Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Metal organic cage-derived cascade antioxidant nanozyme to mitigate renal ischemia-reperfusion injury

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1. General Information

All the reagents and solvents were purchased from commercial sources and used as received.

NMR: The chemical structures of the synthesized MOC and MOC-O were confirmed by nuclear magnetic resonance (NMR) spectra in a Bruker Avance 500 (500 MHz) instrument using an internal deuterium lock for the residual protons in DMSO at ambient probe temperature.

Mass spectra: The precise molecular weights of the synthesized MOC and MOC-O were recorded through mass spectra. Fourier transtom ion cyclotron resonance (FT-ICR) mass spectra were performed on SolariX 7.0T (USA). Matrx-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were pertormed on a Bruker Speed MALDI-TOF 7090 (Bruker, Germany).

FT-IR: Fourier transform infrared (FT-IR) spectra were recorded on a Bruker Tensor 27 FT-IR using ATR measurements for solids as neat samples.

EPR: The Bruker A300 Electron Paramagnetic Resonance (EPR) instrument is mainly used for the detection of free radicals and magnetic metal ions and their compounds, and the detection of certain localized lattice defects in solids.

2. Synthetic Details of MOC and MOC-O

MOC: The 2,2'-Bipyridine-5,5'-dicarboxylic acid (36 mg, 0.15 mmol, 1 eq.) was dissolved in 15 ml of DMSO using sonication. Subsequently, Bis(cyclopentadienyl)zirconium dichloride (150 mg, 3.33 eq.) and 1 ml H_2O were separately added and sequentially reacted at a temperature of 65 °C for a duration of 6 hours. The system was gradually cooled to room temperature resulting in the formation of white crystals that were collected through centrifugation.

Purification of m-CPBA: m-CPBA (25 g) was dissolved in ethanol (200 ml) and washed with buffered aqueous solution (pH 7, 3x100 ml). The organic phase was dried with $MgSO_4$. The solvent was removed under reduced pressure to give pure white crystals of m-CPBA. To avoid decomposition, the purified substance was stored in the dark at -30 °C under inert atmosphere.

MOC-O: The MOC (30 mg, 1 eq.) was added to 20 ml of isopropyl acetate under homogeneous stirring at 0 °C. Subsequently, freshly purified m-CPBA (70 mg, 50 eq.) was added and the reaction proceeded at 0 °C for 18 h. After completion of the reaction, Na_2SO_3 was introduced and stirred for 1 h to quench any excess m-CPBA. The resulting white product was obtained by filtration, followed by washing with H_2O and isopropyl acetate. Finally, it was dried in a vacuum oven at 45 °C.

3. Operational Details of Biological Testing

EPR signal detection: The MOC-O powder (1 mg) was dispersed in 1 mL of DMSO, followed by sonication and shaking. Subsequently, 200 μL of the resulting mixture was added to a DMPO solution (100 mM) at a concentration of 100 μL, which was then mixed thoroughly in capillary tubes before being subjected to testing.

SOD-like activity assays: The SOD-like activity was determined using the nitrogen blue tetrazolium (NBT) method. Under UV irradiation, riboflavin and methionine generate $\cdot O_2^-$, which reduces NBT to form blue methylhydrazone with a maximum absorption at 560 nm. However, SOD scavenges $\cdot O_2^-$ and inhibits the formation of methylhydrazone. Consequently, higher SOD-like activity leads to reduced levels of the reduction product (blue methylhydrazone) and lower absorbance at 560 nm. Thus, the decrease in absorbance at 560 nm confirms the effect of SOD-like activity. The samples (2 mg/mL, 100 μL), NBT (0.6 mg/ml, 0.3 ml), Met (20 mg/ml, 0.3 ml), EDTA-Na_2 (0.4 mg/ml, 0.3 ml), riboflavin (0.06 mg/ml, 0.3 ml), and phosphate buffer solution (pH=7.4) were incubated together under constant intensity UV irradiation for intervals of 20 s to test the SOD-like activity. We set up four sets of experiments, (1) Blank (DMSO), (2) MOC, (3) 2,2'-BPDO, and (4) MOC-O to estimate the SOD-like activity. In addition, we also measured the SOD-like activity by varying the concentration of MOC-O under UV irradiation for 240 s to observe the MOC-O concentration-dependent SOD-like activity.

CAT-like activity assays: The CAT-like activity of MOC-O was determined at room temperature by measuring the oxygen produced using a specific oxygen electrode on a Speedwell Split Dissolved Oxygen Meter (SW9403). In a typical test, 60 μL of 30% H_2O_2 solution was added to 14.94 mL of buffer solution (pH = 7.2), followed by 100 μL of sample, and the solubility of the oxygen generated (in mg/L) was recorded from 0 to 10 minutes. Four groups, (1) Blank (DMSO),

(2) MOC, (3) 2,2'-BPDO, and (4) MOC-O, were set up to test for CAT-like activity. In addition, the concentration-dependent CAT-like activity was determined by adding different amounts (50, 100, 150, and 200 μL) of MOC-O solution to a final volume of 15 mL of buffer solution.

Hemolysis test: Whole blood from healthy mice was collected in 2 mL EP tubes and centrifuged at 14,000 rpm for 15 minutes. The erythrocytes were then washed three times with PBS, and 0.5 mL of erythrocytes were diluted with 10 mL of PBS. The MOC-O solution was diluted into six concentration gradients by double dilution. Then, 0.5 mL of diluted erythrocyte suspension was mixed with 0.5 mL of different concentrations of MOC-O, and PBS and double diluted water were used as negative and positive controls, respectively.

MTT assay: The viability of HK-2 cells was assessed using a commercially available kit, and the cytotoxic effect of MOC-O was evaluated accordingly. PBS solution of MOC-O (0-800 μg/mL, 10 μL) was added to each well of a 96-well microtiter plate containing 9000 cells and incubated at 37°C, 5% CO_2, 90% humidity for 24 hours. Then 10 μL of MTT solution was added and incubated at 37°C, 5% CO_2, 90% humidity for 4 h. All supernatant in the wells was aspirated. 100 μL of DMSO was added to each well and incubated, placed on a shaker at 300 rpm/min until the purple crystals were completely dissolved. Wells with only PBS were blanks and wells with only cells were controls. The absorbance of each sample at 570 nm was measured using a microplate reader. Finally, the survival rate of HK-2 cells was calculated based on the comparison with the blank and control groups.

Cellular uptake: For in vitro cellular uptake assays, HK-2 cells were seeded onto 10 mm tissue plates at a density of 10000 cells/mL and incubated for 24 h. Subsequently, MOC-O (2 mg/mL, 100 μL) was added to each plate and cultured for 2 h, 4 h, 8 h or 12 h. For in vitro cellular retention assays, HK-2 cells were seeded onto 10 mm tissue plates at a density of 10000 cells/mL and incubated for 24 h. Then, MOC-O (2 mg/mL, 100 μL) was added to each plate and cultured

for an additional period of 12h. After that, the medium was extracted and replaced with fresh medium. The cells were subsequently collected at time points of both "12+4" hours and "12+8" hours. ICP-MS analysis was employed to quantify the cellular uptake of MOC-O. Following trypsinization and centrifugation steps, the cell pellets were washed thrice with PBS buffer solution. For conventional ICP-MS analysis purposes, the samples underwent digestion using a solution containing HNO_3 (68%) (0.25 mL) and HCl (38%) (0.75 mL), which lasted for four hours at a temperature of 110 °C. After cooling down, the samples were diluted with HCl (2%) to reach a final volumeof10mL. Zr contents were then determined by ICP-MS.

Intracellular ROS: DCFH-DA can emit fluorescence upon intracellular ROS oxidation; the intensity of fluorescence is directly proportional to the level of ROS content. HK-2 cells were cultured in 6-well microtiter plates (7000 cells per well) at 37 °C for 12 hours. To induce hypoxia, a solution of 2 mM Na_2S_2O4 was added to the culture medium and the cells were placed in a CO_2 incubator. Four experimental groups were designed as follows: (1) Vehicle; (2) H/R; (3) H/R+MOC; and (4) H/R+MOC-O. The experimental group was treated with an additional 10 μL of MOC-O and incubated at 37 °C for another 6 h. Subsequently, the cells were incubated at 37 °C for 30 min followed by three washes with PBS. Fluorescence microscopy and flow cytometry were employed to analyze the results. Except for the Vehicle group, all other groups experienced hypoxic conditions while keeping other parameters unchanged.

Animal and ethics statement: C57BL/6 mice (female, age of 7-8 weeks) were obtained from Shanghai Laboratory Animal Center (China). All animal experiments were performed in accordance with the guidelines of the National Institute of Health for the Care and Use of Laboratory Animals and approved by the Scientific Investigation Board of Shanghai Changzheng Hospital (No. 2023091501).

Induction of renal I/R model: Mice were anesthetized with sodium pentobarbital

(50 mg/kg, intraperitoneally). After a right flank incision, the right renal pedicle was clamped for 30 minutes with a nontraumatic microaneurysm clamp (Shanghai Medical Instruments, Shanghai, China). The left contralateral kidney was considered as sham surgery. The incision was temporarily closed during ischemia. Upon removal of the microaneurysm clamps, the flank was closed after visual verification of reperfusion. Body temperature was maintained using an adjustable heating pad. All mice received intraperitoneal injection of 0.5 mL isotonic saline post-surgery and were sacrificed at specified reperfusion time points.

Serum biochemistry analysis: The mice were sacrificed at the designated time points. All serum samples were collected and subjected to centrifugation at 5000 rpm for 10 minutes. ALT, AST, CRE, and BUN levels were quantified using an automated analyzer (Cobas 8000, Roche, Germany) equipped with the c702 module.

Histopathological evaluation: The mice were sacrificed at the indicated time points. The mouse kidneys were cut coronally, fixed in 10% buffered formalin, embedded in paraffin, and sectioned at a thickness of 3 μm. The sections were stained with hematoxylin and eosinto assess histological injury. Kidney sections were labeled blindly and randomly observed by two investigators. A semiquantitative pathological assessment on a scale of 0 to 4 was performed to grade the degree of renal tubular injury: 0 = no identifiable injury; 1 = necrosis of individual cells; 2 = necrosis of all cells in adjacent proximal convoluted tubules, with the survival of surrounding tubules; 3 = necrosis confined to the distal third of the proximal convoluted tubules, with a band of necrosis extending across the inner cortex; and 4 = necrosis affecting all three segments of the proximal convoluted tubule.

Real-time PCR analysis：The total renal RNA was extracted using TRIzol reagent (Invitrogen, Shanghai, China) according to the manufacturer's instructions. cDNA was transcribed using a Superscript III Reverse Transcriptase Kit (Invitrogen) and oligo d(T) (Applied Biosystems, Waltham, MA, USA). Quantitative RT-PCR analysis was performed with a BeyoFast™ SYBR Green One-Step qRT-PCR Kit (Beyotime, Shanghai, China). All reactions were conducted in a 20 μL reaction volume in triplicate.

The relative expression levels for a target gene were normalized against GAPDH.

Immunofluorescence assay: The frozen sections of kidneys were processed following a standardized protocol. Subsequently, the sections were incubated overnight with primary antibodies against DHE or TUNEL obtained from Abclonal (Wuhan, China). Following this step, the slides underwent three washes with PBS and were then incubated with secondary antibodies at room temperature for 1 hour. Nuclei staining was performed using DAPI. Finally, the slides were examined and imaged under a fluorescent microscope (Nikon 80i, Tochigi, Japan).

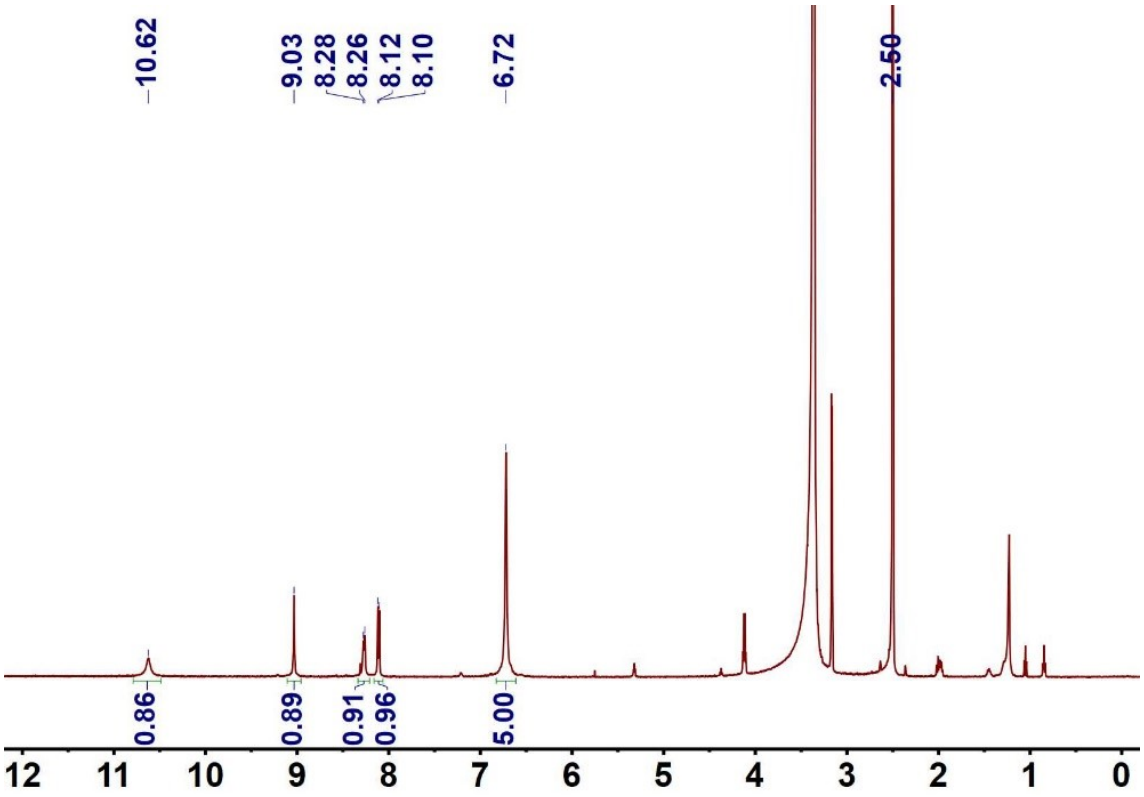

Fig. S1 The ^{1}H-NMR spectra of **MOC** in DMSO-d_6.

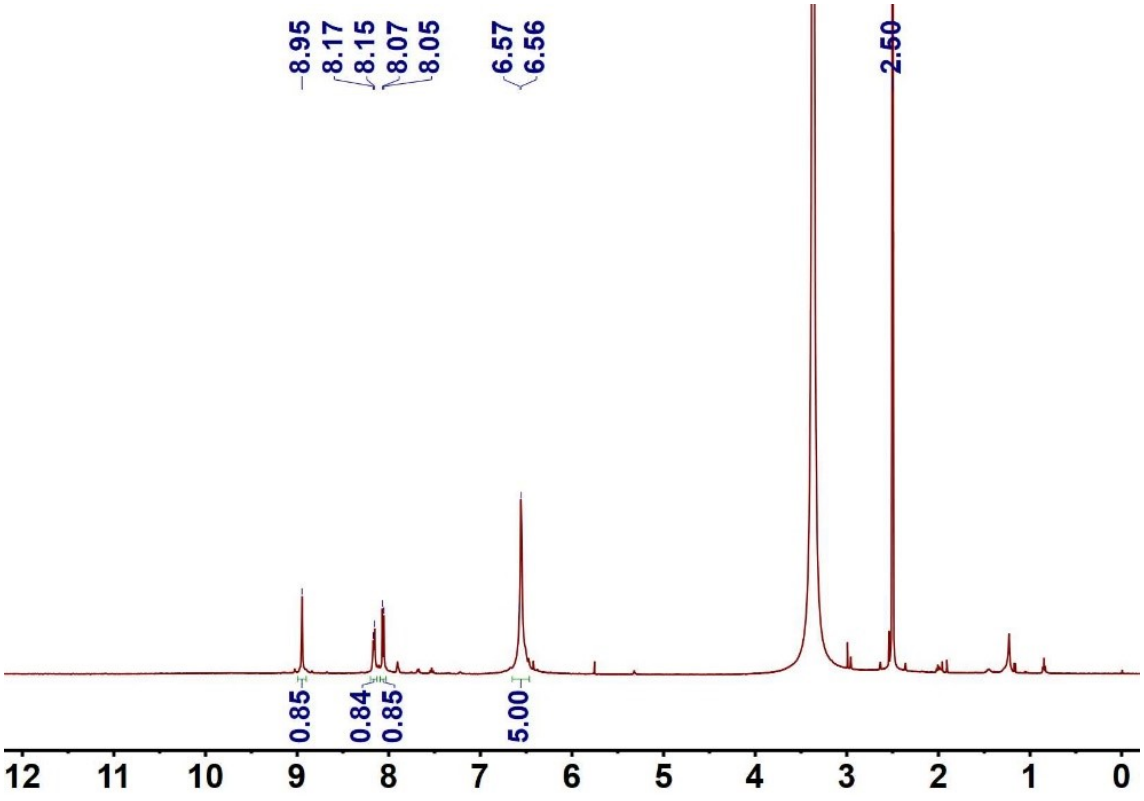

Fig. S2 The ^{1}H-NMR spectra of **MOC-O** in DMSO-d_6.

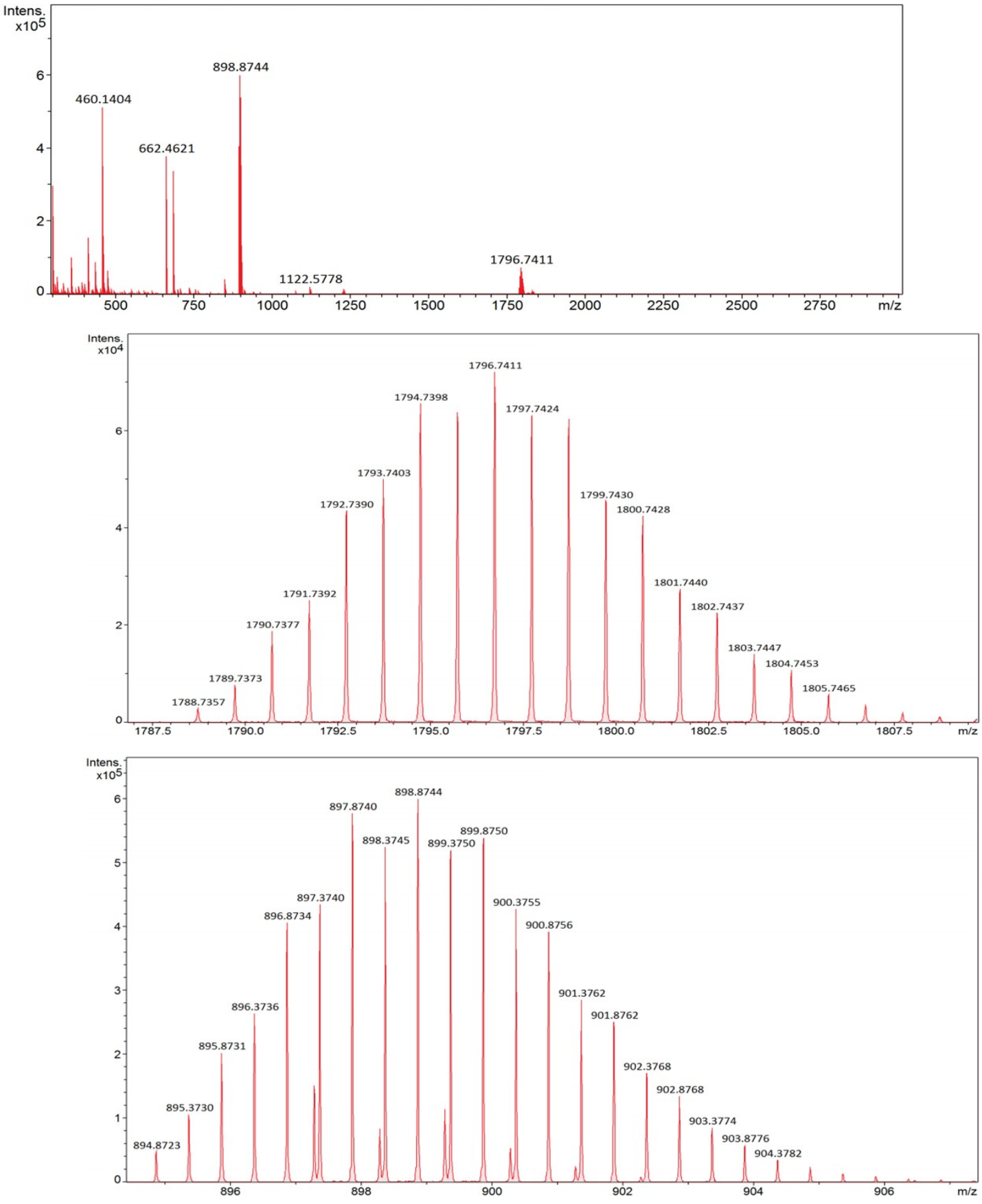

Fig. S3 The ESI mass spectra of **MOC**.

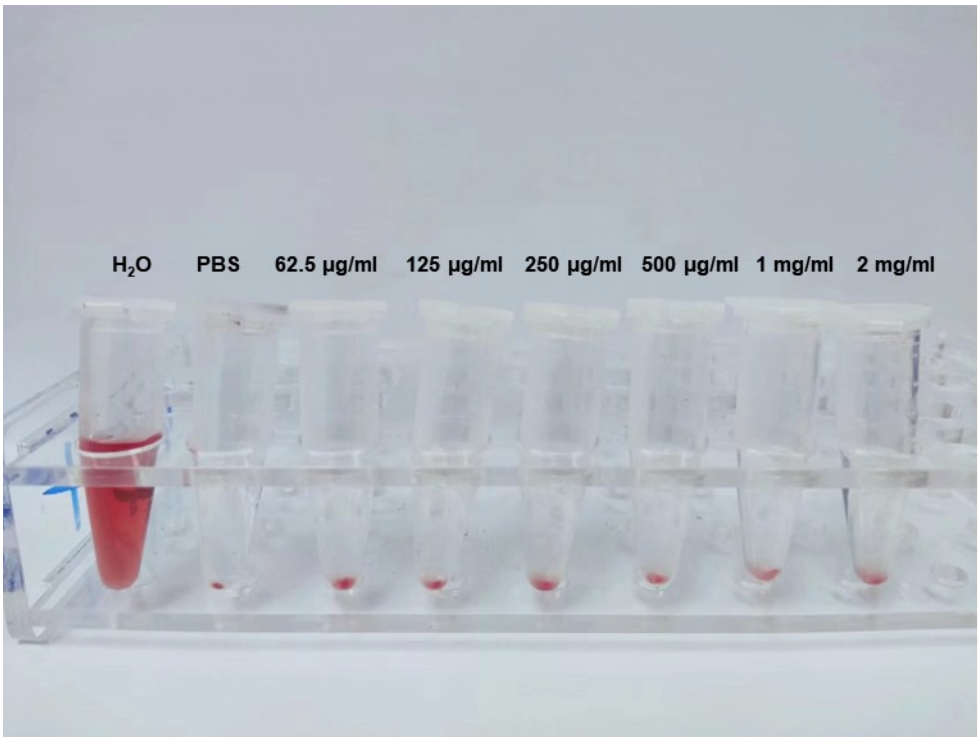

Fig. S4 The Hemolysis assay of **MOC-O**.