Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Metabolic iron detection through divalent metal transporter 1 and ferroportin mediated cocktail fluorogenic probes

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Materials and Methods

General information

Reagents were purchased as follows: ampicillin (Beyotime, Shanghai, China) and isopropyl 1-thio-β-D-galactopyranoside (IPTG) (damas-beta, Shanghai, China). Unless otherwise noted, all reagents were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel (200-300 mesh). ^{1}H NMR spectra were recorded using BRUKER DRX 500 spectrometer and ^{13}C NMR was recorded using 1 BRUKER DRX 500 spectrometer. Chemical shifts were expressed in ppm and coupling constants (J) in Hz. Mass spectrometry was recorded with Agilent-1100 HPLC/TOF mass spectrometer. The UV-Vis absorption spectrum was collected from the UV-240IPC spectrophotometer by the dual light path method. The fluorescence spectrum is obtained from an F-380 spectrophotometer (GANGDONG SCI.&TECH.CO, LTD, Tianjin, China), the emission and detection slits are 5 nm and the voltage is 650 V during the test. All cell and Caenorhabditis elegans (*C. elegans*) fluorescence confocal imaging was taken from sted ultra high-resolution confocal laser microscope (TCS SP8 STED, Leica, Germany). In order to avoid light drift, all incubation processes with added probes or dyes are carried out in the dark. General information and methods. The solution used for in vitro assessment under-went freeze-thaw degassing treatment thrice to avoid the interference of dissolved oxygen.

Synthesis process

Synthesis of compound 5, 6, 7 and 2

Compound **5, 6, 7** and **2** were synthesized according to the literature we have reported previously. [1]

Compound **2:**^{1}H NMR (500 MHz, DMSO) δ 8.622-8.611 (d, 1H, *J*=5.28 Hz), 8.459 (s, 1 H),8.303-8.281(d, 1H, *J*=8.83 Hz),7.977-7.896 (m, 3 H), 7.691-7.672 (d, 1H, *J*=7.86 Hz), 7.647-7.555 (m, 2 H), 7.478 (s,2 H), 7.430-7.339 (t, 1 H, *J*=6.62 Hz), 7.244–7.223 (q, 1 H), 7.052–7.033 (d, 1 H, *J*=7.53 Hz), 6.357 (s, 2H), 6.256 (s, 2H), 5.387 (s, 2 H), 5.121-5.095 (t,2 H, *J*=5.24 Hz), 3.104-3.064 (t, 4 H, *J*=7.58 Hz), 1.835 (s, 6H), 1.174-1.138 (t, 6 H, *J*=7.58 Hz); HRMS: [M+H] calcd for $C_{42}H_{39}N_6O_3^+$: 675.3078; found, 675.3078. [M+Na] calcd for $C_{42}H_{38}N_6NaO_3^+$: 697.2898; found, 697.2900.

Synthesis of compound 4

Compound **4** was prepared according to the literature method. [2]

Synthesis of compound 3

Compound **4** (111 mg, 0.6 mmol) and 4-(diethylamino)benzaldehyde (106 mg, 0.6 mmol) were dissolved in toluene (10 mL) with piperidine (0.15 mL) and acetic acid (0.15 mL) under nitrogen protection at room temperature. The resulting mixture was refluxed for 4 hours. Then the reaction mixture was cooled to

room temperature and condensed. The obtained crude product was purified by silica gel column chromatography (PE:CH_2Cl_2 = 2:1 to 1:1) to yield compound 2 as a green solid (165 mg, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (d, *J* = 8.9 Hz, 2H), 7.04 (d, *J* = 15.9 Hz, 1H), 6.84 – 6.68 (m, 4H), 3.44 (q, *J* = 7.1 Hz, 4H), 2.59 (s, 2H), 2.47 (s, 2H), 1.23 (t, *J* = 7.1 Hz, 6H), 1.09 (s, 6H).^{13}C NMR (126 MHz, CDCl3) δ 169.09, 155.40, 138.28, 129.75, 123.77, 121.13, 114.30, 113.53, 111.60, 110.60, 44.52, 43.04, 39.33, 31.96, 29.67, 28.04, 12.62. HRMS: [M+H] calcd for $C_{23}H_{27}N_3$, 346.2278; found, 346.2277.

Synthesis of compound 1 [3,4]

To a solution of compound **3** (207 mg, 0.6 mmol) in EtOAc (15 mL) was added $NaHCO_3$ (104 mg, 1.2 mmol) and m-CPBA (182 mg, 0.74 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 50:1 to 20:1, v/v) to afford 108 mg as a red solid (30% yield).

Compound **1**: ^{1}H NMR (500 MHz, DMSO) δ 7.97 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 16.3 Hz, 1H), 7.33 (d, J = 16.2 Hz, 1H), 6.93 (s, 1H), 3.88 (dd, J = 11.8, 6.6 Hz, 2H), 3.53 (d, J = 4.5 Hz, 2H), 2.64 (s, 2H), 2.56 (s, 2H), 1.03 (s, 6H), 0.94 (t, J = 6.8 Hz, 6H).^{13}C NMR (126 MHz, DMSO) δ 170.74, 155.89, 136.42, 131.25, 128.36, 123.77, 123.16, 114.19, 113.40 77.45, 66.37, 55.34, 42.80, 38.69, 32.15, 27.89, 8.40. HRMS: [M+H] calcd for $C_{23}H_{27}N_3O$, 362.2227; found, 362.2227.

Spectrophotometric experiments

Accurately weigh a certain amount of compound **1** and compound **2** then dissolved in a certain volume of DMSO or absolute alcohol to prepare a probe stock solution with a concentration of 10 mM to prepare for the subsequent experiments. Prepare several 5.0 mL buffer solutions of DMSO-PBS (0.01M, pH = 7.4). When preparing for the test, take 5.0 uL of the probe stock solution and dilute it into the buffer solution to prepare a final concentration of 10 μM test solution. Then add different amounts of Fe^{2+}/Fe^{3+} or excess (1.0 mM) of various analytes to shake well for 5 minutes, then transfer appropriate amounts to a standard quartz cuvette of 1.0 cm × 1.0 cm for UV absorption and fluorescence emission tests. The stock solutions of **1** and **2** were diluted into the mixed solutions of DMSO/PBS (0.01M, pH = 7.4, v/v = 8/2), and the final concentration of **1** and **2** was maintained at 10 μM, respectively to construct the dual-fluorescent sensor system [**1+2**]. The solutions were then divided into two groups. One group was added with different concentrations of Fe^{2+} (0-650 μM $FeCl_2{\cdot}4H_2O$), and the other group was added with different concentrations of Fe^{3+} (0-740 μM $FeCl_3{\cdot}6H_2O$). For monitoring the dynamic conversion process from Fe^{3+} to Fe^{2+}, 5.0 μL stock solution of $FeCl_3{\cdot}6H_2O$ was added into a 5.0 mL mixed solution, containing 10.0 μM **1** and 10.0 μM **2**. Five min later, sodium ascorbate was added into the mixed solution to obtain the final concentration to be 1.0 mM. Finally, the emission spectra were recorded at 1, 2, 3, 4, 5, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, and 100 min, respectively in the range of 500-800 nm by a F-380 spec-trophotometer (Ex=480 nm, slit=5 nm).

Compound **1** and fluorophore (compound **3**) were dissolved in DMSO to prepare stock solutions (10 mM), respectively. $FeCl_2 \cdot 4H_2O$ was dissolved in degassed water to prepare a stock solution (1.0 M). The stock solutions of compound **1** and fluorophore were diluted to 10 μM with DMSO/PBS (0.01M, pH = 7.4, v/v = 8/2), respectively. Next, 5.0 μL stock solution of $FeCl_2 \cdot 4H_2O$ was added into the 5.0 mL mix solutions containing 10 μM compound **1.** Five minutes later, the emission spectrum was measured in the range of 600-780 nm by a F-380 spectrophotometer (Ex=535 nm, slit=5 nm). Spectra of 10 μM compound **1** and fluorophore were collected under identical conditions.

Compound **1** titration with Fe^{2+}: Different concentrations (0-650 μM) of $FeCl_2 \cdot 4H_2O$ were sequentially diluted into the mix solutions containing 10 μM compound **1** After 5 min of reaction, the emission spectra were measured in the range of 600-780 nm in a F-380 spectrophotometer (Ex=535 nm, slit=5 nm).

Compound **2** titration with Fe^{3+}: compound **2** was solubilized in absolute ethanol to prepare a stock solution (10 mM), and the $FeCl_3 \cdot 6H_2O$ were dissolved in degassed water to prepare stock solutions (1.0 M). The compound **2** stock solutions was diluted to 10 μM with DMSO/PBS (0.01M, pH = 7.4, v/v = 8/2). Next, different concentrations (0-740 μM) of $FeCl_3 \cdot 6H_2O$ were sequentially diluted into the mix solution containing 10 μM compound **2**. After 5 min of reaction, the emission spectra were measured in the range of 500-700 nm in a F-380 spectrophotometer (Ex=480 nm, slit=5 nm).

For the Selectivity experiment, interferences of various bio-analytes towards **[1+2]** was also monitored, including common metal cations (Na^+, K^+, Zn^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}), amino acid (His, Glu, Arg, Ile, Val, Tyr, Leu, Gln), phosphatase (ALP and ACP), redox stress related species: ascorbic acid (Vitamin C), hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), GSH glutathione (GSH), Homocysteine (Hcy), Nicotinamide adenine dinucleotide (NADH) and cholesterol. Various interference analytes (final concentrations of all analytes were 1.0 mM) were added to **[1+2]** solution, separately. After 5 min of reaction, the emission spectra were measured in the range of 500-800 nm in a F-380 spectrophotometer (λ_{ex}=480 nm, slit=5 nm).

The Limit of detection (LOD) calculation method and details are basically the same as those reported.[1]

To analyze the effect of pH, the fluorescence intensity was conducted under physiological pH ranges (pH 6.0-8.0). The experiment was carried out at the pH range 6.0-8.0 in DMSO/PBS (v/v = 8/2, 0.01 M PBS buffer). The range of pH from 6.0-8.0 (0.5 interval) was determined by an accurate range pH test paper.

Cell culture

HeLa cells were cultured in high glucose DMEM (Gibco) medium supplemented with FBS (10% V/V Gibco), 1% penicillin-streptomycin at 37 °C in a 5% CO_2 and 95% air incubator. HepG2 cells were cultured in high glucose DMEM (Gibco) medium supplemented with FBS (15% v/v Gibco),1% penicillin-streptomyci at 37 °C in a 5% CO_2 and 95% air incubator.

Cytotoxicity

Hepatoma cells (HepG-2), lung cancer cells (A-549), human cervical cancer cells (HeLa), human colorectal adenocarcinoma cells (Caco2), human normal lung cells (LO2) and human normal lung epithelial cells (BEAS-2B) were prepared into single cell suspension in a medium (DMEM or RMPI1640) containing 10% fetal bovine serum (FBS). Afterwards, 3000 to 5000 cells per well (100 μL) were inoculated into 96-well plates for 12 to 24 hours in advance. The compounds (compound **2** and compound **1**) were dissolved in DMSO to prepare a stock solution of a certain concentration, and an appropriate volume of the compounds stock solution is added to a 96-well plate so that the final concentration of the compounds in each well is 40 μM, and the final volume of each well is 200 μL. After 48 hours of incubation in a cell constant temperature incubator at 37 °C, discard the culture medium in the 96-well plate, add 20 μL MTS solution and 100 μL fresh culture medium to each well, and then incubate for 3 hours. After the MTS is fully reacted, use the multi-function microplate reader (MULTISKAN FC) to record the absorbance value of each well at 492 nm.

Cell CLSM imaging of compound 1/compound 2

Compound **1:** For the CLSM imaging, cells were seeded onto Cell Culture Dish (170 μm, 35 x 10 mm) 24 hours before imaging. The culture medium was replaced by 1 ml PBS. Then the cells were incubation with 100 μM glutathione ethyl ester for 30 min at 37 °C to consumes the intracellular oxidant. $FeCl_2 \cdot 4H_2O$ (final concentration at 50 μM, 100μM, 200μM) was added and then the cells were incubated for 1 h under argon at 37°C. The cells were washed twice with 2.0 mL PBS at room temperature. Following fixed with formaldehyde (4%), the cells were incubation with final concentration of 5.0 μM of Compound **1** (PBS/DMSO, 100/1, v/v) for 1 h at 37 °C. Then HeLa cells were stained with DAPI (1.0 μM) for 30 min. The cells were washed three times with 2 mL PBS at room temperature before fluorescence imaging. Finally, use a STED ultra high-resolution confocal laser microscope (TCS SP8 STED) to perform fluorescence imaging with a 40 × objective lens. Channel selection: DAPI (λ_{ex}=359 nm, λ_{em}=461 nm); Compound **1** (λ_{ex} =480 nm, λ_{em}=670-750 nm);

Compound **2:** Following medium was replaced by 1.0 ml PBS, $FeCl_3 \cdot 6H_2O$ (final concentration at 50 μM, 100 μM, 200 μM) was added and then the cells were incubated for 1 h at 37°C. After that, cells were washed twice with 2.0 mL PBS at room temperature. Following fixed with formaldehyde (4%), the cells were incubation with final concentration of 5.0 μM of compound **2** (PBS/EtOH, 100/1, v/v) for 1 h at 37 °C. Nuclei were stained with DAPI and imaging was performed on the same microscope with a 40 × objective lens. Channel selection: DAPI (λ_{ex}=359 nm, λ_{em}=461 nm); compound **2** (λ_{ex}=480 nm, λ_{em}=500-580 nm);

Cell CLSM imaging of [1+2]

For exogenous iron (Fe^{2+}/Fe^{3+}) imaging with **[1+2]**, one group of HeLa cells was treated with $FeCl_2 \cdot 4H_2O$ (100 μM) or $FeCl_2 \cdot 4H_2O$ (100 μM) and $FeCl_3 \cdot 6H_2O$ (100 μM) in air. The other group of HeLa cells was

treated with 100 μM GSH-ester and then incubated with $FeCl_2 \cdot 4H_2O$ (100 μM) or $FeCl_2 \cdot 4H_2O$ (100 μM) and $FeCl_3 \cdot 6H_2O$ (100 μM) in argon. Following fixed with formaldehyde (4%), the cells were incubation with final concentration of compound **1 (**5.0 μM) and compound **2 (**5.0 μM) for 1 h at 37 °C. All cells were stained with DAPI and imaging was performed on the same microscope with a 40 × objective lens. Channel selection: DAPI (λ_{ex}=359 nm, λ_{em}=461 nm); green (λ_{ex}=480 nm, λ_{em}=500-580 nm); red (λ_{ex}=480 nm, λ_{em}=670-750 nm).

For endogenous iron imaging with **[1+2],** the adherent HeLa cells were cultured and grown in a medium supplemented with 100 μM ferric ammonium citrate (AFC) for 24 h. The cells were fixed with 4 % formaldehyde for 20 min and then stained with **1 (**5.0 μM) and **2** (5.0 μM) for 1 h at 37 °C. All cells were stained with DAPI, and the imaging was performed by the Stimulated Emission Depletion Microscopy (STED) ultrahigh-resolution confocal laser microscope (TCS SP8 STED) with a 40 × objective lens. Channel selection: DAPI (λ_{ex}=359, λ_{em}=461 nm); green (λ_{ex}=480, λ_{em}=500-580 nm); red (λ_{ex}=480, λ_{em}=670-750 nm).

For cross-color interference of **[1+2],** cells were categorized into two groups, one was incubated with $FeCl_2 \cdot 4H_2O$ (100 μM) and **1** (5.0 μM), the other one was incubated with $FeCl_3 \cdot 6H_2O$ (100 μM) and **2** (5.0 μM).

Cell RNA interference and CLSM imaging.

The siRNA (SLC40A1; DMT1) were purchased from GenePharma (Shanghai, China). The following four siRNA sequences were used: SLC40A1: 5'-GCU GCU AGA AUC GGU CUU UTT-3' (F), 5'-AAA GAC CGA UUC UAG CAG CTT-3'(R); DMT1: 5'-GCU AGA CUG GGA GUG GUU ATT-3' (F), 5'-UAA CCA CUC CCA GUC UAG CTT-3'(R). The siRNA transfection of HepG2 cells was performed in a serum-free and antibiotic-free DMEM medium after 12 hours of plating. Briefly, the siRNA (20 μM) was premixed with Lipofectamine 2000 and added to the cell culture medium dropwise. 12 h later, the transfection mixture was changed to a normal medium supplemented with 100 μM AFC, and then the cells were cultivated for 24 h at 37 ℃. Finally, the imaging was performed according to the above cell imaging method**.**

Protein expression levels assay by western blotting

The interfered cells were harvested 48 hours after transfection. Following washed with PBS buffer, cells were lysed on ice for 30 minutes in lysis buffer RIPA (Solarbio, Beijing) containing ~1% (V/V) PMSF. The supernatant was collected from lysis buffer by centrifugation at 12,000 rpm for 18 minutes at 4 °C. Loading Buffer was added according to the concentration that was measured with BCA Protein Assay kit (Tiangen, Beijing), Each well was loaded with 5.0 μg protein for SDS-polyacrylamide gel electrophoresis. The Fpn and DMT1 rabbit polyclonal antibodies obtained from Novusbio Inc. (USA). The primary antibody used for internal control and the secondary antibody refer to the previous method [5].

C. elegans culture and CLSM imaging

For C. elegans culture, *wild-type* C*. elegans strain* (N2) were cultured on Nematode Growth Medium (NGM) at 20 °C. All NGMs were mixed with OP50 as nematode food. After washing with M9 buffer, gravid *C. elegans* was lysed by 20% alkaline hypochlorite solution. Then, eggs were collected to acquire age-synchronized nematodes. For Prior to CLSM imaging, imaging, transfer the nematodes of synchronized L4 stage using sterile M9 buffer to a centrifuge tube. Following centrifuged the nematodes and discard the supernatant, divide the nematodes into different 1.5 mL centrifuge tubes as required by the experiment.

For the imaging of exogenous iron, the *C. elegans* were incubated in de-aerated M9 buffer with $FeCl_2{\cdot}4H_2O$ (1.0 mM) or $FeCl_2{\cdot}4H_2O$ (1.0 mM) and $FeCl_3{\cdot}6H_2O$ (1.0 mM) under argon for 3 h. Following fixed with formaldehyde (4%), nematodes were stained with the **[1+2]** (20 μM Compound **1** and 20 μM Compound **2**) for 2 h. Ultimately, fluorescence imaging was performed by the STED ultra high-resolution confocal laser microscope (TCS SP8 STED). Channel selection: red ((λ_{ex}=480 nm, (λ_{ex}=670-750 nm); green ((λ_{ex}=480 nm, (λ_{ex}=500-580 nm).

For cross-color interference of **[1+2],** C. *elegans* were divided into two groups, one group was incubated in de-aerated M9 buffer with $FeCl_2{\cdot}4H_2O$ (1.0 mM) under argon for 3 h. After washing thrice with de-aerated M9 buffer, nematodes were fixed with formaldehyde (4 %) for 30 min and finally stained with 20 μM of compound **1** for 2 h. Analogously, the other group was incubated in de-aerated M9 buffer with $FeCl_3{\cdot}6H_2O$ (1.0 mM) under argon for 3 h. After washing thrice with de-aerated M9 buffer, nematodes were fixed with formaldehyde (4 %) for 30 min and stained with 20 μM of compound **2** for 2 h. For the imaging of endogenic iron, nematodes were cultured on NGM supplemented with 2.5 mg/mL AFC at 20 ℃ for about 2 days. Afterward, nematodes were fixed with formaldehyde (4 %) for 30 min and stained with **1** (20 μM) and **2** (20 μM) for 2 h. Finally, fluorescence imaging was performed by the STED ultrahigh-resolution confocal laser microscope (TCS SP8 STED). Channel selection: red ((λ_{ex}=480, (λ_{ex}=670-750 nm); green ((λ_{ex}=480, (λ_{ex}=500-580 nm).

C. elegans RNA interference and CLSM imaging.

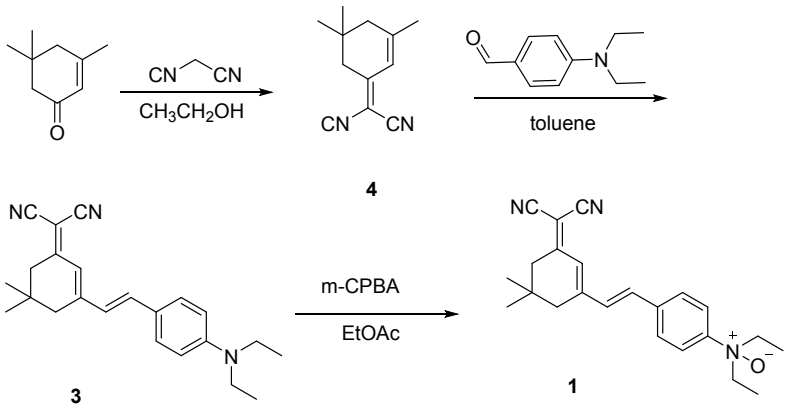
RNAi bacterial strains with the carriers of expressing double-stranded RNA (dsRNA) of target genes were obtained from the State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan.[22] Firstly, *E. coli* HT115 was cultured in a standard Luria–Bertani medium (broth) (LB) supplemented 100 μg/ml ampicillin at 37 ℃ for 8-12 h as a negative control. Besides, 100-300 μL bacterial suspension was allocated upon NGM plates incorporated with 100 μg/ml ampicillin and 5.0 mM IPTG. These dsRNA-expressing bacteria were permitted to grow for 18 h at 37 ℃. Then, *C. elegans* at L1 stage were inoculated on NGM containing different mutant strains at 20 ℃. When *C. elegans* reached L4 stage, the nematodes were transferred into NGM supplemented with 2.5 mg/mL AFC for 8-10 h at 20 ℃. *C. elegans* with RNA interference were fixed with formaldehyde (4 %) and stained with 20 μM of **1** for 2 h.

Finally, the fluorescence imaging was performed by the STED ultra high-resolution confocal laser microscope (TCS SP8 STED). Channel selection: red (λ_{ex}=480, λ_{em}=670-750 nm); green (λ_{ex}=480, λ_{em}=500-580 nm).

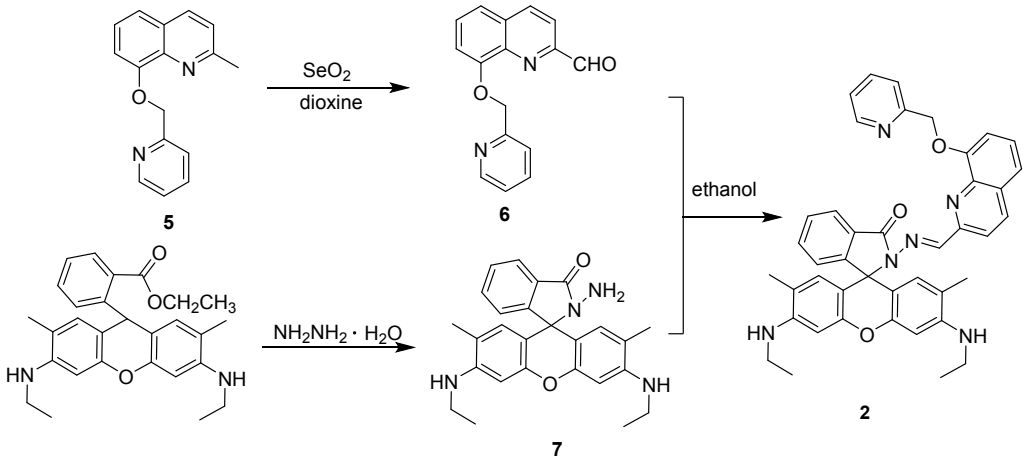
mRNA assay by RT-qPCR

Total RNA was extracted from *C.elegans* with Total RNA Extraction Kit (Solarbio, Beijing). The cDNAs were obtained from the reverse transcription of total RNA samples with PrimeScript™ RT reagent kit (TaKaRa, Dalian, China). A RT-qPCR analysis was performed on a Roche LightCycler 480 System (Roche Applied Science, Penzberg, Germany). using TB Green Premix Ex TAq™ II (Takara, Dalian, China). The relative amount of smf-1, fpn-1.1, fpn-1.2 and fpn-1.3 mRNA to act-1 mRNA was calculated using the method described previously.[22] The primers used for PCR were as follows: smf-1: 5'- CGG TAT GGC ATC GTC TAA-3' (F), 5'- TTT CCT GGG TCC AAA TAG-3' (R); fpn-1.1: 5'- ATA ACC TCG CCG CAT CCT -3' (F), 5'- GAA CCG AAA CTC CGC ACA -3' (R); fpn-1.2: 5'-GAC GGG AAT AAC GCT ACA-3'(F), 5'- TTC TGG GAT GGT GAC TTG-3' (R); fpn-1.3: 5'- ACC ATT CAA CGC CAC CAC -3'(F), 5'- CGC CAA AGC CTG ATC TTC-3' (R).

Synthesis schemes



Scheme S1. Synthetic routine of compound **1.**



Scheme S2. Synthetic routine of compound **2**

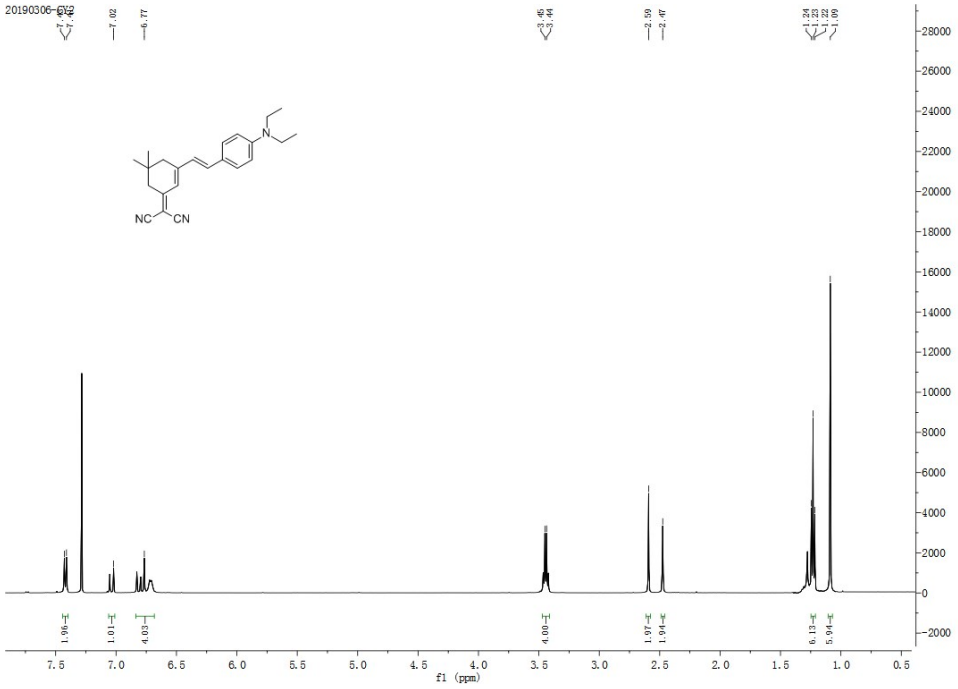


Fig. S1 ^{1}H NMR (500 MHz, $CDCl_3$, 298 K) spectra of compound **3**.

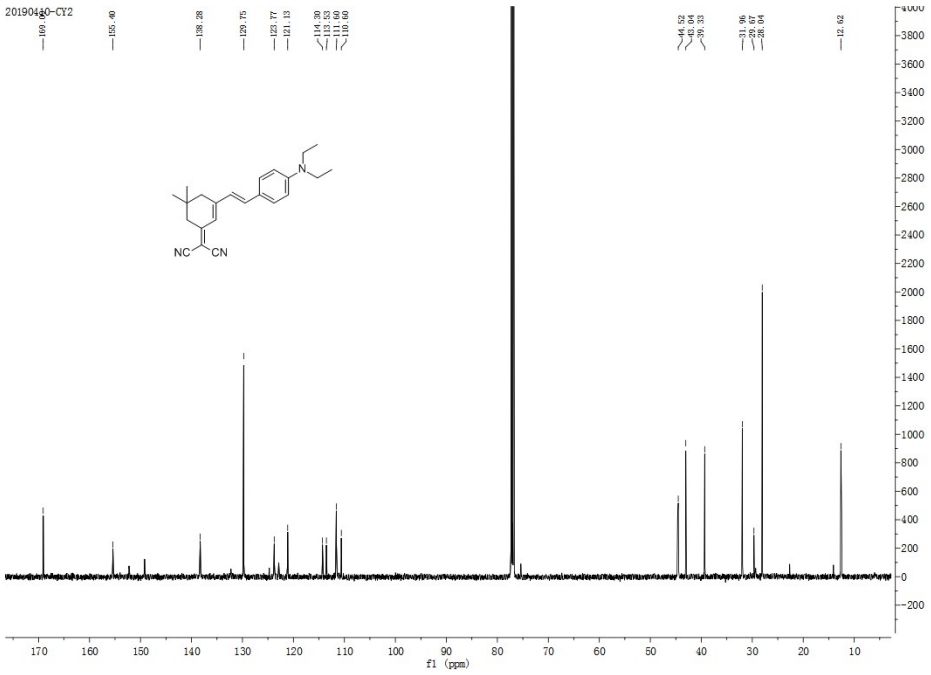


Fig. S2 ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) spectra of compound **3**.

Fig. S3 ^{1}H NMR (500 MHz, DMSO, 298 K) spectra of compound **1**.

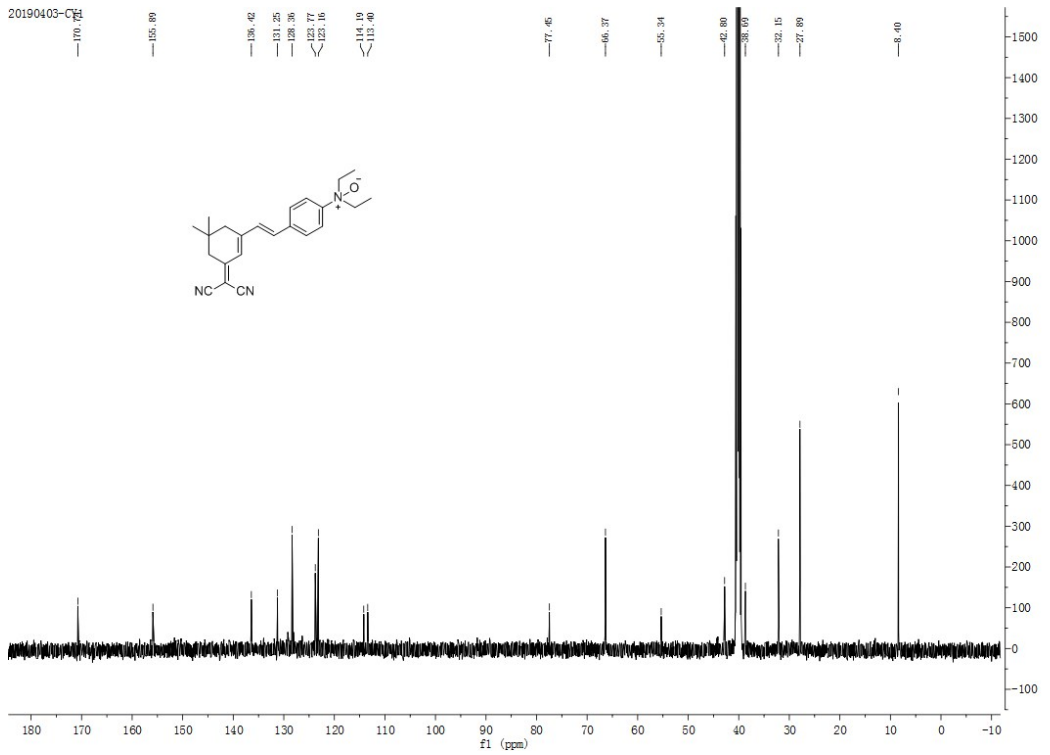


Fig. S4 ^{13}C NMR (125 MHz, DMSO, 298 K) spectra of compound **1**.

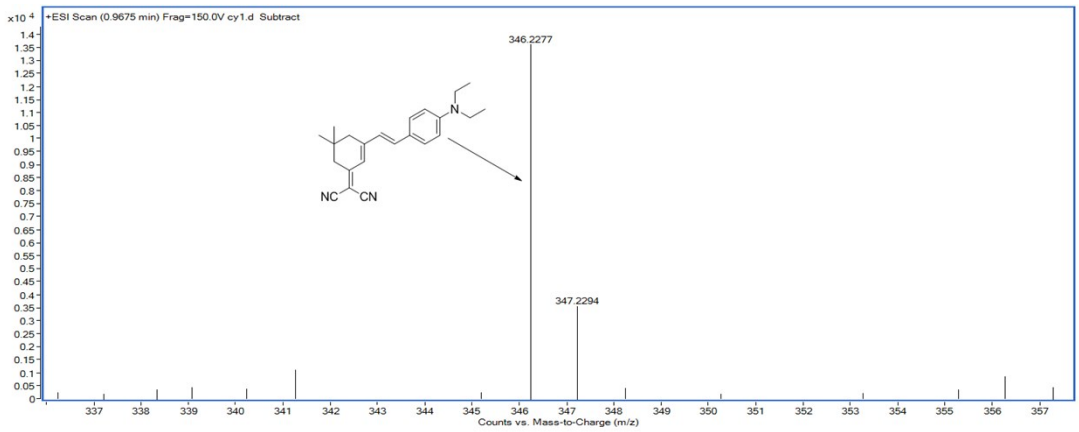


Fig. S5 High resolution mass spectrum (HRMS) of compound **3.**

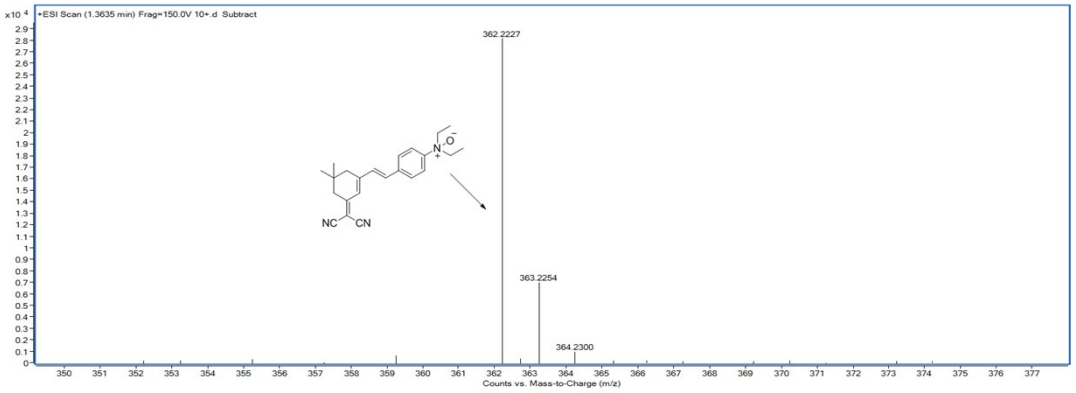


Fig. S6 High resolution mass spectrum (HRMS) of compound **1**.

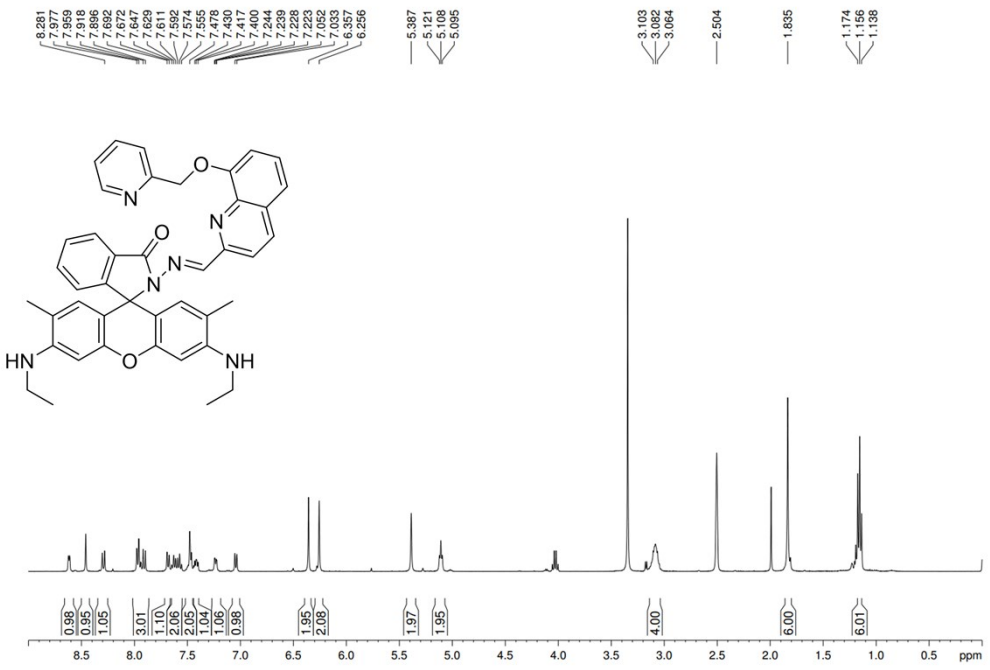


Fig. S7 ^{1}H-NMR (400 MHz, DMSO, 298 K) spectra of compound **2**.

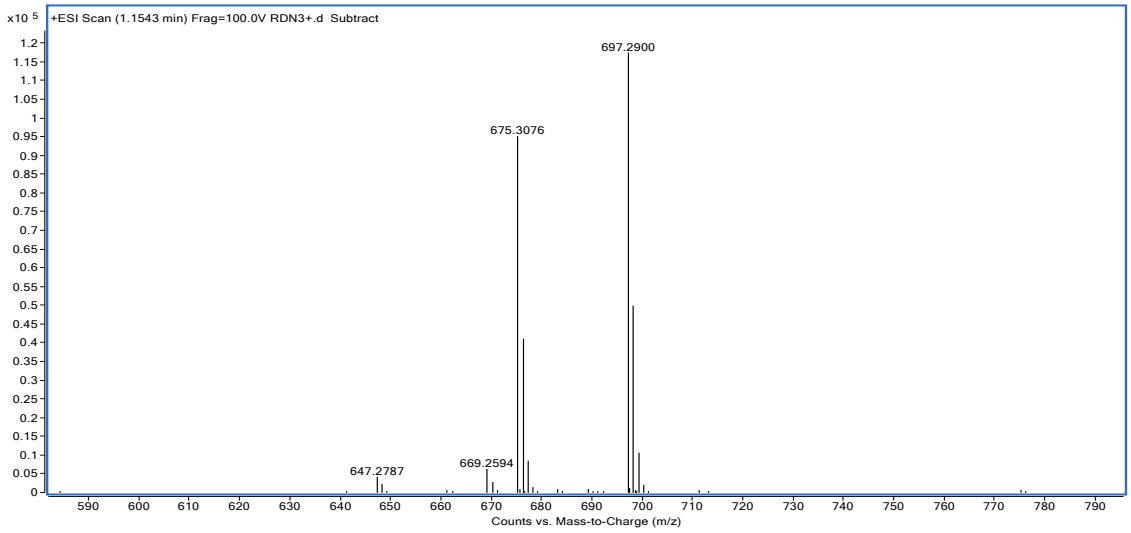


Fig. S8 High resolution mass spectrum (HRMS) of compound **2**.

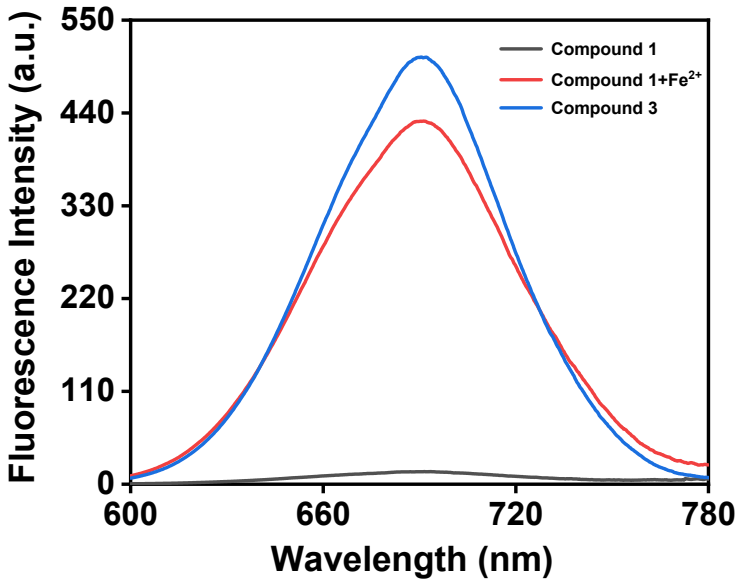


Fig. S9 Fluorescence intensity changes for compound **1** (10 μM) in the absence (black line) and presence (red line) of Fe^{2+}, The blue line represent compound **3** (10 μM). The spectra are recorded in DMSO: PBS buffer (v/v, 8/2, 0.01 M, pH 7.4), under the protection with argon. (λ_{ex}=533 nm, slit=5 nm).

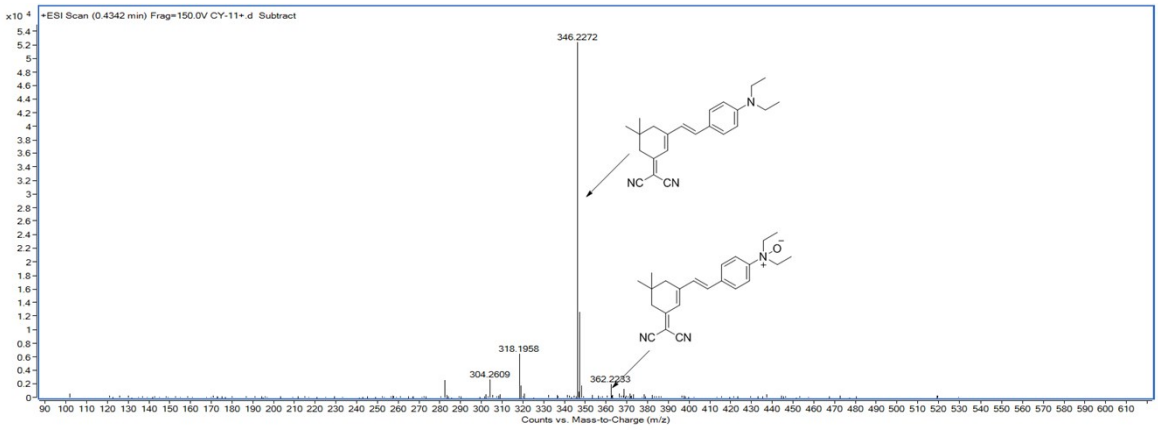


Fig. S10 High resolution mass spectrum (HRMS) of the mixture of compound **1**and Fe^{2+} after stirring for 2 min in DMSO: PBS buffer (v/v, 8/2, 0.01 M, pH 7.4). Compound **3**: [M+H] calcd for $C_{23}H_{27}N_3O$, 346.2278; found, 346.2272. compound **1**: [M+H] calcd for $C_{23}H_{27}N_3$, 362.2227, found, 362.2283.

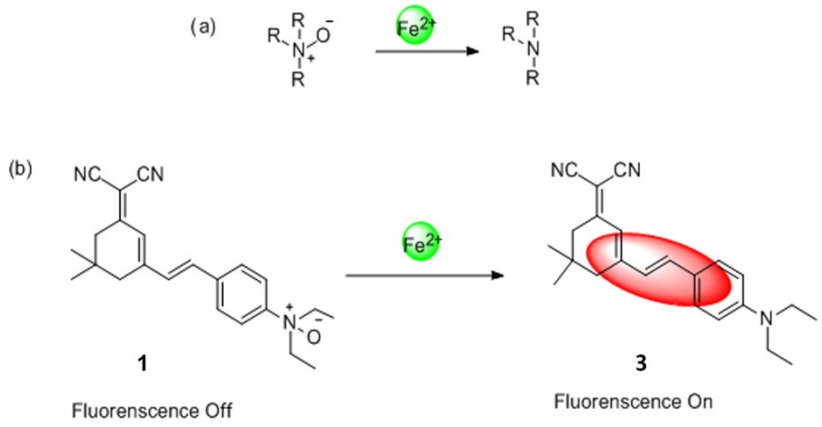


Fig. S11 Proposed response mechanism of compound **1** to Fe^{2+}

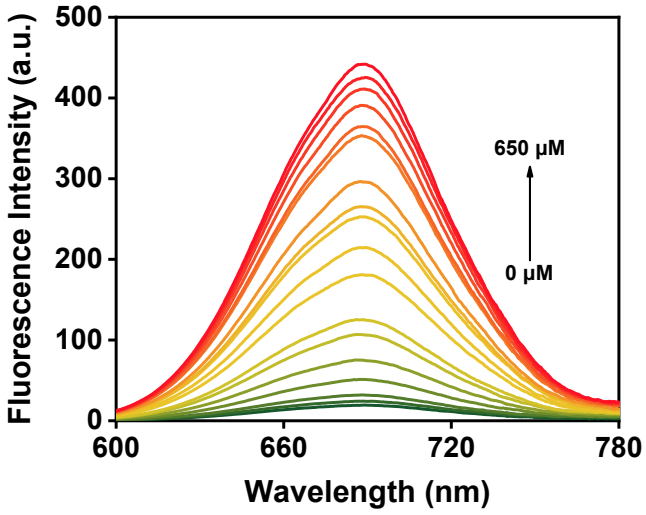


Fig. S12 Fluorescence spectra of compound **1** (10 μM) in the presence of Fe^{2+} (0-650 μM), spectra are recorded in DMSO: PBS buffer (v/v, 8/2, 0.01 M, pH 7.4). λ_{ex}=530 nm, slit=5 nm.

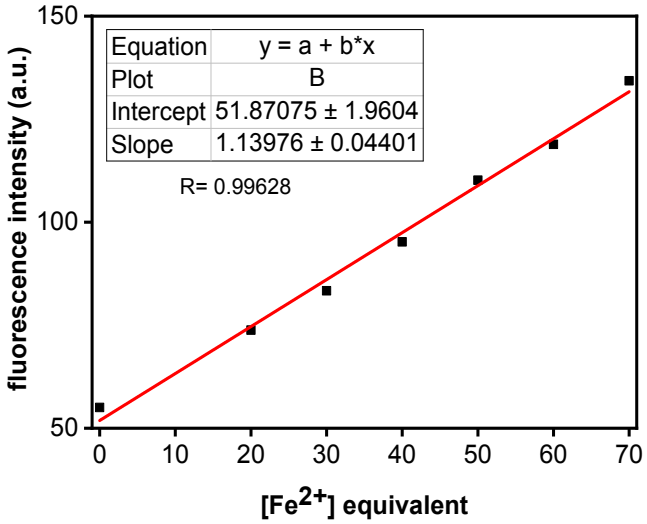


Fig. S13 Fluorescence intensity of compound **1** (1.0 μM) in the absence and presence of Fe^{2+} in DMSO: PBS buffer (v/v, 8/2, 0.01 M, pH 7.4), under the protection with argon. (λ_{ex}=533 nm, slit=5 nm). The average value of the 20 blank is 55.03, the standard deviation S = 1.51, the correlation coefficient R = 0.99628 (y = 1.13976x +51.87075), and the LOD = 3S / k × 10^{-6} mol/L = 3.97 μM.

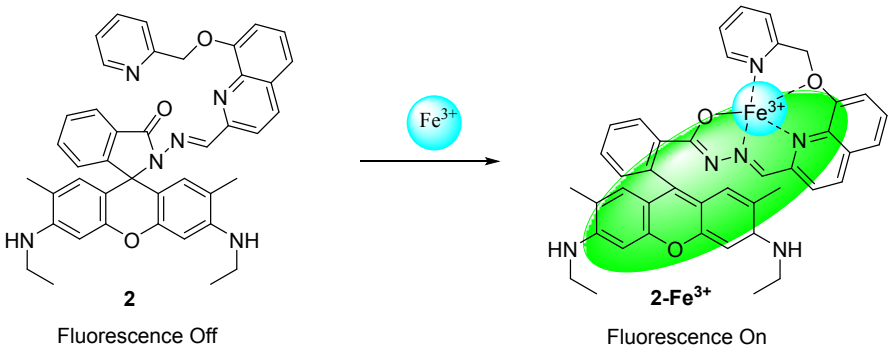


Fig. S14 Proposed response mechanism of Compound **2** to Fe^{3+}.

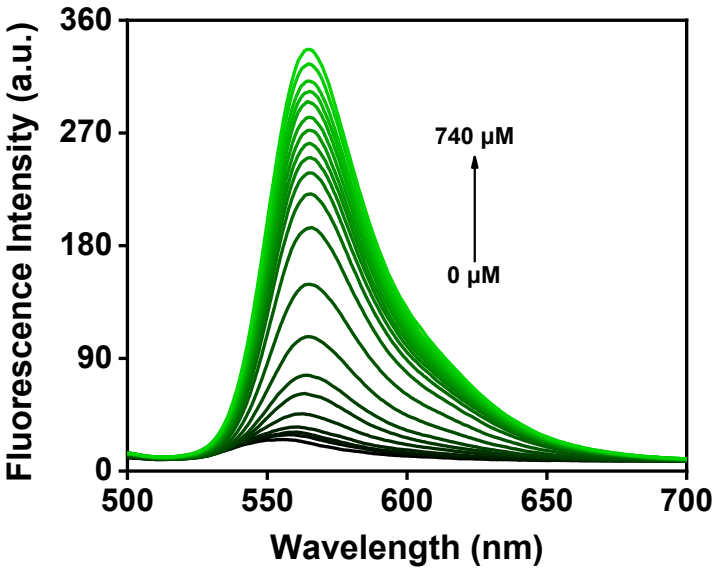


Fig. S15 Fluorescence spectra of compound **2** (10 μM) in the presence of Fe^{3+} (0-740 μM), spectra are recorded in DMSO: PBS buffer (v/v, 8/2, 0.01 M, pH 7.4). λ_{ex}=480 nm, slit=5 nm.

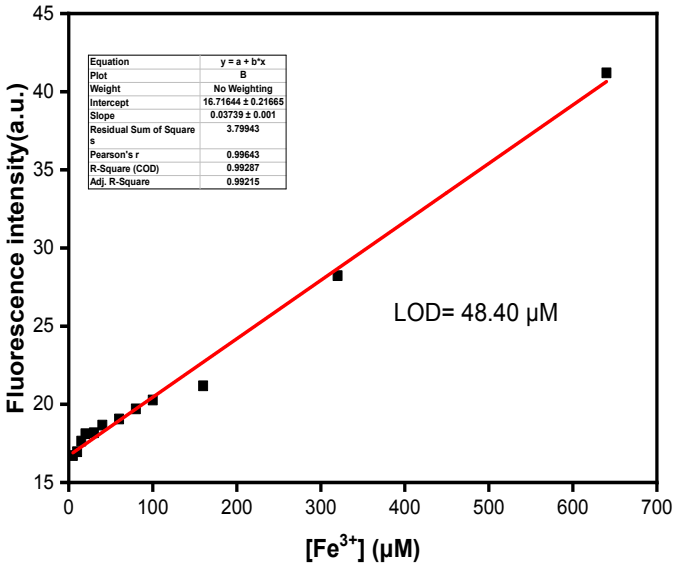


Fig. S16 Fluorescence intensity of compound **2** (1.0 μM) in the absence and presence of Fe^{3+} in DMSO: PBS buffer (v/v, 8/2, 0.01 M, pH 7.4), under the protection with argon. (λ_{ex}=480 nm, slit=5 nm). The average value of the 20 blank is 14.83, the standard deviation S = 0.60, the correlation coefficient R = 0.99215 (y = 0.03739x +16.71644), and the LOD = 3S / k × 10^{-6} mol/L =48.40 μM.

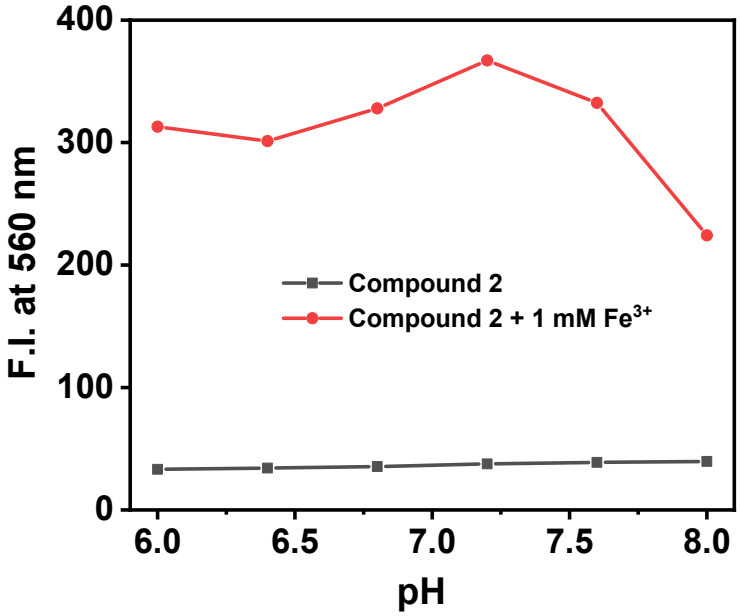


Fig. S17 Fluorescence intensity variety at 560 nm of compound **2** (with/without excess Fe^{3+}) under different pH conditions. λ_{ex}=480 nm slit=5 nm.

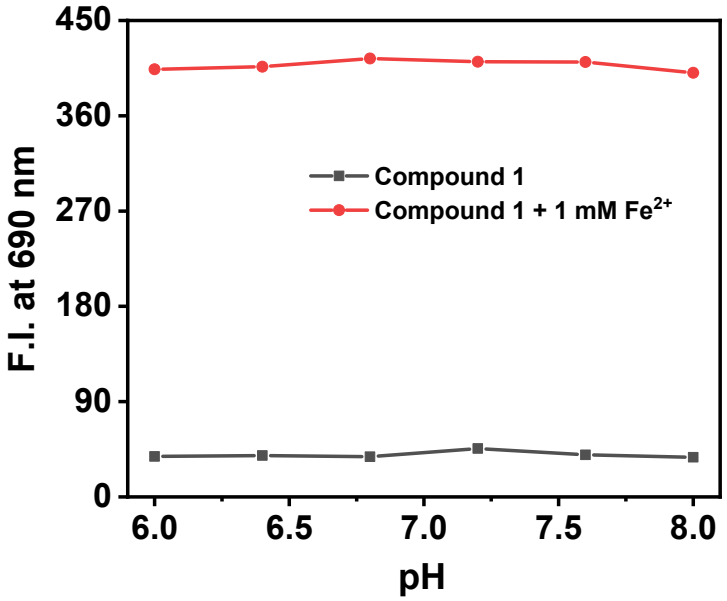


Fig. S18 Fuorescence intensity variety at 690 nm of compound **1** (with/without excess Fe^{2+}) under different pH conditions. λ_{ex}=535 nm slit=5 nm.

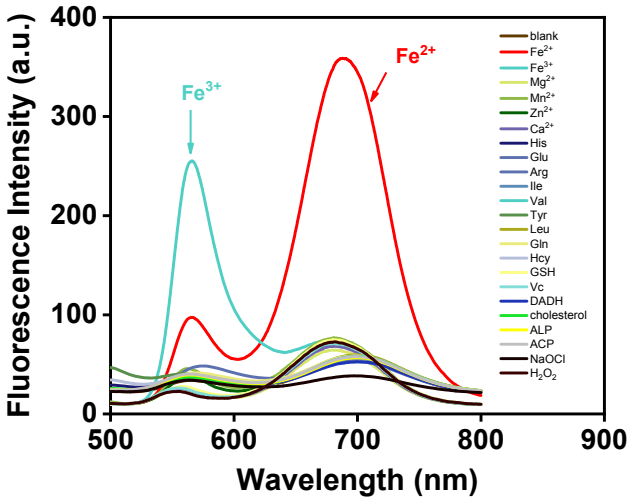


Fig. S19 Fluorescence spectrum of compound **1** (10 μM) at 690 nm and compound **2** (10 μM) at 560 nm in the presence of various analytes (1 mM), intensities are recorded in DMSO: PBS buffer (v/v, 8/2, 0.01 M, pH 7.4). λ_{ex}=480 nm, slit=5 nm.

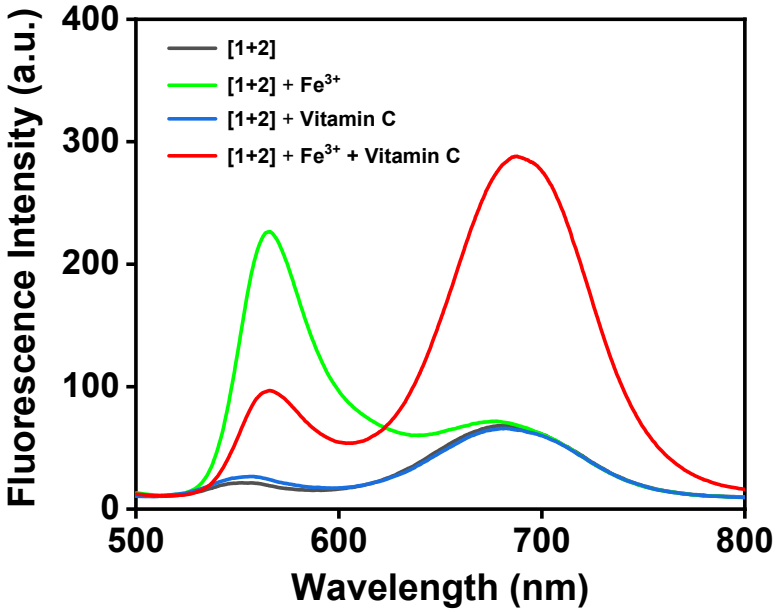


Fig. S20 Fluorescence spectra of compound **1** (10 μM) and compound **2** (10 μM) at 100 min after addition of Fe^{3+} (1 mM) / Vitamin C (1 mM) for 100 min in DMSO/PBS (0.01 M, pH 7.4, 8/2, v/v) buffer under the protection with argon. λ_{ex}=480 nm, slit=5 nm.

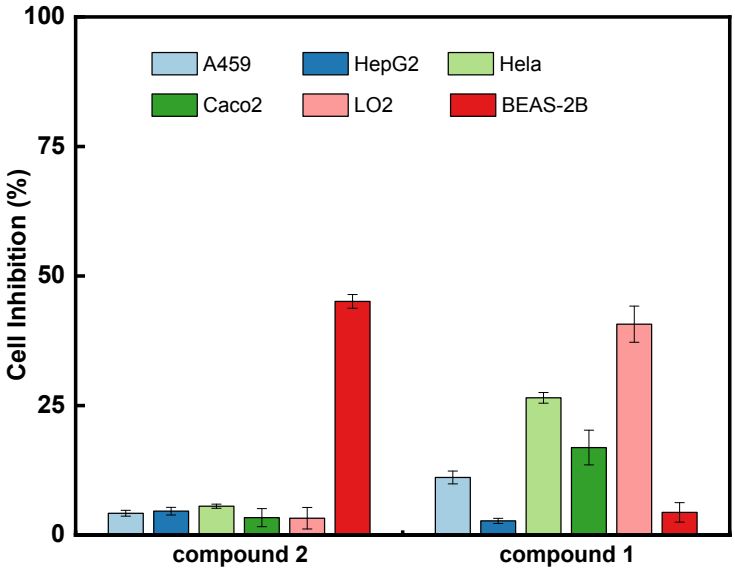


Fig. S21 The respective cytotoxicity of 40 μM compound **2** and compound **1** to different cell lines. human normal (pulmonary epithelial cell BEAS-2B, liver cells L02) and cancer cells (lung cancer cells A594, liver cancer cells, cervical cancer cell HeLa, and colorectal adenocarcinoma cells Caco-2).

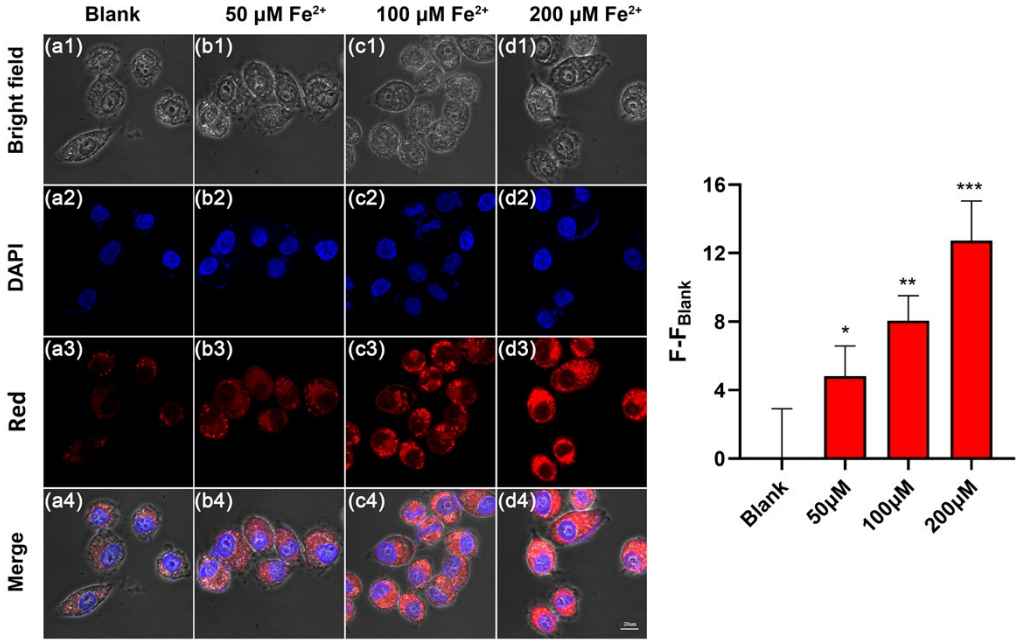


Fig. S22 Confocal fuorescent imaging of HeLa cells, incubated with 5.0 μM compound 1 for 1 h with exogenous Fe^{2+} (under the protection with argon); The red channel were collected at 670-750 nm upon excitation at 480 nm. The histograms show the mean fluorescence intensity of compound **1** in cells (calculated by ImageJ). These results are means ± SD of three independent experiments. Scale bar: 20 μm, *p < 0.05, **p < 0.01, ***p< 0.001. Scale bar: 20 μm.

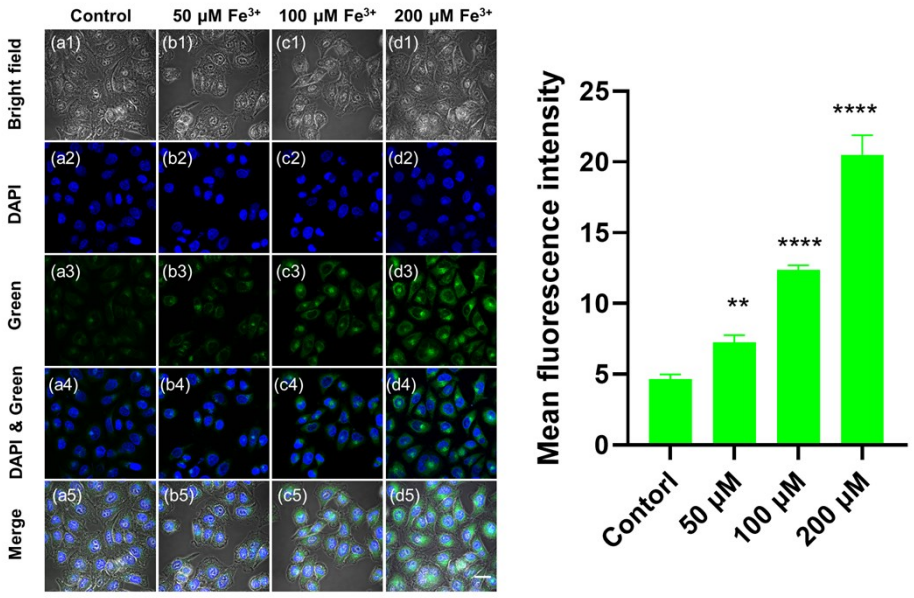


Fig. S23 Confocal fluorescent imaging of HeLa cells, incubated 5.0 μM compound **2** for 1 h with exogenous Fe^{3+}. The green channel were collected at 500-580 nm upon excitation at 480 nm. The histograms show the mean fluorescence intensity of compound **2** in cells (calculated by ImageJ). These results are means ± SD of three independent experiments. Scale bar: 20 μm, *p < 0.05, **p < 0.01, ***p< 0.001. Scale bar: 20 μm.

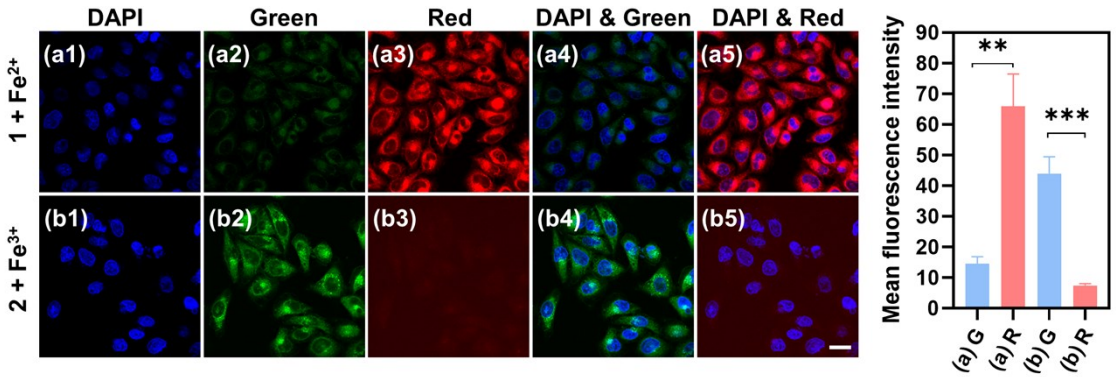


Fig. S24 (a) Confocal fluorescent imaging of HeLa cells, incubated with 5.0 μM compound **1** for 1 h with exogenous Fe^{2+} (100 μM under the protection with argon); The red channel were collected at 670-750 nm upon excitation at 480 nm. (b) Confocal fluorescent imaging of HeLa cells, incubated 5.0 μM compound **2** for 1 h with exogenous Fe^{3+}(100 μM); The green channel were collected at 500-580 nm upon excitation at 480 nm. The histograms show the mean fluorescence intensity in cells (calculated by ImageJ). These results are means ± SD of three independent experiments. Scale bar: 20 μm, *p < 0.05, **p < 0.01, ***p< 0.001. Scale bar: 20 μm

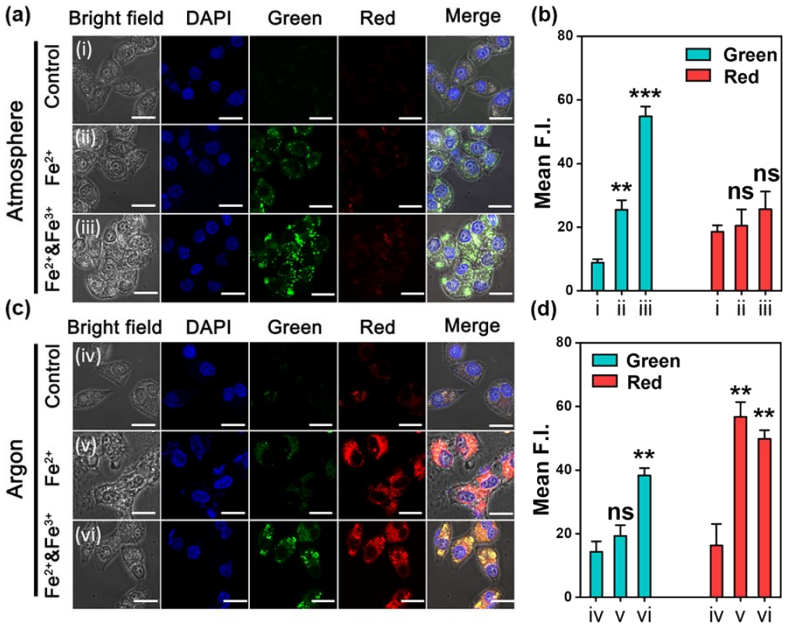


Fig.S25 (a) CLSM imaging of HeLa cells was incubated with **[1+2]** (5.0 μM **1** and 5.0 μM **2**) for 1 h after incubating with Fe^{2+}/Fe^{3+} for 1 h under air; (b) The histograms show the mean fluorescence intensity in cells under air. (c) CLSM imaging of HeLa cells was incubated with **[1+2]** (5.0 μM **1** and 5.0 μM **2**) for 1 h after incubating with Fe^{2+}/Fe^{3+} for 1 h under argon; (d) The histograms show the mean fluorescence intensity in cells under argon. These results are means ± SD of three independent experiments. Scale bar: 20 μm, *p < 0.05, **p < 0.01, ***p< 0.001

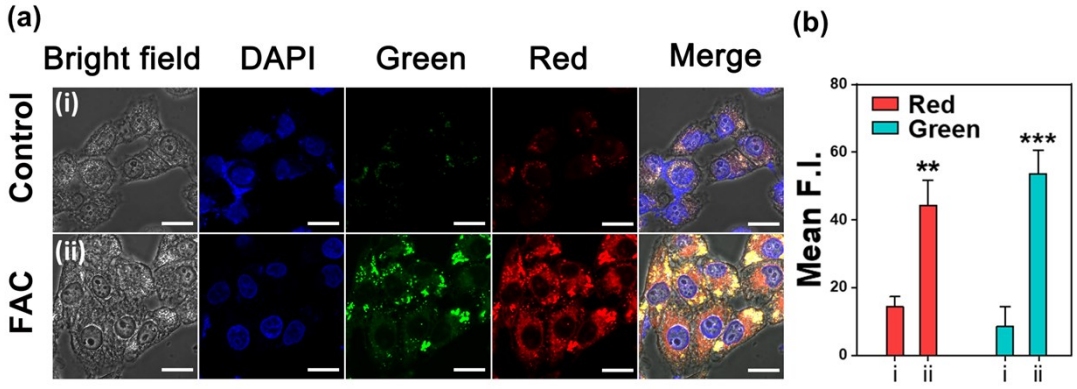


Fig.S26 (a) CLSM images of HeLa cells were incubated with [1+2] (5.0 μM 1 and 5.0 μM 2) for 1 h after incubating without or with ferric ammonium citrate (AFC,100 μM) for 24 h. (b) The histograms show the mean fluorescence intensity in cells These results are means ± SD of three independent experiments. Scale bar: 20 μm, *p < 0.05, **p < 0.01, ***p< 0.001

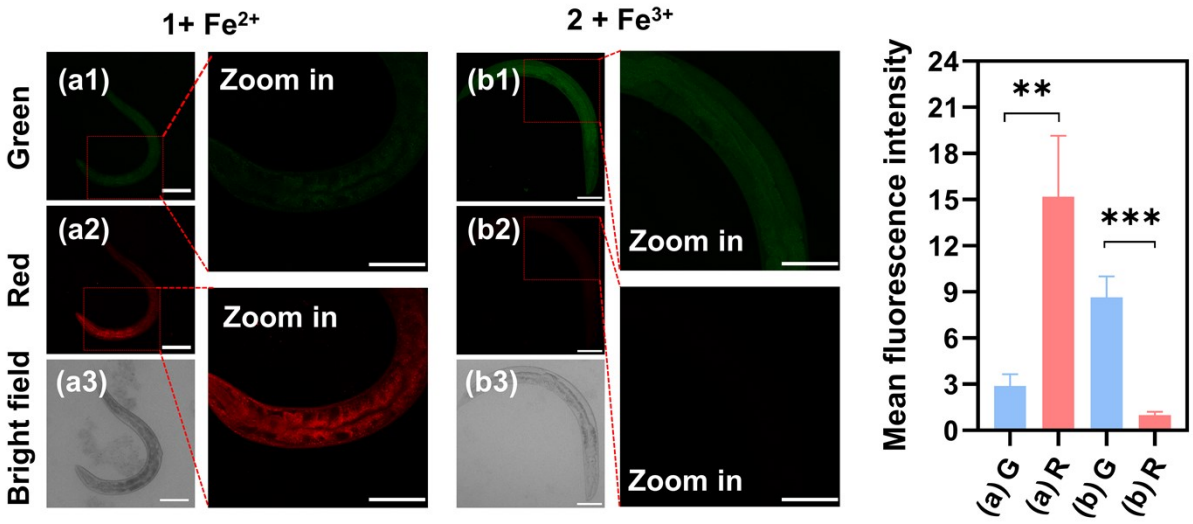


Fig. S27 (a) Confocal fluorescent imaging of *C.elegans*, incubated with 20 μM compound **1** for 2 h with exogenous Fe^{2+} (1 mM under the protection with argon); The red channel were collected at 670-750 nm upon excitation at 480 nm. (b) Confocal fluorescent imaging of *C.elegans*, incubated 20 μM compound **2** for 1 h with exogenous Fe^{3+}(1 mM); The green channel were collected at 500-580 nm upon excitation at 480 nm. The histograms show the mean fluorescence intensity in *C.elegans* (calculated by ImageJ). These results are means ± SD of three independent experiments. Scale bar: 20 μm, *p < 0.05, **p < 0.01, ***p< 0.001. Scale bar: 200 μm

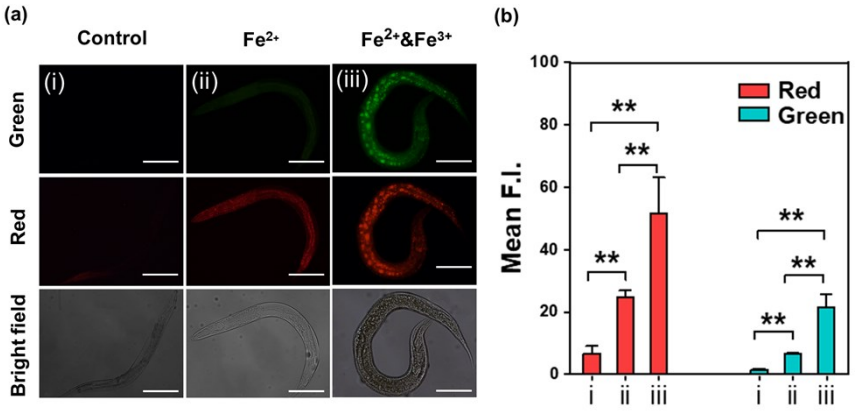


Fig. S28 (a) CLSM imaging of *C.elegans* (N2,L4 larva) was incubated with **[1+2]** (20 μM **1** and 20 μM **2**) for 3 h after incubating without/with exogenous 1.0 mM Fe^{2+}/Fe^{3+} under the argon; (b) The histograms show the mean fluorescence intensity in *C.elegans*. These results are means ± SD of three independent experiments. Scale bar: 200 μm.*p < 0.05, **p < 0.01, ***p<0. 001

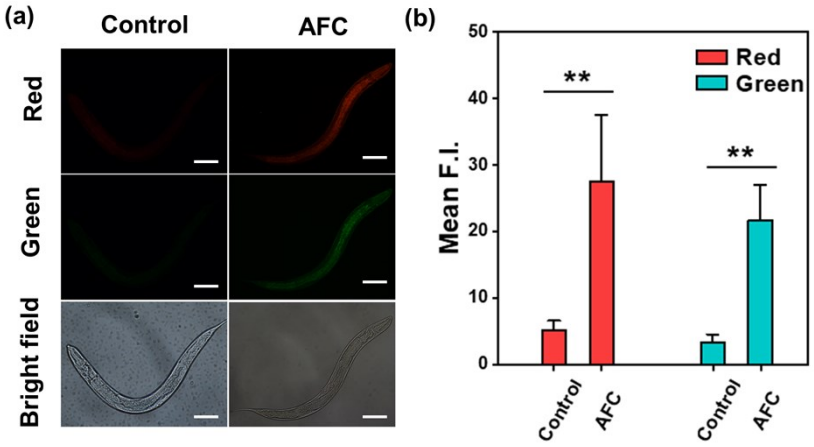


Fig. S29. (a) CLSM imaging of *C.elegans* (N2) was incubated with **[1+2]** (20 μM **1** and 20 μM **2**) for 3 h after incubating without or with ferric ammonium citrate (AFC, 2.5 mg/mL) from L1-L4 on the nematode growth media (NGM). (b) The histograms show the mean fluorescence intensity in *C.elegans*. These results are means ± SD of three independent experiments. Scale bar: 200 μm, *p < 0.05, **p < 0.01, ***p< 0.001

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