Development of a fused imidazo[1,2-a]pyridine based fluorescent probe for Fe^{3+} and Hg^{2+} in aqueous media and HeLa cells

Suman Srivastava,*[a] Neha Thakur,[b] Ashutosh Singh,[d] Poonam Shukla,[c] Vipin Kumar Maikhuri,[c] Neha Garg,[d] Ashok Prasad[c] and Rampal Pandey*[b]

[a]Department of Applied Sciences, National Institute of Technology Delhi, India
[b]Department of Chemistry, National Institute of Technology Uttarakhand, India
[c]Department of Chemistry, University of Delhi, India
[d]Indian Institute of Technology, Mandi

EXPERIMENTAL SECTION

General methods: All solvents, reagent, were commercially available from Sigma-Aldrich and used without further purification. The metal nitrates like $NaNO_3$, $Ca(NO_3)_2{\cdot}4H_2O$, KNO_3, $Mg(NO_3)_2{\cdot}6H_2O$, $Mn(NO_3)_2{\cdot}4H_2O$, $Fe(NO_3)_2.9H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $AgNO_3$, $Pb(NO_3)_2$ and $Hg(NO_3)_2{\cdot}H_2O$ were used as a source of metal ions which were also obtained from Sigma Aldrich. Silica gel (60-120) was used for column chromatography. ^1H and ^{13}C NMR spectra were recorded on Jeol alpha-400 MHz spectrometer at 400 MHz and 100.6 MHz respectively by using TMS as internal standard in $CDCl_3$. The mass spectrometric identification of the products has been carried out by an Agilent G6530AA LC Q-TOF mass spectrometer. Melting points were detected with a Stuart SMP30 Melting Point Measurer (Designed in UK) without correction. Electronic absorption spectra were procured from Perkin Elmer double beam UV/Vis Spectrophotometer: 2201. Fluorescence spectra were recorded on Fluorescence spectrophotometer RF-5301. The cervical cell line HeLa was procured from National Centre for Cell Sciences, Pune, India and was maintained in DMEM supplemented with 10% fetal bovine serum and 1% Antibiotic-Antimycotic. The cells were grown in a humid environment with 5% CO_2.

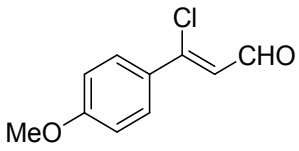
General procedure for one-pot synthesis of 3-chloro-3-(4-methoxyphenyl) acrylaldehyde (2):[i] Phosphorus oxychloride (50 mL) was added drop wise to DMF (200 mL) at 0°C. The mixture was allowed to warm to room temperature, stirred for 30 min, and cooled to 0°C, and a solution of **1** (25g, 167mmol) in DMF (50 mL) was added in 15 min. After addition, the mixture was heated at 50°C until TLC indicated complete consumption of the starting material. The reaction mixture was poured into a 20% sodium acetate solution (1L), allowing the mixture to

heat to 60°C. After cooling overnight, the crude intermediate product was filtered from the solution. The Yellow solid product (90% yields) was used for the next reaction without further purification. A small sample was crystallized from ethanol for NMR analysis.

General procedure for one-pot synthesis of 3-(4-methoxyphenyl)-5-phenylpent-2-en-4-ynal (4):[ii] A mixture of **2** (0.25 mmol), phenyl acetylene (0.26 mmol), $Pd(PPh_3)Cl_2$ (4mol%) CH_3CN (4 mL) and Triethylamine (0.5 mmol) was stirred under N_2 at 80˚ C for 4h (as monitored by TLC). The reaction mixture was concentrated in vacuo and residue was purified by column chromatography on silica gel using EtOAc/hexane as eluent.

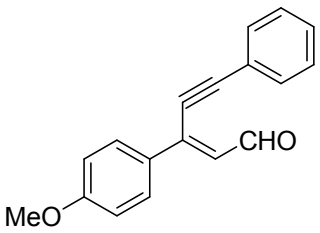
General procedure for one-pot synthesis of fused Imidazo[1,2-a]pyridines (5). A solution of 3-(4-methoxyphenyl)-5-phenylpent-2-en-4-ynal **3** (1 mmol), 1,2-phenylenediamine **4** (1 mmol), acetic acid (0.2 ml) in DMF (100 ml) was heated to 120 °C for 12h. The reaction was monitored by TLC. After the reaction was completed, water was added to the reaction mixture and extracted with ethyl acetate. The combined organic solution was washed with the saturated NaCl solution, dried over anhydrous Na_2SO_4, and concentrated at the reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with Hexane:Ethyl acetate (8:1) to give compound as pale yellow needles. The spectral characterization of the compounds is described below.

3-Chloro-3-(4-methoxphenyl)acrylaldehyde (2):



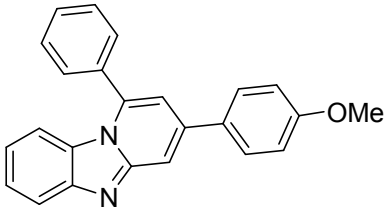
^{1}H NMR (400 MHz, $CDCl_3$): 3.85 (s, 3H), 6.60 (d, *J* = 6.9 Hz, 1H), 6.94 (d, *J* = 9.0 Hz, 2H), 7.71 (d, *J* = 9.0 Hz, 2H), 10.17 (d, *J* = 6.9 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 55.68, 114.36, 122.58, 127.73, 129.12, 152.30, 162.78, 191.72.

3-(4-methoxyphenyl)-5-phenylpent-2-en-4-ynal (4):



^{1}H NMR (400 MHz, $CDCl_3$): 3.86 (s, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 1H), 7.45-7.38 (m, 3H), 7.60-7.58 (m, 2H), 7.82 (d, *J* = 8.8 Hz, 1H), 10.37 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 55.61, 84.40, 101.94, 114.41, 121.83, 127.98, 128.77, 129.03, 129.15, 129.89, 132.06, 142.13, 162.27, 193.33.

3-(4-Methoxyphenyl)-1-phenylbenzo[4,5]imidazo[1,2-a]pyridine (5)



Pale yellow needles; mp 204-208 °C.^{1}H NMR (400 MHz, $CDCl_3$): 3.87 (s, 3H), 6.56 (d, J = 8.5 Hz, 1H), 6.97-6.93 (m, 2H), 7.02 (d, J = 8.8 Hz, 2H), 7.39 (t, J = 7.7 Hz, 1H), 7.70-7.59 (m, 7H), 7.89-7.85 (m, 2H). ^{13}C NMR (100.6 MHz, $CDCl_3$): 160.54, 141.55, 141.16, 134.63, 130.55, 130.26, 129.28, 128.33, 125.32, 120.41, 119.46, 114.72, 114.61, 112.10, 112.00, 55.57. HRMS (EI): m/z = 351.1496

Copy of ^{1}H and ^{13}C spectra of 2, 4 and 5:

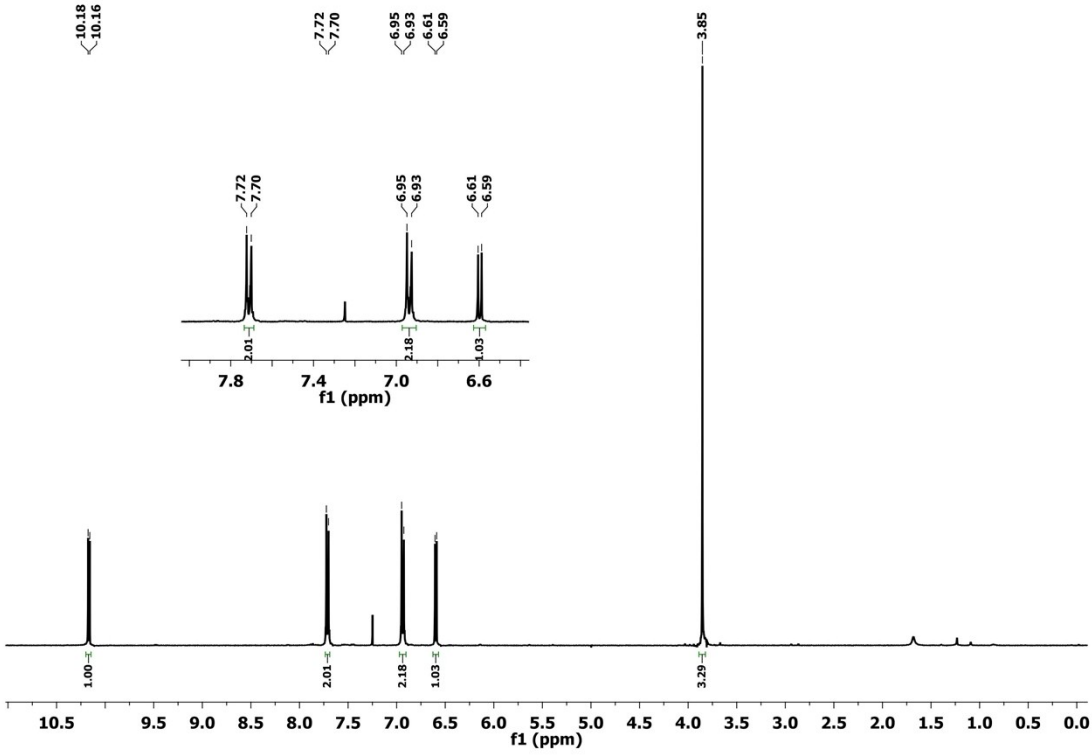


Fig S1. ^{1}H NMR spectra of 2

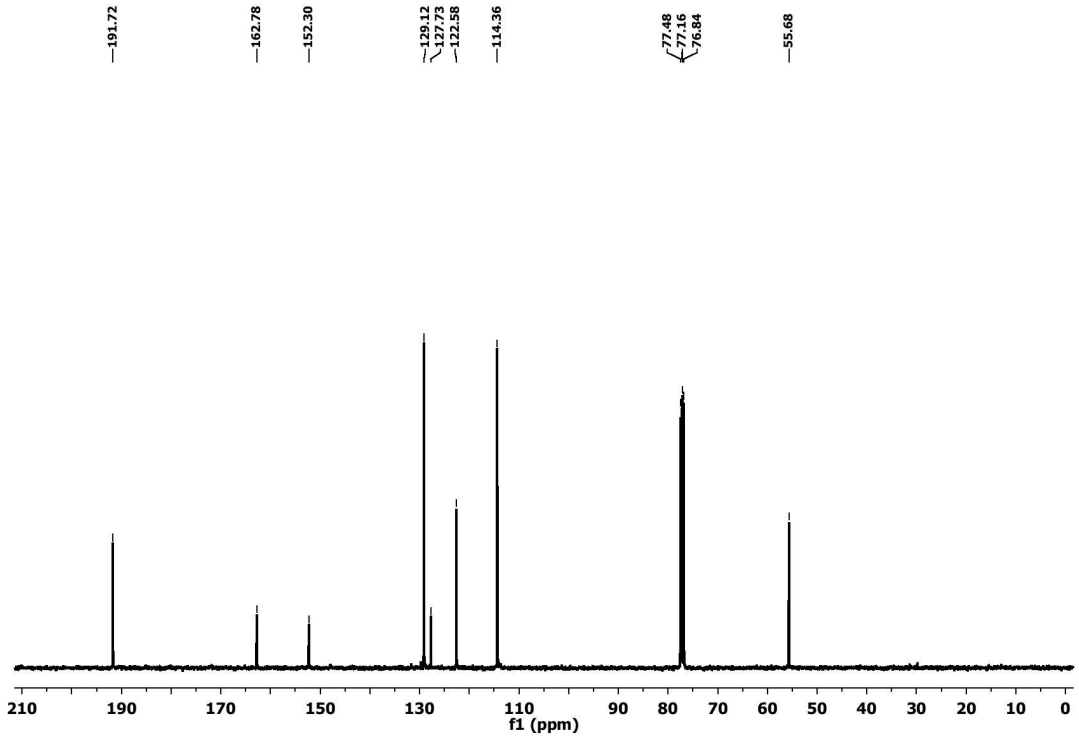


Fig S2. ^{13}C NMR spectra of 2

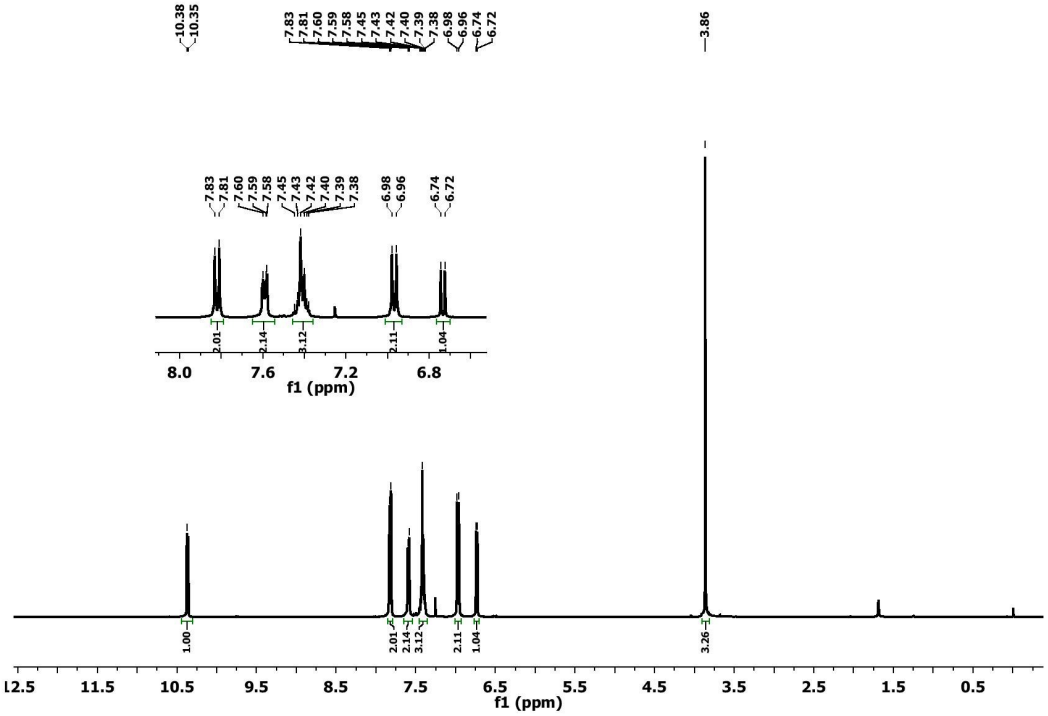


Fig S3. ^{1}H NMR spectra of 4

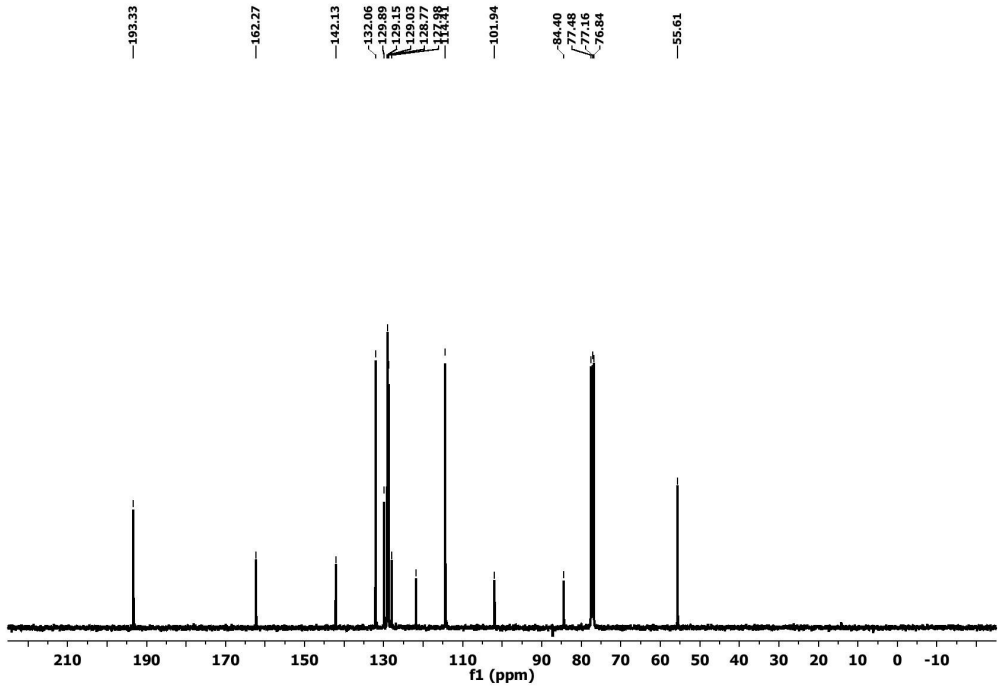


Fig S4. ^{13}C NMR spectra of 4

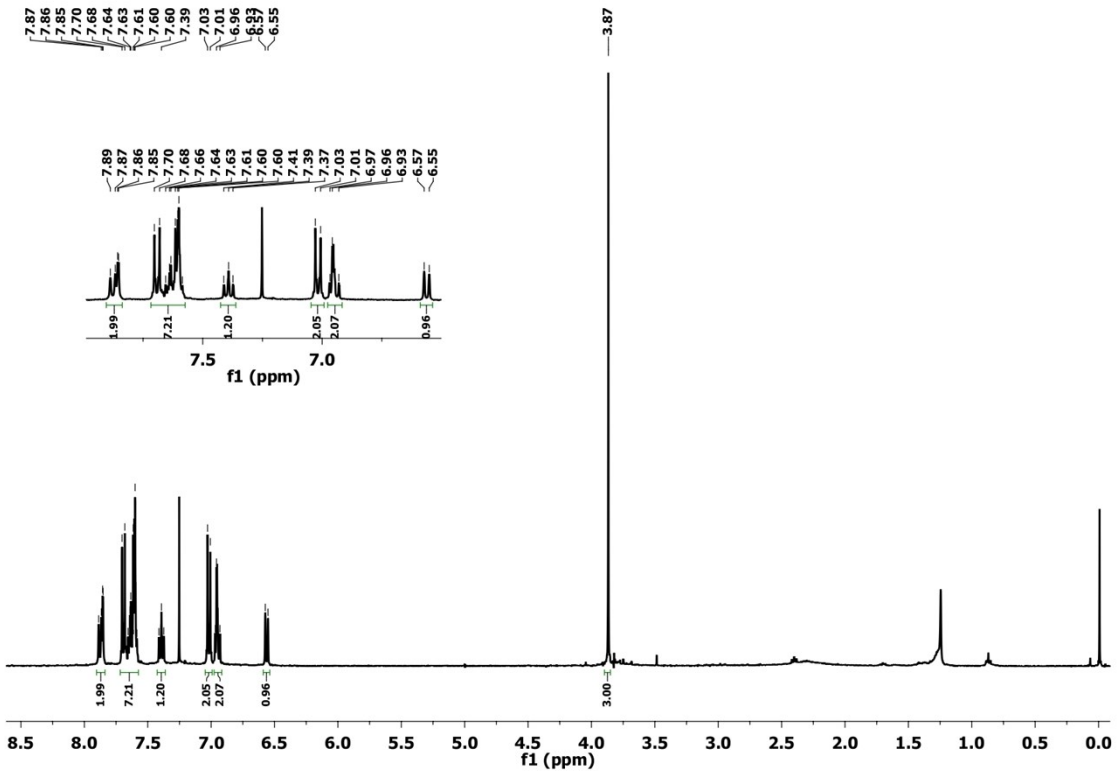


Fig S5. ^{1}H NMR spectra of 5

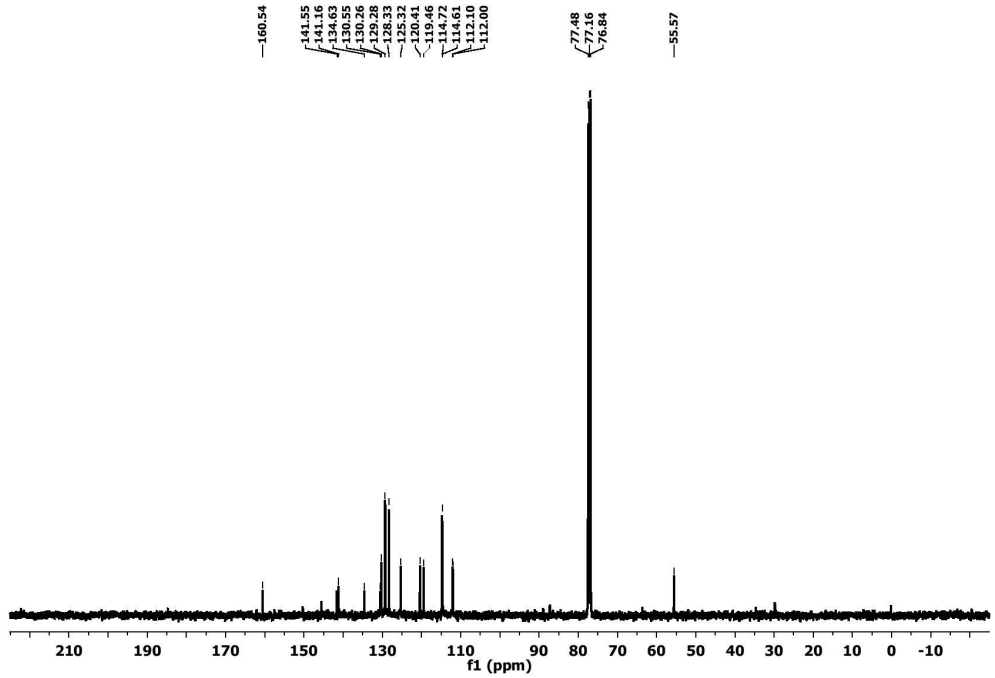


Fig S6. ^{13}**C NMR spectra of 5**

UV-Vis and fluorescence Studies. To perform the absorption and emission spectral studies 10 μM stock solution of **1** was prepared in H_2O/EtOH (8: 2, v/v) media. Solutions of metal ions namely Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^+ and Pb^{2+} of concentration 100 mM (for individual addition) and 10 mM (for titration experiments) were prepared from their nitrate salts in double distilled water and their stock solution used as a source of metal ions. A 2.5 mL solution of **5** was taken in a quartz cell with 1 cm optical path length. The fluorescence experiments at rt were recorded with excitation (λ_{ex}) of **5** at 480 nm. In a typical titration, metal ions in fixed fractions were gradually added with the help of micropipette to a solution of **5** followed by thorough mixing.

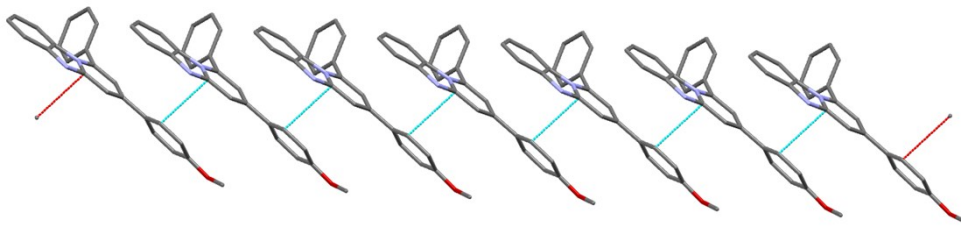


Fig. S7 1D chain formation in **5** through edge-to-edge π···π stacking interaction.

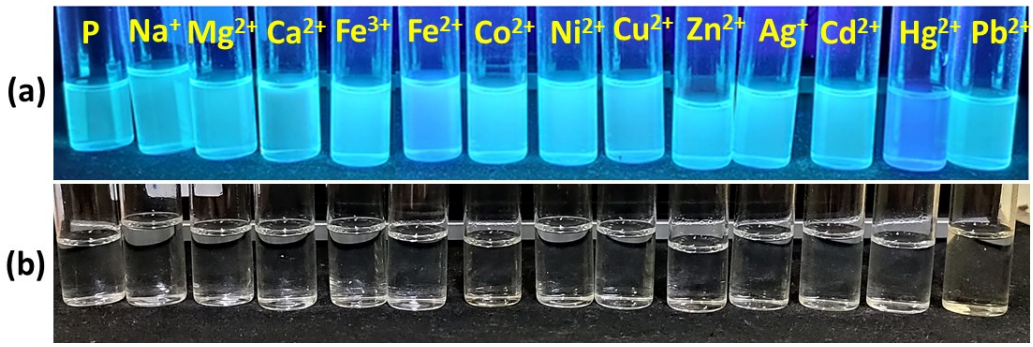
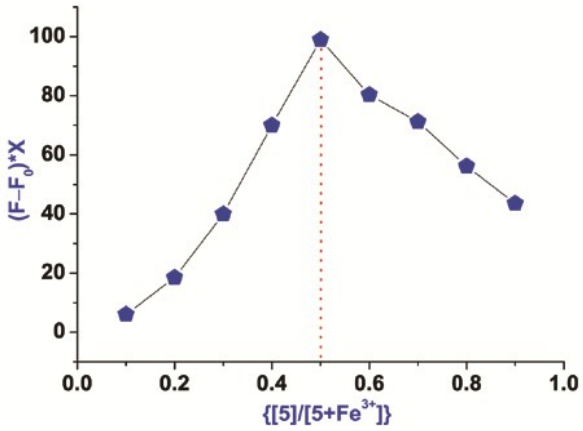
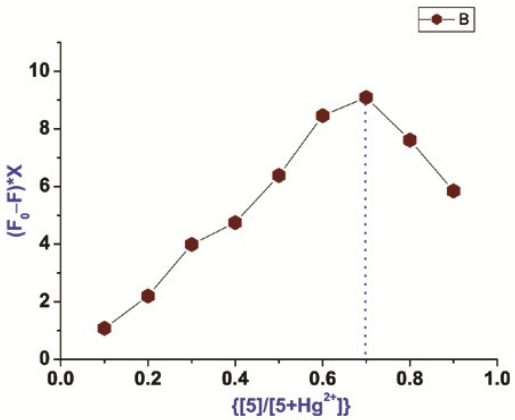


Fig. S8 Images of **5** (10 μM) under UV-light (a) and natural light (b) in presence of various cations.



(a)



(b)

Fig. S9 Job's plot of **5** with Fe^{3+} (a) and Hg^{2+} (b).

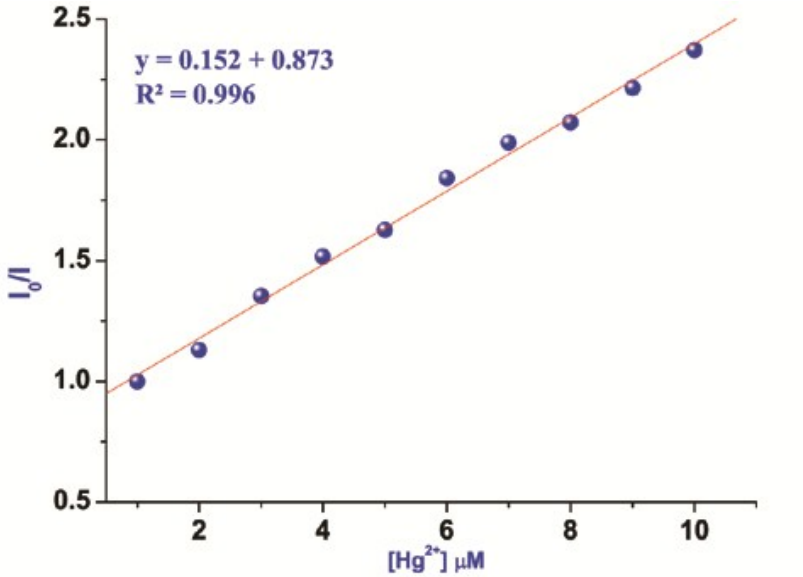
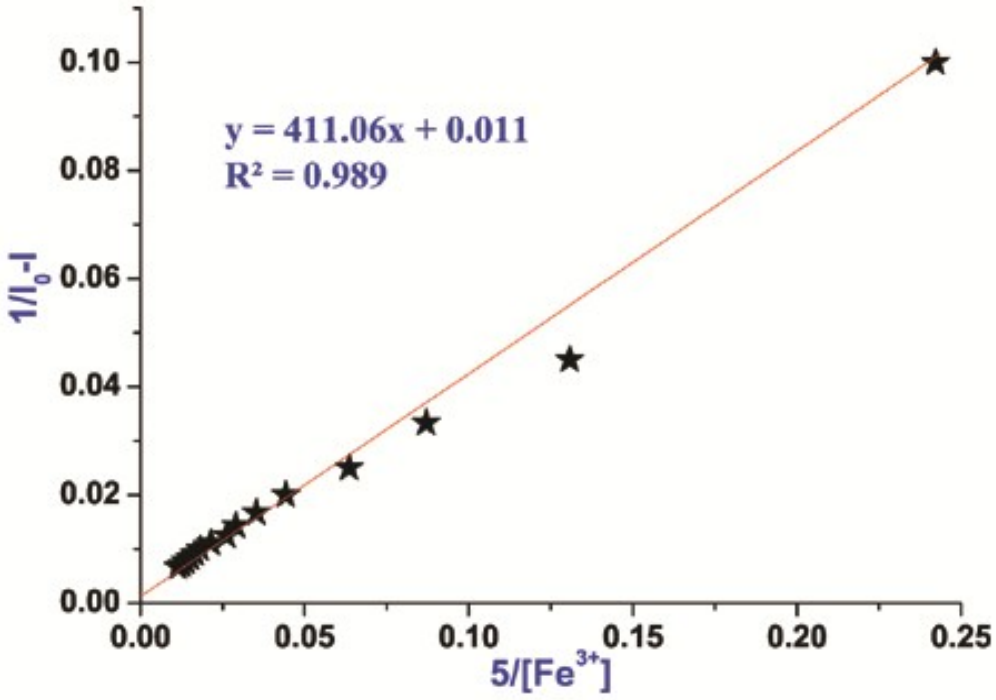
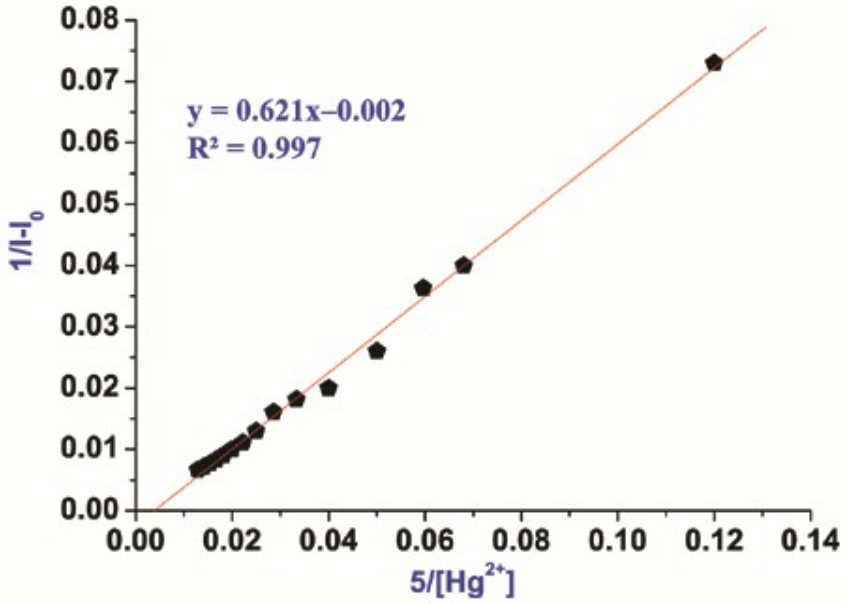


Fig. S10 Stern-volmer plot for quenching in **5** upon interaction with Hg^{2+}.

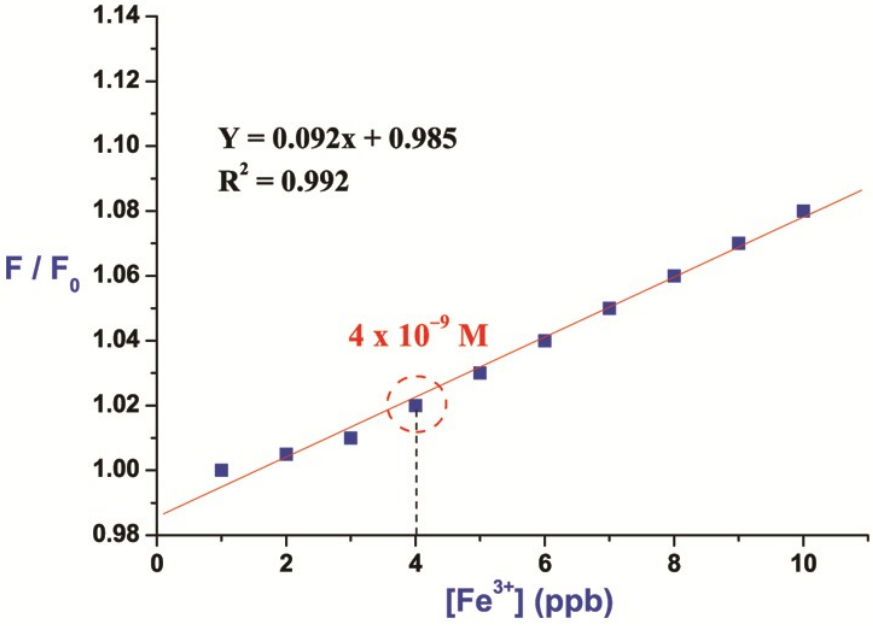


(a)

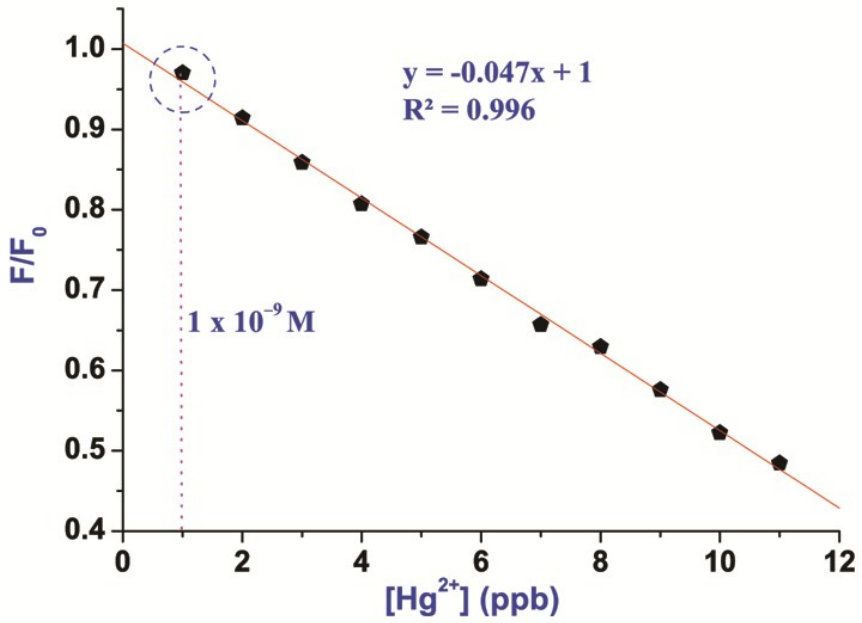


(b)

Fig. S11 Benesi-Hildebrand plots for **5** + Fe^{3+} upon interaction with Hg^{2+}.

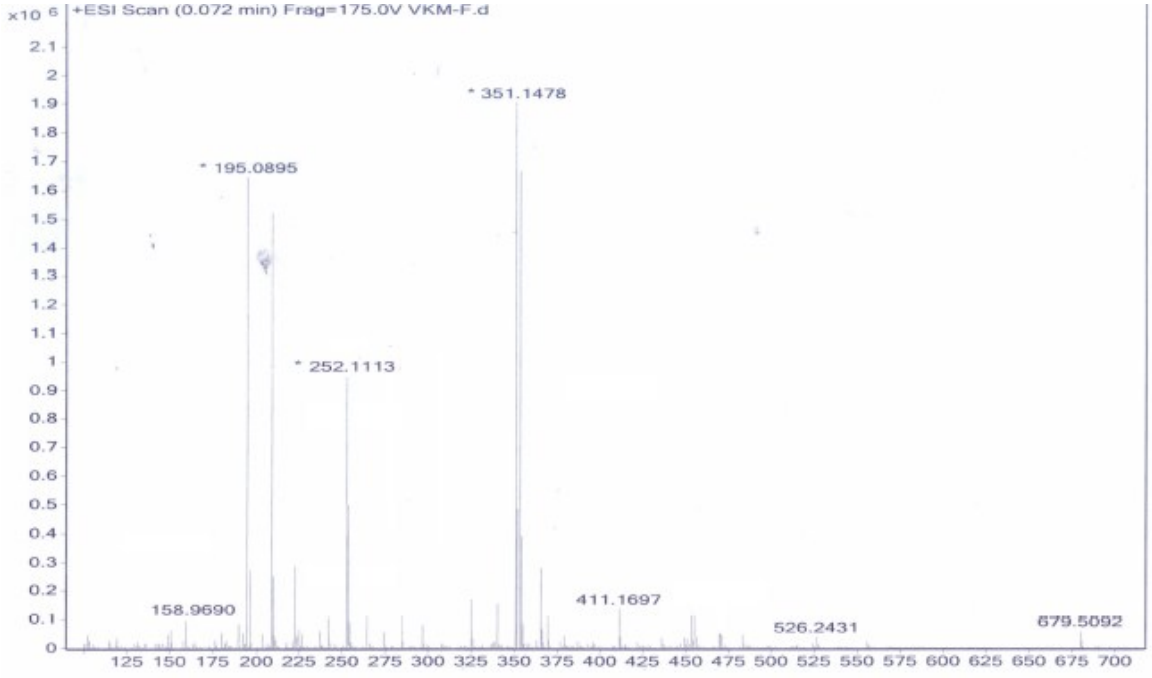


(a)

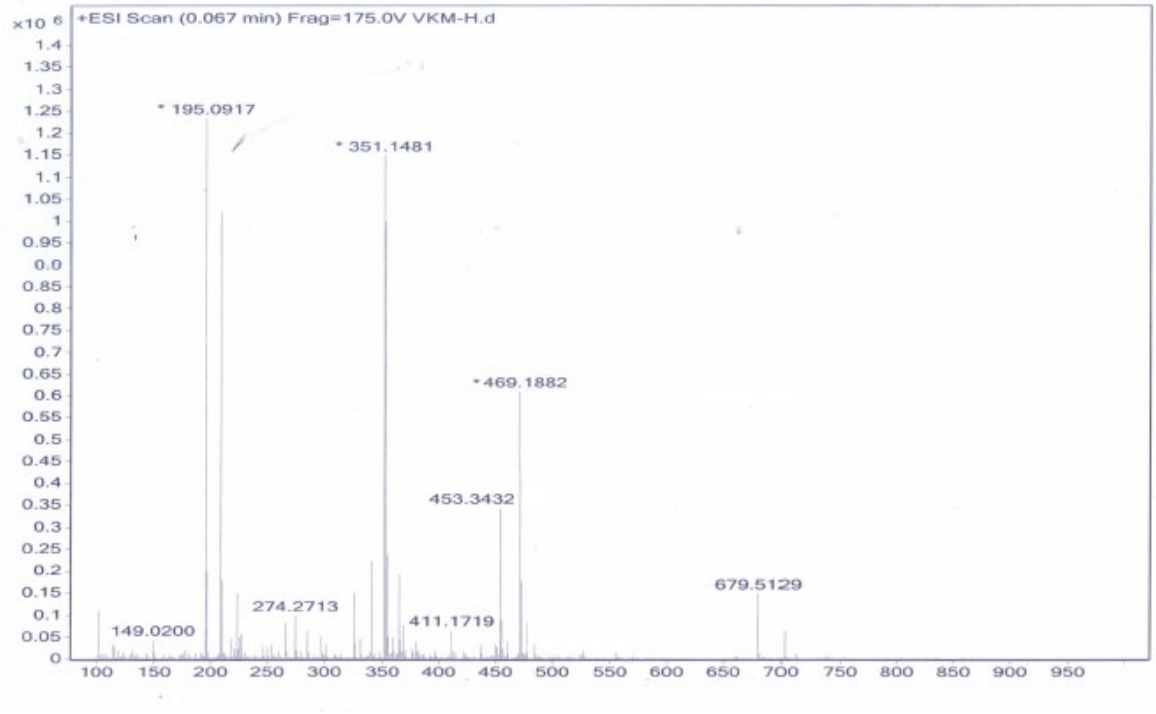


(b)

Fig. S12 Linearity plots for LODs of **5** with (a) Fe^{3+} and (b) Hg^{2+}.



(a)



(b)

Fig. S13 ESI-Mass spectra of (a) **5** + Fe^{3+} and (b) **5** + Hg^{2+}.

Confocal microscopy: **Cell Culture**: The cervical cell line HeLa was maintained in DMEM supplemented with 10% fetal bovine serum and 1% Antibiotic-Antimycotic. The cells were grown in a humid environment with 5% CO_2. The cells were seeded over a coverslip and allowed to adhere for overnight. The cells were then treated with different concentration of Ferric chloride and Mercuric chloride (5uM, 10uM and 30uM) for 10 mins. There after the cells were washed twice with Phosphate buffer saline and fixed with 4% formaldehyde, The cells were later incubated with 2μM of sample MB for 20 mins and then mounted over a glass slide and imaged under Nikon Eclipse Ti-U inverted microscope.

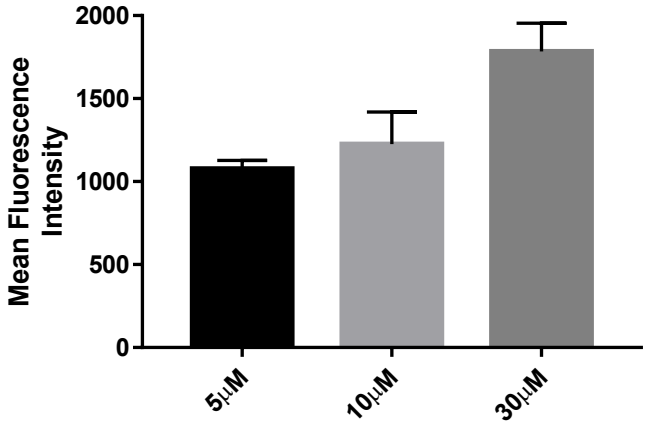


Fig. S14 Mean Fluorescence intensity plot of HeLa cells after incubation with different concentration of Fe^{3+}.

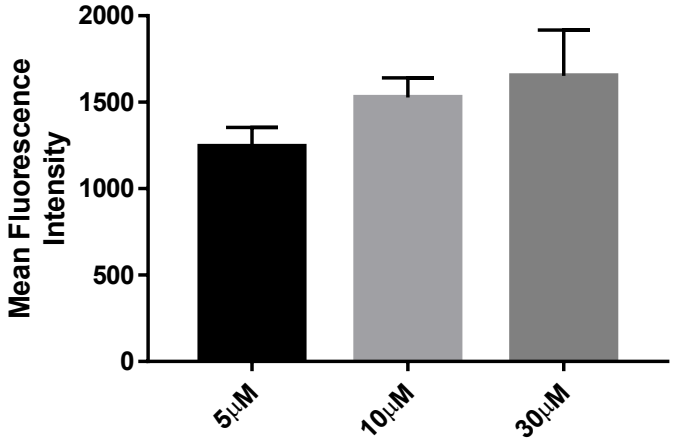


Fig. S15 Mean Fluorescence intensity plot of HeLa cells after incubation with different concentration of Hg^{2+}.

---

[i] Paul H. J. Kouwer,† Wolter F. Jager, Wim J. Mijs, and Stephen J. Picken Macromolecules 2002, **35**, 4322-4329

[ii] Atish Chandra, Bhawana Singh, Shraddha Upadhyay, Radhey M. Singh, Tetrahedron 2008, **64**, 11680–11685