Supplementary Information

Gemcitabine-Camptothecin Conjugates: a Hybrid Prodrug for Controlled Drug Release and Synergistic Therapeutic

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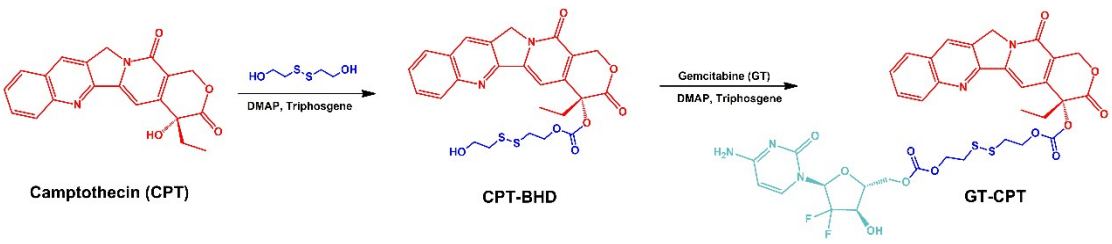

Figure S1. Synthetic route of GT-CPT prodrug.

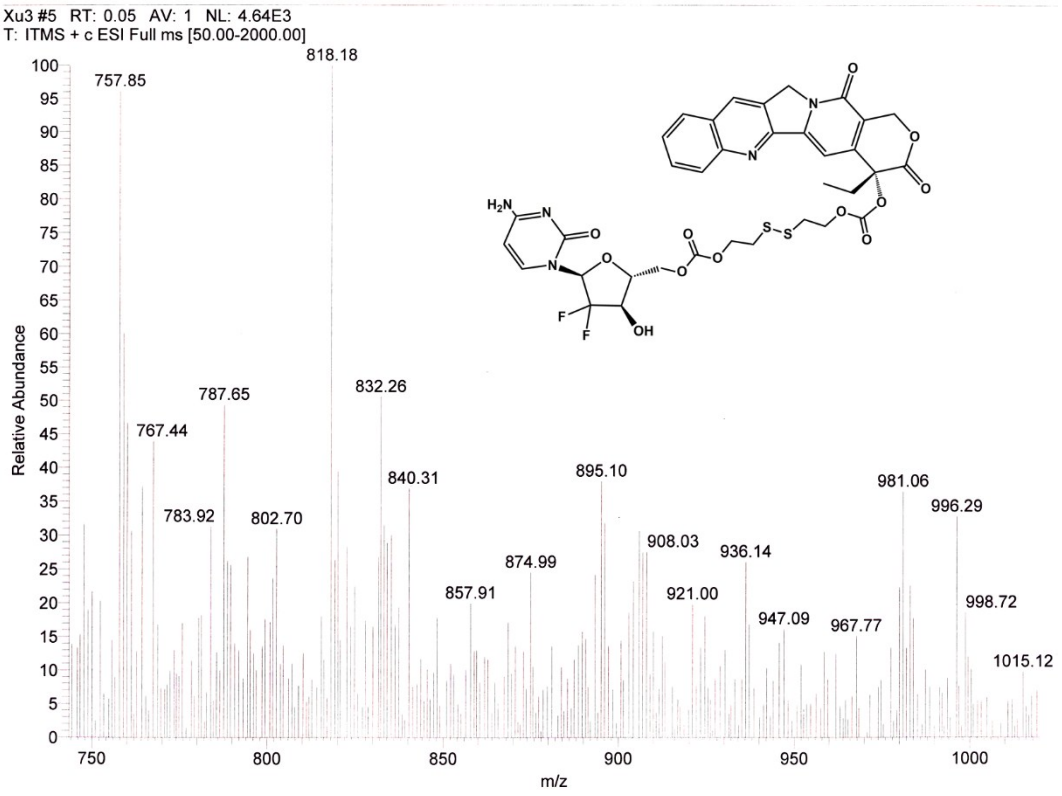

Figure S2. Mass spectra of GT-CPT prodrug.

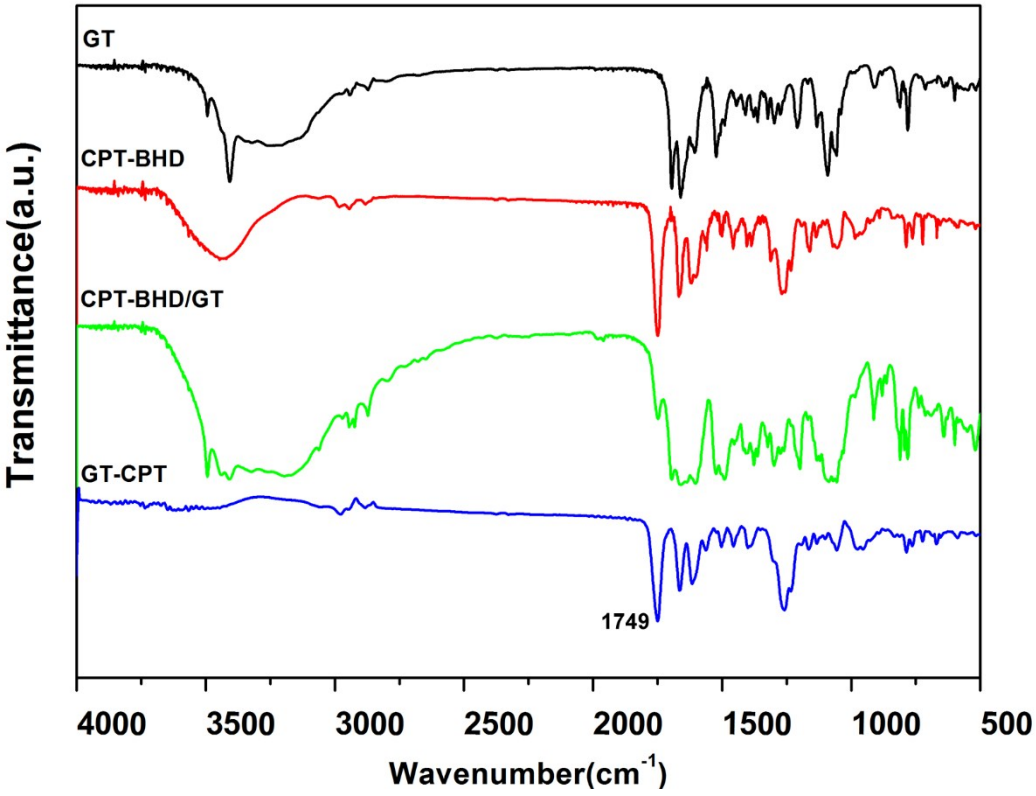

Figure S3. FTIR of GT, CPT-BHD, CPT-BHD/GT and GT-CPT prodrug.

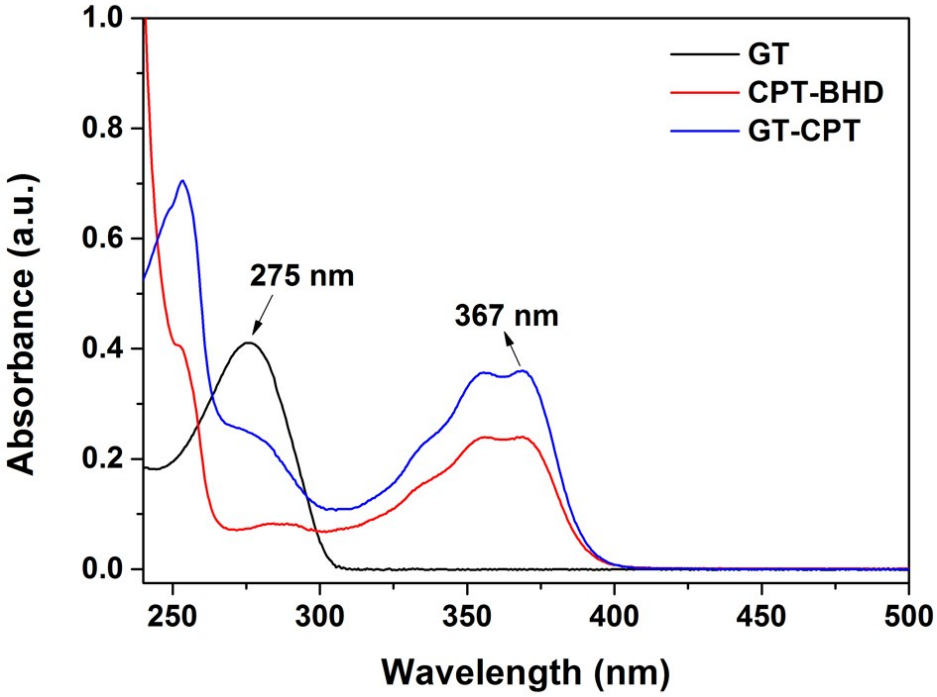

Figure S4. UV-vis spectroscopy of GT, CPT-BHD and GT-CPT.

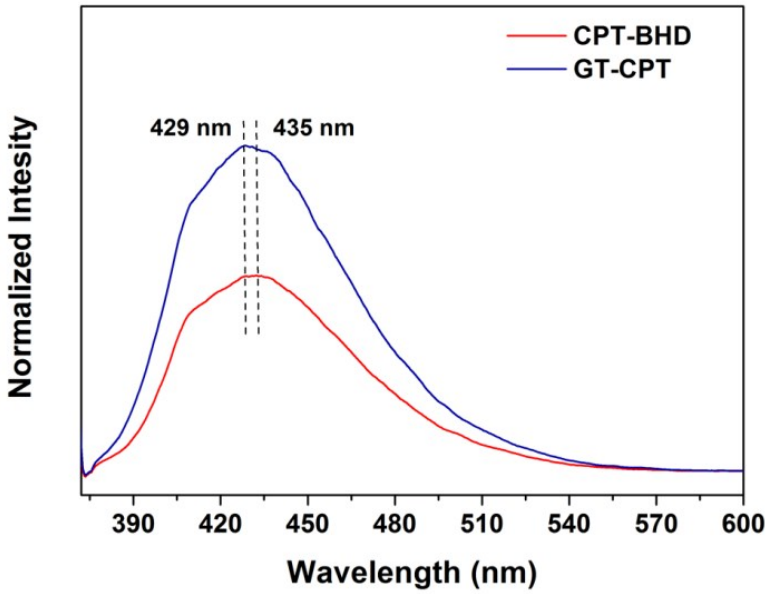

Figure S5. Fluorescence spectroscopy of CPT-BHD and GT-CPT in DMF.

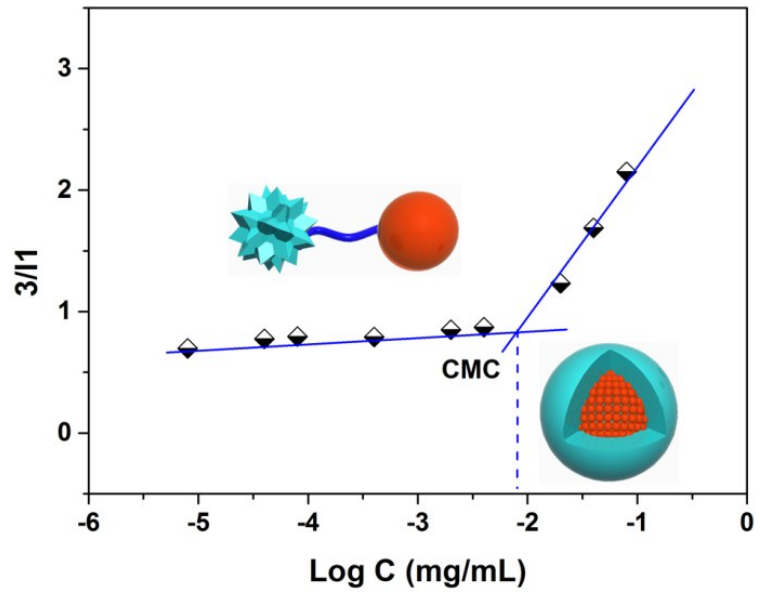

Figure S6. The fluorescence intensity ratio I_3/I_1 of pyrene as a function of GT-CPT concentration. The critical aggregation concentration (CAC) in aqueous medium was determined as approximately 7.94 μg/mL.

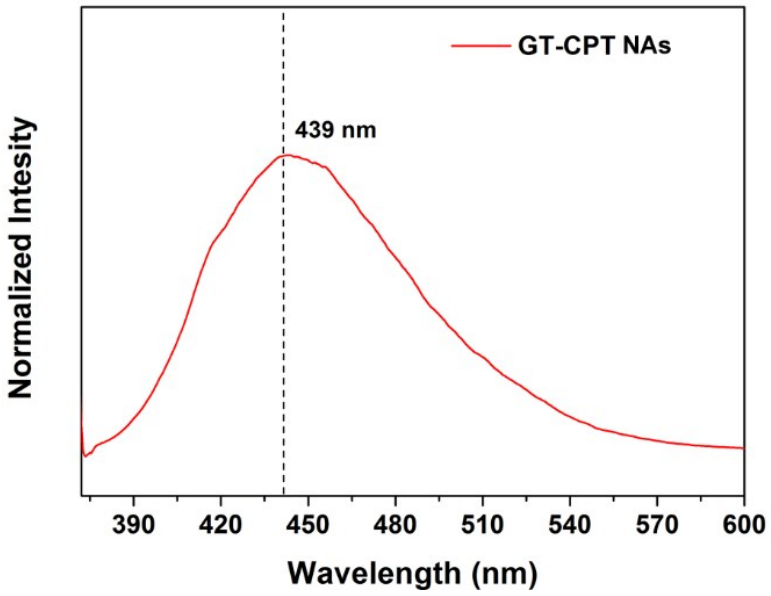

Figure S7. Fluorescence spectra of GT-CPT NAs in water.

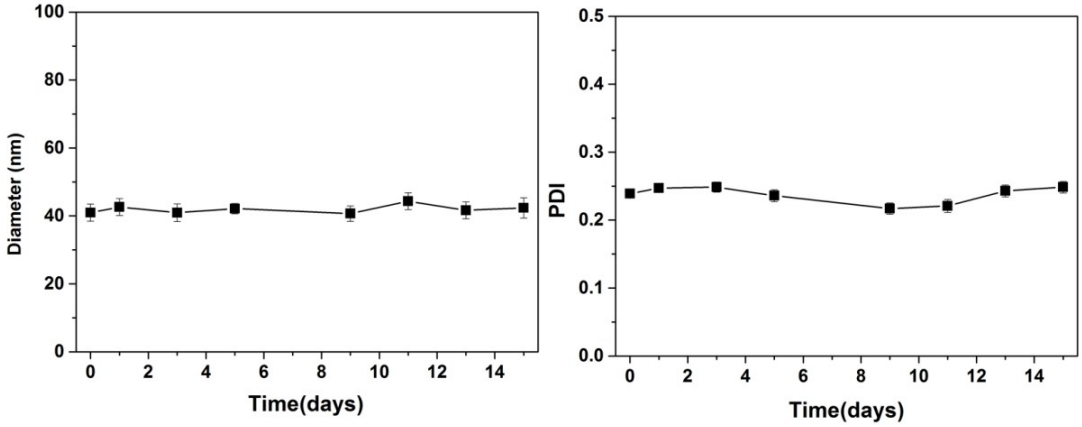

Figure S8. The stability of GT-CPT NAs stored at room temperature for two weeks. The average diameter and PDI were determined at different time points (0, 1, 3, 5, 9, 11, 13 and 15 days). Data are presented as mean ± SD (n = 3).

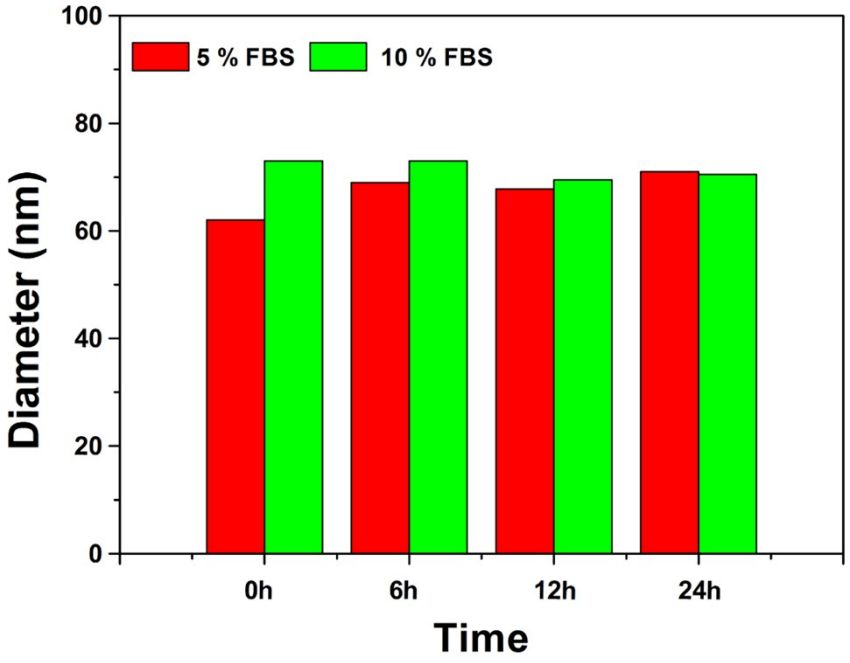

Figure S9. The size of GT-CPT NAs in PBS (pH = 7.4) containing 5 % or 10 % FBS at different time points.

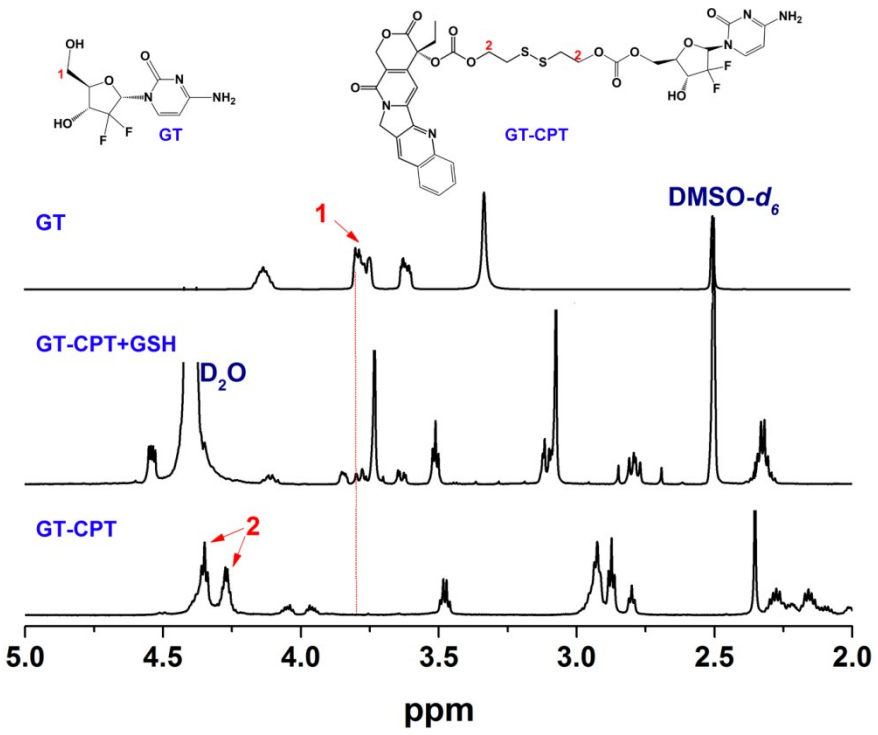

Figure S10. ^{1}H NMR of GT, GT-CPT and GT-CPT NAs treated with 10 mM GSH for 12 h.

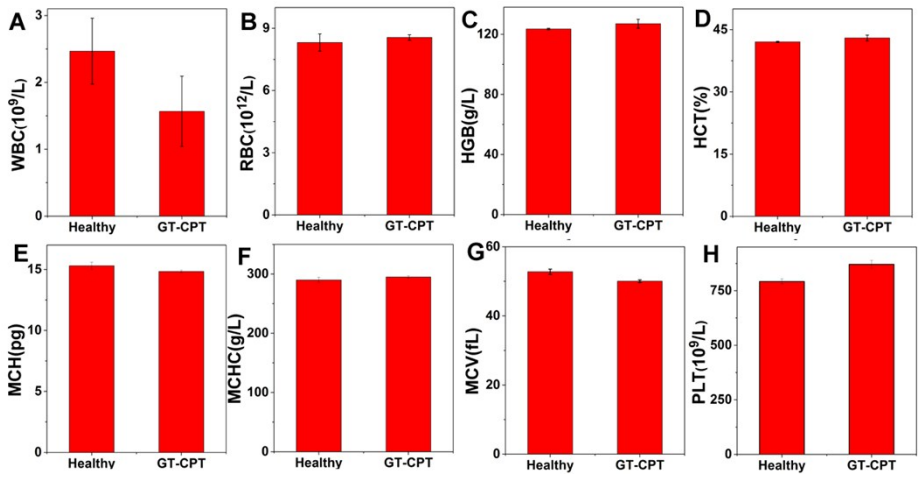

Figure S11. The blood test on the treated mice and healthy controls for: white blood cells (WBC, A), red blood cells (RBC, B), haemoglobin (HGB, C), haematocrit (HCT, D), mean corpuscular haemoglobin (MCH, E), mean corpuscular haemoglobin concentration (MCHC, F), mean corpuscular volume (MCV, G), and platelets (PLT, H). Data were shown as means ± SD (n = 3).

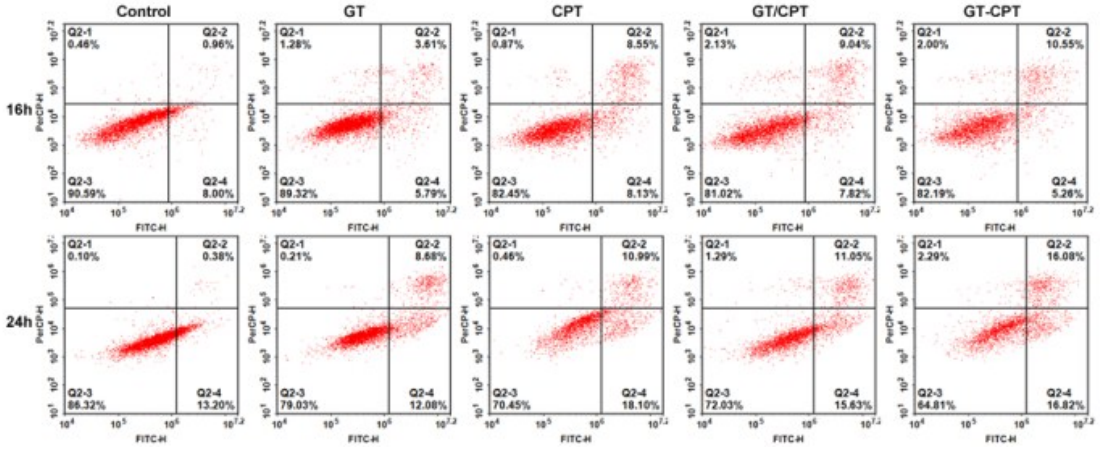

Figure S12. Apoptosis analysis of MCF-7 cells treated with free CPT, free GT, GT/CPT mixture and GT-CPT NAs for 16 h and 24 h. In each flow cytometry plot, the upper right quadrant represents the late stage of apoptotic cells, the percentages in each quadrant represent the percentage of cells in the corresponding stage.

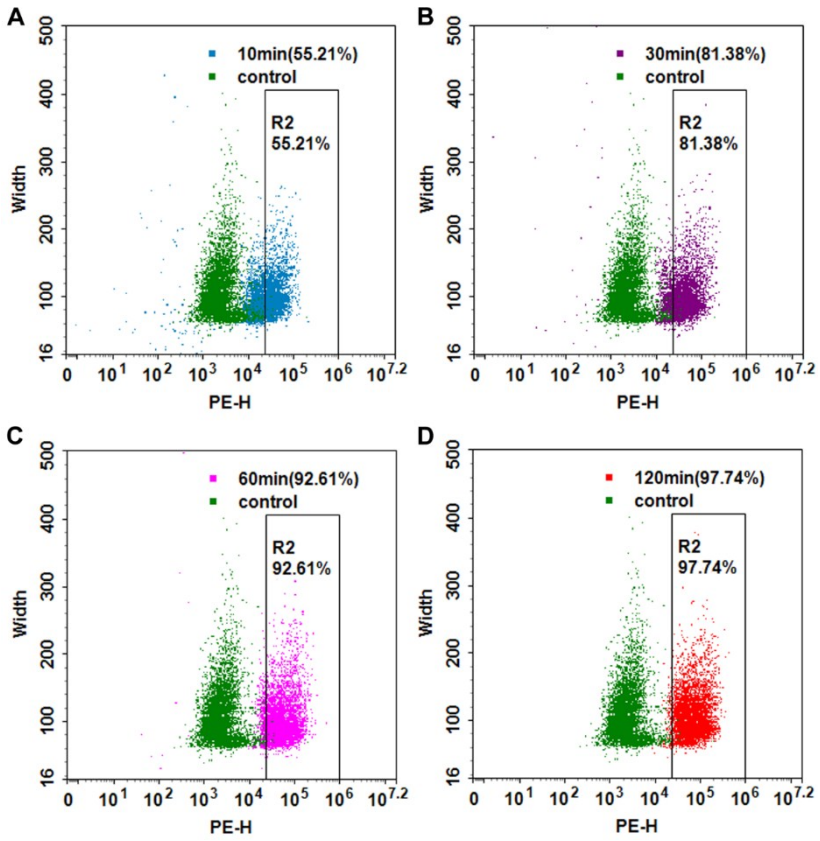

Figure S13. Flow cytometry analysis of HeLa cells after treatment with Nile red-labelled GT-CPT NAs for (A) 10 min, (B) 30 min, (C) 60 min and (D) 120 min.

Table S1. Combination index (CI) value for GT/CPT mixture and GT-CPT NAs incubated with HeLa cells at different time.

Sample (HeLa cells)	IC_{50}(24h) μM	IC_{50}(48h) μM	IC_{50}(72h) μM
Free CPT	37.28	2.96	0.23
Free GT	>50	49.14	8.75
CPT/GT mixture	24.30	2.25	0.33
GT-CPT	8.96	0.24	0.19
CI (GT/CPT)	<0.57	0.40	0.73
CI (GT-CPT)	<0.27	0.06	0.30

Table S2. Combination index (CI) value for GT/CPT mixture and GT-CPT NAs incubated with MCF-7 cells at different time.

Sample (MCF-7 cells)	IC_{50}(24h) μM	IC_{50}(48h) μM	IC_{50}(72h) μM
Free CPT	6.42	2.48	0.84
Free GT	>50	>50	12.45
CPT/GT mixture	19.11	1.50	0.47
GT-CPT	10.72	0.82	0.24
CI (GT/CPT)	<1.69	0.32	0.30
CI (GT-CPT)	<0.39	0.28	0.27

*Note: CI < 1, indicating a synergistic effect; CI = 1, indicating an additive effect; CI > 1, indicating an antagonism effect.