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Supplementary data for

Novel Albumin-binding Photodynamic Agent EB-Ppa for targeted Fluorescent Imaging Guided Tumour Photodynamic Therapy

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Experiment

Materials

All commercial chemicals were purchased from commercial suppliers and used without further purification. Breast cancer cell line 4T1 was purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Dulbecco's modified Eagle's medium (DMEM) was purchased from Sigma-Aldrich. Fetal bovine serum (FBS) was obtained from Hyclone Laboratories Inc. Hydrogen peroxide 30% was bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Fluorescent pictures were captured using a Kodak *In vivo* FX Pro Imaging System. Electrospray ionization mass spectra (ESI-MS) were collected on the LTQ Orbitrap MS instrument.

Synthesis

Compound 1 was prepared according to previous work. To a solution of pyropheophorbide-a (0.53 g, 1mmol), N, N-Diisopropylethylamine (0.26 g, 2 mmol) and O-(7-Azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate (0.38 g, 1 mmol) in DMF (10 mL) was added compound 1 (0.59 g, 1 mmol). The reaction mixture was stirred at room temperature for 6 h. DMF was removed under a high vacuum and the residue was re-dissolved in methanol-H2O (2:1) and purified on an Interchim puriFlash 4250 system (0.69 mg, 63% yield). MS-ESI: m/z calculated for

MS-ESI: m/z calculated for C59H57N9O10S2 [M-H]- 1114.37, found1114.50. The UV/Vis absorption spectra and fluorescence spectra are evaluated by the instrument.

Measurement of singlet oxygen

The singlet oxygen generated by different concentrations and times of EB-Ppa upon laser irradiation was measured using the p-nitrosodimethylaniline (RNO) and imidazole (ID) methods. Typically, 0.225 mg of RNO and 16.34 mg of ID were separately dissolved in 30 mL of DI water. These solutions were air saturated by sufficient air bubbling for 20 minutes. The sample solution was prepared by taking 1 mL of RNO, 1 mL of ID, and 1 mL of EB-Ppa. Then, the absorption of the solution was monitored using a Shimadzu UV-2450 spectrophotometer (Kyoto, Japan at 440 nm after each irradiation). The control experiment was carried out following the same procedure except DI water was used instead of EB-Ppa.

Cell viability assay

In vitro cell viability profile of EB-Ppa was evaluated using the mouse breast cancer cell line (4T1). Briefly, 1×10^4 4T1 cells were seeded at the density of 1×10^5 cells mL^{-1} into a 96-well plate and incubated at 37 °C and 5% carbon dioxide (CO_2) for 24 h. Subsequently, the culture medium was aspirated, samples were added at different concentrations, and the plates were further incubated at 37 °C for 24 h. Then the cell viability profile was quantified using the CCK-8 assay, as the manufacturer's protocol.

In vitro anti-tumor efficacy

Standard CCK-8 assay was conducted to assess the anti-tumor efficacy of EB-Ppa *in vitro*. 4T1 cells were cultured in 96-well plates with a density of 1×10^4 cells mL^{-1} for 24 h and incubated with the samples for 12 h. The concentration of the samples was adjusted from 0 to 200 μg mL^{-1}. Then the cells were irradiated by 660 nm laser with 0.5 W cm^{-2} for 5 minutes. After that, the cells were treated with fresh medium containing 10 μl of CCK-8 solution for an additional 1 h. The solution absorption at 450 nm was measured by a microplate reader. The relative cell viability (%) was calculated by the following equation: Cell viability (%) = $(A_{sample} - A_{blank})/ (A_{control} - A_{blank}) \times 100\%$. Where A_{sample} is the absorbance values obtained in the presence of the samples, A_{blank} is the absorbance at 450 nm without cells, and $A_{control}$ is the absorbance values in the absence of the samples.

Tumor-targeted fluorescence imaging and biodistribution *in vivo*

Transplanted tumor model of mouse breast cancer was established by subcutaneously inoculating 4T1 cells (1×10^7 cells ml^{-1}) at the right underarm of female BALB/c nude mice (6 weeks, 20 g, n=3). After the tumor volume reached 200-300 mm^3, EB-Ppa and Ppa were intravenously injected. At predetermined time intervals (Pre, 2 h, 4 h, 8 h, 12 h, 24 h, and 48 h post-injection), the mice were anesthetized using 1.5% pentobarbital sodium and imaged using Kodak *In vivo* FX Pro Imaging System. At 48 h after injection, the mice were sacrificed and their tumors and major organs were harvested for fluorescence imaging.

In vivo photodynamic therapy

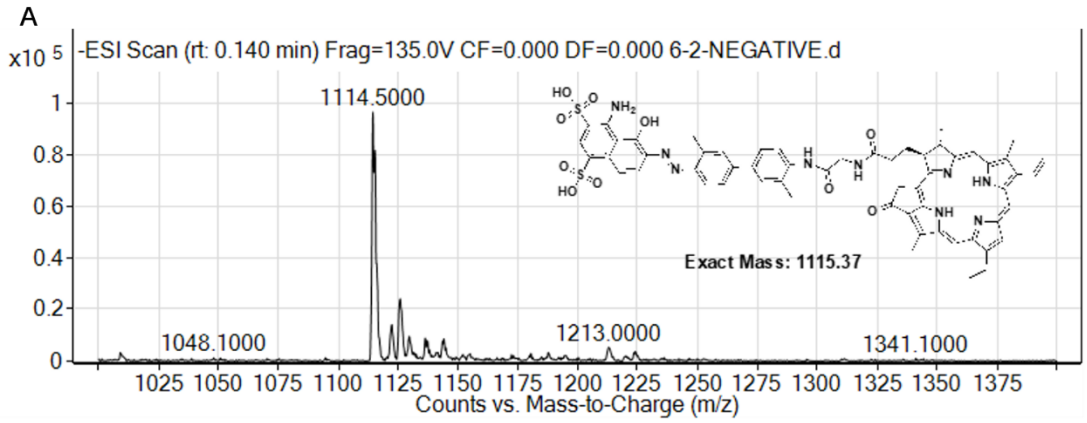
The treatment study was carried out on the 4T1 tumor xenograft model. After the tumor reached about 6-8 mm in diameter, the BALB/c nude mice were randomly divided into four groups (n = 3): PBS, laser, EB-Ppa, and EB-Ppa plus laser groups. The laser treatment groups were irradiated locally by 660 nm laser (1 W cm^{-2}, 10 minutes) on the tumor site at 24 h post-injection. During the treatments, the tumor volume and body weight of the mice were monitored every two days, and the tumor volume was analyzed by the following equation: tumor size = (length × $width^2$)/2. Mice would be euthanized for ethical consideration if tumor volume reached 2000 mm^3. The body weights of all mice were measured every other day. At the end of treatment (day 15), the mice were sacrificed and their blood samples were collected via eye puncture. The treated tumors and major organs (heart, liver, spleen, lung, and kidney) were harvested and photographed. Finally, H&E, TUNEL, and PCNA staining were conducted to further investigate tumor suppression.

Biosafety

The biosafety of EB-Ppa was assessed by histological and serum biochemistry analysis. Major organs were excised from the 4T1 tumor xenograft mice at 24 h after PDT and then were stained with H&E for histological analysis. Meanwhile, serum was analyzed to evaluate the hepatotoxicity, and nephrotoxicity of the samples. Hepatic function indicators included aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). Renal function indicators included creatinine (CRE) and blood urea nitrogen (BUN).

Statistics

Group variation is expressed as mean ± standard deviation. Differences between experimental results are assessed by One-way ANOVA and considered statistically significant at $p < 0.05$.



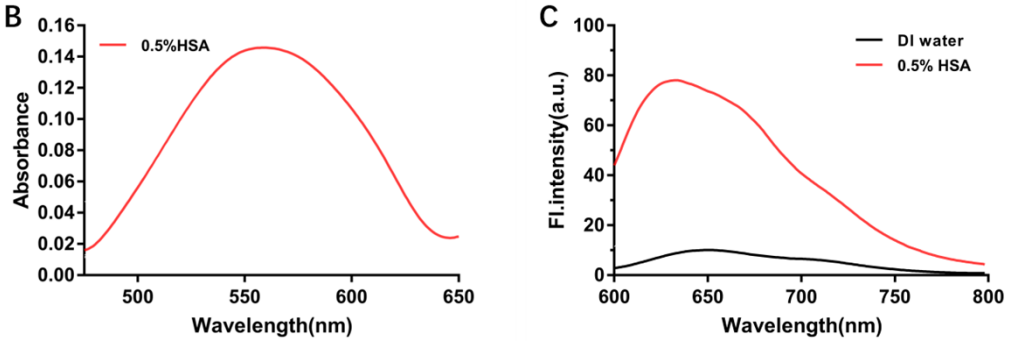


Figure S1. The MS spectroscopy(A), UV/Vis absorption spectra (B), and fluorescence spectra (C) of EB-Ppa (5μM).

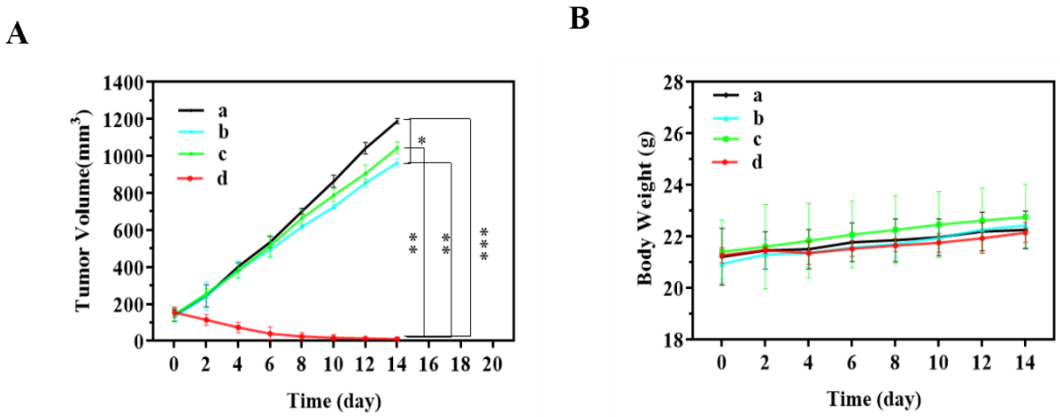


Figure S2 Variations in tumor volume (A), body weight (B) of the mice treated with PBS, laser, EB-Ppa with or without laser irradiation (660 nm, 1.0 W cm^{-2}, 10 minutes). (a) PBS, (b) laser, (c) EB-Ppa, and (d) EB-Ppa + laser, respectively. Data are given as the mean ± S.D. (n = 3). Scale bar =100 μm. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

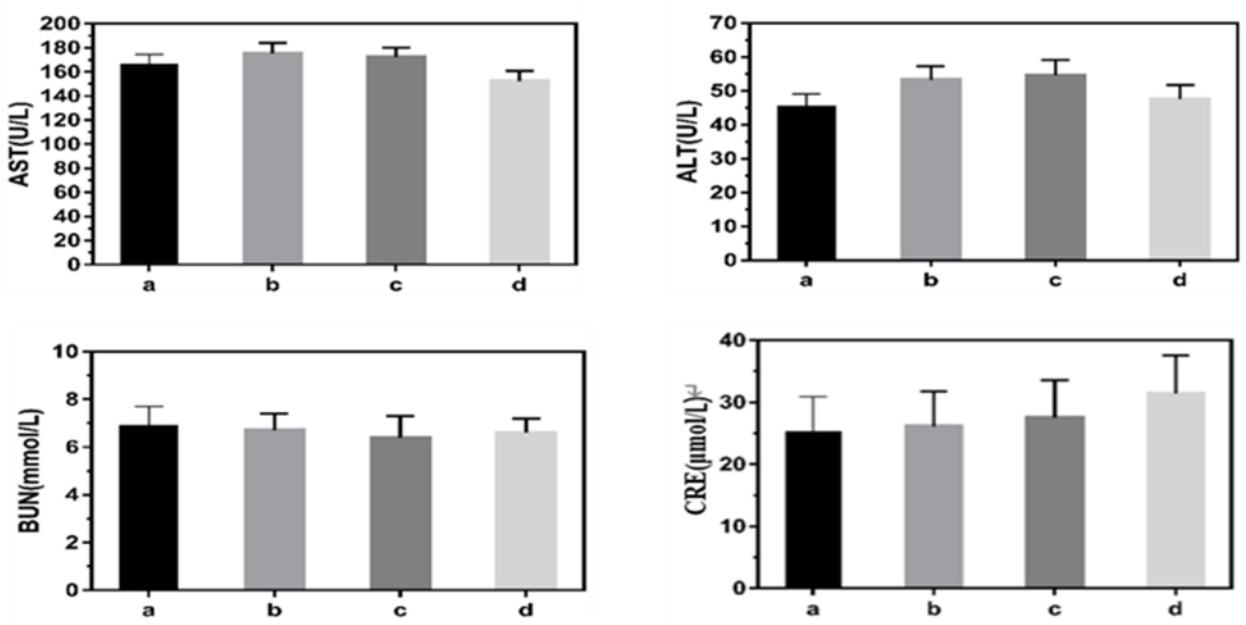


Figure S3 The blood chemistry data of the mice treated with PBS as control (a), laser only（b） and AST are 2.00−7.70 mmol L^{-1}, 22.0−97.0 μmol L^{-1}, 33.0−99.0 U L^{-1}, and 69.5−210.0 U L^{-1}, respectively.