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

Supporting Information

A Thiocoumarin-Based Colorimetric and Ratiometric Fluorescent Probe for Hg^{2+} in Aqueous Solution and Its Application in Live-Cell Imaging

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Photophysical properties of MS4

Table S1 Photophysical properties of the probe.

entry	λ_{ab} (nm)	λ_{em} (nm)	Φ^a	ε / M^{-1} cm^{-1}
MS4	485	543	0.079	33273
MS4+Hg^{2+}	386	477	0.081[b]	28521

(a) The quantum yield (Φ) of **MS4** and **MS4**-Hg^{2+} system were determined according to the literature.[1] (b) Φ was determined in the present of 2.0 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 Rhodamine 6G in EtOH as standard, which has the quantum yield of 0.95.[2]

Additional spectroscopic data

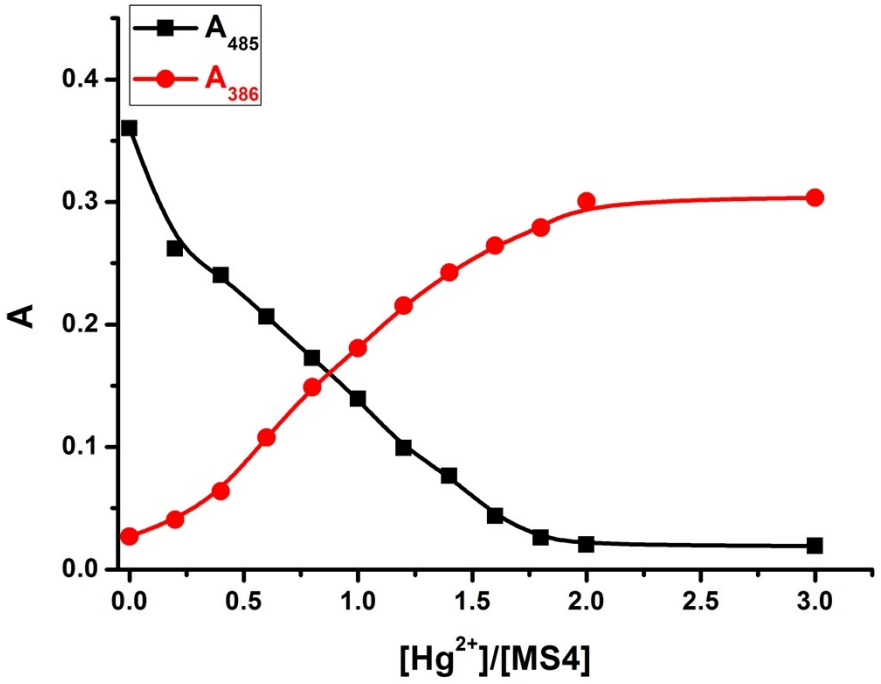


Fig. S1 The UV-vis absorption of **MS4** (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) at 485 nm and 386 nm as a function of Hg^{2+} concentration (0-3.0 equiv.).

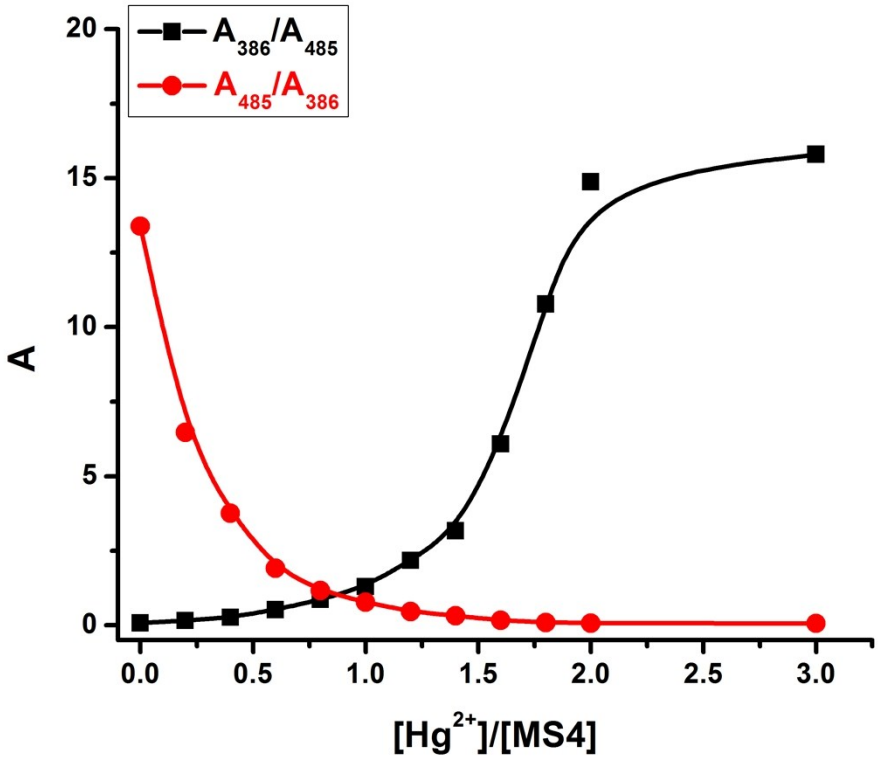


Fig. S2 The ratio of UV-vis absorption of **MS4** (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) at 485 nm and 386 nm (A_{386} nm/A_{485} nm and A_{485} nm/A_{386} nm) as a function of Hg^{2+} concentration (0-3.0 equiv.).

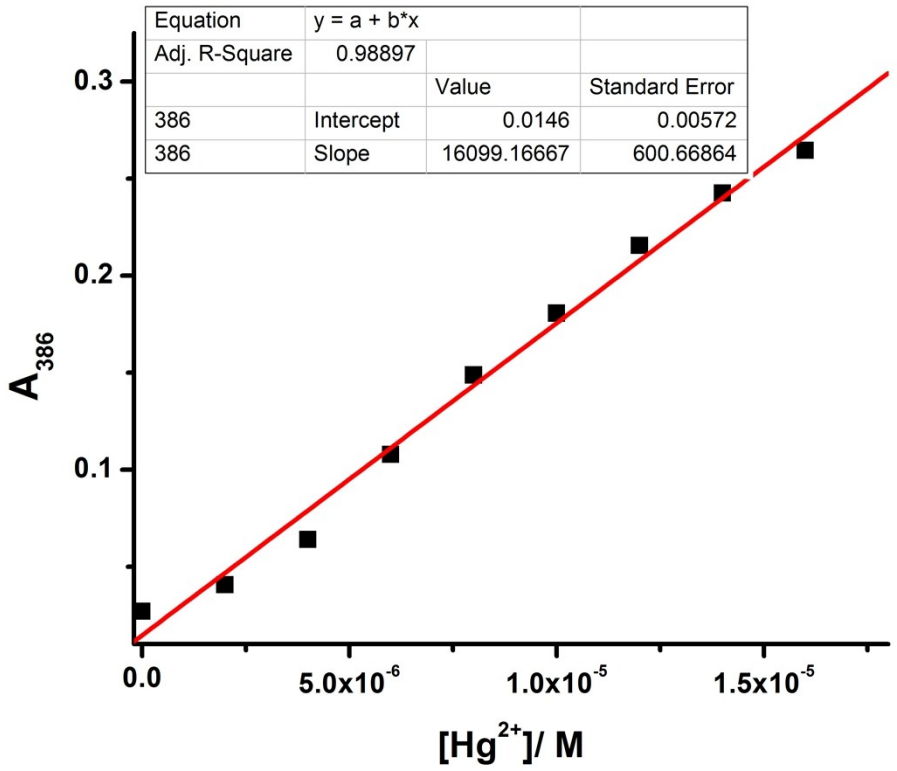


Fig. S3 The absorbance at 386 nm of UV-vis absorption of **MS4** (10.0 μM) as a function of Hg^{2+} concentration (0-16.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO).

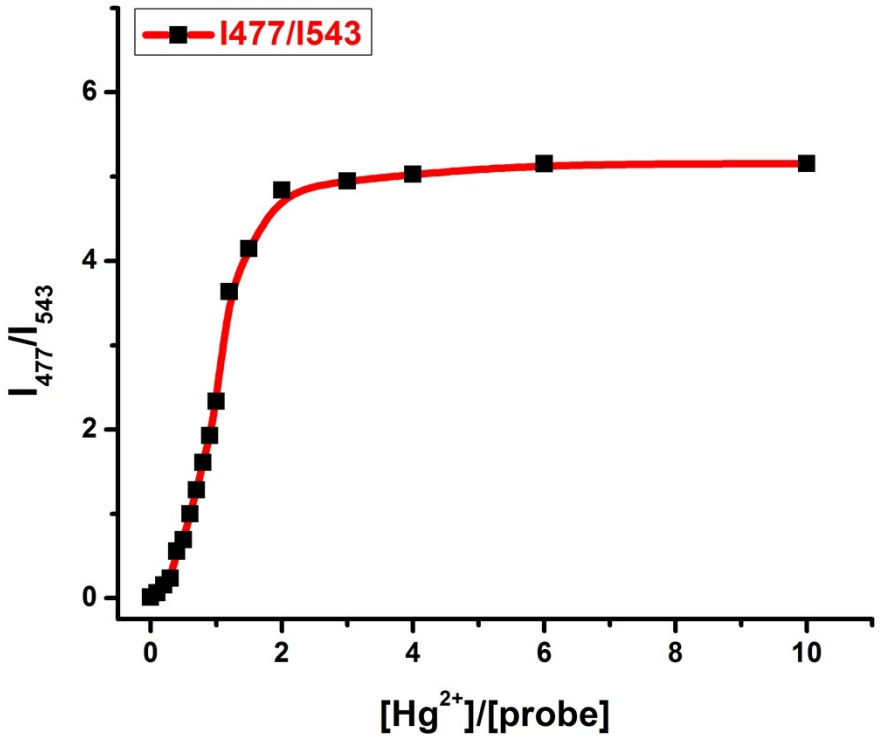


Fig. S4 The ratio of the fluorescent intensity of **MS4** (5.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) at 477 nm and 543 nm (I_{477} nm/I_{543} nm) as a function of Hg^{2+} concentration (0-10.0 equiv.) (λ_{ex} = 420 nm).

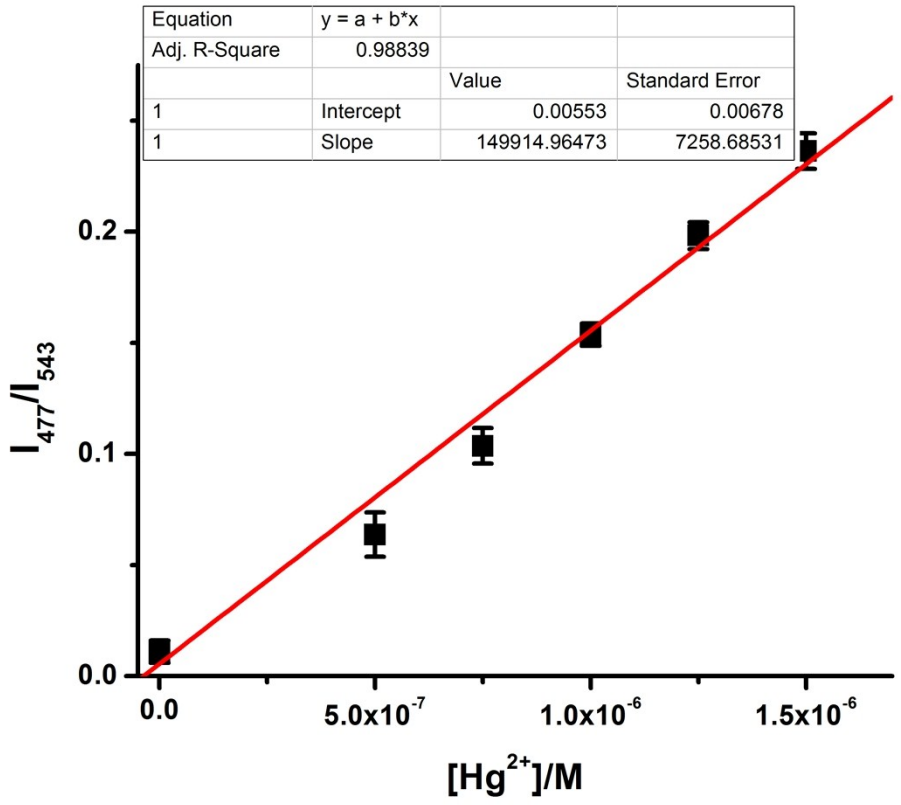


Fig. S5 The ratio of the fluorescent intensity of **MS4** (5.0 μM) at 477 nm and 543 nm (I_{477} nm/I_{543} nm) as a function of Hg^{2+} concentration (0-1.5 μM) under the same condition as the Hg^{2+} titration.

The detection limit (DL) of Hg^{2+} using **MS4**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.

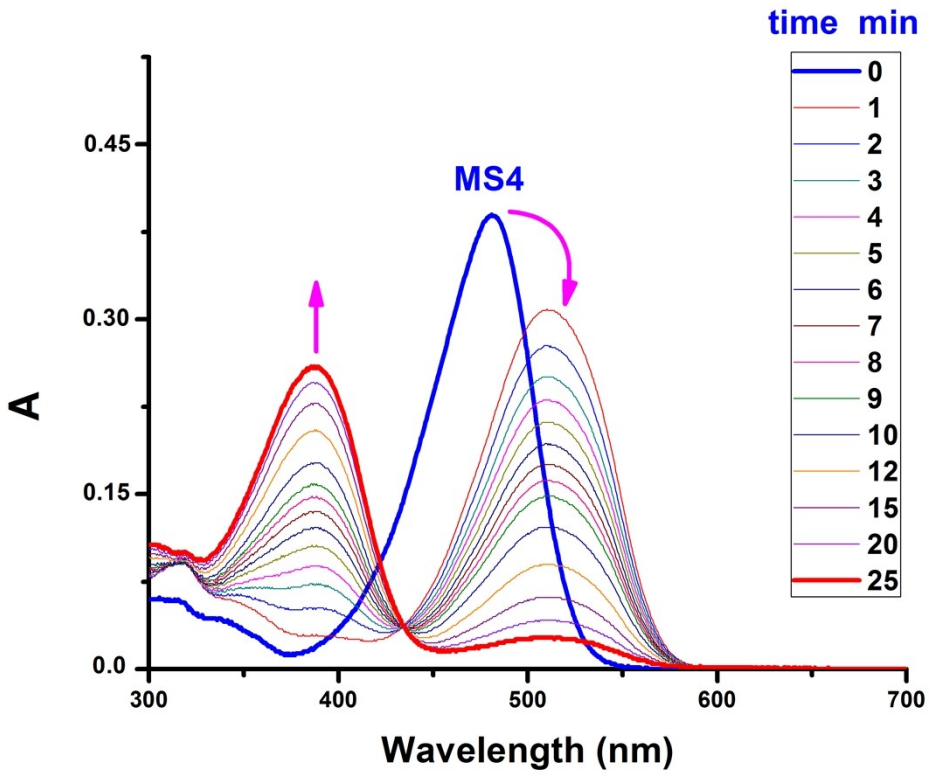


Fig. S6 Time-dependent Absorption spectra of **MS4** (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) in the presence of 2.0 equiv. of Hg^{2+}.

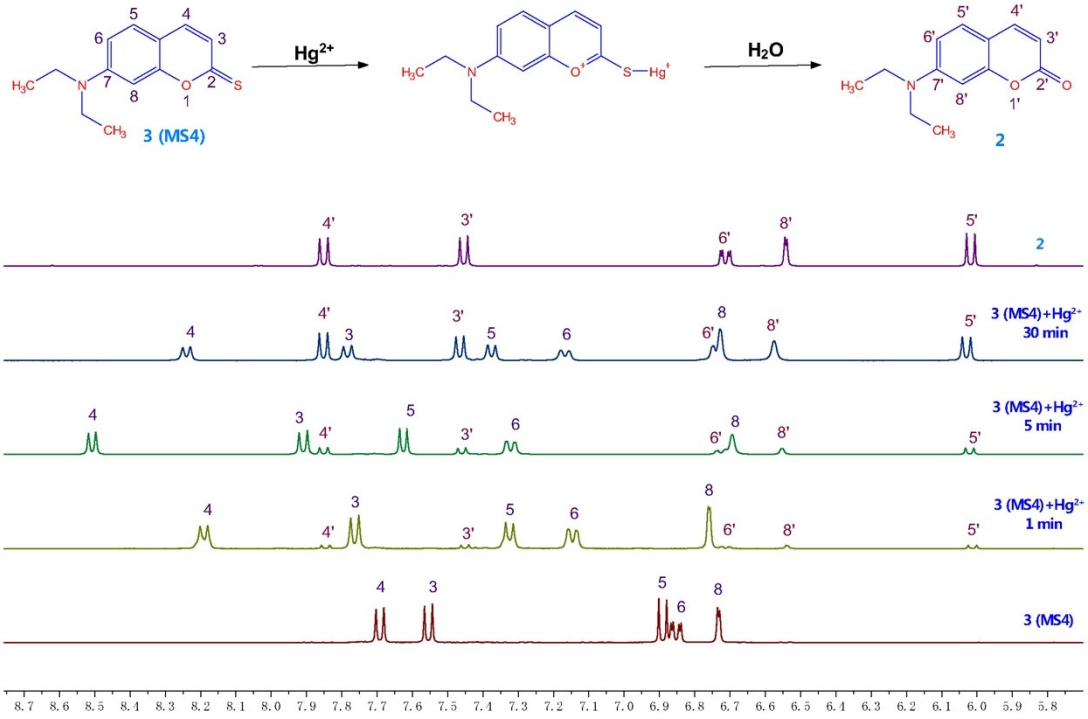


Fig. S7 ^{1}H NMR titration experiment of **MS4** in the present of Hg^{2+} in d_6-DMSO.

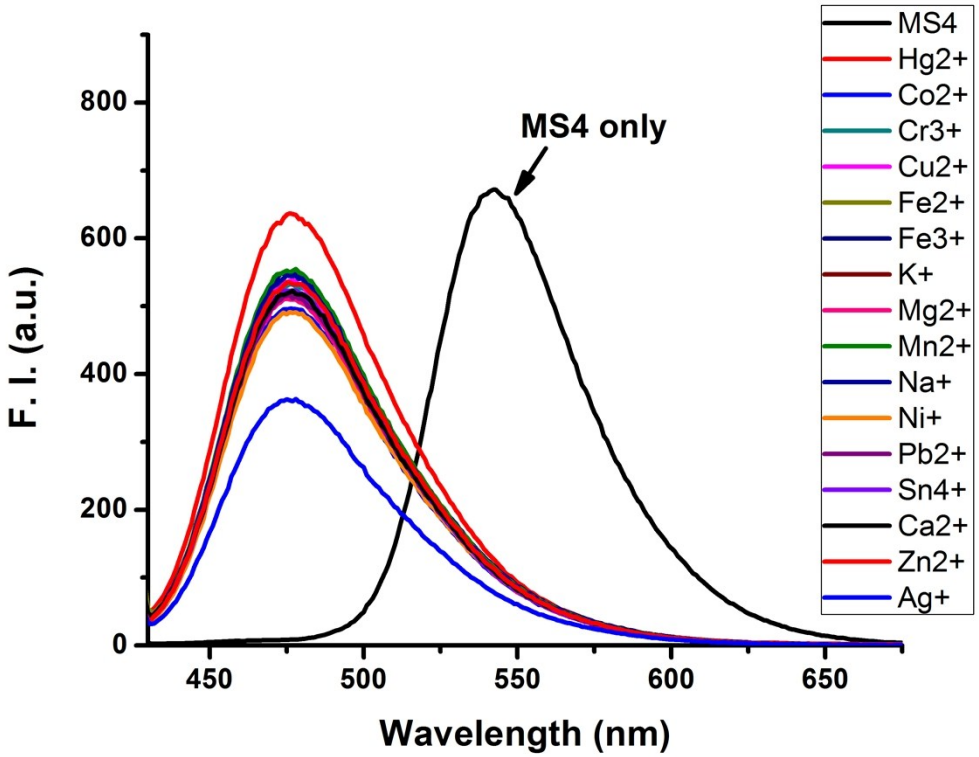


Fig. S8 Fluorescence responses of **MS4** (5.0 μM) with various metal ions (including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Sn^{4+}, Ag^{+}, and Hg^{2+}) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λ_{ex} = 420 nm).

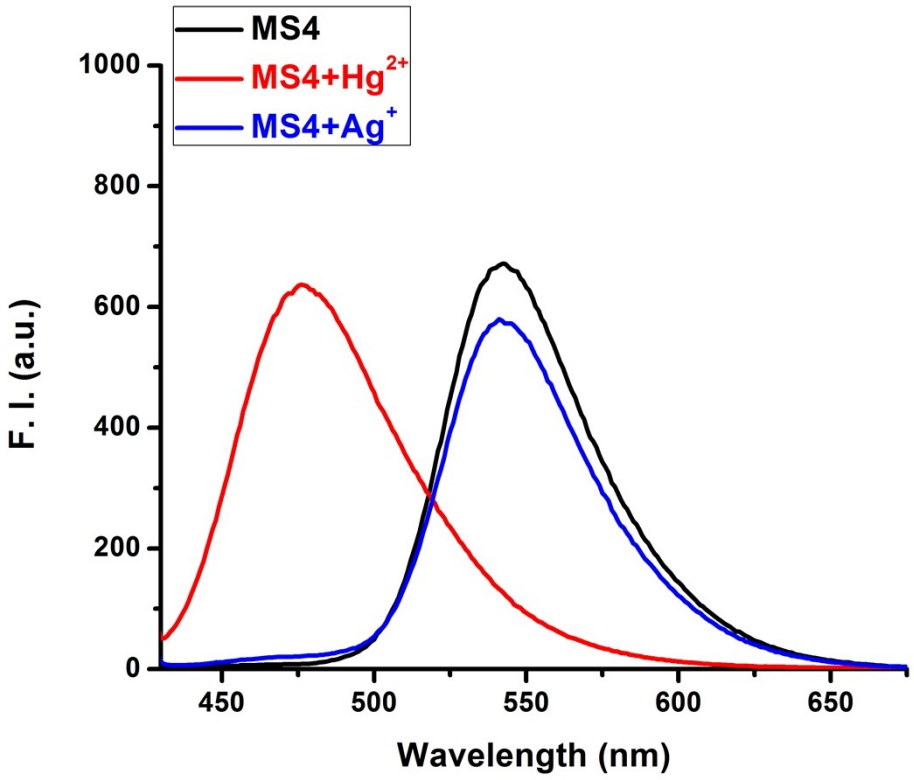


Fig. S9 Fluorescence responses of **MS4** (5.0 μM) with Ag^{+}, and Hg^{2+} in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO and 100 mM NaCl) (λex = 420 nm).

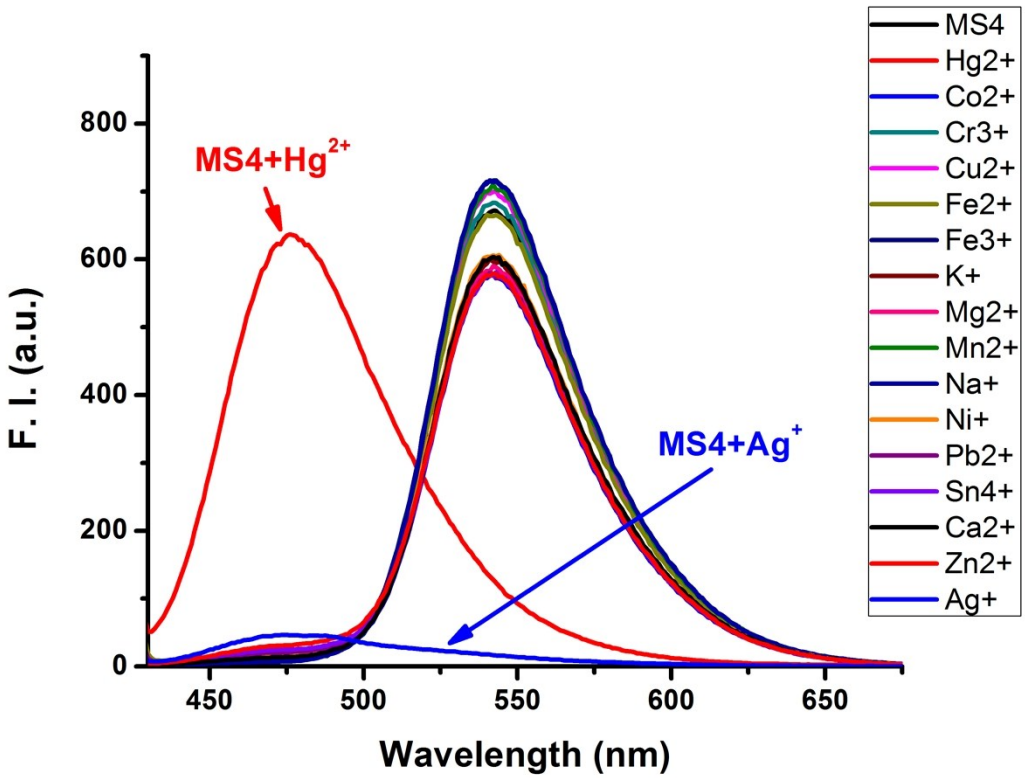


Fig. S10 Fluorescence responses of **MS4** (5.0 μM) in the presence of 3.0 equiv. of various metal ions (including Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Pb^{2+}, Sn^{4+}, and Ag^{+}) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), followed by 2.0 equiv. of Hg^{2+} (λ_{ex} = 420 nm).

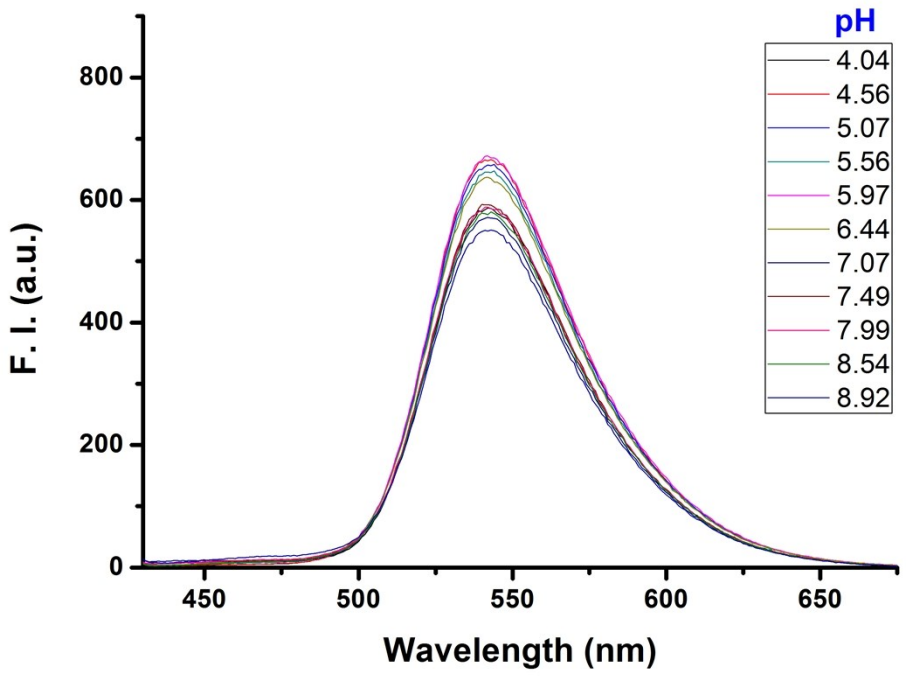


Fig. S11 pH-dependent experiment of **MS4** (5.0 μM) (λ_{ex} = 420 nm).

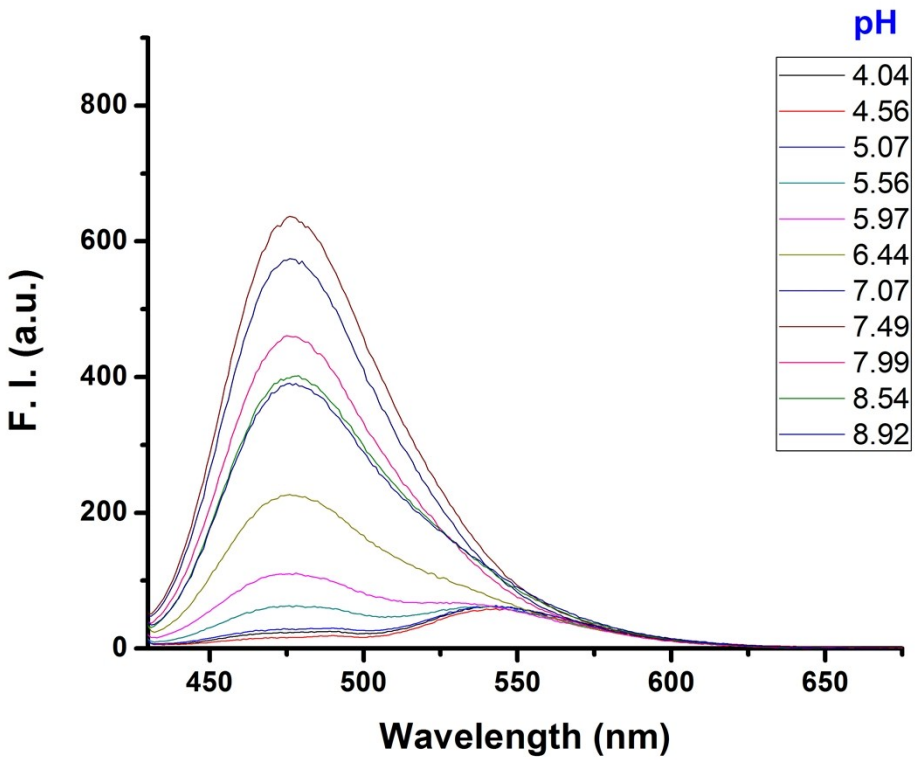


Fig. S12 pH-dependent experiment of **MS4** (5.0 μM) in the presence of Hg^{2+} (2.0 equiv.) (λ_{ex} = 420 nm).

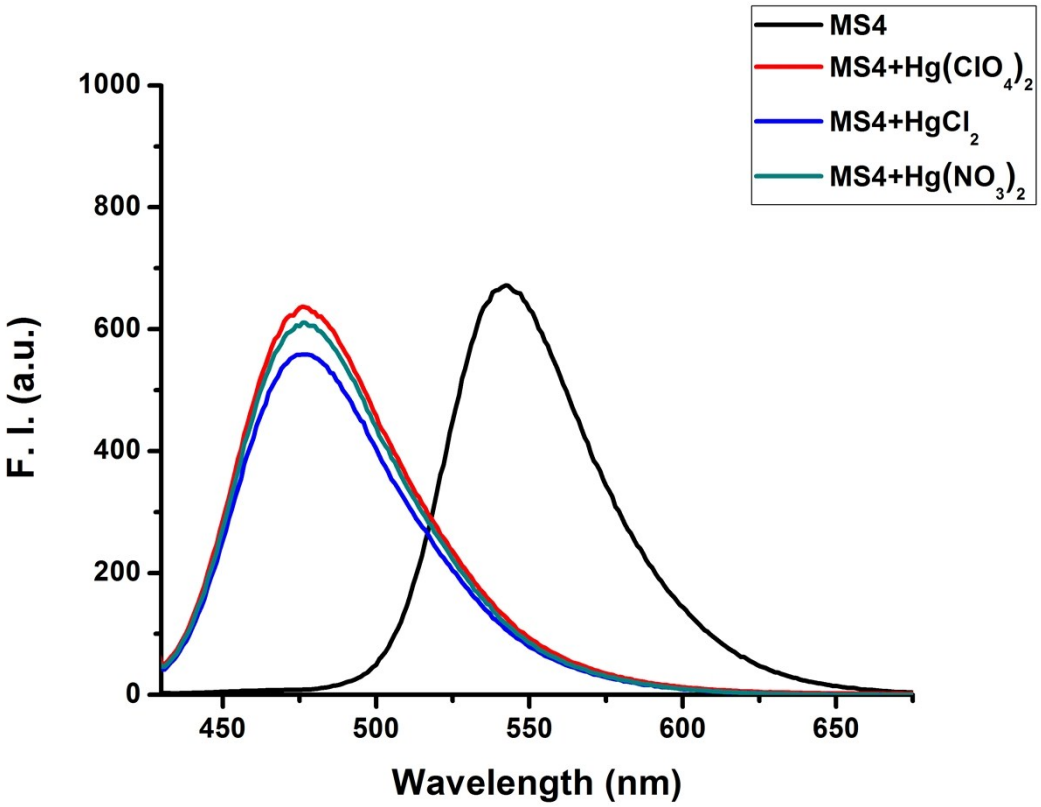
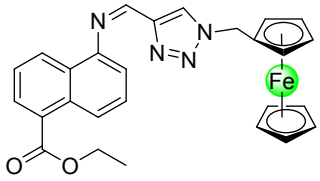
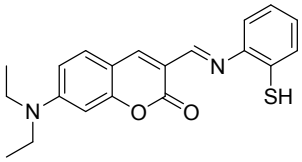
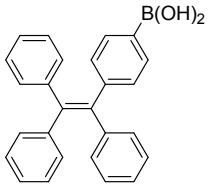
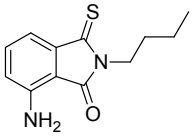
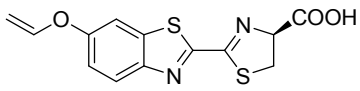
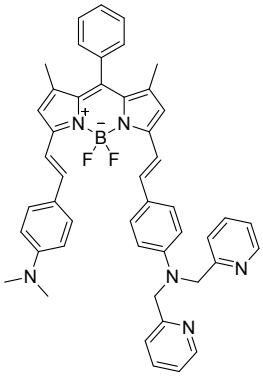
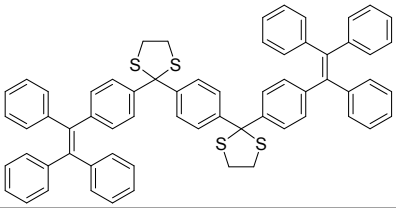
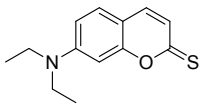


Fig. S13 The experiments between **MS4** (5.0 μM) with different source of Hg^{2+} (2.0 equiv. $HgCl_2$, $Hg(NO_3)_2$ and $Hg(ClO_4)_2$, respectively) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), (λ_{ex} = 420 nm).

Table S2 Comparison of the properties of **MS4** and other mercury sensors.

Structure	Solvent	Incubation time	LOD	Imaging in vivo	References
	MeCN-H_2O 2:8	30 min	2.7 nM	-	*Inorg. Chem*., 2017, 56, 11577−11590
	MeCN-HEPES 3:7			-	*Ind. Eng. Chem. Res.*, 2017, 56, 6358−6368
	THF-H_2O 1:9	10 min	0.6 μM	+	*Anal. Chem*., 2017, 89, 12698−12704
	PBS-DMSO 99:1	5 min	7.5 nM	+	*New J. Chem*., 2018, 42, 1181--1186
	Tris-HCl	30 min		+	*Org. Biomol. Chem*., 2018, 16, 2388–2392
	CH_3CN-HEPES 3:7		0.17 μM	+	*Inorg. Chem*., 2016, 55, 12052−12060
	THF-H_2O 2:98		10 μM	-	*J. Mater. Chem. C*, 2018, 6, 773-780
	PBS-DMSO 99:1	< 5 min	9.23 nM	+	*This work*

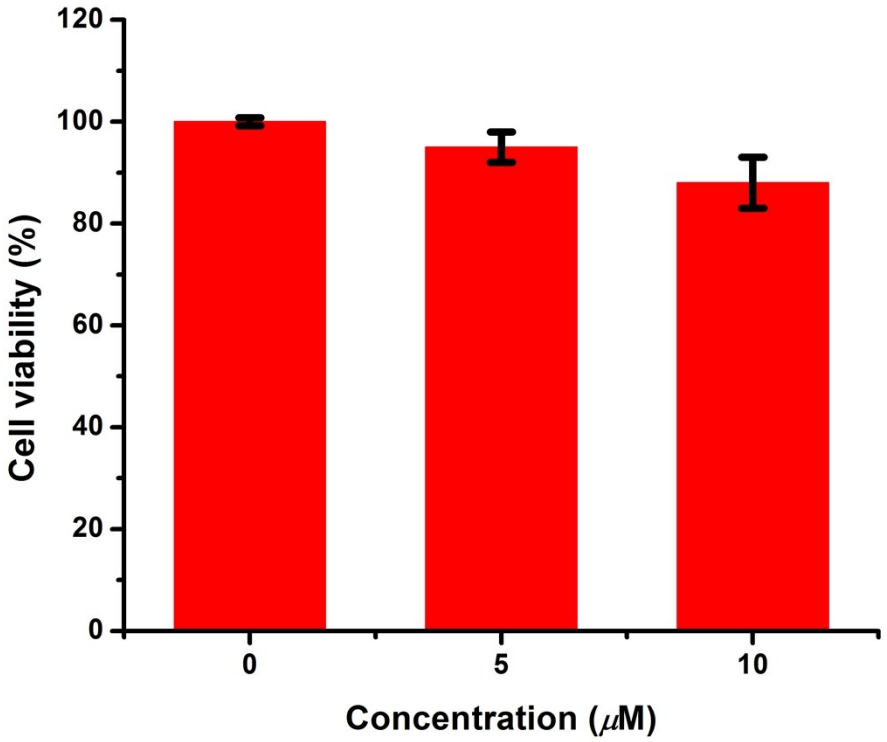


Fig. S14 Cell viability of HeLa cells treated with different concentration of **MS4** for different time periods. No cytotoxic effect was observed for the cells incubated with **MS4** at 10 μM even for 24 h.

CheckCIF/PLATON report of 3

Bond precision: C-C = 0.0034 A Wavelength=0.71073

Cell: a=8.7754(8) b=10.9150(9) c=12.9559(11)
alpha=90 beta=96.034(9) gamma=90
Temperature: 293 K

	Calculated	Reported
Volume	1234.09(19)	1234.09(19)
Space group	P 21/c	P 1 21/c 1
Hall group	-P 2ybc	-P 2ybc
Moiety formula	C13 H15 N O S	C13 H15 N O S
Sum formula	C13 H15 N O S	C13 H15 N O S
Mr	233.32	233.32
Dx,g cm-3	1.256	1.256
Z	4	4
Mu (mm-1)	0.241	0.241
F000	496.0	496.0
F000'	496.67	
h,k,lmax	10,13,15	10,13,15
Nref	2261	2256
Tmin,Tmax	0.906,0.930	0.919,1.000
Tmin'	0.906	

Correction method= # Reported T Limits: Tmin=0.919 Tmax=1.000
AbsCorr = MULTI-SCAN

Data completeness= 0.998 Theta(max)= 25.350

R(reflections)= 0.0496(1576) wR2(reflections)=

0.1296(2256) S = 1.045 Npar= 147

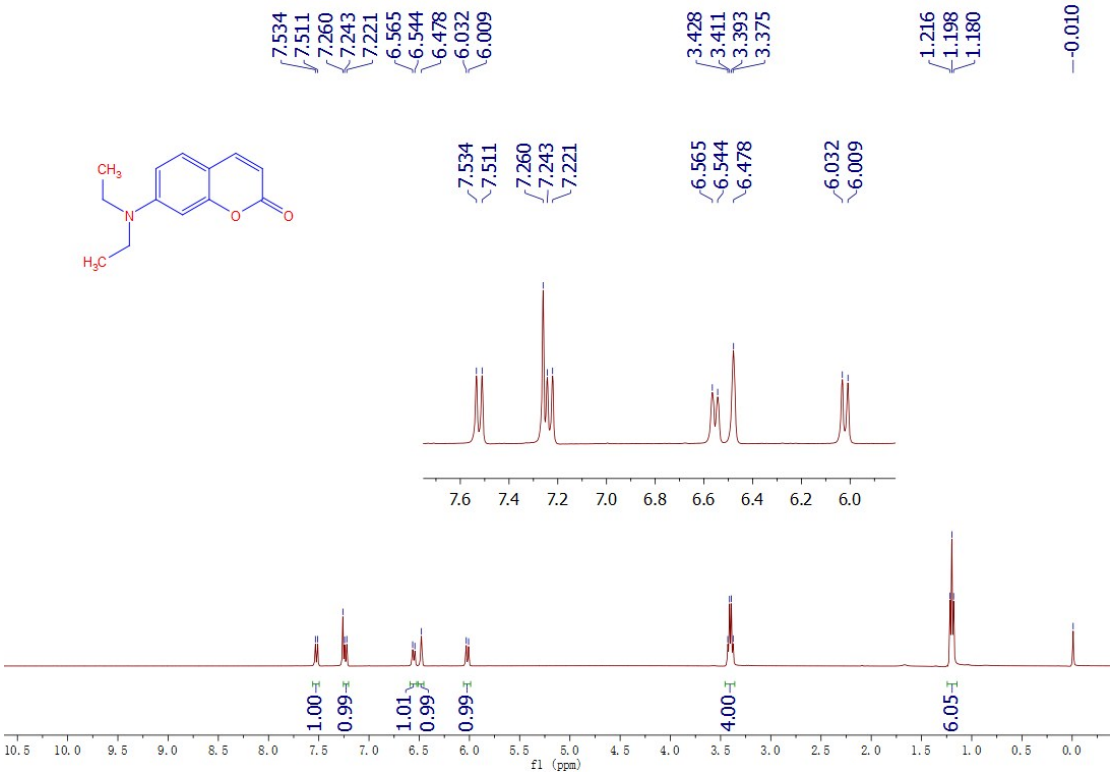
Cell culture and imaging

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, **e**xponentially 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.

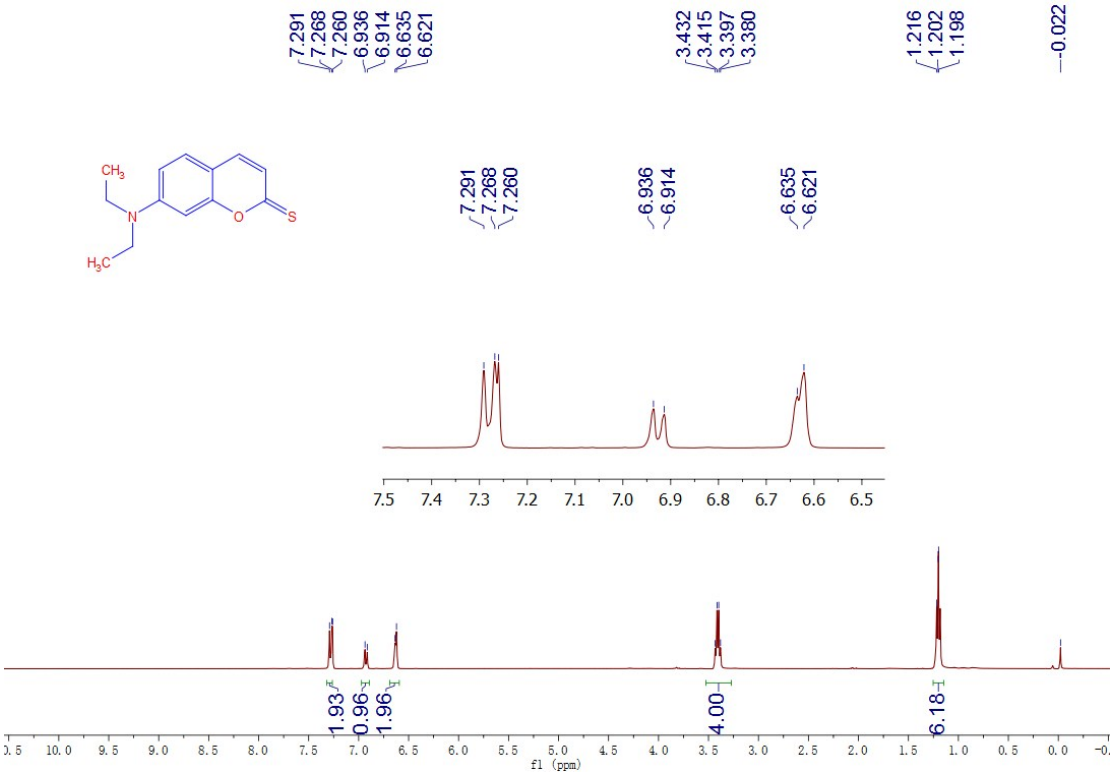
For labeling, the medium was removed and cells were rinsed three times with PBS. Then HeLa cells were incubated with **MS4** (10 μM) in PBS (containing 1% EtOH) at 37 ^oC for 30 min as control. For Hg^{2+} imaging, another set of HeLa cells was preloaded with **MS4** (10 μM) in PBS (containing 1% EtOH) at 37 ^oC for 30 min, rinsed three times with PBS and further treated with Hg^{2+} (20 μM) 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.

The characterization data of MS4

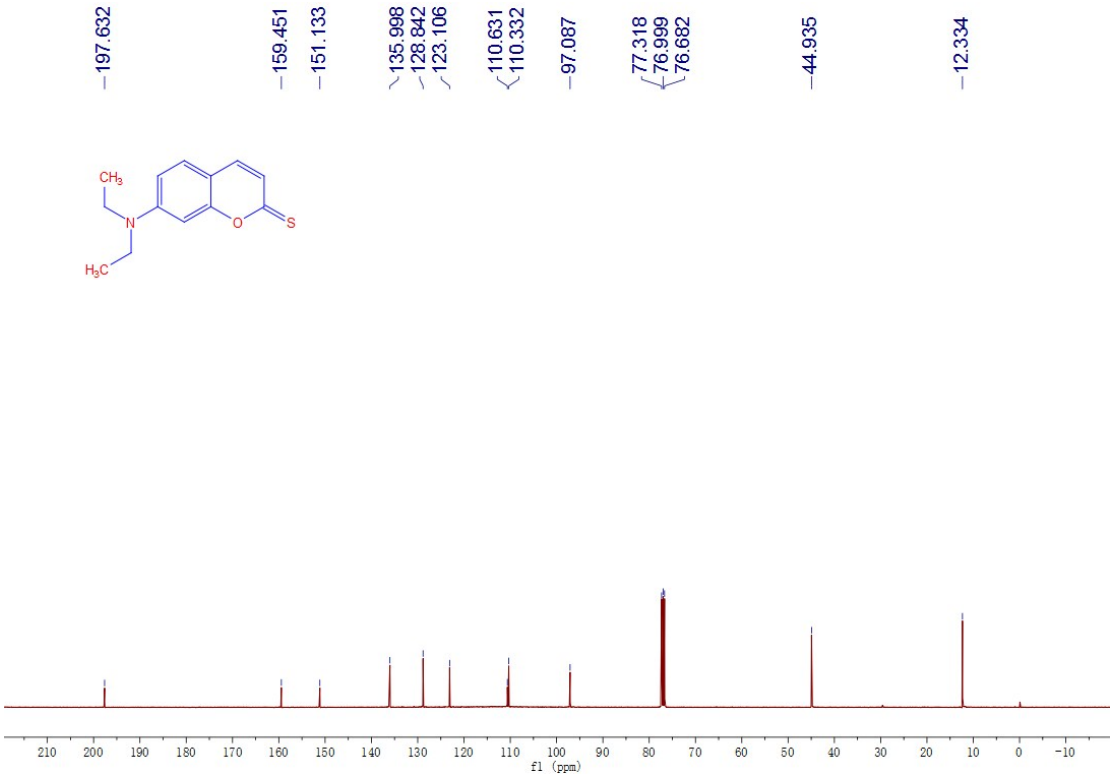
^{1}H NMR of **2**



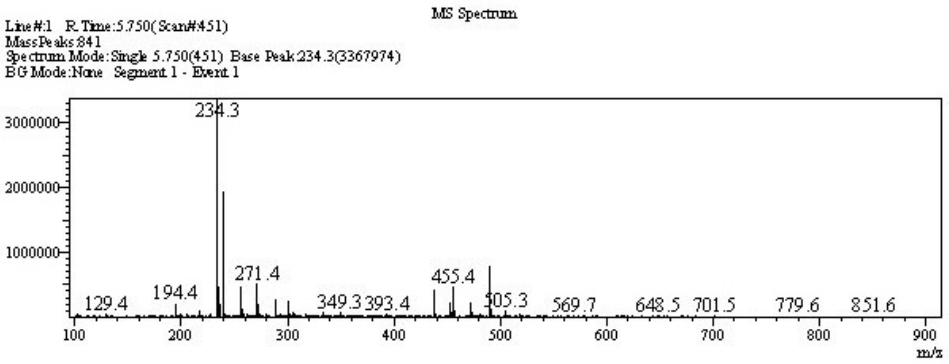
^{1}H NMR of **3**



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

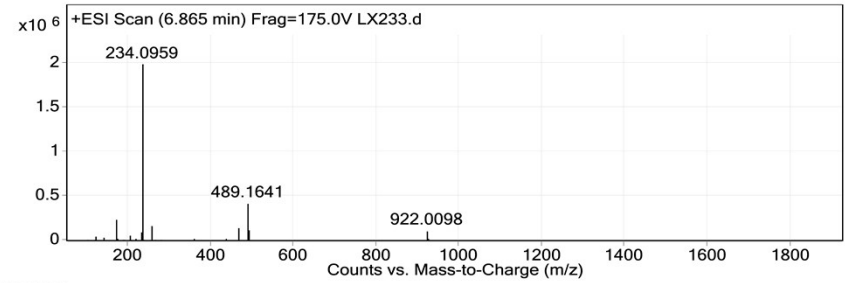


MS of **3**



HR-MS of **3**

Qualitative Analysis Report



Peak List

m/z	z	Abund
172.0943		237790.6
234.0959	1	1985181.9
234.233		105335.6
235.0978	1	291619.2
236.0927	1	100420
256.0765		164040.8
465.1661		137990.9
489.1641	1	417858.3
490.1666	1	123724.1
922.0098		110956.7

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.