Supporting Information

Clickable Amino acid Tuned Self-Assembly of Nucleus-selective Multi-component Nanoplatform for Synergistic Cancer Therapy

Lan Yang,[a] Xiao He,[a] Zhiying Zeng,[a] Jiakun Tang,[a] Dongmei Qi,[a] Huijie Ma,[a] Hui Chen,*[a] Xinghai Ning *[b] and Xuli Feng *[a]

[a] Chongqing Key Laboratory of Natural Product Synthesis and Drug Research, School of Pharmaceutical Sciences , Chongqing University, Chongqing, 401331, P.R. China

[b] National Laboratory of Solid State Microstructures, Chemistry and Biomedicine Innovation Center, College of Engineering and Applied Sciences, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University, Nanjing, 210093, P.R. China

Experimental Section

Materials and Reagents

All chemical reagents were used as supplied without further purification unless otherwise specified. Chlorin e6 (Ce6) was purchased from Frontier Scientific, Inc. (Salt Lake City, UT, USA). Doxorubicin hydrochloride (DOX·HCl) was purchased from Macklin Biological Co., Ltd (Shanghai, China). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium (MTT) and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). Singlet oxygen sensor green (SOSG) were purchased from Thermo Fishcer Scientific (Waltham, MA, USA). Sodium azide (NaN_3), methyl-β-cyclodextrin (M-β-CD), Ivermectin, genistein was purchased from Bide Pharmatech Ltd.

Instruments

The morphology of nanoparticles was observed by transmission electron microscope (TEM, Talos F200S, Holland). Hydrodynamic sizes and zeta potentials were measured by multi angle particle size and high sensitive zeta potential analyzer (Omni, USA). Ultraviolet-visible light spectrophotometry (UV-Vis, Agilent Cary60, USA) and Fluorescence Spectrometer (Hitachi) was used to demonstrate the assembly behavior of Ce6 and nanomaterials.

Synthesis of Compound 1

Ethylenediamine (670 mg, 11.16 mmol) was added to a solution of N, N'-Di-Boc-L-lysine (500 mg, 1.13 mmol) in DCM (40 mL). After stirring overnight at room temperature, the solvent was evaporated and the residue was purified by column chromatography with dichloromethane/methanol (v/v = 20:1) as eluent to afford compound **1** as a white solid.

Synthesis of Compound 2

Compound **1** (201 mg, 0.52 mmol) was added to a solution of 4-nitrophenyl chloroformate-substituted DIBO (100 mg, 0.26 mmol) in 10 mL DCM, then trimethylamine (56 μL, 0.40 mmol) was added. The reaction mixture was stirred overnight at room temperature and the solvent was removed in vacuum. The residue was dissolved in EtOAc, washed with water (2

x 50 mL), filtration and solvent removal to afford the crude product, which was further purified by column chromatography with petroleum ether/ethyl acetate (v/v = 1:1) as eluent to get compound **2** as white solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.2-7.6 (m, 8H), δ 6.61 (t, 1H), δ 5.75 (t, 1H), δ 5.46 (t, 1H), δ 5.19 (t, 1H), δ 4.63 (t, 1H), δ 3.98 (t, 1H), δ 3.2-3.6 (m, 4H), δ 3.06 (m, 2H), δ 2.86 (m, 2H), δ 1.73 (m, 4H), δ 1.42 (m, 18H), δ 1.31 (m, 2H).^{13}C-NMR (100 MHz, $CDCl_3$): δ173.2, 156.5, 156.4, 156.1, 152.1 ,151.1, 130.0, 128.1, 127.2, 126.3, 126.0, 123.9, 121.3, 113.0, 110.0, 80.3, 79.3, 54.9, 46.2, 41.1, 39.8, 38.7, 29.7, 28.5, 28.3, 22.6. LC-MS (m/z): $[M+H]^+$, calcd for 635.77, found: 635.60.

Synthesis of Compound D-K

The compound **2** was dissolved with MeOH contain 2 M HCl and stirred for 2 h at room temperature to remove Boc to get the final product **D-K** with a yield of 56.3%. ^{1}H-NMR (400 MHz, D_2O): chemical shifts (δ) in ppm: δ 8.03 (s, 2H), δ 7.71 (m, 2H), δ 7.55 (m, 6H), δ 5.44 (t, 1H), δ 5.11 (s, 4H), δ 3.68 (m, 1H), δ 3.38-3.45 (m, 4H), δ 2.99 (d, 2H), δ 2.88 (t, 2H), δ 1.83 (dd, 2H), δ 1.52 (m, 2H), δ 1.37 (m, 2H). ^{13}C-NMR (100 MHz, D_2O): 169.52, 169.19, 155.75, 152.43, 151.32, 130.67, 128.96, 127.78, 126.55, 126.22, 124.41, 123.29, 120.71, 94.18, 75.92, 52.46, 38.72, 38.66, 38.02, 36.96, 30.58, 26.71, 21.59. LC-MS (m/z): $[M+H]^+$, calcd 435.53, found 435.46.

Synthesis of Compound 4-A

To a solution of tetraazacyclododecane (115 mg, 0.67 mmol) in CH_3CN (20 mL) was added Cs_2CO_3 (977 mg, 3 mmol). Compound **3** (699 mg, 3 mmol) in anhydrous CH_3CN was added slowly. The reaction mixture was stirred at room temperature for 8 h, filtered and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic phase was collected and dried over Na_2SO_4. After filtered, the solvent was removed in vacuo and the residue was purified via flash chromatography on silica gel with methanol/dichloromethane (V/V = 1:10) to get compound

4-A. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.46-3.69 (t, 8H), δ 2.49-3.08 (m, 16H), δ 1.88-2.30 (m, 8H), δ 1.60-1.83 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 51.00, 49.15, 47.76, 21.86. FT-MS (m/z) : $[M + H]^+$, calcd 504.36, found 504.76.

Singlet oxygen ($^1O_2$) detection by SOSG

To measure $^1O_2$ generation efficiency, samples including free Ce6, D-KC, D-KC/A and D-KCD/A nanoparticles were incubated in water or NaCl solution, respectively. After 6 h incubation, SOSG (2 μM) was added to each sample and the absorbance was then recorded at 504 nm after irradiation for

2 min (660 nm, 100 mW cm^{-2}).

Cell culture

Mouse breast cancer 4T1 cells were obtained from Shanghai Ek-Bioscience and cultured in complete RPMI-1640 medium (10% FBS, 1% penicillin/streptomycin) at 37 °C in the presence of 5% CO_2.

Cellular uptake

4T1 cells (density of 1×10^4 cells/well) were seeded in 96-well plates and incubated for 12 h at 37 °C under 5% CO_2. 100 μL fresh medium containing Ce6, D-KC, D-KC/A or D-KCD/A (30 μg/mL for Ce6 and 30 μg/mL for DOX) was then added. After 2 h incubation, the cells were fixed with 4% paraformaldehyde, stained with DAPI and imaged with fluorescent microscopy (ix51, Japan).

Endocytosis mechanism

4T1 cells (density of 1×10^4 cells/well) were seeded in 96-well plates and incubated 12 h at 37 °C under 5% CO_2. The cells were incubated with different endocytosis inhibitor (10 mM NaN_3, 10 mM M-β-CD or 200 μM genistein) or placed at 4 °C for 1 h, and then D-KCD/A

was added and incubated at 37 °C or 4 °C for another 2 h. After that cells were trypsinsized and collected for flow cytometry analysis.

Nucleus transport mechanism

To explore the nuclear transport mechanism of D-KCD/A nanoparticles, 4T1 cells were preincubated with ivermectin (25 μM) for 1 h and then D-KCD/A (30 μg/mL for Ce6) was added for further incubating another 2 h. After that, the cells were fixed with 4% paraformaldehyde, stained with DAPI and imaged with fluorescent microscopy.

In vitro cytotoxicity

4T1 cells (density of 1×10^4 cells/well) were seeded in 96-well plates and incubated for 12 h at 37 °C under 5% CO_2. Then the medium was replaced with fresh medium containing free Ce6, Ce6 + DOX, D-KC, D-KC/A or D-KCD/A (2 μg/mL for Ce6, 2 μg/mL for DOX). After 12 h incubation, the cells were illuminated with a laser (660 nm, 100 mW cm^{-2}) for 2 min and then incubated for another 12 h. The cell viability was measured by performing MTT assays. For cytotoxicity study of D-K, overnight incubated 4T1 cells were treated with different concentrations of D-K (0, 10, 20, 40, 60 μg/mL) for 24 h, and MTT assay was used to determine the cell viabilities.

Animal Model

All animal experiments were carried out in compliance with the requirements of the National Act on the Use of Experimental Animals (People's Republic of China) and were approved by the Experimental Animal Ethical Committee of Chongqing University Cancer Hospital. Female BALB/c-nude mice (6-8 weeks) were supplied by the Animal Center of Chongqing Medical University (Chongqing, China).

Biodistribution

4T1 tumor-bearing mice were injected intravenously with free Ce6, D-KC/A or D-KCD/A (4 mg/kg for Ce6, 4 mg/kg for DOX). The mice were imaged at different time points (2, 4, 8, 12, 24 h) postinjection by small animal imaging system (IVIS Lumina III, USA). 24 h after administration, the mice were sacrificed, major organs (heart, liver, spleen, lung and kidney) and tumors were imaged and analyzed by an IVIS lumina imaging system.

In vivo antitumor activity

For anti-tumor therapy studies, breast cancer 4T1 cells (2×10^6) were injected subcutaneously into female BALB/c-nude mice. When the tumor volume grew to approximately 60 mm^3, the mice were randomly divided into 6 groups (n=5): (1) PBS control group, (2) DOX group, (3) Ce6 with laser group, (4) D-KC/A with laser group, (5) D-KCD/A group and (6) D-KCD/A with laser group. 150 μL of different samples with equal amount of Ce6 (2 mg/kg) and DOX (2 mg/kg) were intravenously injected into the mice every three days for a total of three times. The tumor region of mice was irradiated by 660 nm laser (200 mW cm^{-2}, 10 min) at 12 h post each injection. The tumor volumes and weight were measured every two days for 20 days. The tumor volumes value was calculated as the following equation: tumor volumes = length × $width^2$ × 0.5. At the end of experiment, the tumor was excised and weighed. For histological examination, tumor tissues and major organs (heart, liver, spleen, lung and kidney) were collected for hematoxylin and eosin (H&E) staining.

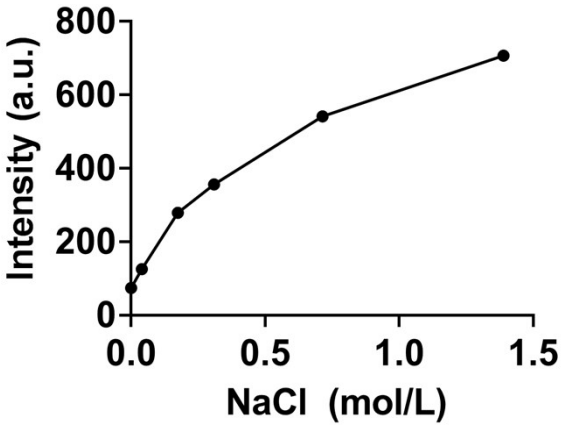

Figure S1. Fluorescence intensity of Ce6 in D-KCD/A nanoparticles in different salt solutions. The excitation wavelength of Ce6 is 400 nm.

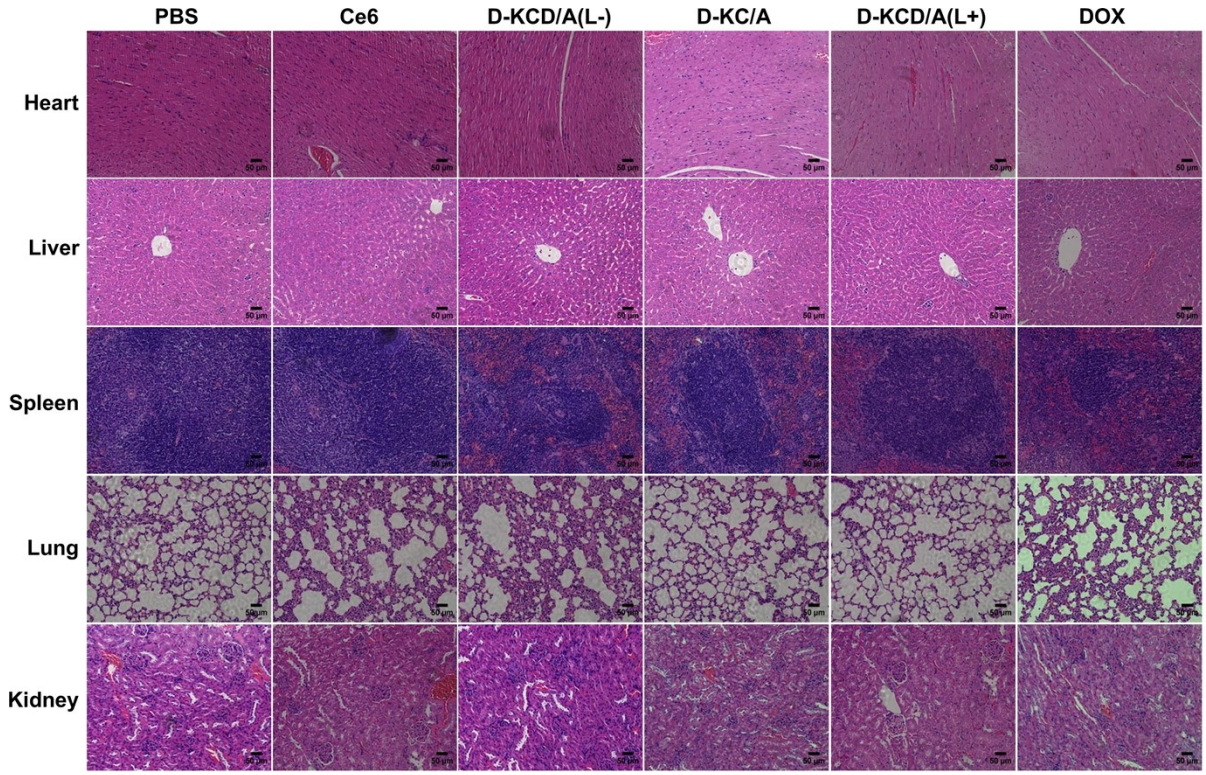

Figure S2. H&E staining of main organs after different treatments. Scale bars: 50 μm.