In vivo metabolizable branched poly(ester amide) based on inositol and amino acids as drug nanocarrier for cancer therapy

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Chemical and Materials

L-Arginine, L-Phenylalanine, hexanediol, inositol, *p*-toluene sulfonic acid monohydrate and *p*-nitrophenol were all purchased from Aladdin and used without further purification. Solvent like triethylamine, acetone, methylbenzene (toluene), isopropanol, ethyl acetate, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), methyl alcohol and dichloromethane (CH_2Cl_2) of analytical grade was obtained from Guangzhou Chemical Reagent Co. Ltd (China) and used without further purification. Deionized water was prepared using the Milli-Q Plus System (Millipore Co., Billerica, MA, USA). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT), Hoechst 33324 were purchased from Sigma-Aldrich (Beijing, China). Coumarin 6 was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Deuterated dimethyl sulfoxide (DMSO-d6) was purchased from Sigma-Aldrich (Steinheim, Germany). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-poly (ethylene glycol) 2000 (DSPE-PEG2000) was purchased from Avanti Polar Lipids (New York, USA). Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, phosphate buffer saline (PBS), fetal bovine serum (FBS), 0.25% trypsin, 0.25% trypsin-EDTA and antibiotic (penicillin and streptomycin) were obtained from Gibco (Guangzhou, China). Acridine orange hemi (zinc chloride) salt (AO) and ethidium bromide solution (EB) were purchased from Sigma-Aldrich (Steinheim, Germany).

The synthesis of monomer Arg-6

Arg-6 monomer was prepared according to the previous study.[1] The procedure is recorded as follow: L-Arginine (0.04mol), 1, 6-hexanediol (0.02mol), and p-toluene sulfonic acid monohydrate (0.082mol) was dispersed in 250 mL methylbenzene with magnetic stirring. The esterification reaction system was stirred for 24 h at 130 °C with the continuously removing of generated water. After the reaction was completely finished, the solution was cooled down to room temperature and the solvent was poured out. Thereafter, the monomer product was purified through dissolving in isopropanol at 70 °C and precipitating at 4 °C three times. The purified product Arg-6 was dried in vacuum oven for 48 h. Finally, the chemical structure of the product was confirmed with ^{1}H NMR.

The synthesis of monomer Phe-ino

The procedure of the synthesis of monomer Phe-ino was shown as follow: L-Phenylalanine (0.122 mol), inositol (0.02 mol), and p-toluene sulfonic acid monohydrate (0.122 mol) was

dispersed in 300 ml methylbenzene with magnetic stirring. The esterification reaction system was stirred for 24 h at 130 °C with the continuously removing of generated water. After the reaction was completely finished, the solution was cooled down to room temperature and the solvent was poured out. Thereafter, the monomer product was purified by extracting the impurity with distilled water three times. The purified Phe-ino production was dried in vacuum oven for 48 h to remove the water. Finally, the chemical structure of the derived production was confirmed with ^{1}H NMR.

The synthesis of N4

N_4 was synthesized by the reacting of p-nitrophenol and dicarboxylic acyl chloride with four methylenes as shown in the formula.[1] The procedure was described briefly as follow: adipic acid chloride (0.20 mol) and triethylamine (0.45 mol) was dissolved in acetone in an ice/water bath to keep the temperature at 0 °C. Then, p-nitrophenol (0.42mol) which was diluted in cold acetone was added to the reaction system dropwise with magnetic stirring at 0 °C for 2 h and room temperature for another 24 h. The derived production of N4 was purified by precipitating in distilled water at least 3 times to remove the byproduct. After that, the production was dried in vacuum oven at room temperature for 24 h. Finally, it was recrystallized in ethyl acetate/ dimethylformamide (4:1, v/v) for three times before dried in in vacuum again. The chemical structure of the product was confirmed with ^{1}H NMR.

Synthesis of BPEA

The synthesis of the amino acid based BPEAs was conducted through the polycondensation of di-p-nitrophenyl ester of dicarboxylic acids monomer (N4), tetra- p-toluenesulfonic acid salts of bis(L-arginine) alkylene diester monomer (Arg-6), and tetra-p-toluenesulfonic acid salts of multi(L-phenylalanine) inositol ester monomer (Phe-ino) in the presence of triethylamine. The monomers N4, Arg-6, and Phe-ino were synthesized through esterification reaction. For the synthesis of BPEA, predetermined amount of N4 (0.338 g), Arg-6 (0.774 g) and Phe-ino (1.776 g) was dissolved in DMSO with stirring at 75 °C. Then, calculated amount of trimethylamine was added to the solution after the monomer completely dissolved. The polycondensation reaction was carried out at 75 °C for 48 hours. Subsequently, the product was purified by dissolving in methanol and then precipitating in ethyl acetate for three times. The purified product was finally dried in vacuum oven for 24 h. The chemical structures of the monomers and the BPEA were confirmed with ^{1}H NMR before further use.

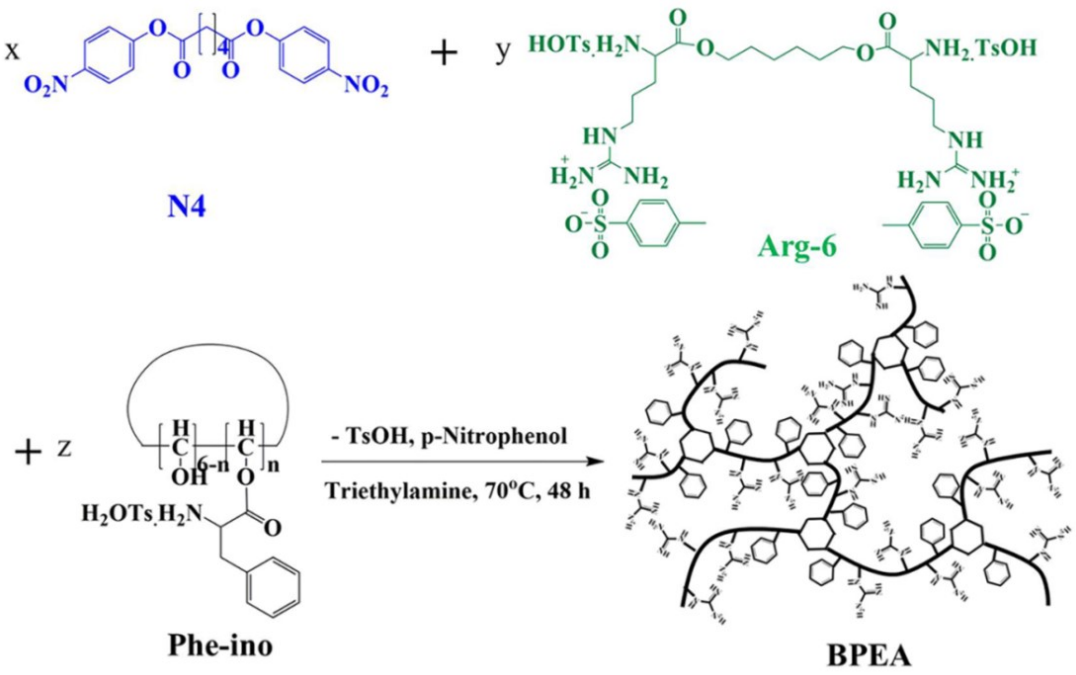

Figure S1. The synthesis scheme of the amino acid based BPEA.

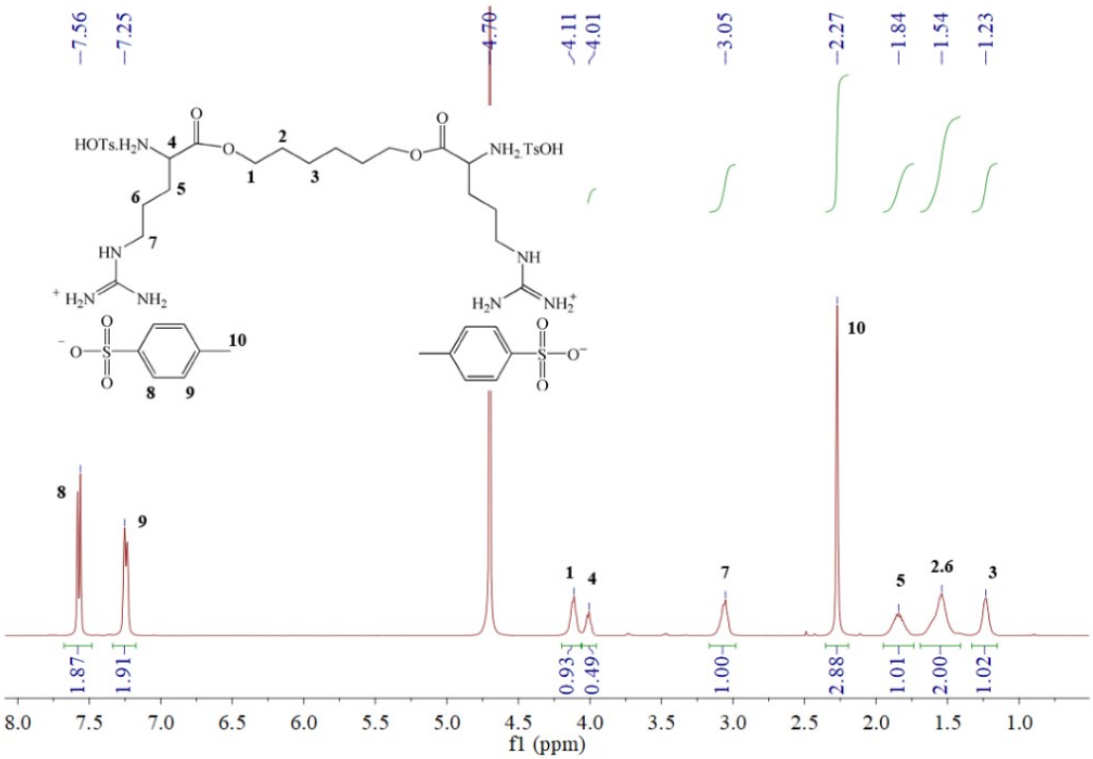

Figure S2. ^{1}H NMR spectra of Arg-6 in D_2O.

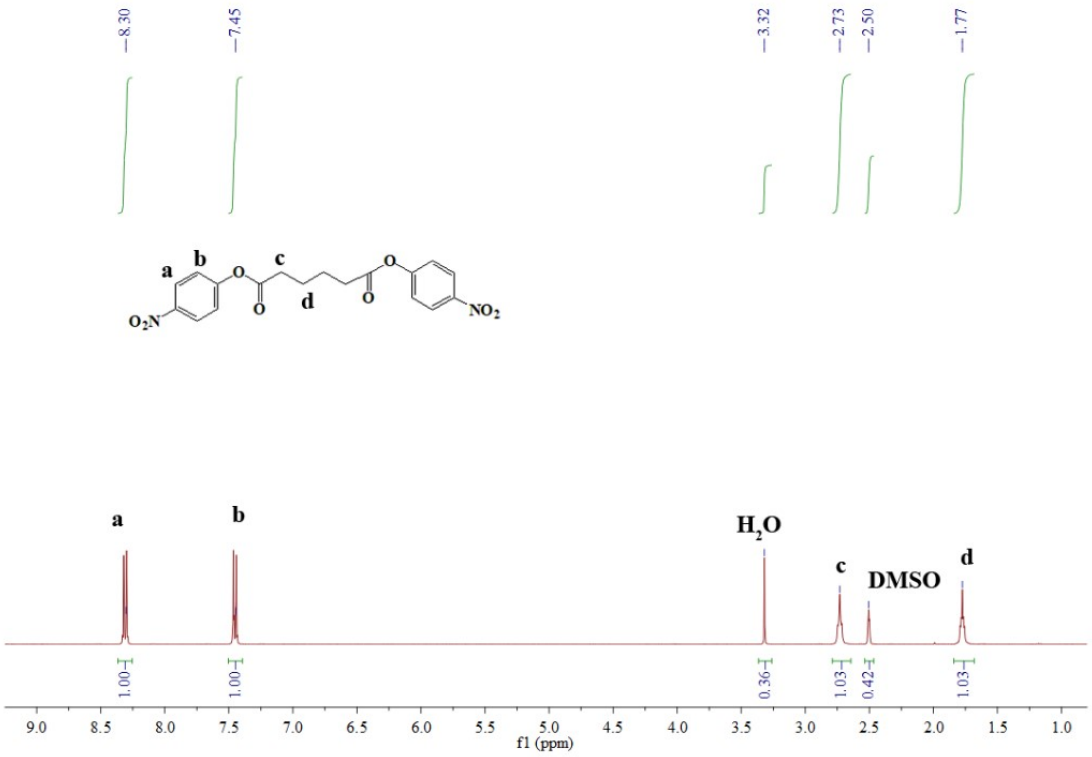

Figure S3. ^{1}H NMR spectra of N4 in DMSO-d_6.

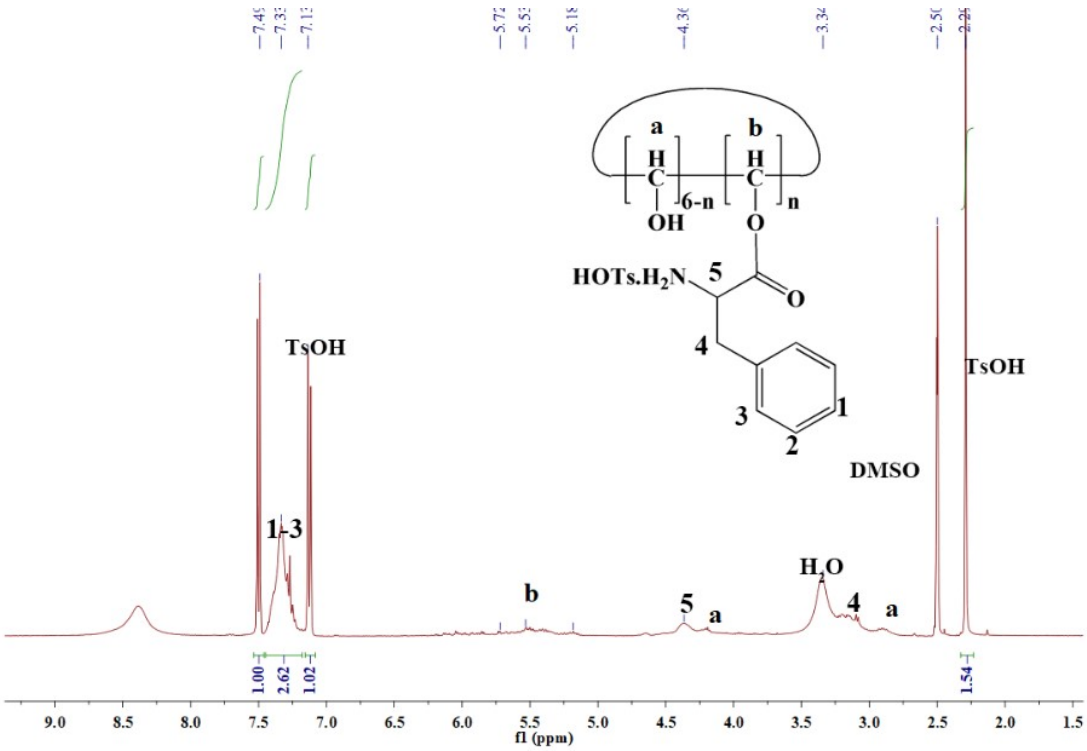

Figure S4. ^{1}H NMR spectra of Phe-ino 6.1 in DMSO-d_6.

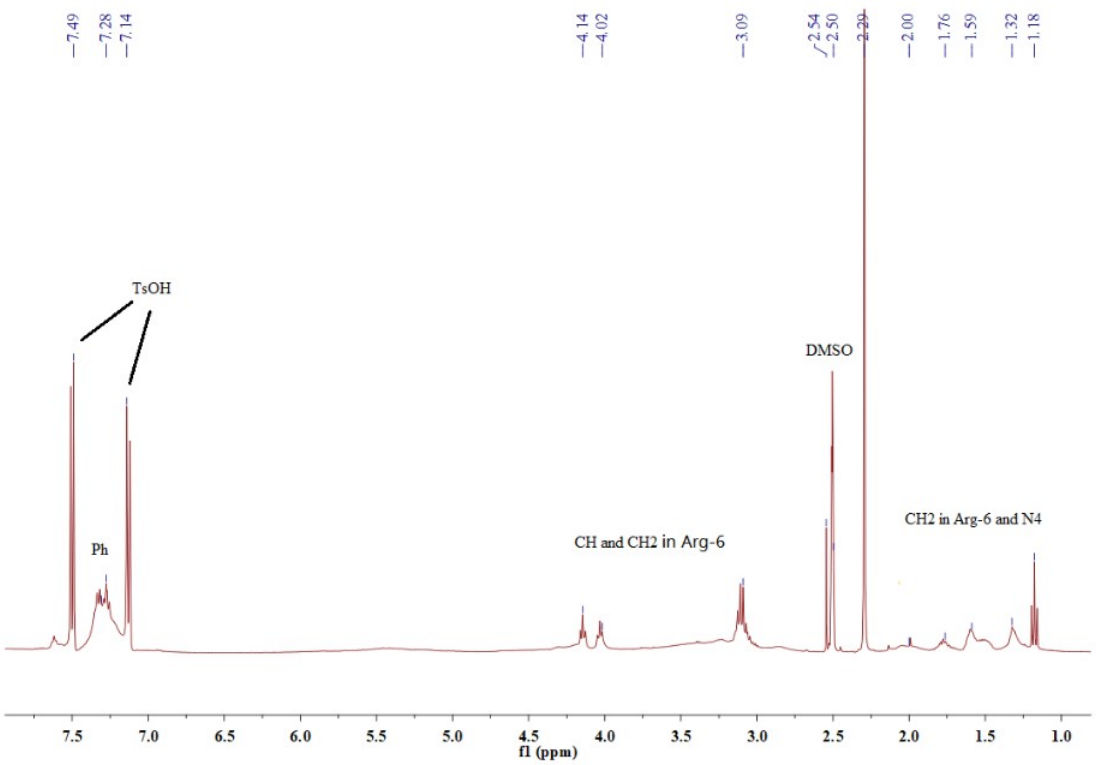

Figure S5. ^{1}H NMR spectra of BPEA in DMSO-d_6.

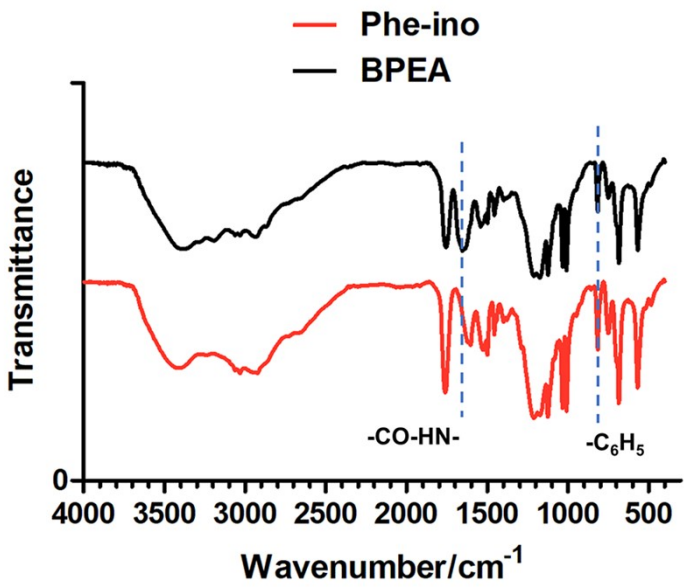

Figure S6. The FTIR spectra of Phe-ino and BPEA.

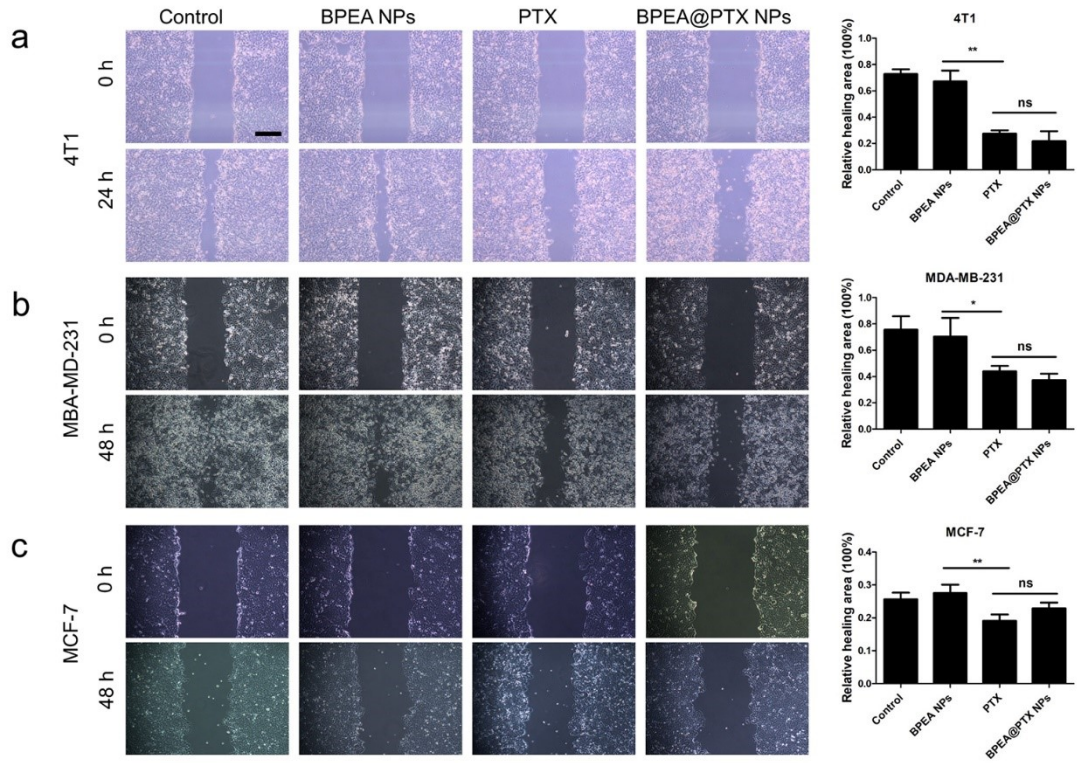

Figure S7. Migration ability of tumor cells upon treatment with BPEA NPs, PTX and BPEA@PTX NPs. (a) The migration ability of 4T1 cells upon treatment with BPEA NPs, PTX and BPEA@PTX NPs for 48 h. (b) The migration ability of MDA-MB-231 cells upon treatment with BPEA NPs, PTX and BPEA@PTX NPs for 24 h. (c) The migration ability of MCF-7 cells upon treatment with BPEA NPs, PTX and BPEA@PTX NPs for 48 h. Scale bar, 400 μm. *P<0.05, **P<0.01, ***P<0.001, comparation of the groups as marked (n=3).

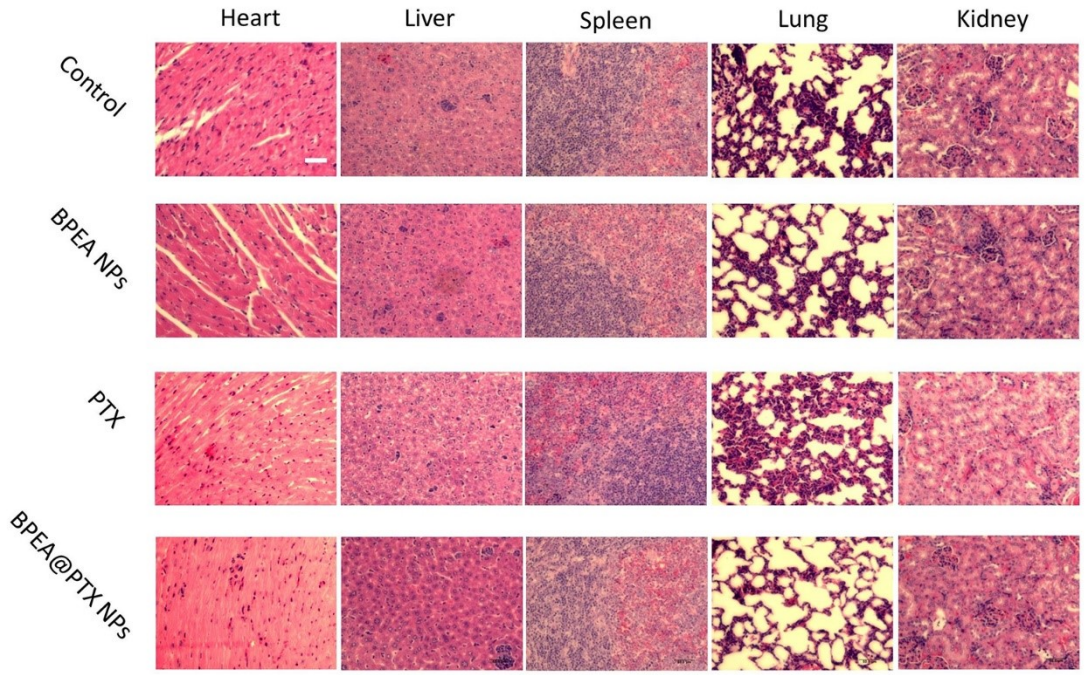

Figure S8. H&E stain of major organs after the mice administrated with BPEA NPs, PTX and BPEA@PTX NPs. Scale bar, 50 μm.

Table S1. The pharmacokinetic parameters of free PTX and BPEA@PTX NPs.

Parameters	PTX	BPEA@PTX NPs
$T_{1/2}$ (h)	1.17 ± 0.69	5.69 ± 0.81
AUC_{last} (h·ug/mL)	2.53 ± 0.89	6.99 ± 1.88
MRT_{last} (h)	0.92 ± 0.46	1.74 ±0.41
CL_obs (mL/h/Kg)	1276.40 ±500.20	435.04 ±106.13