Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Materials and measurements. All the reagents and solvents were commercially available and used as received unless otherwise specified purification. Fetal bovine serum (FBS), 1640 Medium, 0.25% Tryspin-EDTA (1X) and Penicillin-Streptomycin (5,000 U/mL) were purchased from Thermo Fisher Scientific. Cell Counting Kit-8 (CCK-8) was purchased from Beyotime Biotechnology. Aldoxorubicin (AlDox) was purchased from MedChemExpress (MCE). ^{1}H NMR spectra were recorded with a AVANCE III HD 400 MHz spectrometer (Bruker) in the indicated solvents at 25 °C. Chemical shifts were referenced to the residual solvent peaks. Dynamic light scattering (DLS) experiments were conducted on a Malvern Zetasizer Nano ZS90 using a monochromatic coherent He-Ne laser (633 nm) as the light source and a detector that detected the scattered light at an angle of 90°. Scanning electron micrographs were obtained on a JSM-6330F Field Emission SEM microscopy. UV-visible spectra were recorded with a PerkinElmer LAMBDA 650 UV/Vis/NIR spectrometer. The cells were observed by confocal laser scanning microscopy (CLSM) (Zeiss LSM880). Cell viability was measured by a Microplate Reader (BioTek Epoch 2). Compounds **3** and **4** were prepared according to reported method.[1]

Synthesis of SOF-p1 and **SOF-p2**. Previous study established that the 1:2 mixtures of compound **T1** and cucurbit[8]uril (CB[8]) in water give rise to 3D homogeneous regular diamondoid **SOF-p1** and **SOF-p2**.

Synthesis of SOF-AlDox1. Adjust the pH of **SOF-p1** to 6-7 with 1M $NaHCO_3$ solution, and add AlDox to it and react for another 24 hours in the dark. The resulting solution is used directly without further purification.

Synthesis of SOF-AlDox2. **SOF-p2** was prepared as the previous assembly method. After obtaining **SOF-p2**, adjust the pH to 6-7 and add Traut's reagent which is one-fourth of the amount of **SOF-p2** substance to react for 18 hours, and then add AlDox to react for 24 hours in the dark.

Dialysis experiments (AlDox conjugation). **SOF-AlDox1** was prepared in 5% glucose ([**SOF-p1**] = 0.27 mg/mL, [AlDox] = 0.055 mg/mL) and transferred in dialysis bags (1 mL, MWCO: 1000 Da). The dialysis bags were dialyzed against 40 mL aqueous solution of 5% glucose with pH 7.4 at 37 ℃. The drug content of dialysate was determined by UV-Vis absorption in 72 hours. Calibration curves of DOX at pH 7.4 was measured. By recording the adsorption of DOX at 480 nm in the outside solution, the amount of the leaked DOX was calculated.

Dialysis experiments (in vitro DOX release) SOF-AlDox1 and **SOF-AlDox2** were prepared in 5% glucose ([**SOF-p1**] = 0.27 mg/mL, [**SOF-p2**] = 0.21 mg/mL, [AlDox] = 0.055 mg/mL) and transferred in dialysis bags (1 mL, MWCO: 1000 Da). The dialysis bags were dialyzed against 40 mL aqueous solution of 5% glucose with different pH (5.5, 6.0, 6.5 and 7.4) at 37 ℃. The drug content of dialysate was determined by UV-Vis absorption in 72 hours. Calibration curves of DOX at different pH were also measured. By recording the adsorption of DOX at 480 nm in the outside solution, the amount of the leaked DOX was calculated.

Cell line and cell culture. DOX-resistant MCF-7/ADR cells were supplied by Beina Culture Collection (Beijing, China) and incubated in 1640 medium with 10% FBS, 1% penicillin-streptomycin and 1 μg/mL DOX at 37 °C in a humidified atmosphere containing 5% CO_2.

MCF-7, H9C2 and Ana-1 cells were incubated in 1640 medium with 10% FBS and 1% penicillin streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. L02 cells were incubated in 1640 medium with 20% FBS and 1% penicillin streptomycin at 37 °C Before experiments, the cells were pre-cultured until confluence was reached.

Cytotoxicity text. The *in vivo* cytotoxicity was evaluated using the Cell Counting Kit-8 (CCK-8) assay in human breast carcinoma cell line MCF-7/ADR. In brief, MCF-7/ADR cells were seeded in 96-well plates at an appropriate density of 2×10^4 cells per well and then incubated for 24 h at 37 °C under 5% CO_2. After adherence, the cells were treated with the **SOF-AlDox1** or **SOF-AlDox2** with different AlDox concentrations ranging from 0 to 128 μM. The negative control group was filled with PBS (100 μL/well). After 24 hours of incubation, the medium was replaced with 100 μL fresh medium containing 10 μL CCK-8. Then, after incubating for 1 hour, the absorbance was measured at 450 nm using a microplate reader (Bio-Tek, Synergy H1, USA). The relative cell viability was calculated as: cell viability = (OD450 (samples)/OD450 (control)) × 100%, where OD450 (control) was obtained in the absence of **SOF-AlDox1** or **SOF-AlDox2**, and OD450 (samples) was obtained in the presence of **SOF-AlDox1** or **SOF-AlDox2**. Each value was averaged from three independent experiments. The statistical evaluation of data was performed using a two-tailed unpaired Student's *t*-test. A p-value of less than 0.05 was considered statistically significant. Each data point is represented as mean ± standard deviation (SD) of six independent experiments (n = 6, n indicates the number of wells in a plate for each experimental condition).

Confocal laser scanning microscopy (CLSM). For CLSM observations, MCF-7/ADR cells (1×10^6 cells per dish) were seeded in coverglass bottom dishes (35 mm × 35 mm), and then treated with **SOF-AlDox1** or **SOF-AlDox2** at the final concentration ([AlDox] = 20 μmol/L, [**SOF-p1**] = 45 μmol/L, [**SOF-p2**] = 45 μmol/L). After 30, 60, 90 and 120 min of incubation with 1640 medium, the cells were softly washed twice to remove excessive prodrug and SOFs. Then the cells were treated with 5 μg/mL Hoechst 33342 for 30 min and 50 nmol/L Lysotracker Green DND-26 for 60 min at 37 °C, and then washed with PBS (1 mL) five times. The cells were imaged on Zeiss LSM880 fluorescent microscope. MCF-7 cells were also experimented with the same drug dose.

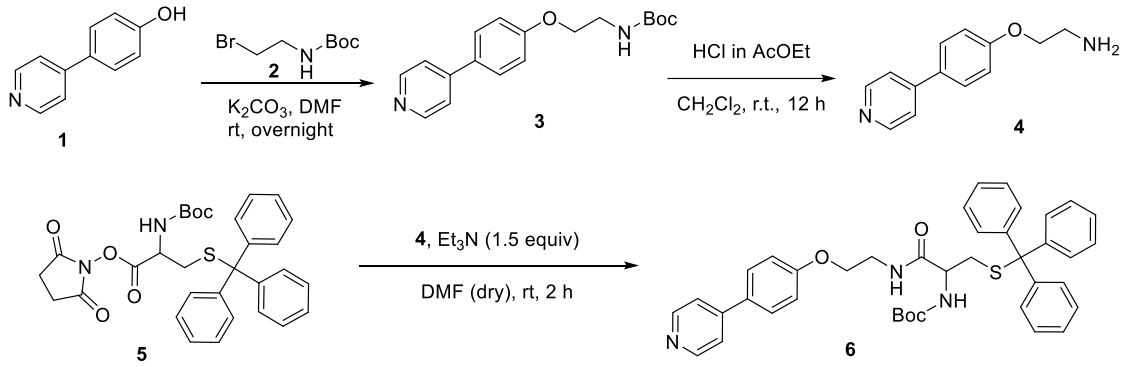
Hemolysis assay. The fresh red blood cells (RBC) in Alserver's solution from rats and human was purchased from Guangzhou Hongquan Biological Science and Technology Co., Ltd. (Guangzhou, China), and centrifuged at 2000 rpm for 10 min. The obtained red blood cells were washed with saline to instead of Alserver's solution. Then, RBC were resuspended in saline at 1% hematocrit. Afterward, the RBC suspension (0.63 mL) was separately incubated with 0.07 mL of SOFs at a series of concentrations, saline (negative control), and ultrapure water (positive control) at 37 ℃ for 1 h. Subsequently, the mixtures were centrifuged at 3000 rpm for 10 min. The supernatant (400 μL) of each sample was separately added into a 96-well plate and the absorbance at 540 nm was measured using a microplate reader. The hemolysis rate was calculated according to the following formula:

$$\text{Hemolysis (\%)} = (A_{sample} - A_{negative}) / (A_{positive} - A_{negative}) \times 100\%$$

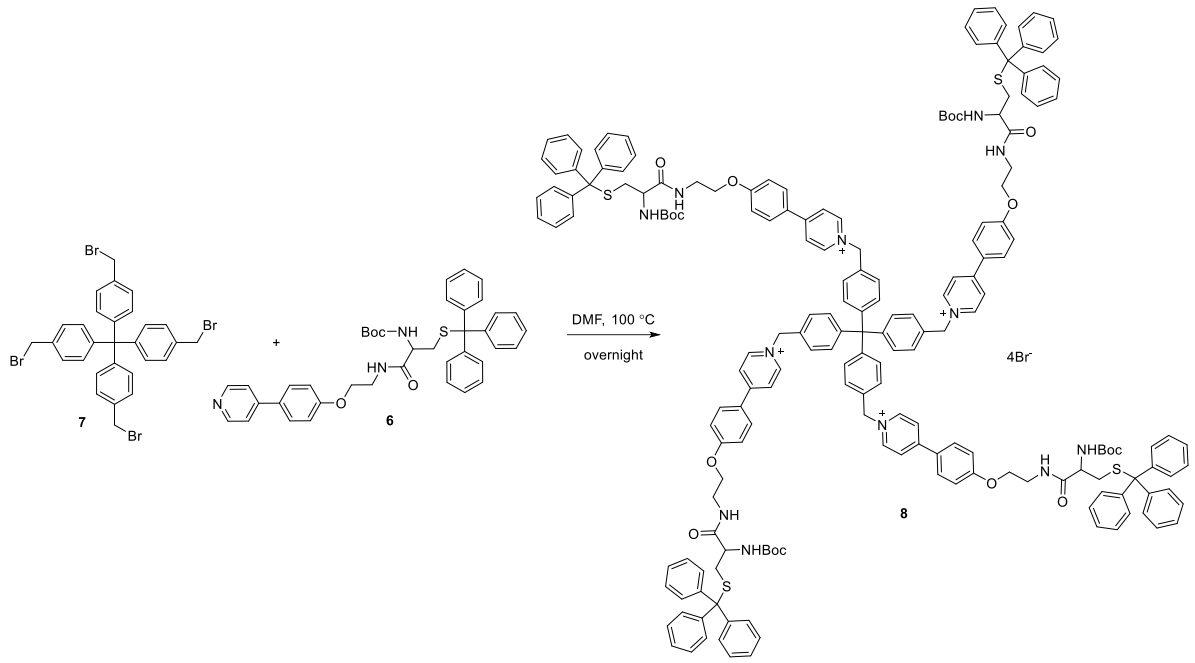
Where A_{sample} represents the absorbance of the test samples. $A_{positive}$ and $A_{negative}$ represent the absorbance of the positive control and negative control, respectively.

Electrophysiological experiments. Experiments were conducted at 25°C. hERG K^+ currents were recorded by the perforated patch-clamp technique with an Axopatch 200B patch-clamp amplifier. Micropipettes were created from glass tubing using a puller. Micropipettes producing resistances of 2-5 MΩ in the external solution were used. Voltage pulse generation and data acquisition were controlled with the software pClamp 10.0.

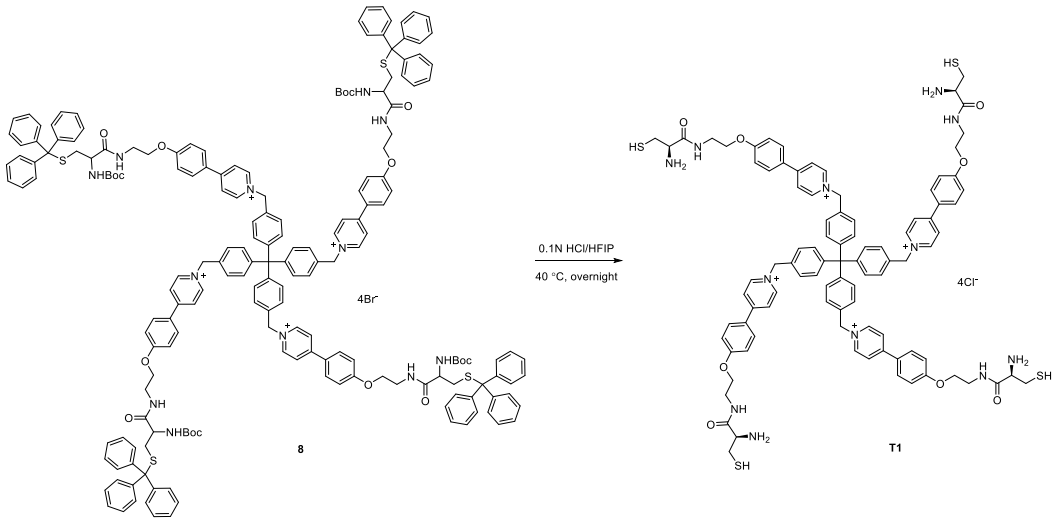
A549/ADR tumor inoculation and treatments. Female BALB/c nude mice bearing A549/ADR tumour (5 weeks old, average body weight ~18 g) were purchased from Shanghai Meixuan Biological Technology Company. When the tumor tissue reached 0.4~0.6 cm in diameter (~7 days after implant), saline, DOX, AlDox, **SOF-p1**, **SOF-p2**, **SOF-AlDox1** or **SOF-AlDox2** were injected through caudal vein in 0.2 mL dose ([AlDox] = [Dox] = 5.0 mg/kg, [**SOF-p1**] = 62.5 mg/kg, [**SOF-p2**] = 62.5 mg/kg) every three days. Tumors were measured with a digital caliper at the indicated time points, and volumes were calculated as ellipsoids, where V = (tumor length) × (tumor width)^2/2.



Compound 6. Dissolve compound **5** (5.1 g, 1.5 equiv, 9.1 mmol) and dry triethylamine (1.3 mL, 1.5 equiv, 9.1 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF, 30 mL) at ambient protection. Then add compound **4** (1.3 g, 1.0 equiv, 6.1 mmol) to continue the reaction for half an hour. After the reaction was completed, add water to quench the reaction and a white solid was precipitated. The white solid was directly filtered to obtain the crude product. Dissolve the crude product in ethyl acetate (EtOAc, 30 mL) and water (30 mL) under stirring then the organic layer was separated but still has solvent residue. Use water (3 × 30 mL) to extract triethylamine and DMF from the organic phase. The organic layers were combined and washed with 1 N HCl (1 × 30 mL), H_2O (2 × 30 mL), dried over $MgSO_4$, and then filtered and concentrated under vacuum to give a yellowish solid (3.7 g, 93%). M.p. 150-152 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (m, J = 4.4 Hz, 2H), 8.01 (d, J = 6.0 Hz, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 4.0 Hz, 2H), 7.34-7.21 (m, 15H), 7.01 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.8 Hz, 1H), 4.00 (s, 3H), 3.49-3.44 (m, 1H), 3.31 (s, 1H), 2.33 (d, J = 4.0 Hz, 2H), 1.35 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 159.4, 154.9, 150.1, 146.4, 144.3, 129.3, 129.1, 128.0, 126.7, 120.5, 115.1, 78.4, 66.3, 53.4, 38.2, 34.0, 28.1. HRMS (ESI): Calcd for $C_{40}H_{42}N_3O_4S^+$: 660.2891 [M + H]$^+$. Found: 660.2897.

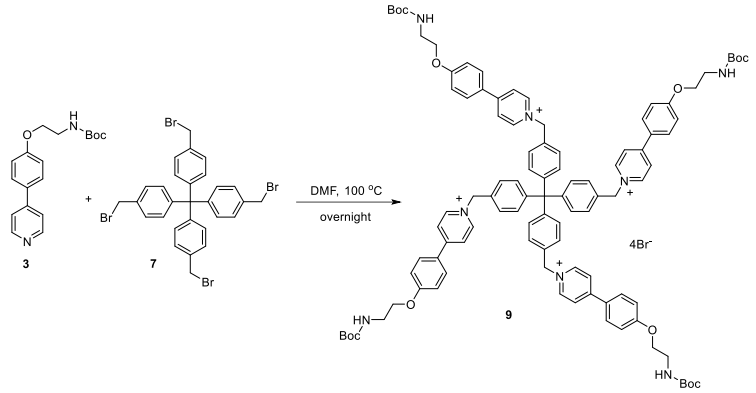


Compound 8. A mixture of compounds **6** (100 mg, 1.0 equiv, 0.1 mmol) and **7** (571.7 mg, 6.0 equiv, 0.9 mmol) in DMF (10 mL) were stirred under reflux for 12 hours and then cooled to room temperature. The solvent was removed by rotary evaporation and EA (30 mL) was added to dissolve the unreacted compound **5**, and the solid obtained by filtration was the target product. The resulting solid was further recrystallized from acetonitrile to give the pure product as bromide salt as a light yellow solid (209.8 mg, 45%). M.p. > 300 °C. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.10 (t, J = 6.4 Hz, 8H), 8.46 (d, J = 6.4 Hz, 8H), 8.05 (d, J = 8.0 Hz, 12H), 7.41 (d, J = 7.6 Hz, 8H), 7.33-7.15 (m, 66H), 7.11 (d, J = 8.4 Hz, 9H), 6.96 (d, J = 8.4 Hz, 4H), 5.71 (s, 8H), 4.06 (s, 8H), 3.95 (d, J = 8.0 Hz, 4H), 3.47 (s, 7H), 2.33 (s, 9H), 1.35 (s, 36H). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ 170.5, 154.9, 144.5, 144.3, 130.8, 130.1, 129.1, 128.2, 128.0, 126.7, 123.5, 115.6, 78.4, 66.6, 65.9, 53.4, 34.0, 28.1. HRMS (ESI): Calcd for $C_{189}H_{188}Br_4N_{12}O_{16}S_4$: 752.8296 $[M\text{-}4Br]^{4+}$. Found: 752.8298.

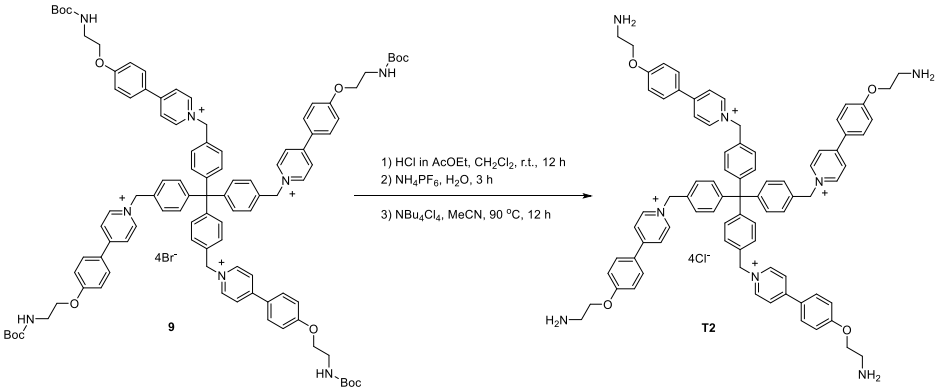


Compound T1. Dissolve compound **8** (100 mg, 1.0 equiv, 0.03 mmol) in the mixture of 0.1 N (*ca*. 0.37% w/v) HCl in HFIP (0.1 mL of *ca*. 37% w/v aq HCl per 9.9 mL of HFIP) were stirred

under 40 ℃ for 12 hours and then cooled to room temperature. The solution was removed by rotary evaporation and acetone (20 mL) was added to dissolve the protecting group product and the solid obtained by filtration was the target product. The resulting solid was further purified by acetonitrile to give the main product as chlorine salt as a light yellow solid (55.6 mg, 80%). M.p. > 300 °C. ^{1}H NMR (400 MHz, Deuterium Oxide) δ 8.58 (d, J = 6.4 Hz, 8H), 7.31-6.92 (m, 40H), 5.23 (s, 8H), 4.14 (t, J = 5.2 Hz, 4H), 3.96 (s, 8H), 3.75-3.70 (m, 4H), 3.43-3.38 (m, 4H), 2.96 (d, J = 5.6 Hz, 8H). ^{13}C NMR (100 MHz, Deuterium Oxide) δ 168.0, 161.6, 154.4, 147.9, 143.0, 132.4, 131.4, 129.3, 128.6, 124.6, 123.3, 115.4, 66.4, 62.5, 54.3, 38.8, 25.0. HRMS (ESI): Calcd for $C_{93}H_{100}Cl_4N_{12}O_8S_4$: 855.8035 $[M-2Cl]^{2+}$. Found: 855.7919.



Compound 9. A solution of compounds **3** (1.0 g, 3.0 mmol) and **7** (0.43 g, 0.6 mmol) in DMF (60 mL) was stirred at 100 °C for 12 h and then concentrated with a rotavapor. The resulting residue was triturated in ethyl acetate (10 mL) for for 30 min. The solid was filtered and recrystallized in acetonitrile to afford compound **5** as a yellow solid (0.93 g, 80%). M.p. > 250 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (d, J = 6.4 Hz, 8H), 8.48 (d, J = 6.4 Hz, 8H), 8.08 (d, J = 8.8 Hz, 8H), 7.43 (d, J = 8.4 Hz, 8H), 7.21-7.16 (m, 16H), 7.07 (s, 4H), 5.74 (s, 8H), 4.09 (s, 8H), 1.38 (s, 36H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 155.7, 154.4, 146.7, 144.5, 130.8, 131.2, 128.2, 125.3, 123.6, 115.7, 77.9, 66.9, 64.0, 61.3, 28.2. HRMS (ESI): Calcd for $C_{101}H_{112}N_8O_{12}Br_2$: 894.3370 $[M-2Br]^{2+}$. Found: 894.3389.



Compound T2. To a solution of compound **9** (0.78 g, 0.3 mmol) in dichloromethane (25 mL) was added a solution of hydrochloric acid in ethyl acetate (6 M, 10 mL). The solution was stirred at room temperature for 24 h and then filtered to afford a yellowish solid. The solid was dissolved in water (10 mL). To the solution was added dropwise the saturated solution of

NH_4PF_6 in water. The mixture was stirred at room temperature for 3 h and the precipitate formed was filtrated off and dried to afford a yellowish solid as hexafluorophosphate salt. The solid was then dissolved in acetonitrile of minimum volume. Saturated solution of tetrabutylammonium chloride in acetonitrile was added dropwise. The mixture was stirred at 90 °C for 24 h and then cooled to room temperature. The precipitate formed was filtered, washed with acetonitrile (3 mL × 3) and dried to afford **T2** as a yellowish solid (0.55 g, 64%). M.p. > 260 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.22 (d, J = 6.4 Hz, 8H),8.50 (d, J = 6.4 Hz, 8H), 8.40 (s, 10H), 8.12 (d, J = 8.4 Hz, 8H), 7.46 (d, J = 8.4 Hz, 8H), 7.22-7.18 (m, 16H), 5.78 (s, 8H), 4.34 (s, 8H), 3.24 (s, 8H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.8, 154.7, 147.0, 145.1, 133.1, 131.2, 130.6, 128.6, 126.3, 124.1, 116.3, 65.1, 61.7, 38.5. MS calcd for $C_{81}H_{80}N_8O_4$ [M-4Br]$^{4+}$: 307.157; found: 307.179.

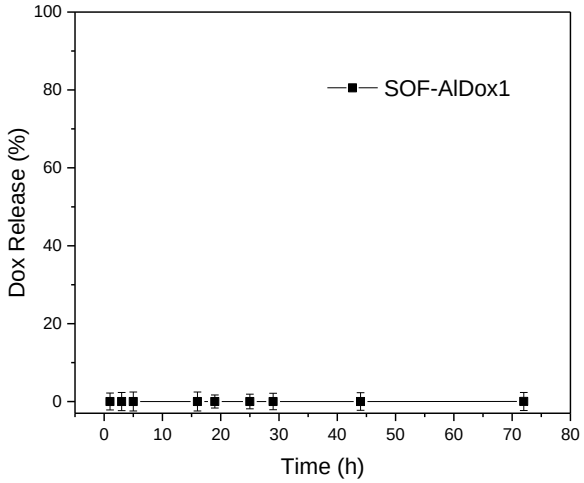


Fig. S1 Dialysis of the solution (1.0 mL, 5% glucose) of **SOF-AlDox1** ([AlDox] = 0.055 mg/mL, [**SOF-p1**] = 0.27 mg/mL) to 5% glucose solution (40 mL) at 37 ℃ (molecular weight cutoff: 1000 Da).

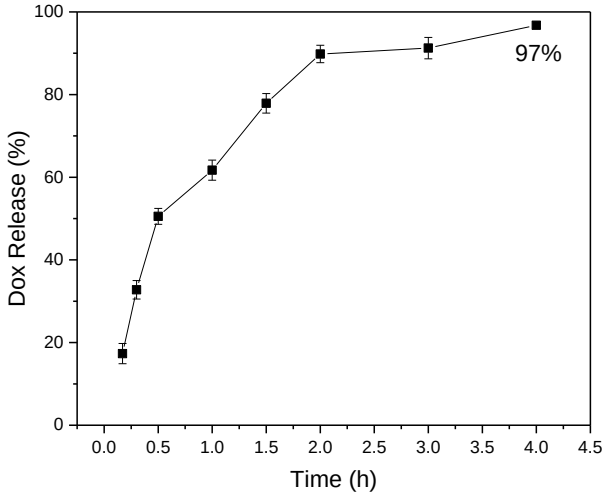


Fig. S2 Dialysis of the solution (1.0 mL, 5% glucose) of AlDox (0.055 mg/mL) to 5% glucose solution (40 mL) at 37 ℃ (molecular weight cutoff: 1000 Da).

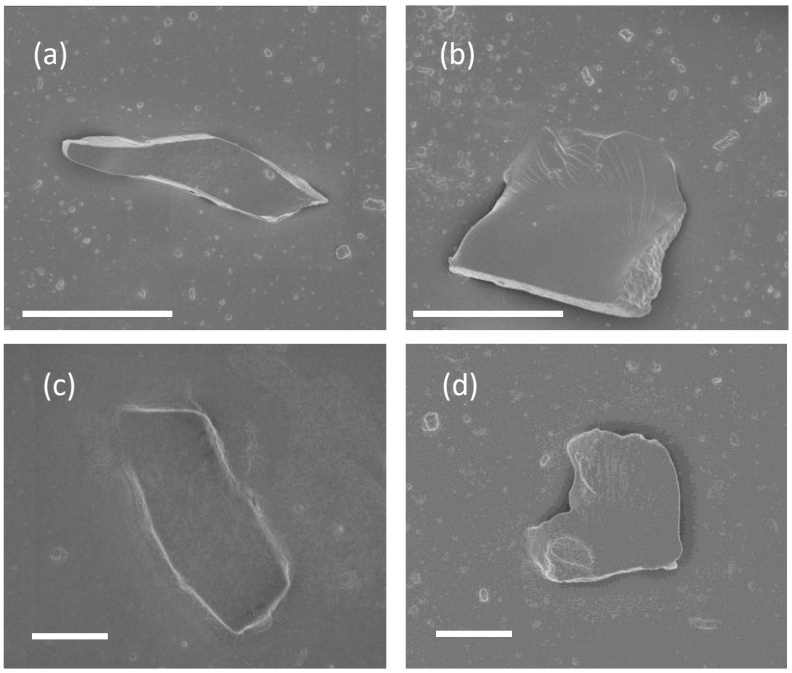


Fig. S3 Scanning electron microscope (SEM) images for (a) **SOF-p1**, (b) **SOF-AlDox1**, (c) **SOF-p2** and (d) **SOF-AlDox2** (scale bar: 4 μm).

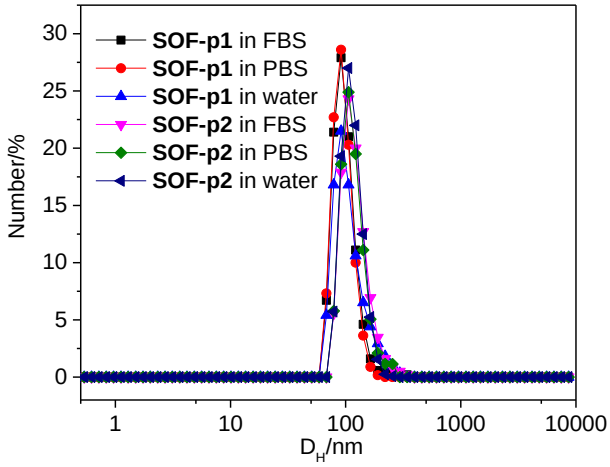


Fig. S4 DLS profile of **SOF-p1** (1.5 mM) and **SOF-p2** (1.5 mM) in different aqueous media.

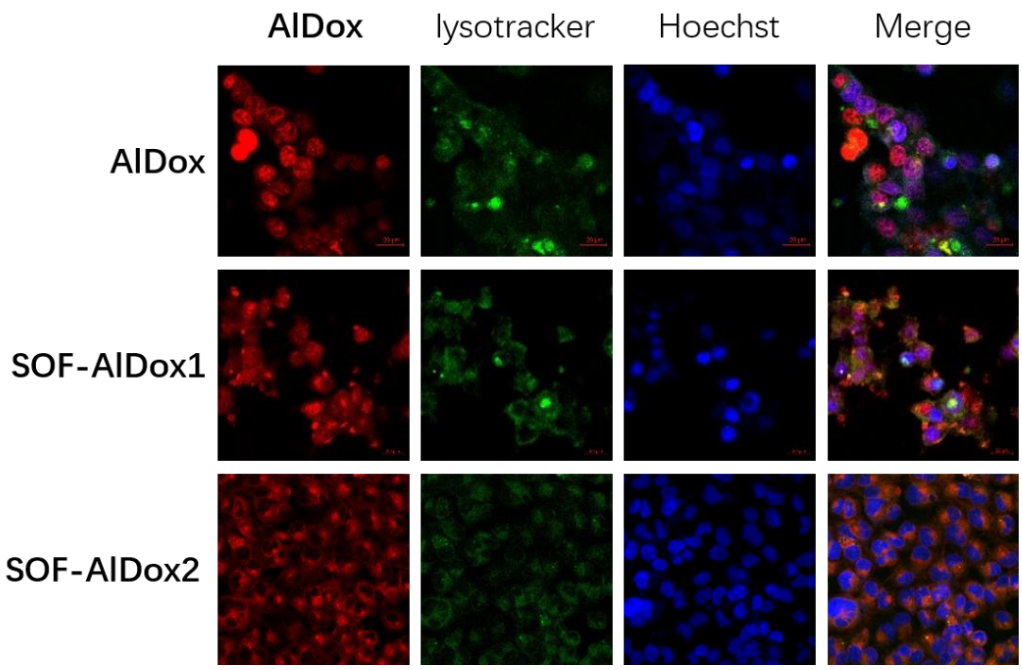


Fig. S5 Confocal microscopic images of MCF-7 cells after incubation with **AlDox**, **SOF-AlDox1** and **SOF-AlDox2** for 2 hours at 37 ℃. [**AlDox**] = 20 μM for all the samples. The nuclei and lysosomes were stained with Hoechst 33342 and Lyso-Tracker Green.

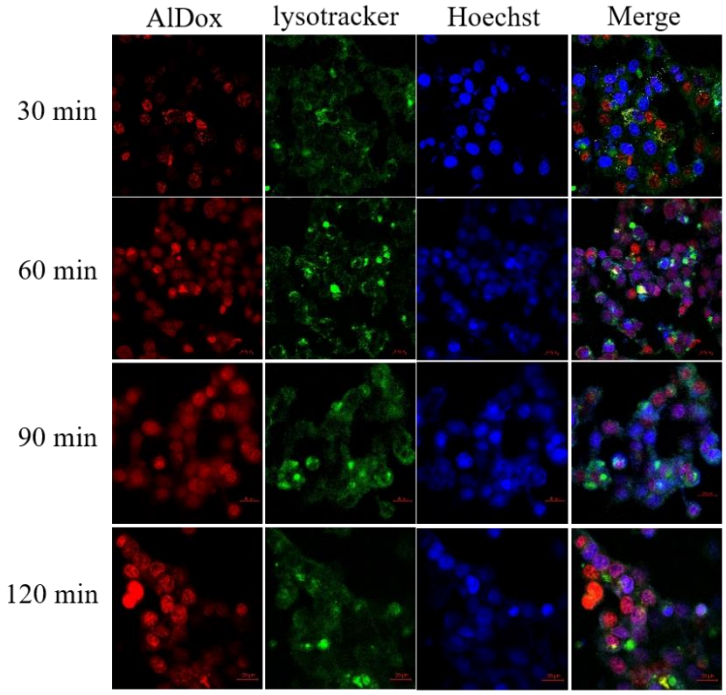


Fig. S6 Confocal microscopic images of MCF-7 cells after incubation with **AlDox** (20 μmol/L for 0.5, 1.0, 1.5 and 2 hours at 37 ℃.

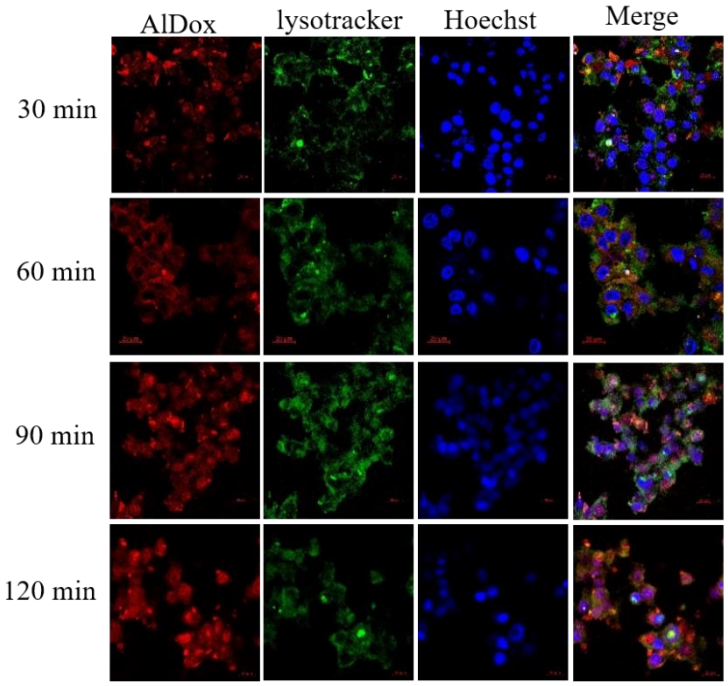


Fig. S7 Confocal microscopic images of MCF-7 cells after incubation with **SOF-AlDox1** ([AlDox] = 20 μmol/L, which corresponded to [**T1**] = 45.0 μmol/L) for 0.5, 1.0, 1.5 and 2 hours at 37 ℃.

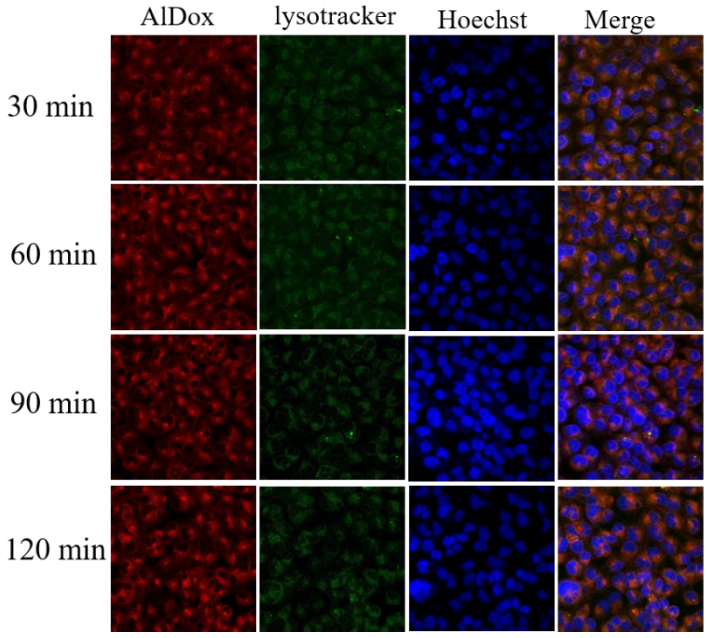


Fig. S8 Confocal microscopic images of MCF-7 cells after incubation with **SOF-AlDox2** ([AlDox] = 20 μmol/L, which corresponded to [**T2**] = 45.0 μmol/L) for 0.5, 1, 1.5 and 2 hours at 37 ℃.

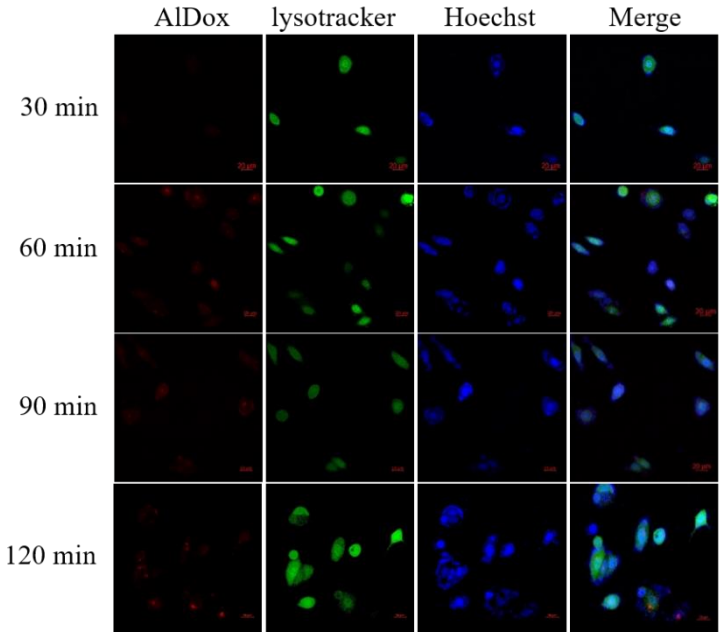


Fig. S9 Confocal microscopic images of MCF-7/ADR cells after incubation with **AlDox** (20 μmol/L) for 0.5, 1, 1.5 and 2 hours at 37 ℃.

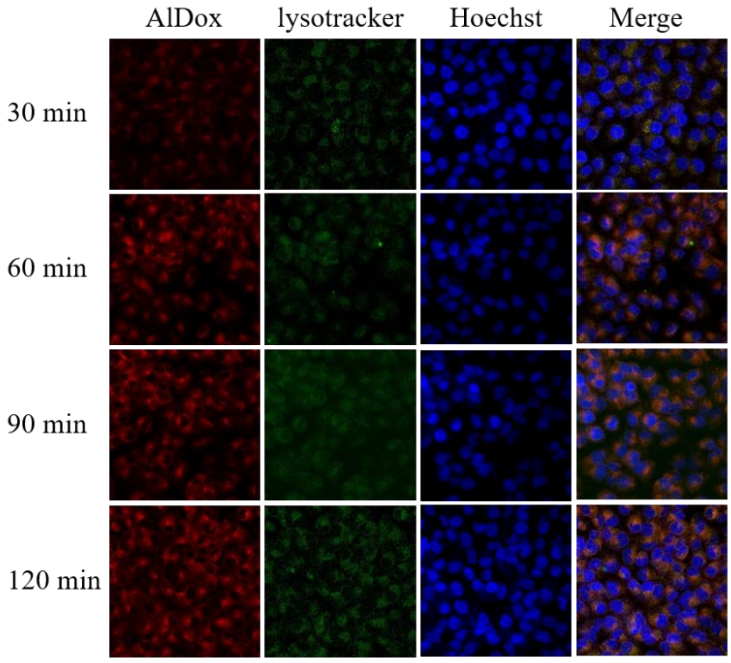


Fig. S10 Confocal microscopic images of MCF-7/ADR cells after incubation with **SOF-AlDox1** ([AlDox] = 20 μmol/L, which corresponded to [**T1**] = 45.0 μmol/L) for 0.5, 1, 1.5 and 2 hours at 37 ℃.

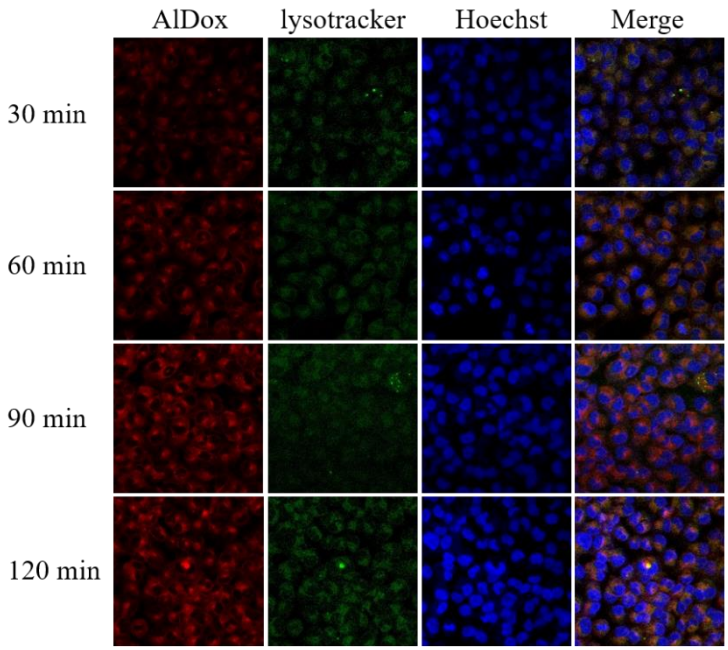


Fig. S11 Confocal microscopic images of MCF-7/ADR cells after incubation with **SOF-AlDox2** ([AlDox] = 20 μmol/L, which corresponded to [**T2**] = 45.0 μmol/L) for 0.5, 1, 1.5 and 2 hours at 37 ℃.

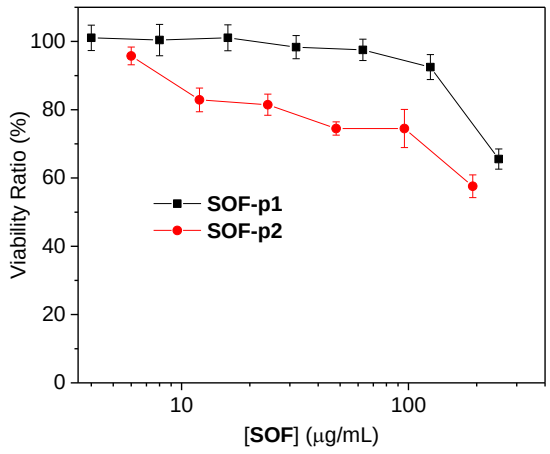


Fig. S12 Viability ratios of MCF-7/ADR cell lines using CCK-8 proliferation array versus the concentration of **SOF-p1** and **SOF-p2**. The cells (~2 × 10^4 per well) were incubated with the two polymeric agents at 37 ℃ for 24 h.

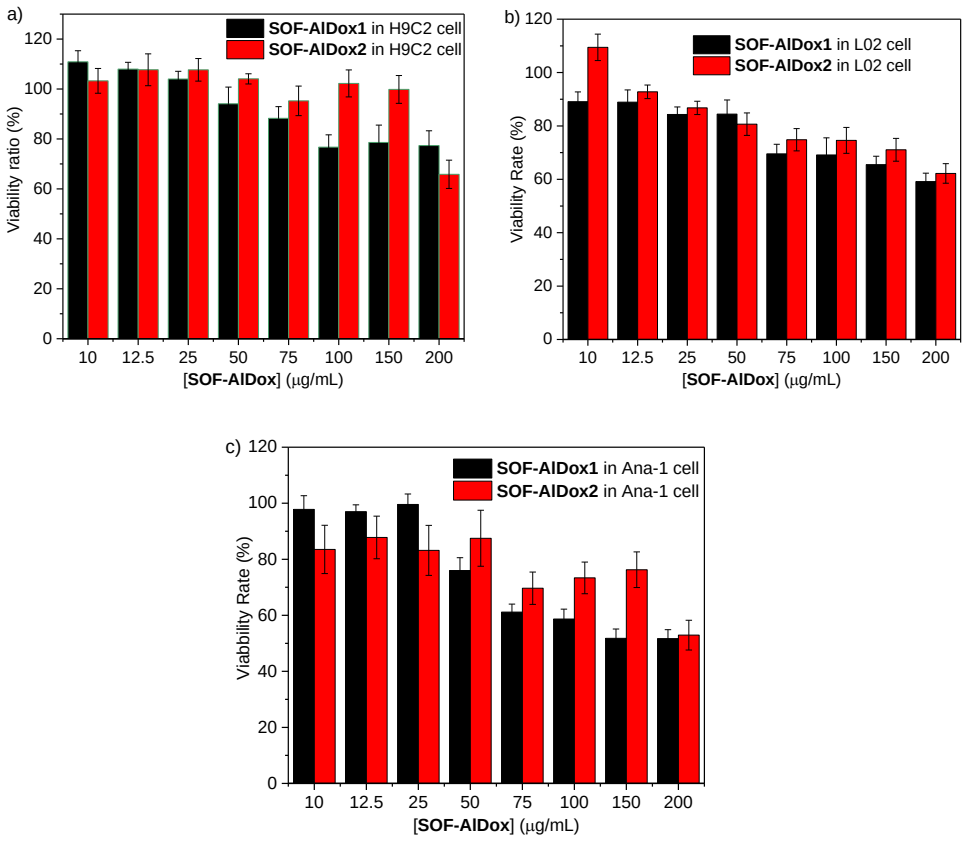


Fig. S13 Viability rate (%) of a) H9C2, b) L02 and c) Ana-1 cell lines at different incubation concentration of **SOF-AlDox1** and **SOF-AlDox2** determined using CCK-8 proliferation tests. The cells (~2 × 10^4 per well) were incubated with the prodrugs at 37 ℃ for 24 h. Error bars represent the s.d. of uncertainty for each point.

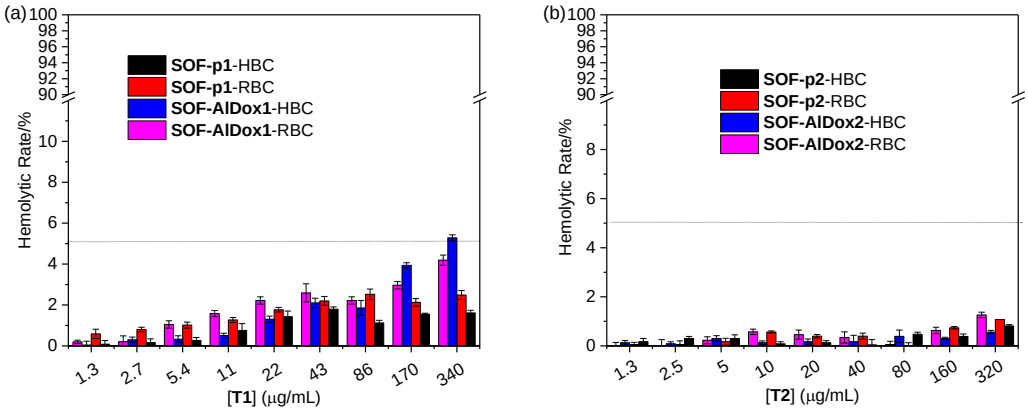


Fig. S14 (a) Hemolysis rates of **SOF-p1** and **SOF-AlDox1** in human red blood cells and rats red blood cells. (b) Hemolysis rates of **SOF-p2** and **SOF-AlDox2** in human red blood cells and rats red blood cells.

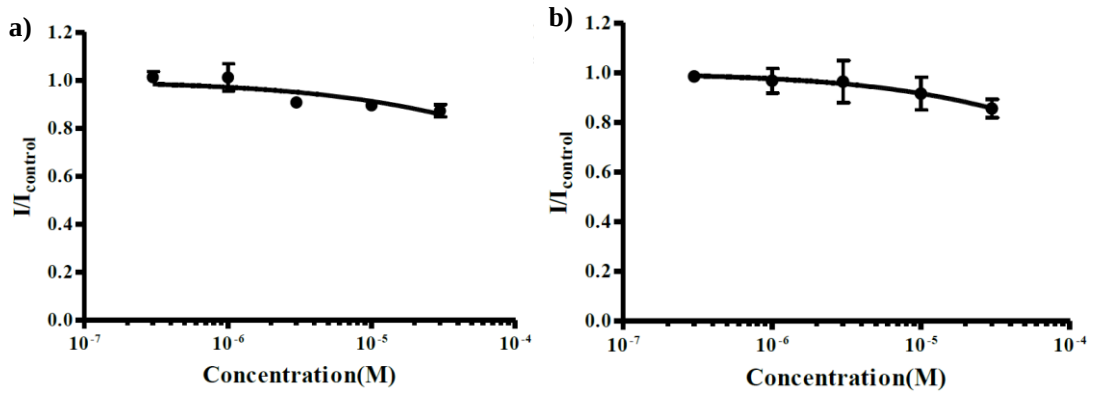


Fig. S15 Dose-response curve of hERG current inhibition by **SOF-AlDox1** and **SOF-AlDox2**, highlighting the low inhibition activity of both prodrugs. The concentrations referred to that of AlDox conjugated to the SOF.

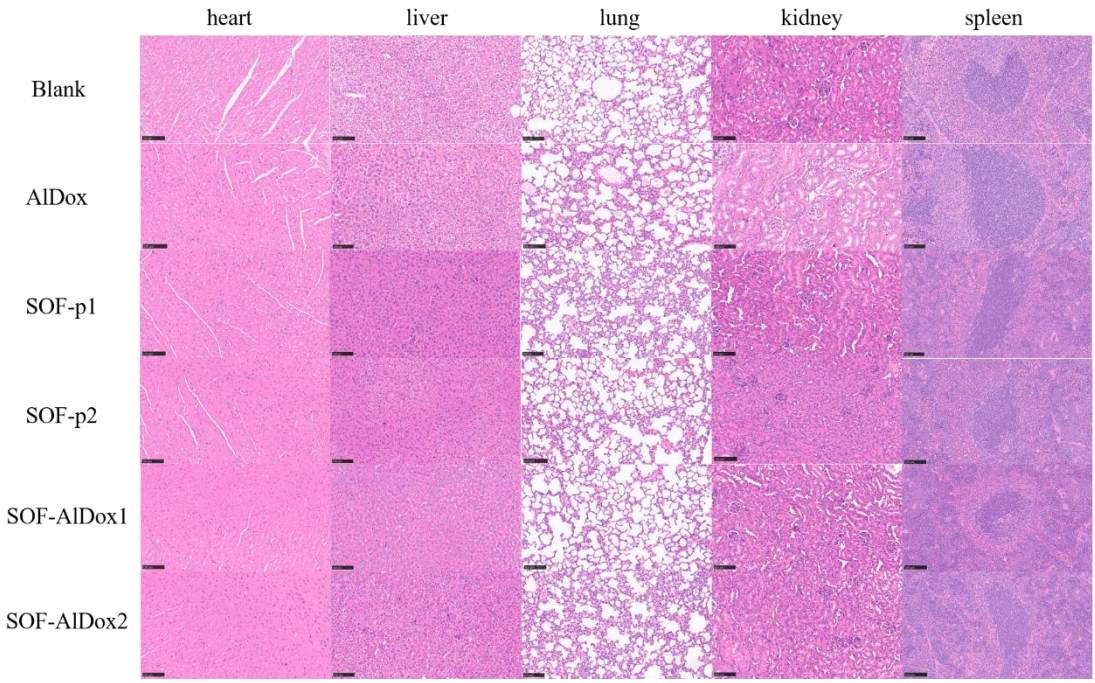


Fig. S16 Slice micrographs of H&E-stained main organs collected from each group (scale bar: 100 μm).

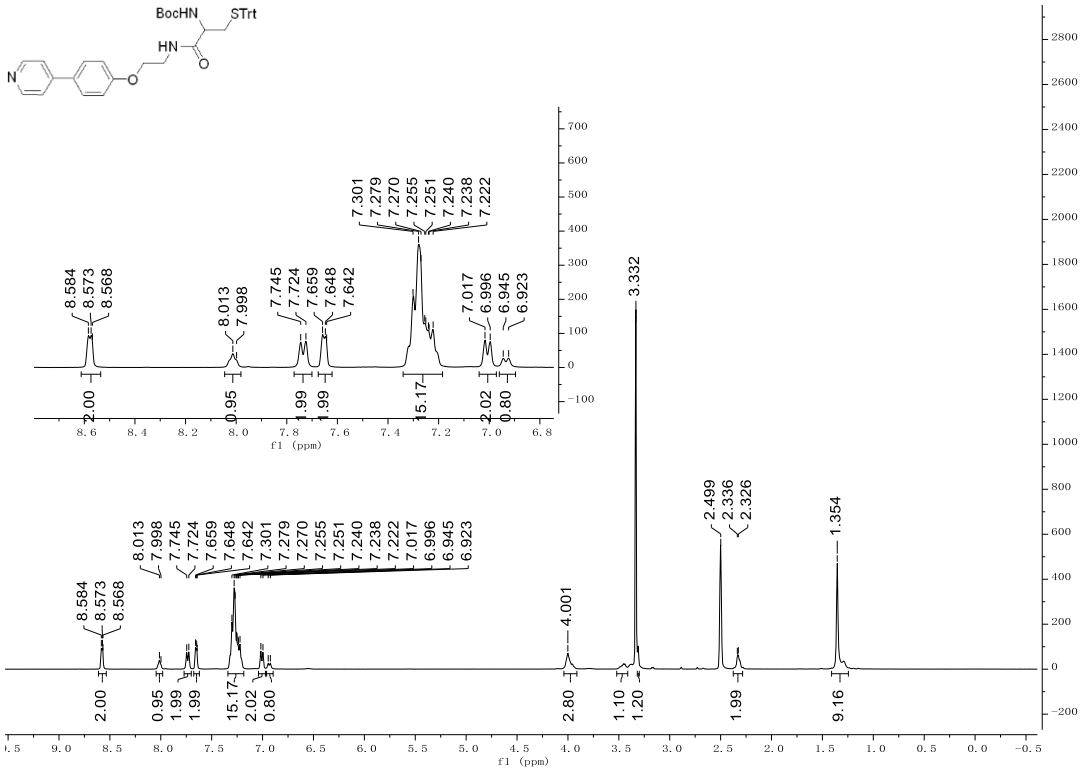


Fig. S17 ^{1}H NMR spectra of compound **6** in DMSO-d_6 (400 MHz, 25 ℃).

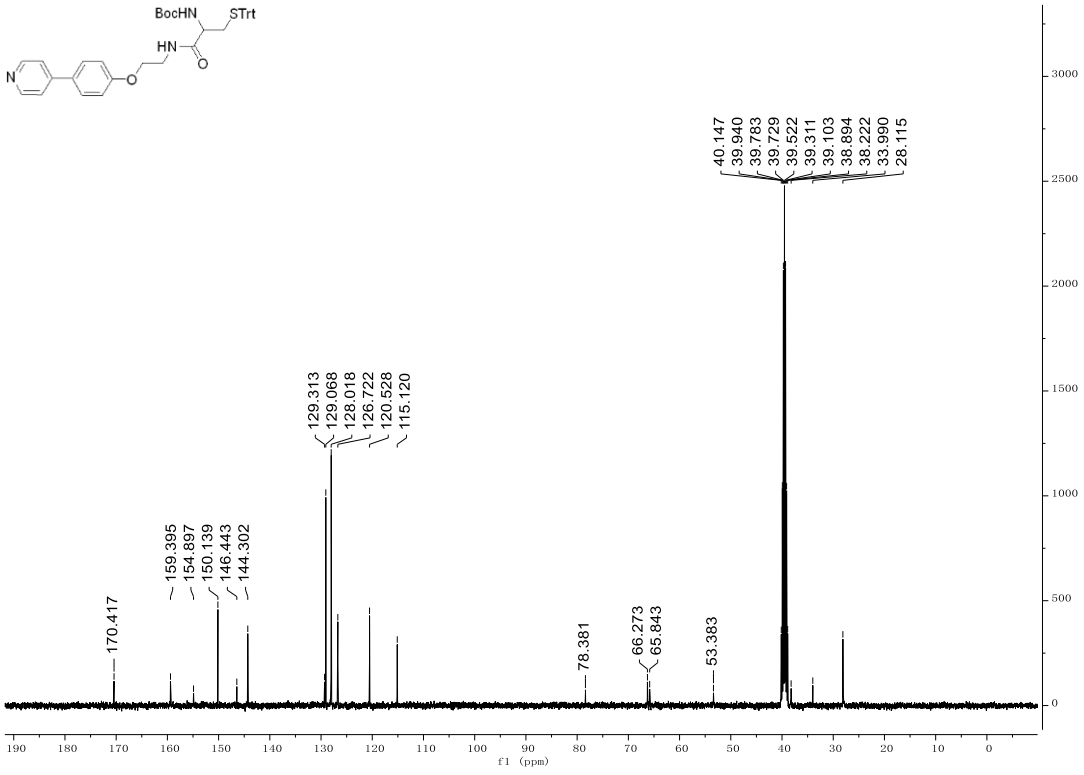


Fig. S18 ^{13}C NMR spectra of compound **6** in DMSO-d_6 (400 MHz, 25 ℃).

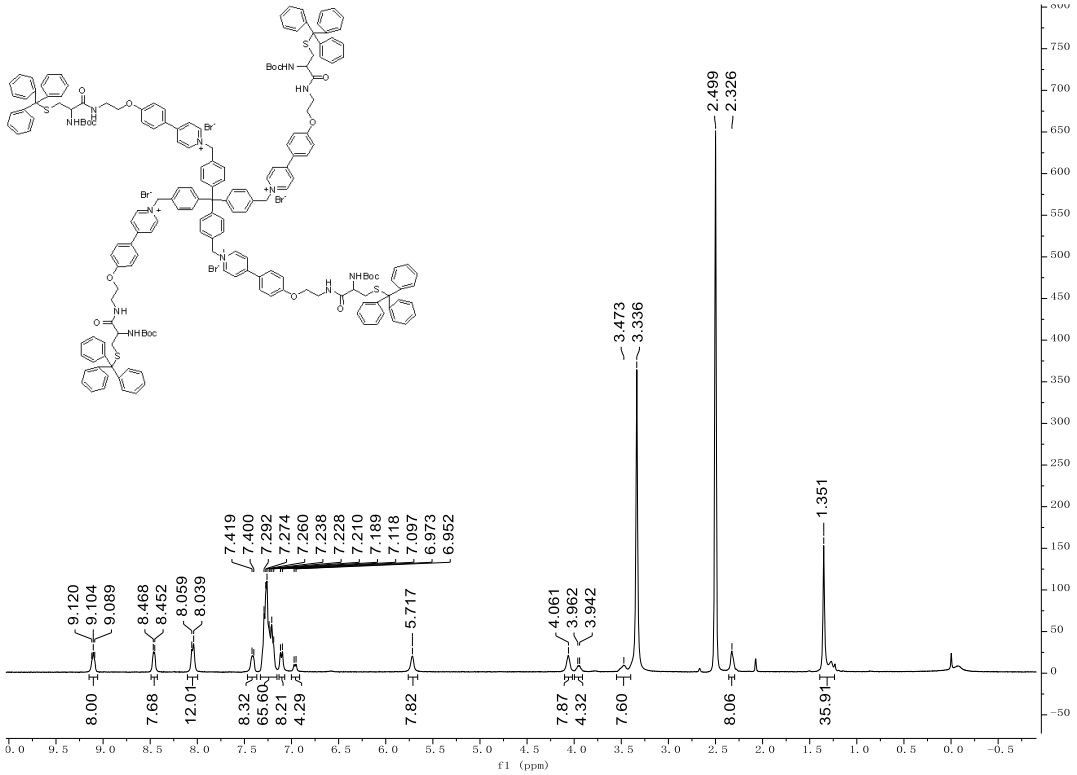


Fig. S19 ^{1}H NMR spectra of compound **8** in DMSO-d_6 (400 MHz, 25 ℃).

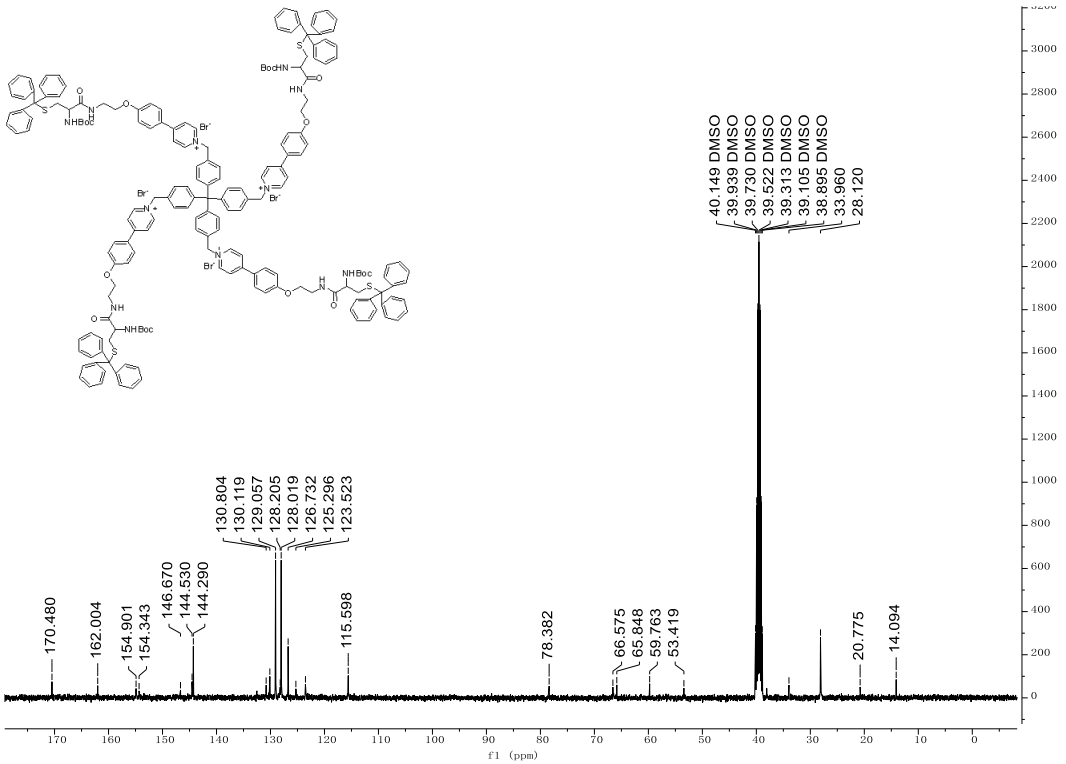


Fig. S20 ^{13}C NMR spectra of compound **8** in DMSO-d_6 (400 MHz, 25 ℃).

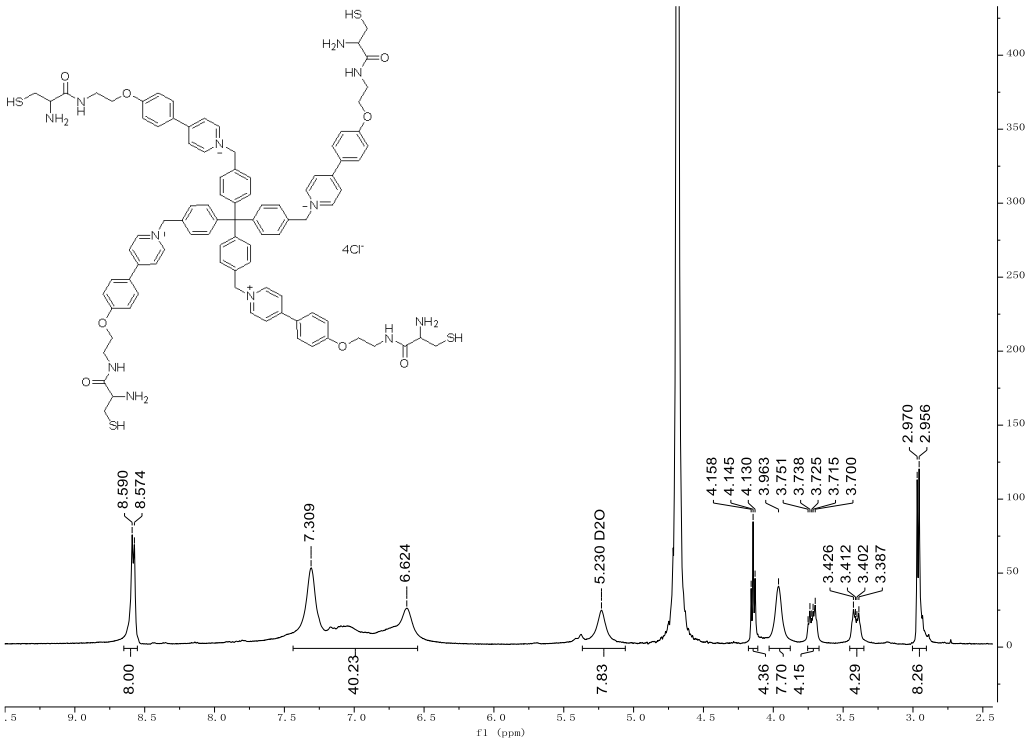


Fig. S21 ^{1}H NMR spectra of compound **T1** in D_2O (400 MHz, 25 ℃).

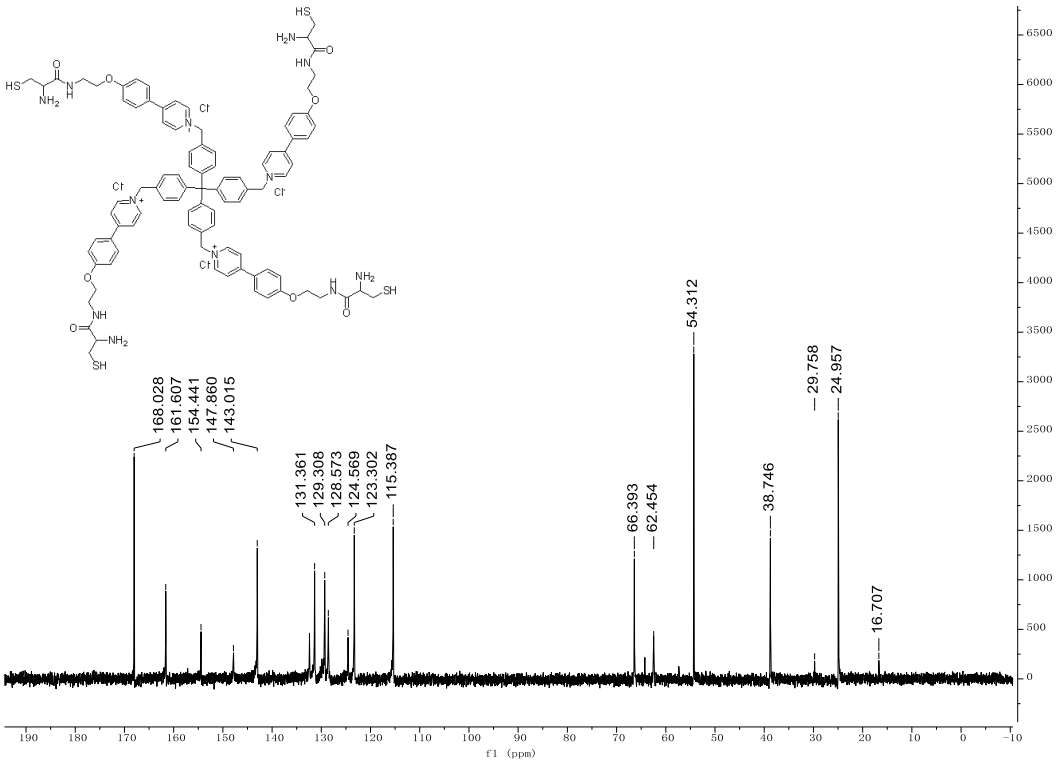


Fig. S22 ^{13}C NMR spectra of compound **T1** in D_2O (400 MHz, 25 ℃).

Reference:

1. Y.-X. Li, Y. Liu, H. Wang, Z.-T. Li, D.-W. Zhang, Water-Soluble Porphyrin-Based Nanoparticles Derived from Electrostatic Interaction for Enhanced Photodynamic Therapy. *ACS Appl. Bio Mater.*, 2022, **5**, 881-888.