Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Ratiometric Real-Time Monitoring of Hydroxyapatite-Doxorubicin Nanotheranostic Agents for On-Demand Tumor Targeted Chemotherapy

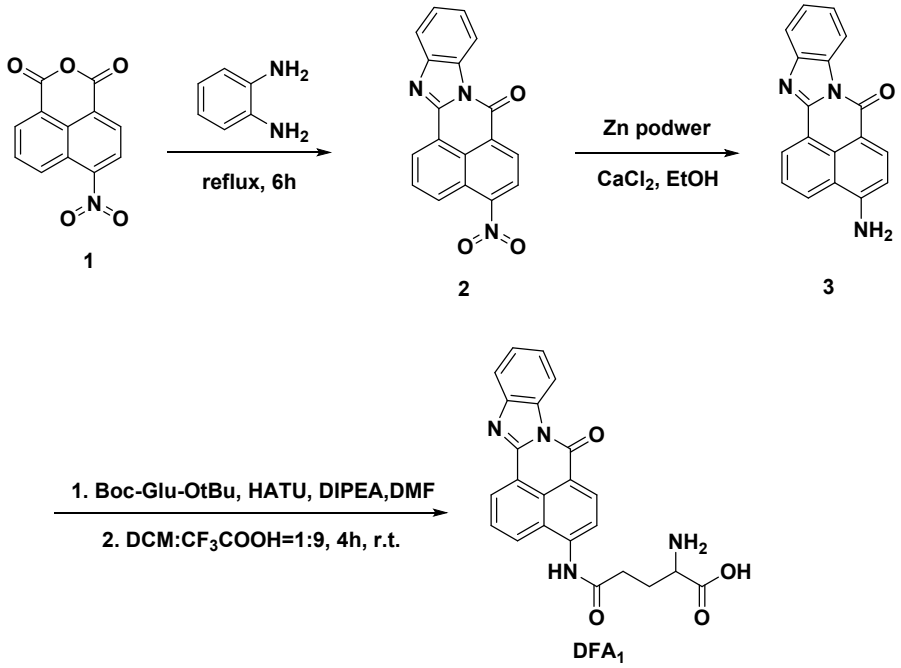
Yao Kang[+a], Wen Sun[+a], Jiangli Fan*[a], Zimu Wei [a], Suzhen Wang [a], Mingle Li [a], Zhen Zhang [a], Yahui Xie [a], Jianjun Du [a], Xiaojun Peng [a]

State Key Laboratory of Fine Chemicals, Dalian University of Technology, 2 Linggong Road, Dalian 116024, China. E-mail: fanjl@dlut.edu.cn.

Materials.

2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluoro-phosphate (HATU) malononitrile (99%), 6-nitrobenzo[de]isochromene-1,3-dione (98%), *o*-phenylenediamine (99%) and N,N-diisopropylethyl amine (DIPEA, 99.5%) were purchased from Energy Chemical. Salts of metal ions ($NaHCO_3$, $PbCl_2$, $CoCl_2$, KCl, $BaCl_2$, NaOAc, NaSCN, NaBr, NaCl, Na_2SO_4, $CuCl_2$, $NiCl_2$, NaF, Na_2SO_3, $CrCl_3$, Na_2CO_3, $NaBaO_3$, $MnCl_2$, $FeCl_3$), HSA, RNA, DNA, triacylglycerol acylhydrolase (PPL), lysozyme (LZM), proteinase k, histone, collagen, hemoglobin (HGB), BSA, β-amylase, trypsin, γ-Glutamyl transpeptidase (γ-GGT), glutathione (GSH), cysteine (Cys), homocysteine (Hcy), ascorbic acid (AA), glycine (Gly), arginine (Arg), phenylalanine (Phe), Histidine (His), Boc-L-glutamic acid 1-tert-butyl ester (Boc-Glu-OtBu), 6-diazo-5-oxo-L-norleucine (DON), and γ-Glutamyl transpeptidase (γ-GGT) from equine kidney were purchased from Aladdin Reagents or Energy Chemicals. Commercial apoptosis detection kits [Propidium Iodide (PI) and Annexin V-FITC] was supplied by KeyGEN Biotech Co. Ltd (Nanjing, China) and used in flow cytometry as received.

Synthesis of GGT targeting sensor DFA_1



Scheme S1. Synthesis of GGT targeting sensor **DFA_1.**

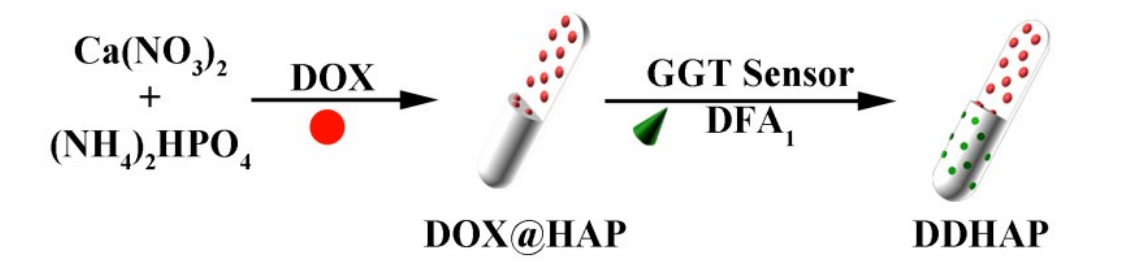
Synthesis of 4-nitro-7H-benzo[de]benzo[4,5]imidazo[2,1-a] isoquinolin-7-one (2). 4-Nitro-1,8-naphthalic anhydride (2.43 g, 10 mmol) was refluxed with ophenylenediamine (1.1 g, 10 mmol) in methoxyethanol (50 mL) for 12 h. The reaction mixture was put into 0 °C water to get a yellow precipitate as the crude product. The crude product was recrystallized by using EtOAc/CH_2Cl_2 mixture to yield the product (2.67 g, 84.8 %). Melting point (m.p.): 247 – 249 °C. ^{1}H NMR (300 MHz, DMSO) δ: 7.56 (m, 2H), 7.97 (d, J=4.2 Hz, 1H), 8.19 (t, J=4.5 Hz, 1H), 8.46 (d, J=4.9 Hz, 1H), 8.65 (d, J= 5.3 Hz, 1H), 8.86 (m, 3H); ^{13}C NMR (75 MHz, DMSO) δ: 122.9, 123.7, 126.7, 127.9, 128.9, 129.3, 129.7, 130.3, 131.5, 131.9, 132.1, 133.2, 133.4, 136.3, 142.3, 147.2, 148.7, 163.6. HR MS: m/z calcd. for $C_{18}H_{10}N_3O_3^+$ $[M]^+$: 315.0644, found: 315.0643.

Synthesis of 4-amino-7H-benzo[de]benzo[4,5]imidazo[2,1-a]-isoquinolin-7-one (3). Compound **2** (1.58 g, 5 mmol), Zn powder (0.98 g 15 mmol) and $CaCl_2$ pellets were dissolved in ethanol (50 mL) under nitrogen. After being stirred for 4 h at 90 °C, the mixture was cooled to room temperature. The solvent was removed under reduced pressure and the product was purified through silica column chromatography with dichloromethane/methanol (20:1) as the eluent to get compound **3** as a yellow solid (153.8 mg), yield: 78.4 %. ^{1}H NMR (400 MHz, DMSO) δ 8.76 (t, J = 7.2 Hz, 1H), 8.70 (d, J = 7.3 Hz, 1H), 8.59 (d, J = 8.4 Hz, 1H), 8.48 (d, J = 8.4 Hz, 1H), 8.42 (d, J = 8.5 Hz, 1H), 7.91 – 7.69 (m, 3H), 7.44 (dd, J = 15.9, 7.2 Hz, 1H), 7.39 – 7.30 (m, 2H), 7.02 – 6.87 (m, 1H).. HR MS: m/z calcd for $C_{18}H_{12}N_3O^+$ $[M]^+$: 286.0975, found: 286.0977.

Synthesis of DFA_1. Under a nitrogen atmosphere, Boc-Glu-OtBu (100 mg, 0.33 mmol), HATU (151 mg, 0.396 mmol) and DIPEA (86 mg, 0.658 mmol) were dissolved in dry dichloromethane. Then compound **3** (188 mg, 0.66 mmol) was added to the above solution. The solution was stirred at room temperature for 24 h. The organic phase was washed with water and then dried over Na_2SO_4. After filtration and concentration, the crude product was purified by silica column chromatography (DCM : MeOH = 80 : 1 in v/v) to furnish a pale yellow solid (87 mg, 62.8 %). And then the product was dissolved in a mixture of trifluoroacetic acid and CH_2Cl_2 (v/v = 1:1, 5 mL) at 0 °C. The solution was kept in the dark and stirred for 1 h at 0 °C before being allowed to room temperature and stirred for another 2 h. The solvent was removed under reduced pressure to get a yellow solid (60 mg, 95.2 %). ^{1}H NMR (400 MHz, $CDCl_3$): 8.56 (m, J = 8.0 Hz, 4H), 8.32 (t, J = 9.3 Hz, 2H), 7.71 (t, J = 7.4 Hz, 1H), 7.26 (d, J = 7.9 Hz, 1H), 4.32 (s, 2H), 3.68 (s, 8H), 3.35 (s, 4H), 2.65 (d, J = 50.5 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 164.22, 163.76, 154.46, 150.03, 148.41, 137.14, 132.78, 132.25, 131.32, 129.81, 129.38, 126.28, 123.47, 119.91, 118.17,

115.84, 66.99, 56.16 , 53.79, 52.72, 46.34, 37.12, 35.91, 31.89, 29.50, 29.35, 29.29, 27.21, 25.52, 22.67, 14.09. HR MS: m/z calcd for $C_{28}H_{28}N_6O_9S^+$ $[M]^+$: 415.1401, found: 415.1404.

Synthesis of DDHAP.



Scheme S2. Preparation and synthesis route of hydroxylapatite nanoparticles DDHAP.

Synthesis of HAP: DOX@HAP was synthesized in aqueous solutions using a precipitation method. These solutions were produced by adding $(NH_4)_2HPO_4$ (0.198 g) and $Ca(NO_3)_2•4H_2O$ (0.596 g) to mixed solution of anhydrous ethanol/deionized water H_2O (5:1, v/v, 35 mL), respectively. First, the pH of the $Ca(NO_3)_2•4H_2O$ solution was adjusted to approximately 10.5 using ammonia water solution, and this alkaline environment (pH = 10.5) was maintained for 2 h. The solution of $(NH_4)_2HPO_4$ was then slowly added dropwise to the above stable $Ca(NO_3)_2•4H_2O$ solution, and the pH of the mixture was adjusted again to >10. After stirring the mixture for 72 h at room temperature, a milky white agitated liquid was obtained; the hydroxyapatite was then collected by washing in deionized water and ethanol at 12, 000 rpm (three times for each solvent) and finally suspended in deionized water. This procedure can remove the surface absorbed DOX to avoid undesired drug leakage. The synthetic method and aftertreatment of DOX@HAP determines that the drug was loaded inside the HAP nanoparticles.

Synthesis of DOX@HAP: DOX@HAP was synthesized using a similar method to that for HAP. First, the pH of the $Ca(NO_3)_2•4H_2O$ solution was adjusted to approximately 7, and this neutral environment (pH = 7) was maintained for 0.5 h. The aqueous DOX solution (50 mg DOX dissolved in 5 mL deionized water) and stabilizer PVP (5 mg, 0.1 % wt) was then slowly added dropwise to the above stable $Ca(NO_3)_2•4H_2O$ solution, stirring for 0.5 h to stabilize the mixture. After adjusting to alkaline (pH = 10), the solution of $(NH_4)_2HPO_4$ was then slowly added dropwise similar to that for the synthesis of HAP.

Synthesis of DDHAP: DDHAP was synthesized in aqueous solution using a self-assembly method involving hydrogen bonds (Figure S10). Specifically, GGT tracer sensor, DFA_1 (10 mg, 2.4 mmol), and HAP doped with anticancer drug, DOX@HAP (200 mg), were added to 50 mL mixed solution of anhydrous ethanol and deionized water (1:4, v/v), and then stirred at 2, 000 rpm for 1 h, followed by 600 rpm for 24 h at 25 °C (room temperature). The product DDHAP was then collected by washing in deionized water and ethanol at 12,000 rpm (every one with three times) to remove excess sensors and finally suspended in deionized water.

Spectrometric experiments

The DFA_1 solution (10 mM) was prepared by using PBS (10 mM) as the solvent. Then, different analytes in stock solutions were added to the above solution. The changes of spectrum in absorption and emission were obtained every 30 min in standard quartz cells. The stock solution of various competitive species were prepared by dissolving salts of metal ions or bioactivators ($NaHCO_3$, $PbCl_2$, $CoCl_2$, KCl, $BaCl_2$, NaOAc, NaSCN, NaBr, NaCl, Na_2SO_4, $CuCl_2$, $NiCl_2$, NaF,

Na_2SO_3, $CrCl_3$, Na_2CO_3, $NaBaO_3$, $MnCl_2$, $FeCl_3$, GSH, Cys, Hcy, AA, Gly, Arg, Phe, His, BSA, and γ-GGT) in double-distilled water.

The DDHAP solution (50 mg DOX/L) was prepared using 10 mM PBS as the solvent containing 2.5 U/L GGT. Then, different analytes in stock solution were added to the above solution. The changes of spectrum in emission were measured after reacting for 30 min in standard quartz cells. The stock solution of various competitive bioactivators were prepared by dissolving HSA, RNA, DNA, LZM, PPL, histone, collagen, trypsin, hemoglobin, proteinase k, BSA, *β*-amylase, and γ-GGT in double-distilled water.

Aqueous electrophoresis studies

The three nanoparticle solution including HAP, HAP@DOX and DDHAP were prepared as a concentration of 5 mg DOX/L. Mean zeta potentials (ζ) of above three dispersions were measured by an ethanol electrophoresis method using a Zetasizer Nano Zetasizer Nano ZS90. All measurements were means of three separate measurements.

Drug release studies in vitro

To simulate drug release in a bioenvironment, the DDHAP nanoparticles (10 mg DOX/L) were prepared with 10 mM PBS, and different analytes were added to the solution respectively. First, emission spectra were measured after adding different concentrations of GGT enzyme until there was no significant change in emission (from 1 U to 50 U); the reaction time interval was 30 min. The pH of the PBS was then adjusted to 6 to create an acidic environment, and emission spectra were measured every 6 min.

Normal/tumor cell lines and cell culture

In this study, four cancer cell lines and two normal cell lines were used: COS-7 (normal cell, African green monkey kidney cell, provided by KeyGEN Biotech Co. Ltd), HL-7702 (normal liver cell, human, provided by Shanghai Institute of Biochemistry and Cell Biology), MCF-7 (cancer cell, epithelial, breast adenocarcinoma cell, provided by Shanghai Institute of Biochemistry and Cell Biology), HCT-116 (cancer carcinoma cell, epithelial, colon carcinoma cell, provided by KeyGEN Biotech Co. Ltd), A2780 (cancer carcinoma cell, epithelial-like, ovarian cell, provided by KeyGEN Biotech Co. Ltd), HepG2 (cancer cell, epithelial, hepatocellular carcinoma cell, provided by KeyGEN Biotech Co. Ltd). The above six cell lines were incubated using DMEM or RPMI 1640 medium (Gibco) as the culture medium, containing 10% fetal bovine serum (Gibco), 0.03% L-glutamine, penicillin and streptomycin (1%, v/v). All cells were cultured at 37 °C in 5% CO_2.

Cytotoxicity experiments

The cytotoxicity of each nanoparticles were measured and evaluated by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan crystals using mitochondrial dehydrogenases.[56] Human breast cancer cells (MCF-7), with a density of 1×10^5 cells/mL, were seeded in microplates (96-well, Corning, America), in which every well contained 100 μL medium containing 10% FBS. After 24 h, the cells were washing with 100 μL/well PBS and then cultured in medium with different drug content (0.625, 1.25, 2.5, 5, 10, 15 and 20 mg DOX/L) of DDHAP nanoparticles for 24 h. The control group (reference group, set as 100 % cell viability) cultured in the medium without DDHAP. Every control and test concentration were set up six replicate wells. Then MTT (5 mg/mL, 10 μL) was added to each well and the 96-well

microplates were incubated at 37 °C. After 4 h incubation, we carefully removed the medium liquid in every well, and added 200 μL DMSO to lyse the purple crystals. The OD value (optical density) was measured by using a microplate reader (Thermo Fisher Scientific) at 492 nm. The cell viability was calculated using the following equation:

$$Cell\ viability\ \% = \frac{OD_{sample} - OD_{blank}}{OD_{control} - OD_{blank}} \times 100\ \%$$

Live cell imaging experiments

From each cell line, the cells were seeded in a cover glass-bottomed culture well with a number of 5×10^4, and then incubated at 37 °C for 24 h. Nanoparticles DDHAP (5.0 mg DOX/L) was added and cells were further incubated for a certain time (120 min, 75 min, or 60 min). Cell imaging experiments was carried out by using OLYMPUS FV-1000 inverted fluorescence microscope with a 100 × objective lens. DDHAP was excited at two laser wavelength (458 nm, 488 nm), and the emission was collected at 510 – 530 nm (green channel) and 590 – 620 nm (red channel).

Tissue imaging experiments

The fluorescence imaging of DDHAP in different organs (heart, liver, stomach, kidney and cancer) was performed in CLSM. The excitation wavelength was 458 nm, and all images were collected at 520 – 560 nm (green channel) and 590 – 620 nm (red channel).

Hemolysis experiments

Firstly, we collected about 2 mL of fresh blood from female Bal B/C nude mice through centrifugation at a speed of 10, 000 × *g* for 3 min. Red blood cells (RBCs) were isolated from it and then washed with PBS for several times until no red color can be observed from the

supernatant. Eight groups (negative and positive control groups, and samples incubated with DDHAP at six different concentrations) were prepared: RBC (0.10 mL) mixed with 0.90 mL PBS or 0.90 mL distilled water used as the negative and positive control groups; a mixture of 0.10 mL of the RBC and 0.90 mL PBS containing DDHAP nanoparticles (final concentrations: 1.3, 2.5, 5.0, 10, 20, 40 μg/mL). After gently shaking, these samples were intensive mixed, which were left at *r. t.* for 2 h, followed by centrifugation at 10,000 × *g* for 3 min to precipitate the red cells. To determine the absorbance of hemoglobin, we take 100 μL of every supernatant to a 96-well plate, and detected the absorbance changes at 540 nm. The hemolysis was evaluated via the following equation:

$$Hemolysis\ \% = \frac{A_{sample} - A_{negative\ control}}{A_{positive\ control} - A_{negative\ control}} \times 100\ \%$$

Flow cytometry and cell apoptosis experiment

Flow cytometry experiment was carried out on an Attune NxT flow cytometer (Thermo Fisher Scientific). To study the localized therapeutic efficiency of DDHAP nanoparticles, an annexin V–FITC/PI method was conducted.[57] HepG2 cells (approximately 2×10^5 cells) were seeded in a 6-well plate. After incubated for 12 h, the pervious medium was removed and a new fresh DMEM medium containing special concentrations of free DOX, DOX@HAP, and DDHAP was added to each well of the plate, which was further incubated overnight. Afterwards, cells were collected and washed with cold PBS (3 times). The collected HepG2 cells were suspended in binding buffer (400 μL), stained with apoptosis staining kit (5 μL of annexin V–FITC and 10 μL of PI). The apoptosis effect in HepG2 cells was finally determined by analyzing 1×10^4 cells with FCM (flow cytometer, Thermo Fisher Scientific).

Anticancer efficiency in vivo

Xenograft tumors were established in female athymic nude mice (6 – 7 weeks old, 14 – 18 g; Dalian Medical University Animal Services Core) by subcutaneous injecting of 1×10^6 HepG2 cells to left flanks. After about two weeks, when the tumor volume reached 83 ± 5 mm^3, mice were divided into four groups randomly (five mice per group) to conduct with different treatments. All mice received a tail vein injection with administration of either 200 μL PBS (control group), 5 mg/kg free DOX, 5 mg DOX/kg of DOX@HAP, or 5 mg DOX/kg DDHAP nanoparticles. The tumor volume was measured every 2 – 3 days and calculated using the formula $V = (a \times b^2)/2$, where a and b are the longest and shortest length of tumors, respectively. Tumor growth in the different treatment groups was monitored. Besides, the body weights were also recorded during the treatment. After treatment, all mice was were scarified by carbon dioxide overdose.

H&E staining

Four-micrometer paraffin sections of different organs were incubated for 40 min at 95 °C, then slides were deparaffinized twice for 10 min and rehydrated with 100% ethanol for 6 min and 95 % (v/v) ethanol for 5 min. After rinsing in tap water for 3 min, then in distilled water for 2 min, the slides were stained with Mayer's acid hematoxylin for 5 min. After washing in water for 2 min, the sections were differentiated with phloxine B for 30 s. After washing in running water for 2 min and dipping thrice in 100% ethanol for 2 min, the slides were stained in saffron (food powder) for 7 min. After dehydration and differentiation in 100 % ethanol for 30 s twice, the slides were cleared twice in Histolemon and mounted with aqueous mounting media. Photomicrographs were taken using a Nikon Eclipse E400 microscope coupled with a digital camera.

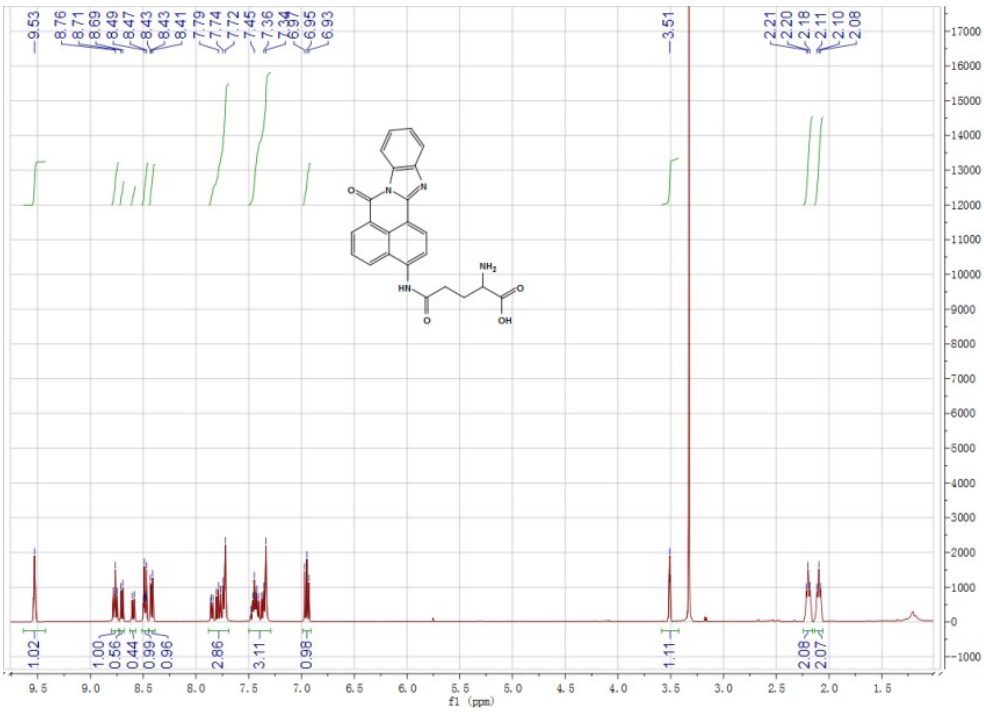


Figure S1. ^{1}H NMR spectrum of DFA_1.

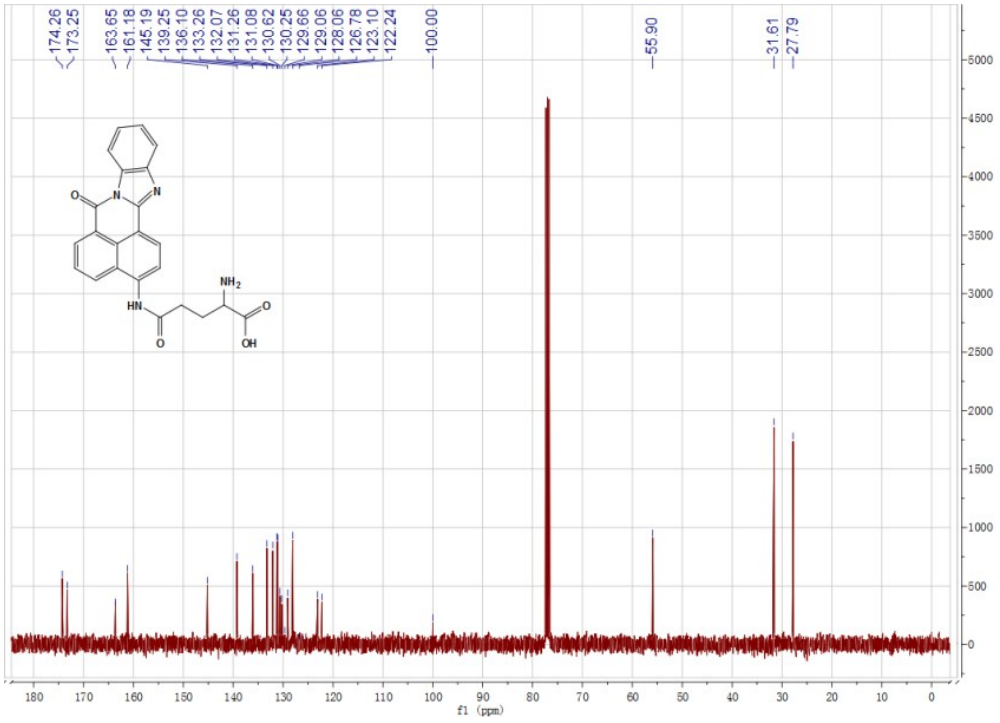


Figure S2. ^{13}C NMR spectrum of DFA_1.

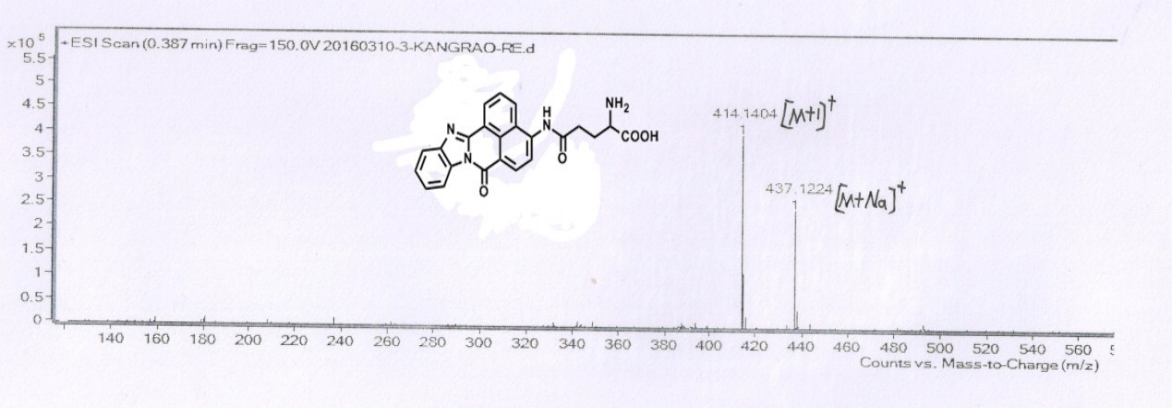
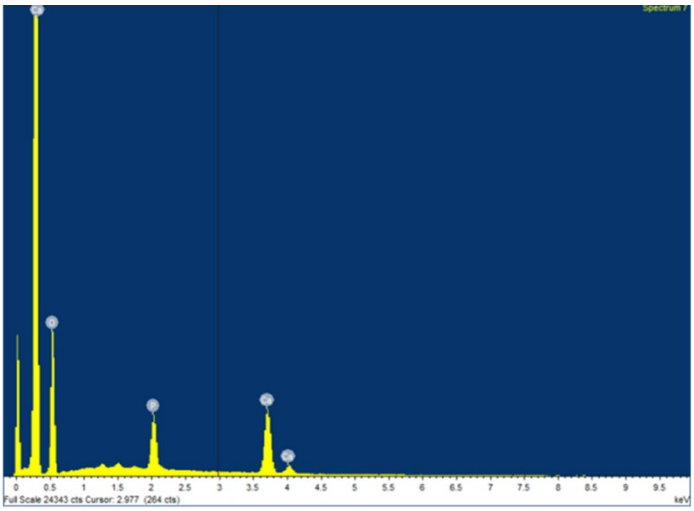


Figure S3. ESI-MS spectrum of DFA_1.

a



b

Element	App Conc.	Intensity Corn.z	Weight%	Weight% Sigma	Atomic%
O K	37.67	0.7327	71.08	0.27	81.91
P K	7.68	1.2442	9.14	0.18	5.44
Ca K	13.17	1.3329	19.78	0.27	9.08
Totals			100.00		

Figure S4. (a) EDX spectrum of HAP. (b) Data analysis of EDX spectrum on three elements: O, P, Ca.

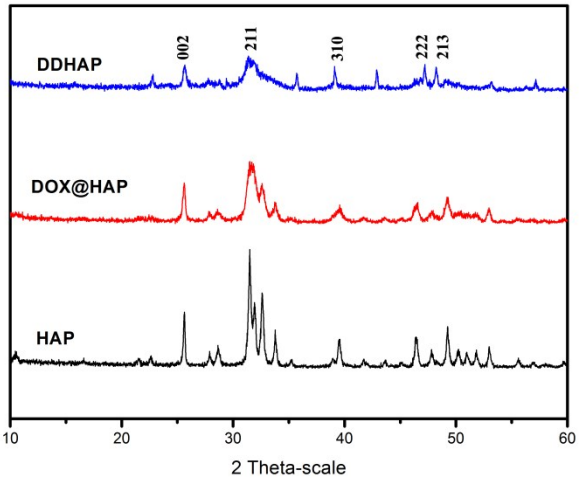


Figure S5. XRD spectra of HAP (Black), DOX@HAP (Red) and DDHAP (Blue).

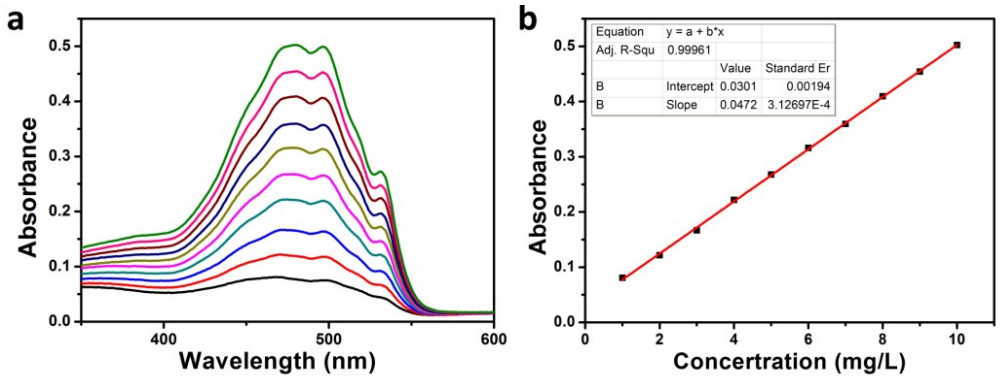


Figure S6. (a) UV spectra of DOX over concentrations. (b) The linear relationship between DOX absorption (λ_{ab} = 479 nm) and concentrations: [A] = 0.0472[c_{DOX}]+0.0301.

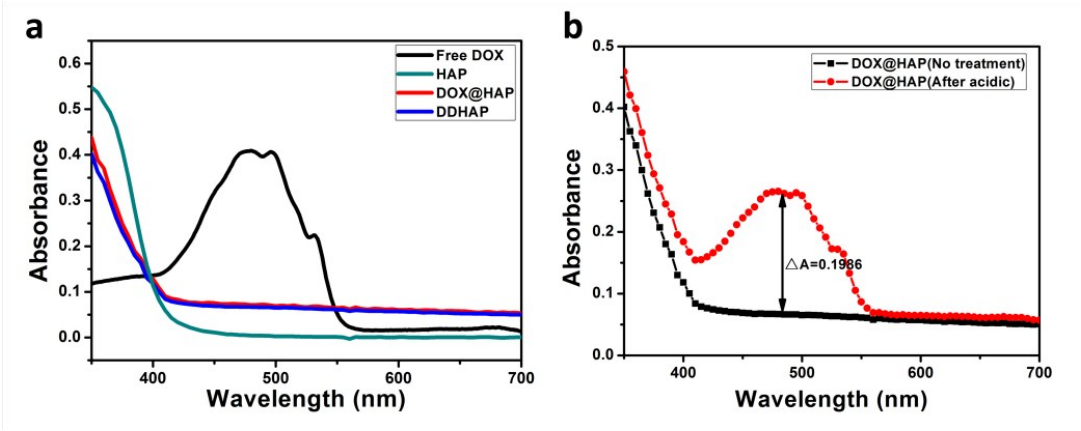


Figure S7. (a) UV spectra of DOX and nanoparticles (HAP, DOX@HAP and DDHAP). (b) UV spectra of DOX@HAP before and after acidolysis. The absorption of DOX@HAP (λ_{ab} = 479 nm) after acidolysis was used for calculating DOX loading content in the nanoparticles.

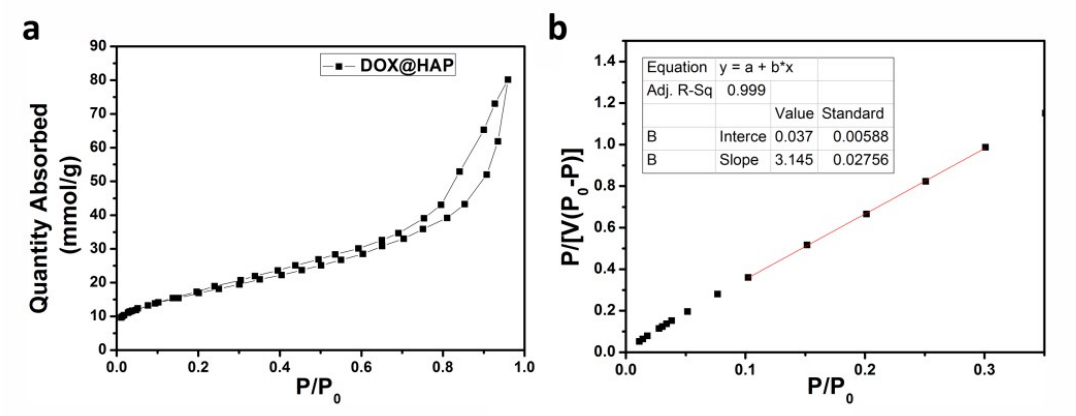


Figure S8. (a) N_2 adsorption-desorption isotherms of DOX@HAP. (b) BET calculation and its linear relationship: $P/[V(P_0-P)] = 3.145[P/P_0]+0.037$ ($S = 4.354Vm = 4.354 \times 24.91 = 108.45$ m^2/g).

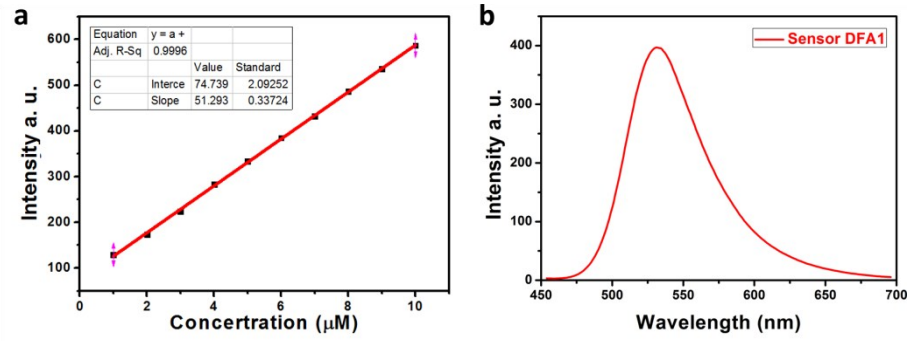


Figure S9. (a) The linear relationship between the fluorescence intensity ($\lambda_{em} = 531$ nm) of DFA_1 over concentrations (pH = 7.4). (b) DFA_1 fluorescence intensity after heating (at 50 °C for 15 min). High temperature breaks the hydrogen bond between DFA_1 and DDHAP. The fluorescence intensity of DFA_1 was used for calculating DFA_1 grafting content in DDHAP.

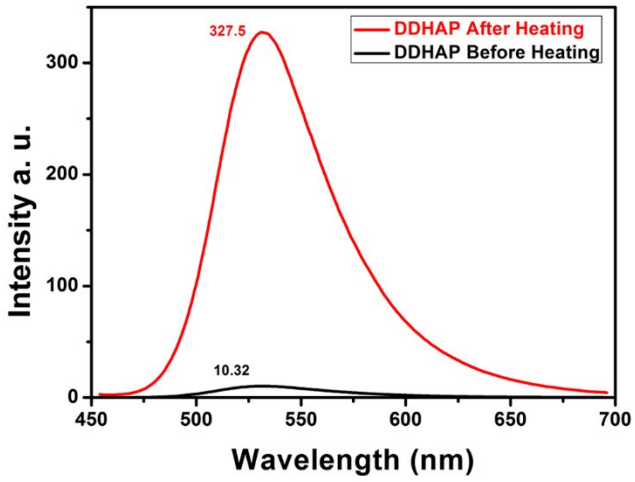


Figure S10. Fluorescence intensity of DDHAP before and after heating (at 50 °C for 15 min). High temperature breaks the hydrogen bond between DFA_1 and DDHAP. Thus the fluorescence of DFA_1 (red curve) was detected.

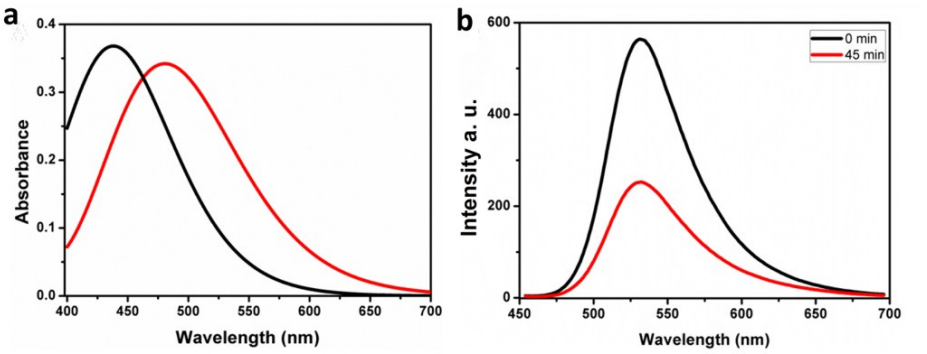


Figure S11. (a) Absorption and (b) fluorescence emission (λ_{ex} = 440 nm) spectra of DFA_1 (10 μM) before (black) and after (red) incubation with GGT (80 U/L) at 37 °C for 45 min.

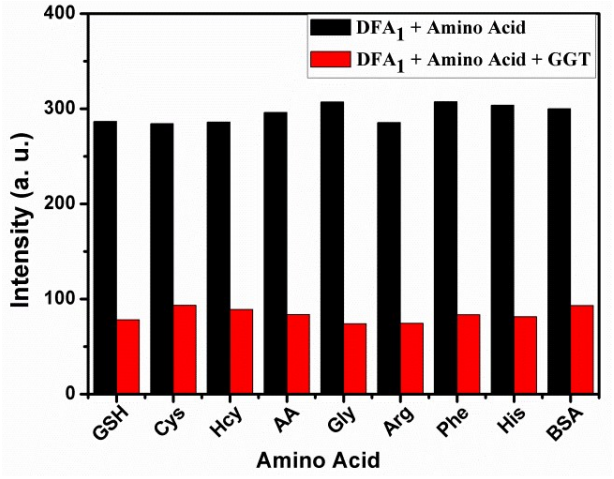


Figure S12. Fluorescence response of DFA_1 (10 μM) to GGT with or without different amino acids.

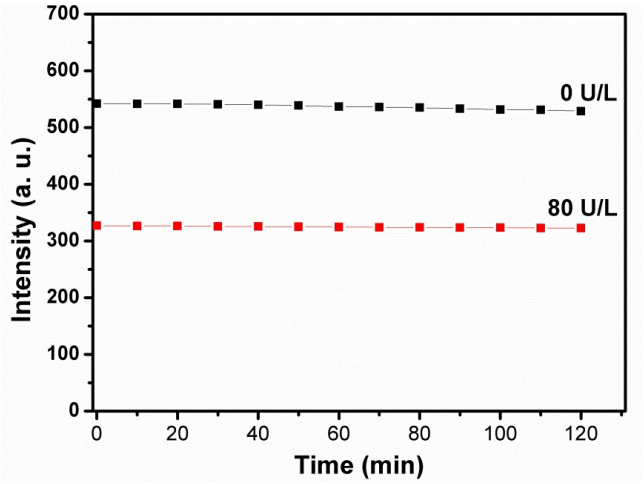


Figure S13. Photostability test of sensor DFA_1 (10 μM) in the absence and presence (80 U/L) of GGT in pH 7.4 PBS. The change of the fluorescence intensity ratio for the sensor is less than 5 % under continuous light irradiation (15 W 365 nm UV lamp), demonstrating the good photostability of DFA_1.

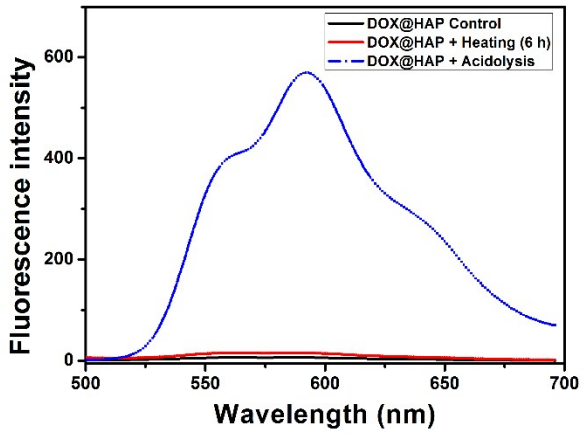


Figure S14. Fluoresce intensity of DOX@HAP after different treatment: black curve: control; red curve: heating in 75 ºC for 6 h; blue curve: incubation in acidic condition (pH = 6.0) for 120 min.

Table S1. IC_{50} value of different groups in cells (HL 7702 & HepG 2)

	HL-7702	HepG2
DDHAP	N/A	3.617
DOX	N/A	3.587
DOX@HAP	N/A	12.323

Table S2. IC_{50} value of DDHAP in different cells

	COS-7	HL-7702	A2780	HepG2	MCF-7	HCT-116
DDHAP	NA	NA	3.5454	3.617	3.899	3.582

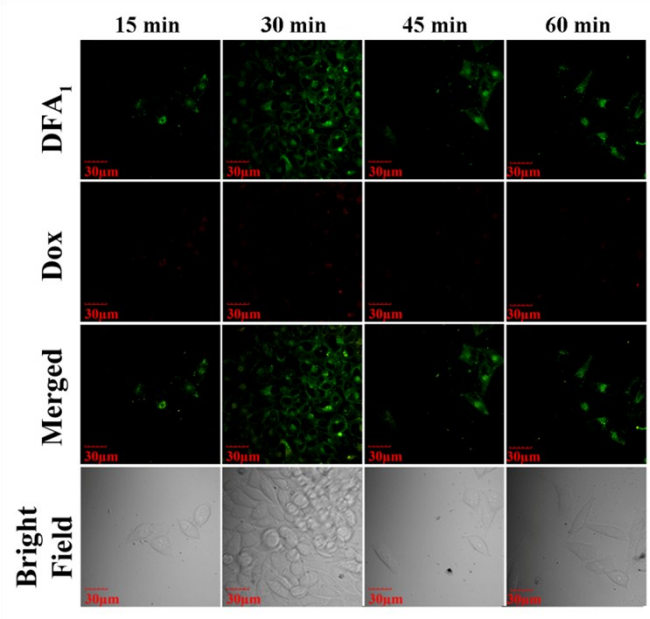


Figure S15. CLSM images of HL-7702 normal cells treated with DDHAP for 15 - 60 min. (Green channel: λ_{ex} = 458 nm, λ_{em} = 510-530 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm)

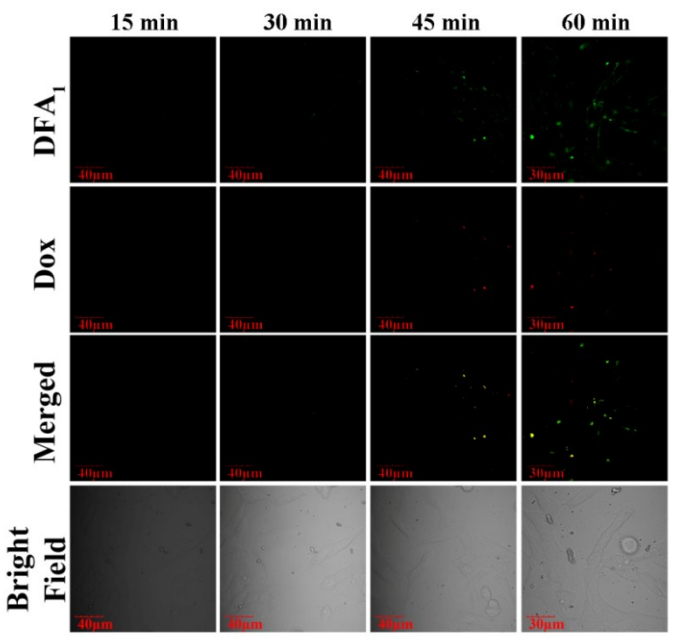


Figure S16. CLSM images of COS-7 cells treated with DDHAP for 15 - 60 min (Green channel: λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm).

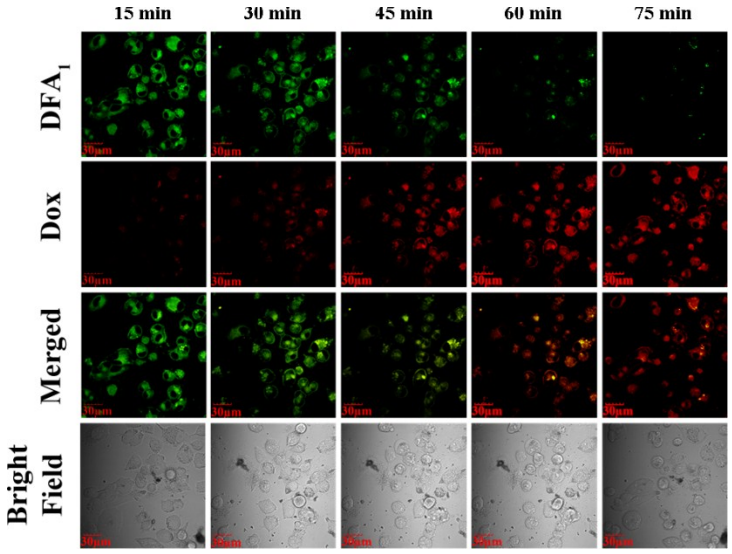


Figure S17. CLSM images of A2780 cells treated with DDHAP for 15 - 75 min (Green channel: λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm).

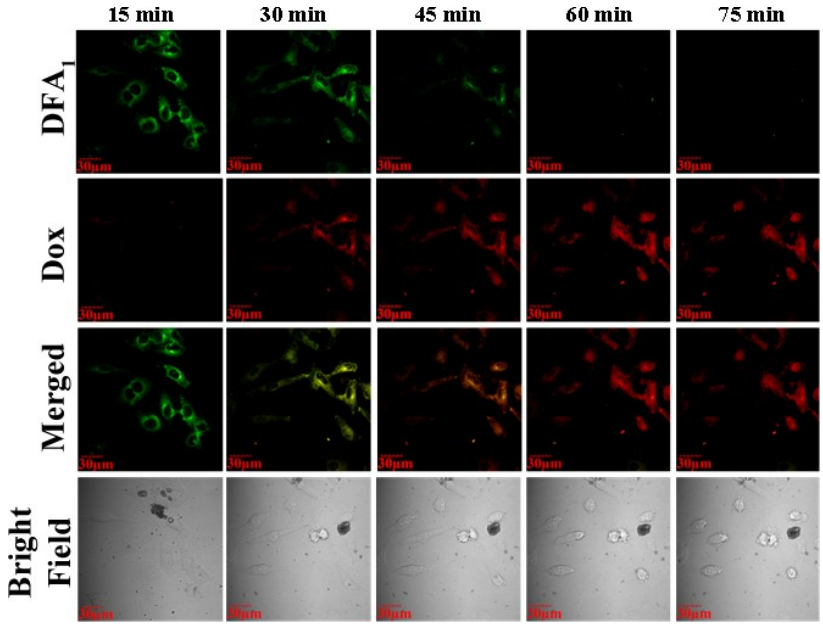


Figure S18. CLSM images of HCT-116 cells treated with DDHAP for 15 - 75 min (Green channel: λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm).

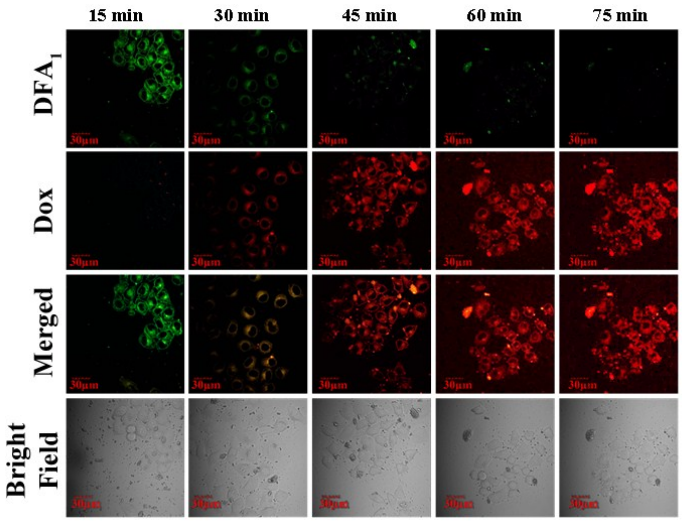


Figure S19. CLSM images of MCF-7 cells treated with DDHAP for 15 - 75 min (Green channel: λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm; Red channel: λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm).

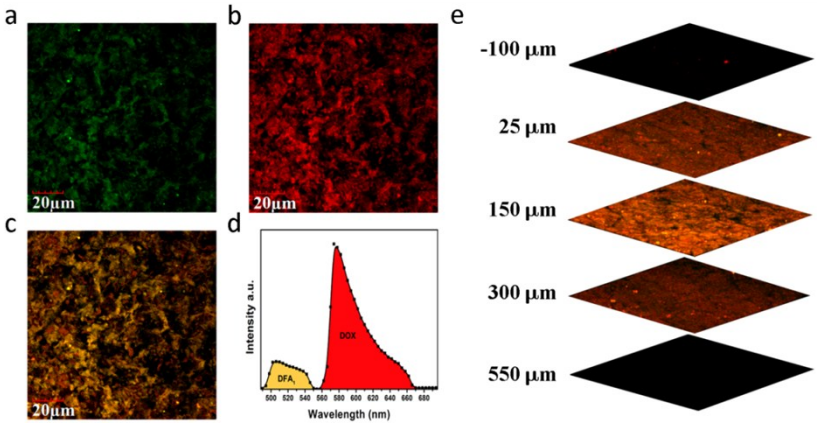


Figure S20. Confocal microscope observations of DDHAP nanoparticles internalized by tumor tissues (a-d). Tumors were incubated with DDHAP (10 mg DOX/L) for 4 h and the images were taken under confocal microscopy. (a) Green channel: DFA_1 (λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm); (b) Red channel: DOX (λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm); (c) Merged. (d) Fluorescence spectra of tumor tissues under the excitation of 458 nm. (e) Depth fluorescent imaging of tumor tissues (λ_{ex} = 458 nm).

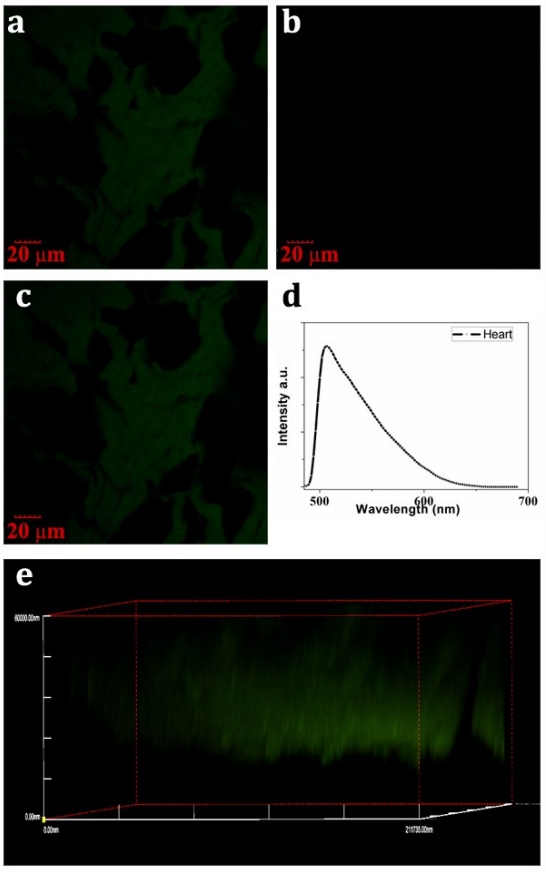


Figure S21. CLSM observations of DDHAP nanoparticles internalized by heart tissues (a-d). Tissues were incubated with DDHAP (10 mg DOX/L) for 4 h and the images were taken under confocal microscopy. (a) Green channel: DFA_1 (λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm); (b) Red channel: DOX; (λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm); (c) Merged. (d) Fluorescence spectrum of heart tissue under the excitation of 458 nm. (e) Depth fluorescent imaging of heart tissue (λ_{ex} = 458 nm). Images were taken with a 100× objective.

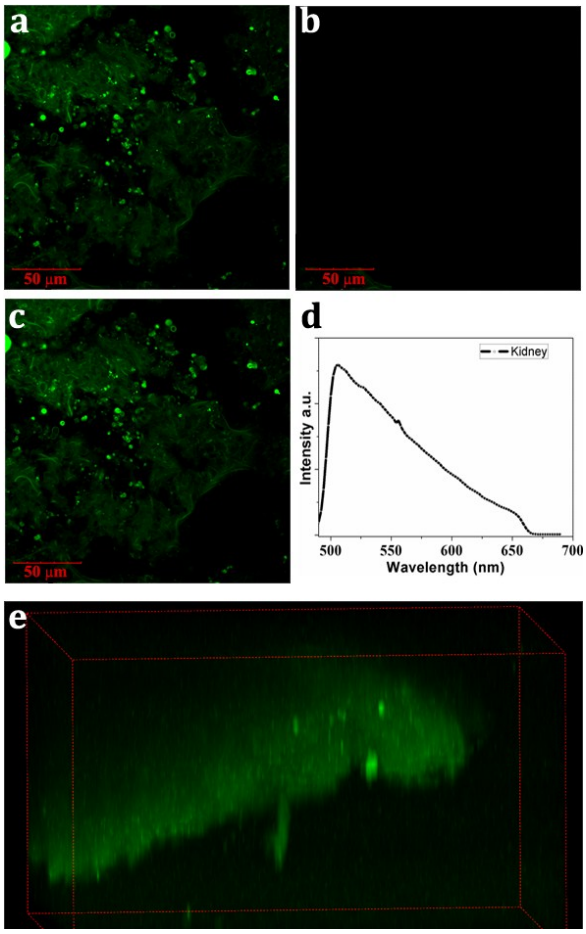


Figure S22. CLSM observations of DDHAP nanoparticles internalized by kidney tissues (a-d). Tissues were incubated with DDHAP (10 mg DOX/L) for 4 h and the images were taken under confocal microscopy. (a) Green channel: DFA_1 (λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm); (b) Red channel: DOX; (λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm); (c) Merged. (d) Fluorescent spectrum of kidney tissue under the excitation of 458 nm. (e) Depth fluorescence imaging of kidney tissue (λ_{ex} = 458 nm). Images were taken with a 100× objective.

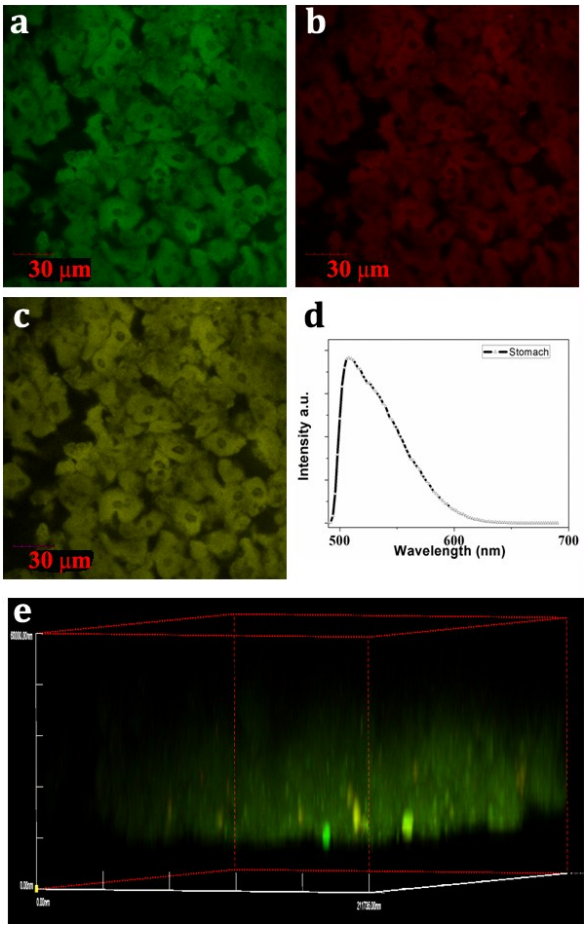


Figure S23. CLSM observations of DDHAP nanoparticles internalized by stomach tissues (a-d). Tissues were incubated with DDHAP (10 mg DOX/L) for 4 h and the images were taken under confocal microscopy. (a) Green channel: DFA_1 (λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm); (b) Red channel: DOX; (λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm); (c) Merged. (d) Fluorescence spectrum of stomach tissue under the excitation of 458 nm. (e) Depth fluorescence imaging of stomach tissue (λ_{ex} = 458 nm). Images were taken with a 100×objective.

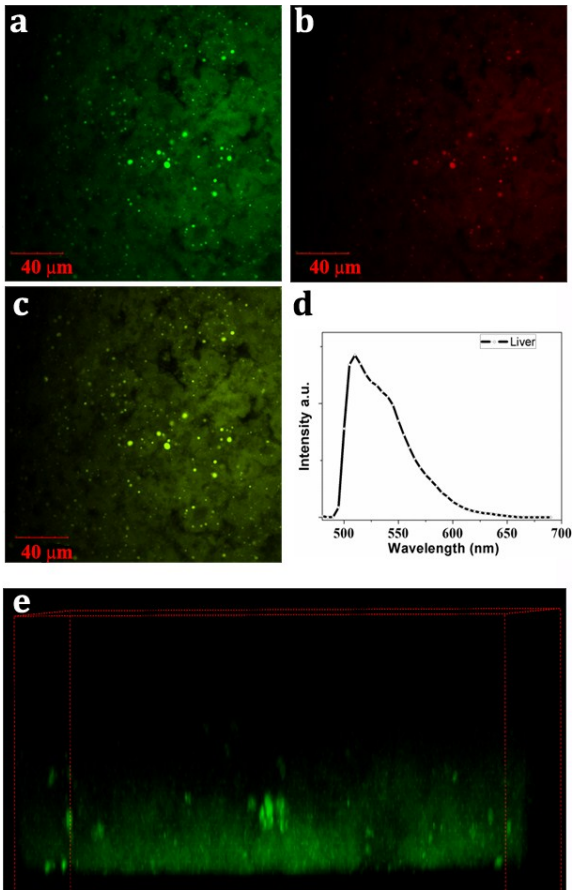


Figure S24. CLSM observations of DDHAP nanoparticles internalized by liver tissues (a-d). Tissues were incubated with DDHAP (10 mg DOX/L) for 4 h and the images were taken under confocal microscopy. (a) Green channel: DFA_1 (λ_{ex} = 458 nm, λ_{em} = 510 - 530 nm); (b) Red channel: DOX; (λ_{ex} = 488 nm, λ_{em} = 590 - 620 nm); (c) Merged. (d) Fluorescence spectrum of liver tissue under the excitation of 458 nm. (e) Depth fluorescence imaging of liver tissue (λ_{ex} = 458 nm). Images were taken with a 100× objective.

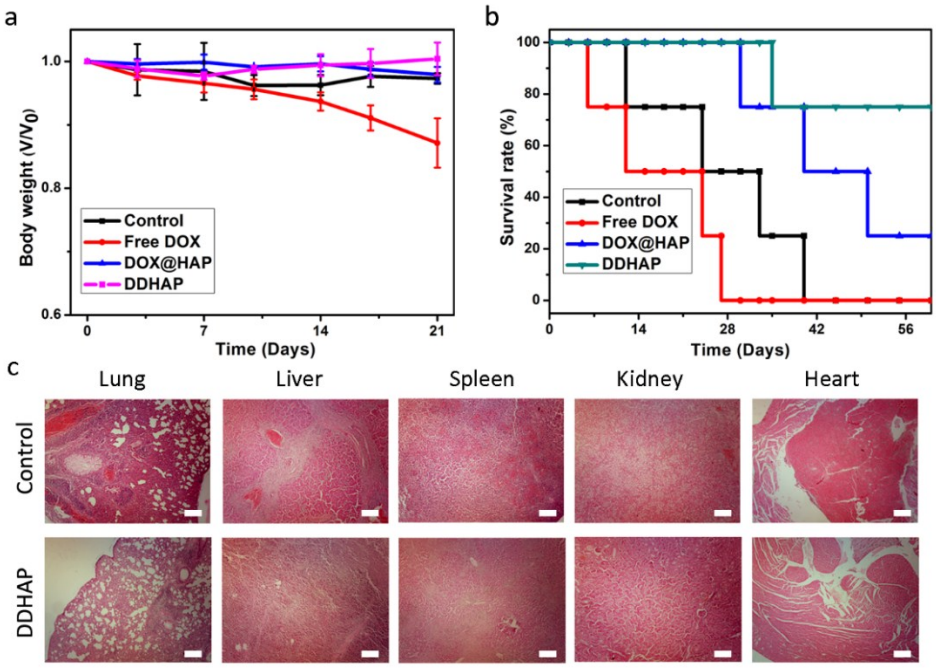


Figure S25. a) Body weights of HepG2 tumor-bearing mice during different treatment. Data are given as mean ± SD (N = 4). b) Survival rate of HepG2 tumor-bearing mice of each group over time. One mouse in the DDHAP group was killed at day 35th for the *in vivo* anticancer evaluation. c) H&E staining images of important organs of the Control and the DDHAP groups after treatment. The mice were sacrificed, and the organs were isolated for staining. Scale bar: 100 μm.

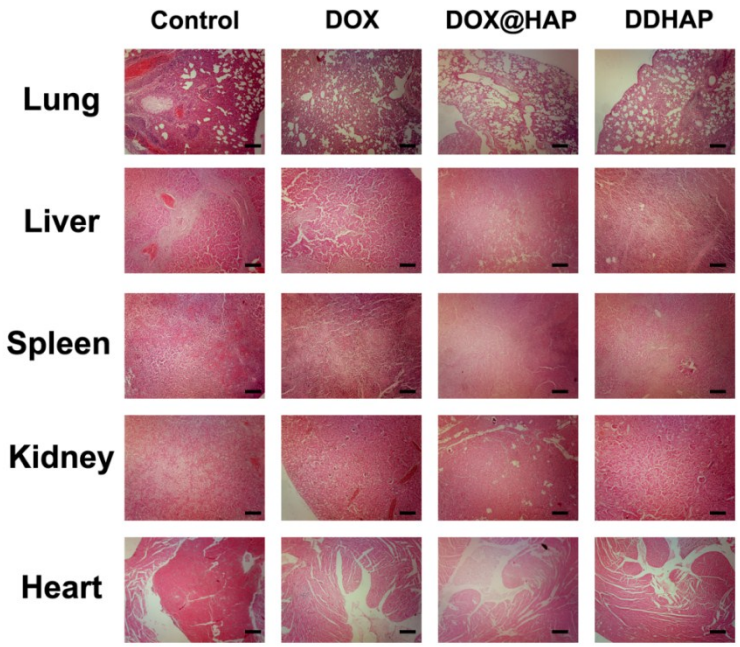


Figure S26. H&E staining of organ tissues in different groups. Scale bar: 100 μm

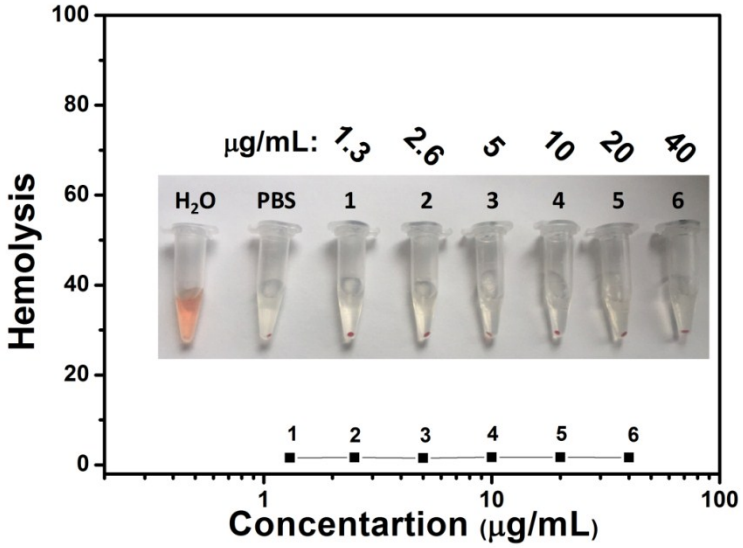


Figure S27. Blood hemolysis using DDHAP at different concentrations (sample 1 to 6). Water was used as a positive control and saline was used as a negative control. No hemolysis was observed even when the concentration of DDHAP was 40 μg/mL, which demonstrated that the nanoparticles are safe to the blood.