Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Hypochlorous acid-activated two-photon fluorescent probe for evaluation of anticancer drugs-induced cardiotoxicity and screening of antioxidant drugs

Kaiqiang Liu,† Jian Zhang,*,† Xinxin Li,† Yingying Xie,† Yong Li,† Xu Wang,*,† Xiaoyun Jiao,† Xilei Xie and Bo Tang*,†

†College of Chemistry, Chemical Engineering and Materials Science, Collaborative Innovation Center of Functionalized Probes for Chemical Imaging in Universities of Shandong, Key Laboratory of Molecular and Nano Probes, Ministry of Education, Shandong Provincial Key Laboratory of Clean Production of Fine Chemicals, Shandong Normal University, Jinan 250014, P. R. China.

E-mail: tangb@sdnu.edu.cn, wangxu@sdnu.edu.cn, zhangjian12b@mails.ucas.ac.cn

1. Materials and Instruments

All chemicals were purchased from Adamas Reagent, Ltd. (China), and analytical grade solvents were used without further purification. All aqueous solutions were prepared using ultrapure water (ultrapure water, 18 MΩ cm^{-1}). MTT was purchased from Sigma Corporation, column chromatography silica gel (200-300 mesh) was purchased from Qingdao Haiyang Reagent Co., Ltd. The DMEM medium, penicillin/streptomycin and fetal calf serum was purchased from Gibco Corporation. LysoTracker Green (C1047S) was purchased from Beyotime Biotechnology Co., Ltd, Shanghai.

H9C2 cells were purchased from Procell Life Science & Technology Co., Ltd.

Fluorescence data was measured by a F-4700 fluorescence spectrophotometer (Hitachi) at room temperature (slit for **THPIC**: 2.5 nm, 5.0 nm). The absorption spectra were measured on a UV-1700 spectrophotometer (Shimadzu, Japan). The mass spectra were obtained by Maxis MHR-TOF ultra-high resolution quadrupole time of flight mass spectrometer (Bruker Germany). The ^{1}H NMR and ^{13}C NMR spectra were acquired on a nuclear magnetic resonance spectrometer (400 MHz, Bruker Co., Ltd., Germany). The δ value represents the shift of the spectrum relative to TMS ($(CH_3)_4Si$ = 0.00 ppm). Two-photon images (Fig 3, 4, 6, S21, S22) were acquired with the Zeiss LSM 880 NLO (Zeiss, Germany) with a 20× water objective from Key Laboratory of Molecular and Nano Probes, Ministry of Education,Shandong Normal University, Jinan 250014, P. R. China. Two-photon images (fig 5) were acquired with the Zeiss LSM 880 NLO (Zeiss, Germany) with a 40× water objective from Experimental Center, Shandong University of Traditional Chinese Medicine, Jinan 250355, PR China. A Ti: sapphire laser was used to excite the specimen at 720 nm with a laser power of 70 mW. MTT assay was measured with a microplate reader (TRITURUS).

Preparation of various interference substances. All reagents were used right after they were ready. Cys, Hcy, GSH and vitamin C (Vc) were all used as received. HS^-, S^{2-} and HSO_3^- were all used as their sodium salt and prepared as the stock solutions.

K^+, Na^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+} and Ag^+ were all used as their chloride salt and prepared as the stock solutions. All these compounds were commercial

available with analytical purity and used directly.

We prepared reactive oxygen species (ROS) as follows:

Peroxynitrite ($ONOO^-$): hydrochloric acid (0.6 M) was added to the mixture of $NaNO_2$ (0.6 M) and H_2O_2 (0.7 M), then NaOH (1.5 M) was added. The resulted faint yellow solution was split into small aliquots and stored at lower than -20 °C. The concentration of the prepared peroxynitrite was determined by testing the absorption of the solution at 302 nm. The extinction coefficient of $ONOO^-$ solution is 1670 M^{-1} cm^{-1} at 302 nm. $C_{ONOO^-} = Abs_{302nm}/1.67$ (mM).

Hydroxyl radical (•OH) was prepared by the reaction of Fe^{2+} with H_2O_2 (1:6), and the concentration of •OH is equal to the concentration of Fe^{2+}.

Singlet oxygen ($^1O_2$) was prepared in situ by addition of the H_2O_2 stock solution into a solution containing 10 eq of NaClO.

Superoxide solution ($O_2^{\bullet-}$) was prepared by adding KO_2 to dry dimethylsulfoxide and stirring vigorously for 10 min.

Hydrogen peroxide (H_2O_2) was diluted appropriately in water. The concentration of H_2O_2 was determined by measuring the absorbance at 240 nm with a molar extinction coefficient of 43.6 M^{-1} cm^{-1}.

Nitric oxide (NO) was used from a stock solution prepared by sodium nitroprusside.

Hypochlorous acid (HClO) was obtained by diluting commercial aqueous solutions. The concentration was determined by measuring the absorbance at 292 nm with a molar extinction coefficient of 391 M^{-1} cm^{-1}.

2. Fluorescence analysis

Fluorescence titration profiles of the probe were constructed by mixing **THPIC** (5.0 μM) with different level of HClO (0-27.0 μM) in PBS buffer solution (1 % DMSO, 100 mM, pH=7.4, 37 °C). The measurement was carried out at $\lambda_{ex}/\lambda_{em}$ = 370/501 nm for **THPIC**. The specificity experiments of the probe towards HClO were carried out by incubation of the probe with HClO and other biorelevant species, including reactive oxygen and nitrogen species (•OH, $^1O_2$, $O_2^{\cdot-}$, H_2O_2, NO, $ONOO^-$), reactive sulfur species (GSH, Cys, Hcy, HSO_3^-, S^{2-} and SH^-), reductive species ($HSO_3^-$and Vc), and metal ions (K^+, Na^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+} and Ag^+) in PBS buffer solution (1 % DMSO, 100 mM, pH=7.4, 37 °C). The kinetic studies of fluorescence responses were performed by incubating the probe (5.0 μM) with HClO (27.0 μM) at $\lambda_{ex}/\lambda_{em}$ = 370/501 nm for **THPIC**.

Determination of Fluorescence Quantum Yield. The absorbance of **THPIC** and the corresponding product was adjusted to ca. 0.05. The emission spectra were obtained by exciting with corresponding maximal excitation wavelength and the integrated areas of the fluorescence spectra were calculated. The fluorescence quantum yield was determined by comparing the integrated emission intensity of the test samples with that of a solution of quinine sulfat (the fluorescence standard, Φ_F = 0.54 in 0.1 M H_2SO_4) and calculated by following equation.

$$\Phi_{F(X)} = \Phi_{F(S)} \times \left(\frac{F_X}{A_X}\right) \times \left(\frac{A_S}{F_S}\right) \times \left(\frac{n_X}{n_S}\right)^2$$

where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and the samples to be tested, respectively.

Measurement of Two-Photon Absorption Cross-Section. The TPA cross-sections (δ) of **THPIC** and the corresponding product were measured by using the TPA imaging method. Using fluorescein as a reference, the fluorescence intensity of **THPIC** and the corresponding product were measured by two-photon excitation at 720 nm. The fluorescence intensity of **THPIC** and the corresponding product were measured by the

same method as above. The parameters of the substances used are as follows.

THPIC (100.0 μM), HClO (270.0 μM), fluorescein (1×10^{-7} M).

The two-photon absorption cross sections (δ) was calculated by two-photon excitation fluorescence spectra, according to the equation:

$$\frac{\delta_x}{\delta_s}=\frac{F_x C_s n_s \varphi_s}{F_s C_x n_x \varphi_x}$$

where δ stands for the two-photon absorption cross-section, F stands for the two-photon fluorescence intensity, φ stands for the fluorescence quantum yield, C stands for molar concentration, n stands for the solvent refractive index, and the subscripts *X* and *S* denote the evaluated sample (**THPIC** and the corresponding product) and the standard reference (fluorescein, in NaOH of 0.1 M), respectively.

3. Synthesis and characterization of THPIC

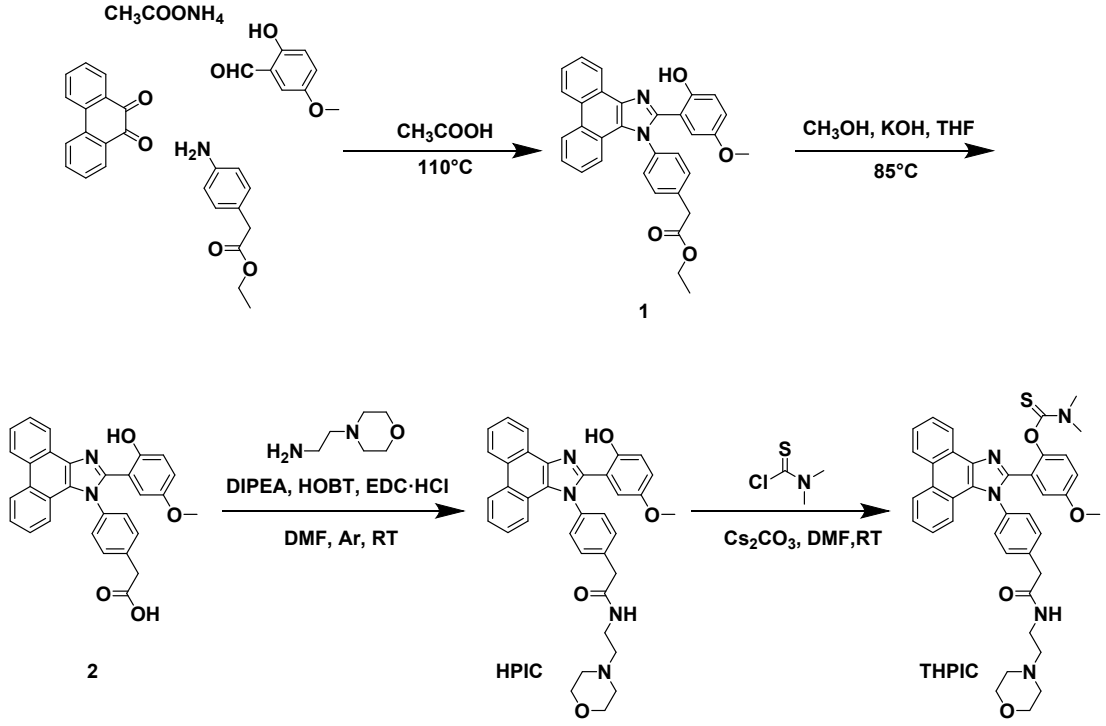

Fig. S1 Synthesis procedure of **THPIC**.

Synthesis of compound 1

Adapted from[1]. The flask was purged with argon gas three times. 9,10-Phenanthraquinone (2.0 g, 9.59 mmol, 1.0 eq), ammonium acetate (5.18 g, 67.13 mmol, 7.0 eq), Ethyl 4-aminophenylacetate (1.72 mL, 9.59 mmol, 1.0 eq), 2-Hydroxy-5-methoxybenzaldehyde (1.47 mL, 9.59 mmol, 1.0 eq) and acetic acid (150 mL) were added to a 500 mL two neck flask equipt with a magnetic stir bar. The solution was heated to 120 ^{o}C. After 12 h the solution was cooled to room temperature and quenched with water. The brown precipitate was collected by vacuum filtration and rinsed with pure water. The crude product was dissolved in CH_2Cl_2 and rinsed with brine, dried over Na_2SO_4 and filtered. The collected crude was dried in a rotary evaporator and subjected to column chromatography (SiO_2, v/v CH_2Cl_2/ Petroleum ether = 1:4) producing a white solid. Yield 1.93 g (40.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 – 8.75 (m, 2H), 8.70 (d, J = 8.2 Hz, 1H), 7.76 (t, J = 7.5 Hz, 1H), 7.70 (d, J = 6.9 Hz, 1H), 7.67 – 7.60 (m, 4H), 7.55 (t, J = 7.7 Hz, 1H), 7.29 (d, J = 7.1 Hz, 1H), 7.08 (t, J = 8.7 Hz, 2H), 6.82 (dd, J = 9.0, 3.0 Hz, 1H), 6.40 (d, J = 3.0 Hz, 1H), 4.25 (q, J = 7.2 Hz, 2H), 3.85 (s, 2H), 3.31 (s, 3H), 1.33 (t, J = 7.2 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$)

δ 170.75, 153.31, 151.41, 148.30, 138.04, 137.25, 132.00, 129.68, 129.39, 128.61, 127.72, 127.10, 126.72, 126.33, 125.68, 125.52, 124.36, 123.33, 122.83, 122.61, 120.97, 119.09, 112.54, 109.63, 61.43, 55.29, 41.25, 14.37. HRMS (ESI) (m/z): calculated for $C_{32}H_{26}N_2O_4$ $[M+H]^+$:503.1965; found 503.1976.

Synthesis of compound 2

Compound 1 (1.0 mL, 1.99 mmol, 1.0 eq) was added to a 250 mL round bottom flask with 25 mL of THF. 3 M KOH in methanol (21 mL, 99.5 mmol, 50 eq) was added to the THF. The solution was heated to 85°C and reacted overnight. The solvents were removed in a rotary evaporator. The resulting residue was dissolved in 200 mL of water and quenched with 3 M H_2SO_4 till the solution was acidic (pH = 4.0) and a white solid had crashed out of the solution. The precipitate was collected by vacuum filtration and rinsed with water and cold methanol. Yield 0.850 g (90%).^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (s, 1H), 12.12 (s, 1H), 8.95 (d, J = 8.4 Hz, 1H), 8.90 (d, J = 8.3 Hz, 1H), 8.59 (dd, J = 8.0, 1.5 Hz, 1H), 7.81 (t, J = 7.5 Hz, 1H), 7.77 – 7.69 (m, 3H), 7.65 (d, J = 8.2 Hz, 2H), 7.58 (ddd, J = 8.3, 7.0, 1.3 Hz, 1H), 7.36 – 7.30 (m, 1H), 7.06 (dd, J = 8.3, 1.2 Hz, 1H), 6.91 (d, J = 8.9 Hz, 1H), 6.83 (dd, J = 8.9, 3.0 Hz, 1H), 6.55 (d, J = 3.0 Hz, 1H), 3.83 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.30, 151.85, 150.88, 148.47, 137.77, 136.71, 134.20, 131.79, 128.69, 127.81, 126.81, 126.14, 125.58, 124.60, 123.82, 121.81, 120.34, 118.53, 117.93, 113.67, 110.83, 67.02, 54.92, 25.13. HRMS (ESI) (m/z): calculated for $C_{30}H_{22}N_2O_4$ $[M+H]^+$: 475.1652; found 475.1682.

Synthesis of HPIC

Under the protection of argon atmosphere, compound 2 (100 mg, 0.21 mmol, 1.0 eq), 2-morpholin-4-ylethanamine (82 mg, 0.63 mmol, 3.0 eq), HOBT (43 mg, 0.32 mmol, 1.5 eq) and EDC·HCl (61 mg, 0.32 mmol, 1.5 eq) were dissolved in dry DMF (5 mL) with DIPEA (110 mg, 0.32 mmol, 1.5 eq). Then the mixture was reacted 4 h at room temperature. The solvent was removed under reduced pressure, and then the crude product was purified by column chromatography (SiO_2, v/v CH_2Cl_2/MeOH = 66.7:1) producing a white solid. Yield 86 mg (69.9%).^{1}H NMR (400 MHz, $CDCl_3$) δ 13.29 (s, 1H), 8.77 (d, J = 8.3 Hz, 1H), 8.70 (d, J = 9.5 Hz, 2H), 7.79 – 7.73 (m, 1H), 7.72 – 7.60 (m, 6H), 7.52 (t, J = 7.1 Hz, 1H), 7.27 (d, J = 5.9 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H),

7.04 (d, *J* = 8.9 Hz, 1H), 6.81 (dd, *J* = 9.0, 3.0 Hz, 1H), 6.32 (d, *J* = 3.0 Hz, 1H), 3.78 (s, 2H), 3.72 (s, 4H), 3.47 (q, *J* = 5.4 Hz, 2H), 3.29 (s, 3H), 2.61 (s, 2H), 2.55 (s, 4H).^{13}C NMR (101 MHz, $CDCl_3$) δ 169.68, 153.34, 151.09, 148.32, 138.15, 138.04, 134.60, 131.74, 129.51, 129.36, 128.42, 127.53, 127.01, 126.55, 126.11, 125.79, 125.30, 124.24, 123.20, 122.60, 122.56, 120.81, 118.76, 118.49, 112.47, 109.65, , 66.50, 57.06, 55.28, 53.26, 43.26, 35.68. HRMS (ESI) (m/z): calculated for $C_{36}H_{34}N_4O_4$ $[M+H]^+$:587.2653; found 587.2660.

Synthesis of THPIC

Under the protection of argon atmosphere, HPIC (80 mg, 0.14 mmol, 1.0 eq), dimethylcarbamyl chloride (52 mg, 0.42 mmol, 3.0 eq) and cesium carbonate(182.5 mg, 0.56 mmol, 4.0 eq) were dissolved in DMF (5 mL) and stirred 3 h at room temperature. The solvent was removed under reduced pressure, and then the crude product was purified by column chromatography (SiO_2, v/v CH_2Cl_2/MeOH = 66.7:1) producing a white solid. Yield 48.2 mg (51.1%).^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 7.9 Hz, 2H), 8.72 (d, *J* = 8.2 Hz, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 3H), 7.57 – 7.47 (m, 3H), 7.29 (d, *J* = 3.7 Hz, 2H), 7.15 (d, *J* = 9.0 Hz, 1H), 6.88 (dd, *J* = 8.9, 3.0 Hz, 1H), 6.48 (s, 1H), 6.26 (s, 1H), 3.71 (s, 4H), 3.68 (s, 2H), 3.49 (s, 3H), 3.43 (s, 4H), 3.15 (s, 3H), 2.52 (d, *J* = 15.5 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.25, 170.20, 162.66, 156.32, 146.76, 146.43, 137.41, 137.03, 130.78, 129.41, 128.44, 127.39, 126.51, 125.69, 125.57, 125.22, 124.61, 124.22, 123.38, 123.10, 122.44, 121.43, 116.10, 115.66, 66.80, 57.20, 55.61, 53.43, 43.51, 43.22, 39.23, 36.61, 35.90. HRMS (ESI) (m/z): calculated for $C_{39}H_{39}N_5O_4S$ $[M+Na]^+$: 696.2615; found 696.2735.

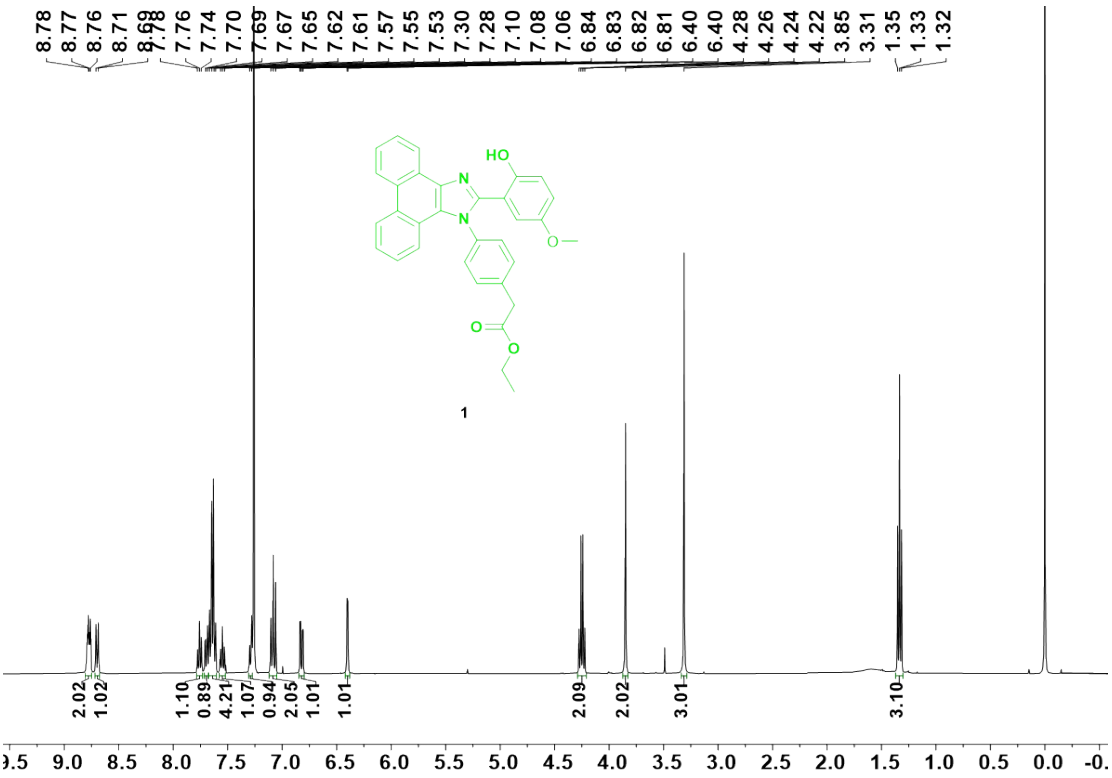

Fig. S2 ^{1}H NMR spectrum of compound 1 in $CDCl_3$.

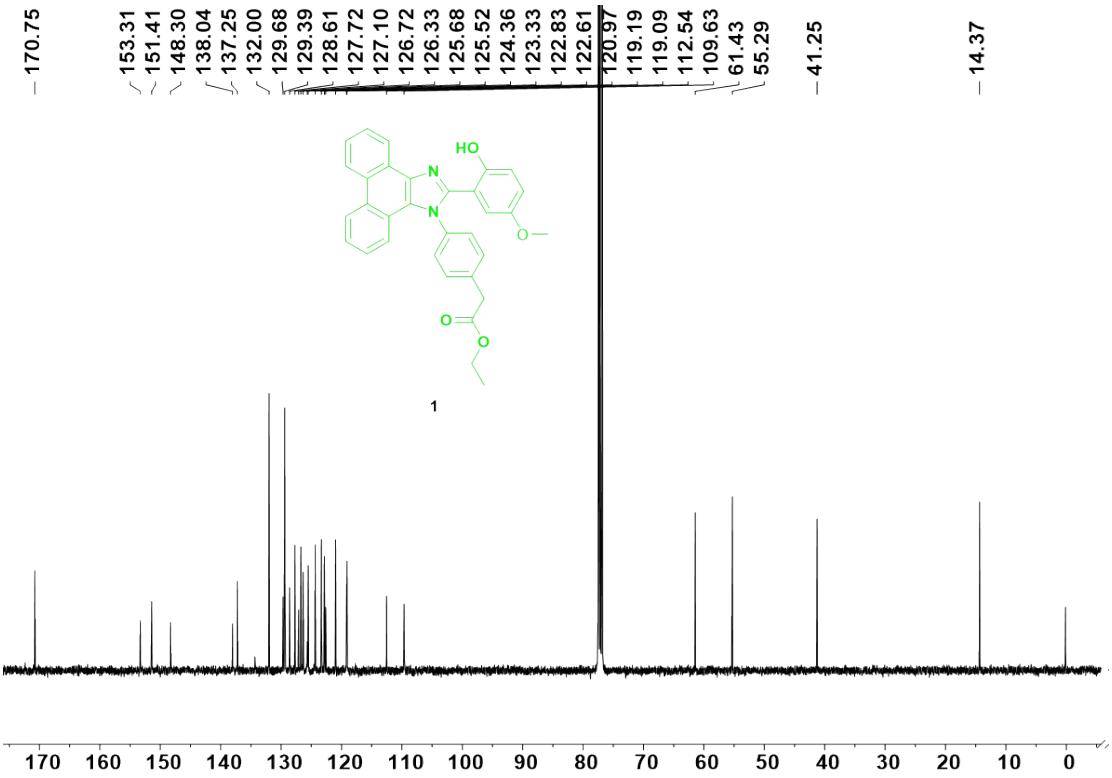

Fig. S3 ^{13}C NMR spectrum of compound 1 in $CDCl_3$.

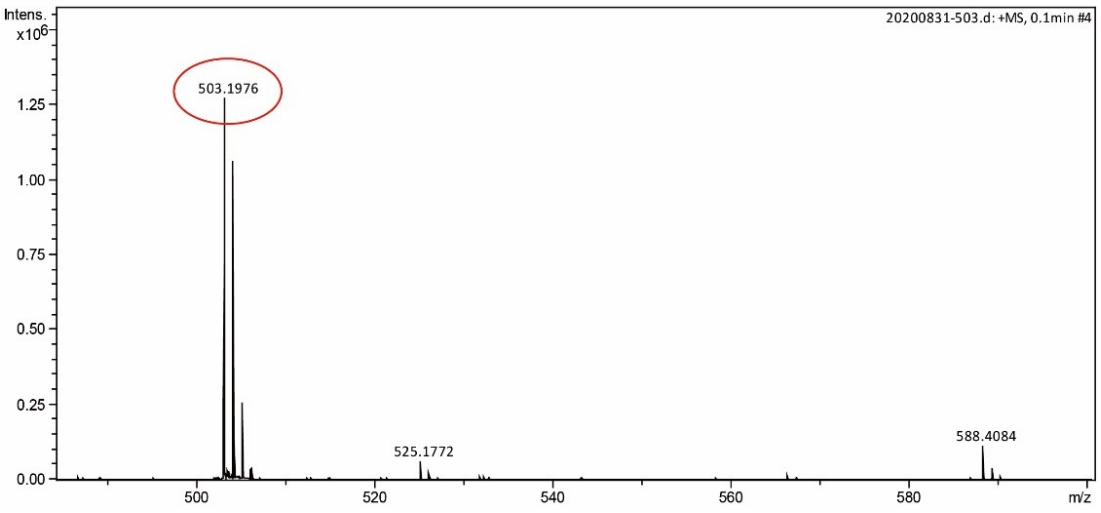

Fig. S4 HRMS of compound 1.

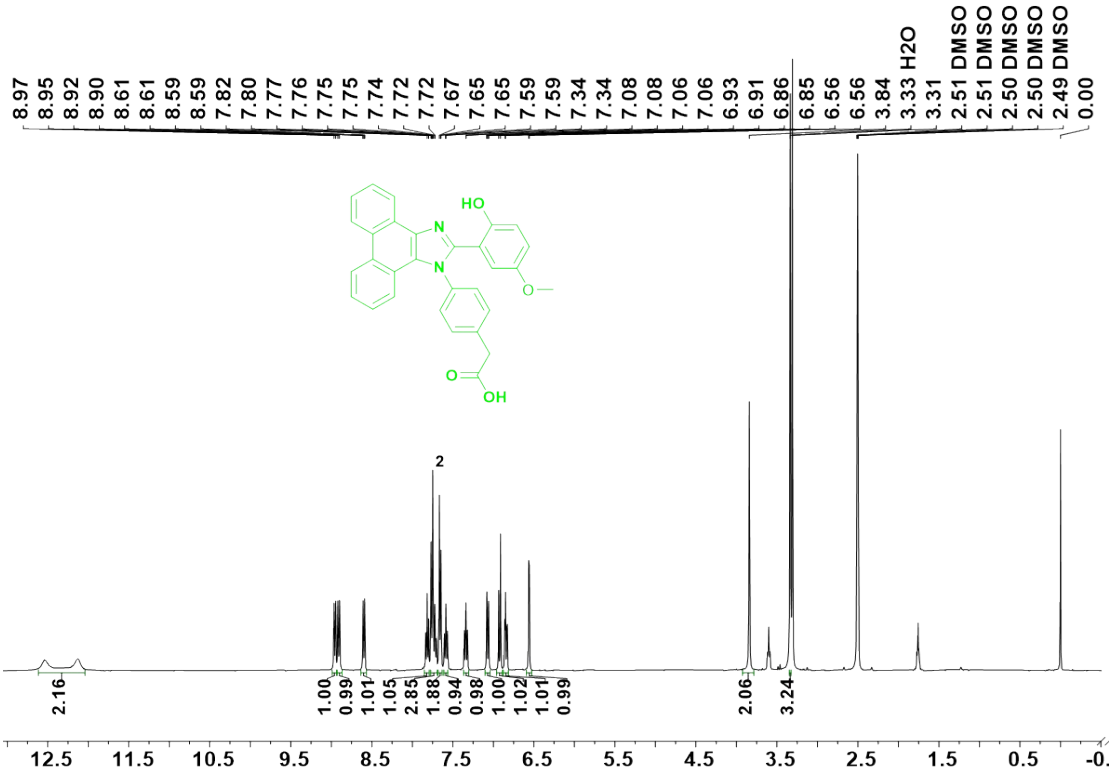

Fig. S5 ^{1}H NMR spectrum of compound 2 in $(CD_3)_2S{=}O$.

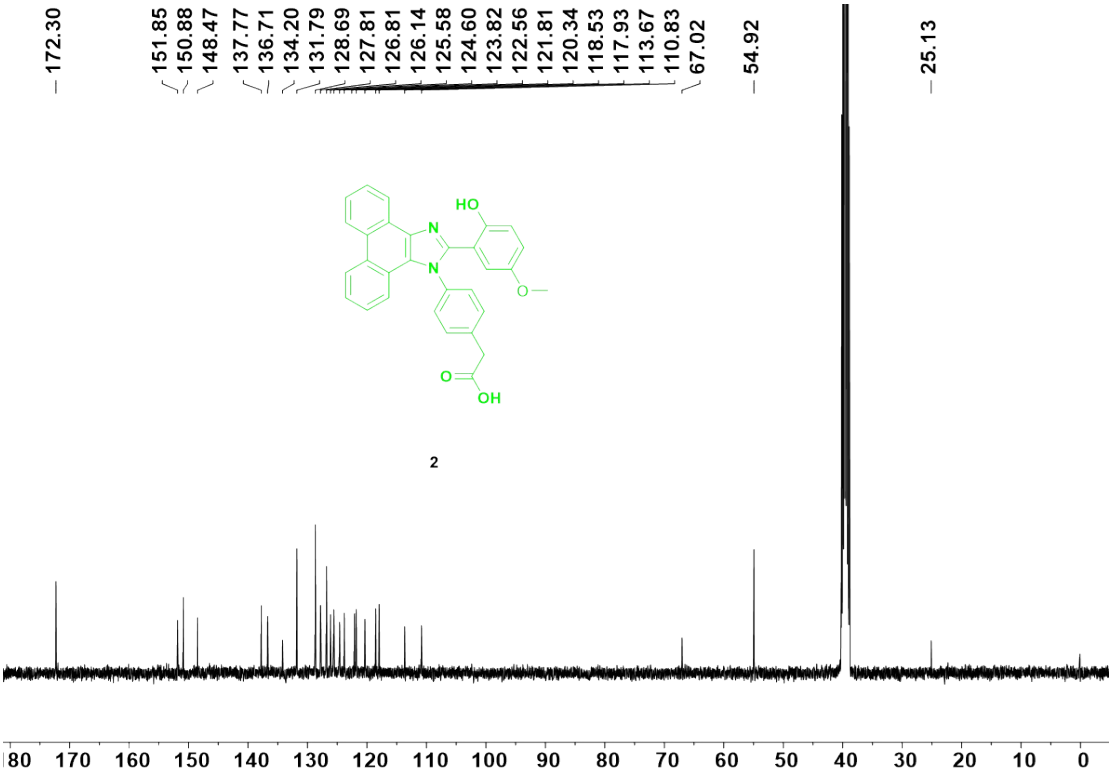

Fig. S6 ^{13}C NMR spectrum of compound 2 in $(CD_3)_2S=O$.

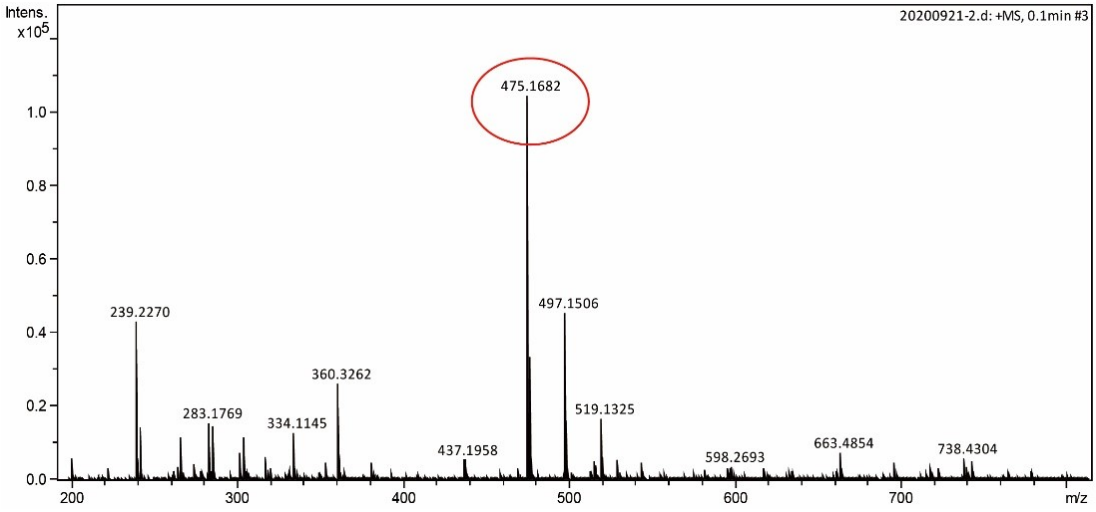

Fig. S7 HRMS of compound 2.

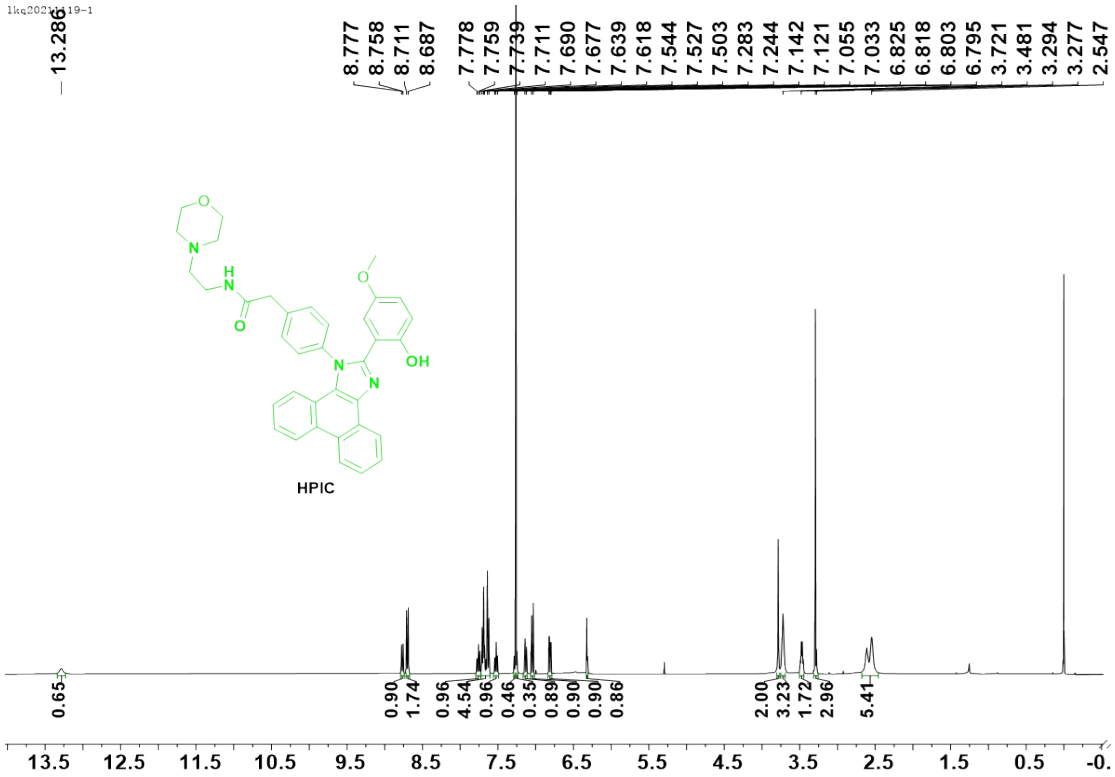

Fig. S8 ^{1}H NMR spectrum of HPIC in $CDCl_3$.

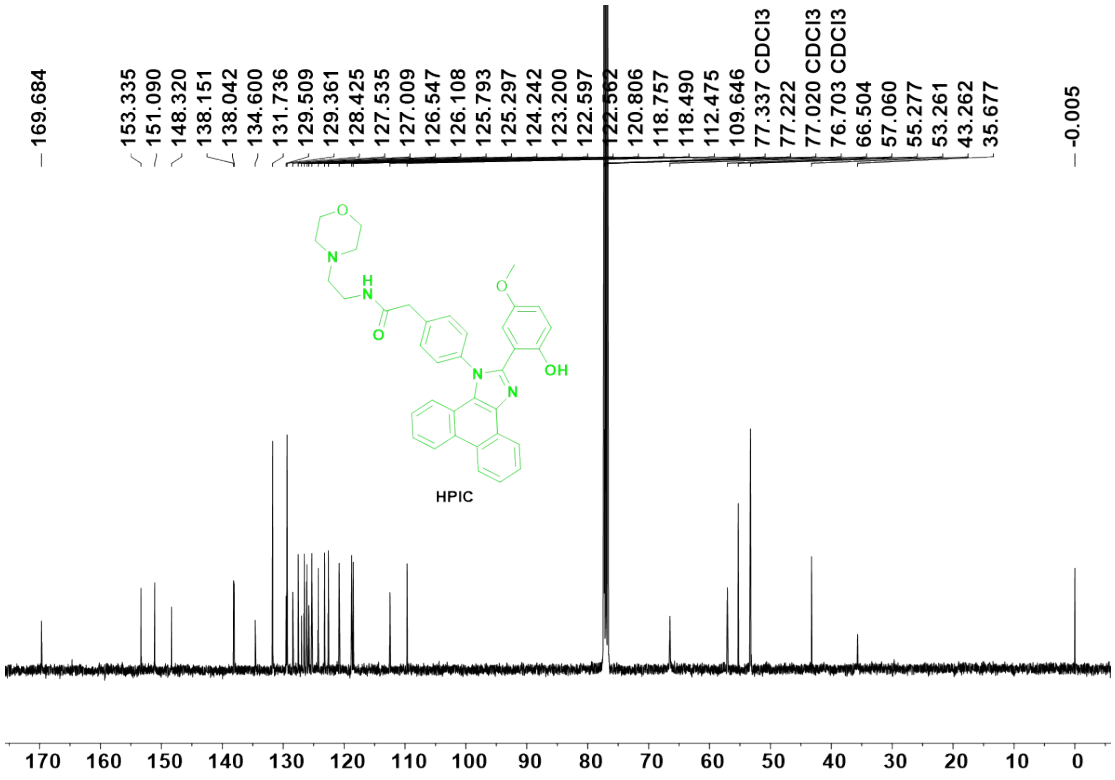

Fig. S9 ^{13}C NMR spectrum of HPIC in $CDCl_3$.

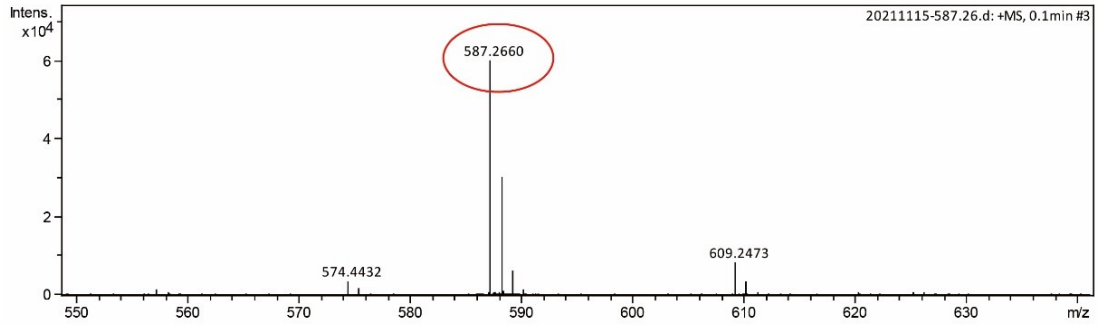

Fig. S10 HRMS of HPIC.

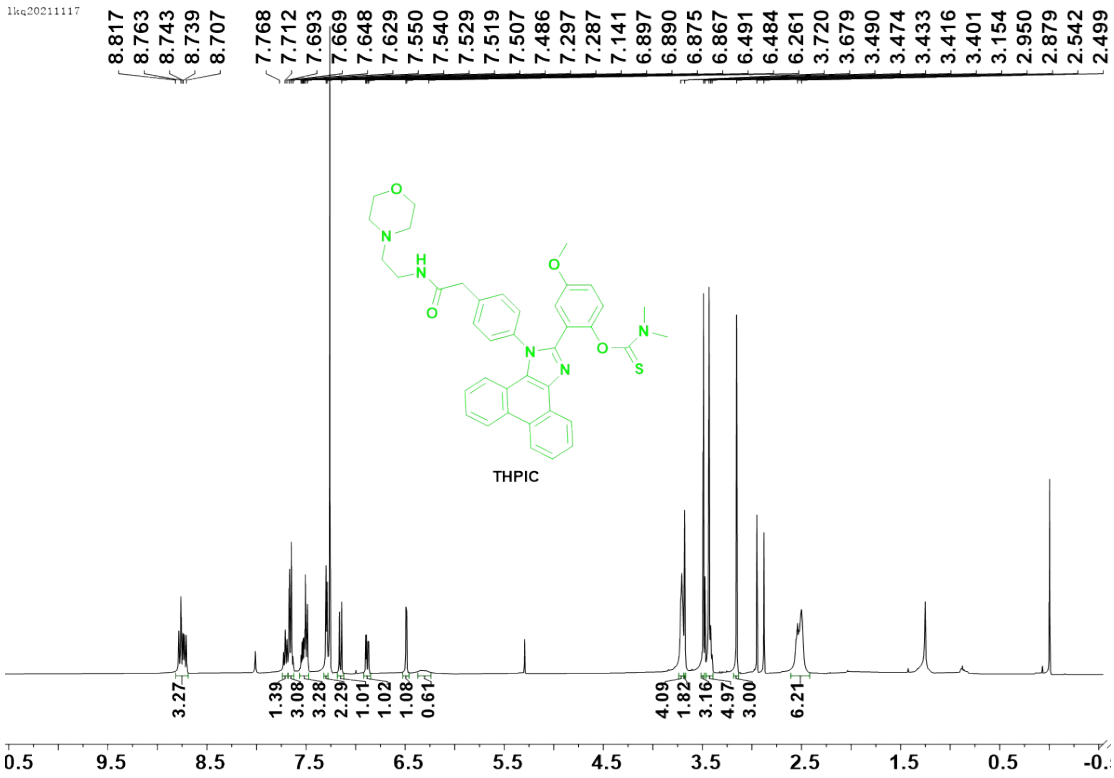

Fig. S11 ^{1}H NMR spectrum of **THPIC** in $CDCl_3$.

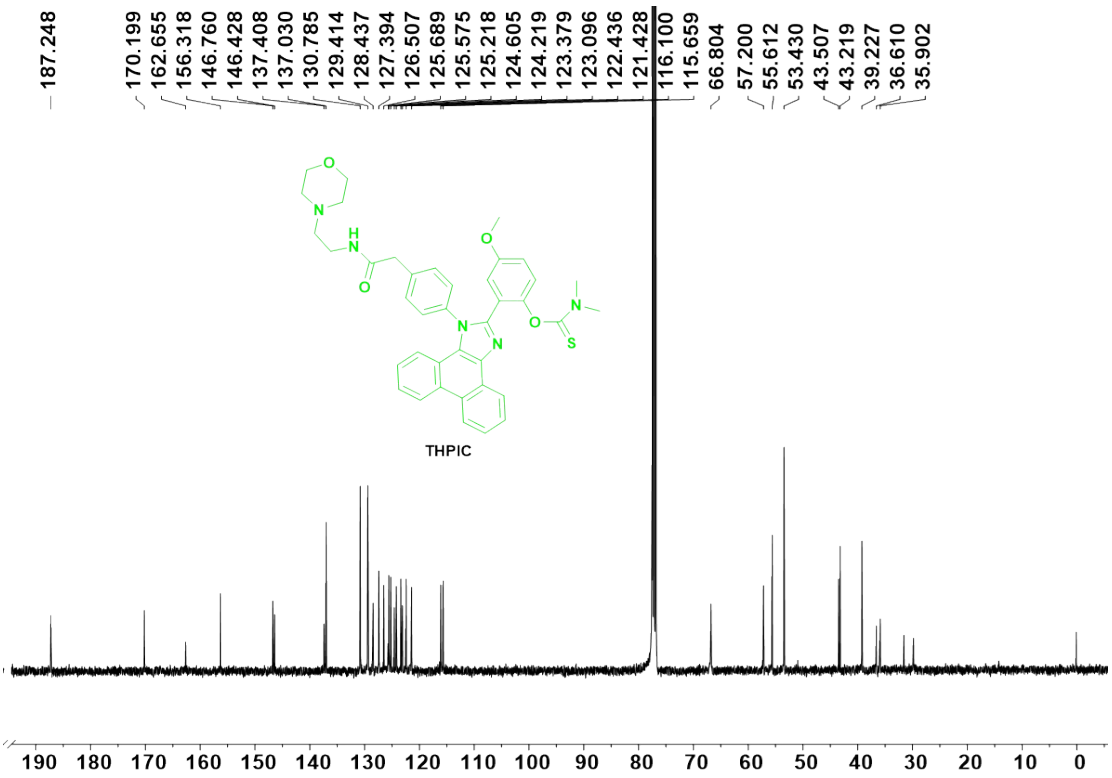

Fig. S12 ^{13}C NMR spectrum of **THPIC** in $CDCl_3$.

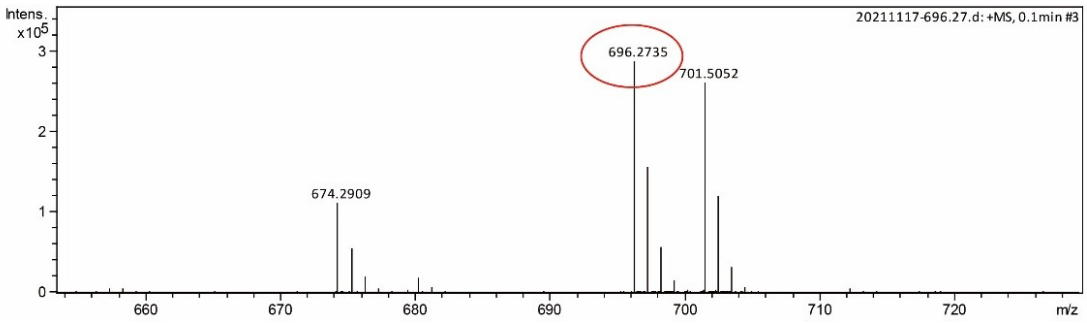

Fig. S13 HRMS of **THPIC**.

3. Cell culture

The H9C2 cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) with 1 % 100 U mL^{-1} antibiotics penicillin/streptomycin and 10 % fetal bovine serum (FBS) at 37 °C under a humidified atmosphere containing 5 % CO_2.

4. MTT assay

The cytotoxicity of the probe was tested in H9C2 cells using a standard MTT assay. The IC_{50} value was calculated according to the method of Huber and Koella[2]. It was displayed that the value of IC_{50} was 256 μM for **THPIC** (Fig. S19), which indicated the good biocompatibility for cell imaging.

5. Cell imaging

H9C2 cells were employed to perform the imaging.

Colocalization assay: H9C2 cells were washed with PBS three times and then co-incubated with Lyso-Tracker Green (3.0 μM) and **THPIC** (10.0 μM) for 15 min at 37 °C. After washing with PBS again, the cells were imaged by two-photon confocal microscopy. Lyso-Tracker Green: $\lambda_{ex}/\lambda_{em}$=488 nm/500-540 nm; **THPIC**: $\lambda_{ex}/\lambda_{em}$=720 nm/440-480 nm.

Two-photon confocal fluorescent imaging of exogenous HClO in H9C2 cells with THPIC:

The cells were washed with PBS for three times firstly. Group a: H9C2 cells were incubated with **THPIC** (5.0 μM) alone for 30 min, after removing **THPIC** solution, PBS was added again to image as control group. Group b: H9C2 cells were pretreated with HClO (20 μM) for 30 min and washed with PBS for three times; After culturing with **THPIC** (5.0 μM) for 30 min, the **THPIC** solution was removed and the imaging was carried out. Group c: H9C2 cells were pretreated with HClO (50 μM) for 30 min and washed with PBS for three times; After culturing with **THPIC** (5.0 μM) for 30 min, the **THPIC** solution was removed and the imaging was carried out. Group d: H9C2 cells were pretreated with HClO (100 μM) for 30 min and washed with PBS for three times; After culturing with **THPIC** (5.0 μM) for 30 min, the **THPIC** solution was removed and the imaging was carried out. Group e: H9C2 cells were pretreated with HClO (100.0 μM) and NAC (1.0 mM) for 30 min and washed with PBS for three times;

After culturing with **THPIC** (5.0 μM) for 30 min, the **THPIC** solution was removed and the imaging was carried out.

Two-photon confocal fluorescent imaging of endogenous HClO in H9C2 cells with THPIC:

The cells were washed with PBS for three times firstly. Group a: H9C2 cells were incubated with **THPIC** (5.0 μM) alone for 30 min, after removing **THPIC** solution, PBS was added again to image as control group. Group b: H9C2 cells were pretreated with PMA (2.0 μg/mL) for 30 min and washed with PBS for three times; After culturing with **THPIC** (5.0 μM) for 30 min, the **THPIC** solution was removed and the imaging was carried out. Group c: H9C2 cells were pretreated with PMA (2.0 μg/mL) and NAC (1.0 mM) for 30 min and washed with PBS for three times; After culturing with **THPIC** (5.0 μM) for 30 min, the **THPIC** solution was removed and the imaging was carried out.

Two-photon confocal fluorescent imaging of HClO in H9C2 cells treated with broad-spectrum anti-cancer drugs by THPIC:

The cells were washed with PBS for three times firstly. Group a: H9C2 cells were incubated with **THPIC** (5.0 μM) alone for 30 min, after removing **THPIC** solution, PBS was added again to image as control group. Group b-j: H9C2 cells were pretreated with difference anticancer drugs (20.0 μM) for 30 min and washed with PBS for three times; After culturing with **THPIC** (5.0 μM) for 30 min, the **THPIC** solution was removed and the imaging was carried out. Cyclophosphamide, Imatinib mesylate, Camptothecin, Cisplatin, Gefitinib, 5-Fluorouracil, Paclitaxel, Erlotinib, and Doxorubicin were used in groups b-j, respectively.

The screening of drugs that treat or prevent doxorubicin-induced cardiotoxicity in cardiomyocytes:

The cells were washed with PBS for three times firstly. Group a: H9C2 cells were pretreated with DMSO for 14 h, and then incubated with **THPIC** (5.0 μM) for another 30 min; After removing **THPIC** solution, PBS was added again to image. Group b: H9C2 cells were pretreated with DMSO (8 h) followed by doxorubicin (20.0 μM, 6 h), and then incubated with **THPIC** (5.0 μM) for another 30 min, and then the **THPIC**

solution was removed and the imaging was carried out. Group c-j: H9C2 cells were pretreated with various drugs (50.0 μM, 8 h) that treat or prevent doxorubicin-induced cardiotoxicity followed by doxorubicin (20.0 μM, 6 h), and then incubated with **THPIC** (5.0 μM) for another 30 min, and then the **THPIC** solution was removed and the imaging was carried out. Glutathione, Coenzyme Q10, Vitamin C, Kaempferol, Baicalein, Mevastatin were used in groups c-j, respectively.

6. Mouse models

Adult Female C57BL/6J mice (10-week-old) were purchased from the Experimental Animal Center of Shandong University (Jinan, PR China). All animal care and experimental protocols complied with the Animal Management Rules of the Ministry of Health of the People's Republic of China and were approved by the Animal Care Committee of Shandong Normal University.

Mice were housed in a facility with a 12-hour/12-hour light/dark cycle and were given free access to water and standard rodent chow. The room was kept specific pathogen-free.

Dox HCl (Anhui Zesheng Technology Co., Ltd.) was dissolved in saline and administered by intraperitoneal injection at a dose of 20 mg/kg.[3]

7. Two-photon imaging for the section of heart of mice

Mice were treated with saline or GSH (1 injection every other day for a total of 3 over a 5 days period, n=3) by i.p, and then Dox HCl (20 mg/kg) induced cardiac dysfunction for 5 days, followed by **THPIC** (50 μM) were injected into mice via i.p. 12 h after injection, the mice were anesthetized and dissected to isolate the hearts, which were then fixed with 4% paraformaldehyde for 12 h. Last, the samples were dehydrated with ethanol and embedded in paraffin before 6 μm sectioning of the left ventricles. Two-photon fluorescence images of HClO were acquired using a Zeiss LSM 880 confocal laser scanning microscope. The concentration of GSH was 100 mg/kg.

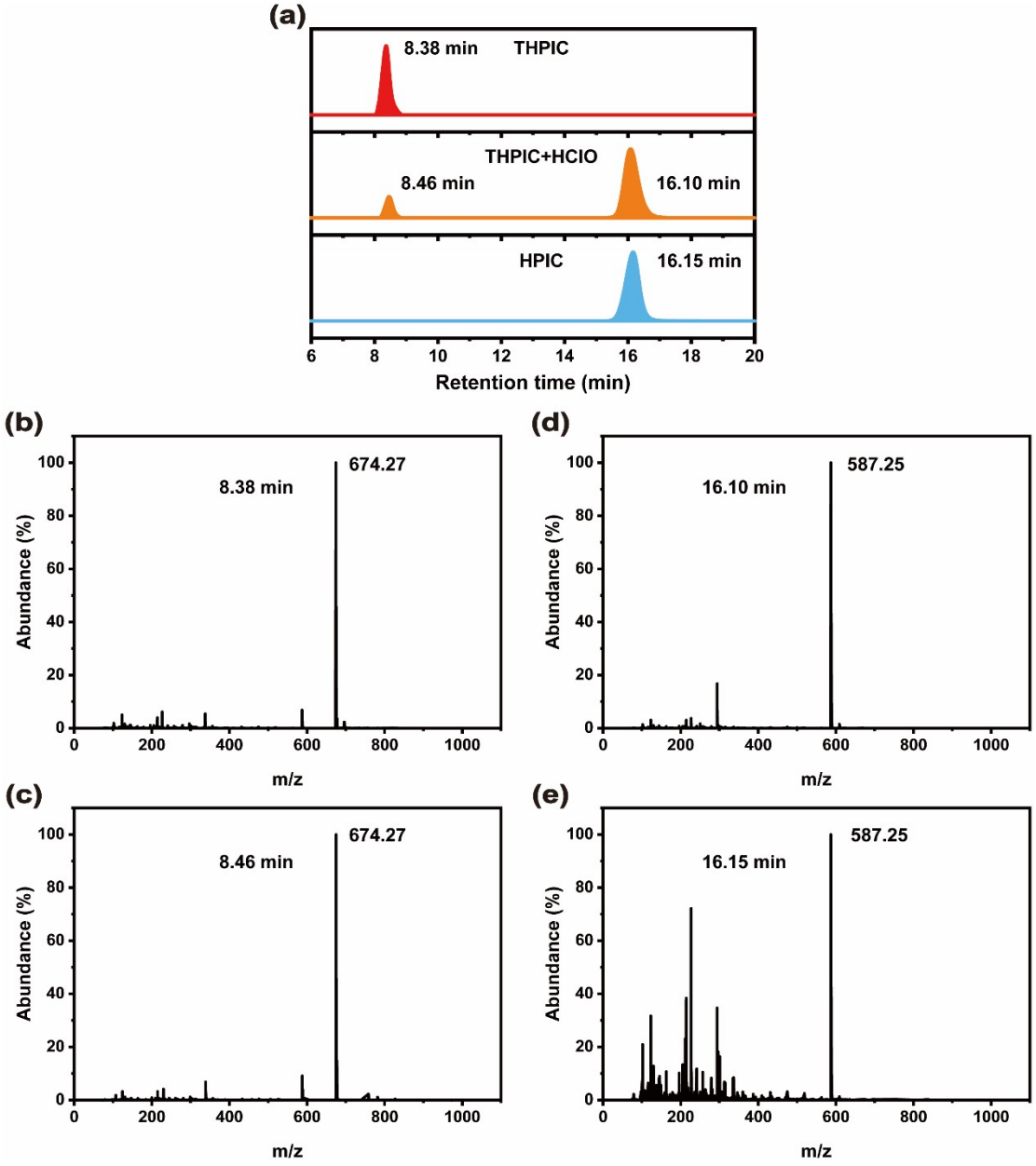

Fig. S14 (a) Validation of the proposed mechanism for **THPIC**. (b-e) The HPLC-MS analysis of **THPIC**, **THPIC** + HClO, HPIC. The mobile phase was the mixture of methanol/ H_2O (v/v, 8/2); conditions: flow rate, 0.6 mL/min; temperature, 37 °C; detection wavelength, 370 nm. The concentration of **THPIC**, HClO, HPIC were 300 μM.

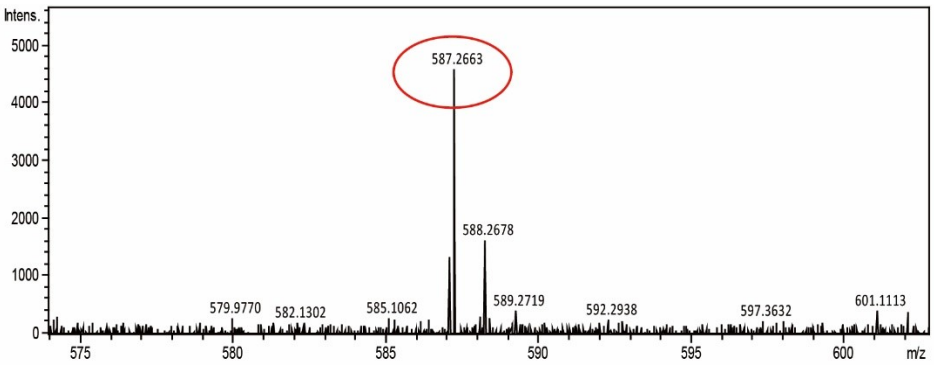

Fig. S15 The HRMS spectra of the solution containing **THPIC** (5.0 μM) and HClO (27.0 μM).

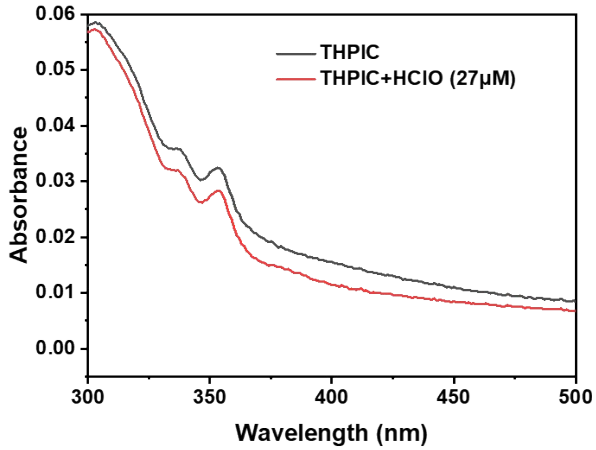

Fig. S16 Absorption spectra of **THPIC** (5.0 μM) in PBS buffer (1 % DMSO 100 mM, pH = 7.4) before (black) and after (red) the addition of HClO (27.0 μM).

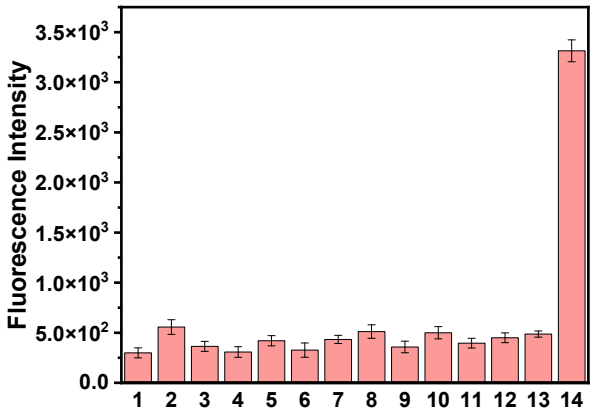

Fig. S17 Fluorescence of **THPIC** (5.0 μM) in the presence of various metal ions: 1. K^+、 2. Na^+、3. Ag^+、4. Ca^{2+} 、5. Mg^{2+}、6. Zn^{2+}、7. Cu^{2+}、8. Fe^{2+}、9. Hg^{2+}、10. Co^{2+}、11. Al^{3+}、12. Fe^{3+}、13. blank、14. HClO (27.0 μM). The concentration of metal ions used were 1 mM.

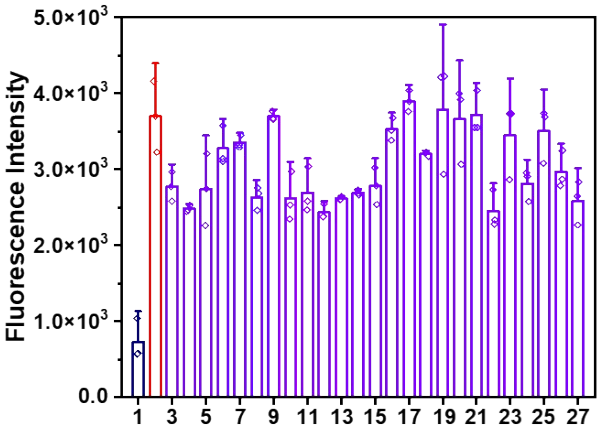

Fig. S18 Fluorescence of **THPIC** (5.0 μM) in the co-presence of HClO (27.0 μM) and various analytes (27.0 μM). 1. Blank, 2. HClO, 3. Hcy, 4. GSH, 5. Cys, 6. Vc, 7. H_2O_2, 8. $O_2^{\bullet-}$, 9. $^1O_2$, 10. •OH, 11. NO, 12. $ONOO^-$, 13. $NaHSO_3$, 14. Na_2S, 15. NaHS, 16. K^+, 17. Na^+, 18. Ag^+, 19. Ca^{2+}, 20. Mg^{2+}, 21. Zn^{2+}, 22. Cu^{2+}, 23. Fe^{2+}, 24. Hg^{2+}, 25. Co^{2+}, 26. Al^{3+}, 27. Fe^{3+}. $\lambda_{ex}/\lambda_{em}$=370 nm/501 nm.

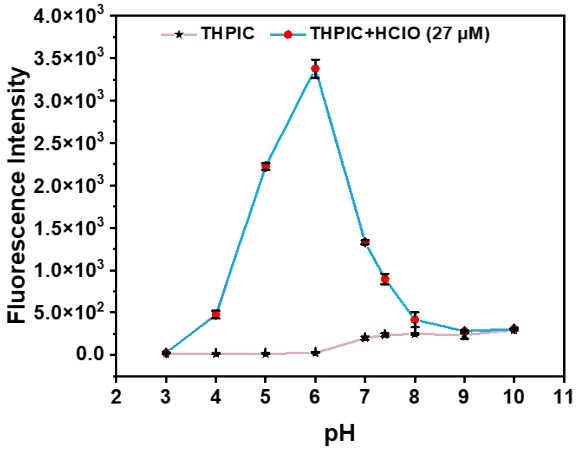

Fig. S19 Effects of pH on the fluorescence intensity of **THPIC** (5.0 μM) in the absence (pink) and presence (blue) of HClO (27.0 μM) at room temperature. $\lambda_{ex}/\lambda_{em}$=370 nm/501 nm.

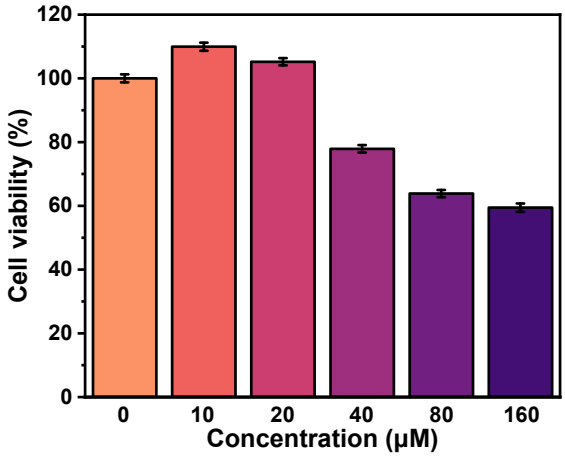

Fig. S20 MTT assay of H9C2 cells with different concentrations of **THPIC** (0 μM, 10 μM, 20 μM, 40 μM, 80 μM, 160 μM).

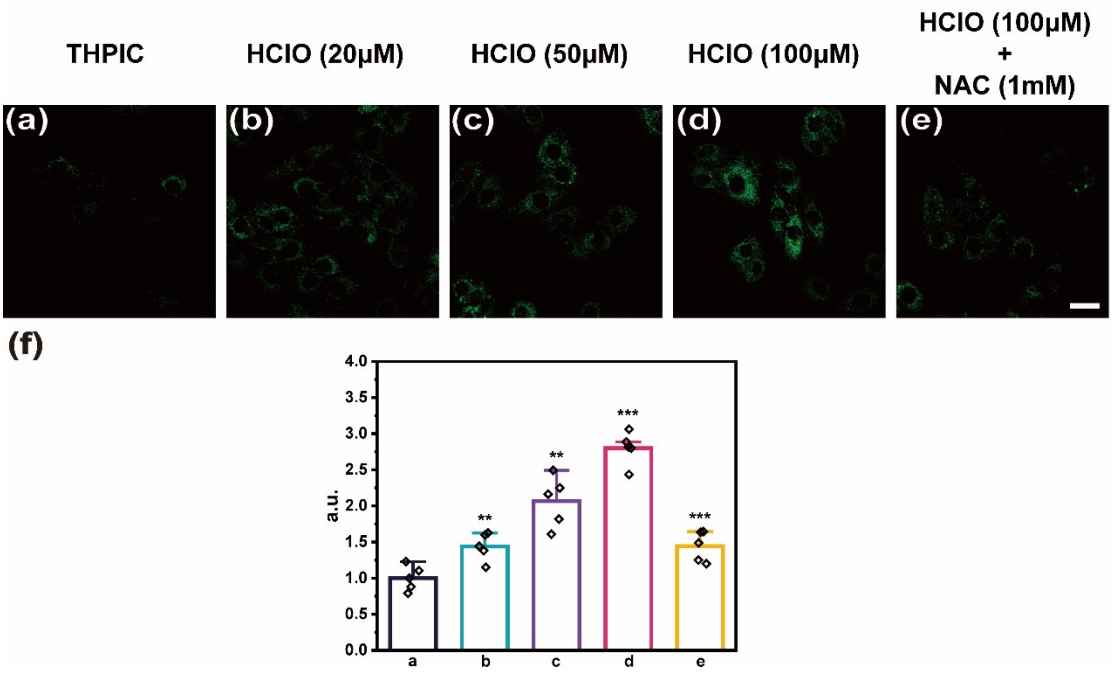

Fig. S21 Two-photon fluorescence imaging of exogenous HClO with **THPIC** (5.0 μM) in H9C2 cells. $\lambda_{ex}/\lambda_{em}$=720 nm/500-540 nm. The values are the mean ± s.d. for n =5, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar: 40 μm.

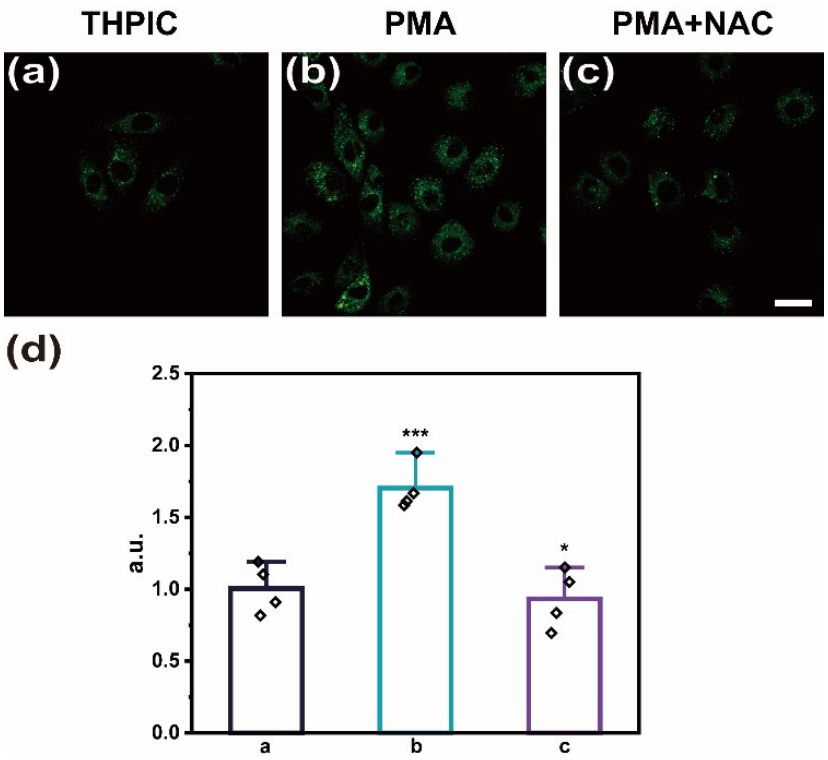

Fig. S22 Two-photon fluorescence imaging of endogenous HClO with **THPIC** (5.0 μM) in H9C2 cells. λex/λem=720 nm/500-540 nm. The values are the mean ± s.d. for n =4, *p < 0.05, **p < 0.01, ***p < 0.001. Scale bar: 40 μm.

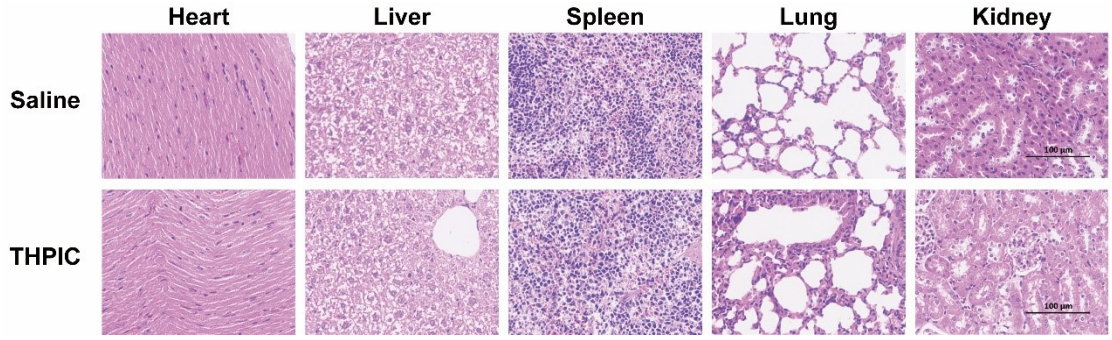

Fig. S23 Representative photomicrographs of H&E staining in paraffin-embedded different organs sections from different experimental groups (salaine, **THPIC**).

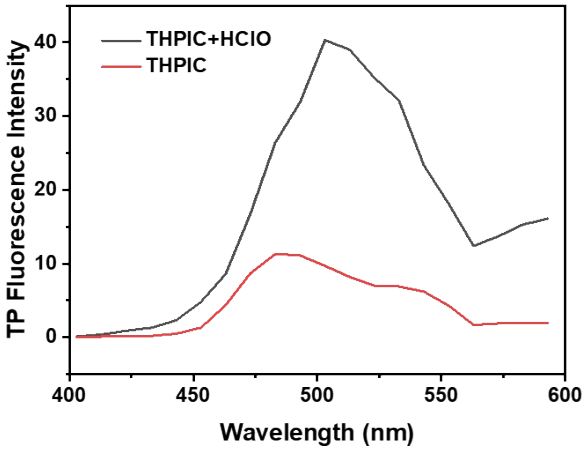

Fig. S24 The two-photon fluorescence spectrum of **THPIC** (red) and **THPIC**+HClO (black). The two-photon absorption cross section was determined as 7.3 GM for **THPIC** (100 μM) and 8.6 GM for **THPIC**+HClO (270 μM). **THPIC** emitted at approximately 510 nm and was excited at 720 nm (TP).

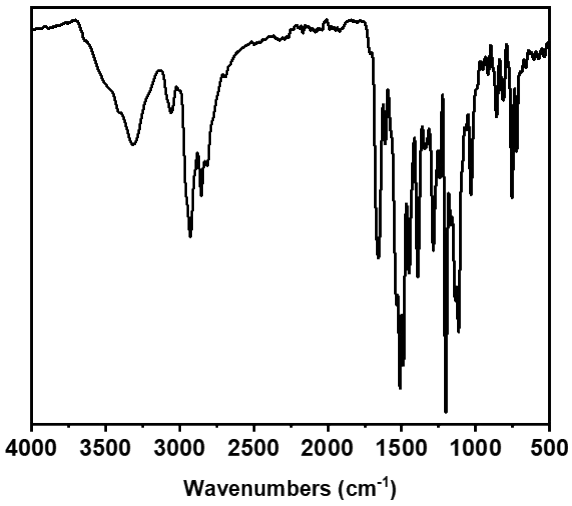

Fig. S25 The FTIR of **THPIC**.

1. W. J. Newsome, S. Ayad, J. Cordova, E. W. Reinheimer, A. D. Campiglia, J. K. Harper, K. Hanson, F. J. Uribe-Romo, Solid State Multicolor Emission in Substitutional Solid Solutions of Metal-Organic Frameworks. *J Am Chem Soc*., 2019, **141**, 11298-11303.

2. T. Bulus, AB. Ahmed, Determination of IC50 and IC90 Values of Ethanolic Extracts of Some Medicinal Plants against Trypanosoma brucei.

3. A. Riad, S. Bien, D. Westermann, P. M. Becher, K. Loya, U. Landmesser, H. K. Kroemer, H. P. Schultheiss, C. Tschöpe, Pretreatment with statin attenuates the cardiotoxicity of Doxorubicin in mice, *Cancer Res*., 2009, **69**, 695–699.