Cascade amplifying synergistic effects of chemo-photodynamic therapy using ROS-responsive polymeric nanocarriers

Chun-Yang Sun[1,I], Ziyang Cao[2,I], Xue-Jun Zhang[3], Rong Sun[4], Chun-Shui Yu[1,*], and Xianzhu Yang[2,*]

[1] Department of Radiology and Tianjin Key Laboratory of Functional Imaging, Tianjin Medical University General Hospital, Tianjin 300052, P.R. China

[2] Institutes for Life Sciences, School of Medicine and National Engineering Research Center for Tissue Restoration and Reconstruction, South China University of Technology, Guangzhou, Guandong 510006, P. R. China

[3] School of Medical Imaging, Tianjin Medical University, Tianjin 300203, P.R. China

[4] School of Biological and Chemical Engineering, Zhejiang University of Science & Technology, Hangzhou, Zhejiang 310023, P. R. China

[I] These authors contributed equally.

E-mail: chunshuiyu@tijmu.edu.cn (C. S. Yu), yangxz@scut.edu.cn (X. Z. Yang)

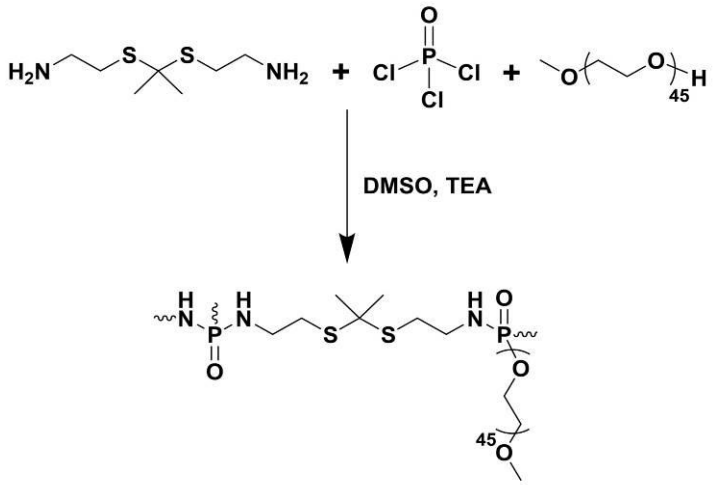

Figure S1. Synthetic route of hyperbranced polyphosphate (RHPPE).

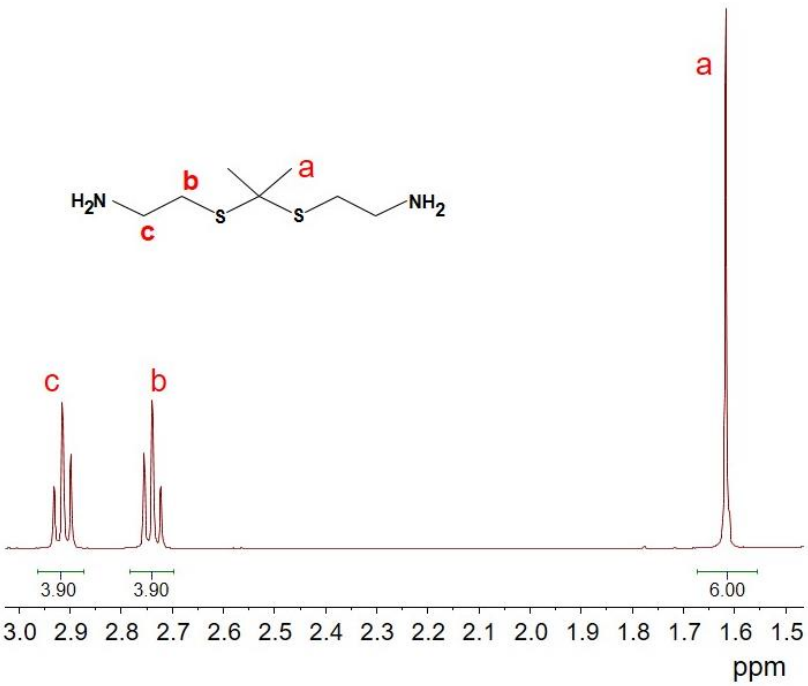

Figure S2. ^{1}H NMR spectrum of RHPPE in $CDCl_3$ recorded on an AVANCE III 400 MHz spectrometer at 25 ℃ (ppm).

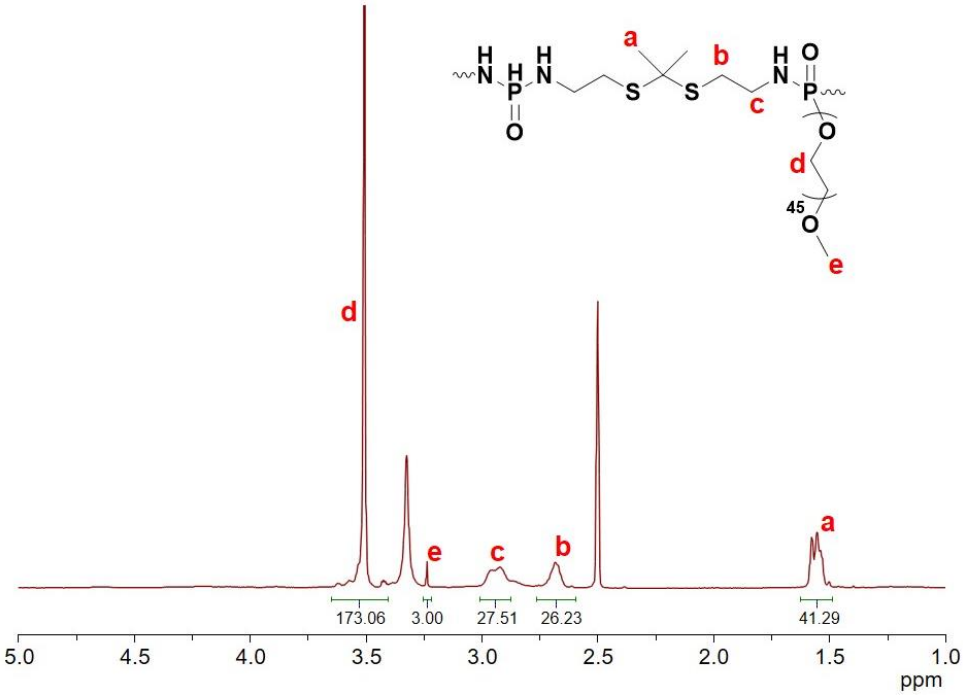

Figure S3. ^{1}H NMR spectrum of RHPPE in d_6-DMSO recorded on an AVANCE III 400 MHz spectrometer at 25 ℃.

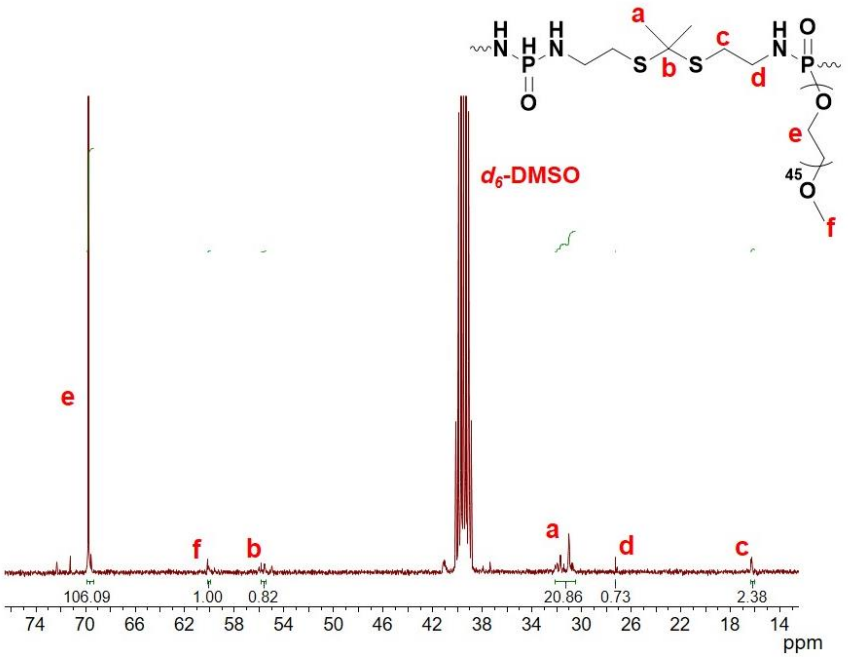

Figure S4. ^{13}C NMR spectrum of RHPPE in d_6-DMSO recorded on an AVANCE III 400 MHz spectrometer at 25 ℃.

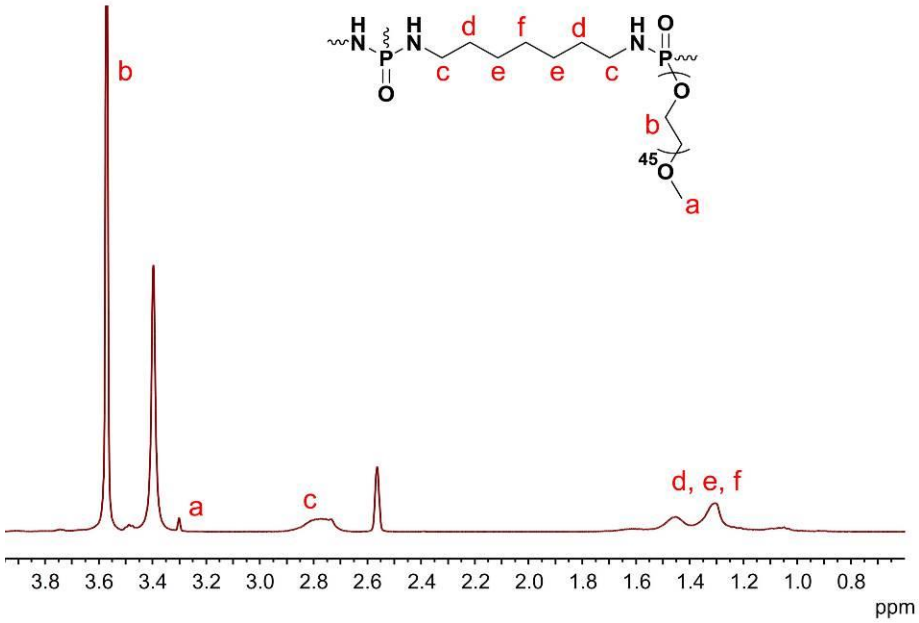

Figure S5. ^{1}H NMR spectrum of non-responsive hyperbranced polyphosphate (HPPE) in d_6-DMSO recorded on an AVANCE III 400 MHz spectrometer at 25 ℃.

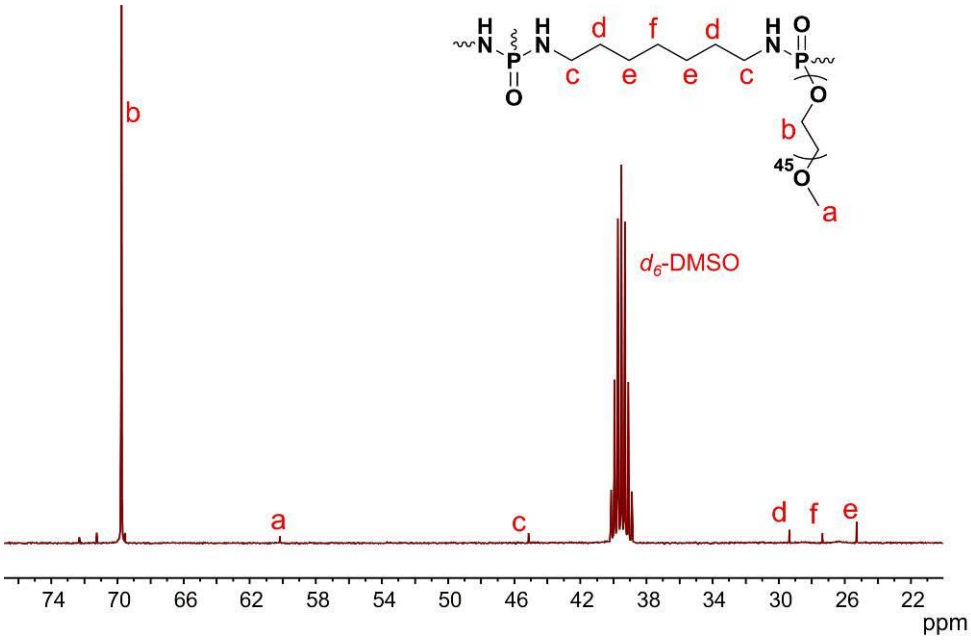

Figure S6. ^{13}C NMR spectrum of HPPE in $CDCl_3$ recorded on an AVANCE III 400 MHz spectrometer at 25 ℃.

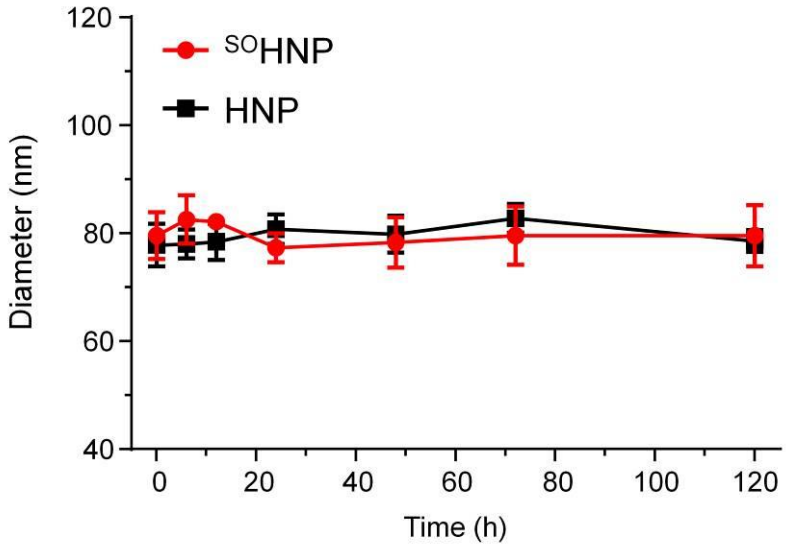

Figure S7. Diameter changes of ^{SO}HNP or HNP as a function of incubation time in PB buffer (pH 7.4).

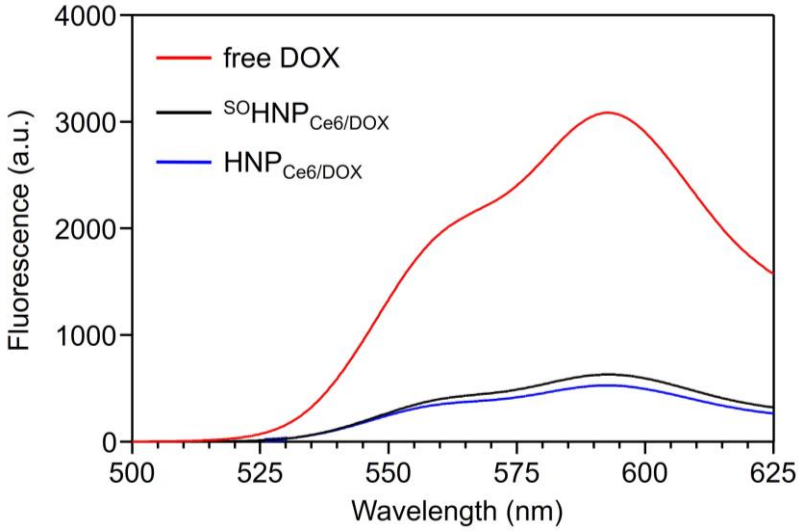

Figure S8. Emission spectra of free DOX, $^{SO}HNP_{Ce6/DOX}$ or $HNP_{Ce6/DOX}$ in aqueous solution (Ex=460 nm).

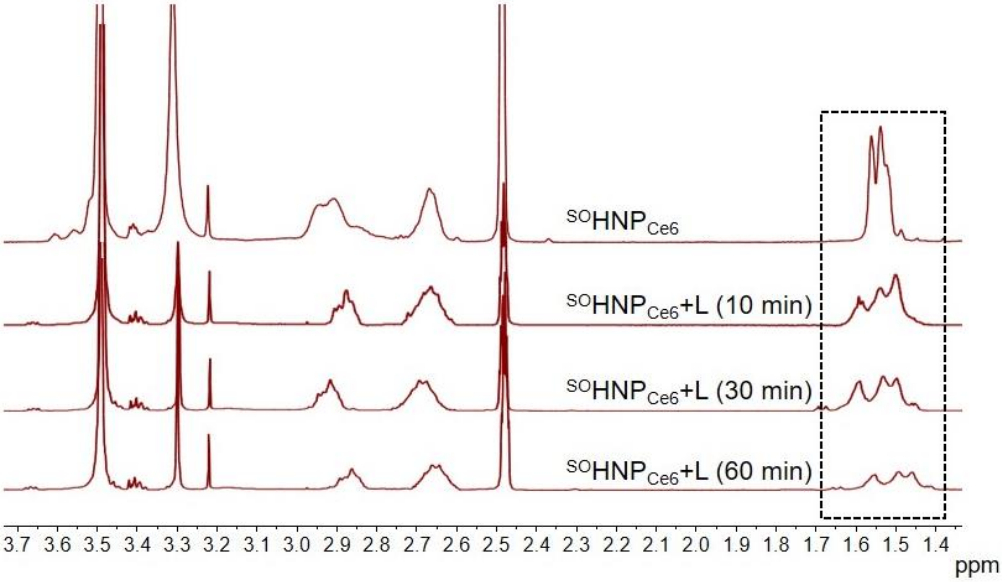

Figure S9. ^{1}H NMR spectra of $^{SO}HNP_{Ce6}$ after 660 nm laser irradiation for different times (10, 30, and 60 min) at power intensity of 0.2 W/cm^{2}.

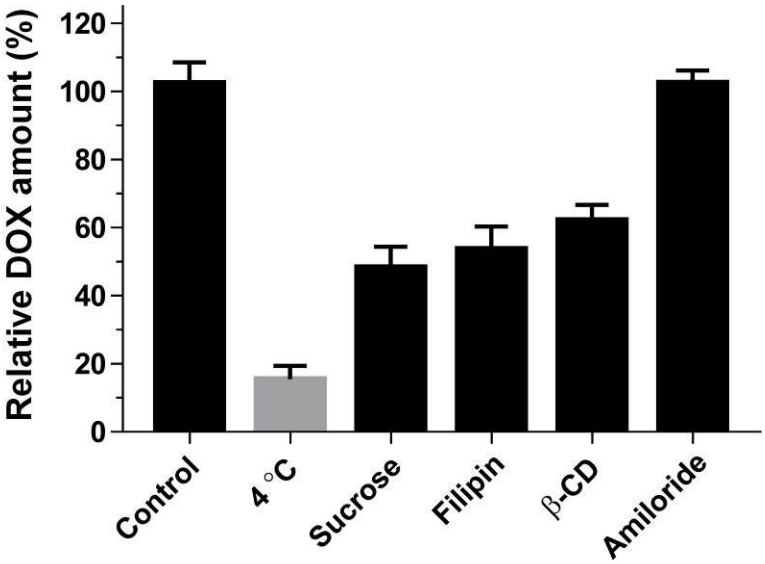

Figure S10. Cellular amount of DOX in MCF-7/ADR cells after 6 h of incubation with $^{SO}HNP_{Ce6/DOX}$.

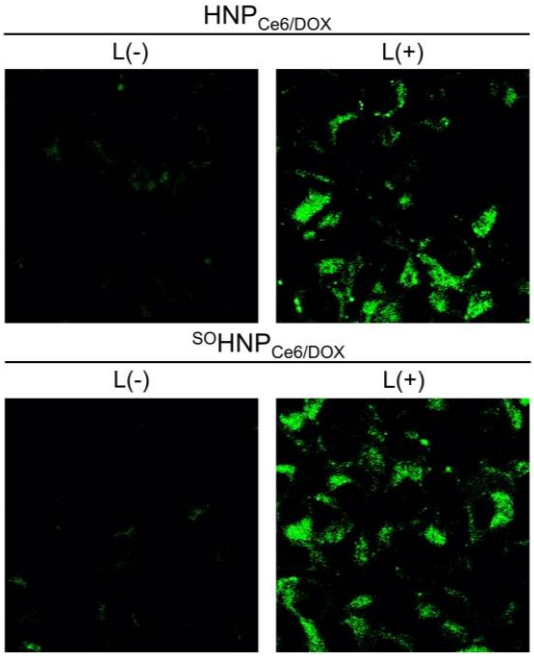

Figure S11. Fluorescence microscopy image of cells incubated with DCF-DA and then treated with $HNP_{Ce6/DOX}$ and $^{SO}HNP_{Ce6/DOX}$ with or without 660 nm laser irradiation (0.2 W/cm^2, 15 min).

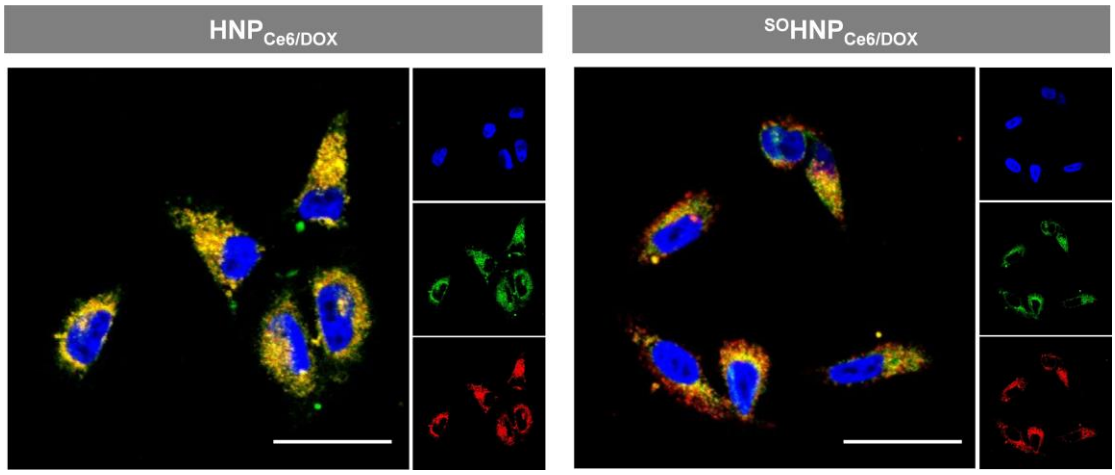

Figure S12. Assessment of the intracellular DOX release and biodistribution of $HNP_{Ce6/DOX}$ or $^{SO}HNP_{Ce6/DOX}$ in MCF-7/ADR cells without continuous 660-laser irradiation (0.1 W/cm^2). The concentration of DOX in the cell culture was 6 μg/mL. Acidic endosomes/lysosomes and cell nuclei were stained with LysoTracker^{TM} Green

(green) and DAPI (blue), respectively.

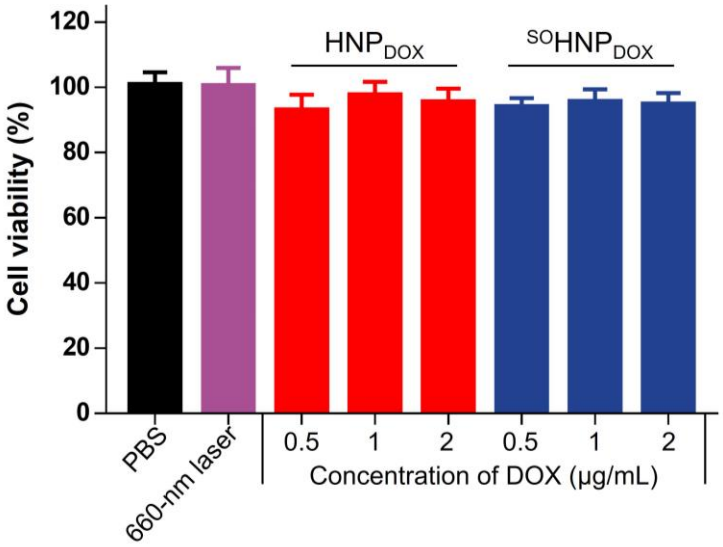

Figure S13. Cytotoxicity of laser, HNP$_{DOX}$ and ^{SO}HNP$_{DOX}$ against MCF-7/ADR cells for 72 h. The power density of 660-nm laser (NIR) was 0.1 W/cm^2.

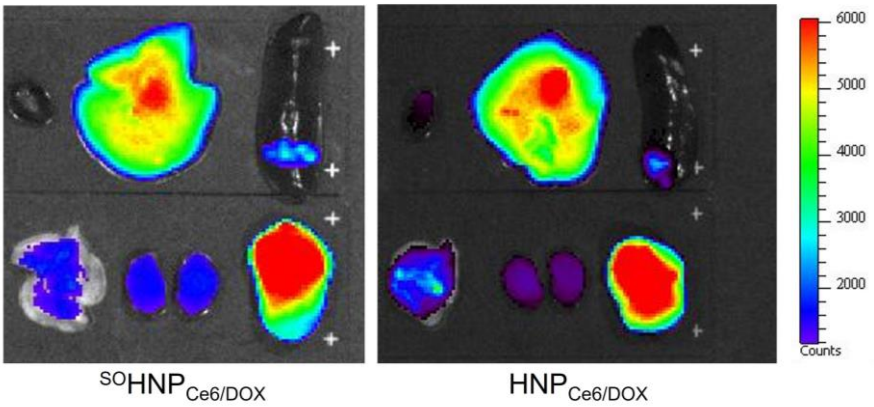

Figure S14. Fluorescence image of Ce6 in major organs 24 h post systemic injection. The quantification of fluorescence intensity calculated by the software is shown in Figure 6C and 6D.

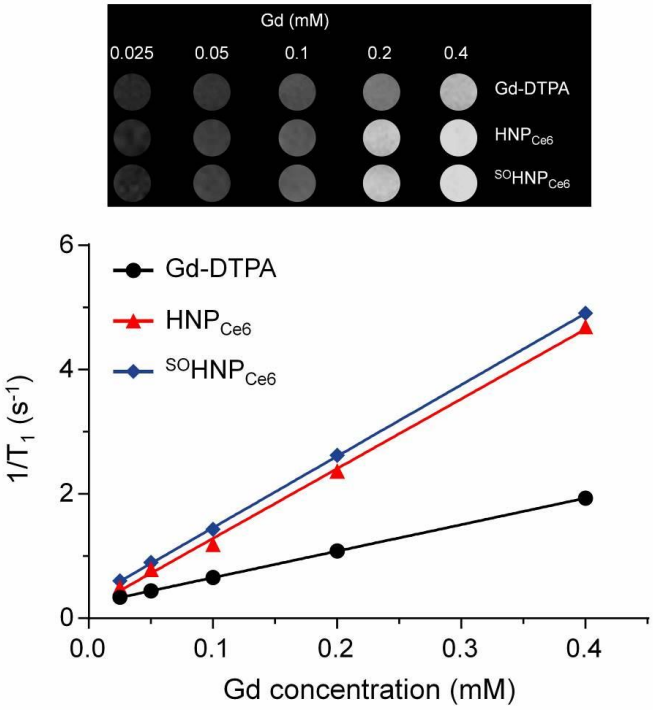

Figure S15. Water proton longitudinal relaxation rate ($1/T_1$) of HNP_{Ce6} and $^{SO}HNP_{Ce6}$ in aqueous solution as a function of Gd^{3+} concentration. The small molecular Gd-DTPA complex was used as a control.

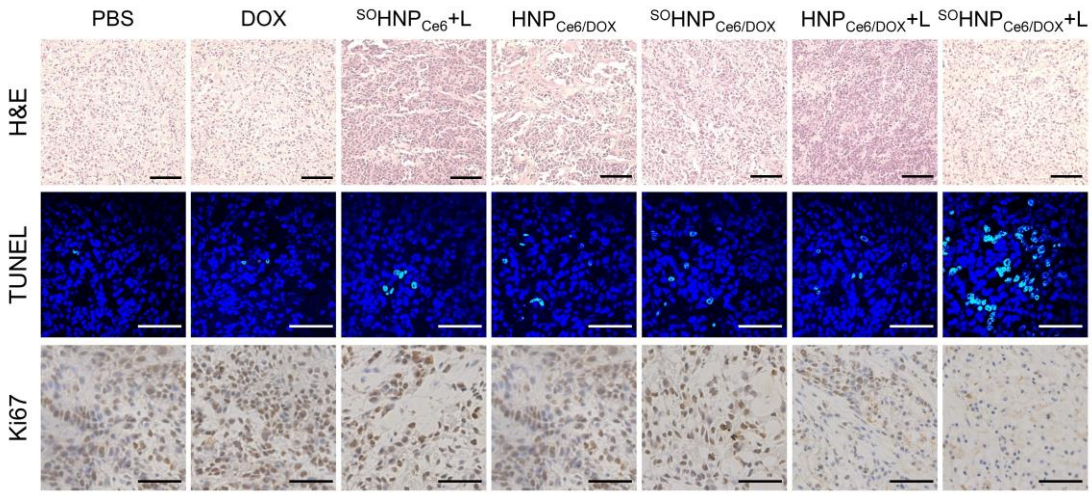

Figure S16. H&E, TUNEL and Ki67 analyses of tumor tissues from mice treated with the indicated formulations. The scale bar for H&E analyses was 200 μm. The scale bar for both TUNEL and Ki67 was 100 μm. Ki67-positive proliferating cells are stained brown.

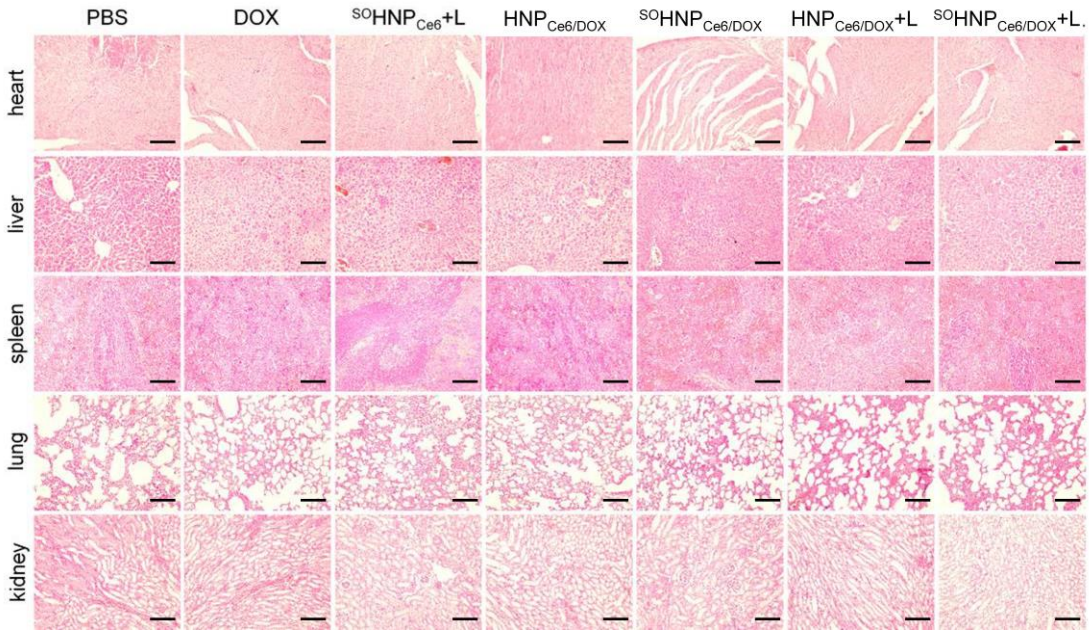

Figure S17. Histopathology analyses of visceral organ sections from MCF-7/ADR xenografted female mice after the tumor growth inhibition experiment (scale bar: 200 μm).

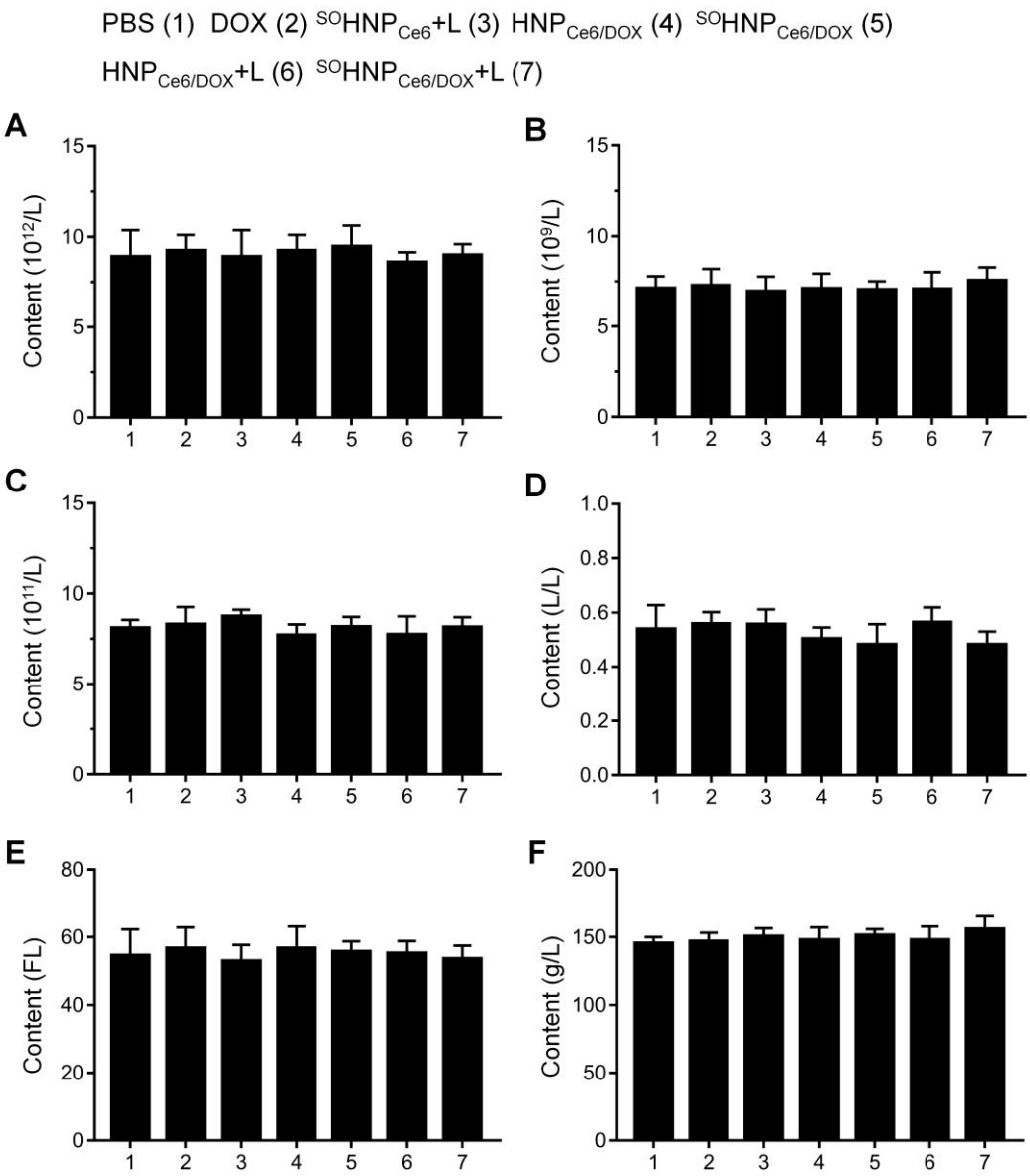

Figure S18. Hematology analysis of the mice after different treatments: (A) red blood cell (RBC), (B) white blood cell (WBC), (C) platelet (PLT), (D) hematocrit (HCT), (E) mean corpuscular volume (MCV), and (F) hemoglobin (HGB), respectively.

Table S1. Drug loading content (DLC) and encapsulation efficiency (EE) of Ce6 and DOX for ^{SO}HNP and HNP.

Parameter	DLC (%)		EE (%)	
	Ce6	DOX	Ce6	DOX
$HNP_{Ce6/DOX}$	3.51	3.27	35.1	32.7
$^{SO}HNP_{Ce6/DOX}$	3.39	3.13	33.9	31.3

Table S2. Pharmacokinetic parameters of these formulations after intravenous administration.

Parameter	$AUC_{0\text{-}48h}$ (μg/L*h)	$t_{1/2z}$ (h)	C_{max} (μg/L)	CI
DOX	64.70	18.46	14.15	21.53
$HNP_{Ce6/DOX}$	810.79	26.47	124.77	1.64
$^{SO}HNP_{Ce6/DOX}$	728.83	27.74	140.84	2.82

AUC, area under curve; $t_{1/2z}$, elimination half-life; C_{max}, peak concentration; CI, clear rate.