Supporting Information

1. Experimental Section

1.1. Materials and reagents

Polyethylene glycol monomethyl ether methacrylate (PEGMA, Mn of 475, 98%) was provided by Aladdin Chemistry Co. Ltd. It was used after purification with a basic alumina column to remove any inhibitor. Doxorubicin hydrochloride (DOX□HCl, 99.4%) was bought from Beijing Huafeng United Technology Co. Ltd. N, N, N', N'-pentamethyl diethylenetriamine (PMDETA, 99%), 2-bromoisobutyryl bromide (BIBB, 98%) and copper(I) bromide (CuBr, 98%) were purchased from Aldrich and used without any pretreatment. Methacryloyl chloride (98%) was purchased from HEOWNS. 2-Hydroxyethyl disulfide (98%) was provided by Shanghai Dibai Biological Technology Co. Ltd. 4-Nitrophenyl chloroformate (98%) and 4-dimethylaminopyridine (DMAP, 99%) were provided by J&K Chemical Co. Ltd. Triethylamine (TEA, 99%) and dichloromethane (DCM, 99.5%) were obtained from Lanzhou Gongchuang Technology E-Commerce Co. Ltd. Other reagents were all analytical grade and used directly as received. Double distilled water was used throughout.

1.2. Characterizations

^{1}H NMR spectra were recorded on a JEOL ECS (400 M, JEOL, Japan) spectrometer. Mass spectrometry analysis was obtained with a micrOTOF II (Bruker) MS spectrometer. The DOX-based prodrug monomer was analyzed on a Waters 2690D HPLC system with C18 reverse phase column (4.6 × 100 mm, 5 mm particle size) at 25 °C with methanol/Milli-Q water/acetic acid (60/36/4, v/v) as the flow phase at a flow rate of 0.8 mL/min at 480 nm. The relative molecular weight of the hyperbranched polymer prodrugs was measured on a gel permeation chromatograph (GPC) equipped with a Waters 1515 pump and a Waters 2414 differential refractive index detector, using DMF as the eluent at 35°C. The UV-vis spectra and drug content were detected using a TU-1901 UV/vis spectrometer (Beijing Purkinje General Instrument Co. Ltd, Beijing, China) at room temperature. The morphology of the unimolecular micelles was observed on a JEM1200 EX/S transmission electron microscope (TEM, JEOL, Japan), sampling with the aqueous dispersion. The hydrodynamic diameter and diameter distribution of the unimolecular micelles were measured with the dynamic light scattering (DLS) technique using a BI-200SM instrument (Brookhaven). The zeta potentials of the GSH-sensitive hyperbranched polymer prodrug (HBPP-DOX) were determined using a NanoBrook 90Plus PALS (Brookhaven Instruments Co., Holtsville, NY, USA) in pH 7.4 PBS with 0.050 mg/mL.

1.3. Drug content and in vitro drug controlled release

The DOX content in HBPP-DOX was obtained by measuring the absorbance at 480 nm of its DMSO solution and calculating with the standard curve of DOX in DMSO (Fig. S7).

The *in vitro* cumulative DOX release was assessed under different conditions: pH 7.4 PBS with 10 μM GSH (blood circulation), pH 6.8 PBS with 10 μM GSH (extracellular environment), pH 5.0 ABS with 2 mM GSH (normal intracellular

microenvironment) or 10 mM GSH (tumor intracellular microenvironment) at 37°C. Generally, 2 mg of HBPP-DOX unimolecular micelles and 120 mL of release medium were adopted to carry out the above release experiment. The cumulative DOX release was calculated with the standard curves of DOX in the corresponding releasing media (Fig. S8).

1.4. In vitro cellular toxicity and uptake

The MTT assay was used to evaluate the cytotoxicity of the HBPP-DOX unimolecular micelles against the L02 and HepG2 cells. The cells were incubated in a 96-well plate with a concentration of 1×10^5 per well at 37°C for 48 h. Then, different concentrations of the HBPP-DOX unimolecular micelles or free DOX were added for a co-incubation of 48 h. After that, MTT (5.0 mg/L) was added into each well, followed by incubation for another 4 h. Finally, the cell viability was measured using the Enzyme-linked Immunosorbent Assay Appliance at 490 nm, after removing the crystals by dissolving in 150 μL of DMSO for 20 min. All the data were presented as the mean value of six measurements.

The cellular uptake and intracellular distribution in the HepG2 cells was evaluated by an inverted fluorescence microscope (OLYMPUS, IX71) after 48 h of incubation. Generally, the cell nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI), and the fluorescent images were obtained at 405 nm for DAPI, 480 nm for DOX, respectively.

2. Supporting figures

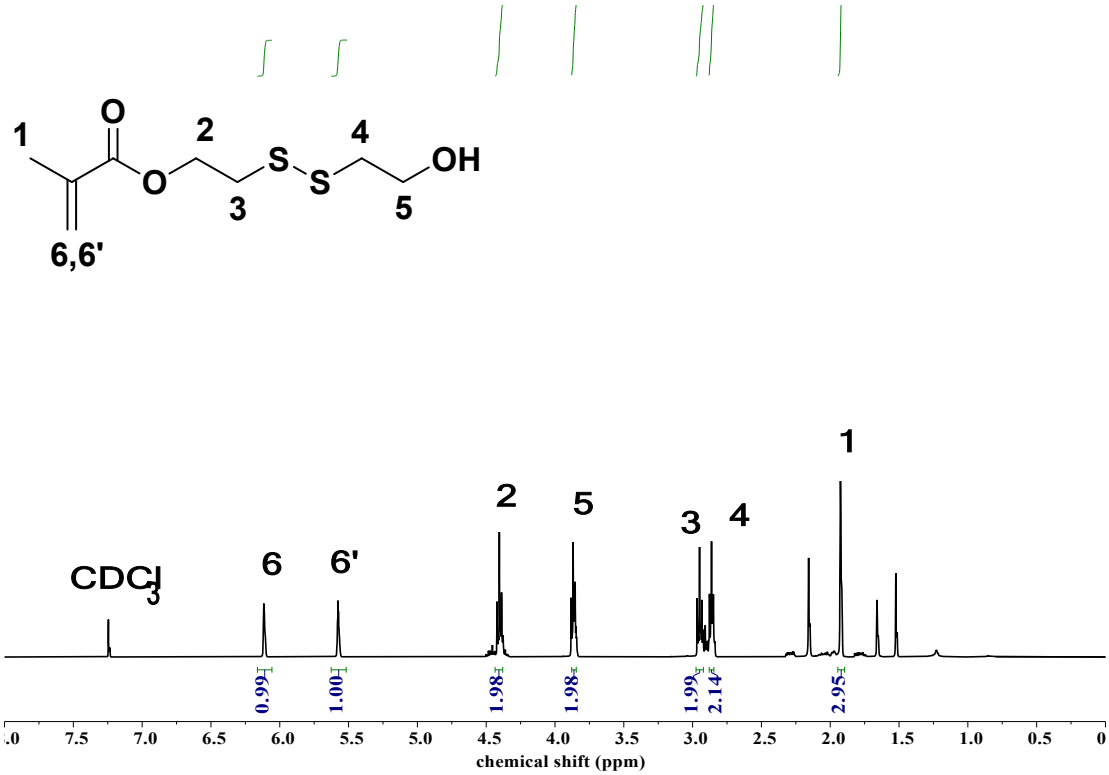

Fig. S1. ^{1}H-NMR spectrum of MA-SS-OH in $CDCl_3$.

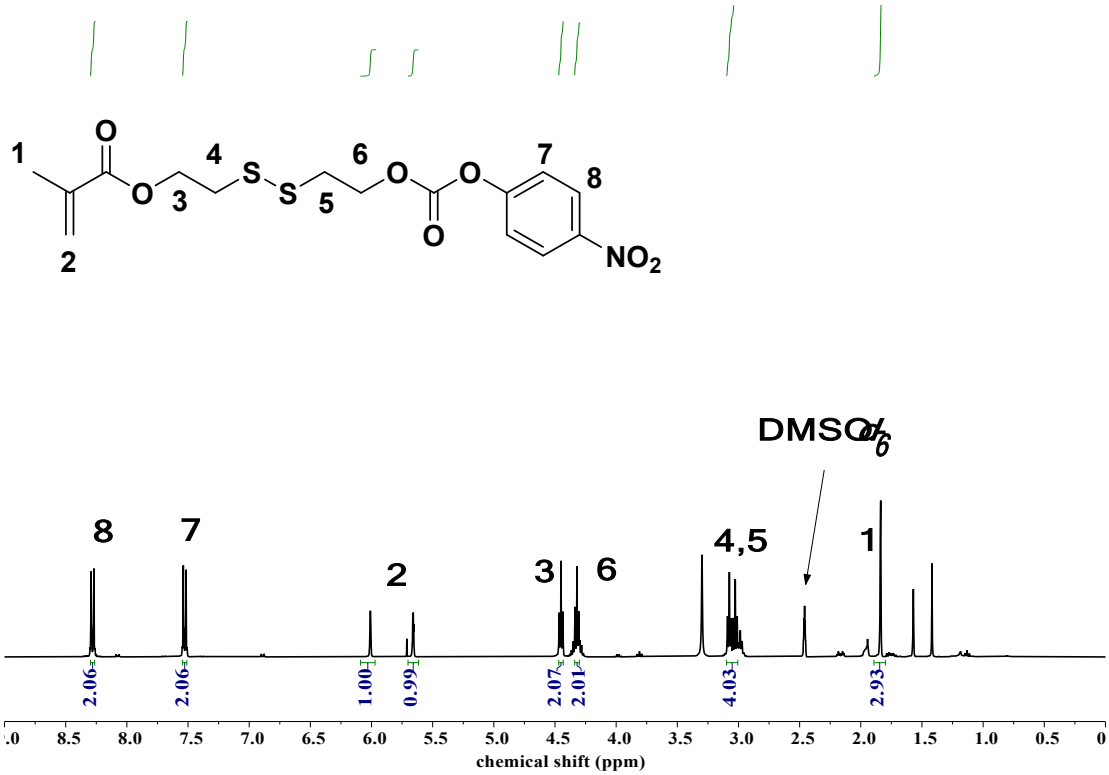

Fig. S2. ^{1}H-NMR spectrum of MA-SS-PNP in DMSO-d_6.

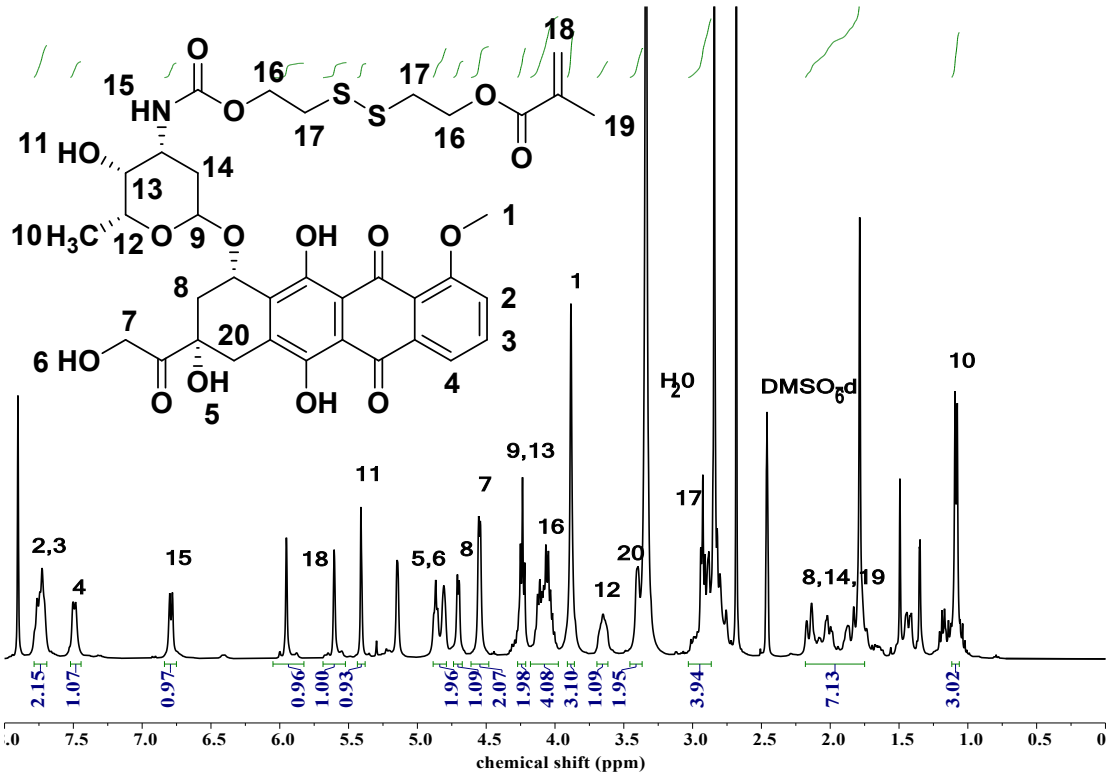

Fig. S3. ^{1}H-NMR spectrum of the DOX-based prodrug monomer MA-SS-DOX in DMSO-d_6.

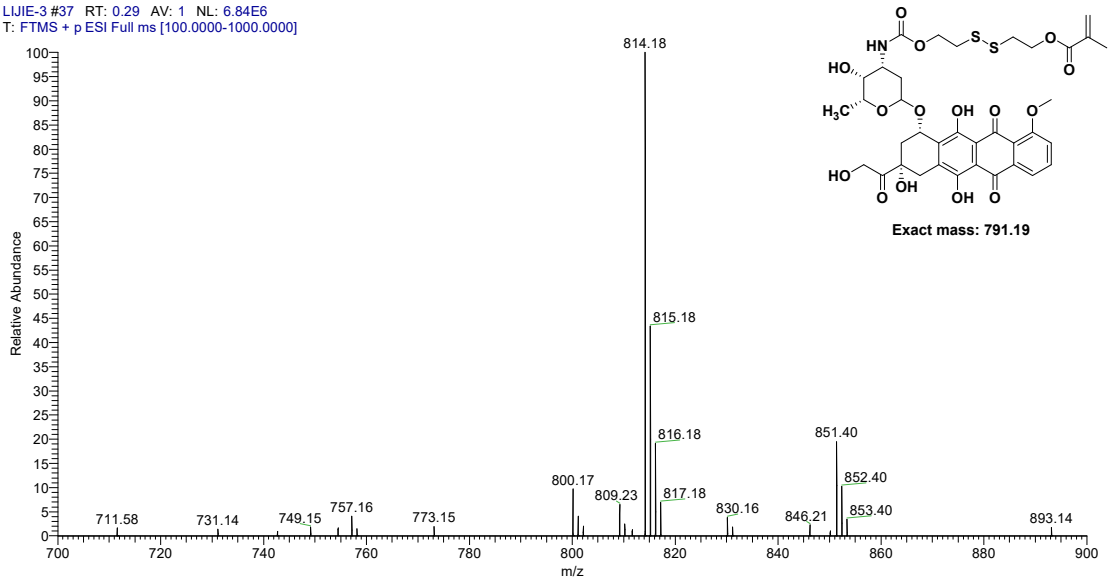

Fig. S4. ESI-MS spectrum of the DOX-based prodrug monomer MA-SS-DOX.

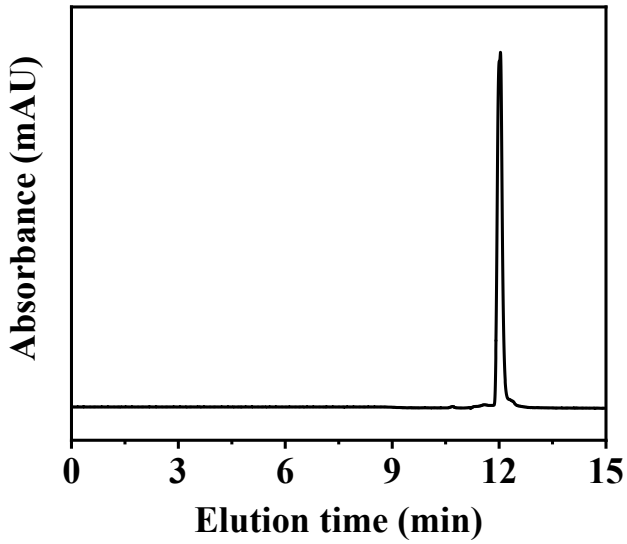

Fig. S5. HPLC profile of the DOX-based prodrug monomer MA-SS-DOX.

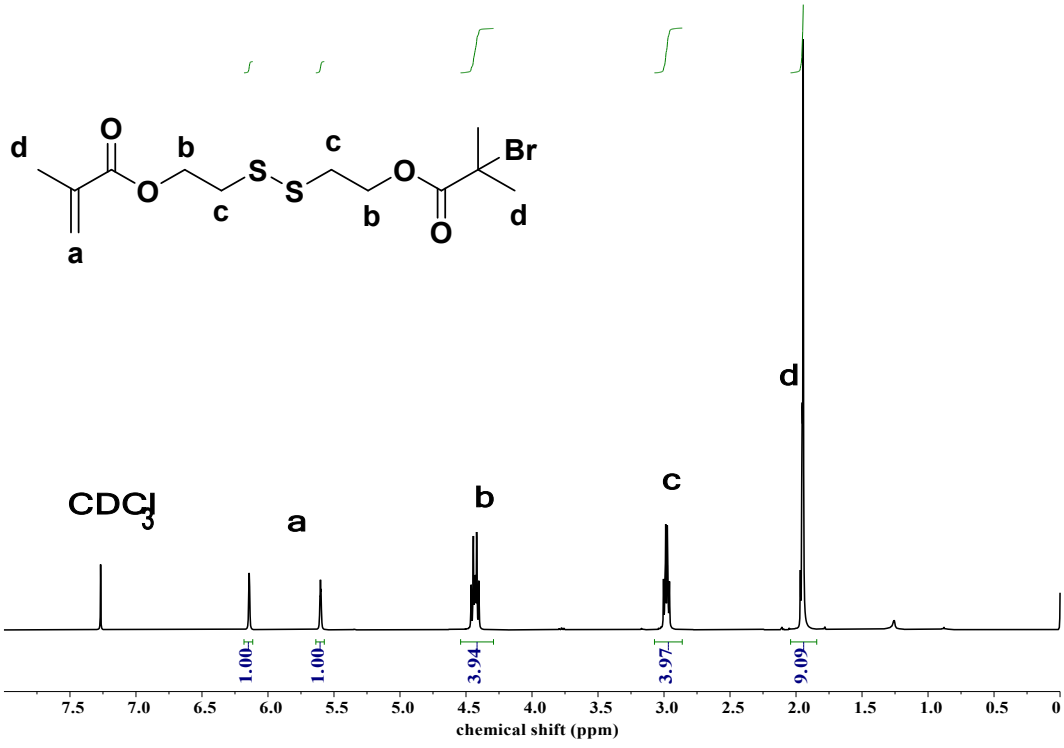

Fig. S6. ^{1}H-NMR spectrum of the inimer MA-SS-Br in $CDCl_3$.

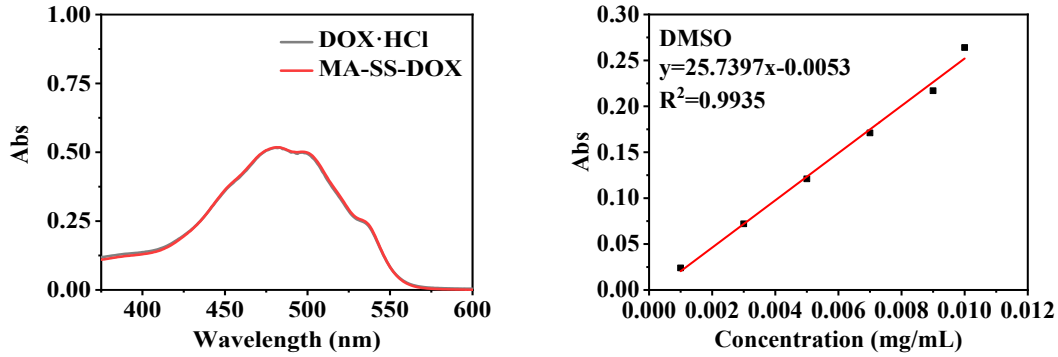

Fig. S7. UV-vis spectra of DOX·HCl (34.48 ×10^{-5} mmol/mL) and the MA-SS-DOX (34.48 ×10^{-5} mmol/mL) in DMSO and Calibration curves of DOX·HCl in DMSO.

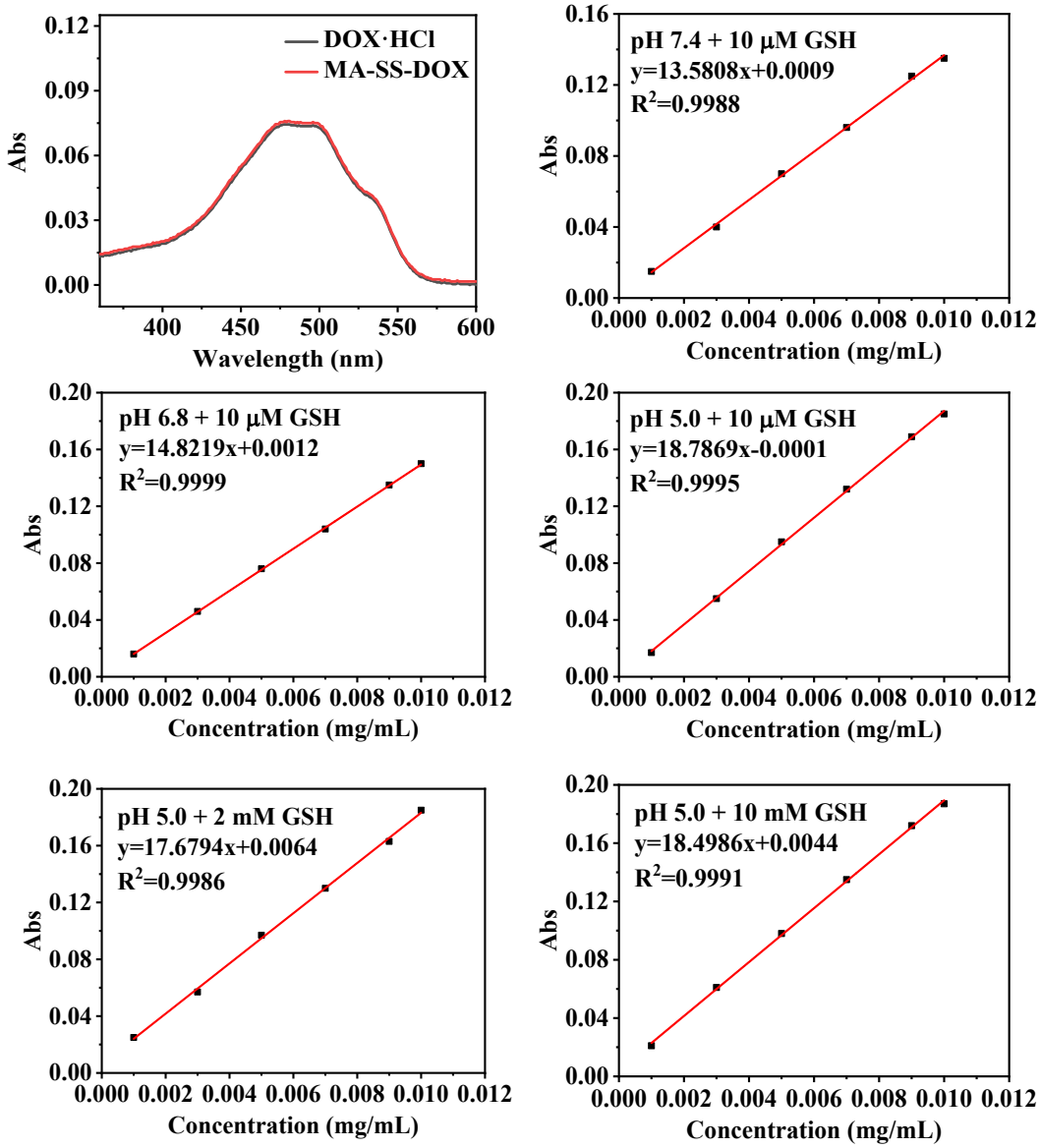

Fig. S8. UV-vis spectra of DOX·HCl (57.46×10^{-6} mmol/mL) and the MA-SS-DOX (57.46×10^{-6} mmol/mL) in water and the calibration curve of DOX·HCl in different solutions.

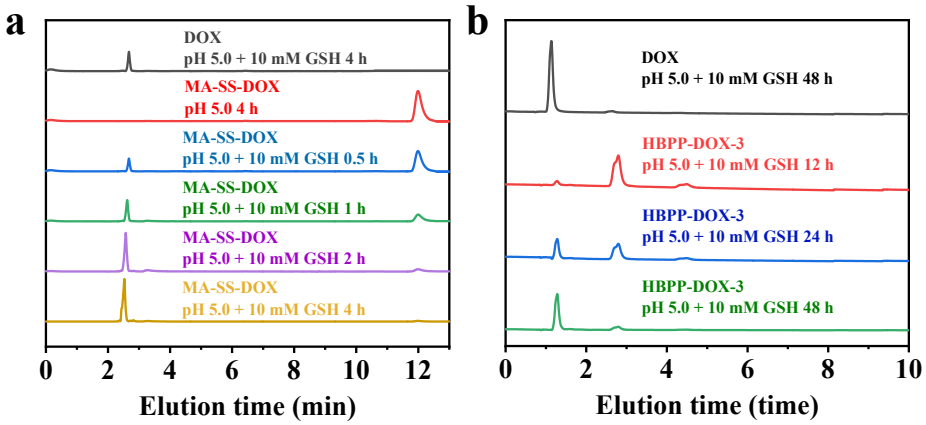

Fig. S9. (a) HPLC profiles of DOX and MA-SS-DOX after treating with pH 5.0 + 10 mM GSH for 0.5, 1, 2 and 4 h, and MA-SS-DOX after treating with pH 5.0 for 4 h, respectively. ($CH_3OH/H_2O/CH_3COOH$ = 60:36:4, detected at 480 nm); (b) HPLC profiles of HBPP-DOX-3 after treating with pH 5.0 + 10 mM GSH for 12 h, 24 h and 48 h, and DOX after treating with pH 5.0 + 10 mM GSH for 48 h, respectively. ($CH_3OH/H_2O/CH_3COOH$ = 80:16:4, detected at 480 nm).

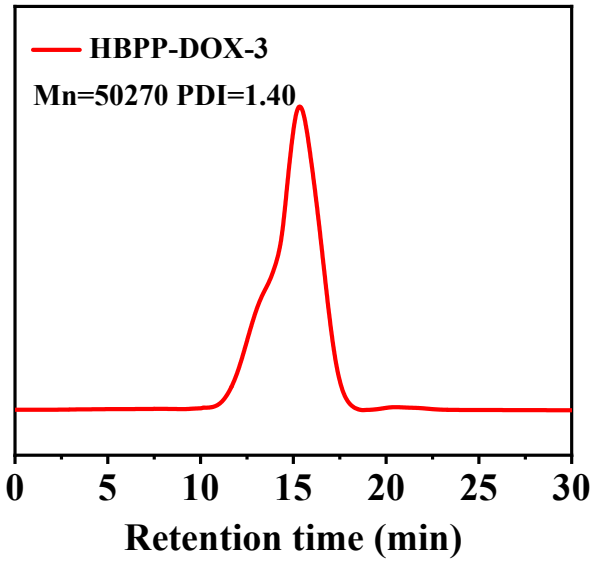

Fig. S10. GPC trace of the HBPP-DOX-3 in DMF.

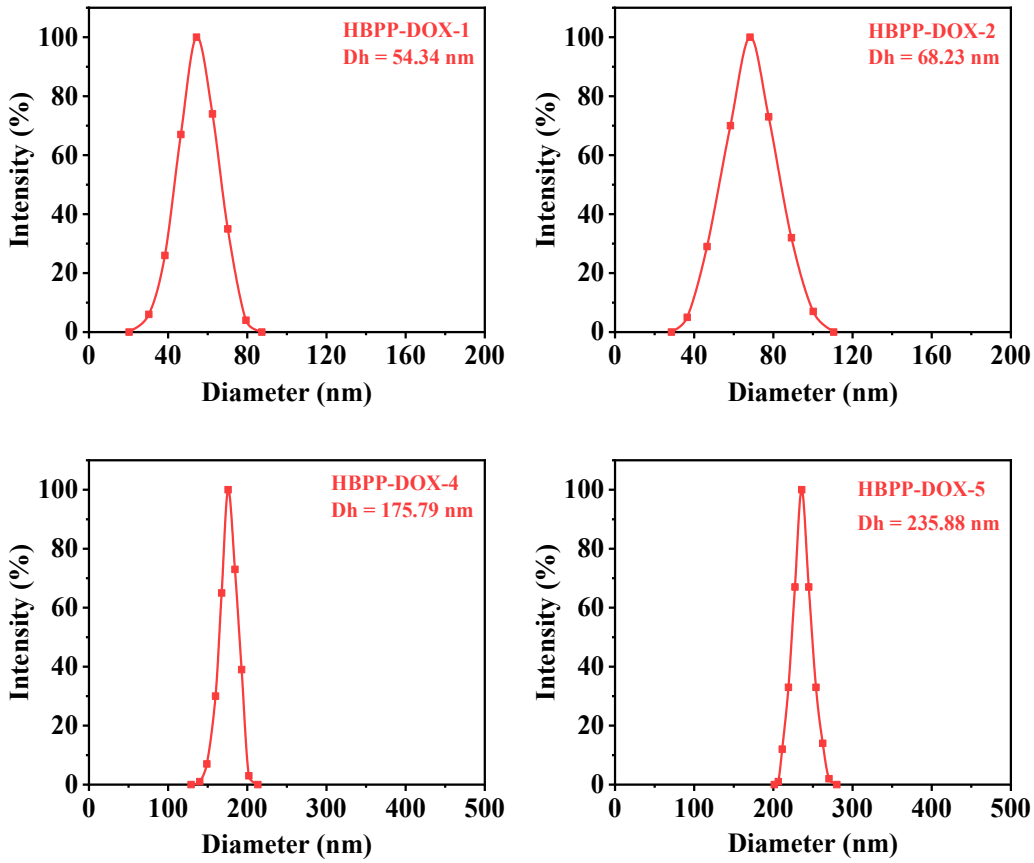

Fig. S11. DLS results of the HBPP-DOX micelles synthesized with different MA-SS-Br/MA-SS-DOX/PEGMA feeding molar ratios in pH 7.4 PBS (0.01 mg/mL).

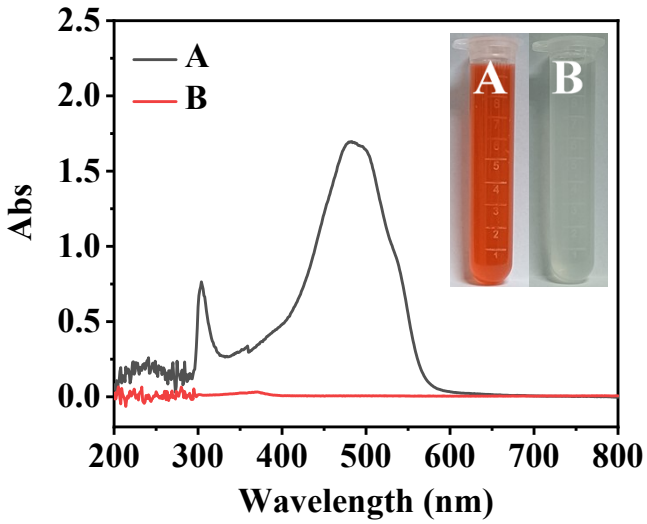

Fig. S12. UV-vis spectra of the GSH-triggered disintegrable HBPP-DOX-3 unimolecular micelles in pH 7.4 + 10% BSA (0.100 mg/mL) for five days (A) and the supernatant (B). Inset: digital images of the dispersions of unimolecular micelles in pH 7.4 + 10% BSA for five days (A) and the supernatant (B) under daylight.

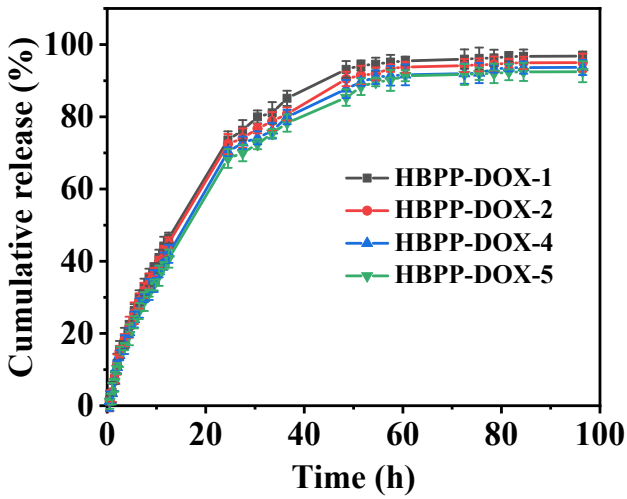

Fig. S13. Cumulative release of DOX from the GSH-triggered disintegrable HBPP-DOX-1, HBPP-DOX-2, HBPP-DOX-4 and HBPP-DOX-5 unimolecular micelles in the simulated tumor intracellular microenvironment (pH 5.0 + 10 mM GSH). Error bars were based on three repetitions at each time point.

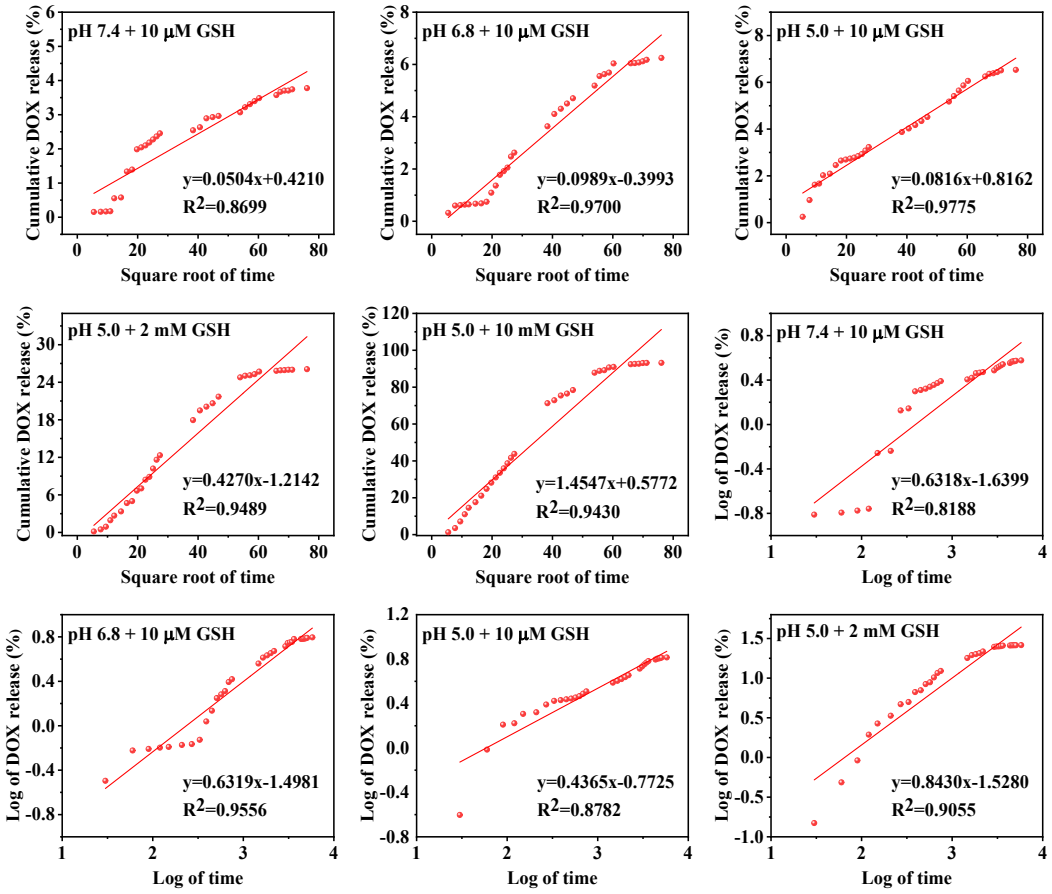

Fig. S14. Fitted plots of the DOX release from the HBPP-DOX-3 unimolecular micelles in different releasing media with the Higuchi and Korsmeyer-Peppas models.

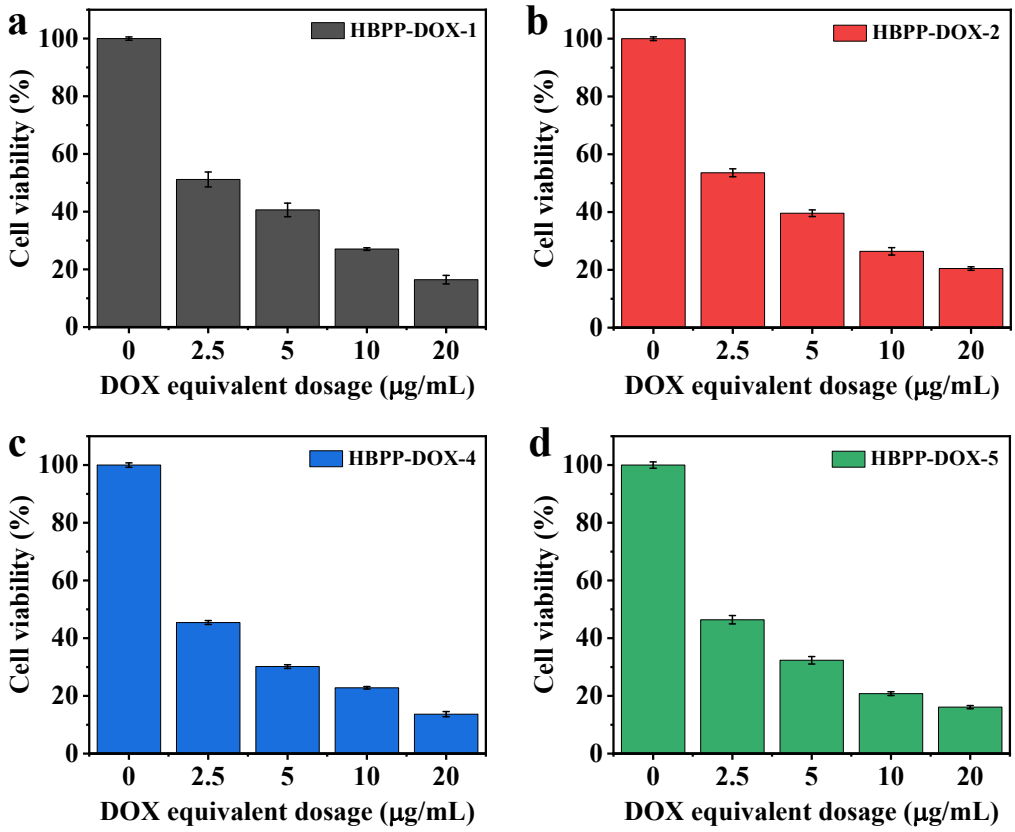

Fig. S15. Cell viability assay in HepG2 cells of (a) HBPP-DOX-1, (b) HBPP-DOX-2, (b) HBPP-DOX-4 and (d) HBPP-DOX-5 unimolecular micelles with different concentrations for 48 h, respectively. Values are expressed as mean ± SD (n = 6).