Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

An activatable fluorescent prodrug of Paclitaxel and BODIPY

Jinxiu Xia [a], Qing Pei [b], Min Zheng [a,*] and Zhigang Xie [b,*]

[a] School of Chemistry and Life Science, Advanced Institute of Materials Science, Changchun University of Technology, 2055 Yanan Street, Changchun, Jilin 130022, P. R. China

[b] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun, Jilin 130022, P. R. China

*Corresponding author. E-mail: zhengm@ciac.ac.cn; E-mail: xiez@ciac.ac.cn

Experimental Section

Fig. S1 ^{1}H NMR spectrum of PTX (a) and PTX-S-BDP (b) in $CDCl_3$.

Fig. S2 ^{1}H NMR spectrum of BDP-OH in $CDCl_3$.

Fig. S3 ^{1}H NMR spectrum of compound 1 in $CDCl_3$.

Fig. S4 ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

Fig. S5 ^{1}H NMR spectrum of compound 3 in $CDCl_3$.

Fig. S6 Mass spectrum of PTX-S-BDP.

Fig. S7 (a) Particle size change of PTX-S-BDP NPs in PBS containing 10% FBS. (b) The photograph of PTX-S-BDP NPs stored at 4 ^{o}C for two months.

Fig. S8 HPLC analysis of PTX-S-BDP. The elution time of PTX-S-BDP is 7.9 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

Fig. S9 HPLC analysis of 10 mM H_2O_2. The elution time of H_2O_2 is 3.01 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

Fig. S10 HPLC analysis of 10 mM DTT. The elution time of DTT is 3.3 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

Fig. S11 HPLC analysis of 10 mM GSH. The elution time of GSH is 2.9 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

Fig. S12 The dynamic degradation process of PTX-S-BDP monitored by HPLC, treated with 10 mM of GSH.

Fig. S13 The mass spectra of PTX-S-BDP after treated with 10 mM H_2O_2 for 120 h.

Fig. S14 Degradation mechanism of PTX-S-BDP triggered by H_2O_2.

Fig. S15 The mass spectra of PTX-S-BDP after treated with 10 mM DTT for 120 h.

Fig. S16 Degradation mechanism of PTX-S-BDP triggered by DTT.

Fig. S17 CLSM images of Hela cells cultured with PTX-S-BDP NPs (concentration: 1 μM) at 4 and 37 ^{o}C for 2, 4, 6 h. (scale bar: 20 μm).

Table S1. DLC, DLE and Zeta potential of PTX-S-BDP NPs with different feed ratios.

Materials. Paclitaxel (PTX) was acquired from Dalian Meilun Biotechnology Co., Ltd. Tert butyl acrylate and mercaptopropionic acid were purchased from Heowns Technology Co., Ltd. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl, GL Biochem) and 4-dimethylaminopyridine (DMAP, Aladdin) were used as received. Cell viability (live dead cell staining) assay kit was purchased from Shanghai Beyotime Biotechnology Co., Ltd. Tubulin-Tracker Red was purchased from Jiangsu KeyGEN Biotechnology Co., Ltd. Chloroform-d ($CDCl_3$) was purchased from Qingdao Tenglong Weibo Technology Co., Ltd. Ultrapure water was prepared from a Milli-Q system (Millipore, USA). Solvents for chemical synthesis were purified by distillation.

Characterizations. Ultraviolet-visible (UV-vis) absorption spectra were recorded on Shimadzu UV-2450 PC UV-vis spectrophotometer. Fluorescence spectra were obtained by using a PerkinElmer LS-55 spectro fluorophotometer. The size and size distribution of nanoparticles were determined by dynamic light scattering (DLS) using a Malvern Zeta-sizer Nano. High performance liquid chromatography (HPLC) were recorded on Shimadzu CBM-20A chromatography to determine the concentration of PTX-related preparations. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AV400 M in $CDCl_3$. The mass spectrum (MS) analyses were performed on a LTQ ion trap mass spectrometer (Finnigan, USA) equipped with an electrospray source. The morphology of nanoparticles was measured on a JEOL JEM-1011 electron microscope, at an acceleration voltage of 100 kV. MTT assays were measured at 490 nm by a microplate reader (BioTek, EXL808). Intracellular endocytosis was observed under confocal laser scanning microscope (CLSM) (Zeiss LSM 700, Zurich, Switzerland).

Synthesis of BDP-OH. 4-Hydroxymethylbenzylaldehyde (10 mmol) and dichloromethane (400 mL) were added to a 500 mL of three neck flask. After degassed with nitrogen for 30 min, 2,4-dimethylpyrrole (20 mmol) and five drops of TFA were added in sequence. The reaction mixture was stirred for overnight under N_2, at room temperature. Then DDQ (10 mmol) was added to the reaction mixtureand stirred for 4 h. After that, 10 mL of Et_3N and 12 mL of $BF_3{\cdot}OEt_2$ were add in turn, and stirred for another 2 h. Finally, the reaction mixture was rinsed with water (300 mL) and then extracted into dichloromethane. The organic layer is dried on anhydrous $MgSO_4$. The solvent was evaporated and the residue was purified by silica gel column chromatography to get a orange solid with the yield of 20%. δ_H (500 MHz, $CDCl_3$) 7.49

(d, *J* = 8.2 Hz, 2 H), 7.30-7.27 (m, 2 H), 5.98 (s, 2 H), 4.81 (s, 2 H), 2.56 (s, 6 H), 1.38 (s, 6 H).

Synthesis of the linker (compound 1). Mercaptopropionic acid (0.5 g, 4.71 mmol), tert butyl acrylate (0.602 g, 4.71 mmol) and triethylamine (TEA, 0.5 mL) were added to a round bottom flask. The mixture was stirred at 60 ^{o}C for 12 h. After cooled to room temperature, the crude product was diluted with dichloromethane (100 mL) and washed with water (100 mL). The organic layer was dried on anhydrous sodium sulfate, and evaporated under reduced pressure to obtain colorless oil-like compounds with the yield of 70%. δ_H (500 MHz, $CDCl_3$) 10.63 (s, 1 H), 3.45 (q, *J* = 7.1 Hz, 1 H), 2.91 (ddt, *J* = 14.5, 13.2, 6.6 Hz, 2 H), 2.55 (ddd, *J* = 11.8, 5.7, 1.6 Hz, 2 H), 1.46 (t, *J* = 3.7 Hz, 12 H).

Synthesis of compound 2. EDC·HCl (0.098 g, 0.51 mmol) was added to the anhydrous dichloromethane (5 mL) solution of compound 1 (0.10 g, 0.426 mmol), The mixture was stirred at room temperature for 30 min, then, BDP-OH (0.166 g, 0.426 mmol) and DMAP (Catalysis) were added. After stirred at room temperature for 24 h, the mixture was diluted with dichloromethane, and washed with sodium bicarbonate aqueous solution (100 mL×3). The combined organic extracts were collected, dried on anhydrous sodium sulfate, concentrated and decompressed. The crude product was purified on silica gel column to obtain compound 2. Yield 50%. δH (500 MHz, $CDCl_3$) 7.50 (d, *J* = 8.1 Hz, 2 H), 7.29 (d, *J* = 8.1 Hz, 2 H,), 5.98 (s, 2 H), 5.25 (dd, *J* = 18.4, 12.7 Hz, 2 H), 3.51 (q, *J* = 6.8 Hz, 1 H,), 2.87-2.79 (m, 2 H), 2.55 (d, *J* = 6.5 Hz, 6 H), 2.51 (td, 2 H, *J* = 7.4, 2.2 Hz), 1.48 (d, *J* = 7.1 Hz, 3 H), 1.45-1.43 (m, 9 H), 1.36 (s, 6 H).

Synthesis of compound 3. Trifluoroacetic acid was added dropwise to the dichloromethane (50 mL) solution of compound 2 (0.150 g, 0.246 mmol). The resulting mixture was stirred at room temperature for overnight. Finally, the solvent was evaporated under reduced pressure to obtain a colorless solid with the yield of 60%. δ_H (500 MHz, $CDCl_3$) 7.53-7.46 (m, 2 H), 7.34-7.26 (m, 2 H), 5.98 (s, 2 H), 5.31-5.22 (m, 2 H), 3.53-3.49 (m, 1 H), 2.93-2.84 (m, 2 H), 2.69-2.61 (m, 2 H), 2.63-2.41 (m, 6 H), 1.48 (d, *J* = 7.1 Hz, 3 H), 1.39-1.31 (m, 6 H).

Synthesis of the PTX-S-BDP. Compound 3 (0.12 g, 0.217 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL), added EDC·HCl, then stirred the mixture for 30 min. Paclitaxel (0.093 g, 0.217 mmol) and DMAP (catalytic capacity) were added to the mixture and stirred overnight. Finally, CH_2Cl_2 (100 mL) was added to dilute the

reaction, and the reaction mixture was washed with $NaHCO_3$ aqueous solution (50 mL x 3), then extracted into dichloromethane. The organic layer is dried on anhydrous magnesium sulfate. The solvent was evaporated and the residue was purified by silica gel column chromatography to obtain orange solid with a yield of 25%. δ_H (400 MHz, $CDCl_3$) 8.17-8.12 (m, 2H), 7.78-7.73 (m, 2H), 7.60 (d, *J* = 7.4 Hz, 1H), 7.57-7.27 (m, 14H), 7.04 (d, *J* = 9.2 Hz, 1H), 6.30-6.23 (m, 2H), 6.02-6.93 (m, 3H), 5.69 (d, *J* = 7.1 Hz, 1H), 5.51 (dd, *J* = 3.0, 2.0 Hz, 1H), 5.25-5.16 (m, 2H), 4.97 (d, *J* = 8.2 Hz, 1H), 4.47-4.41 (m, 1H), 4.32 (d, *J* = 8.5 Hz, 1H), 4.23-4.19 (m, 1H), 3.81 (d, *J* = 7.0 Hz, 1H), 3.51-3.43 (m, 1H), 2.89-2.78 (m, 2H), 2.76-2.65 (m, 2H), 2.55 (s, 6H), 2.50 (d, *J* = 3.9 Hz, 1H), 2.45 (d, *J* = 0.9 Hz, 3H), 2.40-2.34 (m, 1H), 2.23 (s, 3H), 2.19-2.12 (m, 1H), 2.03 (d, *J* = 11.6 Hz, 1H), 1.93 (d, *J* = 8.2 Hz, 3H), 1.86 (d, *J* = 19.3 Hz, 2H), 1.68 (s, 3H), 1.42 (dd, *J* = 7.2, 3.4 Hz, 3H), 1.33 (s, 6H), 1.22 (s, 3H), 1.14 (s, 3H). Electrospray ionization (ESI)/MS for PTX-S-BDP is 1350.3.

Preparation of PTX-S-BDP NPs. A mixture of PTX-S-BDP and F-127 (1:1, 1:2, 1:5, 1:8, 1:10, mass ratio) in THF was slowly dropped to deionized water and stirred for 12 h. Unassembled PTX-S-BDP was removed by centrifugation at 10000 rpm for 10 min. The centrifuged supernatant was dialyzed against water for 24 h. Then yellow liquid was placed in a dialysis bag (cutoff Mn: 3.5 kDa) and dialyzed against water for 24 h to remove small molecules and THF. The water was replaced every 6 h, and finally, NPs in the dialysis bag were collected.

The drug loading content (DLC) and drug loading efficiency (DLE) were calculated by the following equations, respectively:

$$\text{DLC (wt\%)} = \frac{\text{the free drug weight in the nanoparticles}}{\text{the weight of nanoparticles}} * 100\%$$

$$\text{DLE (\%)} = \frac{\text{the free drug weight in the nanoparticles}}{\text{the weight of feeding drug}} * 100\%$$

H_2O_2 and DTT initiated hydrolysis of PTX-S-BDP. PTX-S-BDP (50 μg) was cultured in 0.5 mL of release medium (methanol/acetonitrile, v/v = 1/1,) with or without 10 mM of H_2O_2/DTT at 37 ^oC. The in vitro cleavage of monosulfide bond in PTX-S-BDP and the hydrolysis of PTX precursor were studied. After incubation at different

time intervals, centrifuged at 14000 rpm for 10 min. HPLC and LTQ-MS were used to detect the cleavage and hydrolysis of PTX-S-BDP in the supernatant.

Cell lines and cell culture. Human cervical carcinoma (HeLa) and Mouse fibroblasts (L929) cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) with 10% (v:v) heat-inactivated fetal bovine serum (FBS, GIBCO). Cells were cultured in a humidified incubator at 37 ^{o}C with 5% CO_2, and the culture medium was replaced once every day.

Cell viability assays. MTT assay was used to detect the cytotoxicity of PTX-S-BDP NPs to cancer cells and normal cells at different incubation times. In brief, HeLa and L929 cells harvested in logarithmic growth phase were inoculated in 96-well plates at an initial density of 2×10^3 cells per well and incubated in DMEM for 24 h. Then PTX-S-BDP NPs with different concentrations (0, 0.0001, 0.001, 0.01, 0.1, 0.5, 1.0 μM) were used to replace the culture medium. After incubation for 48 h, 20 μL of MTT solution was added, and the plate was incubated at 37 ^{o}C for another 4 h, then the culture medium containing methotrexate was removed, and 150 mL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formed methyl crystal. Finally, the plate was shaken for 5 min, and the absorbance of PTX-S-BDP NPs was measured at 490 nm with microplate reader. Taxol was used as the control group, and all of the operations were same as the experimental group.

Cellular uptake. The cellular uptake of PTX-S-BDP NPs was examined by a confocal laser scanning microscope (CLSM). HeLa cells were seeded in 6-well plates (a clean cover slip was put in each well) at the density of 2×10^5 cells per well and allowed to adhere for 24 h. And then the medium was replaced with PTX-S-BDP NPs and diluted with fresh culture medium to a final concentration of 1.0 μM. Thereafter, cells were incubated for additional 2, 4, and 6 h, at 4 and 37 ^{o}C, respectively. Subsequently, the supernatant was removed and the cells were washed gently three times with PBS (pH=7.4), fixed with 4% paraformaldehyde (1 mL/well) for 15 min and washed thrice with cold PBS. Hoechst was employed to stain the cell nuclei. In the blocking assay, HeLa cells were incubated with EIPA (20 μg/mL) for 1 h, with M-β-CD (10 μg/mL) for 30 min, with CPZ (10 μg/mL) for 30 min.

Calcein-AM/PI staining tests. To further verify the in vitro anticancer efficacy of NPs, HeLa cells were stained with the calcein-AM/propidium iodide (PI) to identify dead (red) and live (green) cells, respectively. In brief, HeLa cells harvested in

logarithmic growth phase were inoculated in 96-well plates at an initial density of 2×10^3 cells per well and incubated in DMEM for 24 h. Then, Hela cells were incubated with different concentrations of PTX-S-BDP NPs and Taxol (0, 0.5, 1.0 μM) for another 48 h. After incubation for 48 h, the medium was removed and cells were washed gently. Then cells were incubated with Calcein-AM/PI for 30 min at room temperature, subsequently imaged by a NikonC1si laser scanning confocal microscopy.

Microtubule staining. To explore the anticancer mechanism of PTX, microtubular disorder was stained and imaged by CLSM. The planting density of HeLa cells was the same as that in the cellular uptake experiment. HeLa cells were incubated with 0.5, 1.0 μM of Taxol and PTX-S-BDP NPs for 12 h. The PBS group was set as the no-treatment control. At the end of the incubation, the medium supernatant was discarded, the cells were washed gently, fixed with 4% paraformaldehyde (1 mL/well) for approximately 10 min, and washed 5 times with PBS (pH 7.4) containing 0.1% Triton X-100. 0.1% Triton X-100 was then employed to wash cells to increase the cell membrane permeability. The cells were then incubated with immunostained tubulin second antibody (diluted with PBS (pH 7.4) solution containing 0.1% Triton X-100 and 3% BSA) at room temperature for 45 min in the dark. The cells were washed three times with 0.1% Triton X-100. Finally, the red fluorescence signal was measured using Alexa Fluor 555 channel.

Figures

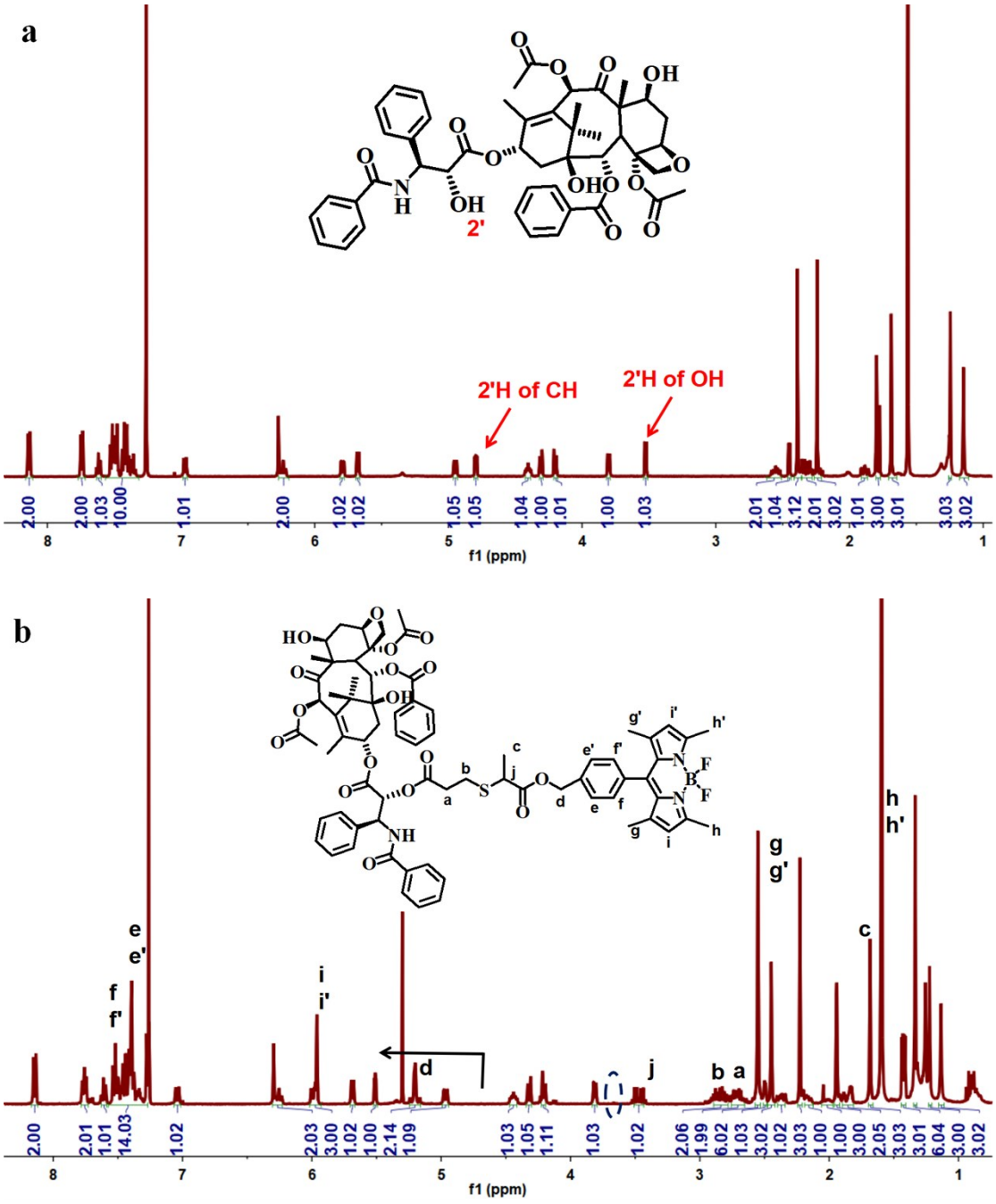

Fig. S1 ^{1}H NMR spectrum of PTX (a) and PTX-S-BDP (b) in $CDCl_3$.

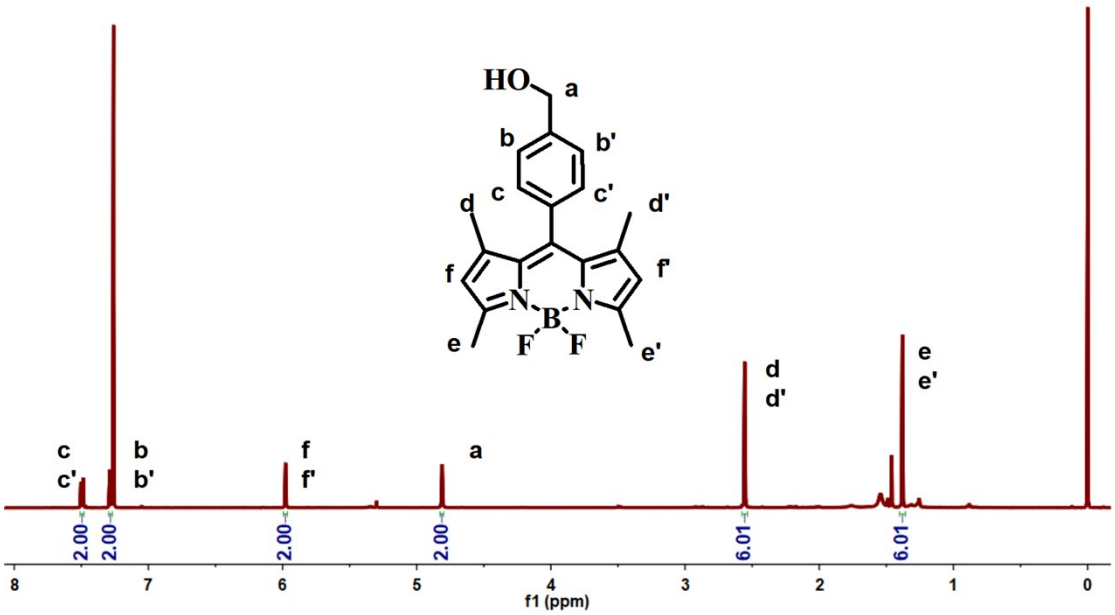

Fig. S2 ^{1}H NMR spectrum of BDP-OH in $CDCl_3$.

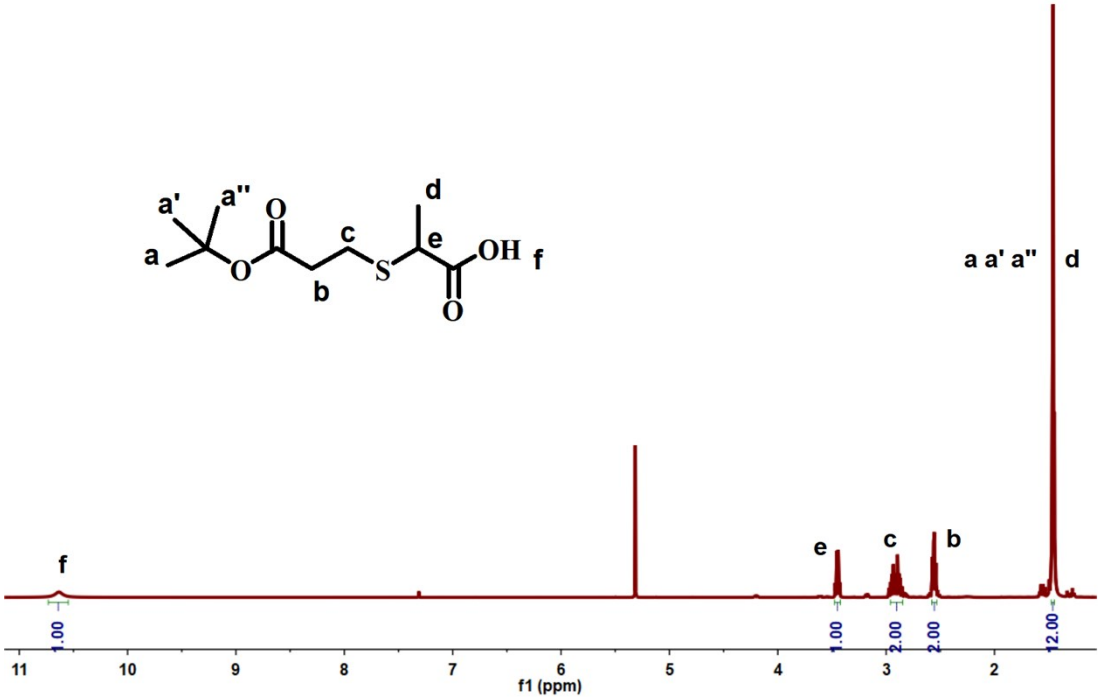

Fig. S3 ^{1}H NMR spectrum of compound 1 in $CDCl_3$.

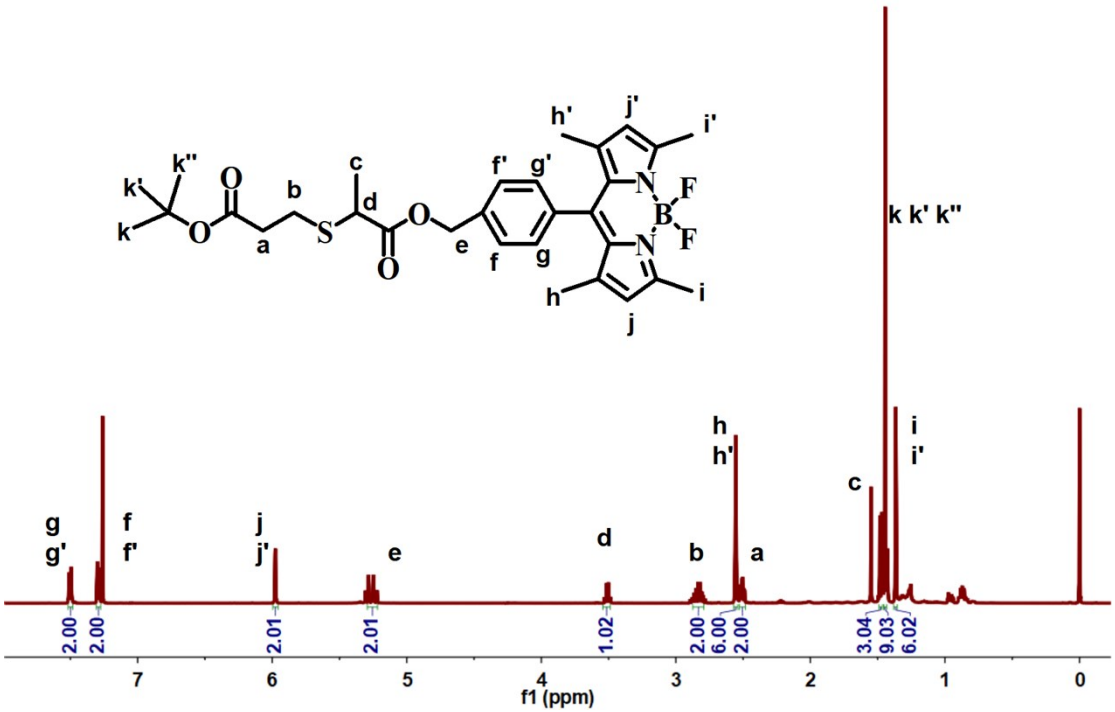

Fig. S4 ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

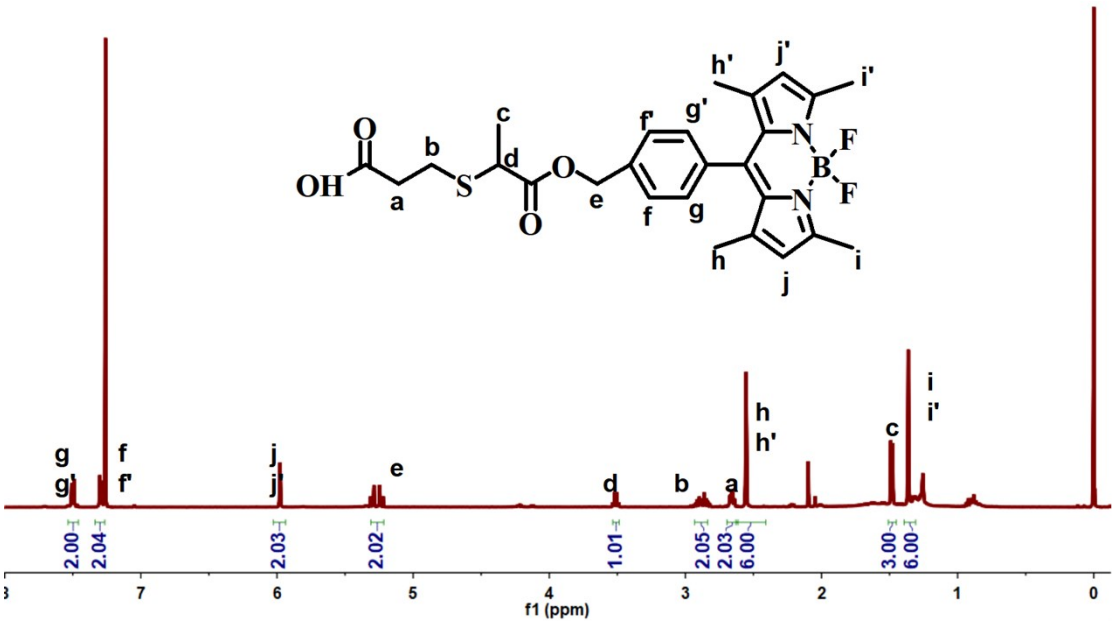

Fig. S5 ^{1}H NMR spectrum of compound 3 in $CDCl_3$.

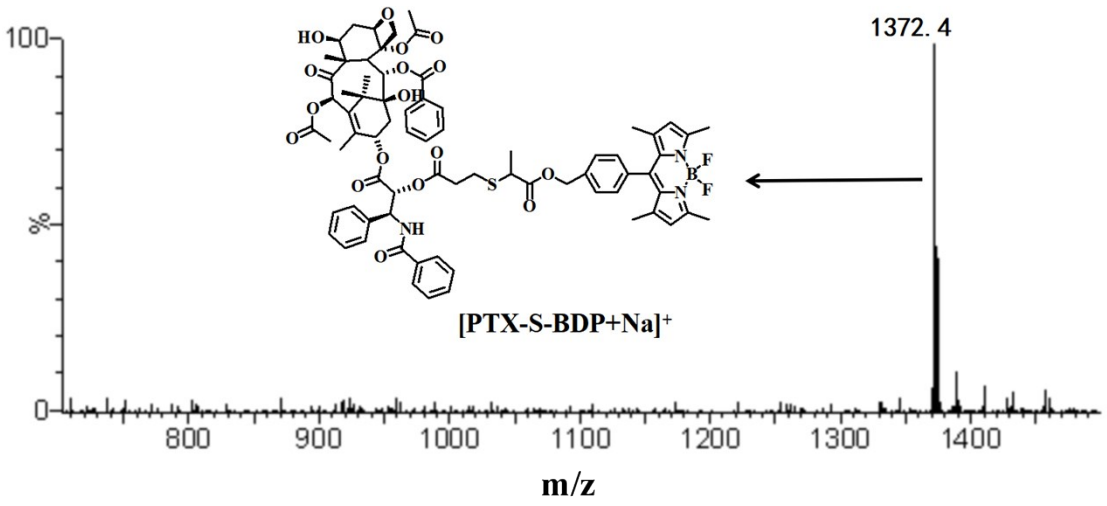

Fig. S6 Mass spectrum of PTX-S-BDP.

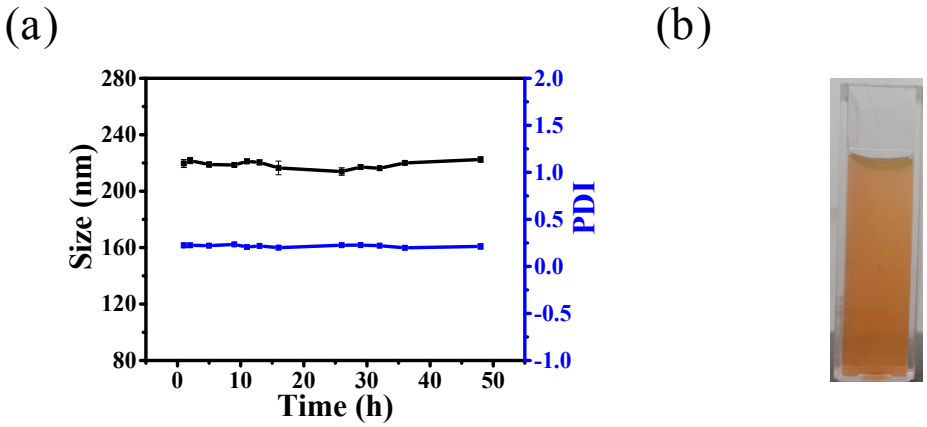

Fig. S7 (a) Particle size change of PTX-S-BDP NPs in PBS containing 10% FBS. (b) The photograph of PTX-S-BDP NPs stored at 4 ^{o}C for two months.

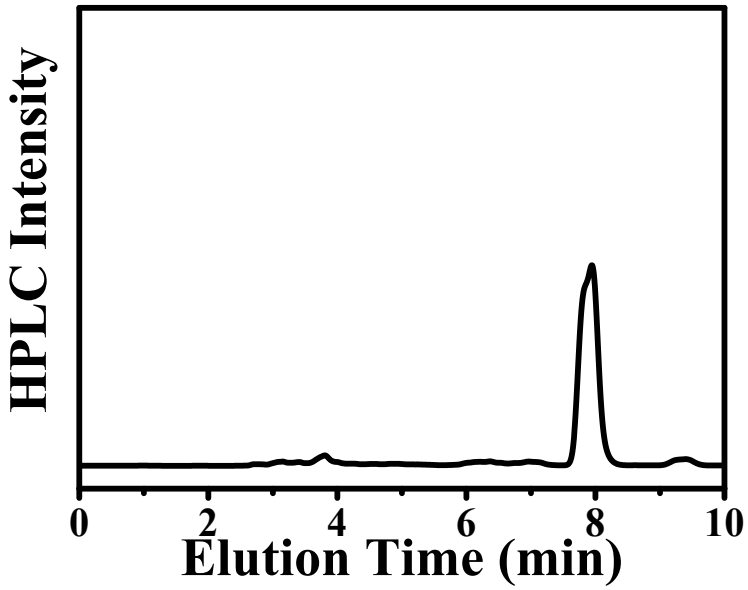

Fig. S8 HPLC analysis of PTX-S-BDP. The elution time of PTX-S-BDP is 7.9 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

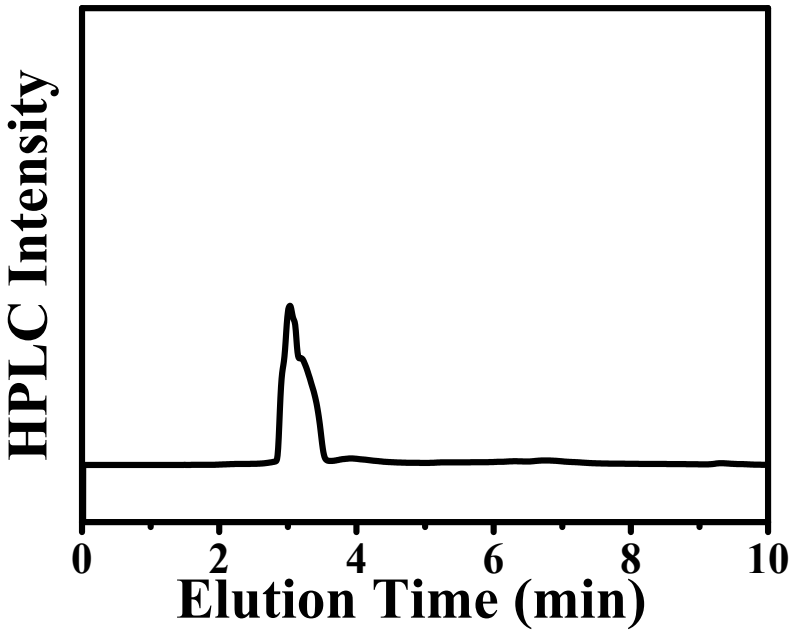

Fig. S9 HPLC analysis of 10 mM H_2O_2. The elution time of H_2O_2 is 3.01 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

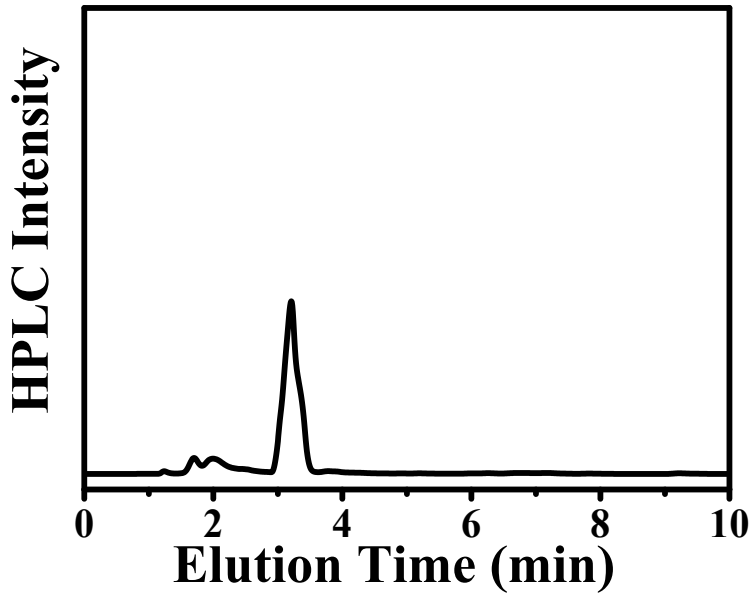

Fig. S10 HPLC analysis of 10 mM DTT. The elution time of DTT is 3.3 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

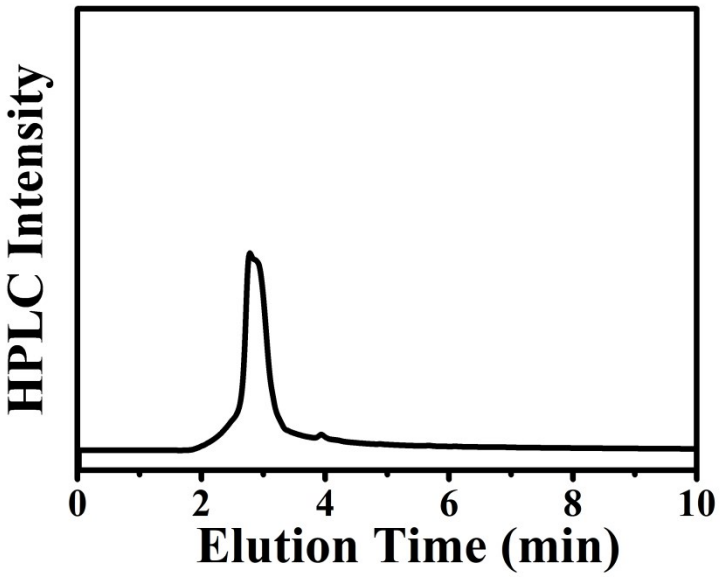

Fig. S11 HPLC analysis of 10 mM GSH. The elution time of GSH is 2.9 min. Elution solvent: methanol/acetonitrile/H_2O, v/v/v = 45/45/10.

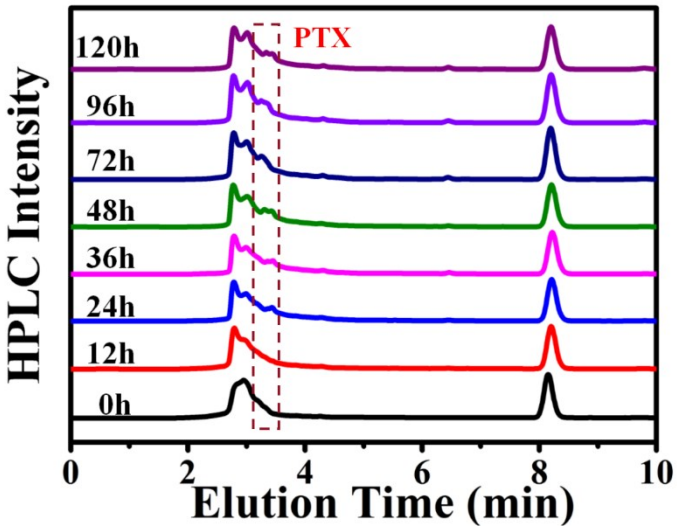

Fig. S12 The dynamic degradation process of PTX-S-BDP monitored by HPLC, treated with 10 mM of GSH.

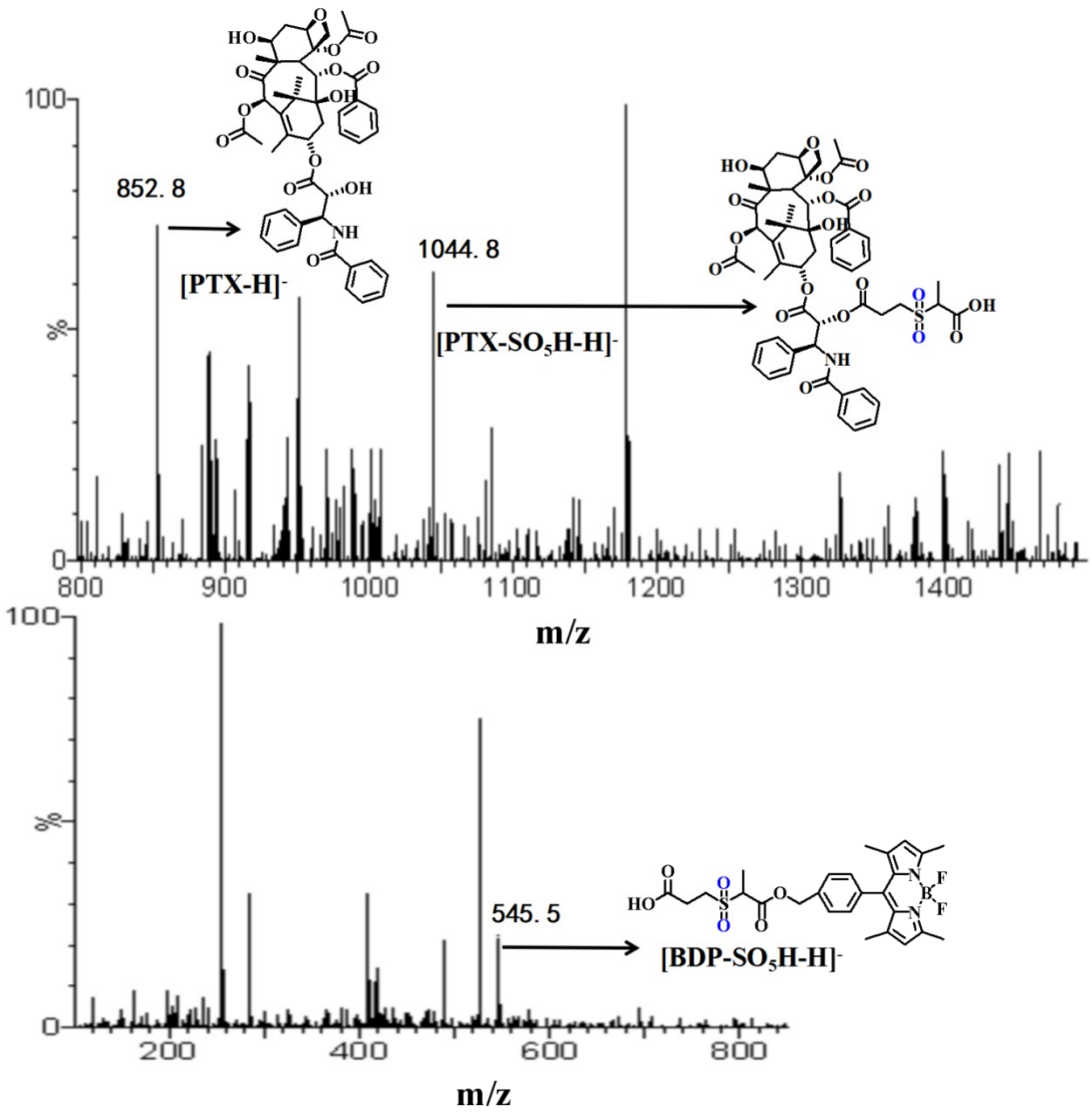

Fig. S13 The mass spectra of PTX-S-BDP after treated with 10 mM H_2O_2 for 120 h.

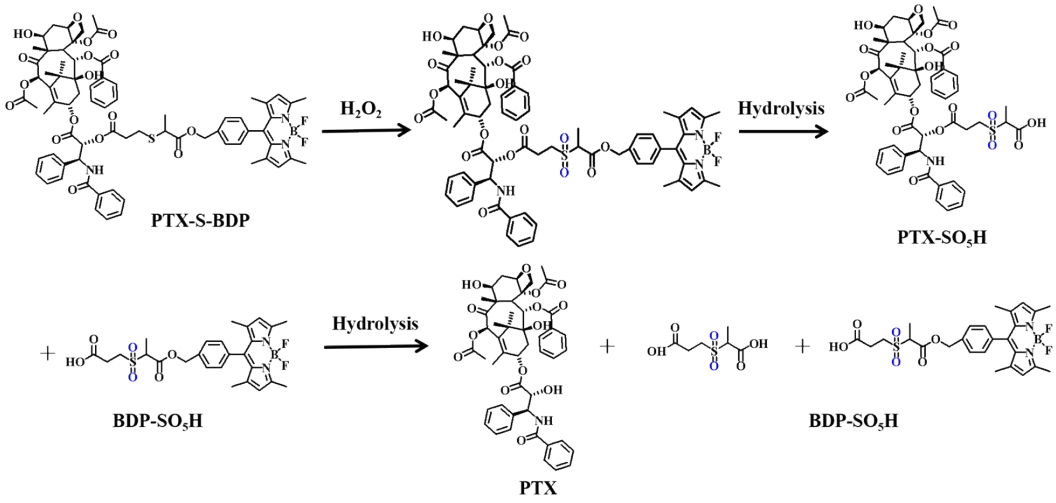

Fig. S14 Degradation mechanism of PTX-S-BDP triggered by H_2O_2.

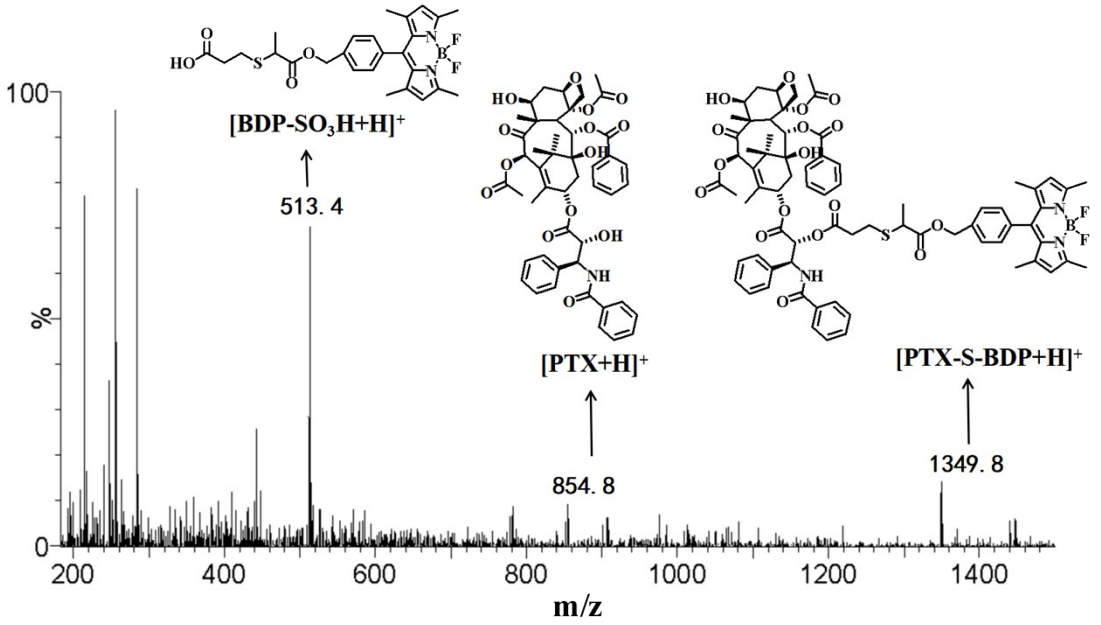

Fig. S15 The mass spectra of PTX-S-BDP after treated with 10 mM DTT for 120 h.

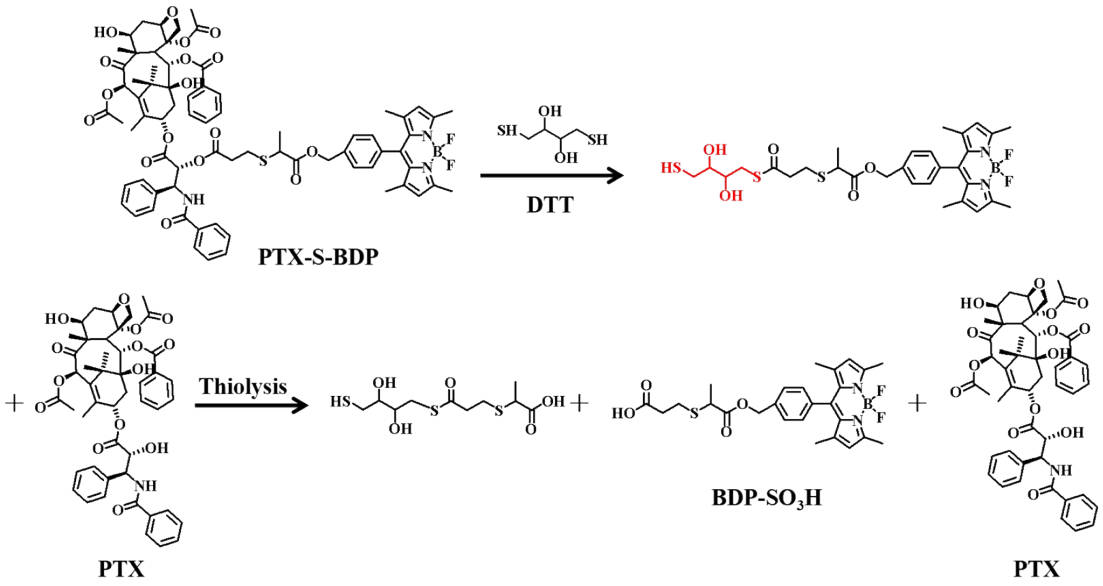

Fig. S16 Degradation mechanism of PTX-S-BDP triggered by DTT.

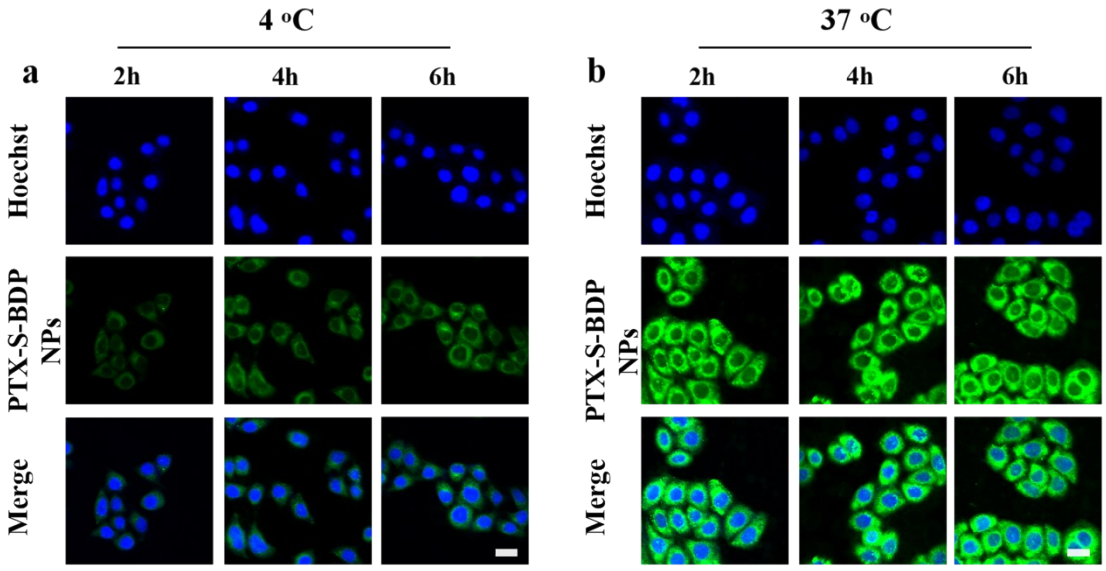

Fig. S17 CLSM images of HeLa cells cultured with PTX-S-BDP NPs (concentration: 1.0 μM) at 4 and 37 °C for 2, 4, 6 h. (scale bar: 20 μm).

Table S1. DLC, DLE and Zeta potential of PTX-S-BDP NPs with different feed ratios.

	Feed ratio (w/w) PTX-S-BDP/F-127				
	1:1	1:2	1:5	1:8	1:10
DLC (wt%)	13.02%	15.1%	11.82%	6.86%	5.42%
DLE (wt%)	22.79%	44.60%	69.37%	56.48%	56.23%
Zeta potential (mV)	-21.1	-20.2	-18.9	-22.3	-21.3