Supporting Information Available

Carrier free nanomedicine for enhanced photodynamic tumor therapy through glutathione S-transferase inhibition

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Experimental Section

1. Materials.

Coniferyl ferulate (Con) was obtained from Shanghai yuanye Bio-Technology Co., Ltd (China). Chlorin e6 (Ce6) was provided by Frontier Scientific (Shanghai, China). Cell apoptosis detection kit (Annexin V-FITC), reactive oxygen species assay kit (DCFH-DA), methylthiazolyldiphenyl-tetrazolium bromide (MTT), radio immunoprecipitation assay (RIPA) lysis buffer (weak), and SDS-PAGE gel preparation kit were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). Calcein-AM/PI double stain kit was obtained from Yeasen Biotech. Co., Ltd. (Shanghai, China). Singlet oxygen sensor green (SOSG) and Hoechst 33342 were provided by Invitrogen Corp. Glutathione S-transferase (GST) activity detection kit was purchased from Beijing Solarbio Science & Technology Co., Ltd (China). Anti-GST3/GST pi antibody was acquired from Abcam.

2. Preparation of CeCon.

Ce6 and Con were respectively dissolved into DMSO to prepare the stock solutions with the concentration of 10 g/L and 20 g/L. Among which, 40 μL Ce6 and 36 μL Con were mixed and stirred for 4 h in the dark. After adding 2 mL pure water, the mixed solution was stirred for another 4 h and then on dialysis (1000 KD) overnight. Next, the solution was centrifuged (3000 rpm) for 5 min and the supernatant was collected to get CeCon. At different molar ratios, Ce6 and Con were assembled using the same method to obtain various formulations.

3. Cell Culture.

Human lung cancer cells (A549) were cultured in Dulbecco's modified eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% antibiotics upon a humidified atmosphere containing 5% CO_2 at 37°C.

4. ROS Detection.

Singlet oxygen ($^1O_2$) production was detected by using SOSG as the indicator. In brief, SOSG (5 μM) was incubated with CeCon (4 mg/L), Ce6 (2 mg/L) or Con (2 mg/L). In the presence or absence of light (0.38 W), the fluorescence changes of SOSG was monitored to evaluate the $^1O_2$ production. Besides, the intracellular ROS was also measured by using DCFH-DA as the sensor. A549 cells were seeded and cultured with CeCon (4 mg/L), Ce6 (2 mg/L) or Con (2 mg/L) for 6 h. Afterwards, the cells were washed with PBS and treated with DCFH-DA for 20 min at 37°C. Then the cells in light groups were irradiated by light (638nm, 29.8 mW cm^{-2}) for 3 min. The other cells were cultured in the dark. Finally, the intracellular fluorescence was observed by CLSM to detect the ROS generation.

5. Cell Internalization.

To evaluate the cellular uptake behavior, A549 cells were seeded and cultured in 6-well plates. Subsequently, the cells were treated with CeCon (4 mg/L) or Ce6 (2 mg/L) for 2, 4, and 8 h respectively. Then the cells were washed with PBS and then collected for flow cytometry analysis. Moreover, A549 cells were also incubated with CeCon (4 mg/L) or Ce6 (2 mg/L) for 2, 4 and 8 h respectively. Then the cells were stained with Hoechst 33342 for 10 min. After being washed by PBS, the cells were detected by CLSM to observe the intracellular fluorescence.

6. MTT Assay.

Cell viability was determined by MTT assay. A549 cells were respectively seeded and cultured in 96-well plates. After that, the cells were treated with gradient concentrations of CeCon, Ce6, Con, or Ce6 + Con. 20 h later, the cells in light groups were exposed to light (638nm, 29.8 mW cm^{-2}) for 3 min. The other cells were incubated without light. Afterwards, all of the cells were incubated for another 4 h in the dark. Then 20 μL MTT (5 g/L) was added into every well. After 4 h, the cell medium of each well was replaced by DMSO (150 μL). The optical density (OD) of DMSO solution at 570 nm was measured by using a microplate reader. Cell viability was calculated by comparing the OD values in the presence and absence of therapeutic agents.

7. Cell Apoptosis Test.

A549 cells were seeded and cultured in 6-well plates. Subsequently, the cells were treated with CeCon (4 mg/L), Ce6 (2 mg/L) or Con (2 mg/L) for 6 h. Then the cells were irradiated with light (638nm, 29.8 mW cm^{-2}) for 15 s or cultured in the dark. Next, A549 cells were cultured in the dark for another 2 h at 37°C. After being washed by PBS, the cells were harvested and stained with Annexin V-FITC and PI for 20 min. The cellular fluorescence was analyzed by flow cytometry to test the cell apoptosis.

8. Live/Dead Cell Staining Assay.

A549 cells were seeded and cultured with CeCon (4 mg/L), Ce6 (2 mg/L) or Con (2 mg/L) for 6 h at 37°C in the shield of light. Subsequently, the cells were irradiated

with light (638nm, 29.8 mW cm^{-2}) for 5 min or cultured in the dark. Then A549 cells were cultured in the dark for another 2 h at 37°C. After being washed by PBS, the cells were stained with Calcein-AM and PI for 20 min. The fluorescence of A549 cells were observed by CLSM.

9. Western Blot Analysis.

A549 cells were seeded and cultured in 6-well plates. Then the cells were treated with CeCon (20 mg/L) or Con (10 mg/L) for 20 h. Subsequently, the cells were irradiated with light (638nm, 29.8 mW cm^{-2}) for 30 s or cultured in the dark. And cells were cultured in the dark for another 4 h at 37°C. After being washed by PBS, the cells were treated with cell lysis buffer RIPA (100 μL) for 30 min, which were then centrifuged (10000 rpm) for 30 min at 4°C. The protein contents were determined by using BCA protein assay. 20 μg protein was used and performed for electrophoretic analysis. Then the protein in gel was transferred to nitrocellulose membrane (300mA，70min), which was washed with TBST for three times. TBST containing 5% skim milk was used for blocking. After being washed with TBST, the membrane was incubated with Anti-GST 3/GST Pi overnight at 4°C. Then the membrane was washed with TBST again and incubated with Goat Anti-Rabbit IgG H&L (HRP) for 2 h at room temperature. Chemiluminescence was used for development to evaluate the protein levels. Gray values were counted by Image J.

10. Glutathione S-Transferase (GST) Activity Measurement.

A549 cells were cultured with CeCon (20 mg/L) or Con (10 mg/L) for 20 h. Then the cells were irradiated with light (638nm, 29.8 mW cm^{-2}) for 30 s or cultured in the

dark. After incubation for another 4 h, the cells were washed with PBS and collected by centrifugation (1500 rpm, 3 min). The amount of cells was confirmed by a cell count meter. The cells were diluted by reagent 1 to the density of 5×10^6 cells per milliliter, which were then broken under ultrasound (300 W). After that, the fragmentized liquid was centrifuged (8000 rpm) for 10 min at 4°C. The supernatant (100 μL) was mixed with reagent 2 (900 μL) and reagent 3 (100 μL). The absorbance at 340 nm was recorded as A_3 at the moment. After incubation for 5 min at 37°C, the absorbance at 340 nm was recorded as A_4. As the control, 100 μL reagent 1 was mixed with 900 μL reagent 2 and 100 μL reagent 3. The absorbance at 340 nm was recorded as A_1 at the moment. After incubation for 5 min at 37°C, the absorbance at 340 nm was recorded as A_2. The GST activity (U/10^4 cells) was calculated as 0.23×[(A4-A3)-(A2-A1)].

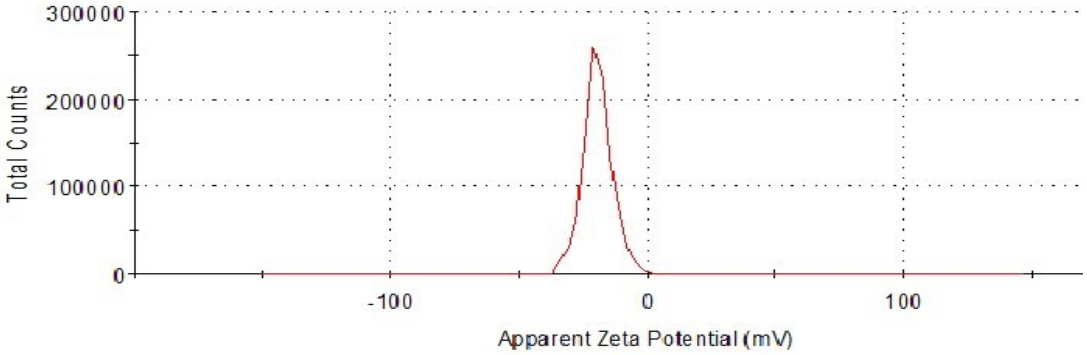

Fig. S1 The zeta potential of CeCon.

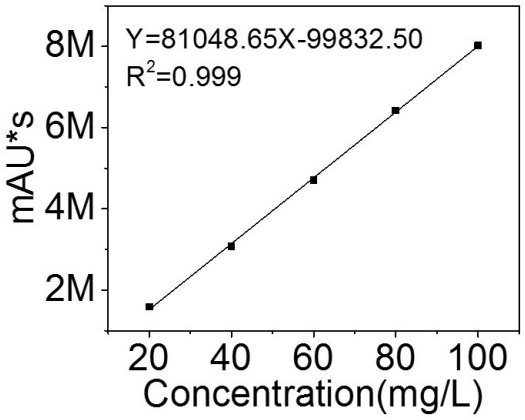

Fig. S2 The standard curve of Con by using HPLC.

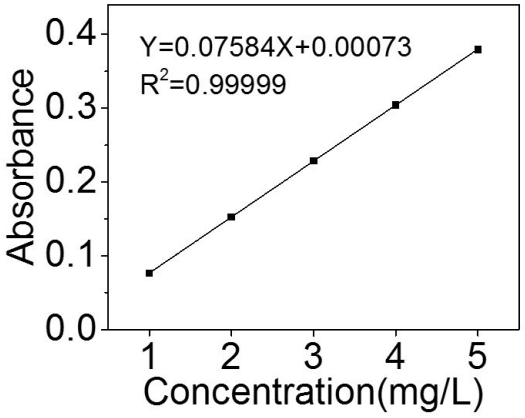

Fig. S3 The standard curve of Ce6 by using UV-vis spectrum.

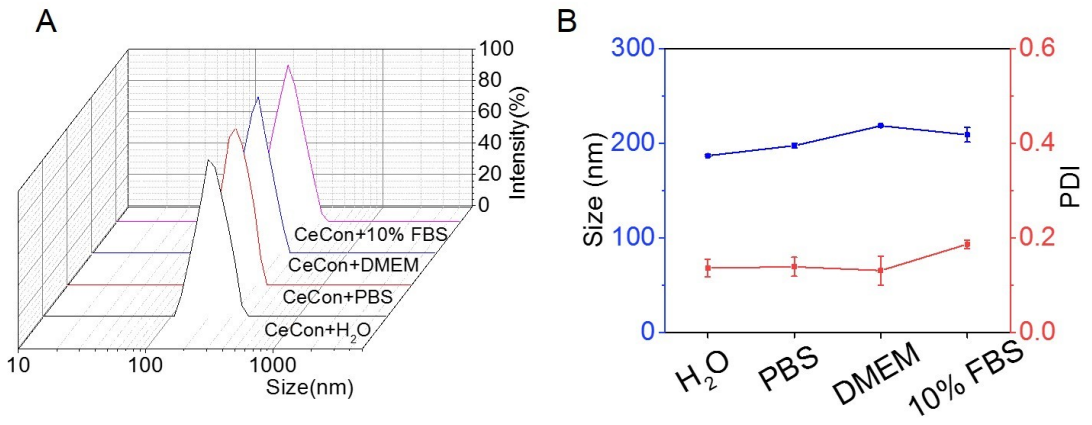

Fig. S4 (A) The particle size distributions, (B) the size and PDI values of CeCon in H_2O, PBS, DMEM and 10% FBS.

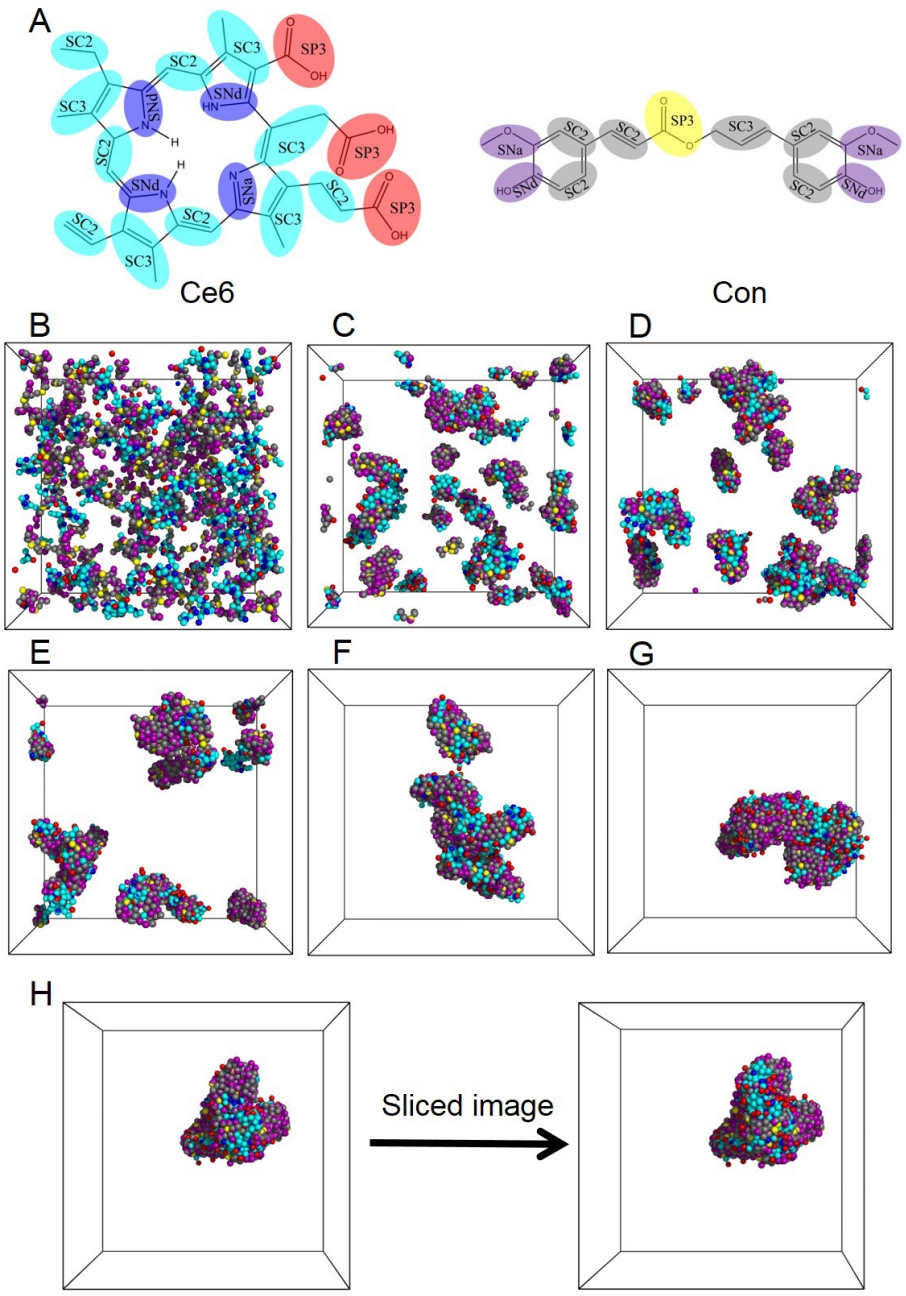

Fig. S5 Molecular simulation of the self-assembly process of Ce6 and Con. (A) Coarse-grained models of Ce6 and Con. CGMD simulations on the self-assembly of Ce6 and Con in solution. (B) Initial state, (C) 30 ns, (D) 50 ns, (E) 100 ns, (F) 200 ns, (G) 500 ns, (H) Terminal structure and the cross-sectional view of one CeCon nanoparticle. Water beads were omitted for clarity.

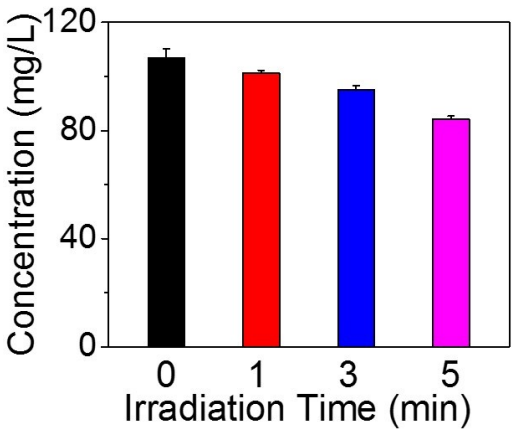

Fig. S6 The concentration of Con in CeCon after irradiation for different time.