Electronic supplementary information

Giant Macrocycle Overcomes the Post-Treatment Phototoxicity of Photofrin through Host-Guest Complexation

Shujie Lin,‡[a] Xiang Yu,‡[b] Ziliang Zhang,[a] Yahan Zhang,[a] Junyi Chen,*[ab] Chunju Li*[b] and Qingbin Meng*[a]

[a] State Key Laboratory of National Security Specially Needed Medicines, Beijing Institute of Pharmacology and Toxicology, Beijing 100850, P. R. China

[b] Academy of Interdisciplinary Studies on Intelligent Molecules, Tianjin Key Laboratory of Structure and Performance for Functional Molecules, College of Chemistry, Tianjin Normal University, Tianjin 300387, P. R. China

‡ These authors contributed equally to this work.

Table of Contents

1. General materials and methods

1.1 Materials

All the reagents and solvents were commercially available and used as received unless other specified purification. Cationic pentaphen[3]arene (CPP3) was synthesized according to a literature method.[1] Photofrin was purchased from Hubei Chenghai Chemical Industry Co. LTD. Dulbecco's modified eagle medium (DMEM), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) were purchased from Gibco (Thermo Fisher Scientific). Fetal bovine serum, penicillin-streptomycin and phosphate buffered saline (PBS) were purchased from Invitrogen (Carlsbad, CA, USA). The Cell Counting Kit-8 was purchased from Dojindo China Co. Ltd. (Shanghai, China). The human non-small cell lung cancer (A549) cell line was purchased from the cell bank of Chinese Academy of Science.

1.2 Instruments

Fluorescence spectroscopic studies were carried out using a FL-6500 fluorescence spectrophotometer, Perkin Elmer Co. Ltd. Cytotoxicity Assay was performed on Spectra Max® iD5 plate reader, Molecular Devices. The production of ROS was monitored by confocal laser scanning microscopy (LSM 510 META, Carl Zeiss, Germany). *In vivo* fluorescence imaging was performed with a Bruker Optical and X-ray Small Animal Imaging System (in-vivo Xtreme, Germany).

1.3 Cell and animals

A549 cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin and 1% streptomycin. Then cells were incubated at 37 ^{o}C under 5% CO_2 and 90% relative humidity, and passaged every 2 days.

Balb/C nude mice (~20 g body weight) were purchased from the SPF (Beijing) Biotechnology Co. Ltd and maintained at 25 ^{o}C in a 12 h light/dark cycle with free access to food and water. Animals were allowed to acclimate to environment for at least one week before experiments. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the AAALAC, and were approved by the Animal Care and Use Committee of the National

Beijing Center for Drug Safety Evaluation and Research (IACUC-2024-001A). Best efforts were made to minimize the number of animals used and their suffering.

1.4 Fluorescence titration

To quantitatively investigate the complexation behavior of between CPP3 and Photofrin, fluorescence competitive titration was performed at 298 K in a solution state. [1]

1.5 Cytotoxicity assay

The relative cytotoxicity of CPP3, Photofrin and Photofrin/CPP3 against A549 cells was assessed *in vitro* using CCK-8 according to the manufacturer's instructions. Cells were seeded into 96-well plates at a density of 8000 cells/well in 100 μL of DMEM supplemented with 10% FBS, 1% penicillin, and 1% streptomycin and cultured for 24 h in 5% CO_2 at 37 ºC. The culture medium was replaced with 100 μL of a fresh medium containing various concentrations of CPP3 (1.25, 2.5, 5, 10, 20, 40, and 80 μM), Photofrin (1.25, 2.5, 5, 10, 20, 40, and 80 μM) or CPPS/Photofrin (1.25, 2.5, 5, 10, 20, 40, and 80 μM). 5 wells were arranged in parallel for each concentration group, and a control group was set. Upon incubation at 37 ºC under 5% CO_2 for 24 h, the cells were exposed to a solar simulator (100 mW/cm^2) for 30 min. Subsequently, 100 μL of CCK-8 was added into each well and incubated for another 0.5 h in the dark condition. The plates were then measured at 450 nm using a plate reader. All experiments were carried out five independent times. Cell viability was calculated as follows:

$$\text{Cell Viability} = \frac{\text{OD}_{\text{test}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100\%$$

Where OD_{blank} is the optical density of blank well (medium and CCK-8 reagent), OD_{test} is the optical density of the test group and $OD_{control}$ is the optical density of the control group.

1.6 Reactive oxygen species Assay

A549 cells were seeded into 6-well plates at a density of 3 × 10^5 cells/well in 1.5 mL of DMEM supplemented with 10% FBS, 1% penicillin, and 1% streptomycin and cultured for 24 h under 5% CO_2 at 37ºC. PBS, CPP3 (10 μM), Photofrin (10 μM) and Photofrin/CPP3 (10/10 μM) were respectively added to each well in parallel for each

solution group, and the plates were further incubated at 37 °C under 5% CO_2 for 24 h. Then the cells were exposed to a solar simulator (100 mW/cm^2, 30 min). After treatment, the cells were carefully washed three times with PBS. The cells were stained with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) for 30 min in the dark condition and then washed three times with PBS. The production of ROS was monitored by CLSM.

1.7 Safety evaluations of CPP3

In a preliminary study of the safety profile of CPP3, six Balb/C nude mice (~20 g) were randomly assigned to two groups, with three mice in each group. Specifically, CPP3 (12.17 mg·kg^{-1}) was injected intravenously and the mice were exposed to sunlight for 14 days. For control group, mice were administrated with PBS. Body weight of the mice were monitored and their general behaviors were observed at defined time point. The mice were euthanized after 2 weeks. Blood samples were taken for hematological analysis. The main organs were separated and weighed, including heart, liver, spleen, lung, and kidney. The organ index was determined by calculating the ratio of organ weight to the body weight of each mouse.

1.8 Measurement of *in vivo* therapeutic efficacy

Six female nude Balb/C mice were randomly divided into 3 groups (n = 2, each group). Mice of group 1 were intravenously injected with PBS. Mice of groups 2 and 3 were intravenously injected with Photofrin (4.00 mg·kg^{-1}). After being kept in dark for 24 h, mice of group 3 were intravenously injected with CPP3 (12.17 mg·kg^{-1}). After being kept in dark for another 24 h, the mice were exposed to a solar simulator (100 mW/cm^2, 30 min). After 24 h, the mice from each group were imaged to monitor the skin damage of their right hind legs and then sacrificed. Skin samples were collected from the right hind legs (1.5 cm × 1.5 cm) for weighing. H&E staining was performed for skin damage evaluation.

1.9 Mechanism studies of CPP3

In a preliminary study of mechanism of sequestration agent CPP3, 2 mL of 1.0 μM Photofrin in PBS buffer solution was added to fluorescent cuvette and scanned from 640-700nm to measure the fluorescence spectra by fluorescence spectrophotometer. Upon adding Photofrin/CPP3 (1.0/1.0 μM) in PBS buffer solution, the solution was

scanned again over the spectral range to observe the change of fluorescence intensity of Photofrin. Meanwhile, Photofrin (1.0 μM) and Photofrin/CPP3 (1.0/1.0 μM) in PBS buffer solution are imaged by Optical and X-ray Small Animal Imging System to further verify the fluorescence quenching.

To observe the distribution of Photofrin and evaluate the *in vivo* photosensitivity of Photofrin with or without the treatment of CPP3, healthy female Balb/C mice were randomly divided into two groups (n = 3, each group). Mice in groups 1 and 2 were intravenously injected with Photofrin (4.00 $mg \cdot kg^{-1}$) in the dark condition. After two minutes, group 1 and group 2 were intravenously injected with PBS and CPP3 (12.17 $mg \cdot kg^{-1}$), separately. Then two groups were kept in dark and imaged by Optical and X-ray Small Animal Imaging System at predetermined intervals (1, 4 and 8 h).

2. Supporting results and experimental raw data

2.1 The determination of molecular weight of Photofrin by GPC.

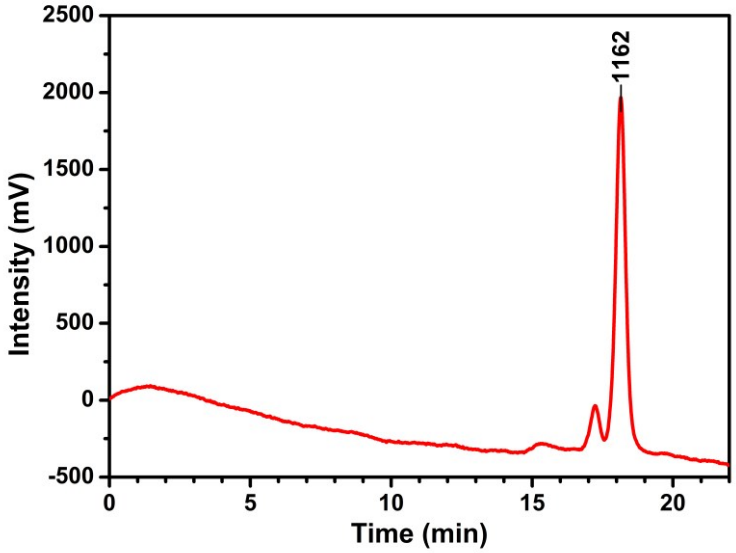

Fig. S1 The peak molecular weight of Photofrin was determined by gel permeation chromatography.

References

1. X. Yu, Y.-H. Zhang, L. Tian, F. Zhang, Z.-L. Zhang, L.-M. Chen, J.-Y. Chen, C.-J. Li and Q.-B. Meng, *Cell Rep. Phys. Sci.*, **2024**, 5, 102044.