Electronic Supplementary Material (ESI) for RSC Advances.

Supplemental information for

Small molecular nanomedicines formed from camptothecin dimer containing disulfide bond

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Experimental details

Materials. All starting materials were purchased from commercial suppliers and used without further purification unless otherwise noted. Solvents for chemical synthesis were purified by distillation.

Synthesis of CPT-s-s-CPT. It has been reported by J. Cheng's group previously,[1] so it is not described here.

Characterization of CPT-s-s-CPT. ^{1}H NMR spectra was recorded on a Bruker AV400 M in $CDCl_3$ at 25 °C. Chemical shifts were given in parts per million from that of tetramethylsilane (TMS) as an internal reference.

Preparation of CPT nanoparticles (CPT-NPs). The CPT-NPs were prepared as follows: 1.45 mg of CPT-s-s-CPT was dissolved in 4 mL of N, N-dimethylformamide (DMF) and stirred for half an hour. Then the solution was added dropwise to 10 mL of deionized water. DMF and unself-assembled CPT-s-s-CPT were removed by dialysis against distilled water (MWCO=1000).

The concentration of CPT-NPs was determined by the UV absorbance at 364 nm for CPT-s-s-CPT, according to the standard calibration curve of CPT-s-s-CPT in DMF. The solution of CPT-NPs was diluted 10 times with DMF, then was measured by UV-vis spectrophotometer peformed on Shimadzu UV-2450 PC.

CPT-NPs characterization. Size distribution of the CPT-NPs was determined by dynamic light scattering (DLS) with a vertically polarized He-Ne laser (DAWN EOS, Wyatt Technology, U.S.A.). The scattering angle was fixed at 90° and the measurement was carried out at 25 °C. The morphology of the CPT-NPs was

measured by Transmission Electron Microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. To prepare the specimen for TEM, a drop of nanoparticles solution was deposited onto a copper grid with a carbon coating. The specimen was air-dried and measured at room temperature. It was also measured by Scanning Electron Microscopy performed on JEOL JXA-840 under an accelerating voltage of 15 kV.

Cell culture. The human cervical cancer cell line HeLa and human hepatocarcinoma cell line HepG2 were routinely grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) containing 10% FBS (Hyclone). Cells were cultured in a humidified incubator at 37 °C with 5% CO_2.

Confocal laser scanning microscopy (CLSM). HeLa and HepG2 cells were seeded on the coverslip in 6-well plates at a density of 5×10^5 cells per well in 1 mL of DMEM medium and incubated in a humidified 5% CO_2 atmosphere for 24 h. For cellular internalization observation, cells were incubated with CPT-NPs at the concentration of 20 μg mL^{-1} in fresh culture medium. After incubation for 0.5 h and 2 h at 37 °C, cells were washed twice with ice-cold PBS and fixed with fresh 4% (w/v) paraformaldehyde for 15 min at room temperature. The cellular localization was visualized under a confocal laser scanning microscope (Carl Zeiss LSM 780).

In vitro cytotoxicity. The cytotoxicity of CPT-NPs was measured via MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, HeLa and HepG2 cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 2×10^3 cells/well and incubated in DMEM for 24 h. The medium was then

replaced with CPT-NPs at various concentrations from 0.0005 to 5 μg/mL. At the designated time intervals (48 h), 20 μL of MTT solution in PBS with the concentration of 5 mg/mL was added and the plate was incubated for another 4 h at 37 °C. After that, the medium containing MTT was removed and 150 μL of DMSO was added to each well to dissolve the MTT formazan crystals. Finally, the plates were shaken for 3 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader (BioTek, EXL808). Data are presented as means ± SD (standard deviation) (n=4).

The reduction-responsiveness of disulfide-based nanomedicines was conducted as follows: HeLa cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 2×10^3 cells/well and incubated in DMEM for 24 h. GSH with the final concentration of 10 mM was added and cultured with cells for 4 h. Then the medium was removed and the 96-well plates were washed two times, followed by addition of fresh medium and CPT-NPs at various concentrations from 0.0005 to 5 μg/mL. Subsequently cells were further incubated for 48 h, and cell viability was determined by MTT assay. Cells without GSH treatment were used as control.

Statistics. All experiments were performed at least three times and all results are expressed as mean ± SD.

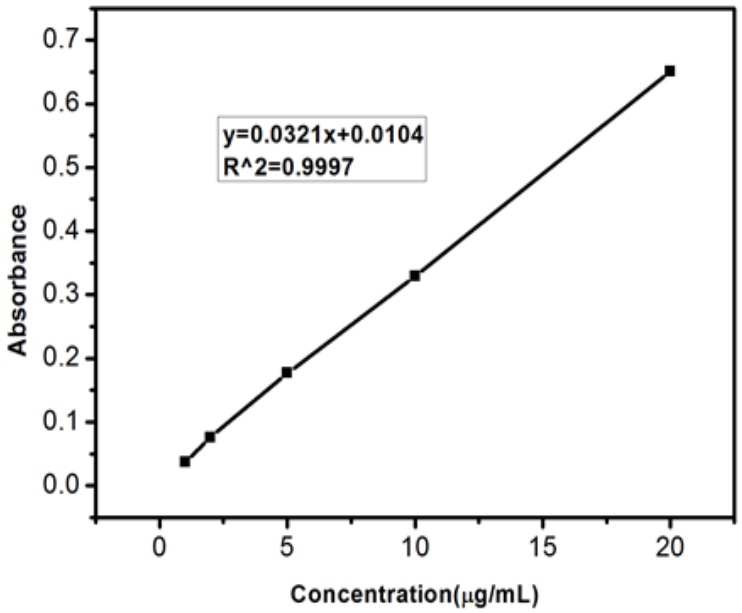

Fig. S1 The UV-vis absorption standard curve of CPT-s-s-CPT in DMF.

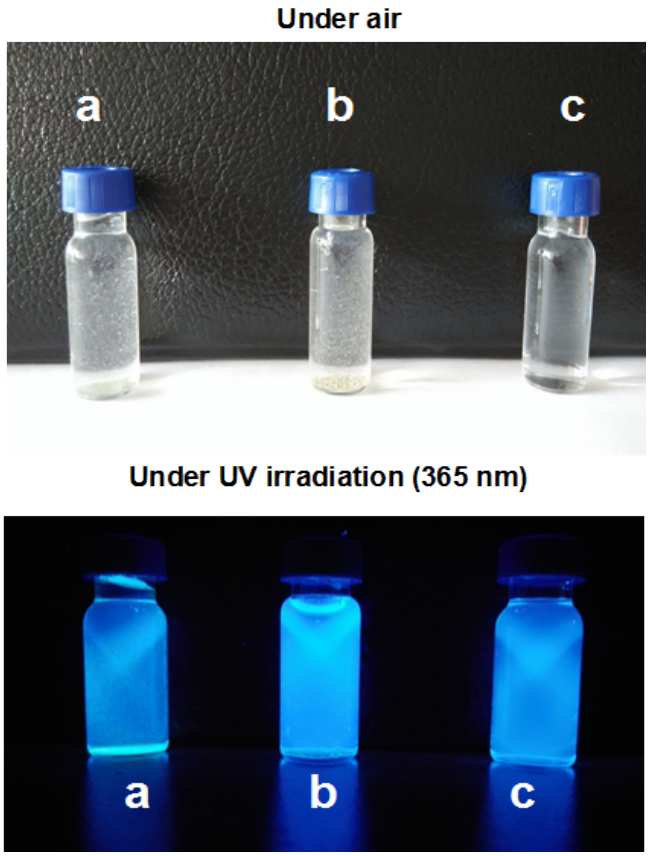

Fig. S2 The picture of CPT (a), CPT-s-s-CPT (b) and CPT-NPs (c) in water.

Fig. S3 SEM image of CPT-NPs.

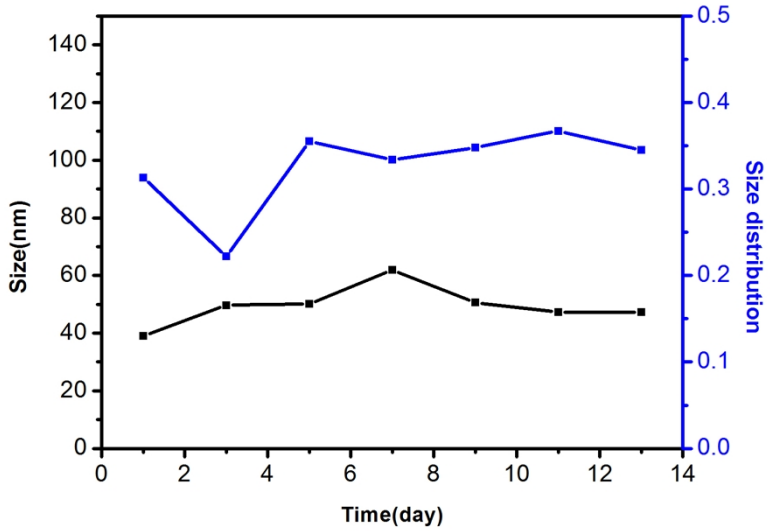

Fig. S4 change of size and size distribution of CPT-NPs with different times determined by DLS.

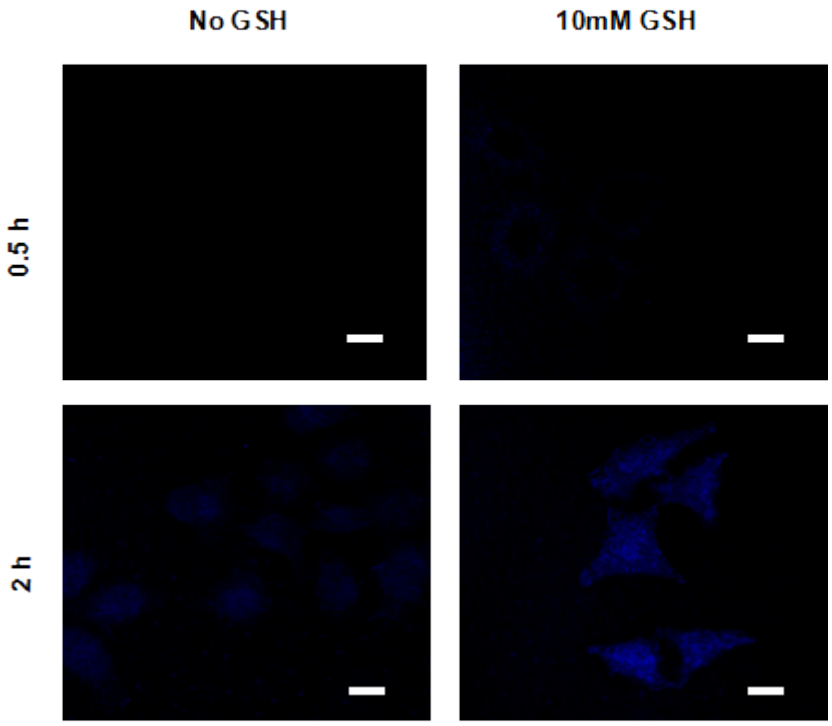

Fig. S5 The cell uptake of CPT-NPs. All scale bars stand for 20 μm.

1 H. Wang, M. Xu, M. Xiong and J. Cheng, *Chemical Communications*, 2015, **51**, 4807-4810.