Supplementary Information

A biomimetic ZIF nanoagent for synergistic regulation of glutamine metabolism and intracellular acidosis of cancer

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Chemicals.

$Zn(COOH)_2 \cdot 6H_2O$, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), imidazole-2-formaldehyde (ICA) and Rhodamine 123 were purchased from Sigma. Calcein-AM/PI Double Stain Kit, pH probe 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein,acetoxymethyl ester (BCECF AM) and Annexin V-FITC Apoptosis Detection Kit were purchased from Beyotime (Nantong, China). MCF-7 cells were obtained from Aoluo Biotechnology Co. Ltd. (Shanghai, China). Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) was purchased from APExBIO Technology LLC. and 3,4-dihydroxy-N'-(2-hydroxybenzylidene)benzohydrazide (KM91104) was purchased from Shanghai Bepharm Science&Technology Co. Ltd. All the other reagents and solvents were analytical grade and used directly without further purification. Sartorius ultrapure water (18.2 $M\Omega \cdot cm^{-1}$) was used for experiments.

Measurement and characterization.

Transmission electron microscopy (TEM, HT7700, Japan) was employed to characterize the morphologies of the nanomaterials. Powder X-ray diffraction (XRD) pattern was obtained on a Rigaku Smart Lab SE X-Ray Powder Diffractometer with Cu Kα line focused radiation (λ = 1.5405 Å). Absorption spectra were measured on the UV-1700 UV-visible spectrophotometer (Shimadzu, Japan). Fourier infrared spectrometer (Nicolet iS50 FT-IR) was used to characterize the infrared spectrum. The textural properties of the nanoparticles were obtained by N_2 adsorption-desorption isotherms at -196 °C using an ASAP 2020 automatic Micromeritics. The specific surface area was derived using Brunaner-Emmett-Teller (BET) method and the distribution of pore volume was calculated using Barrette-Joynere-Halenda (BJH) method. In the MTT assay, the absorbance was measured using a microplate reader (Synergy 2, Biotek, USA). Cell disruption was performed using a homogenizer (IKA, Germany, T10 basic ultra-turrrax). All pH measurements were performed with a digital pH-meter (pH-3e, LeiCi, China). Confocal fluorescence images were captured using TCS-SP8 confocal laser scanning microscope (Leica, Germany). In vivo fluorescence images were captured using live animal imaging system (IVIS Lumina III, US).

Synthesis of ZIF.

Imidazole-2-formaldehyde (ICA, 40.00 mg, 0.42 mmol) was dissolved into N, N-dimethyl formamide (DMF) (8 mL) under 50 °C and stirred. When the temperature of solution cooled to room temperature, $Zn(COOH)_2 \cdot 6H_2O$ (15.8 mg, 0.05 mmol) solution in DMF (0.25 mL) was dropwise added into ICA solution. The mixture was stirred for 10 min, then trioctylamine (TOA, 500 μL) was added very slowly. After stirring at room temperature for 24 h, ZIF were isolated by centrifugation at 12000 revolutions per minute (rpm) for 15 min. Finally, the obtained ZIF was completely washed with DMF and EtOH for three times.

Synthesis of BPTES/KM91104@ZIF (BKZ), KM91104@ZIF (KZ) and BPTES@ZIF (BZ).

ICA (40.00 mg, 0.42 mmol) was dissolved into N, N-dimethyl formamide (DMF) (9 mL) in 50 °C under stirring, then the solution was cooled into room temperature. BPTES (5mg) and KM91104 (5mg) were dissolved in the above solution. $Zn(COOH)_2 \cdot 6H_2O$ (15.8 mg, 0.05 mmol) solution in DMF (0.25 mL) was dropwise added into the ICA solution. The mixture was stirred for 10 min, then TOA (500 μL) was added very slowly. After stirring at room temperature for 24 h, the nanoparticles were isolated by centrifugation at 12000 revolutions per minute (rpm) for 15 min and completely washed with DMF and EtOH for three times to obtain BKZ. KZ and BKZ were synthesized in a similar method according to the above steps.

UV-vis absorbance curves of BPTES and KM91104.

BPTES and KM91104 with a concentration from 1 nM to 45 nM were prepared, and the UV-vis absorbance spectra of each solution were recorded.

Cell culture.

MCF-7 cells were incubated in cell culture dishes with a diameter of 10 cm containing DMEM supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5 % CO_2.

Prepared of BPTES/KM91104@ZIF@CM (BKZ@CM), KM91104@ZIF@CM (KZ@CM), BPTES@ZIF@CM (BZ@CM) and ZIF@CM (ZIF@CM).

First, MCF-7 cells were digested and centrifuged for future use. The cell pellet was washed with Tris-$MgCl_2$ buffer (pH = 7.4) for three times, and re-dispersed in tris buffer containing protease inhibitor. Next, the cells disruption experiment was carried out by homogenizer to obtain the cell membrane debris. The whole experiment was done on an ice-water bath. The cell membrane debris were finally received by differential centrifugation for several times. The cell membrane fragments were compounded with BKZ at a moderate speed stir for 24 h. Finally, the nanomaterials were wrapped with cytomembrane and then washed with H_2O for three times and resuspended in H_2O for the next experiments. The KZ@CM, BZ@CM and ZIF@CM were prepared with the same procedure.

MTT assay.

MCF-7 cells were dispersed in 96-well plate and incubated for 24 h. Then ZIF@CM, KZ@CM, BZ@CM or BKZ@CM with different concentrations (5, 15, 25, 50, 75 μg/mL) in DMEM culture medium were added into the well and incubated for 6 h. The control group was PBS group. Subsequently, fresh culture medium was added and future incubate for another 12 hours. Then the culture medium was removed from the 96-well, and 150 μL of MTT solution (0.5 mg/mL) was added. After incubated for 4 h, the MTT solution was removed, and 150 μL of DMSO was added. The absorbance at 490 nm was monitored by a microplate reader.

Live/dead cell staining assay.

MCF-7 cells were inoculated into confocal dishes and incubated for 24 hours. Then the cells were treated with PBS, ZIF@CM, KZ@CM, BZ@CM or BKZ@CM with a concentration of 75 μg/mL and incubated for 4 h. Furtherly, the fresh culture medium was added and continue incubated for 12 h. Subsequently, calcein-AM/PI probe was added to the confocal dishes and incubated for another 15 min. Finally, the cells were washed three times with PBS and imaged with CLSM (Ex: 488 nm, Em: 500-560 nm).

Combination index calculation

The combination index (CI) for the viability of cells equal to 50% were calculated from the equation:

$CI = (D)_1/(DX)_1+(D)_2/(DX)_2$

The effect of drug combinations could be synergistic (CI < 1), summation (CI = 1), or antagonistic (CI > 1). $(Dx)_1$ and $(Dx)_2$ are the doses of drug 1 and 2 that inhibit cell growth to 50%, and $(D)_1$ and $(D)_2$ are the respective doses of drug 1 and 2 in combination therapy to inhibit 50% cell growth.[1]

When the inhibition of cell growth reaches 50%, the dosage of KZ@CM is about 81.34 μg/mL (containing 17.19 μg/mL KM91104) and the dosage of BZ@CM is about 98.50 μg/mL (containing 25.81μg/mL BPTES) and for BKZ@CM group, the dosage of BKZ@CM is about 38.94 μg/mL (containing 8.23 μg/mL KM91104 and 10.20 μg/mL BPTES). According to the above equation, CI=8.23/17.19+10.20/25.81=0.87.

Evaluating the mitochondrial membrane potential.

MCF-7 cells were dispersed into confocal dishes and incubated for 24 h. Then the cells were treated with PBS, ZIF@CM, KZ@CM, BZ@CM or BKZ@CM with a concentration of 75 μg/mL and incubated for 4 h. After that, the fresh culture medium was added and continue incubated for 12 h. Subsequently, the cells were treated with Rhodamine 123 (1 μM) at 37°C for 15 min and washed for three times. Finally, the fluorescence intensity of each group was assessed with CLSM (Ex: 488 nm, Em: 500-560 nm).

Determination of intracellular pH value.

MCF-7 cells were inoculated into confocal dishes and incubated for 24 hours. Then the cells were treated with PBS, ZIF@CM, KZ@CM, BZ@CM or BKZ@CM with a concentration of 75 μg/mL and incubated for 4 h. After that, the culture medium was replaced with fresh medium and the confocal dishes continue incubated for 12 h. Subsequently, cells were stained with BCECF AM probe (5 μM) at 37°C for 15 min. Finally, all the cells were washed with PBS and analysed with CLSM (Ex: 488 nm, Em: 500-560 nm).

Flow cytometry analysis of apoptosis.

MCF-7 cells were cultured in cell dishes with DMEM for 24 h and randomly divided into five groups: PBS, ZIF@CM, KZ@CM, BZ@CM and BKZ@CM. Each group of cells incubated with the corresponding nanomaterials (75μg/mL) for 4 h. Subsequently, the fresh culture medium was added

and cell dishes were continue incubated for 12 h. Each group of cells were collected through the centrifugal. After that, the cells were treated with Annexin V-FITC/PI for 20 min and washed with PBS for three times. Finally, MCF-7 cells were resuspended in 50 μL PBS for flow analysis.

Establishment of tumor model.

All animal experiments were conducted and agreed with the Principles of Laboratory Animal Care (People's Republic of China) and the Guidelines of the Animal Investigation Committee, Biology Institute of Shandong Academy of Science, China. Female nude mice (6 weeks old, ~16 g) were raised under normal circumstances of 12 h light and dark cycles and given access to food and water optionally. 1×10^7 MCF-7 cells in 100 μL of serum-free RPMI DMEM medium were injected subcutaneously into the right axillary region of the nude mice. After the tumor volume had reached approximately 75-100 mm^3, the mice were used into subsequent therapy experiments.

In vivo fluorescence imaging.

The preparation of IR808@ZIF and IR808@ZIF@CM was as follows: 1 mg of ZIF NPs and 20 μL of IR808 (10 mg/mL) were mixed in 2 mL of aqueous solution and stirred for 12 h. Then the obtained IR808@ZIF was collected by centrifugation and washed three times for future use. Subsequently, the cytomembrane of MCF-7 cancer cells was coated on the surface of IR808@ZIF to obtain IR808@ZIF@CM. Equal amount (50 μL, 4 mg/mL) of IR808@ZIF or IR808@ZIF@CM were injected into each group of nude mice through the caudal vein. At different time points of 0, 12, 24, 36 h after injection, the fluorescence intensity of the mice tumor was recorded through a live body imaging system.

In Vivo Blood Circulation.

The tumor-bearing nude mice were random divided into five groups and intravenously injected normal saline, ZIF@CM, KZ@CM, BZ@CM and BKZ@CM. After 24h, the primary organs and tumor tissues were collected and digested with aqua regia for the ICP-MS measurements to quantify the zinc concentration.

Evaluation of antitumor effect in vivo.

The tumor-bearing nude mice were random divided into five groups, and injected intravenously with 10 mg/kg of PBS, ZIF@CM, KZ@CM, BZ@CM or BKZ@CM. The tumor volume and body weights of the nude mice were recorded every other day for 14 days after treatment (tumor volume = $W^2\times L/2$, W = width, L = length). After the different treatment of 14 days，the nude mouse were sacrificed, and five major organs (liver, lung, spleen, kidney, and heart) were harvested for H&E staining.

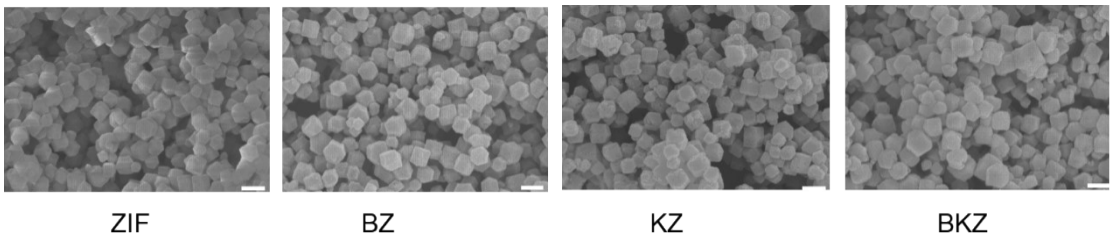

Fig.S1.SEM images of ZIF, BZ, KZ, BKZ and BKZ@CM. All scale bars are 100 μm.

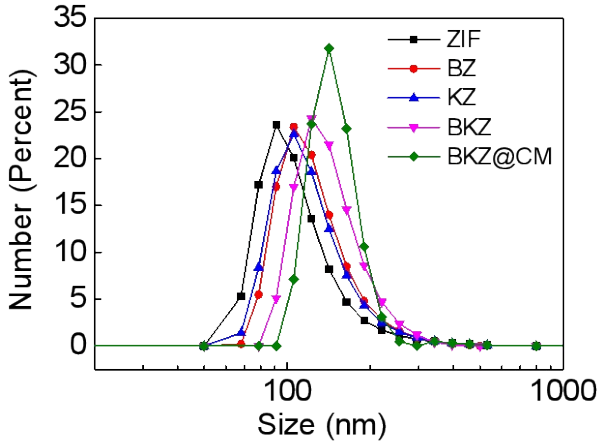

Fig.S2. DLS of ZIF, BZ, KZ, BKZ, and BKZ@CM.

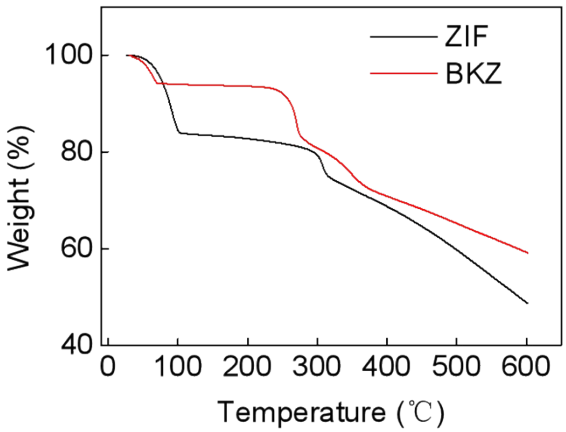

Fig.S3. TGA curves of ZIF and BKZ.

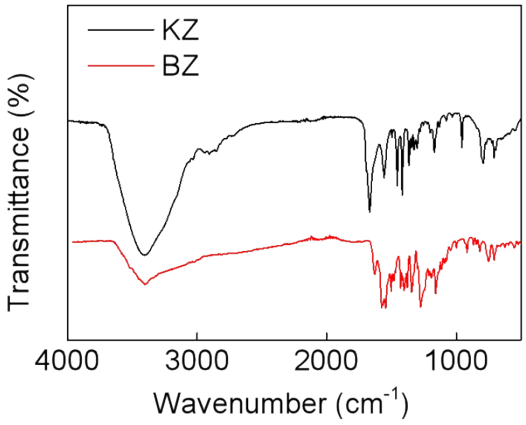

Fig.S4. FT-IR spectra of KZ and BZ.

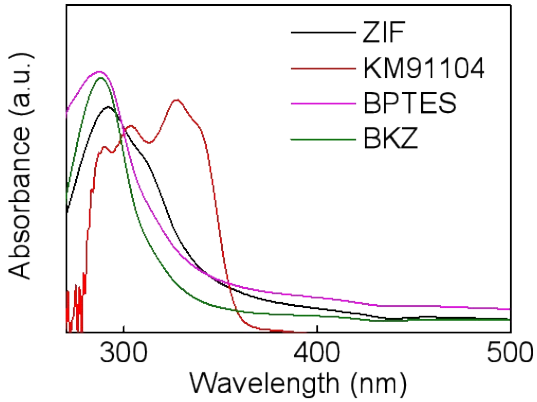

Fig. S5. UV-vis spectrum of ZIF, BPTES, KM91104, and BKZ.

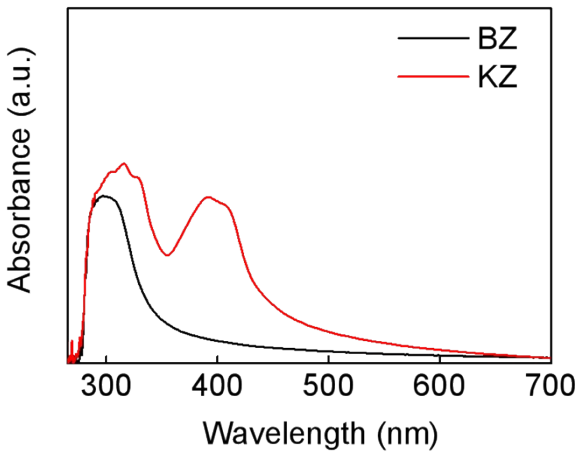

Fig. S6. UV-vis spectra of BZ and KZ.

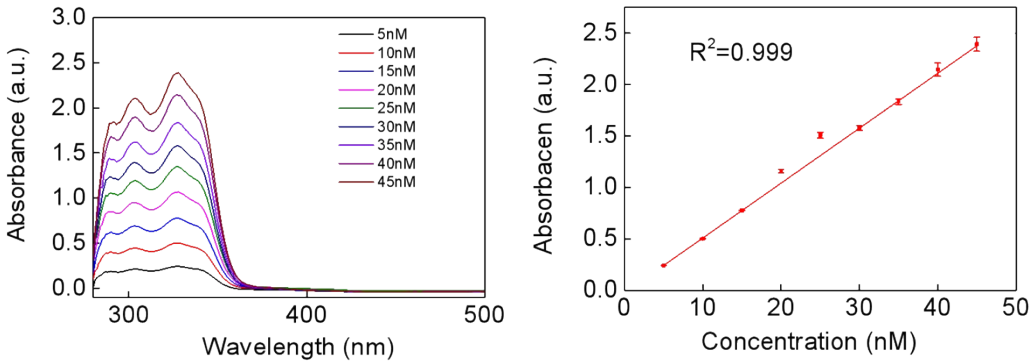

Fig. S7. UV-vis spectra and linear standard curve of KM91104 from 5-45 nM.

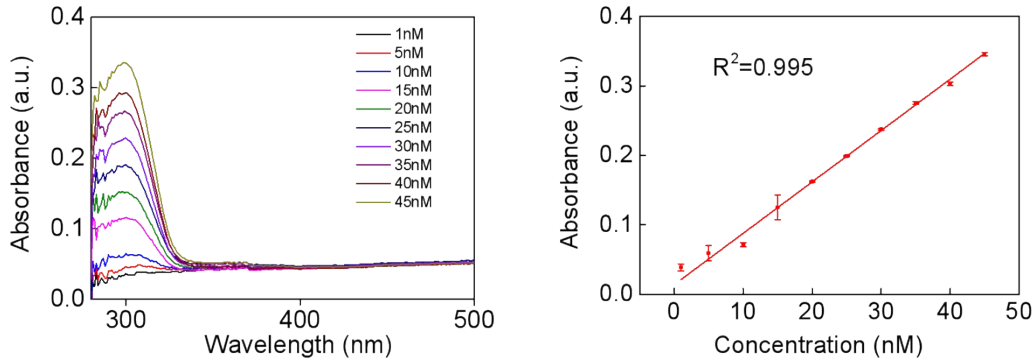

Fig. S8. UV-vis spectra and linear standard curve of BPTES from 1-45 nM.

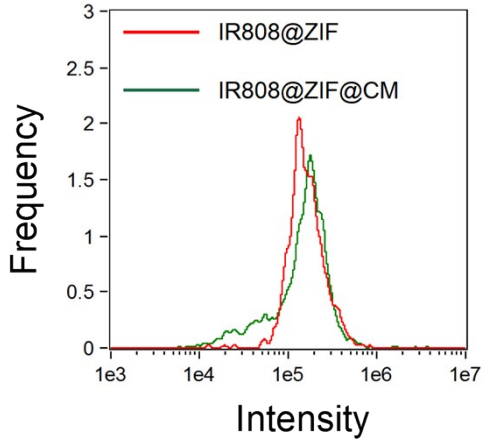

Fig.S9. Flow cytometry analysis of the cells treated with IR808@ZIF and IR808@ZIF@CM.

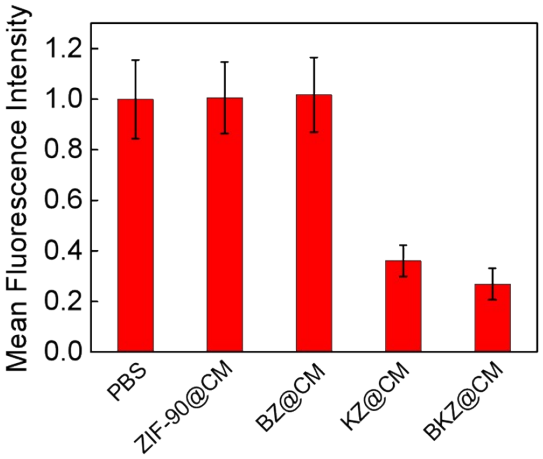

Fig.S10. The quantitative fluorescence intensity analysis of BCECF AM-stained MCF-7 cells with different treatments.

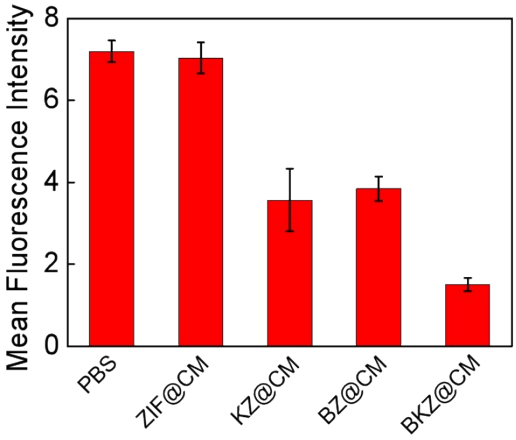

Fig.S11. The quantitative fluorescence intensity analysis of Rhodamine 123-stained MCF-7 cells with different treatment.

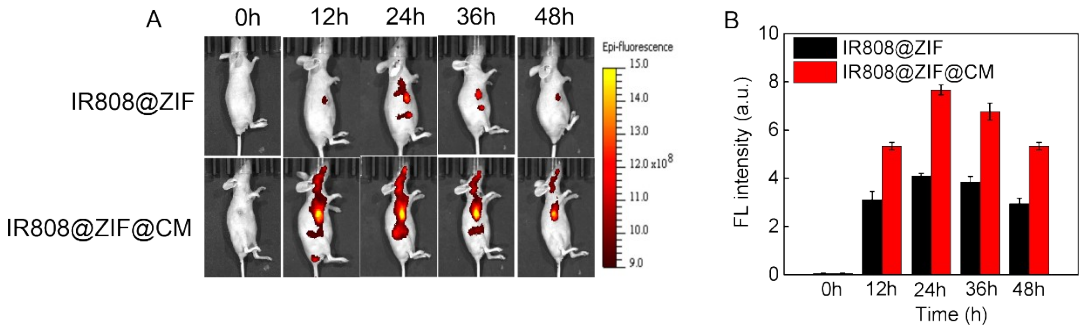

Fig.S12. (A) In vivo fluorescence imaging of MCF-7 tumor-bearing nude mice with intravenous injection of IR808@ZIF or IR808@ZIIF@CM after different time. (B) The quantization of fluorescence intensity of (A).

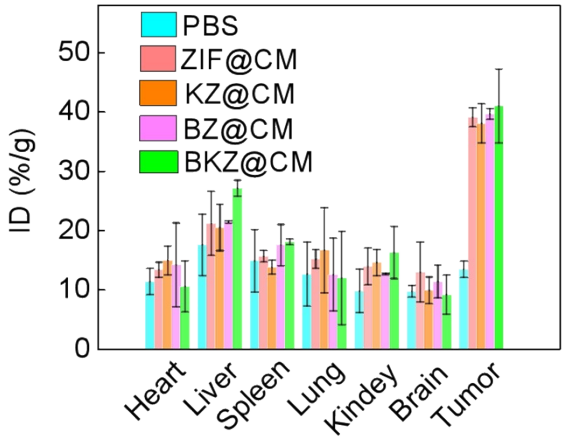

Fig.S13. Quantification of Zn^{2+} in different organs and tumor tissue.

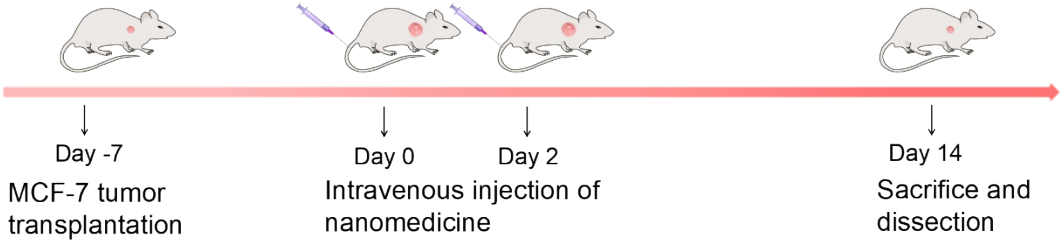

Fig.S14. Diagrammatic instruction of the therapeutic process.

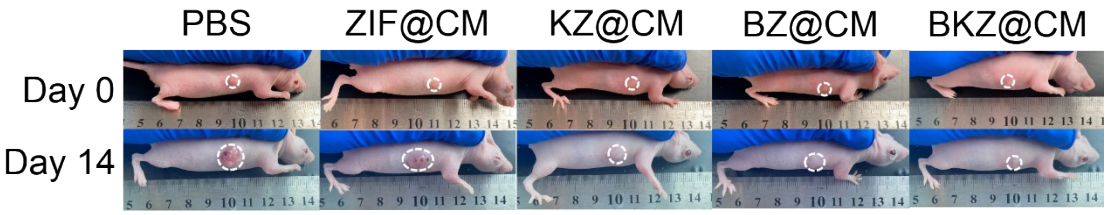

Fig.S15. Pictures of MCF-7 tumor bearing nude mice taken at day 0 and day 14 in different groups.

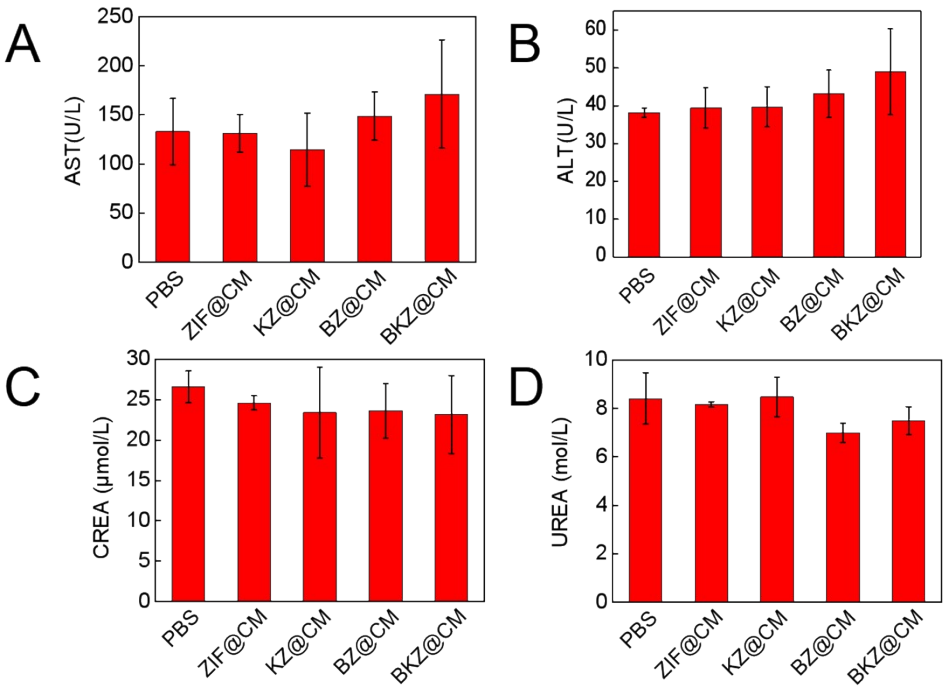

Fig.S16. Blood routine examination of the mice with different treatments. (A) aspartate aminotransferase (AST), (B) alanine aminotransferase (ALT), (C) creatinine and (D) UREA.

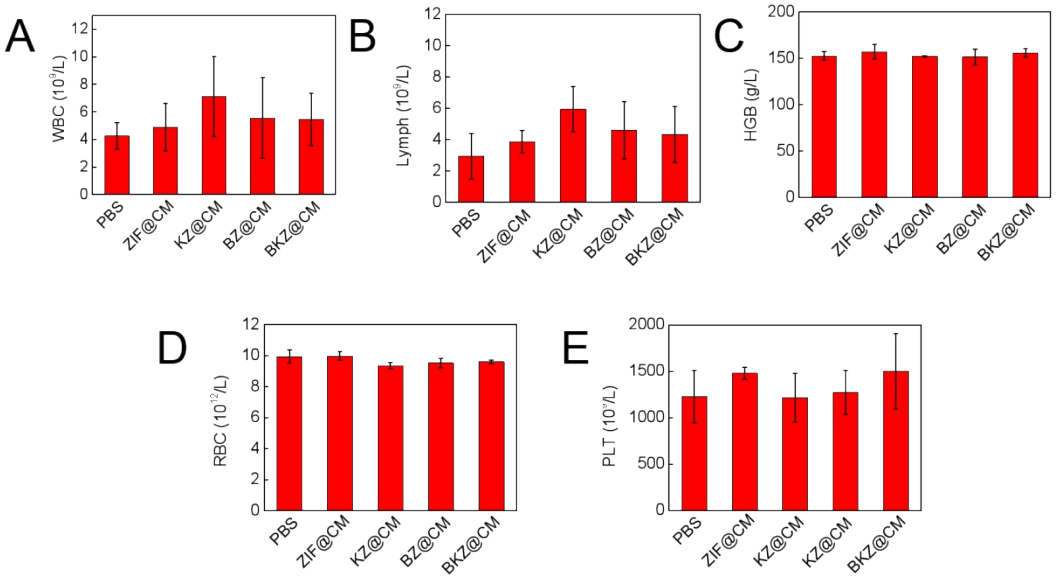

Fig. S17. Haematological data of the mice in different group. (A) white blood cells (WBC), (B) lymphocyte (Lymph), (C) hemoglobin, (D) red blood corpuscle, and (E) platelets (PLT).

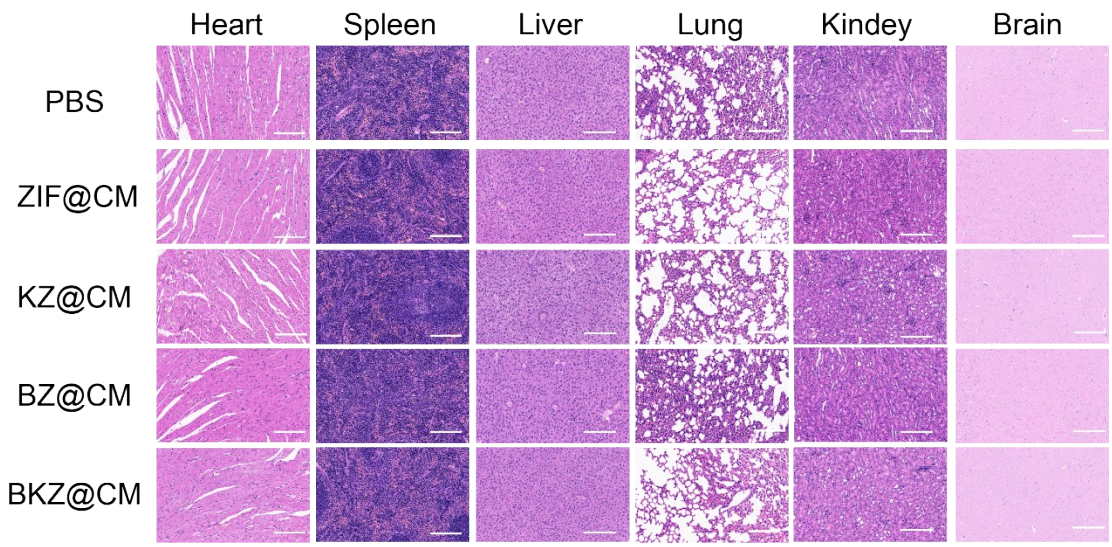

Fig.S18. H&E staining of major organs (heart, liver, spleen, lung and kidney) with different treatments. Scale bars are 200 μm.

Table S1. Textural data (from N_2 adsorption) of ZIF and BKZ.

	Specific surface area (m^2/g)	Pore volume (cm^3/g)
ZIF	903.06	0.34
BKZ	190.44	0.10

Notes and references

[1] T. C. Chou, P. Talalay, *Advances in Enzyme Regulation,* 1984, **22**, 27-55.