Indole-Based Chemosensor for Hg^{2+} and Cu^{2+} Ions: Applications in Molecular Switches and Live Cell Imaging

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Electronic Supplementary Information

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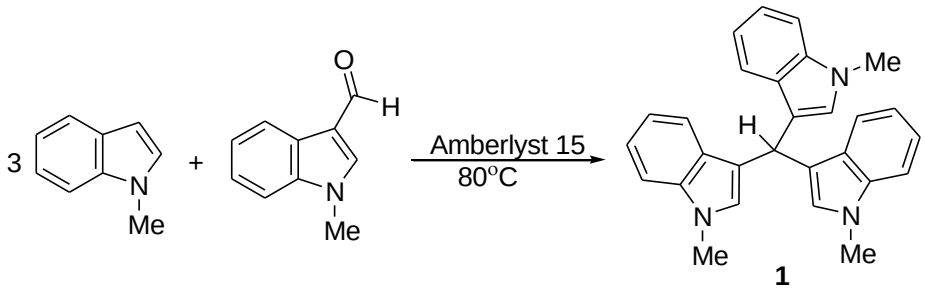
S1. Instrumentation Details

UV-vis spectra were recorded on a SHIMADZU 1601 PC spectrophotometer, with a quartz cuvette (path length, 1 cm) and studies were performed in AR grade CH_3CN and double distilled water. The cell holder of the spectrophotometer was thermostatted at 25 ^oC for consistency in the recordings. The fluorescence spectra (excitation λ_{max} 497 nm) were recorded on a Varian Cary Eclipse fluorescence spectrophotometer, with a quartz cuvette (path length, 1 cm and slit width, 10 nm). The ^1H and ^{13}C NMR spectra were recorded on BRUKER Avance II 400 MHz spectrophotometer using $CDCl_3$ and CD_3CN as solvent and tetramethylsilane ($SiMe_4$) as internal standard. Data are reported as follows: chemical shifts in ppm (δ), multiplicity (s = singlet, m = multiplet), integration, coupling constant J (Hz) and assignment. The mass spectra were recorded with Waters Micromass Q-Tof Micro mass spectrometer. Elemental analyses were performed with a Thermo Flash EA 1112 analyser and were within ±0.4% of the theoretical values. IR spectra were recorded on Varian 660-IR Fourier-Transform IR Spectrophotometer in range 400-4000 cm^{-1} using KBr as medium. Titration isotherms generated from UV-vis changes were fit in HypSpec fitting programme to establish the stoichiometry of the complex and to determine the binding constant. The pH titrations were performed with the Equip-Tronics Digital pH meter model-EQ 610 and electrode was calibrated using standard buffers. Electrochemical studies were carried out on CHI 660C Electrochemical Workstation with a conventional three-electrode configuration consisting of platinum working (2 mm diameter) and counter electrodes and Ag/AgCl as reference electrode. The experiments were carried out in solution of sample in CH_3CN containing tetrabutylammonium perchlorate (TBAP) as supporting electrolyte at room temperature. Deoxygenation of the solutions was achieved by bubbling nitrogen for 10 min and the working electrode was cleaned after each run. The voltammograms were recorded with a scan rate of 100 mVs^{-1}. The imaging experiments of live cells were performed with a laser scanning confocal microscope (Olympus Fluorview, FV 1000) with excitation at 488 nm and dual emission at 571±20 and 590±20 nm.

S2. Experimental details

The UV-vis, fluorescence and cyclic voltametric titrations were carried out by addition of aliquots of metal ion solutions into the solution of compound **1**. The pH titrations were performed by each addition of 0.1 μM of acid and base.

S3. Synthesis of 1.



1

A mixture of N-methyl indole (3 mmol) and N-methyl indolecarboxaldehyde (1 mmol) and amberlyst 15 (20 mg) was stirred at 80°C for 10-15 min. After completion (TLC) the reaction mixture was allowed to cool to room temperature and diluted with DCM, filtered and concentrated under vacuum. The residue was chromatographed on silica gel 60-120 mesh to isolate the compound **1** in good yield (96 %).[1]

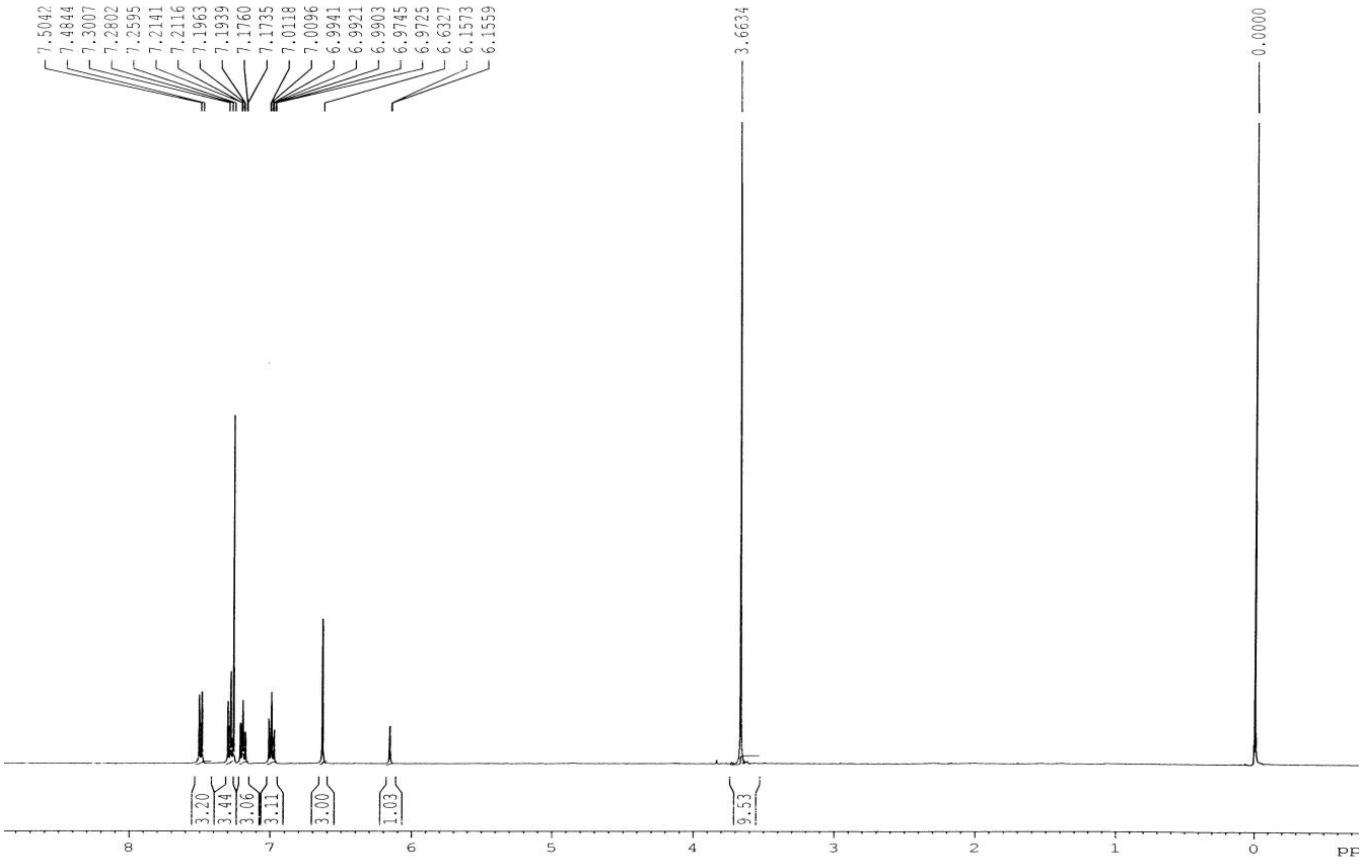
Yield: 96%; m.p. 223°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (3H, d, *J* 8.1 Hz, ArH), 7.25-7.30 (3H, m, ArH), 7.17-7.22 (3H, m, ArH), 6.97-7.01 (3H, m, ArH), 6.63 (3H, s, ArH), 6.15 (1H, s, *meso*-CH), 3.66 (9H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 137.4, 127.9, 127.5, 121.2, 120.2, 118.4, 118.2, 108.9, 32.7, 30.8; ν_{max}/cm^{-1}: 1639, 1615 (C-N), 1472 (C=C), 1234, 1152, 1057, 1011 (CH, in-plane), 803, 739 (CH, out-plane); Anal. Calcd. (%) for $C_{28}H_{25}N_3$: C, 83.37; H, 6.20; N, 10.42; Found: C, 83.24; H, 6.56; N, 10.41; *m/z* (EI): 402.3 (M^+-1).

This method is different from the previously reported literature[2] where $La(OTf)_3$), AcOH has been used as a catalyst for the preparation of **1**.

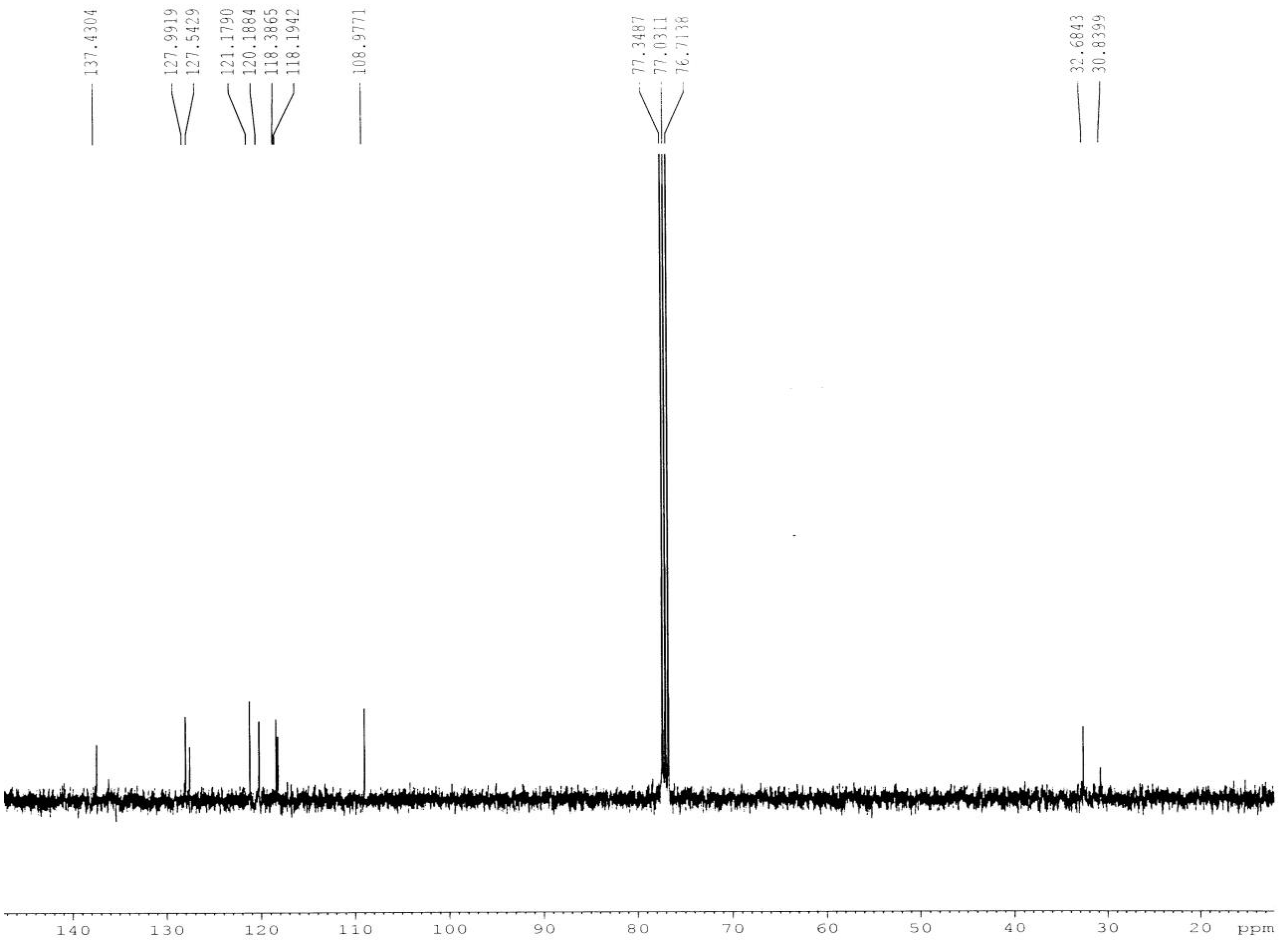
1 K. Singh, S. Sharma, A. Sharma, *J. Mol. Catal. A: Chem.*, 2011 (Accepted).

2 S. N. Lavrenov, Y. N. Luzikov, E. E. Bykov, M. I. Reznikova, E. V. Stepanova, V. A. Glazunova, Y. L. Volodina, V. V. Tatarsky Jr., A. A. Shtil, M. N. Preobrazhenskaya, *Bioorg. Med. Chem.*, 2010, **18**, 6905.

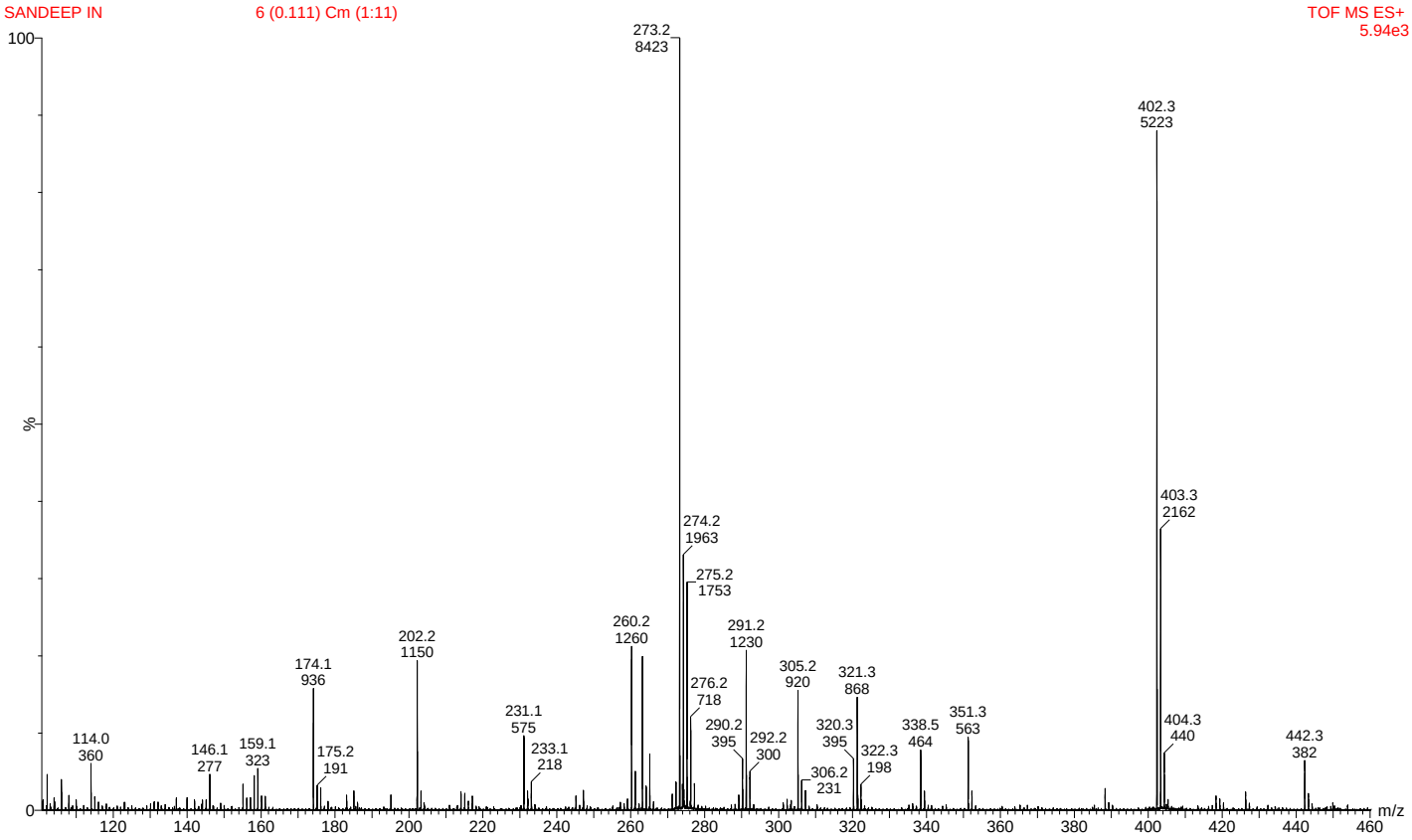
S4. Spectroscopic Analysis of 1.



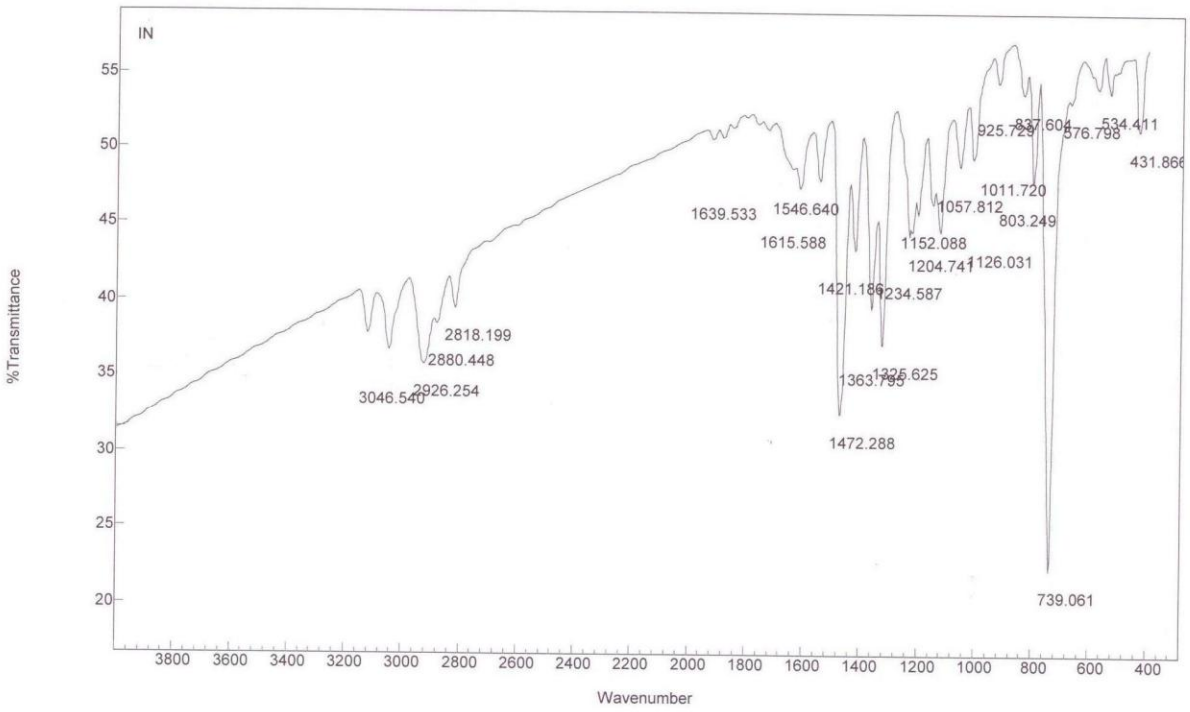
^{1}H NMR of **1**


137.4304
127.9919
127.5429
121.1790
120.1884
118.3865
118.1942
108.9771
77.3487
77.0311
76.7136
32.6843
30.8399
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

^{13}C NMR of **1**



EI Mass Spectrum of **1**



IR Spectrum of 1

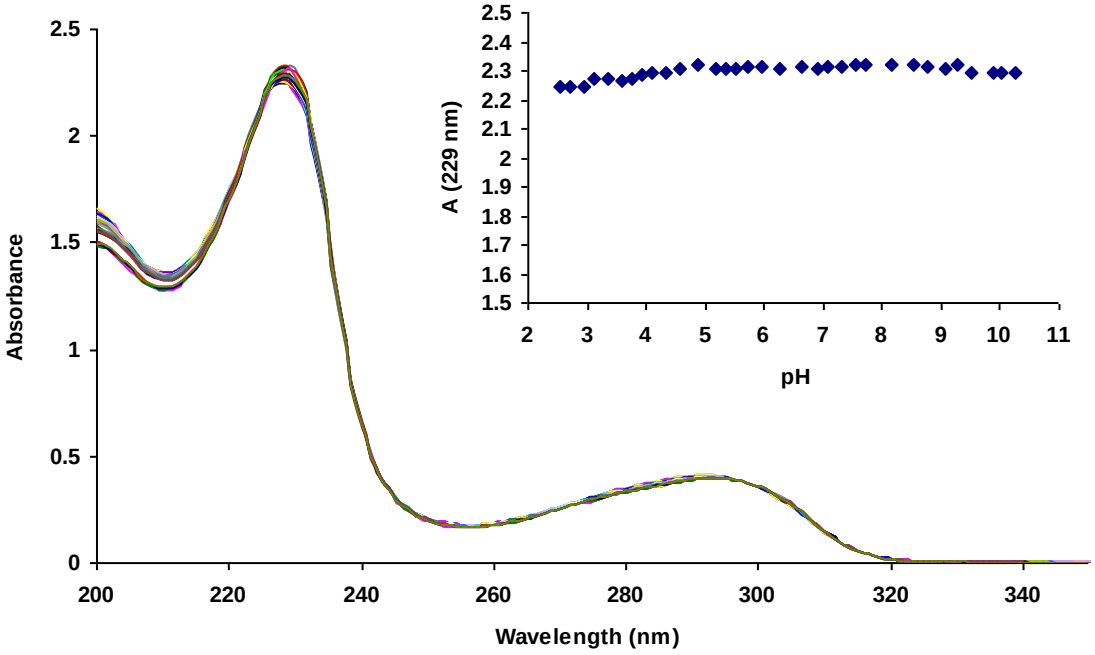


Fig. S5. Changes in the UV-vis spectrum of pH titration of **1** [3 x 10^{-5} M, in CH_3CN] with both HCl and $NaHCO_3$ (0.01 M). Inset: Changes in the UV-vis spectrum of pH titration of **1** at 229 nm.

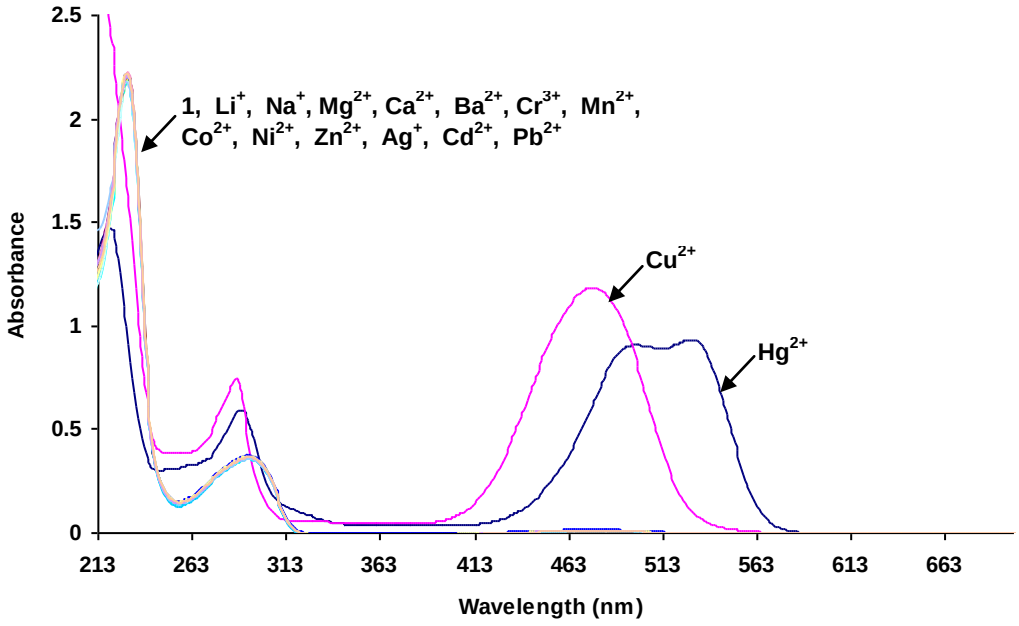


Fig. S6. Changes in UV-vis spectrum of **1** [3 x 10^{-5} M, in CH_3CN] after the addition of aqueous solution of Hg^{2+}, Cu^{2+} and other metal ions [8 x 10^{-5} M] recorded immediately after mixing.

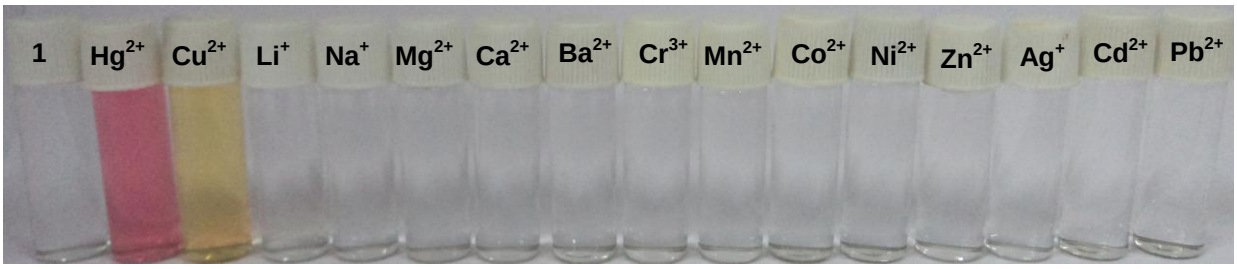


Fig. S7. Observed color changes of solutions of **1** [3 x 10^{-5} M, in CH_3CN] in the presence of aqueous solution of Hg^{2+}, Cu^{2+} and other metal ions [8 x 10^{-5} M].

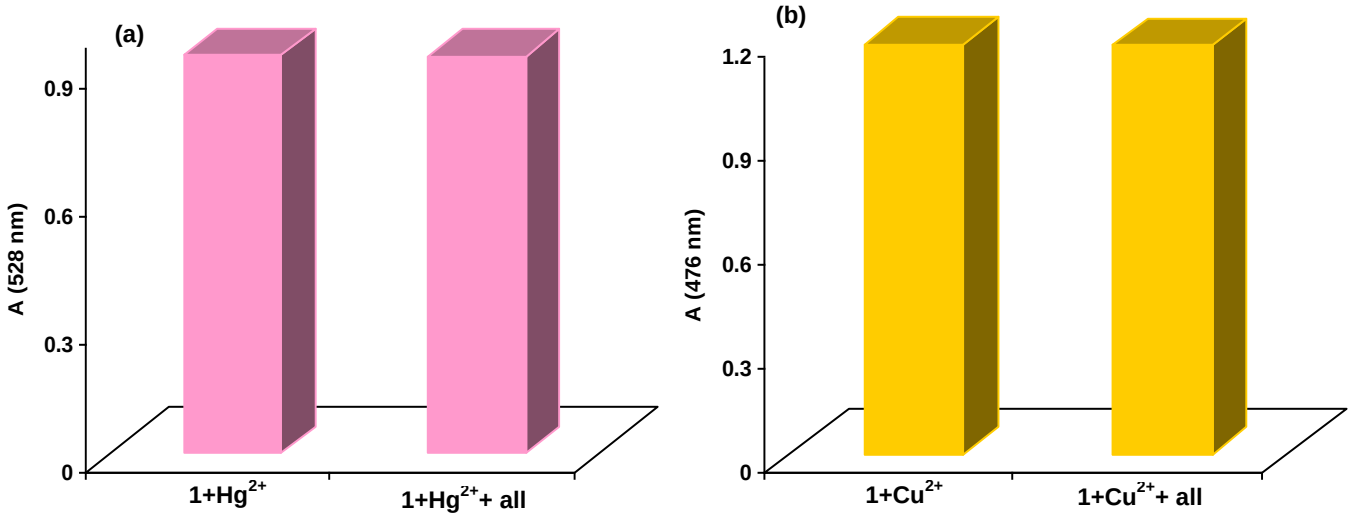


Fig. S8. Changes in absorbance of **(a) 1**+Hg^{2+} [3 x 10^{-5} M + 7.42 x 10^{-6} M] **(b) 1**+Cu^{2+} [3 x 10^{-5} M + 1.31 x 10^{-5} M] in the presence of interfering metal ions. The term 'all' include Li^{+}, Na^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+} and Pb^{2+} [4 x 10^{-5} M].

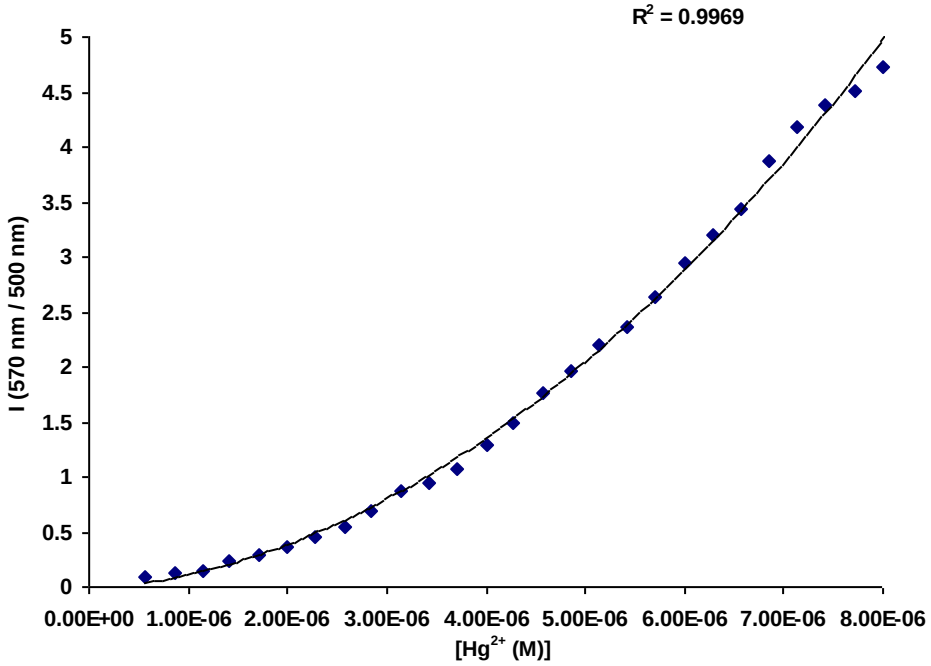


Fig. S9. The ratiometric calibration curve I (570 nm/500 nm) as function of Hg^{2+} ions concentration.

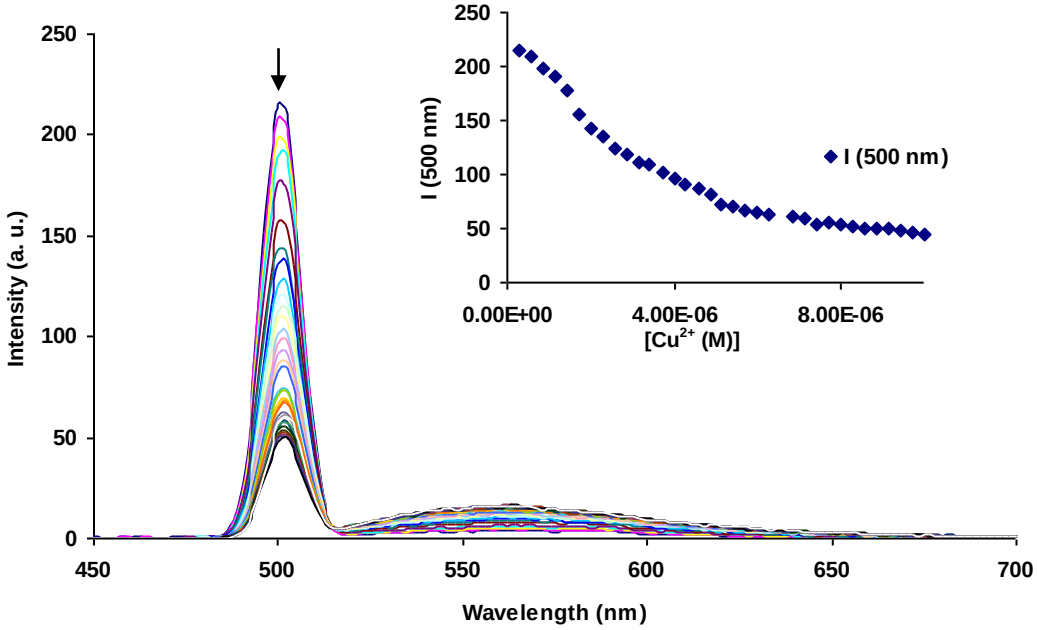


Fig. S10. Changes in the fluorescence spectrum of **1** [3 x 10^{-5} M in CH_3CN] upon titration with aqueous solution of Cu^{2+} (2.85 x 10^{-7} to 1.14 x 10^{-5} M). Inset: The response of intensity at 500 to the increasing concentration of Cu^{2+}.

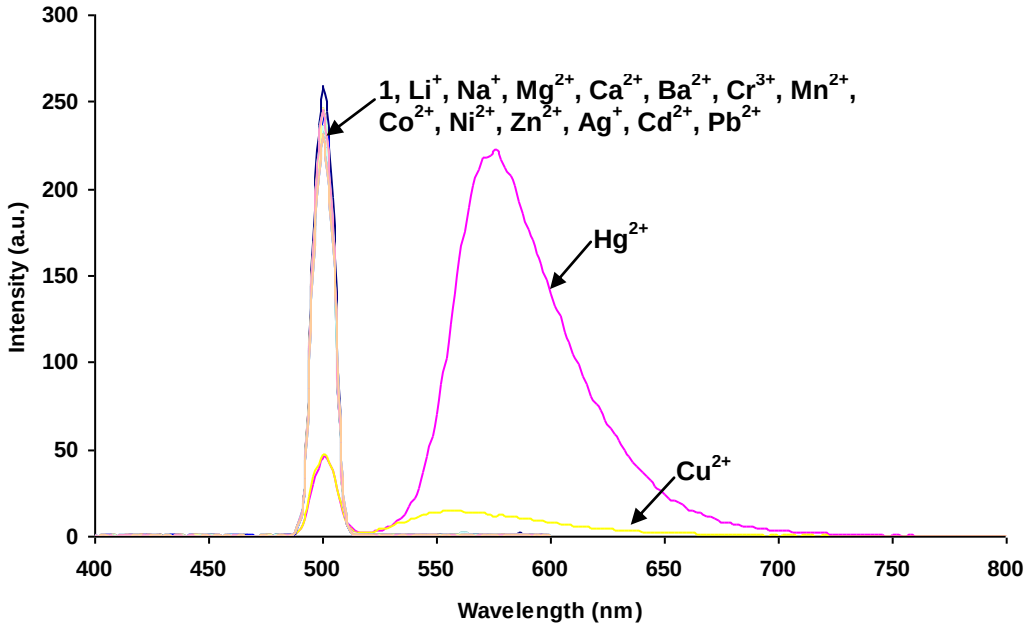


Fig. S11. Changes in the fluorescence spectrum of **1** [3 x 10^{-5} M in CH_3CN] after the addition of aqueous solution of Hg^{2+}, Cu^{2+} and other metal ions (8 x 10^{-5} M) recorded immediately after mixing.

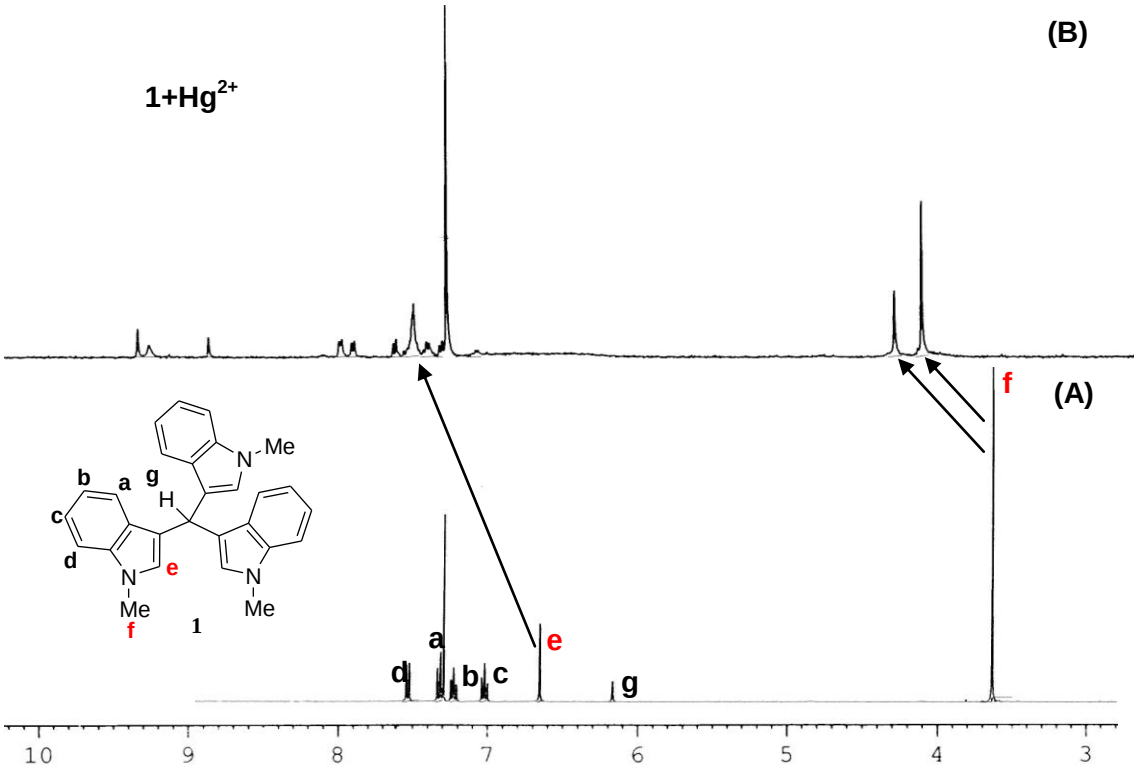


Fig. S12. ^{1}H NMR spectrum of **1** (**A**) before and (**B**) after addition of Hg^{2+} ions.

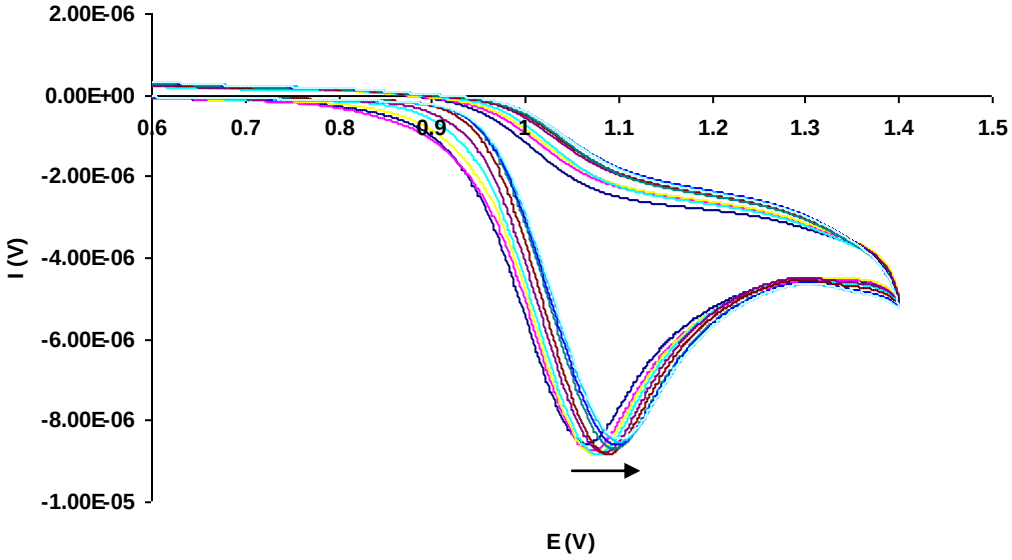


Fig. S13. Cyclic Voltammetry of **1** [3 x 10^{-5} M in CH_3CN] upon titration with aqueous solution of Hg^{2+} (2.5 x 10^{-8} to 2.25 x 10^{-7} M).

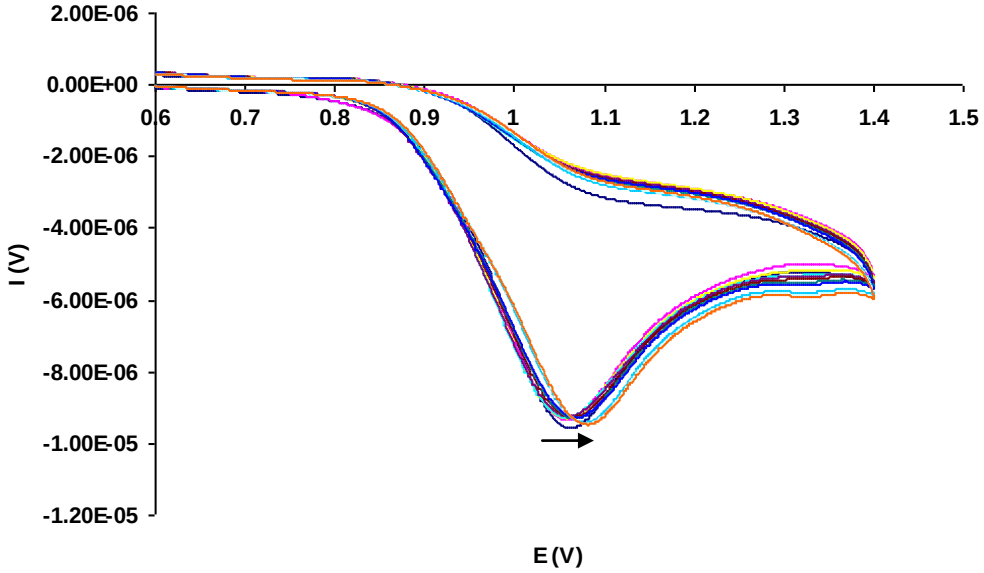


Fig. S14. Cyclic Voltammetry of **1** [3 x 10^{-5} M in CH_3CN] upon titration with aqueous solution of Cu^{2+} (2.5 x 10^{-8} to 2.75 x 10^{-7} M).

S15. Preparation of HeLa Cells Culture

HeLa Cells were cultured in Minimum Essential Medium Eagle (Sigma) supplemented with 10% fetal bovine serum and 1% penicillin solution. One day before imaging, cells were trypsinized and plated on glass cover slips. The imaging experiments of live cells were performed with a laser scanning confocal microscope (Olympus Fluorview, FV 1000) with excitation at 488 nm and dual emission at 571±20 and 590±20 nm. The cells were visualized with a 60X/1.4NA oil objective lens. The data were collected with two photomultiplier tubes.