Supporting information

Molecular Recognition-Driven Supramolecular Nanoassembly of Hydrophobic Uracil Prodrug and Hydrophilic Cytarabine for Precise Combination Treatment of Solid and Non-solid Tumors

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Experimental Section

Materials

Ara-C, DOX, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) and DAPI was purchased from Dalian Meilun Biotechnology Co., Ltd, China. The raw materials for the synthetic prodrugs were purchased from Shanghai Bide pharmaceutical technology Co., Ltd. 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2k})was bought from Shanghai Advanced Vehicl Technology Co. Ltd, China. Reduced glutathione assay kit was obtained from Nanjing Jiancheng Bioengineering Institute. Annexin V-FITC Apoptosis Assay Kit was purchased from Solarbio Technology Co. Ltd. (Beijing, China). Cell culture medium, penicillin-streptomycin and fetal bovine serum were obtained from GIBCO, Invitrogen Corp. (Carlsbad, California, USA). Cell-culture dishes were commercially available from NEST Biotechnology (Wuxi, China). Cellular GSH Detection assay kit was purchased from Nanjing Jiancheng Bioengineering Institute. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma-Aldrich, USA. Dithiothreitol (DTT) was purchased from Aladdin, Shanghai, China. Other regents and chemicals applied in the article were all analytical or HPLC grade.

Synthesis of prodrugs (U-SS-DOX)

Synthesis of Cmpd 3:

Cmpd 1 was dissolved in DMF with *Cmpd 2***,** then a small amount of NaOH was added to form an alkaline environment. After reaction, the crude product *Cmpd 3* was recrystallized and purified. (Yield：90%)

Synthesis of Cmpd 4:

Cmpd 3 and 2,2'-dithiobisacetic acid anhydride were dissolved in 100 ml THF, then DIPEA was added and stirred at rt for 4 h. Then contrated under high vacumn to afford crude *Cmpd 4***.** It was purified by semi-preparative HPLC to aford 2 g white soild product. (Yield：23%)

Synthesis of Cmpd 6:

Cmpd 4 and *Cmpd 5* were fully dissolved in 40 ml DMF, then HATU was added. The reaction

mixture was stirring under r.t. for 2 h. The reaction mixture was concentrated under the vacuum, the crude product to purified by semi-preparative HPLC to aford 500 mg heme soild product. (Yield：29%)

Optimum proportion screening of U:C NPs

In order to verify the effect of hydrogen bonding force on the performance of nanoparticles, nine molar ratios of U-SS-DOX and Ara-C were mixed in the same method as the part of ***Preparation and characterization of U:C NPs***. The mean size and (polydispersity index) PDI of the supramolecular nanoassembly were measured using Zetasizer (Nano ZS, Malvern Co., UK), and the measurements were repeated in triplicate.

Preparation and characterization of U:C NPs

Briefly, 6.875 mg of U-SS-DOX, 1 mg of Ara-C and DSPE-PEG_{2k} (20%, w/w) were dissolved in 1 mL dimethyl sulfoxide (DMSO) in ep tubes. The solution was ultrasonic dissolved at room temperature for 5 min. 50 μL of sodium hydroxide solution (2 mg/mL) was added to 2 mL deionized water. The mixture solution (128 μL) was added dropwise into deionized water under stirring (800 rpm). After stirred for 20 min, the solution was centrifuged at 3000 rpm for 10 min by ultrafiltration centrifuge tube to remove free drugs. Finally, the co-assembled U:C NPs was obtained. U-SS-DOX, Ara-C and U:C NPs were scanned by UV-Vis at 200~800 nm. The size, size distribution, and zeta potential of the U:C NPs were measured using Zetasizer (Nano ZS, Malvern Co., UK), and the measurements were repeated in triplicate. Additionally, the morphology of the nanoparticles was observed by JEOL 100CX II transmission electron microscopy (TEM) (JEOL, Japan). Samples were stained with 1% hosphotungstic acid. In order to study the hydrogen bond interaction between U-SS-DOX and Ara-C in U:C NPs, different concentrations of urea were used to treat non-PEGylated U:C NPs.

Preparation and characterization of DiR-loaded U:C NPs.

The DiR-loaded U:C NPs were prepared similarly by co-assembling DiR with the mixture in aqueous medium. To be specific, 6.875 mg of U-SS-DOX, 1 mg of Ara-C, 156 μL of DiR ethanol solution (10 mg/mL) and DSPE-PEG_{2k} (20%, w/w) were dissolved in 1 mL dimethyl sulfoxide

(DMSO) in ep tubes. The solution was ultrasonically dissolved at room temperature for 5 min. 50 μL of sodium hydroxide solution (2 mg/mL) was added to 2 mL deionized water. The mixture solution (128 μL) was added dropwise into deionized water under stirring (800 rpm). The size, size distribution of the DiR-loaded U:C NPs were measured using Zetasizer (Nano ZS, Malvern Co., UK), and the measurements were repeated in triplicate. The morphology of the nanoparticles was observed by JEOL 100CX II TEM (JEOL, Japan). Samples were stained with 1% hosphotungstic acid.

Molecular docking simulations

The structure of U-SS-DOX and Ara-C was created using ChemBioDraw Ultra 14.0. The 3D structures of were built by optimizing containing the structural minimization and the structural dynamics optimization with the Sybyl 6.9.1 software (Tripos Associates: St.Louis, MO, 2003). The parameters of optimizing were: Energy Change 0.005 kcal/mol and Max Iterations 10000, and assigned charges using the Gasteiger–Huckel method and minimized with the Powell method (Tripos force field) to an energy change of 0.005 kcal/(mol*Å) (Powell, 1977). All other parameters were maintained at the default values. Complexes between U-SS-DOX and Ara-C were predicted by molecular docking using AutoDock 4.0 software. The optimized AutoDocking parameters were as follows: the maximum number of energy evaluations was 25,000,000 per run; the iterations of Solis and Wets local search were 3000; the number of generations was 100, and the number of individuals in population was 300. Results differing by less than 2 Å in a positional root mean square deviation were clustered together.

In vitro drug release study

The *in vitro* release of Ara-C and DOX-SH from U:C NPs was determined by PBS (pH 5.5, 6.5 or 7.4) containing DMSO (10% v/v%) as the release medium. The U:C NPs were placed in dialysis bag and incubated with 30 mL release medium with (10 mM) or without (0 mM) DTT at 37°C. At predetermined timepoints, 1 mL release medium was removed and 1 mL fresh medium was added. The concentration of Ara-C and DOX-SH was determined by HPLC at the absorbance of 272 nm and 232 nm, respectively. The mobile phase system of DOX was methanol: distilled

water containing 10 mM $NH_4H_2PO_4$ plus 0.1% glacial acetic acid = 70:30. The mobile phase system of Ara-C was methanol: distilled water containing 10 mM Na_2HPO_4 and 5 mM NaH_2PO_4 = 20:80.

Activation kinetics of DOX-SH from U-SS-DOX and U:C NPs

The activation kinetics of DOX-SH from U-SS-DOX and U:C NPs was determined by PBS (pH 7.4) containing DMSO (10% v/v%) as the release medium. The U-SS-DOX and U:C NPs were placed in dialysis bag and incubated with release medium with 10 DTT at 37℃, respectively. The activation of DOX-SH from U-SS-DOX and U:C NPs was measured every 0.5 h by HPLC.

Cell culture

MCF-7 cells (a human breast adenocarcinoma cell line), L1210 cells (a mouse lymphocytic leukemia cell line) were grown in Dulbecco's Modified Eagle's medium (DMEM) and 4T1 cells (a mouse breast cancer cell line) were grown in 1640 RPMI medium supplemented with 10% (v/v) FBS and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell uptake of U:C NPs

The cell uptake of U:C NPs was investigated in 4T1 cancer cells by flow cytometry and confocal laser scanning microscopy (CLSM) respectively.

HPLC: 4T1 cells were seeded into Petri dish at 1.0×10^6 cells per well in 1 mL of complete 1640 RPMI and incubated for 24 h at 37 °C. Then, the same molar concentration of free U-SS-DOX and Ara-C dissolved in fresh serum-free culture medium were added into different dishes. The cells were further incubated for 12, 24, 48 and 72 h, respectively. After that, the culture medium was removed and the 4T1 cells were rinsed with cold PBS, treated with trypsin and prepared for HPLC analysis.

Flow cytometry: 4T1 cells were seeded into 12-well plates at 1.0×10^5 cells per well in 1 mL of complete 1640 RPMI and incubated for 24 h at 37 °C. Then, the free DOX, free U-SS-DOX and U:C NPs dissolved in fresh serum-free culture medium were added into different wells at the equivalent DOX concentration (10 μg/mL) and the cells were further incubated for 1, 2, 4 and 6 h,

respectively. After that, the culture medium was removed and the 4T1 cells were rinsed with cold PBS, treated with trypsin and prepared for flow cytometry analysis. Data for 1.0×10^4 gated events were collected and analyzed by means of FlowJo software.

CLSM: 4T1 cells were seeded into 12-well plates with the slides at a density of 1.0×10^5 cells per well in 1 mL of complete 1640 RPMI and incubated for 12 h at 37°C. Subsequently, the culture medium was removed and replaced with fresh 1640 RPMI culture medium containing the free DOX, free U-SS-DOX and U:C NPs at the equivalent DOX concentration (10 μg/mL). The cells were further incubated at 37 °C for 2 and 6 h, respectively. After that, the 4T1 cells were washed with cold PBS for three times and fixed with 4% paraformaldehyde for 10 min at room temperature. Thereafter, the cells were washed with PBS and the nucleus were stained with hoechst for 10 min. The slides were mounted and the fluorescence of cells was visualized with a CLSM (TCS SP2/AOBS, LEICA, Germany).

Multimode Reader: 4T1 cells were incubated with the free DOX, U-SS-DOX, U:C NPs or PBS (control) for 2 h, respectively. Then the free medium was discarded, the cells were destroyed and the intracellular drugs were extracted with methanol. Since methanol can destroy U:C NPs, the uptake of intracellular U:C NPs could be determined by measuring the fluorescence intensity of intracellular prodrugs.

Analysis of cellular cytotoxicity synergy

The cellular cytotoxicity synergy of Ara-C and DOX was explored by MTT viability assay on MCF-7 and 4T1 cell lines. Two free drug mixtures with 5 different molar ratios were set from 1:5 to 5:1. The rest of the steps were as follow in ***In vitro cellular cytotoxicity and intracellular drug release***. The cellular cytotoxicity synergy was analyzed using the CompuSyn software developed by Chou and Talalay. The drug cytotoxicity synergy was expressed as combination index (CI). In the application of the CI approach, synergy is defined as a combined effect that is statistically significantly greater than the purely additive effect of the individual components. Whereas antagonism is defined as a combined effect that is statistically significantly less than the purely additive effect of the individual components[1]. Generally, CI = 1 means a purely additive effect, CI

> 1 means antagonism, and CI < 1 means synergy. According to the CompuSyn software, CI values have been defined as follows: CI > 1.1, antagonistic effect; CI = 0.9-1.1, nearly additive effect; CI = 0.85-0.9, slightly synergy; CI = 0.7-0.85, moderate synergy; CI = 0.3-0.7, synergy; CI = 0.1-0.3, strong synergy; and CI < 0.1, very strong synergy[2].

In vitro cellular cytotoxicity and intracellular drug release

In the same way, the *in vitro* cytotoxicity of U:C NPs was investigated by MTT viability assay on MCF-7, 4T1 and L1210 cell lines. The free drug U-SS-DOX, Ara-C, DOX, the mixture of U-SS-DOX and Ara-C (U/C mixture) and the mixture of DOX and Ara-C (D/C mixture) were used as controls. Briefly, MCF-7 and 4T1 cells were incubated in 100 μL of complete culture medium in 96-well plates at a density of 1000 cells/well for 24 h. Then the culture medium was replaced with a fresh medium, and the cells were treated with 200 μL of medium containing serial concentrations of U:C NPs, free U-SS-DOX, free Ara-C, free DOX, the U/C mixture and the D/C mixture for 48 and 72 h. Untreated cells were utilized as control. At the end of the incubation, 20 μL of MTT (5 mg/mL) was added, and the plates were incubated for additional 3 h at 37 °C. The medium was then discarded, and the formed formazan crystals were dissolved in DMSO (150 μL). L1210 cells were incubated in 100 μL of complete culture medium in 96-well plates at a density of 3000 cells/well for 24 h. The other process was the same as above, except that the solution of formazan was replaced by triple solution (10% SDS, 5% Isopropanol 0.012 mol/L HCl), and all the solution were added directly instead of removing the original medium. The absorbance in each individual well was determined at the wavelength of 490 nm with a multi-detection microplate reader (Plate CHAMELEON V-Hidex). Each drug concentration was tested in three wells and all data were presented as mean ± SD.

Next, we determined whether the free DOX-SH could release from the prodrugs in the cell. Firstly, for quantitative determination DOX-SH release from the prodrugs in intracellular, the 4T1 cells were seeded into 24-well plates at a density of 5000 cells per well for 24 h attachment. Then we collected 1 mL 4T1 cells and culture medium containing two kinds of concentration of U:C NPs (DOX-SH equivalent concentrations: 2 μg/mL and 4 μg/mL) at pre-determined time intervals

(2, 4, 8, 12, 24, 48, 72 h). Then, centrifuged and discarded the supernatant, 1 mL PBS was added to the resuspended cells. After crushed by Cell Ultrasonic Crusher, 100 μL of samples were taken for analysis by UPLC-MS-MS. Briefly, 50 μL diazepam solution as the internal standard was added into the mixture of 50 μL cellular samples and 50 μL methanol and vortex mixed for 3 min. Then adding 3 mL ethyl acetate, vortex mixed and centrifuged at 4000 rpm for 5 min to extract the analytes. Next removed the upper layer and dried under nitrogen gas flow at 37 °C. Finally, 100 μL methanol was applied to redissolve the dried extracts and centrifuged at 13000 rpm for 5 min. The supernatant was collected to analyze using UPLC-MS/MS. All the experiments were performed in triplicate and the results were expressed as the concentration of released DOX-SH.

Chromatographic conditions: ACQUITY UPLCTM BEH C18 (50 mm × 2.1 mm, 1.7 μm, Waters) Corp, Milford, MA, USA); Mobile phase: methanol (A) - Water containing 0.5% formic acid (B); Flow rate: 0.2 mL /min; The gradient ratio was 0 min 10% A, 0.5min 10% A, 0.51 min 80% A, 1.0 min 80% A, 3.0 min 70% A; 3.5 min 10% A; Injection volume: 10 μL; Injector temperature: 7 ℃; Column temperature: room temperature.

Ion source: ESI source; Detection method: positive ion detection; Capillary voltage: 3.4 KV; Taper hole: voltage 16 V; Ion source temperature :120 ℃; Solvent removal temperature: 350 ℃; Solvent removal gas flow: 550 L/h; Taper hole airflow quantity: 50 L/h; Scanning mode: multiple reactive ion monitoring (MRM).

Detection of cellular GSH

4T1, MCF-7, L1210 and L02 cells were collected at 3.0×10^5 cells respectively. The cells were repeated freeze-thaw cycles thrice with liquid nitrogen, and supernatants were collected by centrifugation at 3000 rpm for 5 min. Then the GSH concentration in cells were measured based on Reduced Glutathione Assay Kit.

Apoptosis analysis with flow cytometry

4T1 cells were seeded in 6-well plates at 3.0×10^5 cells per well in 2 mL of complete 1640 RPMI and cultured for 24 h. Then they were exposed to U:C NPs, U/C mixture at DOX dose of 3 μg/mL for 24 h. 4T1 cells without the treatment were used as control groups. For quantitative

measurement of apoptosis, both floating and attached cells were harvested, rinsed three times with cold PBS and stained with Annexin V-FITC and propodium iodide (PI). Finally, they were analyzed by flow cytometry (BD FACS Calibur).

Animals and tumor models

All the animal were obtained from the Laboratory Animals Center of Shenyang Pharmaceutical University. All the animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals of Shenyang Pharmaceutical University and approved by the Institutional Animal Ethical Care Committee (IAEC) of Shenyang Pharmaceutical University (reference number: SYPU-IACUC-C2020-9-2-118).

In vivo biodistribution

Sprague-Dawley rats (190-210 g) were used to evaluate the blood retention time by IVIS imaging of U:C NPs. All rats were randomly assigned to two treatment groups (n = 3). DiR sol and DiR-loaded U:C NPs (equivalent DiR dose of 1 mg/kg) were intravenously injected via the tail vein. At prescribed time points (5 min, 15 min, 30 min, 1, 2, 4, 8, 12 and 24 h) after the injection, about 200 μL blood samples were collected and placed in 96-well black opaque plates. The mean radiation efficiency of DiR was analyzed by fluorescence imaging.

The biodistribution of supramolecular nanoparticles, was investigated in 4T1 tumor-bearing mice (n = 3). 4T1 cells (5.0×10^6 cells in 100 μL) were inoculated subcutaneously. Until the tumor volume reached ≈ 300 mm^3, mice were intravenously administrated with DiR sol, DiR-labeled U:C NPs at an equivalent DiR dose of 1 mg/kg. After 12 h or 24 h post injection, the mice were sacrificed and the fluorescence signals in the major organs and tumors were detected using IVIS imaging system (n = 3).

In vivo imaging in 4T1 tumor-bearing mice

Non-invasive optical imaging systems were used to observe the real-time distribution and tumor accumulation ability of fluorescent DiR-loaded U:C NPs in 4T1 cell xenografts in mice. When tumors reached approximately 200-300 mm^3 in volume, the mice were randomly divided into two treatment groups (3 animals each). Then tumor-bearing mice were given an intravenous

injection via the tail vein of DiR sol or DiR-loaded U:C NPs nanoparticles at the same DiR concentration (1 mg/kg, diluted in PBS). The mice were anesthetized by inhaling isoflurane and scanned at 4, 8, 12 and 24 h using IVIS imaging system.

Studies on the breast cancer model

The tumors were produced in Balb/c female mice as described above. After the inoculated tumor volume reached 100-150 mm^3, the mice were randomly divided into 7 groups (6 mice per group): untreated control (Saline), free DOX (3 mg/kg), free Ara-C (3 mg/kg), U:C NPs, free U-SS-DOX, the U/C mixture and the D/C mixture (with an equivalent DOX dose of 3 mg/kg). These formulations were intravenously administrated every other day for a total of four injections. The tumor volume and body weight were measured every day. The tumor volume and the body weight of mice were measured before every treatment and the fourth day after the last administration to mice. Tumor volumes were calculated using the formula $V = 1/2ab^2$ (a, long diameter; b, short diameter). Mice were sacrificed 6 days post final injection, and serum was collected. The activities of aspartate transaminase (AST), alanine transaminase (ALT), blood urea nitrogen (BUN), and creatinine were assayed as indicators of hepatic and renal function. The major organs and tumor tissues were collected and sectioned for further pathological study. The formalin-embedded organ and tumor tissues were stained by H&E to investigate the pathological change of major organs.

Treatment experiment in murine leukemia tumor model

L1210 cells (3.0×10^6 cells in 200 μL of PBS) were injected intraperitoneally into the left abdomen of female eight-week-old mice, DBA/2 (huafukang biotechnology co. Ltd, Beijing) after a minimum of 7 days of acclimation time prior to experiments. After tumor cell inoculation, weight and survival rate were monitored continuously. One day after cell inoculation, animals were randomly divided into 8 groups (n = 6). Intravenous treatments were performed four times (on days 1, 3, 5 and 7). Animals were injected via tail vein injection with 200 μL of high dose U:C NPs (U:C NPs-H, 5 mg/kg Ara-C, 35 mg/mL U-SS-DOX), low dose U:C NPs (U:C NPs-L, 2.5 mg/kg Ara-C, 17.5 mg/kg U-SS-DOX), free drug U-SS-DOX (17.5 mg/kg), Ara-C(2.5 mg/kg), DOX(5.6 mg/kg), U/C mixture (2.5 mg/kg Ara-C, 17.5 mg/kg U-SS-DOX) and D/C mixture (2.5 mg/kg Ara-

C, 11.25 mg/kg DOX), saline. All of the mice in each group were normally fed for 30 days to assess survival rate. The statistics were stopped when fewer than three mice survived. Healthy animals were injected via tail vein injection with 200 μL of high dose U:C NPs, low dose U:C NPs, free Ara-C, U-SS-DOX, DOX, U/C mixture, D/C mixture and saline. The body weight of mice was measured every two days. In other to further investigate the treat effect of hematology, the parameters of blood routine check including white blood cell, the number of monocytes, neutrophile granulocytes, leukomonocytes and percent monocytes, neutrophilic granulocyte percentage and percentage of lymphocytes were detected for hematology index on the 8th day.

Statistical Analysis

Data were presented as mean ± SD. Comparison between groups was analyzed with Student's t-test and one-way analysis of variance (ANOVA), and statistical differences were considered significant at $p < 0.05$.

Synthesis of prodrugs (U-SS-DOX)

Synthesis of Cmpd 3:

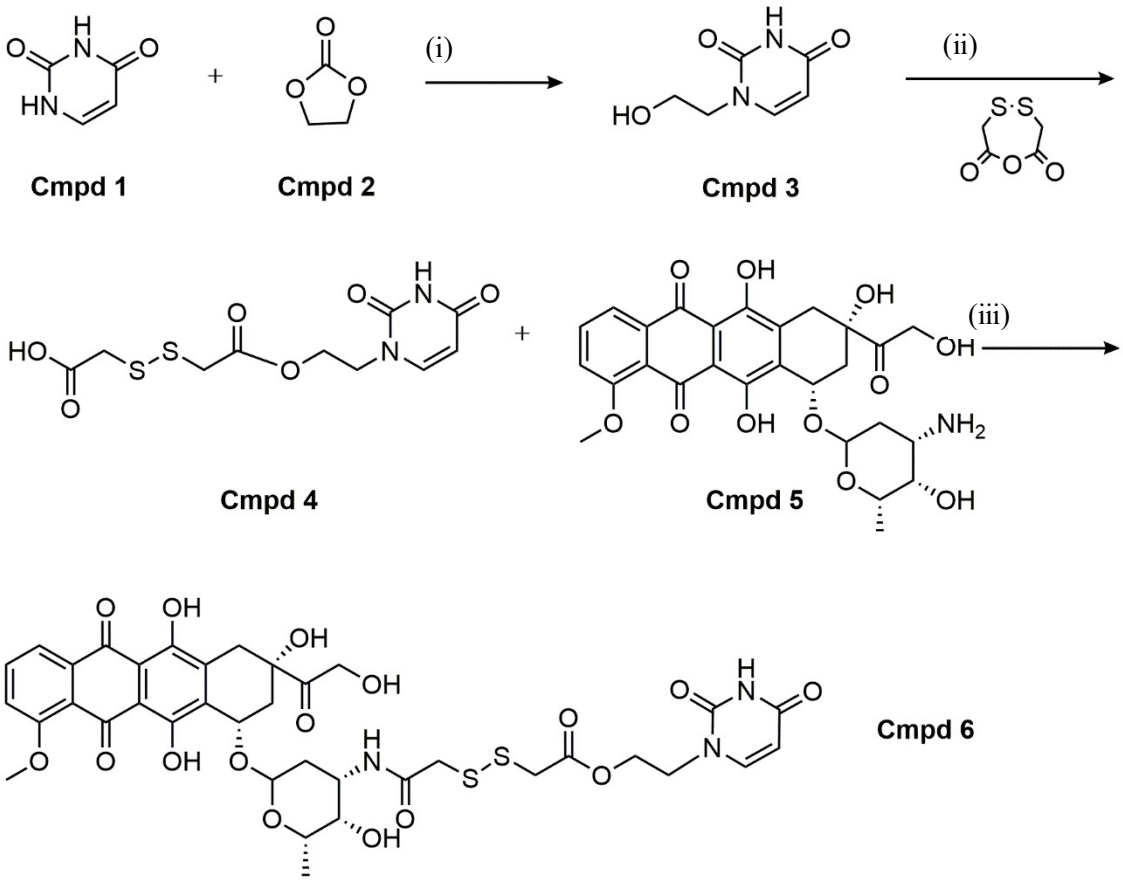
Cmpd 1 was dissolved in DMF with *Cmpd 2***,** then a small amount of NaOH was added to form an alkaline environment. After reaction, the crude product *Cmpd 3* was recrystallized and purified. (Yield：90%)

Synthesis of Cmpd 4:

Cmpd 3 and 2,2'-dithiobisacetic acid anhydride were dissolved in 100 ml THF, then DIPEA was added and stirred at rt for 4 h. Then contrated under high vacumn to afford crude *Cmpd 4***.** It was purified by semi-preparative HPLC to aford 2 g white soild product. (Yield：23%)

Synthesis of Cmpd 6:

Cmpd 4 and *Cmpd 5* were fully dissolved in 40 ml DMF, then HATU was added. The reaction mixture was stirring under r.t. for 2 h. The reaction mixture was concentrated under the vacuum, the crude product to purified by semi-preparative HPLC to aford 500 mg heme soild product. (Yield：29%)



Scheme S1 Synthesis routes of U-SS-DOX. (i) DMF, NaOH, r.t.; (ii) DIPEA, THF, r.t.; (iii) HATU, DIPEA, DMF, r.t.;

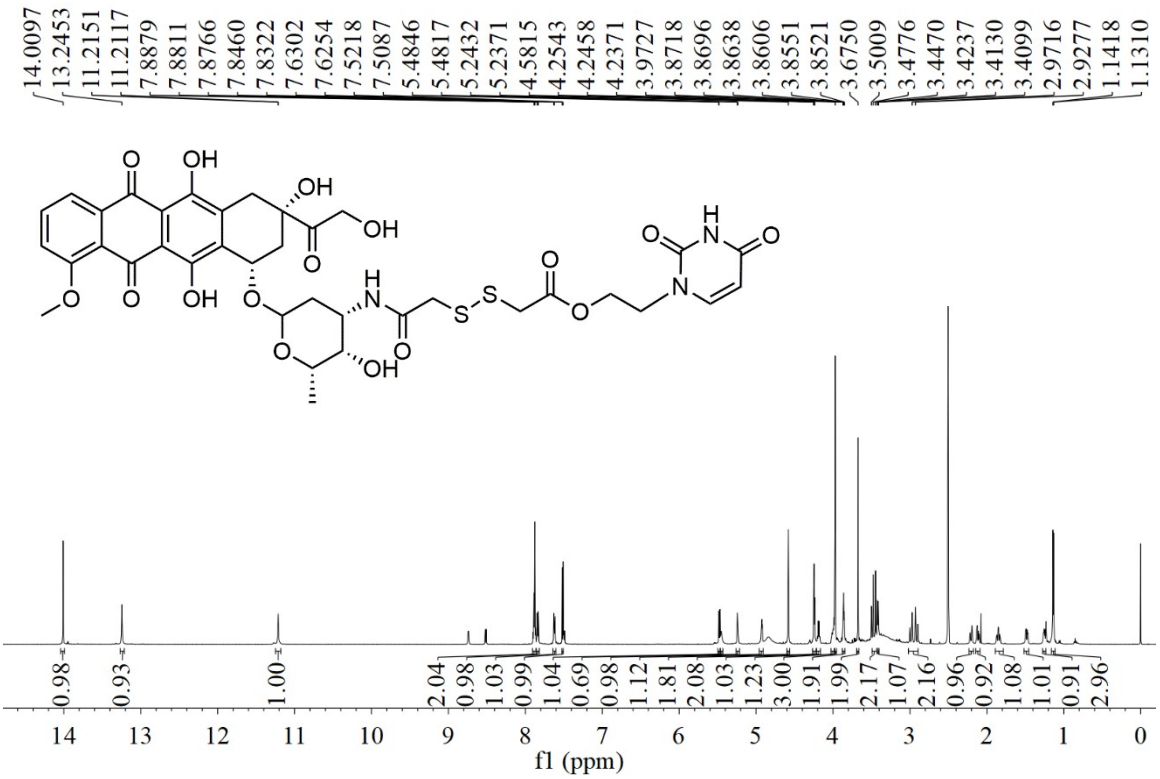


Fig. S1. 1H NMR spectrum of U-SS-DOX in DMSO. ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.01 (s, 1H), 13.25 (s, 1H), 11.23 – 11.20 (m, 1H), 7.92 – 7.85 (m, 2H), 7.84 (d, J = 8.3 Hz, 1H), 7.62 (d、d, J = 6.9, 2.9 Hz, 1H), 7.52 (d, J = 7.9 Hz, 1H), 5.48 (dd, J = 7.8, 1.7 Hz, 1H), 5.46 – 5.43 (m, 1H), 5.24 (d, J = 3.6 Hz, 1H), 4.93 (dd, J = 5.5, 3.4 Hz, 1H), 4.58 (s, 2H), 4.25 (t, J = 5.2 Hz, 2H), 4.19 (q, J = 6.6 Hz, 1H), 4.02-3.99 (m, 1H), 3.97 (s, 3H), 3.86 (td, J = 4.9, 1.5 Hz, 2H), 3.67 (s, 2H), 3.52 – 3.41 (m, 2H), 3.43 – 3.39 (m, 1H), 3.03 – 2.89 (m, 2H), 2.21 (dt, J = 13.8, 3.0 Hz, 1H), 2.12 (dd, J = 14.2, 5.7 Hz, 1H), 1.85 (td, J = 12.9, 4.0 Hz, 1H), 1.48 (dd, J = 12.5, 4.6 Hz, 1H), 1.29 – 1.24 (m, 1H), 1.14 (d, J = 6.5 Hz, 3H).

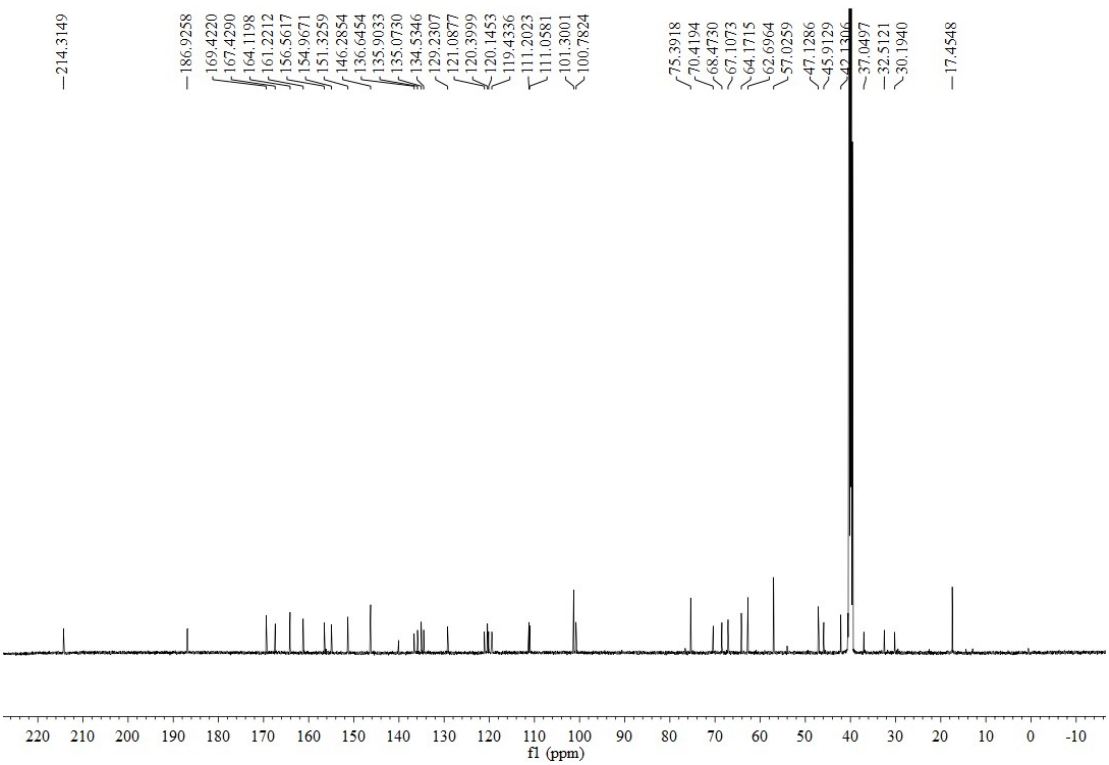


Fig. S2. 13C NMR spectrum of U-SS-DOX in DMSO (151 MHz, DMSO) δ 214.31, 186.93, 169.42, 167.43, 164.12, 161.22, 156.56, 154.97, 151.33, 146.29, 136.65, 135.90, 135.07, 134.53, 129.23, 121.09, 120.40, 120.15, 119.43, 111.20, 111.06, 101.30, 100.78, 75.39, 70.42, 68.47, 67.11, 64.17, 62.70, 57.03, 47.13, 45.91, 42.13, 37.05, 32.51, 30.19, 17.45.

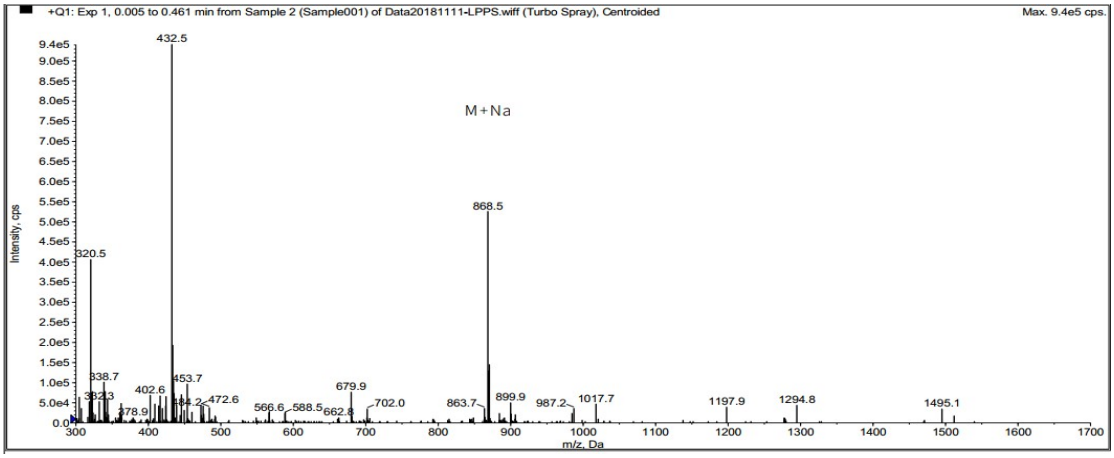


Fig. S3. Mass spectrum (ES^+) of U-SS-DOX.

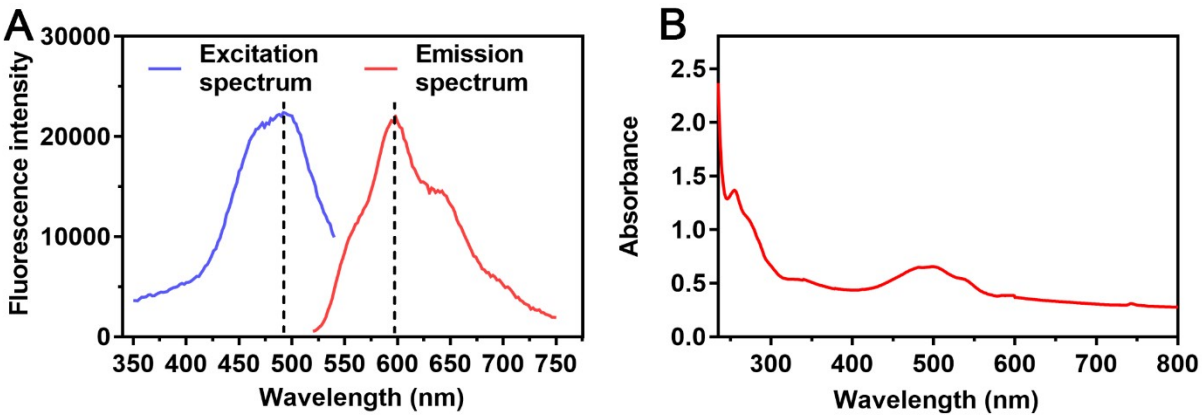


Fig.S4. Spectrogram of U-SS-DOX. (A) Fluorescence spectra (B) UV-vis spectra.

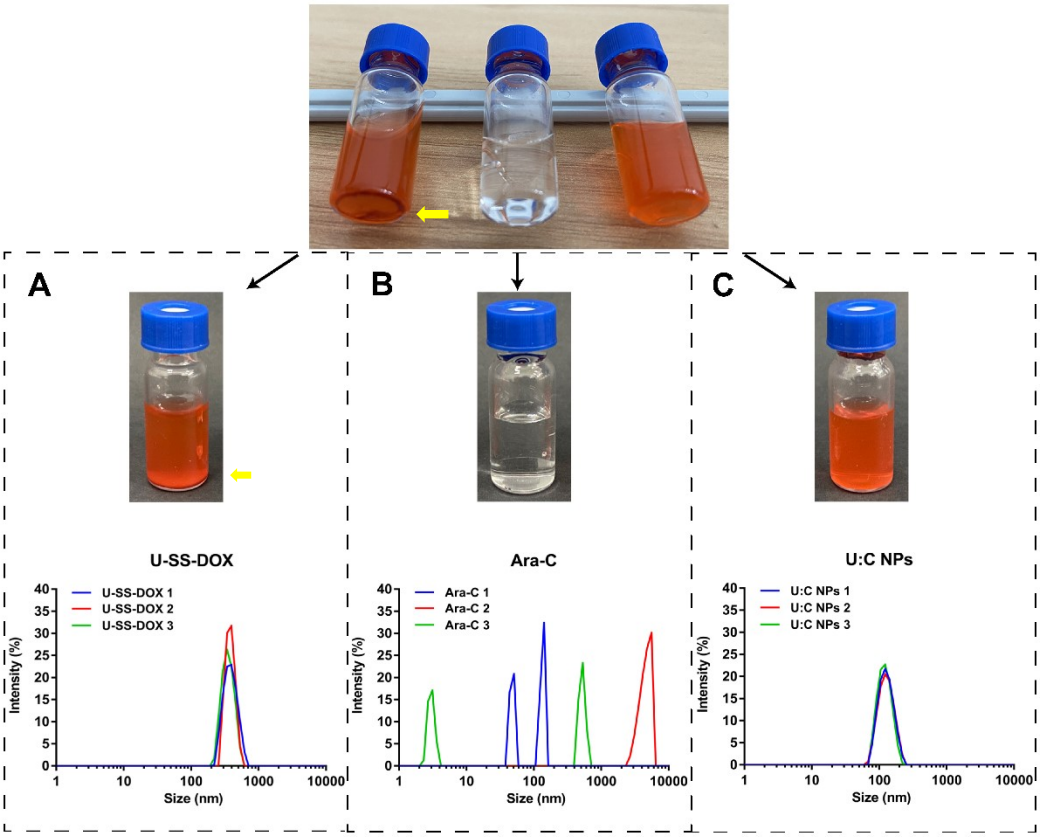


Fig. S5. Appearance pictures and particle size diagrams of self- and co-assembly of (A)U-SS-DOX, (B)Ara-C, (C)U:C NPs.

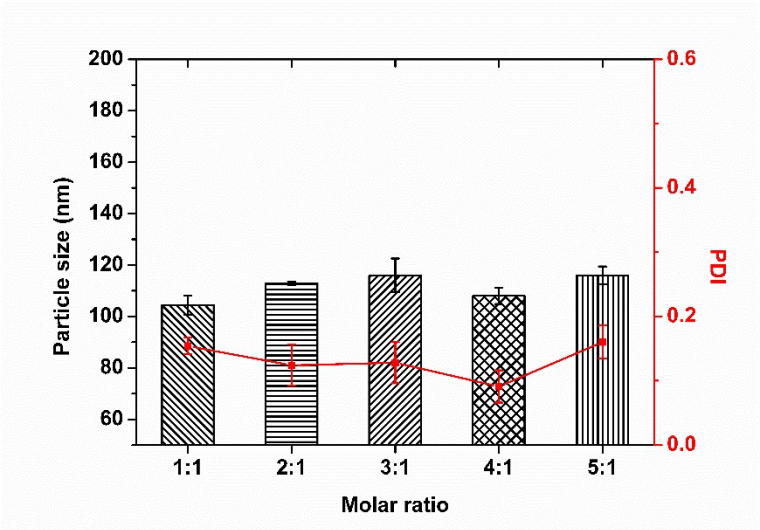


Fig. S6. Size and PDI of differences molar ratio of U-SS-DOX to Ara-C. Data are presented as the mean ± SD (n = 3).

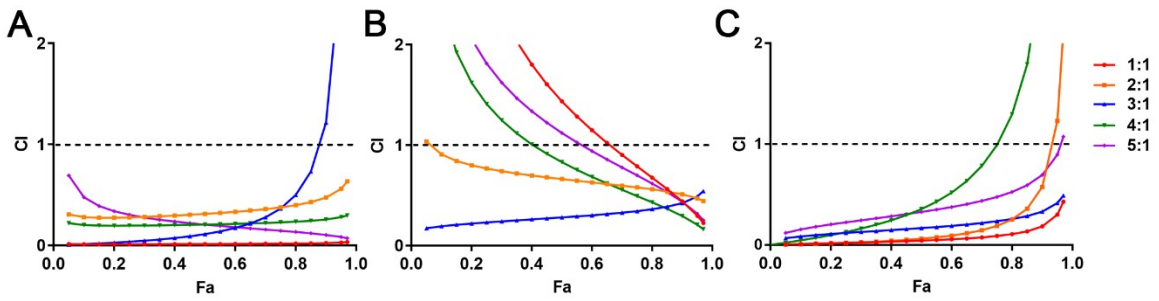


Fig. S7. Combination index of the mixture of DOX and Ara-C in (A) MCF-7 cells, (B) 4T1 cells and (C) L1210 cells.

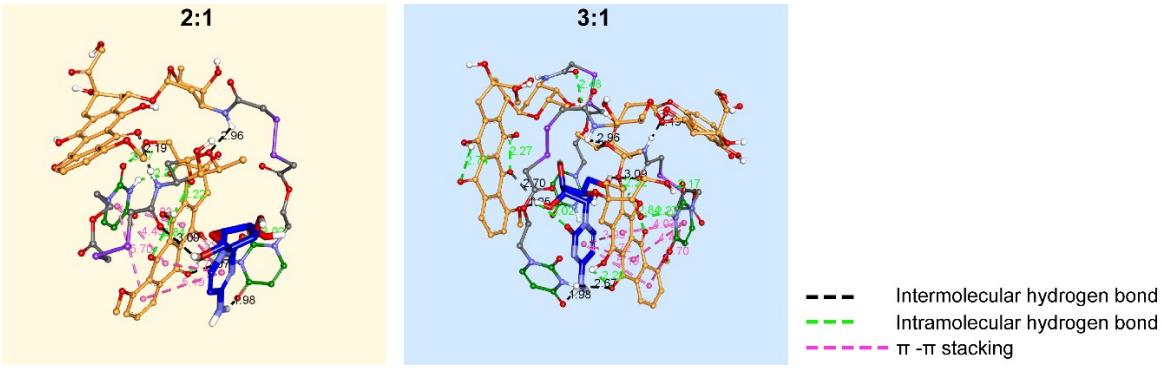


Fig. S8. The interaction diagram of U-SS-DOX and Ara-C with molar ratios of 2:1 and 3:1 respectively. The color of the atoms in U-SS-DOX and Ara-C are the same as that of the structural formula in Figure 1. The oxygen atoms of both drugs are red and nitrogen atoms are wathet blue.

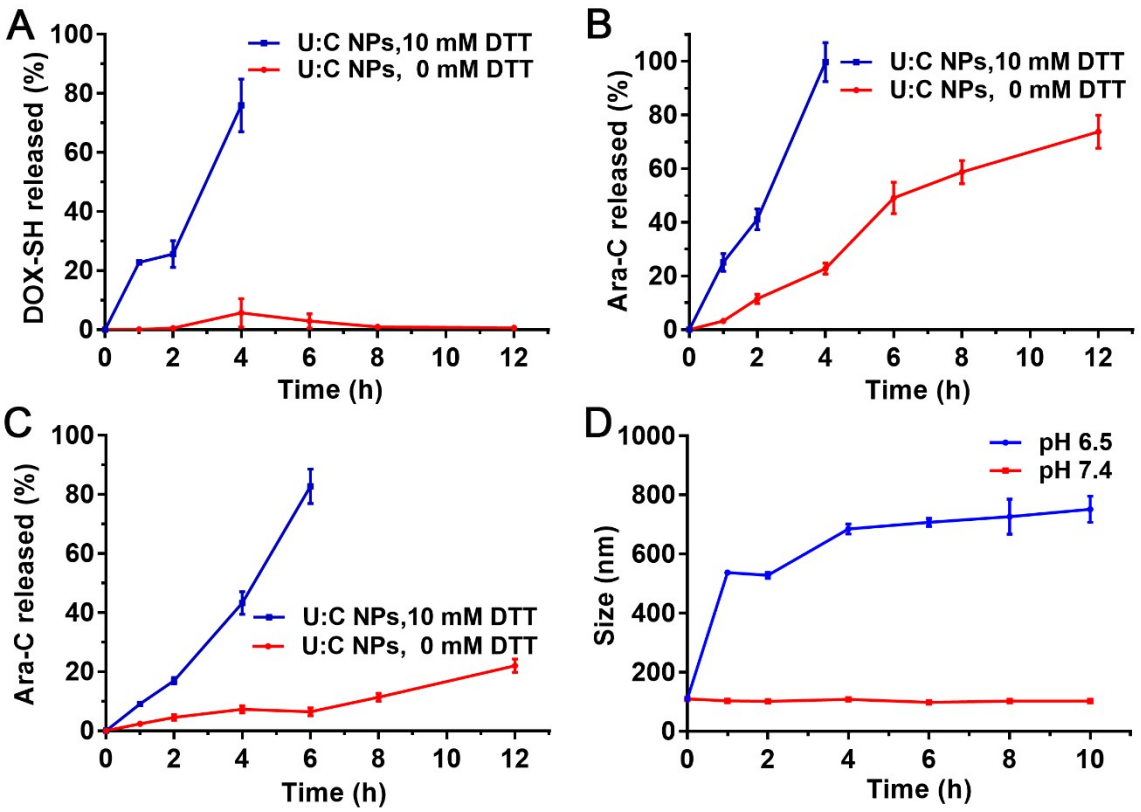


Fig. S9. (A) *In vitro* cumulative drug release profiles of DOX-SH from U:C NPs at pH 6.5. (B) *In vitro* cumulative drug release profiles of Ara-C from U:C NPs at pH 6.5. (C) *In vitro* cumulative drug release profiles of Ara-C from U:C NPs at pH 7.4. (D) Particle size variation profiles of U:C NPs at different pH values (6.5 and 7.4). Data are presented as the mean ± SD (n = 3).

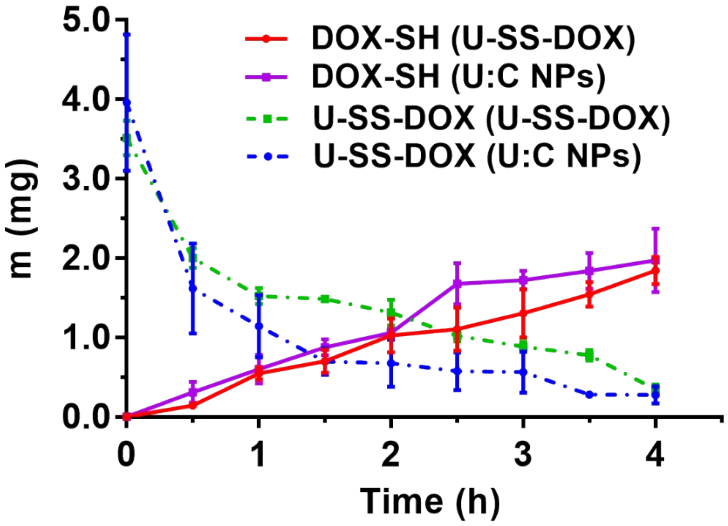


Fig. S10. Activation kinetics of DOX-SH from U-SS-DOX and U:C NPs. Data are presented as the mean ± SD (n = 3).

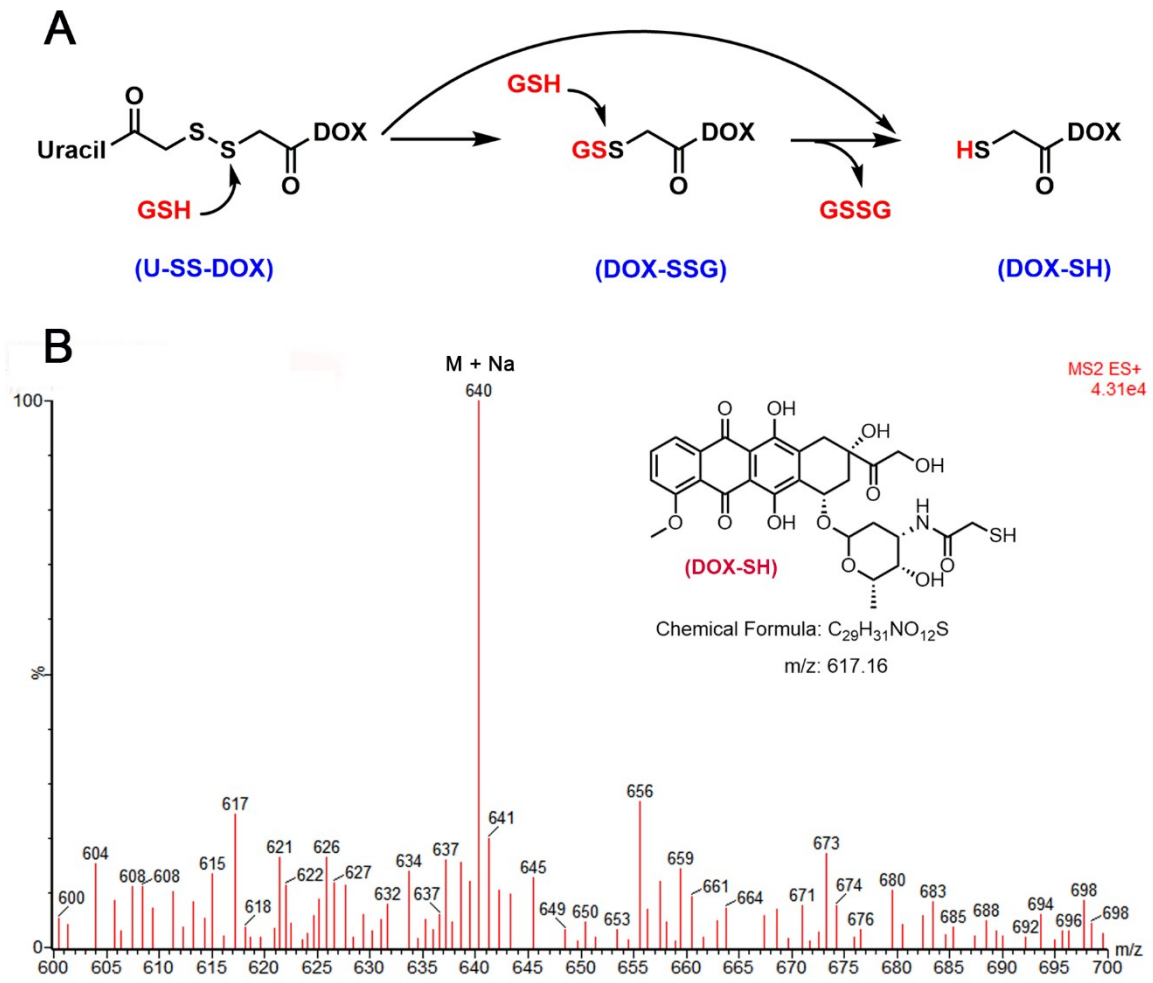


Fig. S11. (A) GSH-responsive mechanism of U:C NPs. (B) Mass spectra of U:C NPs after incubated with 10 mM DTT-containing release media.

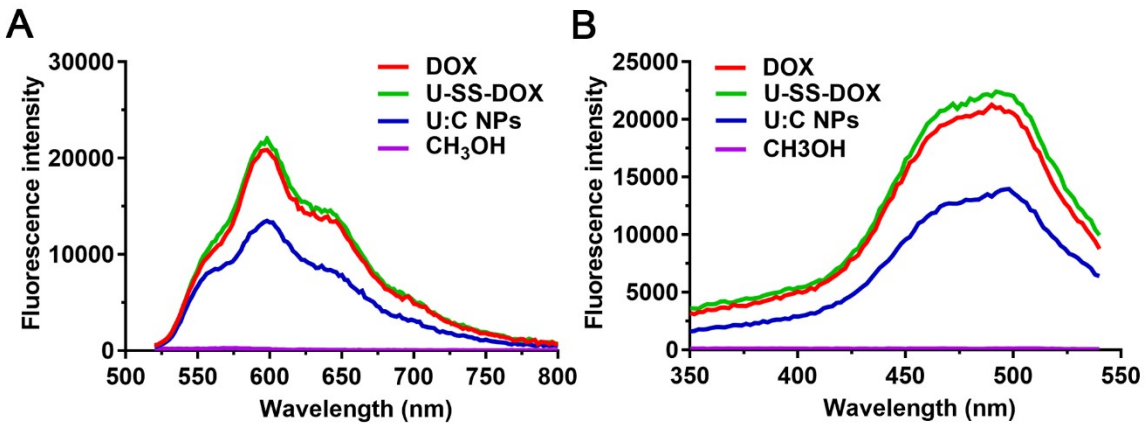


Fig. S12. Fluorescence spectra of DOX, U-SS-DOX and U:C NPs at DOX equivalent concentration of 1 μg/mL. (A) Emission spectra. (B) Excitation spectra.

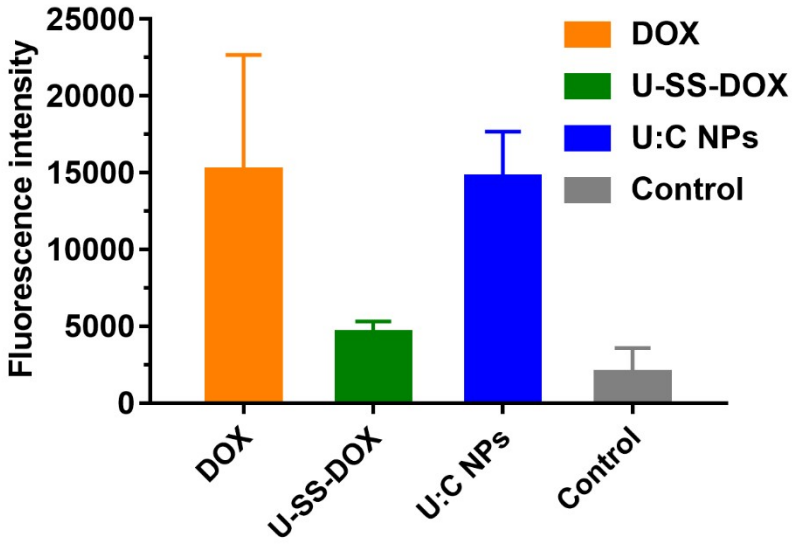


Fig. S13. Fluorescence intensity of 4T1 cells incubated with free DOX, U-SS-DOX, U:C NPs or PBS (Control) for 2 h. Error bars represent ± S.D, ns, not significant, *P < 0.1, **P < 0.01, ***P < 0.001, ****P < 0.0001.

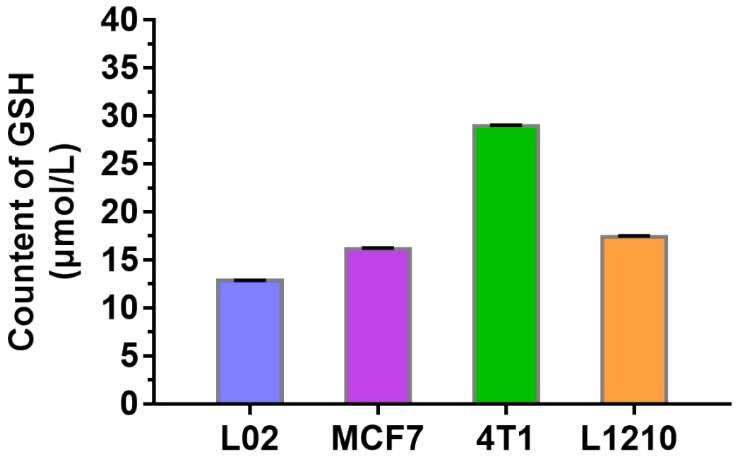


Fig. S14. Determination of GSH concentration in four types of cells. Data are presented as the mean ± SD (n = 3).

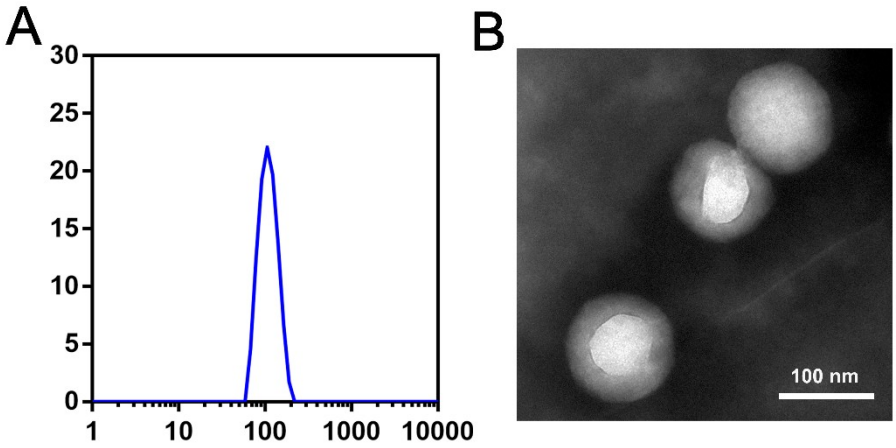


Fig. S15. (A) Particle size distribution profile of DiR-loaded U:C NPs. (B) TEM image of DiR-loaded U:C NPs.

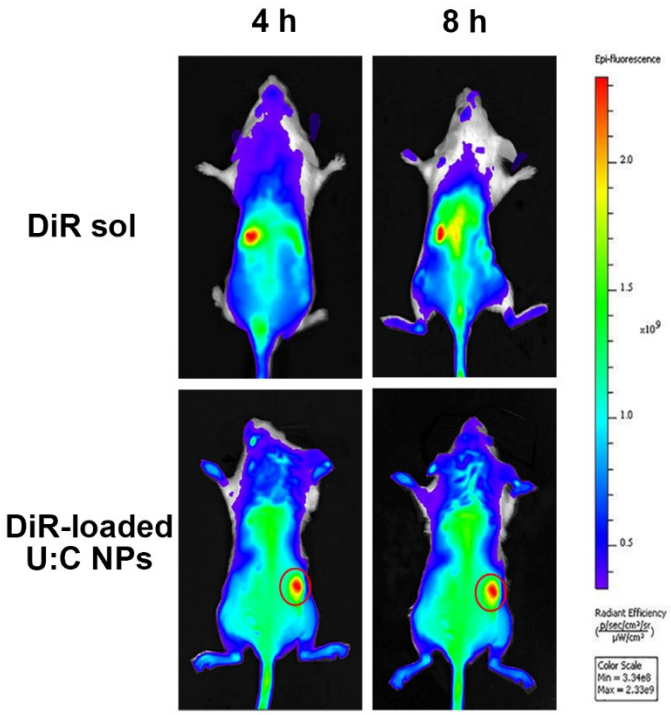


Fig. S16. In vivo IVIS whole bodies imaging of time dependent of 4T1 tumor-bearing micce after intravenous injection of DiR sol and DiR-loaded U:C NPs at 4 h and 8 h. The tumor is circled in red.

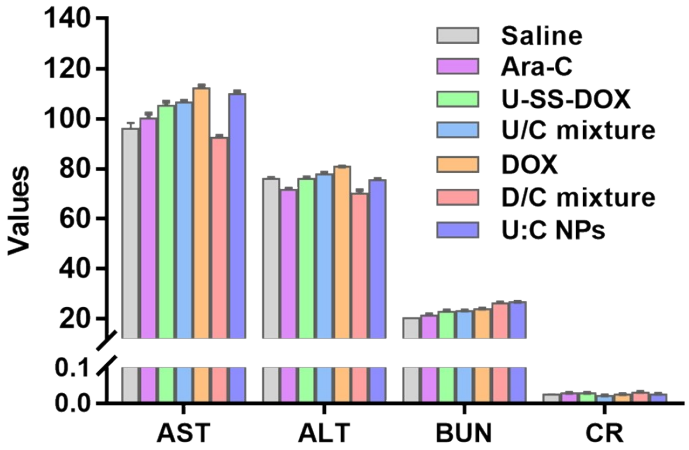


Fig. S17. Hepatic and renal function indicators of mice bearing 4T1 tumor after treatment. Data are presented as the mean ± SD (n = 5).

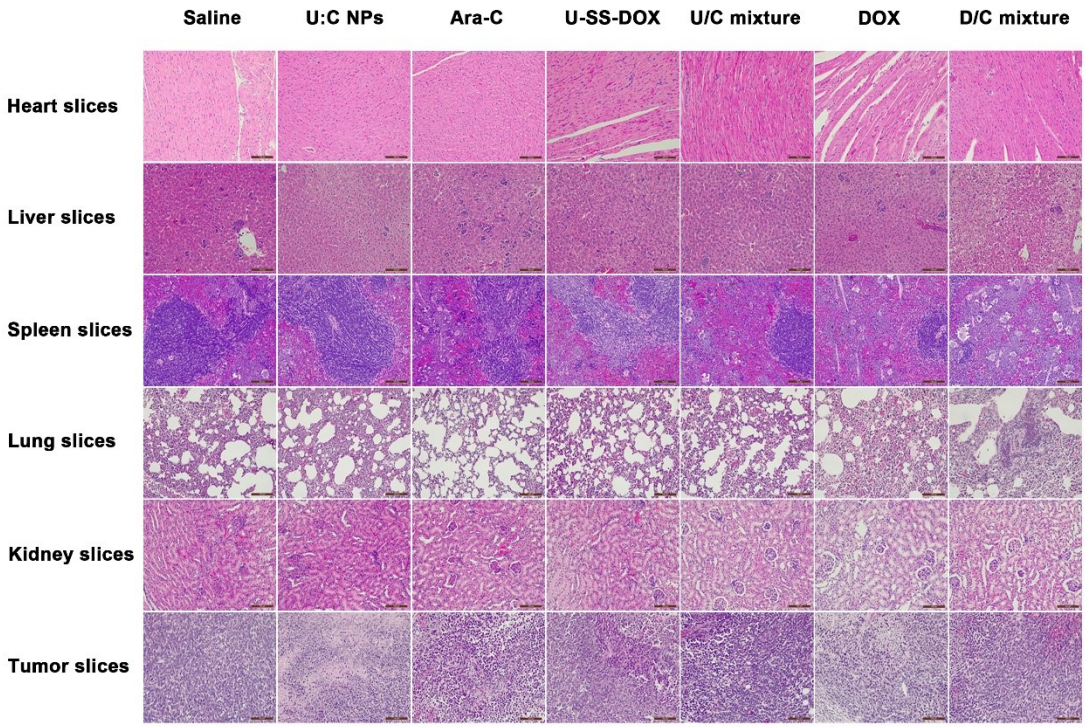


Fig. S18. H&E staining of the major organs and tumors after treatments. Scale bar represents 100 μm.

Table S1 Optimization of molar ratio of U-SS-DOX to Ara-C.

Molar ratio of U-SS-DOX to Ara-C	Z-average (nm±SD)	PDI±SD
1:1	104.4 ± 3.666	0.154 ± 0.013
1:2	231.2 ± 73.02	0.654 ± 0.069
1:3	1026 ± 130.4	1.000
1:4	1202 ± 906.8	1.000
1:5	-	-
2:1	112.9 ± 0.7234	0.124 ± 0.032
3:1	116 ± 6.527	0.128 ± 0.032
4:1	108 ± 3.153	0.091 ± 0.025
5:1	116 ± 3.430	0.160 ± 0.026

Table S2 A list of the number and length of hydrogen bond and π-π stacking contained in the computer-simulated interaction diagram. (Black represents intermolecular hydrogen bonds, green represents intramolecular hydrogen bonds, and pink represents π-π stacking)

U-SS-DOX: Ara-C = 2:1

Hydrogen bond	Serial number	1	2	3	4	5	6	7	8	9	10	11	
	Color	■	■	■	■	■		■	■	■	■	■	■
	Distance (Å)	1.98	3.09	2.19	2.96	2.67	3.02	2.27	2.83	2.22	2.17	2.28	
π-π stacking	Serial number	1	2	3	4	5	6						
	Color	■	■	■	■	■	■						
	Distance (Å)	5.70	4.43	4.03	5.19	3.72	3.99						

U-SS-DOX: Ara-C = 3:1

Hydrogen bond	Serial number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	Color	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	Distance (Å)	1.98	2.70	1.25	3.09	2.19	2.96	2.67	3.02	2.27	2.84	2.22	2.17	2.28	2.48	2.27	2.74
π-π stacking	Serial number	1	2	3	4	5	6										
	Color	■	■	■	■	■	■										
	Distance (Å)	5.19	3.72	3.99	5.70	4.43	4.03										

Table S3 The percentage of free DOX released by U:C NPs at different time points after incubation with 4T1 cells at different timepoints.

	Time (h)	2	4	8	12	24	48	72
DOX release (%)	2 (μg/mL)	11.69	19.99	31.44	33.53	40.58	41.27	38.27
	4 (μg/mL)	12.59	16.13	30.26	27.41	31.51	35.45	35.18

References

1. Ye, M.; Han, Y.; Tang, J.; Piao, Y.; Liu, X.; Zhou, Z.; Gao, J.; Rao, J.; Shen, Y., A Tumor-Specific Cascade Amplification Drug Release Nanoparticle for Overcoming Multidrug Resistance in Cancers. *Adv Mater* **2017,** *29* (38).
2. Wang, D.; Yu, C.; Xu, L.; Shi, L.; Tong, G.; Wu, J.; Liu, H.; Yan, D.; Zhu, X., Nucleoside Analogue-Based Supramolecular Nanodrugs Driven by Molecular Recognition for Synergistic Cancer Therapy. *J Am Chem Soc* **2018,** *140* (28), 8797-8806.