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Supporting Information

Development of diselenide-based fluorogenic system for the selective and sensitive detection of the Hg(II) in aqueous media

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Keywords: Diselenide, Detection of Hg(II), Turn-on fluorescence, Fluorogenic probes, Rhodamine based organoselenium

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Experimental Section

Quantum yield

Quantum yield was calculated according to the formula (1),

$$\phi = (\phi_R)\ (I/I_R)\ (A_R/A)\ (\eta^2/\eta_R^2) \quad (1)$$

Where,

ϕ is the quantum yield,

I is integrated area under the corrected emission spectra,

A is absorbance at excitation wavelength,

η is refractive index.

The subscript R refers to the reference fluorophore of known quantum yield. Fluorescein diluted with 0.1 M NaOH used as a standard, which has quantum yield of 0.95.[1] The excitation and emission slit width used for the experiment was 1.5 nm/1.5 nm. Emission spectra were obtained maintaining nearly equal absorbance (0.2).

pH study

The probe (**3**) (2.5 µM) was added with Hg(II) (50 equiv, 125 µM) in 10 mM PBS. PBS buffer of 10 mM concentration was used with a pH range from 2-12. The solutions were incubated for 30 min at room temperature and fluorescence spectra were recorded at λ_{ex} 559 nm.

Time dependent study

The time dependent study of the probe (**3**) was performed using 2.5 µM of the probe (**3**) solution and 125 µM of Hg(II) solution. In the probe solution (**3**), analyte Hg(II) was added and the spectrum was recorded for 75 min at λ_{ex} 559 nm and λ_{em} 579 nm with slit width 3 nm/3 nm.

Detection limit

Increasing concentration study was performed to calculate detection limit of the probe. To the probe (**3**) solution, 0 – 50 equivalent of Hg(II) solution was added in increasing fashion. The solution was incubated for 30 min at room temperature. Detection limit was calculated using the following equation (2).

$$\text{Detection Limit} = 3\sigma/k \quad (2)$$

Where σ is standard deviation

k is slope.

Standard deviation was calculated by taking 10 readings of the probe solutions.

Interference study

For this experiment, the probe solution **3** (2.5 µM) was incubated with 125 µM of Hg(II) solution and then the other metal ions (Hg^{2+},Cu^{2+}, Ca^{2+}, Na^{+}, Co^{2+}, K^{+}, Zn^{2+}, Al^{3+}, Fe^{2+}, Cd^{2+}, Mn^{2+}, Ag^{+}, Fe^{3+}, Pb^{2+}, Au^{3+}, Pt^{4+}) (50 equiv, 125 µM) in water were added. The fluorescence measurements were obtained after incubation of 30 min at room temperature at an excitation maxima 559 nm.

Job's plot

Job's plot experiment was performed using continuous variation method of the probe in presence of Hg(II). The probe (**3**) (100 µM) was taken as 747.1 µL, 672.4 µL, 597.7 µL, 523.0 µL, 448.3 µL, 373.5 µL, 298.8 µL, 224.1 µL, 149.4 µL, 74.7 µL, 0.0 µL in different vials and was diluted with water, to that Hg(II) (100 µM) was added as 0.0 µL, 74.7 µL, 149.4 µL, 224.1 µL, 298.8 µL, 373.5 µL, 448.3 µL, 523.0 µL, 597.7 µL, 672.4 µL, 747.1 µL to each vial, respectively. The solutions were incubated for 30 min and the absorbance readings were recorded.

Reversibility study

To check the reversibility of the probe (**3**) with Hg(II) in presence of biothiols, the probe (**3**) were reacted with 125 µM of Hg(II) and then 125 µM of biothiols (DL-homocysteine, L-cysteine, glutathione, N-acetyl-L-cysteine, and Na_2S) were added to the solution and incubated for another 30 min. Then, the spectra were recorded. Further, to check the redox cycle of the probe (**3**), the same experiment was performed continuously with 125 µM of Na_2S and 125 µM of Hg(II) in the same sample vial.

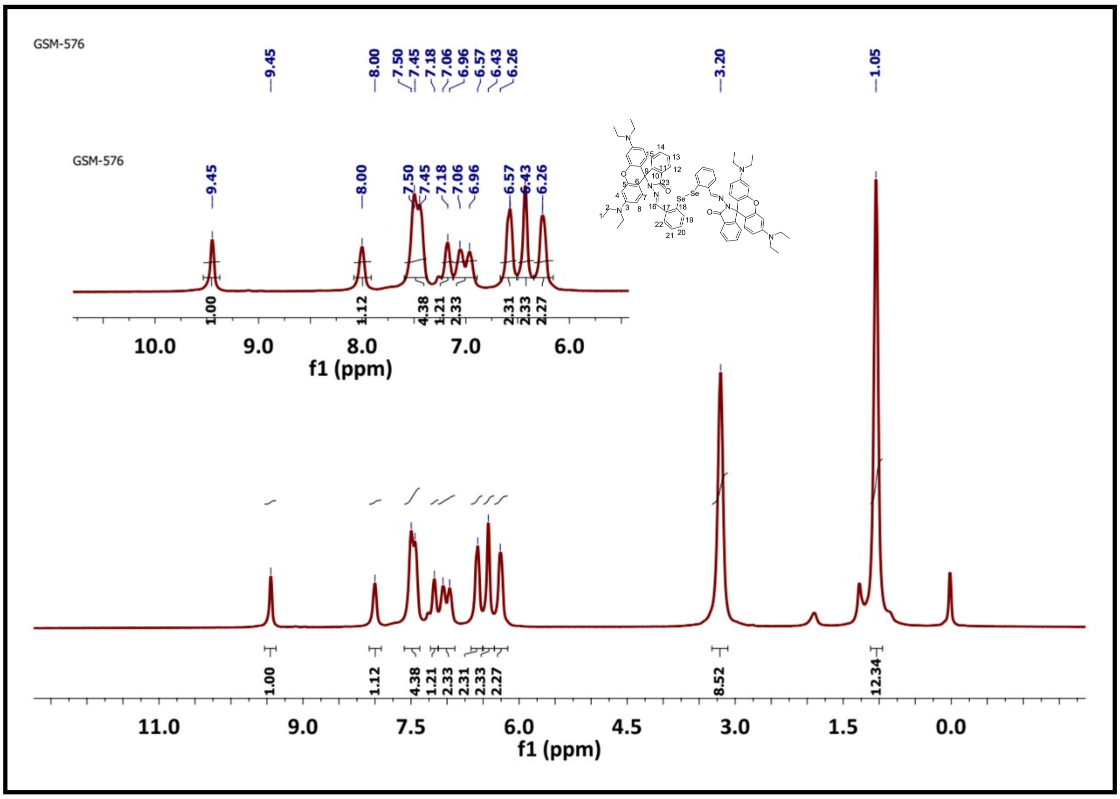


Fig. S1. ^{1}H NMR spectrum of probe **3** in $CDCl_3$.

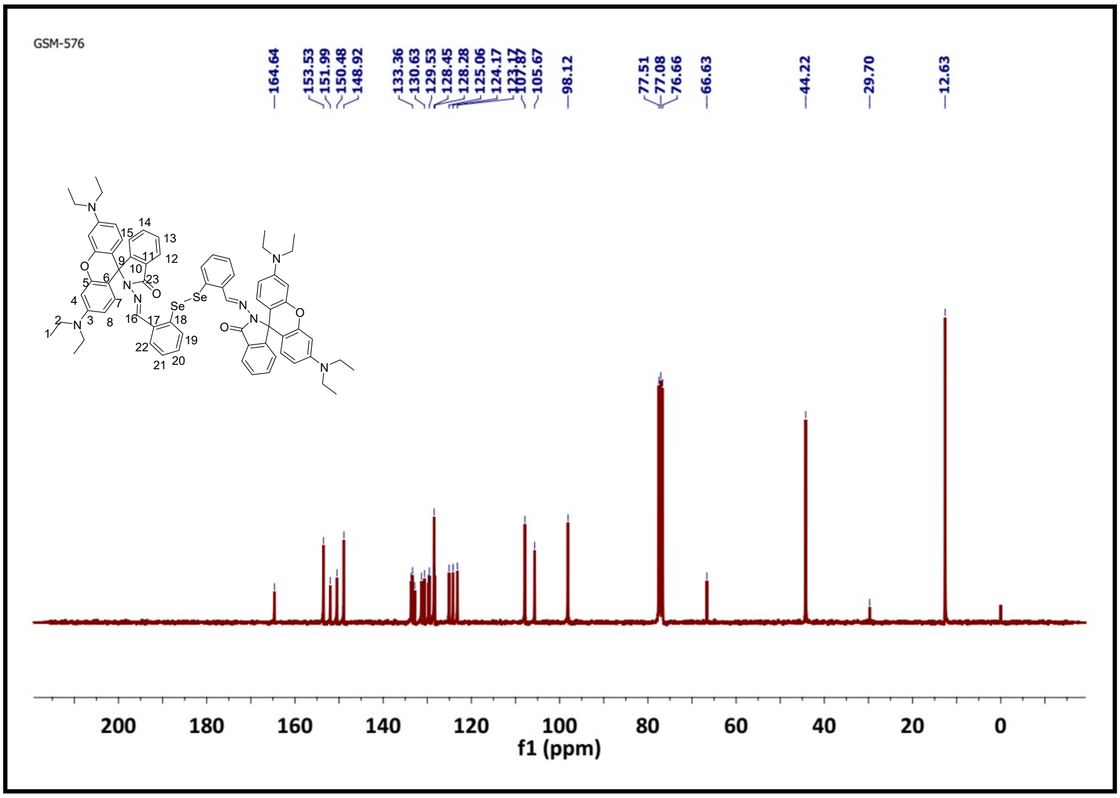


Fig. S2. ^{13}C NMR spectrum of probe **3** in $CDCl_3$.

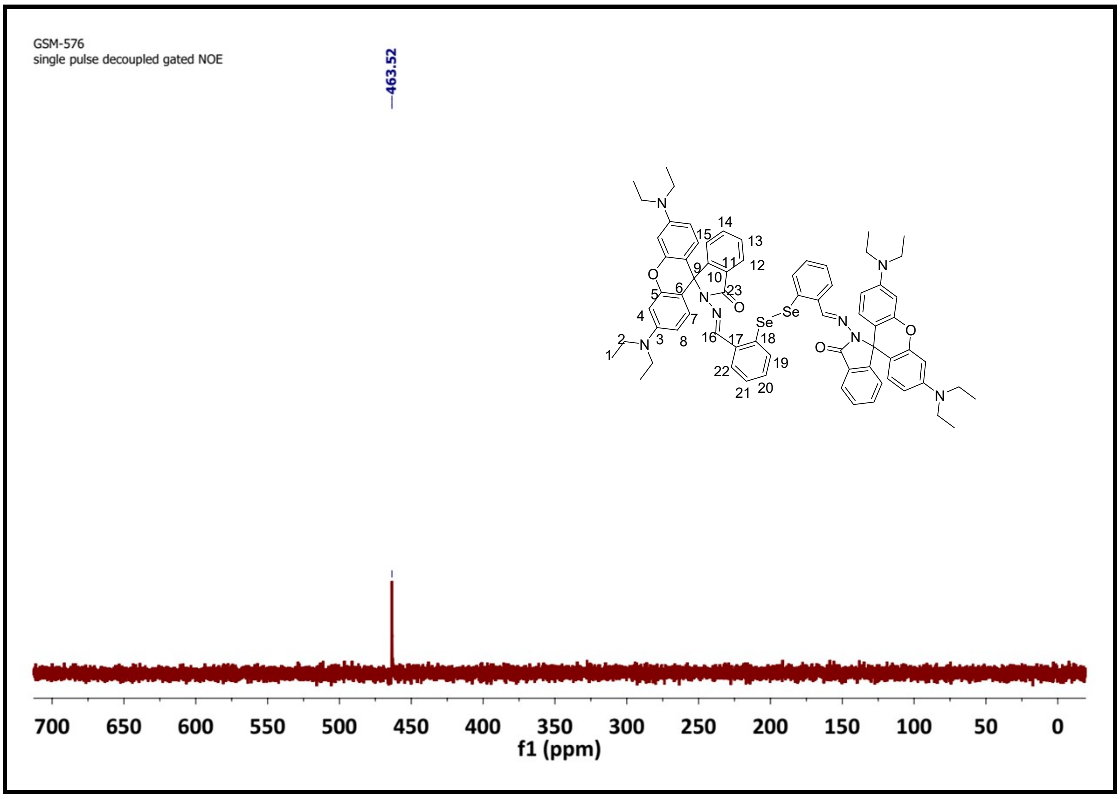
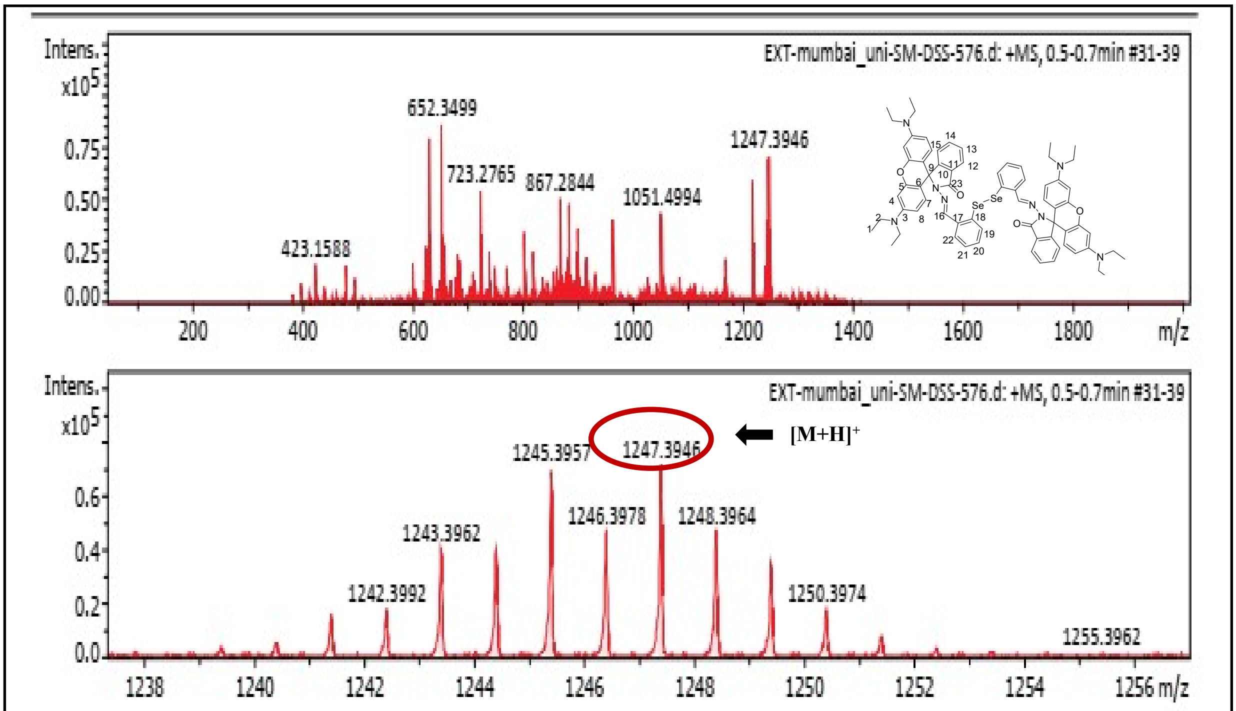


Fig. S3. The ^{77}Se NMR spectrum of probe **3** in $CDCl_3$.



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
1247.3946	1	C70H71N8O4Se2	1247.3923	-0.1	24.3	1	100.00	44.0	even	ok

Fig. S4. Mass spectrum of probe **3**.

Table S1. Refinement details of X-ray structure of the probe **3** (#CCDC: 2393067).

Compound	**Probe 3**
Formula	$C_{70}H_{70}N_8O_4Se_2$
Crystal System	Tetragonal
Space Group	P-4b2
T/K	100(2)
a [A^0]	21.474(4) Å
b [A^0]	21.474(4) Å
c [A^0]	13.425(3) Å
α [^{0}]	90.00(3)°
β [^{0}]	90.00(3)°
γ [^{0}]	90.00(3)°
V [Å^3]	6191(3) Å^3
Z	4
ρ_{cal}Mg/m^3	1.336
μ(mm^{-1})	1.251
F(000)	2584
Crystal Size [mm^3]	0.260 x 0.250 x 0.210
GOF	1.029
***2θ* range (deg)**	2.025 to 28.294°
Reflections collected	39151
Independent reflections	7614
Parameters	479
R_{int}	0.0491
R_1,wR2[I>2σ(I)]	R1 = 0.0334, wR2 = 0.0736
R_1,wR2[I>2σ(I)]	R1 = 0.0482, wR2 = 0.0796

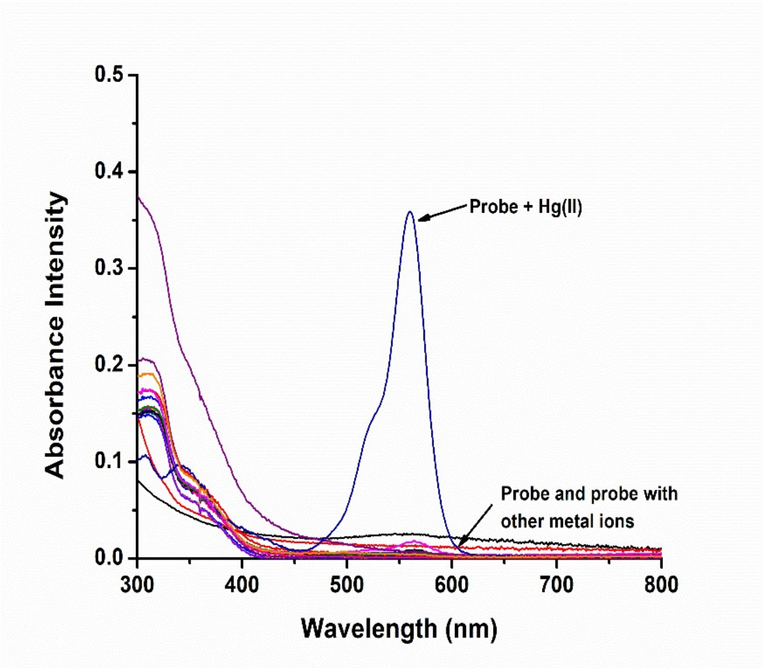


Fig. **S5.** Absorption spectra of the probe (**3**) (2.5 μM) with metal ions (Hg^{2+}, Cu^{2+}, Ca^{2+}, Na^{+}, Co^{2+}, K^{+}, Zn^{2+}, Al^{3+}, Fe^{2+}, Cd^{2+}, Mn^{2+}, Ag^{+}, Fe^{3+}, Pb^{2+}, Au^{3+}, Pt^{4+}, 50 equiv) in water incubated for 30 min at rt.

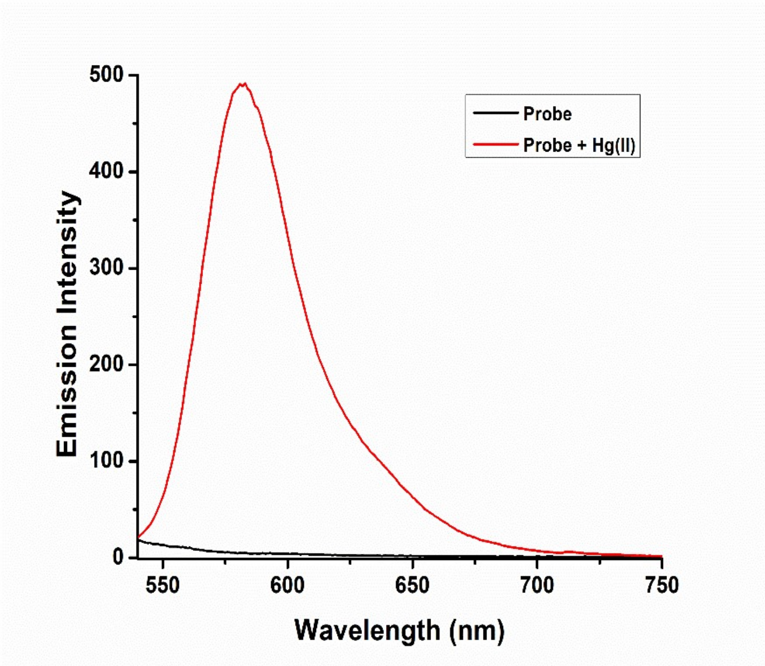


Fig. S6. Fluorescence spectra of probe **3** (2.5 μM) with Hg(II) (50 equiv) in water incubated for 30 min. λ_{ex} = 500 nm, λ_{em} = 579 nm,

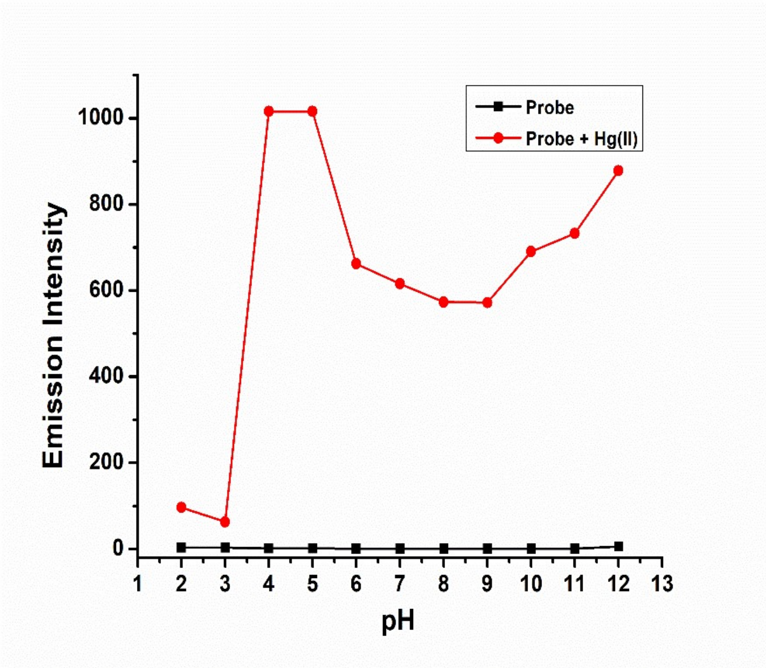


Fig. S7. Fluorescence intensity changes of probe **3** (2.5 µM, black) and probe **3** (2.5 µM) with 50 equiv of Hg^{2+} (red) in the solution (10 mM PBS) incubated for 30 min. λ_{ex} = 559 nm, λ_{em} = 579 nm, slit width 3 nm/3 nm, under different pH range.

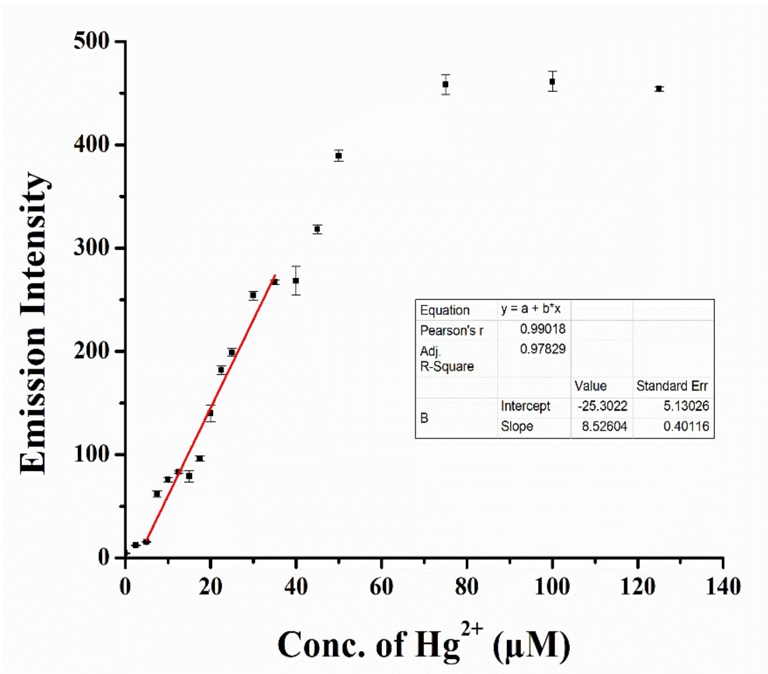


Fig. S8. Plot for the calculation of limit of detection from the emission of probe **3** (2.5 µM, water) with increasing concentration of Hg^{2+} (0 to 50 equiv) incubated for 30 min at rt; λ_{ex} = 559 nm, λ_{em} = 579 nm, slit width 3 nm/3 nm (average of three experiments).

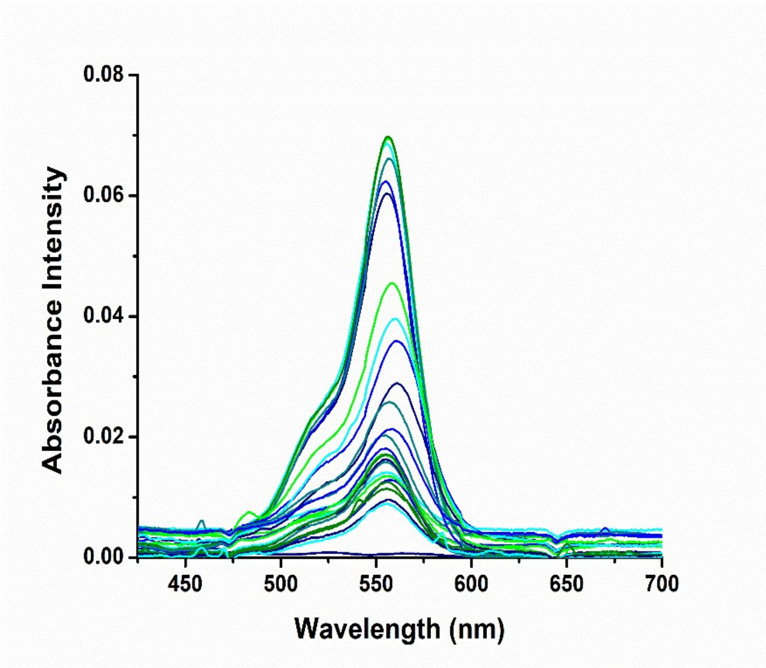


Fig. S9. Absorbance spectral changes of probe **3** (2.5 μM) with various concentrations of Hg(II) (0 - 50 equiv) in water incubated for 30 min.

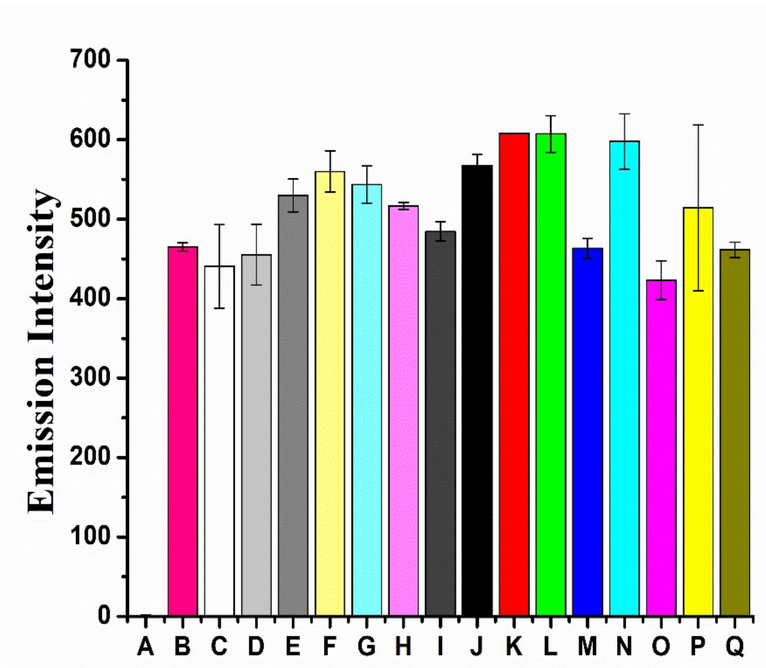


Fig. S10. Fluorescence intensity of probe **3** (2.5 μM) and Hg^{2+} (50 equiv) with metal ions (Hg^{2+},Cu^{2+}, Ca^{2+}, Na^{+}, Co^{2+}, K^{+}, Zn^{2+}, Al^{3+}, Fe^{2+}, Cd^{2+}, Mn^{2+}, Ag^{+}, Fe^{3+}, Pb^{2+}, 50 equiv) (A = probe **3**, B = probe **3** + Hg^{2+}, C = probe **3** + Hg^{2+} + Cu^{2+}, D = probe **3** + Hg^{2+} + Ca^{2+} , E = probe **3** + Hg^{2+} + Na^{+}, F = probe **3** + Hg^{2+} + Co^{2+}, G = probe **3** + Hg^{2+}+ K^{+}, H = probe **3** + Hg^{2+}+ Zn^{2+}, I = probe **3** + Hg^{2+} + Al^{3+}, J = probe **3** + Hg^{2+} + Fe^{2+}, K = probe **3** + Hg^{2+} + Cd^{2+}, L =

probe **3** + Hg^{2+} + Mn^{2+}, M = probe **3** + Hg^{2+} + Ag^{+}, N = probe **3** + Hg^{2+} + Fe^{3+}, O = probe **3** + Hg^{2+} + Pb^{2+}, P = probe **3** + Hg^{2+} + Au^{3+}, Q = probe **3** + Hg^{2+} + Pt^{4+} in water incubated for 30 min. λ_{ex} = 559 nm, λ_{em} = 579 nm, slit width 3 nm/3 nm.

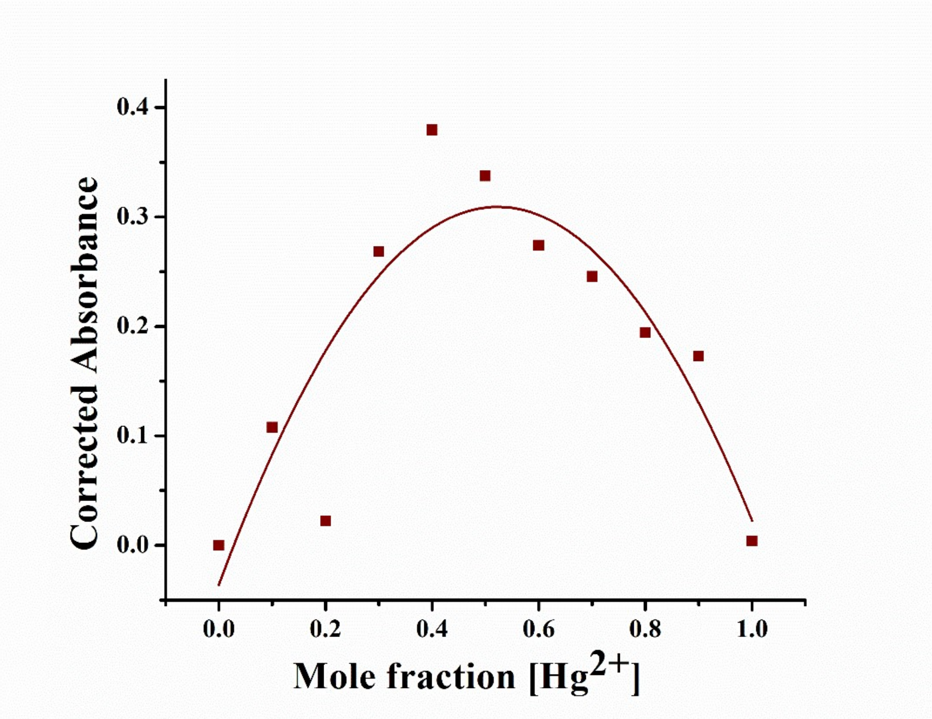


Fig. S11. Job's plot of the probe (**3**) with Hg^{2+} metal ion complex in water incubated for 30 min. The total concentration of the probe and Hg^{2+} was 100 µM. The monitored wavelength was 559 nm.

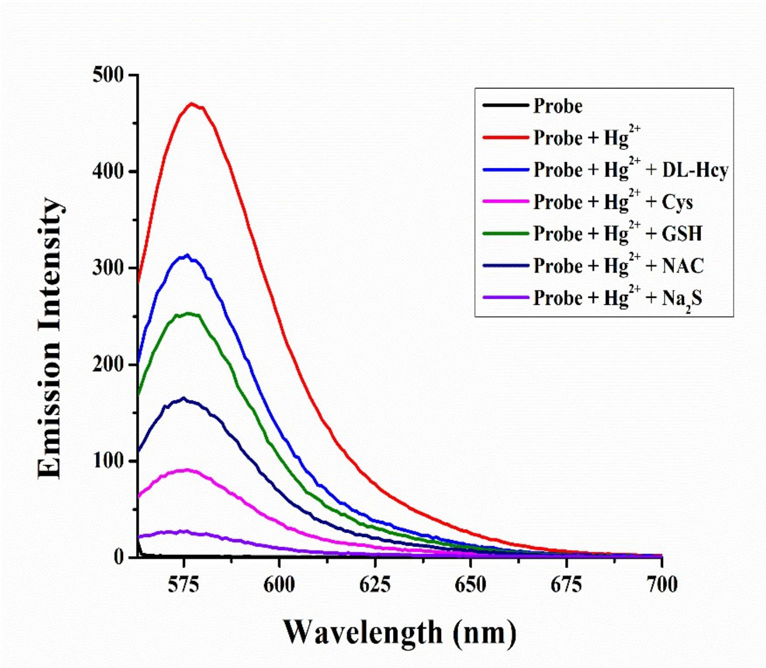


Fig. S12. Fluorescence response of probe **3** (2.5 μM) with Hg^{2+} (50 equiv) in water incubated for 30 min and after addition of biothiols (DL-homocysteine, L-cysteine, glutathione, N-acetyl-L-cysteine, and Na_2S, 50 equiv) incubated for 30 min at rt, λ_{ex} = 559 nm, λ_{em} = 579 nm, slit width 3 nm/3 nm.

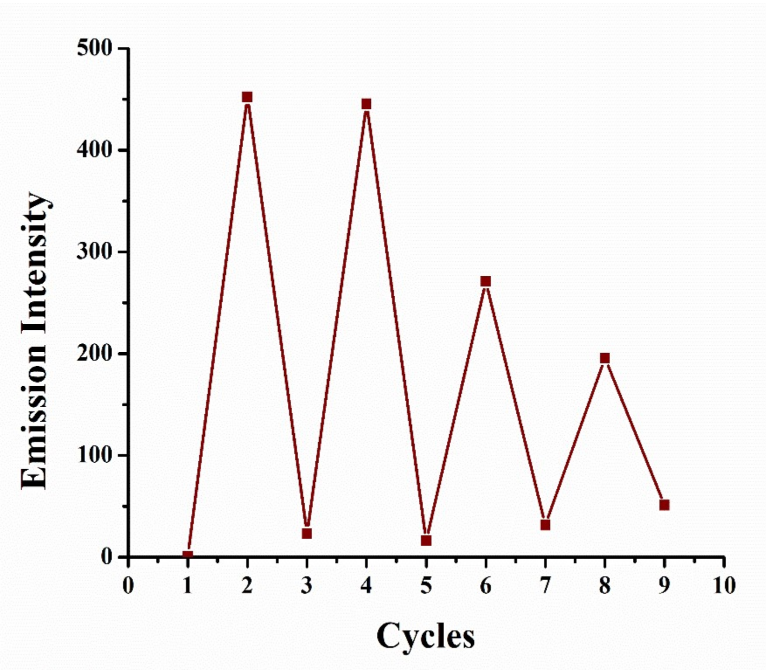


Fig. S13. Redox cycles of probe **3** (2.5 μM) with 50 equiv of Na_2S in water λ_{ex} = 559 nm, λ_{em} = 579 nm, slit width 3 nm/3 nm.

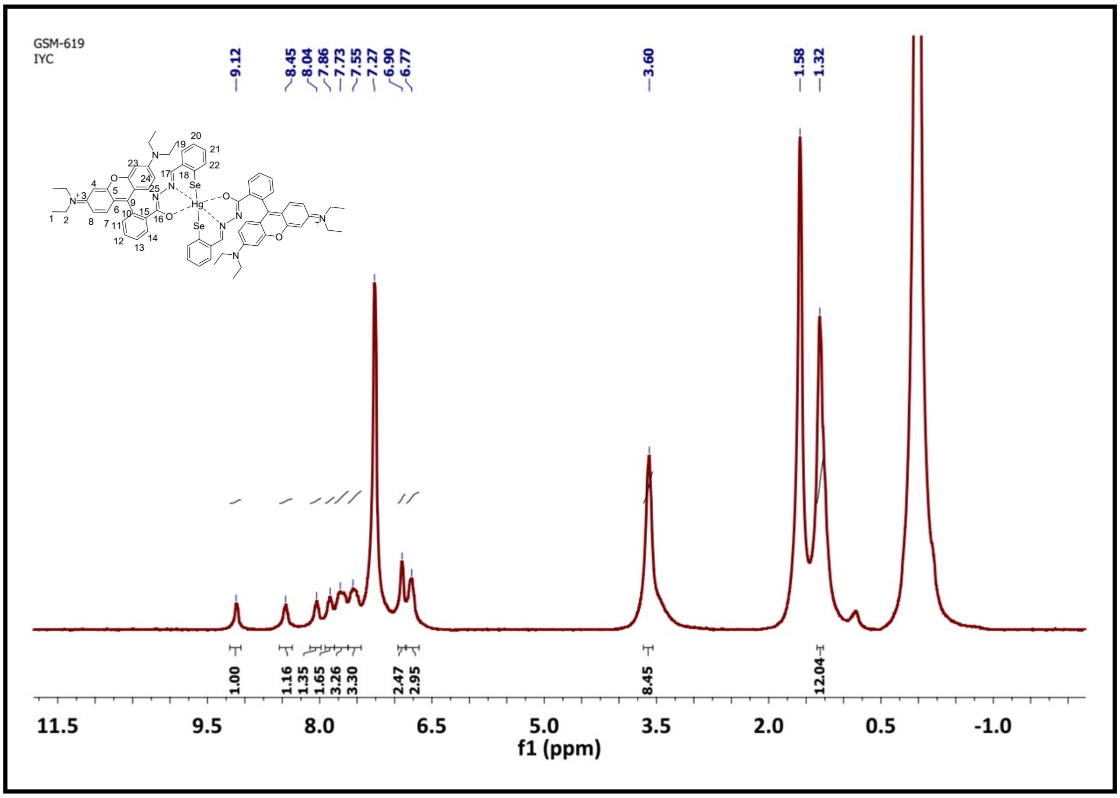


Fig. S14. ^{1}H NMR spectrum of compound **4** in $CDCl_3$.

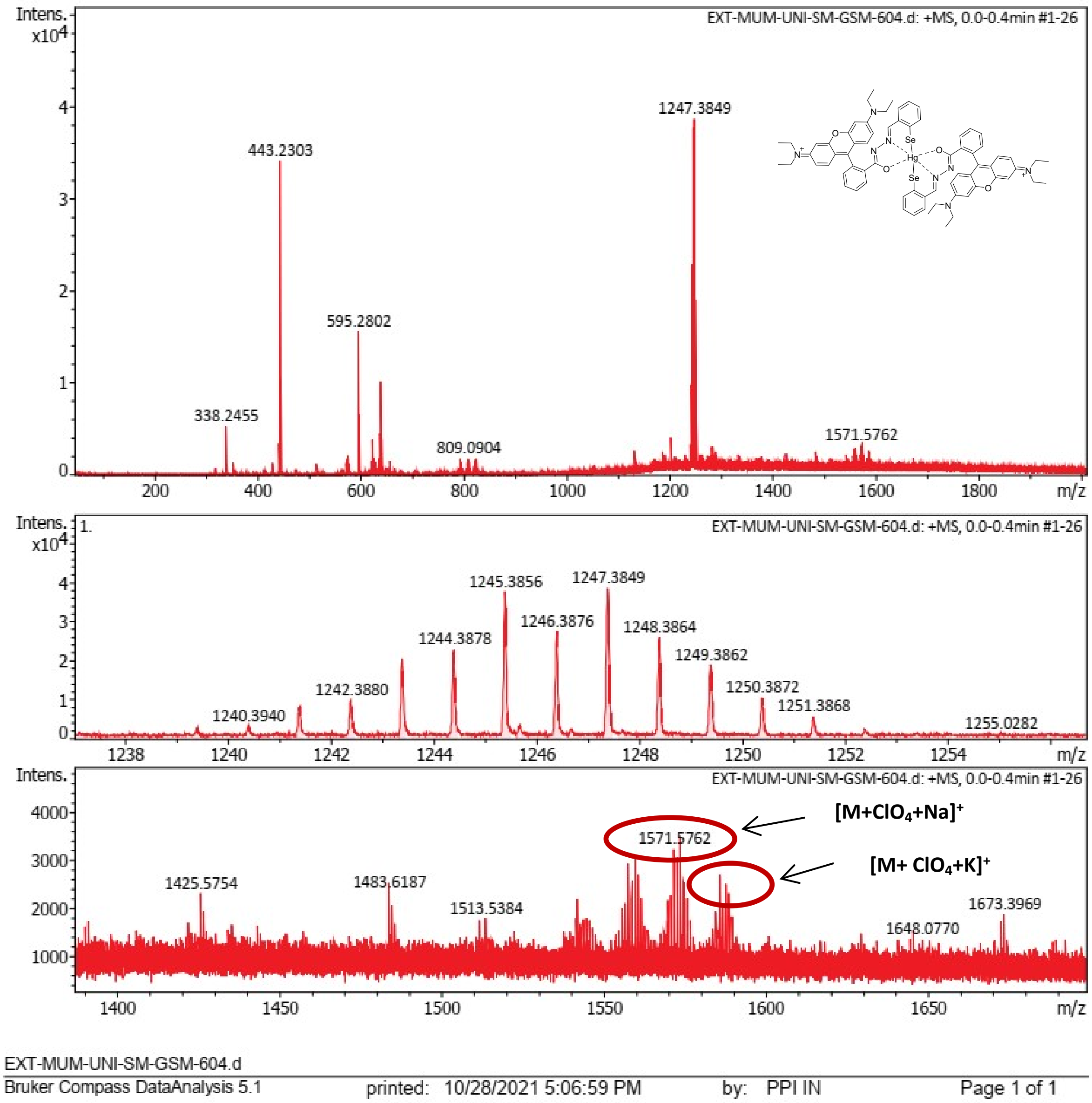


Fig. S15. Mass spectrum of compound **4**.

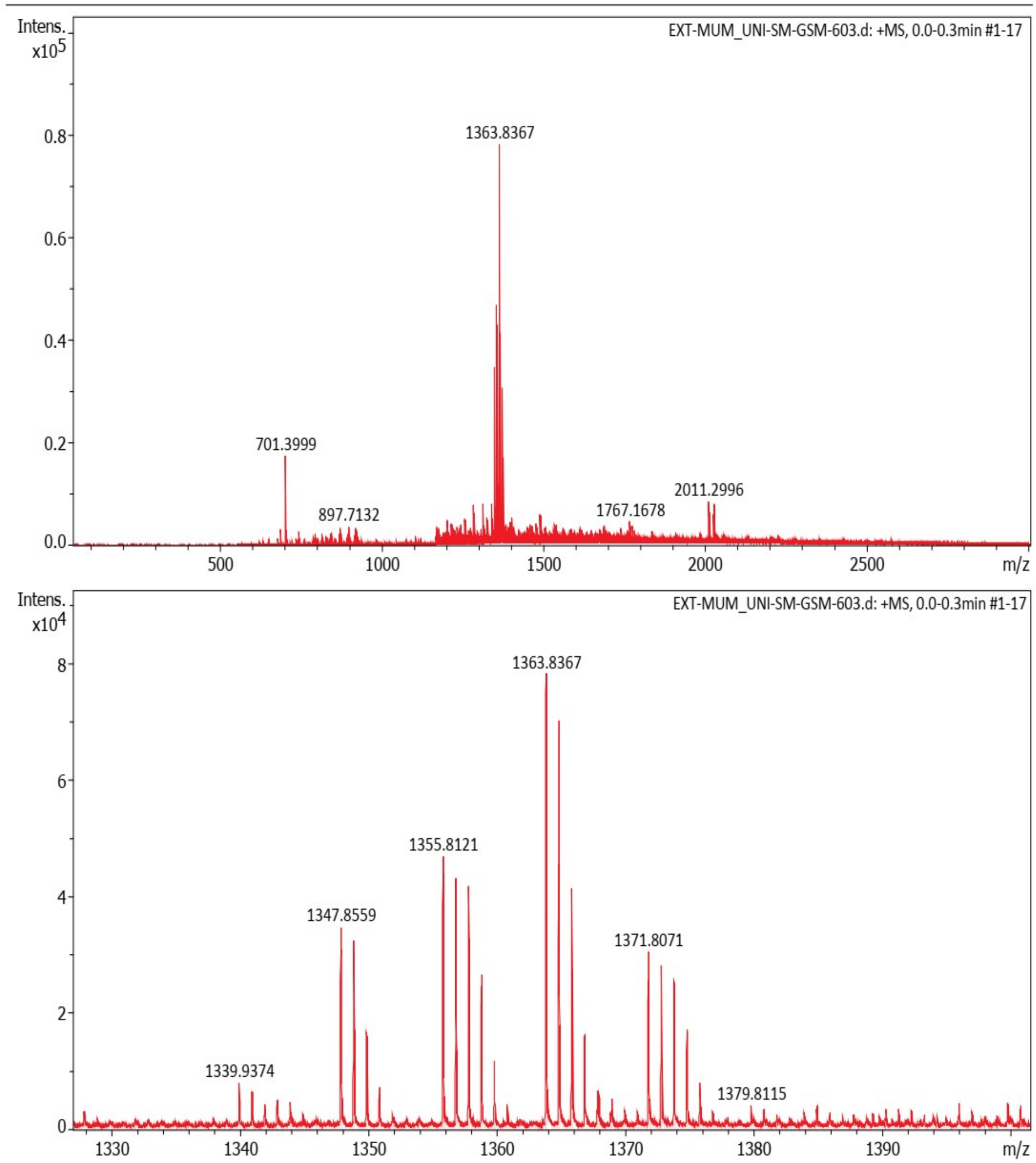


Fig. S16. Mass spectrum of compound **4**.

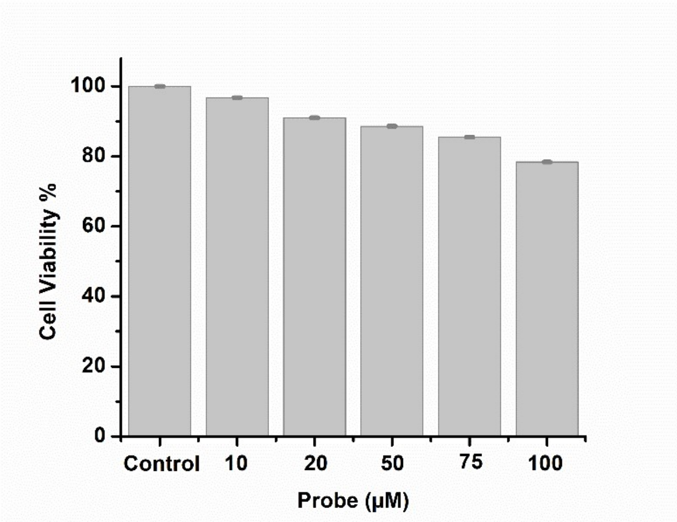


Fig. S17. MTS assay U2-OS cell line

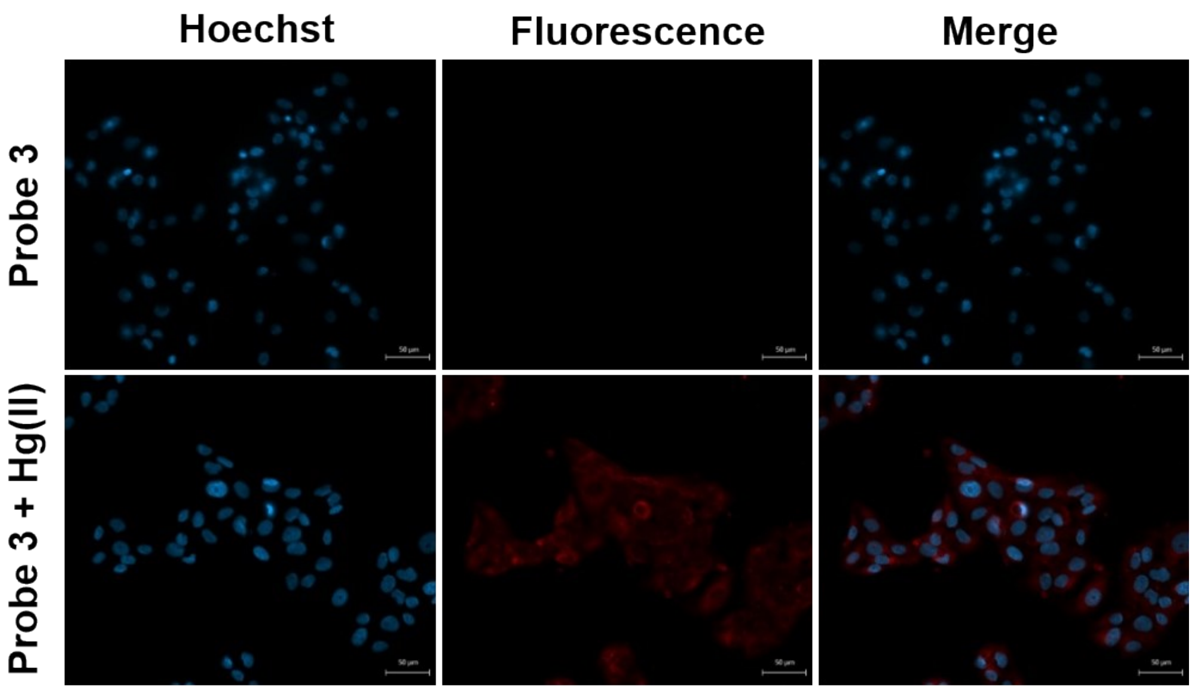


Fig. S18 Confocal images of U2-OS cell line using 20x objective. Probe (50 μM) and Hg(II) (50 μM). Scale bar is 50 μm.

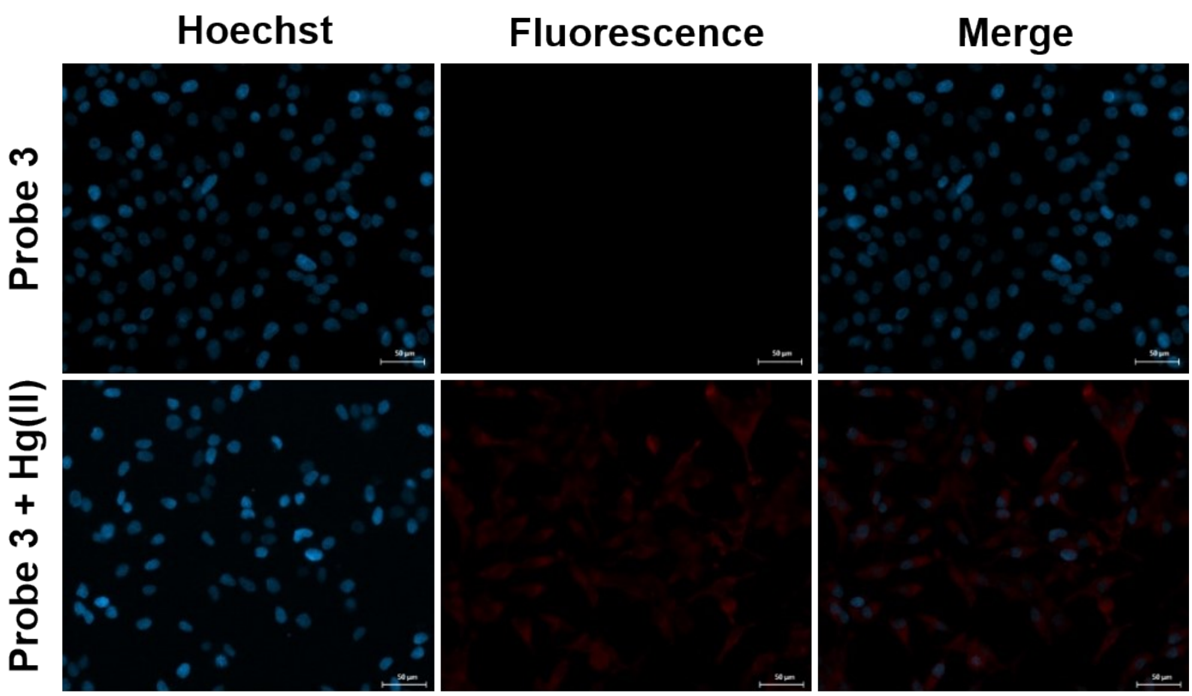


Fig. S19. Confocal images of C4-2 cell line using 20x objective. Probe (20 µM) and Hg(II) (50 µM). Scale bar is 50 µm.

Table S2. Comparison of the current method with other recently reported rhodamine probes selective for Hg(II).

Sr. No.	Ref No.	LOD	Response time	λex (nm)	λem (nm)	Potential Applications
1	2	2.1×10^{-9} mol/L	40 min	616	672	HepG2 cells
2	3	0.015 µM	<10 s	566	596	A549 cells, Zebrafish and mice
3	4	9.4×10^{-9} M	-	546	567	A549 cancer cells
4	5	10.0 nM	40 min	525	F649/F463	HeLa cells
5	6	0.26 µM	210 s	620	685	HeLa cells, zebrafish.
6	7	0.14 µM	10s	568	594	Determination of mercury residues in aquatic samples.
7	8	9.67×10^{-8} M	30 min	566	594	HeLa cell
8	9	2.76 nM	7 min	540	585	smartphone APP Color Recognizer
9	10	8×10^{-9} M	-	537	559	living HeLa cells and the organs of live mice

10	11	491 nM	-	365	584	SiHa cells
11	12	17.26 nM	<1 min	543	-	-
12	13	17.5 nM	<1 min	613	665	HeLa cells, Zebrafish, water samples.
13	14	334 nM	12 min	558	582	-
14	15	0.12 μM	5 min	520	550	MCF-7
15	16	2.43×10^{-8} M (DMSO-Water) 4.54×10^{-8} M (Ethanol-Water)	-	320 (DMSO-Water) 326 (Ethanol-Water)	526 (DMSO-Water) 540 (Ethanol-Water)	HeLa cells, soil sample detection
This Work		**62.3 nM**	**30 min**	**559**	**579**	**C4-2 cells**

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