Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A ratiometric fluorescent probe of methionine sulfoxide reductase with improved response rate and emission wavelength

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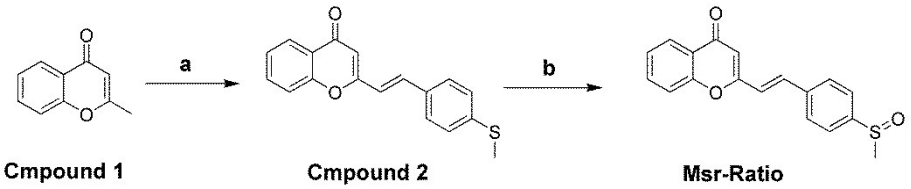
1. Synthesis of probe Msr-Ratio



Scheme S1 Synthetic route of the probe Msr-Ratio. Reagents and conditions: (a)_4-(methylthio)benzaldehyde/ piperidine/AcOH/toluene, reflux, 29%; (b) *m*-CPBA/ DCM, rt, 72%.

Synthesis of compound 2

To the mixture of compounds **1** (80 mg, 0.5 mmol) and 4-(methylthio)benzaldehyde (76 mg, 0.5 mmol) in toluene (5 mL), a few drops of piperidine and AcOH were added. Then the mixture was stirred under refluxing for 3 h. After the mixture cooled to room temperature, the crude product was purified by silica gel column chromatography with a yield of 29%.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 7.9 Hz, 1H), 7.67 (t, *J* = 7.7 Hz, 1H), 7.54 – 7.45 (m, 4H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.27 – 7.21 (m, 2H), 6.73 (d, *J* = 16.0 Hz, 1H), 6.31 (s, 1H), 2.52 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 178.35, 161.78, 155.96, 141.38, 136.28, 133.63, 131.53, 127.99, 126.11, 125.66, 124.92, 124.12, 119.25, 117.77, 110.39, 15.21. EI-MS (m/z, %): 294(M+, 100), 247(46), 44(17).

Synthesis of Msr-Ratio

Compound **2** (59 mg, 0.2 mmol) was dissolved in DCM (20 mL), and *m*-CPBA (35 mg, 0.2 mmol) was added successively to the solution. The mixture was stirred for another 15 min at room temperature. Upon solvent evaporation, the crude product was purified by silica gel column chromatography (72% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.80 – 7.68 (m, 5H), 7.66 (s, 1H), 7.62 (s, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.45 – 7.38 (m, 1H), 6.89 (d, *J* = 16.0 Hz, 1H), 6.38 (s, 1H), 2.78 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 178.37, 160.93, 155.95, 147.02, 137.76, 135.14, 133.92, 128.36, 125.74, 125.16, 124.21, 124.09, 122.43, 117.84, 111.42, 43.89. HRMS (m/z) $[M+H]^+$ calcd for 311.0670, found 311.0736.

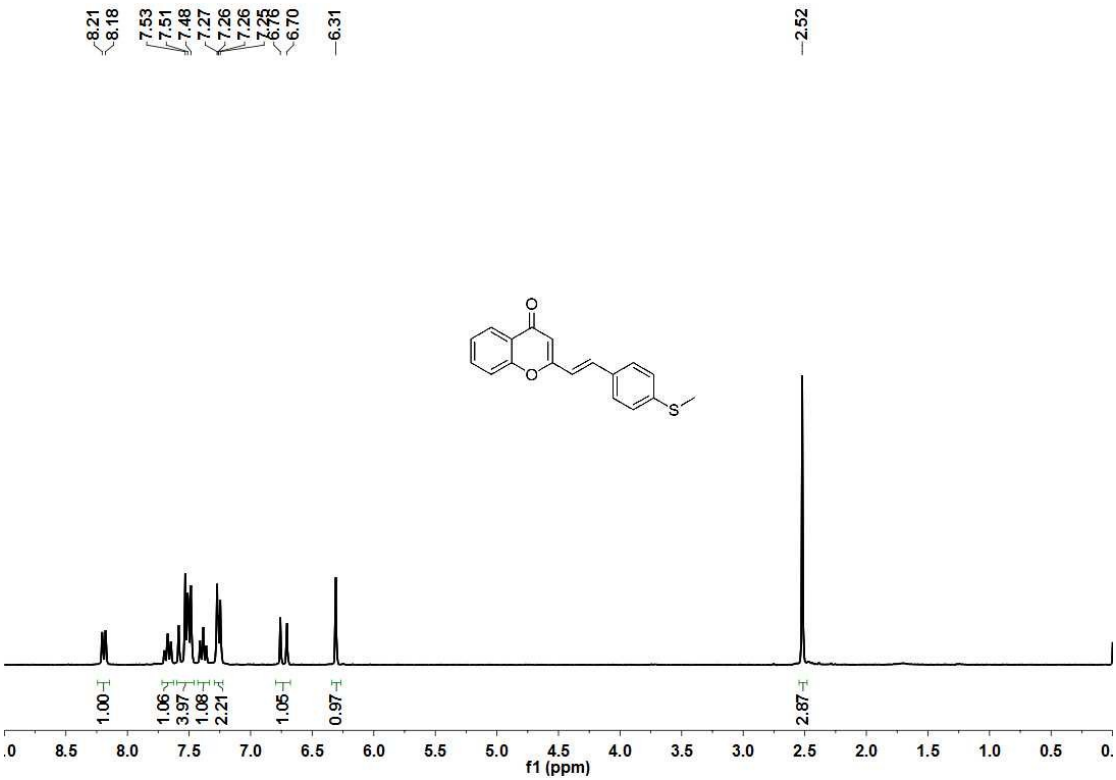


Fig. S1 ^{1}H NMR spectra of Compound 2

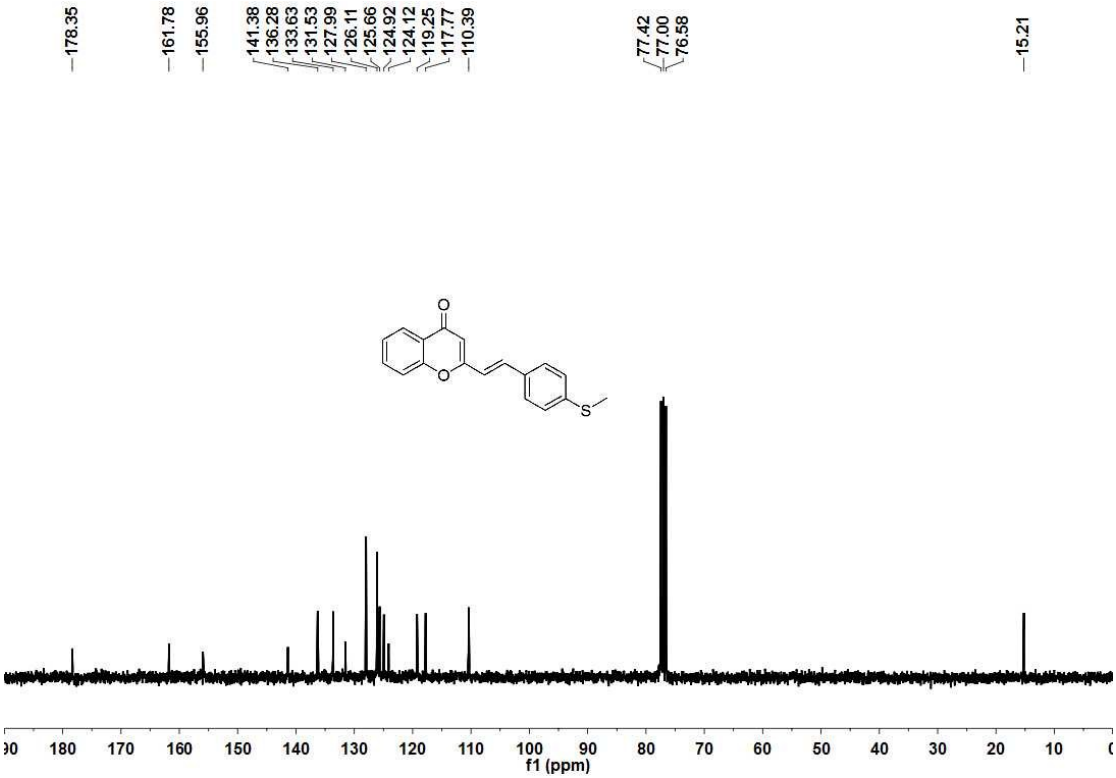


Fig. S2 ^{13}C NMR spectra of Compound 2

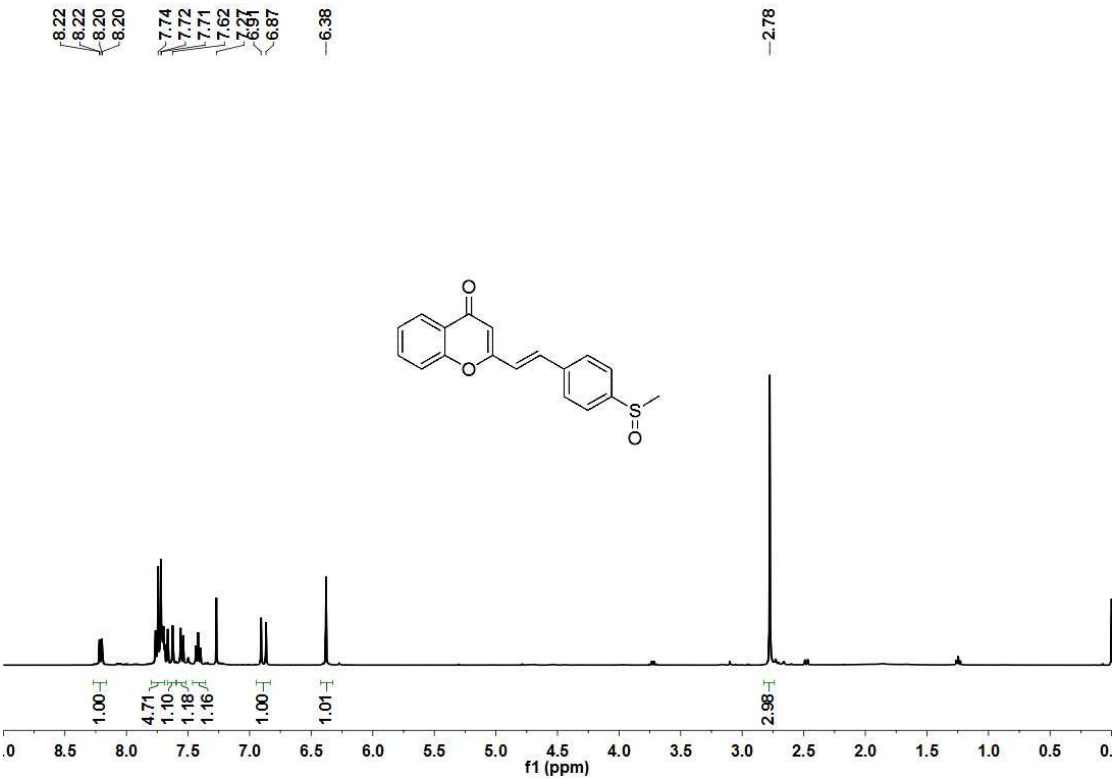


Fig. S3 ^{1}H NMR spectra of Msr-Ratio

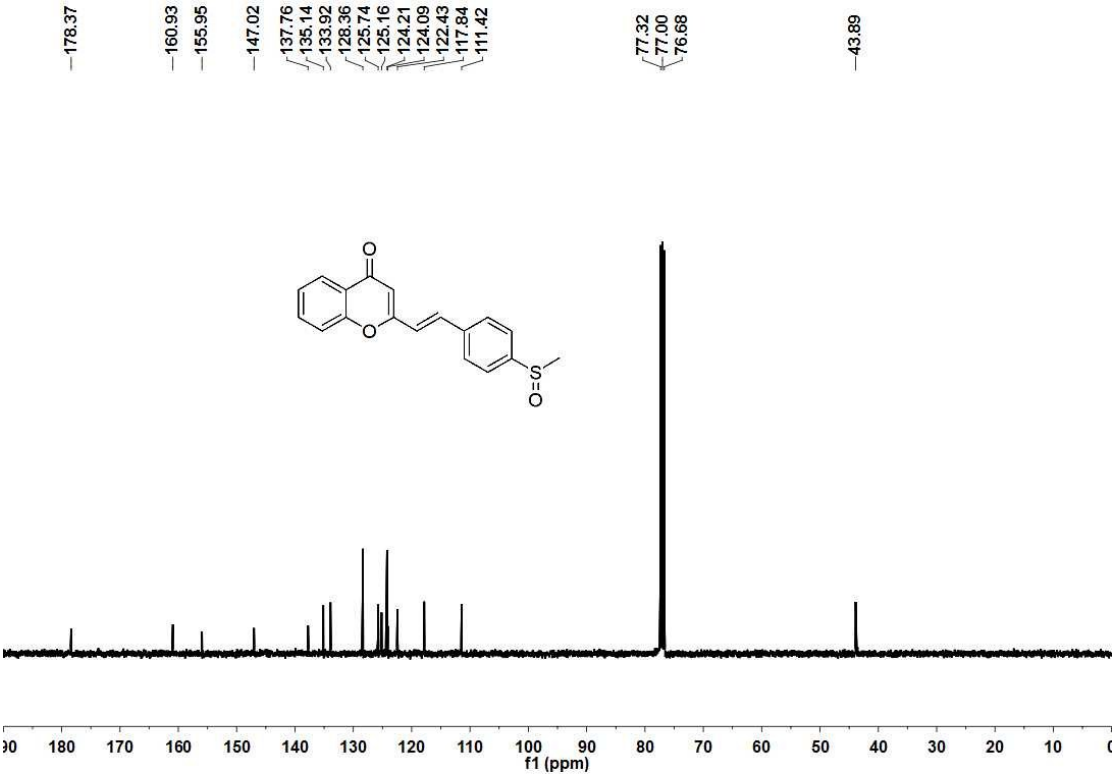


Fig. S4 ^{13}C NMR spectra of Msr-Ratio

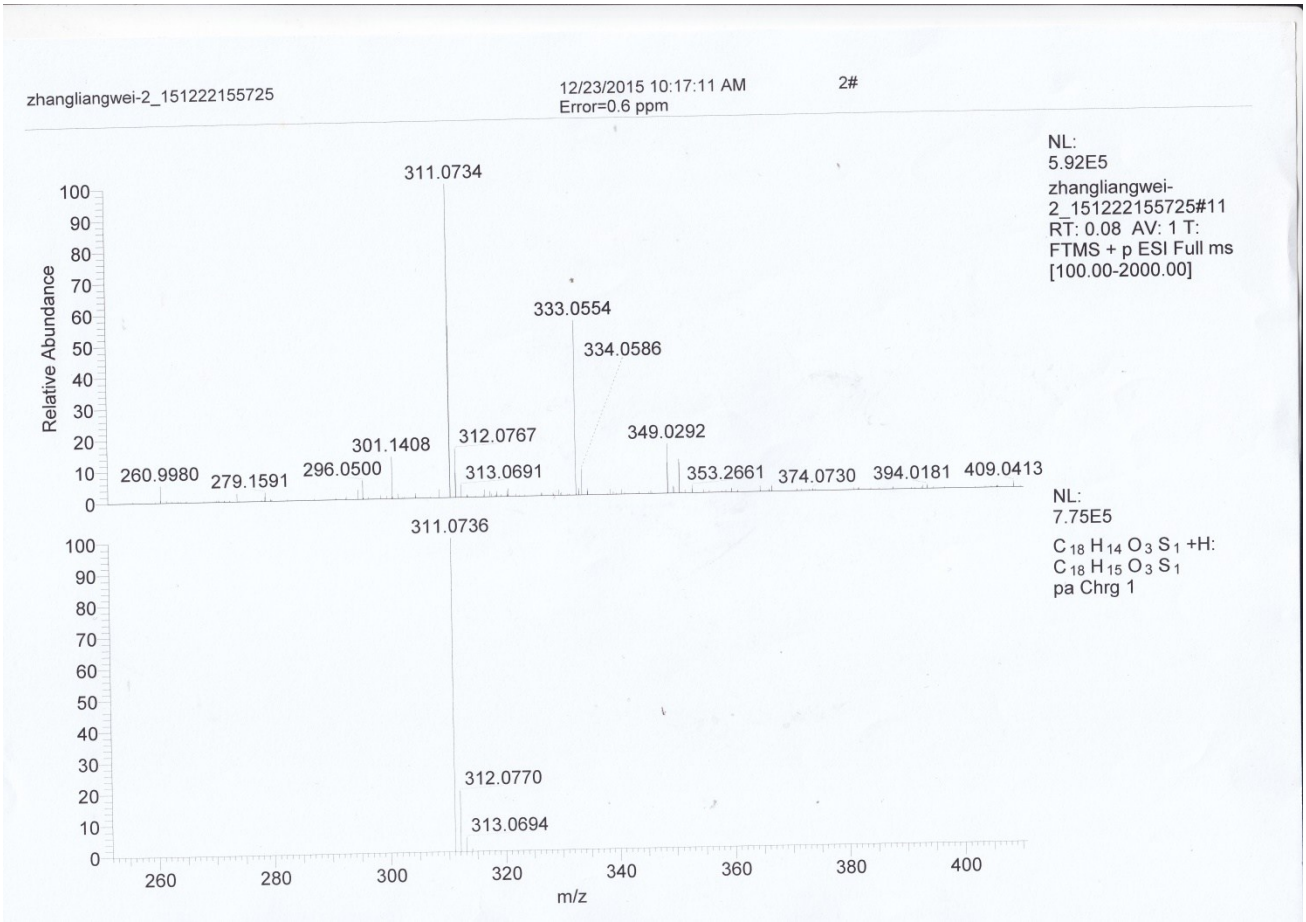


Fig. S5 HRMS spectra of Msr-Ratio

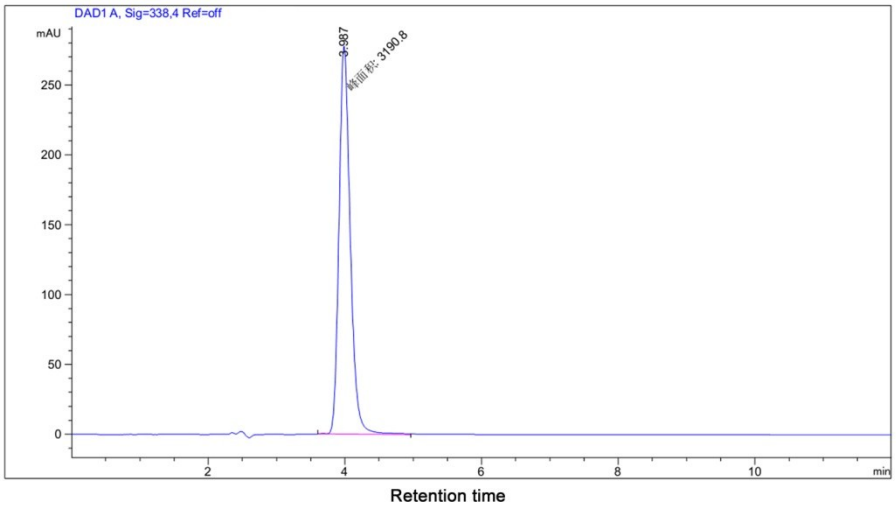


Fig. S6 Analysis of the purity of Msr-Ratio by HPLC (99%).

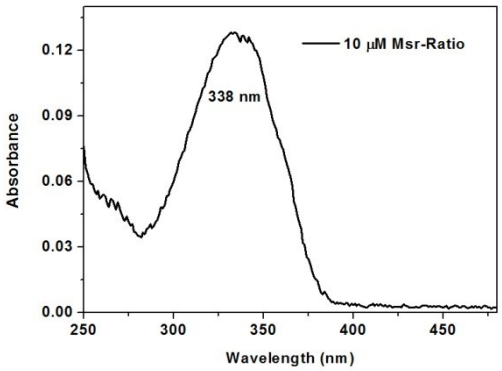


Fig. S7 UV spectrum of probe Msr-Ratio in TE buffer (50 mM Tris-HCl, 1 mM EDTA buffer, pH=7.40, 37 ^{o}C).

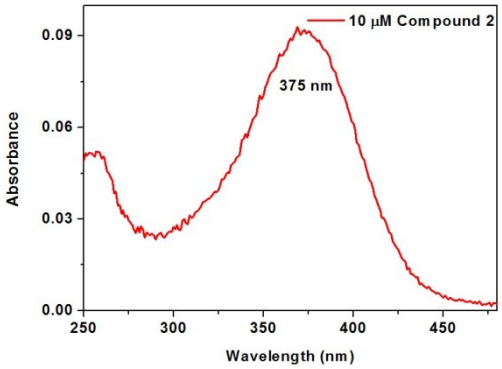


Fig. S8 UV spectrum of compound **2** in TE buffer (50 mM Tris-HCl, 1 mM EDTA buffer, pH=7.40, 37 ^{o}C).

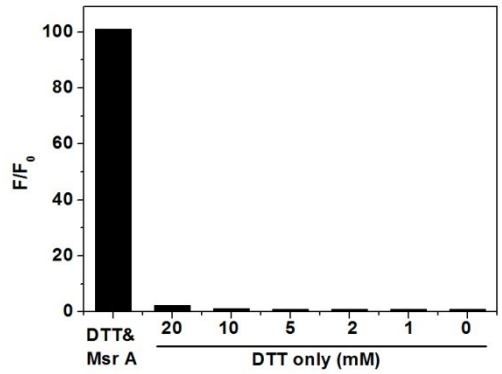


Fig. S9 Fluorescent response of the probe Msr-Ratio (10 μM) towards different concentration of DTT (1 mM, 2mM, 5mM, 10 mM, 20 mM) and DTT/Msr A (5 mM/120 nM), recording at 0.5 h (λ_{ex}=375 nm, λ_{em} = 550 nm).

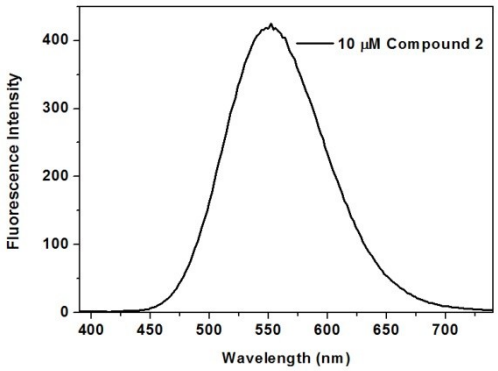


Fig. S10 The fluorescent spectrum of compound **2** in TE buffer.

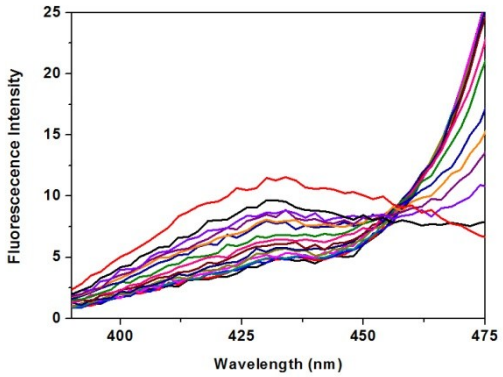


Fig. S11 The amplified image of Fig.1A.

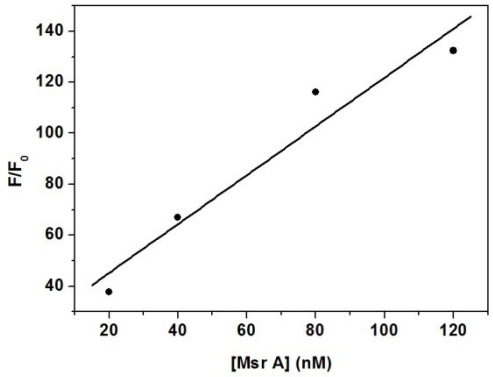


Fig. S12 Linear fitting of F/F_0 versus Msr A (0-120 nM), and F/F_0 was determined (λ_{ex}=375 nm, λ_{em}=550 nm). The detection limit of Msr-Ratio was calculated to be 2.07 nM by the equation ($3\sigma/k$).

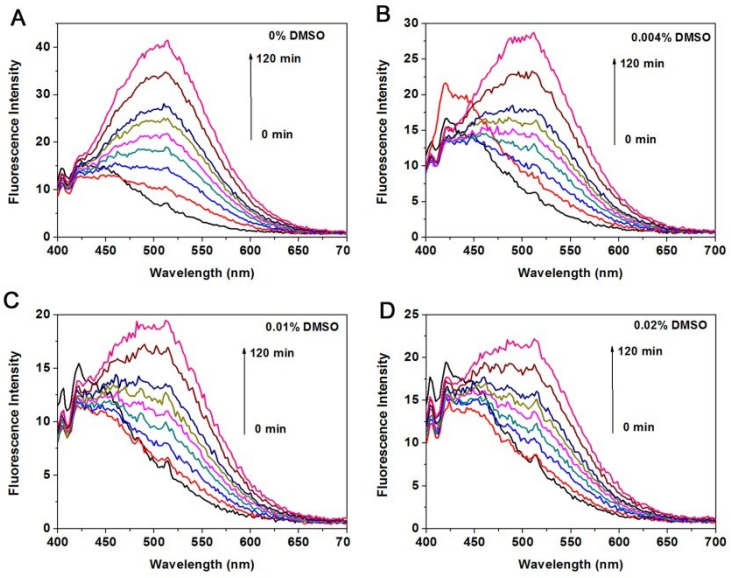


Fig. S13 Inhibition of the HL60 lysate-mediated reduction of Msr-Ratio (10 μM) by different concentration of DMSO.

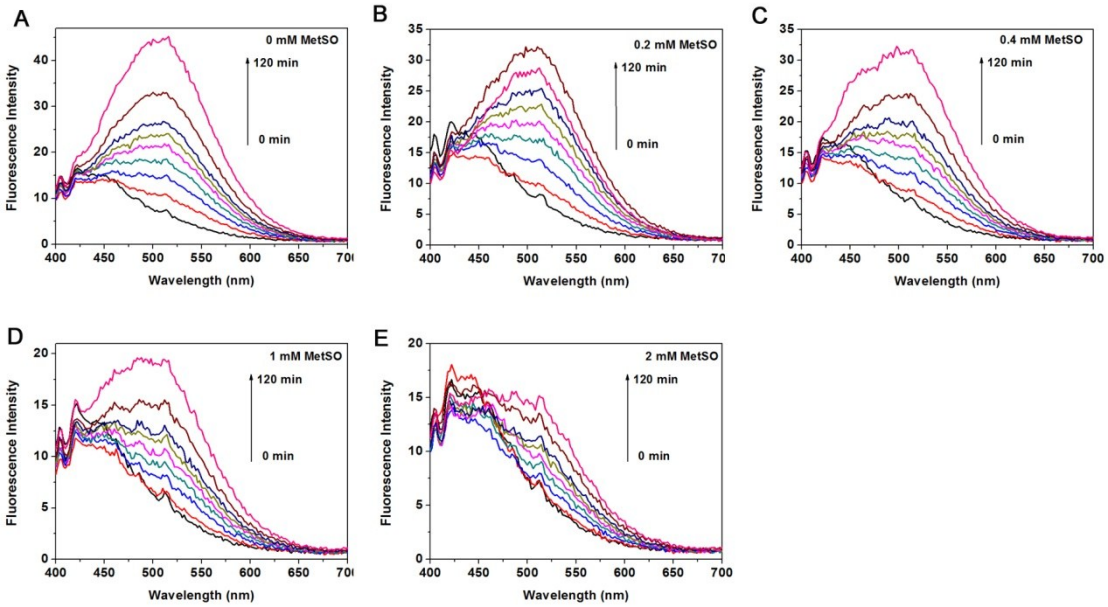


Fig. S14 Inhibition of the HL60 lysate-mediated reduction of Msr-Ratio (10 μM) by different concentration of MetSO.

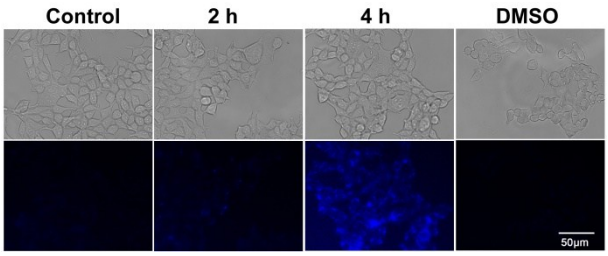


Fig.S15 Imaging Msr activity in 293T cells. Left to right: the cells without the probe, the cells incubated with Msr-Ratio (10 μM) for 2h, the cells incubated with Msr-Ratio (10 μM) for 4h, and the cells treated with DMSO (0.2 %) followed incubation with Msr-Ratio (10 μM) for 4h.

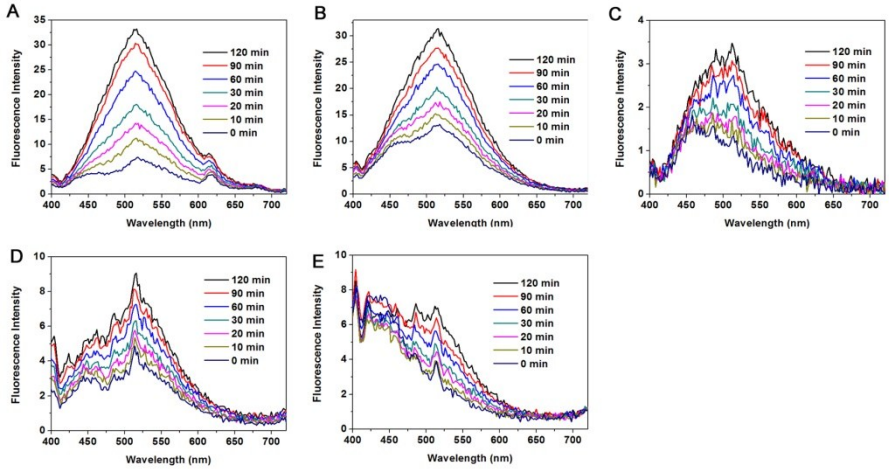


Fig.S16 Fluorescent response of Msr-Ratio (10 μM) incubated with Msr activity in mouse organs. (A) Kidney lysate, (B)Liver lysate, (C) Spleen lysate, (D) Heart lysate, (E) Brain lysate.

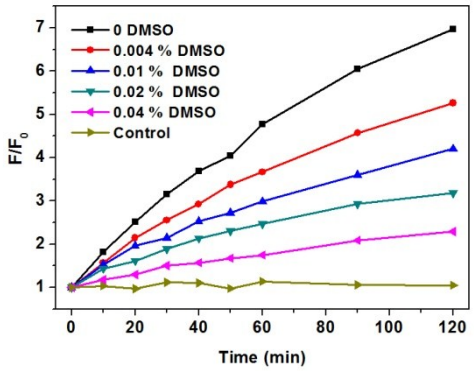


Fig.S17 Time course of the fold of fluorescence increment of the probe. The mouse kidney lysate was incubated with DTT (5 mM), varying concentrations of DMSO and Msr-ratio (10 μM), and the fold of fluorescence increment (λ_{ex}=375 nm, λ_{em} =514 nm) was recorded.

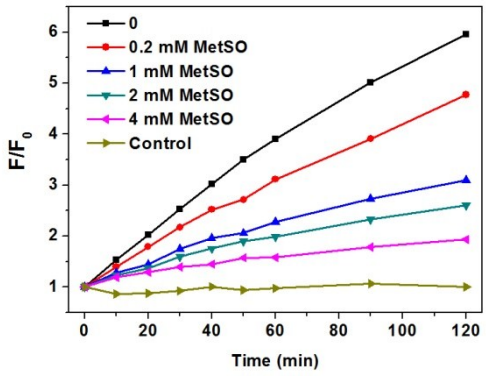


Fig.S18 Time course of the fold of fluorescence increment of the probe. The mouse kidney lysate was incubated with DTT (5 mM), varying concentrations of MetSO and Msr-ratio (10 μM), and the fold of fluorescence increment (λ_{ex}=375 nm, λ_{em} =514 nm) was recorded.

2 Materials and instruments

All reagents were of analytical grade and were purchased from commercial supplies without further purification. NMR spectra were recorded on Bruker 400 MHz instruments or Agilent Mercury plus 300 BB. MS spectra were recorded on Trace DSQ GC-MS spectrometer. HRMS was obtained on Orbitrap Elite (Thermo Scientific). Live cell imaging was carried out on inverted fluorescent microscope (Leica DMI 4000B). Fluorescence studies were carried out using an Agilent Cary Eclipse Fluorescence Spectrophotometer (the silt wide was 5 nm for both excitation and emission). UV-vis absorption spectra were accomplished on an Evolution 220 UV-Visible spectrometer. The fluorescent quantum was determined on FL sp920 by absolute value method.

3 HPLC analysis

HPLC assays of Msr A-mediated Msr-Ratio reduction was performed on an Agilent 1100 series HPLC system. Msr-Ratio (10 μM) was incubated with DTT (5 mM) and Msr A (200 nM) at 37 ^{o}C in TE buffer. The reaction mixture was dried under reduced pressure, and the residue was extracted by ethyl acetate. The combined solvent was removed under vacuum and the residue was reconstituted in the small volume of methanol. Msr-Ratio and the pure compound **2** were both prepared as a 10 μM solution and were used as standard samples. All samples were passed through a 0.22 μm filter, and 20 μL of each sample was loaded onto Agilent ZORBAX SB-C18, reversed-phase column (5 μm, 4.6×150 mm). The column was eluted with methanol/water (80:20). The flow rate was set at 0.6 mL min^{-1}. A UV/vis detector was used to monitor the desiring product at wavelength at 338nm and 375 nm, respectively. The purity of Msr-Ratio was carried out on Agilent 1260 Infinity II HPLC equipped with Agilent reversed

phase column SB-C18 (5 μm, 4.6×150 mm), using the same conditions above.

4. Live cell imaging.

HL60 cells ($4x10^5$) and 293T cells ($4x10^5$) were cultured in 6-well plates and allowed to grow overnight. Then Msr-ratio (10 μM) was added to the plate and continued culture for different time. With difference, the inhibition experiment is addition of 0.2% (V/V) DMSO 30 minutes ahead the addition of the probe. The cells were visualized and photographed on inverted fluorescent microscope (Leica DMI 4000B).

5. Flow cytometric analysis

Flow cytometric analysis was carried out on Flow Cytometer (LSRFortessa). The HL60 cells were handled same as above procedure of live cell imaging. And then the cells were washed by PBS buffer for three times prior to the Flow cytometric analysis.

6. Measuring Msr activity in mouse tissues.

Kunming Mice, purchased from the Laboratory Animal Center of Lanzhou University, were sacrificed by decapitation, and different organs, *i. e.*, heart, brain, kidney, liver and spleen were collected. These organs lysate were prepared according to our reported literature and its protein concentration was determined by the Bradford method, and all samples were adjusted to 15 mg/mL. Generally, Msr activity analysis in samples was handled as the following description, Msr-Ratio (10 μM), DTT (5 mM) and 50 μL organs lysate was mixed in a final volume of 500 μL and incubated under 37 ^{o}C. The increase of fluorescence was monitored.

7. Immunoprecipitation

The protein extract from mouse kidney was prepared as described above, and the total protein concentration was adjusted to 15 mg/mL determined by the Bradford method. The samples were incubated with anti-Msr A antibody (Santa Cruz Biotechnology) for 8 h at room temperature and further incubated with Protein A/G beads (Santa Cruz Biotechnology) for 8 h at room temperature. The beads were pelleted by centrifugation (1000 g for 5 min), and the supernatants were collected and immediately used to measure Msr activity with Msr-Ratio.