Supporting Information

Nanogels co-loading paclitaxel and curcumin prepared *in situ* through photopolymerization at 532 nm for synergistically suppressing breast tumor

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1. Methods

1.1 Experimental parameters

In the fabrication process, each time a 5 mL solution was taken for photopolymerization. The proportion of compounds in 5ml of solution is as follows.

Named	PEGDA (wt%)	PEGMA (wt%)	Tween 80	Drug (mg)
NG-PC21	30%	10%	1.2%	PTX 14 mg + Cur 7 mg
NG-PC11	30%	10%	1.2%	PTX 10 mg + Cur 10 mg
NG-PC12	30%	10%	1.2%	PTX 7 mg + Cur 14 mg
NG-T	30%	10%	/	/
NG-PTX	30%	10%	1.2%	PTX 10 mg

1.2 Measurements

The morphology of nanogels was observed using a scanning electron microscope (SEM, Hitachi, S-4800, Japan) with an acceleration voltage of 5.0 kV and a current of 10 μA. The dynamic light scattering (DLS) including hydrodynamic diameter and polydispersity index (PDI) were recorded on Zeta Sizer Nano ZS (Malvern, UK). The zeta potential (ζ) of the nanogels was measured by phase analysis light scattering (PALS) using the Zeta Sizer Nano ZS. The nanogels were analyzed by Fourier transform infrared spectroscopy (FT-IR, Tianjin Gangdong Science and Technology Co, Ltd., China) to identify the functional groups, and the wavenumber ranged from 4000 to 400 cm^{-1}. FT-IR using the KBr disk method for qualitative analysis. The molecular composition of nanogels was characterized using proton nuclear magnetic resonance (^1H NMR, AVANCE IIITM HD 400 MHz NanoBAY, Switzerland) with D_2O as deuterated solvent for quantitative analysis. Spectra width was 8012.8 Hz, pulse width was 9.44 μs, pulse sequence was zg30, sampling time (AQ) was 4.0 s, and relaxation time(D1) was 1s. X-ray diffraction (XRD) patterns of the NG-PC, NG-T/PTX/Cur mixture, NG-PTX, NG-T, PTX and Cur powder were investigated on MiniFlex 600 X-ray (Rigaku, Japan) diffractometer. The specimens were tested in a 2θ ranges from 5° to 50° at a scanning rate of 3°/min.

1.3 Drug loading of nanogels

The drug loading capacity (DLC) of nanogels was measured using an ultraviolet-visible spectrophotometer (Shimadzu, UV-1800, Japan) and high performance liquid chromatography (HPLC, Agilent Technologies, 1200 Series). The wavelength used for the detection of curcumin was 425 nm and the standard curve in ethanol (The concentration of curcumin is 2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L and 10 mg/L, respectively). The wavelength used for the detection of PTX was 227 nm and the standard curve in acetonitrile (The concentration of PTX is 0.1 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 5 mg/L, 10 mg/L and 50 mg/L, respectively). The DLC of nanogels calculated according to the following equation:

$$DLC = \frac{Weight\ of\ loaded\ drugs}{Weight\ of\ NG-C} \times 100\% \qquad (Equation\ S1)$$

1.4 The double bond conversion by internal standard

Hydroquinone (Acros, 99.5%) was purchased from Thermo Fisher Scientific (Beijing, China).

The compound weight $m_{(x)}$ using Hydroquinone (HQ) as the internal standard was calculated following equation:

$$m_{(x)} = \frac{I_{(x)} \times N_{(HQ)} \times M_{(x)} \times m_{(HQ)}}{I_{(HQ)} \times N_{(x)} \times M_{(HQ)}} \times HQ\ purity \qquad (Equation\ S2)$$

where $I_{(X)}$ is the integral intensity of the studied compound, $M_{(X)}$ is the molecular weight test item [g/mol], $N_{(X)}$ is the number of protons for the integrated signal in the molecule of an analyte, $I_{(HQ)}$ is the integral intensity of protonated hydroquinone, $M_{(HQ)}$ is the molecular weight standard [g/mol], $N_{(HQ)}$ is the number of protons for the integrated signal in the molecule of standard, and $m_{(HQ)}$ is the sample weight of standard [mg]. $I_{(HQ)} = 4$, $M_{(HQ)} = 110.1$ g/mol, $N_{(HQ)} = 4$, $m_{(HQ)} = 5.5$ mg, HQ purity = 99.5%.

1.5 HPLC test

The amount of PTX and Cur was quantified by HPLC. In detail, the analysis was performed with HPLC system (Agilent Technologies, 1200 Series, Japan) and a C18 column (4.6 mm × 250 mm, 5 μm). For both drugs, the injection volume was 20 μL and the elution rate was adjusted at 1.0 mL/min with column temperature set at 25 °C. The PTX detection wavelength was 227 nm and the phase mobile consisted of acetonitrile-water (60:40, v/v). Cur was detected at 425 nm using acetonitrile-2% acetic acid (50:50, v/v) as the mobile phase. Mobile phases were filtered through a 0.45 μm nylon membrane filter and ultrasonically degassed prior to use.

1.6 combination index (CI)

The curcumin and paclitaxel combination was appraised by calculating the CI value using the CompuSyn software, with the method utilized by Chou and Talalay:

$$\mathrm{CI} = \frac{a}{A} + \frac{b}{B} \qquad (Equation\ S3)$$

where *a* is the IC_{50} value of PTX in combination with Cur, while *b* is the IC_{50} value of Cur in combination with PTX, and *A* and *B* is the IC_{50} value of free PTX alone and free Cur alone, respectively. According to the Chou and Talalay equation, when CI < 1, the interaction between the two drugs is synergistic; when CI = 1, the interaction between the two drugs is additive; and when CI > 1, the two drugs are antagonistic.

2. Characterizations

Table S1. Characterization of nanogels

Item	Size/nm	PDI	ζ/mV	DLC-PTX/%	DLC-Cur/%
NG-PC21	183.9 ± 2.3	0.153 ± 0.021	−13.5 ± 0.5	9.82 ± 0.28	3.59 ± 0.31
NG-PC11	178.9 ± 1.0	0.146 ± 0.013	−15.3 ± 1.3	6.37 ± 0.15	6.82 ± 0.11
NG-PC12	173.5 ± 0.5	0.128 ± 0.038	−22.9 ± 0.9	3.71 ± 0.12	10.07 ± 0.15
NG-T	193.5 ± 2.2	0.085 ± 0.032	−30.9 ± 0.4	/	/
NG-PTX	186.2 ± 1.1	0.101 ± 0.015	−11.6 ± 0.6	13.46 ± 0.65	/

Note: Data are represented as mean ± SD (n = 3).

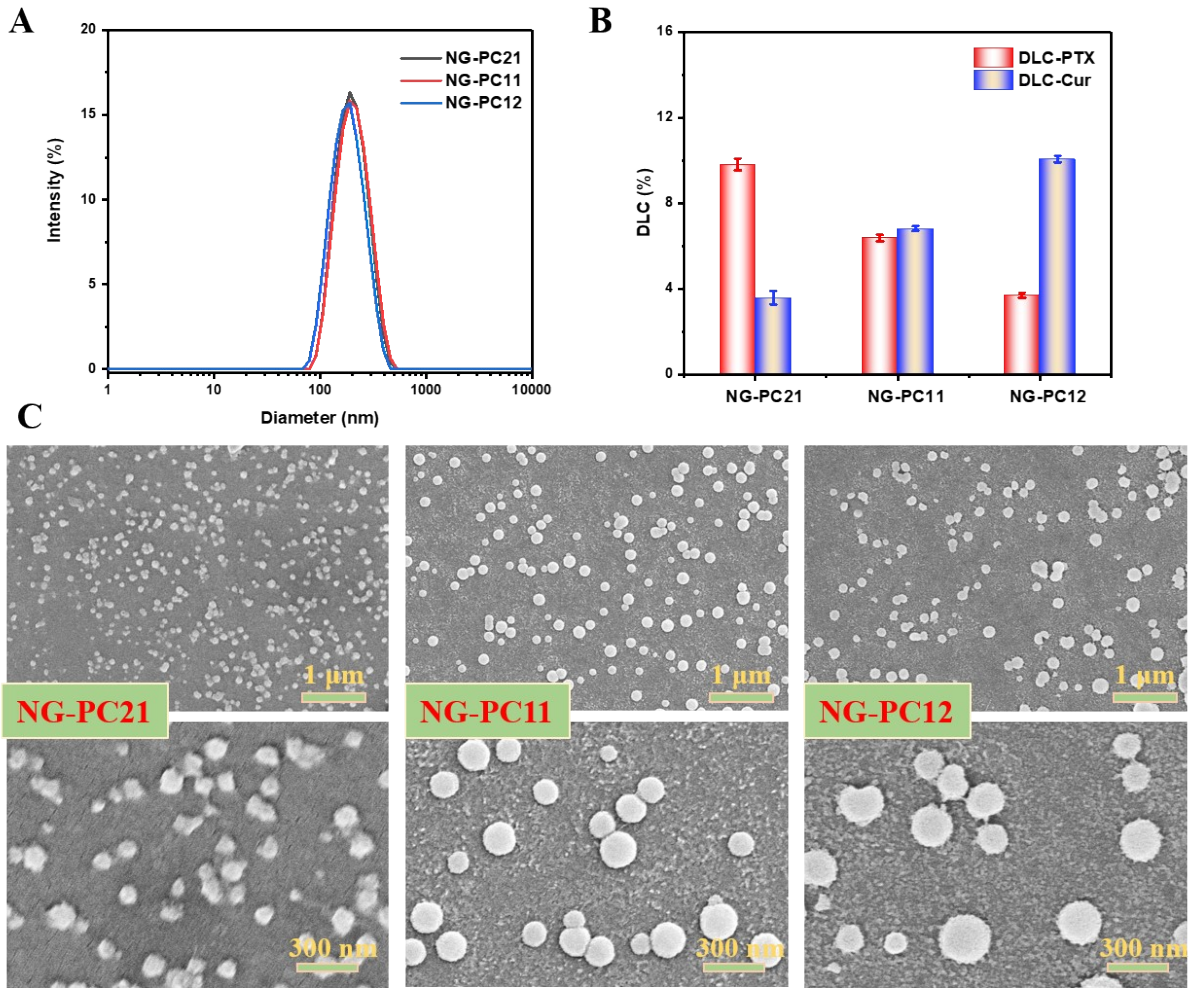

Figure S1. Preparation and characterization of NG-PC with different drug ratio. (A) DLS, (B) DLC, (C) SEM characterization of NG-PC, respectively. (Note: Data of DLC are represented as mean ± SD, n = 3)

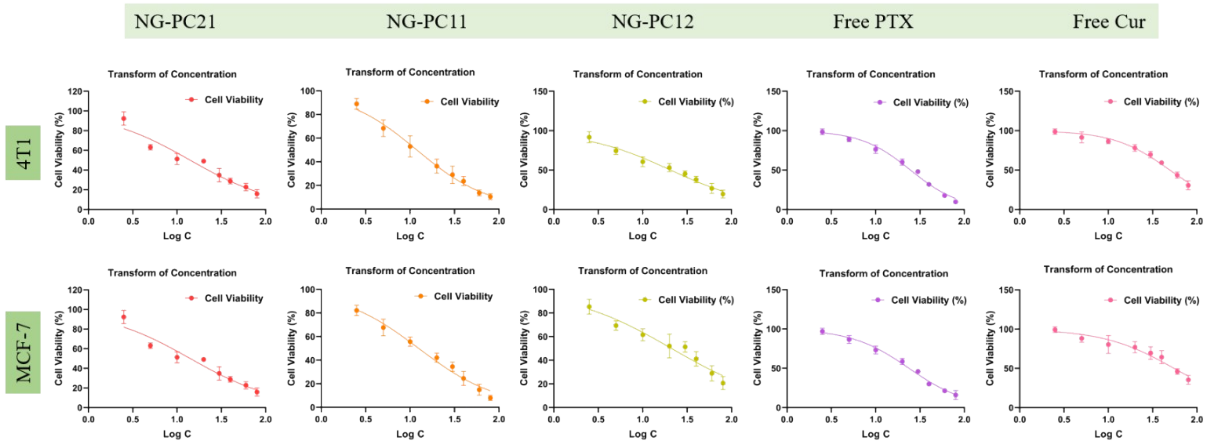

Figure S2. Curves of *in vitro* cell viability for calculating IC_{50} of free drug and co-loaded nanogels in 4T1 and MCF-7 cells, respectively. Data are represented as mean ± SD (n = 5).

Table S2. IC_{50} of free drug and co-loaded nanogels in 4T1 and MCF-7 cells (panel to Figure S2)

Item	4T1 cell		MCF-7 cell	
	IC_{50} (μg/mL)	R^2	IC_{50} (μg/mL)	R^2
Free PTX	24.71 ± 1.028	0.9793	23.79 ± 1.030	0.9764
Free Cur	49.53 ± 1.034	0.9539	55.30 ± 1.071	0.8682
NG-PC21	14.10 ± 1.069	0.9101	15.92 ± 1.059	0.9273
NG-PC11	11.93 ± 1.051	0.9523	12.50 ± 1.050	0.9524
NG-PC12	20.59 ± 1.055	0.9329	21.11 ± 1.074	0.8808

Note: Data are represented as mean ± SD (n = 5).

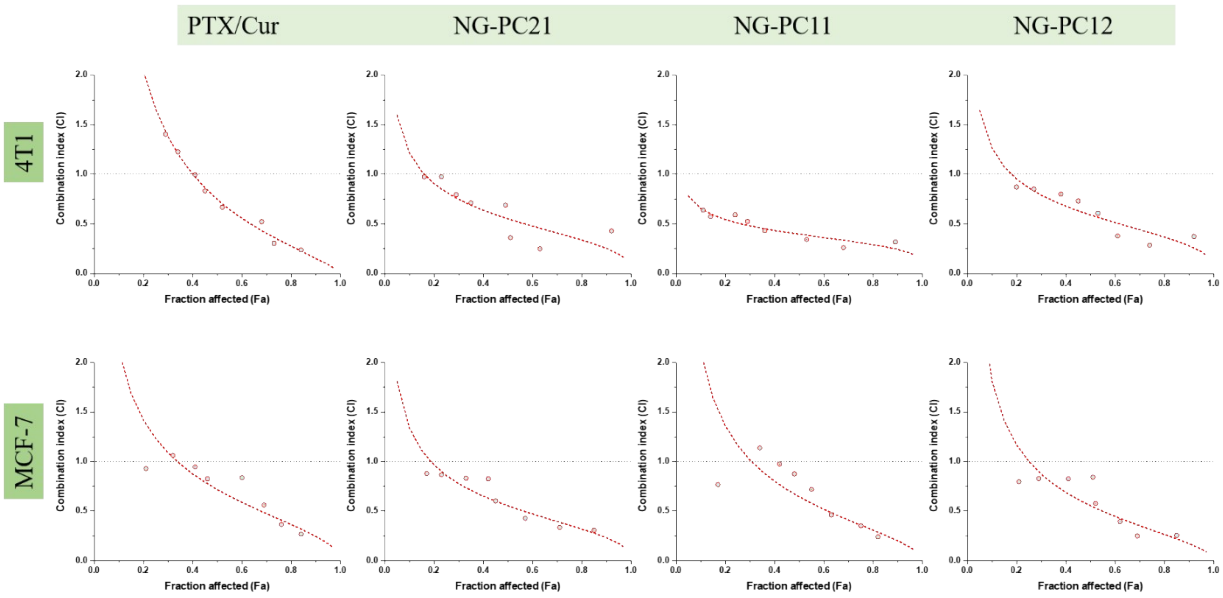

Figure S3. CI values were calculated by Chou-Talalay's equation analysis of the Fraction affected (Fa).

PTX/Cur, NG-PC21, NG-PC11 and NG-PC12 in 4T1 and MCF-7 cells, respectively.

Table S3. Paclitaxel and curcumin combination index (CI) against 4T1and MCF-7 cells (panel to Figure S3)

Combination type	4T1 cell		MCF-7 cell	
	CI	Interaction type	CI	Interaction type
NG-PC21	0.86	synergistic	0.96	synergistic
NG-PC11	0.72	synergistic	0.75	synergistic
NG-PC12	1.25	antagonistic	1.27	antagonistic

Note: CI < 1, synergistic; CI = 1, additive; CI > 1, antagonistic.

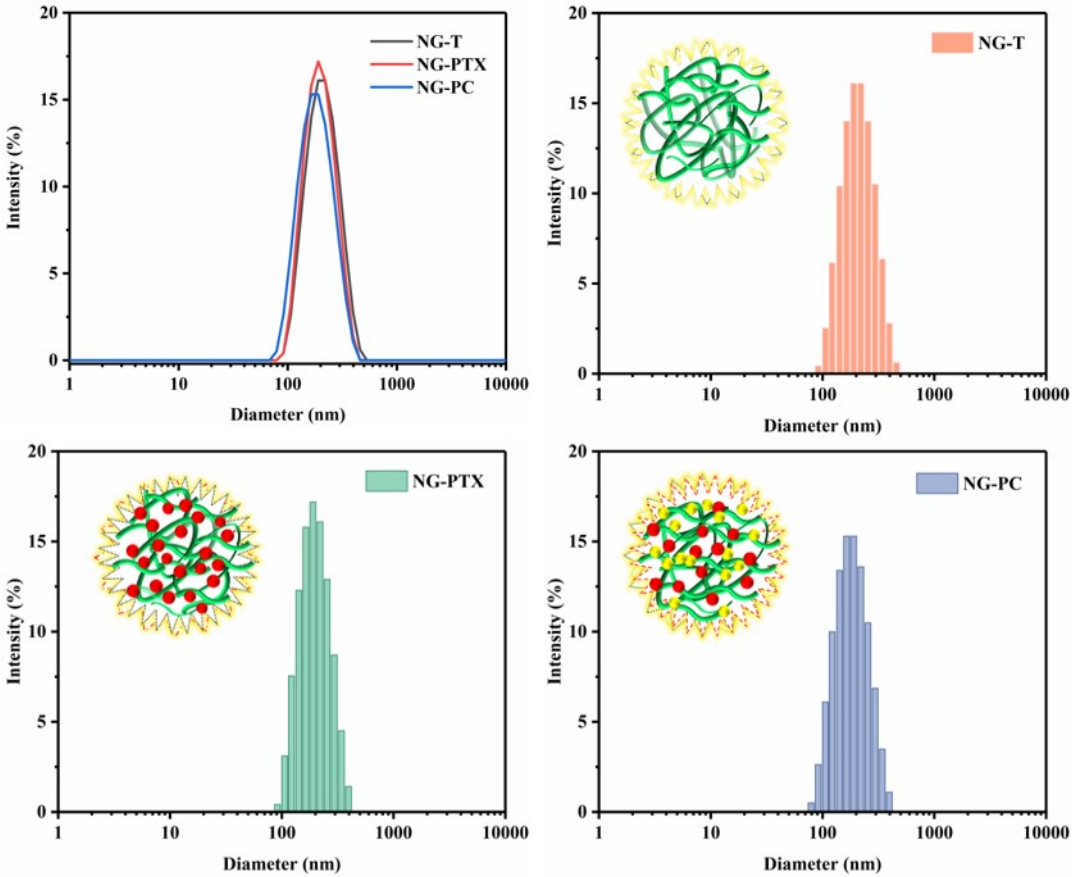

Figure S4. DLS characterization of NG-T, NG-PTX and NG-PC, respectively

Table S4. The characterization of double bond conversion of NG-T/NG-PTX/NG-PC

Item	Conv% PEGMA [a]	Conv% PEGDA [b]	Conv% total [c]
NG-T	59.09	95.14	93.12
NG-PTX	57.14	91.11	86.18
NG-PC	62.50	98.26	97.44

$$= \frac{n_{(after\ polymerization)}}{n_{(before\ polymerization)}} \times 100\%$$

a,b. Double bond conversion, Conv%

$n_{(after\ polymerization)}$ can be calculated by Eq. S2 based on the integral intensity of the studied functional groups, where is carbon-carbon double bond (C=C), respectively.

$n_{(before\ polymerizatin)}$ can be calculated by $m_{(PEGDA)}$ & $m_{(PEGMA)}$ in the nanogels, respectively.

c. Total Conv% , learn from the formula of weighted average in mathematics, as following.

$$\text{Conv\% total} = \frac{n_{(before\ polymerization,\ PEGDA)} \times Conv\%\ PEGDA + n_{(before\ polymerization,}}{n_{(before\ polymerization,\ PEGDA)} + n_{(before\ polymerization,\ P}} \times 100\%$$

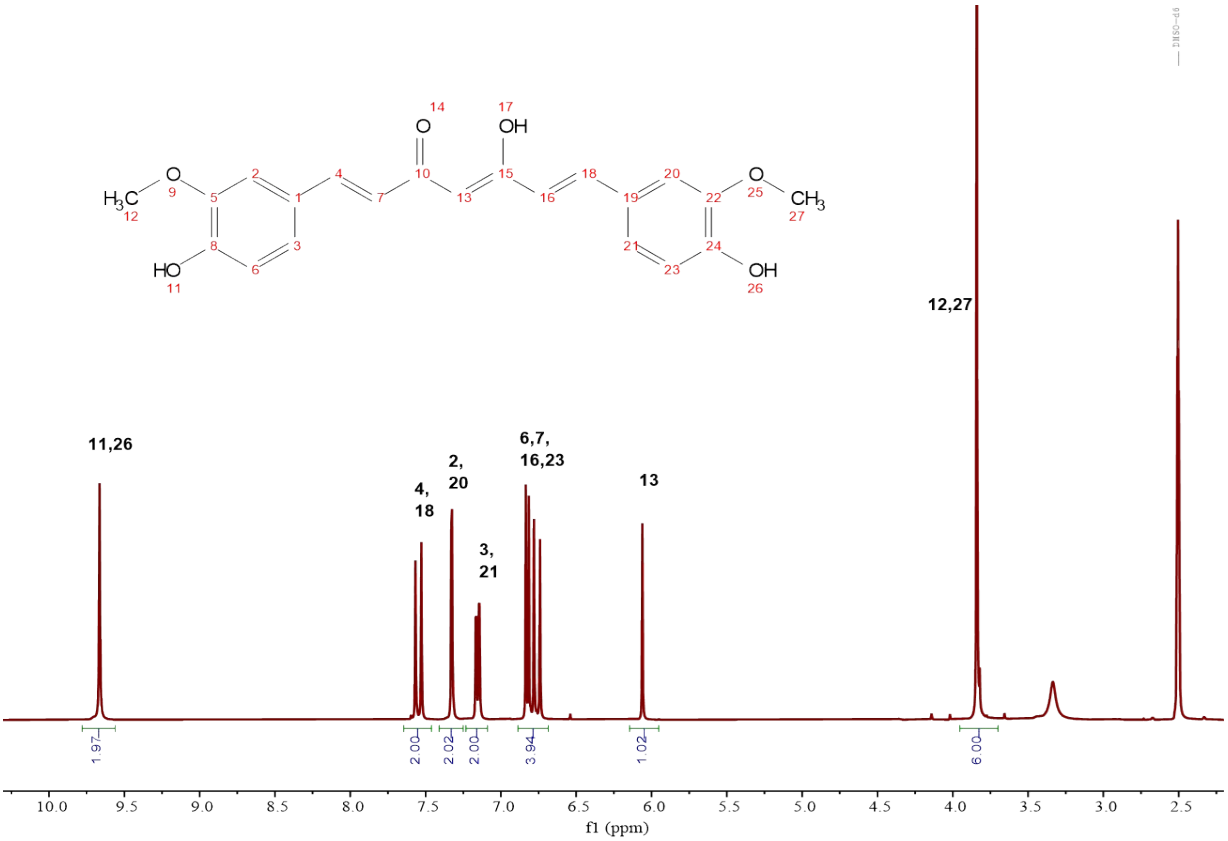

Figure S5. ^{1}H NMR (400 MHz, 298 K, DMSO-d_6) spectrum of curcumin. δ 9.66 (s, 2H), 7.55 (d, J = 15.8 Hz, 2H), 7.33 (d, J = 2.0 Hz, 2H), 7.15 (dd, J = 8.3, 2.0 Hz, 2H), 6.90 - 6.68 (m, 4H), 6.06 (s, 1H), 3.84 (s, 6H).

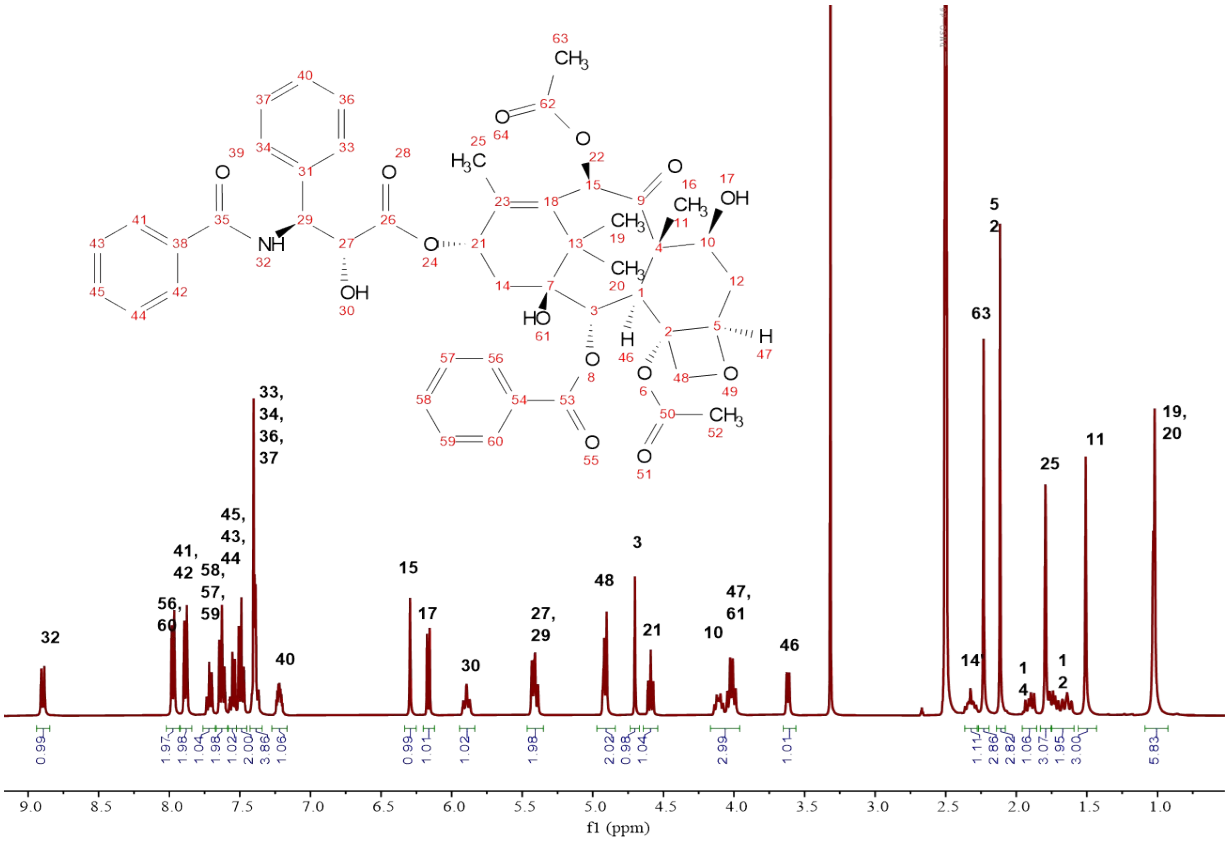

Figure S6. ^{1}H NMR (400 MHz, 298 K, DMSO-d_6) spectrum of PTX. δ 8.90 (d, J = 8.6 Hz, 1H), 8.02 - 7.93 (m, 2H), 7.92 - 7.84 (m, 2H), 7.71 (ddt, J = 6.8, 5.5, 2.7 Hz, 1H), 7.63 (dd, J = 8.2, 6.8 Hz, 2H), 7.58 - 7.52 (m, 1H), 7.52 -7.45 (m, 2H), 7.43 - 7.34 (m, 4H), 7.22 (tt, J = 5.7, 2.7 Hz, 1H), 6.29 (s, 1H), 6.16 (d, J = 7.6 Hz, 1H), 5.89 (t, J = 9.1 Hz, 1H), 5.47 - 5.35 (m, 2H), 4.91 (d, J = 7.1 Hz, 2H), 4.70 (s, 1H), 4.59 (t, J = 7.7 Hz, 1H), 4.17 - 3.96 (m, 3H), 3.62 (d, J = 7.2 Hz, 1H), 2.36 - 2.28 (m, 1H), 2.23 (s, 3H), 2.11 (s, 3H), 1.90 (dd, J = 15.4, 9.2 Hz, 1H), 1.79 (d, J = 1.4 Hz, 3H), 1.75 - 1.59 (m, 2H), 1.51 (s, 3H), 1.03 (d, J = 4.4 Hz, 6H).

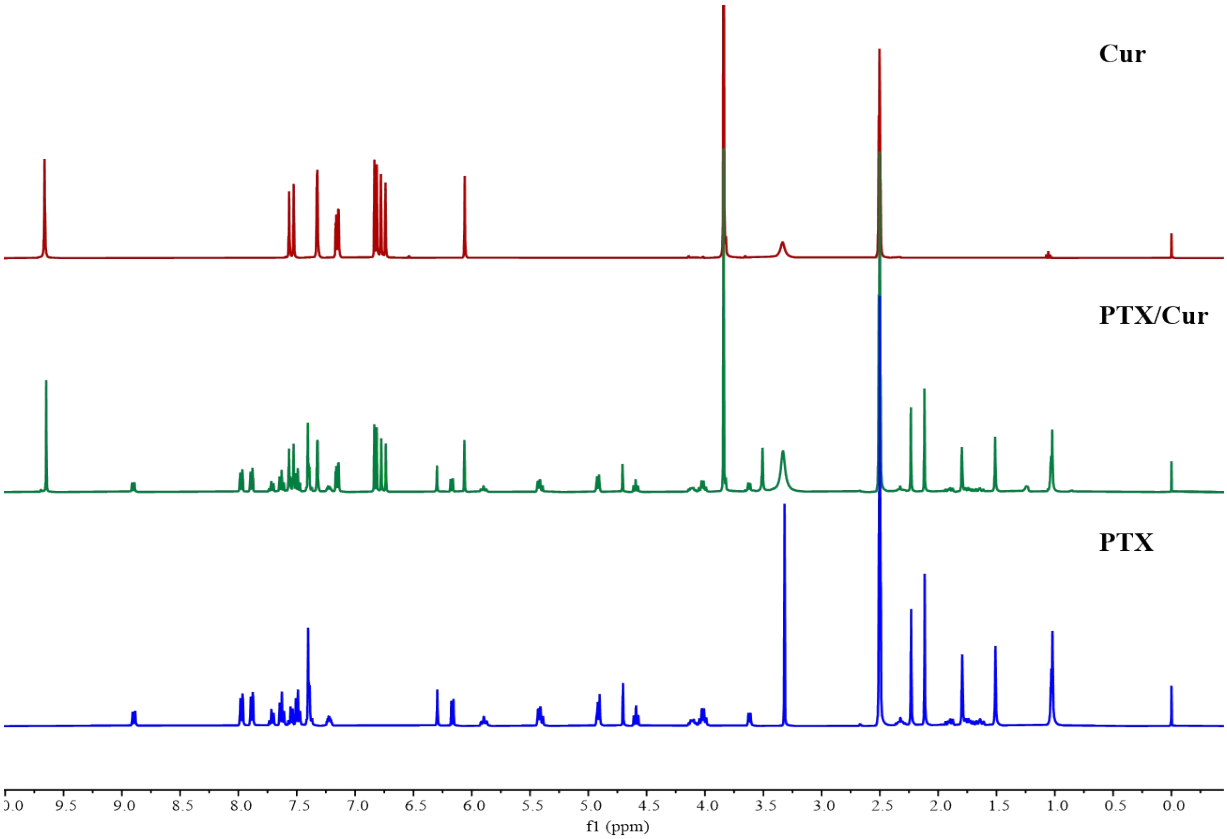

Figure S7. ^{1}H NMR (400 MHz, 298 K, DMSO-d_6) spectra of PTX and Cur before irradiation, and PTX/Cur after irradiation, respectively.

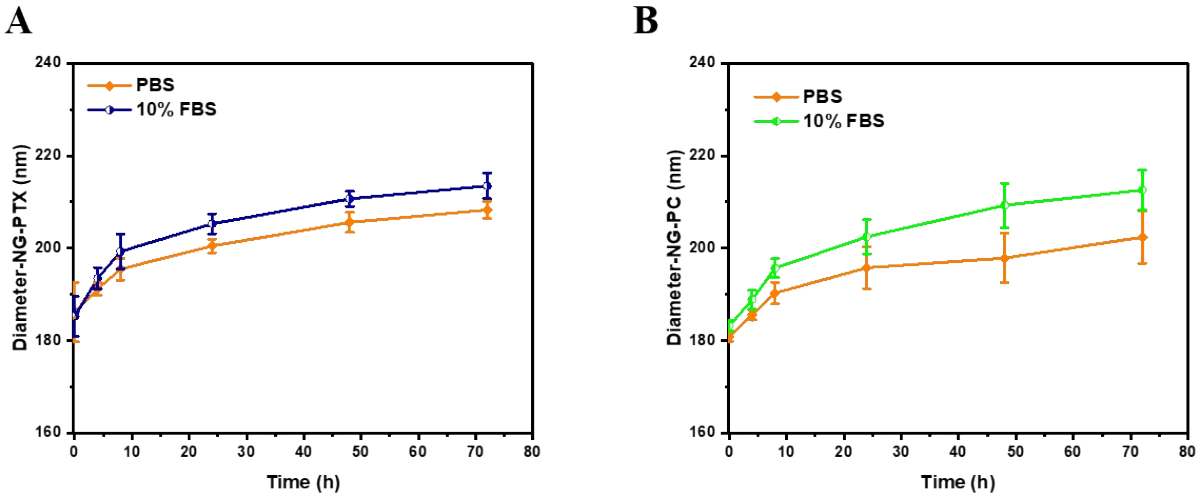

Figure S8. Size stability of (A) NG-PTX and (B) NG-PC incubated in PBS with or without 10 wt% FBS, respectively. The concentration of NG-PTX or NG-PC was 1 mg/mL. Results are expressed as means ± SD (n=3).

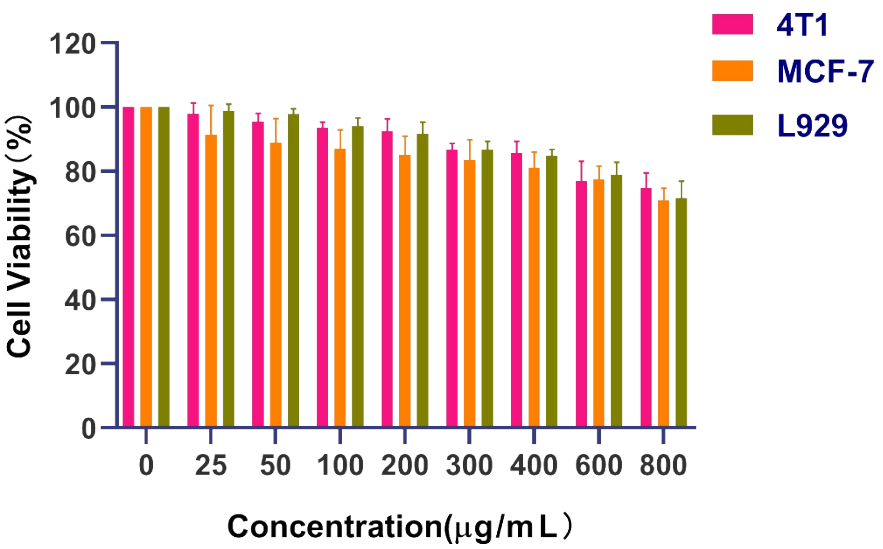

Figure S9. *In vitro* relative cell viability of NG-T in L929, 4T1 and MCF-7 cells after 24 h of incubation, respectively (n = 5).

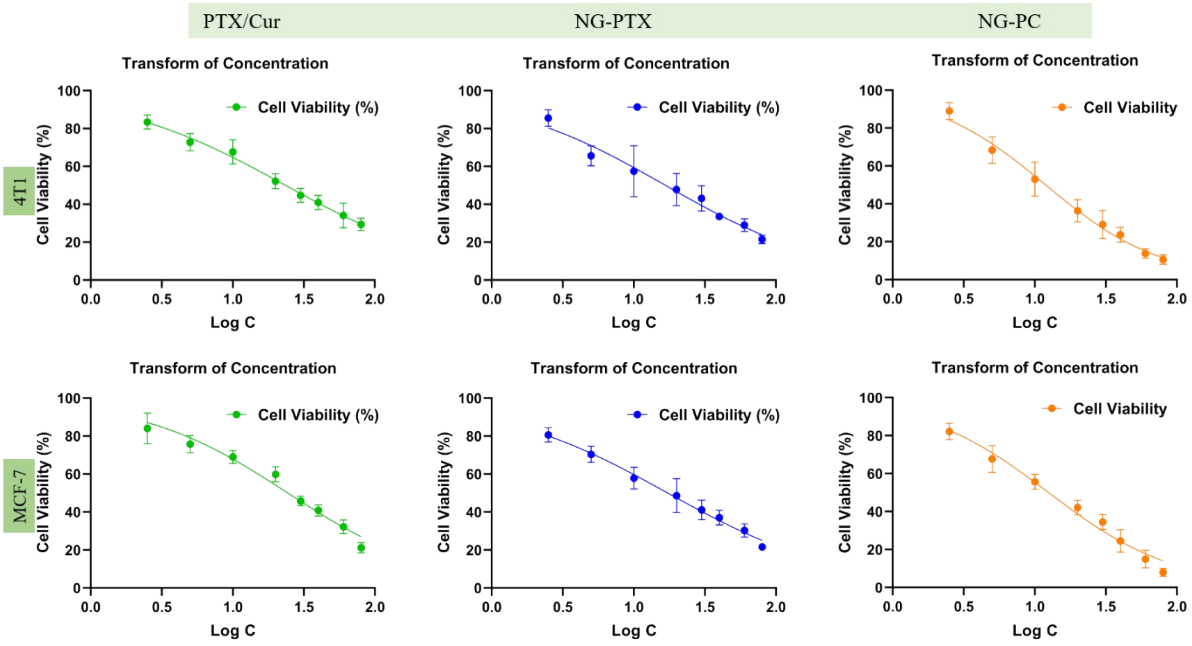

Figure S10. Curves of *in vitro* cell viability for calculating IC_{50} of PTX/Cur, NG-PTX and NG-PC in 4T1 and MCF-7 cells, respectively (n = 5).

Table S5. IC_{50} of PTX/Cur, NG-PTX and NG-PC in 4T1 and MCF-7 cells (panel to Figure S10)

Item	4T1 cell		MCF-7 cell	
	IC_{50} (μg/mL)	R^2	IC_{50} (μg/mL)	R^2
PTX/Cur	23.52 ± 1.047	0.9451	24.41 ± 1.048	0.9415
NG-PTX	16.73 ± 1.073	0.8898	17.34 ± 1.052	0.9393
NG-PC	11.93 ± 1.051	0.9523	12.50 ± 1.050	0.9524

Note: Data are represented as mean ± SD (n = 5).

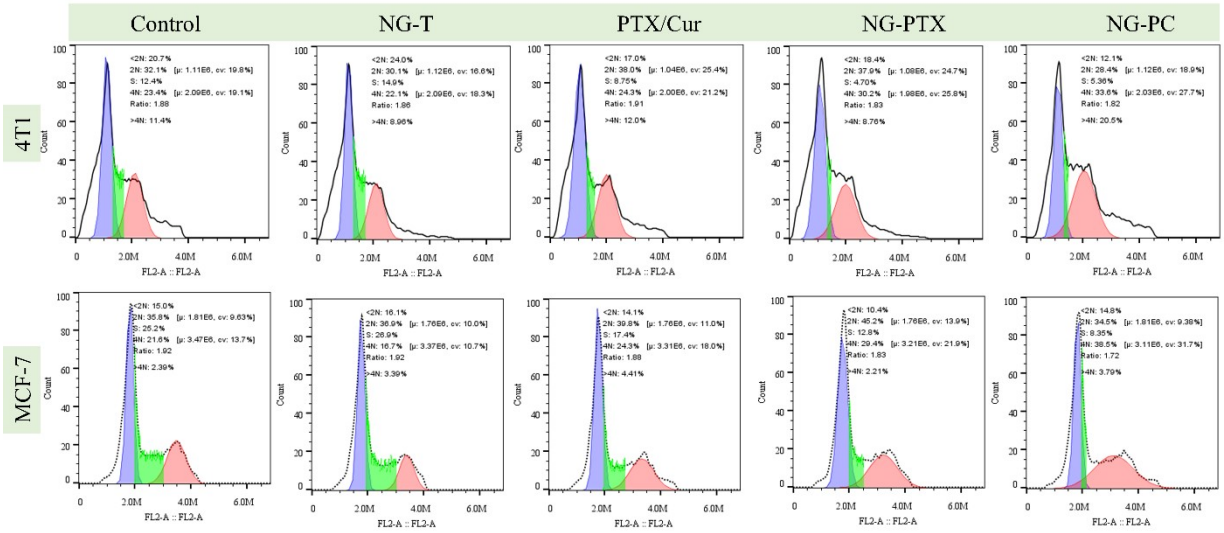

Figure S11. Representative histograms of cell cycle distribution in control, NG-T, PTX/Cur, NG-PTX and NG-PC groups in 4T1 or MCF-7 cells after treatment for 72 h, respectively.

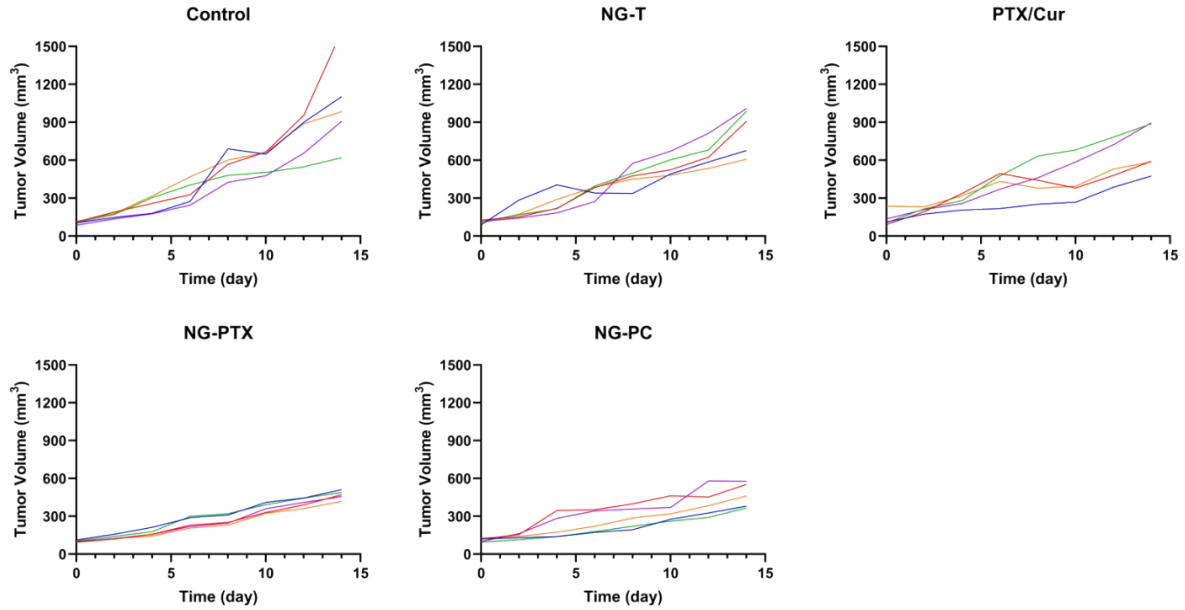

Figure S12. Individual tumor growth curves of 4T1 tumor-bearing mice after triple (on day 0, 4 and 8) injection of saline, NG-T, PTX/Cur, NG-PTX and NG-PC, respectively. (total dose: PTX= 7.5 mg·kg^{-1}, Curcumin = 7.5 mg·kg^{-1}, n = 5)

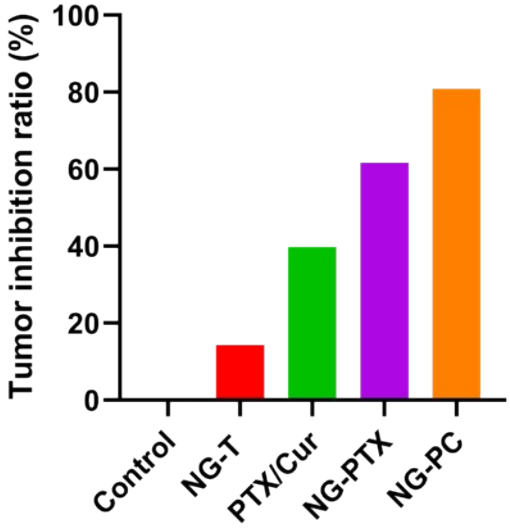

Figure S13. Histogram of tumor inhibition ratio after treatment with saline, NG-T, PTX/Cur, NG-PTX and NG-PC, respectively.