Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

A Novel ESIPT-Based Fluorescent Chemodosimeter for Hg^{2+} and Its Application in Live-Cell Imaging

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Photophysical properties of MS3

Table S1 Photophysical properties of the probe.

entry	λ_{ab} (nm)	λ_{em} (nm)	Φ^a	ε / M^{-1} cm^{-1}
MS3	438	501	0.01	7520
MS3+Hg^{2+}	386	501	0.49[b]	7931

(a) The quantum yield (Φ) of **MS3** and **MS3**-Hg^{2+} system were determined according to the literature.[1] (b) Φ was determined in the present of 15.0 equiv. of Hg^{2+}.

$$\Phi_{Sample} = \frac{\Phi_{QS} \cdot A_{QS} \cdot F_{Sample} \cdot \lambda_{exQS} \cdot \eta^2_{Sample}}{A_{Sample} \cdot F_{QS} \cdot \lambda_{exSample} \cdot \eta^2_{QS}}$$

Where Φ is quantum yield; A is absorbance at the excitation wavelength; F is integrated area under the corrected emission spectra; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the Sample and QS refer to the sample and the standard, respectively. We chose fluorescein in 0.1 M NaOH as standard, which has the quantum yield of 0.95.[2]

Additional spectroscopic data

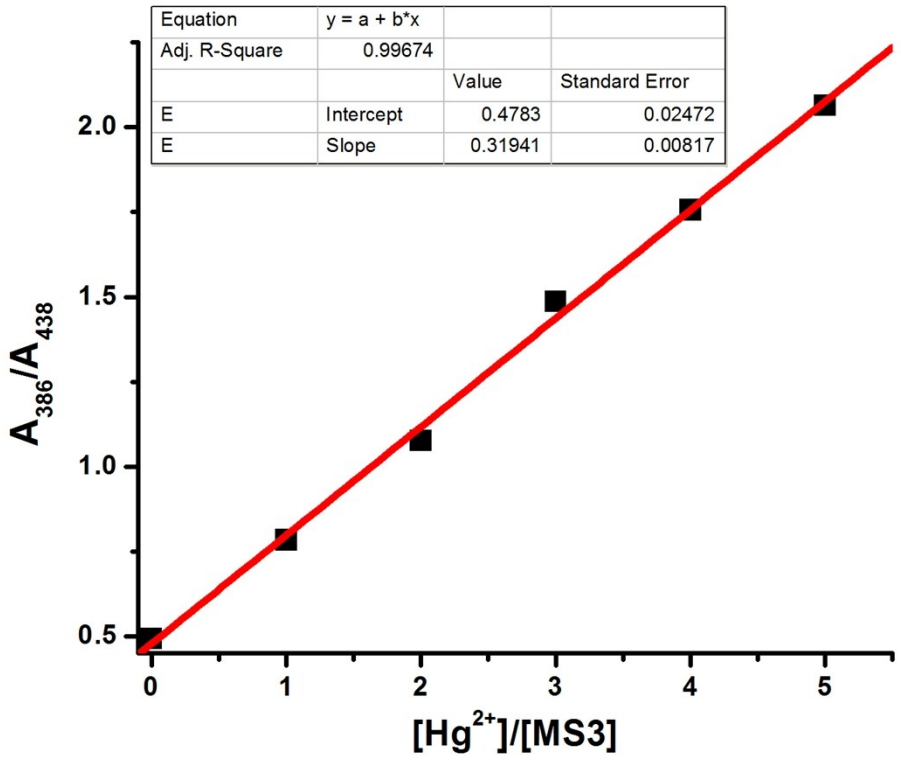


Fig. S1 The ratio of the UV-vis absorption of **MS3** (20.0 μM) at 386 nm and 438 nm as a function of Hg^{2+} concentration in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO).

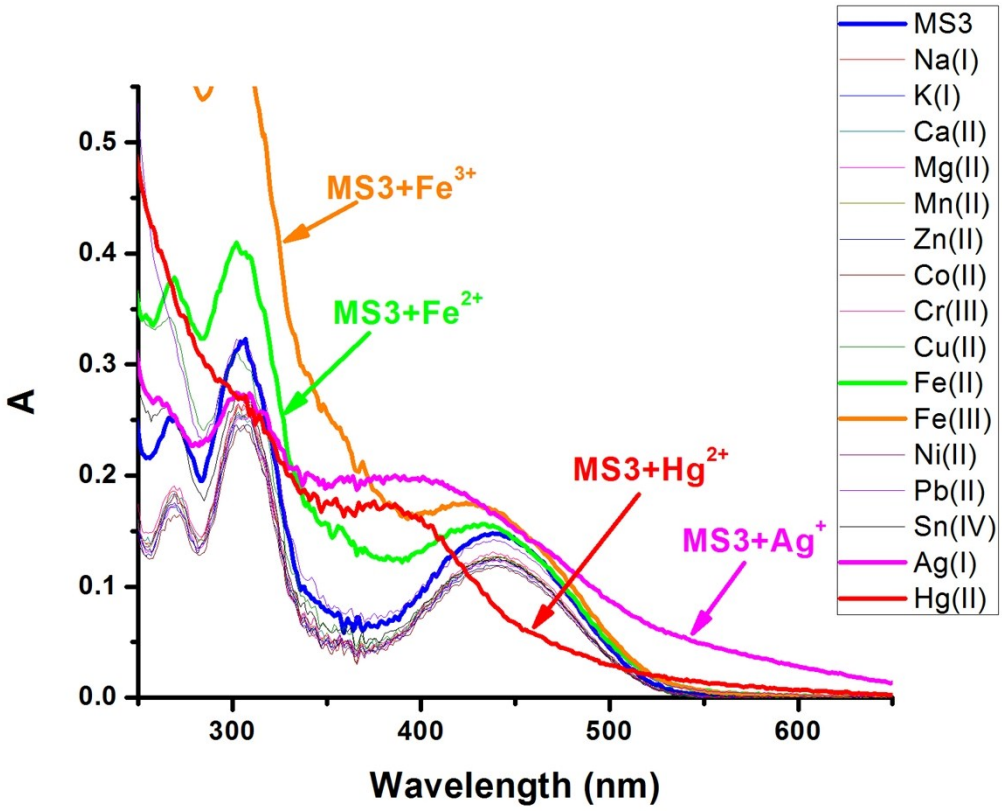


Fig. S2. The UV-vis absorption of **MS3** to various metal ions (5.0 equiv. of each, including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Sn^{4+}, Ag^{+}, and Hg^{2+},) in PBS buffer solution, 10 mM, pH 7.4, containing 1% DMSO.

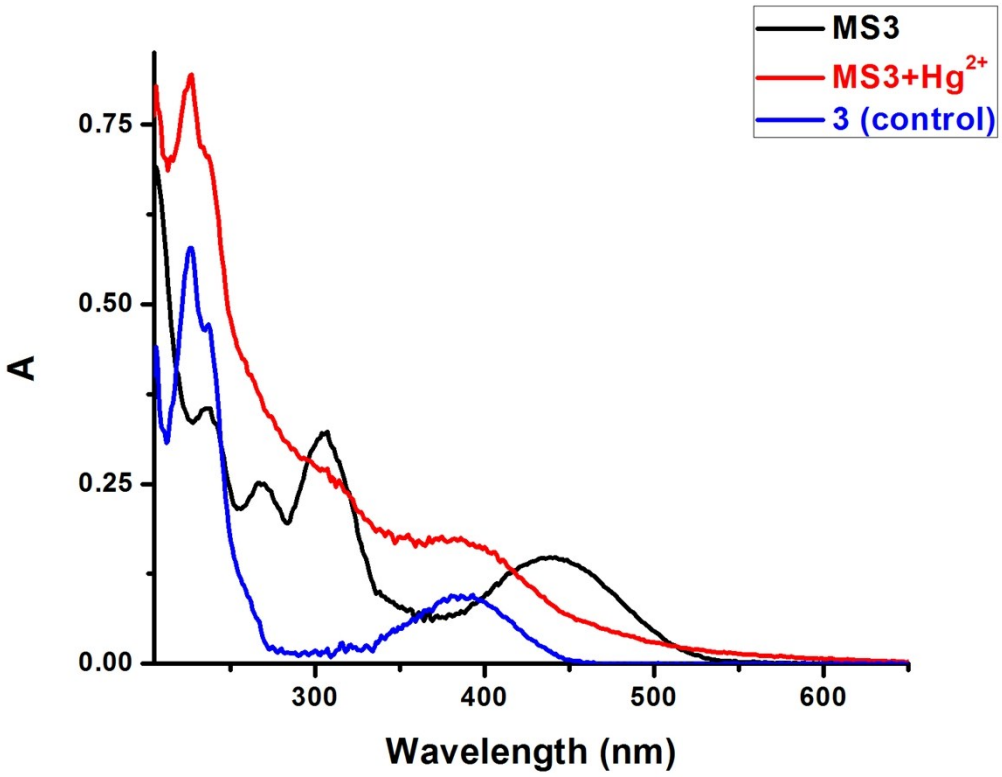


Fig. S3. The UV-vis absorption of **MS3**, **MS3**+Hg^{2+} system, and compound **3** (control) in PBS buffer solution, 10 mM, pH 7.4, containing 1% DMSO.

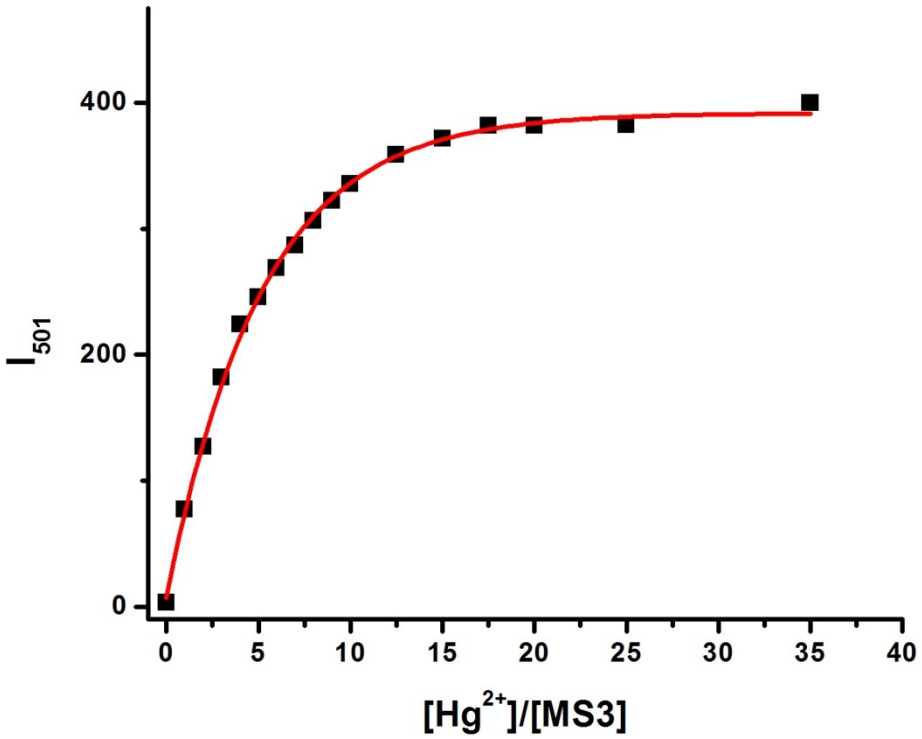


Fig. S4 Fluorescent intensity of **MS3** (10.0 μM) at 501 nm as a function of Hg^{2+} concentration (0-35.0 equiv.) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO). (λ_{ex} = 392 nm).

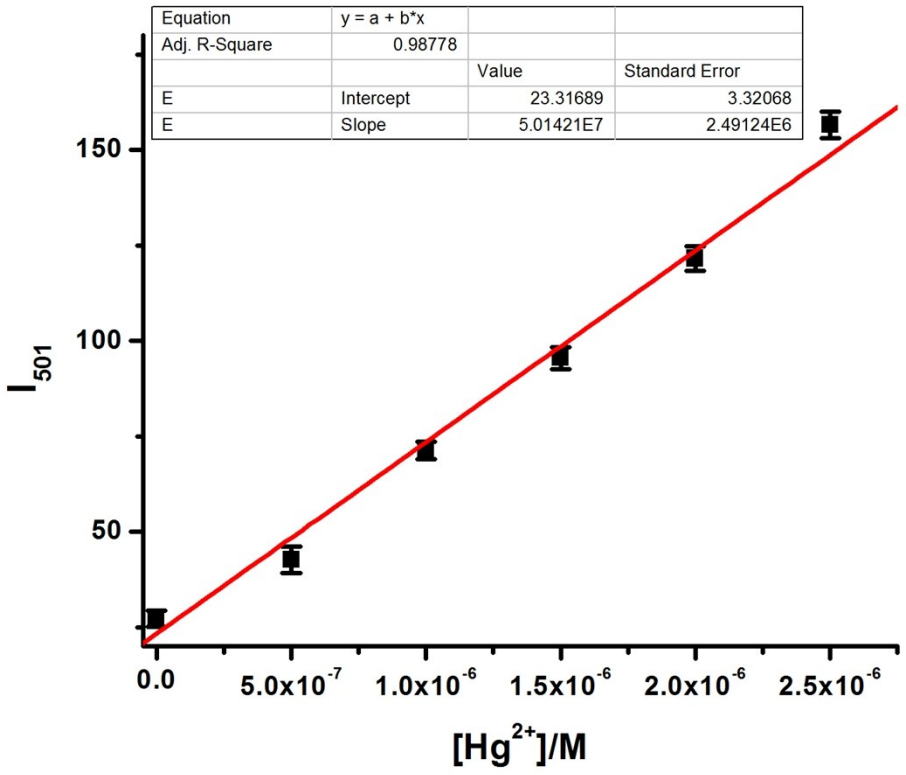


Fig. S5 The fluorescent intensity of **MS3** (1.0 μM) at 501 nm as a function of Hg^{2+} concentration (0-2.5 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), (λ_{ex} = 392 nm).

The detection limit (DL) of Hg^{2+} using **MS3** was determined from the following equation: [3]

$$DL = 3*\sigma/K$$

Where σ is the standard deviation of the blank solution; K is the slope of the calibration curve.

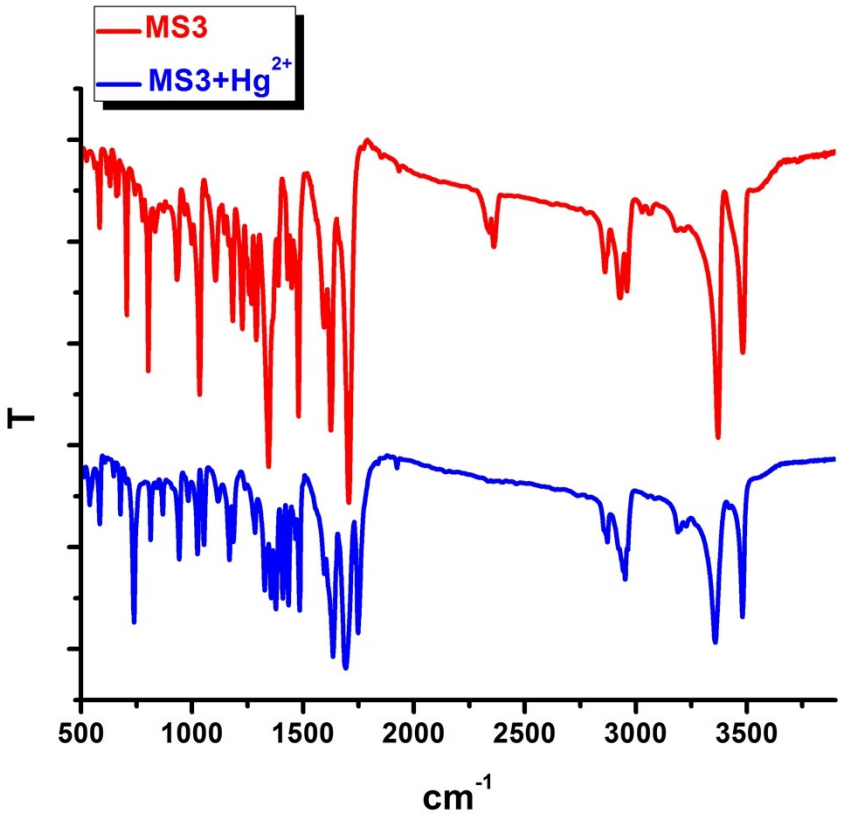
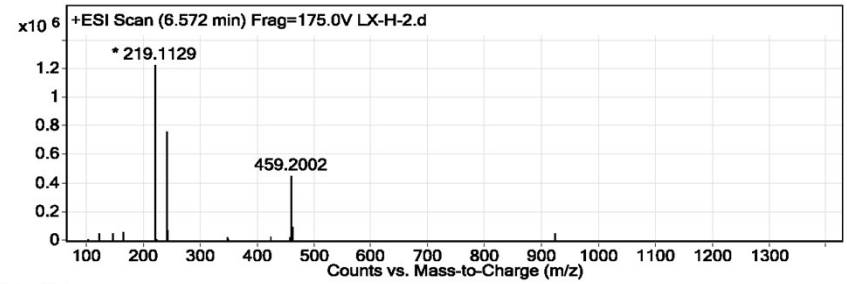


Fig. S6 FT IR spectra of **MS3** and **MS3**+Hg^{2+}.

Qualitative Analysis Report



Peak List

m/z	z	Abund
163.0501		67458.9
219.1129	1	1230246
219.1577		69750.8
220.1157	1	138012.5
241.0946	1	762472.6
242.0977	1	84057.4
459.2002	1	455256.4
460.2037	1	104230.6

Fig. S7 HR-MS spectra of the isolated product of the **MS3**–Hg2+ solution. HR-MS (TOF-ESI): Calcd. for $([M+H])^+$, 219.1134; Found, 219.1129.

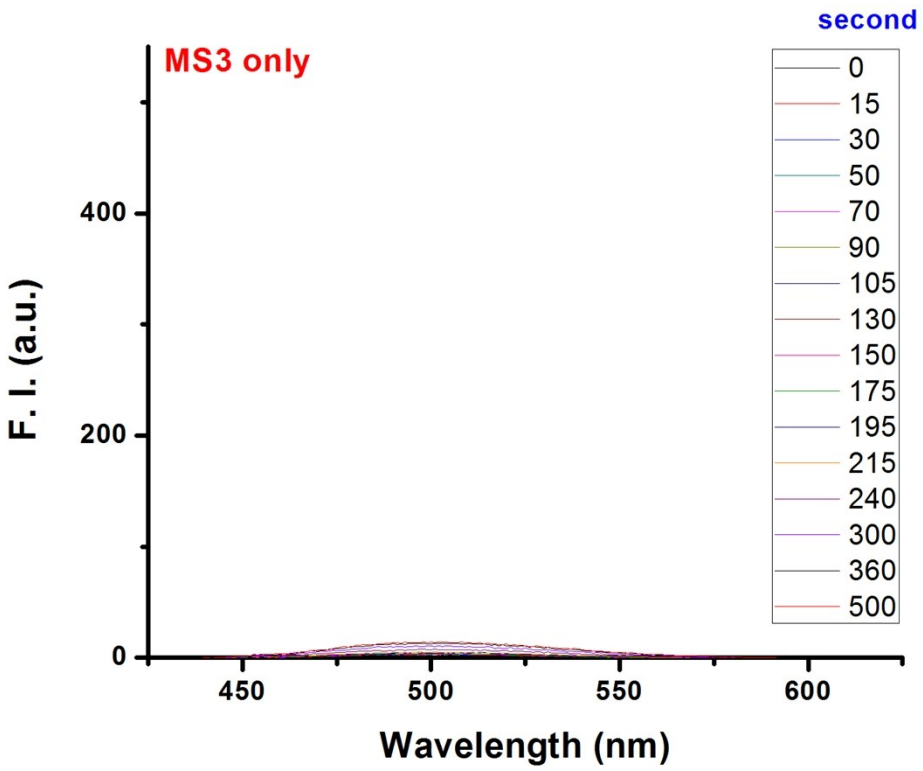


Fig. S8 Time-dependent fluorescence intensity changes of **MS3** (10.0 μM) alone in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λ_{ex} = 392 nm).

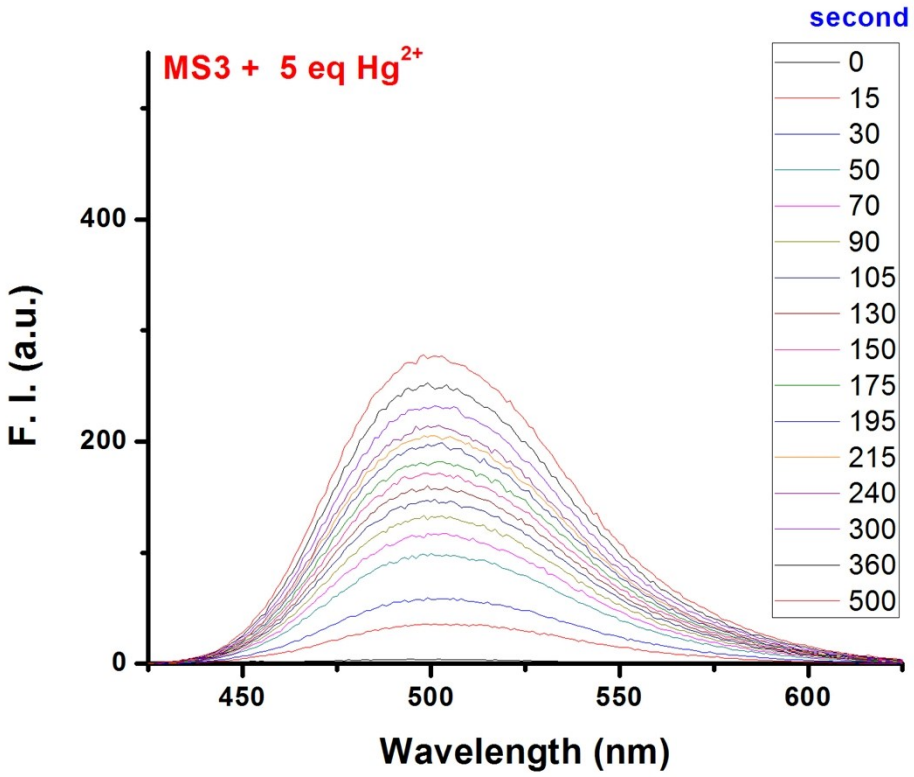


Fig. S9 Time-dependent fluorescence intensity changes of **MS3** (10.0 μM) upon addition of 5.0 equiv. of Hg^{2+} in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λ_{ex} = 392 nm).

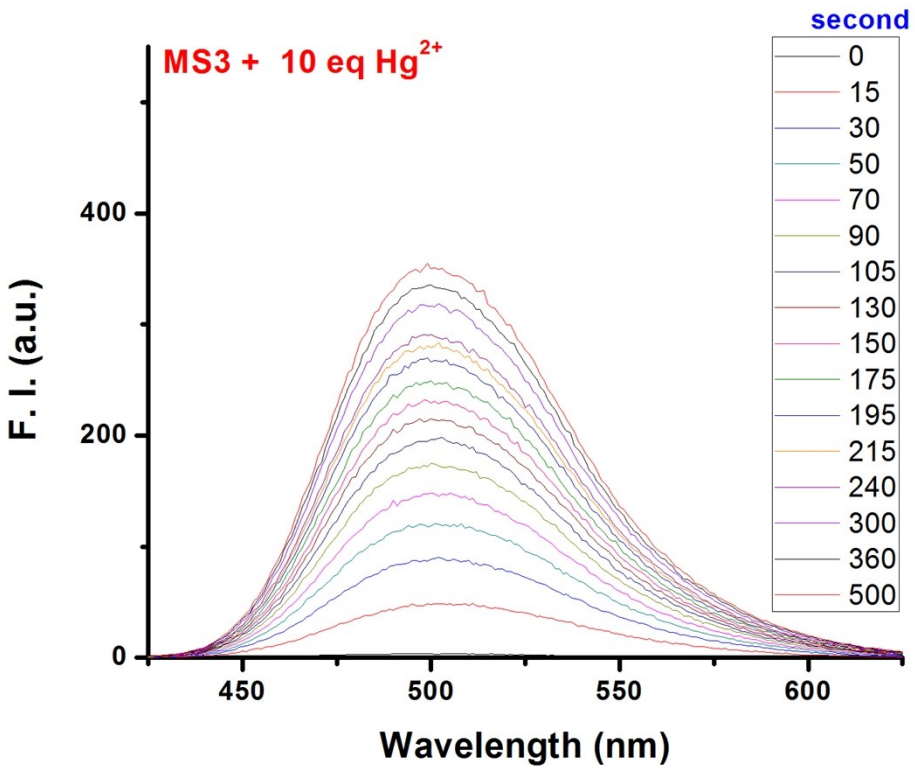


Fig. S10 Time-dependent fluorescence intensity changes of **MS3** (10.0 μM) upon addition of 10.0 equiv. of Hg^{2+} in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λ_{ex} = 392 nm).

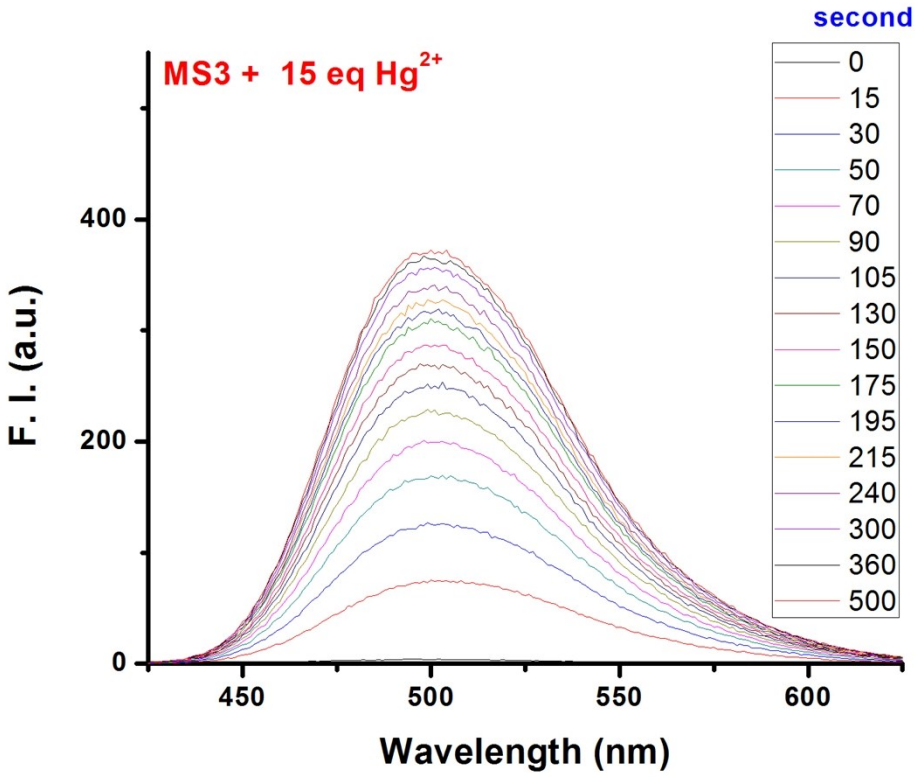


Fig. S11 Time-dependent fluorescence intensity changes of **MS3** (10.0 μM) upon addition of 15.0 equiv. of Hg^{2+} in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λ_{ex} = 392 nm).

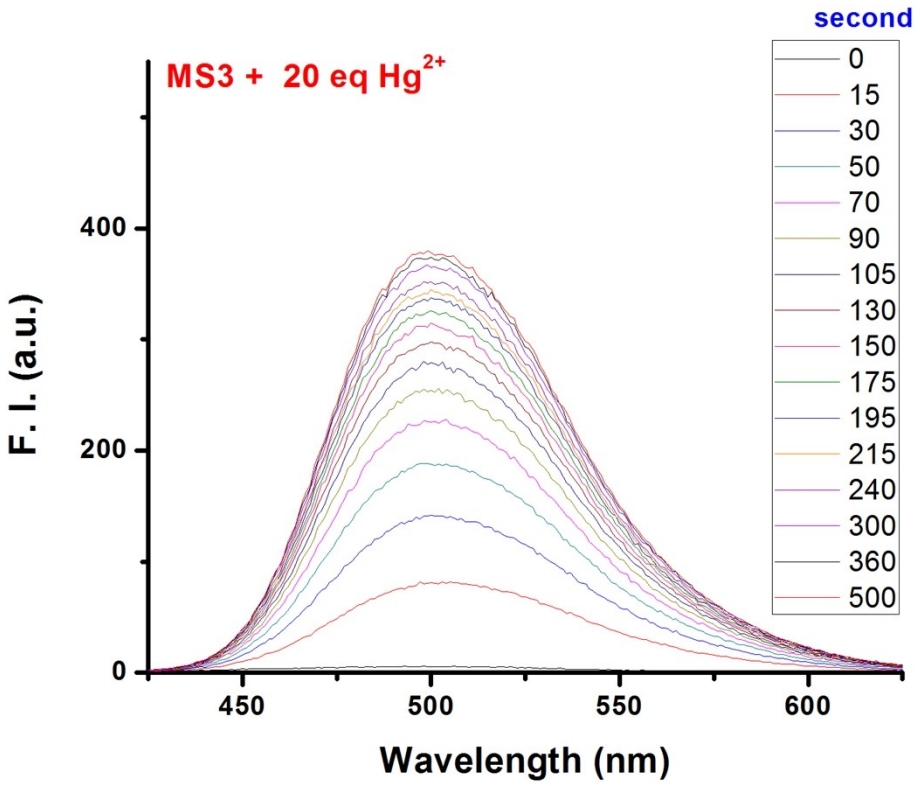


Fig. S12 Time-dependent fluorescence intensity changes of **MS3** (10.0 μM) upon addition of 20.0 equiv. of Hg^{2+} in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λ_{ex} = 392 nm).

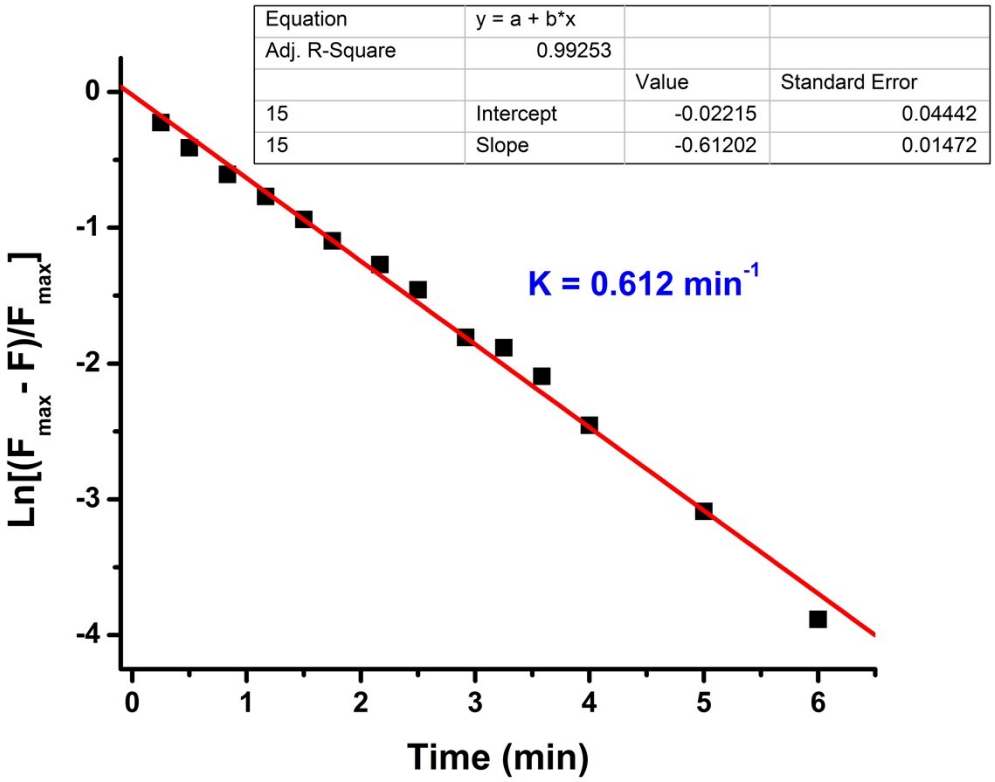


Fig. S13 The pseudo-first-order kinetic plot of the reaction of the probe **MS3** (10.0 μM) with Hg^{2+} (150.0 μM) at 501 nm in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λ_{ex} = 392 nm). Slope = 0.612 min^{-1};

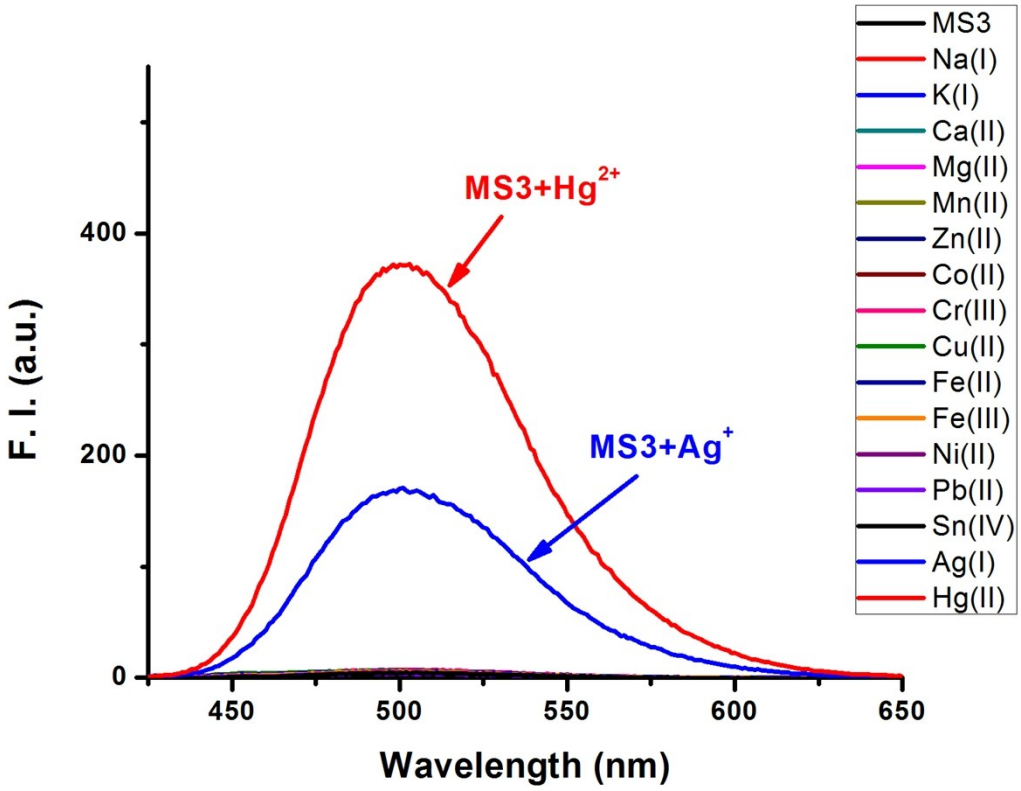


Fig. S14 Fluorescence responses of **MS3** (10.0 μM) to various metal ions (15.0 equiv. of each metal ions, including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Sn^{4+}, Ag^{+}, and Hg^{2+}) in PBS buffer solution, 10 mM, pH 7.4, containing 1% DMSO (λ_{ex} = 392 nm).

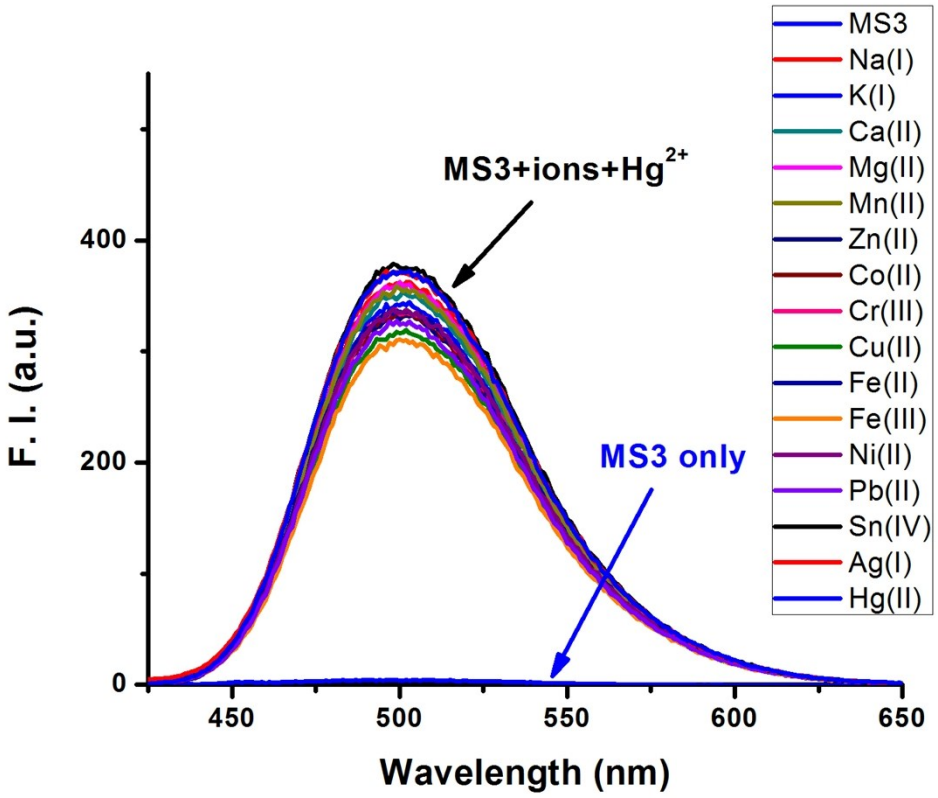


Fig. S15 Fluorescence responses of **MS3** (10.0 μM) in the presence of 15.0 equiv. of metal ions (including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Sn^{4+}, Ag^{+}, and Hg^{2+}) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), followed by 15.0 equiv. of Hg^{2+} (λ_{ex} = 392 nm).

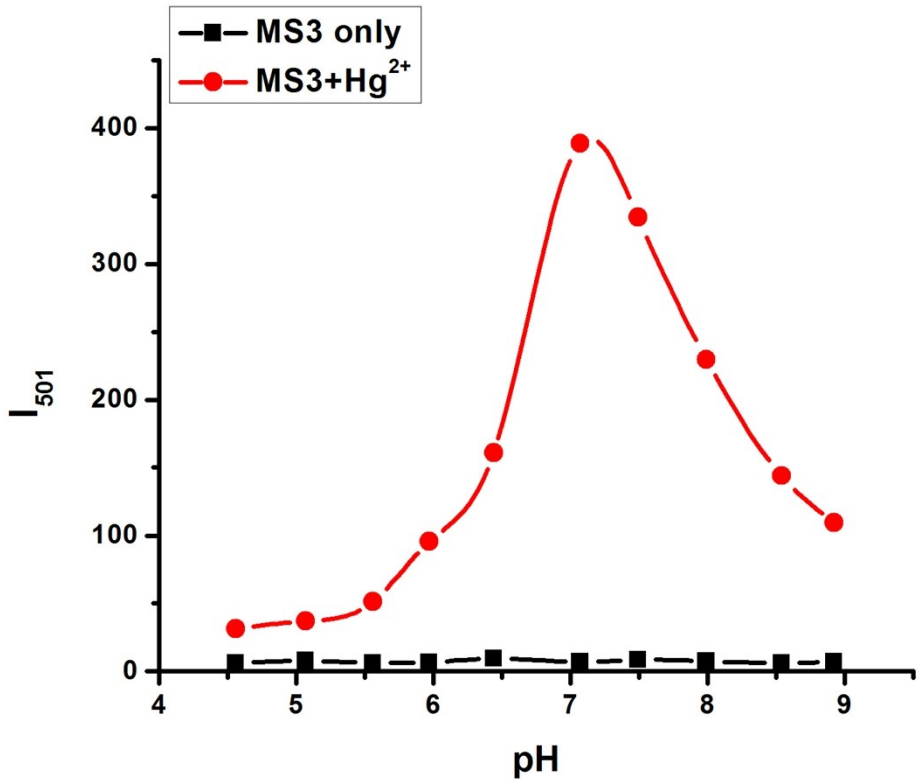


Fig. S16 Effect of the pH on the fluorescence intensity at 501 nm of **MS3** (10.0 μM) alone and in the presence of Hg^{2+} (15.0 equiv.) (λ_{ex} = 392 nm).

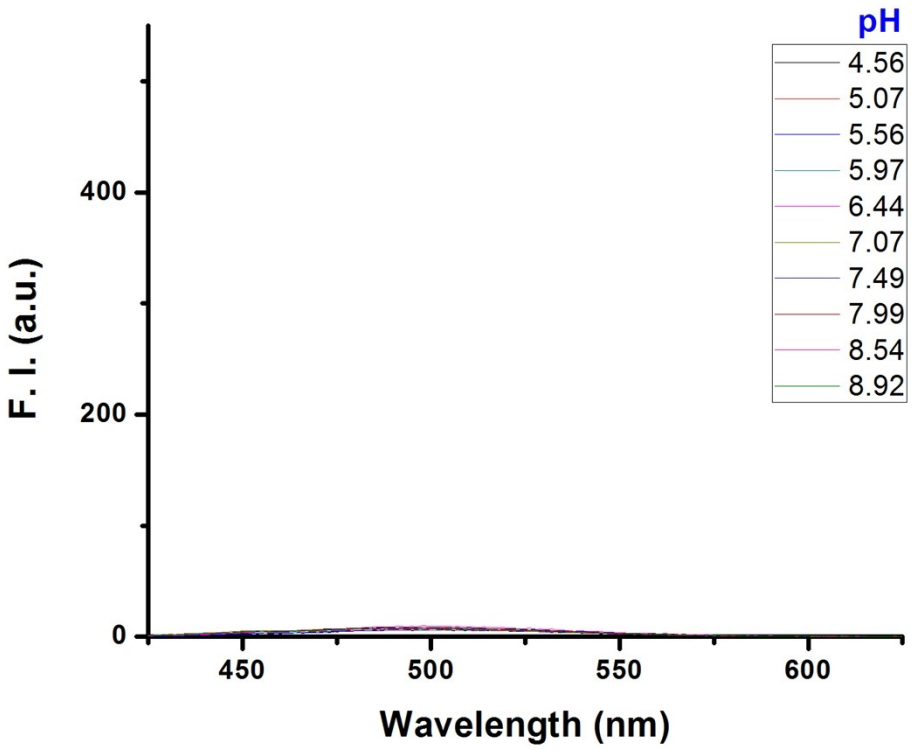


Fig. S17 Effect of the pH on the fluorescence intensity at 501 nm of **MS3** (10.0 μM) alone (λ_{ex} = 392 nm).

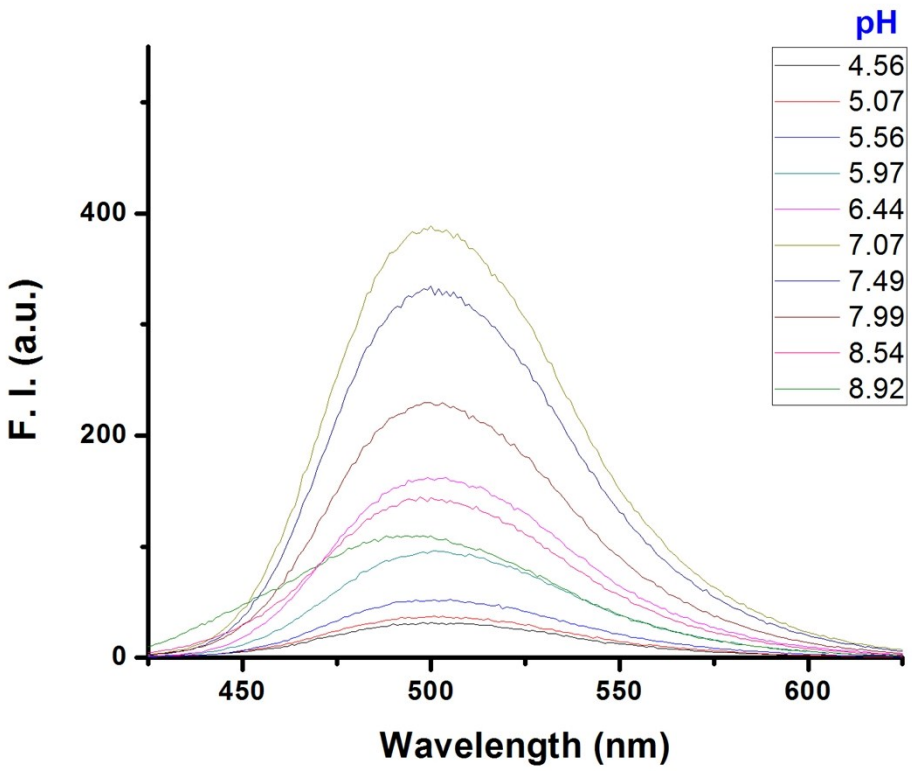


Fig. S18 Effect of the pH on the fluorescence intensity at 501 nm of **MS3** (10.0 μM) in the presence of Hg^{2+} (15.0 equiv.) (λ_{ex} = 392 nm).

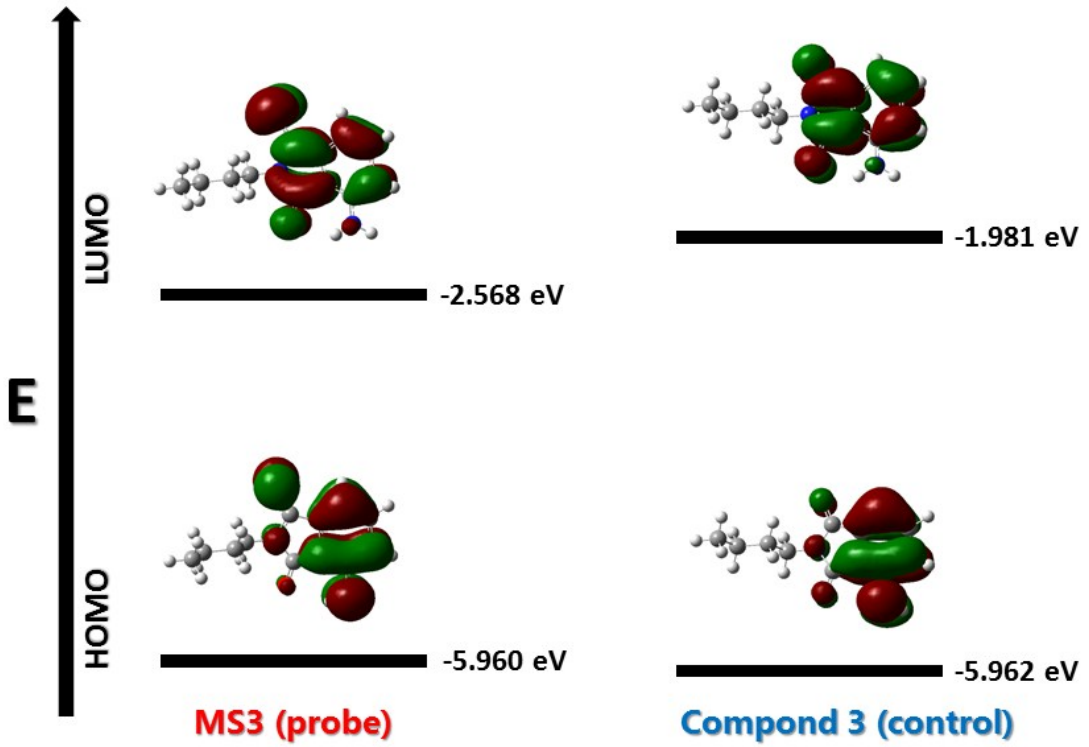


Fig. S19 HOMO and LUMO energy levels of the frontier molecular orbitals of **MS3** (probe) and compound **3** (3-aminophthalimide).

Cell lines and imaging experiments

HeLa cells were cultured in DMEM (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37 ^oC. For imaging experiments, exponentially growing cells were seeded in 24-well plate. Cells were cultured at 37 ^oC in a 5% CO_2 atmosphere for 24 h before they were exposed to reagents.

For labeling, the medium was removed and cells were rinsed three times with PBS. Then HeLa cells were incubated with **MS3** (10 μM) in PBS (containing 1% EtOH) at 37 ^oC for 30 min as control. For Hg^{2+} imaging, another set of HeLa cells was preloaded with **MS3** (10 μM) in PBS (containing 1% EtOH) at 37 ^oC for 30 min, rinsed three times with PBS and further treated with different concentrations of Hg^{2+} (10, 15, and 20 μM, respectively) in PBS at 37 ^oC for additional 30 min. Cells were rinsed three times with PBS and bathed in it, then imaging was carried out. Images were acquired using an inverted fluorescence microscope and fluorescence imaging was performed in a blue channel.

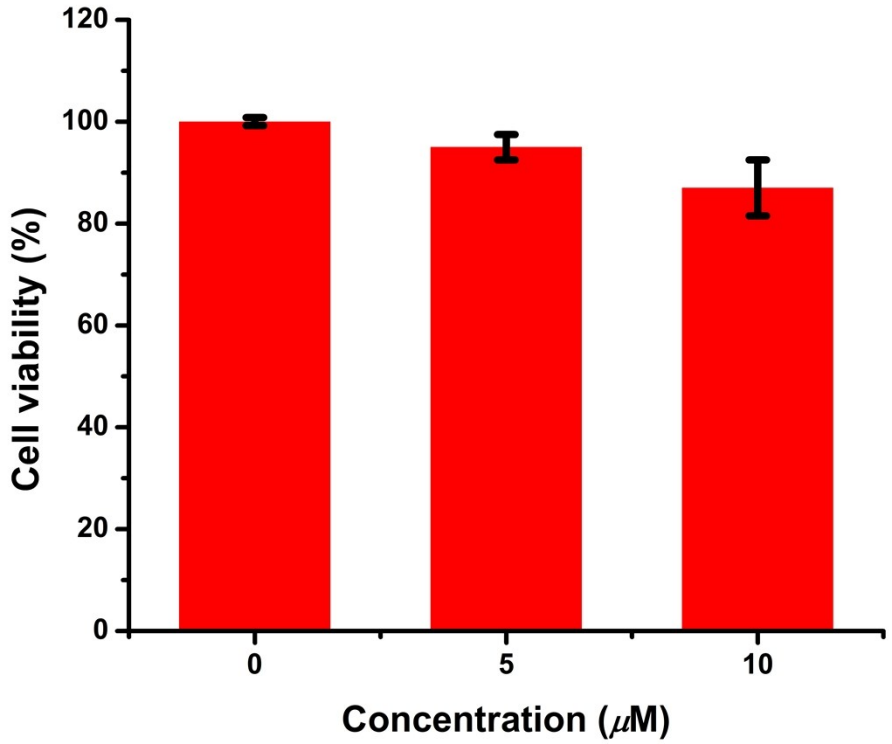
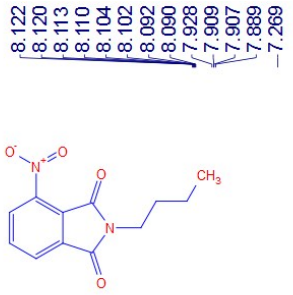
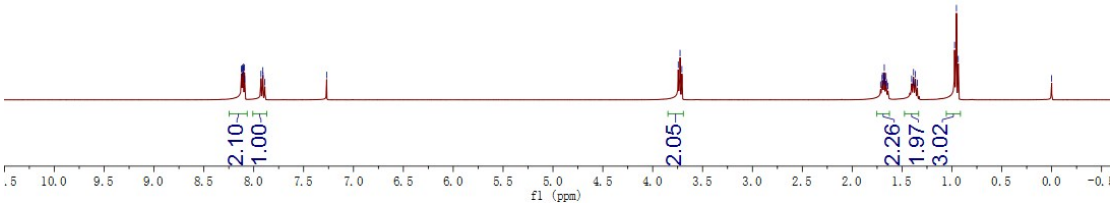


Fig. S20 Cell viability of HeLa cells treated with different concentration of **MS3** for different time periods. No cytotoxic effect was observed for the cells incubated with **MS3** at 10 μM even for 24 h.

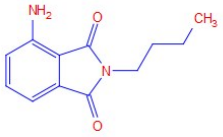
The characterization data of MS3

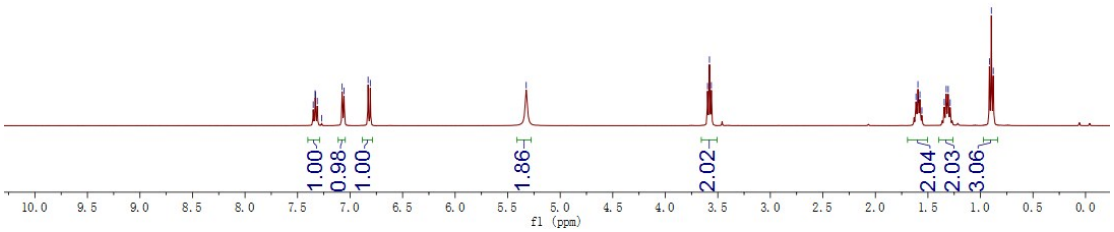
^{1}H NMR of **2**



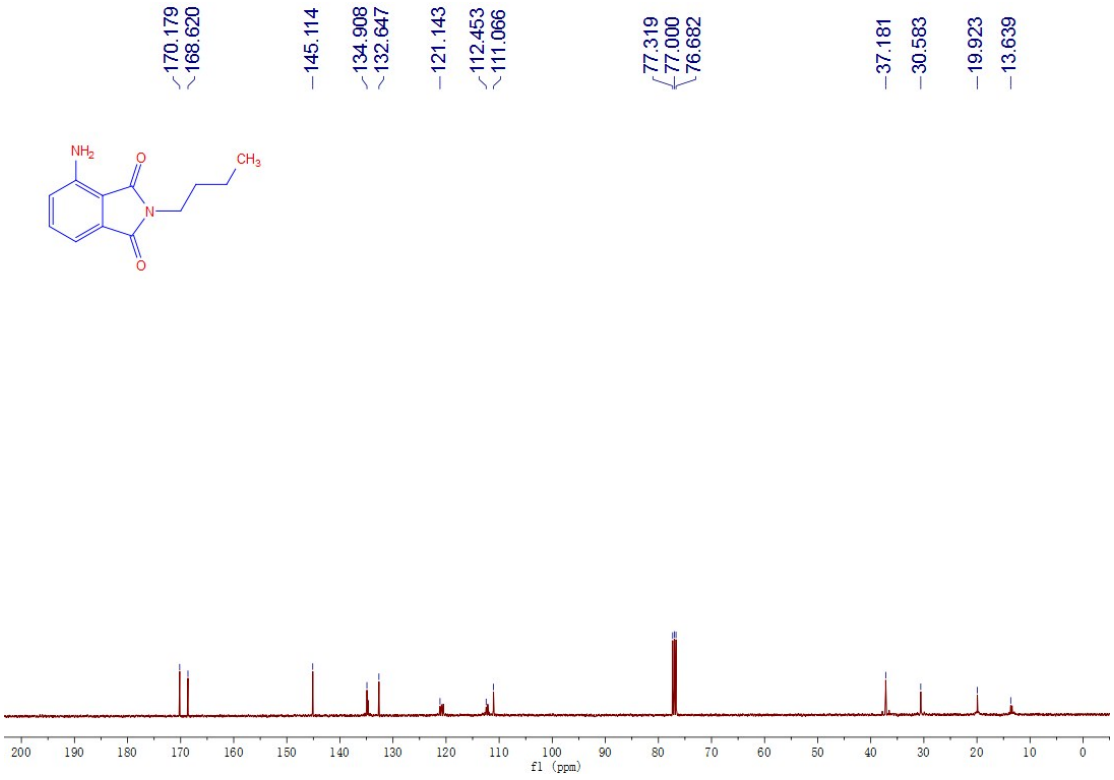


^{1}H NMR of **3**

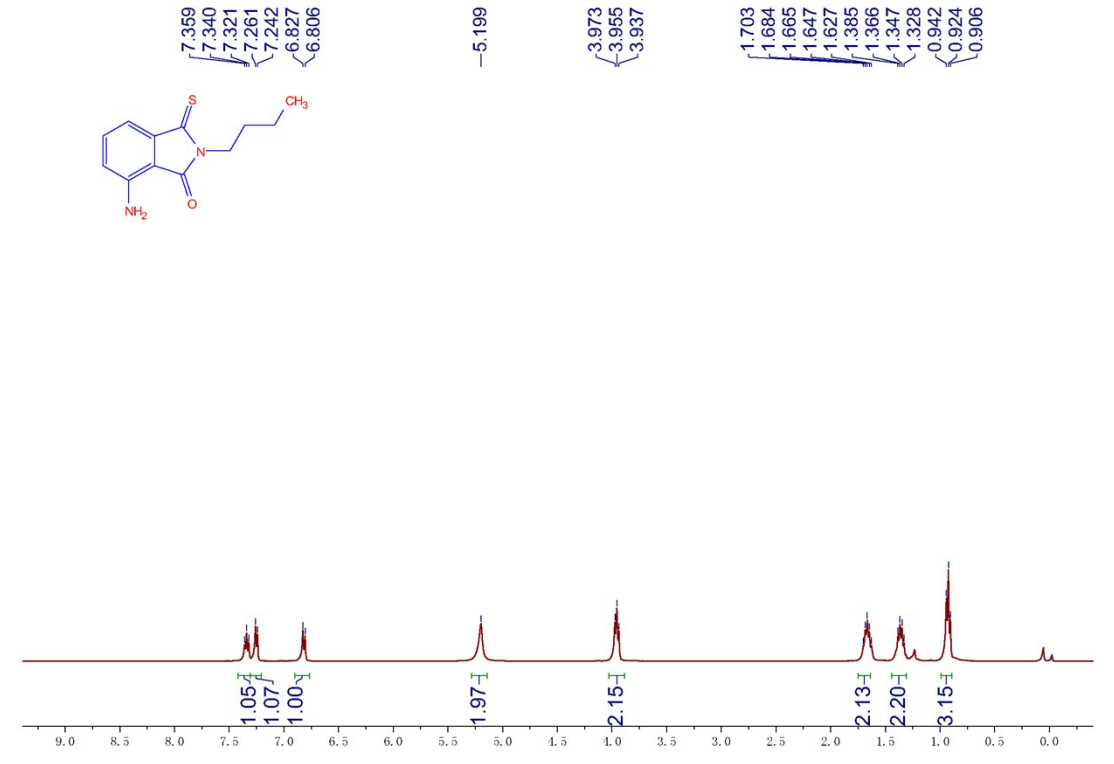




^{13}C NMR of **3**



^{1}H NMR of **4 (MS3)**



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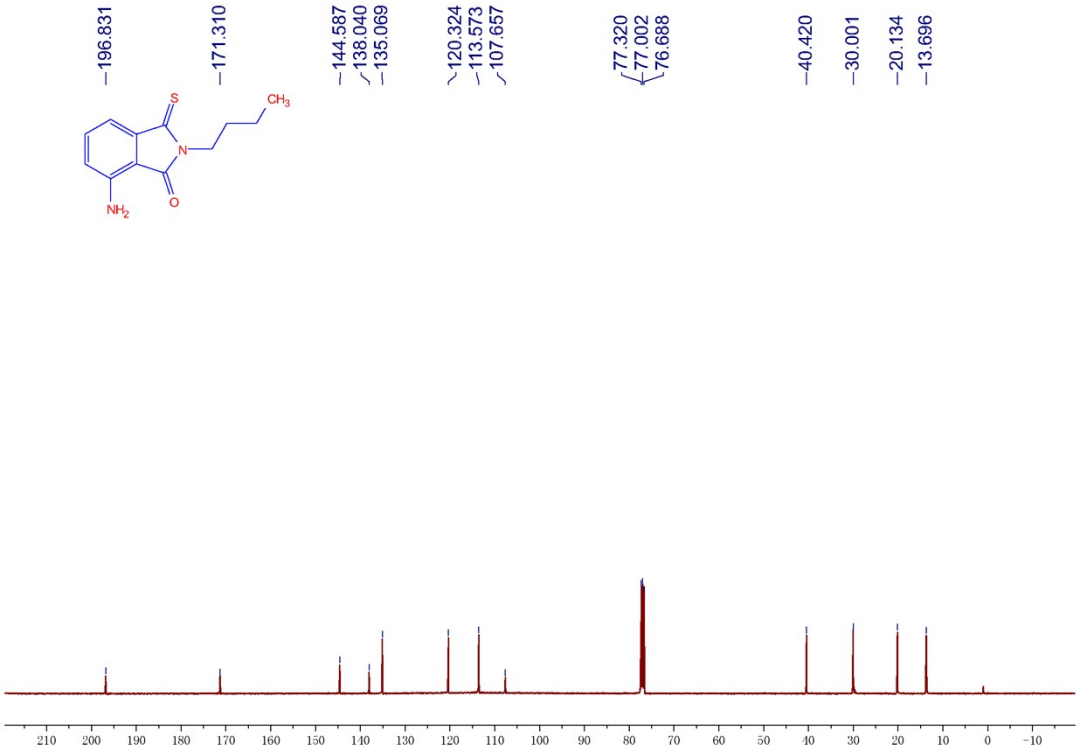
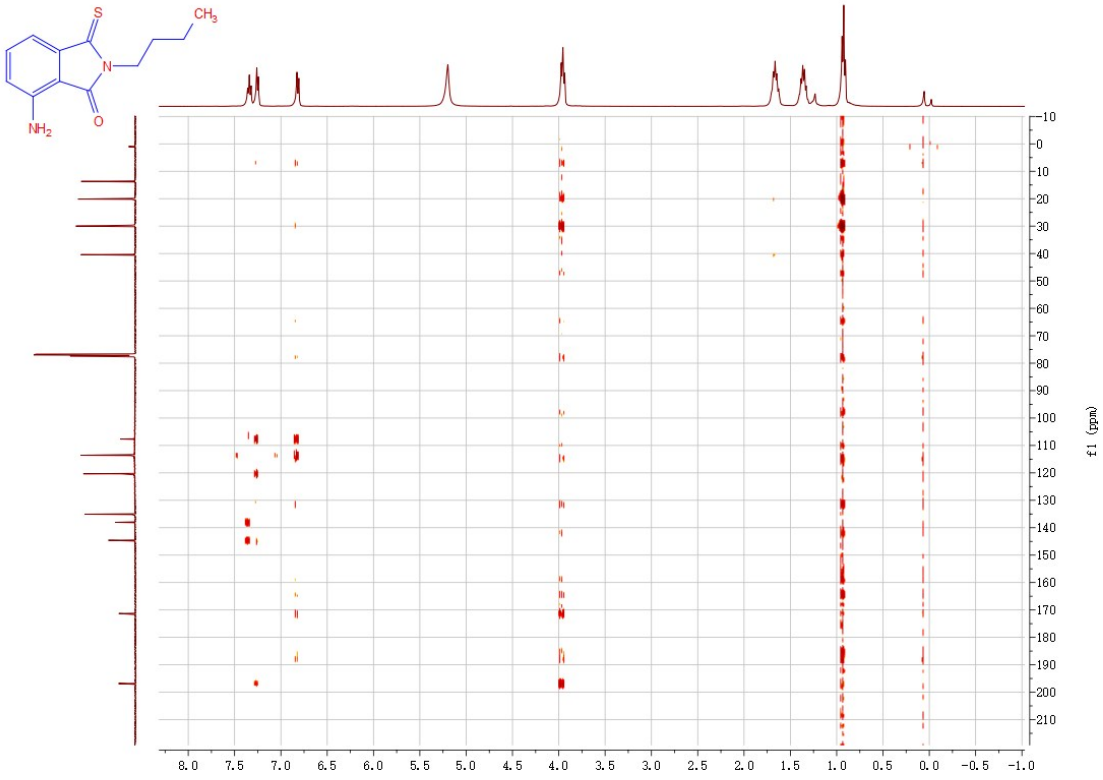
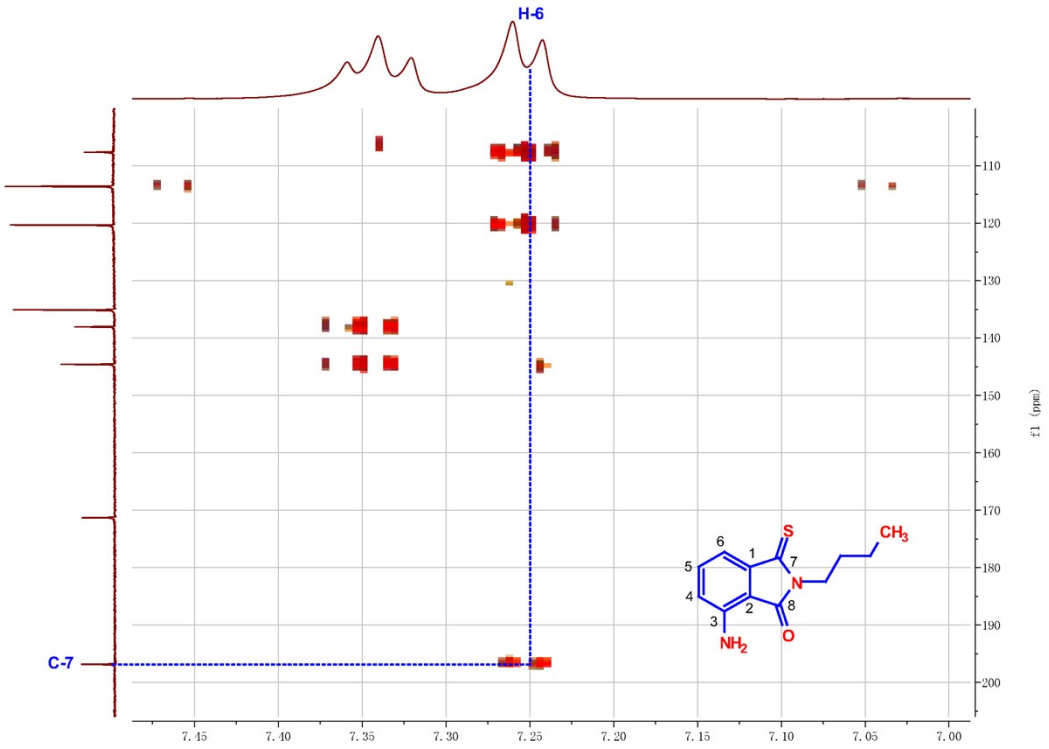


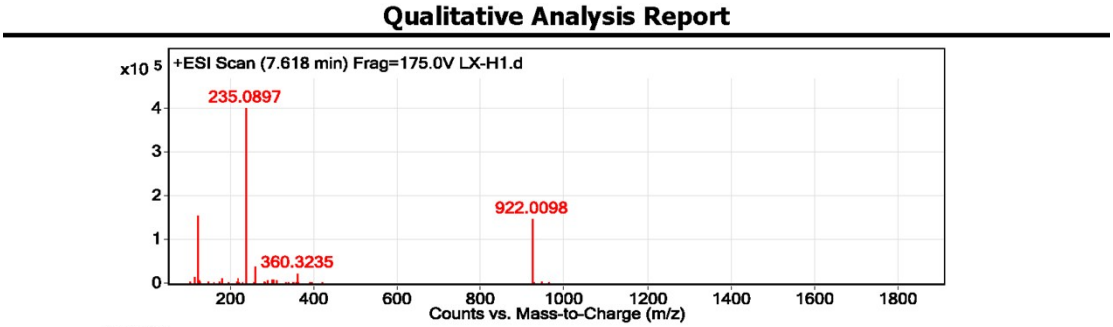
2D NMR (HMBC) of **4 (MS3)**



Partial enlarge of HMBC spectrum of **4 (MS3)**



HRMS of **4 (MS3)**



Peak List

m/z	z	Abund
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235.1354		28787.5
236.093	1	48276.7
257.0721		40137
360.3235		24592.8
922.0098	1	148511.5
923.0127	1	27007.8

References

1 R. A. Velapoldi, and H. H. Tønnesen, *J. Fluoresc*., 2004, **14**, 465-472.

2 (*a*) D. F. Eaton, *Pure Appl. Chem*., 1988, **60**, 1107-1114; (*b*) D. Magde, R. Wong, and P. G. Seybold, *Photochem. Photobiol*., 2002, **75**, 327-334.

3 (*a*) J. T. Yeh, P. Venkatesan and S. P. Wu, *New J. Chem*., 2014, **38**, 6198-6204. (*b*) A. Roy, D. Kand, T. Saha and P. Talukdar, *Chem. Commun*., 2014, **50**, 5510-5513.