Electronic Supplementary Material (ESI) for Biomaterials Science.

Bifunctional liposome reduces chemotherapy resistance of doxorubicin induced by Reactive Oxygen Species

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Supporting Information

Table.S1 Feed Ratio and Obtained Ratio of DOX: ACF in DOX-ACF@Lipo

Feed Ratio (ACF: DOX)	DOX (μg/mL)	ACF (μg/mL)	Obtained ratio (ACF: DOX)	Diameter (nm)
2.1:1	478.9	1018.8	2.13:1	136.46
2.33:1	543.6	1156.7	2.12:1	148.21
2.5:1	565.4	1203.3	2.12:1	152.73
3:1	527.6	1012.9	1.92:1	161.36

	Diameter(nm)	PDI
DOX@Lipo	**136.71**	**0.100**
ACF@Lipo	**139.19**	**0.094**
DOX-ACF@Lipo	**141.72**	**0.103**

Fig.S1 Polydispersity index of DOX@Lipo, ACF@Lipo and DOX-ACF@Lipo

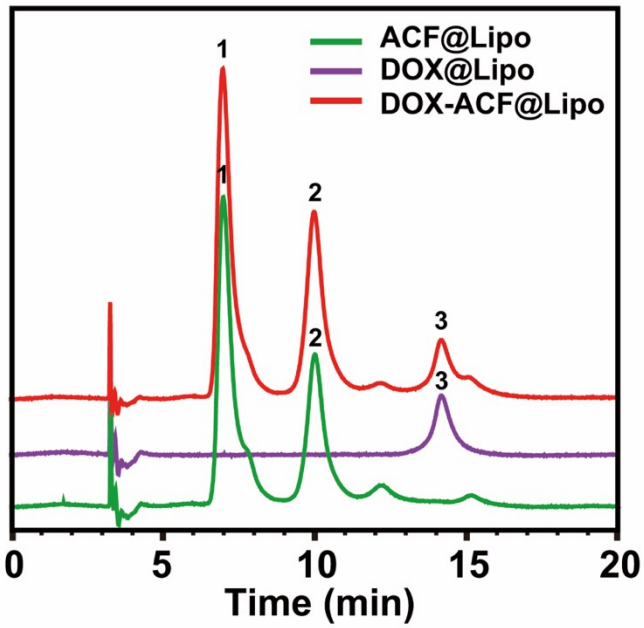

Fig.S2 High Performance Liquid Chromatography (HPLC) data of DOX-ACF@Lipo (Red), DOX@Lipo (purple) and ACF@Lipo (green). The peak 1 and peak 2 belong to two components of ACF (3,6-Diamino-10-methylacridinium and 3,6-Diaminoacridine) and the peak 3 belongs to DOX.

DOX Concentration (μg/mL)	Peak area at 480nm
0	0
1	2175
10	92036
20	225927
50	589928
80	1009350
100	1294387

ACF Concentration (μg/mL)	Peak area at 468nm
0	0
1	22891
10	494992
20	1089958
50	2729218
80	4369534
100	5394433

Fig.S3 The standard curve value of DOX and ACF.

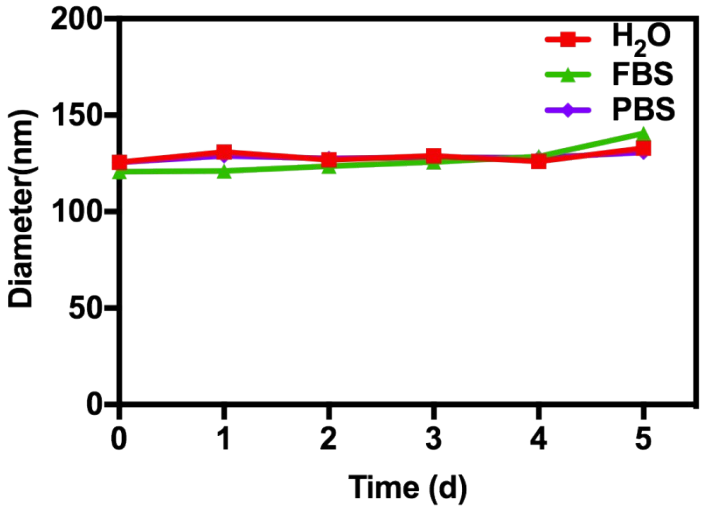

Fig.S4 Size distribution of DOX-ACF@Lipo after 5 days of storage at 4 °C

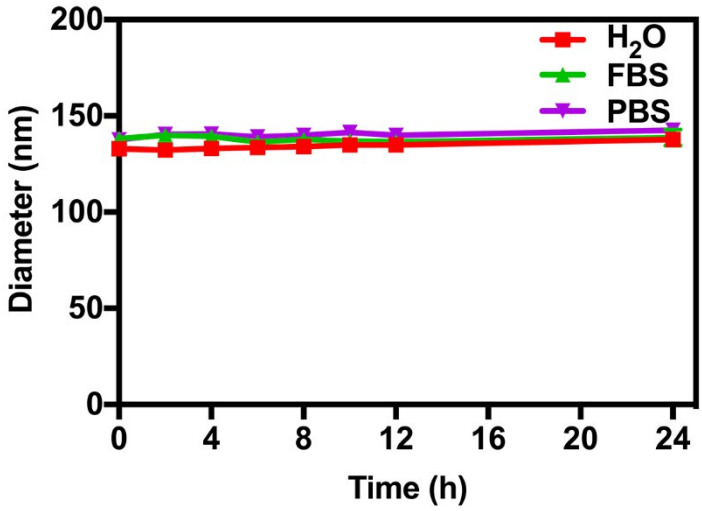

Fig.S5 Size distribution of DOX-ACF@Lipo after 24 h of storage at room

temperature.

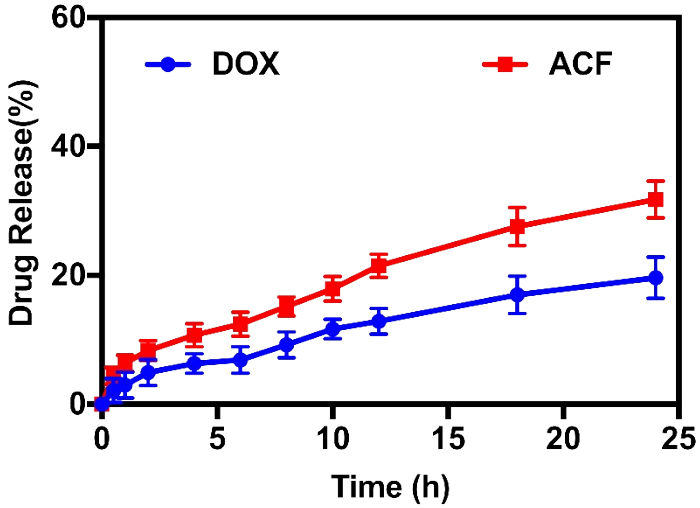

Fig.S6 Drug release curves of DOX-ACF@Lipo.

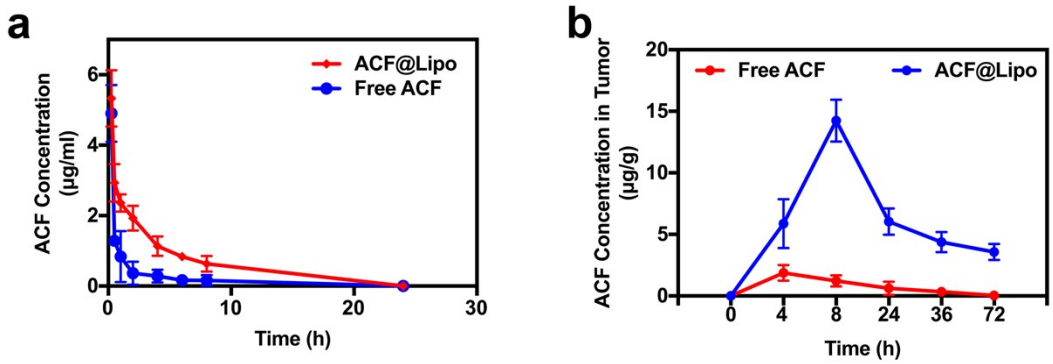

Fig.S7 Accumulation and the pharmacokinetic behavior of ACF. (a) Pharmacokinetic curves of acriflavine in vivo ([acriflavine] = 3 mg/kg). Blood samples were collected and analyzed with HPLC at different time points (0.25, 0.5, 1, 2, 4, 8, 24 h). (b) The dynamic concentrations of acriflavine accumulated in the tumor tissues after intravenous injection of free acriflavine or ACF@Lipo ([acriflavine] = 3 mg/kg). Data are shown as mean ± SD (n = 3).

Table.S2 Pharmacokinetic parameters.

	AUC_{0-t} (mg/L*h)	$AUMC_{0-t}$ (h*h*mg/ml)	MRT_{0-t} (h)	$t_{1/2}$ (h)	C_{max} (mg/L)
Free ACF	4.98±0.23	7.78±2.05	1.55±0.35	3.01±2.58	4.91±0.81
ACF@Lipo	12.41±1.17	32.58±2.25	2.63±0.07	3.70±0.42	5.33±0.80

* Data was shown as mean ±SD (n=3). The pharmacokinetic parameters of acriflavine were analyzed by DAS 3.2 software.

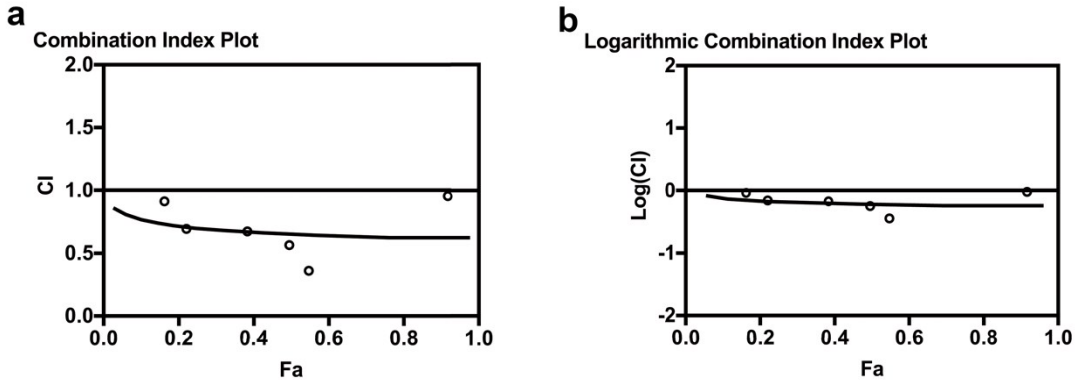

Fig.S8 The combination index (CI) of DOX-ACF@Lipo. (a)Combination Index Plot. (b) Logarithmic Combination Index Plot.

Table.S3 CI values for actual experimental points*.

Total Dose	Fa	CI Value
0.147	0.917	0.95394
0.735	0.547	0.35934
1.47	0.495	0.56614
2.94	0.383	0.67359
7.35	0.22	0.69430
14.7	0.162	0.91293

* The CI values for actual experimental points were analyzed by the CompuSyn v1.0 software.

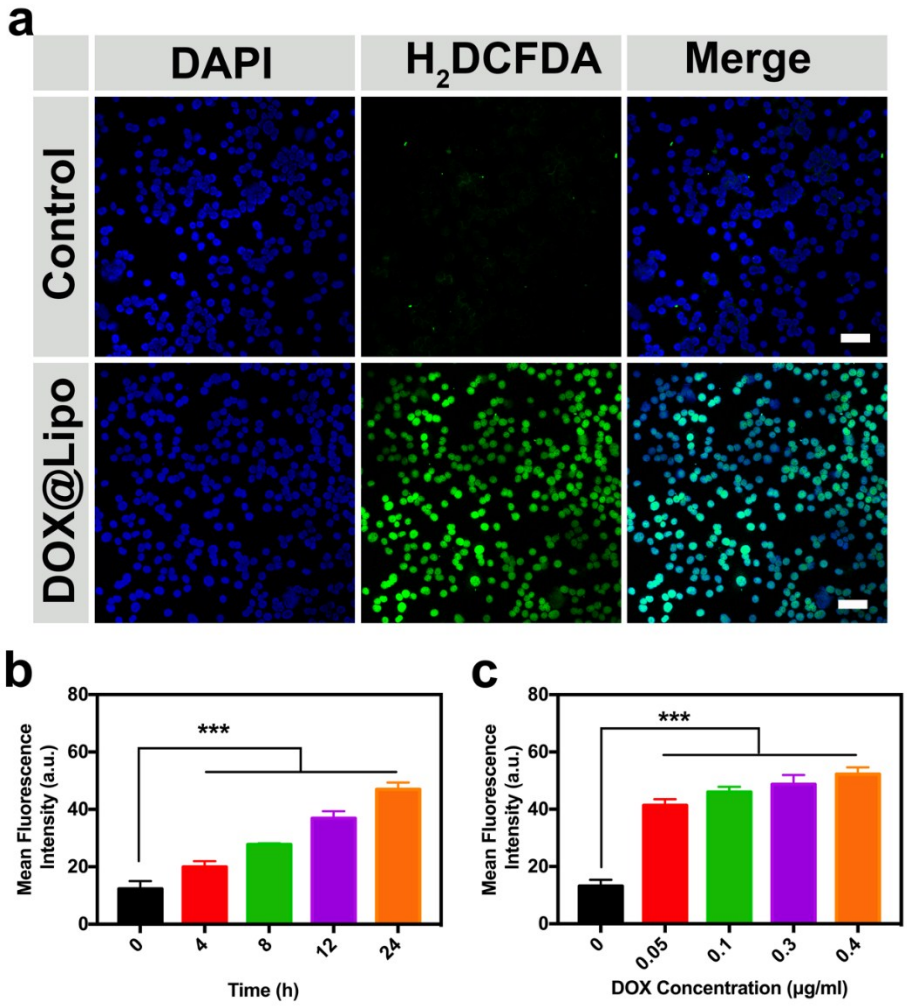

Fig. S9 Generation of hydrogen peroxide in vitro. (a) Images of CT26 cells stained with H_2DCFDA and DAPI after indicated treatments, scale bar= 50 μm. (b) The ROS content of CT26 cells analyzed with flow cytometer after different treatments with the indicated time. (c) Dynamic changes of ROS content as a function of DOX concentrations. Data are shown as mean ± SD; *p < 0.05; **p < 0.01; ***p < 0.001.