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Electronic Supporting Information for Cu^{2+} selective fluorescent chemosensor based on coumarin and its application in bioimaging

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1. Instruments, reagents and experimental procedures

^{1}H and ^{13}C NMR spectra were taken on a Varian mercury-400 spectrometer with

TMS as an internal standard and d^6-DMSO as solvent. Absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. Fluorescence spectra measurements were performed on a Hitachi F-4500 spectrofluorimeter. All pH measurements were made with a pH-10C digital pH meter. HRMS were determined on a Bruker Daltonics APEXII 47e FT-ICR spectrometer.

All the materials for synthesis were purchased from commercial suppliers and used without further purification. Methanol for spectra detection was HPLC reagent without fluorescent impurity.

Procedures of metal ion sensing

Stock solutions of the metal ions (2.5 mM) were prepared in deionized water. A stock solution of **L1**(1 mM) was prepared in DMSO. The solution of **L1** was then diluted to 5 μM with water/ DMSO (9:1 v/v). In titration experiments, each time a 2 mL solution of **L1** (5μM) was filled in a quartz optical cell of 1 cm optical path length, and the Cu^{2+} stock solution was added into the quartz optical cell gradually by using a micro-pippet. Spectral data were recorded at 2 min after the addition. In selectivity experiments, the test samples were prepared by placing appropriate amounts of metal ion stock into 2 mL solution of **L1** (5μM). For fluorescence measurements, excitation was provided at 450nm, and emission was collected from 460 to 670 nm.

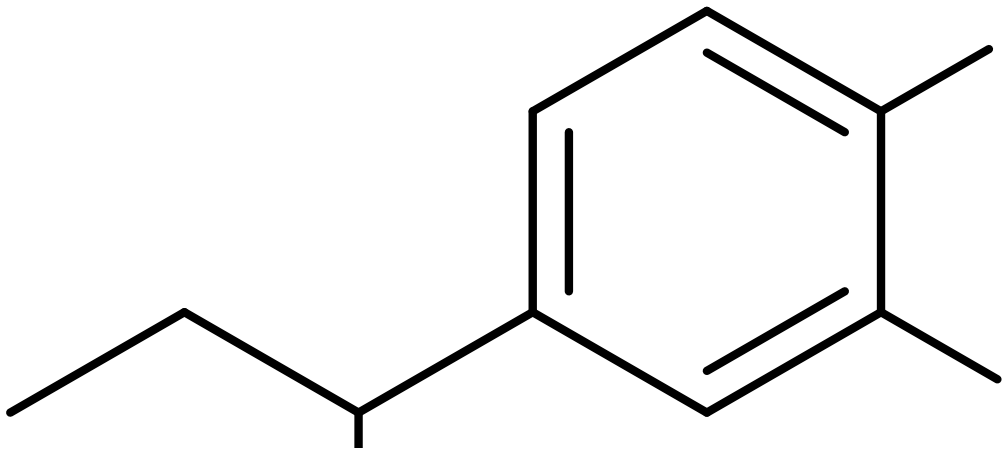
Cell Culture

The HEP G2 cell line was provided by Institute of Biochemistry and Cell Biology (China). Cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10 % FBS (Fetal Bovine Serum) in an atmosphere of 5 % CO_2, 95 % air at 37°C. Cells (5×10^8/L) were plated on 18 mm glass coverslips and allowed to adhere for 24 hours. Experiments to asses Cu^{2+} uptake were performed in the same media supplemented with 20 μM $CuCl_2$ for 0.5h.

Fluorescence Imaging

Fluorescent pictures were taken on Zeiss Leica inverted epifluorescence /reflectance laser scanning confocal microscope. Excitation of 1-loaded cells at 450 nm was carried out with a HeNe laser. Emission was collected using a 520nm long-pass filter. Emission was collected from 480 to 625 nm. Before the experiments, cells were washed with PBS buffer and then incubated with 5 μM **L1** in DMSO-PBS (1:49, v/v) for 5h at 37 °C. Cell imaging was then carried out after washing cells with PBS.

2. Synthesis routes and characteristic data



Scheme S1 Synthesis of Chemosensors **L1** and **L2**.

Compound **L4** was prepared according to the literature method.[1]

Synthesis of compound **L1**:

Compound **L4** (0.245g, 0.1mmol) and benzhydrazide (0.136g, 0.1mmol) were mixed in boiling ethanol with stirring for 4 hours, then brown precipitates obtained were filtered off, washed with ethanol and dried over P_2O_5 under vacuum. Yield: 82 %. ^{1}H NMR (d6-DMSO, 400 MHz) δ (ppm): 8.513 (s, 1H), 8.372 (s, 1H), 7.917-7.935 (d, 2H, *J*=7.2), 7.503-7.622 (m, 4H), 6.746-6.773 (m, 1H) 6.572-6.577 (d, 1H),

3.440-3.492 (q, 4H, *J*=6.8), 1.120 -1.155 (t, 6H, *J*=7.2). ^{13}C NMR (d6-DMSO, 50 MHz) δ (ppm):164.89,160.76, 159.56, 156.61, 156.50, 151.42, 151.28, 143.45, 138.96, 133.84, 133.55, 131.66, 130.95, 130.80, 128.41, 128.15, 127.60, 118.80, 117.31, 115.38, 112.59, 112.23, 109.74, 108.04, 96.38, 44.23, 12.33. ESI-MS m/z = 364.3 [M + H]$^+$, calc. for C21H21N3O3 =363.16. Elemental Analysis. Found (calculated) for $C_{21}H_{21}N_3O_3$ (%): C, 68.96 (69.41); H, 5.79 (5.82); N, 12.01 (11.56).

Synthesis of compound **L2:**

Compound **L4** (0.245g, 0.1mmol) and salicylhydrazide (0.152g, 0.1mmol) were mixed in boiling ethanol with stirring for 4 hours, then brown precipitates obtained were filtered off, washed with ethanol and dried over P_2O_5 under vacuum. Yield: 76 %. ^{1}H NMR (d6-DMSO, 400 MHz) δ (ppm): 12.00 (s, 1H), 11.929 (s, 1H), 8.503 (s, 1H), 8.372 (s, 1H), 7.886-7.903 (d, 1H, *J* = 7.2 Hz), 7.424-7.506 (m, 3H) 7.630-7.652 (d, 1H, *J* = 8.8 Hz) 7.416-7.457(q, 1H) 6.924-6.971 (m, 2H), 6.733-6.761 (q, 1H,), 6.560-6.564 (d, 1H), 3.428-3.479 (q, 4H, *J* = 6.8 Hz), 1.109-1.144 (t, 6H, *J* = 6.8 Hz). ^{13}C NMR (d6-DMSO, 50 MHz) δ (ppm):164.88, 160.76, 159.54, 156.67, 151.42, 143.43, 138.97, 133.86, 130.95, 128.15, 118.86, 117.29, 115.37, 112.27, 109.74, 108.04, 96.37, 44.24, 12.33. ESI-MS m/z = 380.3 [M + H]$^+$, calc. for C21H21N3O4 =379.15. Elemental Analysis. Found (calculated) for $C_{21}H_{21}N_3O_4$ (%): C, 66.13 (66.48); H, 5.49 (5.58); N, 11.27 (11.08).

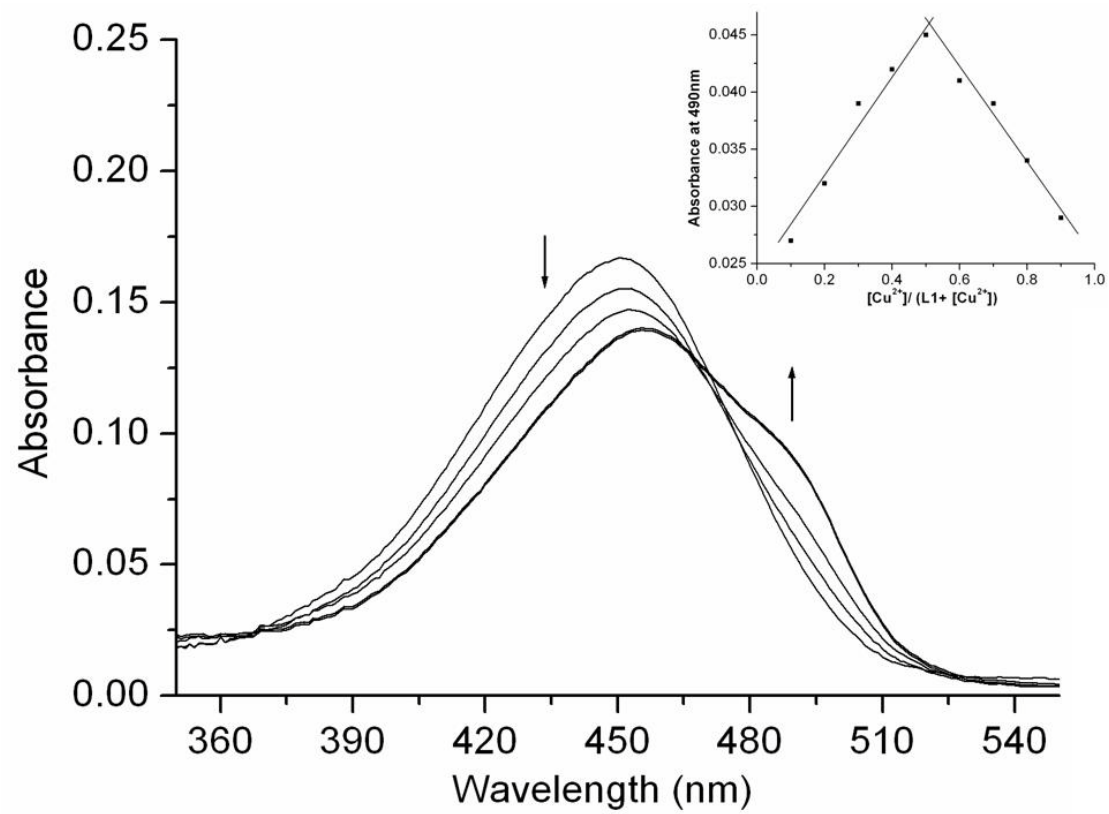


Fig. S1 Changes in the absorption spectra of **L1** (5 μM) in the presence of Cu^{2+} (10μM) in H_2O/ DMSO (9: 1 v/v). Inset: Job's plots according to the method for continuous variations, indicating the 1: 1 stoichiometry for **L1**-Cu^{2+} (the total concentration of **L1** and Cu^{2+} is 5μM, absorbance at 490nm).

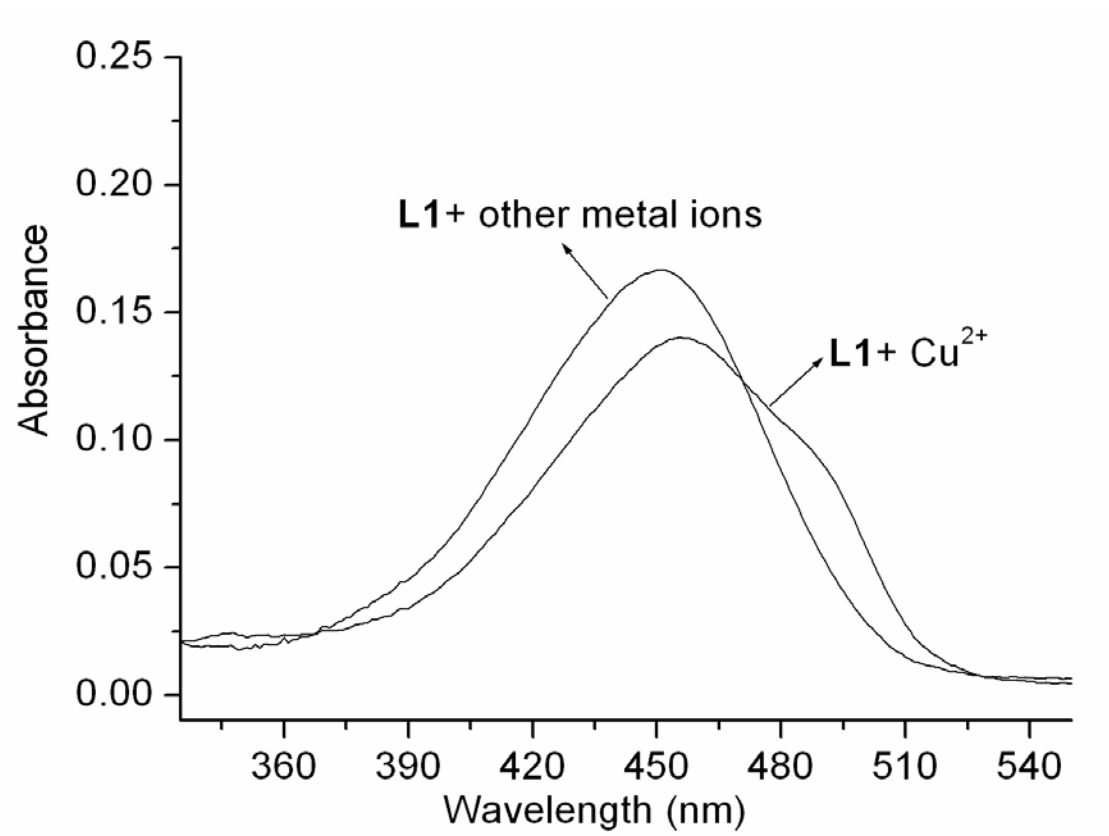


Fig. S2 Fluorescence spectra of **L1** (5 μM) upon the addition of various metal ions (5 μm for all metal ions) in H_2O/ DMSO (9:1, v/v) solution. Other metal ions: K^+, Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}.

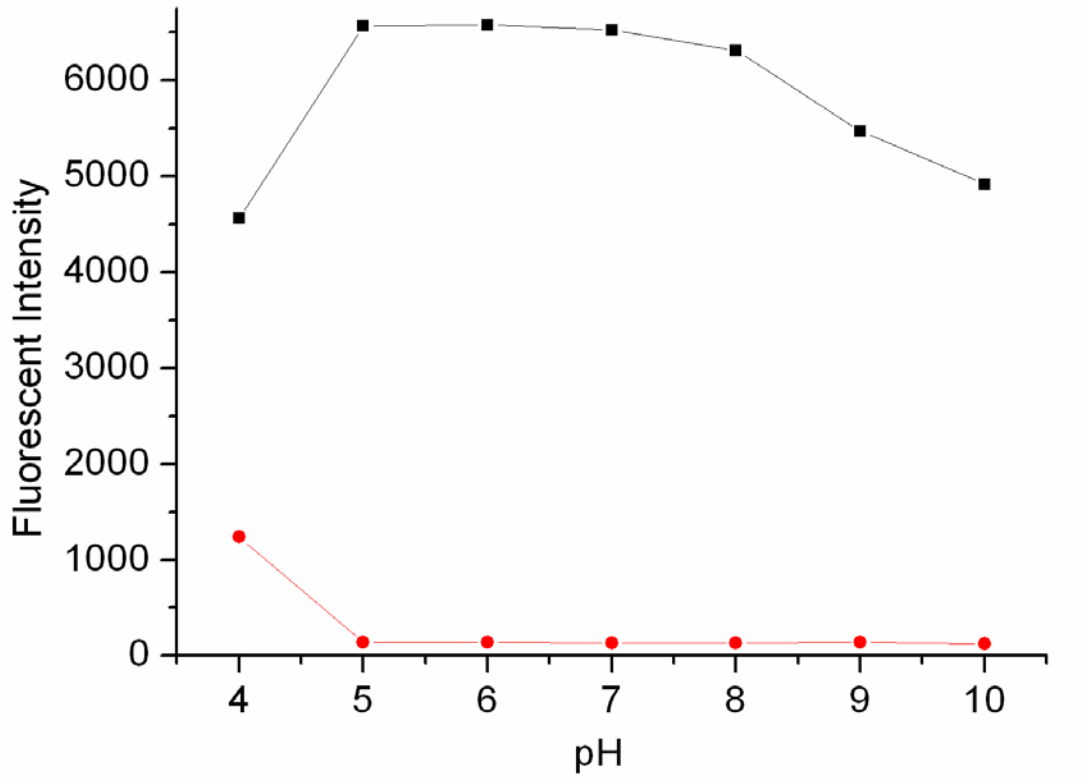


Fig. S3 Variation of fluorescence spectra of **L1** (5 μM) in aqueous solution H_2O/ DMSO (9:1, v/v) with and without Cu^{2+} (1.0 equiv) ion as a function of pH. (Fluorescence intensity (au) at 523 nm)

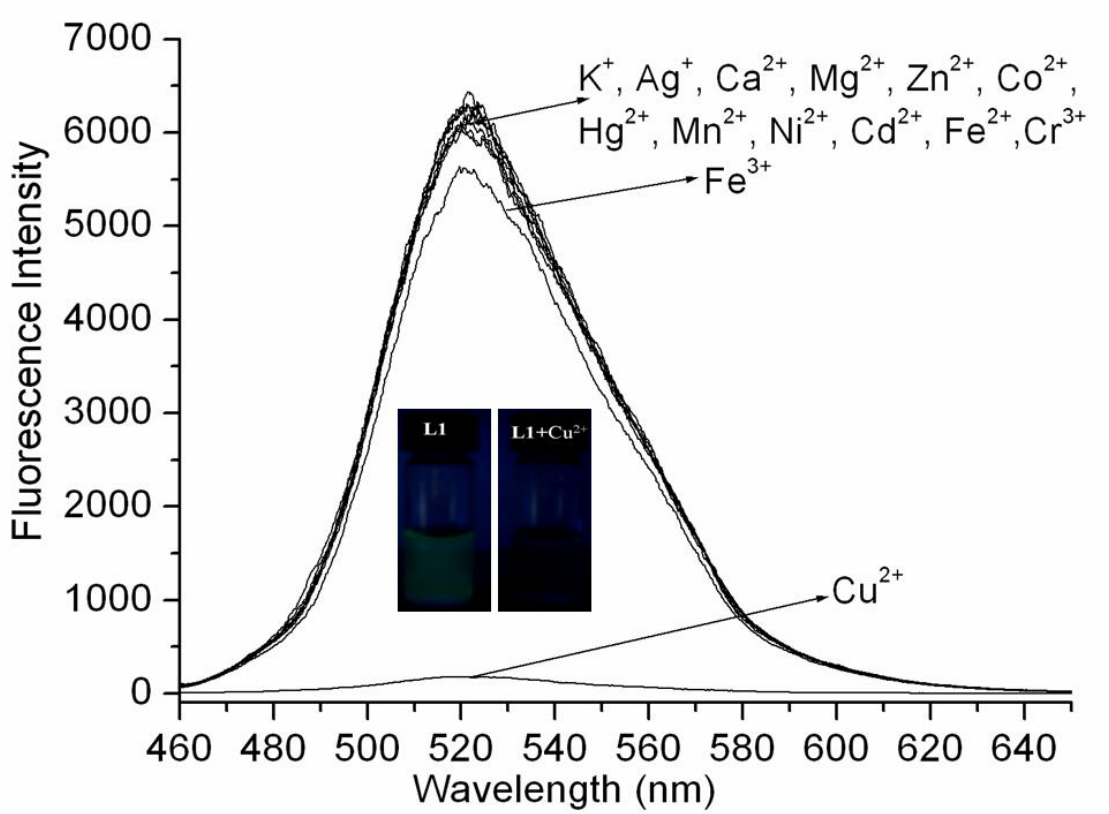


Fig. S4 Fluorescence spectra of **L1** (5 μM) upon the addition of various metal ions (5 μm for all cations) in H_2O/ DMSO (9:1, v/v) solution.

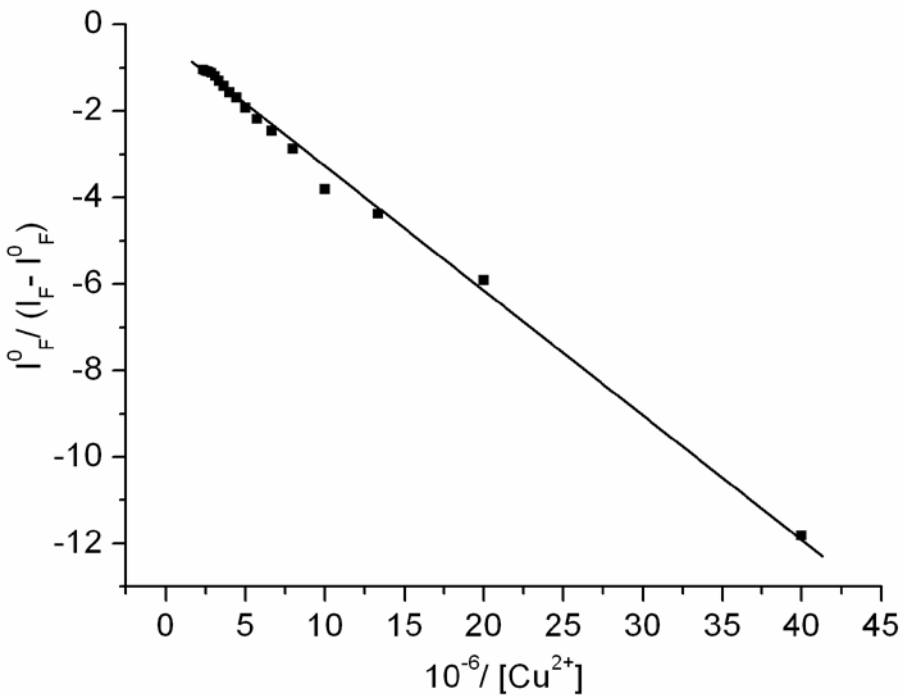


Fig. S5 Fitting of fluorescence titration curve of **L1** in H_2O/ DMSO (9:1, v/v) solution.

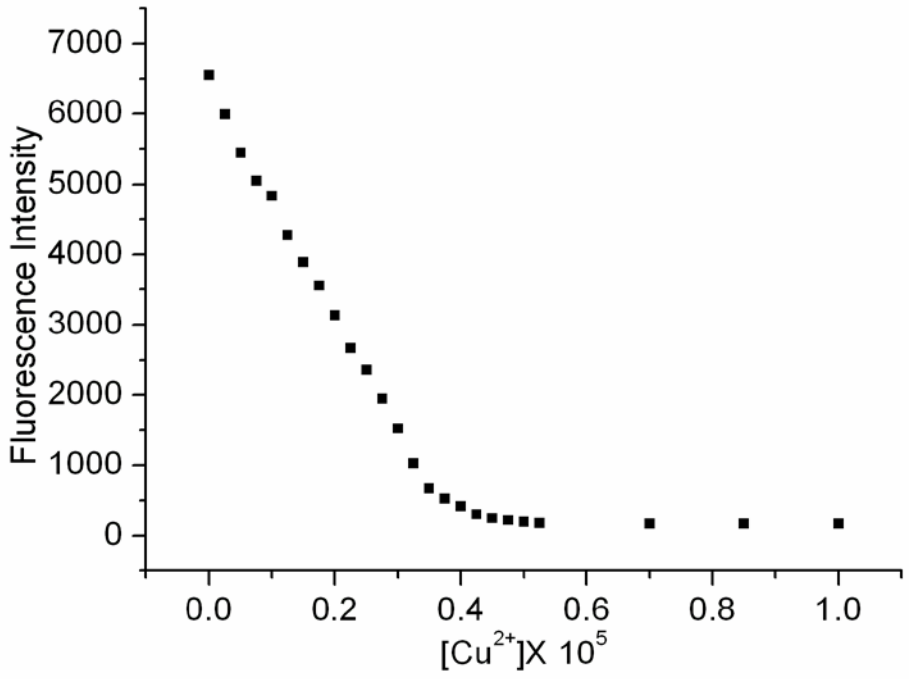


Fig. S6 Fluorescence intensity at 523 nm of **L1** (5 μM) as a function of Cu^{2+} (10 μM) concentration.

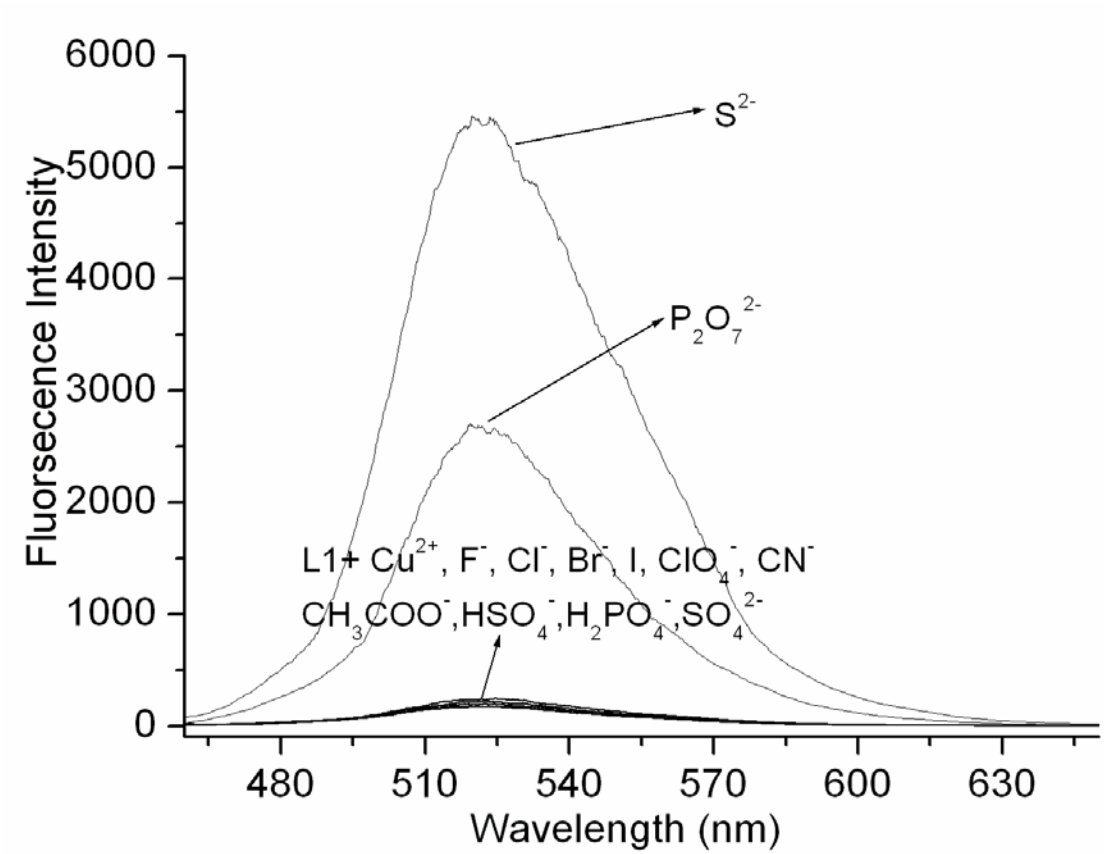


Fig. S7 Fluorescence enhancement responses of **L1**-Cu^{2+} to different anions (as their $[Bu_4N]$ salts) in H_2O/ DMSO (9:1, v/v) solutions. λ_{ex} = 450 nm, [**L1**] = 5μM, $[Cu^{2+}]$ = 5 μM, $[Bu_4NX]$ = 10 μM.

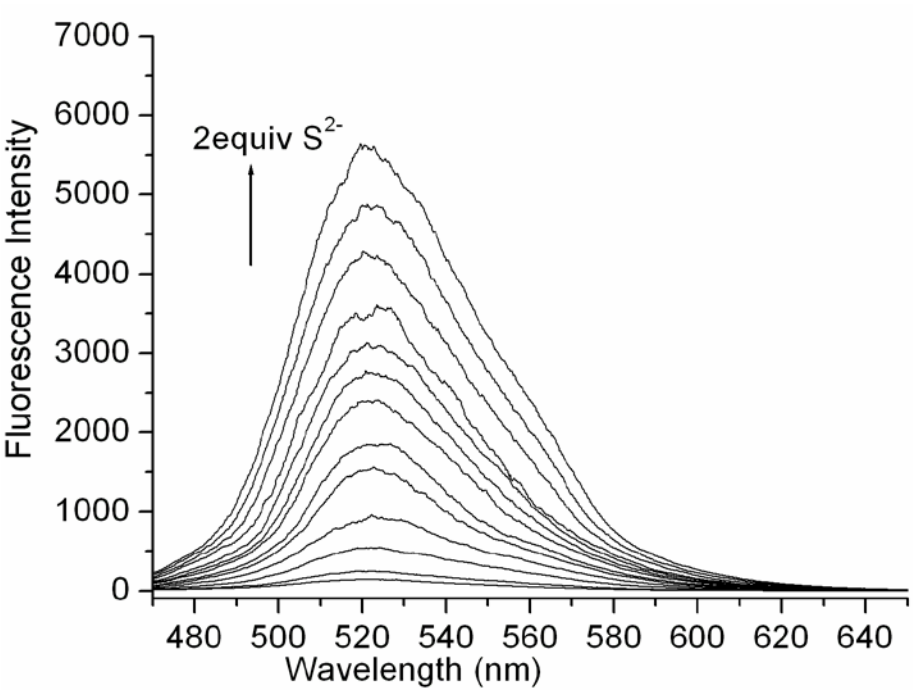


Fig. S8 Fluorescence titration of the **L1**–Cu^{2+} complex with sulfide ions In H_2O/ DMSO (9:1, v/v) solution. [**L1**-Cu^{2+}] = 5μM, λ_{ex} = 450 nm.

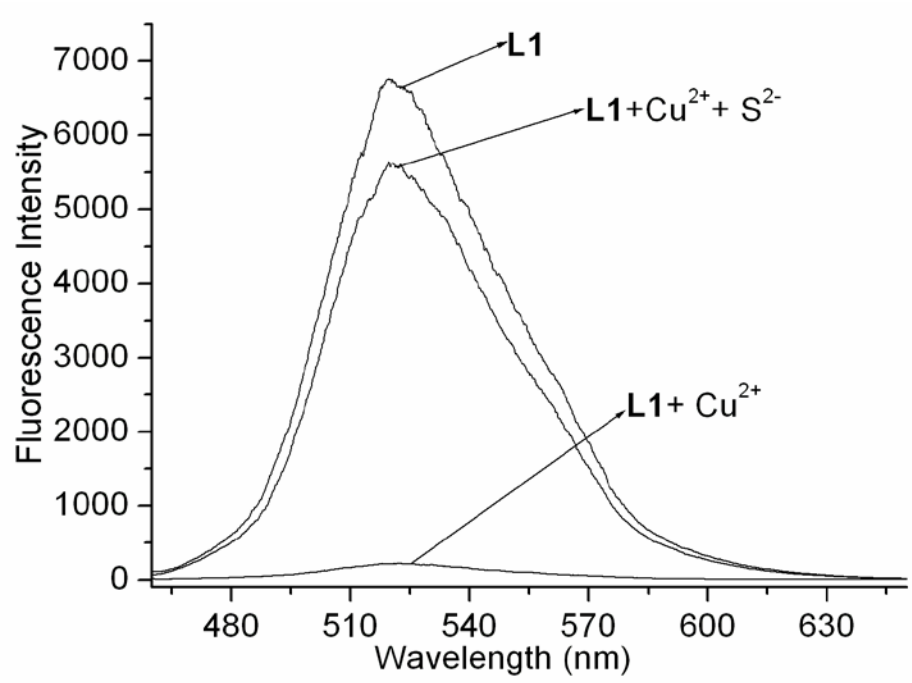


Fig. S9 Fluorescence spectra of **L1** (5μM) upon the addition of 1 equiv Cu^{2+} in H_2O/ DMSO (9:1, v/v) solution, and then on addition 2.0 equiv S^{2-}.

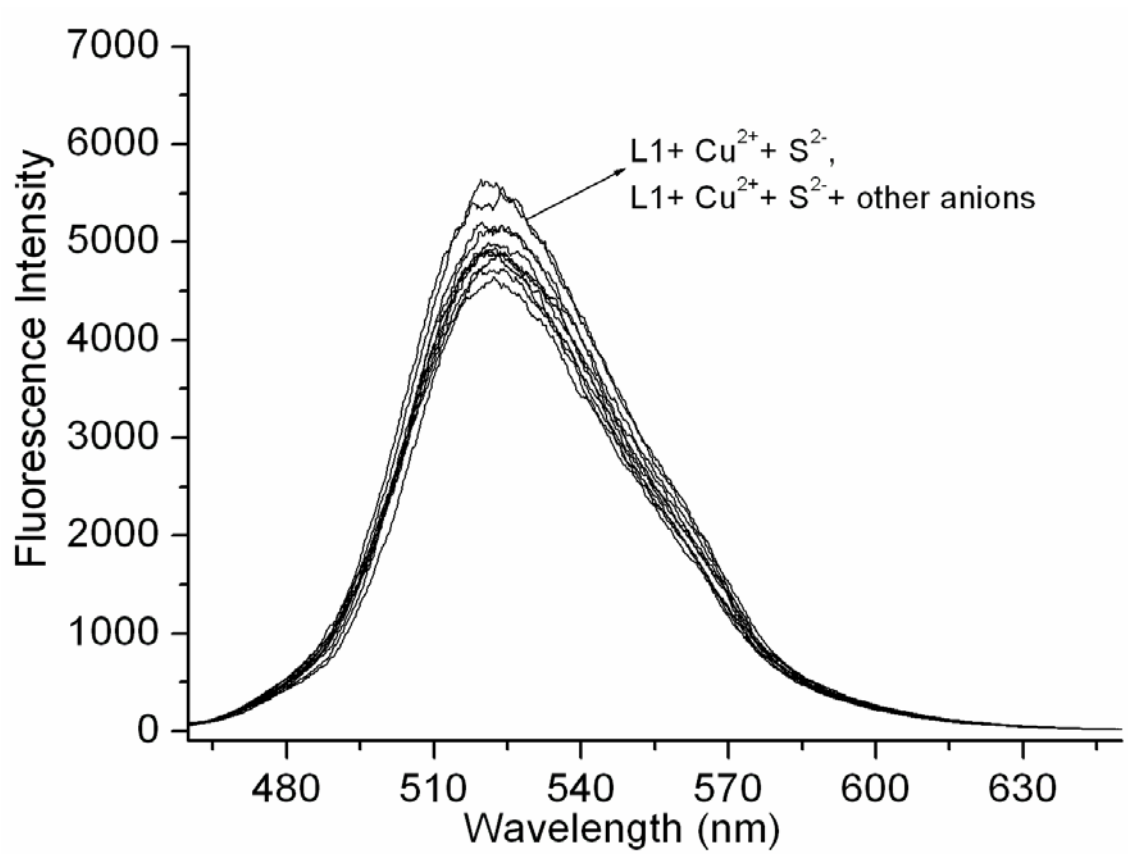


Fig. S10 Signaling of S^{2-} ions by the **L1**-Cu^{2+} system in the presence of 2 equiv of possibly interfering anions (10 μM for F^-, Cl^-, Br^-, I^-, ClO_4^-, NO_3^-, CN^-, HPO_4^-, HSO_3^-) as background in H_2O/ DMSO (9:1, v/v) solution. [**L1**] = 5 μM, [Cu^{2+}] =5 μM, [S^{2-}] = 10μ M, [A^{n-}] = 10 μM, λ_{ex} = 450 nm.

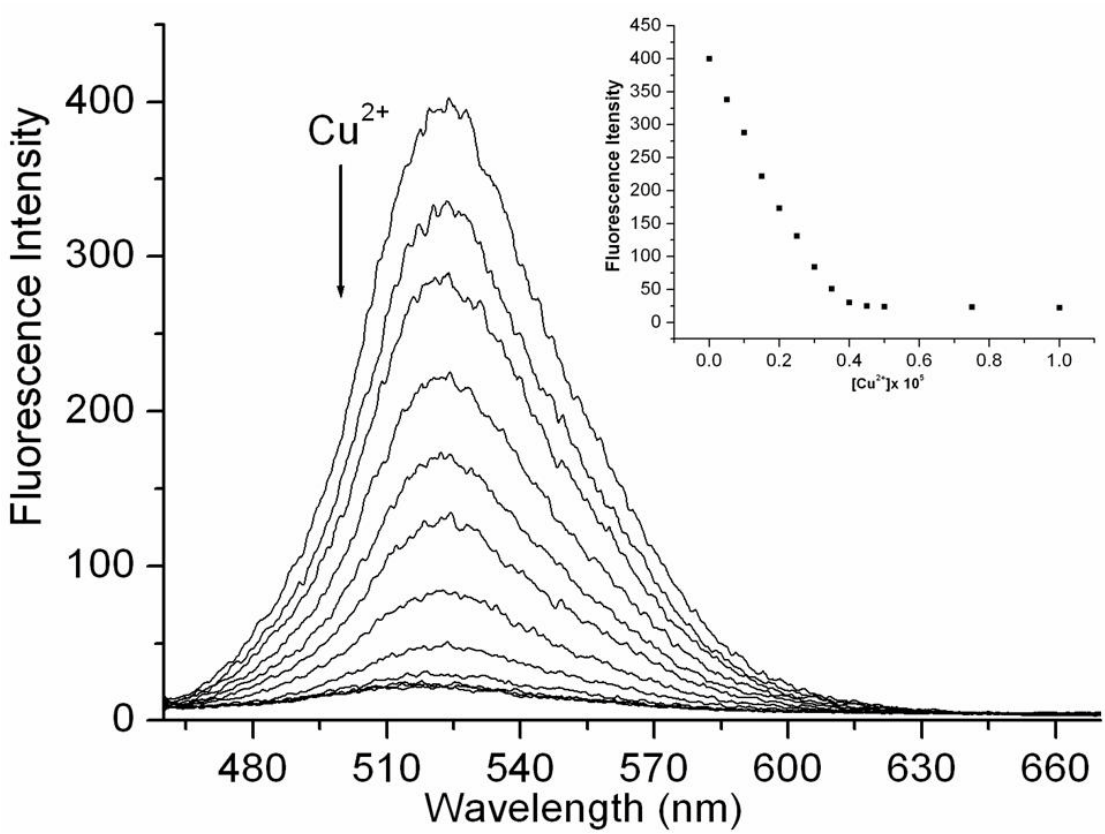


Fig. S11 Fluorescence spectra of **L2** (5μM) in H_2O/ DMSO (9:1, v/v) upon addition of Cu^{2+} (0–2 equiv), Inset shows fluorescence intensity at 523 nm of **L2** as a function of Cu^{2+} concentration.. (λ_{ex} = 450 nm).

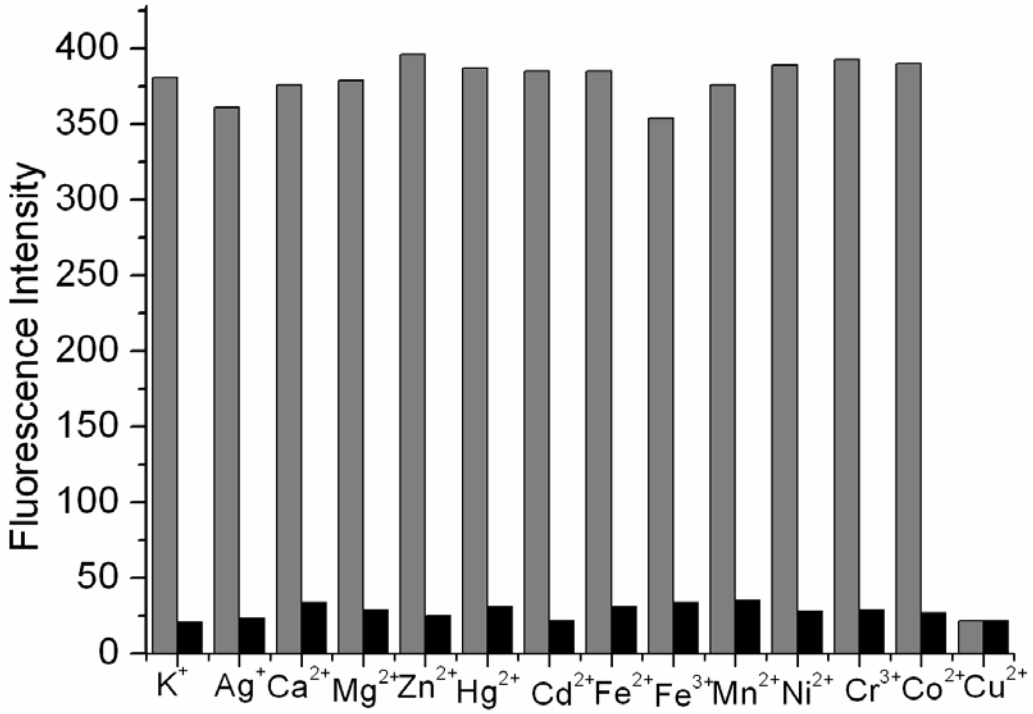


Fig. S12 Fluorescence intensities of 5μM **L2** upon the addition of various metal ions in H_2O/DMSO (9: 1 v/v) solution. Gray bars represent the fluorescence response of **L2** to the metal ion of interest (5 mM for K^+, Ag^+ and Ca^{2+}; 5Mm for other metal ions). Black bars represent the addition of Cu^{2+} (5μM) to the solution. (λ_{ex} = 450 nm).

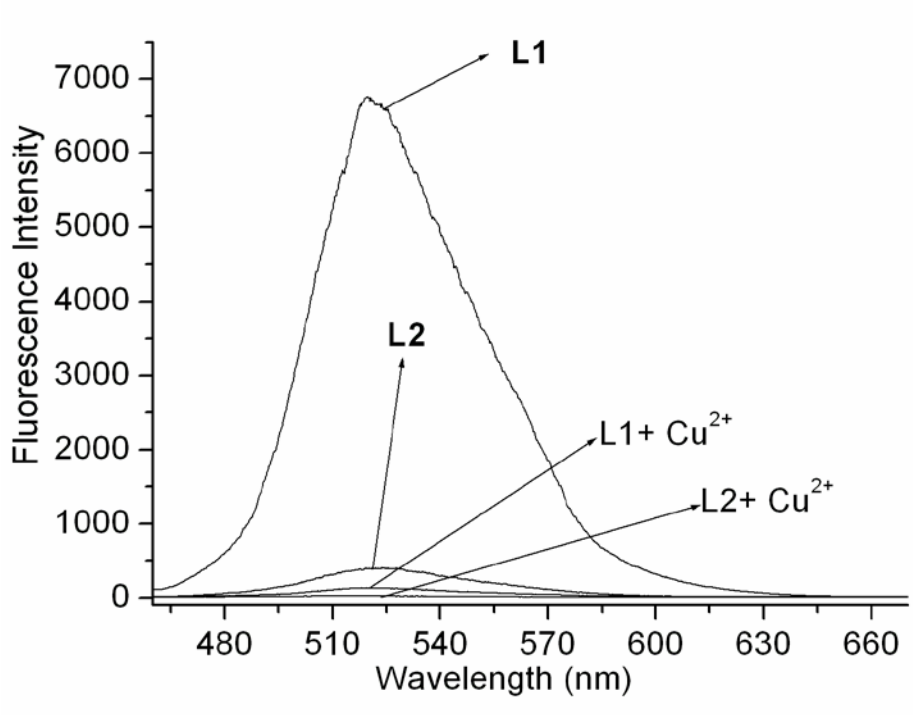


Fig. S13 Fluorescence spectra of **L1**, **L2** (5μM) in H_2O/ DMSO (9:1, v/v) upon addition of Cu^{2+} (5 μM).

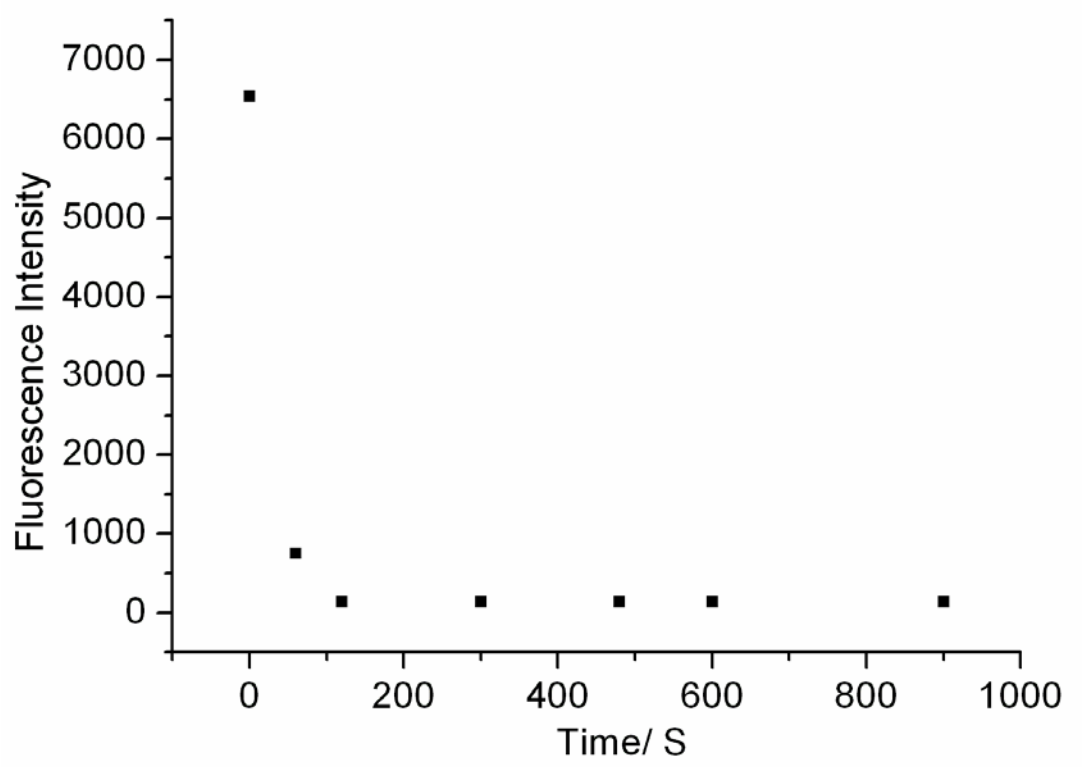


Fig. S14 Time course of the response of **L1** (5 μM) to 1.0 equiv Cu^{2+} in H_2O/ DMSO (9:1, v/v) solution.

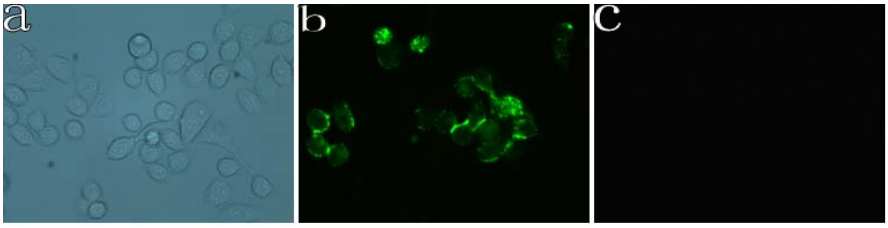


Fig. S15 Confocal fluorescence images of Cu^{2+} in Hep G2 cells (Zeiss LSM 510 META confocal microscope, 40× objective lens). (a) Brightfield transmission image of Hep G2 cells. (b) Fluorescence image of Hep G2 cells incubated with **L1** (5 μM). (c) Cells supplemented with 1 μM **L1** in the growth media for 0.5 h at 37 ℃ and then incubated with 3 equiv $CuCl_2$ for 2h at 37 ℃.

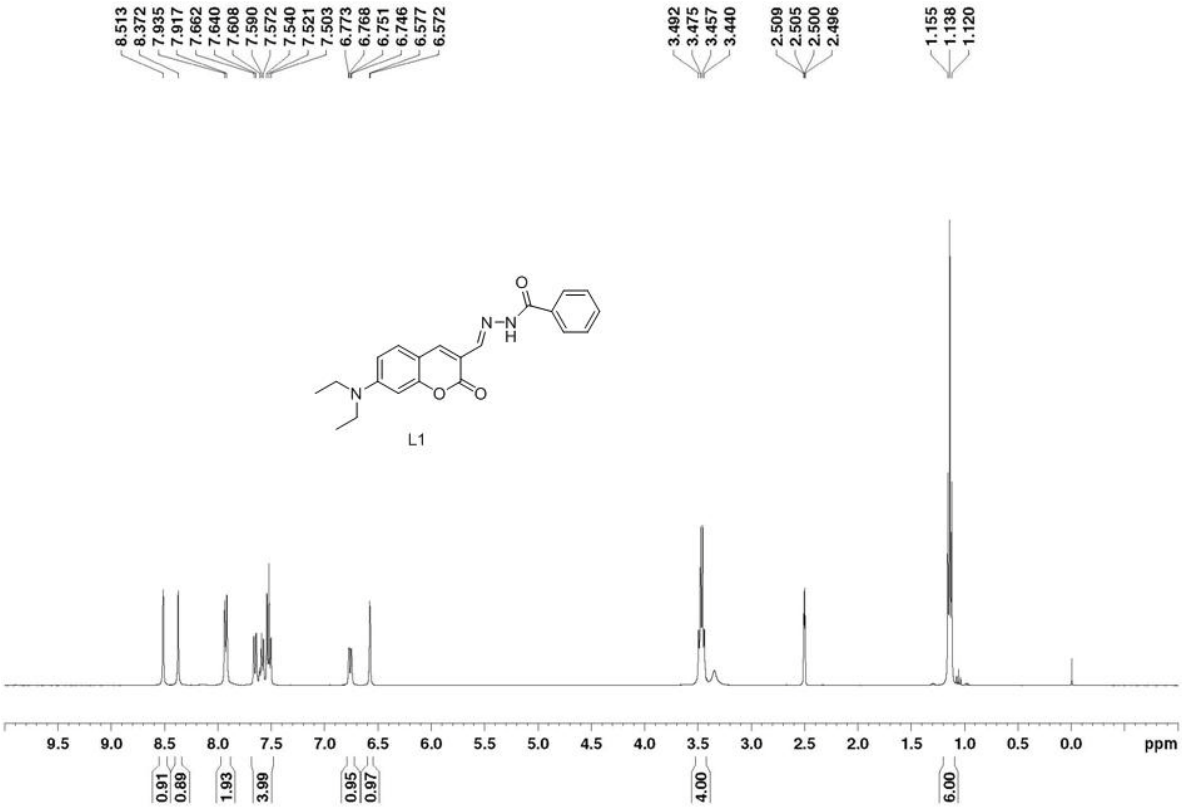


Fig.S16 ^{1}H NMR (d6-DMSO, 400 MHz) spectrum of **L1**.

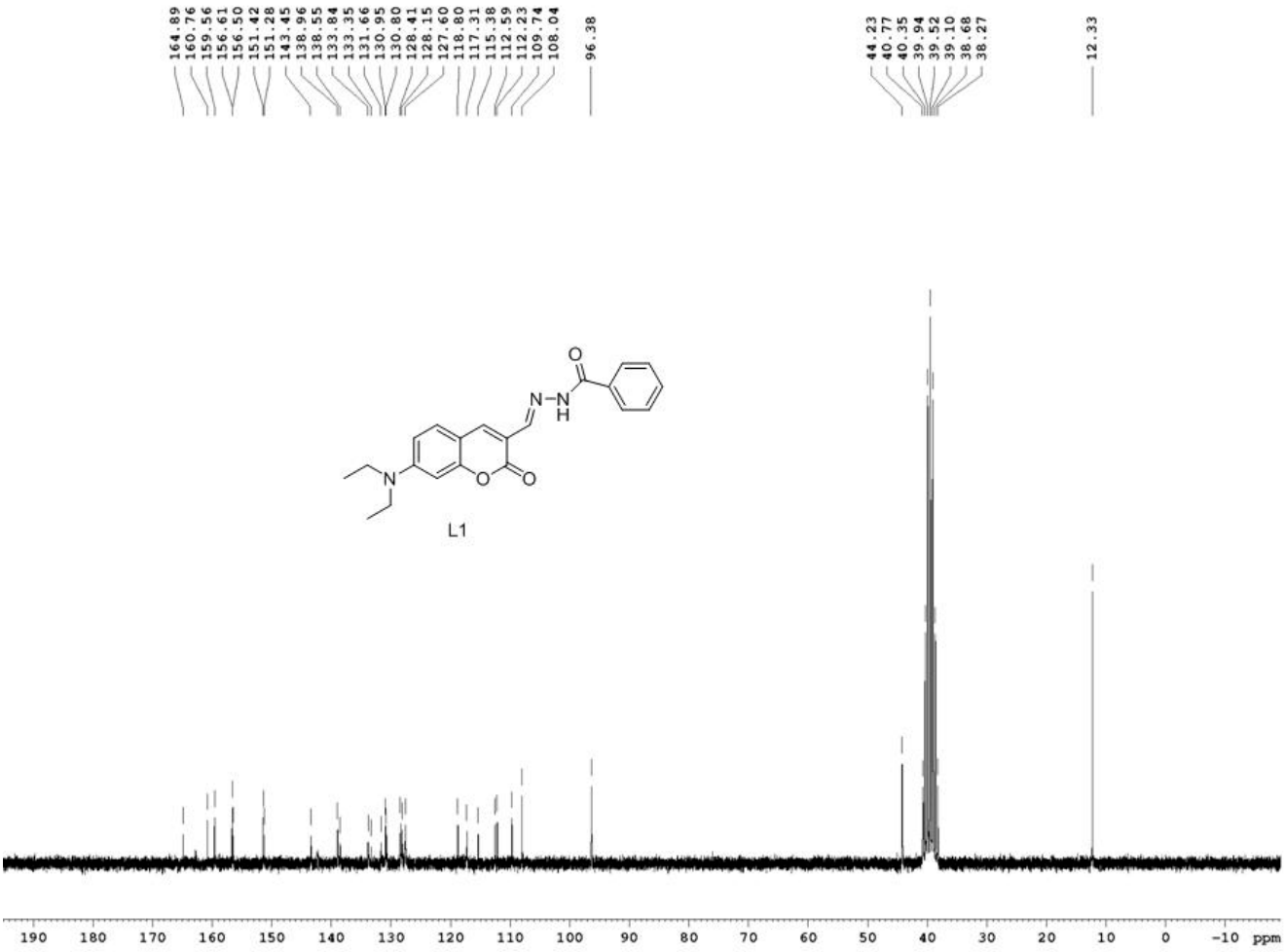


Fig. S17 ^{13}C NMR (d6-DMSO, 50MHz) spectrum of **L1**.

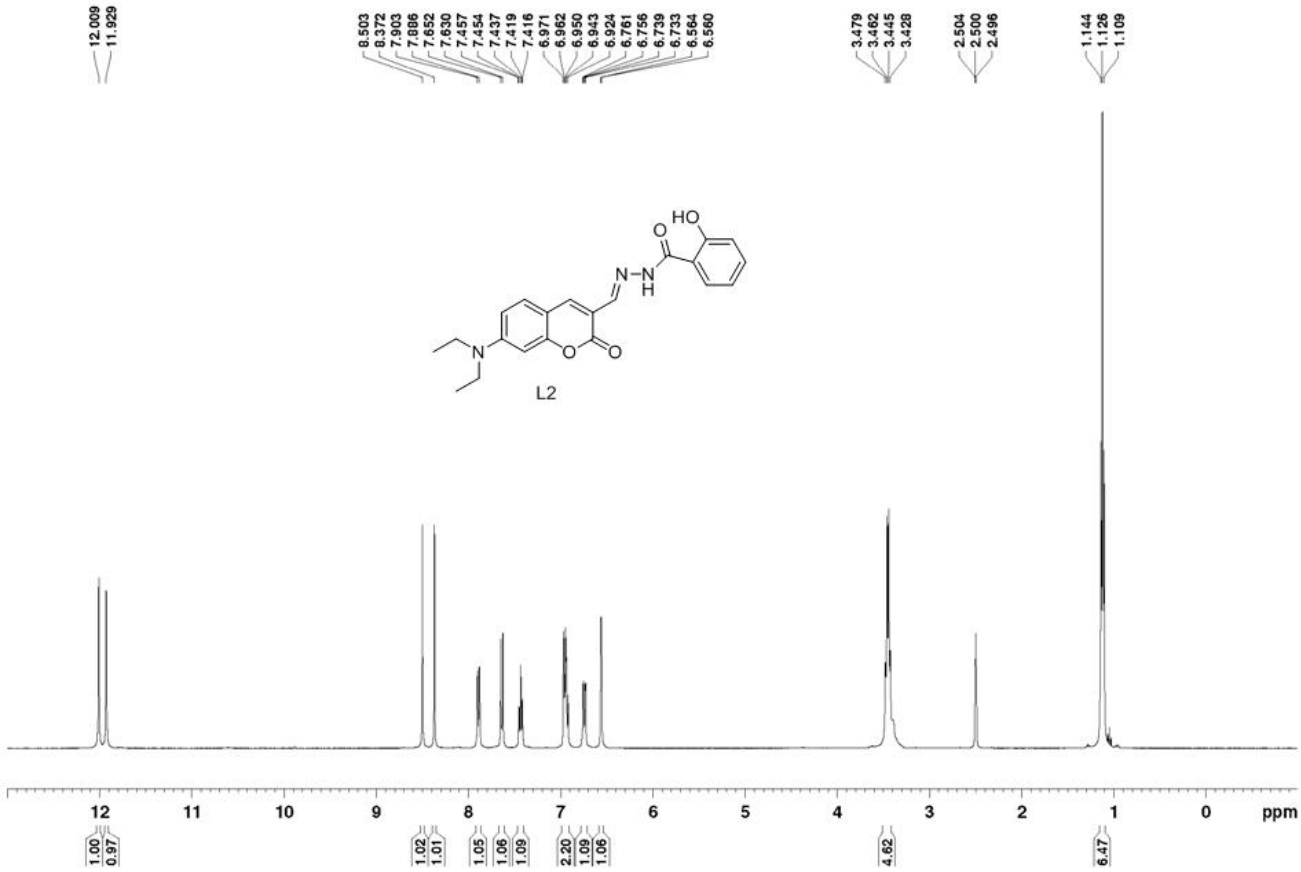


Fig. S18 ^{1}H NMR (d6-DMSO, 400 MHz) spectrum of **L2**.

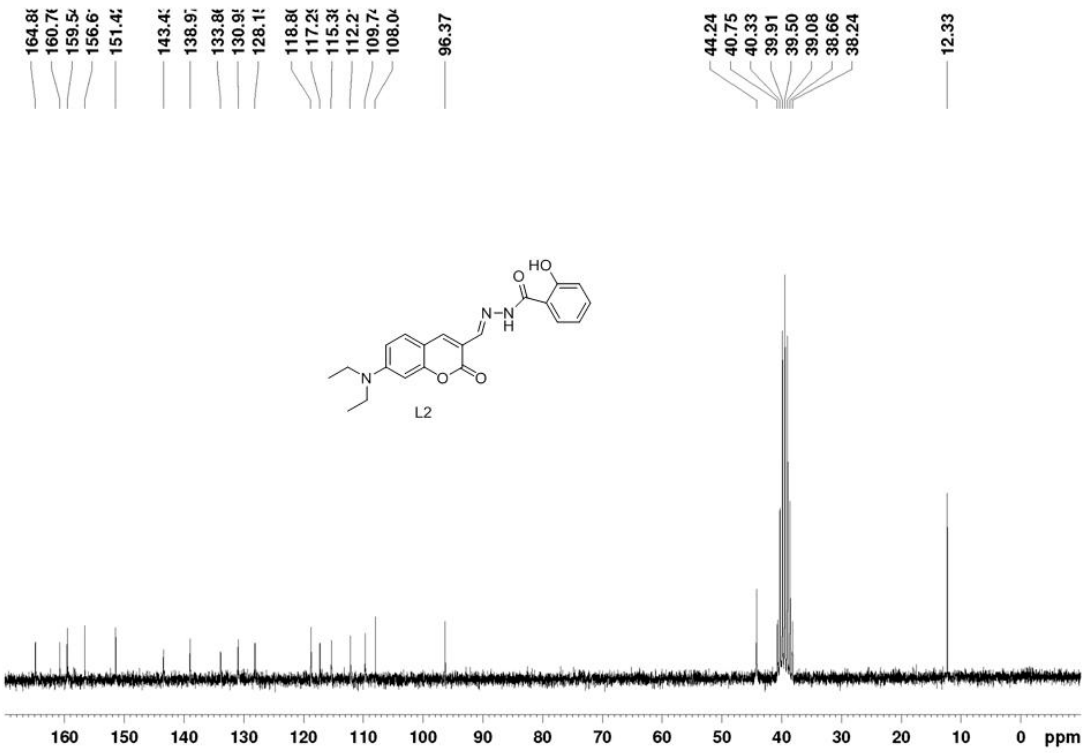


Fig. S19 ^{13}C NMR (d6-DMSO, 50MHz) spectrum of **L2**.

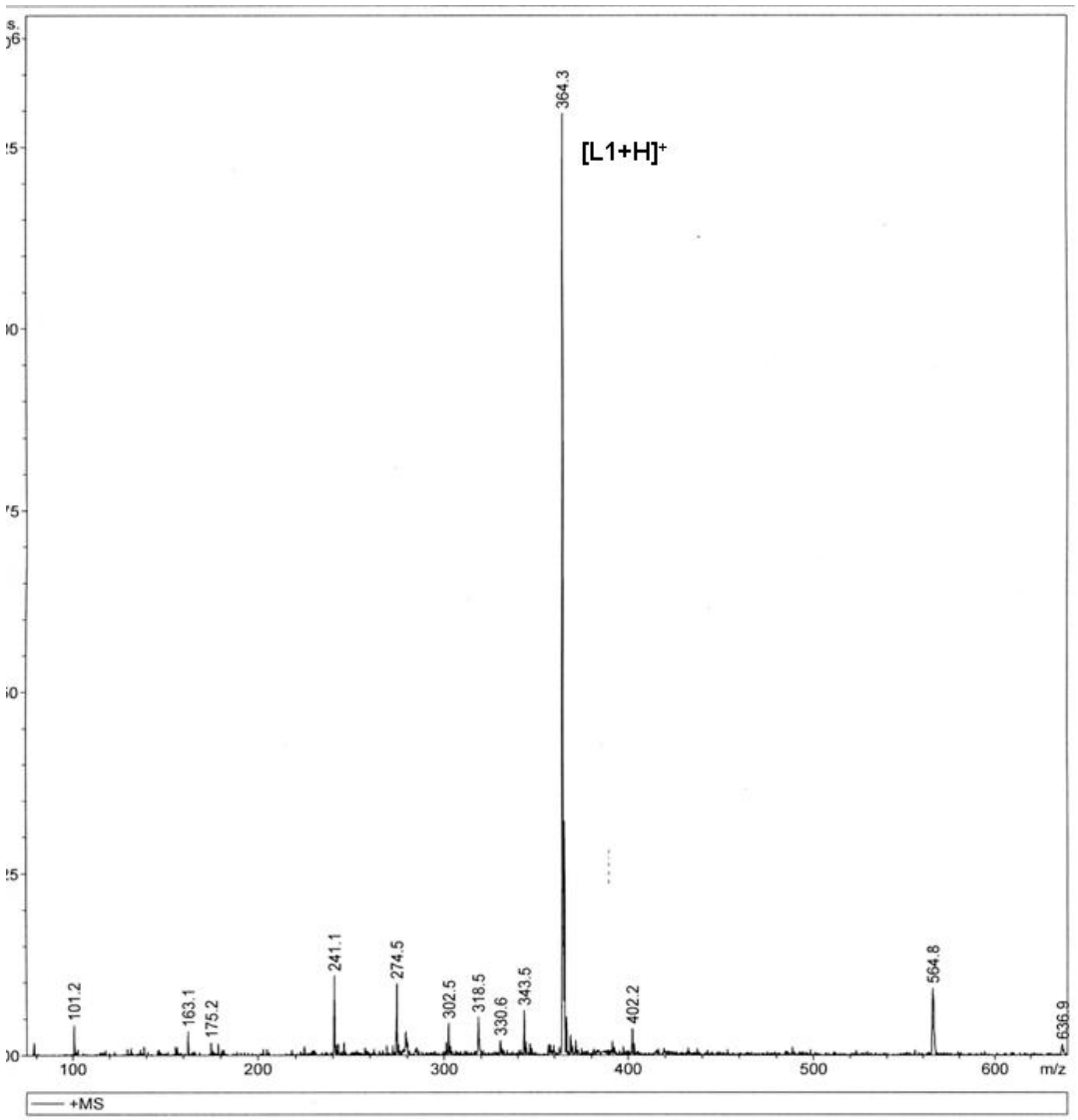


Fig. S20 ESI mass spectrum of **L1.**