Supporting Information

Prodrug-based Cascade Self-assembly Strategy for Precisely Controlled Combination Drug Therapy

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Chemicals. Monomethoxy polyethylene glycol with a molecular weight of 1900 was purchased from Alfa Aesar. Camptothecin (CPT) was purchased from Sichuan Jiangyuan Natural Products Co. (China). 5-Fluorouracil (5-FU) was purchased from Shanghai Shaoyuan Co. Triphosgene was purchased from TCI (Shanghai) Development Co., Ltd. (China). 2-Hydroxyethyl disulfide (HEDS) was purchased from Alfa Aesar China (Tianjin) Co., Ltd. (China). α-cyclodextrin (α-CD, purity ≥ 98.0%) and 4-Dimethylaminopyridine (DMAP, purity > 99.0%) were purchased from Aladdin Chemical Co. Ltd. N,N′-dicyclohexylcarbodiimide (DCC) were purchased from Shanghai GL Biochem Ltd. (China). Dichloromethane (analytical reagent) was used after drying through CaH_2. All other reagents and solvents were of reagent grade or purified according to standard methods before use. Ultrapure water was obtained by a water purification system, which was purchased from Shanghai Laikie Instrument Co., Ltd.

Materials Characterization. NMR spectra were obtained on a Bruker AVANCE III-400 spectrometers using $CDCl_3$ as the solvent. Fluorescence spectra of CyNH-S-S-PEG in HEPES (pH 7.4, containing 10% DMSO as a co-solvent) were determined by Perkin-Elmer LS-55 fluorescence spectrophotometer with a slit width of 10.0 and 5.0 nm for excitation and emission. The morphologies of PEG-S-S-CPT prodrug micells were investigated on an FEI-Tecnai G2 Transmission Electron Microscope (TEM). The morphologies of self-assembled supramolecular hydrogel were investigated and analyzed on a JSM-5600LV electron microscope after the samples were freeze-dried and coated with gold vapor. The X-ray diffraction measurements of hydrogels were performed by a PHILP X'Pert PRO, using Cu Kα (λ = 1.542 Å) irradiation (40 kV, 40 mA) in the range of 2θ=5-80°.The rheological behavior of the hydrogels was investigated by a HAAKE RS6000 rotational rheometer using a 35 mm parallel-plate geometry at 20 °C. The gap distance between the two plates was fixed at 1 mm. Oscillating stress was fixed at 1 Pa for all dynamic tests. Fluorescence imaging experiments were performed on an Olympus Fluoview

1000 confocal laser scanning microscope with excitation at 633 nm. HPLC was performed on an Agilent ZORBAX SB-C18 column (4.6 × 150 mm, 5 μm) at 30 ℃ with methanol and 0.1% phosphoric acid aqueous solutions (68:32, v/v) as a mobile phase at a flow rate of 1.0 mL/min. A wavelength of 372 nm was used to detect PEG-S-S-CPT, and 265 nm to detect 5-FU.

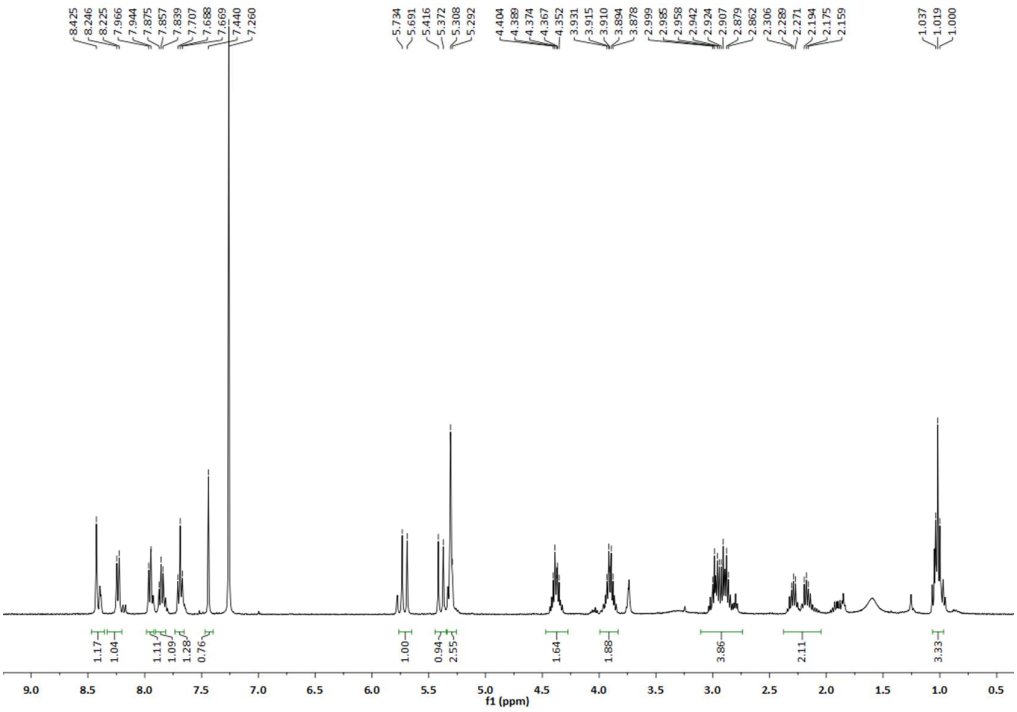

Figure S1. ^{1}H NMR spectrum of compound CPT-S-S-OH

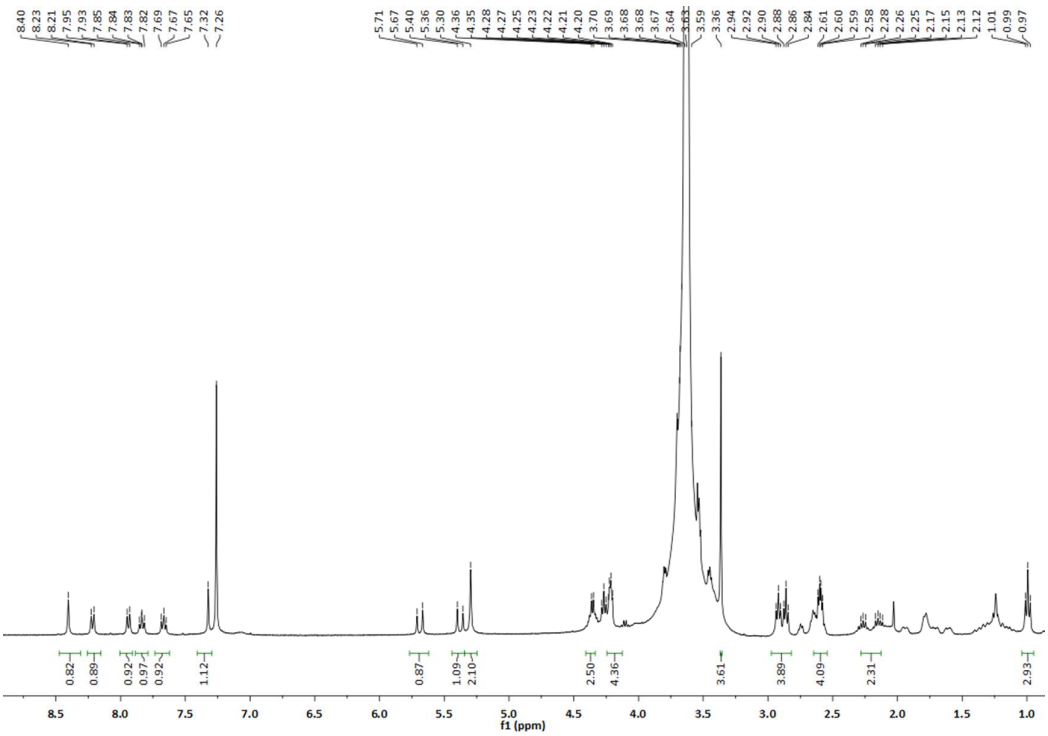

Figure S2. ^{1}H NMR spectrum of compound PEG-S-S-CPT

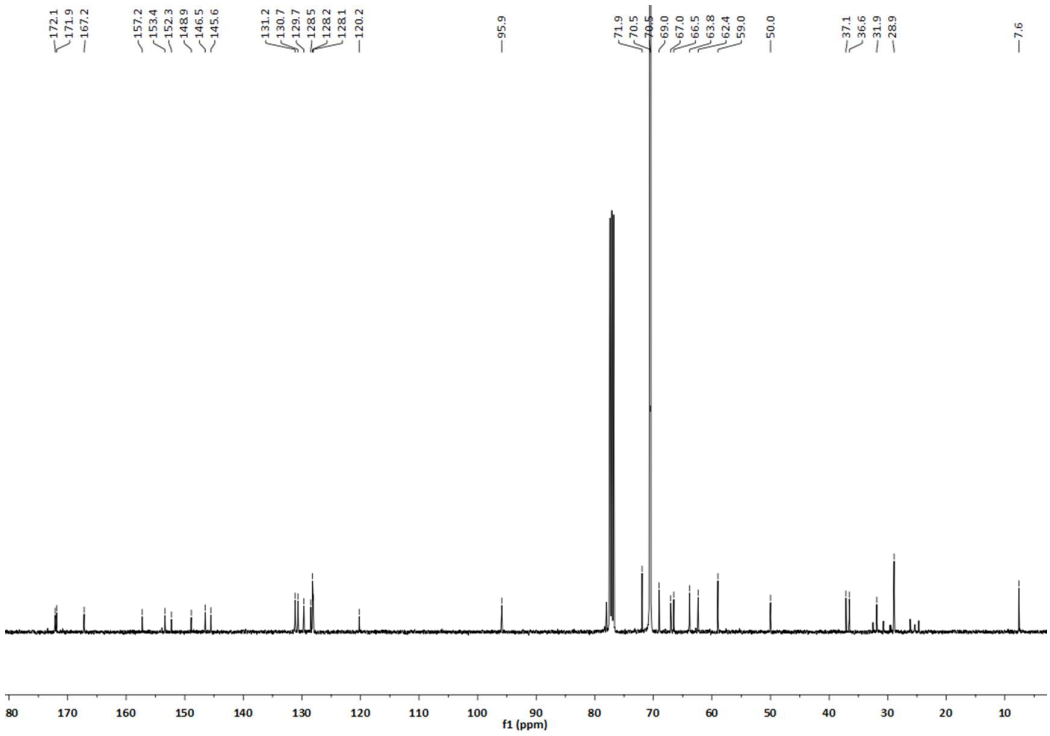

Figure S3. ^{13}C NMR spectrum of compound PEG-S-S-CPT

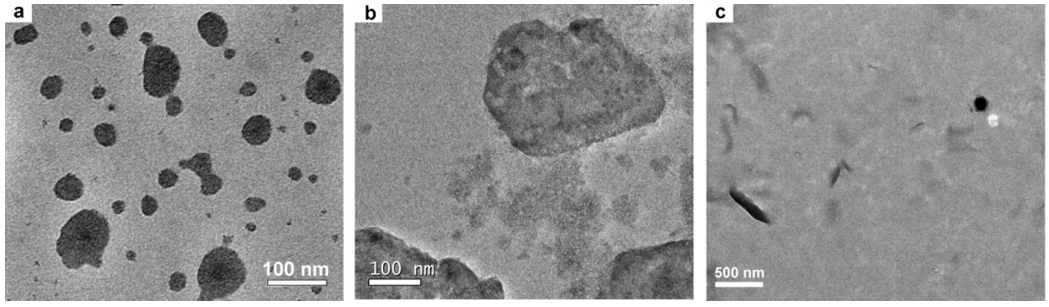

Figure S4. The TEM images of PEG-S-S-CPT prodrug micelles solution (a) and after adjusting the pH to 6.0 (b) or introducing 10 mM GSH (c) to the solution.

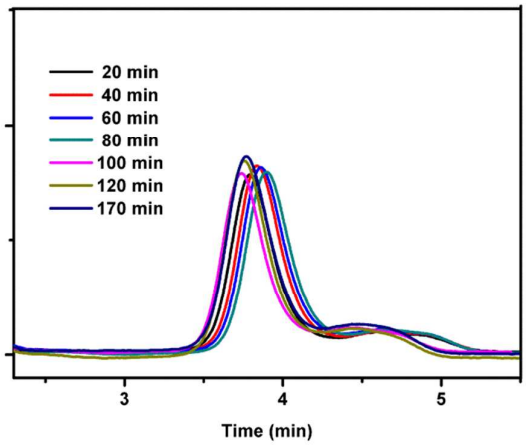

Figure S5. The HPLC curves of PEG-S-S-CPT prodrug micelles during the entirely release process.

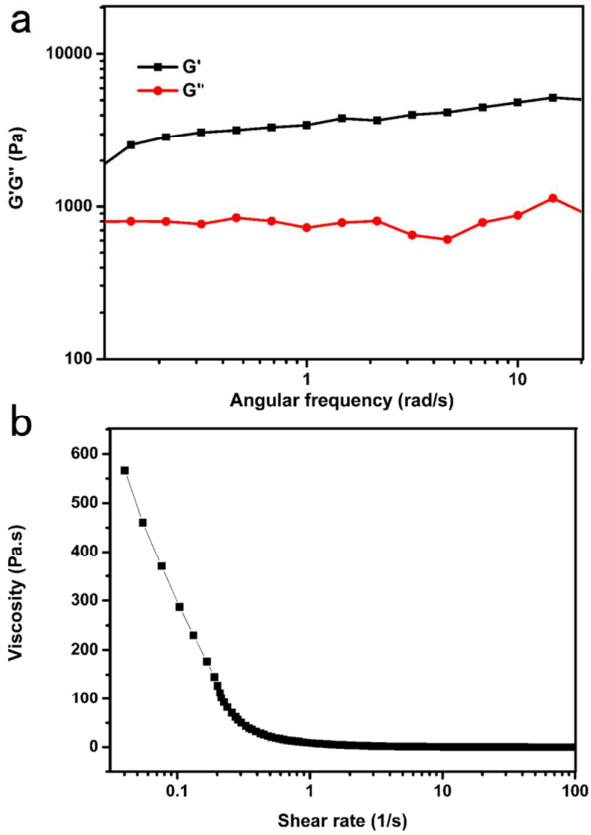

Figure S6. (1) Dynamic and (2) steady rheological behaviors of the supramolecular hydrogel made of PEG-S-S-CPT (20 mg/mL) and α-CD (100 mg/mL).

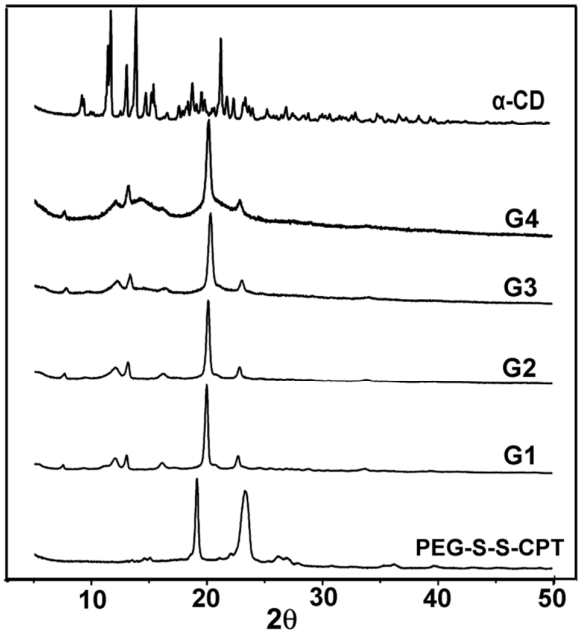

Figure S7. X-ray diffraction patterns for dried α-CD, PEG-S-S-CPT and G1-G4.

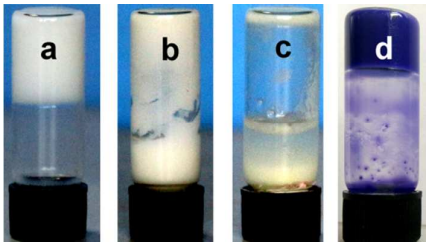

Figure S8. Optical photos of the PEG-S-S-CPT/α-CD supramolecular hydrogels before (a) and after introducing 10 mM GSH (b) or DTT (c). Optical photo of the CyNH-S-S-PEG (20 mg/mL)/α-CD (100 mg/mL) supramolecular hydrogels.

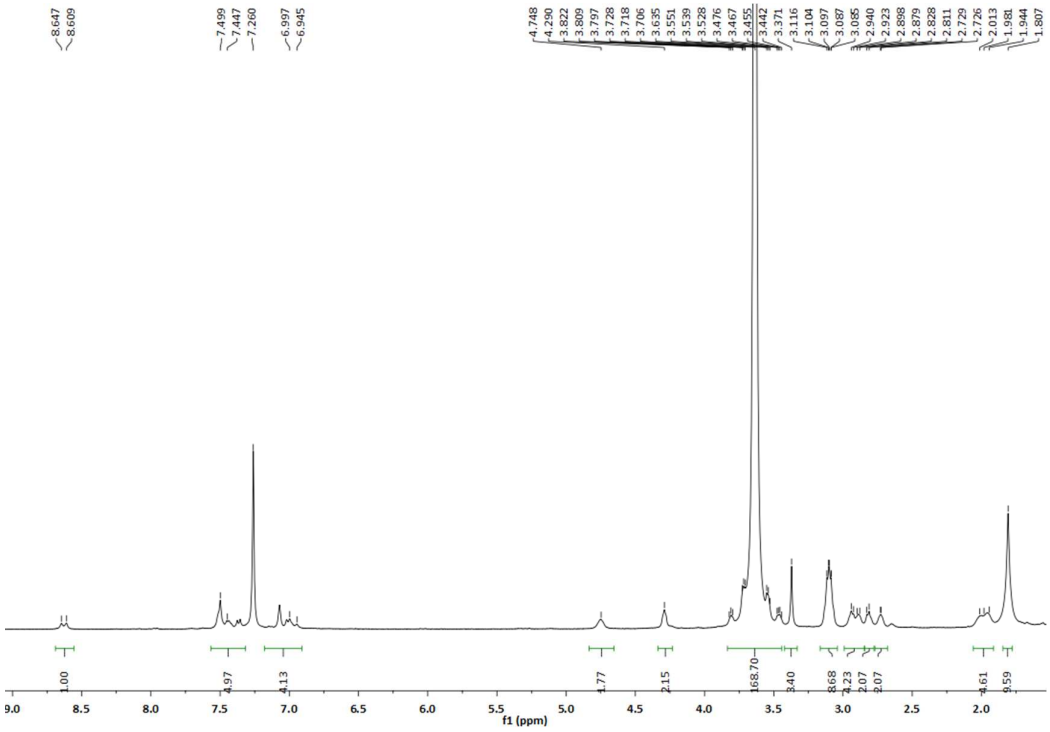

Figure S9. ^{1}H NMR spectrum of compound CyNH-S-S-PEG.

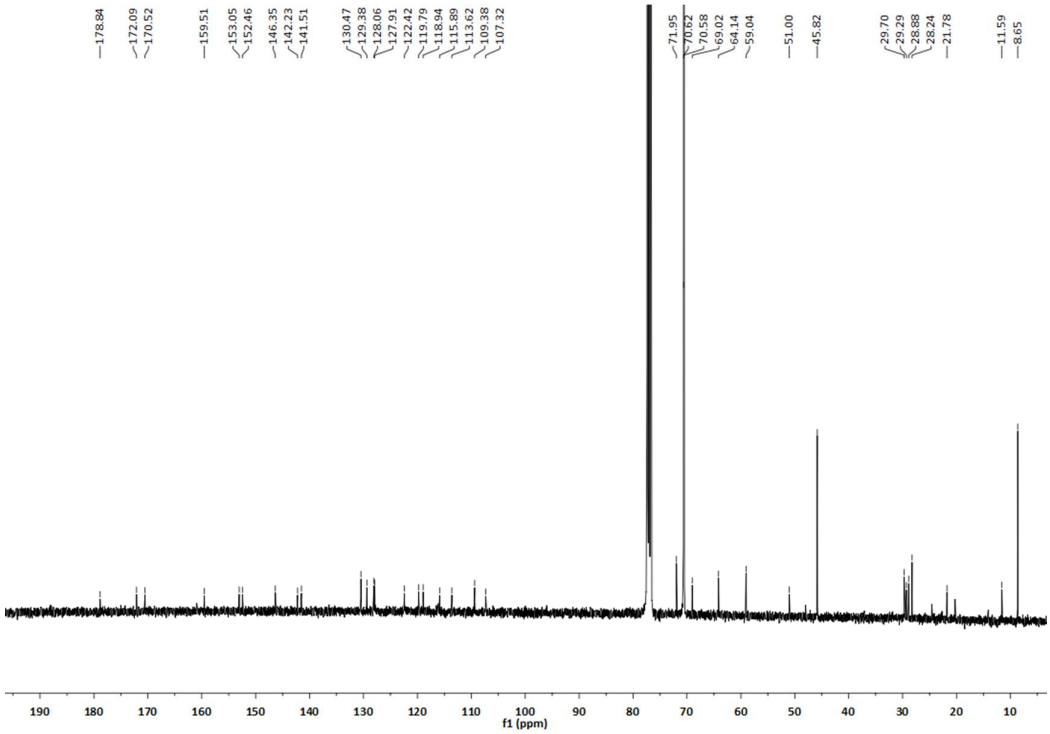

Figure S10. ^{13}C NMR spectrum of compound CyNH-S-S-PEG.

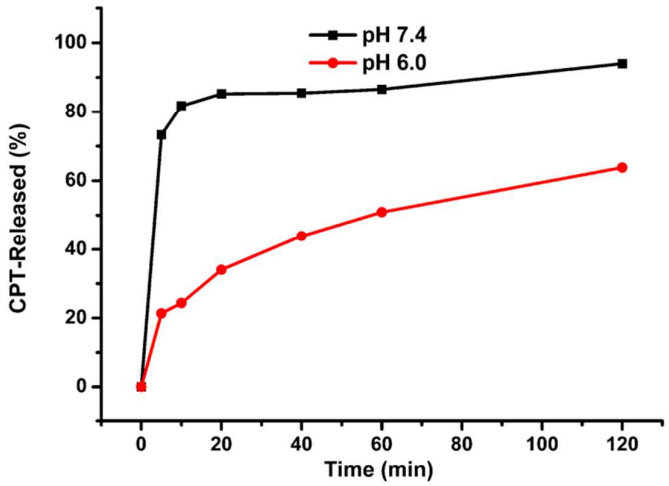

Figure S11. The CPT release kinetics of the released samples from hydrogel in the first stage with addition of 10 mM GSH.

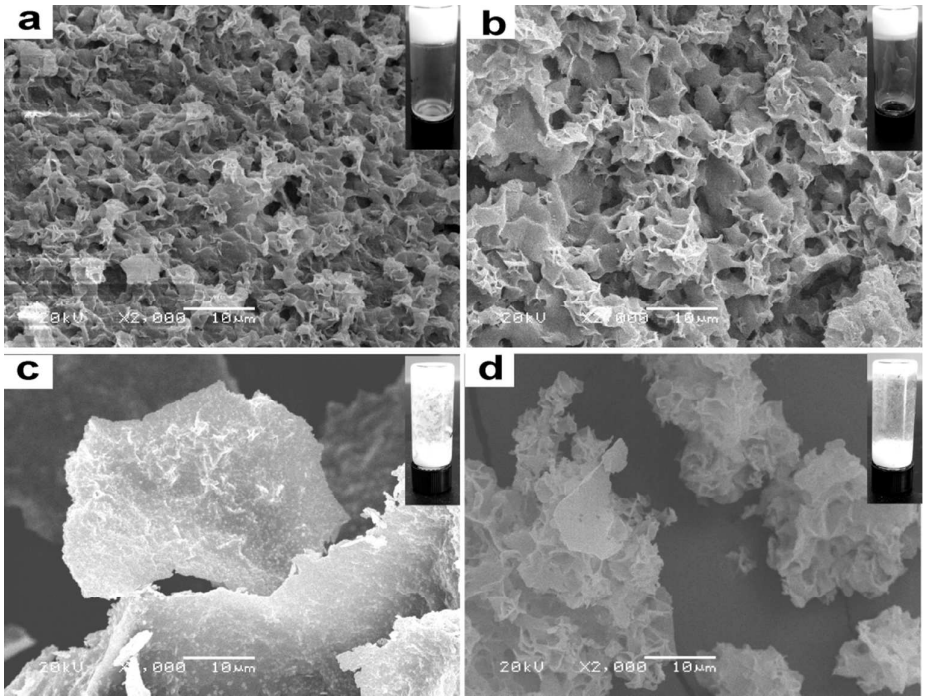

Figure S12. SEM images and optical photos of the 5-FU loaded hydrogel incubated for 20 h under different release conditions. (a) blank, (b) pH 7.4, (c) pH 6.0 and (d) in the presence of 10 mM GSH.

Table S1 Cytotoxicity activity of CPT, PEG-S-S-CPT and 5-FU in different method of administration against two human tumor cell lines

Method of Administration	drug	IC_{50}（μM）	
		HCT-116 cell	HL-60 cell
single modality treatment	CPT	0.07	0.01
	PEG-S-S-CPT	0.17	0.04
	5-FU	4.19	3.60
Drug combination	PEG-S-S-CPT	0.13	0.02
	5-FU	0.67	0.11

Median-effect principle for dose-effect analysis and the combination index studies: The multiple drug effect analysis based on the median-effect principle was used to examine drug interactions. This involves plotting dose effect curves for each agent and for multiple diluted, fixed ratio combinations of agents using the median effect equation:

$$f_a/f_u = (D/D_m)^m$$

In this equation, *D i*s the dose, D_m is the dose required for 50% effect (e. g. 50% inhibition of cell growth), f_a is the fraction effected by D, f_u is the unaffected fraction, (1-f_a), and m is the coefficient of sigmoidicity of the dose-effect curve; The dose-effect curve is plotted using a logarithmic conversion of this equation to: $\log^{f_a/f_u} = m\log^{(D)} - m\log^{(D_m)}$ for the median-effect plot:$x = \log^{(D)}$ versus $y = \log^{f_a/f_u}$, which determines the m (slope) and Dm (anti-log of x intercept) values. A combination index (*CI*) is then determined with the classic isobologram equation of Chou-Talalay:

$$CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2$$

Where $(D_x)_1$ is the dose of agent 1(PEG-S-S-CPT) required to produce× percentage effect alone and $(D)_1$ is the dose of agent 1 required to produce the same× percentage effect in combination with $(D)_2$. Similarly, $(D_x)_2$ is the dose of agent 2 (5-FU) required to produce× percentage effect alone and $(D)_2$ is the dose required to produce the same effect in combination with $(D)_1$. The denominators of the *CI* equation above, $(D_x)_1$ and $(D_x)_2$ can be determined by $D_x = D_m \left[f_a / (1 - f_a) \right]^{1/m}$. Different values of *CI* may be obtained for solving the equation for different values of f_a. *CI* values of <1 indicate synergy, >1 indicate antagonism and =1 indicates additive effect.

Table S2. Dose-effect relationship parameters for PEG-S-S-CPT and 5-FU in cancer model

Cell type	PEG-S-S-CPT			5-FU			(4:1) PEG-S-S-CPT:5-FU		
	D_m (μM)	*Linear equation*	*r*	D_m	*Linear equation*	*r*	D_m	*Linear equation*	*r*
HCT-116	0.17	y = 0.9125x + 0.7037	0.99	4.19	y = 1.5436x - 0.9608	0.97	0.13+0.67	y = 0.9099x + 0.0858	0.99
HL-60	0.04	2.0979x + 2.964	0.95	3.60	y = 1.2837x - 0.7147	0.93	0.02+0.11	y = 2.7809x + 2.4534	0.95

Shape (sigmoidicity) and conformity of dose-effect curve (linear correlation coefficient) are represented by *Dm*, linear equation, *r*, respectively, where D_m is the antilog of *x*-intercept in μM,*r* is the linear correlation coefficient of the median-effect plot.

The *CI* values were calculated by the Chou-Talalay method based on the median-effect equation and the classic isobologram equation.

Table S3. Interaction of PEG-S-S-CPT and 5-FU combinations in cells at different stage of carcinogenesis: combination indices at different effect levels

Cell type	Combination index (*CI*) at:								
	$f_{a0.1}$	$f_{a0.2}$	$f_{a0.3}$	$f_{a0.4}$	$f_{a0.5}$	$f_{a0.6}$	$f_{a0.7}$	$f_{a0.8}$	$f_{a0.9}$
HCT-116	0.82	0.85	0.87	0.89	0.92	0.93	0.98	1.07	1.20
HL-60	0.72	0.64	0.60	0.56	0.53	0.50	0.47	0.44	0.40

CI value <1, =1, >1 indicates synergism, additive effect, and antagonism, respectively. f_a is the fraction effected.