Electronic supplementary information

Treatment of hyperphosphatemia based on specific interaction between phosphorus and active center Zr(IV) of nano-MOFs

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Experimental Section：

Materials:$ZrCl_4$ was purchased from Bellingwell (Beijing, China) and 2-aminoterephthalic acid was purchased from Shanghai Siyu (Shanghai, China). Adenine, chitosan, cesium carbonate, potassium antimony tartrate, ascorbic acid, ammonium heptamolybdate and adenosine triphosphate (ATP) were purchased from Macklin (Shanghai, China).BSAprotein,pyrrole, 4-formylbenzoic acid, L-cysteine Acid,glutathione and MTT were purchased from Sigma-Aldrich (Shanghai, China). Other related inorganic compounds:$MgSO_4$, $CaCO_3$, $Al(OH)_3$, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, Na_2SO_4, Na_2CO_3, $NaHCO_3$, $NaHSO_3$, NaCl, etc. were purchased from Sinopharm (Shanghai, China). Analytical grades of all other chemical reagents were used directly without further purification. Ultrapure water (18.2 MΩ·cm) was obtained from a Water Pro water purification system (Labconco Corp., Kansas City, MO).

Instruments:The FT-IRspectra were determined on a Nicolet Magna 750 FT-IR spectrometer. Transmission electron micrographs and scanning electron micrographs were obtained using a HITACHI HT7700 electron microscope and a SUPRA 55 electron microscope, respectively. Powder X-ray diffraction patterns were measured at room temperature (298K) on aD8 ADVANCE X-ray powder diffractometer. UV-visible absorption was measured on a TU-1900 dual beam UV-Vis spectrophotometer. Dynamic light scattering (DLS) and Zeta potentials of the MOF material were obtained on a Malvern instruments Nano-ZS90. The fluorescence spectra were obtained with a spectrofluorometer with a xenon lamp (FLS-920, Edingburgh). In the MTT assay, the absorbance was measured in a microplate reader (Synergy 2, Biotek, USA), and

thesamples were measured for phosphorus content using a Thermo Fisher ICP 7000 emission spectrometer. The fluorescence imaging of mouse living and kidney organs was obtained using a Caliper IVIS Lumina Series III small animal *in vivo* imager. The fourparameters of kidney function in mouse blood were determined by an automatic biochemical analyzer (Qilu Medical Laboratory, Jinan, China). All pH measurements were performed using a pH-3c digital pH meter (Shanghai Leici, China) and a glass calomel electrode combination.

Synthesis of H_2TCPP:5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinwassynthesized according to previousliterature.[S1] First, 4-formylbenzoicacid(3.0 g, 0.02 mol) was dissolvedinto themixture of propionic acid (200 mL) and pyrrole (1.39 mL, 0.02 mol). Next, the mixture washeated to reflux for 1 h followed by cooling with the formation of brown-purpleprecipitate. The precipitate was isolated by filtration, washed with CH_2Cl_2 for 3 times, and then dried under vacuum for 6 h.

Synthesis of the MOFs: Zirconium tetrachloride (0.164 g, 0.7 mmol) and 2-aminoterephthalic acid (0.127 g, 0.7 mmol) were dissolved in a mixture of 20 mL of DMF, 3 mL of acetic acid, and 0.2 mL of H_2O at room temperature. After 5 min sonication to allow the solid fully dissolve, 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (36 mg) was added and the solution, which changed the solution color from yellow to green. With another 5 min sonication, the solution was transferred to a 20 mL Teflon-lined stainless steel autoclave and incubated at 120 °C in oven for 24 hours. After cooling to room temperature, the solution was centrifuged at 8,000 rpm for 15 min to obtain the red-brown

precipitate.Afterwards, the product was washed three times with DMF and then well dispersed in 50 mL of DMF for 12 hours. The solid product that not dissolved in DMF was centrifuged and collected to thoroughly disperse in 50 mL of ethanol for 24 hours. The final product was washed with ethanol for 3 times and dried in a vacuum oven for 12 hours.

Synthesis of the BSA-MOFs:50 mg of the MOFs material was sufficiently dissolved in 100 ml H_2O, followed by addition of 250 μL of glutaraldehyde (25%, 1:4). The reaction was stirred at 30 ℃ for 1 h and centrifuged at 10,000 rpm for 5 min. The product was re-dissolved completely in 100 mLH_2O and added with 10 mg of BSA for 2 h at 30 ℃. The product was collected by centrifugation at 10,000 rpm for 5 min and freeze-dried at low temperature.

Cytotoxicity Test: HL-7702 cells harvested at log phase were seeded at a density of 5 × 10^4 cells/well in 96-well microtiter plates and incubated in DMEM at 37 ℃, 5% CO_2, 95% air for 24 h (The cell counter recorded 100 μL of cells).The cells were incubated with different concentrations of MOFs (10, 50, 100, 200, 300 and 400 mg/L) for another 24 hours. With additionof 20 μL MTT solution (0.5 mg/mL) to each well and 4 h incubation, the remaining MTT solution was removed and 150 μL of DMSO was added to each well to dissolve the formazan crystals in the dark with slightly shaken. Finally, the plate was shaken for 10 min to dissolve the formazan crystals. Absorbance was measured at 490 nm with a microplate reader.

Explore the Effect of Reducing Phosphorus in the MOFs.1 mg of the MOFs was added into 10 mL phosphate solution (100 μmol/L) and the sample was incubated on

aincubator at 30 ℃ for 4 h. Centrifuged at 10,000 rpm for 5 min, the supernatant solution was obtained. Phosphomolybdenum blue spectrophotometric methodwas used to determine the phosphate content of pre-adsorption sample 1 and post-adsorption sample 2.[S2] The formula for the effect of reducing phosphorus: adsorption capacity (μg/mg) = $(C_2 - C_1) \times V \times M_0 / M$,$C_1$ and C_2 are the concentration of PO_4^{3-} before and after adsorption of the MOFs, respectively; V is the sample's volume; M_0 is the molar mass of PO_4^{3-}; and M is the mass of the MOFs material.

Study on Effect of Rat Serum Phosphorus Reduction *in vitro*: 4 mL of wistar rat heart blood was allowed to stand at room temperature for 10 min and centrifuged at 3,500 rpm for 10 min. Upper serum is diluted 10 times. The control group, the group 1 & 2 each took 2 mL of the above serum dilution solution, then 1 mg of the MOFs was added to the group 1, and 1 mg of MOFs material modified with the BSA protein was added to the group 2. The samples were incubated for 4 h in aincubator at 30°C and centrifuged for 5 min at 10,000 rpm. 1 ml of upper serum is diluted 10 times. The three groups of samples'phosphorus content were measured by ICP 7000 emission spectrometer and phosphorus molybdenum blue spectrophotometry.

Mice Model for Chronic Kidney Disease:[S3]Adenine solution with a concentration of 10 mg/mL was prepared with H_2O at 80 ˚C. Thirty Kunming mice were divided into control groups and model groups with 15 per group. The model group was established by adenine gavage induction method to establish a chronic renal failure mouse model and the intragastric administration dose was 0.4 mL (The average dose per gavage is approximately 100-150 mg/kg). There were 12 does for each mousewith every other

day and a total model time for 24 days. Both the model group and the control group had normal diet and water. The mice were obtained from Shandong University Laboratory Animal Center. The experiments were approved by the institutional committee. All the animal experiments were carried out in accordance with the relevant laws and guidelines issued by the Ethical Committee of Shandong University.

Treatment of Mouse Chronic Renal Failure Model Group:The mice that established the chronic renal failure model and normal mice were divided into control groups, model groupsand treatment group with 6 per group.The mice in the treatment group were intragastrically administered 0.4 mL of the BSA-modified MOF per day (concentration of 1 mg/mL). The treatment period was 10 days and normal diet and water. The control groups and model groupswere served with normal diet and water.

Evaluation of Blood Phosphate Levels Treated by MOFs:Each mouse was bled with 0.2 mL of blood and kept for 10 min followed by centrifugation at 3,500 rpm for 10 min, and 20 μL of upper serum was sampled. Serum samplesin the normal group, the model group, and the treated group were collected with 40 μL per group (Each group of serum samples provided by two mice). Three groups of serum samples were collected three times in parallel. By diluting 25 times with H_2O, 0.25 mL of the serum diluentwas again diluted 40 times and the phosphorus levels were determined by ICP 7000 emission spectrometer. Finally, 1 mL of serum diluent was characterized by the blood institution for the four indicators of renal function.

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86,11459-11463.

(S2) P. Worsfold, I. McKelvie and P. Monbet, *Anal.Chim.Acta*, 2016, **918**, 8-20.

(S3) B. H. Ali, I. Inuwa, M. Al Za'abi, S. Al Bahlani, H. Al Issaei, A. Ramkumar and R. Zatz, *Cell. Physiol.Biochem.,* 2014,**34**, 818-828.

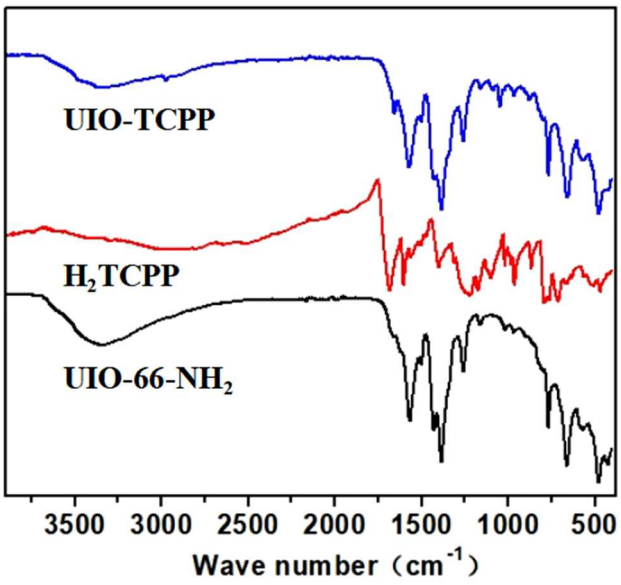

Fig.S1 FTIR spectra of the UIO-TCPP, H_2TCPP and UIO-66-NH_2.

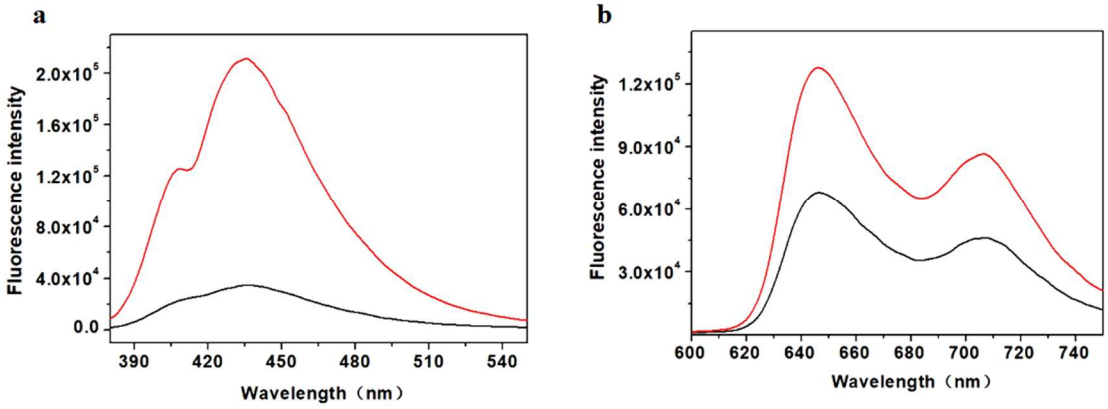

Fig. S2 Fluorescence response of MOF solution incubated with 100 μM phosphate ion for 2 h with different excitation wavelengths. (a) NH_2-H_2BDC; (b) H_2TCPP; Experimental details: C_{MOFs}= 25 μg/mL, λ_{ex} = 330 nm, λ_{ex} = 420 nm.

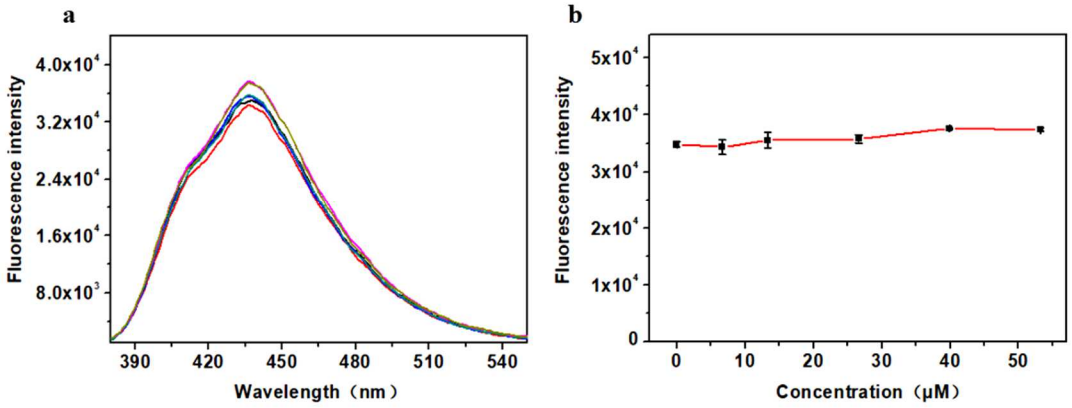

Fig. S3 Fluorescence emission spectra of MOFs solution incubated with Zr^{4+} (20μM) and phosphate (0-50μM). Experimental details: C_{MOFs}= 25 μg/mL, λ_{ex} = 330 nm.

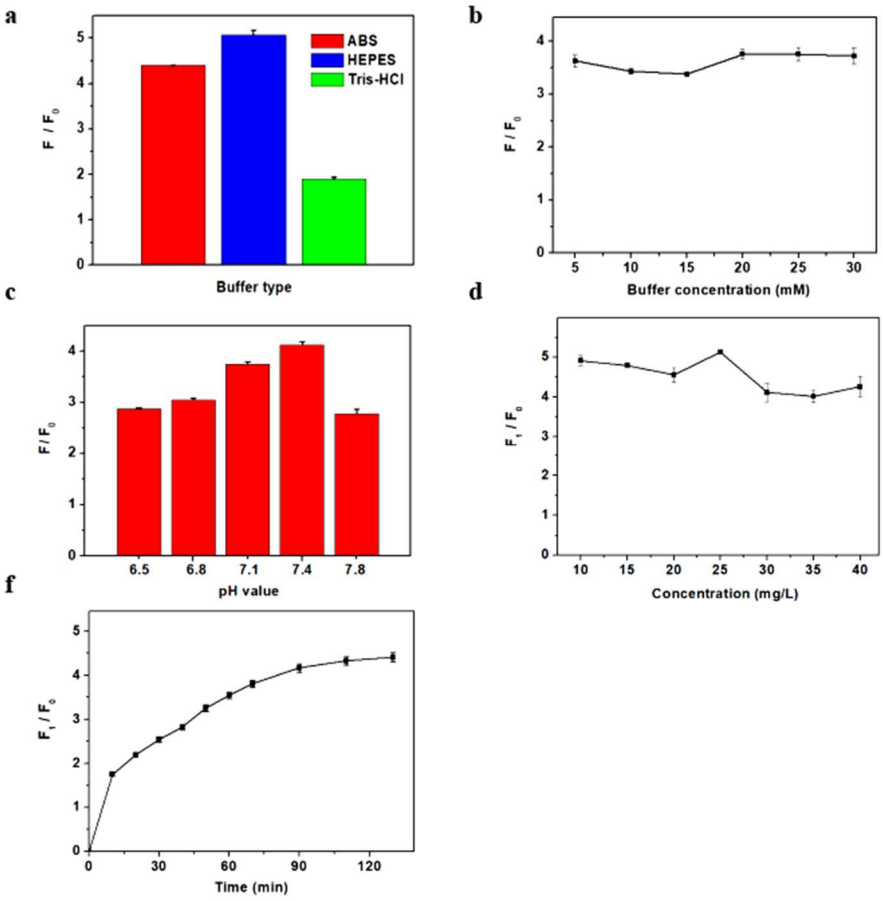

Fig.S4 Conditional optimization experiment: (a) Optimized type of buffer; (b) Optimized concentration of HEPES buffer; (c) Optimize pH of the HEPES buffer; (d) Optimization of MOFs concentration and (e) Optimization of the reaction time.

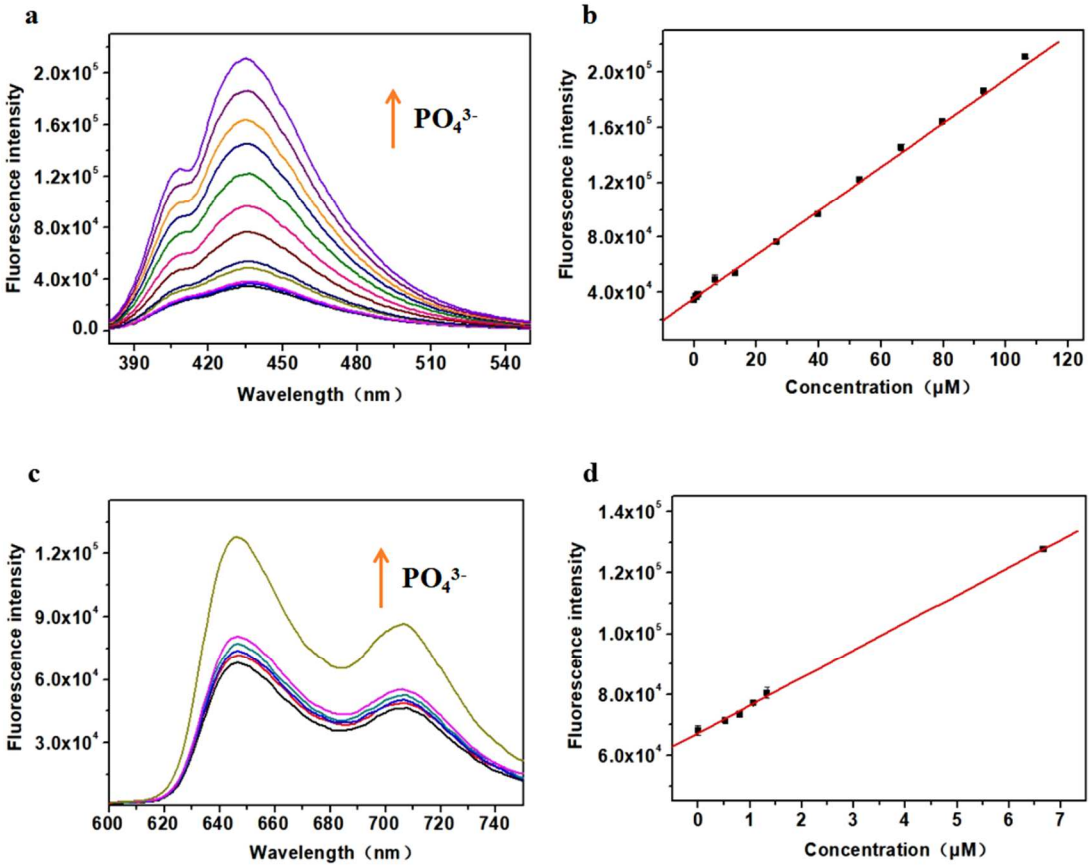

Fig.S5 Fluorescence emission spectra of the MOFs (25μg/mL) uponthe addition of a solution of the phosphate at the indicated concentration; (a) Fluorescence emission spectra of phosphate from 440 nm;(b) linear correlation in the range of 0.5-105μM phosphate; (c) Fluorescence emission spectra of phosphate from 650 nm; (d) linear correlation in the range of 0.5-7μM phosphate; Experimental details: 20 mMHEPES buffer, pH 7.40,λ_{ex} = 420 nm.

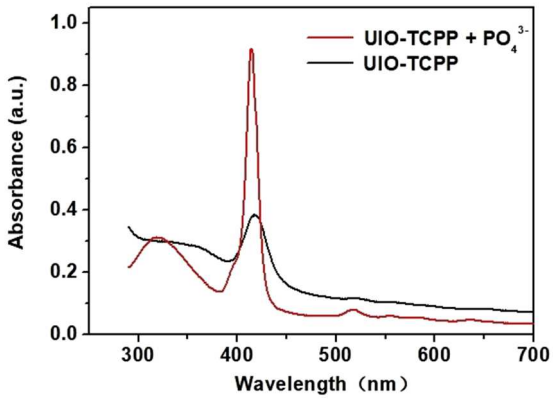

Fig.S6 UV-Vis absorption spectra of UIO-TCPP and UIO-TCPP+PO_4^{3-}. Experimental details: 20 mMHEPES buffer, pH 7.40;C_{MOFs}= 60 μg/mL.PO_4^{3-} (250μM).

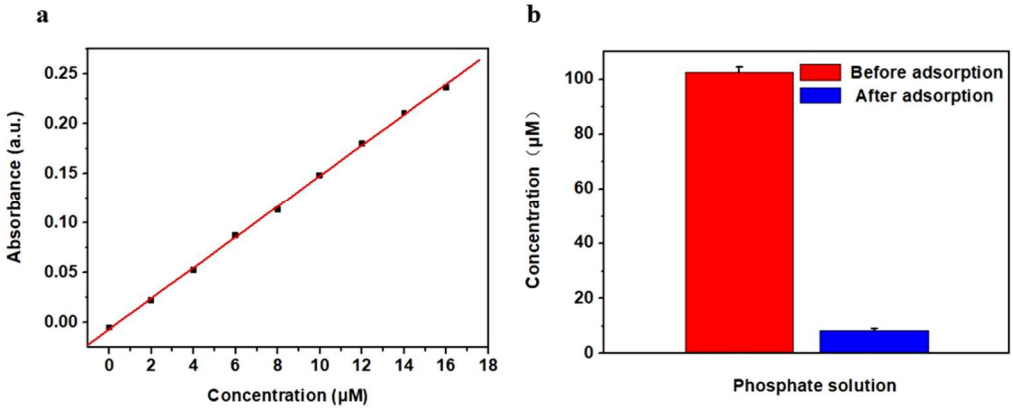

Fig.S7 (a) Linear relationship between absorbance and phosphateconcentration;(b) Adsorptioncapacity of the MOFs for the phosphate.Experimental details:C_{MOFs}= 0.1mg/mL; PO_4^{3-} (100μM), 10mL.

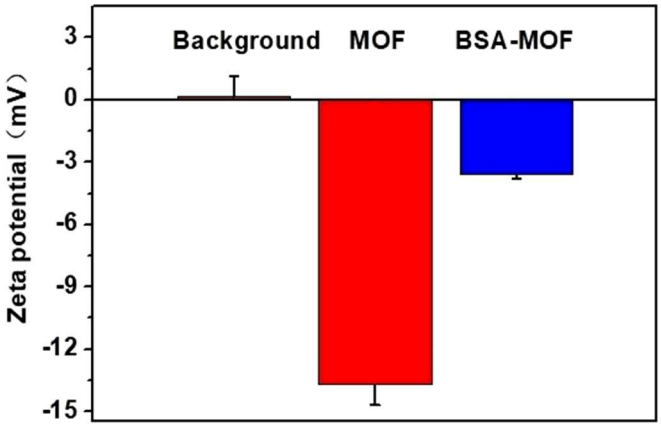

Fig.S8 Zeta potentials of the as-prepared MOFs.Red bar refers to the MOF and blue bar refers to the BSA-MOF.

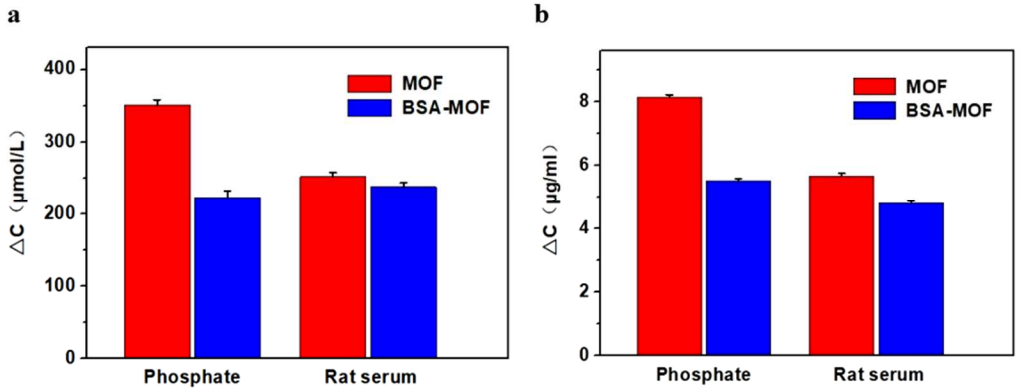

Fig.S9 Adsorptioncapacity of the MOF and BSA-MOF for the phosphate measured by (a) phosphomolybdic blue spectrophotometric method and (b)ICP emission spectrometer. Experimental details:Phosphate concentration(1mM), Rat serum (10-fold dilution), C_{MOF}= 0.5 mg/mL, $C_{BSA\text{-}MOF}$ = 0.5 mg/mL.

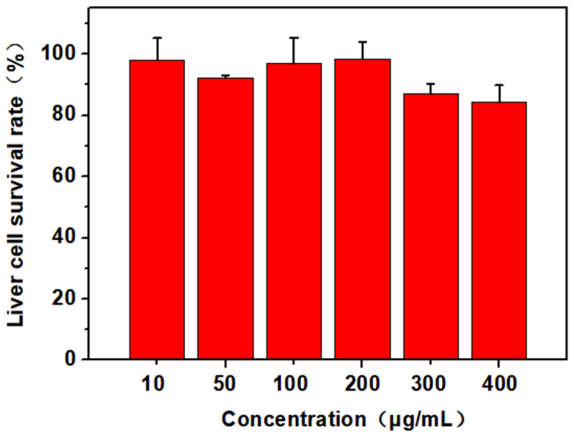

Fig.S10 Cell viability of HL-7702 cells treatment with MOFs.Experimental details: the cells were incubated with different concentrations of MOFs (10, 50, 100, 200, 300 and 400 mg/L) respectively for 24 hours.

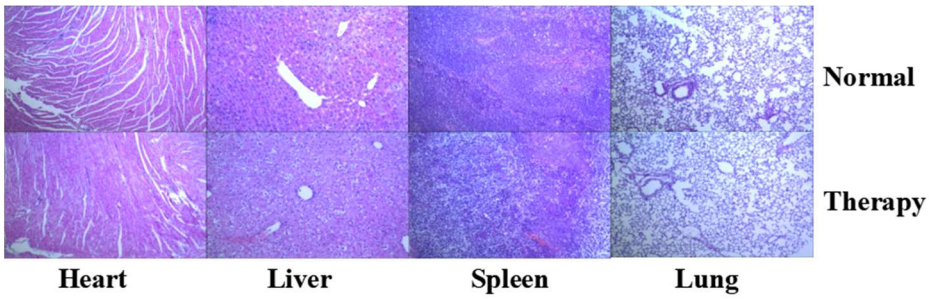

Fig.S11 Experiments of tissue slides (Heart, Liver, Spleen, Lung, respectively). Upper: the normal group with normal diet and drinking water. Under: renal failure mice were treated with MOFs by gavage.