20~25 ℃) and protected from light, the cells were then detected by flow cytometry.

Intracellular ROS Detection. After **Pt-TB** (0, 2, 4, 6, 8, 16 μM) treatment, when the cell confluence reached about 60%, HepG2 cells were washed with PBS to remove the residual DMEM medium. Incubate HepG2 cells with DCFH-DA (10 μM) for 30 min at 37 °C in the dark. Tests were performed using a FACS Aria II flow cytometer (BD Biosciences, USA), Ex = 488 nm. flow cytometry was performed by digesting the cells in 6-well plates with a similar manipulation method to the protocol described above.

Western blotting. HepG2 and HL-7702 cells were inoculated into 6-well plates at a density of 2×10^5 per well and cultured overnight. Then the medium in each well was replaced with 2 ml of culture medium containing Pt-TB (8 μM, 16 μM). After 6 h of incubation, the plates were exposed to light (λ_{em} = 532 nm; 200 mw/cm^2) for 1 min, and then the total proteins were extracted using RIPA lysis buffer (Beyotime) and PMSF (Beyotime), and the protein concentration was determined using a Thermo Fisher Scientific Ultra-Micro Nucleic Acid Protein Assay. Proteins were added to different Western blotting lanes by SDS-PAGE and transferred to PVDF membranes (Beyotime). The membrane was closed with 5% skimmed milk for 1-2 hours, then incubated with primary antibody at 4°C overnight, then with secondary antibody for 2 hours at room temperature, and finally the blot was detected by chemiluminescence reagent (Biosharp) and the labeled bands were scanned with ChemiScope S7 (Qinxiang, China). Protein expression levels were analyzed using Adobe Photoshop analysis software. The following antibodies were used in this study: β-Actin (1:1000, Chengdu Zhengneng), PARP1 (1:1000, Chengdu Zhengneng), Bax (1:1000, Chengdu Zhengneng), Bcl-2 (1:1000, Chengdu Zhengneng), Caspase-3 (1:1000, Chengdu Zhengneng) and Cleaved Caspase-3 (1:1000, Chengdu Zhengneng).

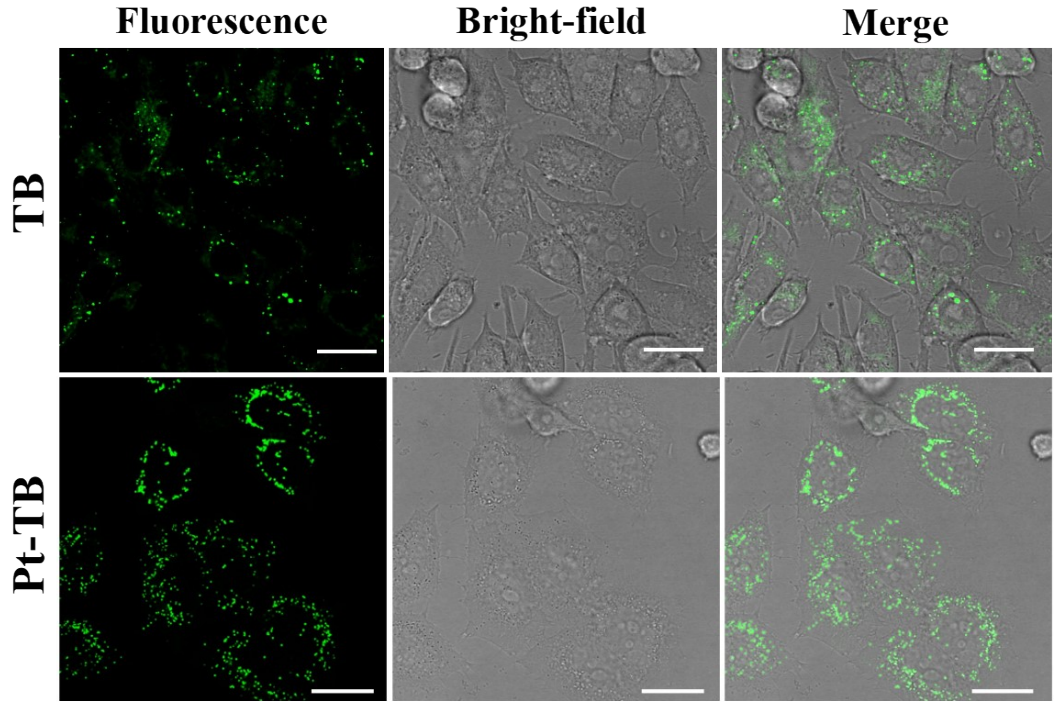


Fig. S14 Cellular uptake of compounds **TB** and **Pt-TB** analyzed by confocal microscopy in HepG2 cells at 30 min. Scale bar = 10 μm.

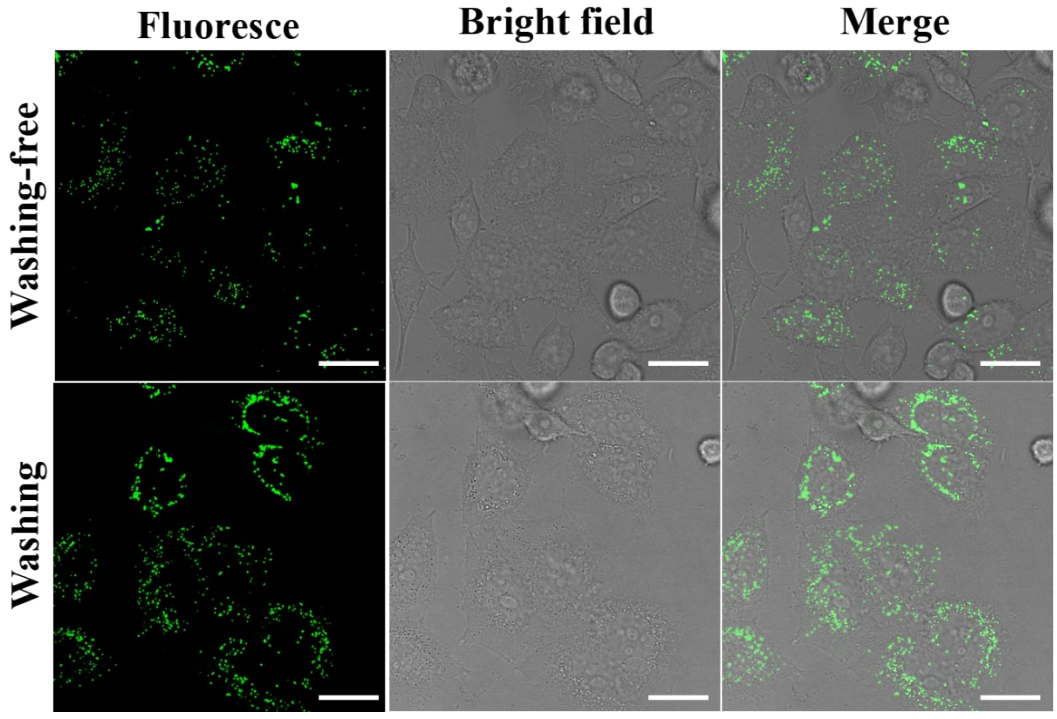


Fig. S15 Confocal images of living HepG2 cells after **Pt-TB** cultivation, imaged without washing or after washing. For **Pt-TB**, λ_{ex} = 488 nm; λ_{em} = 490-540 nm. Scale bar: 10 μm.

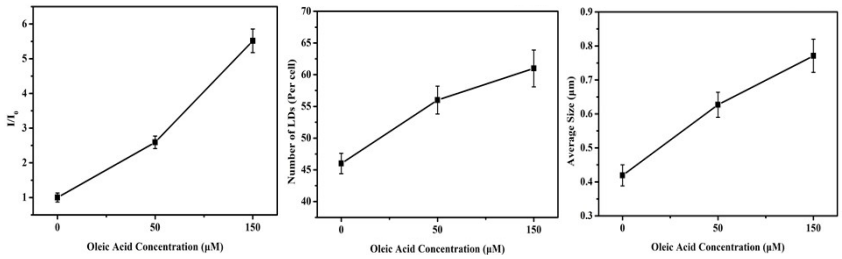


Fig. S16 Changes in the relative fluorescence intensity, number of LDs and average diameter at different OA concentrations.

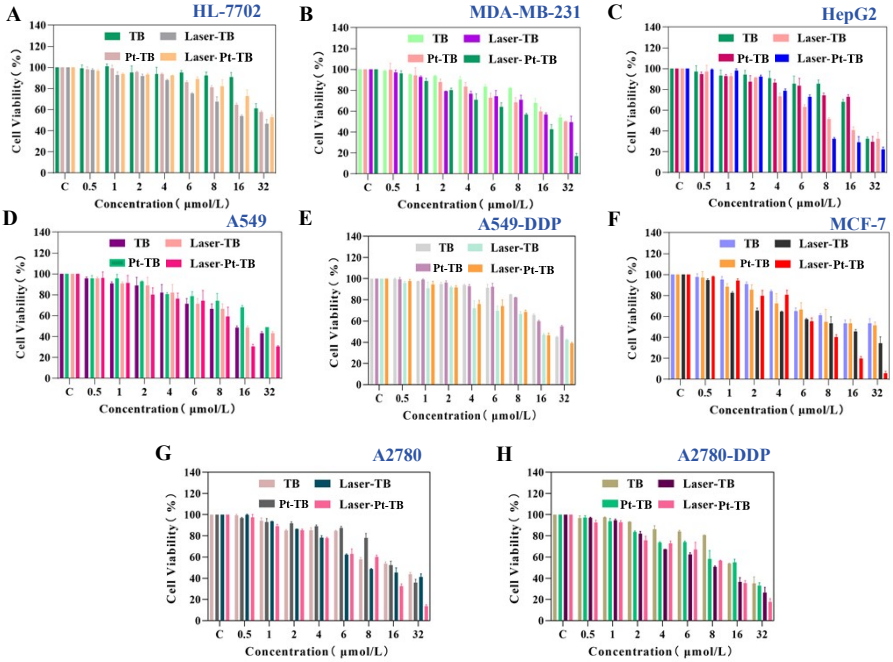


Fig. S17 Cell viability of **TB** and **Pt-TB** in light (λ_{em} = 532 nm; 200 mw/cm^2, 1 min) and dark conditions on eight selected human cells for 24 h.

Table. S1 IC_{50} (μM) values of **TB**、**Pt-TB** and **DDP** (Cisplatin) on the eight selected human cells for 24 h.

Cell line	TB		Pt-TB		DDP
	Dark	Light	Dark	Light	Dark
HL-7702	41.44±2.36	21.47±0.89	38.32±1.45	37.24±2.17	23.56±1.94
MDA-MB-231	35.49±0.42	28.74±2.23	31.08±1.40	9.96±0.08	29.49±0.92
HepG2	22.44±0.59	11.35±0.54	19.66±0.77	8.44±0.38	27.16±1.20
A2780	20.19±0.37	13.55±0.61	20.15±0.6	9.77±0.30	11.07±0.26
A2780-DDP	19.91±0.14	10.43±0.98	15.46±2.49	7.81±1.55	34.81±2.04
MCF-7	24.69±2.52	10.67±0.87	22.16±2.45	6.89±0.17	28.64±1.65
A549	22.73±1.12	18.87±1.36	33.20±0.30	11.33±0.62	26.33±2.06
A549-DDP	21.70±2.31	14.28±1.97	33.06±1.74	17.22±0.20	27.51±0.70

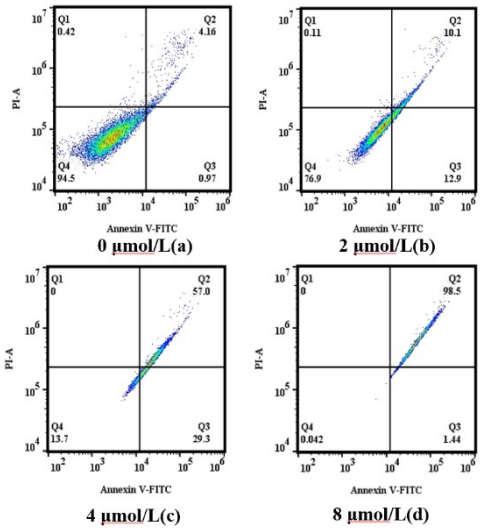


Fig. S18 Effect of different concentrations of Pt-TB on apoptosis of HepG2 cells under light irradiation (532 nm; 200 mw/cm^2, 1 min).

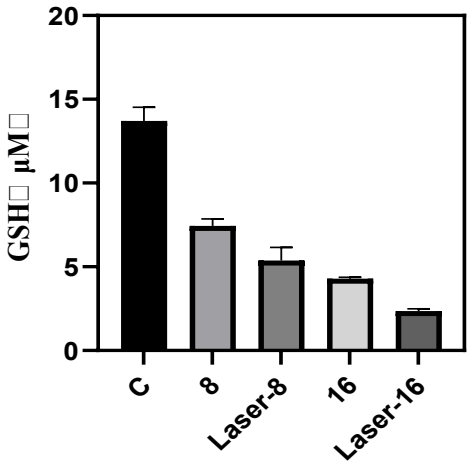


Fig. S19 Changes in GSH levels after **Pt-TB** treatment of HepG2 cells under different conditions.

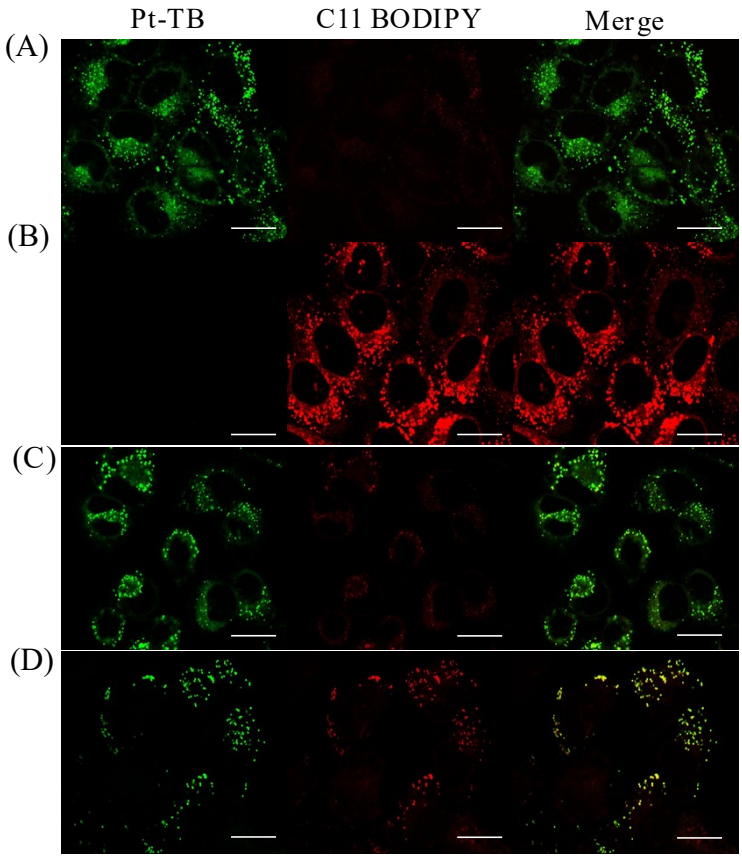


Fig. S20 (A) HepG2 cells treated with **Pt-TB** only; (B) HepG2 cells treated with the LPO probe C11 BODIPY only; (C) Experimental group: HepG2 cells treated with **Pt-TB** under light illumination, followed by the addition of the C11 BODIPY probe; (D) Experimental group: HepG2 cells treated with **Pt-TB** in the dark, followed by the addition of the C11 BODIPY probe. Scale bar: 10 μm.

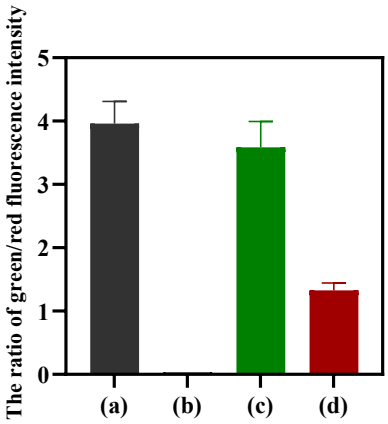


Fig. S21 Representation of the ratio of green/red fluorescence intensity obtained respectively from the confocal images in **Fig. S20** (A), (B), (C), and (D).

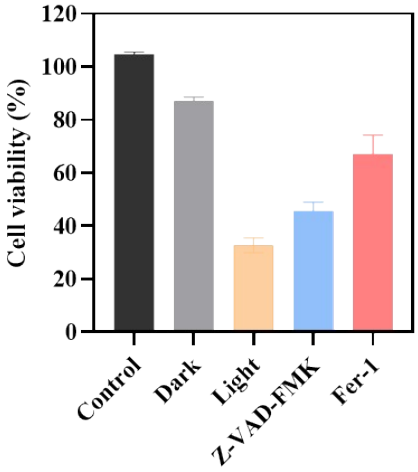


Fig. S22 Viability of HepG2 cells co-incubated with 5 μM Pt-TB under dark, light, light + apoptosis inhibitor (Z-VAD-FMK, 30 μM), and light + ferroptosis inhibitor (Fer-1, 30 μM) conditions (532 nm, 100 mW/cm^2, 4 min).

The raw images for all Western blot: Western blotting analysis showed that HepG2 cells and HL-7702 normal hepatocytes were treated with different concentrations of Pt-TB (8 μM, 16 μM) under dark or light conditions (λem = 532 nm, 200 mW/cm², 1 min). From left to right, they are the control group, treated with 8 μM **Pt-TB** under dark, the 8 μM **Pt-TB** with light exposure, treat with 16 μM **Pt-TB** under dark, and the 16 μM **Pt-TB** with light exposure, respectively.

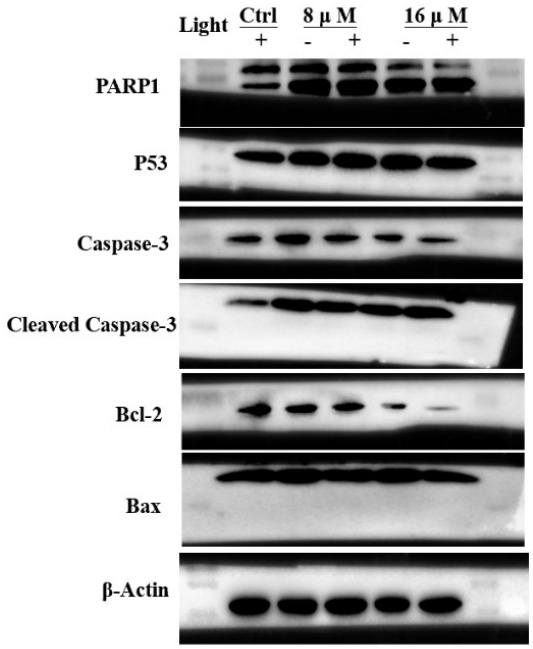


Fig. S23 The uncropped western blots for Figure **4A$_1$**.

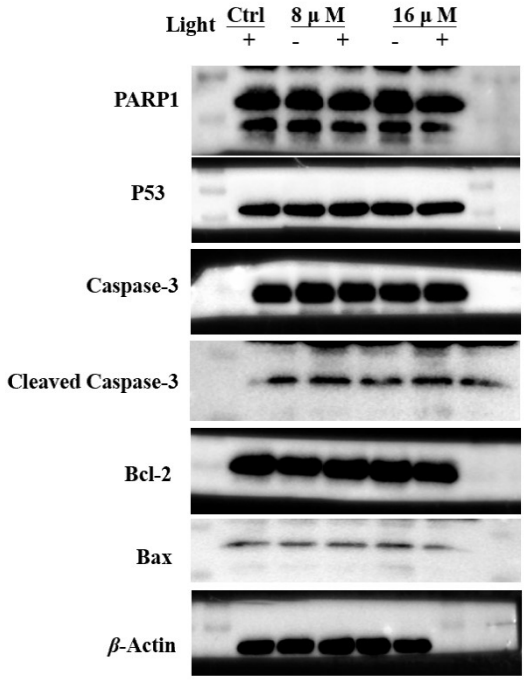


Fig. S24 The uncropped western blots for Figure **4A$_2$**.

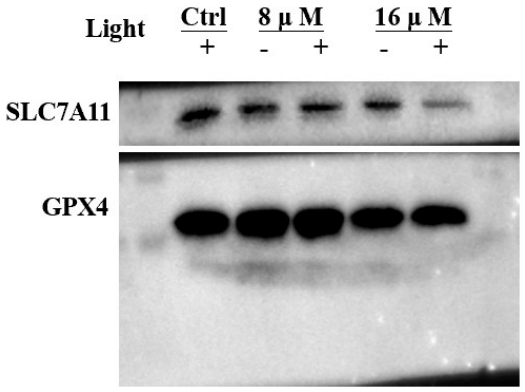


Fig. S25 The uncropped western blots for Figure **4B$_1$**.

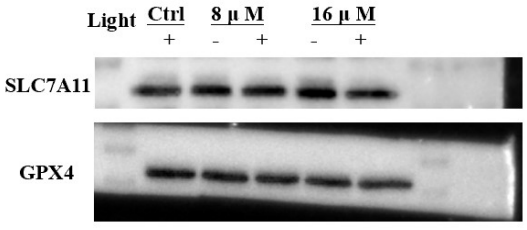


Fig. S26 The uncropped western blots for Figure **4B$_2$**.

5. References

[1] J. Husson, J. Dehaudt and L. Guyard, *Nat. Prot.*, 2014, **9**, 21-26.

[2] N. Boechat, J. C. S. da Costa, J. de S. Mendonça, K. C. Paes, E. L. Fernandes, P. S. M. de Oliveira, T. R. A. Vasconcelos and M. V. N. de Souza, *Synth. Commun.*, 2005, **35**, 3187-3190.

[3] I. S. Turan, G. Gunaydin, S. Ayan and E. U. Akkaya, *Nat. Commun.*, 2018, **9**, 805.

[4] K. Wang, C. Zhu, Y. He, Z. Zhang, W. Zhou, N. Muhammad, Y. Guo, X. Wang and Z. Guo, *Angew. Chem. Int. Ed.*, 2019, **58**, 4638-4643.