Supporting Information

A water soluble FRET-based ratiometric chemosensor for Hg(II) and S^{2-} applicable in living cell staining

Buddhadeb Sen,[a] Manjira Mukherjee,[a] Siddhartha Pal,[a] Koushik Dhara,[b] Sushil Kumar Mandal,[c] Anisur Rahman Khuda-Bukhsh,[c] and Pabitra Chattopadhyay*,[a]

[a] Department of Chemistry, Burdwan University, Golapbag, Burdwan, 713104, India,

[b]Department of Chemistry, Sambhu Nath College, Labpur, Birbhum 731303, West Bengal, India

[c]Molecular Biology and Genetics Laboratory, Department of Zoology, Kalyani University, India

Corresponding author: pabitracc@yahoo.com

CONTENTS

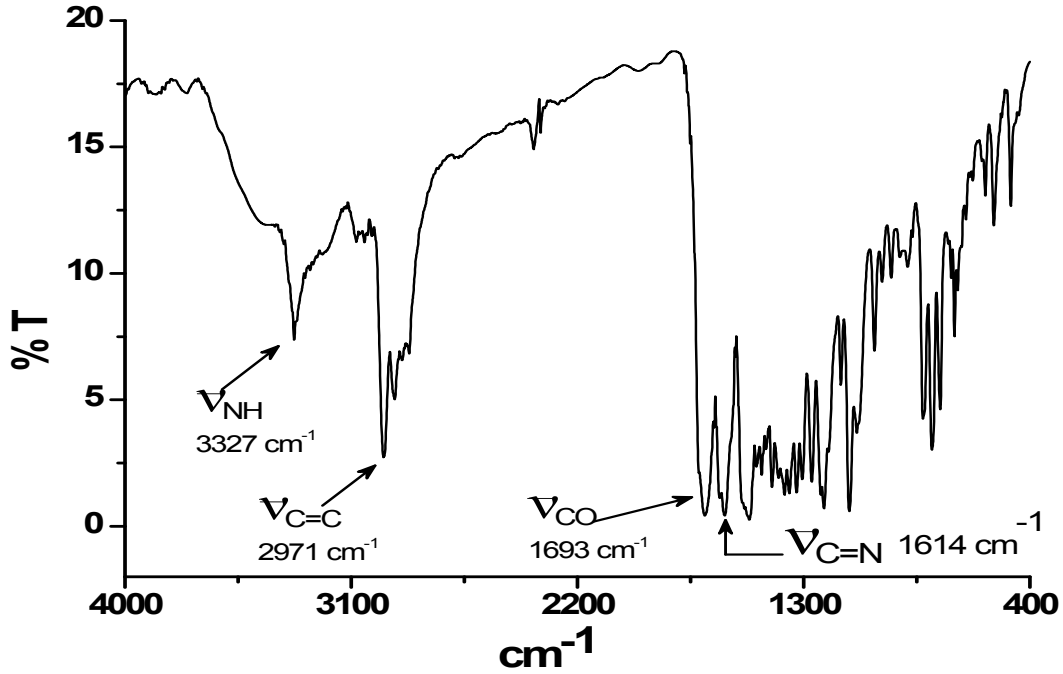


Fig. S1A FTIR spectrum of Hg-probe (**L[1]**)

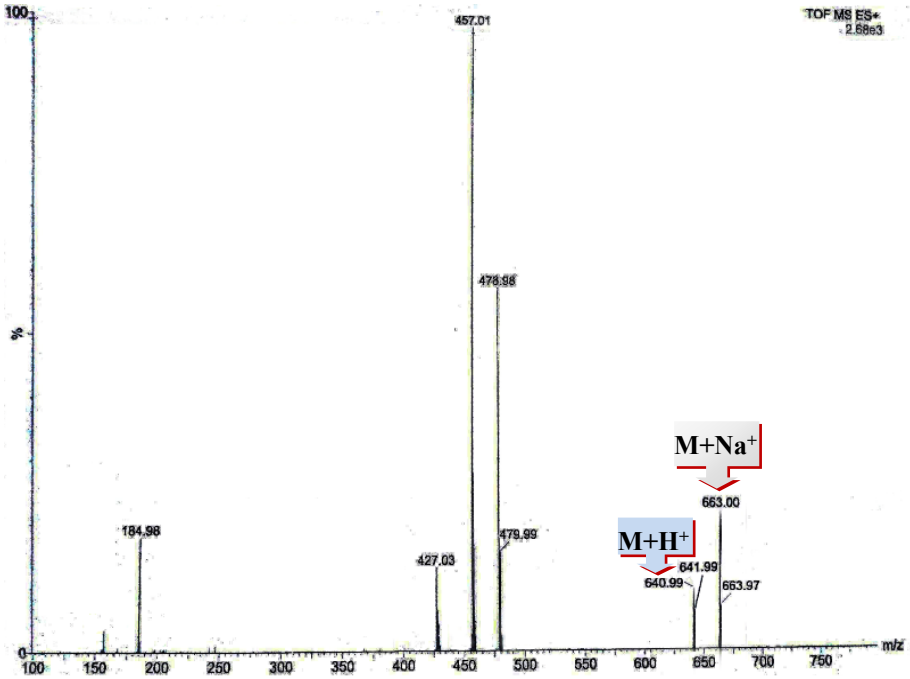


Fig. S1B ESI-MS spectrum of probe (**L[1]**)

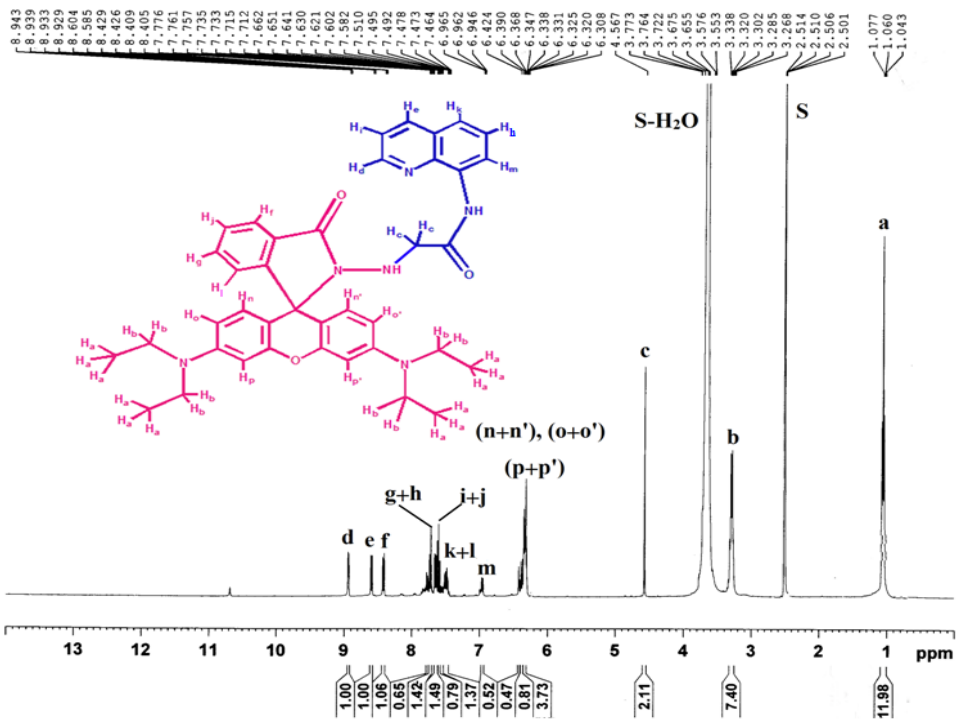


Fig. S1C ^{1}H NMR of the probe (**L^{1}**) in DMSO-d$_6$

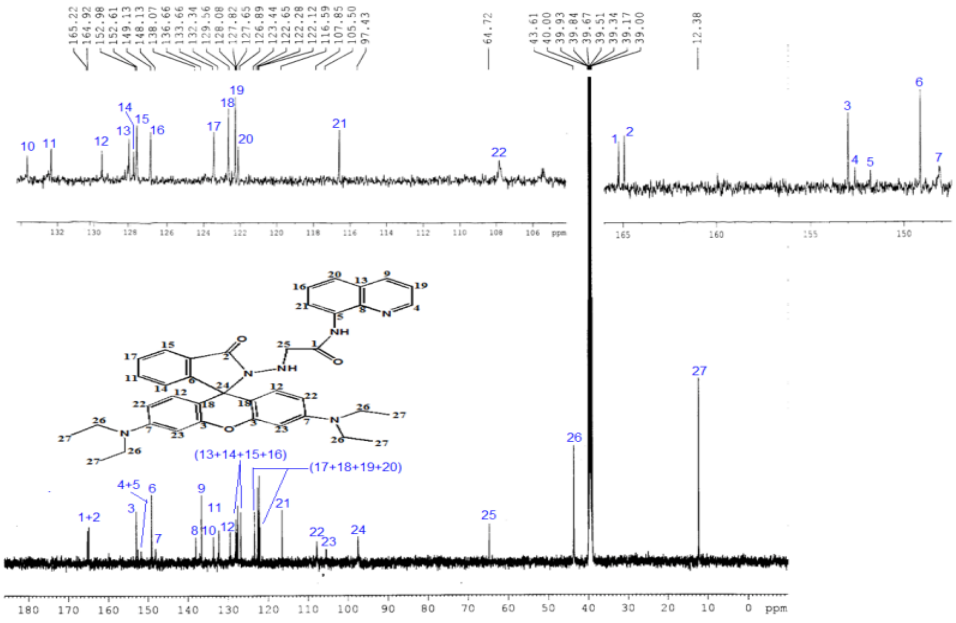


Fig. S1D ^{13}C NMR of the probe (**L^{1}**) in DMSO-d$_6$

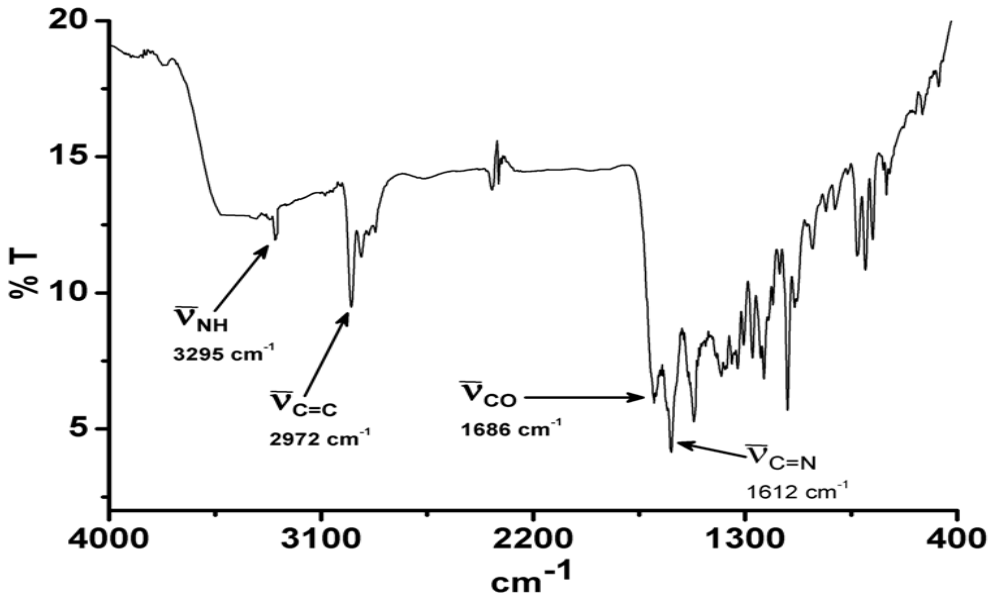


Fig. S1E FTIR spectrum of $[Hg(L)Cl_2]$

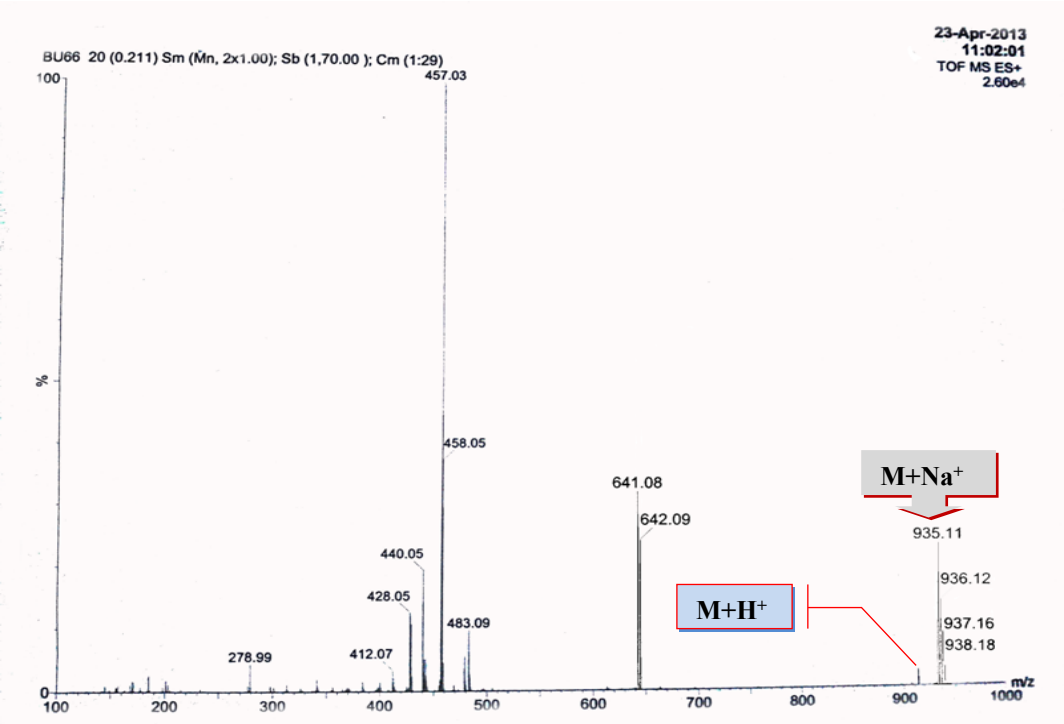


Fig. S1F ESI-MS spectrum of $[Hg(L)Cl_2]$

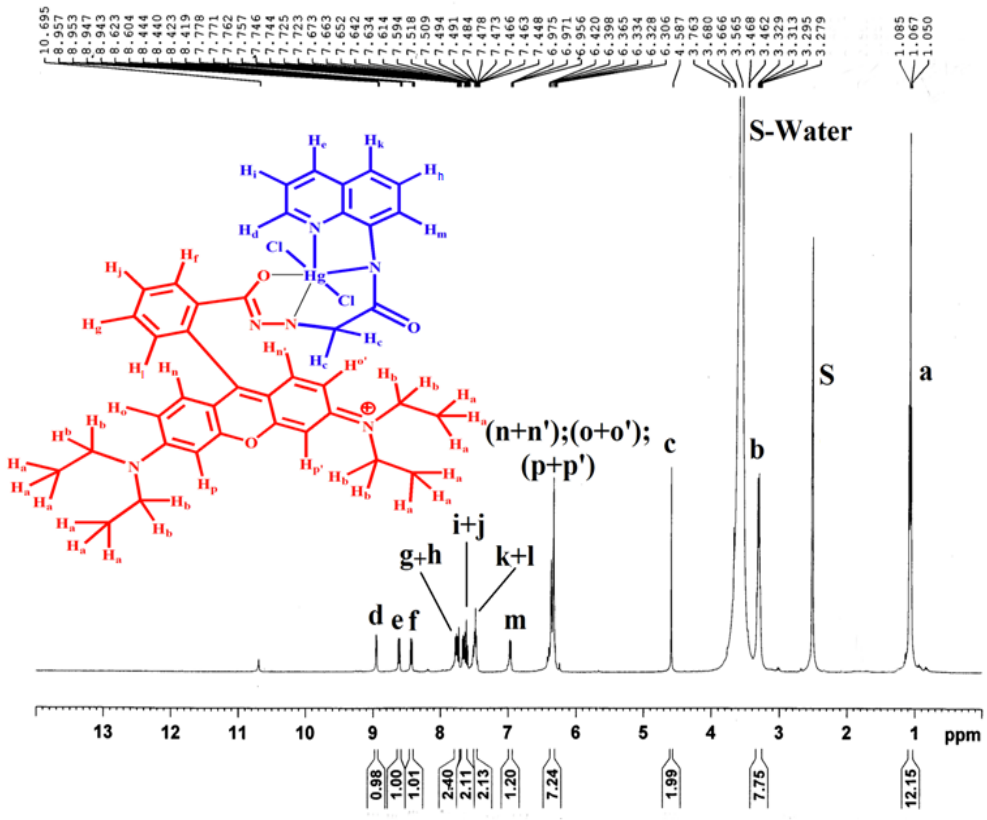
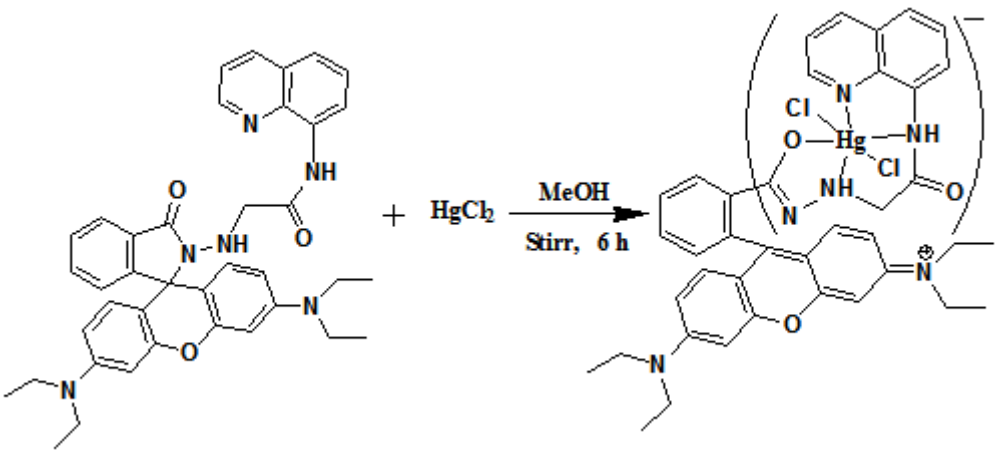


Fig. S1G ^{1}H NMR of $[Hg(L)Cl_2]$



Scheme S1 Synthesis of L-Hg Complex as $[Hg(L)Cl_2]$

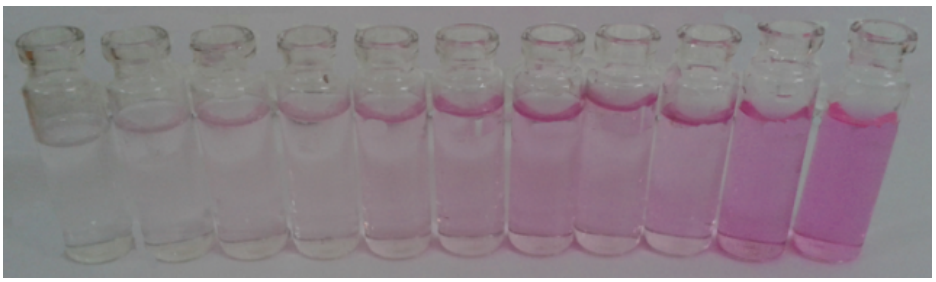


Fig. S2 Visual color change with increase of concentration of Hg(II) ions added (0-30 μM) in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C

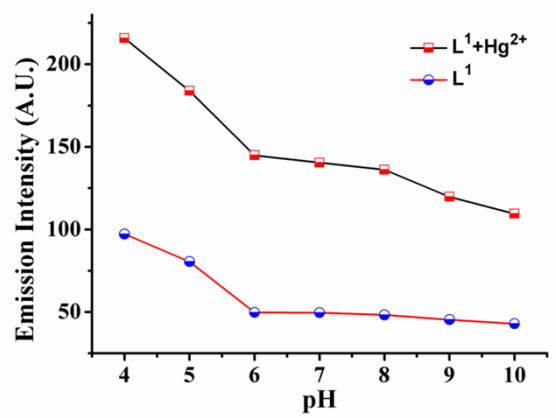


Fig. S3 Effect of pH on the probe, **L**[1] in absence of Hg(II) and in presence of Hg(II) in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C at λ_{em} = 575 nm

Detection Limit

The detection limit was determined from the fluorescence titration data at λ_{ex} 550 nm.[1]

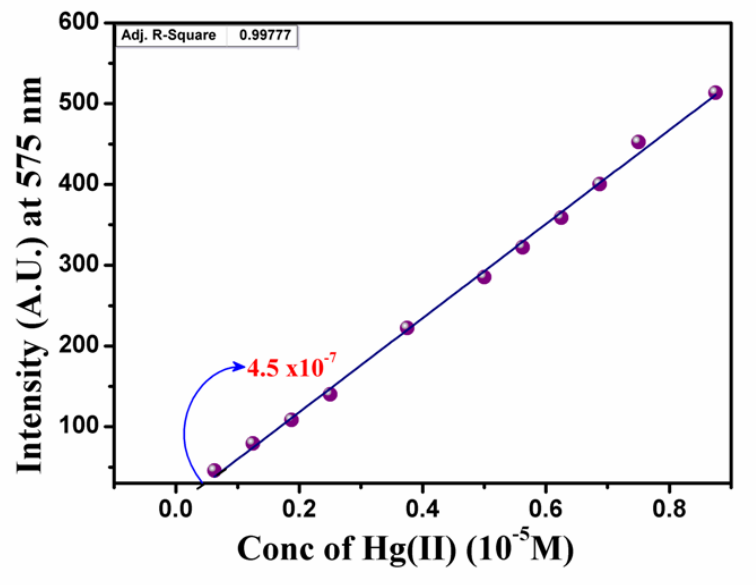


Fig. S4 Detection limit of Hg(II) (4.5 x 10^{-7} M ≡ 90 ppb) in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C

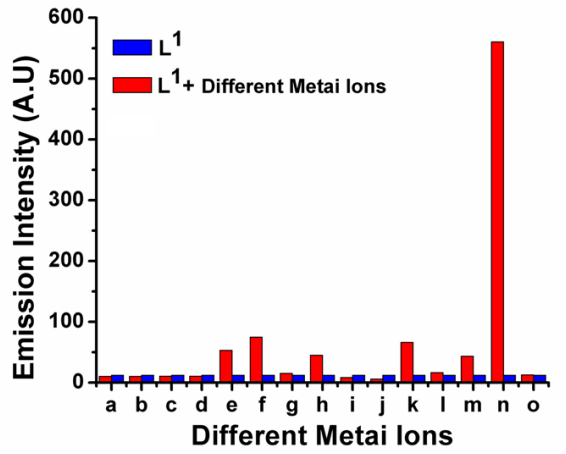


Fig. S5 Fluorescence intensity assay of **L[1]** in presence of different metal ion salts in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25 °C (λ_{ex} = 550 nm), a) Na(I), b) K(I), c) Ca(II), d) Mg(II), e) Al(III), f) Cr(III), g) Mn(II), h) Fe(III), i) Co(II), j) Ni(II), k) Cu(II), l) Zn(II), m) Cd(II), n) Hg(II), and o) Pb(II) at λ_{em} = 575 nm.

Fig. S6 Visual color change of the probe due to the addition of different cations in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C

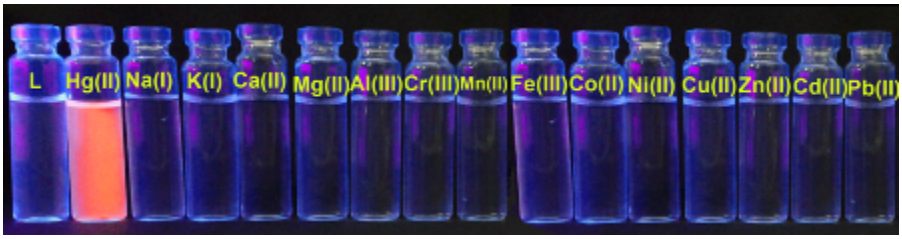


Fig. S7 Fluorescence color of the probe in absence and presence of different metal ions in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C

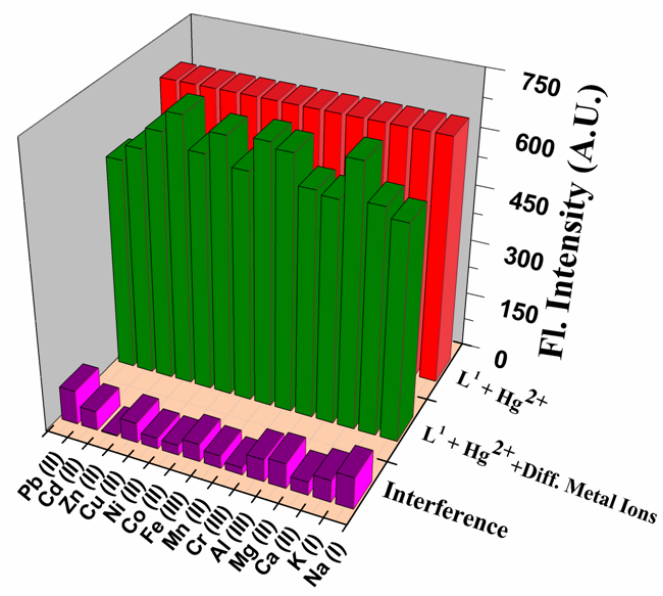


Fig. S8 Signaling of Hg(II) ions by $\mathbf{L^1}$ in the presence of 10-50 eq. of competitive ions in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C at λ_{ex} = 550 nm

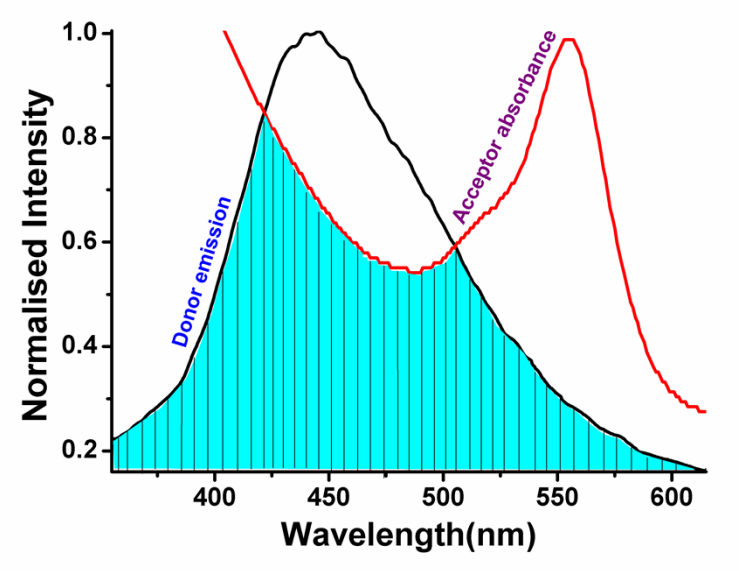


Fig. S9 Overlap spectra of donor emission and acceptor absorbance of $\mathbf{L^1}$ (10 µM) in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C

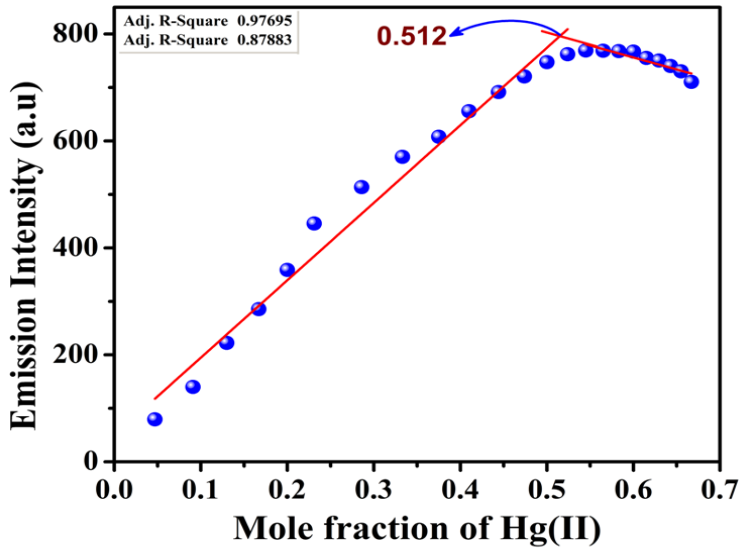


Fig. S10 Job's plot analysis from emission intensity showing maximum emission at 1:1 ratio [$\mathbf{L^1}$: Hg(II)] at λ_{em} = 575 nm

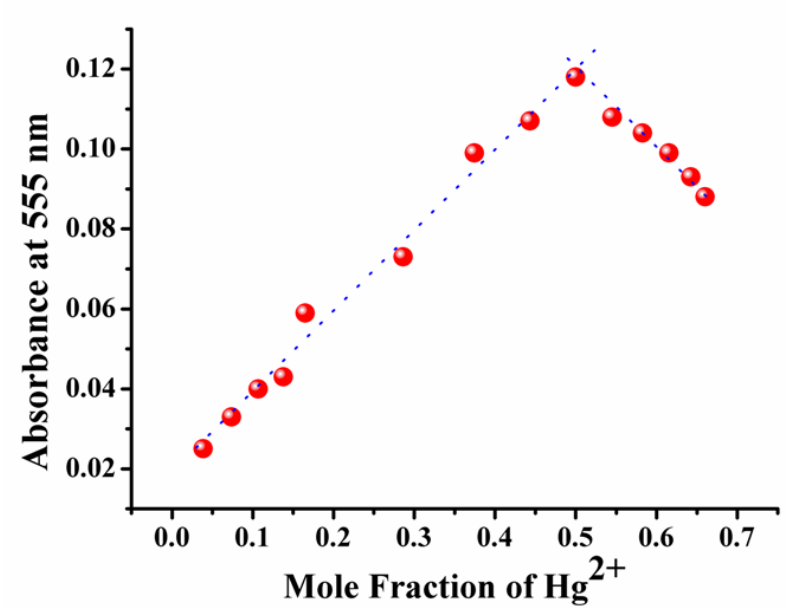


Fig. S11 Job's plot analysis from UV-vis data showing maximum absorption at 1:1 ratio [$\mathbf{L^1}$: Hg(II)]

Binding Constant: The binding constant value was determined from the emission intensity data following the modified Benesi-Hildebrand equation.[2]

$$1/\Delta F = 1/\Delta F_{max} + (1/K[C])(1/\Delta F_{max}),\ \Delta F = F_x - F_0,\ \Delta F_{max} = F_\infty - F_0$$

$$\text{i.e. } (F_\infty - F_0)/(F_x - F_0) = 1 + 1/K[C]$$

where F_0, F_x, and F_∞ are the emission intensities of organic moiety considered in the absence of Hg(II) ion, at an intermediate Hg(II) concentration, and at a concentration of complete interaction, respectively, and where K is the association constant and [C] is the Hg(II) concentration. K value (2.5 x 10^6 M^{-1}) was calculated from the intercept/slope using the plot of $(F_\infty - F_0)/(F_x - F_0)$ against $[C]^{-1}$.

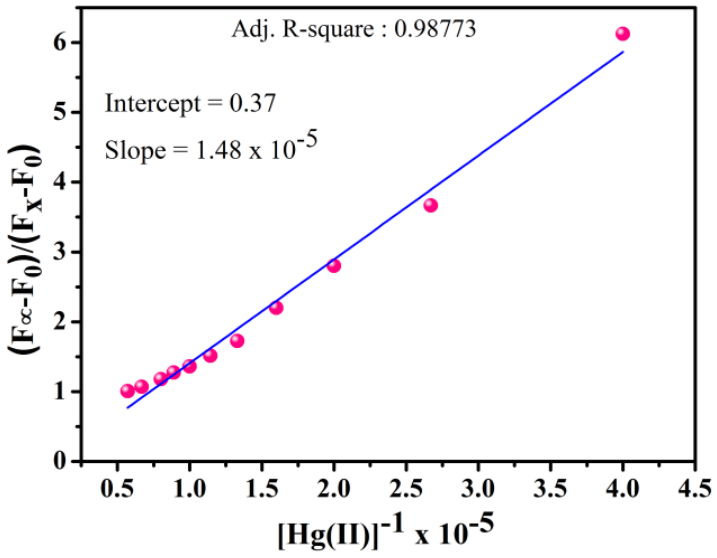


Fig. S12 Binding constant (K) value 2.5×10^6 M^{-1} determined from the intercept/slope of the plots resulting in the interactions of $\mathbf{L^1}$ with Hg(II)

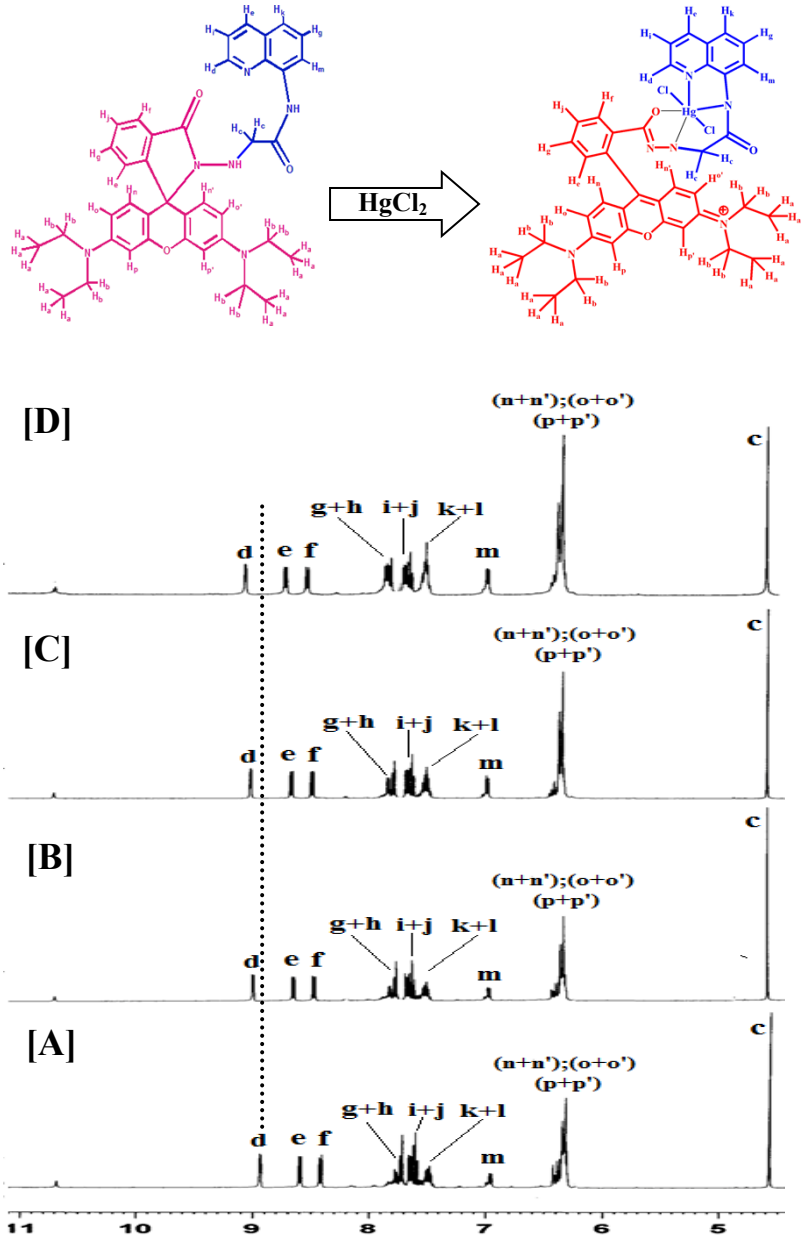


Fig. S13 Partial ^{1}H NMR spectra for **L^{1}** (10 mM) in presence of varying [Hg(II)] [A) 0 mM, B) 3.33 mM, C) 6.67 mM, and D) 10 mM] in DMSO-d_6

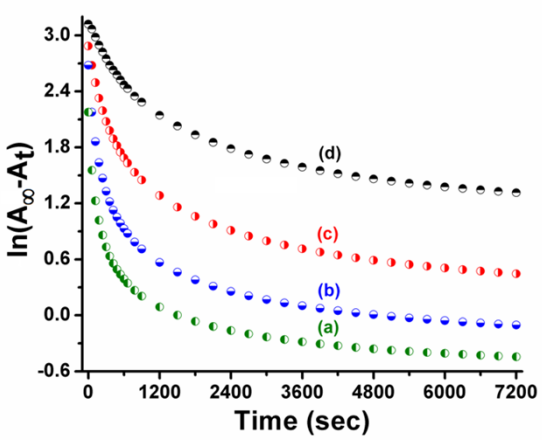


Fig. S14 Plot of $\ln(A_\infty-A_t)$ vs time (sec) of $\mathbf{L^1}$ (10 μM) for different [Hg(II)] (0.2, 0.4, 0.6, 0.8 mM) in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 40°C

Table S1. k_1 and k_2 values for complexation reaction[3] and ring opening

[Hg(II)] (mM)	$10^3\, k_1$ (s^{-1})[a]	$10^5\, k_2$ (s^{-1})[a]
0.2	1.11	8.79
0.4	1.94	8.62
0.6	2.61	8.46
0.8	3.15	8.38

[a]rate data are the average of duplicate runs, and reproducible within ± 4%

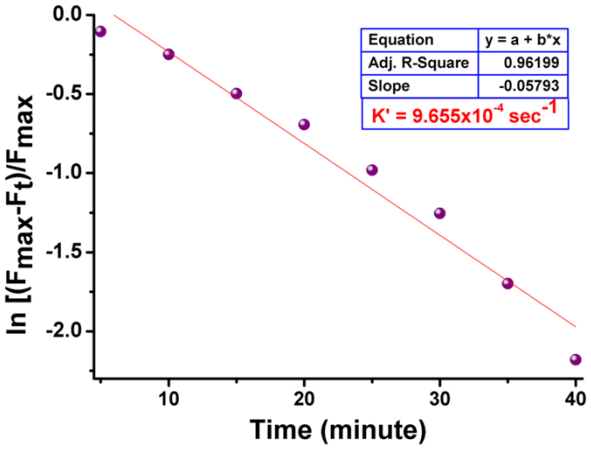


Fig. S15 P*seudo* first-order (overall) kinetic plot of the reaction of $\mathbf{L^1}$ (10 μM) with Hg(II) (0.1-1.0 mM) in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C, Slope = -0.05793 min^{-1}

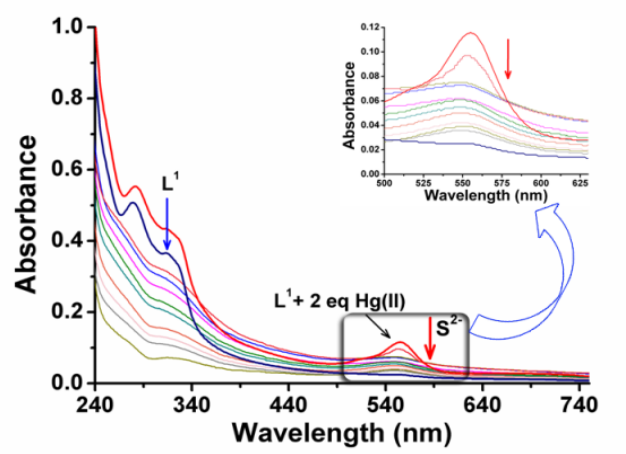


Fig. S16 UV-vis titration spectra of L-Hg (1:1 complex, 10 μM) upon incremental addition of S^{2-} (0-30 μM) in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C

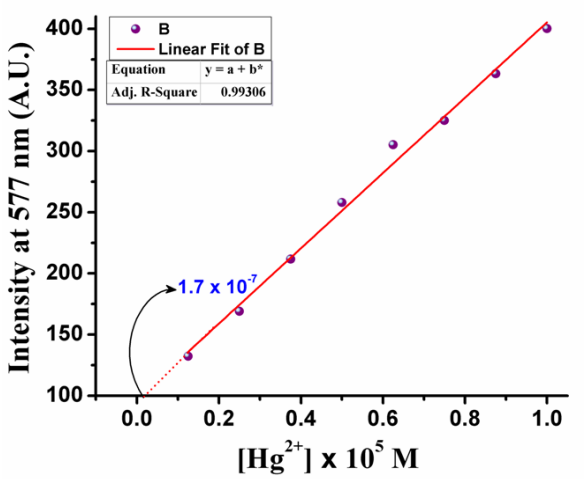


Fig. S17 Detection limit of S^{2-} in HEPES buffer (1 mM, pH 7.4; 2% EtOH) at 25°C

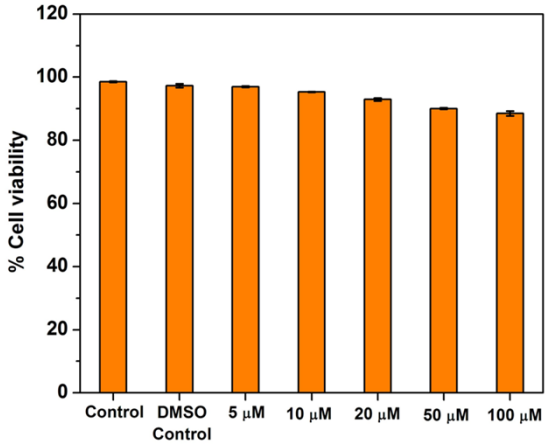


Fig. S18 Cytotoxic effect of $\mathbf{L^1}$ (5, 10, 20, 50 and 100 μM) in HeLa cells incubated for 6 h

Reference

(1) (a) M. Shortreed, R. Kopelman, M. Kuhn, B. Hoyland, *Anal. Chem.* 1996, **68**, 1414; (b) A. Caballero, R. Martinez, V. Lloveras, I. Ratera, J. V. Gancedo, K. Wurst, A. Tarraga, P. Molina, J. Veciana, *J. Am. Chem. Soc.,* 2005, **127**, 15666.
(2) H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703.
(3) H.Y. Lee, J. Jo, H. Park and D. Lee, *Chem. Commun.*, 2011, **47**, 5515.