Supporting Information

Experimental Section

Materials

Paclitaxel (PTX) was purchased from Beijing Maisuo Chemical Technology Co. Ltd. (Beijing, China). Vitamin E succinate was obtained from Chengdu Gracia Chemical Technology Co. Ltd. (Chengdu, China). 4-Dimethylaminopyridine (DMAP), p-nitrophenyl chloroformate were bought from Aladdin Industrial Corporation (Shanghai, China). 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) were purchased from Energy Chemical Co. Ltd. (Shanghai, China). 2-distearoyl-snglycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (DSPE-PEG2000) was obtained from Shanghai Advanced Vehicle. The human lung cancer cells (A549) and breast cancer cells (MCF-7) were purchased from the cell bank of Chinese Academy of Sciences (Beiijng, China). Dulbecco's Modified Eagle Medium (DMEM, high glucose), trypsin and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT) were obtained from Gibco (Beijing, China). Fetal bovine serum (FBS) and CS were purchased from Hyclone (Beijing, China) and KeyGEN (Nanjing, China), respectively. Dimethylsulfoxide (DMSO) was purchased from Kemeng (Tianjin, China). 1, 1-dioctadecyl-3, 3, 3, 3-tetramethylindotricarbocyanine iodide (DiR) was brought from AAT Bioquest (Beiijng, China). All other reagents and solvents used in this article were of analytical grade. The kunming mice were purchased from Laboratory

Animal Center of Shenyang Pharmaceutical University (Shenyang, China).

Synthesis of 6-*O*-[4-oxo-4-[dihydroxyethyl]amino]butyryl]Vitamin E (2)

Compound 2 was synthesized by reaction of Vitamin E succinate (6.23 mmol) with diethanolamine (7.48 mmol) dissolved in appropriate dry dichloromethane with HBTU (7.48 mmol), DMAP (9.35 mmol) and DIEA (9.35 mmol) at room temperature under nitrogen atmosphere for 5 h. The pH of reaction liquid was adjusted to 3-4 by hydrochloric acid, which was then washed by saturated sodium chloride (NaCl) solution for three times. The organic layer was dried by anhydrous sodium sulfate (Na_2SO_4). Next, compound 2 was obtained as transparent oily liquid with yield of 60.5% by silica gel column chromatography.

Synthesis of 6-*O*-[4-oxo-4-[bi[2-[*O*-2'-*O*-taxolformyloxy]ethyl]amino]butyryl] Vitamin E (PC)

Compound 2 (0.79 mmol) dissolved in dry dichloromethane was mixed with pyridine (3.97 mmol) at -30 ^{o}C under nitrogen atmosphere. Then *p*-Nitrophenyl chloroformate (4.72 mmol) in dry dichloromethane was added to the mixture and stirred for 5 h. The crude product was washed by saturated NH_4Cl solution and NaCl solution respectively. The organic layer was then dried by anhydrous Na_2SO_4. The crude product was purified using silica gel column chromatography to obtain compound 3 (yield 69.2%), which reacted with PTX (1.12 mmol) in the presence of DMAP (1.34 mmol) at room temperature under nitrogen atomosphere for 24 hours. The water layer was washed with saturated NH_4Cl solution to pH 5-6 and the organic layer was dried by anhydrous Na_2SO_4. After filtration and evaporation, the crude product was purified

by high preparative performance liquid chromatography (acetonitrile, 8 mL min^{-1}, and UV 226 nm) following the initial isolation of silica gel column chromatography to obtain the white solid, PC (yield 57.4%). **^{1}H-NMR (δ/ppm $CDCl_3$):** 8.14 (t, *J* = 7.4 Hz, 4H, *o*-Ph1), 7.72 (d, *J* = 7.7 Hz, 2H, *o*-Ph3), 7.67 (d, *J* = 7.7 Hz, 2H, *o*-Ph3), 7.63-7.59 (m, 2H, *p*-Ph1), 7.54-7.50 (m, 4H, *m*-Ph1), 7.46-7.41 (m, 6H, *p*-Ph3, *o*-Ph2), 7.38-7.35 (m, 8H, *m*-Ph2, *m*-Ph3), 7.32 (m, 2H, *p*-Ph2), 7.23-7.14 (m, 2H, 3'-NH), 6.29-6.25 (m, 4H, H-10, H-13), 6.03 (d, *J* = 9.5 Hz, 2H, H-3'), 5.67 (dd, *J* = 13.2, 6.8 Hz, 2H, H-2), 5.40 (d, *J* = 2.5 Hz, 2H, H-2'), 4.97 (d, *J* = 9.4 Hz, 2H, H-5), 4.46-4.39 (m, 4H, H-7, -NCH_2CH_2O-), 4.31 (t, *J* = 8.0 Hz, 2H, H□-20), 4.21-4.06 (m, 4H, H□-20, -NCH_2CH_2O-), 3.81 (d, *J* = 7.0 Hz, 2H, H-3), 3.64-3.46 (m, 4H, -NCH_2CH_2O-), 2.83 (br.s, 2H, VE-4-CH_2-), 2.78-2.69 (m, 2H, VE-6-$OCOCH_2CH_2$-), 2.52 (m, 2H, H□-6), 2.47 (s, 6H, 4-OAc), 2.41-2.35 (m, 4H, H□-14, -$NCOCH_2CH_2$-), 2.21 (s, 6H, 10-OAc), 2.17 (m, 2H, H□-14), 2.06 (s, 3H, VE-CH_3), 1.98 (m, 1H, VE-3-CH_2-), 1.92 (s, 3H, VE-CH_3), 1.89 (m, 1H, VE-3-CH_2-), 1.84(s, 3H, VE-CH_3), 1.76(m, 4H, H□-6, VE-2-CH_2-), 1.68 (d, *J* = 5.9 Hz, 6H, Me-18, Me-19), 1.55-1.49 (m, 3H, 2-VE-$CH_2CH_2CH_2CH(CH_3)CH_2$-), 1.36-1.23 (m, 16H, 2-VE-$CH_2CH_2CH_2CH(CH_3)CH_2$-), 1.19 (s, 6H, Me-17), 1.12 (d, *J* = 10.7 Hz, 9H, Me-16, VE-2-CH_3), 0.87 (s, 3H), 0.85 (s, 6H), 0.83 (d, *J* = 3.4 Hz, 3H). **IR (KBr, cm^{-1}):** υ_{CH} = 2926, 2852, $\upsilon_{C=O}$ = 1730, 1661, $\upsilon_{C=C}$ = 1603, 1485, δ^{as}_{CH} = 1452, δ^{s}_{CH} = 1373, υ_{C-O} = 1243, 1070, δ=CH = 776, 711. **HRMS (m/z):** 1211.0322 $[M + 2Na]^{2+}$.

Synthesis of 6-*O*-[4-oxo-4-[bi[2-[4-oxo-[*O*-2'-*O*-taxol]butyryloxy]ethyl]amino] butyryl]Vitamin E (PE)

Compound 2 (0.32 mmol), succinic anhydride (1.62 mmol) and DMAP (1.62 mmol) were dissolved in dry dichloromethane. The mixture was stirred for 2 h at room temperature under nitrogen atmosphere. Thereafter, the obtained crude product was subjected to the purification of silica gel column chromatography to give compound 4 as transparent oily liquid (yield 69.0%). Compound 4 (0.19 mmol), EDCI (0.46 mmol), HOBT (0.46 mmol), and DMAP (0.11 mmol) were dissolved in anhydrous dichloromethane and TEA was added to adjust the pH to 7 ~ 8. The mixture was stirred at -5 ~ -8 ^{o}C for 1 h under nitrogen atmosphere, then PTX (0.38 mmol) in anhydrous dichloromethane was added to the reaction liquid, and stirred at room temperature overnight. The obtained reaction liquid was extracted by diluted hydrochloric acid for three times and the organic layer was dried by anhydrous Na_2SO_4 to give yellow solid, which was further purified by high preparative performance liquid chromatography (acetonitrile, flow rate: 8 mL min^{-1}, and UV 226 nm) to obtain the target product PE (yield 60.9%) as white solid. **^{1}H-NMR (δ/ppm $CDCl_3$):** 8.14 (d, *J* = 8.1 Hz, 4H, *o*-Ph1), 7.77 (d, *J* = 7.5 Hz, 4H, *o*-Ph3), 7.60 (t, *J* = 7.4 Hz, 2H, *p*-Ph1), 7.51 (m, 4H, *m*-Ph1), 7.46 (m, 2H, *p*-Ph3), 7.42-7.36 (m, 12H, *o*-Ph2, *m*-Ph2, *m*-Ph3), 7.32 (m, 2H, *p*-Ph2), 7.19 (t, *J* = 8.6 Hz, 2H, 3'-NH), 6.29 (s, 2H, H-10), 6.21 (t, *J* = 8.9 Hz, 2H, H-13), 5.98 (dd, *J* = 9.1, 2.9 Hz, 2H, H-3'), 5.68 (d, *J* = 7.1 Hz, 2H, H-2), 5.47 (br.s, 2H, H-2'), 4.96 (d, *J* = 9.3 Hz, 2H, H-5), 4.43 (q, 2H, H-7), 4.30 (d, *J* = 8.5 Hz, 2H, H-20), 4.20 (d, *J* = 8.5 Hz, 2H, H-20), 4.06 (t, 4H, -NCH_2CH_2O-), 3.80 (d, J =7.0 Hz, 2H, H-3), 3.51-3.46 (dt, 4H, -NCH_2CH_2O-), 2.90 (t, 2H, VE-4-CH_2-), 2.67 (m, 6H, VE-6-$OCOCH_2$-, 2'-$OCOCH_2CH_2$-), 2.57 (m, 6H, -

$NCOCH_2CH_2$-, 2'-$OCOCH_2CH_2$-), 2.47 (m, 2H, H-6), 2.44 (s, 6H, 4-OAc), 2.35 (m, 2H, H-14), 2.20 (s, 6H, 10-OAc), 2.15 (m, 2H, H-14), 2.09 (m, 1H, VE-3-CH_2-), 2.07 (s, 3H, VE-CH_3), 1.99 (s, 3H, VE-CH_3), 1.94(s, 3H, VE-CH_3), 1.91 (s, 6H, Me-18), 1.87 (m, 2H, H-6), 1.84 (m, 1H, VE-3-CH_2-),1.76(m, 2H, VE-2-CH_2-), 1.68 (s, 6H, Me-19), 1.53 (m, 3H, 2-VE-$(CH_2)_3CH$-), 1.37-1.26 (m, 16H, 2-VE-$(CH_2)_3$-), 1.21 (s, 6H, Me-17), 1.13 (s, 9H, Me-16, VE-2-CH_3), 0.87 (s, 3H), 0.85 (d, J =2.0 Hz, 6H), 0.83 (s, 3H). **IR (KBr, cm^{-1}):** υ_{CH} = 2921, 2851, $\upsilon_{C=O}$ = 1739, 1656, $\upsilon_{C=C}$ = 1582, 1520, δ^{as}_{CH} = 1452, δ^{s}_{CH} = 1384, υ_{C-O} = 1243, 1071, $\delta_{=CH}$ = 777, 711. **HRMS (m/z):** 1267.0584 $[M+2Na]^{2+}$.

Characterization of prodrugs

^1H NMR spectra were recorded on a Bruker ARX-400 NMR spectrometer operating at 400 MHz and using deuterated chloroform ($CDCl_3$) as a solvent. The chemical shifts were corrected against residual solvent signals. The accurate mass of the samples were performed using a Bruker microTOF-Q timeof-flight mass spectrometer with ESI +, and mass range of m/z was 50−3000. Different functional groups in the prodrugs were examined to get the FTIR spectra by Bruker IR-IFS-55 infrared spectrophotometer (Bruker, Switzerland). Each sample was blended with KBr and then compressed to form a small flake. Each absorbance spectrum was obtained at the wavenumber ranging from 400 to 4000 cm^{-1}.

Preparation and characterization of nanoassemblies

Prodrug nanoparticles (NPs) were prepared according to the nanoprecipitation method. Briefly, ehanol solution of a PTX conjugate was added dropwise to deionized water

containing 10% DSPE-PEG2000 (w/w) under continuous stirring (~800 rpm) at room temperature with a final concentration of about 10% ethanol. Consquently, the PTX conjugate self-assembled into nanoparticles with light blue opalescence. Finally, it was essential to remove the remaining ethanol by evaporation under reduced pressure. PC and PE self-assembling nanoparticles were termed as PCNPs and PENPs, respectively. To prepare DiR loaded prodrug NPs, DiR was co-assembled with the prodrug by injecting the mixture of DSPE-PEG2000, DiR and prodrug in ethanol into water.

The particle size, surface charge and polydispersity index were evaluated using a ZetaSizer (Nano ZS, Malvern Co., UK). The measurements were performed in triplicate and the results were expressed by mean ± standard deviation (SD). The morphology of nanoparticles was observed via transmission electron microscopy (TEM, JEM2100, JEOL, Japan). The sample was prepared by dropping about 10 μL of suspension of nanoparticles on a carbon-coated copper grid and excess solution was tapped with filter paper. The grid was then dried at room temperature and stained with 1% uranyl acetate (5 μL) for 30 sec and air dried before TEM visualization.

Physical and chemical stability of nanoassemblies

The aqueous suspension of the self-assembly nanoparticles (equivalent to 1 mg/mL PTX) was stored at 4 °C for 3 months to investigate the long-term stability. In addition, nanoparticles were also suspended in DMEM medium (pH 7.4) containing 10% of FBS under the shaking of 100 rpm at 37 ^{o}C for 24 h. The particle size and PDI of nanoparticles were evaluated at predetermined intervals (0, 2, 4, 6, 8, 10, 12, and

24 h) using the ZetaSizer to examine the physical stability of the nanoparticles.

The chemical stabilities of PCNPs and PENPs in rat plasma and PBS (pH 7.4 and 5.0) were studied. Briefly, PCNPs and PENPs were incubated in rat plasma and PBS containing 30% ethanol at the final concentration of 10 μg PTX equiv/mL. Then the samples were placed in an air bath under the shaking of 100 rpm at 37 ^{o}C. At selected time intervals, for plasma samples, aliquots (100 μL) were removed and diluted in acetonitrile to 400 μL, which was vortex mixed and centrifuged at 13000 rpm for 10 min. On the another hand, 200 μL of sample was removed in PBS and directly centrifuged at 13000 rpm for 10 min. The supernatants were respectively collected for measurement of PTX and PTX conjugates by HPLC with Waters e2695 Separations Module and Waters 2489 UV/Visible Detector on a reverse ODS Kromasil-C8 column (150 mm×4.6 mm, 5 μm) thermostated at T = 30 ^{o}C with UV detection at 227 nm. Use mixtures of acetonitrile and water with the ratio of 55:45 and 95:5 (v/v) as the mobile phases to determine PTX and PTX conjugates, respectively, with a flow rate of 1 mL/min. All the samples were analyzed in triplicate, and the results were expressed as percentage of PTX and PTX conjugates.

Cell culture

MCF-7 (breast adenocarcinoma cells) and A549 (lung adenocarcinoma cells) were cultured in routine medium consisted of DMEM, 10% FBS, penicillin (100 units mL^{-1}) and streptomycin (100 μg mL^{-1}). All cells were maintained in a humidified atmosphere of 5% CO_2 at 37 °C.

Cell viability assay

The antitumor activity of formulations was evaluated by MTT assays. A549 and MCF-7 cells were seeded in a 96-well plate (3000 cells/100 μL/well and 2800 cells/100 μL/well, respectively) and cultured at 37 °C, 5% CO_2. Next day, the medium was removed and then replaced with fresh media containing series of concentrations of formulations. PTX and PTX conjugates were dissolved by DMSO and then diluted by fresh medium, respectively. The final concentration of DMSO was less than 0.3%. PCNPs and PENPs were directly diluted by fresh medium. When the cells were cultured for 48 h and 72 h, the medium was replaced with another 100 μL fresh medium, and then, 20 μL of MTT solution (5 mg mL^{-1}) was added to the medium and the cells were incubated for another 4 h. Then the medium was replaced with 200 μL DMSO and the 96-well plates were vibrated for 10 min. The absorbance at 570 nm or 490 nm was selected for measurement on a microplate reader. Equation 1 was employed to calculate the cell inhibition rate:

$$\text{Cell viability (\%)} = (A_s - A_{blank}) / (A_c - A_{blank}) \quad (1)$$

In Equation 1, A_s is the mean absorbance of the experimental group, and A_{blank} is the mean absorbance of the blank group. A_c represents the mean absorbance of the control group. IC50 values were calculated by SPSS software.

To quantitatively determine the free PTX released from prodrug NPs after incubating with A549 cells for 24, 48 and 72 h, the cells together with the drug-containing culture medium (the initial concentration of PTX equivalent to 10 μg mL^{-1}) were collected, and the cells were broken by ultrasonication. The subsequent steps of extraction and measurement were the same as those to treat plasma samples. The

supernatants were respectively collected for measurement of PTX by the same HPLC conditions as the measurement for PTX conjugates, except using mixtures of acetonitrile and water with the ratio of 55:45 (v/v) as the mobile phase.

In vivo fluorescence imaging

The tumor biodistribution of PCNPs was assessed in kunming mice bearing A549 tumor (Laboratory Animal Center of Shenyang Pharmaceutical University, Shenyang, Liaoning, China) using the IVIS in vivo imaging system (PerkinElmer). The near infrared fluorescence dye DiR was loaded in PCNPs for near-infrared (NIR) fluorescence imaging. Tumor-bearing mice model was established by injecting a suspension of 1×10^6 A549 cells in PBS into the right axillary flank of female kunming mice. Mice with subcutaneous tumors of approximate 100 mm^3 were subjected to treatment. DiR loaded PCNPs and DiR solution were injected intravenously via the tail vein, and then anesthetized by 2% isoflurane delivered via a nose cone system. There are two mice for each group. The tumor accumulation and biodistribution of DiR loaded PCNPs and free DiR was traced at 4, 8, 12 and 24 h. One mice in each group was sacrificed at 24 h and the major organs (heart, liver, spleen, lung and kidney) and tumor were harvested to detect the near-infrared fluorescence.

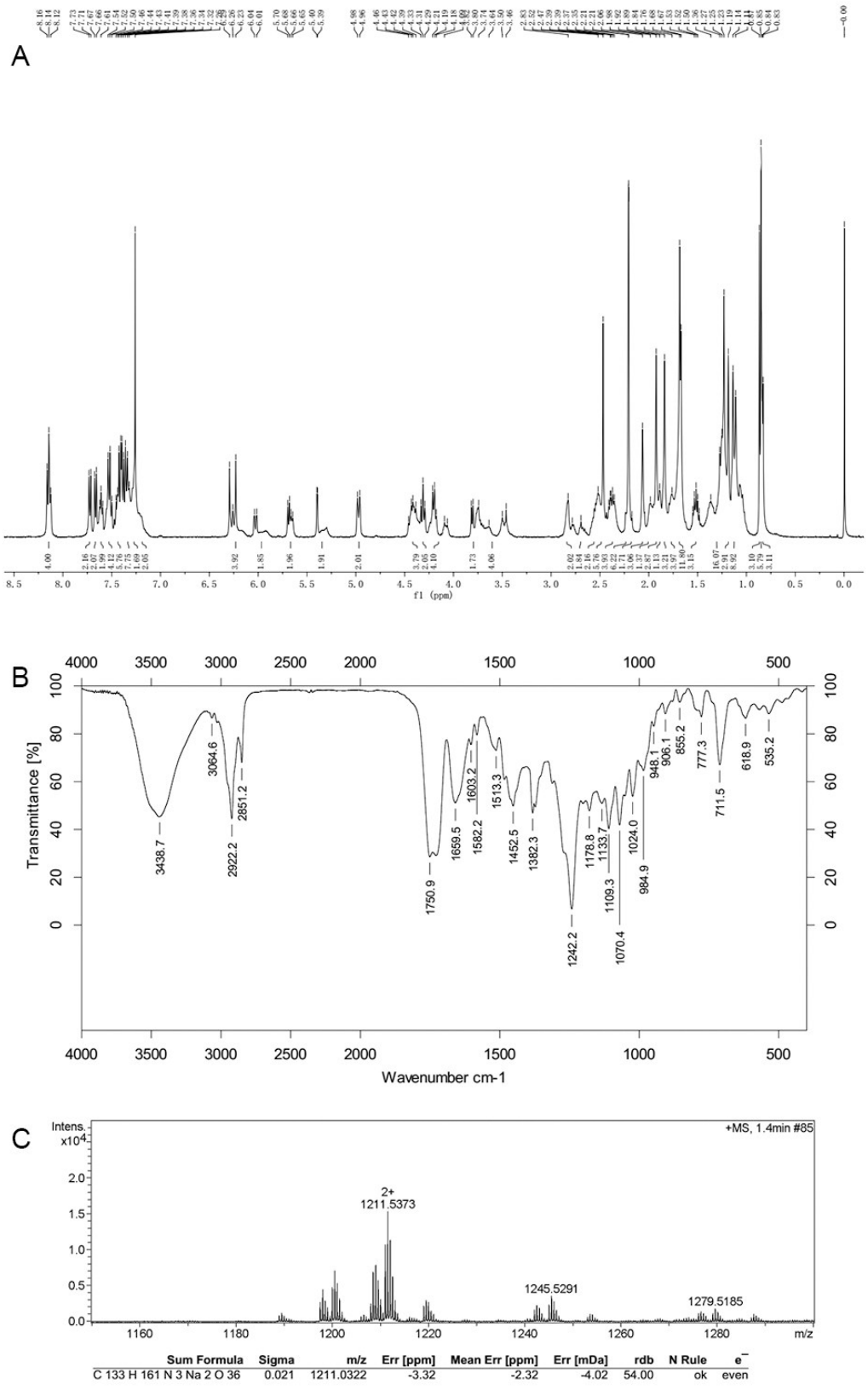

Figure S1. Spectra of PC. (A) ^{1}H-NMR spectrum. (B) FTIR spectrum. (C) Mass spectrum.

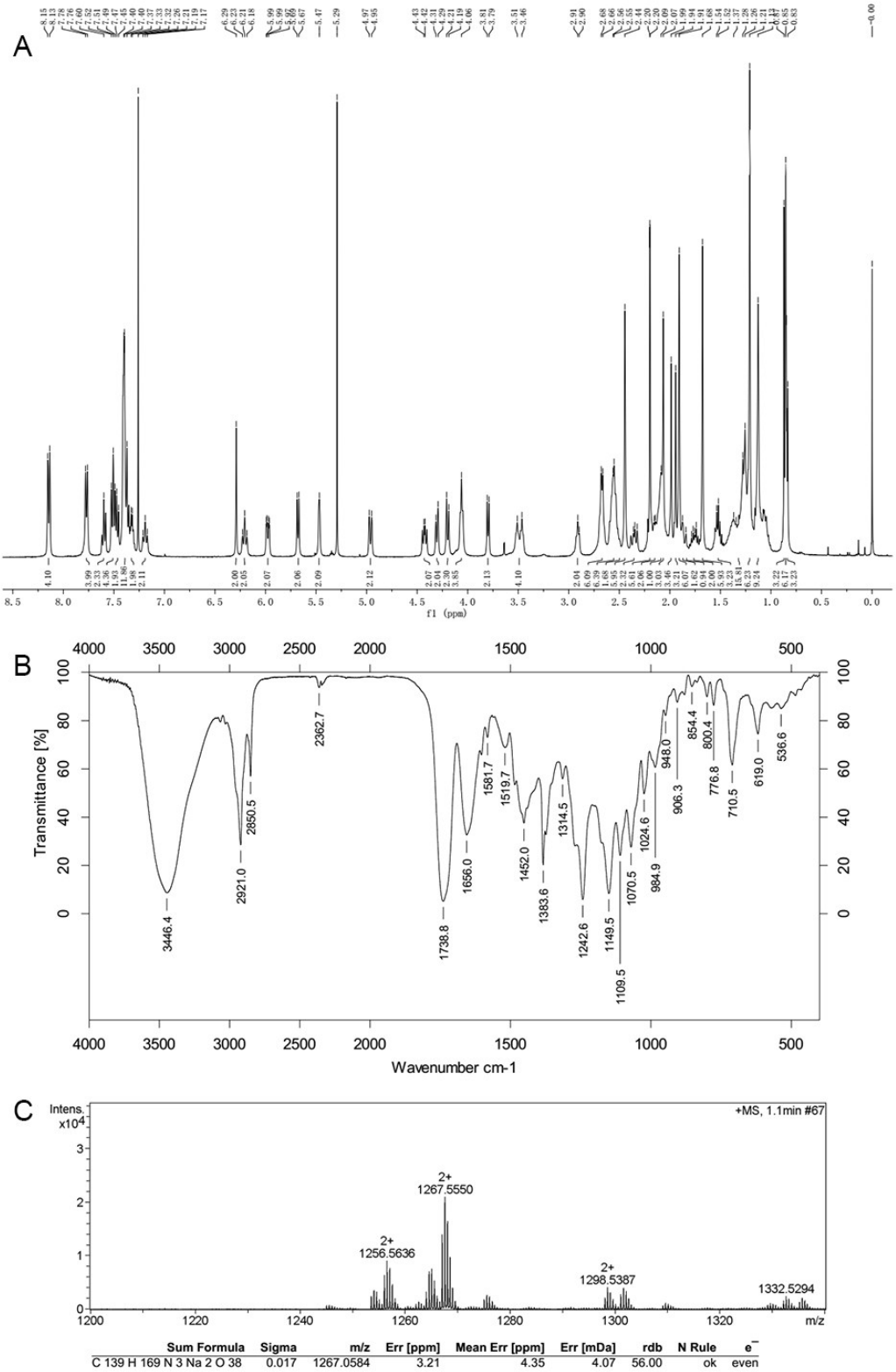

Figure S2. Spectra of PE. (A) ^{1}H-NMR spectrum. (B) FTIR spectrum. (C) Mass spectrum.

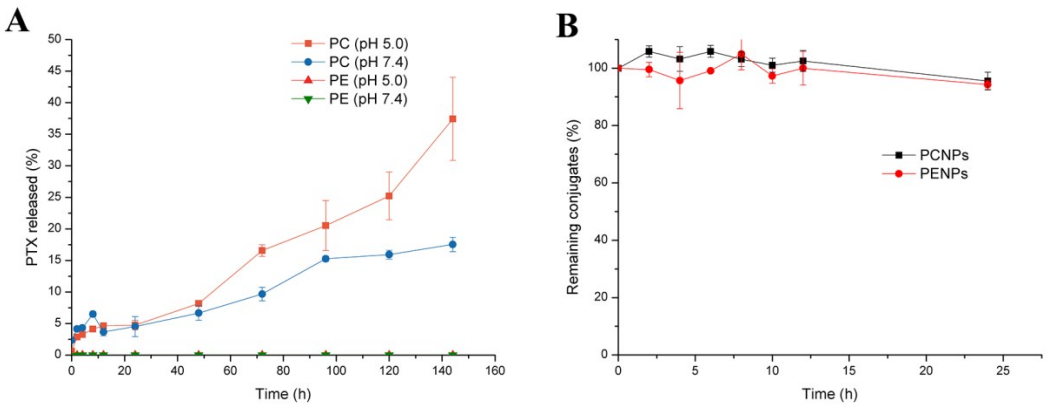

Figure S3. Chemical stability profiles of PCNPs and PENPs in PBS (pH 7.4 and pH 5.0) and rat plasma at 37 ^{o}C (n = 3).

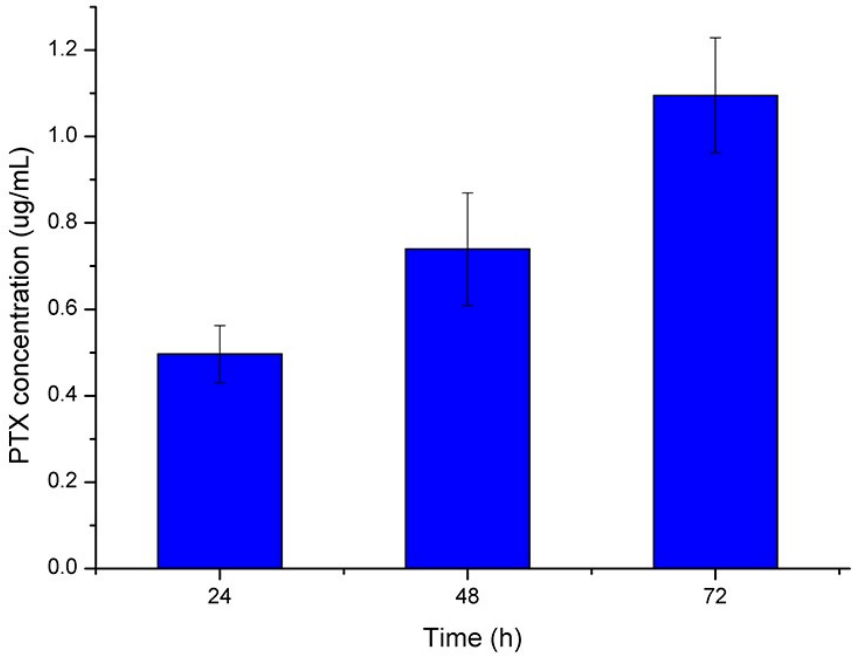

Figure S4. Free PTX released from PCNPs at 24, 48 and 72 h in the *in vitro* cytotoxicity assays (n=3).

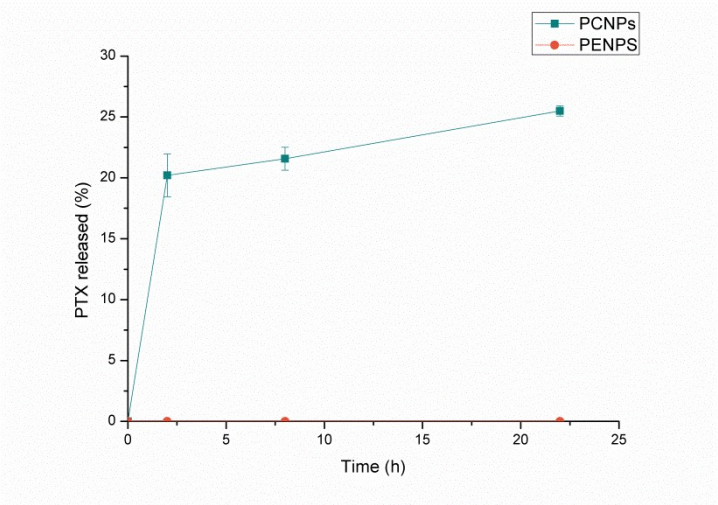

Figure S5. The released PTX from PC and PE in homogenate of A549 cells at 37 ^{o}C incubated with PCNPs and PENPs during 24 h

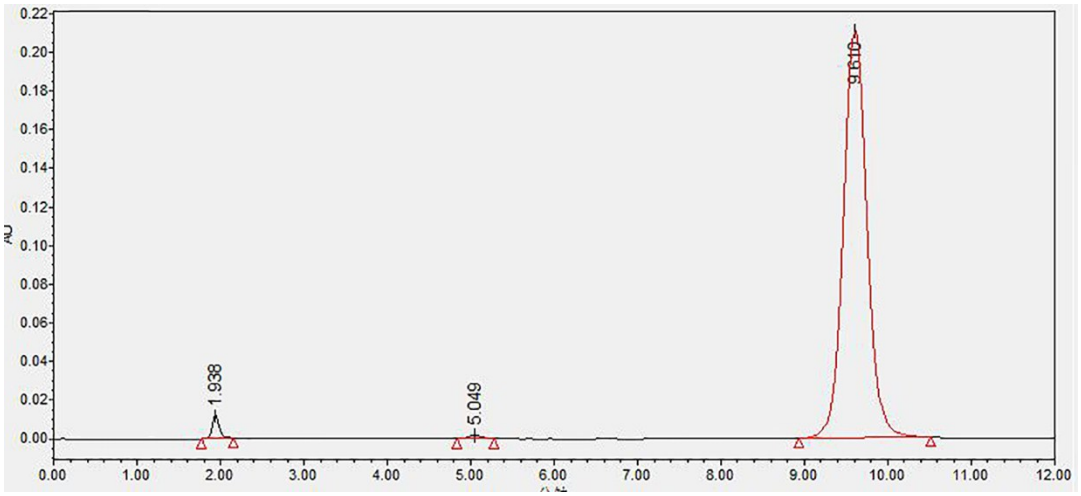

Figure S6. High performance liquid chromatogram of the final product of PC

Table S1. Characterization of prodrugs NPs (n = 3, mean ± SD).

NPs	Size[a)] (nm)	Zeta potential [a)] (mV)	PDI [a)]	DL[b)] (%)
PCNPs	113.1 ± 2.4	-30.7 ± 1.1	0.19 ± 0.01	65.3
PENPs	124.5 ± 1.7	-18.7 ± 1.8	0.13 ± 0.04	62.2

[a)] Particle size, zeta potential and polydispersity index of prodrug NPs were measured by ZetaSizer.

[b)] Drug loading was calculated by the weight percentage of PTX in these conjugates, the amount of conjugates, and the amount of DSPE-PEG2000.