Supporting Information

Light-Activatable Dual-Source ROS-Responsive Prodrug Nanoplatform for Synergistic Chemo-Photodynamic Therapy

Bin Yang[a], Kaiyuan Wang[a], Dong Zhang[a], Bingjun Sun[a], Bin Ji[b], Lin Wei[c], Zhenbao Li[a], Menglin Wang[a], Xuanbo Zhang[a], Haotian Zhang[d], Qiming Kan[d], Cong Luo[*,a], Yongjun Wang[a], , Zhonggui He[a] and Jin Sun[*,a]

[a]Department of Pharmaceutics, Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang, Liaoning, 110016, China

[b]Department of Laboratory Medicine, The First Affiliated Hospital, China Medical University, Shenyang, Liaoning, 110001, China

[c]Key Laboratory of Microbiology, School of Life Science, Heilongjiang University, Harbin, 150080, China

[d]School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Wenhua Road, No. 103, Shenyang 110016, China

*Corresponding authors:

Prof. Jin Sun, Ph.D.

Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang, Liaoning, 110016, P. R. China

Tel: +86-24-23986325; Fax: +86-24-23986325

E-mail address: sunjin@syphu.edu.cn

Dr. Cong Luo.

Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang, Liaoning, 110016, P. R. China

Tel: +86-24-23986320; Fax: +86-24-23986320

E-mail address: luocong_0312@163.com.

PTX- OA 1H-NMR (400MHz, CDCl3):

δ8.12 (2H, t), 7.60 (1H, d), 7.49-7.53 (2H, m), 7.43 (2H, m), 7.30-7.34 (3H, m), 6.28 (1H, d, -NH), 5.66 (1H, d, 3'-H), 5.45 (1H, d, 2-H), 4.98-5.36 (4H, m, 2'-H, -CH=CH-, 5-H), 4.84 (1H, t, 7-H), 4.32-4.34 (1H, d, 20α-H), 4.20 (1H, d, 20β-H), 3.88-3.92 (2H, d, 15α-H, 15β-H), 3.86 (3H, d, 3-H), 3.32-3.34 (4H, m, 6α-H, 4-COCH3), 2.72 (1H, m, 13-H), 2.38-2.46 (3H, t, 14α-H, -CH2CO-), 2.34 (4H, s, 14β-H, 10-COCH3), 2.02 (6H, s, -CH2CH=CHCH2-), 1.81-1.84 (4H, s, 18-H), 1.51-1.61 (t, 5H, 6β-H), 1.23-1.37 (10H, s, 16-H, 19-H, -CH2CH2CO-), 1.22 (24H, t, 17-H), 0.90 (3H, t, -CH3). ESI-MS (m/z): calcd for $[C_{63}H_{89}NO_{15}SNa]$ $[M+Na]^+$ = 1122.7; found: 1099.7.

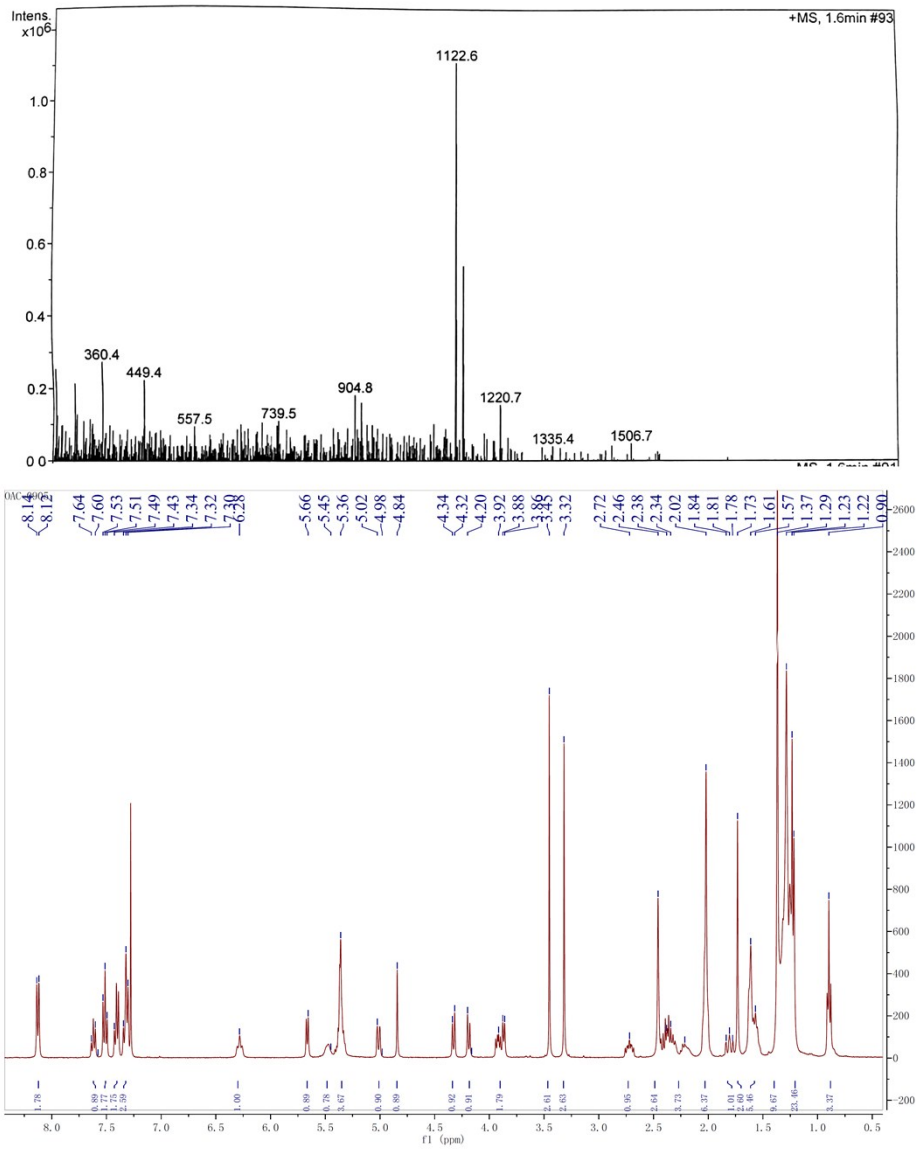
PTX-S-OA 1H-NMR (400MHz, CDCl3):

δ 8.15 (2H, d, 3″7″), 7.58 (1H, m, 5″), 7.49 (2H, m, 4″6″), 7.38 (2H, m, 5′6′), 7.35 (3H, m, 7′8′9′), 6.27 (1H, s,13-H), 5.64-5.69 (2H, d, 2-H, 3'-H), 5.50 (1H, d, 2′-H), 5.34 (3H, m, 3-H, -CH=CH-), 4.98 (1H, d, 4-H), 4.82 (1H, m, 5-H), 4.36 (1H, d, 20α-H), 4.34-4.30 (4H, m, -OCH2CH2O-), 4.16 (1H, d, 20β-H), 3.84 (1H, d, 7 -H), 3.83 (1H, d, 8-H), 3.44-3.50 (4H, m, -CH2-S-CH2-), 3.37 (1H, d, 18-H), 3.23-3.30 (4H, m, 6α-H, s, 4-$COCHCH_2$), 2.71 (1H, m,14α-H), 2.33 (3H, m, 14β-H, 15α-H, 15β-H), 2.29 (2H, t, -CH2CO-), 2.21 (3H, s, 10-COCH3), 2.01 (6H, s, -CH2CH=CHCH2-), 1.80 (1H, t, 6β-H), 1.71 (3H, s, 19-H), 1.60 (6H, m, -$CH_2CH_2CH_2CO$-), 1.29-1.34 (25H, t, 17-H), 1.20-1.26 (8H, s, 16-H), 0.88 (3H, t, -CH3). ESI-MS (m/z): calcd for $[C_{69}H_{97}NO_{19}SNa]$ $[M+Na]^+$ = 1298.7; found: 1275.7.

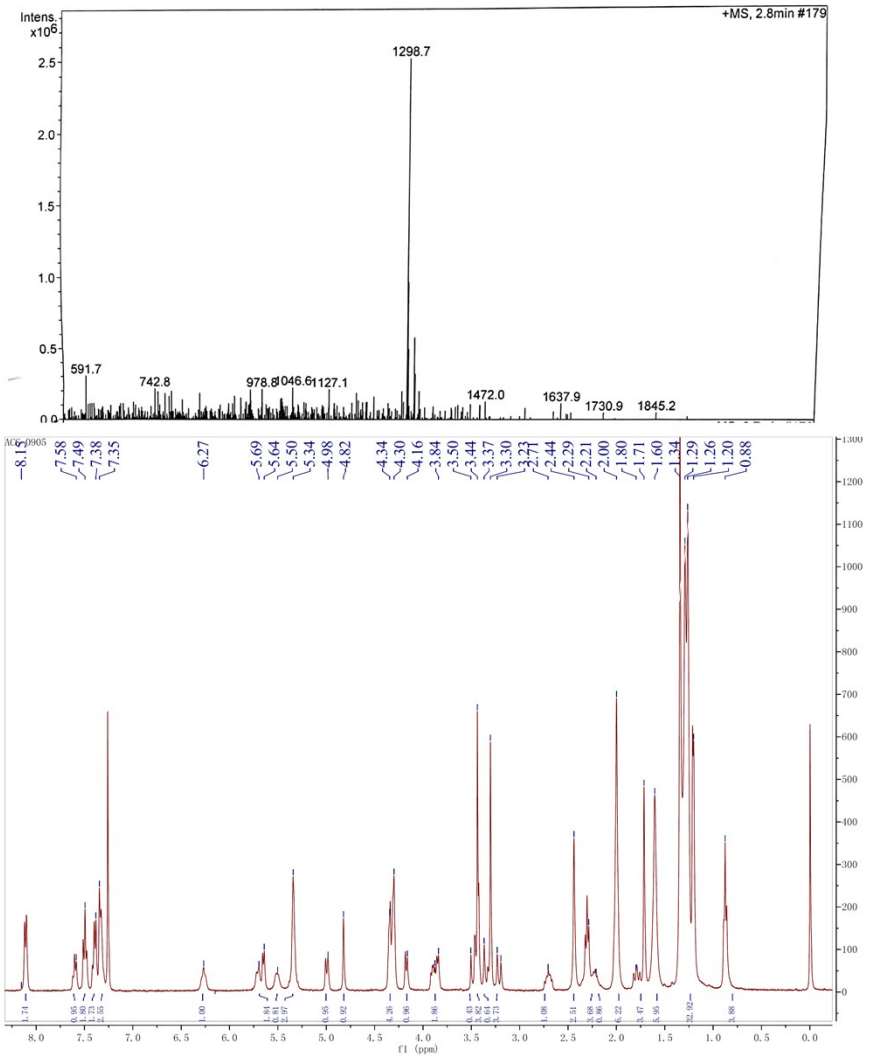
PTX-Se-OA 1H-NMR (400MHz, CDCl3):

δ 8.14 (2H, d, 3″7″), 7.62 (1H, m, 5″), 7.51-7.53 (2H, m, 4″6″), 7.38 (2H, m, 5′6′), 7.32-7.34 (3H, m, 7′8′9′), 6.29 (1H, s,13-H), 5.52-5.66 (2H, d, 2-H, 3'-H), 5.35 (1H, d, 2′-H), 5.31 (3H, m, 3-H, -CH=CH-), 4.99 (1H, d, 4-H), 4.83 (1H, m, 5-H), 4.36 (1H, d, 20α-H), 4.20-4.32 (4H, m, -OCH2CH2O-), 3.86 (1H, d, 7 -H), 3.66 (1H, d, 8-H), 3.44(4H, m, -CH2-S-CH2-), 3.32 (4H, m, 6α-H, s, 4-$COCHCH_2$), 3.17 (1H, d, 18-H), 2.71 (1H, m,14α-H), 2.32-2.46 (2H, m, 14β-H, 15α-H), 2.28 (3H, t, 15β-H, -CH2CO-), 2.03 (7H, t, 6β-H, -CH2CH=CHCH2-), 1.73 (5H, s, 19-H, 10-COCH3), 1.61-1.62 (6H, m, -

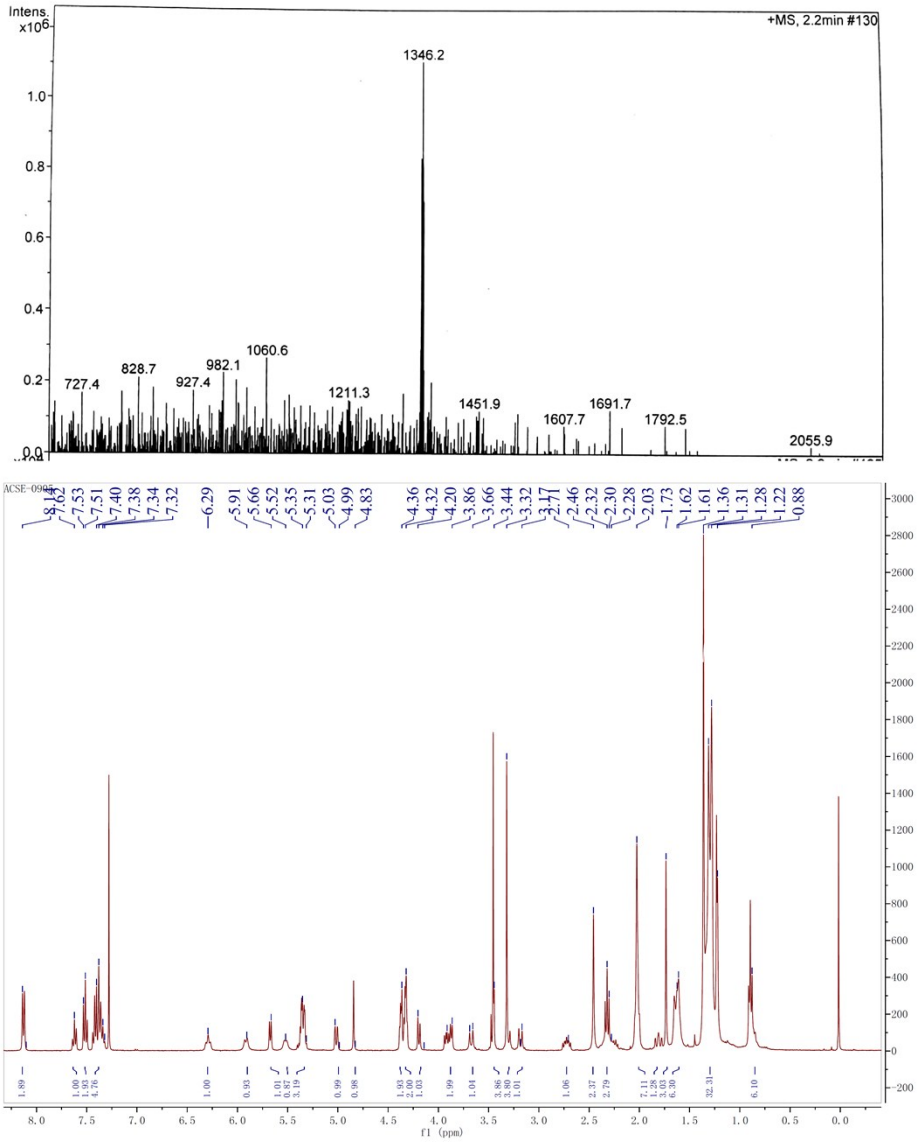
$CH_2CH_2CH_2CO$-), 1.31-1.36 (25H, t, 17-H), 1.22-1.28 (7H, s, 16-H), 0.88 (6H, t, -CH3). ESI-MS (m/z): calcd for $[C_{69}H_{97}NO_{19}SeNa]$ $[M+Na]^+$ = 1346.2; found: 1323.2.



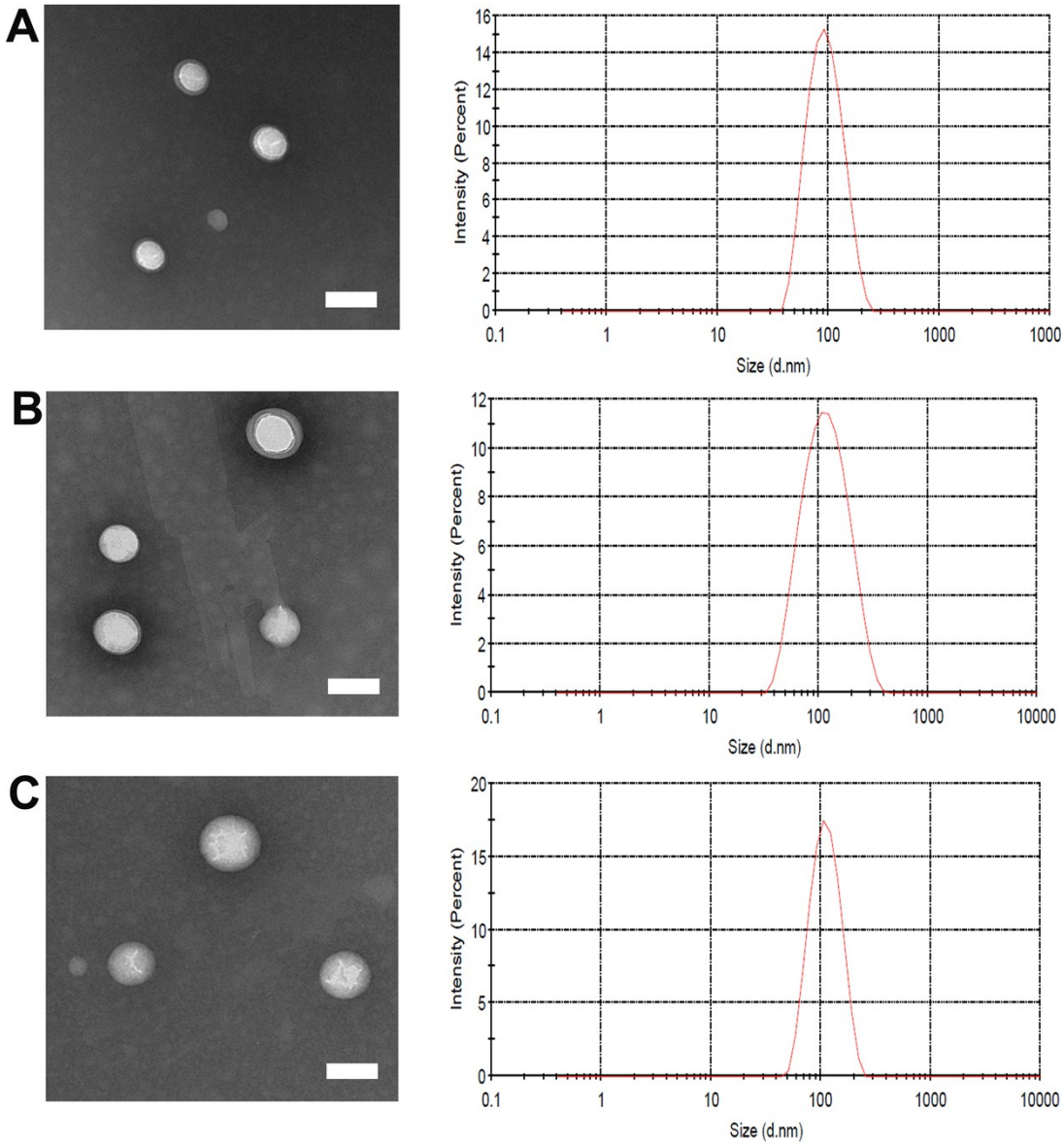
FigS1. Mass spectrum of CTX-S-OA and 1H NMR spectrum of CTX- OA



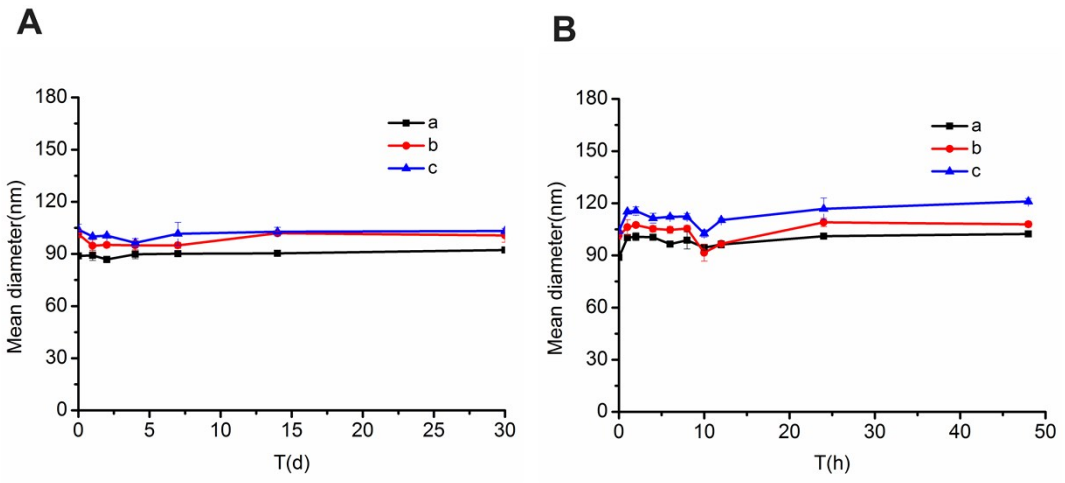
FigS2. Mass spectrum of CTX-S-OA and 1H NMR spectrum of CTX-S-OA.



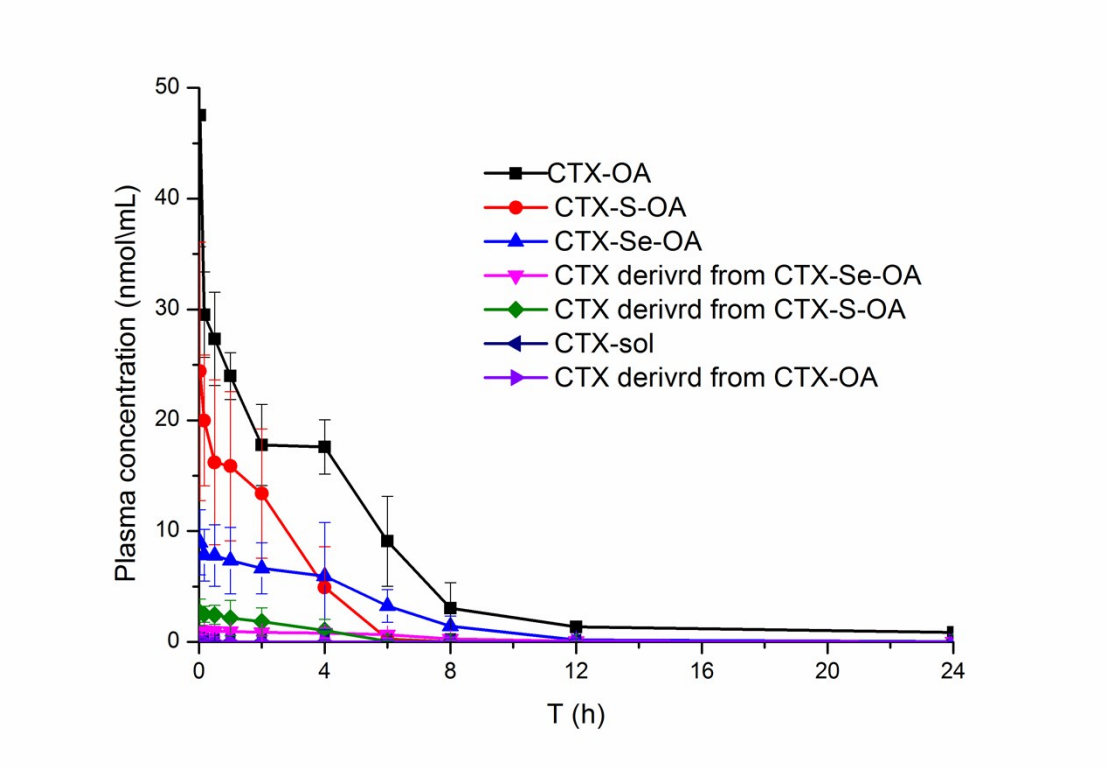
FigS3. Mass spectrum of CTX-Se-OA and 1H NMR spectrum of CTX-Se-OA.



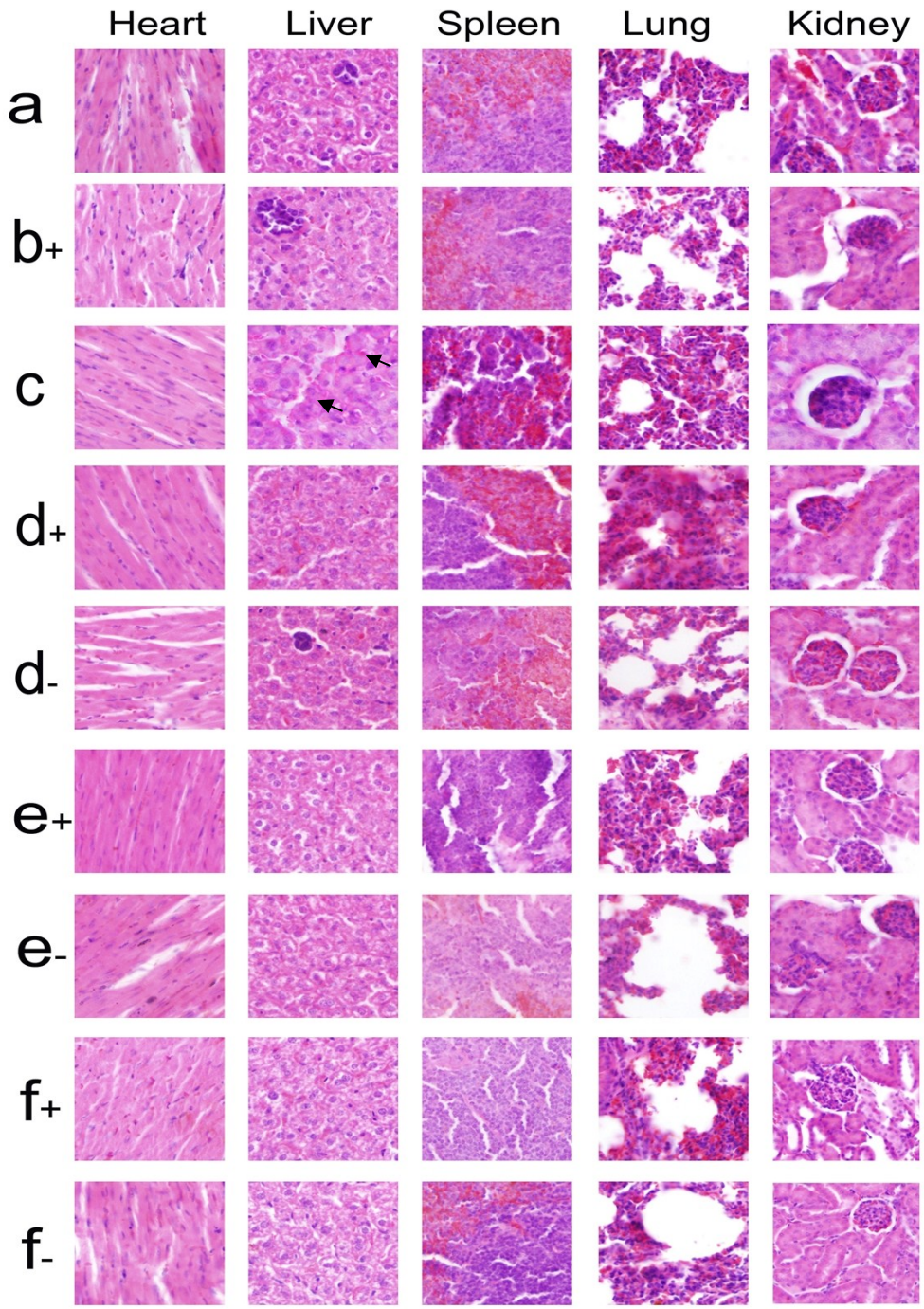
FigS4. The characterization of PPa@prodrug NPs. TEM image and DLS results of （A）PPa@CTX-OA/DSPE-PEG2k, (B) PPa@CTX-S-OA/DSPE-PEG2k, (C) PPa@CTX-Se-OA/DSPE-PEG2k. Scale bars: 100 nm.



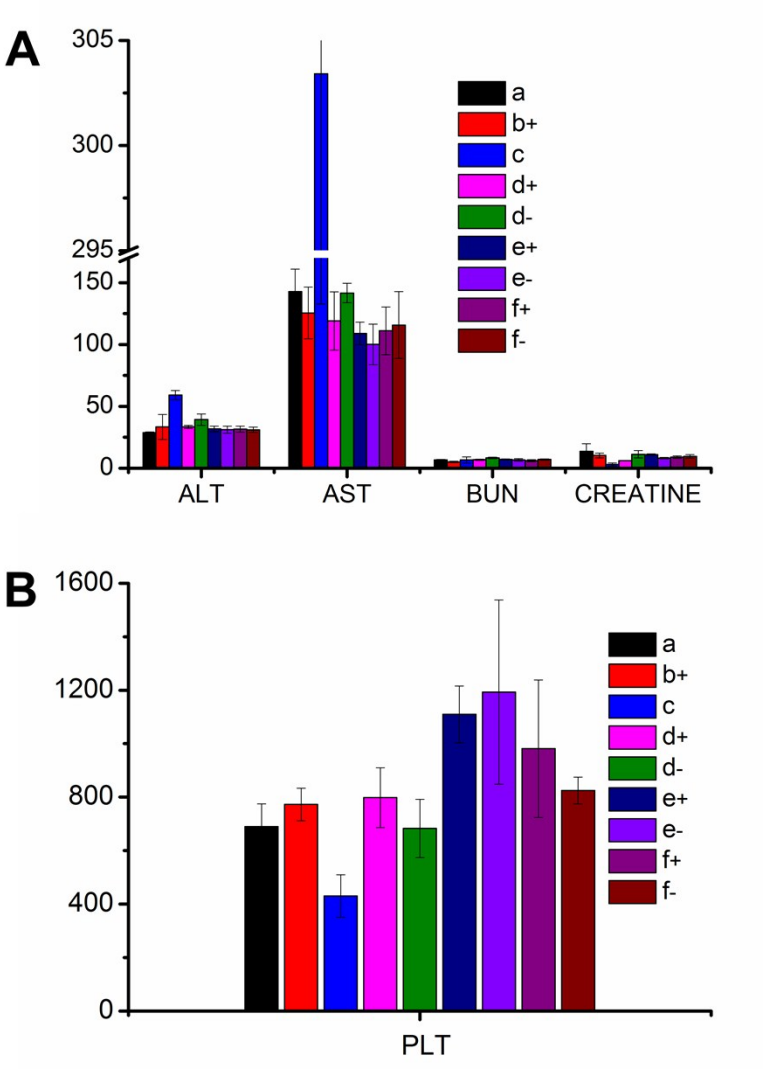
FigS5. (A) Long-term stability of a, b and c after store at 4 °C. (B) Colloidal stability of a, b and c after incubation in PBS (pH 7.4) supplemented with 10% FBS at 37 °C. (a:PPa@CTX-OA/DSPE-PEG2k), b:PPa@CTX-S-OA/DSPE-PEG2k and c:PPa@CTX-Se-OA/DSPE-PEG2k)



FigS6. *In vivo* plasma concentration-time profiles of the formulations following a single i.v. administration at a CTX equivalent dose of 2.5mg/kg. (n=5)



FigS7. H&E stained images of major organs; Heart, Liver, spleen, lung and kidney of healthy mice. The mice were sacrificed and an abnormality was observed in liver (The black arrow). (a:PBS, b:PPa-sol, c:CTX-sol, d:PPa@CTX-OA/DSPE-PEG2k, e: PPa@CTX-S-OA/DSPE-PEG2k, f:PPa@CTX-Se-OA/DSPE-PEG2k)



FigS8. Hematology and blood biochemistry result of female BALB/c mice treated with different formulations. Results illustrated mean and standard deviation of (A) blood biochemistry and (B) platelet. (a:PBS, b:PPa-sol, c:CTX-sol, d:PPa@CTX-OA/DSPE-PEG2k, e:PPa@CTX-S-OA/DSPE-PEG2k, f:PPa@CTX-Se-OA/DSPE-PEG2k)

Table S1. Characteristics of PPa/NPs

PPa/NPs	Size[a] (nm)	Zeta[b] (mv)	PDI[c]	DL[d]	EE[e]
OAC	88.85 ± 1.6	-23.8 ± 1.86	0.162 ± 0.023	60.3%	82.1%
ACS	101.4 ± 2.2.	-25.2 ± 2.97	0.173 ± 0.012	51.9%	85.6%
ACSE	104.1 ± 3.1	-28.7 ± 3.04	0.107 ± 0.063	50.1%	91.8%

a) Mean diameters and b) Zeta potential of PPa/NPs obtained by DLS. c) Polydispersity index of the PPa/NPs. d) Drug-loading (CTX wt %) = the molecular weight of CTX/ (the molecular weight of conjugates and the amount of TPGS2k). e) Encapsulation efficiency (PPa %) = amount of drug encapsulated in micelles/total amount of drug added.

Table S2.In vitro cytotoxicity ((IC_{50}) values) of CTX-sol and PPa/NPs to 4T1 cancer cells (MTT assay).

Formulations	4T1 (nM)	
	48 h	72 h
CTX-sol	1.908	1.308
H-PPa	25.48	25.28
OAC-	> 200	> 200
OAC+	> 200	> 200
ACS-	91.42	39.56
ACS+	40.06	21.28
ACSE-	19.09	7.196
ACSE+	5.678	2.493