Supporting Information

FDPP-HA as Theranostic Agent for Cancer-targeted Fluorescence Imaging and Photodynamic Therapy

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Experimental section

Reagents and materials

All the chemical reagents were purchased from Adamas and used without purification. 4, 6-diamino-2-phenyl indole (DAPI), dichlorofluorescein diacetate (DCFH-DA), HCT-116 and A2780 cell lines, Fetal bovine serum, pancreatin, Penicillin-Streptomycin Solution, dulbecco's modified eagle medium, Green Mito-Tracker, Green Lyso-Tracker and Green ER-Tracker dyeing liquid were purchased from Institute of Biochemistry and Cell Biology, SIBS, CAS (China).

Characterization and measurements

The absorption spectra were recorded on UV-3600 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were measured on F-7000 spectrometer (HITACHI, Japan). ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker DRX NMR spectrometer (400 MHz). Cellular fluorescence images were carried out on confocal fluorescence microscope. Flow cytometry were measured on BD FACSCalibur. The

light source was a simulated sunlight Xenon lamp light source system (Beijing Taught Jin Yuan Technology Co., Ltd).

Synthesis of DPP-1

Potassium tert-butoxide (16 g, 142.6 mmol) and tert-amyl alcohol (100 mL) were added into a 250 ml two neck flask under N_2 atmosphere. The reaction mixture was heated to 110 °C. Then, dimethyl succinate (5.84 g, 39.96 mmol) in 4-cyanofuran (10 g, 107.4 mmol) and tert-amyl alcohol (20 mL) was injected. One hour later, methanol (5 mL) was extracted from the mixture and the reaction was cooled to 65 °C. Two hours later, methanol (150 mL) was added to terminate the reaction. A few minutes later, acetic acid (10 ml) was injected and the reaction mixture was stirred at 65 °C overnight. Upon completion of the reaction, the crude product was washed with water and methyl alcohol to obtain DPP-1 (7.1 g, yield: 27%). ^{1}H NMR (500 MHz, DMSO) δ 8.62 (d, J = 4.3 Hz, 2H), 8.58 (d, J = 4.2 Hz, 2H), 8.0 (t, J = 7.6 Hz, 2H), 7.24 (t, J = 7.2 Hz, 2H). ^{13}C NMR (126 MHz, DMSO) δ 161.12, 146.76, 143.74, 131.24, 116.68, 113.56, 112.83, 107.57, 41.03, 37.82.

Synthesis of DPP-2

DPP-1 (5.36 g, 20 mmol), potassium hydroxide (2.8 g, 50 mmol) and 1, 6-dibromide hexane (12.2 g, 50 mmol) were dissolved in N, N-dimethyl formamide (150 mL) and stirred at room temperature for 24 hours. The solvent was removed. Then washed and dried the crude product. At last, the resulted residue was purified by chromatography on a silica column (PE/DCM, V/V=1:6) to obtain DPP-2 (5.865 g, yield: 50%). ^{1}H NMR (500 MHz, CDCl3) δ 8.36 (d, J = 4.3 Hz, 2H), 7.71 (d, J = 1.8 Hz, 2H), 6.75 (d,

J = 3.9, 1.8 Hz, 2H), 4.46 - 3.95 (m, 4H), 3.45 (t, J = 7.1 Hz, 4H), 1.57 - 1.47 (m, 16H). ^{13}C NMR (126 MHz, CDCl3) δ 160.85, 144.92, 133.61, 120.21, 113.51, 106.44, 77.48, 75.95, 42.18, 33.73, 32.63, 29.98, 27.83, 25.97.

Synthesis of FDPP

DPP-2 (1 g, 1.68 mmol) was dissolved in 50 mL chloroform then N-Bromosuccinimide (NBS) (0.66 g, 3.71 mmol) was added quickly. The mixture was stirred at room temperature for 4 hours and the solvent was removed. The resulted residue was purified by chromatography on a silica column (PE/DCM, 1:5) to afford FDPP (1.1 g, yield: 87%). 1H NMR (500 MHz, CDCl3) δ 8.30 (d, J = 4.3 Hz, 2H), 6.69 (d, J = 4.2 Hz, 2H), 4.17 (t, J = 7.6 Hz, 4H), 3.45 (t, J = 7.2 Hz, 4H), 1.96 – 0.91 (m, 16H). 13C NMR (126 MHz, CDCl3) δ 160.51, 146.15, 132.49, 126.40, 122.23, 115.62, 106.32, 77.73, 42.30, 33.56, 32.84, 32.52, 32.04, 29.82, 27.78, 27.30, 26.01, 25.29.

Synthesis of FDPP-HA

FDPP (1 g, 1.33 mmol) and protonated hyaluronic acid (0.625 g, 1.33 mmol) were dissolved in the mixture of 30 mL tetrahydrofuran and 10 mL acetone. Then the mixture was stirred at 45°C for 24 hours. After removing the solvent, the crude product was added into 50 mL deionized water and dissolved by sonication. After that, the mixture was filtered by microporous membrane (diameter 0.45 μm) and dialyzed for two days (cut-off value: 100 kDa). At last, the solvent was removed by freeze drying to obtain FDPP-HA (412 mg, yield: 66%). IR (KBr): ν = 1720.5 cm^{-1}(m), 1654.4 cm^{-1}(m), 1620.5 cm^{-1}(m), 1571.5 cm^{-1}(m), 1486.2 cm^{-1}(m), 1466.9 cm^{-}

$^{-1}$(m),1406.3 cm^{-1}(m), 1380.5 cm^{-1}(m), 1321.2 cm^{-1}(m), 1235.5 cm^{-1}(m), 1205.4 cm^{-1}(m), 1153.1 cm^{-1}(m), 1080.7 cm^{-1}(m), 1039.0 cm^{-1}(m), 945.9 cm^{-1}(m), 886.1 cm^{-1}(m), 798.1 cm^{-1}(m), 740.0 cm^{-1}(m), 697.6 cm^{-1}(m), 669.2 cm^{-1}(m), 613.6 cm^{-1}(m), 564.5 cm^{-1}(m).

Calculation of Singlet Oxygen Quantum Yield

The following equation [16a] was used to calculate the singlet oxygen quantum yield: $\Phi_{\Delta(X)} = \Phi_{\Delta(MB)} \times (S_X/S_{MB}) \times (F_{MB}/F_X)$, where the subscripts X and MB represent the sample and methylene blue, respectively. S represents the slope of absorbance plot of DPBF (416 nm) vs irradiation time. F represents the absorption correction factor calculated by $F = 1\text{-}10^{-OD}$ (OD represents the areas of the sample and MB from 450-600 nm).

Cell Culture and Incubation Conditions

HCT-116 and A2780 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 1% (penicillin + streptomycin) at 37 °C in a humidified atmosphere with 95% air and 5% CO_2.[1]

***In vitro* Cell Cytotoxicity**

To determine the cytotoxicity, HCT-116 cells were seeded onto two 96-well plates at a density of 1×10^5 cells per well in 200 μL complement medium with 10% heat inactivated fetal bovine serum (FBS) and 1% (penicillin + streptomycin). To ensure the 100% humidity, the PBS was added in the wells which around the whole plate and allowed the cells to attach to the plate for 24 hours. After that, the medium was

removed and replaced by the complement medium with different concentrations of FDPP-HA (0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 1.5 mg/mL) added to the 96-well plates. The number of the replication well is five. The cells were incubated for another 24 hours under the dark condition in the incubator. One of the two plates was kept in dark and the other was irradiated by xenon lamp light (510 nm, 40 mW/cm^2) for 10 minutes. The cells were incubated for an additional 12 hours, and all the solution in the wells was discarded. Then 20 μL of 3-(4,5-dimethyl-2-thiazolyl)- 2,5-diphenyl-2-H-tetrazolium bromide (MTT, 5 mg/mL) was added to each well to dissolve the crystals.[2] And the cells were further incubated for 4 hours. After the last incubation, the medium was removed and DMSO (150 μL) was added to dissolve the purple precipitate. After all of these, absorbance intensity of the two plates was read at the optical densities (O.D) of 492 nm using a microplate reader. The viability of controlled group was assumed to be 100% and relative viabilities of the treatment groups were calculated using the followed formula: cell viability (%) = (mean of absorbance value of treatment group/mean of absorbance value of control group) ×100%.

***In vitro* Cellular Uptake of FDPPBr-HA**

HCT-116 and A2780 cells were seeded into confocal culture plates and incubated to adhere for 24 hours. After washed with PBS, the cells were incubated in 2 mL culture medium of 0.6 mg/mL FDPP-HA in dark condition for another 24 hours. At last, the cells were stained with 4', 6-diamidino-2-phenylindole (DAPI). The cell imaging was performed with a laser scanning up-conversion luminescence microscope equipped

(Olympus IX 70). The luminescence signals were detected in the blue channel (450-490 nm), red channel (620-680 nm) and green channel (500-560 nm). The experiment was excited at the wavelength of 559 nm and collected from 570 to 670 nm.

Sub-Cellular Localization

HCT-116 cells were seeded into confocal culture plates and incubated to adhere for 24 hours. After washed with PBS, the cells were incubated in culture medium (2 mL) containing 0.6 mg/ml of FDPP-HA in dark condition for 24 hours. Then green color Lyso-Tracker, Mito-Tracker and ER-Tracker dyeing liquid were respectively added into three culture plates. The cell imaging was performed with a laser scanning up-conversion luminescence microscope equipped (Olympus IX 70). The experiment was excited at the wavelength of 480 nm and collected from 500 to 600 nm.

Intracellular ROS Assay

HCT-116 cells were seeded into confocal culture plates and incubated to adhere for 24 hours. Then cells were incubated in culture medium (2 mL) containing 0.6 mg/mL of FDPP-HA in dark condition for another 24 hours. After that, the cells were incubated in the DCFH-DA in dark condition for 20 min. At last, the cells were also stained with DAPI. The cell imaging was also performed with a laser scanning up-conversion luminescence microscope equipped (Olympus IX 70). This experiment was excited at the wavelength of 405 nm and collected from 420 to 520 nm.

Animal Preparation and Tumor Model

12 nude mice (five weeks old, 15-18 g weight) were obtained from Comparative Medicine Centre of Yangzhou University. The HCT-116 tumors were generated by

the left front leg subcutaneous injection with 0.2 mL of PBS containing 4×10^6 cells. Same sex mice were arranged in the cages in ventilated animal rooms with free access to water and a commercial laboratory complete food.

Fluorescence Images of Tumors and Major Organs

Four nude mice bearing HCT-116 tumors were injected the FDPP-HA (6 mg/mL, 0.1 mL) by the intravenous injection, then the four mice were killed after 24 hours. The tumors and major organs including hearts, livers, spleens, lungs, and kidneys were obtained. Fluorescence images of the tumors and major organs were observed by the Bruker In-Vivo Imaging System Fx Pro.

***In Vivo* Photodynamic Therapy**

The HCT-116 tumors bearing nude mice were divided into three groups (n=4). When the tumor volume was about 60-120 mm3, 200 μL of saline were injected into the mice (the control group) via tail vein. 200 μL of PBS (pH = 7.4, containing FDPP-HA) were also injected into the mice (the no illumination and illumination groups). After 24 hours, the solid tumors of the treatment group were irradiated for 10 min with xenon lamp light source system at a light density of 40 mW/cm^2. The tumor volumes and body weights of each group were evaluated by caliper measurements and balance for 30 days (one time every two days). And tumor volumes were calculated using the formula of length × width× width/2.

Histology Sample Preparation

After treatment of 30 days, the survival mice were killed to collect major organs such as heart, liver, spleen, lung, and kidney for histological analysis. The isolated test

specimens were soaked in 10% formalin solution for 48 hours at the room temperature and embedded with paraffin for haematoxylin and eosin (H&E) staining. Finally, the morphological changes were observed under a microscopy

References

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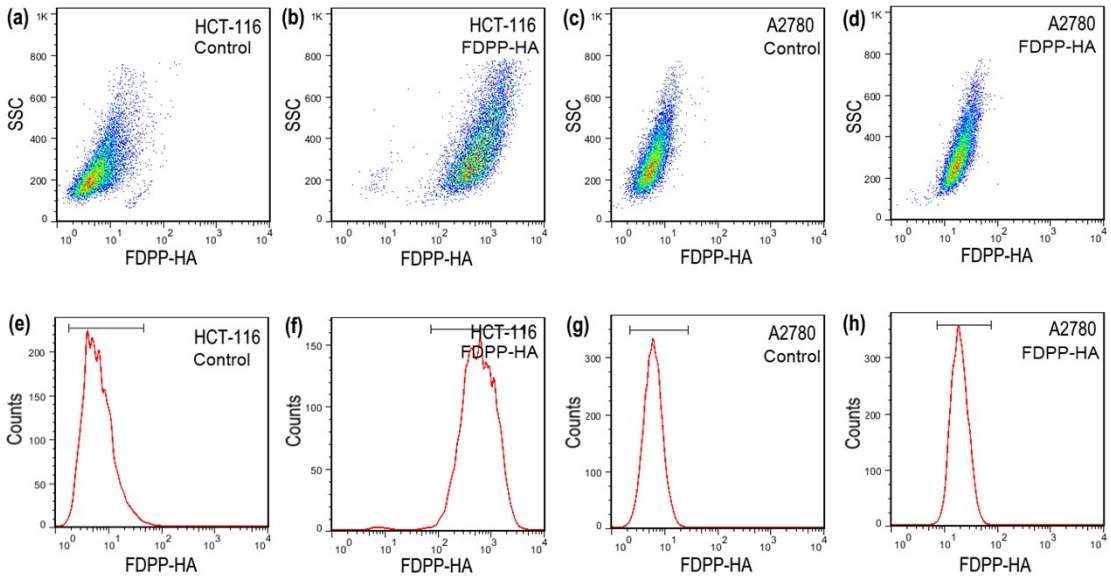

Figure S1. Flow cytometry results for HCT-116 and A2780 cells. (a, c, e) and (g) are control groups (cells only). (b, d, f) and (h) are the cells incubated with FDPP-HA.