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# Supporting Information

**Pt(IV) Prodrug as Potent Nanosonosensitizer Self-Cyclically Amplify Sonodynamic-Chemotherapy with Dually Reversing Cisplatin Resistance**

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## Experimental Section

**Synthesis of aquated asplation (Aspt).** 100 mg of CDDP (0.33 mmol) was dissolved in 5 mL of water, and $H_2O_2$ (3.5 mL 30% w/v, 0.03 mol) was added to the suspension. The mixture was stirred open at 50 ℃ for 1 h, then stirred at room temperature for another 12 h. Freeze dry the solution. Wash the obtained product with cold water, ethanol, and ether, and dry it again in a vacuum to obtain Oxoplation (Oxo). Dissolve 10 mg of Oxo in 1 mL of DMSO, add twice the amount of 20.4 mg of acetylsalicylic anhydride, and stir at room temperature for 24 h. Wash with acetone and ether, centrifuge at 10000 rpm for 5 min to obtain a yellow oily substance. Add acetone and ether (until white precipitate appears), wash and centrifuge, and repeat twice to obtain Aspt. Aspt was dissolved in ultrapure water, added with $AgNO_3$, reacted at room temperature for 24 h, centrifuged at 12000 rpm for 10 min, and filtered through a 0.22 μm filter membrane to obtain aquated Aspt.

**Synthesis of PHPAP.** Dissolved 3.5 mg of PH in 1 mL of ultrapure water, added different molar ratios of aquated Pt(IV), aquated Aspt solutions, and stirred at 300 rpm for 5 h at room temperature. Transferred the sample from the reaction bottle to a centrifuge tube, centrifuged at 14000 rpm for 20 min, removed the supernatant, precipitated and dispersed with ultrapure water to obtain the PHPAP solution. Stored the PHPAP solution at 4 ℃ for future use.

**Synthesis of PHPA.** Dissolved 3.5 mg of PH in 1 mL of ultrapure water, dissolved aspirin (As) in ultrapure water (PHPA1-3, in Table S3) or DMSO (PHPA-4, in Table S3), added different molar ratios of aquated Pt(IV) and aspirin, and stirred at 300 rpm at room temperature for 5 h. Transferred the sample from the reaction bottle to a centrifuge tube, centrifuged at 14000 rpm for 10 min, removed the supernatant, precipitated and dispersed with ultrapure water to obtain the PHPAP solution. Stored the PHPA solution at 4 ℃ for future use.

**Characterization.** Transmission electron microscopes (TEM, HT-7700) were used to study the morphology of PHPt. A Zetasizer Nanoseries instrument from Malvern Instruments was used to measure the hydrodynamic diameter distribution and zeta potential. Fourier transform infrared (FT-IR) spectra were measured using a Nicolet 6700 FTIR spectrometer. X-ray photoelectron spectroscopy (XPS) spectra were analyzed using an ESCALAB 250Xi X-ray high-performance photoelectron spectrometer. Electron spin resonance (ESR) spectra were acquired on an EMX-500 10/12 EPR spectrometer. Confocal laser scanning microscope (CLSM) images were acquired using a Leica TCS SP8 microscope.

**Intracellular Pt content detection by ICP-MS.** 4T1 cells ($3\times10^5$) were seeded in 6-well plates and cultured for 24 h. Afterward, the cells were treated with PHPt (200 μg $mL^{-1}$) and CDDP (32 μg $mL^{-1}$) and incubated for 24 h. Then the cells were separately harvested with trypsin, washed twice with PBS, and digested with aqua regia for 24 h. Then the intracellular Pt content was measured by ICP-MS.

***In vitro* generation of ROS.** 4T1 cells ($2\times10^5$) were seeded in a glass bottom cell culture dish and cultured for 24 h. Afterward, the cells were treated with PBS (without any treatment), US (with US), CDDP (32 μg $mL^{-1}$), PHPt (200 μg $mL^{-1}$), PHPt+US, PHPt+As (100 μM)+US for 24 h, respectively. The US condition was 1.0 MHz, 1.0 W $cm^{-2}$, 2 min, duty cycle 50%. Subsequently, the cells were incubated with DCFH-DA for 25 min and washed with PBS. Afterward, the 4T1 cells were irradiated with US irradiation for 2 min (1.0 MHz, 50% duty cycle, 1 W $cm^{-2}$) Finally, the generation of ROS was measured by CLSM.

**Live/Dead cell staining.** 4T1 cells ($2\times10^5$) were seeded in a glass bottom cell culture dish and cultured for 24 h. Afterward, the cells were treated with PBS (without any treatment), US, CDDP (32 μg $mL^{-1}$), PHPt (200 μg $mL^{-1}$), PHPt+US, PHPt+As (100 μM)+US for 12 h. For the US, PHPt+US, and PHPt+As+US groups, they were exposed to the US (1.0 MHz, 1.0 W $cm^{-2}$,

2 min, duty cycle 50%), followed by further incubation for 12 h. The group without US treatment continued to be incubated for 12 h. Then, the cells were stained with Calcein-AM and PI for 30 min. The green and red fluorescence were observed by CLSM.

**Immunofluorescence detection of γ-$H_2AX$.** 4T1 cells ($1\times10^5$) were seeded in a glass bottom cell culture dish and cultured for 24 h. Afterward, the cells were treated with PBS (without any treatment), CDDP (32 μg $mL^{-1}$), PHPt (200 μg $mL^{-1}$), PHPt+US, PHPt+As (100 μM)+US for 12 h. For the PHPt+US and PHPt+As+US groups, they were exposed to the US (1.0 MHz, 1.0 W $cm^{-2}$, 2 min, duty cycle 50%), followed by further incubation for 12 h. The group without US treatment continued to be incubated for 12 h. Then, the cells were fixed with 4% paraformaldehyde for 15 min at room temperature and washed three times with washing liquid, and then blocked with Immunofluorescence staining blocking solution for 15 min and washed with washing liquid. Afterward, cells were treated by γ-$H_2AX$ rabbit monoclonal antibody for 1 h at room temperature. Then, cells were washed with washing liquid and incubated with Alexa Fluor 488-conjugated antibody for 1 h at room temperature. After being washed with washing liquid again, nuclei were stained with DAPI for 5 min at room temperature. Subsequently, images were collected with CLSM.

**Determination of NF-κB by ELISA kit.** 4T1 cells ($2\times10^5$) were seeded in 6-well plates and cultured for 24 h. Afterward, the cells were treated with DMEM (without any treatment), CDDP (32 μg $mL^{-1}$), CDDP+As (100 μM), PHPt (200 μg $mL^{-1}$) and PHPt+As and incubated for 12 h. The PHPt+US group and PHPt+As+US group were exposed to the US (1.0 MHz, 1.0 W $cm^{-2}$, 2 min, duty cycle 50%), followed by further incubation for 12 h. The group without US treatment continued to be incubated for 12 h. Then the cell culture supernatant was collected and the NF-κB level was detected according to the protocol of ELISA kit.

**Determination of $PGE_2$ by ELISA kit.** 4T1 cells ($2\times10^5$) were seeded in 6-well plates and cultured for 24 h. Afterward, the cells were treated with DMEM (without any treatment), CDDP (32 μg $mL^{-1}$), CDDP+As (100 μM), PHPt (200 μg $mL^{-1}$) and PHPt+As and incubated for 12 h. The PHPt+US group and PHPt+As+US group were exposed to the US (1.0 MHz, 1.0 W $cm^{-2}$, 2 min, duty cycle 50%), followed by further incubation for 12 h. The group without US treatment continued to be incubated for 12 h. Then the cell culture supernatant was collected and the $PGE_2$ level was detected according to the protocol of ELISA kit.

**Immunofluorescence detection of NF-κB.** 4T1 cells ($1 \times 10^5$) were seeded in 6-well plates with clean coverslips and cultured for 24 h. Afterward, the cells were treated with DMEM (without any treatment), CDDP (32 μg $mL^{-1}$), CDDP+As (100 μM), PHPt (200 μg $mL^{-1}$) and PHPt+As and incubated for 12 h. The PHPt+US group and PHPt+As+US group were exposed to the US (1.0 MHz, 1.0 W $cm^{-2}$, 2 min, duty cycle 50%), followed by further incubation for 12 h. The group without US treatment continued to be incubated for 12 h. Then, the cells were fixed with 4% paraformaldehyde for 15 min. After being blocked with immunol staining blocking buffer for 60 min, samples were incubated with rabbit anti-NF-κB p65 (1 : 200) antibodies at 4 ℃ overnight. FITC-labeled goat anti-rabbit IgG (1 : 1000) was used as the secondary antibody. After being washed with washing liquid, nuclei were stained with DAPI for 5 min. A drop of antifade mounting medium was used to seal the coverslip. Subsequently, images were collected with CLSM.

**Assessment of intracellular GSH depletion by fluorescence detection.** 4T1 cells ($1 \times 10^4$) were seeded in 96-well plates for 24 h. Afterward, the cells were treated with DMEM (without any treatment), PHPt (200 μg $mL^{-1}$) and PHPt+As (100 μM) and incubated for 12 h. The PHPt+As+US group was exposed to the US (1.0 MHz, 1.0 W $cm^{-2}$, 2 min, duty cycle 50%), followed by further incubation for 12 h. The group without US treatment continued to be incubated for 12 h. Then, the cells were washed with PBS and incubated with monochlorobimane (MCB, 40 μmol/L) in the dark for 20 min at room temperature. After the cells were solubilized with 1% SDS and 5 mmol/L Tris HCl, fluorescence was measured by microplate reader.

**ROS staining of tumor sections.** After 4T1 tumor-bearing mice were injected with (1) 100 uL PBS (Control), (2) 2 mg $mL^{-1}$, 100 uL of PHPt and 1 mM Aspirin with US irradiation (1.0 MHz, 50% duty cycle, 1.5 W $cm^{-2}$, 5 min) (PHPt+As+US), respectively, the mice were sacrificed immediately and the tumors were removed. Dihydroethidium (DHE) staining of tumor tissues were used to detect ROS production.

**Hemolysis test of PHPt.** Performed biosafety analysis on BALB/c mouse red blood cells. The cells were washed and diluted with PBS. Water and PBS were used as positive and negative controls, respectively. The PHPt solution was adjusted to different concentrations of 12.5, 25,

50, 100, and 200 μg $mL^{-1}$ with PBS. Mixed PHPt with diluted red blood cells of equal volume and incubate at room temperature for 3 h. Finally, cells were obtained by centrifugation (3000 rpm, 10 min) and the supernatant was collected for absorbance test at 540 nm. Hemolysis rate (%) = (sample absorption-negative control absorption)/(positive control absorption-negative control absorption)×100%

**Blood biochemical assay.** Balb/c mice were randomly divided into two groups (n=3): (1) PBS (Control). (2) PHPt and Aspirin (PHPt+As). Next, the mice were injected subcutaneously with 100 uL PBS and 2 mg $mL^{-1}$, 100 uL of PHPt and 1 mM Aspirin, respectively. All mice were sacrificed on the 7th day after injection, the blood of mice was collected to detect the blood biochemical parameters and evaluate the side effects of the preparation.

**Animals and statement.** BALB/c female mice (5 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal experimentation protocols were approved by the local ethics committee of Beijing University of Chemical Technology.

**Statistical analysis.** Data were analyzed by GraphPad Prism software. The significant difference between every two groups was calculated by Student's T-test. Statistical significance is indicated as: *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

## Supplemental Figures and Tables

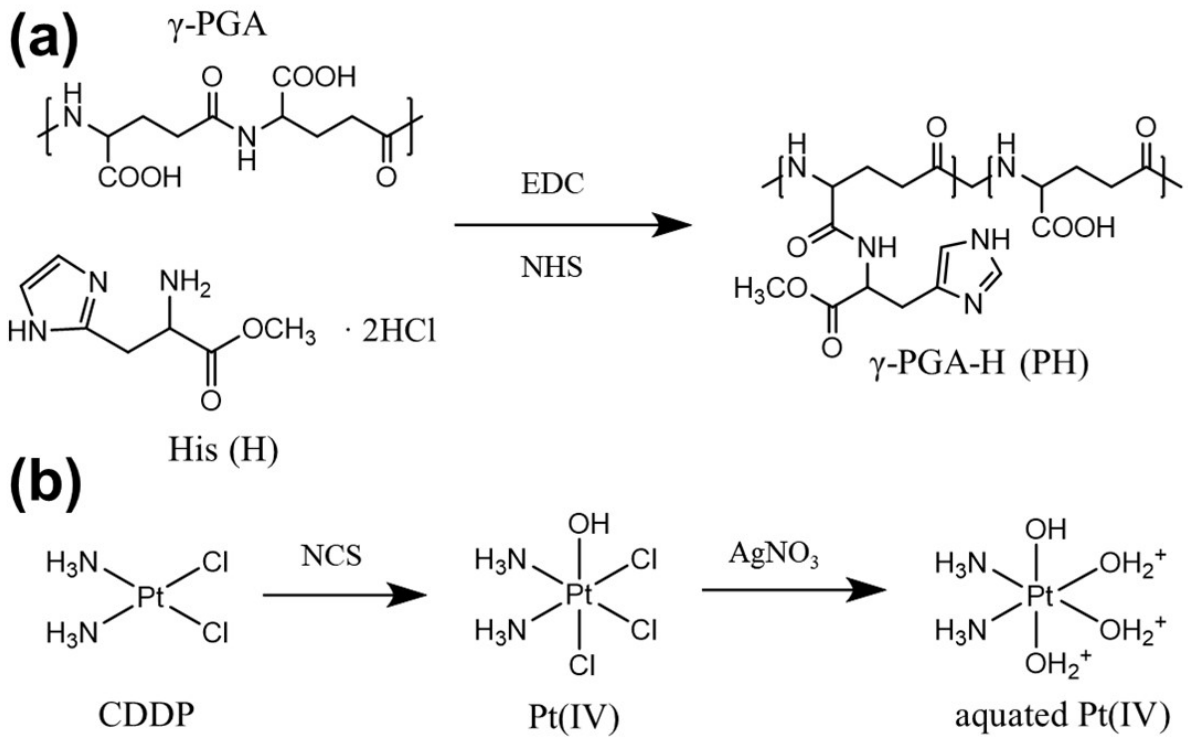


**Figure S1.** (a) Synthesis of γ-PGA-H (PH). (b) Synthesis of aquated Pt(IV).

**Table S1.** Synthesis conditions and particle size of PHPt

| | PH / mmol | aquated Pt(IV) / mmol | Size / nm | PDI |
|---|---|---|---|---|
| PHPt-1 | 0.02 | 0.003 | 112.1 | ND |
| **PHPt-2** | **0.02** | **0.004** | **219.8** | **0.260** |
| PHPt-3 | 0.02 | 0.005 | 193.5 | 0.294 |
| PHPt-4 | 0.02 | 0.006 | 126.3 | ND |
| PHPt-5 | 0.02 | 0.007 | 138.0 | ND |

| | | | | |
|---|---|---|---|---|
| PHPt-6 | 0.02 | 0.008 | ND | 0.220 |

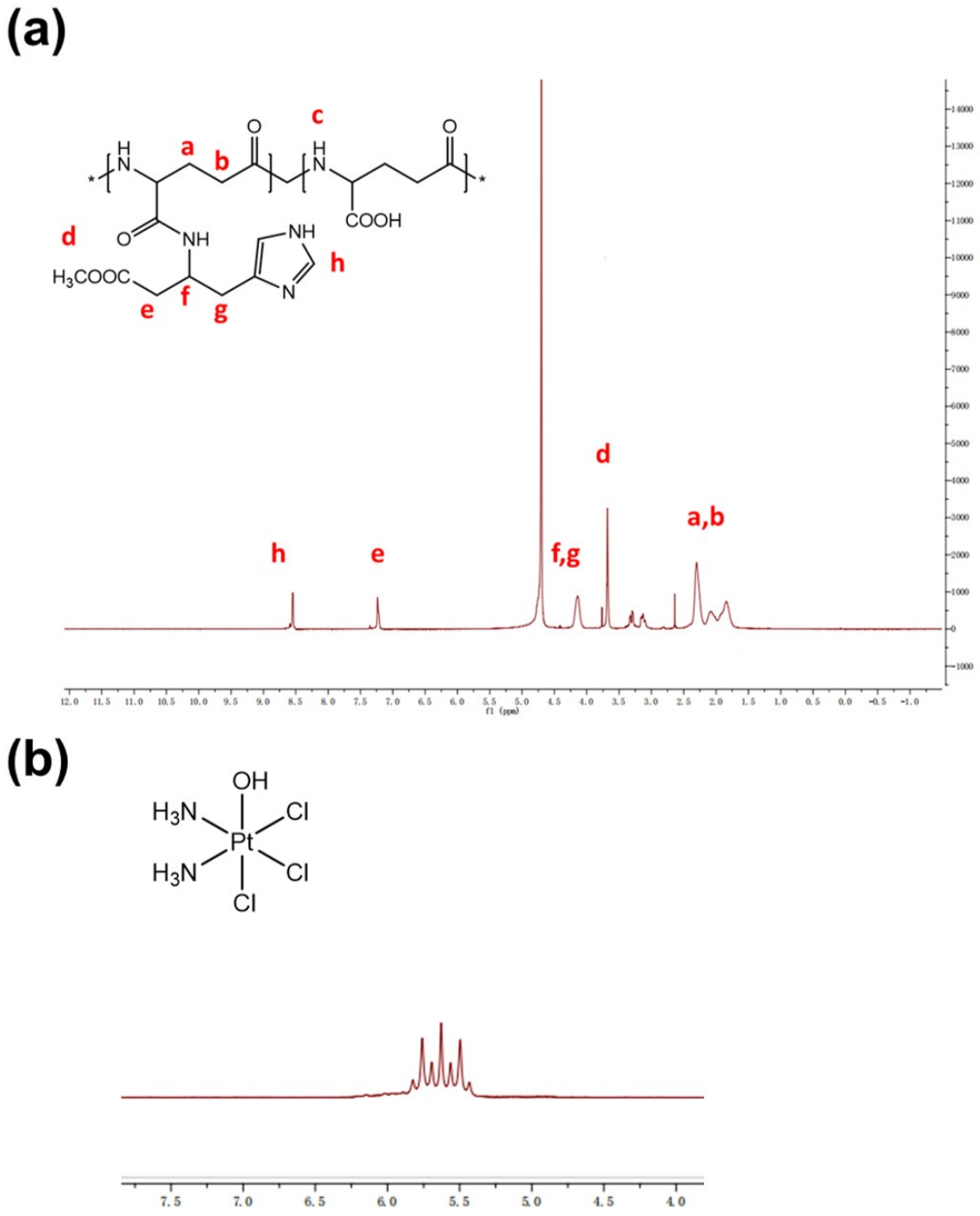


**Figure S2.** (a) $^{1}$H NMR spectrum of PH. (b) $^{1}$H NMR spectrum of Pt(IV).

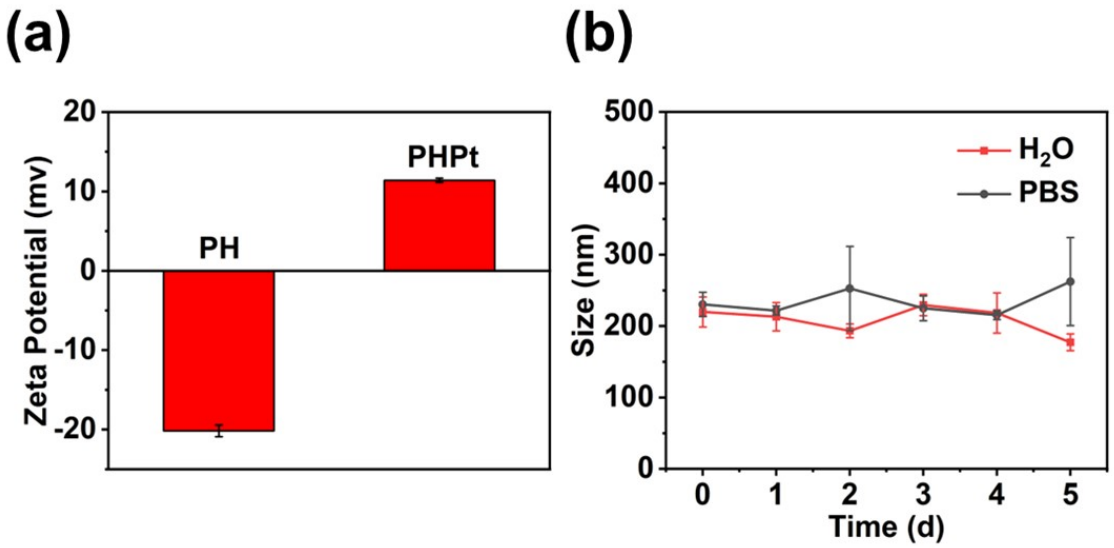


**Figure S3.** (a) Zeta potential of PHPt. (b) Size of PHPt at different days in $H_2O$, PBS. Data are presented as mean ± SD (n = 3).

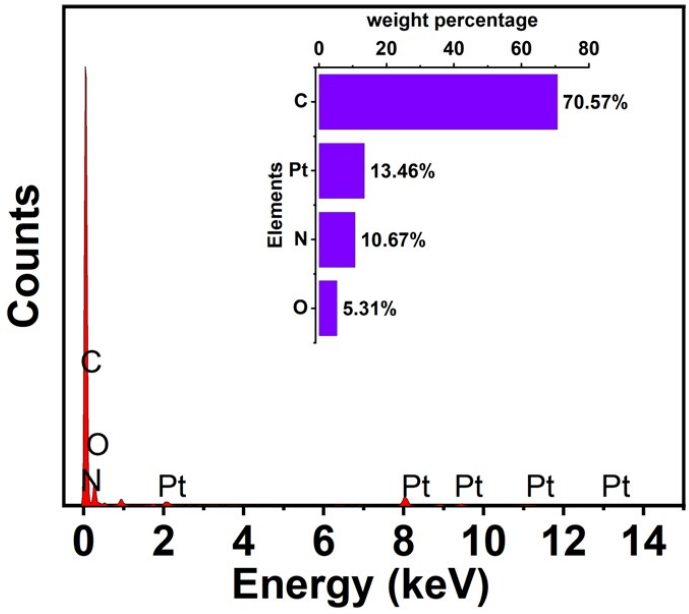


**Figure S4.** EDS spectrum of PHPt.

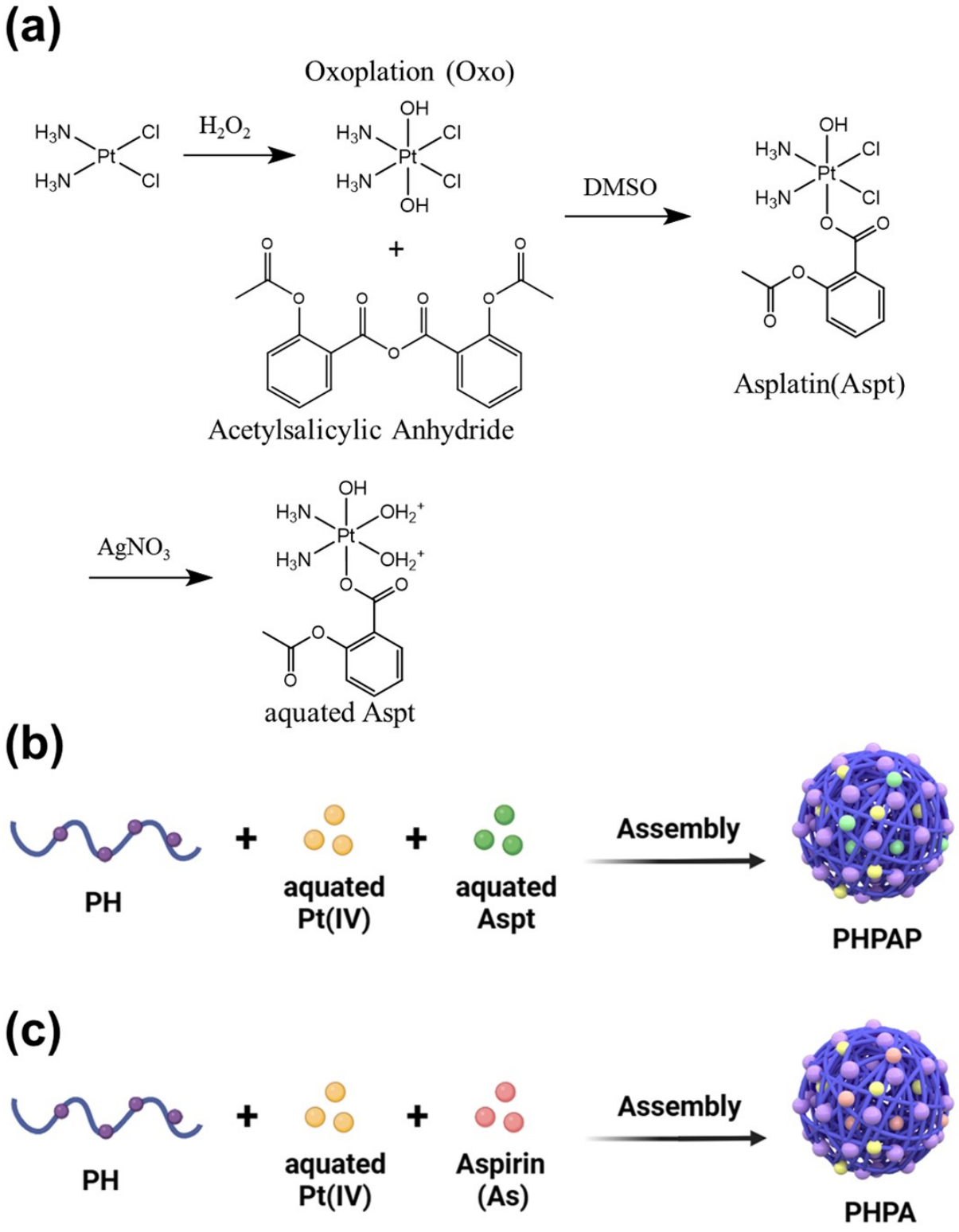


**Figure S5.** (a) Synthesis of aquated Aspt. Synthesis diagram of PHPAP (b) and PHPA (c).

**Table S2.** Synthesis conditions and particle size of PHPAP

| | PH / mmol | aquated Pt(IV) / mmol | aquated Aspt/ mmol | Size / nm | PDI |
|---|---|---|---|---|---|
| PHPAP-1 | 0.02 | 0.002 | 0.002 | ND | ND |
| **PHPAP-2** | **0.02** | **0.003** | **0.001** | **250** | **0.199** |
| PHPAP-3 | 0.02 | 0.001 | 0.003 | ND | ND |

**Table S3.** Synthesis conditions and particle size of PHPA

| | PH / mmol | aquated Pt(IV) / mmol | As / mmol | Size / nm | PDI |
|---|---|---|---|---|---|
| PHPA-1 | 0.02 | 0.004 | 0.004 | ND | ND |
| PHPA-2 | 0.02 | 0.004 | 0.002 | ND | ND |
| PHPA-3 | 0.02 | 0.004 | 0.001 | ND | ND |
| **PHPA-4** | **0.02** | **0.004** | **0.002** | **148.7** | **0.239** |

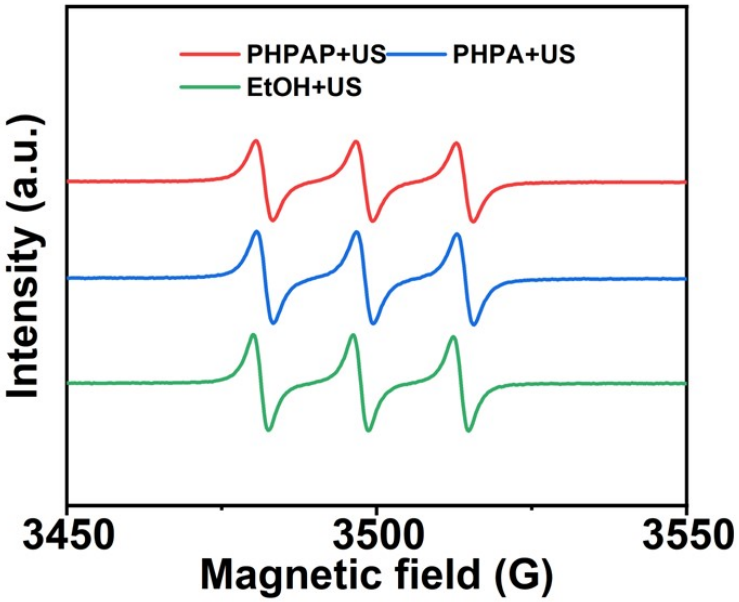


**Figure S6.** ESR detects $^1O_2$ generation of PHPAP+US, PHPA+US, and EtOH+US.

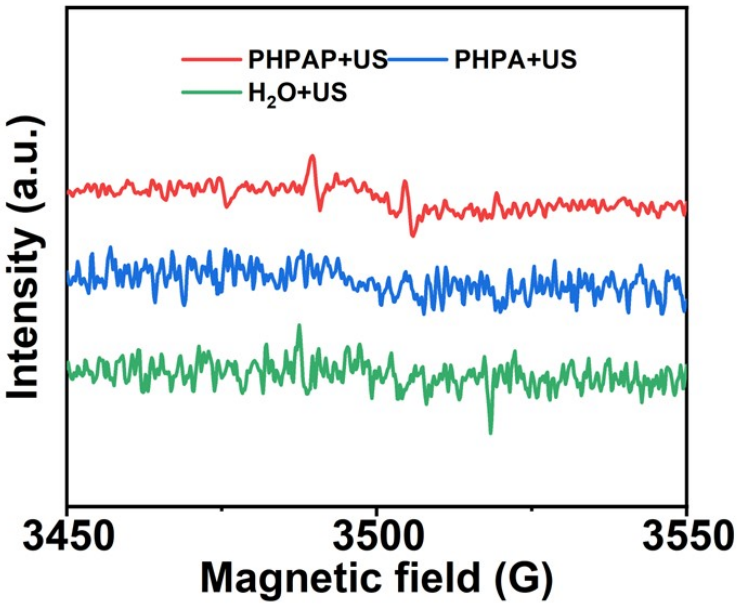


**Figure S7.** ESR detects •OH generation of PHPAP+US, PHPA+US, and $H_2O$+US.

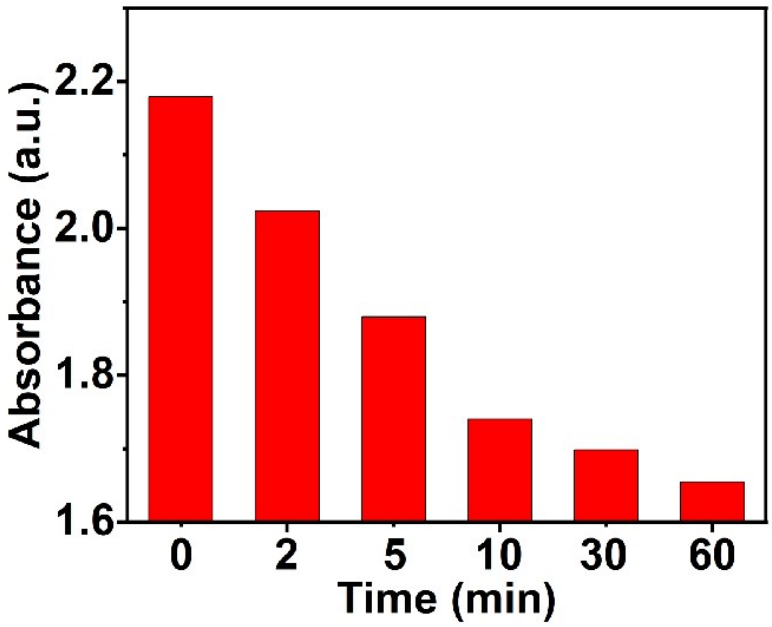


**Figure S8.** The absorbance of DTNB incubating with PHPt at 412 nm for different times.

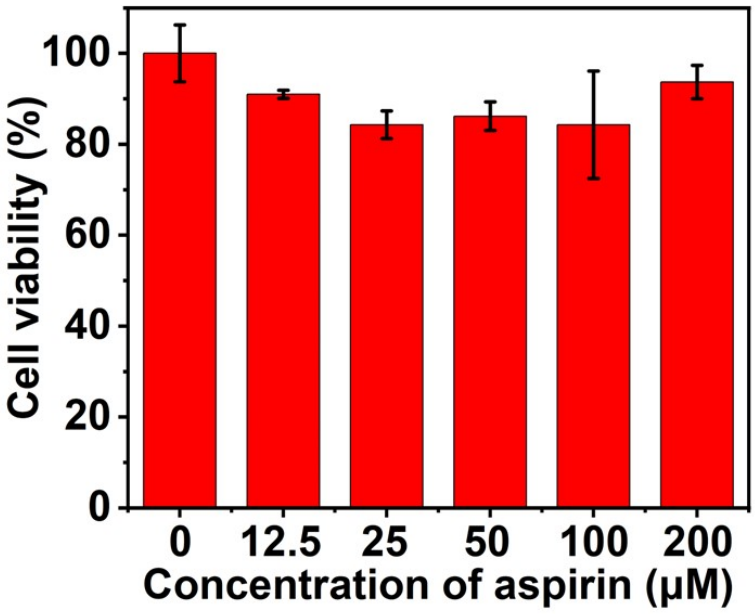


**Figure S9.** Cell viability of 4T1 cells incubated with aspirin for 24 h (n = 3).

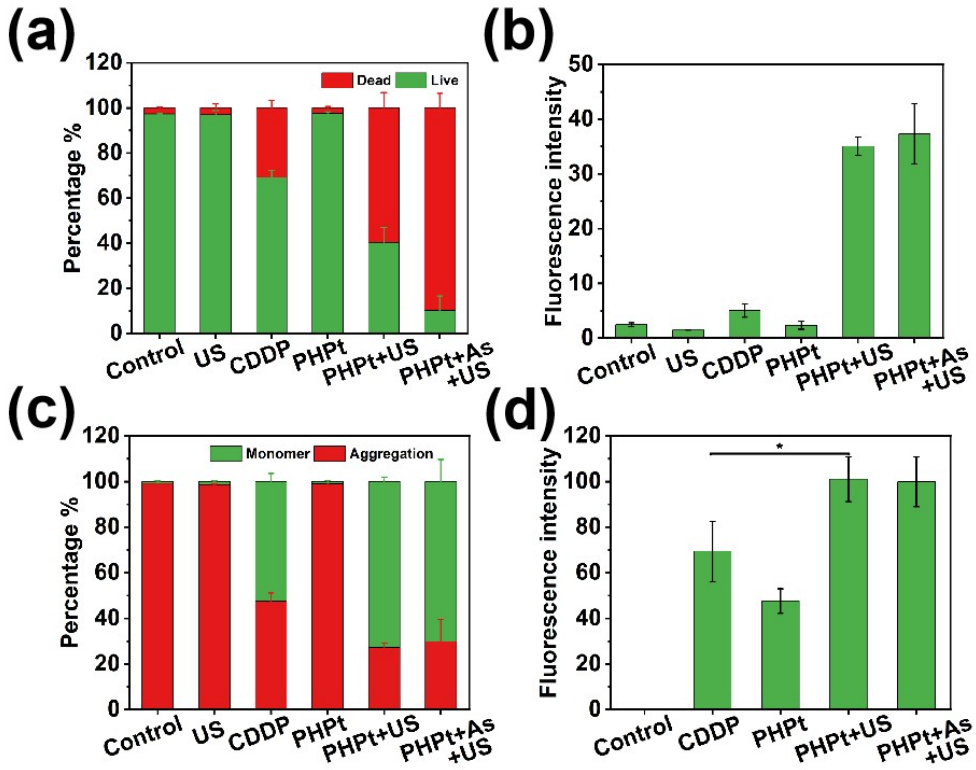


**Figure S10.** The fluorescence intensity of (a) Figure 3d, (b) Figure 3e, (c) Figure 3f and (d) green fluorescence of Figure 4a in each treatment group (n = 3).

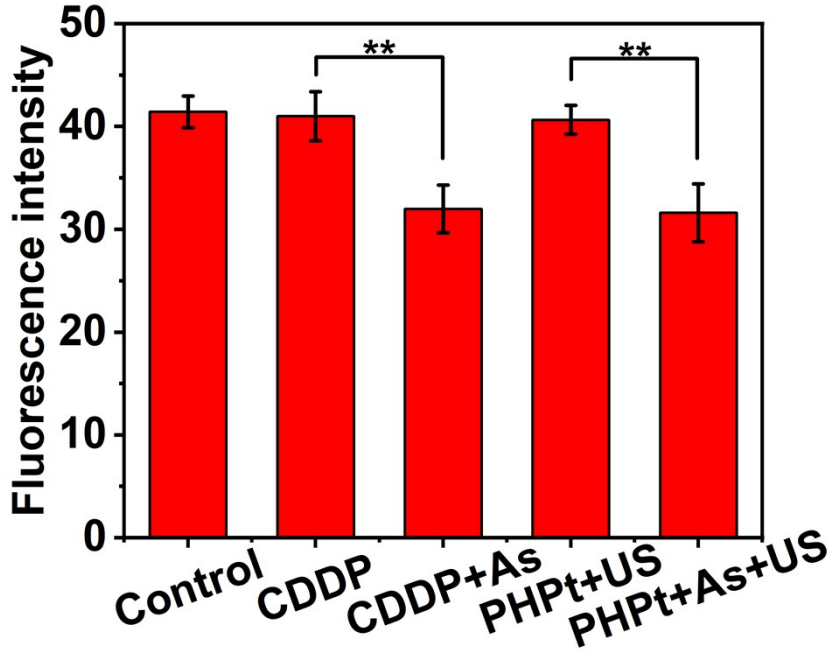


**Figure S11.** The fluorescence intensity of Figure 4 (c) in each treatment group (n = 3).

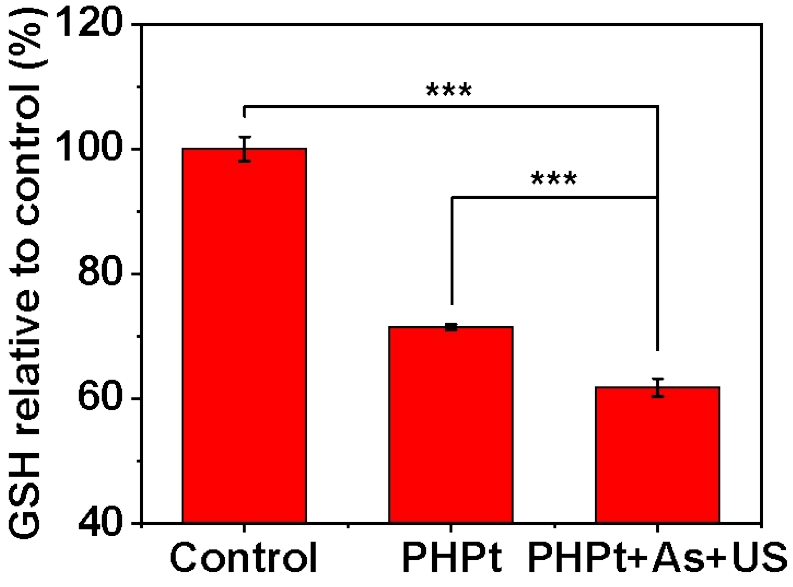


**Figure S12.** Assessment of intracellular GSH depletion by fluorescence detection (n = 3).

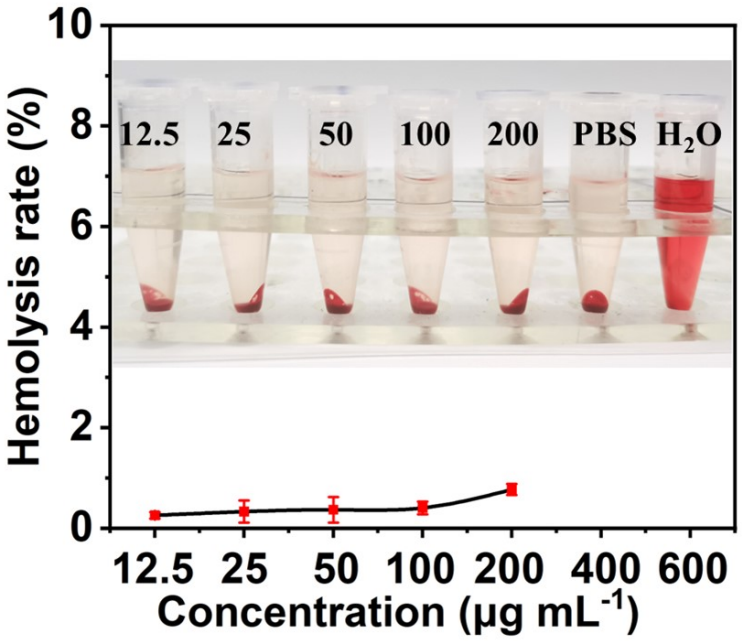


**Figure S13.** Hemolysis rate of PHPt at different concentrations (12.5, 25, 50, 100, 200 μg $mL^{-1}$) PBS, and water.

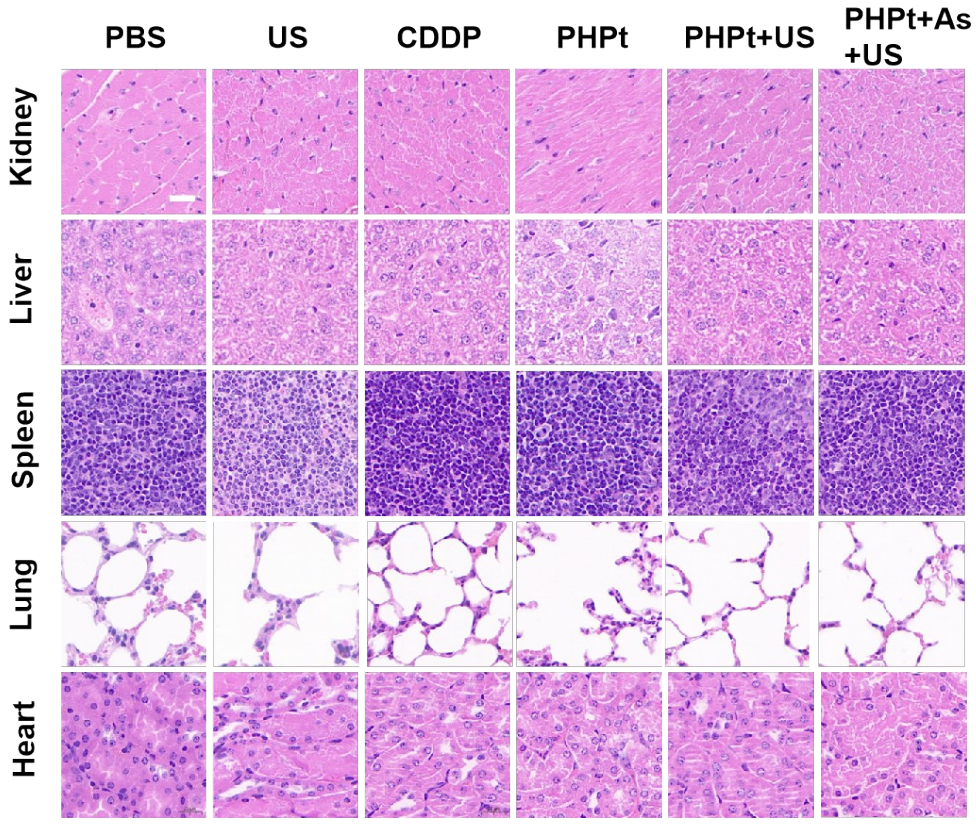


**Figure S14.** H&E-stained major organs (heart, liver, spleen, lung, and kidney) collected from mice with different treatments. Scale bar = 20 μm.