Supporting Information for

Drug-loaded nanoscale metal-organic framework with tumor targeting agent for highly effective hepatoma therapy

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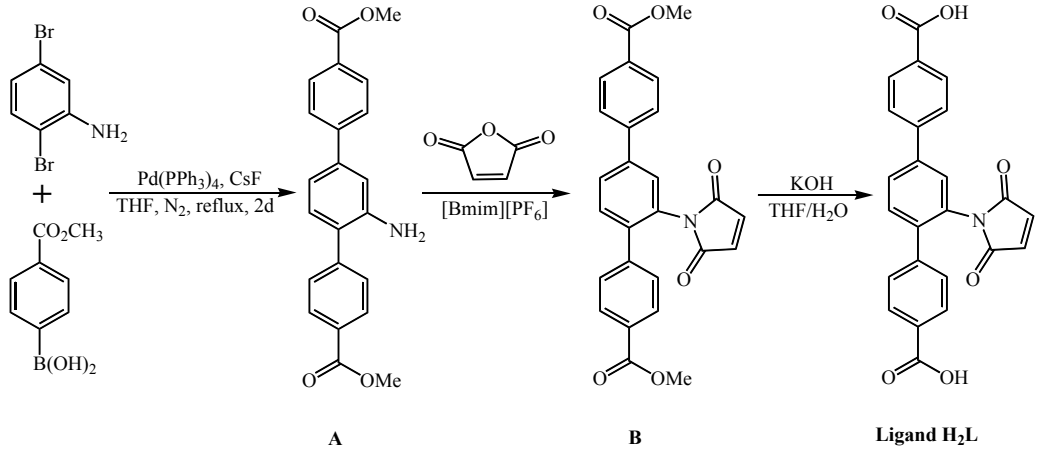
Content

1. Materials and instrumentation

2, 5-Dibromoaniline, 4-(methoxycarbonyl)phenylboronic acid, CsF and [Bmin][PF_6] were purchased from Shanghai Darui Fine Chemical Co., Ltd., unless otherwise noted, and used without further purification. FA-$(CH_2)_2$-SH (DMF solution) was purchased from Qilu Pharmaceutical Co., Ltd. Infrared (IR) samples were prepared as KBr pellets, and spectra were obtained in the 400-4000 cm^{-1} range using a Perkin-Elmer 1600 FTIR spectrometer. ^1H NMR data were collected using an AM-300 spectrometer. Chemical shifts are reported in δ relative to TMS. Fluorescence spectra were obtained with FLS-920 Edinburgh Fluorescence Spectrometer with a Xenon lamp. The scanning electron microscopy (SEM) micrographs were recorded on a Gemini Zeiss Supra TM scanning electron microscope. The X-ray diffraction (XRD) experiments were obtained on a D8 ADVANCE X-ray powder diffractometer with CuKα radiation (λ = 1.5405 Å). Confocal fluorescence imaging studies were performed with a TCS SP5 confocal laser scanning microscopy (Leica Co., Ltd. Germany) with an objective lens (× 20). MS spectra were obtained by Bruker maxis ultra-high resolution-TOF MS system. HRTEM (High resolution transmission electron microscopy) analysis was performed on a JEOL 2100 Electron Microscope at an operating voltage of 200 kV.

2. Synthesis of ligand and 1-3

Synthesis of H_2L.



Scheme 1. Synthesis of **H_2L**.

The 1st step

2,5-Dibromoaniline(10 mmol, 2.51 g), 4-methoxy carbonylphenylboronic acid (30 nmol, 5.4 g), CsF (47.5 mmol, 7.22 g), tetrakis(triphenylphosphine) palladium (3.3 mmol, 3.8 g) and

anhydrous tetrahydrofuran (150 mL) were allowed to reflux for 2 days in nitrogen atmosphere. After cooling to room temperature, the solvent was removed under vacuum. The residue was washed with a large amount of water, the residue was purified by column chromatography on silica gel (CH_2Cl_2 : ethyl acetate = 40 : 1) to give **A** as a yellow solid (2.3 g, yield: 63.9%). IR (KBr pellet cm^{-1}): 3457 (m), 3374 (m), 2946 (m), 1706 (s), 1606 (s), 1434 (s), 1280 (m), 1106 (w), 767 (s), 704 (s). ^{1}HNMR(300 MHz, DMSO-d^6, 25°C,TMS, ppm): 8.05-8.02 (d, 4H, -C6H4-), 7.78-7.75 (d, 2H, -C_6H_4-), 7.64-7.62 (d, 2H, -C_6H_4-), 7.17-7.16 (d, 1H, -C_6H_3-), 7.15 (s, 1H, -C_6H_3-), 7.03-7.00 (d, 1H, -C_6H_3-), 5.10 (s, 2H, -NH_2), 3.88 (s, 6H, -CH_3).

The 2nd step

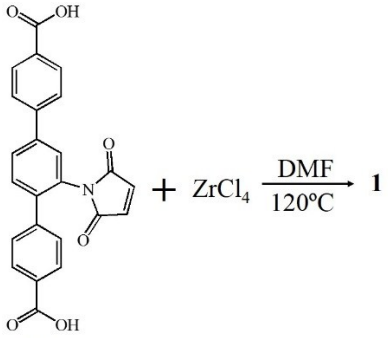
A (2 mmol, 0.722 g) and 2, 5-furandione (2 mmol, 0.196 g) were added to ionic liquid [Bmin][PF_6] (2 mL). The mixture was stirred at 140ºC for 2 h and cooled. The resulted mixture was extracted with Et_2O (3×10 mL). The combined ether phase was evaporated under reduced pressure to give crude product. The product was further purified with column chromatography (silica gel, CH_2Cl_2) to give **B** as white solid (0.80 g, yield: 90.7%). MW = 441. IR (KBr pellet cm^{-1}): 3083 (s), 2954 (s), 1712 (s), 1608 (m), 1435 (s), 1405 (m), 1277 (s), 1109 (m), 832 (m), 770 (w), 700 (m). ^{1}H-NMR(300 MHz, DMSO-d^6, 25°C,TMS, ppm): 8.09-8.07 (d, 2H, -C_6H_4-), 8.01-8.00 (d, 1H, -C_6H_3-), 7.98-7.96 (d, 2H, -C_6H_4-), 7.95-7.93 (d, 1H, -C_6H_3-), 7.91-7.90 (d, 2H, -C_6H_4-), 7.78 (s, 1H, -C_6H_3-), 7.37-7.34 (d, 2H, -C_6H_4-), 7.10 (s, 2H, -CH=CH-), 3.88 (s, 3H, -CH_3), 3.85 (s, 1H, -CH_3). Elemental analysis(%) calcd ($C_{26}H_{19}NO_6$): C 70.74, H 4.34, N 3.17; Found: C 70.43, H 4.56, N 3.39.

The 3rd step

An aqueous solution (10 mL) of KOH (3.0 g, 53.6 mmol) was added to a THF (20 mL)/MeOH (20 mL) solution of **B** (0.74 g, 1.68 mmol) at room temperature. The mixture was refluxed for 12 h. After cooling down to room temperature, the solvent was removed under vacuum. Water was added to the resulting solution and the mixture was heated until the solid was fully dissolved, then the homogeneous solution was acidified with diluted HCl until no precipitate formed (pH < 2). The yellowish powder (**H_2L**) was collected by filtration, washing with water and drying in air. (0.50 g, yield: 72.4%). MW = 413. IR (KBr pellet cm^{-1}): 3072 (s), 2941 (s), 1700 (s), 1608 (m), 1472 (s), 1401 (m), 1147 (s), 1104 (m), 853 (m), 760 (w), 698 (m). ^{1}H-NMR (300 MHz,

DMSO-d^6, 25°C,TMS, ppm): 13.15 (s, 1H, -COOH), 13.09 (S, 1H, -COOH), 10.05 (s, 1H,-C_6H_3-), 8.07-8.04 (d, 2H, -C_6H_4-), 8.02-8.00 (d, 1H, -C_6H_3-), 7.99-7.97 (d, 2H, -C_6H_4-), 7.95-7.93 (d, 1H, -C_6H_3-), 7.84-7.81 (d, 2H, -C_6H_4-), 7.78 (s, 1H, -C_6H_3-), 7.37-7.34 (d, 2H, -C_6H_4-), 6.47-6.43 (d, 1H, -CH=CH-), 6.26-6.22 (s, 1H, -CH=CH-). Elemental analysis(%) calcd for **H_2L** ($C_{24}H_{15}NO_6$): C 69.73, H 3.66, N 3.39; Found: C 69.43, H 3.56, N 3.48.

Synthesis of Mi-UiO-68 (1)



Scheme 2. Synthesis of **1**.

$ZrCl_4$ (9.60 mg, 0.040 mmol) and **H_2L** (0.040 mmol, 16.5 mg) were dissolved in DMF (3.2 mL). After addition of acetic acid (240 μL), the solution was heated at 120ºC for 24 h and then cooled to room temperature. The product was collected by centrifugation and washed with DMF three times. After that, the obtained yellowish powder was soaked in fresh DMF at 80ºC for 6 h, then in alcohol at 60ºC for 2 days with replacing the soaking solvent every 12 h to exchange alcohol. Finally, the product was washed three times with alcohol and dried at 80 ºC in an oven.

Synthesis of DOX@Mi-UiO-68 (2)

Scheme 3. Synthesis of **2**.

Mi-UiO-68 (**1**, 10 mg, 3.2 mmol) was soaked in an aqueous solution (5 mL) of DOX (1 mg/mL). The DOX aqueous solution was refreshed for every six hours. UV-vis spectrum was used to monitor the adsorption process (Figure S1). The resulting DOX loaded Mi-UiO-68 was washed water (three times) to generate **2**. The DOX loading amount in **2** was determined by fluorescence spectroscopy (Figures S2 and S3) to be 4.84 wt %, which was calculated as follows: DLC (%) = (amount of loaded drug) / (amount of drug loaded NPs) × 100%[1, 2]

As shown in Fig. S3, the hydrodynamic diameters of the DOX@UiO-68 (**2**) possess a narrow

size distribution and the size of the particles are centered at 140 ± 21 nm. The Zeta potential is -15.7(±0.9) mV.

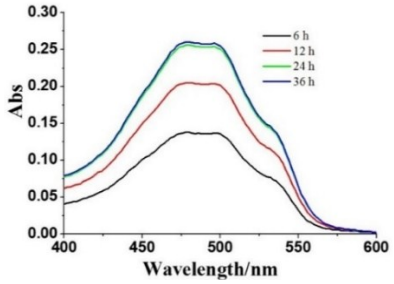


Fig. S1 UV-vis spectrum was used to monitor the DOX adsorption.

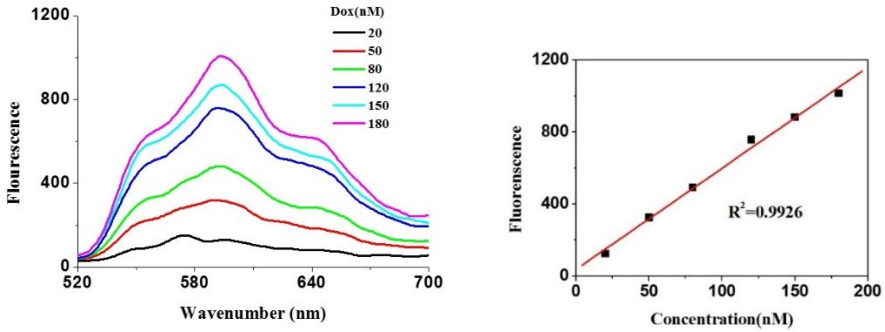


Fig. S2 Free DOX luminescent intensities (λ_{ex} = 485 nm) at different concentrations and the linearity between the relative luminescent intensities and concentrations of DOX.

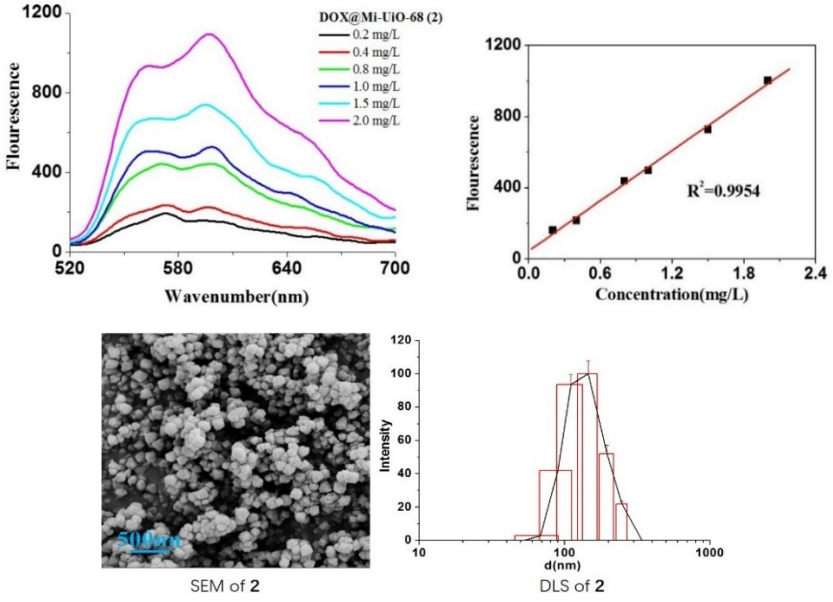


Fig. S3 Top: Luminescent intensities (λ_{ex} = 485 nm) at different concentrations of **2** and the linearity between the relative luminescent intensities and concentrations. The DOX loading amount is 4.84 wt %. Bottom: SEM image and DLS measurement of **2**. The NPs diameter of **2**

is centered at 140±21 nm.

Synthesis of DOX@UiO-68-FA (3)

2 + Thiolated folic acid ⟶ DOX@UiO-68-FA (**3**)

Scheme 4. Synthesis of **3**.

DOX@Mi-UiO-68 (**2**, 10 mg, 3.1 mmol) was immersed in a DMF solution (5 mL) of thiolated FA (0.62 mol/L) for 30 min. The FA decorated **3** was collected by centrifugation. After washed with water, the product was dried in air. Elemental analysis showed that the S content in **3** is up to 0.25 %, indicating the post-synthetic yield of FA is 4.2 mol %.

The reactions of esterified maleimide-containing organic linker $\mathbf{H_2L}$ with thiolated FA was examined before the post-synthetic synthesis of **3** from **2**. The reaction proceeded very smoothly (DMF, r.t., 10 min.) and the expected thiolated product was well confirmed by the MS spectra (Fig. S4).

The DOX loading amount in **3** was determined by fluorescence spectroscopy (Fig. S5) to be 4.79 wt %, which was calculated as follows: DLC (%) = (amount of loaded drug) / (amount of drug loaded NPs) × 100%.[1, 2]

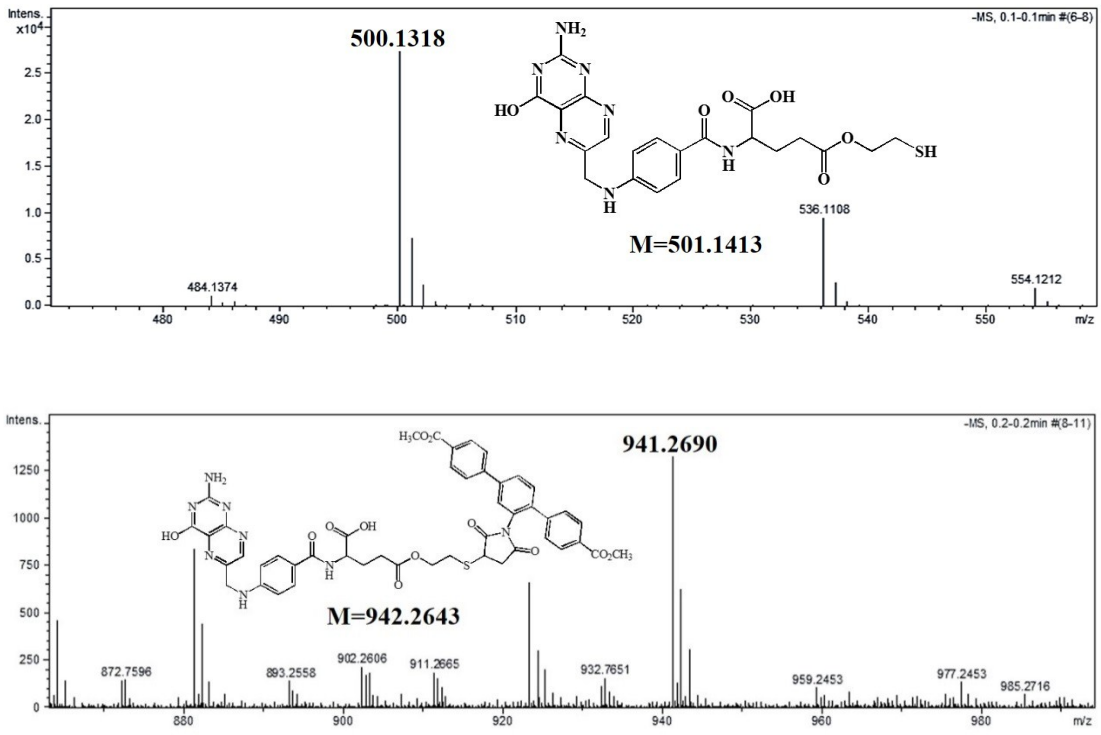


Fig. S4 Top: MS spectrum of the thiolated FA. Bottom: MS spectrum of the product after thiol-maleimide Michael-type addition of the esterified $\mathbf{H_2L}$ and thiolated FA.

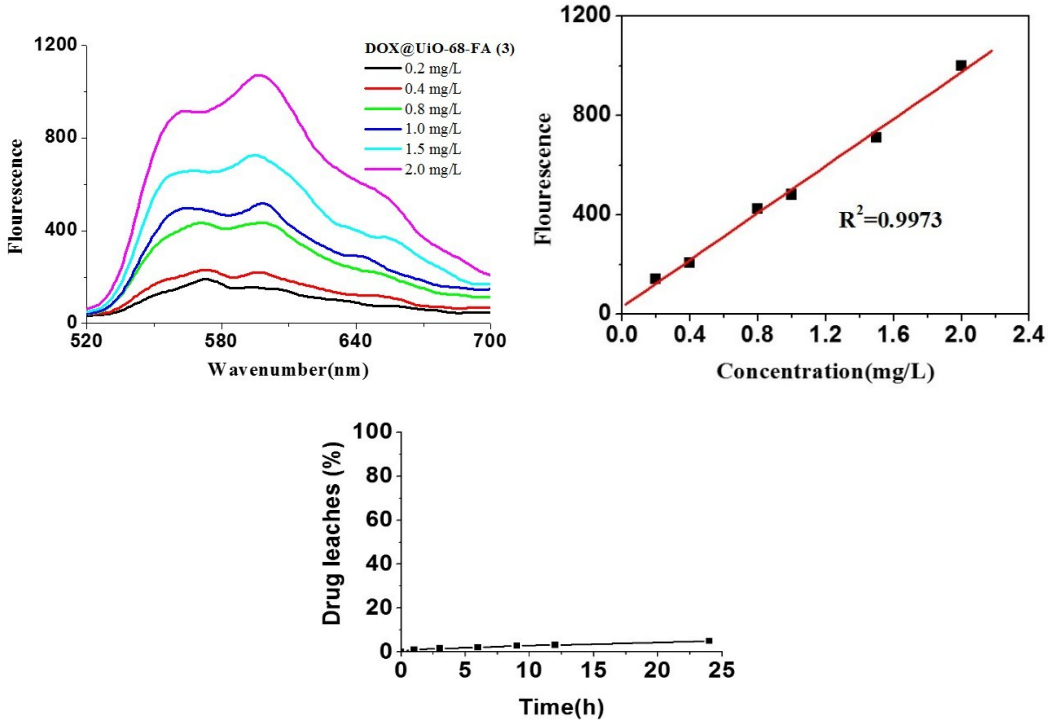


Fig. S5 Top: luminescent intensities (λ_{ex} = 485 nm) at different concentrations of **3** and the linearity between relative luminescent intensities and concentrations. The DOX loading amount is 4.79 wt %. Bottom: the leaching experiment of **3** in PBS during 24 h, and only less than 5% of DOX loss was observed, indicating DOX was encapsulated in MOF pores.

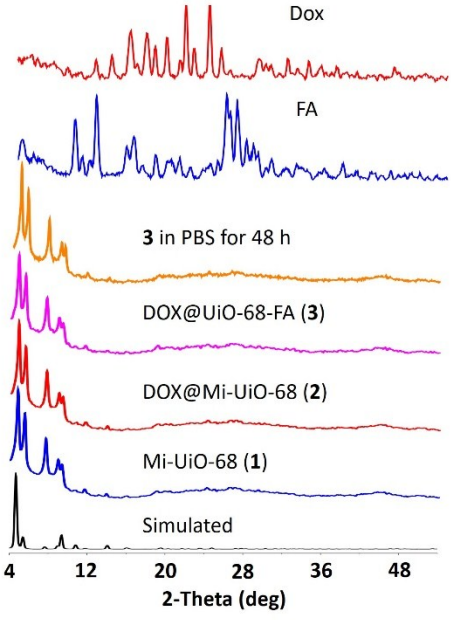


Fig. S6 The XRPD patterns of **1-3**, Dox and FA. Compared to **1**, there are no characteristic differences in the intensities in XRPD peaks of **2** and **3** after Dox loading and FA decoration because of the low included amount of Dox and FA.

3. DOX release measurement

2 (1 mg) and **3** (1 mg) were respectively dispersed in pure water (25 mL, pH = 7.0) and PBS (25 mL, pH = 7.4), and the systems were shaking (100 rpm) at 37°C. The DOX release amount was determined by measurement of the corresponding fluorescent intensity (λ_{ex} = 485 nm) of above DOX solution (1 mL each time) at given time. The sample was returned to the original release systems after the fluorescence measurement.[1-3]

4. Cell lines

The HL-7702 and HepG2 cell lines were provided by Institute of Basic Medicine, Shandong Academy of Medical Sciences (China). All animal experiments were carried out and accorded with the Principles of Laboratory Animal Care (People's Republic of China). Female nude mice (4-6 week old, ~15 g) were raised on normal conditions of 12 h light and dark cycles and given access to food and water *ad libitum*. The HL-7702 cells were grown in RPMI-1640 (Invitrogen, CA, USA) containing 10% heat-inactivated new born calf serum and 100U/mL penicillin, 100 mg/mL streptomycin in an atmosphere of 5 % CO_2, 95 % air at 37ºC, while the HepG2 cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, CA, USA) containing 10 % heat-inactivated new born calf serum and 100 U/mL penicillin, 100 mg/mL streptomycin in an atmosphere of 5 % CO_2, 95 % air at 37 ºC. For confocal fluorescence imaging, cells were incubated in glass bottom dishes for 24 h. Cells were incubated at 37ºC with **2** or **3** (10^{-4} mg/mL) in PBS (Phosphate Buffered Saline) for 1 h in an atmosphere of 5 % CO_2, 95 % air, then washed with PBS and fluorescence images were captured. Samples were excited with two lasers (405 nm for MOF, 488 nm for DOX) and collected with two groups of channels (470-520 nm for green channel, 560-610 nm for red channel), respectively.

5. Cytotoxicity test

For Mi-UiO-68 (**1**): HepG2 cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 4×10^4 cells/well and incubated in DMEM for 24 h in an atmosphere of 5 % CO_2, 95 % air at 37ºC, the medium was then replaced. Mi-UiO-68 (**1**) was incubated with the cells in DMEM culture medium for 24 h/48 h, 20 μL MTT solution (5 mg/mL) was then added to each well. After incubation for 4 h at 37ºC, the MTT solution was removed, and 150 μL of DMSO was added to each well under slight shake in the dark. Finally, the plates were shaken for 10 min., and the absorbance of formazan product was measured at 490 nm by a microplate

reader.

For free DOX, **2** and **3**: HepG2 cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 4×10^4 cells/well and incubated in DMEM for 24 h in an atmosphere of 5 % CO_2, 95 % air at 37ºC, the medium was then replaced. Free DOX, **2** and **3** were respectively incubated with the cells in DMEM culture medium for 48 h, 20 μL MTT solution (5 mg/mL) was then added to each well. After incubation for 4 h at 37ºC, the MTT solution was removed, and 150 μL of DMSO was added to each well under slight shake in the dark. Finally, the plates were shaken for 10 min., and the absorbance of formazan product was measured at 490 nm by a microplate reader.

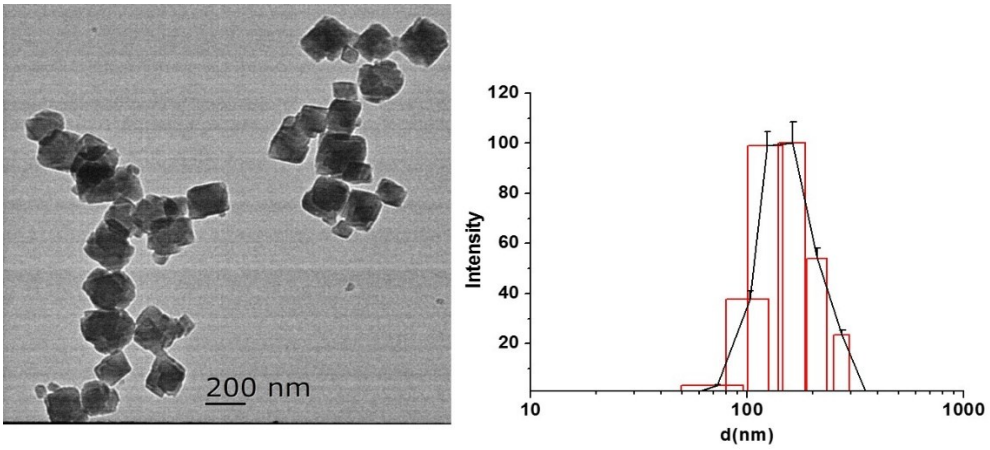


Fig. S7 TEM image and DLS measurement of **3** in PBS. The Zeta potential of **3** in PBS is -12.8(±0.6) mV. These results indicate that **3** is stable in PBS.

6. Animal tumor xenograft models

Xenograft was established from cultured cells. HepG2 cells were suspended *via* trypsinization and collected by centrifugation (1500 rpm, 4 min) and approximately 5×10^6 HepG2 cells in 100 μL DMEM were injected subcutaneously into right of the nude mice. The tumor volume (V) was calculated as $V = L \times W^2/2$ by measuring length (L is the longest diameter) and width (W is the shortest diameter). The relative tumor volumes were calculated for each sample as V_t/V_0 (V_0 was the original tumor volume). The treatments were administrated when the tumor volume reach to about 150 mm^3.

7. *In vivo* anticancer test

When the tumor volume reached to about 150 mm^3 the tumor-bearing mice were weighed and randomly divided into 4 groups (6 mice each group). The mice were subjected with different

treatments: PBS (50 μL) only, free DOX, **2** and **3** *via* tail-vein injection every two days intervals with the same dosage of 5 mg DOX/kg body weight. The tumor size and the body weight of each mouse were measured every two days within 14 days.

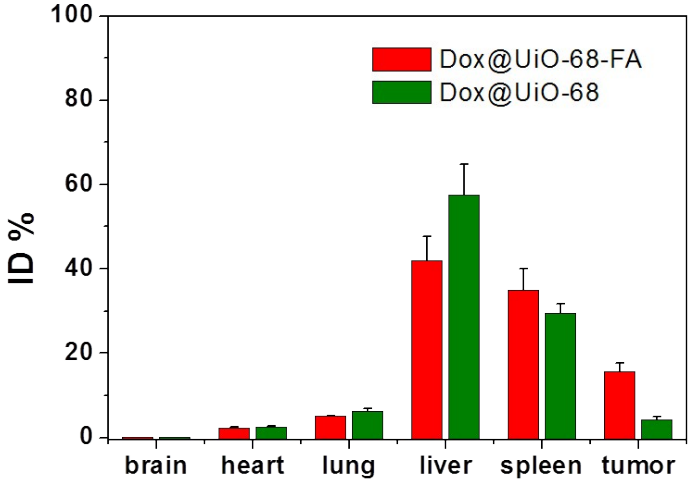


Fig. S8 Biodistribution of **2** and **3** in mice organs bearing Hep G2 tumor sacrificed 24 h after intravenous injection.

8. References

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(3) H. Zheng, Y. Zhang, L. Liu, W. Wan, P. Guo, A. M. Nyström, X. Zou, *J. Am. Chem. Soc.,* 2016, **138**, 962-968.