Supporting Information

Celecoxib Induced Self-assembly of Smart Albumin-Doxorubicin Conjugate for Enhanced Cancer Therapy

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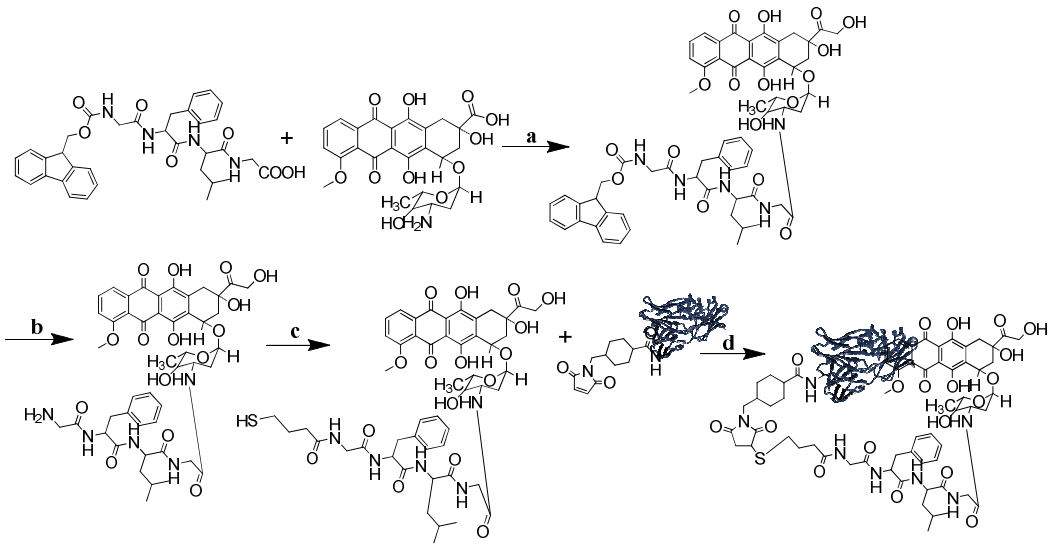

Figure S1. Schematic diagram of synthesis of HSA-GFLG-DOX conjugate. (a) EDCI, NHS, DMF, room temperature. (b) Piperidine, DMF, room temperature. (c) Traut's reagent, PBS buffer, DMSO, room temperature. (d) Click reaction at PBS buffer.

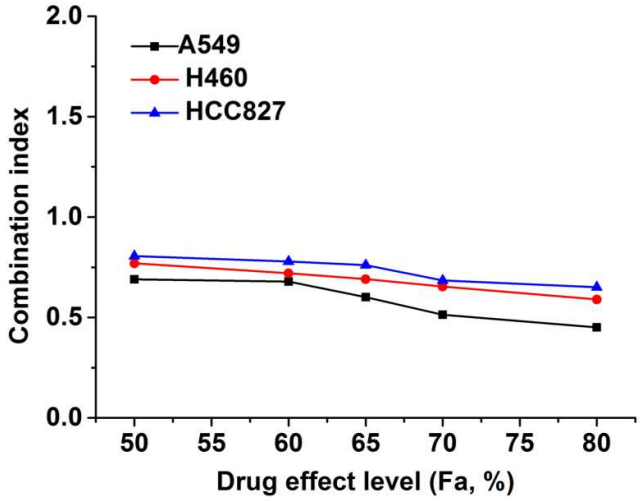

Figure S2: The combination index (CI) plots for different A549, H460 and HCC827 cell lines, respectively.

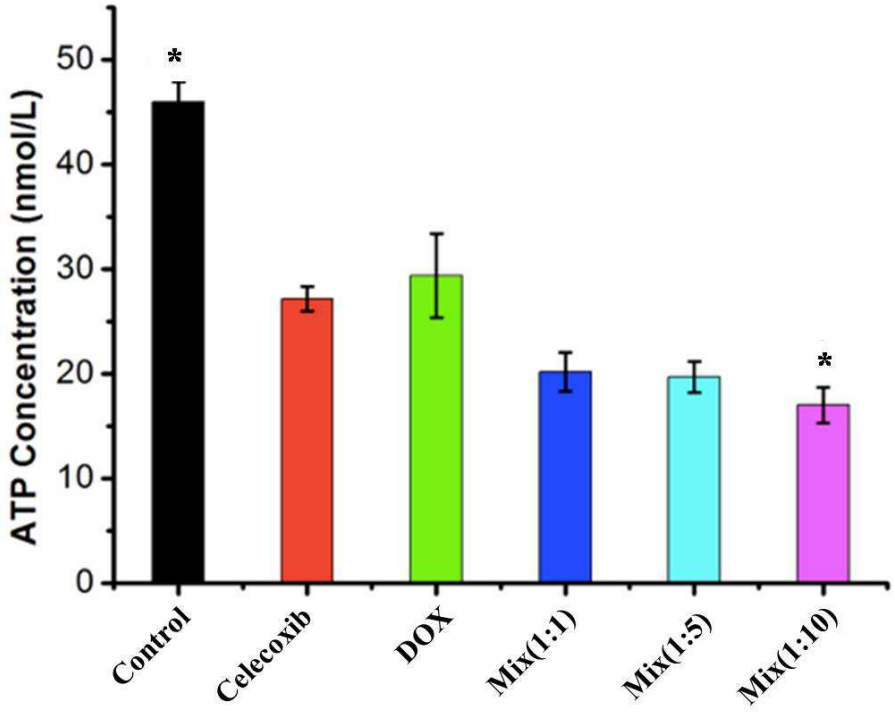

Figure S3. The cellular ATP content in A549 cells induced by drug groups and the control group. Data are presented as average ± standard error (n = 7), and the statistical significance level is *p<0.05.

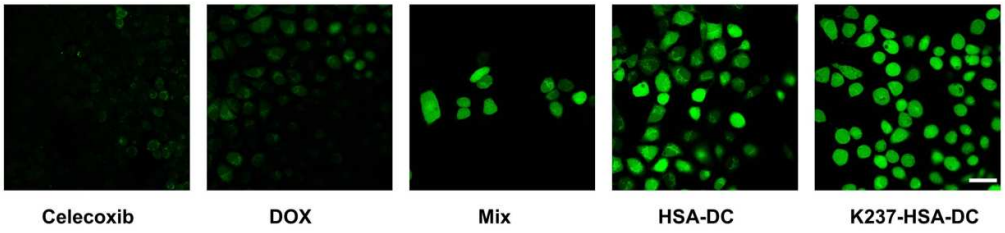

Figure S4. The cellular ROS content in A549 cells induced by drug groups and the control group. Upper: Bright field, middle: the green fluorescence of DCFH-DA probe, bottom, the merged image. The scale bar is 25 μm.

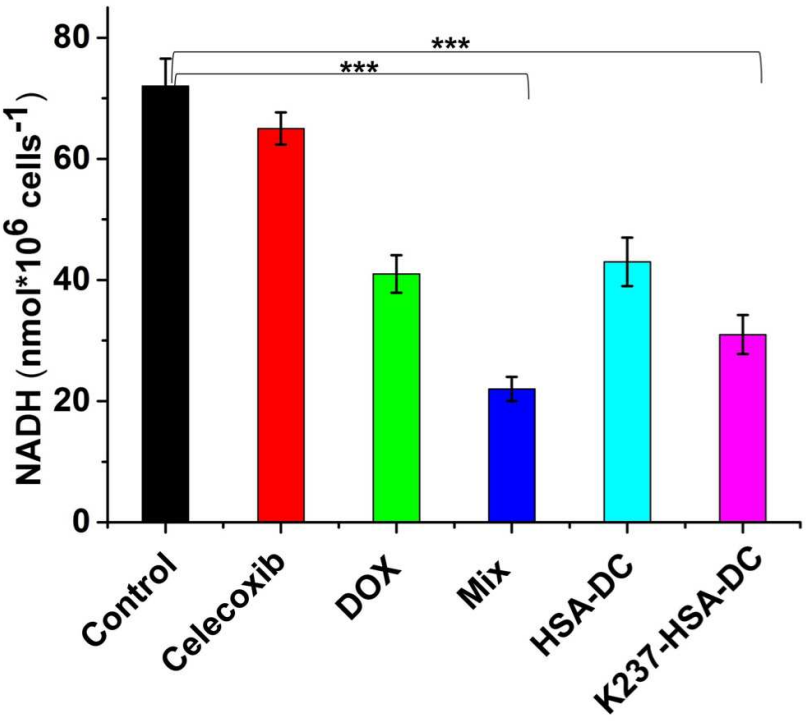

Figure S5. Hexokinase activity measurement in A549 cell lysates after cells were treated by different agents.

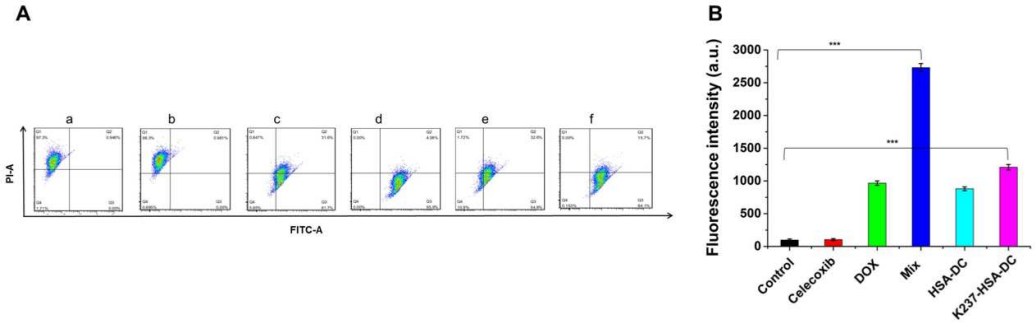

Figure S6. Mitochondria membrane potential analysis of A549 cells with JC-1 (Beyotime) staining after cells were treated by different agents. A: The ratio of red fluorescence and green fluorescence in A549 cells analyzed by flow cytometry (a, Control; b, Celecoxib; c, DOX, d, Mix; e, HSA-DC; f, K237-HSA-DC); B: The intensity of green fluorescence in

A549 cells after cells treated by Celecoxib, DOX, Mix, HSA-DC and K237-HSA-DC, respectively.

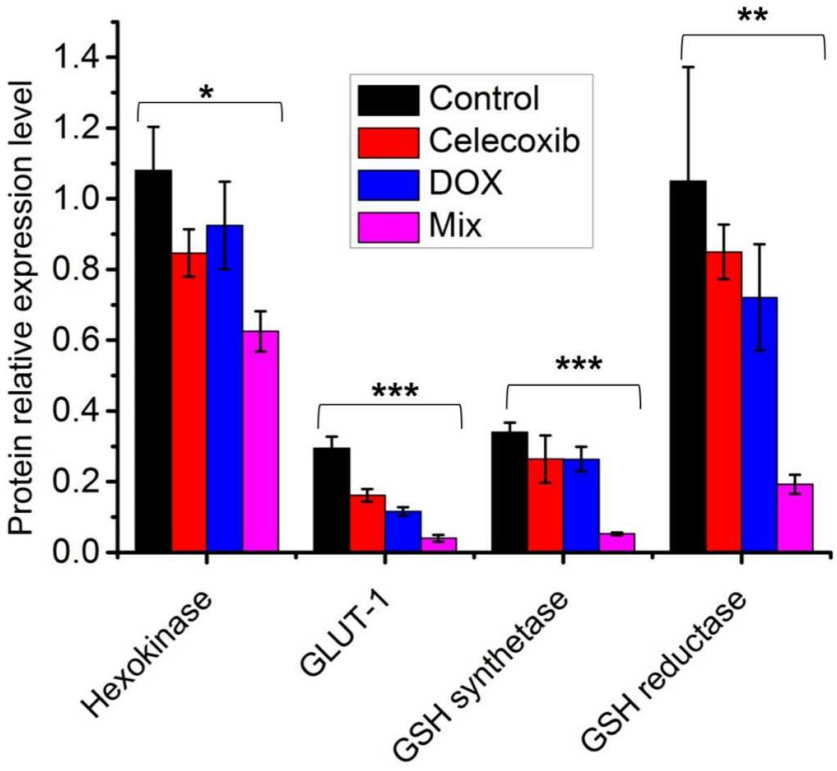

Figure S7. The protein relative expression level in A549 cells induced by celecoxib (20 μM), DOX (2 μM), drug mixture (molar ratio, 1:10) for 24 h. The statistical significance level is *p<0.05, **p<0.01, ***p<0.001.

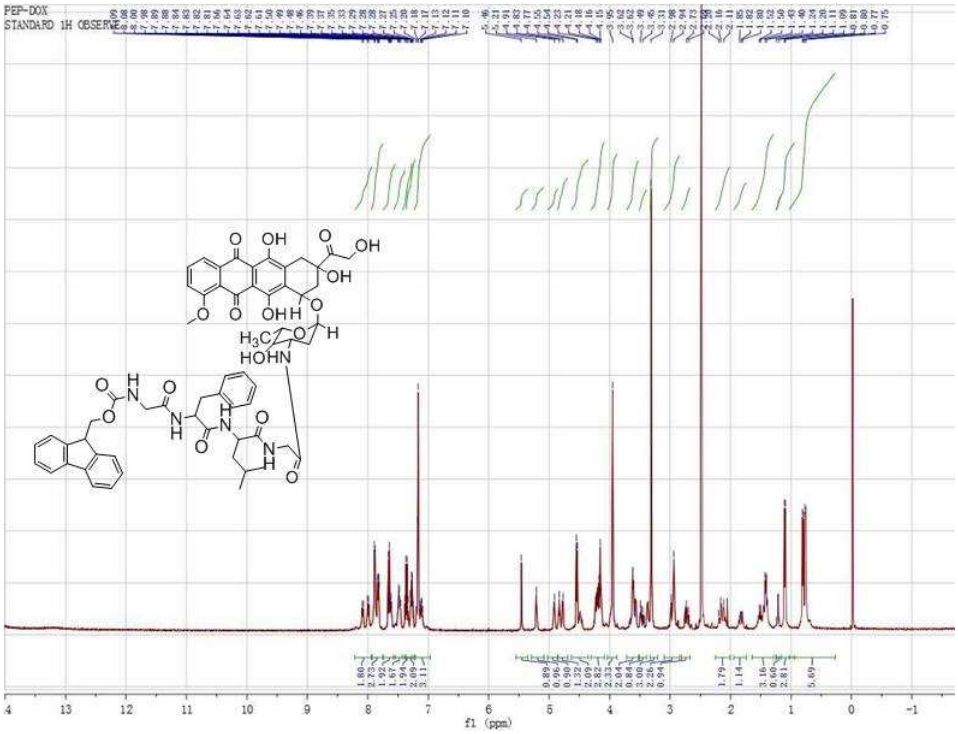

Figure S8. The ^{1}H-NMR of Fmoc-GFLG-DOX (400 MHz, d_6-DMSO).

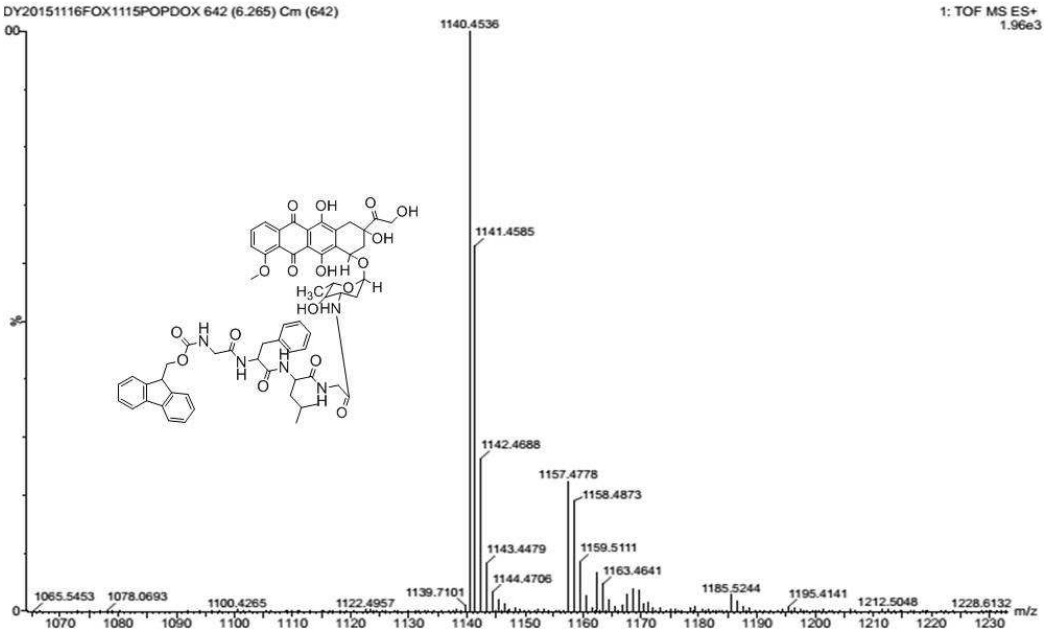

Figure S9. Mass spectrum of Fmoc-GFLG-DOX.

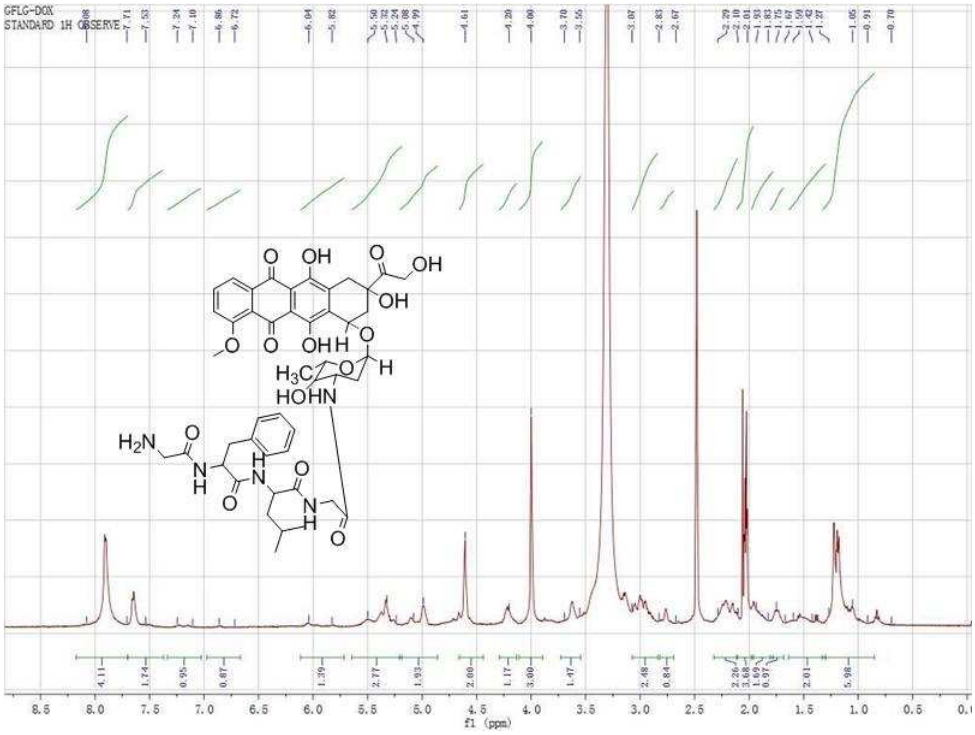

Figure S10. ^{1}H-NMR spectrum of GFLG-DOX (400 MHz, d_6-DMSO).

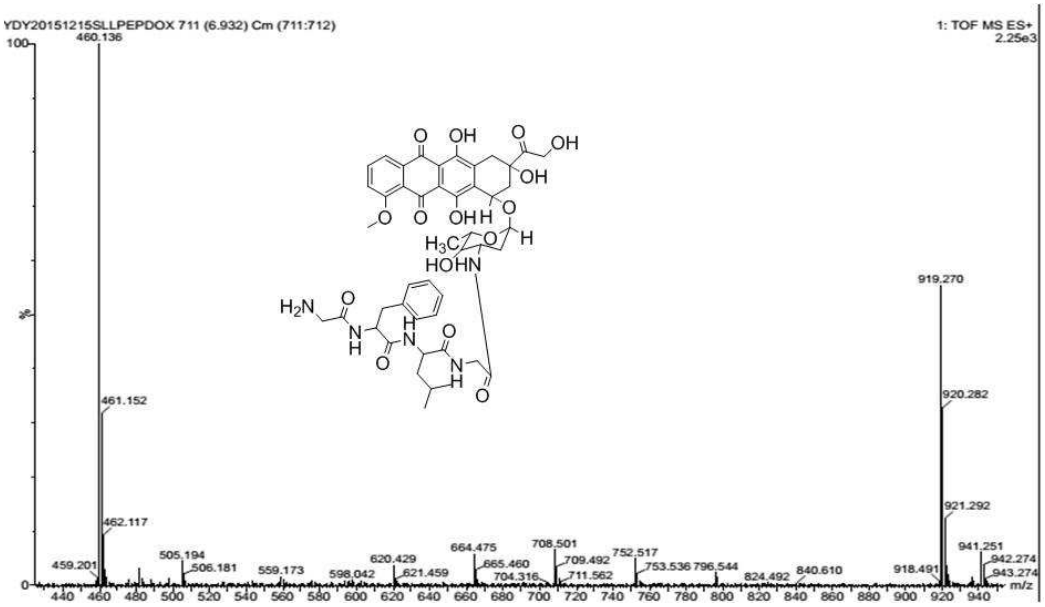

Figure S11. Mass spectrum of GFLG-DOX.

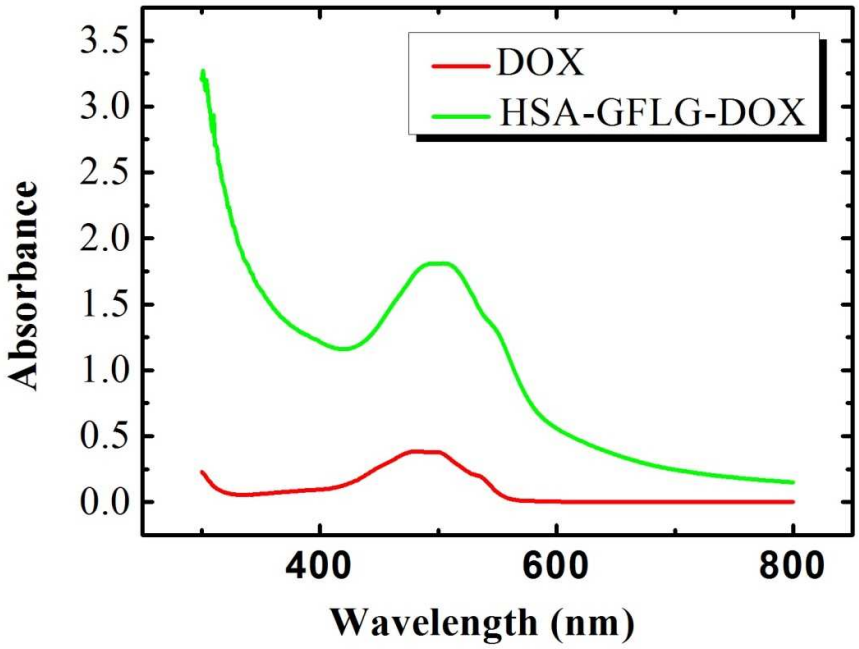

Figure S12. UV-Vis spectra of HSA-GFLG-DOX and DOX, HSA-GFLG and DOX.HCl were dispersed in PBS buffer.

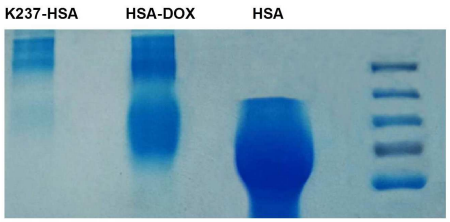

Figure S13: Polyacrylamide gel electrophoresis (PAGE) for HSA, HSA-GFLG-DOX, K237-HSA identification.

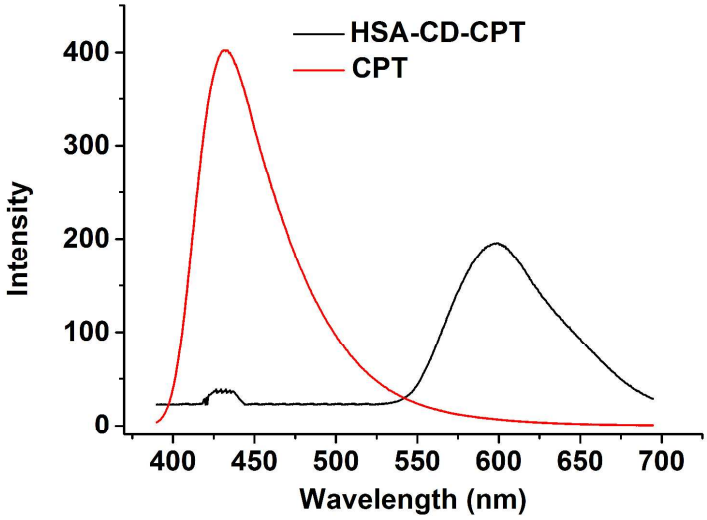

Figure S14: Fluorescence intensity study of HSA-CD-CPT and CPT with excitation wavelength 370 nm.

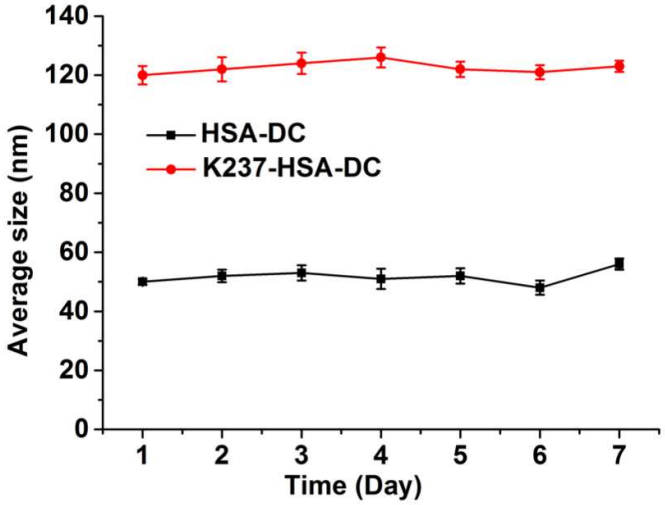

Figure S15: Size stability measurement of HSA-DC and K237-HSA-DC for different time points.

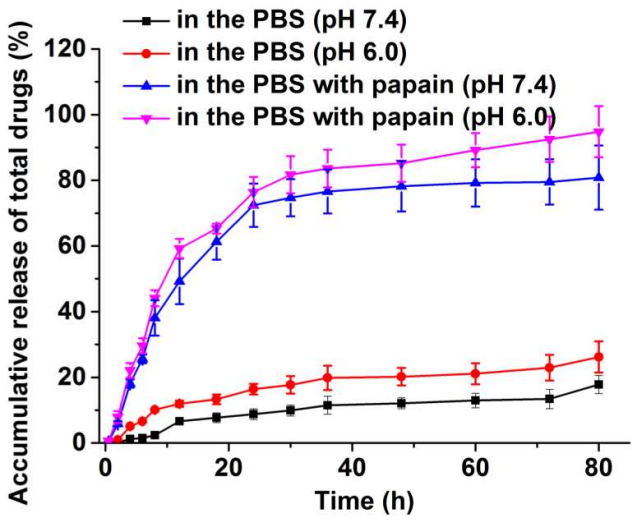

Figure S16. Accumulative drug release from HSA-DC nanoparticles in PBS buffer (pH = 7.4 and 6.0) and PBS buffer containing papain (2 μM).

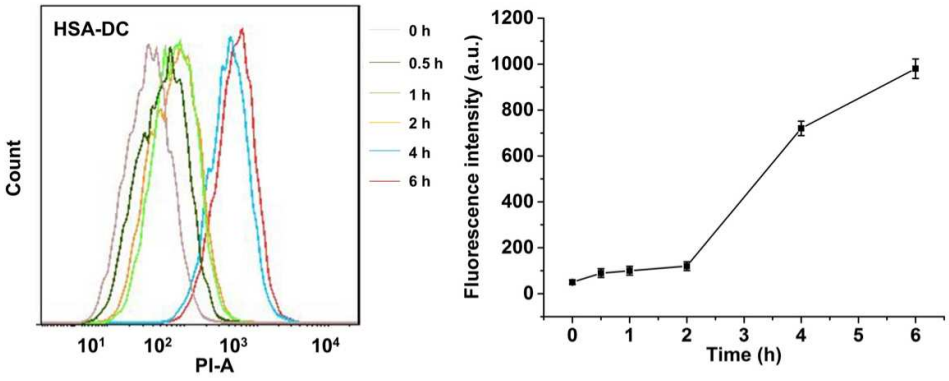

Figure **S17.** Representative flow cytometry histogram profiles of A549 cells cultured with HSA-DC for 0.5 h, 1 h, 2 h, 4 h, 6 h, respectively.

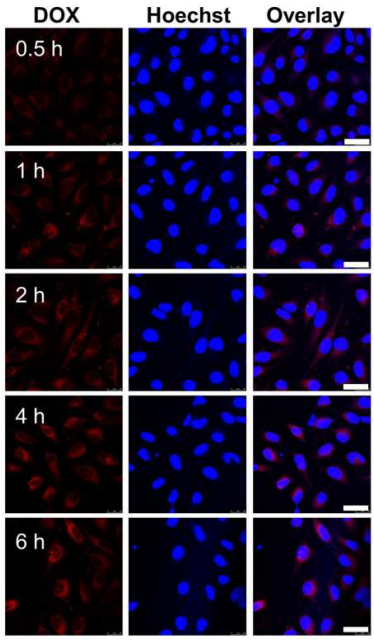

Figure **S18.** Fluorescence microscopy images of A549 cells cultured with HSA-DC for 0.5 h, 1 h, 2 h, 4 h, 6 h, respectively. The blue fluorescence indicates cell nuclei stained by Hoechst. The red fluorescence is fluorescence-emitting DOX. The scale bar is 25 μm.

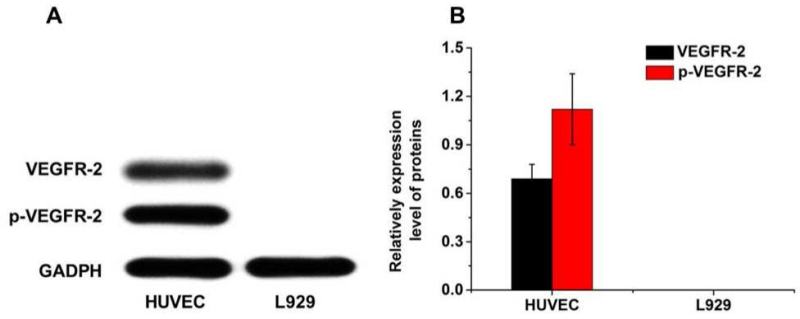

Figure S19. The expression level of VEGFR-2 and phosphorylation of VEGFR-2 in HUVEC and L929 cells, respectively. (a) Western blot analysis of VEGFR-2 and p-VEGFR-2 in HUVEC and L929 cells. (b) Quantification of the western blot band densities.

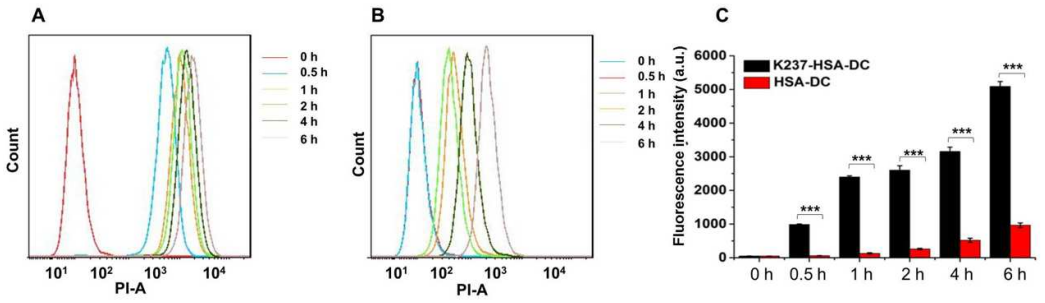

Figure S20. Representative flow cytometry histogram profiles of HUVEC cells cultured with HSA-DC and K237- HSA-DC for 0.5 h, 1 h, 2 h, 4 h, 6 h, respectively.

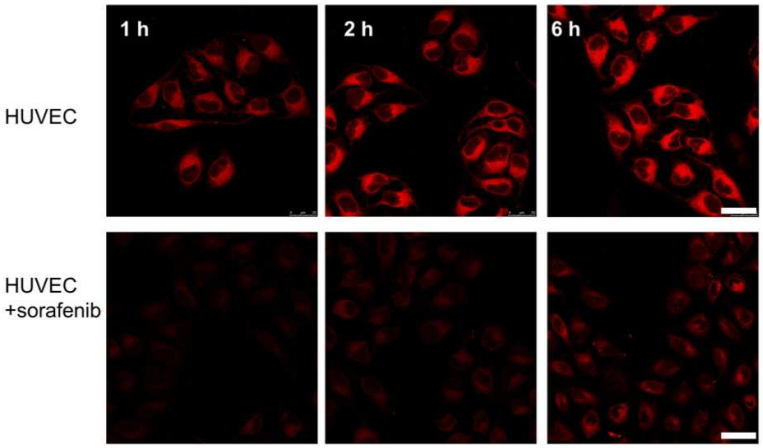

Figure S21. Fluorescence microscopy images of HUVEC cultured with K237-HSA-DC. Upper: HUVEC cultured with K237-HSA-DC for 1 h, 2 h and 6 h, respectively. Bottom: HUVEC was firstly incubated with sorafenib (2 μM) for 24 h to inhibit VEGFR-2, then K237-HSA-DC was incubated with HUVEC for 1 h, 2 h and 6 h respectively. The red fluorescence is fluorescence-emitting of DOX. The scale bar is 25 μm.

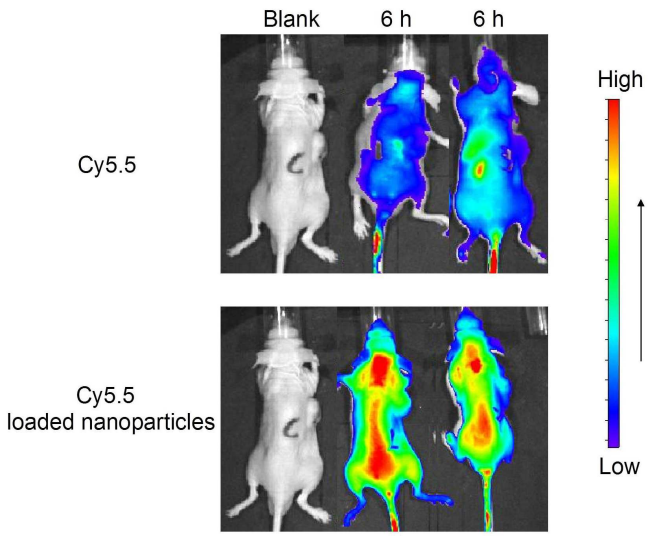

Figure S22. In vivo near-infrared images of whole body imaging of A549 tumor-bearing nude mice after intravenous injection of free Cy5.5 and Cy5.5-loaded K237-HSA-DC for 6 h.

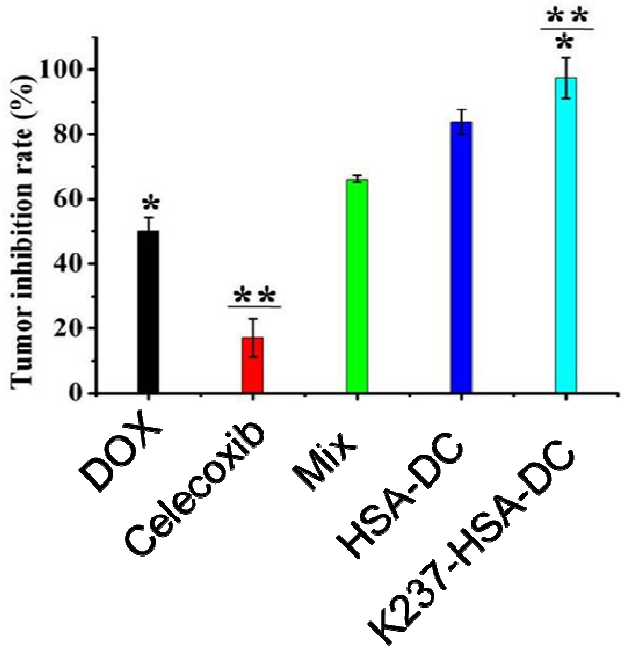

Figure S23. Tumor inhibition rate (TIR) is calculated as the following equation: TIR (%) = 100 × (mean tumor weight of control group - mean

tumor weight of experimental group) / mean tumor weight of control group. Data are represented as average ± standard error (n = 7), and the statistical significance level is *p<0.05, **p<0.01, ***p<0.001.

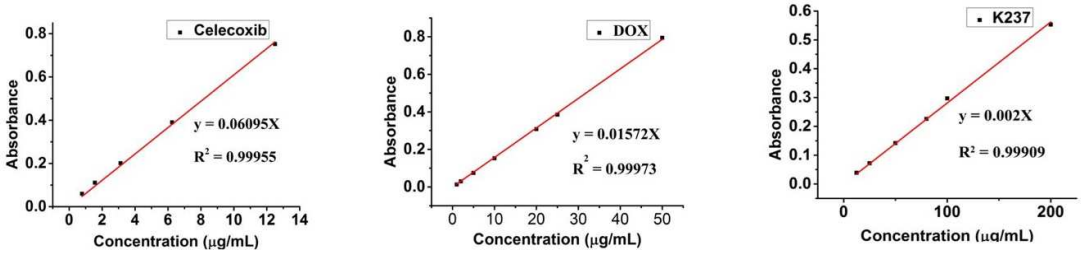

Figure S24. Standard curves of celecoxib, DOX and K237, respectively.

Table S1. Intracellular metabolites induced by celecoxib, DOX, and drug mixture, respectively. Variable importance in the projection (VIP) was obtained from OPLS with a thresold of 1.0. *p* Value was calculated by student's *t*-test.

Biomarker (Celecoxib)	FC	P	VIP	Biomarker (DOX)	FC	P	VIP	Biomarker (Drug mixture)	FC	P	VIP
glutathione	0.039557	2.79E-10	1.42445	glutathione	0.051604	6.57E-11	1.71306	hypoxanthine	0.185977	1.48E-10	1.46438
aspartic acid	3.68389	4.21E-10	1.42344	hypoxanthine	0.314337	8.40E-08	1.67933	creatine	5.26554	2.56E-07	1.42757
phosphate	1.99538	9.39E-07	1.37609	N-(3-aminopropyl)-morpholine	2.59327	3.65E-05	1.57128	proline	0.640977	5.01E-07	1.42066
proline	0.642809	1.59E-06	1.36934	proline	1.20736	9.55E-05	1.53772	N-Methyl-L-glutamic acid	2.1329	1.96E-06	1.40333
Isoleucine	2.52152	3.48E-06	1.35776	Isoleucine	2.14095	0.000103	1.53472	tyrosine	0.38795	2.94E-06	1.39718
tyrosine	0.358391	4.95E-06	1.35193	phosphate	1.40605	0.000308	1.48625	putrescine	0.158046	6.21E-06	1.38436
putrescine	0.139689	5.31E-06	1.35071	uracil	1.41398	0.000351	1.47956	aspartic acid	2.47443	8.23E-06	1.37899
myo-inositol	3.17896	7.75E-06	1.34385	glycine	0.755063	0.000556	1.45474	glutathione	0.188537	2.16E-05	1.35799
hypoxanthine	0.334863	3.82E-05	1.30805	Pyruvic acid	0.301847	0.001062	1.41508	Fluorene	2.68722	2.48E-05	1.35458
Creatine	24.4885	5.16E-05	1.29984	tyrosine	0.698902	0.00172	1.38145	myo-inositol	1.98159	1.31243	1.34456
hydroxylamine	2.247	0.000152	1.26569	glutamic acid	0.697497	0.002277	1.3601	cholesterol	0.631402	0.000145	1.3016
fumaric acid	0.215848	0.000235	1.24934	lactic acid	0.755111	0.002843	1.34218	Isoleucine	1.80559	1.27724	1.2919
L-Malic acid	5.52514	0.00028	1.24232	taurine	1.76039	0.003166	1.33312	fumaric acid	0.249483	0.04693	1.2817
glycerol	1.87219	0.000296	1.24	cholesterol	1.22144	0.003193	1.3324	L-cysteine	1.8132	0.000331	1.2687
uracil	2.7233	0.000648	1.20475	fumaric acid	0.343343	0.003575	1.32258	phosphate	1.36277	1.25923	1.2147
N-acetyl-L-aspartic acid	1.53701	0.00071	1.20025	nicotinamide	1.50057	0.005043	1.29118	oxoproline	1.18444	1.21914	1.2009
Pyruvic acid	0.260113	0.000862	1.19031	D-Glyceric acid	0.490986	0.005752	1.27846	inosine	1.34746	1.21708	1.1988
guanosine	1.34389	0.000864	1.19017	ornithine	0.569355	0.005815	1.27737	hydroxylamine	1.63312	0.001544	1.18894
glutamic acid	0.611903	0.000877	1.18938	pantothenic acid	0.59315	0.013758	1.18251	asparagine	0.335652	0.001775	1.18023
glycine	0.745461	0.00109	1.17765	asparagine	0.530401	0.01706	1.15533	lactic acid	0.725706	0.001992	1.17279
N-(3-aminopropyl)-morpholine 2	2.72816	0.001191	1.17273	glutamine	2.61984	0.027069	1.09144	uracil	1.67062	0.002411	1.16005
inosine	1.26906	0.002722	1.12134	lysine	0.780687	0.032922	1.06183	L-Malic acid	2.86852	0.00427	1.11825
ornithine	0.482705	0.002828	1.1187	aspartic acid	0.796184	0.04647	1.00547	ornithine	0.550992	0.004588	1.11257
serine	1.30477	0.002987	1.11491	hypoxanthine	0.314337	8.40E-08	1.67933	glycerol	1.31681	0.004732	1.1101
asparagine	0.410146	0.00463	1.08257	N-(3-aminopropyl)-morpholine	2.59327	3.65E-05	1.57128	phenylalanine	0.702041	0.005678	1.09513
taurine	1.76912	0.006088	1.06056	L-Malic acid	1.850	0.0076	1.0041	glutamine 3	3.21689	0.006198	1.0877
glutamine	3.87715	0.006101	1.06039					Pyruvic acid	0.532504	0.010849	1.03615
lactic acid	0.735826	0.00733	1.04476								