Supporting Information

Naphthylhydrazone based selective and sensitive chemosensors for Cu^{2+} and their application in bioimaging

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Experimental section

Materials and measurements

1-hydroxynaphthalene-2-hydrazone was synthesized by modified literature procedure.[15] All the materials for synthesis of **R**^{1} and **R**^{2} were purchased from various commercial sources and used without further purification. Spectroscopic grade CH_3CN solvent was used for all titration experiments. ^{1}H and ^{13}C NMR spectra were taken on a Bruker 400 MHz spectrometer. The chemical shifts (δ) in ^{1}H NMR spectra are reported in parts per million (ppm) relative to tetramethylsilane (Me_4Si) as internal standard (0.0 ppm) or proton resonance resulting from incomplete deuteration of the NMR solvent: d_6-DMSO. IR spectra were recorded on a Bruker ALPHA FT-IR spectrometer. HRMS was determined on a Q-TOF micromass spectrometer. Electronic absorption spectral measurements were performed using Perkin-Elmer LAMBDA 750 UV-visible spectrophotometer and fluorescence emission studies were carried out on HORIBA JOBIN YVON Fluoromax-4 spectrometer.

Synthesis of receptor R^1:

15 mL ethanolic solution of Tris(4-formylphenyl)amine (50 mg, 0.15 mmol) was added slowly to 1-hydroxynaphthalene-2-hydrazide (92.1 mg, 0.45 mmol) in ethanol and the mixture was refluxed for 6 h with continuous stirring. An orange solid was formed which was collected by filtration and washed several times with cold ethanol. Finally, obtained orange solid was recrystallized in dicholoromethane. Isolated yield = 102 mg (76%). Anal. Calcd for $C_{54}H_{39}N_7O_6$: C, 73.54; H, 4.46; N, 11.12; Found: C, 73.91; H, 4.58; N, 11.41. IR (neat): υ = 3207s (-OH), 3050s (-NH), 1654s (-C=O), 1587s (-C=N). ^{1}H NMR (DMSO-d^6, 400 MHz): δ (ppm); 11.98 (s, 3H, phenolic-OH), 11.37 (s, 3H, NH), 9.91 (s, 3H, CH=N), 8.46 (d, 6H, *J* = 10.0 Hz, H-phenyl), 7.92 (d, 3H, *J* = 8.4 Hz, H- naphthalene), 7.76 (d, 6H, *J* = 8.4 Hz, H- phenyl), 7.69 (d, 3H, *J* = 6.2 Hz, H- naphthalene), 7.37 (dd, 3H, 6.6 Hz, H-naphthalene), 7.33 (dd, 3H, 6.2 Hz, H-naphthalene), 7.22 (d, 3H, *J* = 6.4 Hz, H- naphthalene), 7.19 (d, 3H, *J* = 6.2 Hz, H-naphthalene).^{13}C NMR (DMSO-d^6, 100 MHz) δ (ppm); 109.3, 119.7, 122.6, 124.6, 124.8, 125.3, 128.7, 128.9, 129.8, 132.3, 133.1, 133.2, 135.7, 151.7, 160.9, 162.1. HRMS: m/z calcd for [$\mathbf{R^1}$ + H]$^+$ 881.93, found = 882.12.

Synthesis of receptor R^2:

The receptor $\mathbf{R^2}$ has been synthesized by following the procedure of synthesis of receptor $\mathbf{R^1}$ using 1-hydroxynaphthalene-2-hydrazone (85 mg, 0.45 mmol) instead of using 1-hydroxynaphthalene-2-hydrazide. Isolated yield = 92 mg (73%). Anal. Calcd for $C_{54}H_{39}N_7O_3$: C, 77.77; H, 4.71; N, 11.76; Found: C, 78.01; H, 4.82; N, 11.86. IR (neat): υ = 3335s (-OH), 1592s (-C=N). ^{1}H NMR (DMSO-d^6, 400 MHz): δ (ppm); 12.89 (s, 3H, phenolic-OH), 9.99 (s, 3H, CH=N), 9.93 (s, 3H, CH=N), 8.64 (d, 6H, *J* = 8.8 Hz, H-phenyl), 8.04 (d, 6H, *J* = 9.2 Hz, H-phenyl), 7.93 (d, 3H, *J* = 6.8 Hz, H- naphthalene), 7.89 (d, 3H, *J* = 2.2 Hz, H- naphthalene), 7.63

(dd, 3H, 6.8 Hz, H-naphthalene), 7.45 (dd, 3H, 6.2 Hz, H- naphthalene), 7.29 (d, 3H, *J* = 6.8 Hz, H-naphthalene), 7.27 (d, 3H, *J* = 6.4 Hz, H-naphthalene). ^{13}C NMR (DMSO-d^6, 100 MHz) δ (ppm); 111.5, 121.1, 124.7, 125.1, 126.7, 127.7, 129.2, 129.6, 129.7, 130.3, 131.1, 136.7, 148.8, 154.9, 164.6. HRMS: m/z calcd for [**R^2**+ H]$^+$ 833.93, found = 833.24.

Procedures for sensing studies:

UV-vis and fluorescence titrations were performed on 1 μM solutions of receptors **R^1** and **R^2** in H_2O:CH_3CN (7:3, v/v) mixture, respectively. Typically, aliquots of freshly prepared metal chloride (Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}) standard solutions (1 ×10^{-3} M) in H_2O were added to record the UV-vis and fluorescence spectra. In titration experiments, each time a 2 mL solution of **R^1** / **R^2** was filled in a quartz cuvette (path length, 1 cm) and metal ions were added into the quartz cuvette by using a micro-pipette. Their corresponding fluorescence spectra were recorded at 298 K. Each titration was repeated at least two times to get consistent value. λ_{exc} were 470 (**R^1**) and 430 nm (**R^2**); and the emission wavelength was monitored from 400–700 nm. Both excitation and emission slit widths were 5 nm. There was no considerable change in shape of the emission spectra except a significant quenching of the initial fluorescence intensity of **R^1** and **R^2** upon the gradual addition of metal cations solution. Analysis of the normalized fluorescence intensity (I_0/I) as a function of increasing guest concentration ([G]) was well described by the Stern-Volmer equation $I_0/I = 1 + K_{SV}[G]$. The K_{SV} values of **R^1** and **R^2** were calculated from the slope of the Stern-Volmer plot.

Cell culture and fluorescence imaging

Human cervical cancer (HeLa) cancer cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. The cancer cells (5 × 10^9/L) were placed on untreated glass coverslips coated with 0.2% gelatin for at least 1 h at room temperature and grown to 90%

confluence in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FCS, glutamine (2 mM), penicillin (100 units/mL), and streptomycin (100 µg/mL). The medium was removed and replaced with DMEM containing 1% serum prior to receptors **R**^{1} and **R**^{2} treatment. PBS-CH_3CN (8:2, v/v) stock solutions (1 mM) of **R**^{1} and **R**^{2} were diluted to 100 µM with DMEM and added to the cells in DMEM containing 1% FCS by bath application for a final dye concentration of 10 µM. The cells were incubated with the receptors **R**^{1} and **R**^{2} for 0.5 h at 37 °C and under 5% CO_2, washed with serum-free DMEM (2 × 2 mL), and bathed in serum free DMEM (2 mL) before imaging. Experiments to asses Cu^{2+} uptake were performed in the same media supplemented with 10 µM $CuCl_2$ for 1 h. Fluorescent images were captured on ApoTome [ZEISS] Fluorescense microscope and analyzed using the Axio vision Rel 4.8 software. Excitation wavelengths of laser were 450 nm (**R**^{1}) and 405 nm (**R**^{2}), respectively, and fluorescence images are recorded over the range 460-545 nm (single channel).

Cytotoxicity Studies

HeLa cells were maintained in a humidified atmosphere containing 5% CO_2 at 37 ºC in DMEM medium supplemented with 100 units of penicillin, 100 µg /mL of streptomycin and 10% Fetal bovine serum. Briefly, HeLa cells with a density 1×10^4 cells per well were precultured in to 96–well microtiter plates for 48 h under 5% CO_2. The Cu^{2+} ion, receptors **R**^{1}, **R**^{2} and their Cu^{2+}complexes ([**R**^{1} + **Cu**$^{2+}$] and [**R**^{2} + **Cu**$^{2+}$]) (0-50 µM) were added in micro wells and then incubated in 5% CO_2 at 37 °C, for 1 day. Then each well was loaded with 10 µL MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg mL^{-1} in PBS pH = 7.4) for 4 h at 37 ºC. The insoluble formazan was dissolved in 100 µL of 4% DMSO and the cell viability was determined by measuring the absorbance of each well at 540 nm using BIORAD ELISA plate reader. All experiments were performed in triplicate and the percentage of cell

viability was calculated according to the following equation. The IC_{50} values were determined by nonlinear regression analysis using Origin 6.0 software.

$$\textbf{Inhibition rate (IR\%)} = \frac{\textbf{OD (control) - OD Drug treated cells}}{\textbf{OD (control)}} \textbf{ X 100\%} \quad \text{.....(1)}$$

Fluorescence staining of HeLa cells

After 24 h, IC_{50} concentrations of the Cu^{2+}, receptors **R^1**, **R^2**, **[R^1 + Cu^{2+}]** and **[R^2 + Cu^{2+}]** were treated and controlled cells were washed twice with 1X PBS buffer and stained with acridine orange/propidium iodide (AO/PI) (1 : 1, 10 µM) in a 4 well Labtek II chambered cells for 15 min and images observed under the microscope. The appropriate amount of binding buffer added should be sufficient to ensure that the slide must not dried out during fluorescence microscopic observation. The cells were examined by using Plan–Neofluar 20x lens and then photographed as described above.

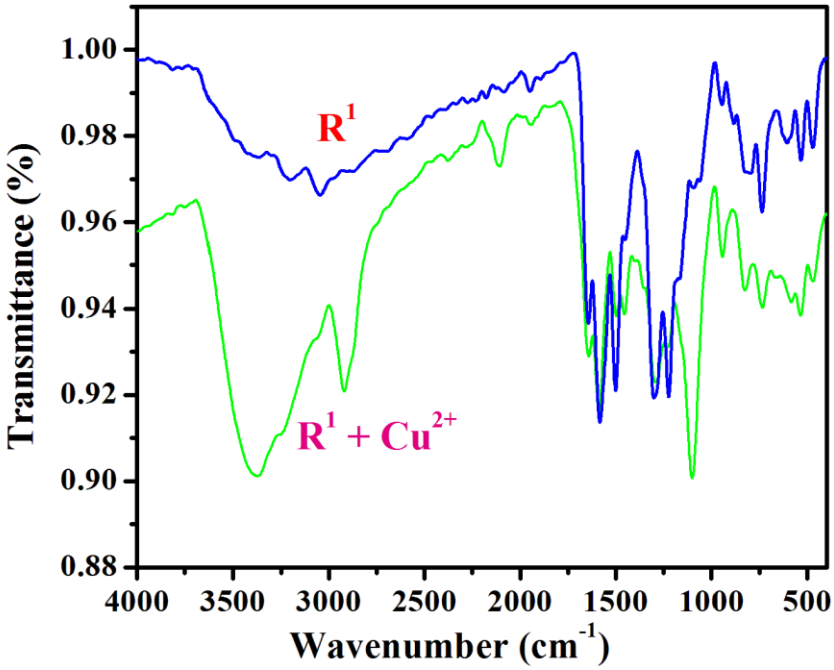

Fig. S1. Infrared spectra of receptors **R^1** and **R^1 + Cu^{2+}**.

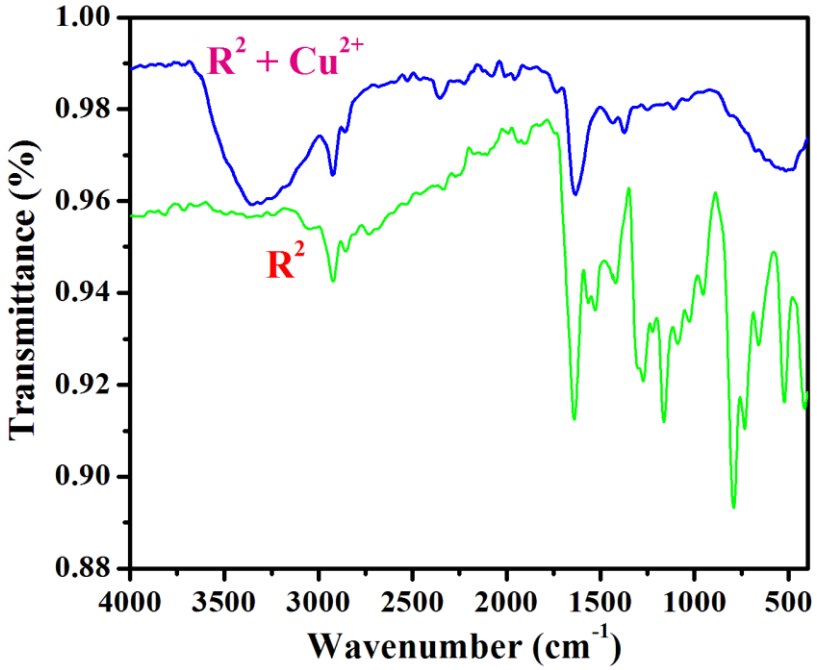

Fig. S2. Infrared spectra of receptors $\mathbf{R^2}$ and $\mathbf{R^2 + Cu^{2+}}$.

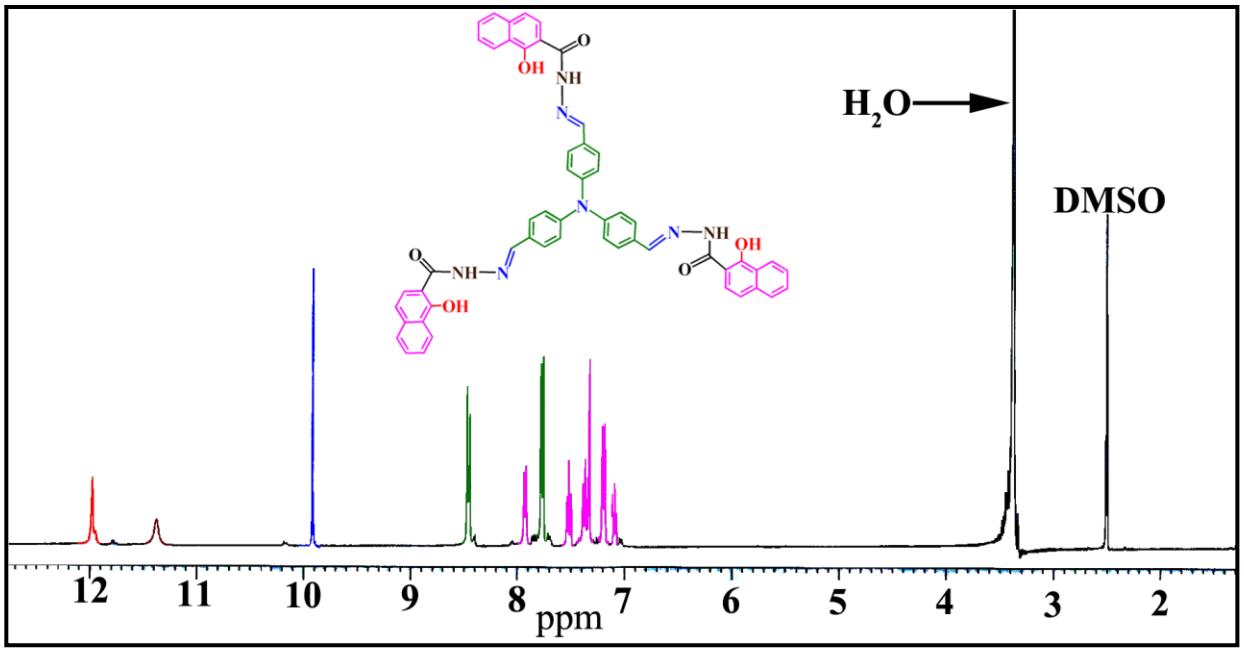

Fig. S3. Partial ^{1}H NMR spectrum of receptor $\mathbf{R^1}$ recorded in d_6-DMSO with peak assignment.

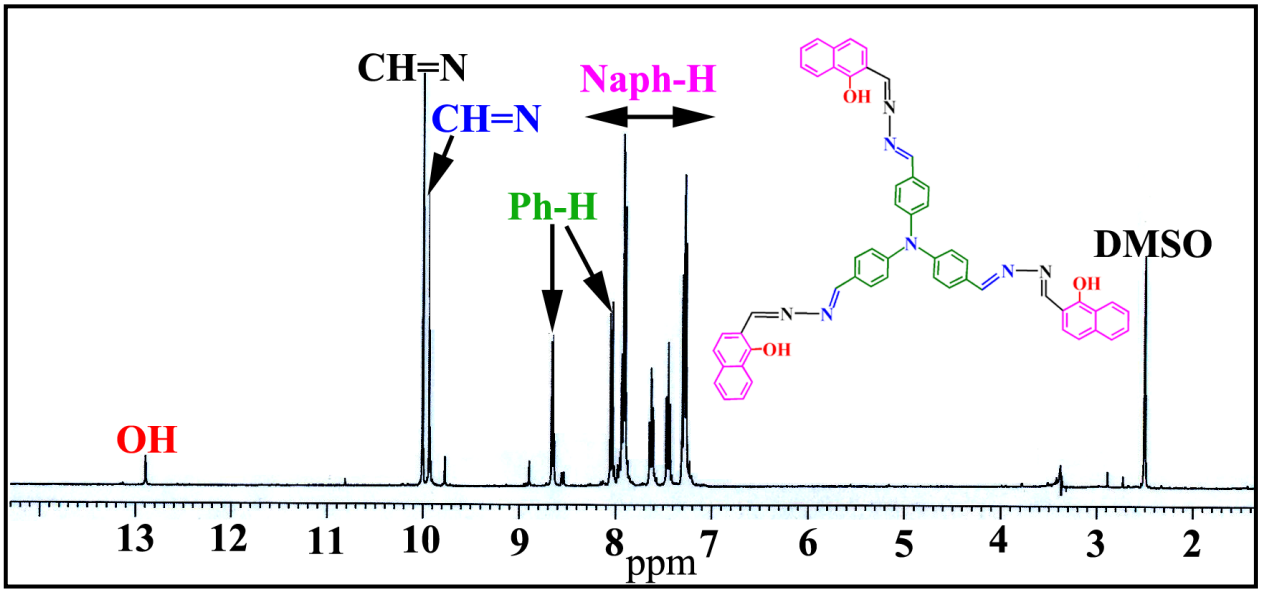

Fig. S4. Partial ^{1}H NMR spectrum of receptor **R**^{2} recorded in d_6-DMSO.

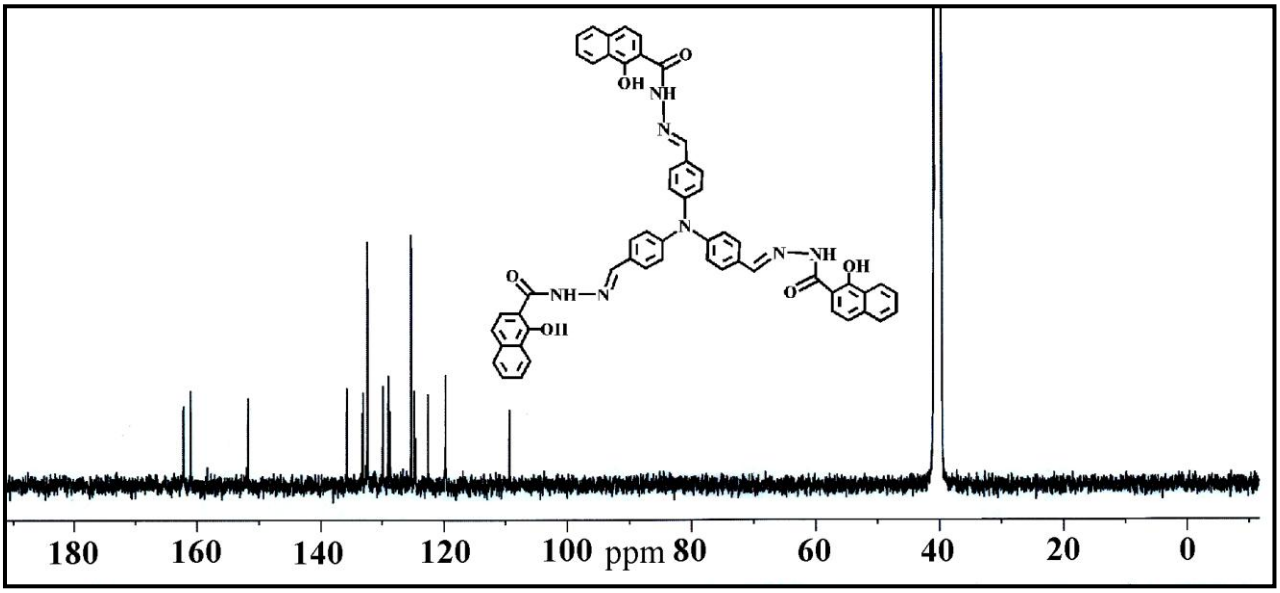

Fig. S5. ^{13}C NMR spectrum of receptor **R**^{1} recorded in d_6-DMSO.

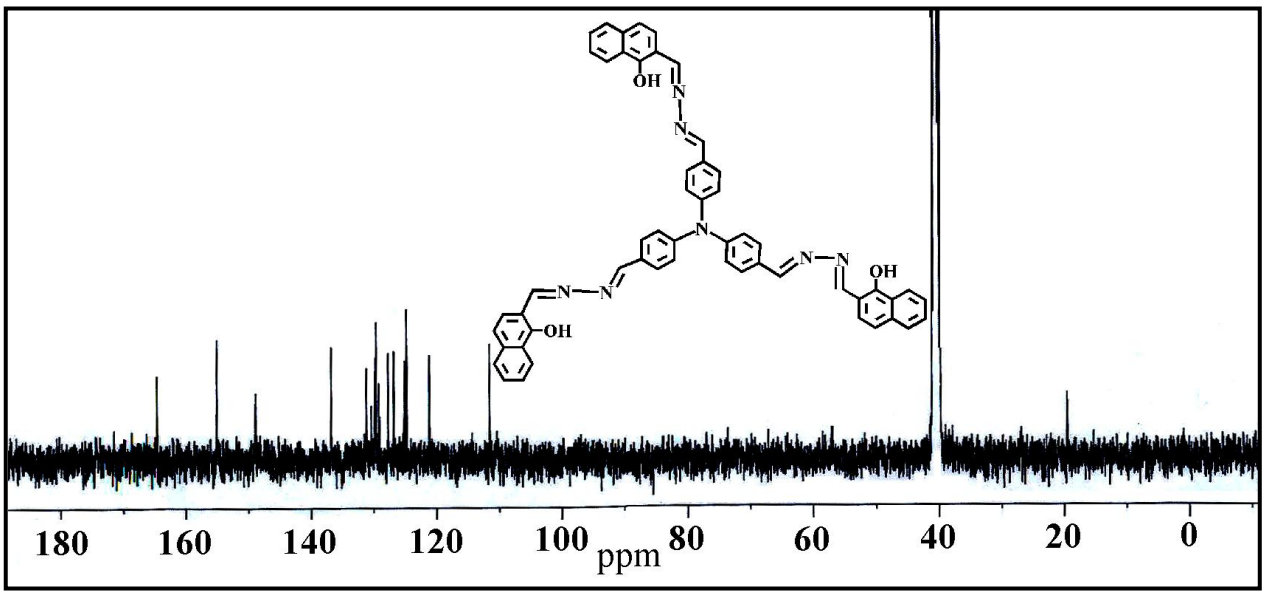

Fig. S6. Partial ^{13}C NMR spectrum of receptor $\mathbf{R^2}$ recorded in d_6-DMSO.

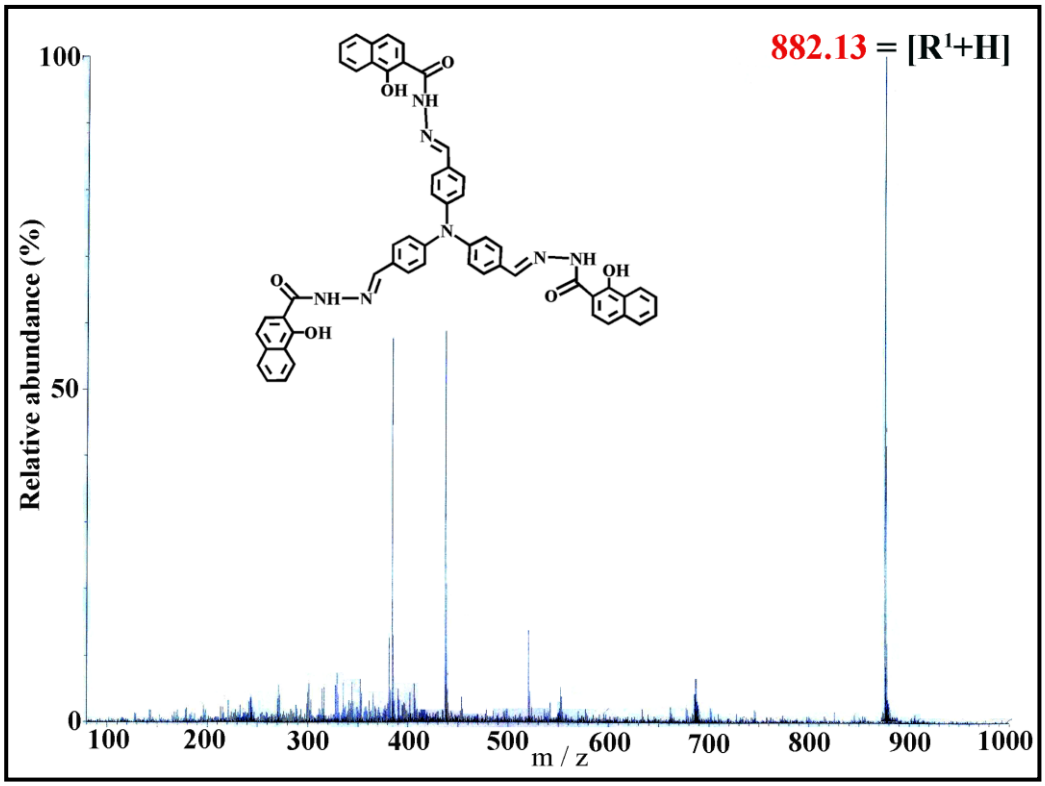

Fig. S7. HRMS spectrum of receptor $\mathbf{R^1}$ recorded by dissolving in CH_3CN.

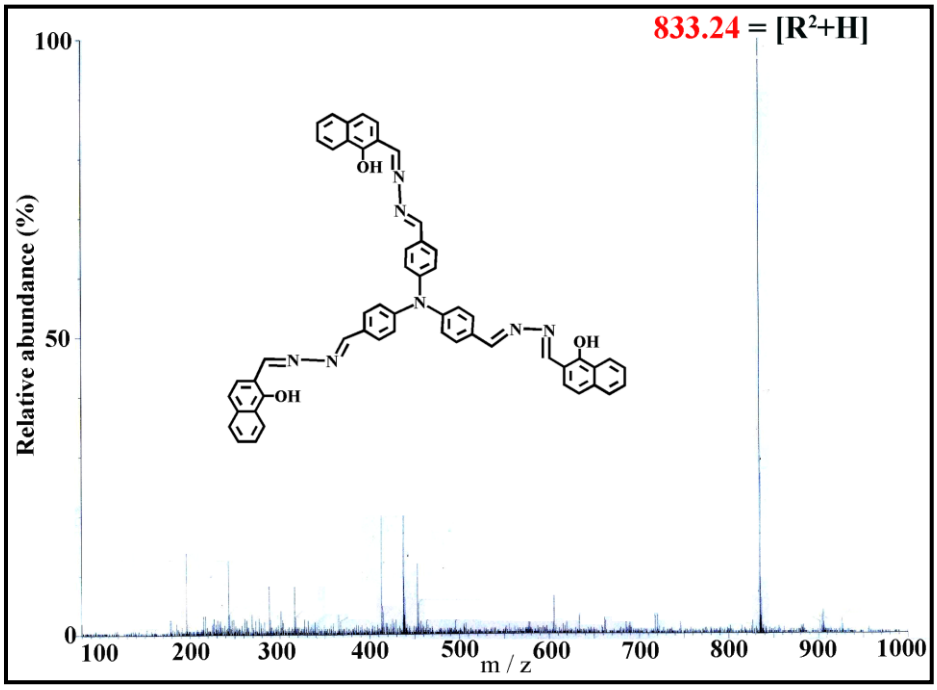

Fig. S8. HRMS spectrum of receptor **R²** recorded by dissolving in CH_3CN.

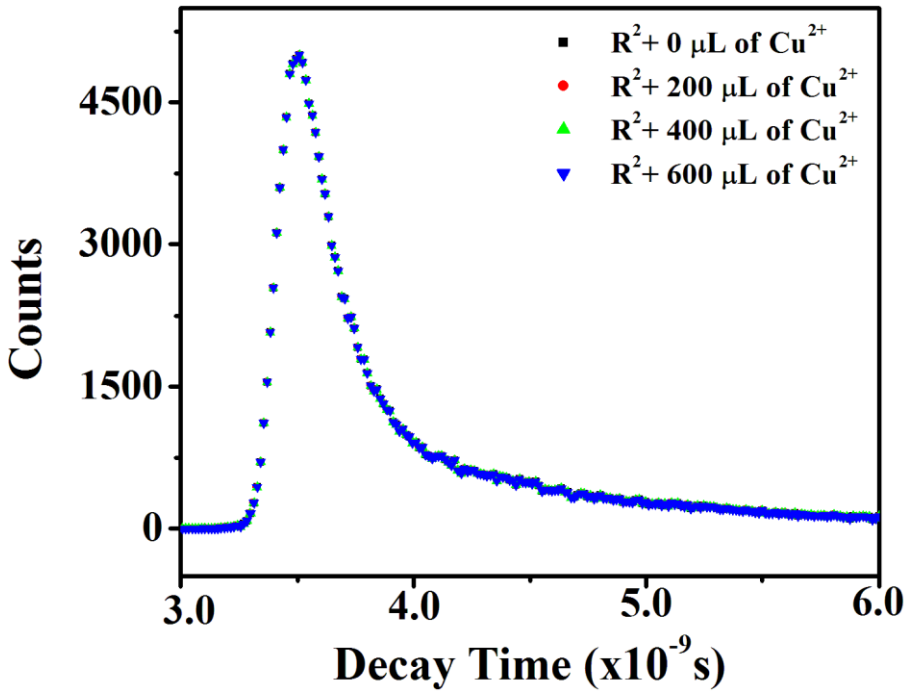

Fig. S11. Time resolved fluorescence study of receptor **R²** with respect to increasing the concentration of Cu^{2+} (0−600 μL) at room temperature.

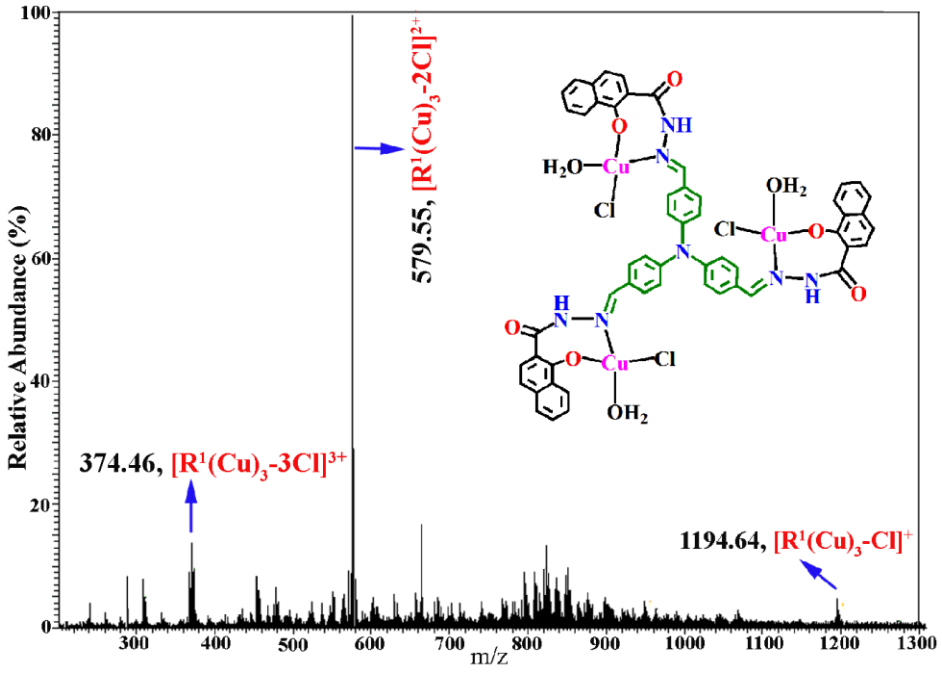

Fig. S10. ES-MS spectrum of trinuclear Cu^{2+} complex of receptor **R**^{1} recorded in CH_3CN.

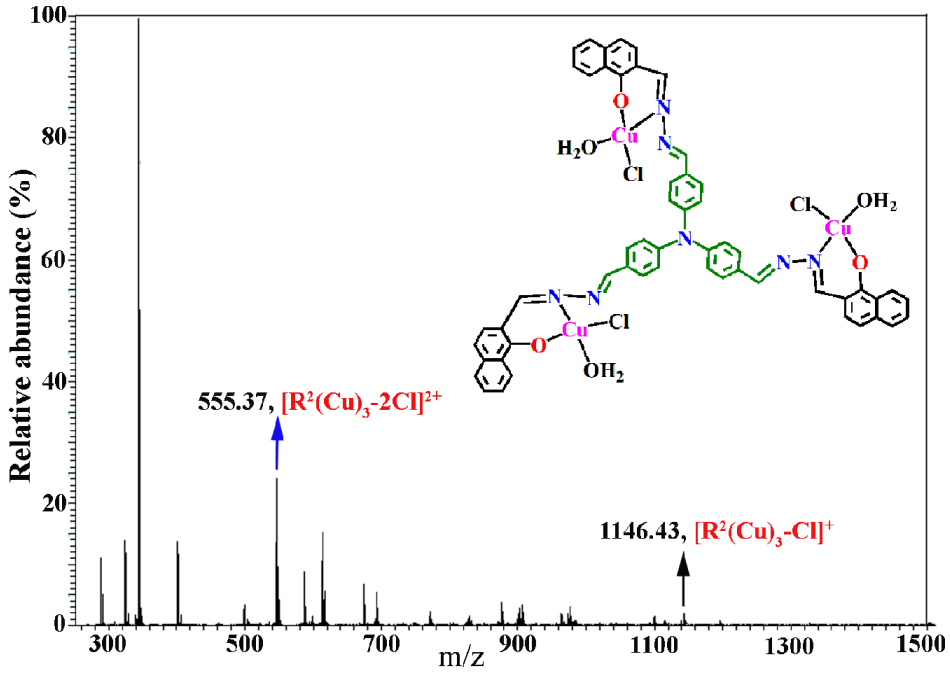

Fig. S11. ES-MS spectrum of trinuclear Cu^{2+} complex of receptor **R**^{2} recorded in CH_3CN.

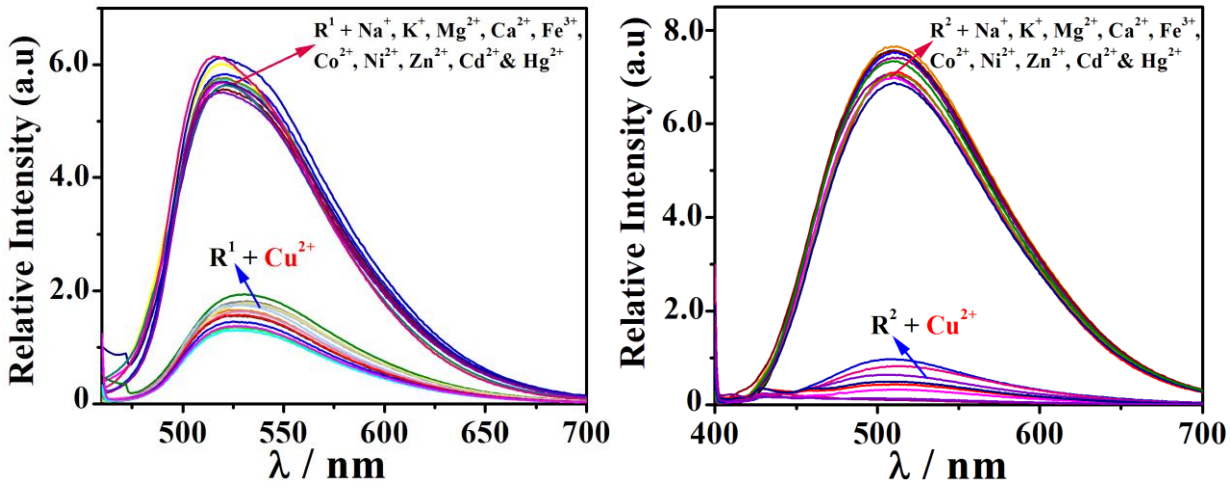

Fig. S12. Competitive selectivity of receptors $\mathbf{R^1}$ (left) and $\mathbf{R^2}$ (right) (1 μM) towards Cu^{2+} ions (10 μM) in the presence of other metal ions (5 μM) in H_2O/CH_3CN (7:3, v/v) medium.