Supporting Information

Magnetite Nanoparticles and Spheres for Chemo- and Photothermal Therapy of Hepatocellular Carcinoma in vitro

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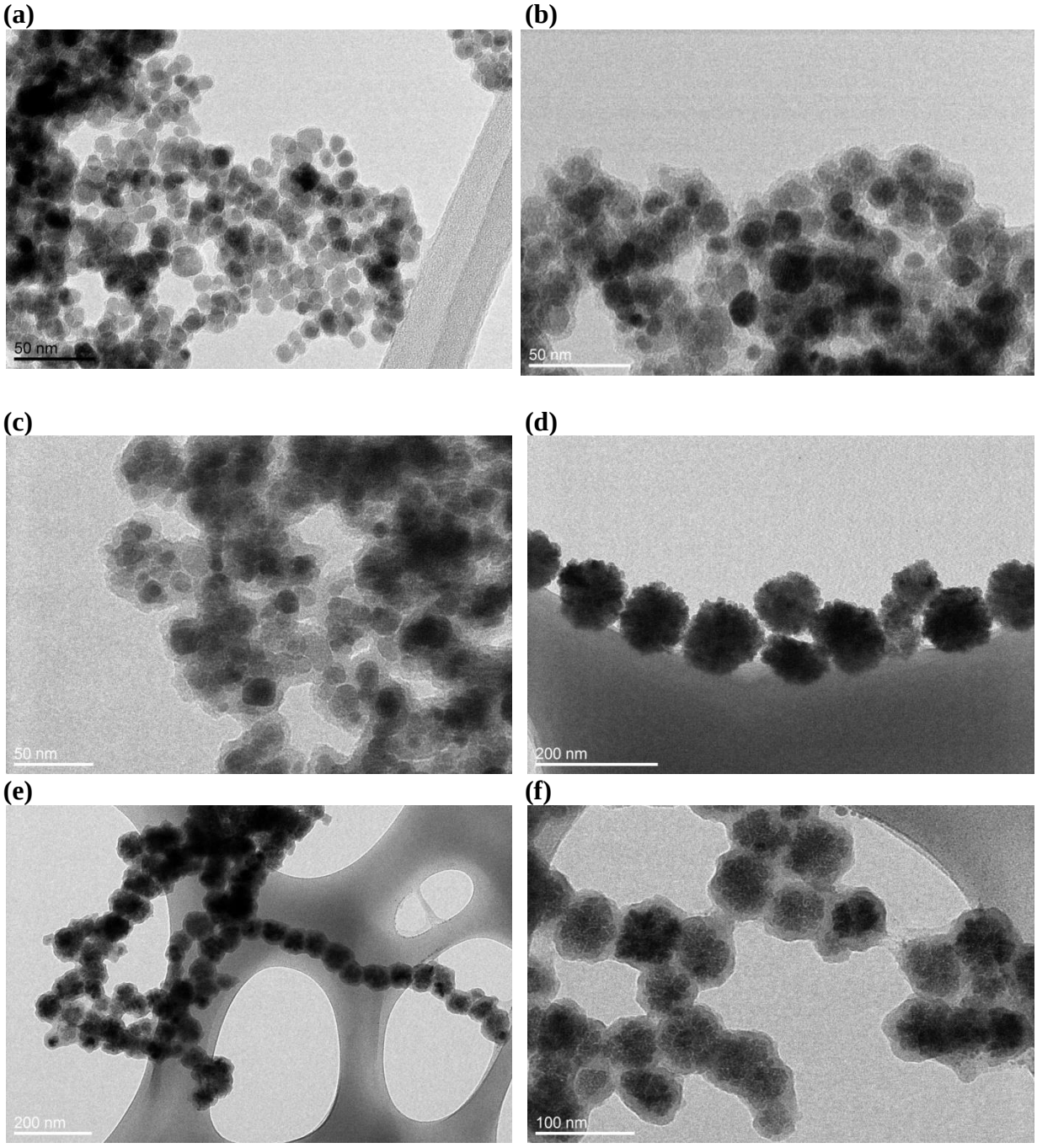
Preparation of SH-FA derivative
1. Folic acid NHS ester.

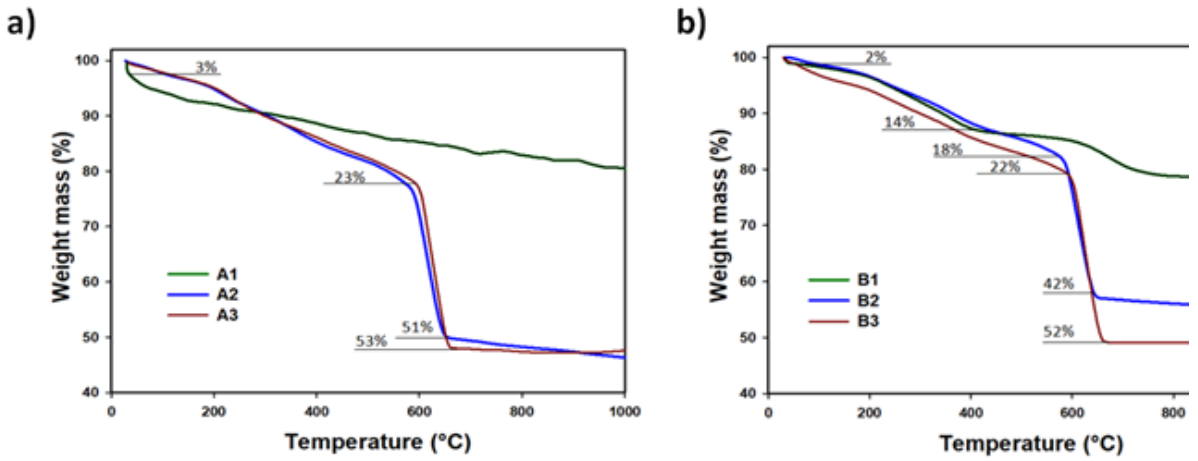
2 g of folic acid (FA) was dissolved in 80 mL DMSO and 10 mL TEA under nitrogen environment till FA was completely dissolved. Then 0.52 g of NHS and 1 g of DCC added to the FA solution and the reaction was carried out at ambient temperature for 48h, protected from light. After filtration, the filtrate was mixed with a copious amount of ethyl acetate to precipitate FA-NHS. The precipitate was collected with filtration and washed with ethanol. Then the filtrate was dissolved in DMSO and re-precipitated with a copious amount of diethyl ether. The precipitate was separated without filtration with repeated diethyl ether washes to remove DMSO, and the residual solvent was removed in vacuo. When FA-NHS did not precipitate from diethyl ether, a mixture of ethyl acetate and petroleum ether was used. After removal of solvents in vacuo, 1.848 g of yellow powder was obtained.

2. Preparation of folic acid thiol derivative of folic acid (FA-SH).

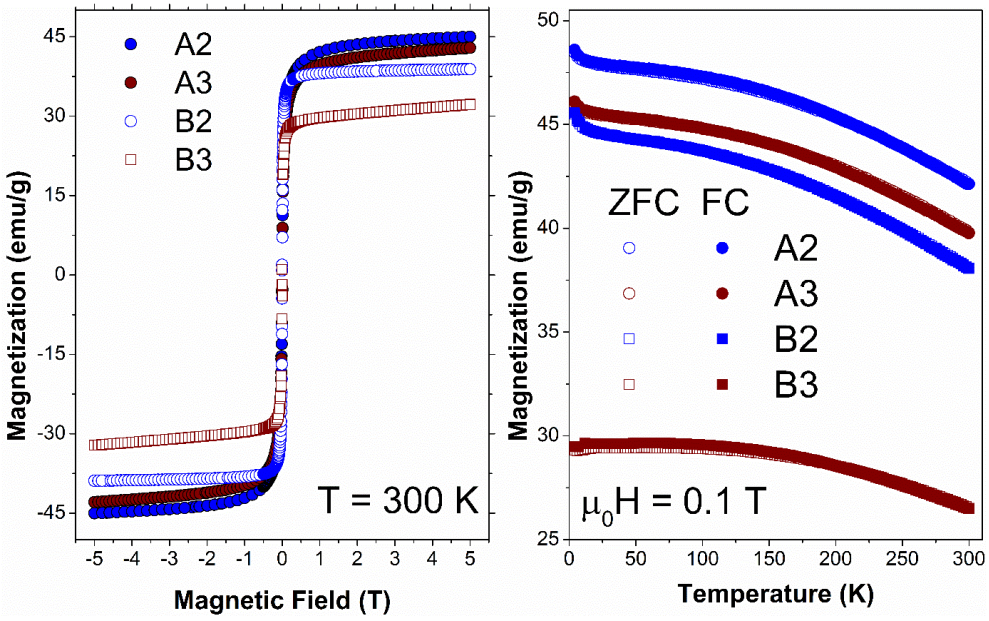
0.381 mg (0.72mmol) of FA-NHS, 3 mL of anhydrous TEA and 89 mg (0.79 mmol) of cysteamine-HCl were added into 18 mL of DMSO. The mixture was stirred for 18h under nitrogen atmosphere in the dark, at ambient temperature. After filtration, the filtrate was first precipitated by copious amount of EtOAc – diethyl ether – petroleum ether mixture to remove the unreacted reactants in DMSO. After final precipitation, the product was dissolved in DMSO and precipitated from ethanol – petroleum ether mixture. After removal of solvents in vacuo, 302 mg of yellow powder was obtained.
Live/Dead cell viability assay.



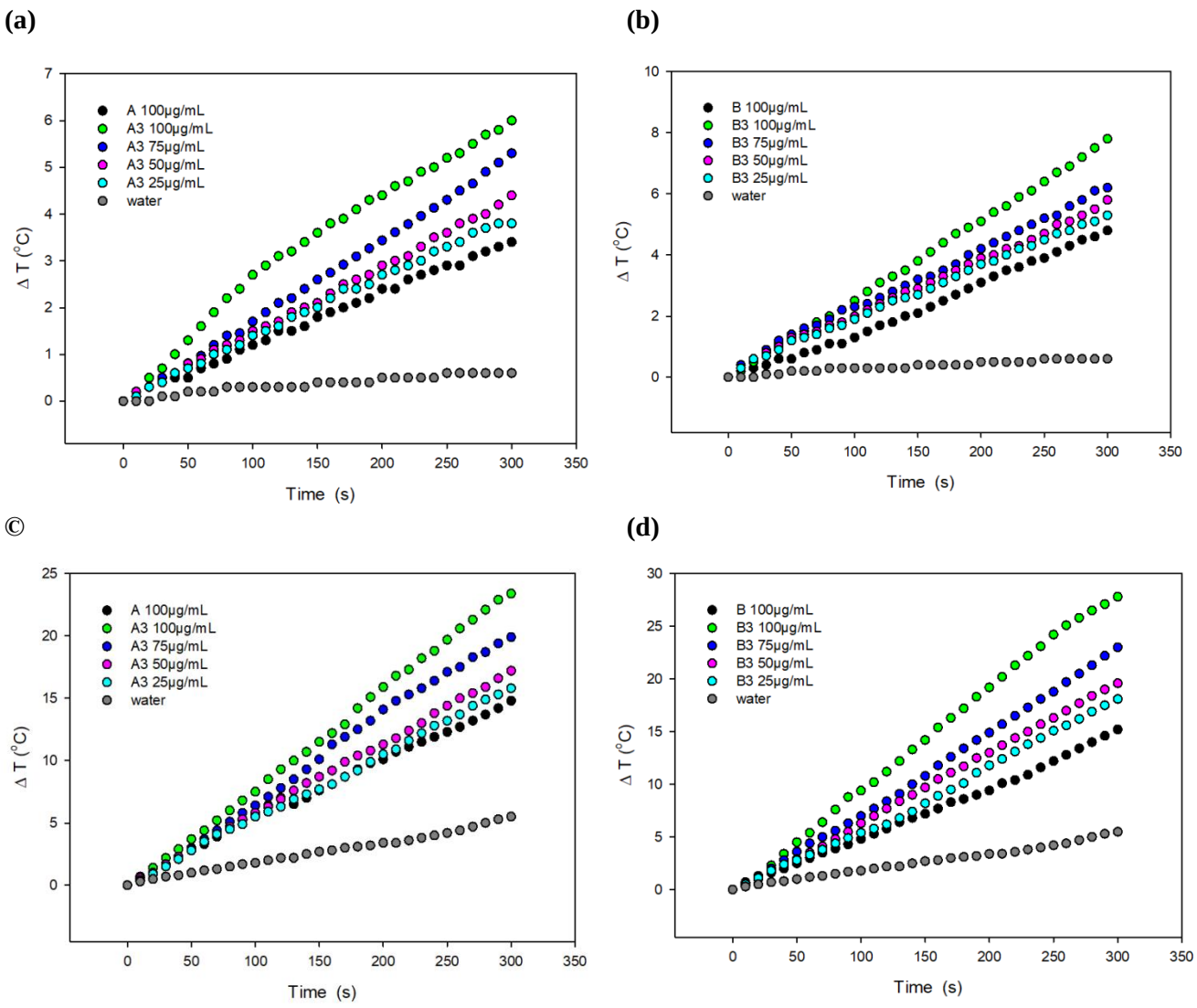
SI Fig. 1. TEM micrographs of materials: A (a) B (b) A1(c) B1 (d) A2 (e) B2 (f)



SI Fig. 2. Thermogravimetric analysis of samples **A** (a) and **B** (b)



SI Fig. 3. Magnetic field variation of the magnetization for **A2**, **A3**, **B2**, and **B3** samples measured at 300 K (a). Temperature dependence of magnetization (zero field cooled (ZFC) and field cooled (FC) modes) for **A2**, **A3**, **B2**, and **B3** samples measured in an applied magnetic field of 0.1 T (b).

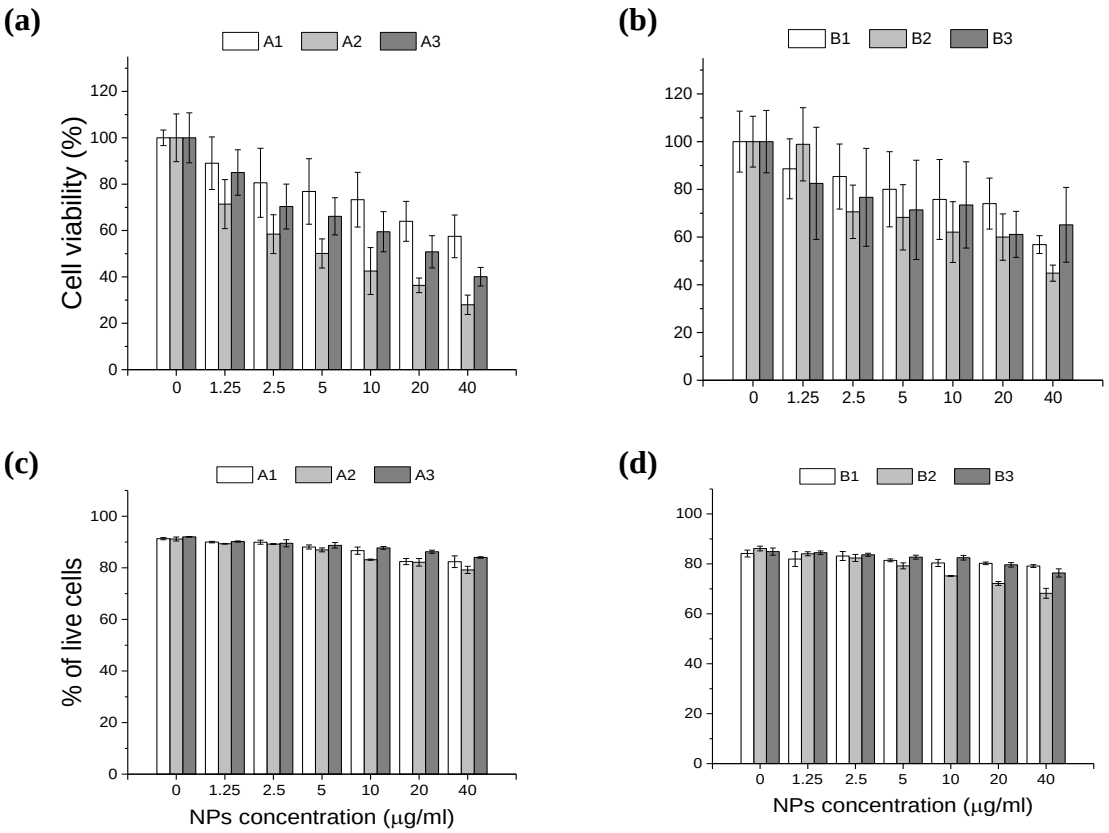


SI Fig.4. Change of medium temperature caused by material **A3** (a) and **B3** (b) at different concentrations vs. materials **A** and **B** (100 μg/mL) respectively under NIR light irradiation 1 W/cm^2; change of medium temperature caused by material **A3** (b) and **B3** (d) at different concentrations vs. material **A** and **B** (100 μg/mL) respectively under NIR light irradiation 3 W/cm^2.

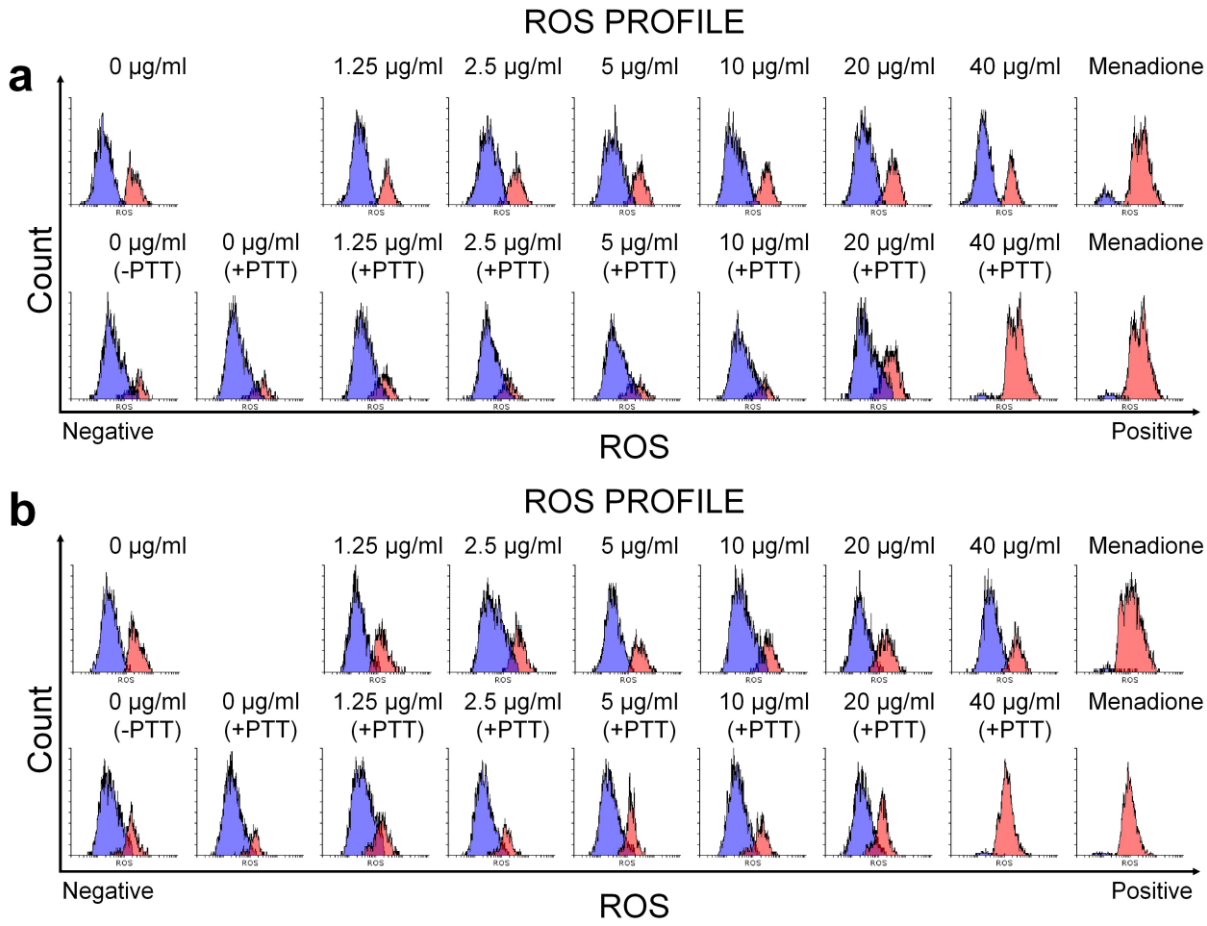
SI Table 1.

Time (h)	A2+Doxo (pH=4.5)	A4 (pH=4.5)	A2+Doxo (pH=7.4)	A4 (pH=7.4)
1	2.82	4.78	0.53	0.59
2	4.77	8.30	0.58	0.88
3	6.21	10.69	0.75	0.98
4	7.24	12.85	0.99	1.00
5	7.87	14.32	1.00	1.04
24	8.73	16.93	1.27	1.71
48	9.13	18.63	1.58	1.96
120	9.48	19.54	1.81	2.11
loading = 93.16%				

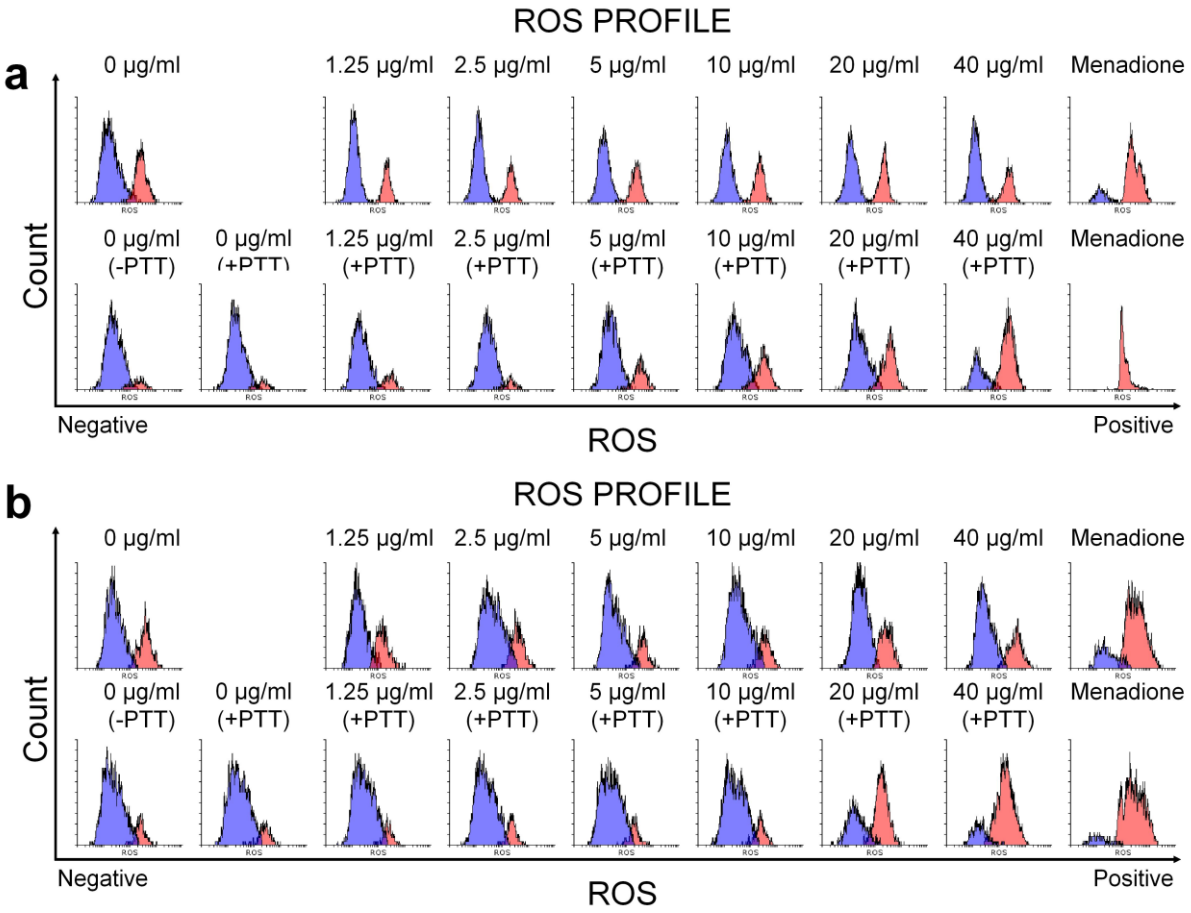
Time (h)	B2+Doxo (pH=4.5)	B4 (pH=4.5)	B2+Doxo (pH=7.4)	B4 (pH=7.4)
1	1.32	3.45	0.51	0.53
2	2.30	5.87	0.63	0.63
3	3.10	7.82	0.76	0.76
4	3.79	9.43	0.87	0.86
5	4.36	10.69	0.99	1.11
24	5.28	12.52	1.10	1.37
48	5.91	13.78	1.33	1.79
120	6.25	14.47	1.76	2.13
loading = 92.04%				



SI Fig. 5. Cell viabilities of THLE-2 cells after being incubated with materials **A1, A2, A3**(a) and **B1, B2, B3** (b) using WST-1 assay; Cell viabilities of THLE-2 cells after being incubated with materials **A1, A2, A3**(c) and **B1, B2, B3** (d) using Live/Dead assay.



SI Fig. 6. ROS profiles of HepG2 cells after being incubated with materials **A3** for 4 h (a) and 24 h (cells non-irradiated (upper raw) and irradiated (lower raw) with laser power of 2W for 5 min).



SI Fig. 7. ROS profiles of HepG2 cells after being incubated with materials **B3** for 4 h (a) and 24 h (cells non-irradiated (upper raw) and irradiated (lower raw) with laser power of 2W for 5 min).