Supporting Information

Pyrene-hydrazone based chemosensors for Cu^{2+} and their application in cancer cell imaging

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Experimental section

Materials and measurements

1-Pyrenecarboxaldehyde-hydrazone was synthesized by following the reported procedure.[1] All the materials for synthesis were purchased from various commercial sources and used without further purification. Spectroscopic grade CH_3CN solvent was used for all titration experiments. ^1H and ^{13}C NMR spectra were taken on a Bruker 400 MHz spectrometer. The chemical shifts (δ) in ^1H 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 measurement was done using Perkin-Elmer LAMBDA 750 UV-visible spectrophotometer and fluorescence emission studies were carried out on HORIBA JOBIN YVON Fluoromax-4 spectrometer.

Synthesis of the receptor R^1: 15 mL ethanolic solution of salicylaldehyde (50 mg, 1.00 mmol) was added slowly to 1-pyrenecarboxaldehyde hydrazone (100 mg, 1.00 mmol) in ethanol and dichloromethane mixture (20 mL, 2:1, v/v). The reaction mixture was refluxed for 3 h with continuous stirring. Yellow spongy solid precipitated at the bottom of the flask was collected by filtration and washed several times with cold ethanol. Finally the product was recrystallized from chloroform solution. Isolated yield = 110 mg (77%). Anal. Calcd for $C_{24}H_{16}N_2O$: C, 82.74; H, 4.63; N, 8.04; Found: C, 82.53; H, 4.74; N, 7.86. IR (neat): υ = 3012s (-OH), 1600s (-C=N). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm); 11.93 (s, 1H, phenolic-OH), 9.64 (s, 1H, CH=N), 8.95 (s, 1H, CH=N), 8.93 (d, 1H, *J* = 12 Hz, H-pyrene), 8.69 (d, 1H, *J* = 10.5 Hz, H-pyrene), 8.27 (m, 4H, H-pyrene), 8.17 (d, 1H, *J* = 11.0 Hz, H- pyrene), 8.10 (d, 1H, *J* = 9.5 Hz, H-pyrene), 8.07 (t, 1H, *J* = 19.0 Hz, H-pyrene), 7.42 (d, 1H, *J* = 9.5 Hz, H-phenol), 7.39 (dd, 1H, *J* = 7.0 Hz, H-phenol), 7.09 (d, 1H, *J* = 10.0 Hz, H-phenol), 6.98 (dd, 1H, *J* = 10.5 Hz, H-phenol). ^{13}C NMR ($CDCl_3$, 121 MHz) δ (ppm): 117.5, 118.3, 120.1, 123.1, 124.9, 125.5, 126.6, 126.8, 126.9, 129.7, 131.0, 131.1, 131.7, 132.9, 133.4, 133.2, 160.4, 161.6, 165.5. HRMS: m/z calcd for $[\mathbf{R^1}+ H]^+$ 348.40, found = 349.14.

Synthesis of the receptor R^2: The receptor $\mathbf{R^2}$ was synthesized by following the procedure of synthesis of receptor $\mathbf{R^1}$ using 2-hydroxy-1-naphthaldehyde (70.5 mg, 1.0 mmol) instead of salicylaldehyde. The product was isolated as orange solid. Isolated yield = 136 mg (83%). Anal. Calcd for $C_{28}H_{18}N_2O$: C, 84.40; H, 4.55; N, 7.03; Found: C, 84.62; H, 4.84; N, 7.31. IR (neat): υ = 3036s (-OH), 1604s (-C=N). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm); 13.54 (s, 1H, phenolic-OH), 9.88 (s, 1H, CH=N), 9.70 (s, 1H, CH=N), 8.95 (d, 1H, *J* = 11.5 Hz, H-phenol), 8.74 (d, 1H, *J* = 10.0 Hz, H-phenol), 8.26 (m, 4H, H-pyrene), 8.17 (d, 2H, *J* = 11.0 Hz, H- pyrene), 8.11 (d, 1H, *J* = 10.0 Hz, H-pyrene), 8.05 (d, 1H, *J* = 9.5 Hz, H-pyrene), 7.89 (d, 1H, *J* = 11.0 Hz, H-

phenol), 7.82 (d, 1H, *J* = 10.0 Hz, H-phenol), 7.61 (dd, 1H, *J* = 12.0 Hz, H-pyrene), 7.41 (dd, 1H, *J* = 10.5 Hz, H-phenol), 7.28 (dd, 1H, *J* = 11.5 Hz, H-phenol). ^{13}C NMR ($CDCl_3$, 121 MHz) δ (ppm); 113.8, 115.5, 117.6, 121.8, 122.0, 123.3, 123.7, 123.8, 125.2, 125.6, 127.1, 127.2, 127.5, 127.8, 128.8, 129.0, 129.9, 130.7, 132.4, 133.0, 149.9, 151.1, 156.6, 156.9. HRMS: m/z calcd for $[\mathbf{R^2}+ H]^+$ 398.46, found = 399.15.

X–ray data collection and refinements

Crystal of receptor R^2 was translucent orange and could be sorted using polarizing microscope (Leica DMLSP). Crystal was cut into suitable size and mounted on Kappa Apex-II CCD diffractometer equipped with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The intensity data were collected using ω and φ scans with frame width of 0.5°. The frame integration and data reduction were performed using Bruker SAINT–Plus (Version 7.06a) software.[1-4] The multi–scan absorption corrections were applied to the data using SADABS (Bruker 1999)[5] program. Both samples were stable at room temperature. The structures were solved using SIR92.[6] Full matrix least squares refinement was performed using SHELXL–97 (Sheldrick, 1997) programs.[7] All the non–hydrogen atoms were refined with anisotropic displacement parameters. The refinement of water hydrogen atoms were restrained such that they remain in the vicinity of the respective difference peak. All the hydrogen atoms could be located in Difference Fourier map. However, they were relocated at chemically meaningful positions and were given riding model refinement with following restraints: secondary CH_2 group (C–H = 0.97 Å, U_{iso}= 1.2 U_{eq} of the parent carbon), aromatic C–H group (C–H = 0.93 Å U_{iso}=1.2 U_{eq} of the parent carbon).

CCDC-896663 (for receptor $\mathbf{R^2}$) contains the supplementary crystallographic data reported in this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Center via *www.ccdc.cam.ac.uk/data_request/cif.*

Procedures for sensing study:

Stock solutions (1 mM) of tested metal cations (1×10^{-3} M) in deionized water and that of receptors $\mathbf{R^1}$ and $\mathbf{R^2}$ (1.0×10^{-3} M) in CH_3CN were prepared. A 2 mL stock solution (5 μM, H_2O/ CH_3CN (2:1 v/v) of $\mathbf{R^1}$ and $\mathbf{R^2}$ were placed in a quartz cell of 1 cm width and tested cation solution was added in an incremental fashion. Their corresponding fluorescence spectra were recorded at 298 K. Each titration was repeated at least two times to get consistent value. For all measurements λ_{exc} = 425 ($\mathbf{R^1}$) and 435 nm ($\mathbf{R^2}$) and the emission wavelength was monitored from 440–700 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 $\mathbf{R^1}$ and $\mathbf{R^2}$ upon gradual addition of metal ion 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 $\mathbf{R^1}$ and $\mathbf{R^2}$ were calculated from the slope of the Stern-Volmer plot.

Cell culture and fluorescence imaging

The HeLa cancer cell line was obtained from National Centre for Cell Science (NCCS), Pune, India. Human cervical cancer (HeLa) cells (5×10^9/L) were placed on untreated glass cover slips 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 $\mathbf{R^1}$ and $\mathbf{R^2}$ treatment. CH_3CN-PBS

(1:2, 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.

Reference

1. D. R. Brown and H. Kozlowski, *Dalton Trans*., 2004, 1907.
2. SMART/SAINT; Bruker AXS, Inc.: Madison, WI, 2004.
3. G. M. Sheldrick, SHELX-97, Program for the Solution and Refinement of Crystal Structures; University of Gottingen: Gottingen, Germany, 1998.
4. L. J. Farrugia, WinGX: An Integrated System of Windows Programs for the Solution, Refinement and Analysis for Single Crystal X-ray Diffraction Data, version 1.65.04; Department of Chemistry: University of Glasgow, 2003. (L. J. Farrugia, *J. Appl. Crystallogr*., 1999, **32**, 837).
5. (a) G. M. Sheldrick, SADABS, Bruker Nonius Area Detector Scaling and Absorption Correction, version 2.05; University of Gottingen: Gottingen, Germany, 1999. (b) A. J. Arvai, C. Nielsen, ADSC Quantum-210 ADX Program, Area Detector System Corporation; Poway, CA, USA, 1983. (c) Z. Otwinowski, W. Minor, Methods in Enzymology, ed. Carter, Jr., C. W.; Sweet, R. M. Academic Press, New York, 1997, **276**, 307. (d) G. M. Sheldrick, SHELXTL-PLUS, Crystal Structure Analysis Package; Bruker Analytical X-ray; Madison, WI, USA, 1997.

6. L. J. Farrugia, ORTEP-3 for Windows, version 1.08. *J. Appl. Crystallogr.*, 1997, **30**, 565.

7. A. L. Spek, *Acta Crystallogr.*, 1990, **A46**, C34.

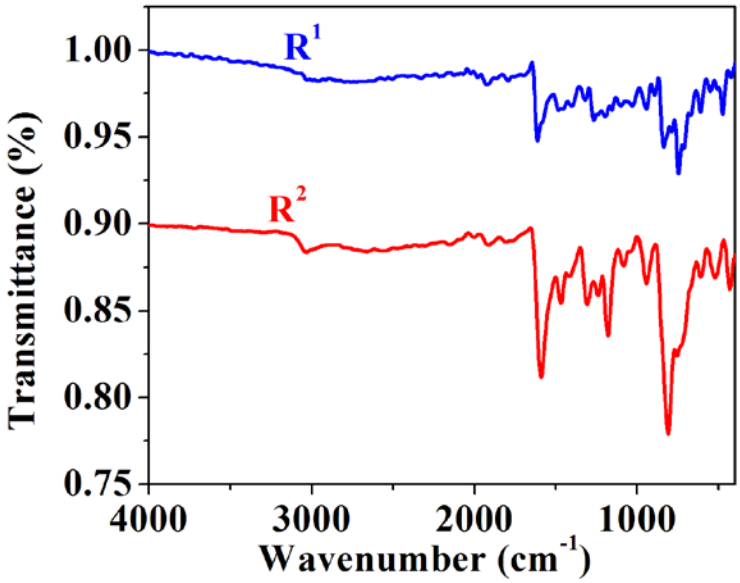

Fig. S1. Infrared spectra of receptors $\mathbf{R^1}$ and $\mathbf{R^2}$.

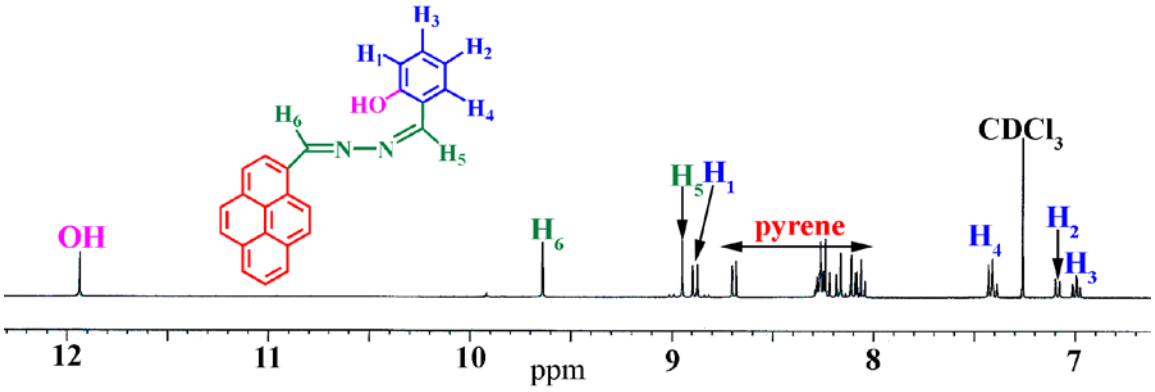

Fig. S2. Partial ^{1}H NMR spectrum of receptor $\mathbf{R^1}$ recorded in d_6-DMSO with peak assignment.

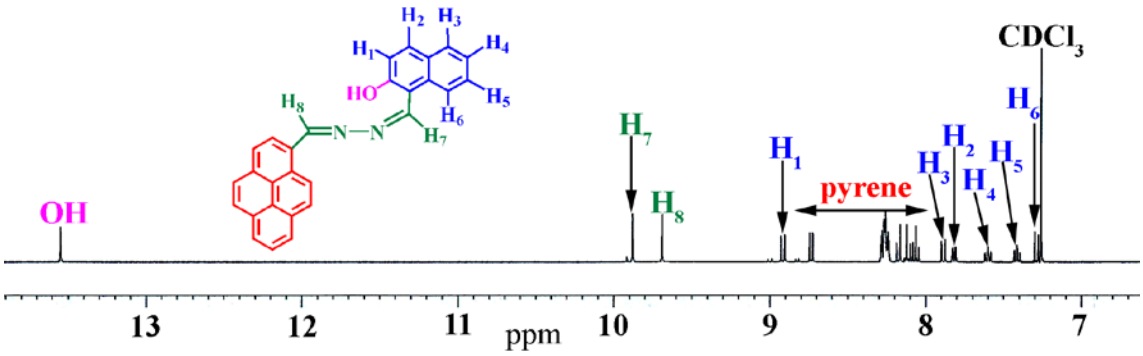

Fig. S3. Partial ^{1}H NMR spectrum of receptor **R^{2}** recorded in d_6-DMSO.

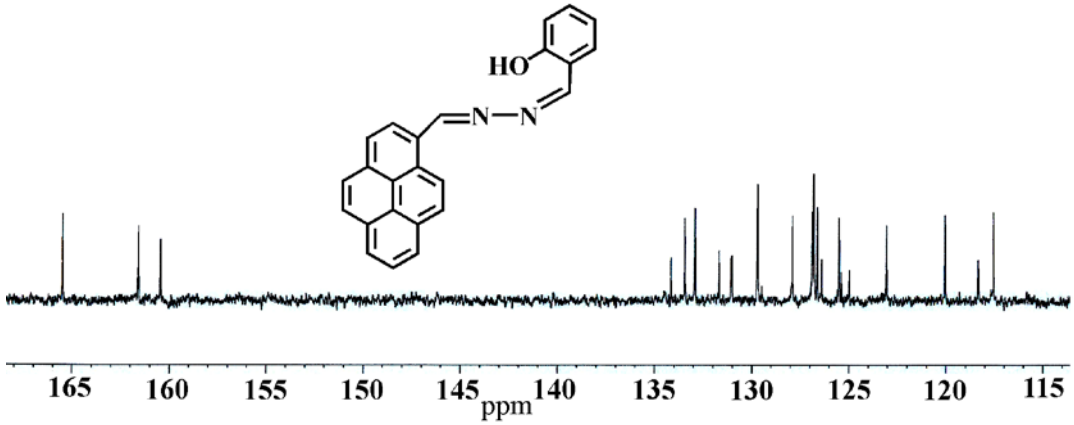

Fig. S4. ^{13}C NMR spectrum of receptor **R^{1}** recorded in d_6-DMSO.

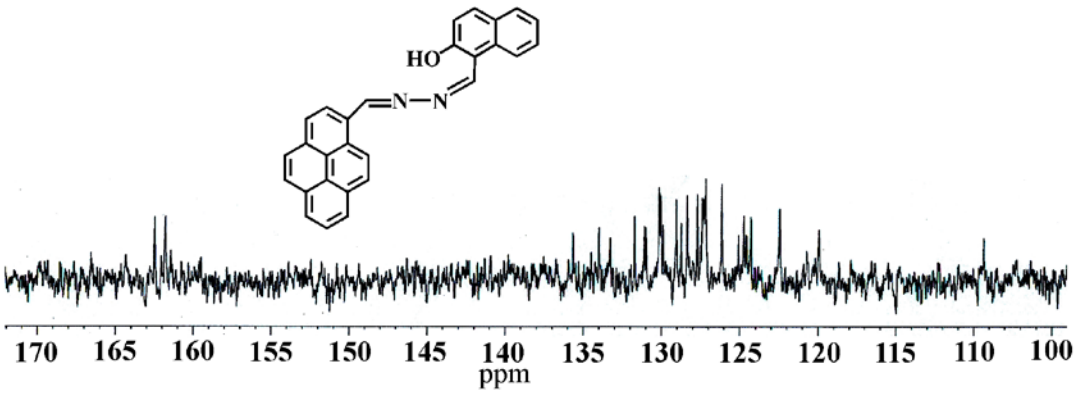

Fig. S5. Partial ^{13}C NMR spectrum of receptor **R^{2}** recorded in d_6-DMSO.

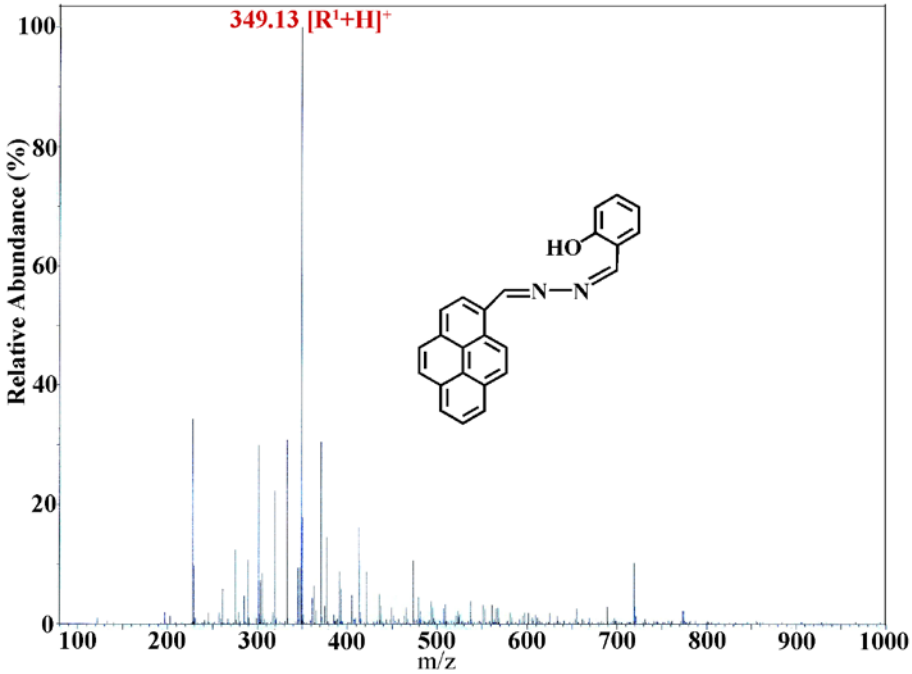

Fig. S6. HRMS spectrum of receptor **R**[1] recorded by dissolving in DMSO.

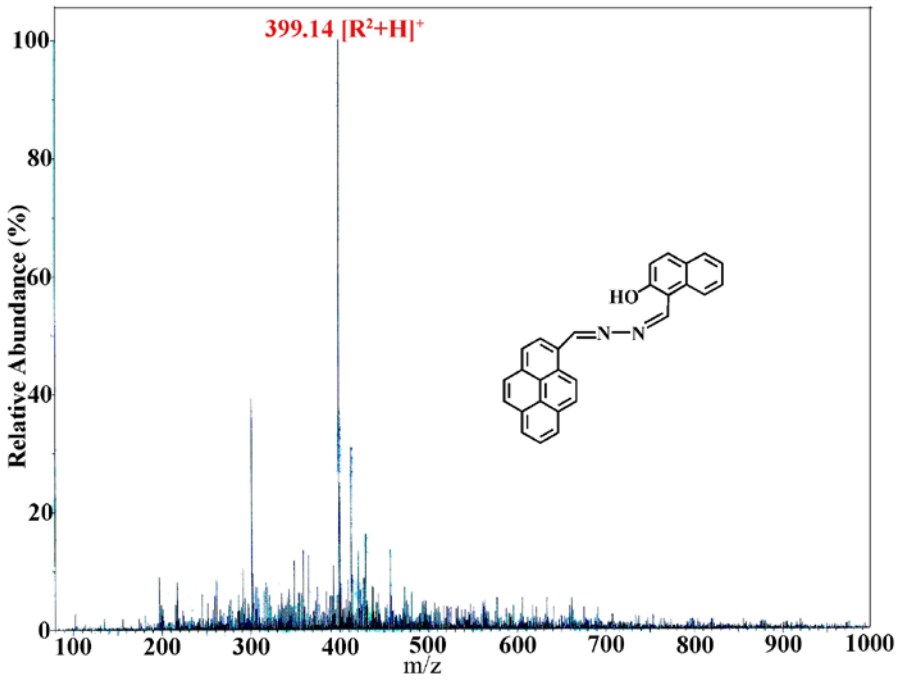

Fig. S7. HRMS spectrum of receptor **R**[2] recorded by dissolving in DMSO.

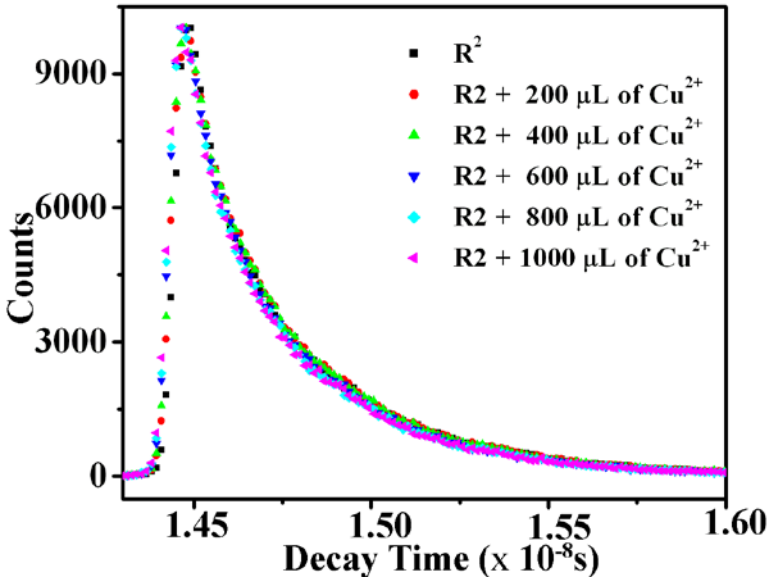

Fig. S8. Time resolved fluorescence study of receptor **R**^{2} with respect to increasing the concentration of picric acid (0−1000 µL) at room temperature.

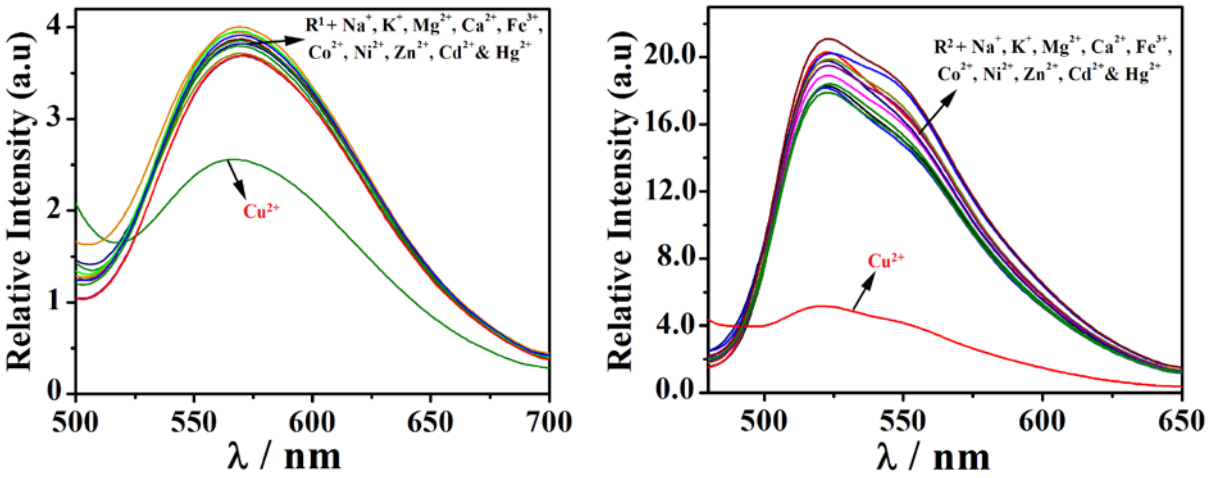

Fig. S9. Relative changes in the fluorescence intensity of receptors **R**^{1} (left) and **R**^{2} (right) (5 µM) upon mixing with various metal ions (10 µM) in H_2O/CH_3CN (2:1, v/v) medium.

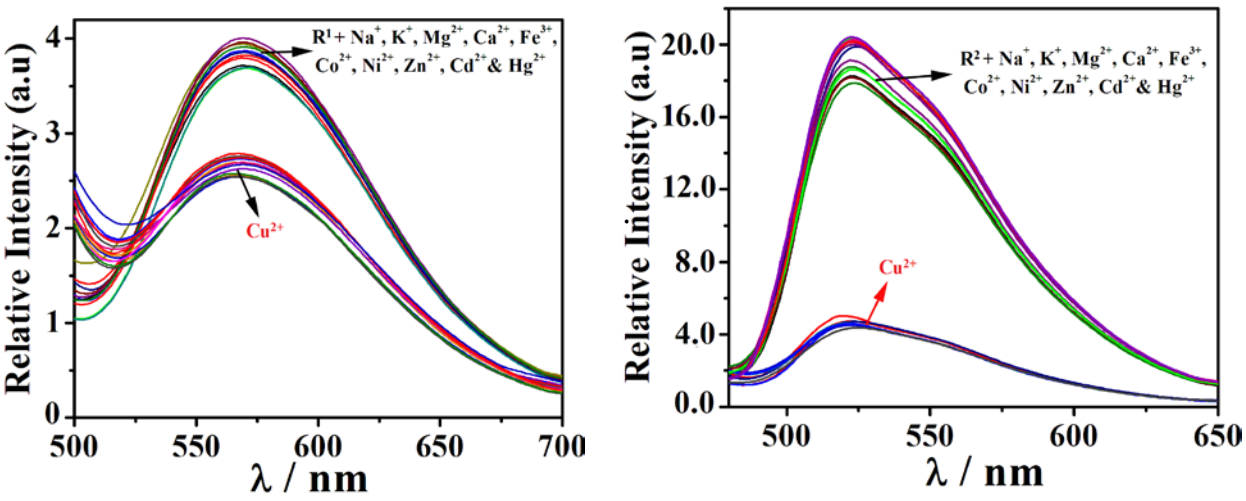

Fig. **S10**. Competitive selectivity of receptors $\mathbf{R^1}$ (left) and $\mathbf{R^2}$ (right) (5 μM) towards Cu^{2+} ions (10 μM) in the presence of other metal ions (10 μM) in H_2O/CH_3CN (2:1, v/v) medium.

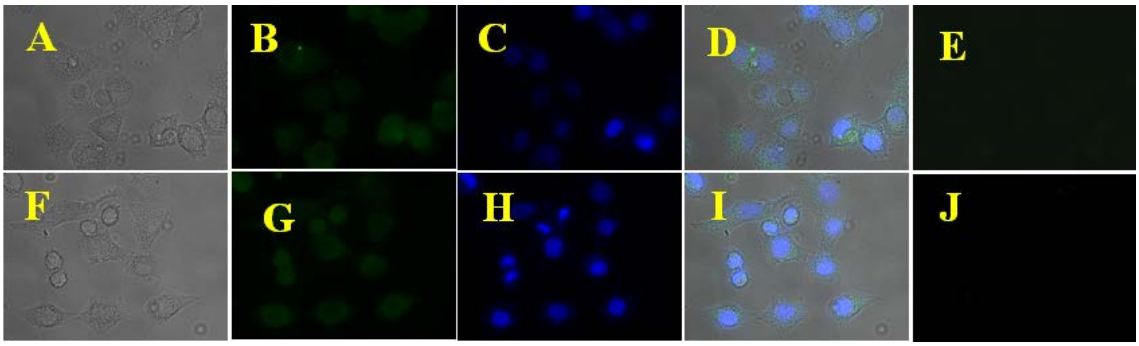

Fig. S11. Confocal fluorescence images of Cu^{2+} in HeLa cells (Zeiss ApoTome Fluorescense confocal microscope). (A, F) Brightfield transmission images of HeLa cells. (B, G) Fluorescence images of HeLa cells incubated with $\mathbf{R^1}$ and $\mathbf{R^2}$ (1 μM), respectively. (C, H) Hoechst 33342 stained fluorescence images of HeLa cells incubated with $\mathbf{R^1}$ and $\mathbf{R^2}$ (1 μM), respectively. (D, I) Merged images of (B & C) and (G & H), respectively. (E, J) Cells supplemented with $\mathbf{R^1}$ and $\mathbf{R^2}$ (1 μM) in the growth media for 0.5 h at 37 °C and then incubated with $CuCl_2$ (5 μM) for 1 h at 37 ºC.

Table S1. Crystallographic data and refinement parameters of **R**[2].

empirical formula	$C_{28}H_{18}N_2O$
formula weight	398.44
crystal system	monoclinic
space group	*P*21
T, K	293
λ (Mo Kα), Å	0.71073
a, Å	9.7010(9)
b, Å	16.3311(15)
c, Å	12.5479(12)
α, °	90.00
β, °	96.057(6)
γ, °	90.00
V, Å^3	1976.8(3)
Z	4
ρ_{calcd}, g cm^{-1}	1.339
μ, mm^{-1}	0.082
GOF[a]	1.012
R1[b] [I > 2σ(I)]	0.0875 (3908)
wR2[c] [I > 2σ(I)]	0.2682 (6830)

[a]GOF = $\{\Sigma [w(F_0^2 - F_c^2)^2]/(n - p)\}^{1/2}$, where n and p denotes the number of data points and the number of parameters, respectively. [b]R1 = $(\Sigma \mathrm{II}F_0\mathrm{I} - \mathrm{I}F_c\,\mathrm{II})/\Sigma\ \mathrm{I}F_0\mathrm{I}$; [c]wR2 = $\{ \Sigma [w(F_0^2 - F_c^2)^2]/\Sigma[w(F_0^2)^2]\}^{1/2}$, where $w = 1/ [\sigma^2(F_0^2) + (aP)^2 + (bP)]$ and $P = [\max (0,F_0^2) + 2F_c^2]/3$.