Supporting Information

A Mitochondria-Localized Fluorescent BODIPY-Platinum Conjugate

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Materials and Methords

Cisplatin (purity = 99.0%) was purchased from Shandong Boyuan Pharmaceutical Co., Ltd. (China). BODIPY and BODIPY-Pt were synthesized according to the literatures. All of the other chemicals and solvents were obtained commercially and were used without further purification, unless otherwise noted. Cisplatin was dissolved in water, while the stock solution of BODIPY and BODIPY-Pt were in methanol for cell biology experiments.

Measurements

^{1}H NMR spectra were recorded on a Bruker NMR 400 DRX spectrometer at 400 MHz at room temperature. UV–Vis absorption spectra were obtained using a Shimadzu UV-2450 PC UV–Vis spectrophotometer. Strengthening experiments were performed using PerkinElmer LS-55 Spectrofluorophotometer. The mass spectra (MS) of the sample was recorded by the German company Bruker autoflex III smartbeam MALDI-TOF/TOF mass spectrometer with smartbeam laser at 355 nm wavelength. The emission quantum yields in MeOH were obtained relative to rhodamine 6G at room temperature. Platinum content was determined by inductively coupled plasma mass

spectrometry (ICP-MS; Xseries II, Thermoscien-tific).

Synthesis of BODIPY

BODIPY was synthesized according to the literature.[1]

Synthesis of BODIPY-Pt

BODIPY-Pt was synthesized according to the literatures.[2-3] Cisplatin (0.387 mmol, 117 mg) and silver nitrate (0.387 mmol, 66 mg) were dissolved in 10 mL of DMF and stirred in the dark at room temperature for 24 h. The resulted turbid solution was centrifuged to remove white silver chloride. The light yellow colored solution was added to a solution of BODIPY (0.387 mmol, 126 mg) in 6 mL DMF and stirred at 55 °C for 48 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in 25 mL of MeOH. Unreacted yellow cisplatin was removed by filtration. The filtrate was stirred vigorously, and diethylether (150 mL) was then added to precipitate the desired compound as a solid. The compound was filtered and washed twice with 50 mL of diethylether. The compound was purified by redissolving in methanol and precipitating by adding it dropwise to vigorously stirred diethyl ether for twice. The final compound was isolated by vacuum filtration and further washed with methanol, dichloromethane and diethyl ether. The solid was further dried in vacuum and 120 mg of the product were obtained in 48% yield.

Absorption and Emission Spectra of BODIPY and BODIPY-Pt

BODIPY-Pt was solubilized directly in water, but the spectra of BODIPY were obtained by adding two drop of its solution (1 mg/mL) in methanol to 3 mL of water, because of its water insolubility.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

Cell lines and cell culture: All cells line were grown in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% heat-inactivated fetal bovine serum (FBS, GIBCO), and the culture medium was replaced once every day.

Cytotoxicity test: Cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 10^5 cells per well and incubated in DMEM for 24 h. The medium was then replaced by BODIPY, cisplatin, and BODIPY-Pt at various concentrations. The incubation was continued for 48 h. Then, 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added and the plates were incubated for another 4 h at 37 °C, followed by removal of the culture medium containing MTT and addition of 150 μL of DMSO to each well to dissolve the formazan crystals formed. Finally, the plates were shaken for 5 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader.

Confocal Laser Scanning Microscopy (CLSM)

Cellular uptake by HeLa cells was examined with CLSM. HeLa cells were seeded in 6-well culture plates (a clean cover slip was put in each well) at a density of 5×10^4 cells per well and allowed to adhere for 24 h. The medium was then replaced by BODIPY and BODIPY-Pt solutions at a final concentration of 5 μM for each. After incubation 2 h at 37 °C, the supernatant was carefully removed and the cells were washed three times with PBS. Subsequently, the cells were fixed with 1 mL of 4% formaldehyde each well for 10 min at room temperature and washed twice with PBS again. Samples

were examined by CLSM using a Zeiss LSM 510 (Zurich, Switzerland). 4',6-diamidino-2-phenylindole (DAPI) was used to stain the nuclei. MitoTracker® Red CM-H2XRos was also used to stain mitochondria.

Flow Cytometry Scanning (FCS)

Cellular uptake by HeLa cells was examined with FCS. The HeLa cells were seeded in six-well culture plates at a density of 1×10^6 cells per well and allowed to adhere for 24 h. After that, the cells were treated with BODIPY and BODIPY-Pt for 1 h at 37 °C. Thereafter, the culture medium was removed, and the cells were washed with PBS three times and treated with trypsin. Then, 1.0 mL of PBS was added to each culture well, and the solutions were centrifuged for 5 min at 1000 rpm. After the removal of the supernatants, the cells were resuspended in 0.5 mL of PBS. Data for the 10,000 gated events were collected, and analyses were performed by flow cytometry (Beckman, California).

Cell Imaging and Flow Cytometry Scanning with Carbonyl Cyanide *m*-Chlorophenylhydrazone (CCCP) Treatment

The HeLa cells were seeded in six-well culture plates at a density of 5×10^4 cells per well for CLSM and 1×10^6 cells per well for FCS, and allowed to adhere for 24 h. The cells were incubated with 20 μM CCCP (stock solution of CCCP in DMSO) for 45 min. The CCCP treated cells were then incubated with BODIPY-Pt for 2 h at 37 °C at a concentration of 5 μM. The next processing is the same as aforementioned operation.

mtDNA Binding of Cisplatin and BODIPY-Pt[4-5]

The HeLa cells were grown in DMEM with 10% fetal bovine serum at 37 °C in 5% CO_2. HeLa cells harvested in a logarithmic growth phase were seeded in petri dishes at a density of 1×10^7 cells/dish (seven petri dishes for each) and incubated in DMEM for 24 h. The medium was then replaced by cisplatin and BODIPY-Pt solutions at a concentration of 5 μM for each. The HeLa cells were harvested 2 h posttreatment, and mtDNA was isolated with a biohao mitochondrial DNA isolation kit (biohao, Wuhan, China). The final DNA pellet was air-dried and then dissolved in 30 μL of TE buffer. The DNA concentration and purity were determined by the measurement of the absorbance at 260/280 nm with a NanoDrop UV spectrometer (NanoDrop Technologies, Inc., Wilmington, DE). The samples were then analyzed by electrophoresis on a 0.8 % agarose gel with ethidium bromide. The Pt concentration was then determined by ICP-MS. The total Pt–mtDNA adducts were expressed as nanograms of Pt per micrograms of DNA.

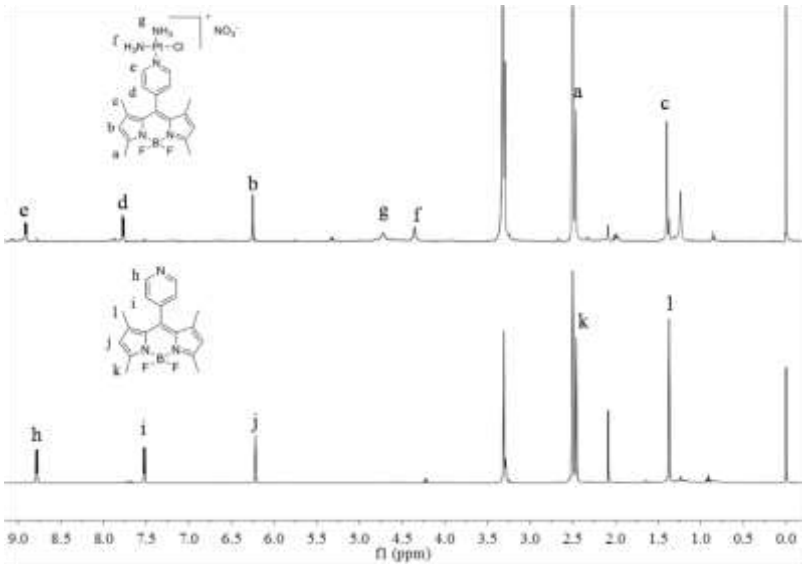

Figure S1. ^{1}H NMR spectra of BODIPY and BODIPY-Pt in DMSO-d_6.

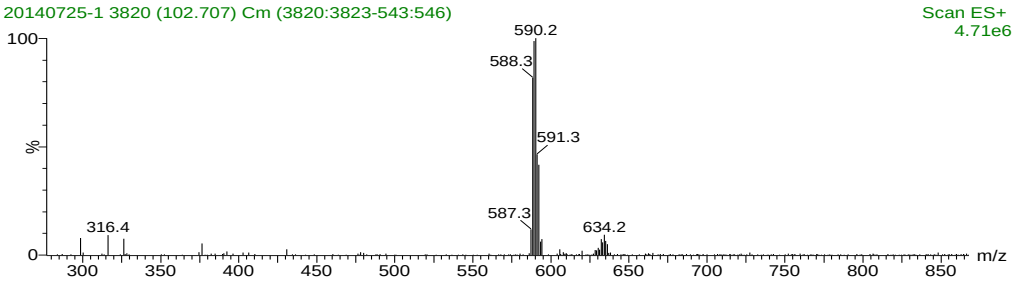

Figure S2. MALDI-TOF MS of BODIPY-Pt.

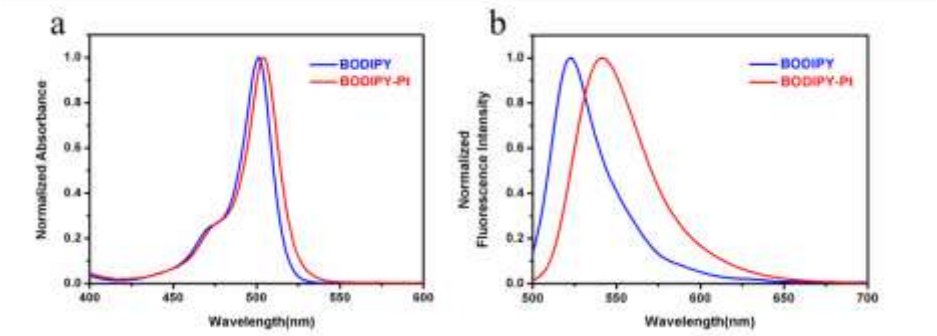

Figure S3. Normalized UV-Vis absorption (a) and emission (b, λ_{ex} = 500 nm for BODIPY and 504 nm for BODIPY-Pt) spectra of BODIPY and BODIPY-Pt in water.

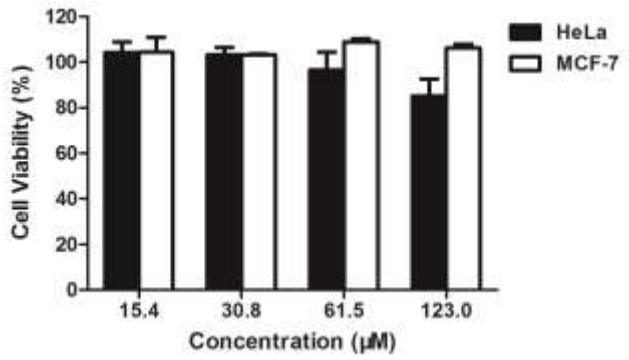

Figure S4. *In vitro* biocompatibility of BODIPY against HeLa and MCF-7 cells at 48 h.

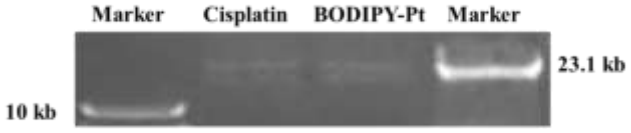

Figure S5. Agarose gel electrophoresis of mtDNA isolated from the mitochondria of HeLa cells.

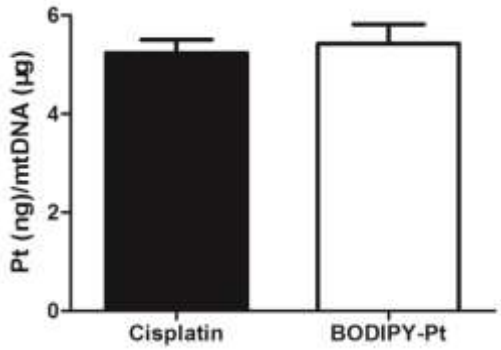

Figure S6. Pt contents in the mtDNA collected from the HeLa cells treated with cisplatin and BODIPY-Pt at a concentration of 5 μM for 2 h.

References

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