Supplementary Information

Combinatorial discovery of Mo-based polyoxometalate clusters for tumor photothermal therapy and normal cell protection

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Materials: $H_3O_{40}PMo_{12}\cdot xH_2O$ (analytical reagent), sodium carbonate anhydrous (Na_2CO_3, > 99.5%), gallic acid (GA, > 99.0%) were purchased from Aladdin. Cell culture medium (DMEM), roswell park memorial institute (RPMI-1640), phosphate buffered saline (PBS), fetal bovine serum (FBS), serum and antibiotic were purchased from Hyclone. Calcein, propidium Iodide (PI), 2,7-dichlorodi hydrofluorescein diacetate (DCFH-DA), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich. All reagents were of analytical grade and used without any purification.

***Instrumentation*:** The morphologies and hydrodynamic diameter distribution of Mo-POM were acquired by a transmission electron microscope (JEM-1400flash, 120 kV, Japan) and NanoBrook-90 Plus instrument (Brookhaven Instrument Corporation, USA), respectively. X-ray photoelectron spectroscopy (XPS, ESCALab 250Xi, Thermo Scientific) and Fourier transform infrared (FTIR) spectroscopy (Nicolet, Thermo Nicolet) were also used to characterize the element valence distribution and composition. The optical property of Mo-POM was confirmed by a spectrophotometer (U-5100, Hitachi). Moreover, the accurate Mo content in obtained Mo-POM was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Atomscan Advantage). Photothermography was recorded on an IR thermal camera (Testo 865, Testo, German).

***Synthesis of Mo-based Polyoxometalate Nanoclusters (Mo-POM)*:** Mo-POM were synthesized according to a modified Folin-Ciocalteu assay. Specially, 100 mg gallic acid were firstly dissolved in deionized water (DI water, 6.0 mL). After a complete dissolution, 70 mg/mL of $H_3O_{40}PMo_{12}\cdot xH_2O$ (1.0 mL) was then added into the gallic acid aqueous solution and stirred intensely for 5 min. Following the injection of 3.0 mL of Na_2CO_3 solution (7.5 wt%), the whole system was allowed to stand for 5.0 h with continuous stirring. After that, the dark green solution was packaged in a dialysis bag (Mw = 3500 Da) for three days to remove any unreacted raw reagent. The purified Mo-POM aqueous solution was then undergone typical lyophilization process and stored at 4 ℃ for further use.

***Photothermal Properties of Mo-POM*:** At various concentrations (0, 50, 100, 200, 300, 400 μg/mL) of Mo-POM, it was irradiated with an 808 nm laser for 5 min (0.5, 1.0, 2.0 W/cm^2). The FLIRTM E50 camera was used to monitor temperature changes. In addition, five NIR laser on/off cycles were further carried out to evaluate the photothermal stability of Mo-POM.

***Cell Culture and Cytotoxicity Assessment*:** Rabbit blood (New Zealand big ear rabbit) were purchased from the Second Affiliated Hospital of Harbin Medical University (Harbin, China). Mice breast cancer 4T1 cell line was purchased from American Type Culture Collection and was cultured in DMEM (Hyclone) with 10% FBS and 1% penicillin–streptomycin. All cells were treated by trypsin (0.05%)/EDTA. Typical MTT and hemolytic assays as well as endogenous ROS generation were systemically evaluated. Mice breast cancer 4T1 cells were cultured in DMEM at 37 ℃ and 5% CO_2 supplemented with 10% fetal calf serum and 1% antibiotic. The cells were seeded into 96-well plates and different concentrations of the drug were added to the cells in the 96-well plate for 24 hours, then stained with MTT solution and finally tested with a microplate reader.

***ROS Scavenging Activities*:** Human umbilical vein endothelial cells (HUVEC) was obtained from American Type Culture Collection and was cultured in RPMI-1640 medium (Hyclone) with 10% fetal bovine serum and 1% penicillin–streptomycin. All cells were treated by trypsin (0.05%)/EDTA. Three typical ROS (H_2O_2, $O_2^{\bullet-}$, $^{\bullet}OH$) were employed here to evaluate the ROS-scavenging activities of as-prepared Mo-POM. For H_2O_2 scavenging evaluation, 300 μg/mL of Mo-POM were incubated with gradient concentrations of H_2O_2 and the related UV-vis-NIR spectra of

mixture were recorded. The superoxide anion ($O_2^{\cdot-}$) and free radical scavenging activities were then assessed with a standard SOD (superoxide dismutase) assay kit and ABTS (2,2′-azinobis (3-ethylbenzothiazoline 6-sul-fonate)) radical cation decolorization assay, respectively. In addition, H_2O_2 (0, 0.3, 3.0 mM) and LPS (0, 50, 100, 150, 200 ng/mL) were further employed to establish the intracellular ROS threats in HUVECs. After incubating with gradient concentrations of Mo-POM, typical cell staining experiments including ROS staining with DCFH-DA and Live/Dead cell staining as well as MTT assay were then carried out for evaluation the ROS-scavenging activities of Mo-POM at cellular levels.

***In Vitro Photothermal Ablation of 4T1 Cells*:** For calcein and PI staining, PBS containing Mo-POM was added to cells in a 6-well plate and an adherent cell solution culture containing different concentrations of Mo-POM was exposed to an 808 nm laser having a power density of 2.0 W/cm^2. Next 3 or 5 minutes, only the Mo- POM and no NIR irradiation group was used as a control. The cells were then stained with a calcein/PI mixed solution for 15 minutes and the cells were washed three times with PBS. The morphology of adherent cells was observed with an inverted optical microscope. The cells stained red are counted as dead cells, and the cells stained green are counted as living cells.

***In Vivo Photothermal Therapy*:** Femal Balb/c mice (5–6 weeks old, 14–16 g of body weight) were purchased from the Second Affiliated Hospital of Harbin Medical University (Harbin, China). All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Harbin Medical University and approved by the Animal Ethics Committee of Harbin Medical University. All in vivo studies were performed in accordance with the ‘‘Rules for experiments animals’’ published by the Chinese Government (Beijing, China). Femal Balb/c mice (~ 20 g) were intravenously injected with 40 μg /mL of Mo-POM and then sacrificed at predetermined intervals (n = 5). Blood, urine, major organs (femal Balb/c mice) were purchased from the Second Affiliated Hospital of Harbin Medical University (Harbin, China). Collected and weighed blood, urine and major organs were digested with nitric acid to get limpid solutions. The accurate contents of molybdenum were determined by ICP-AES and then the corresponding distributions of Mo-POM were calculated as percentages of injected dose per gram of tissue (%ID/g).

For *in vivo* photothermal therapy, BALB/c mice with 4T1 were randomized into 4 treatment groups: the first group received PBS (200 μL); The second group was exposed to PBS and exposed to a power density of 1.5 W /cm^2; The third group was intravenously injected with Mo-POM (4 mg/mL) without NIR laser irradiation; The fourth group of intravenous Mo-POM (4 mg/mL) was exposed to an 808 nm laser with an output power density of 1.5 W/cm^2. The volume of the tumor after treatment was recorded every other day using a caliper for two weeks. Body weight and tumor appearance were monitored relative to tumor volume (V/V0, where V0 represents the initial tumor volume, day 0).

***Histological Assessment*:** The *in vivo* biocompatibility of Mo-POM was assessed using standard H&E staining. The above 4 groups of BALB/c mice were euthanized. Tissues were subjected to H&E staining to monitor histological changes in mice heart, liver, spleen, lung and kidney. Tissue sections were observed under an inverted optical microscope.

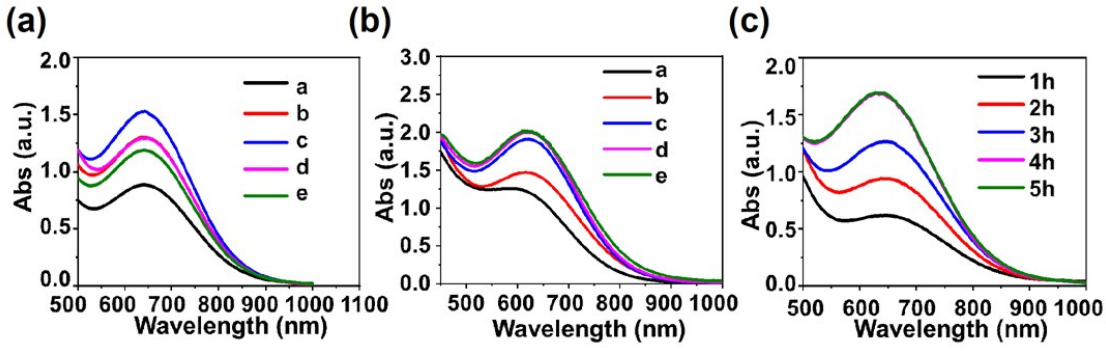

Figure S1. The optimization for the synthesis of Mo-POM. (a) UV-vis spectra of different concentrations (line a-e) of gallic acid (GA), in the solution containing Na_2CO_3 (75 mg/mL). a: 80 mg GA, b: 90 mg GA, c: 100 mg GA, d: 120 mg GA, e: 150 mg GA. (b) UV-vis spectra of different concentrations of phosphomolybdic acid (PMA), in the solution containing Na_2CO_3 (75 mg/mL). a: 30 mg PMA, b: 40 mg PMA, c: 50 mg PMA, d: 60 mg PMA, e: 70 mg PMA. (c) UV-vis spectra of synthetic Mo-POM at different reaction time. Its absorbance gets maximum after 5 h reaction.

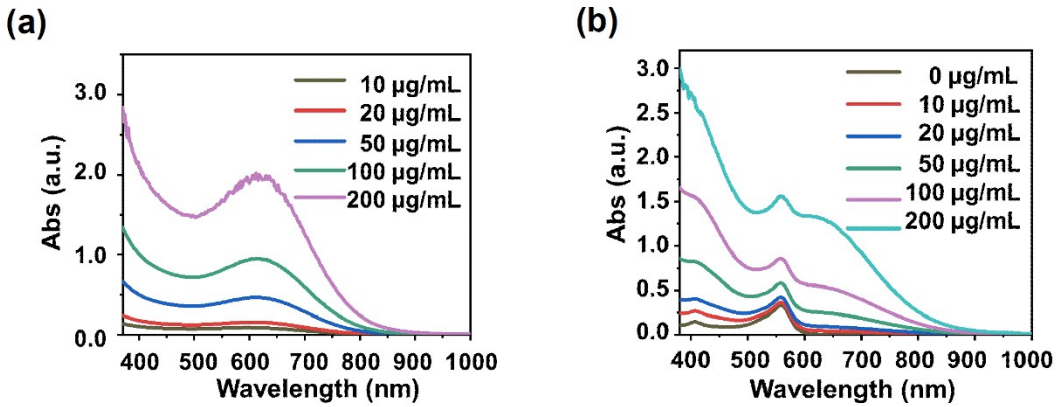

Figure S2. The UV-vis-NIR spectra of Mo-POM solution at different concentrations within deionized water solution (a) and cell culture medium (b) respectively, showing the concentration dependent behavior.

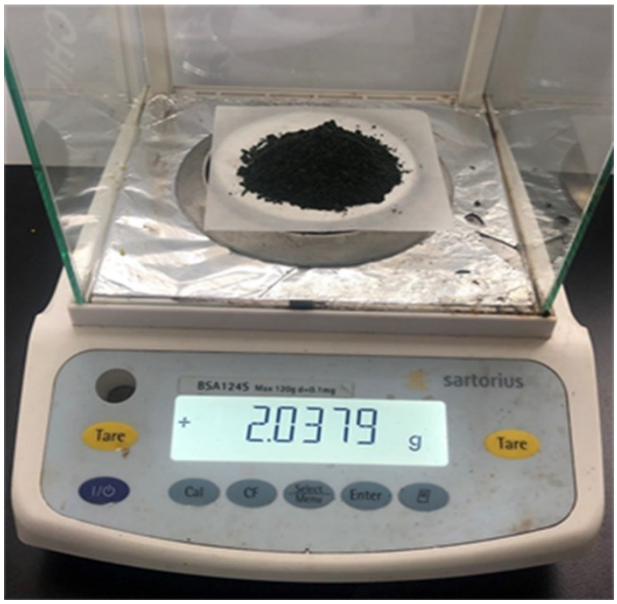

Figure S3. The digital photo showing the as-synthesized Mo-POM with the weight over 2g, which can be further scaled up.

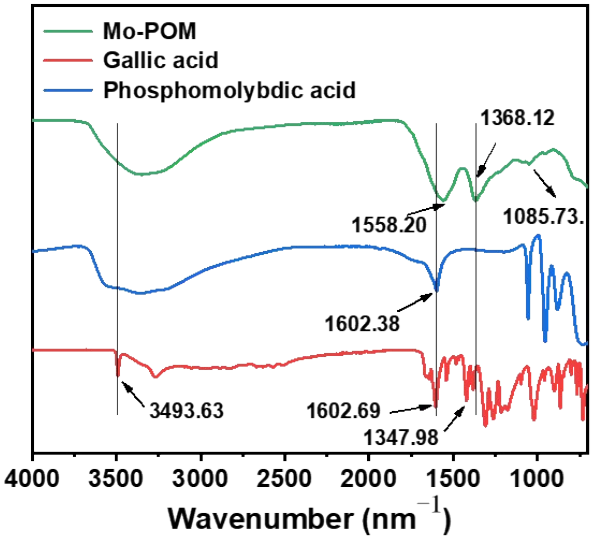

Figure S4. FTIR spectra of Mo-POM (green plot), pure gallic acid (red plot) and pure phosphomolybdic acid (blue plot).

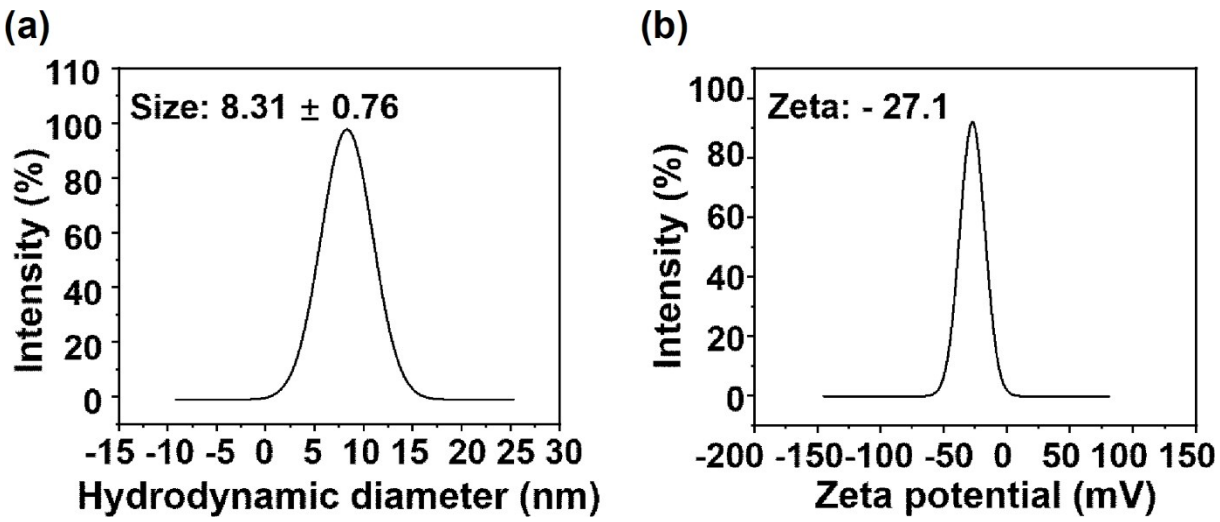

Figure S5. (a) DLS and (b) Zeta potential measurement of the Mo-POM aqueous solution (pH = 7.41). The concentration of Mo-POM solution is 200 μg/mL.

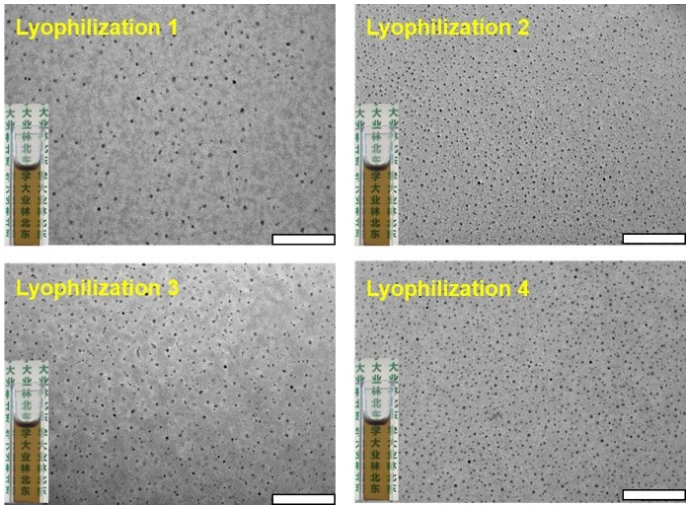

Figure S6. TEM images of Mo-POM after four consecutive freeze-drying treatment, with the scale bar of 50 nm. The sample concentration is 200 μg/mL.

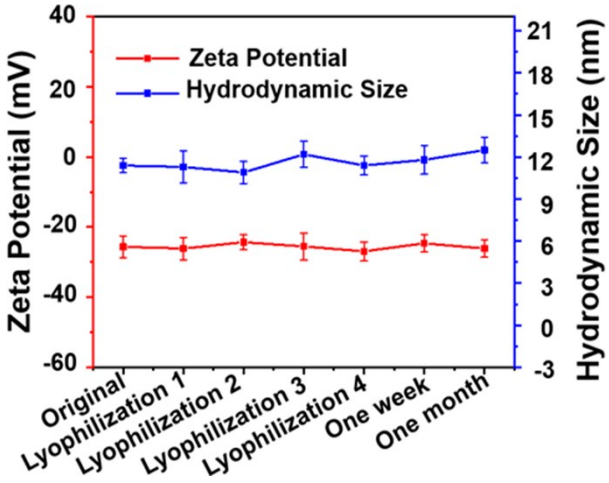

Figure S7. Hydration size and zeta potential (pH = 7.41) of Mo-POM after four consecutive freeze-drying, and stored for one week and one month, respectively. The sample concentration is 200 μg/mL.

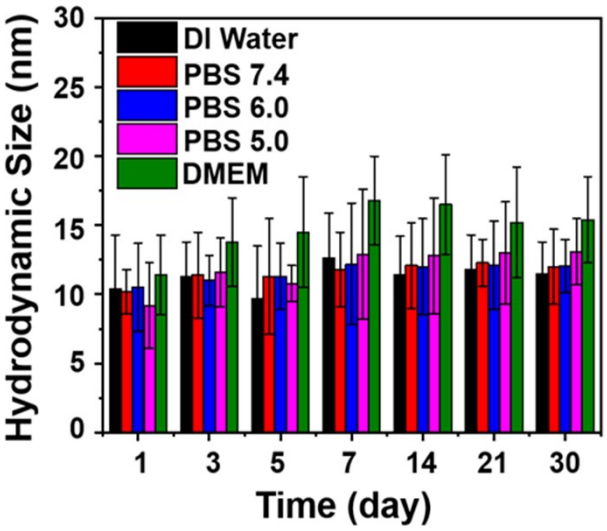

Figure S8. The plot showing the hydrodynamic size of Mo-POM dissolved in various solvents for 1, 3, 5, 7, 14, 21 and 30 days. The sample concentration is 200 μg/mL.

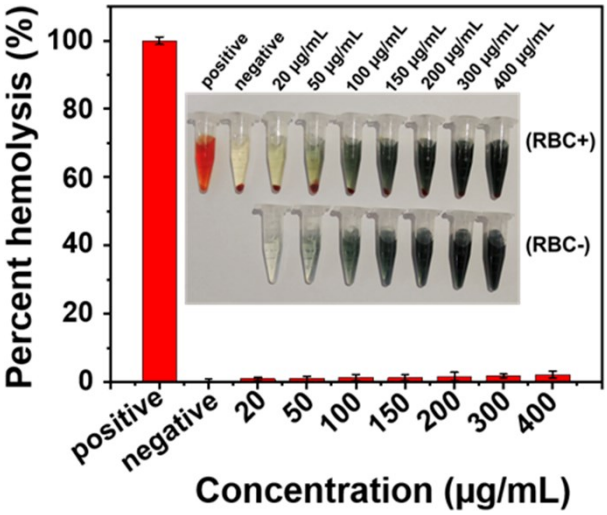

Figure S9. The hemolysis assay of red blood cells incubated with Mo-POM with various concentrations for 2 h, using PBS (pH = 7.4, 0.01 M) and deionized water as negative and positive controls, respectively.

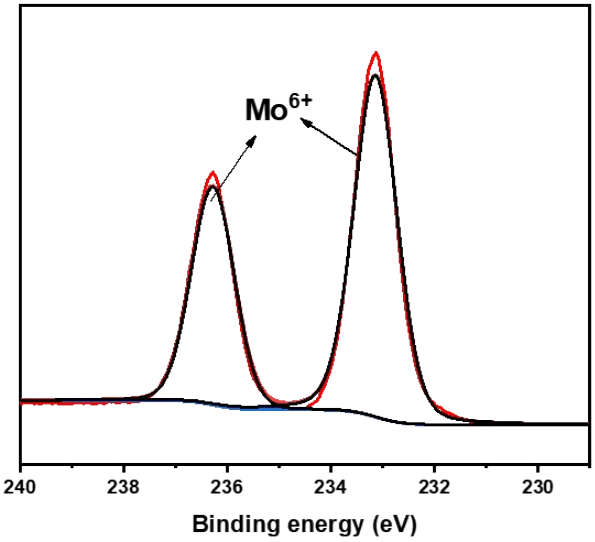

Figure S10. XPS spectrum of Mo after Mo-POM oxidation.

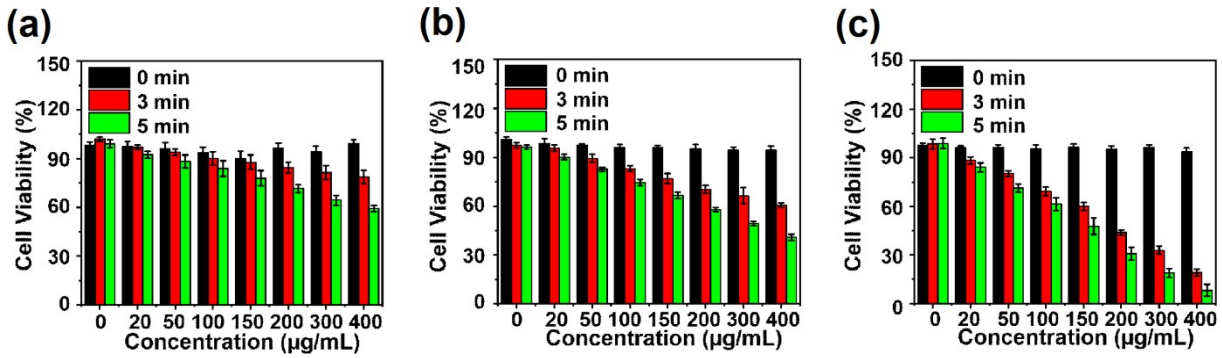

Figure S11. Plots showing the cell viability under different treatments. Incubation of 4T1 cells in different concentrations of Mo-POM for 24 h before laser irradiation (irradiated with 808 nm laser, for 0, 3 and 5 min), with different laser power of 0.5 W/cm^2 (a), 1.0 W/cm^2 (b) and 2.0 W/cm^2 (c), respectively.

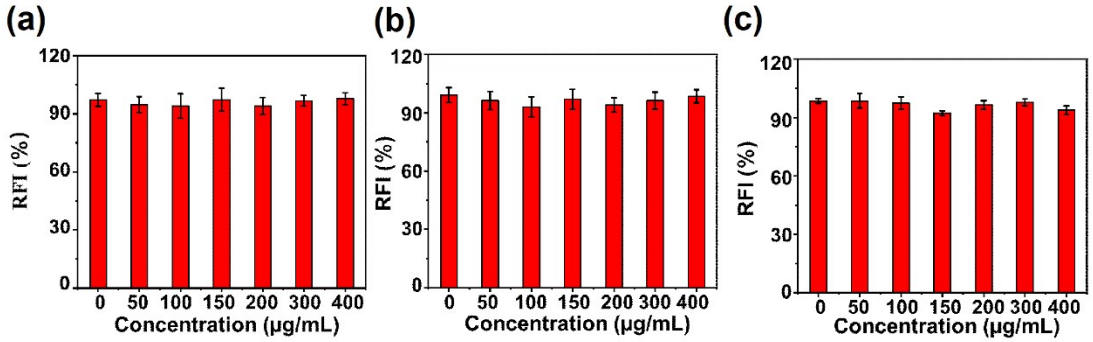

Figure S12. Endogenous ROS level in HUVECs treated with different concentrations of Mo-POM for 6 h (a); 12 h (b); 24 h (c) (RFI: Relative Fluorescence Intensity).

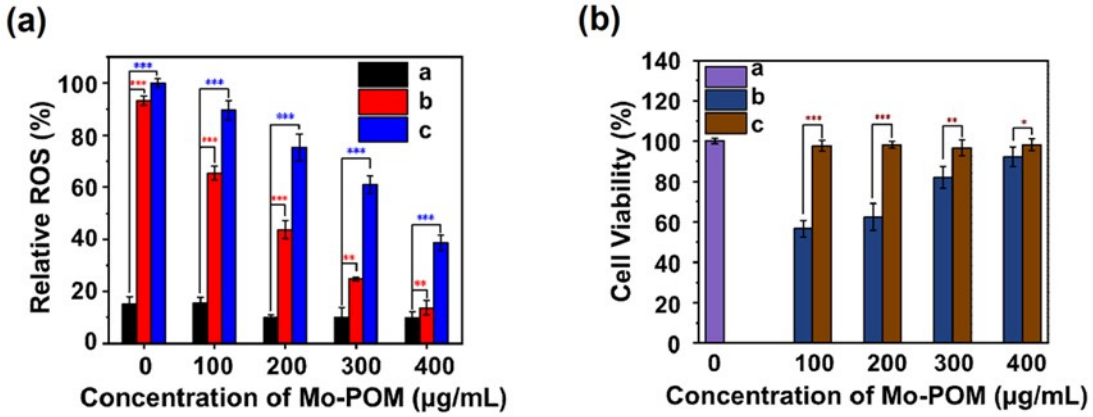

Figure S13. (a) Relative percent ROS levels in HUVECs with different concentrations of Mo-POM and different concentrations of H_2O_2 under different treatments. a: 0 mM H_2O_2, b: 0.3 mM H_2O_2, c: 3 mM H_2O_2. (b) Plots showing the cell viability with different combinations of Mo-POM and different concentrations of H_2O_2 (0, 0.5, 1, 2, 3 mM). a: control, b: H_2O_2 + Mo-POM, c: Mo-POM. P values was calculated by two-way ANOVA test(*P < 0.05; **P < 0.01; ***P < 0.001).

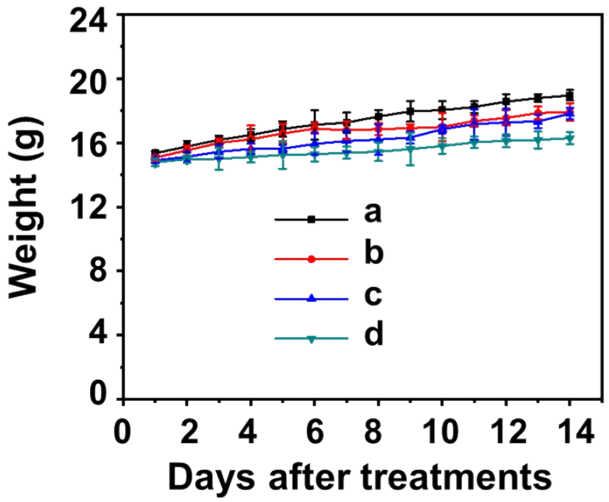

Figure S14. The body weight measurements after various treatments, including Mo-POM (40 mg/kg, 200 μL each time for injection), laser (808 nm, 10 min, 1.5 W/cm^2), PBS (pH = 7.4, 0.01 M), showed a normal growth of the mice with these different treatments, indicating good biocompatibility of Mo-POM. a: PBS, b: PBS + NIR, c: Mo-POM, d: Mo-POM+ NIR.

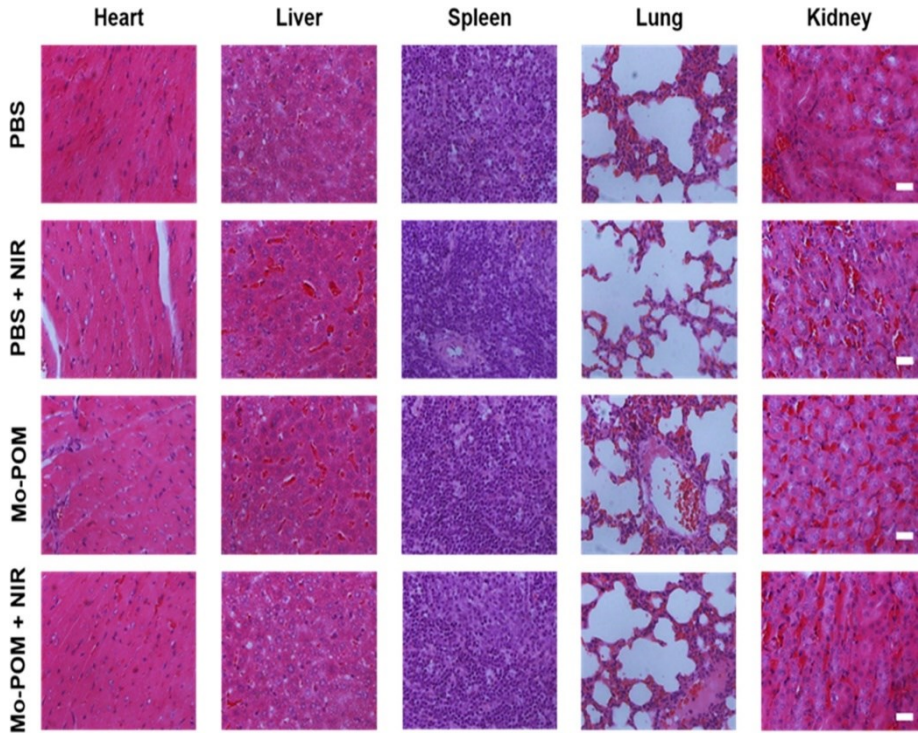

Figure S15. H&E-stained tissues from mice to monitor the histological changes in heart, liver, spleen, lung and kidney prior to 14 days after intravenous injection of Mo-POM (40 mg/kg, 200 μL each time for injection). Scale bar: 50 μm.

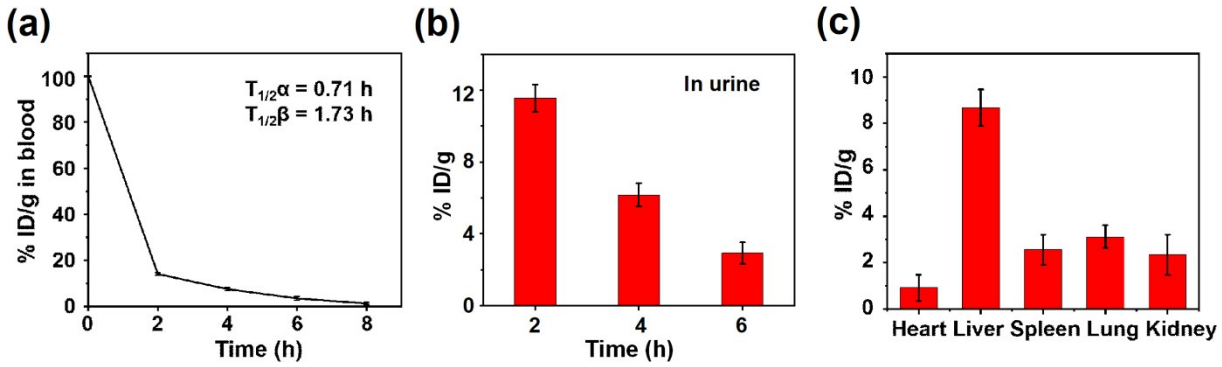

Figure S16. (a) Blood circulation profile of Mo-POM detected by Mo amount in mice. (40 mg/kg, 200 μL each time for injection). (b) Distribution of Mo element in mice urine at different times after tail vein injection (40 mg/kg, 200 μL each time for injection). (c) Distribution of Mo element in different organs of mice after tail vein injection (40 mg/kg, 200 μL each time for injection).