Supporting Information

Supramolecular Organometallic Drug Complex with H_2O_2 Self-Provision Intensifying Intracellular Autocatalysis for Chemodynamic Therapy

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1. Materials and methods

1.1 Materials and structure characterization methods

Gemcitabine (GEM), doxorubicin hydrochloride (DOX·HCl) and ethynylferrocene were purchased from J&K Scientific Ltd., China. β-cyclodextrin (β-CD), hydrogen peroxide (H_2O_2) (30 wt %) and Cesium chloride were purchased from Energy Chemical, China. N, N, N′, N″, N″-Pentamethyldiethylenetriamine was purchased form Adamas, China. CuBr was purchased from Energy Chemical and purified by washing with acetic acid and methanol before use.

^{1}H NMR, ^{13}C NMR and NOESY spectra were obtained from Bruker Avance 400 instruments. Electrospray Ionization Mass Spectrometry were acquired with TOF-Q II 10280 (Varian Inc., USA). Ultraviolet visible (UV-vis) spectra were recorded on a Shimadzu UV-2550 spectrometer. Dynamic light scattering (DLS) experiments were conducted on a Malvern Instrument before sample was filtered. Transmission electron microscopy (TEM) measurement was prepared by a FEI Talos F200X instrument, and atomic force microscopy (AFM) images were obtained by Dimension Fast Scan and Dimension Icon instrument. XPS data were employed by KRATOS Axis ultra-DLD X-ray photoelectron spectrometer. The live cell samples were observed and imaged by Nikon A1R fluorescence microscopy.

1.2 Determination of association constant for GEM-(Fc)$_3$ and β-CD-DOX

We utilized **β-CD-DOX** and **GEM-(Fc)$_3$** to determine the association constants (*K*) in water (with 2% DMSO as cosolvent) by determining UV absorption at 311 nm.[1,2] The concentration of Fc of **GEM-(Fc)$_3$** was kept at 1×10^{-5} M. With the increase of the **β-CD-DOX** concentration, the absorption of Fc enhanced clearly. The usual double reciprocal plot according to the modified Hidebrand-Benesi equation was used:

$$\frac{1}{\triangle A} = \frac{1}{K \triangle \varepsilon[H][G]} + \frac{1}{\triangle \varepsilon[H]}$$

Where H, G, *K* represent host (β-CD of **β-CD-DOX**), guest (Fc of **GEM-(Fc)$_3$**) and association constant, respectively. ΔA denotes the absorbance difference before and after **β-CD-DOX** are added. $\Delta\varepsilon$ indicates the difference of the molar extinction coefficient of the host-guest complex at the same wavelength.[1]

1.3 Preparation of SOMDMs

GEM-$(Fc)_3$ and **β-CD-DOX** (**GEM-$(Fc)_3$** and **β-CD-DOX** with 1 :3 molar ratio) was dissolved in 50 μL of DMSO and added to deionized water (5 mL), and the mixture was ultrasonicated for 5 min and stirred for 24 h.[3]

1.4 *In vitro* drug release from SOMDMs

1mg **SOMDMs** were dispersed in 5 mL for different of PBS buffer solution with a membrance tubing (MWCO = 3500): (1) PBS (pH 7.4); (2) PBS (pH 7.4) + 0.1% (w/w) H_2O_2, (3) PBS (pH 7.4) + 0.3% (w/w) H_2O_2 at 37 ℃ and stirred at 100 rpm. At certain time intervals, 4 mL PBS medium was removed and replaced by 4 mL fresh PBS. The released amount of DOX in the solution was determined by UV-vis spectrometry at λ = 480 nm.

1.5 Generation of hydroxyl radicals by SOMDMs mediated Fenton-like reaction.

SOMDMs were incubated with MB (1.00 μM) and H_2O_2 (100 μM) in a $NaHCO_3$ buffer solution (25 mM) at 37 °C for 30 min.[4] The generation of •OH was monitored by the absorption increase at 650 nm. **SOMDMs** incubated with MB but in the absence of H_2O_2 were used as controls: (I) MB, (II) MB + **SOMDMs**, (III) MB + H_2O_2, (VI) MB + H_2O_2 + **SOMDMs**.

1.6 Cell culture

The MCF-7 cells and 4T1 cells provided by Department of Pharmaceutical Chemistry and Analysis, Air Force Medical University, was cultured in RPMI 1640. The cells were cultured in a standard incubator at 37 °C containing 5% CO_2.

1.6.1 Cellular uptake of SOMDMs by MCF-7 cells

The cellular uptake behaviors were studied in MCF-7 cells through using CLSM. MCF-7 cells were seeded in 6-well plates at 5.0×10^4 cells per well in 2 mL of complete RPMI 1640 and cultured for 24 h. Then the solution of **SOMDMs** was diluted with RPMI 1640 culture medium at concentration of 5 μM. After incubation for another 2 h and 8 h, stained the cells with Hoechst 33342 for 5 min at

37 °C. The resulting slides were observed with Nikon A1R fluorescence microscopy.

1.6.2 *In Vitro* cytotoxicity studies of SOMDMs

The MCF-7 cells were used to evaluate the anticancer activity of **SOMDMs**. The cells were seeded into 96-well plates at 1 × 10^4 cells per well in 100 μL of culture medium. After 24 h incubation, the medium was removed and replaced with 100 μL of a medium containing serial dilutions of free GEM, free DOX, GEM/DOX mixture (with molar ratio 1:3)[5], and **SOMDMs** at a series of concentrations (0.25, 0.5, 1, 3, 6 and 12 μM) for 24 h. After incubation, the wells were rinsed with PBS. Subsequently, 100 μL of culture medium (10% CCK-8) was added into each well and incubated for another 4 h at 37°C. Finally, the group was measured at a wavelength of 450 nm using a SpectraFluor™ Plus microplate reader.

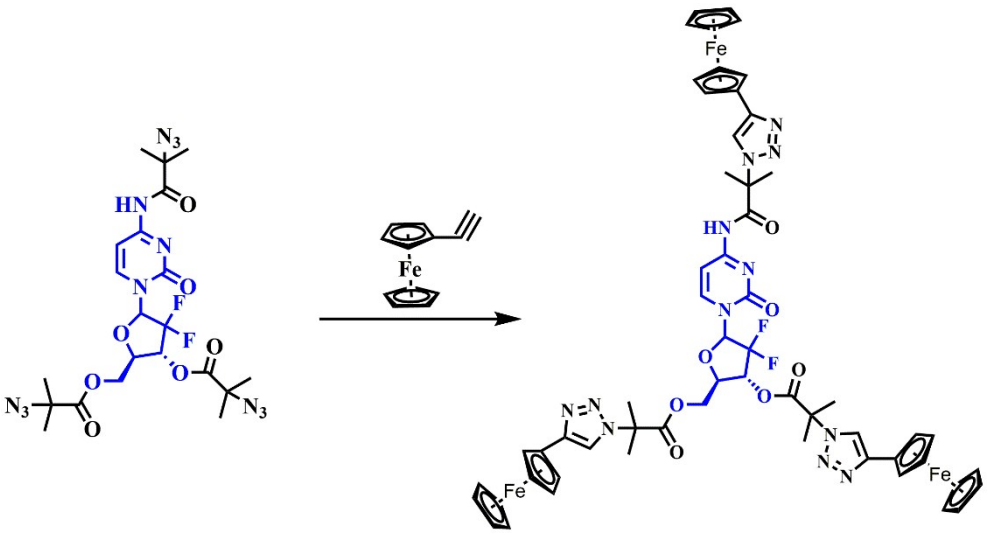
1.6.3 *In vitro* ROS generation by SOMDMs mediated fenton-like reaction.

MCF-7 cells were randomly seeded in 6-well plates at a density of 5×10^5 per well for 24 h. Then the cells were treated with PBS, **SOMDMs** and **SOMDMs** plus sodium pyruvate at the concentration of 5 μM for another 12 h. After that, the cells were washed with PBS twice and incubated with 15 μM of DCFH-DA for 30 min. After rinsed with PBS, the fluorescence intensity of cells was monitored by the CLSM. Green fluorescence indicates the ROS level.

1.7 Animals and Tumor Models.

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of the Air Force Medical University and approved by the Animal Ethics Committee of the Air Force Medical University. 4T1 tumor-bearing mice were randomly divided into five groups and were intravenously injected *via* the tail vein with PBS, free GEM (2 μmol/Kg), DOX (2 μmol/Kg), CPT/Cur mixture (GEM 0.5 μmol/Kg and DOX 1.5 μmol/Kg) [5], and **SOMDMs** (2 μmol/Kg) at 1, 4, 7, 10 days. During 22 days of the corresponding treatments, the length and width of the tumor and the body weight of mice were measured at certain time intervals. At the end of the treatment, all the mice were sacrificed. The major organs of mice and tumors were collected for weighing, H&E and TUNEL staining.

2. Synthesis of GEM-$(Fc)_3$



Scheme S1. Synthetic routes of **GEM-$(Fc)_3$**.

Compound GEM-$(N_3)_3$ was synthesized according to the literature procedure.[6]

Compound GEM-$(N_3)_3$ (0.6 g, 1.0 mmol), ethynylferrocene (0.96 g, 4.50 mmol) and PMDETA (0.60 g, 3.50 mmol) were dissolved in DMF (50 mL). The mixture was degassed *via* three freeze-evacuate-thaw cycles, and CuBr (0.09 g, 0.60 mmol) was added. After 24 h of stirring under an N_2 atmosphere at 50 °C, the reaction was quickly quenched in an ice bath. The solvent was reduced *via* distillation. The combined organic phase was concentrated and purified by flash column chromatography to afford compound **GEM-$(Fc)_3$** as an orange solid 0.85 g.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 11.06 (s, 1H), 8.44-8.34 (m, 3H), 7.93 (s, 1H), 7.36 (s, 1H), 6.29 (s, 1H), 5.50 (s, 1H), 4.74-4.70 (m, 6H), 4.47-4.46 (m, 3H), 4.31-4.27 (m, 6H), 4.07-3.96 (m, 15H), 1.97-1.89 (m, 18H).

^{13}C NMR (100 MHz, $CDCl_3$) δ =170.94, 146.71, 132.30, 117.78, 117.44, 70.11, 69.75, 69.65, 69.26, 68.95, 68.88, 66.89, 66.77, 64.31, 64.16, 25.71, 25.60.

HRMS (ESI-TOF) ($C_{57}H_{56}N_{12}O_7F_2Fe_3$) m/z: for $[M+H]^+$ found: 1227.2172.

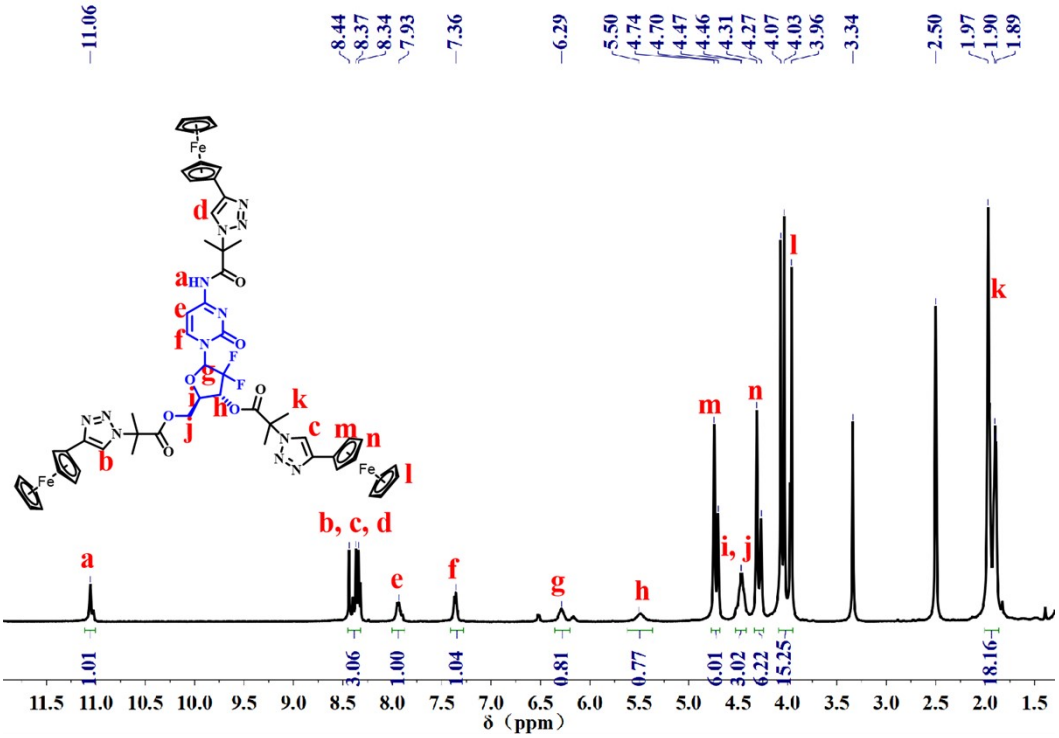


Figure S1. ^{1}H NMR spectrum (400 MHz, DMSO-d_6, RT) recorded for **GEM-(Fc)$_3$**.

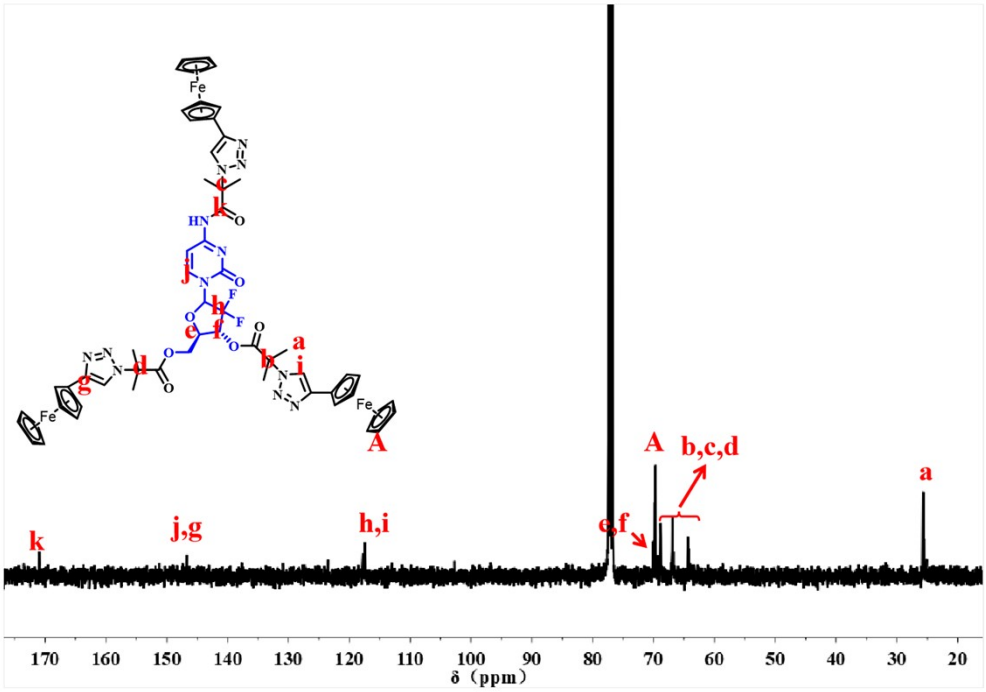


Figure S2. ^{13}C NMR spectrum (100 MHz, DMSO-d_6, RT) recorded for **GEM-(Fc)$_3$**.

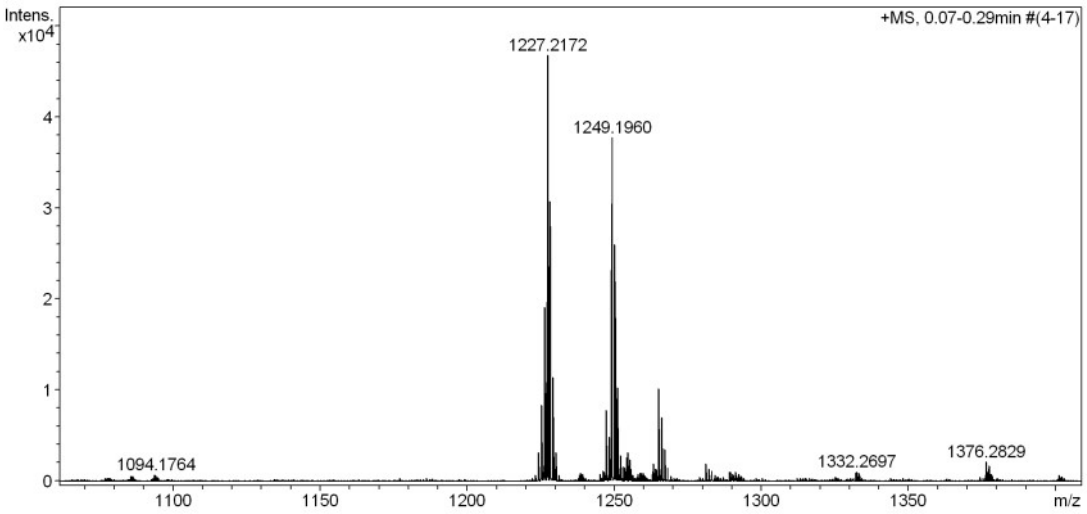


Figure S3. Mass spectrum of **GEM-(Fc)$_3$**.

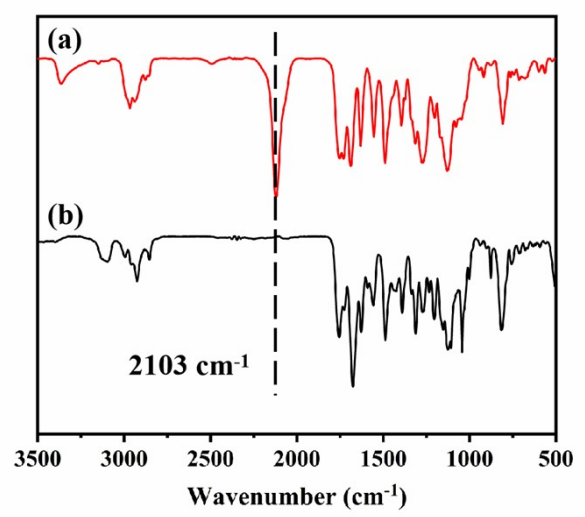
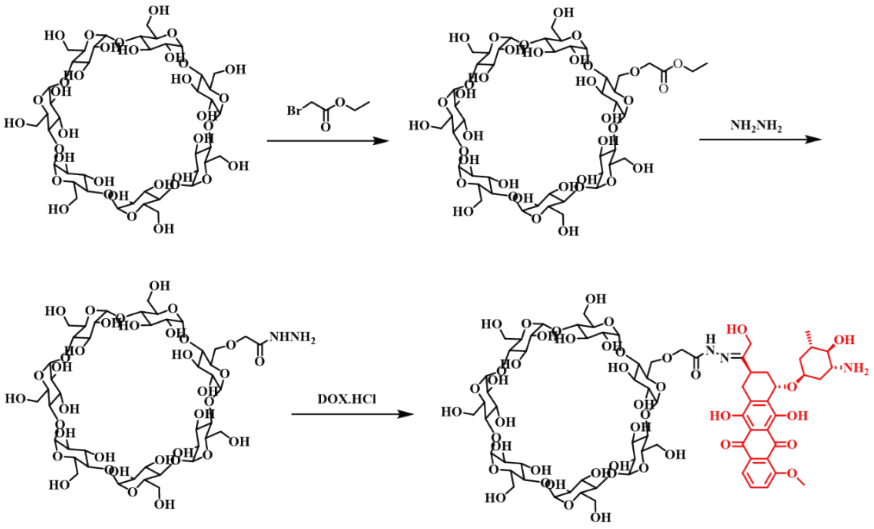


Figure S4. FT-IR spectra of (a) GEM-(N$_3$)$_3$, (b) **GEM-(Fc)$_3$**

GEM-(N$_3$)$_3$ displays a sharp and intense peak at 2103 cm^{-1}. After reacting with ethynylferrocene by click reaction, the 2103 cm^{-1} band is disappeared, suggesting that GEM-(N$_3$)$_3$ reacted with ethynylferrocene to afford **GEM-(Fc)$_3$**.

3. Synthesis of β-CD-DOX[7]



Scheme S2. Synthetic routes of **β-CD-DOX**.

Compound **β-CD-DOX** was synthesized according to the previous work.[7]

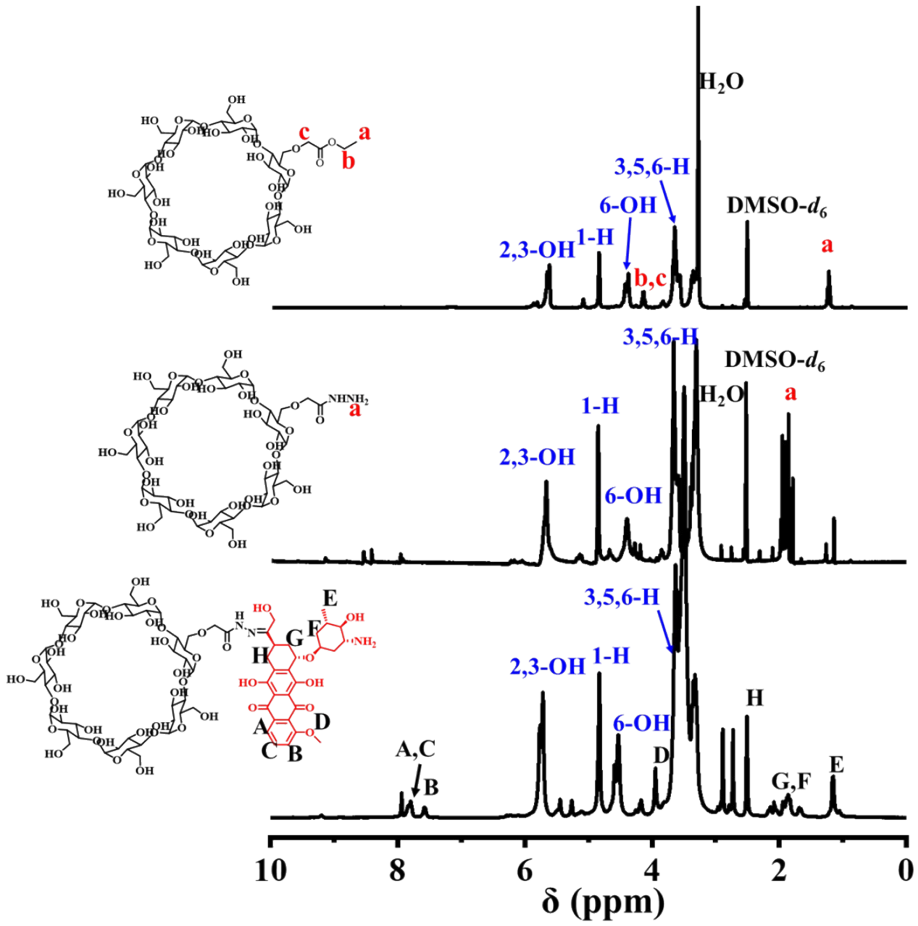


Figure S5. ^{1}H NMR spectrum recorded (400 MHz, DMSO-d_6, RT) for β-CD-ester, β-CD-hydrazide and **β-CD-DOX**.

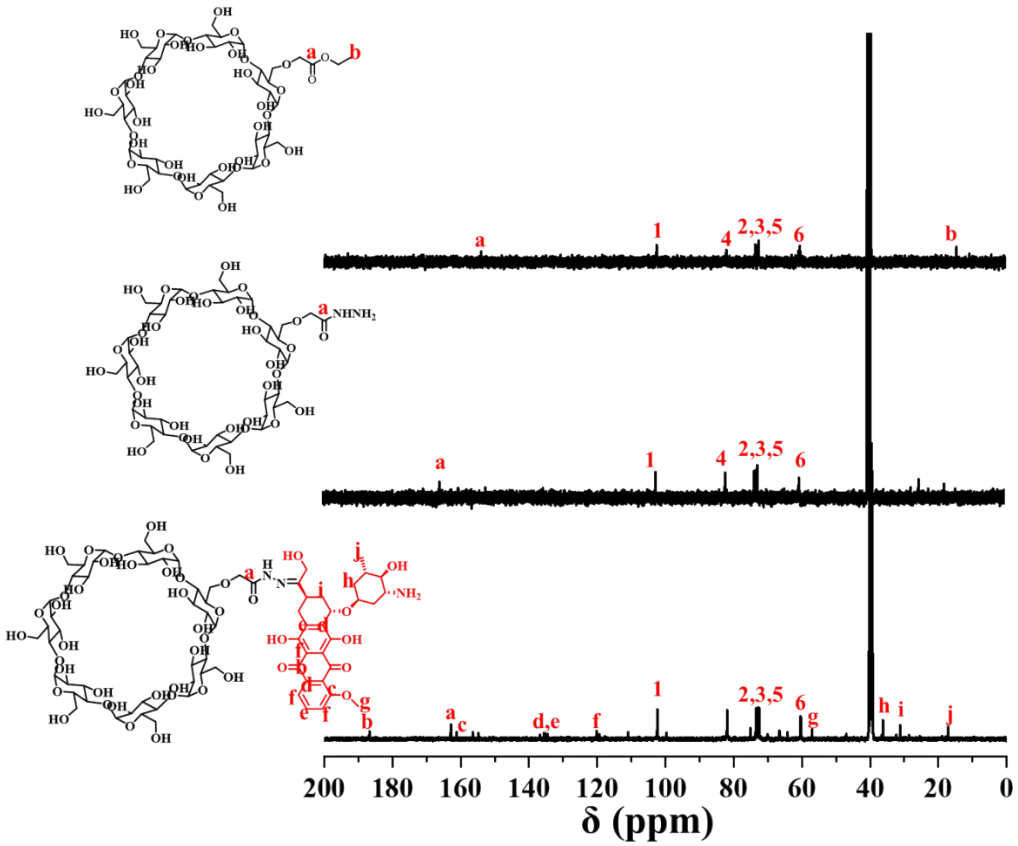


Figure S6. ^{13}C NMR spectrum recorded (100 MHz, DMSO-d_6, RT) for β-CD-ester, β-CD-hydrazide and **β-CD-DOX**.

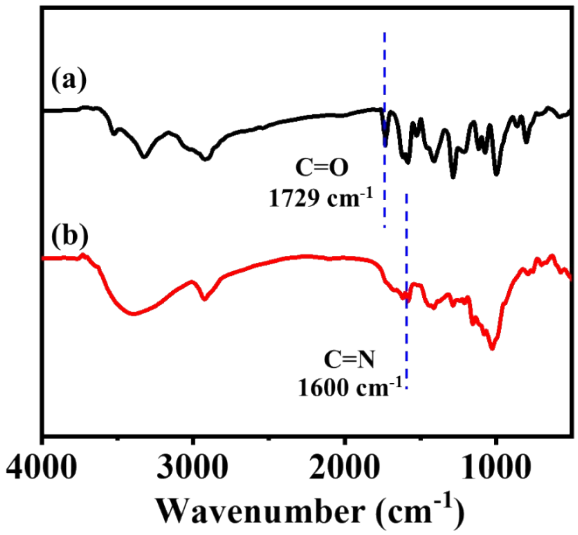


Figure S7. FT-IR spectra of (a) DOX and (b) **β-CD-DOX**

4. Self-assembly behavior of SOMDMs

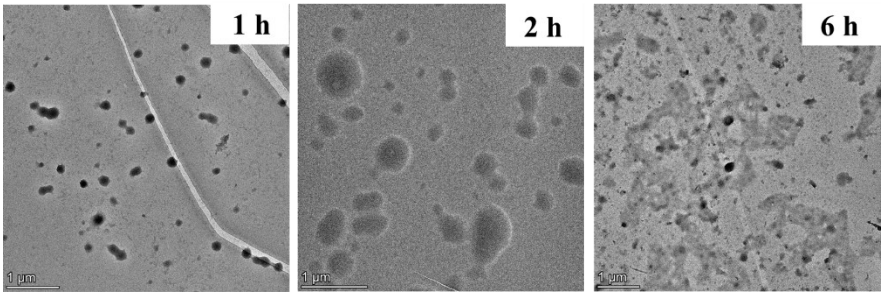


Figure S8. TEM images of **SOMDMs** after 1 h, 2 h and 6 h incubation in the H_2O_2 solution.

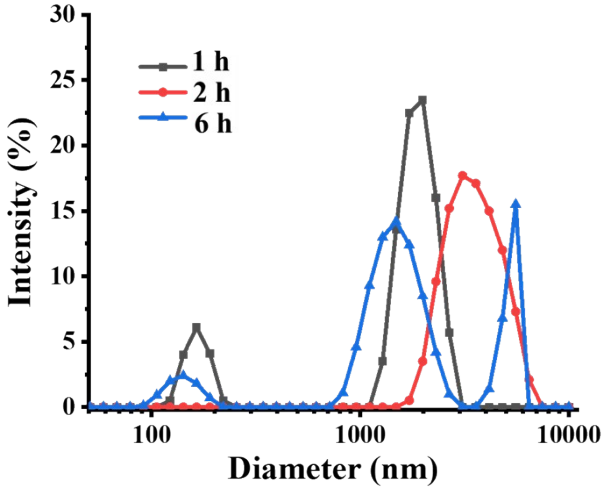


Figure S9. DLS of **SOMDMs** after 1 h, 2 h and 6 h incubation in the H_2O_2 solution.

5. Biological properties of **SOMDMs**

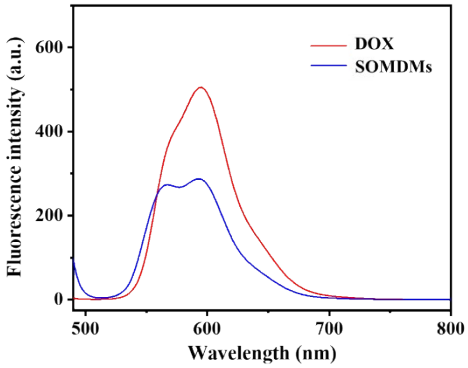


Figure S10. Fluorescence titration experiments. Fluorescence emission spectrum of DOX (1 μM, λ_{ex}=480 nm λ_{max}=580 nm)

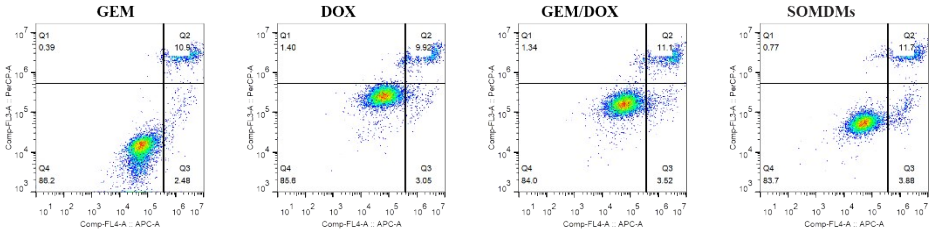


Figure S11. Flow cytometry analysis for the apoptosis of MCF-7 cells induced by GEM, DOX, GEM/DOX mixture and **SOMDMs** at a drug concentration of 15 μM for 24 h.

Table S1. IC_{50} of GEM, DOX, GEM/DOX mixture, and **SOMDMs** in MCF-7. Error barsindicate SD (n = 5)

Sample	MCF-7 (μM)
GEM	>30
DOX	8.35±0.67
DOX/GEM	12.34±0.23
SOMDMs	5.82±1.73

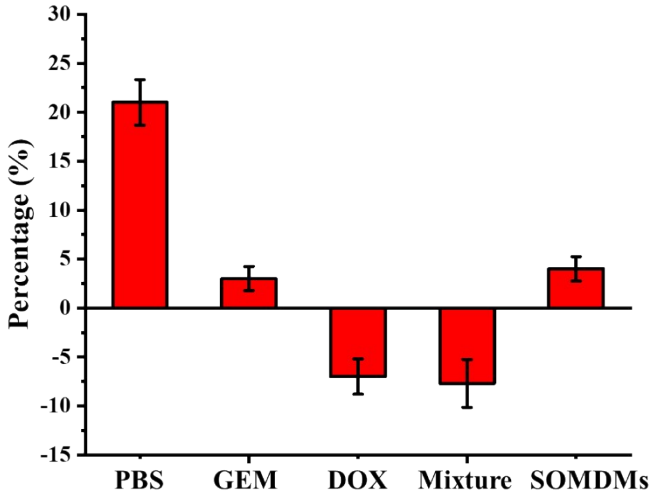


Figure S12. The changed percentage of body weight for the treatment with PBS, GEM, DOX, the GEM/DOX mixture, and **SOMDMs** after 22 days.

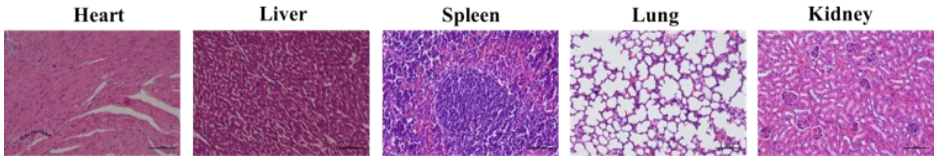


Figure S13. Histopathological analysis by H&E staining of liver, spleen, lung, kidney, and heart for **SOMDMs**.

7. References

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