Supporting information

Ferroptosis Promotes Sonodynamic Therapy: A Platinum(II)-Indocyanine Sonosensitizer

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Contents

Experimental Section

Materials. 2-phenyl-pyridin (ppy), potasium tetrachloroplatinate (I) (K_2PtCl_4), 1,1,2-triMethyl-1H-benzo[e]indole, iodoethane, 3,3'-bipyridine-6,6'-diformaldehyde, 2-ethoxyethanol and hematoporphyrin (HP) were purchased from Aladdin. 9,10-diphenylanthracene (DPA), 2,2,6,6-tetramethylpiperidine (TEMP), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), methylene blue, hemoglobin, RPMI medium, fetal bovine serum (FBS), glutamine and penicillin/streptomycin were purchased from Sigma-Aldrich. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′,7′-dichlorofluorescin diacetate (DCFH-DA) and the calcein AM/PI kit were obtained from Life Technologies. Singlet Oxygen Sensor Green reagent (SOSG) was purchased from Invitrogen.

Instruments. NMR spectra were recorded on a Bruker AV-500MHz spectrometer. UV-visible absorption spectra were recorded on a Shimadzu UV-3600PLUS spectrophotometer. The emission spectra were recorded on an Edinburgh FS5 Fluorimeter. ESR spectra were recorded using a Bruker Model A300 ESR spectrometer equipped with a Bruker ER 4122 SHQ resonator. Confocal images were recorded on a Zeiss LSM 880 confocal microscopy. The cell viability assays were recorded using a Promega microplate reader. DJO-2776 sonicator was used to generate ultrasound during the treatment. The ultrasound used in this article is 10% duty cycle and the working power is 3 W/cm^2, and the marked power is the average output power, which is calculated by multiplying the working power by 10%. The parameters of US used in this work were listed in Table S2. 465 nm LED lamp was used to generate light during the treatment.

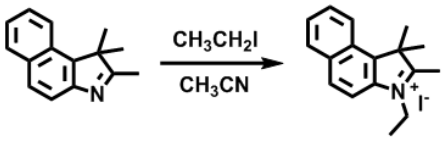
Synthesis

Synthesis of [(ppy)PtCl]$_2$. A mixture containing K_2PtCl_4 (0.913 g, 2.2 mmol) and 2-phenyl-pyridin (0.31 g, 2 mmol) in 20 mL mixed solution of 2-ethoxyethanol and water (v: v=3:1) were refluxed under nitrogen atmosphere and 120 ℃ for 24 h. Cooling it to

room temperature and filtrating the solid under reduced pressure, the yellow precipitate was collected by washing 3 times with diethyl ether. The yield of the yellow solid $[(ppy)PtCl]_2$ was approximately 71.5%. ^{1}H-NMR (500 MHz, DMSO-d^6): δ 9.51 (d, J = 5.8 Hz, 2H), 8.23 (d, J = 7.8 Hz, 2H), 8.16 (d, J = 10.6 Hz, 4H), 7.80 (d, J = 7.6 Hz, 2H), 7.53 (t, J = 7.3 Hz, 2H), 7.20 (t, J = 7.3 Hz, 2H), 7.16 (t, J = 8.3 Hz, 2H).

Synthesis of Pt-CHO. $[(ppy)PtCl]_2$ (1 equiv, 0.231 g, 0.3 mmol) was dissolved in 20 mL 2-ethoxyethanol, and the 3,3 '-bipyridine-6,6'-diformaldehyde (2 equiv, 0.127 g, 0.6 mmol) was then added. The reaction mixture was refluxed for 24 h under nitrogen atmosphere in the dark. After cooling to room temperature, a red brown precipitate was filtered and washed with dichloromethane. The yield of the red brown solid was approximately 35.6%. ^{1}H NMR (500 MHz, DMSO-d^6): δ 10.22 (s, 2H), 9.48 (d, J = 3.0 Hz, 1H), 9.03 (d, J = 3.3 Hz, 1H), 8.83 (s, 1H), 8.21 (d, J = 6.4 Hz, 1H), 8.14 (d, J = 7.2 Hz, 3H), 7.92 (d, J = 3.5 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.51 (s, 2H), 7.20 – 7.11 (m, 3H).

Synthesis of 1-ethyl iodide-2,3,3-trimethylbenz[e]indole. The iodoethane (8.97 g, 0.057 mol) and 1,1,2-trimethylbenz[e]indole (10.06 g, 0.065 mol) were dissolved in 40 mL CH_3CN and then refluxed for 24 h in the dark. After cooling the reaction mixture at room temperature and spinning off the solvent by a rotary evaporator, a grey precipitate was obtained and then washed with diethyl ether. Yield: 40.9%. ^{1}H NMR (500 MHz, DMSO-d^6): δ 8.38 (d, J = 8.3 Hz, 1H), 8.31 (d, J = 8.9 Hz, 1H), 8.23 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 8.9 Hz, 1H), 7.77 (d, J = 28.3, 7.5 Hz, 2H), 4.63 (d, J = 7.4 Hz, 2H), 2.95 (s, 3H), 1.77 (s, 6H), 1.51 (t, J = 7.3 Hz, 3H).



Synthesis of Pt-Cy. **Pt-CHO** (1 equiv, 0.056 g, 0.2 mmol) and 1,1,2-trimethyl-1H-benzo[e]indole (2 equiv, 0.146 g, 0.4 mmol) were dissolved in 20 mL anhydrous ethanol.

The reaction mixture were refluxed for 24 h under nitrogen atmosphere in the dark. After cooling to room temperature, a dark red precipitate was collected by filtering and washing with petroleum ether and ethyl acetate (v: v=3:1). The yield of the dark red solid was approximately 63.7%. ESI-MS (acetone, positive mode): m/z 333.7924 ([M-Cl^--$2I^-$]$^{3+}$/3). Elemental analysis: C, 53.01; H, 4.06; N, 5.42; found: C, 53.05; H, 4.07; N, 5.49. ^{1}H NMR (500 MHz, DMSO-d^6): δ 9.12 (s, 2H), 9.03 (d, J = 5.0 Hz, 2H), 8.72 (d, J = 16.5 Hz, 2H), 8.52 (d, J = 8.3 Hz, 2H), 8.42 – 8.38 (m, 2H), 8.31 – 8.26 (m, 3H), 8.25 (d, J = 8.9 Hz, 2H), 8.19 (d, J = 8.1 Hz, 2H), 8.16 – 8.12 (m, 2H), 8.07 (d, J = 16.6 Hz, 2H), 7.88 (dd, J = 8.2, 7.0 Hz, 2H), 7.81 (dd, J = 11.3, 4.6 Hz, 3H), 7.48 – 7.44 (m, 2H), 7.27 (td, J = 7.4, 1.1 Hz, 2H), 5.00 (dd, J = 14.4, 7.0 Hz, 4H), 2.11 (s, 12H), 1.61 (t, J = 7.3 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d^6): δ 182.65, 165.37, 156.73, 155.89, 150.64, 149.62, 145.30, 144.70, 143.32, 141.59, 140.32, 138.54, 134.06, 133.10, 131.82, 130.57, 129.13, 128.29, 127.15, 125.84, 124.89, 124.19, 123.96, 122.86, 122.38, 120.41, 117.15, 114.01, 55.02, 43.87, 40.54, 25.37, 14.79.

Fluorescence lifetimes

The fluorescence lifetime spectra were achieved with an Edinburgh Instruments FS5 spectrofluorometer equipped with 405 nm pulsed diode laser source using 10 mm path length quartz cuvettes with four transparent polished faces, time-correlated single photon counting (TCSPC) was employed. The sample were measured in DCM at concentration 10 μM. The analysis was performed on Fluoracle software (Edinburgh Instruments). The intensity average lifetimes (τAv,I) and amplitude average lifetimes (τAv,A) were obtained from the fitting parameters according to the following equations:

$$\tau_{Av,I} = \frac{\sum A_I \tau_i^2}{\sum A_i \tau_i}$$

$$\tau_{Av,A} = \frac{\sum A_I \tau_i}{\sum A_i}$$

ROS generation

Analysis of $^1O_2$ generation. The absorbance spectra of 10 μM **Pt-Cy** and 2 μg/mL DPA in H_2O solution (containing 1% DMSO) were measured using UV-vis spectrophotometer after different US (0.3 W/cm^2, 3 MHz) or light (465 nm, 10 W/cm^2)

irradiation durations. The absorbance changes of DPA at 378 nm were recorded to quantify the generation rate of $^1O_2$.

Analysis of •OH generation. 10 μM **Pt-Cy** and 5 μg/mL methylene blue (MB) mixing solution was measured using UV-vis spectrophotometer after different US (0.3 W/cm^2, 3 MHz) irradiation durations.

Cytotoxicity *in vitro*

5×10^3 cells/well 4T1 cells were incubated with different concentrations of **Pt-Cy** for 1 h. After that, the culture media was replaced with fresh culture media, which did not contain the complex. The 96-well plates were in the dark or irradiated by US or 465 nm light, respectively (US: 3 MHz, 0.3 W/cm^2, 20 min; Light: 465 nm, 10 W/cm^2, 30 min). After irradiation, upon further incubation for 43 h and then MTT (25 μL/well, 5 mg/mL) was used to stain the viable cells in the plates for 4 h. The liquid was discard. DMSO (150 μL/well) was added and the optical density was measured at 490 nm by a Promega microplate reader after shaking gently. The wells containing cells incubated without complex were set as control. The cell viability rate (VR) was calculated according to the equation: VR = (A-A_0)/(A_S-A_0) * 100%, where A is the absorbance of the experimental group, A_S is the absorbance of the control group and A_0 is the absorbance of the blank group (no cells).

The live and dead assay was measured using calcein-AM and PI co-staining. 4T1 cells (5×10^3 well^{-1}) were seeded in 96-well plates and incubated overnight at 37 ℃ in a humidified 5 % CO_2 atmosphere. The cells were then incubated with/without **Pt-Cy** (10 μM) for 1 h. After that, the cells were treated or not by US/light irradiation. The cells were stained with calcein-AM for the visualization of live cells and with PI for the visualization of dead/late apoptotic cells according to the manufacturer's suggested protocol.

Intracellular ROS detection

SOSG method: The cells were incubated with 10 μM **Pt-Cy** for 1 h and in the absence of or the presence of 5 mM NaN_3 for 1 h followed by incubation with 2.5 μM Singlet

Oxygen Sensor Green (SOSG) for 30 min. After that, cells were washed with PBS and then irradiated with US (3.0 MHz, 0.3 W/cm^2) for 20 min or light (465 nm, 10 W/cm^2) for 30 min. The fluorescence images were immediately observed using confocal microscopy (λ_{ex} = 488 nm; λ_{em} = 525 ± 30 nm).

DCFH-DA method: The cells were incubated with 10 μM **Pt-Cy** for 1 h, then cells were washed with PBS and irradiated with US (3.0 MHz, 0.3 W/cm^2) for 20 min or light (465 nm, 10 W/cm^2) for 30 min. After that, cells were treated with 10 μM DCFH-DA for 20 min. The fluorescence images were immediately observed (λ_{ex} = 458 nm; λ_{em} = 540 ± 30 nm).

Depletion of GSH in solution

Pt-Cy (10 μM) was mixed with GSH (200 μM) at room temperature. At different US irradiation durations, 50 μL of this solution was added into 450 μL PBS, and then 2 μL 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (10 mg/mL) was added. Then the absorbance spectra of the mixture were measured by UV-vis spectroscopy.

Cellular GSH detection

The **Pt-Cy**-induced cellular GSH concentration variation was determined by the commercial GSH assay kit. The 4T1 cells (1×10^7) in a 13 cm culture dish were treated by **Pt-Cy** at a dose of 10 μM in the dark or under US irradiation. The incubation time was set at 1 h and drugs-free cells were used as the control. Then the harvested cells were suspended in 0.6 mL of PBS and sonicated for 10 min at 4 ^{o}C. After that, based on the product protocol, the absolute GSH level in 4T1 cells with reference to unit protein concentration was calculated based on the product protocol. The relative GSH level in drugs-treated cells was calculated against that in control cells.

GPX4 analysis

4T1 cells were seeded in 6-well plates at a density of 4×10^5 per well. After 24 h, the complex were added at a dose of 10 μM. After 1 h's incubation, the cells were irradiated by US for 20 min. The cells without US irradiation were used as control. All cells were collected, the expression of GPX4 upon formulation treatment was analyzed by western

blotting. The cell lysates containing identical protein (40 μg) were subjected to standard electrophoresis, followed by antibody incubation at 4 ^{o}C. The dilution ratio for the first antibody was 1:2000 (β-actin-specific antibody) and 1:2500 (GPX4-specific antibody). Regarding the secondary antibody, the dilution ratio was 1:5000 for both GPX4 and β-actin. The protein bands were developed via the ECLTM western blotting detection reagents.

Metabolomics analysis

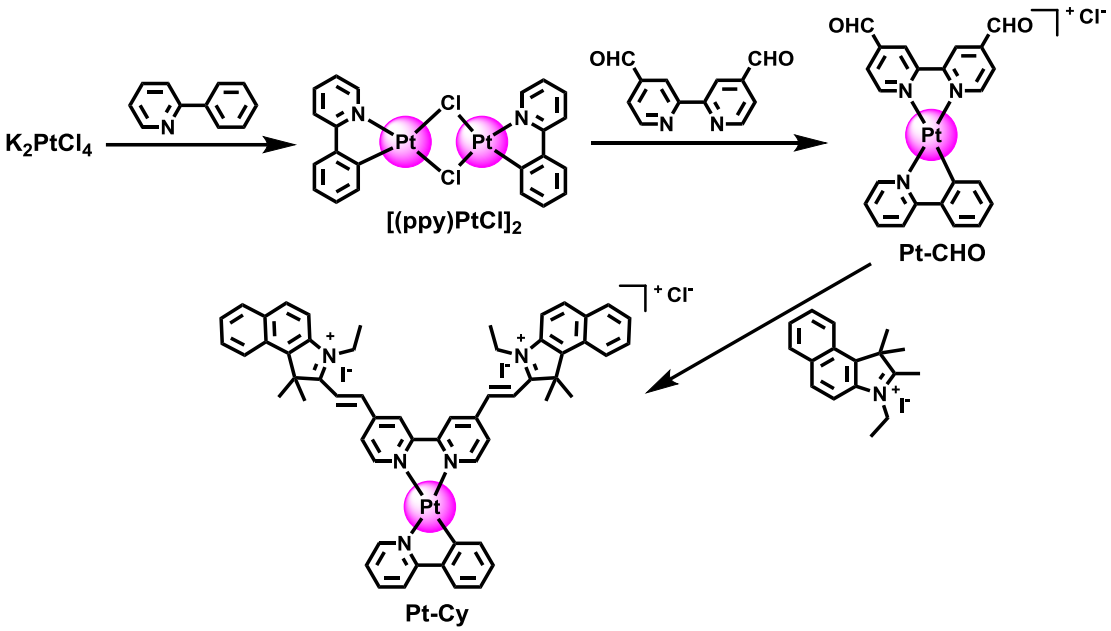
4T1 cells used for metabolomics analysis were treated with different conditions (control, US, **Pt-Cy** and **Pt-Cy** + US). Metabolites from samples (six biological replicates) were extracted and dissolved with acetonitrile : H_2O (v : v, 1 : 1). The solution was injected into the UPLC-MS/MS system analysis. Raw instrument data were exported to Compound Discoverer 3.1 (CD3.1). Identifies compounds using mzCloud (ddMS2) and ChemSpider (formula or exact mass). Performs similarity search for all compounds with ddMS2 data using mzCloud. Applies mzLogic algorithm to rank order ChemSpider results. Maps compounds to biological pathways using Metabolika. QC samples were used for batch standardization. Calculates differential analysis (t-test or ANOVA), determines p-values, adjusted p-values, ratios, fold change, CV, etc. The metabolites with P-value < 0.05 and FC < 0.67 or FC > 1.5 were considered to be differential metabolites. Pathway analysis was performed with MetaboAnalyst 5.0 metabolomics software. Drug does: **Pt-Cy**, 20 μM. US irradiation: 3.0 MHz, 0.3 mW/cm^2.

***In vivo* experiments**

Balb/c mice and nude mice were purchased from Liaoning Changsheng Biotechnology Co. Ltd. This work was conducted in according with Animal Care and Institutional Ethical Guidelines in China. And all animal experiments were carried out under the permission by the Ethic Committee of Shenzhen University (certificate number: SYXK 2014-0140). One million 4T1 cancer cells in 25 μL PBS were subcutaneously injected to the right back of each mouse. About 7 days after injection, the mice with ~100 mm^3

tumor volume were selected for further experiments.

4T1 tumor bearing Balb/c mice were randomly divided into 6 groups (n = 5 per group) for various treatments: (1) Control, (2) Only **Pt-Cy**, (3) Only US, (4) Only Light hv, (5) **Pt-Cy** + hv, (6) **Pt-Cy** + US; **Pt-Cy**: i.t. injection, 500 μM, 25 μL per mice; US: 3 MHz, 0.3 W/cm^2, 20 min. Light: 465 nm, 10 W/cm^2, 30 min. Tumor sizes were monitored every two days for 14 days. The tumor volumes were calculated by the formula: volume = 0.5*length×width^2. 14 days after treatment, the mice were sacrificed and their tumors were collected for photographing and weighing. For histology examination, at 24 h post treatment, tumor tissue was collected from different groups of mice. After fixing in 10% formalin, tumor tissue was paraffin embedded and sectioned for H&E and TUNEL staining.



Scheme S1. The synthetic route of **Pt-Cy**.

Figures

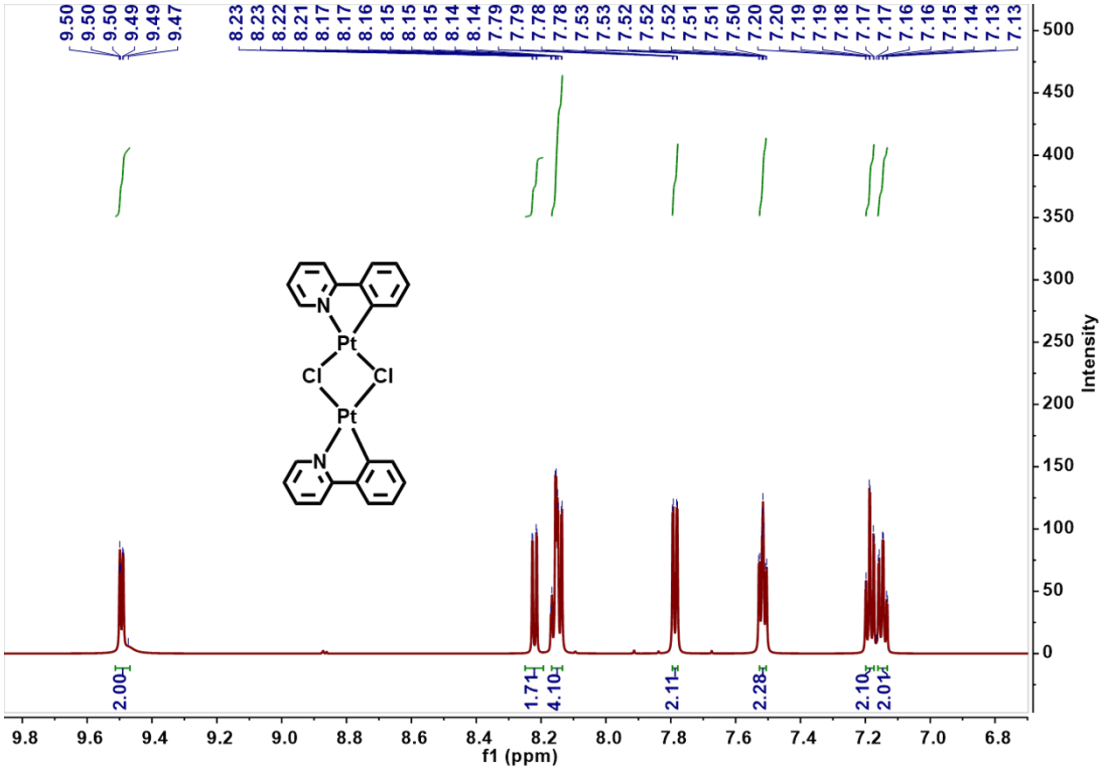


Fig. S1. The 500 MHz ^{1}H NMR spectrum of [(ppy)PtCl]$_2$ in the DMSO-d$_6$ solution.

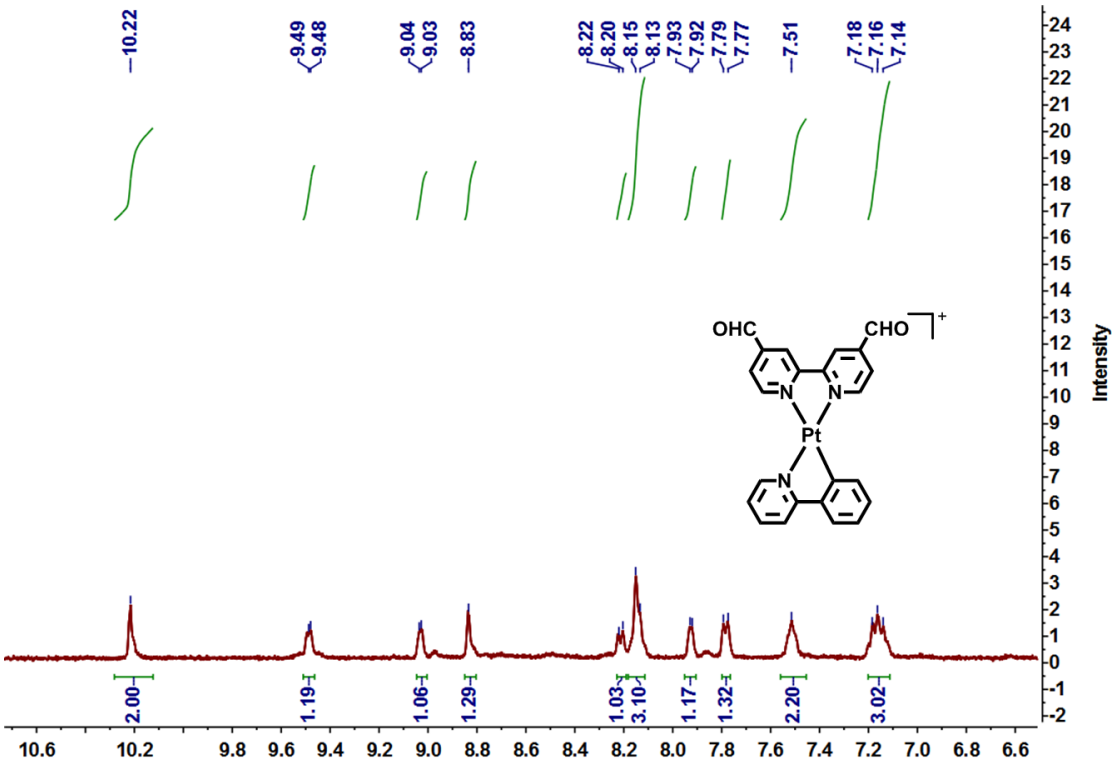


Fig. S2. The 500 MHz ^{1}H-NMR spectrum of **Pt-CHO** in the DMSO-d_6 solution.

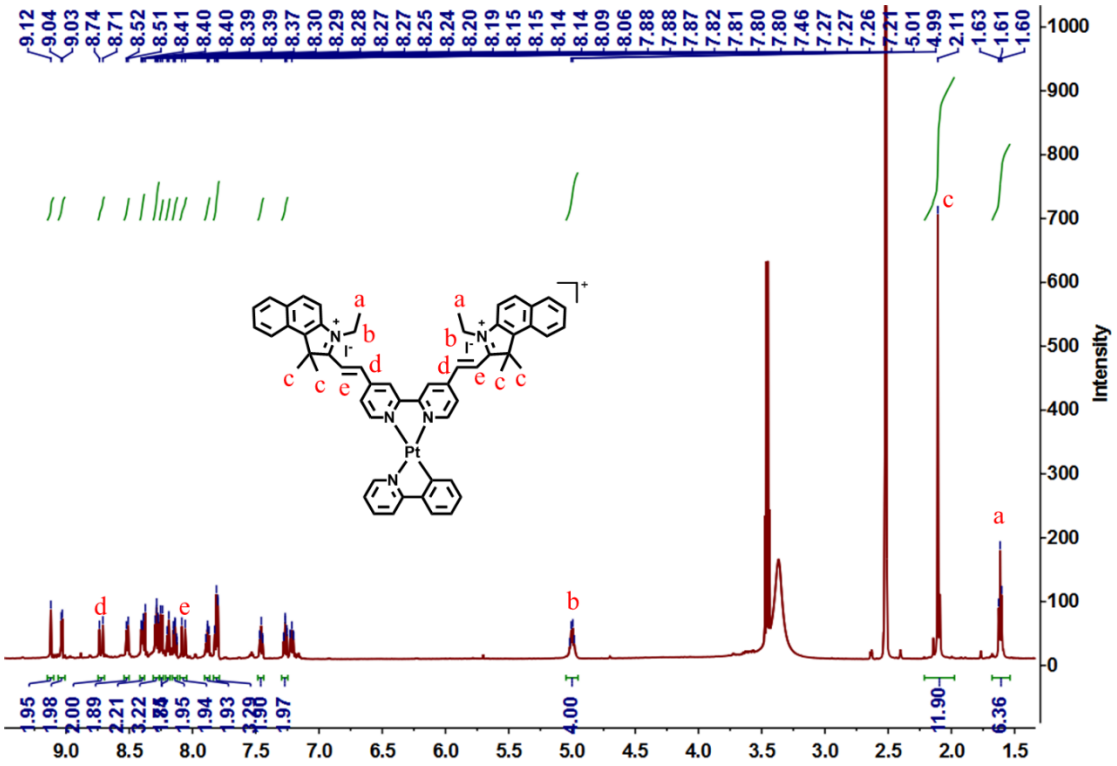


Fig. S3. The 500 MHz ^{1}H NMR spectrum of **Pt-Cy** in the DMSO-d_6 solution.

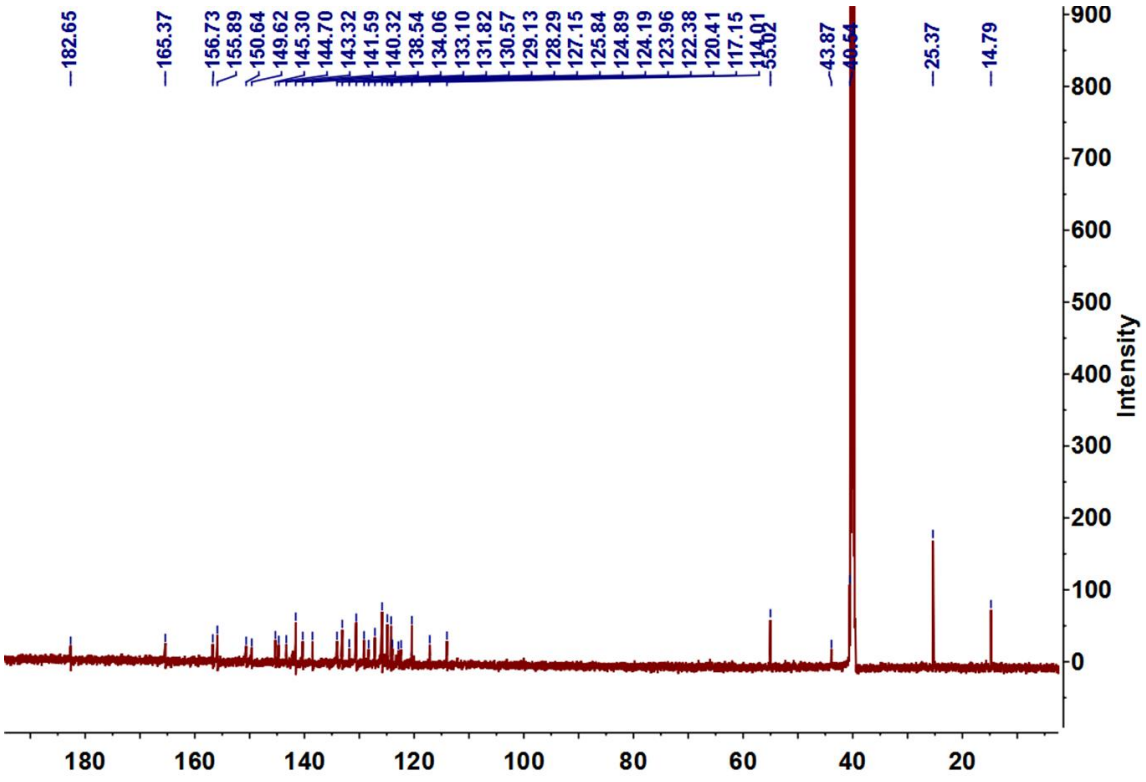


Fig. S4. The ^{13}C NMR spectrum of **Pt-Cy** in the DMSO-d_6 solution.

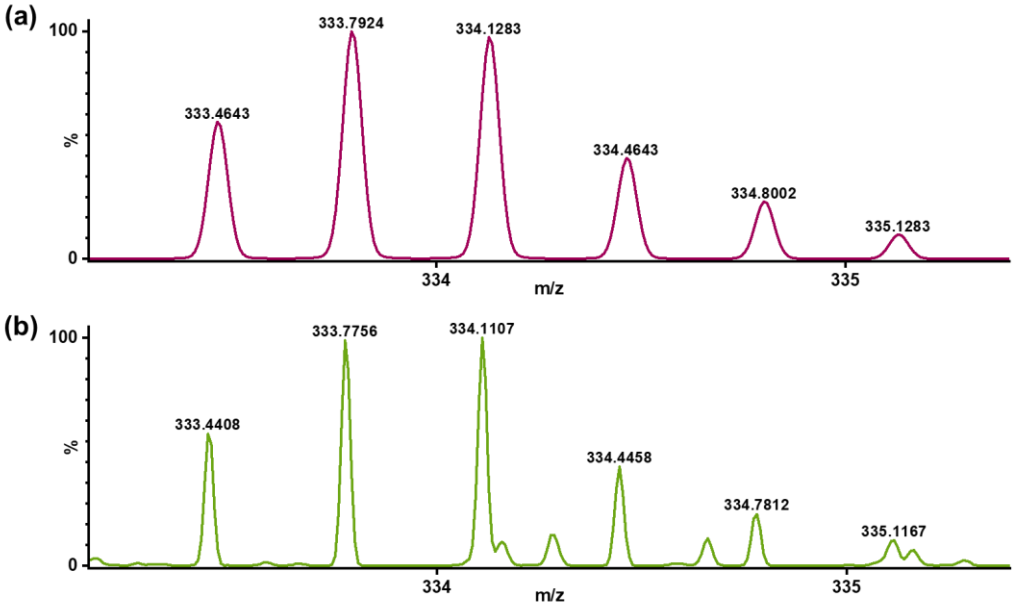


Fig. S5. The enlarged ESI-MS spectrum of (a) the theoretical calculated value and (b) the measured value of **Pt-Cy** (acetone, positive mode). m/z 333.7924 ($[M\text{-}Cl^{-}\text{-}2I^{-}]^{3+}/3$).

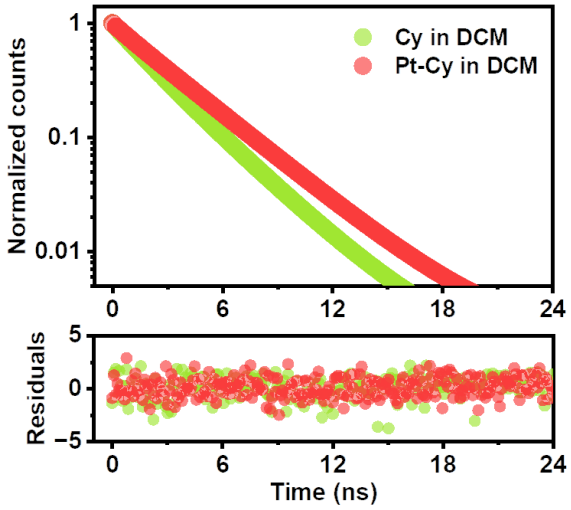


Fig. S6. The fluorescence lifetime decay of Cy and **Pt-Cy** (10 μM) in DCM with fitting and residuals (bottom) (λ_{ex} = 405 nm).

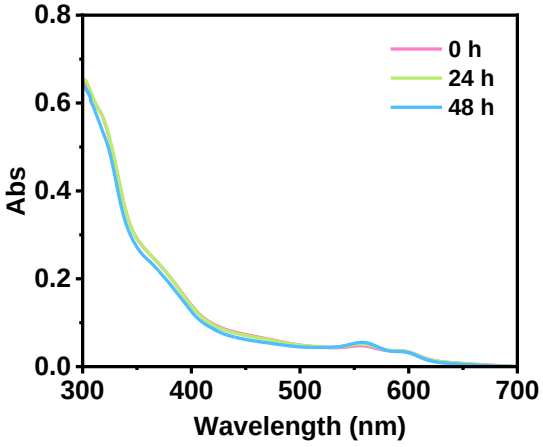


Fig. S7. UV-vis absorption spectra of **Pt-Cy** (20 μM) in the 1640 cell culture medium (containing 10 % FBS) in the dark for 24 h and 48 h, respectively.

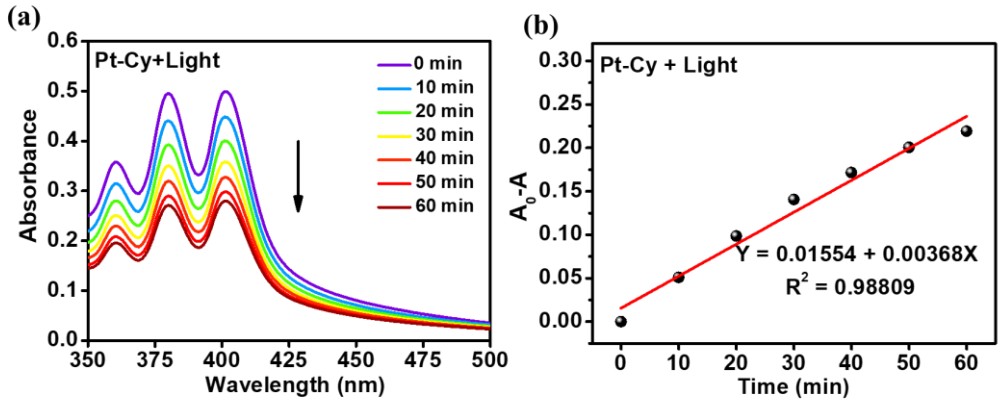


Fig. S8. (a) Time-dependent oxidation of DPA indicating $^1O_2$ generation by **Pt-Cy** (10 μM) under 465 nm light irradiation (10 mW/cm^2). (b) Rate constant for DPA decomposition at 378 nm in (a).

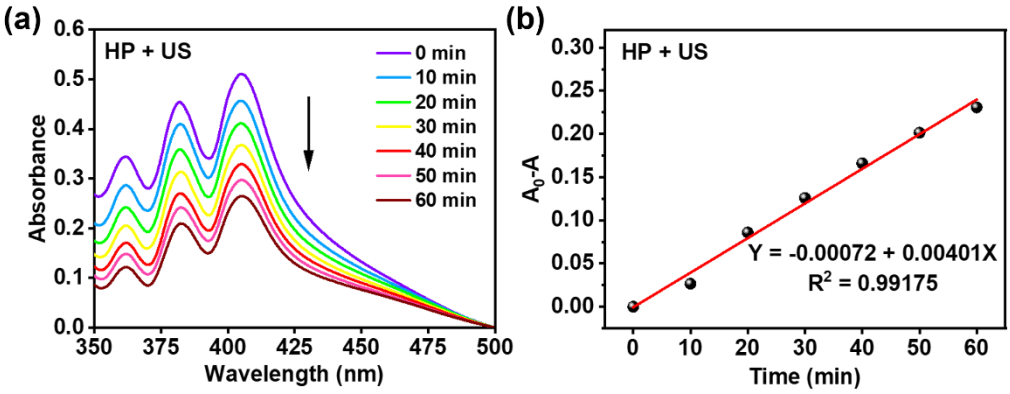


Fig. S9. (a) Time-dependent oxidation of DPA indicating $^1O_2$ generation by hematoporphyrin (HP) (10 μM) under US irradiation (0.3 W/cm^2, 3 MHz). (b) Rate constant for DPA decomposition at 378 nm in (a).

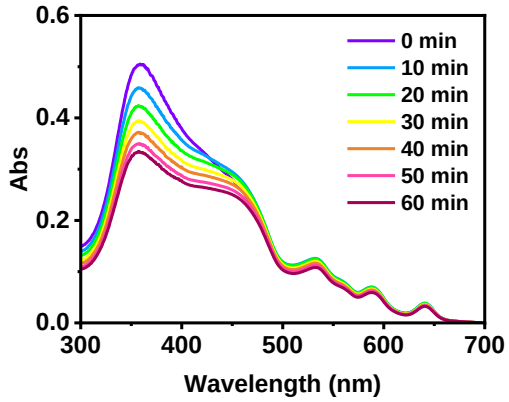


Fig. S10. The stability of hematoporphyrin (HP) (10 μM) by UV-vis absorption spectra under US irradiation (0.3 W/cm^2, 3 MHz).

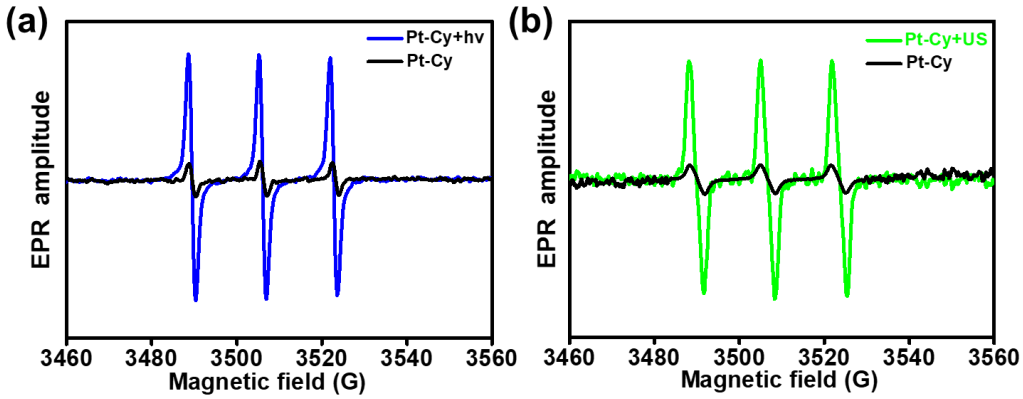


Fig. S11. The ESR signals of TEMP for $^1O_2$ characterization produced by **Pt-Cy** under (a) light irradiation (465 nm, 10 mW/cm^2, 30 min) or (b) US irradiation (3.0 MHz, 0.3 W/cm^2, 20 min).

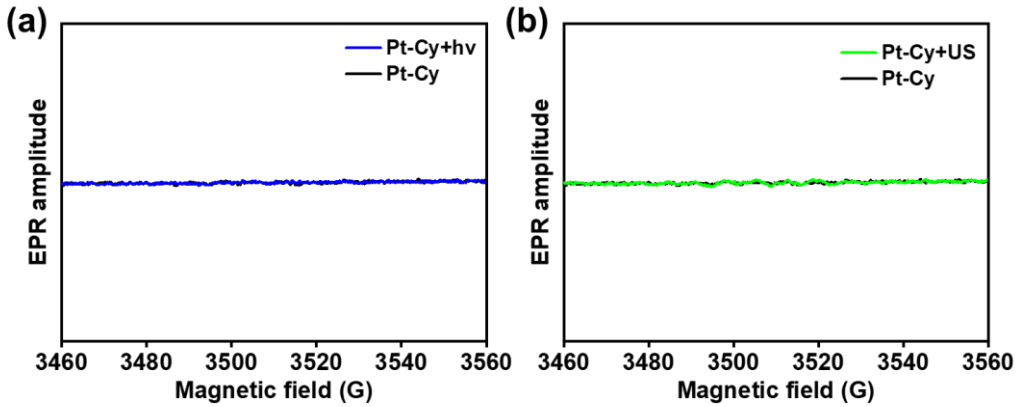


Fig. S12. The ESR signals of DMPO for •OH characterization produced by **Pt-Cy** under (a) light irradiation (465 nm, 10 mW/cm^2, 30 min) or (b) US irradiation (3.0 MHz, 0.3 W/cm^2, 20 min).

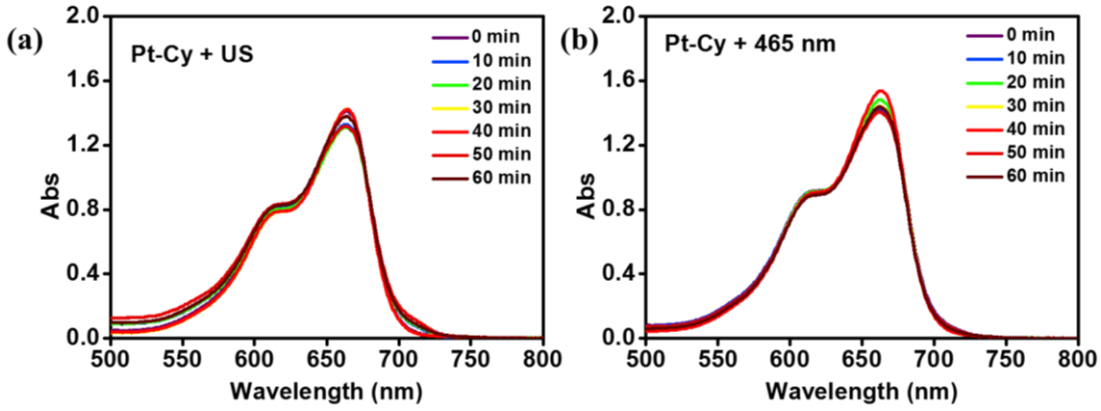


Fig. S13. The absorption spectra of MB (5 μg mL^{-1}) to detect •OH generation by **Pt-Cy** under (a) US irradiation (3.0 MHz, 0.3 W/cm^2) or (b) light irradiation (465 nm, 10 mW/cm^2).

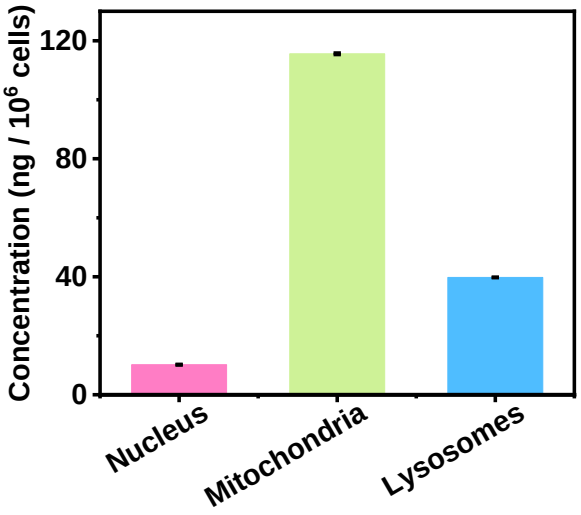


Fig. S14. ICP-MS quantification of the subcellular distribution of Pt in the 4T1 cells treated with **Pt-Cy**.

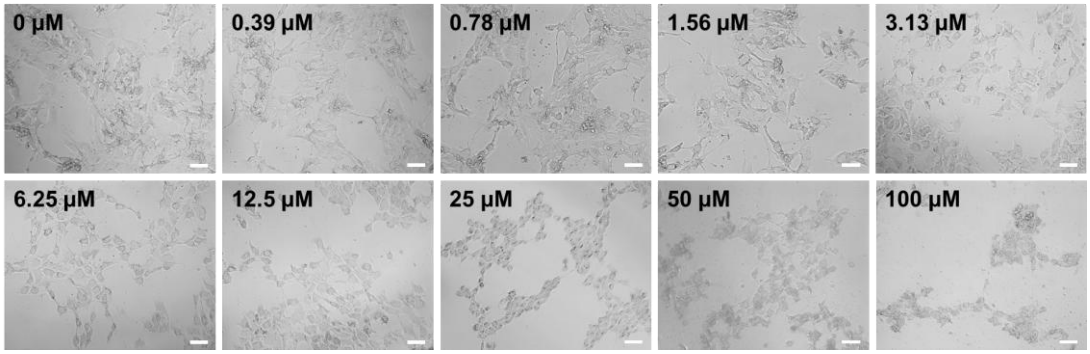


Fig. S15. Bright-field microscopy images of 4T1 cells treated with different concentrations of **Pt-Cy** under US irradiation (3.0 MHz, 0.3 mW/cm^2, 20 min). Scale bar: 50 μM.

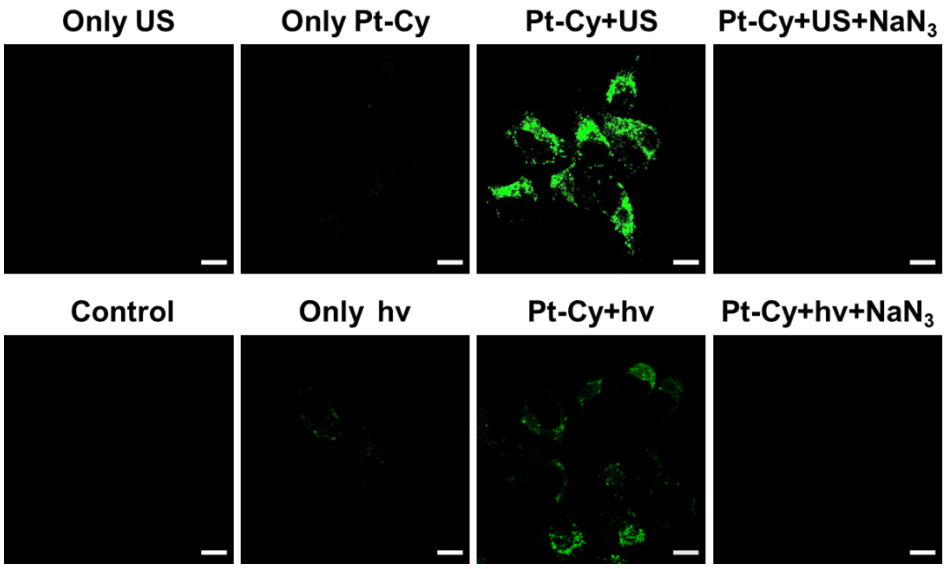


Fig. S16. Confocal microscopy images of 4T1 cells treated with **Pt-Cy** (10 μM, 1 h) and co-stained with SOSG (2.5 μM, 30 min) in the presence or absence of NaN_3 (5 mM, 1 h) under different conditions. US irradiation: 3.0 MHz, 0.3 W/cm^2, 20 min. Light irradiation: 465 nm, 10 mW/cm^2, 30 min. The SOSG probe was excited at 488 nm and the emission was collected at 525 ± 30 nm. Scale bar: 20 μm.

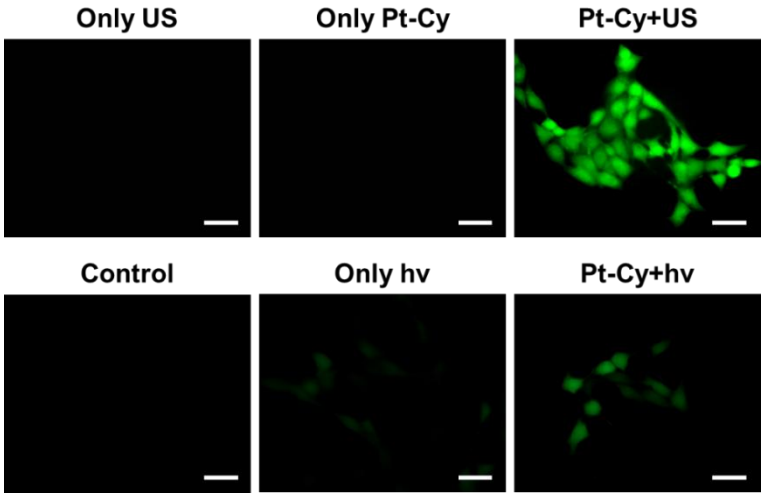


Fig. S17. The fluorescence images of ROS detected by DCFH-DA in 4T1 cells treated with **Pt-Cy** (10 μM, 1 h) after different treatments. US irradiation: 3.0 MHz, 0.3 W/cm^2, 20 min. Light irradiation: 465 nm, 10 mW/cm^2, 30 min. The DCFH-DA probe was excited at 458 nm and the emission was collected at 540 ± 30 nm. Scale bar: 40 μm.

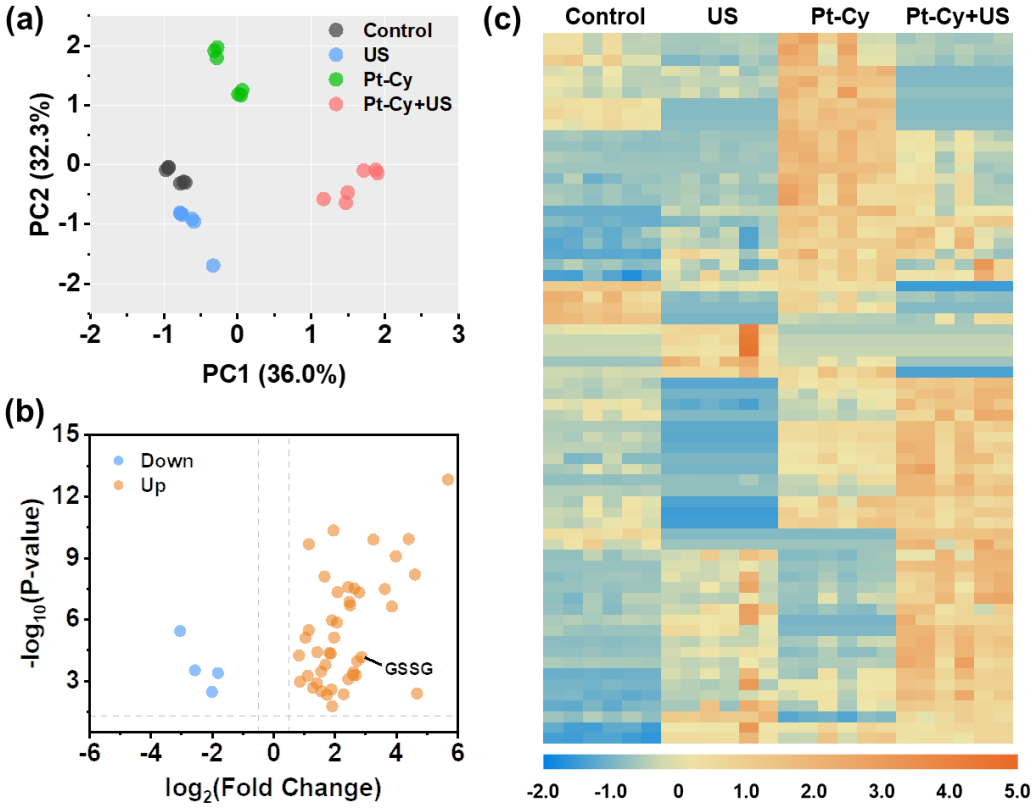


Fig. S18. (a) PCA score plot showing differences between control, US, **Pt-Cy** and **Pt-Cy** + US groups of 4T1 cells. (b) Volcano plot demonstrating altered metabolite levels between control and **Pt-Cy** + US groups. (c) Heatmap of metabolites in each treatment group. US irradiation: 3.0 MHz, 0.3 mW/cm^2, 20 min. Ion mode: negative.

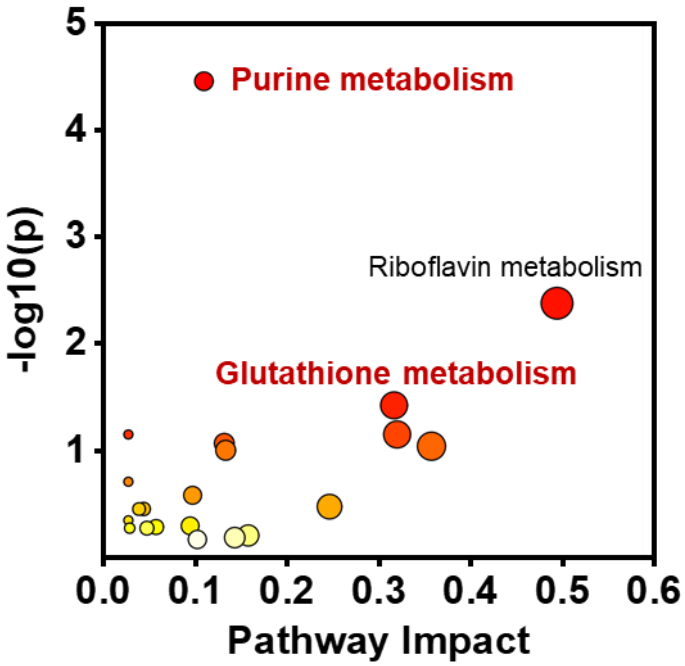


Fig. S19. Pathway analysis of significant differential metabolites between control and **Pt-Cy** + US groups. US irradiation: 3.0 MHz, 0.3 mW/cm^2, 20 min. Ion mode: negative.

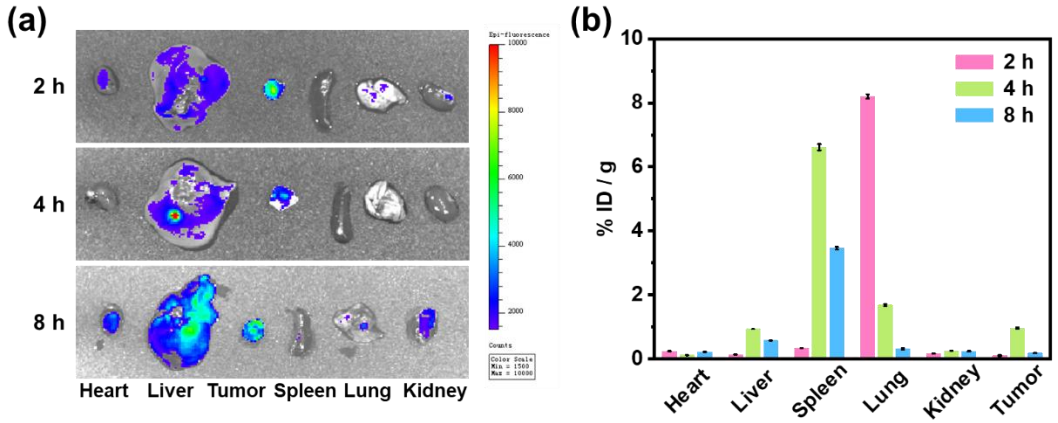


Fig. S20. (a) The fluorescence imaging of the tumor tissue and other organs collected at 2, 4, 8 h post i.v. injection, respectively. (b) The Pt biodistributions of tumor tissue and other organs in (a) were measured by ICP-MS analysis.

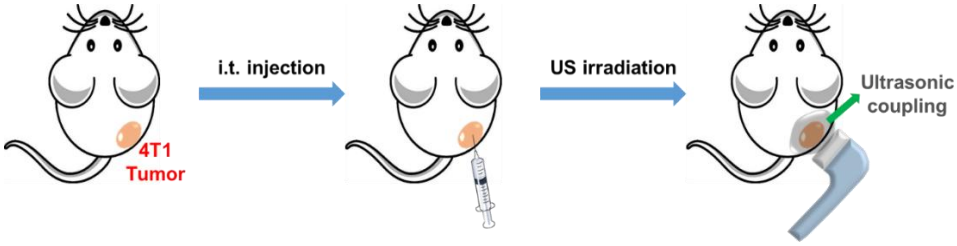


Fig. S21. The schematic diagram of the in vivo therapeutic protocol for SDT.

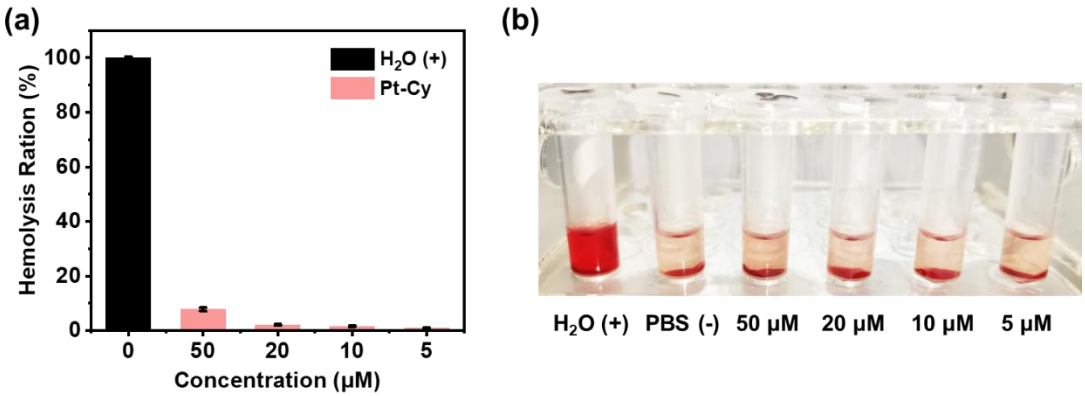


Fig. S22. (a) The hemolysis ration of mouse red blood cells treated with different concentrations of **Pt-Cy**. (b) Images of hemolytic effects of mouse red blood cells treated with different concentrations of **Pt-Cy**.

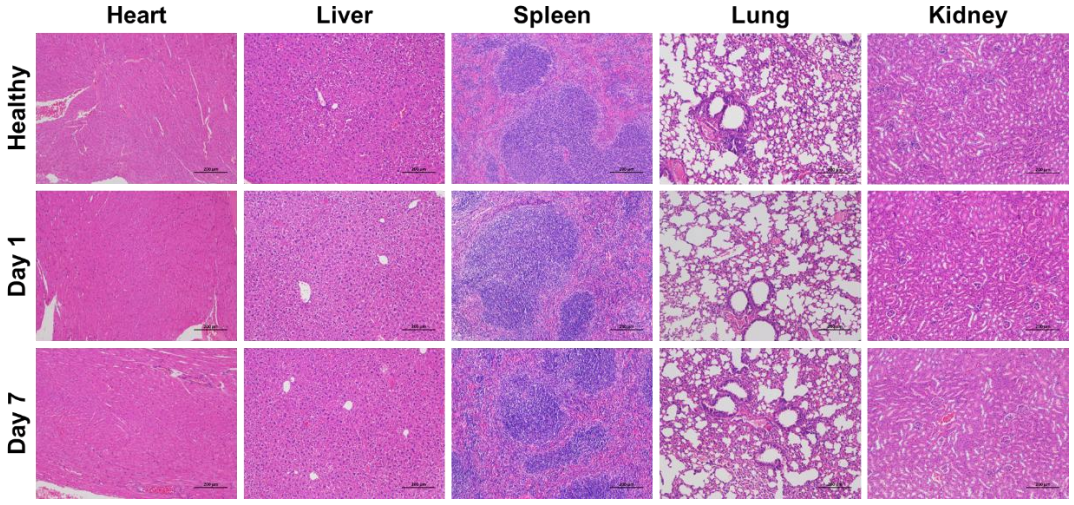


Fig. S23. H&E staining images of the major organs (heart, liver, spleen, lung, and kidney) of healthy Balb/c mice after i.v. injection of **Pt-Cy** (1.99 mg/kg) at different time points (day 1 and 7). Scale bar: 200 μm.

Tables

Table S1. Fluorescence lifetime and the exponential fitting of Cy and **Pt-Cy** (10 μM) in DCM.

	τ / ns	**Std. Dev. / ns**	**Rel / %**	**X^2**	**$\tau_{Av, I}$ / ns**	**$\tau_{Av, A}$ / ns**
Cy	1.81	0.21	25.09	1.22	2.84	2.73
	3.04	0.15	74.91			
Pt-Cy	0.93	0.80	1.35	1.08	3.35	3.33
	3.36	0.02	98.65			

Table S2. Ultrasound parameters involved in the following experiments.

Experiments	**Probe area / cm^2**	**Frequency / MHz**	**Intensity / W/cm^{-2}**	**Duty cycle / %**	**Durations / min**
96-well plates	5	3	3	10	20
6-well plates	10	3	3	10	20
35 mm dishes	5	3	3	10	20
In vivo	1	3	3	10	20

Table S3. Differential metabolites between control and **Pt-Cy** + US groups in positive ion mode.

Name	**Formula**	**Log_2 FC**	**P-value**
Phenylethylamine	$C_8H_{11}N$	-1.44	5.02E-06
Oleic acid	$C_{18}H_{34}O_2$	-0.92	8.01E-05
LysoPC(22:5(7Z,10Z,13Z,16Z,19Z))	$C_{30}H_{52}NO_7P$	0.74	1.05E-03
PC(P-16:0/18:4(6Z,9Z,12Z,15Z))	$C_{42}H_{76}NO_7P$	0.81	1.33E-02
Choline	$C_5H_{13}NO$	0.85	3.03E-06
Prolyl-Glutamine	$C_{10}H_{17}N_3O_4$	0.86	3.76E-03
Aliskiren	$C_{30}H_{53}N_3O_6$	0.94	1.04E-03
cis-5-Tetradecenoylcarnitine	$C_{21}H_{39}NO_4$	0.97	3.72E-04
Monoethylhexyl phthalic acid	$C_{16}H_{22}O_4$	0.99	7.33E-08
7-Dehydrodesmosterol	$C_{27}H_{42}O$	1.00	2.46E-05
PE(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	$C_{45}H_{78}NO_8P$	1.06	1.95E-04
LysoPC(18:3(9Z,12Z,15Z))	$C_{26}H_{48}NO_7P$	1.16	2.34E-05
PC(16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	$C_{46}H_{80}NO_8P$	1.19	1.07E-03
DG(18:2(9Z,12Z)/18:3(9Z,12Z,15Z)/0:0)	$C_{39}H_{66}O_5$	1.21	4.21E-04
LysoPC(P-18:0)	$C_{26}H_{54}NO_6P$	1.23	1.19E-05

Arginyl-Proline	$C_{11}H_{21}N_5O_3$	1.25	4.19E-05
Acetyl tributyl citrate	$C_{20}H_{34}O_8$	1.28	1.18E-09
Prolyl-Lysine	$C_{11}H_{21}N_3O_3$	1.31	3.39E-06
Calcitroic acid	$C_{23}H_{34}O_4$	1.35	8.62E-04
Niacinamide	$C_6H_6N_2O$	1.45	5.35E-05
L-Pipecolic acid	$C_6H_{11}NO_2$	1.47	2.07E-04
Eicosapentaenoic acid	$C_{20}H_{30}O_2$	1.50	1.09E-02
7-Dehydrocholesterol	$C_{27}H_{44}O$	1.51	1.41E-05
Protoporphyrin IX	$C_{34}H_{34}N_4O_4$	1.51	8.26E-06
L-Valine	$C_5H_{11}NO_2$	1.51	1.53E-05
8,11,14-Eicosatrienoic acid	$C_{20}H_{34}O_2$	1.53	8.55E-04
LysoPC(16:0)	$C_{24}H_{50}NO_7P$	1.54	5.32E-05
Glutamylaspartic acid	$C_9H_{14}N_2O_7$	1.55	1.59E-05
7-Sulfocholic acid	$C_{24}H_{40}O_8S$	1.56	1.73E-04
Glutathione	$C_{10}H_{17}N_3O_6S$	1.59	1.07E-07
L-Carnitine	$C_7H_{15}NO_3$	1.68	4.74E-05
Lacto-N-tetraose	$C_{26}H_{45}NO_{21}$	1.69	2.71E-08
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	1.70	3.96E-07
Glutamylserine	$C_8H_{14}N_2O_6$	1.71	1.68E-05
Vitamin A	$C_{20}H_{30}O$	1.72	1.22E-05
PC(14:0/14:0)	$C_{36}H_{72}NO_8P$	1.73	1.44E-04
Mimosine	$C_8H_{10}N_2O_4$	1.76	3.80E-04
18-Hydroxycorticosterone	$C_{21}H_{30}O_5$	1.77	1.40E-10
Methyl nicotinate	$C_7H_7NO_2$	1.78	5.01E-07
Propylene glycol stearate	$C_{21}H_{42}O_3$	1.79	1.98E-06
Glycerophosphocholine	$C_8H_{20}NO_6P$	1.79	4.80E-05
Inosinic acid	$C_{10}H_{13}N_4O_8P$	1.80	2.05E-04
N-Acetylneuraminic acid	$C_{11}H_{19}NO_9$	1.84	4.91E-05
Trimethadione	$C_6H_9NO_3$	1.87	1.81E-10

Cytidine monophosphate	$C_9H_{14}N_3O_8P$	1.88	1.03E-03
L-Cysteinylglycine disulfide	$C_8H_{15}N_3O_5S_2$	1.91	6.91E-05
Abietinol	$C_{20}H_{32}O$	2.03	6.07E-06
Edetic Acid	$C_{10}H_{16}N_2O_8$	2.04	2.53E-05
(3beta,24R,24'R)-fucosterol epoxide	$C_{29}H_{48}O_2$	2.05	2.99E-06
Phosphonoacetaldehyde	$C_2H_5O_4P$	2.06	2.00E-05
L-Arginine	$C_6H_{14}N_4O_2$	2.09	1.01E-04
L-Phenylalanine	$C_9H_{11}NO_2$	2.09	2.26E-06
Uridine diphosphate-N-acetylglucosamine	$C_{17}H_{27}N_3O_{17}P_2$	2.16	3.52E-08
Linoleyl carnitine	$C_{25}H_{45}NO_4$	2.23	1.35E-04
Creatine	$C_4H_9N_3O_2$	2.27	2.67E-07
7a-Hydroxy-cholestene-3-one	$C_{27}H_{44}O_2$	2.28	8.82E-07
CDP	$C_9H_{15}N_3O_{11}P_2$	2.30	5.53E-04
Uridine 5'-diphosphate	$C_9H_{14}N_2O_{12}P_2$	2.36	2.73E-04
Cytidine monophosphate N-acetylneuraminic acid	$C_{20}H_{31}N_4O_{16}P$	2.37	2.81E-05
Kasugamycin	$C_{14}H_{25}N_3O_9$	2.39	4.83E-06
ADP	$C_{10}H_{15}N_5O_{10}P_2$	2.40	4.77E-08
Adenosine monophosphate	$C_{10}H_{14}N_5O_7P$	2.41	9.33E-10
Stearoylcarnitine	$C_{25}H_{49}NO_4$	2.42	8.74E-05
L-Palmitoylcarnitine	$C_{23}H_{45}NO_4$	2.42	5.41E-05
L-Methionine	$C_5H_{11}NO_2S$	2.49	2.24E-06
Nicotinamide ribotide	$C_{11}H_{15}N_2O_8P$	2.52	8.81E-09
NAD	$C_{21}H_{27}N_7O_{14}P_2$	2.52	1.35E-08
Hypoxanthine	$C_5H_4N_4O$	2.54	3.42E-08
lysoPC(26:0)	$C_{34}H_{70}NO_7P$	2.57	1.27E-06
Indoleacrylic acid	$C_{11}H_9NO_2$	2.69	9.18E-09
Cepharadione A	$C_{18}H_{11}NO_4$	2.70	6.02E-03
Guanosine	$C_{10}H_{13}N_5O_5$	2.71	5.89E-07

Adenosine	$C_{10}H_{13}N_5O_4$	2.72	1.57E-04
Xanthine	$C_5H_4N_4O_2$	2.73	1.85E-08
6-Methylquinoline	$C_{10}H_9N$	2.73	1.19E-06
Citicoline	$C_{14}H_{26}N_4O_{11}P_2$	2.81	3.67E-07
3-Methylindole	C_9H_9N	2.84	7.34E-07
gamma-Glutamylleucine	$C_{11}H_{20}N_2O_5$	2.86	9.50E-09
N-Acetylglutamine	$C_7H_{12}N_2O_4$	2.91	7.84E-08
L-Leucine	$C_6H_{13}NO_2$	2.99	2.48E-09
Adenine	$C_5H_5N_5$	3.01	2.54E-10
5'-Methylthioadenosine	$C_{11}H_{15}N_5O_3S$	3.02	3.95E-12
NADP	$C_{21}H_{28}N_7O_{17}P_3$	3.09	1.17E-04
Phenylacetylglycine	$C_{10}H_{11}NO_3$	3.10	3.57E-10
Gibberellin A67	$C_8H_{18}N_2O_4S$	3.13	4.77E-08
1H-Indole-3-carboxaldehyde	C_9H_7NO	3.17	5.16E-08
gamma-Glutamylcysteine	$C_8H_{14}N_2O_5S$	3.18	1.13E-11
L-Proline	$C_5H_9NO_2$	3.19	2.02E-07
Guanine	$C_5H_5N_5O$	3.19	1.05E-07
Benzoic acid	$C_7H_6O_2$	3.23	5.18E-09
5'-Phosphoribosyl-N-formylglycinamide	$C_8H_{15}N_2O_9P$	3.24	7.11E-06
Pyroglutamic acid	$C_5H_7NO_3$	3.24	1.98E-07
Pregabalin	$C_8H_{17}NO_2$	3.25	3.03E-07
Guanosine diphosphate	$C_{10}H_{15}N_5O_{11}P_2$	3.25	2.73E-05
Uridine 5'-monophosphate	$C_9H_{13}N_2O_9P$	3.25	7.45E-07
Uracil	$C_4H_4N_2O_2$	3.32	6.66E-08
Oxidized glutathione	$C_{20}H_{32}N_6O_{12}S_2$	3.36	5.87E-05
Lanosterin	$C_{30}H_{50}O$	3.37	1.73E-06
L-Isoleucine	$C_6H_{13}NO_2$	3.37	1.73E-11
5-Dehydroavenasterol	$C_{29}H_{46}O$	3.43	5.13E-05
Spermidine	$C_7H_{19}N_3$	3.53	3.15E-03

Pantothenic acid	$C_9H_{17}NO_5$	3.60	1.67E-05
2-Aminobenzoylacetate	$C_9H_9NO_3$	3.67	3.93E-09
lysoPC(28:1(5Z))	$C_{36}H_{72}NO_7P$	3.71	3.71E-07
Penicillin G	$C_{16}H_{18}N_2O_4S$	4.07	9.97E-10
22alpha-Hydroxy-campest-4-en-3-one	$C_{28}H_{46}O_2$	4.10	2.49E-07
Adenosine triphosphate	$C_{10}H_{16}N_5O_{13}P_3$	4.36	4.20E-04
4,4-Dimethyl-14alpha-formyl-5alpha-cholesta-8-en-3beta-ol	$C_{30}H_{50}O_2$	4.52	2.76E-04
Indoleacetic acid	$C_{10}H_9NO_2$	4.53	1.03E-09
2,4-Dimethylthiazole	C_5H_7NS	4.75	2.64E-09
Fluconazole	$C_{13}H_{12}F_2N_6O$	4.77	2.61E-09
Adenylsuccinic acid	$C_{14}H_{18}N_5O_{11}P$	4.78	2.47E-12
Cycloeucalenone	$C_{30}H_{48}O$	4.80	2.96E-05
L-Glutamic acid	$C_5H_9NO_4$	4.83	3.27E-09
Bromocriptine	$C_{32}H_{40}BrN_5O_5$	5.11	2.54E-04

Table S4. Differential metabolites between control and **Pt-Cy** + US groups in negative ion mode.

Name	**Formula**	**Log_2 FC**	**P-value**
Prostaglandin F1a	$C_{20}H_{36}O_5$	-3.04	3.80E-06
11(R)-HETE	$C_{20}H_{32}O_3$	-2.56	3.13E-04
Prostaglandin E2	$C_{20}H_{32}O_5$	-2.00	3.53E-03
Prostaglandin F2a	$C_{20}H_{34}O_5$	-1.81	4.23E-04
dGDP	$C_{10}H_{15}N_5O_{10}P_2$	0.83	5.80E-05
Guanosine diphosphate	$C_{10}H_{15}N_5O_{11}P_2$	0.86	1.12E-03
Glutathione	$C_{10}H_{17}N_3O_6S$	1.04	8.06E-06
Edetic Acid	$C_{10}H_{16}N_2O_8$	1.11	5.72E-04
Asparaginyl-Proline	$C_9H_{15}N_3O_4$	1.15	3.40E-06
Chenodeoxycholic acid glycine conjugate	$C_{26}H_{43}NO_5$	1.15	2.17E-10

Linoleyl carnitine	$C_{25}H_{45}NO_4$	1.28	2.19E-03
9,12-Hexadecadienoylcarnitine	$C_{23}H_{41}NO_4$	1.41	1.32E-03
LysoPA(18:0/0:0)	$C_{21}H_{43}O_7P$	1.42	3.97E-05
Cytidine	$C_9H_{13}N_3O_5$	1.55	3.52E-04
4a-Carboxy-4b-methyl-5a-cholesta-8,24-dien-3b-ol	$C_{29}H_{46}O_3$	1.56	3.29E-03
2'-Deoxyguanosine 5'-monophosphate	$C_{10}H_{14}N_5O_7P$	1.66	8.21E-09
Uridine	$C_9H_{12}N_2O_6$	1.69	1.65E-04
Arachidonic acid	$C_{20}H_{32}O_2$	1.74	4.85E-03
Taurocholic acid	$C_{26}H_{45}NO_7S$	1.82	4.45E-05
Uridine 5'-monophosphate	$C_9H_{13}N_2O_9P$	1.86	4.70E-05
4-Amino-2-methyl-5-phosphomethylpyrimidine	$C_6H_{10}N_3O_4P$	1.88	2.68E-03
NAD	$C_{21}H_{27}N_7O_{14}P_2$	1.90	1.13E-06
Wax ester	$C_{16}H_{32}O_4$	1.91	1.74E-02
Cascarillin	$C_{22}H_{32}O_7$	1.95	4.63E-11
UDP-N-acetyl-D-mannosamine	$C_{17}H_{27}N_3O_{17}P_2$	1.97	7.75E-06
FAD	$C_{27}H_{33}N_9O_{15}P_2$	2.06	1.45E-06
GDP-L-fucose	$C_{16}H_{25}N_5O_{15}P_2$	2.08	4.79E-08
Eicosapentaenoic acid	$C_{20}H_{30}O_2$	2.28	4.55E-03
Fructose 1,6-bisphosphate	$C_6H_{14}O_{12}P_2$	2.43	8.44E-04
Inosine	$C_{10}H_{12}N_4O_5$	2.43	2.71E-08
Guanosine	$C_{10}H_{13}N_5O_5$	2.47	1.44E-07
gamma-Glutamylleucine	$C_{11}H_{20}N_2O_5$	2.49	2.11E-07
dGTP	$C_{10}H_{16}N_5O_{13}P_3$	2.59	5.17E-04
Uric acid	$C_5H_4N_4O_3$	2.60	3.82E-04
Flavin Mononucleotide	$C17H_{21}N_4O_9P$	2.63	3.13E-08
Oleic acid	$C_{18}H_{34}O_2$	2.68	5.41E-04
5'-Phosphoribosyl-N-formylglycinamide	$C_8H_{15}N_2O_9P$	2.72	1.10E-04

Coenzyme A	$C_{21}H_{36}N_7O_{16}P_3S$	2.79	4.94E-08
Oxidized glutathione	$C_{20}H_{32}N_6O_{12}S_2$	2.86	7.00E-05
Violacein	$C_{20}H_{13}N_3O_3$	3.25	1.30E-10
LysoPA(18:2(9Z,12Z)/0:0)	$C_{21}H_{39}O_7P$	3.62	3.36E-08
NADPH	$C_{21}H_{30}N_7O_{17}P_3$	3.85	2.41E-07
NADH	$C_{21}H_{29}N_7O_{14}P_2$	3.98	8.46E-10
4-Phosphopantothenoylcysteine	$C_{12}H_{23}N_2O_9PS$	4.40	1.22E-10
Penicillin G	$C_{16}H_{18}N_2O_4S$	4.60	6.53E-09
Uridine diphosphategalactose	$C_{15}H_{24}N_2O_{17}P_2$	4.67	4.16E-03
Adenylsuccinic acid	$C_{14}H_{18}N_5O_{11}P$	5.68	1.53E-13