Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting information

Specific probe for Hg^{2+} to delineate even H^{+} in pure aqueous buffer / Hct116 colon cancer cells: Hg(II)-η^2-arene π-interaction and a TBET-based fluorescence response

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Materials.

Rhodamine B, Ethylenediamine, 9,10- Phenanthrenequinone, Terephthalaldehyde, 4-Methylbenzaldehyde, Trifluoroacetic acid, all metal perchlorate salts such as $NaClO_4$, $KClO_4$, $Mg(ClO_4)_2$, $Ca(ClO_4)_2$, $Cu(ClO_4)_2$, $Zn(ClO_4)_2$, $Co(ClO_4)_2$, $Ni(ClO_4)_2$, $Cr(ClO_4)_2$, $Fe(ClO_4)_2$, $Cd(ClO_4)_2$, $Hg(ClO4)_2$, $Pb(ClO_4)_2$ salts and $PdCl_2$ were obtained from Sigma-Aldrich and were used as received. Solvents used for synthesis of intermediates and final compounds were of AR grade and HPLC grade solvents for spectroscopic studies from S.D. Fine Chemicals in India.

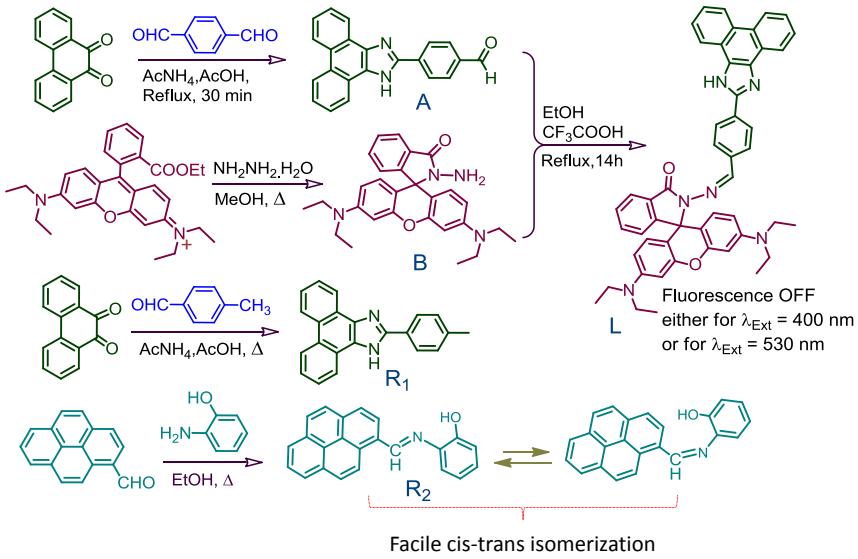
Analytical Methods:

^{1}H NMR spectra were recorded AV 400 MHz or AV-500 MHz Bruker NMR spectrometers using DMSO-d_6 and CD_3CN-d_3 as the solvent and tetra methyl silane (TMS) as an internal standard. ESI-Ms measurements were carried out on a Waters QTof-Micro instrument. Electronic spectra were recorded with a Shimadzu UV-3101 PC spectrophotometer; while fluorescence spectra were recorded using Qunata Master 400, PTI spectrofluorometer.

General experimental procedure for UV-Vis and Fluorescence studies:

$5x10^{-2}$ M solution of the perchlorate salts of the respective ion (Na^+, K^+, Fe^{3+}, Na^+, Mg^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cr^3+ and Hg^{2+}) were prepared in pure aqueous medium, while $PdCl_2$ in brine solution for all studies. The effective final concentrations of all metal salts were maintained at 5.0 x 10^{-5} M. A stock solution of the receptor **L** and $\mathbf{R_1}$ and $\mathbf{R_2}$ (5 x 10^{-3} M) was prepared in dimethylsulphoxide (DMSO) medium and 20 µL of this stock solution was added to 4.98 ml of 0.4 mM TX100 in 10 mM HEPES aqueous buffer medium having solution pH 7.2 to make the effective ligand concentration of 20 µM. For all luminescence measurements, λ_{Ext} = 400 nm with an emission slit width of 1 nm. The relative fluorescence quantum yields (ϕf) were estimated using Rhodamine B (ϕ_f = 0.3 in aqueous medium at RT) as a reference.

Synthesis:



Scheme 1: Methodologies that were adopted for synthesis of **A, B, R_1, R_2** and **L.**

Synthesis of A:[1] A mixture of 9, 10-phenanthroquinone (700 mg, 3.365 mmol), terephthalaldehyde (1352 mg, 10.08 mmol), and ammonium acetate (5182 mg, 67.29 mmol) were stirred in glacial AcOH (15 mL) under reflux condition. After 30 min reaction was stopped, the hot solution was cooled to room temperature, and the resulting yellow solid was collected by filtration and washed with excess amount of water and methanol to remove starting materials. Yield: 850 mg, 78.50 %. ESI- Ms (m/z) calculated for $C_{22}H_{14}N_2O$: 322.11, observed: 323 [M + H^+]. ^{1}H NMR [400 MHz, DMSO-d_6: δ (ppm)]: 13.71 (1H, s, -NH); 10.10 (1H, s, -CHO); 8.67(2H, d, *J* = 8 Hz, ArH); 8.60 (2H, d, *J* = 7.6 Hz, ArH); 8.53 (2H, d, *J* = 8.4 Hz, ArH); 8.13 (2H, d, *J* = 8 Hz, ArH); 7.77 (2H, t, *J* = 7.6 Hz, ArH); 7.67 (2H, t, *J* = 7.6 Hz, ArH). ^{13}C NMR (500 MHz, DMSO-d6, δ (ppm)): 193.00, 172.58, 149.08, 148.74, 13.91, 136.51, 135.90, 130.56, 128.58, 127.76, 127.32, 127.05, 127.00, 126.13, 124.44 and 122.56.

Synthesis of R_1:[1] A mixture of 4- Methylbenzaldehyde (288 mg, 2.4 mmol), 9, 10-phenanthroquinone (500 mg, 2.4 mmol), and ammonium acetate (3696 mg, 48 mmol) were stirred in glacial AcOH (10 mL) under reflux condition for 30 min. After 30 min the hot

solution was cooled to room temperature, and the resulting white solid was collected by filtration and washed with excess amount of water and methanol to remove starting materials. Yield: 600 mg, 81 %. ESI- Ms (m/z) calculated for $C_{22}H_{16}N_2$: 308, observed: 308 [M]. ^{1}H NMR [500 MHz, DMSO-d_6: δ (ppm)]: 13.36 (1H, s, -NH); 8.84 (2H, d, *J* = 8 Hz, ArH); 8.56 (2H, d, *J* = 7.4 Hz, ArH); 8.21 (2H, d, *J* = 8 Hz, ArH); 7.77-7.60 (4H, m, ArH); 7.41 (2H, d, *J* = 8 Hz, ArH); 2.41 (3H, s, -CH_3). ^{13}C NMR (500 MHz, DMSO-d_6, δ (ppm)): 149.77, 139.33, 137.41, 130.00, 128.18, 128.03, 127.54, 126.56, 125.62, 124.50, 124.23, 122.29 and 21.15.

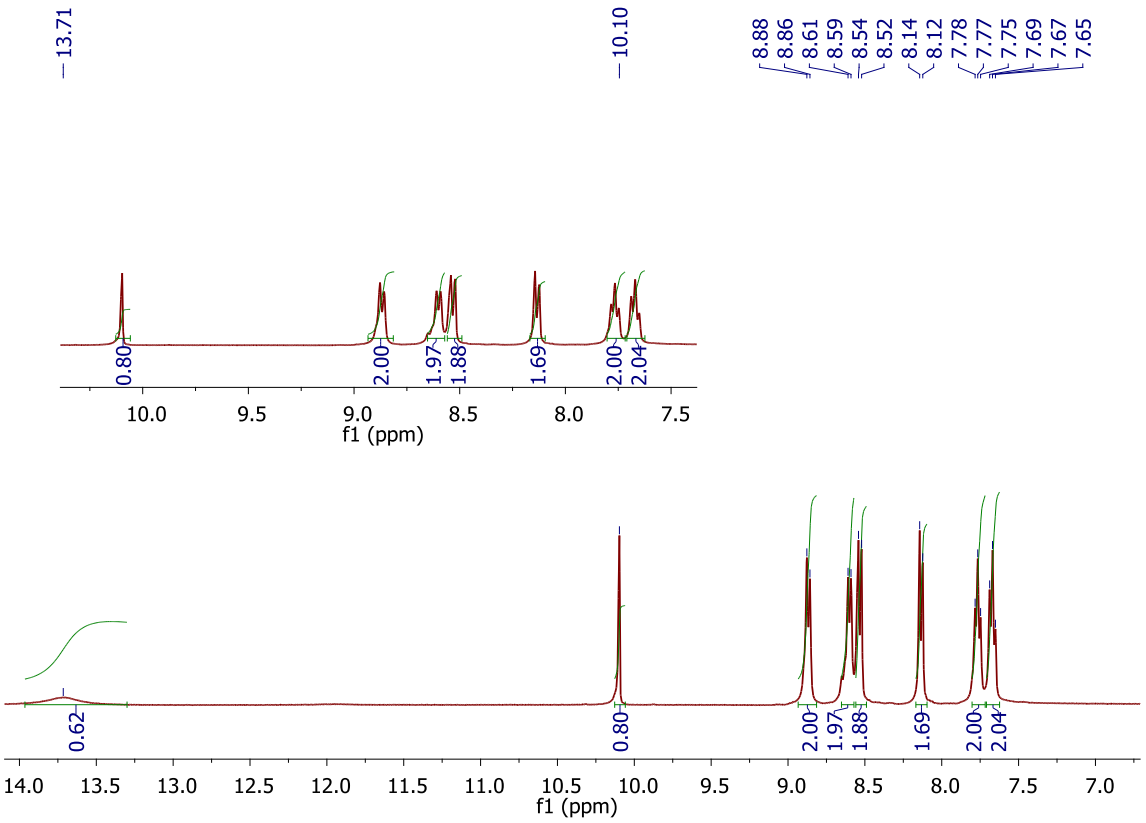
Synthesis of R_2: Reagent R_2 was synthesized following a previously reported procedure.[2]

Synthesis of L: Rhodamine 6G hydrazine (B) (150 mg, 0.33 mmol) and compound **A** (106 mg, 0.33 mmol) were dissolved in 40 mL of ethanol. To this was added approximately 2 drops of acetic acid, and the resulting solution was refluxed for 16 h. The solution was filtered while hot to remove starting materials and yellow precipitate was collected. The residue was washed thoroughly with methanol and acetone to isolate pure form of **L**. Yield: 160 mg, 64 %. ESI- Ms (m/z) calculated for $C_{50}H_{46}N_6O_2$: 762, observed: 763 [M + H^+]. ^{1}H NMR [500 MHz, DMSO-d_6: δ (ppm)]: 8.89 (1H, s, -CH=N-); 8.87 (2H, d, *J* = 10 Hz, ArH); 8.57 (2H, d, *J* = 10 Hz, ArH); 8.26 (2H, d, *J* = 10 Hz, ArH); 7.96 (1H, t, *J* = 9.0 Hz, ArH); 7.76 (3H, t, *J* = 9Hz, ArH); 7.70-7.61 (6H, m, ArH); 7.15 (1H, d, *J* = 9.5 Hz, ArH); 6.55 (1H, s, ArH); 6.50 (2H, d, *J* = 11, Hz, ArH); 6.40 (2H, d, *J* = 9.5, ArH); 3.33 (8H, q, *J* = 8.5 Hz, CH_2); 1.08 (12H, t, *J* = 8.5 Hz, CH3). ^{13}C NMR (500 MHz, DMSO-d_6, δ (ppm)): 189.77, 182.95, 175.76, 173.74, 169.54, 154.73, 152.55, 145.71, 144.87, 144.20, 144.02, 143.45, 142.47, 139.98, 138.87, 137.57, 66.83, 54.98, 39.98 and 31.60.

References

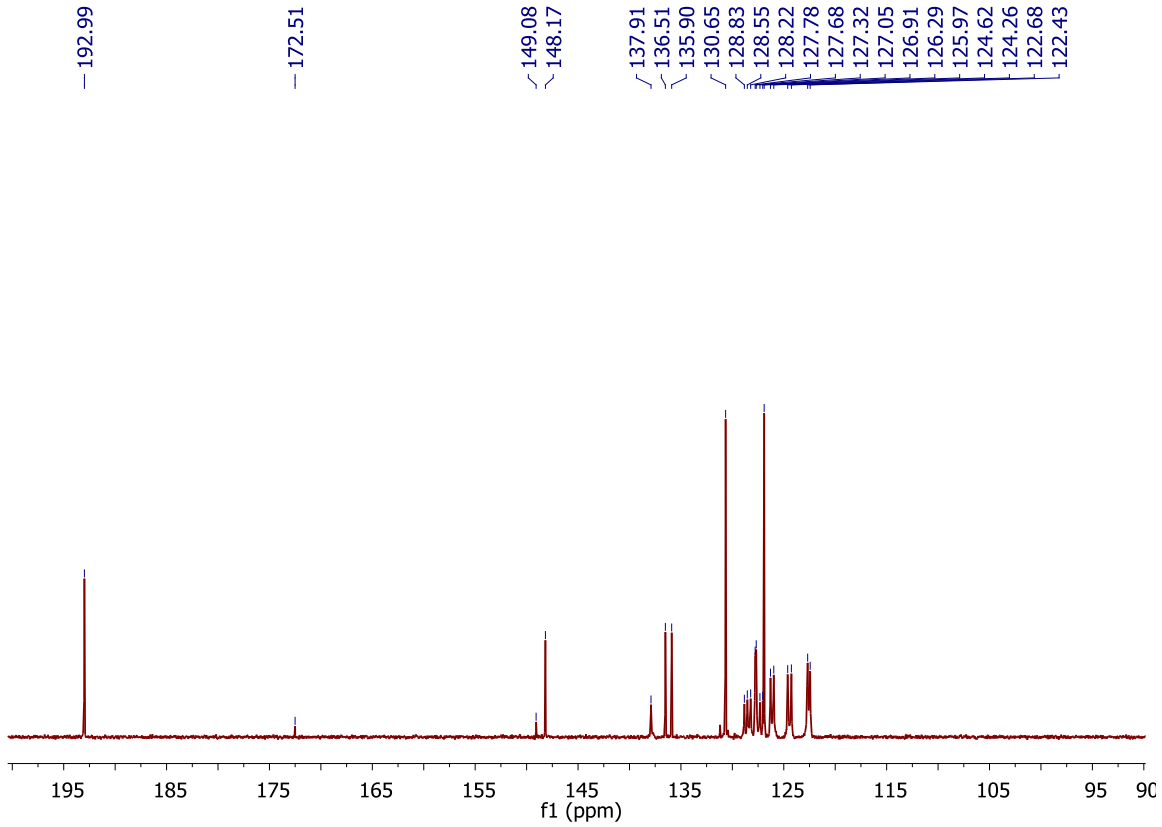
(1) W. Lin, L. Long, L. Yuan, Z. Cao, B. Chen and W. Tan, *Org. Lett*., 2008, **10**, 5577-5580; (2) L. Zang, D.Wei, S.Wang and S. Jiang, *Tetrahedron*., 2012, **68,** 636-641.

[1]HNMR spectra of A



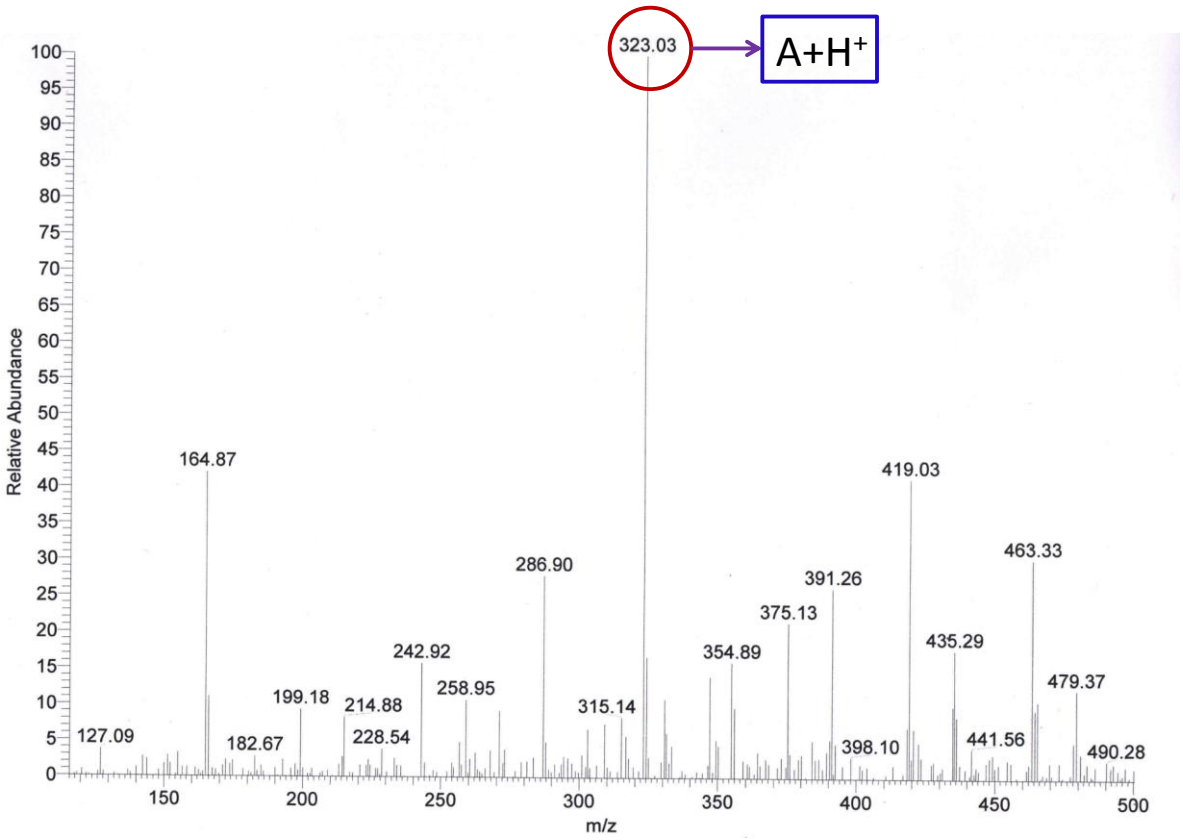
SI Figure 1: ^{1}H NMR spectra of **A** in DMSO-d_6 medium.

^{13}C NMR spectra of A



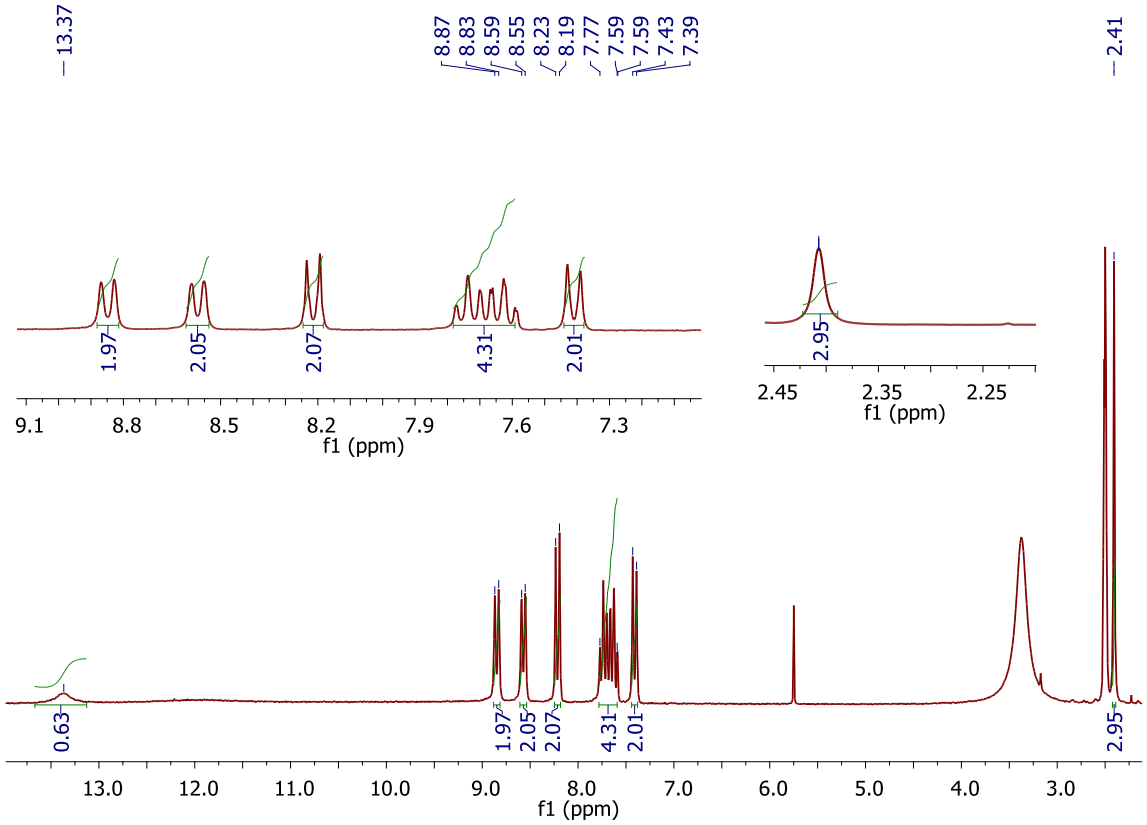
SI Figure 2: ^{13}C NMR spectra of A in DMSO-d_6 medium.

Mass spectra of A



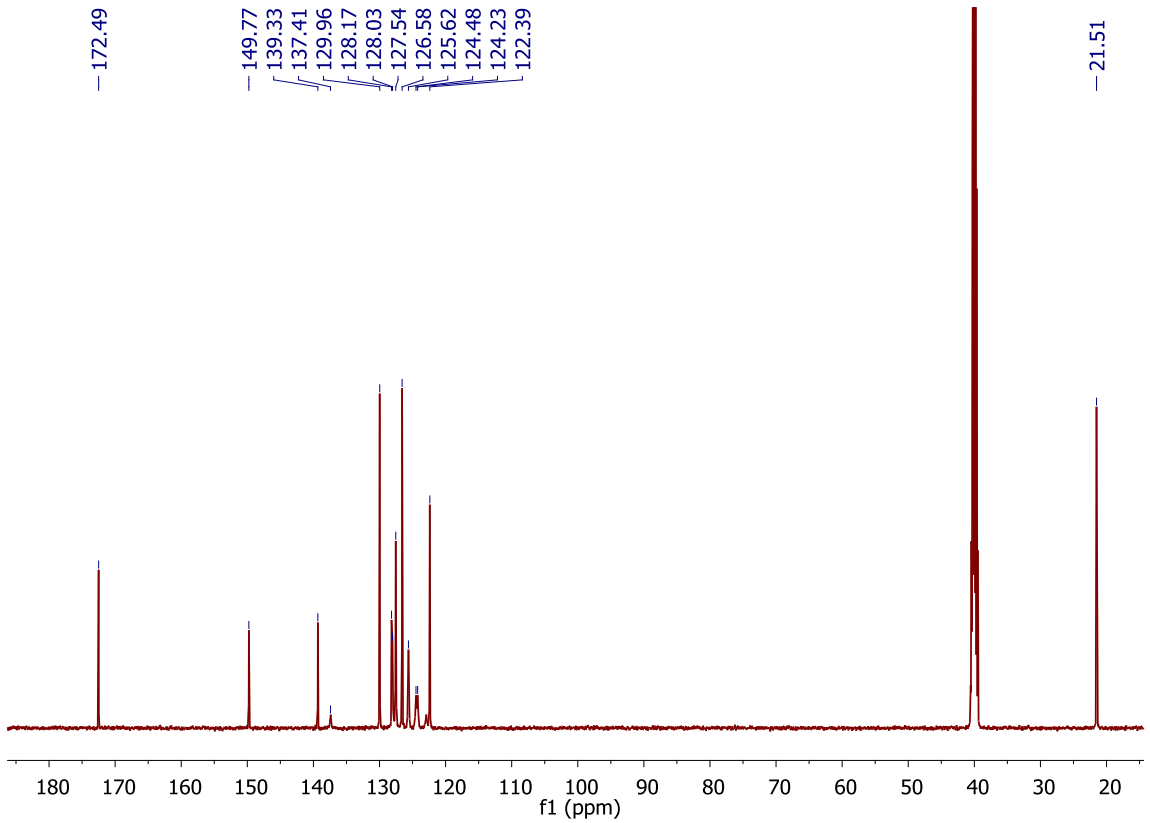
SI Figure 3: ESI- Ms Spectrum of **A** in CH_3OH.

^{1}H NMR spectra of R_1



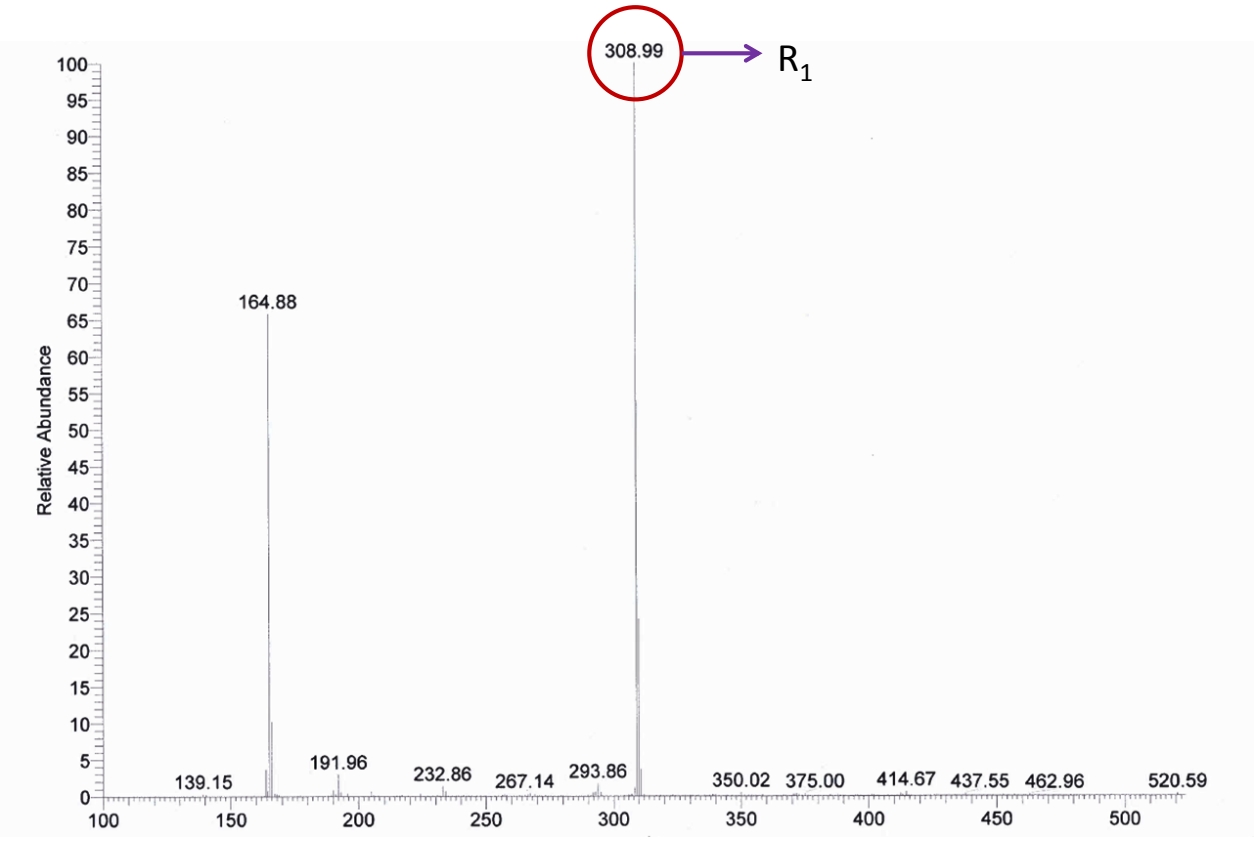
SI Figure 4: ^{1}H NMR spectra of **R_1** in DMSO-d_6 medium.

^{13}CNMR spectra of R_1



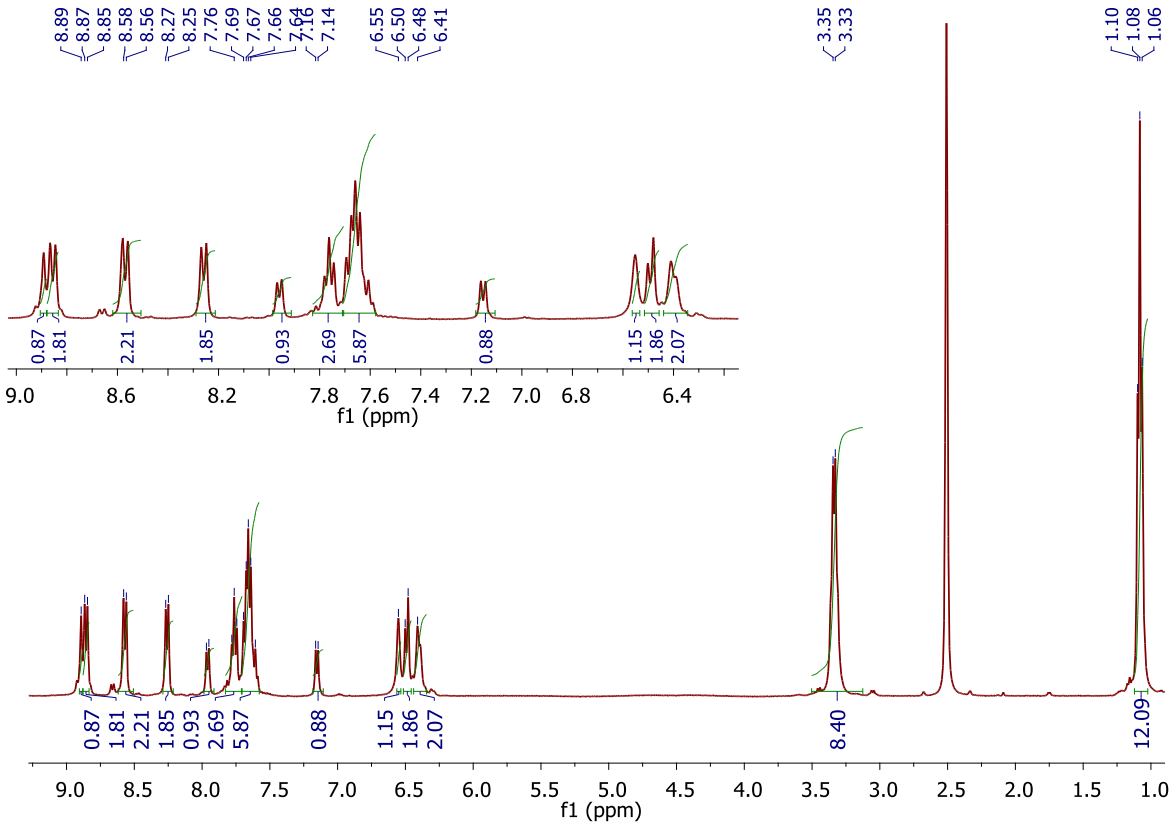
SI Figure 5: ^{13}C NMR spectra of **R_1** in DMSO-d_6 medium.

Mass spectra of R_1



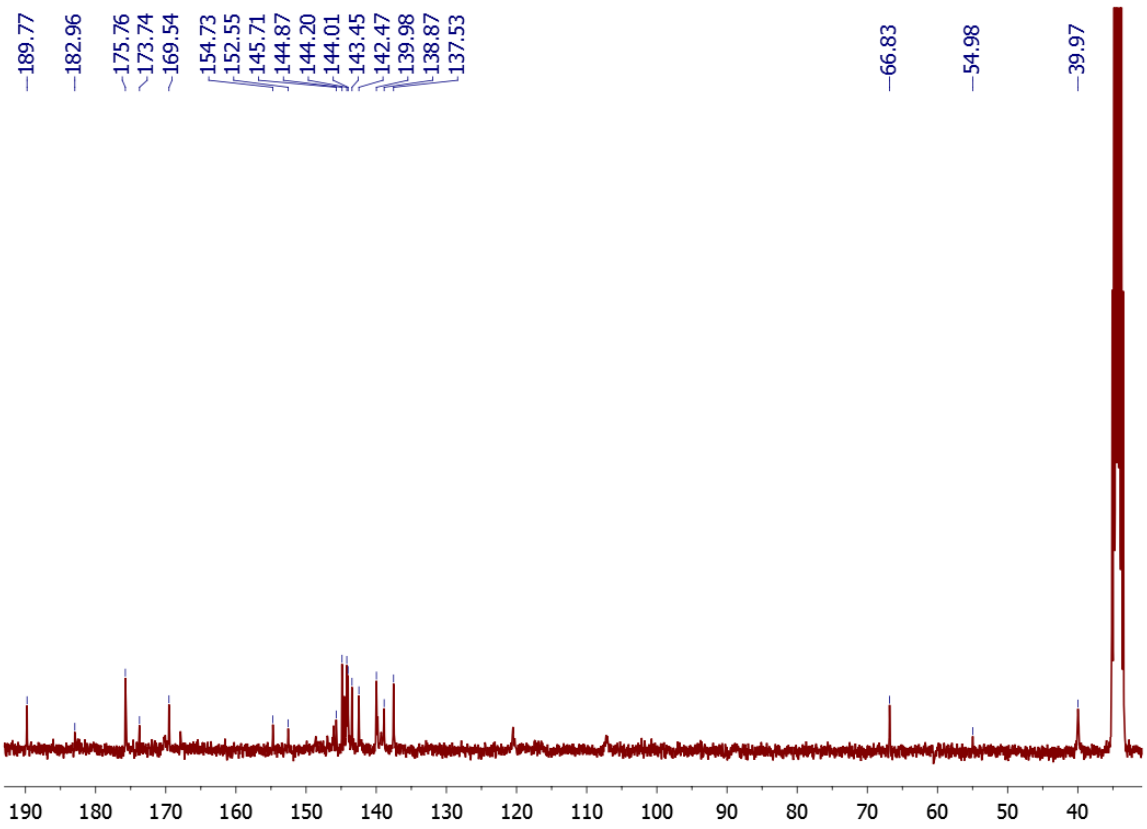
SI Figure 6: ESI- Ms Spectrum of **R_1** in CH_3OH.

^{1}H NMR spectra of L



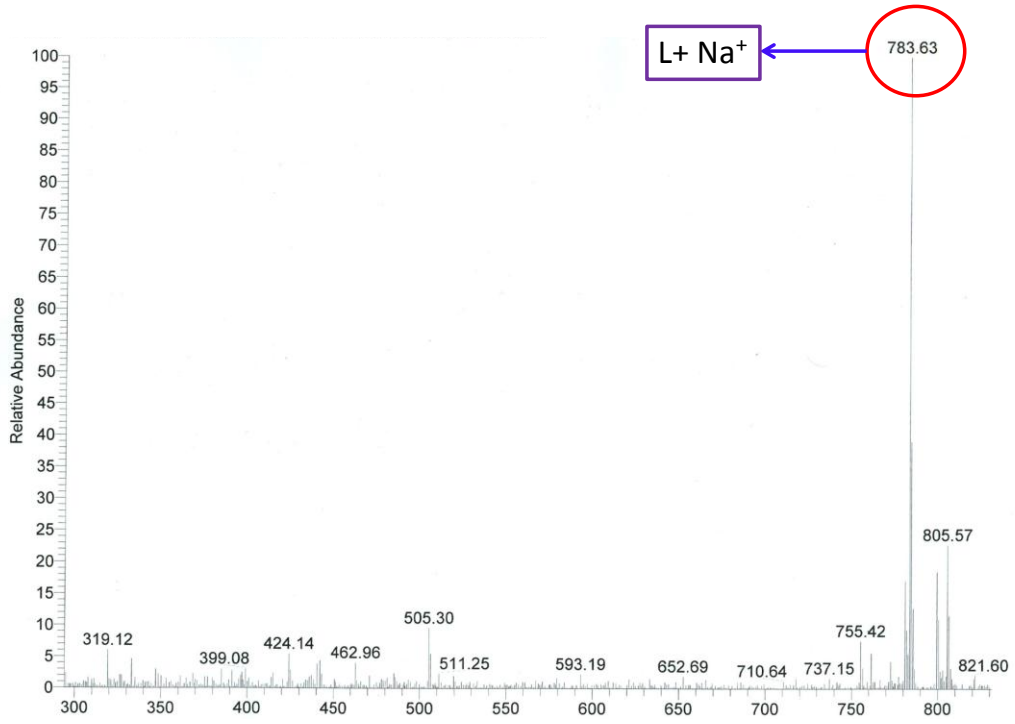
SI Figure 7: ^{1}H NMR spectra of **L** in DMSO-d_6 medium

^{13}C NMR spectra of L



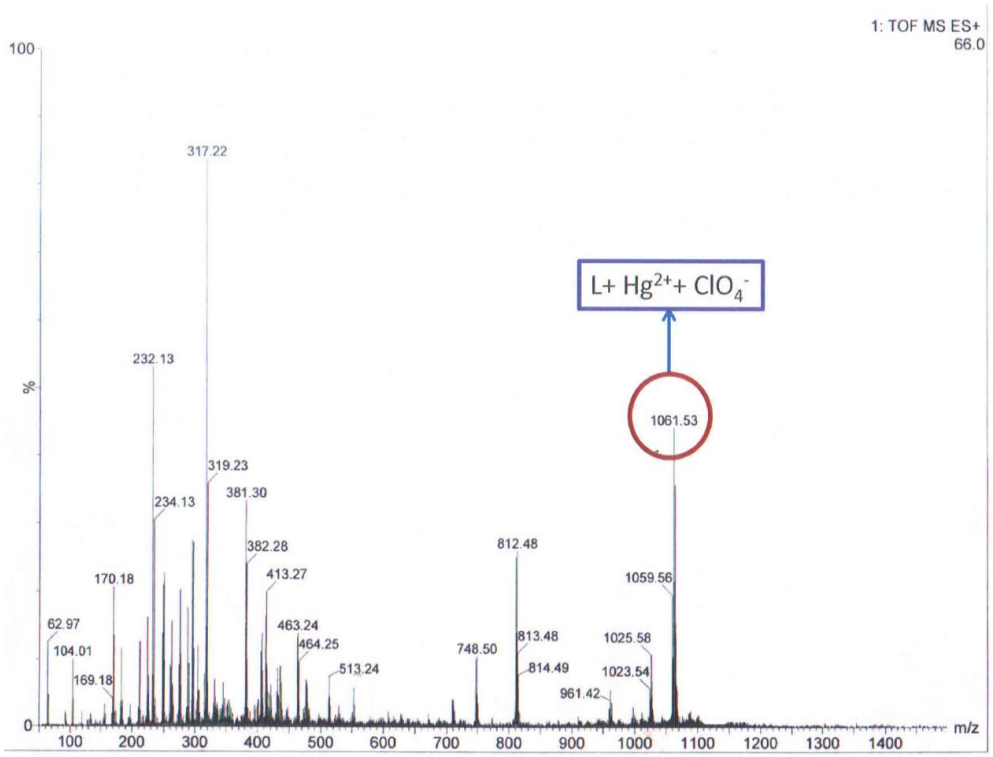
SI Figure 8: ^{13}C NMR spectra of **L** in DMSO-d_6 medium

Mass spectra of L



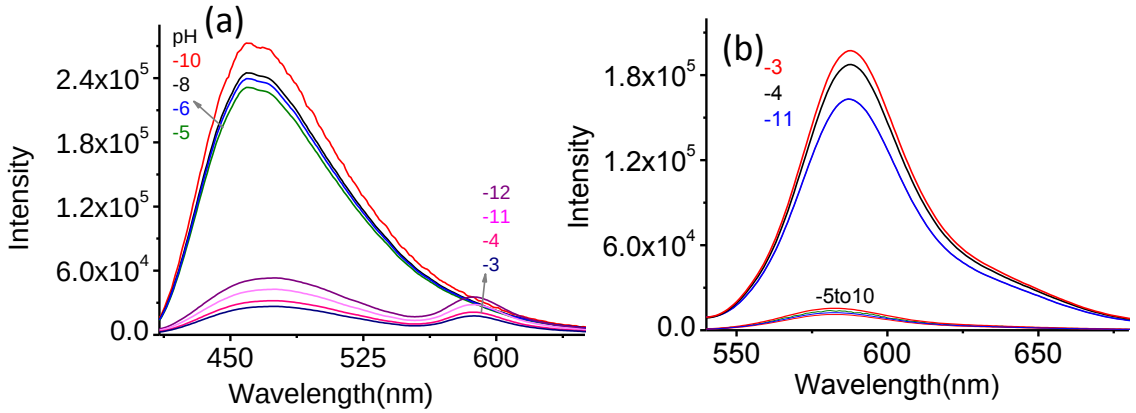
SI Figure 9: ESI- Ms Spectrum of **L** in Acetonitrile.

Mass spectra of L + Hg^{2+} in Acetonitrile



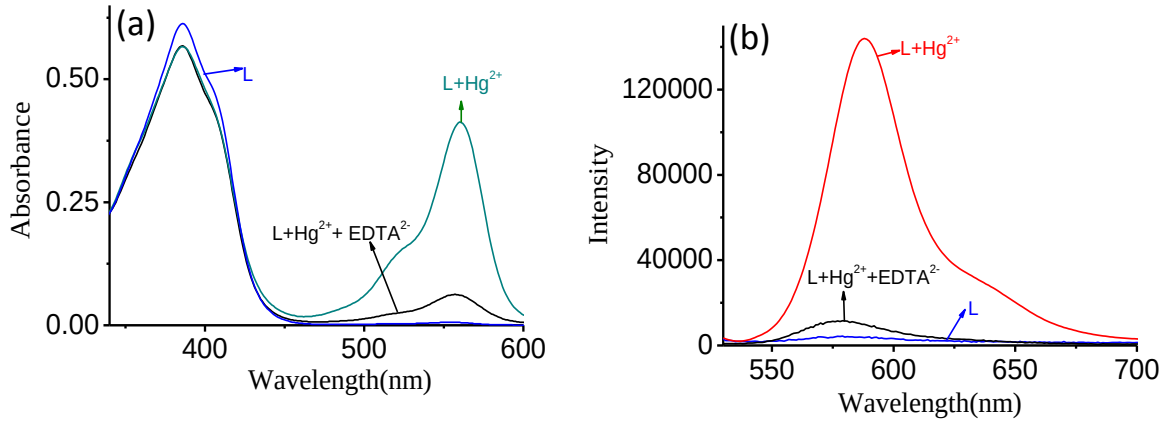
SI Figure 10: ESI- Ms Spectrum of L+ Hg^{2+} in Acetonitrile.

Change of Emission intensity of L as a function of the solution pH:



SI Figure11: Studies were performed in aq. solution of 0.4 mM TX100 and HEPES buffer (10 mM; pH 7.2) by using (a) λ_{Ext} = 400 nm; (b) λ_{Ext} = 530 nm, slit width 1/1nm.

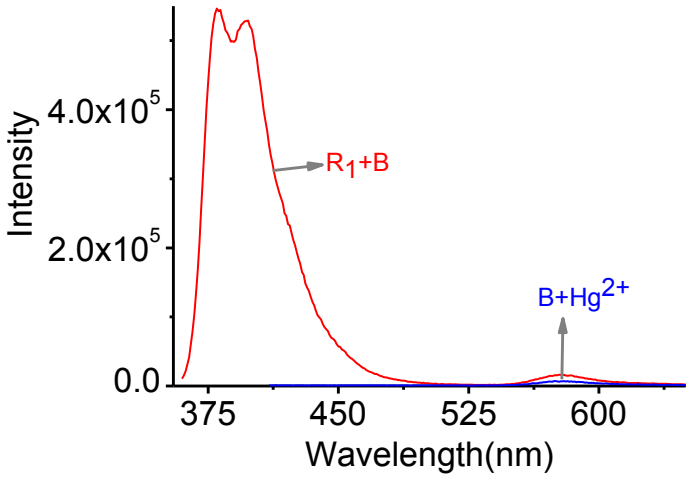
Uv-Vis and Flourescence spectral studies for establishing the reversible binding of Hg^{2+} to the L:



SI Figure 12: (a) Uv and (b)Fluorescence studies for establishing the reversible binding of Hg^{2+}(30 eq) to **L** (20 mM) in presence of $EDTA^{2-}$ (2 x 10^{-3} M) using λ_{Ext} = 530 nm; and slit width 1/1 nm, in aq. Solution of 0.4 mM TX100 and HEPES buffer (10 mM; pH 7.2).

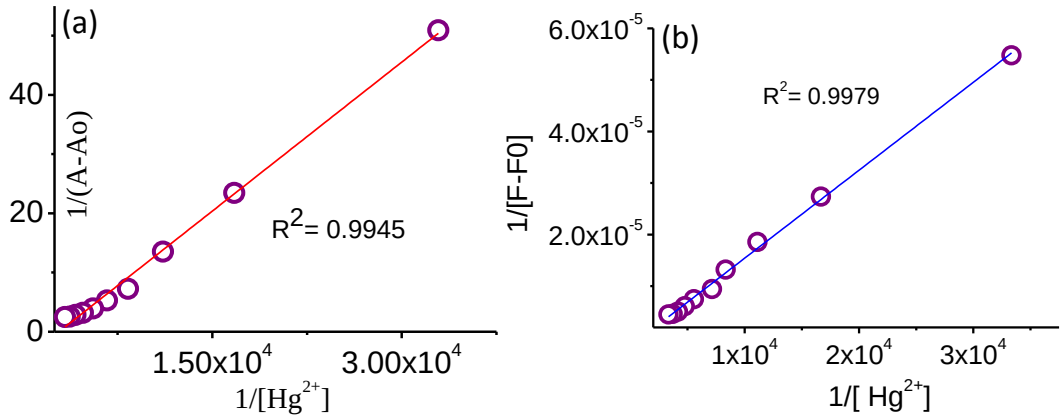
The reversibility of the binding process between L and Hg^{2+} was also established. On addition of aq. solution of $EDTA^{2-}$ to an aq. HEPES buffer solution (20 μM) of Hg^{2+}.L (pH = 7.2), the original spectrum of the spirolactam form of L was restored. Higher binding affinity of Hg^{2+} towards $EDTA^{2-}$ led to the formation of Hg^{2+}–$EDTA^{2-}$ and regeneration of L.

Fluorescence spectra of equimolar mixture of R_1 and rhodamine B + Hg^{2+}



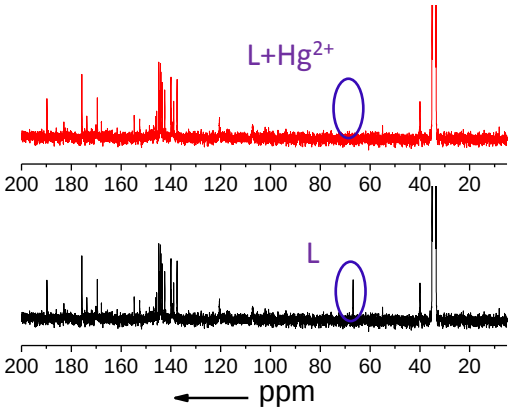
SI Figure 13: Fluorescence spectra of equimolar mixture of **R_1** and rhodamine B in presence of Hg^{2+} (30 equiv.) performed in aq. solution of 0.4 mM TX100 and HEPES buffer (10 mM; pH 7.2). The concentration is 20 μM for both R and rhodamine B using λ_{Ext} = 375 nm; slit width 1/1 nm. This shows there is no inter molecular energy transfer process between acceptor and donor moiety.

Benesi-Hildebrand plot for binding studies of [Hg^{2+}] towards L



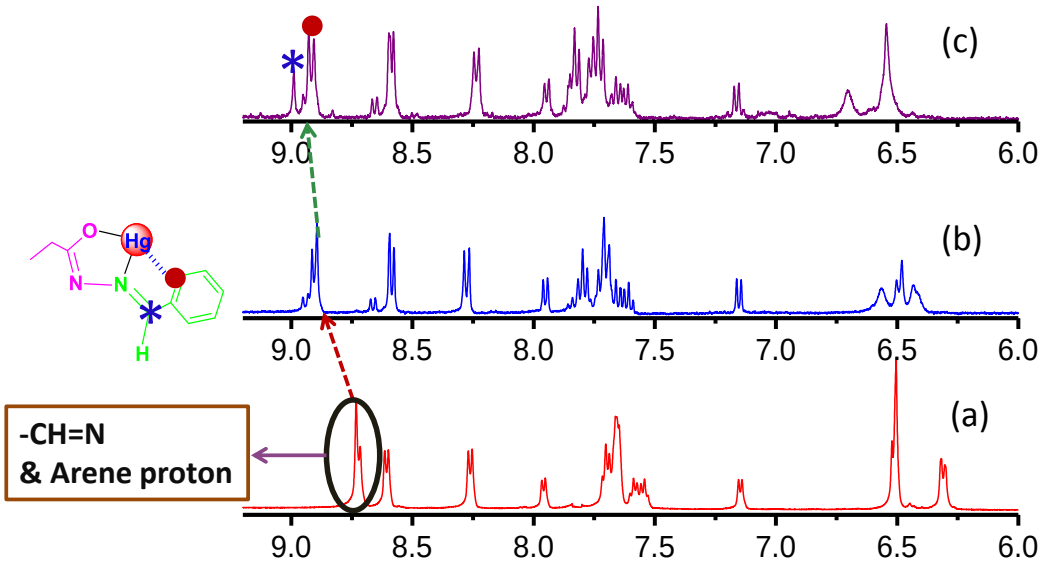
SI Figure 14: Benesi-Hildebrand plot of **L** (20 μM) for varying [Hg^{2+}] (0 to 3.33 x 10^{-5} M) (a) from Uv-Visible titration; (b) Fluorescence titration by using λ_{Ext} = 400 and λ_{Mon} = 587 nm. Good linear fit confirms the 1: 1 binding stoichiometry in aq. solution of 0.4 mM TX100 and HEPES buffer (10 mM; pH 7.2).

^{13}C NMR of L in absence and in presence of Hg^{2+} in DMSO-d6



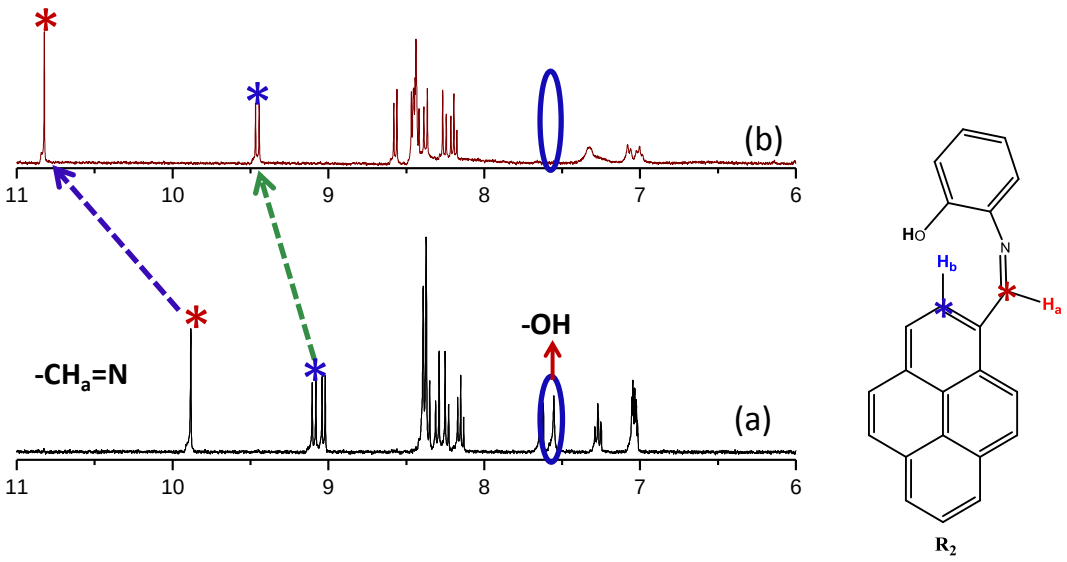
SI Figure 15: ^{13}C NMR of **L** in absence and in presence of Hg^{2+} in DMSO-d_6

^{1}H NMR of L in absence and in presence of Hg^{2+} in DMSO-d_6



SI Figure 16 : ^{1}H NMR spectra of **L** (3mM) in absence and in presence of Hg^{2+} (a) 0 equiv. (b) 50 equiv. (c) 500 equiv. were recorded in DMSO-d_6.

^{1}H NMR of R_1 in absence and in presence of Hg^{2+} in DMSO-d_6



SI Figure 17 : ^{1}H NMR spectra of **R_2** (3mM) in (a) absence and in (b) presence of 50 mole equiv. of Hg^{2+} in CD_3CN.

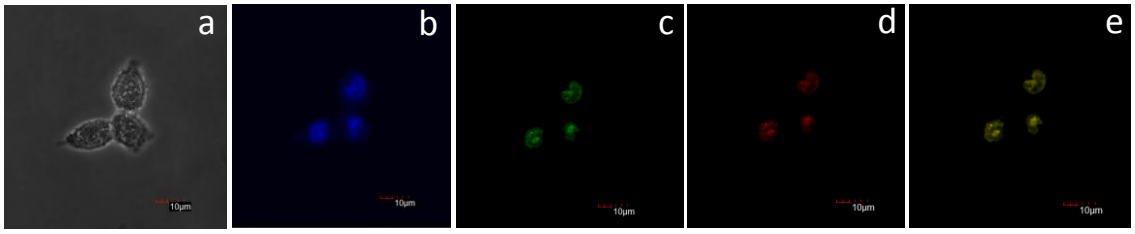
Cell culture and fluorescence imaging

Hct116 cells were seeded on coverslips placed in 6 well plates. After 24 hours cells were treated with **L** (10μM) for 30 minutes. Cells were then washed thrice with Phosphate Buffer Saline (1X PBS) and fixed with 4% PFA for 20 minutes and washed again with 1X PBS. Permeabilization of the cells was done using 0.2% Triton X 100 for 5 minutes. The **L**-stained colon cancer cells Hct116 incubated with Hg^{2+} (4 ppb) for 20 min. Again three washes were given and then coverslips mounted using Fluor shield with DAPI (Sigma) mounting medium. Nail paints was used to seal the coverslips mounted on the glass slides. Images were acquired in Olympus Fluoview Microscope.

The imidazole part of receptor **L** has a pKa of around 5.5 and the predominant LH^+(Scheme 2B in manuscript) form is expected to exist at pH ≤ 4.0. The confocal image shown in Fig. 4b in manuscript clearly reveals that LH^+ became nuclear membrane impermeable.

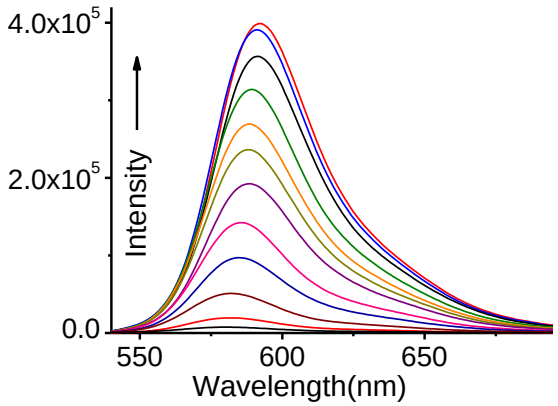
Use of 0.4 mM TX100 in aqueous HEPES buffer solution (10 mM; pH 7.2) allowed reagent **L** to be trapped inside the micellar structure of TX100 and this allowed all recognition, detection and binding studies to be performed in pure aqueous medium having a physiologically relevant pH of 7.2.

Confocal microscopic images of L with Hg^{2+} in Hct116 cells



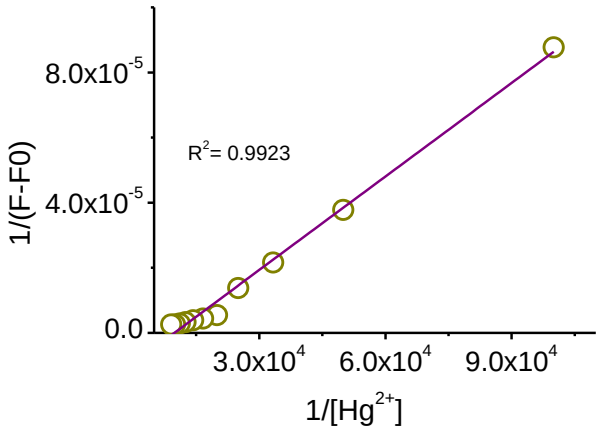
SI Figure 18. Confocal images of Hct116 cells treated with 0.2ppb of Hg^{2+}: Cells were incubated with **L** (10 μM) for 30 min; (a) Bright filed images of Hct116 cells as control; (b) co-staining of **L** with nuclear staining dye DAPI from blue channel, (c - e) These pre-treated cells were exposed to $Hg(ClO_4)_2$ for 4 hr: (c) at green channel, (d) at red channel and (e) overlay images of (c) and (d); λ_{Ext} = 400 nm.

Fluorescence response of L with different Concentrations of Hg^{2+} in Acetonitrile medium



SI Figure 19. Emission spectral responses of L (20 μM) towards varying [Hg $(ClO_4)_2$] (0 to 10 equiv.) in Acetonitrile medium by using λ_{Ext} = 525 nm and λ_{Mon}= 590 nm. Slit width 1/1nm.

Benesi-Hildebrand plot for binding studies of L towards Hg^{2+} in Acetonitrile medium



SI Figure 20. Benesi-Hildebrand plot of **L** (20 μM) for varying [Hg^{2+}] (0 to 10 equiv.) by using λ_{Ext} = 525 nm and λ_{Mon} = 590 nm in Acetonitrile medium. Good linear fit confirms the 1:1 binding stoichiometry with association constant of (8.2 ± 0.5) x 10^4 M^{-1}.