Supporting information

Supramolecular interaction mediated aggregation of anticarcinogen on the triformyl cholic acid functionalized Fe_3O_4 nanoparticles and its dual-targeting treatment for liver cancer

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1. Synthesis information

1. 1 Synthesis of triformyl cholic acid

Triformyl cholic acid was prepared by previously reported method [27]. Briefly, cholic acid (8.34 g, 20 mmol) was dissolved in formic acid (40 mL, 0.81 mol), and the mixture was vigorously stirred at 55 °C for 6 h. After removing the excess formic acid under reduced pressure, the solid was crystallized from an ethanol-water mixture 250 mL (1:1.5). The resulting white precipitate was washed three times and dried under vacuum. ^{1}H NMR ($CDCl_3$, 600 MHz). δ:

0.773 (s, 3H), 0.858 (d, 3H), 0.961 (s, 3H), 1.109-1.131(m, 3H), 1.323-2.396 (m), 4.732 (m, 1H), 5.088 (br s, 1H), 5.285(br s, 1H), 8.040 (s, 1H,), 8.122 (s, 1H,), 8.178 (s, 1H,) (Fig. S1), which consistent with the literature [27].

1.2 Synthesis of APTES-Fe_3O_4 magnetic nanoparticles.

Superparamagnetic Fe_3O_4 nanoparticles (MNPs) were obtained by chemical deposition method according to our previously reported work [28]. Typically, the $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ were added into the three-necked flask with mechanical stirring under N_2 protection and heated to 85^oC. Then, ammonium hydroxide solution was added to the mixture dropwise until the pH raised to 10. The above mixture was kept reacting at 85^oC for another 2 h. After cooling to room temperature, the obtained black sediments were magnetically separated and washed with ethanol and double-distilled water successively. Finally, the prepared MNPs were dried under vacuum at 40 ^oC for 12 h.

500 mg MNPs was ultrasonically dispersed in ethanol for 30 min. 240 μL APTES was added into the solution with rapid stirring under N_2 protection. After mechanically rabble for 8 h, the suspended material was separated with external magnetic field and washed by deionized water and ethanol for 5 times. The resulted powder dried under vacuum at room temperature.

2 Cellular uptake and in vitro anti-cancer activity evaluation

2.1 Cellular uptake

The fluorescence microscopy has been used to observe the cellular uptake of TCA-MNPs/DOX and TCA-MNPs/EPI by these four kinds of cells. The cells were seeded on a 24-well plate at 5×10^3 cells per well and incubated with culture medium at 37 ^oC for 24 h. Subsequently, the cells were incubated with TCA-MNPs/DOX and TCA-MNPs/EPI (10 μg/mL) for 1 h, 4 h and 8 h. After washing with PBS, the fluorescence image of cells was observed at 585–610 nm with excitation at 525 nm through fluorescence microscope.

The cells (5×10^5) were seeded on a 6-well plate and incubated 24 hours sunder normal growing conditions. Then the cells were gently replaced the fresh medium containing TCA-MNPs/DOX (10 μg/mL) and incubated for another 24 hours. After washing with PBS, the cell was treated with trypsin solution and centrifuged at 1000 rpm for 3 min. Finally, the fluorescence expression of each group of cells was detected by flow cytometry.

2.2 *In vitro* anti-cancer activity study

The MTT assay has been used to determine the cytotoxicity of TCA-MNPs/DOX and TCA-MNPs/EPI towards cells (HepG2 cells, K150 cells, H1299 cells and 4T1 cells). The cells were seeded in 96-well microplates and grew in medium at a density of 5×10^3 cells/well for 24 h at 37 ^oC. Subsequently, the medium was replaced with 100 μL of fresh medium, and incubated with different concentrations (0, 10, 20 and 40 μg/mL) of free TCA-MNPs, TCA-MNPs/DOX and TCA-MNPs/EPI for a further 24 h. Then discard the medium and incubated with MTT reagent for 4 h. The dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan crystals. Finally, the absorbance of each well was measured at a wavelength of 490 nm using a microplate reader. The cell viability has been expressed by half-maximal inhibitory concentration (IC_{50}). Repetitive studies were carried out five independent times in each experiment.

Further, the cytotoxicity of TCA-MNPs and TCA-MNPs/DOX for these four kinds of cells has been observed via the fluorescence microscopy. The cells were seeded on a 12-well plate at 5×10^3 cells per well and incubated with culture medium at 37 ^oC for 24 h. Subsequently, the cells were incubated with TCA-MNPs and TCA-MNPs/DOX (10 μg/mL) at 37 ^oC for 4 h. After washing with PBS and fixing the cells with 4% paraformaldehyde for 20 minutes, the cells were treated with One Step TUNEL-FITC apoptosis assay staining kits for 1 hour in the dark. Then, the fluorescence image of cells was observed through fluorescence microscope, while the DOX signal was obtained at 585-610 nm with excitation at 525 nm, and the TUNEL-FITC signal was detected at 505-545 nm with excitation at 488 nm.

3. Correlative calculation formulas

3.1 Formula of drug loading amount

$$Q_e=(M_0-M_e)/M_n \quad (S1)$$

Where M_0 is the feed mass of DOX/EPI in the initial solution, and M_e is the residual mass of DOX/EPI in the supernatant solution. M_n is the mass of the TCA-MNPs (mg).

3.2 Formula of drug release

The percentage of drug release was calculated via the following formula (Eq. (S2)):

$$\text{Drug released (\%)} = \frac{M_r}{M_l} \times 100\ \% \quad \text{(S2)}$$

Where M_r is the released amount of drug, and M_l is the loading amount of drug.

3.3 Formula oftumor volumes and tumor inhibition rate

Tumor volumes were calculated by the formula (Eq. (S3)):

$$\text{Tumor volumes} = 0.5 \times (\text{length}) \times (\text{width})^2 \quad \text{(S3)}$$

Tumor inhibition rate was calculated according to the following formula (Eq. (S4)):

$$\text{Tumor inhibition rate} = \frac{V_{\text{tumor of control group}} - V_{\text{tumor of treatment group}}}{V_{\text{tumor of control group}}} \times 100\% \quad \text{(S4)}$$

3.4TheScherrer formula of MNP core sizescalculation by XRD:

The average MNP core sizes can be evaluated from the XRD results by Scherrer formula:

$$D = \frac{0.9\lambda}{B\cos\theta} \quad \text{(S5)}$$

Where D is the average particle diameter, 0.94 is the Scherrer's constant, λ is the X-ray wavelength (λ=0.154 nm), B (0.770) is the angular line width of half-maximum intensity and θ (35.52) is the Bragg's angle in degree. Here, the (311) peak of the highest intensity was picked out to evaluate the particle diameter of the TCA-MNPs, and the D was calculated to be 10.2 nm which is similar to the results of TEM.

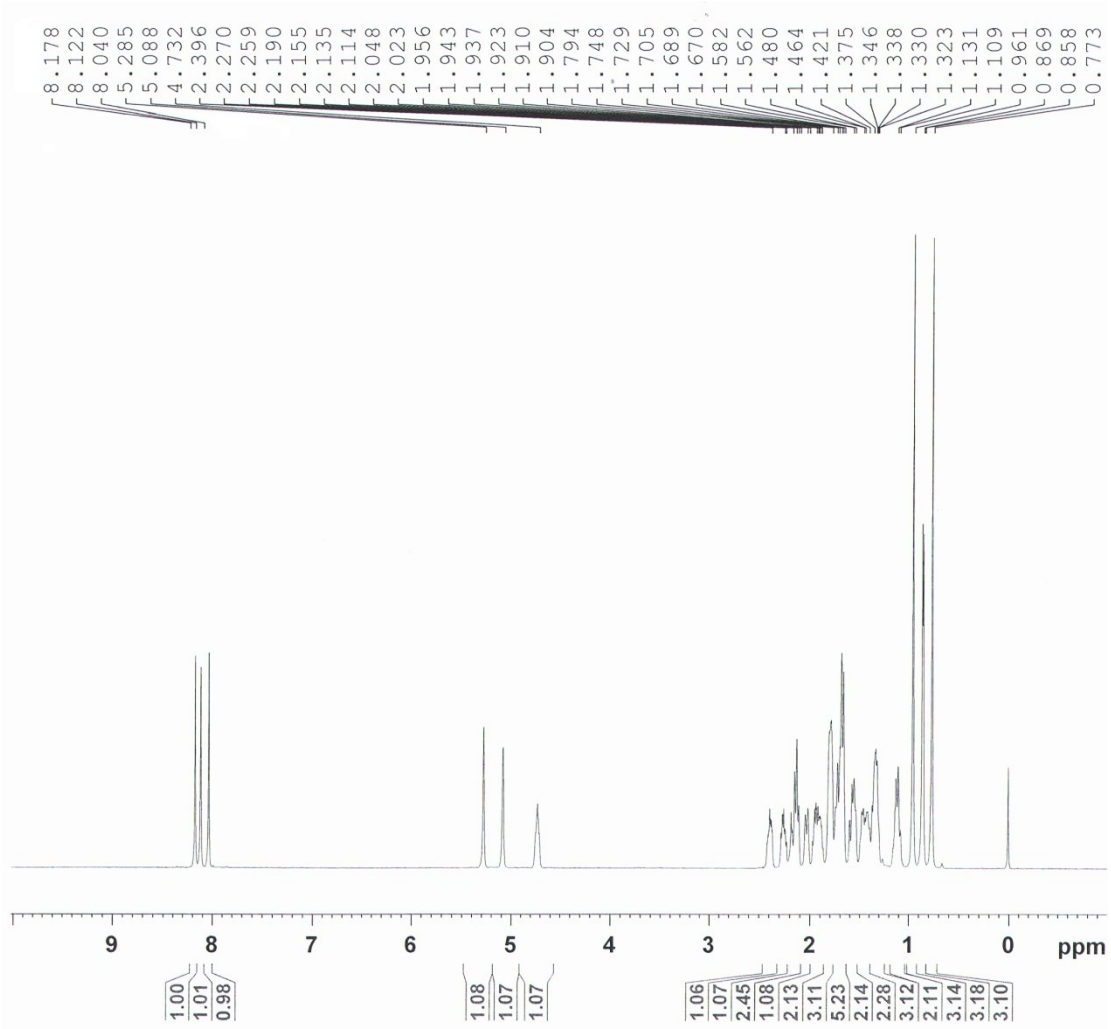
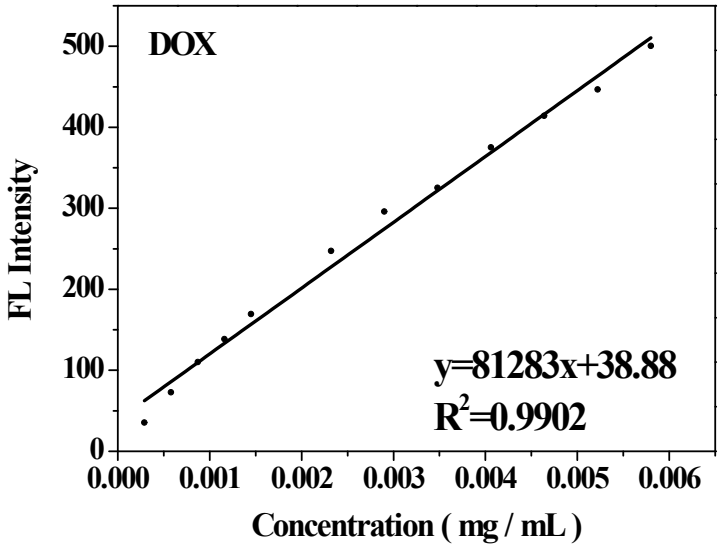


Fig.S1. ^{1}H NMR of TCA (600 MHz, $CDCl_3$).



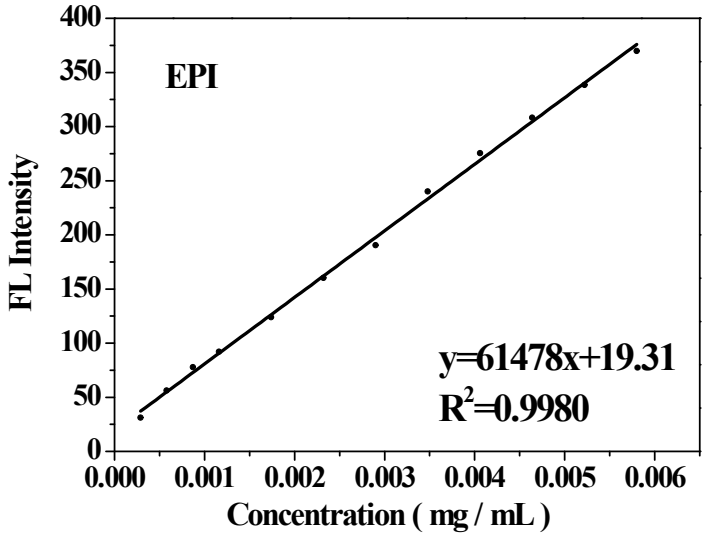


Fig.S2. Plot of calibration curves for DOX and EPI.

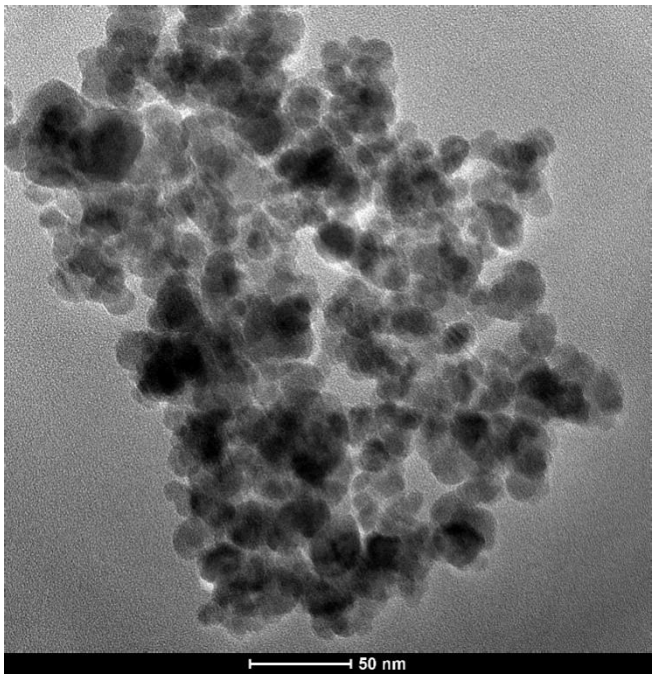


Fig.S3. TEM image of MMPs.

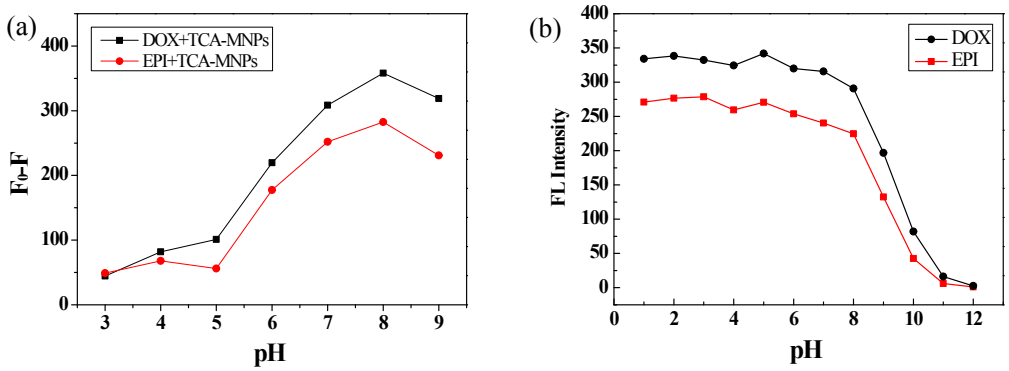


Fig.S4. (a) The influence of pH on absorption between TCA-MNPs and DOX, EPI at PB solution ($c = 5.8\times10^{-3}$ mg/mL); (b) The pH effect on fluorescence intensity of DOX and EPI at PB solution ($c = 5.8\times10^{-3}$ mg/mL).

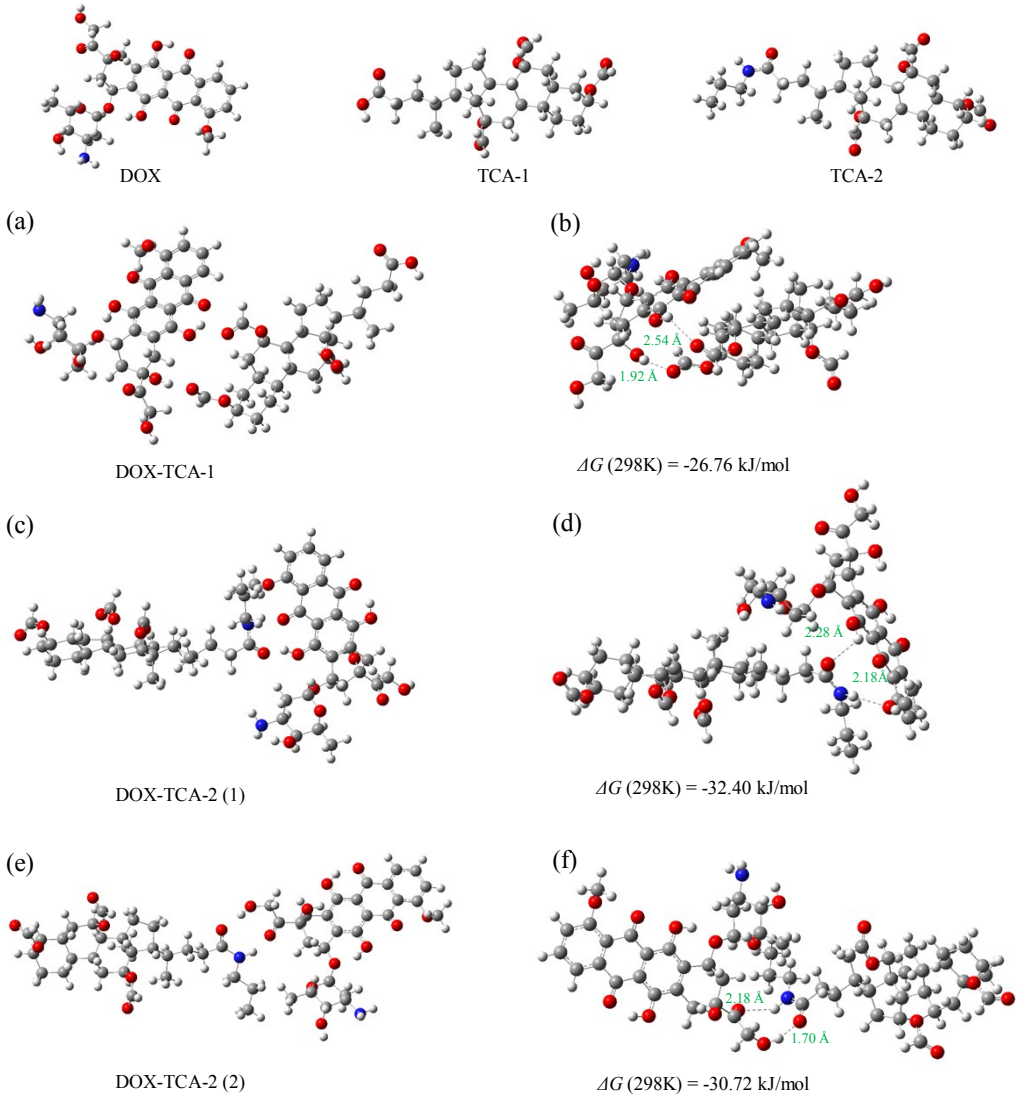


Fig.S5. The possibleH-bonded structures of DOX-TCA complexes (a, c, e); the minimum energy structures and their Gibbs free energy (*ΔG*) (b, d, f). The dashed lines indicate the hydrogen bonding.

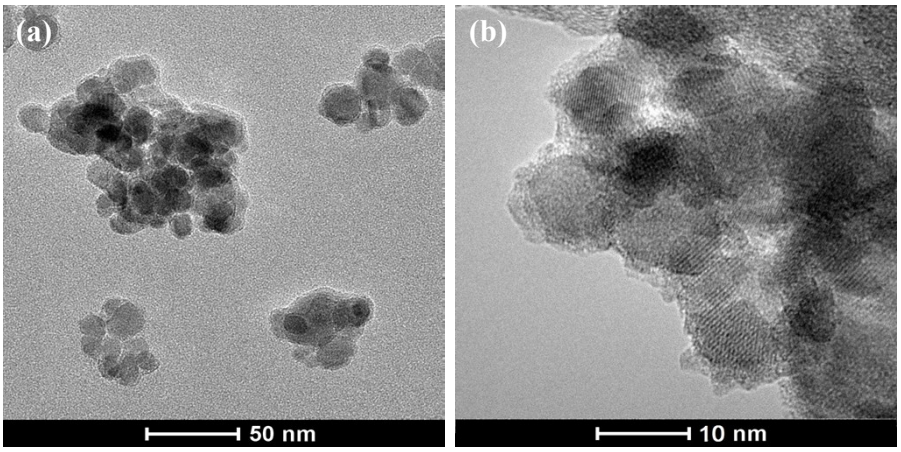


Fig.S6. TEM (a) and HRTEM (b) images of TCA-MMPs/DOX.

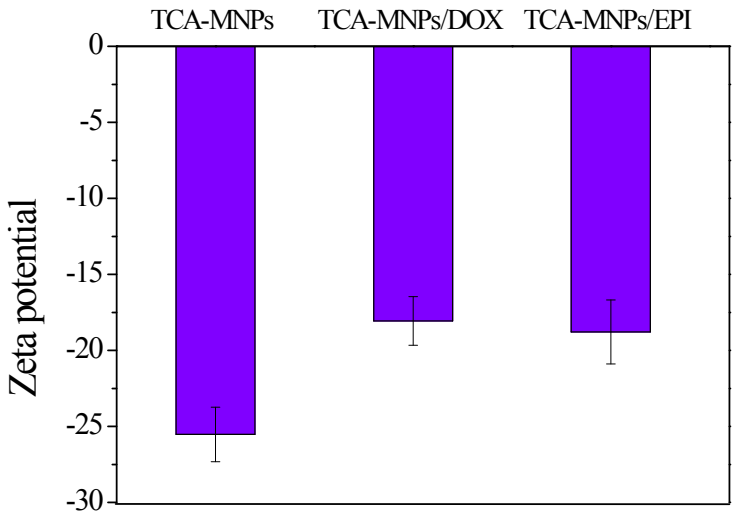


Fig.S7. Zeta potential values of TCA-MNPs, TCA-MMPs/DOX and TCA-MMPs/EPI at pH 7.0.

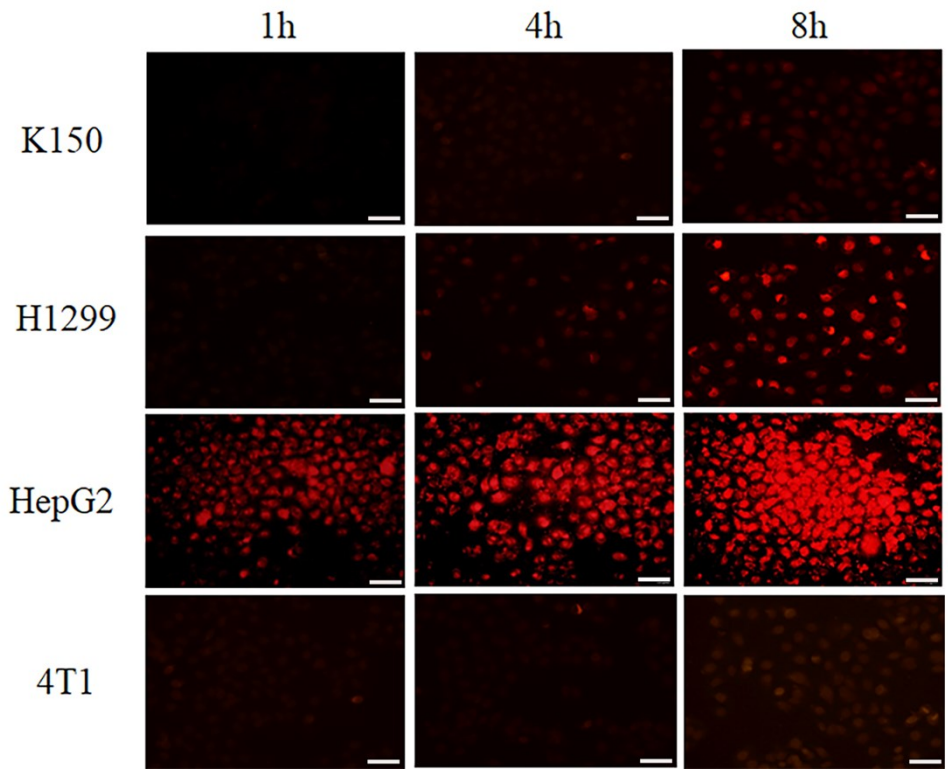


Fig.S8. Fluorescence microscope image of HepG2, K150, H1299, and 4T1 cells incubated with TCA-MNPs/EPI for 1-8 hours, Bar = 50 μm.