Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Electronic Supporting Information

Water switched aggregation/ disaggregation strategies of a coumarin-naphthalene conjugated sensor and its selectivity on Cu^{2+} and Ag^{+} ions along with cell imaging study on human osteosarcoma cells (U-2 OS)

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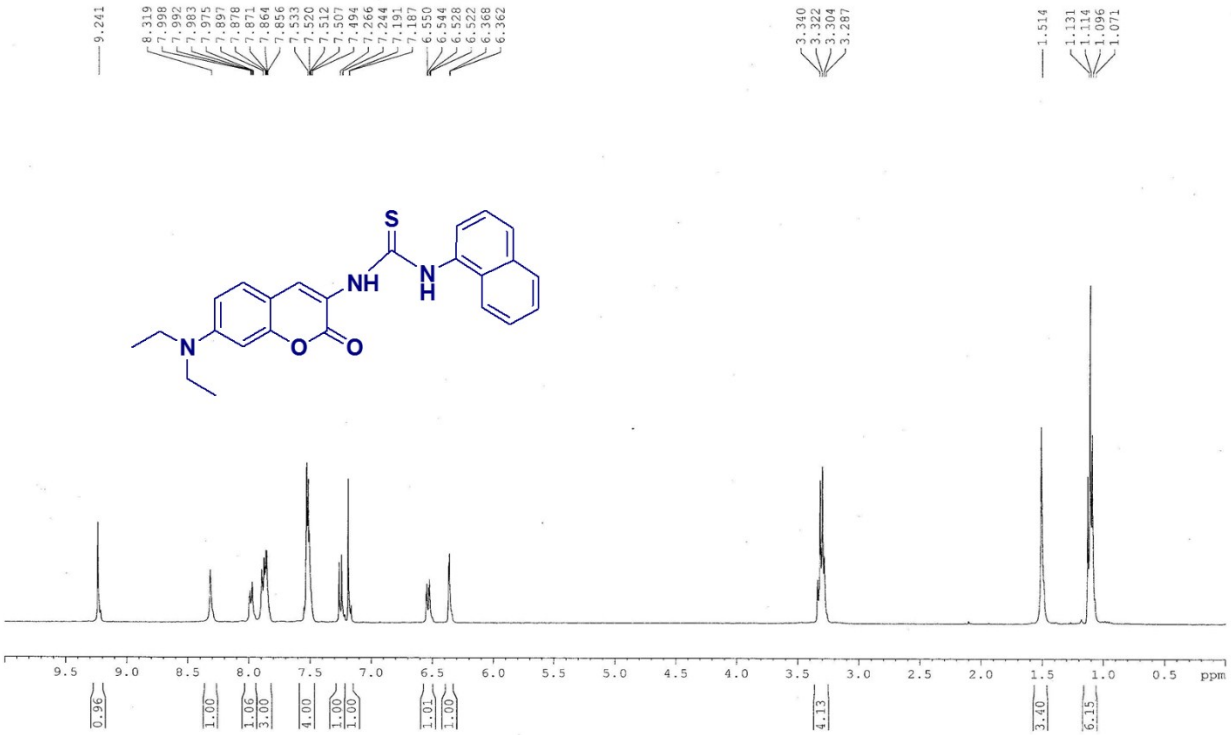


Fig. S1:^{1}H-NMR spectra ($CDCl_3$, 400 MHz) R1:

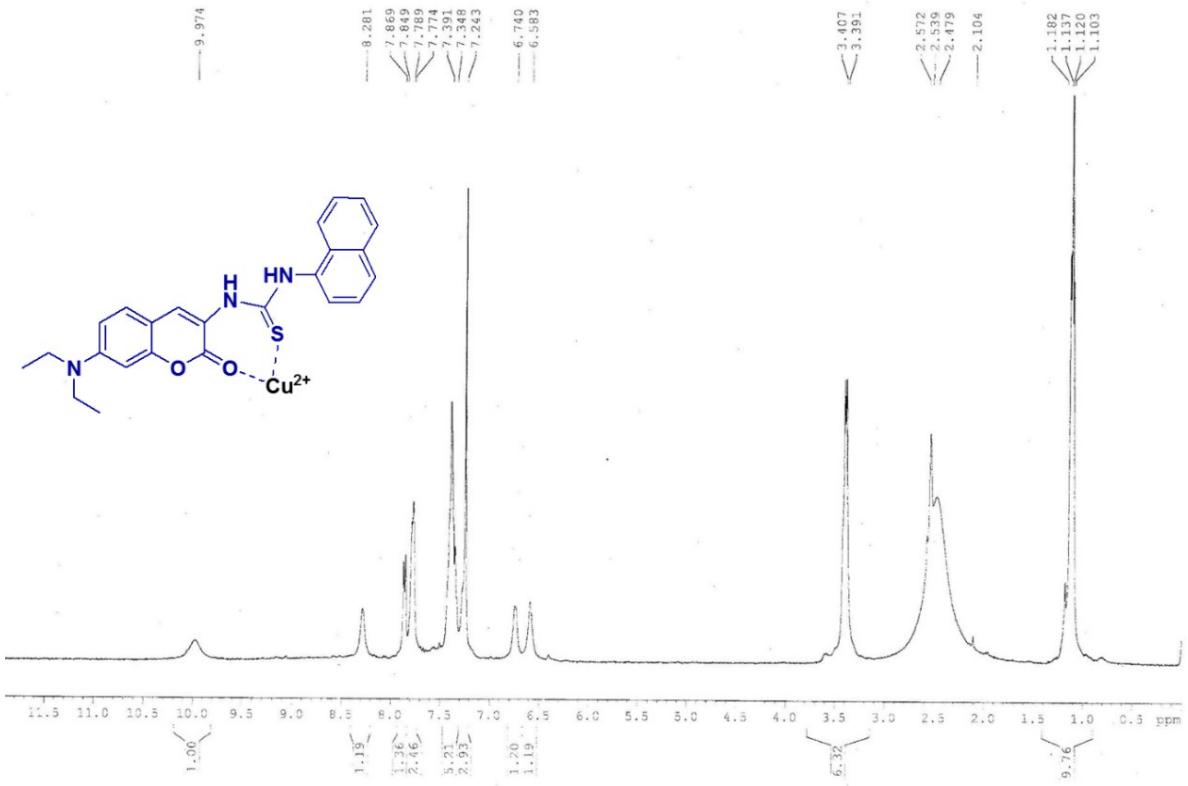


Fig. S2:^{1}H-NMR spectra ($CDCl_3$, 400 MHz) R1:Cu^{2+}:

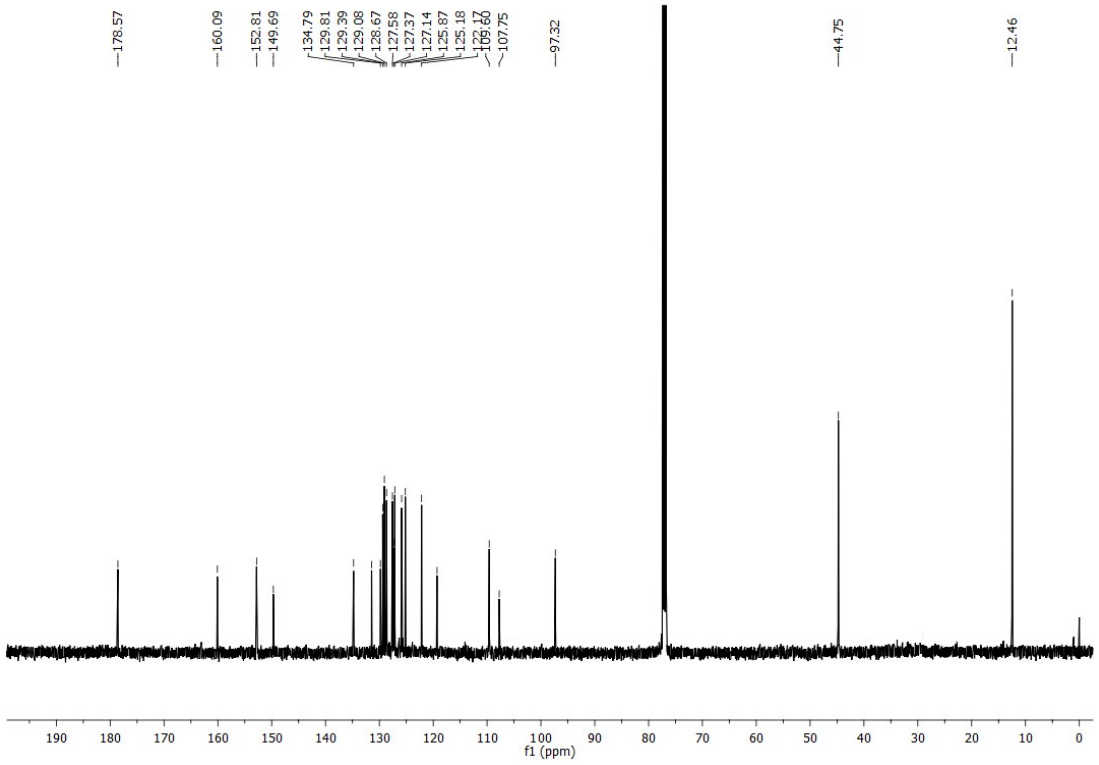


Fig. S3:^{13}C-NMR spectra ($CDCl_3$, 400 MHz) R1:

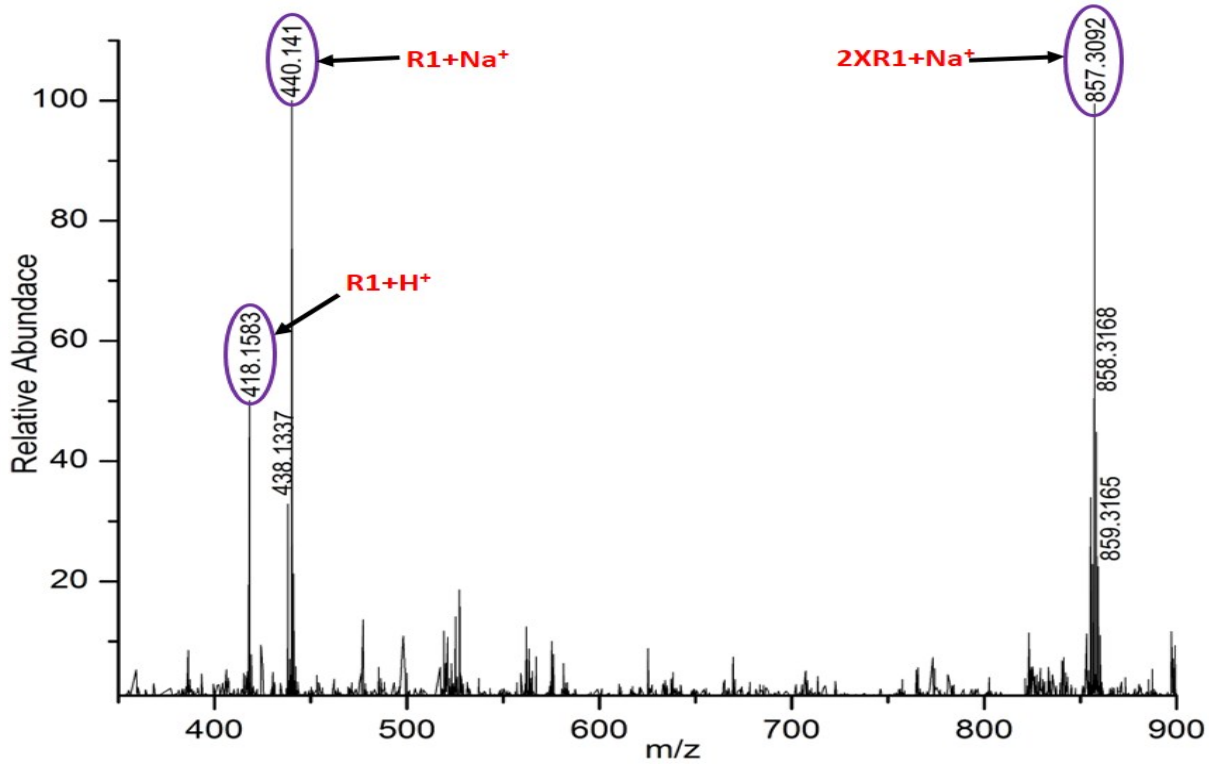


Fig. S4: ESI-MS of R1: m/z: calculated for$C_{24}H_{23}N_3O_2S$: 417.1511, Found: 418.1583 [$M+H^+$, 50], 440.141 [$M+Na^+$, 100], 857.3092 [$2M+Na^+$, 98]

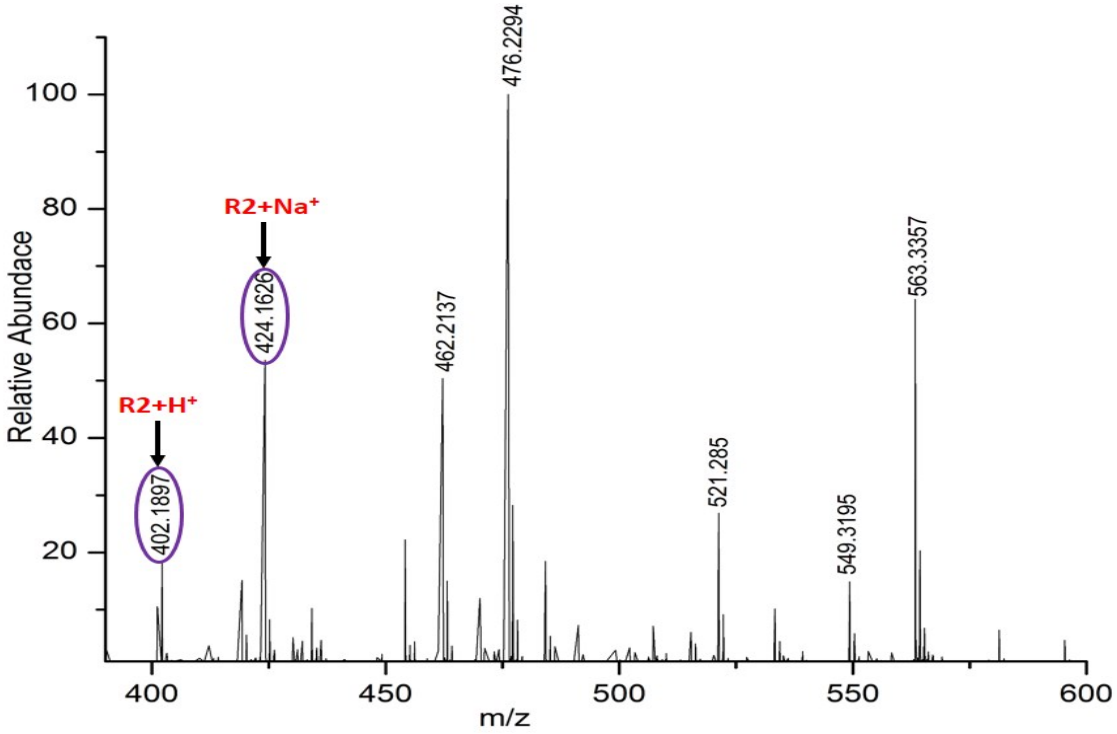


Fig. S5: ESI-MS of R2 (R1:Ag^+): m/z: calculated for $C_{24}H_{23}N_3O_3$: 401.1739, Found: 402.1897 [$M+H^+$, 20], 424.1626 [$M+Na^+$, 51].

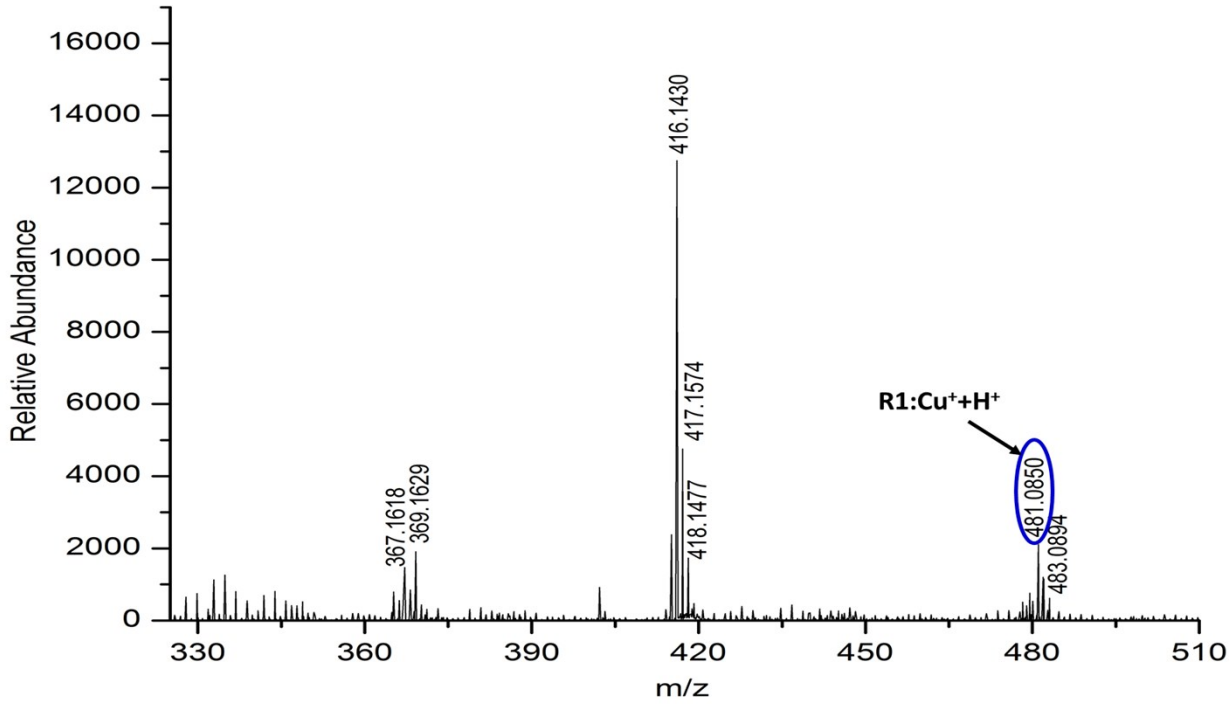


Fig. S6: ESI-MS of R2 (R1:Cu^{2+}): m/z: calculated for $C_{24}H_{23}N_3O_3$+ Cu^{2+} +H^+: 481.0869, Found: 481.0750 [M+Cu^{2+}+H^+].

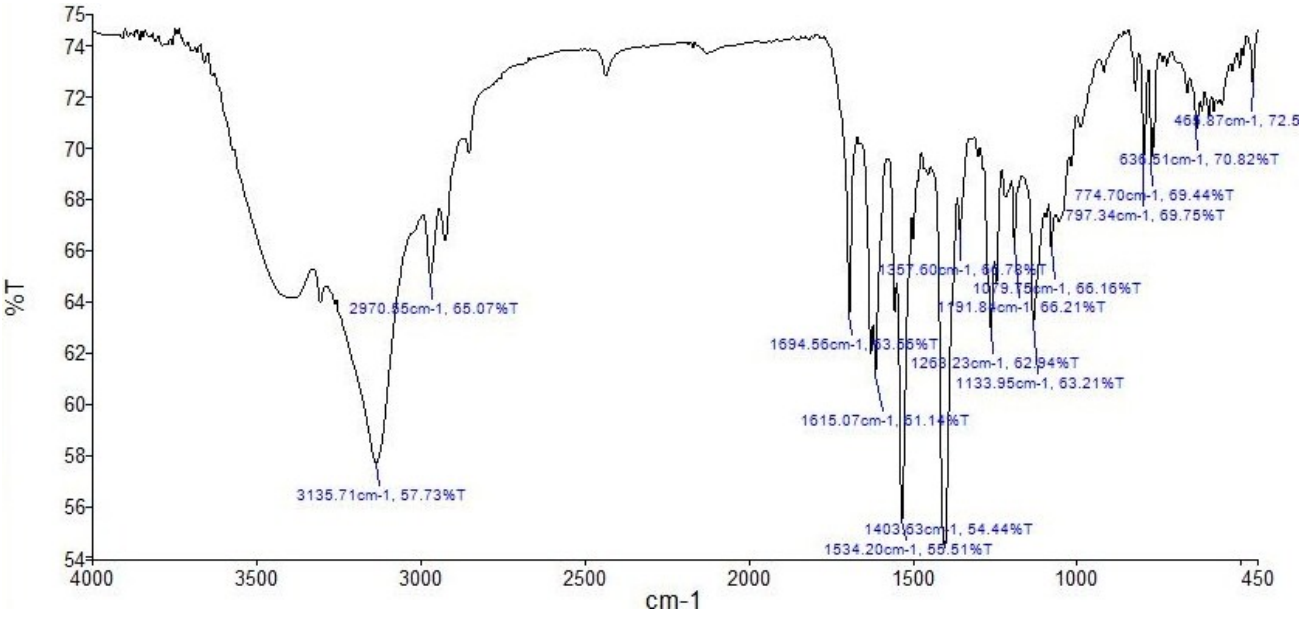


Fig. S7: FTIR spectra of **R1**

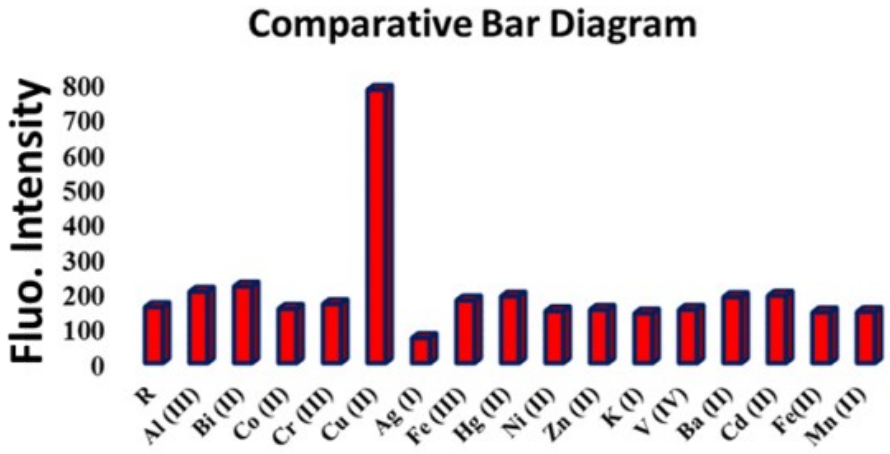


Fig. S8: Comparative studies of **R1** (5.4 μM) with different metal ions (10.0 equiv.)

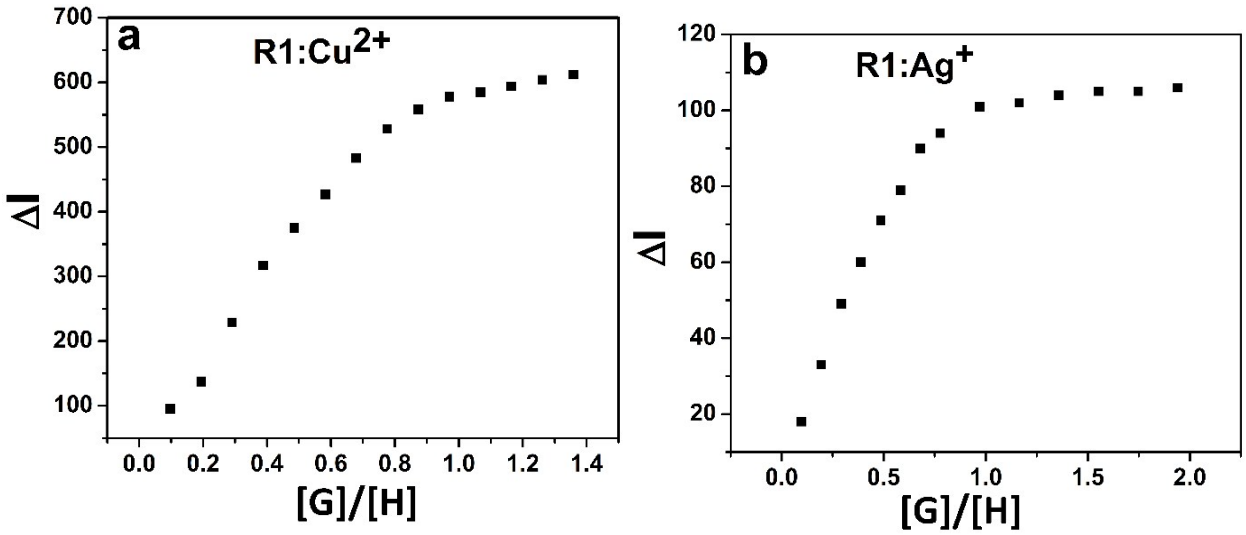


Fig. S9: (a) fluorescence titration curve of **R1** with continuously increased concentration of Cu^{2+} ions (b) with Ag^{+} ions.

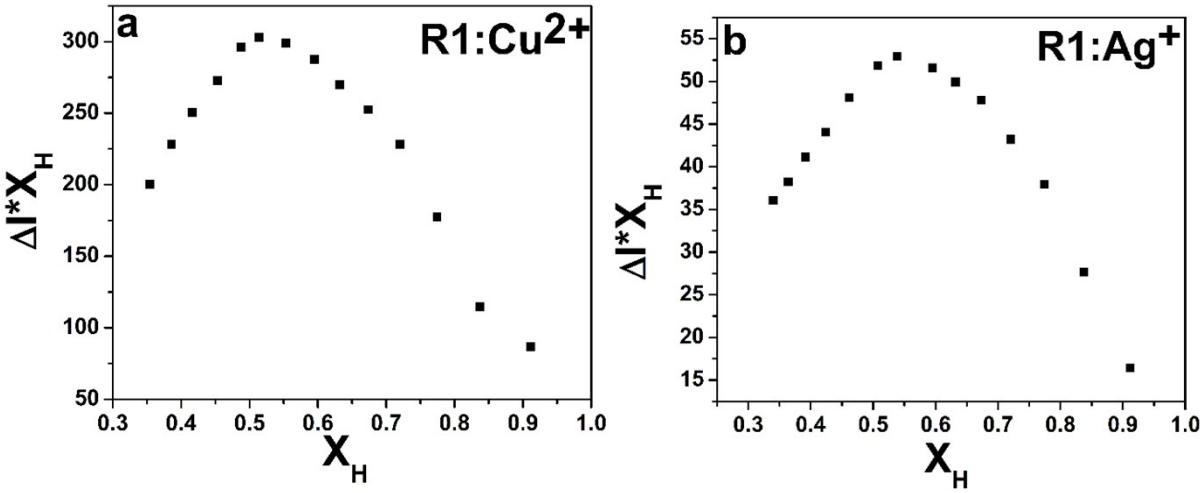


Fig. S10: Ratiometric analysis of complex using fluorescence titration data by using Job's plot (a) with Cu^{2+} (b) with Ag^{+}.

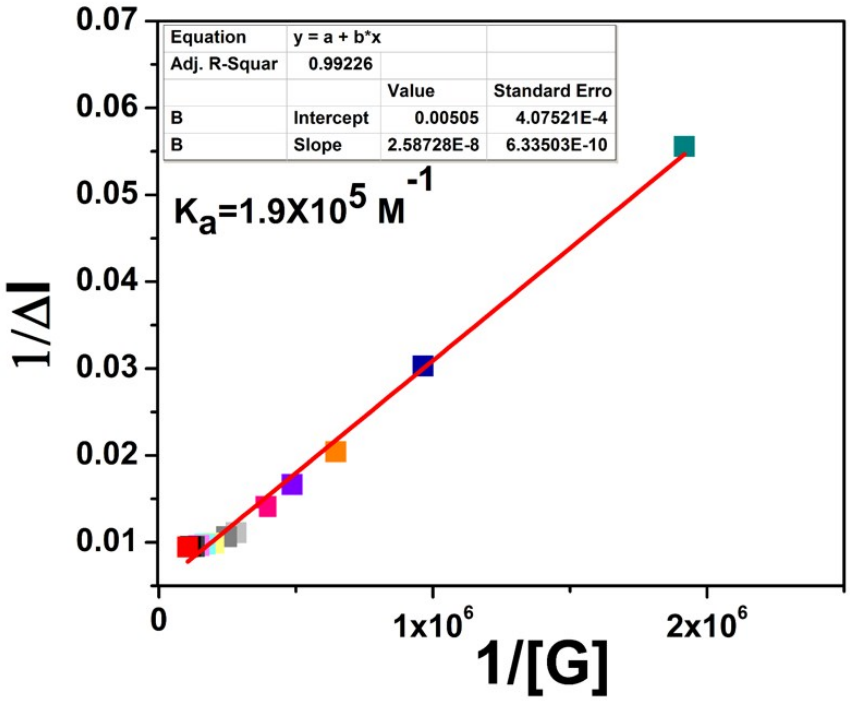


Fig. S11: Binding constant calculation was calculated using fluorescence spectra of complex R1:Cu^{2+}

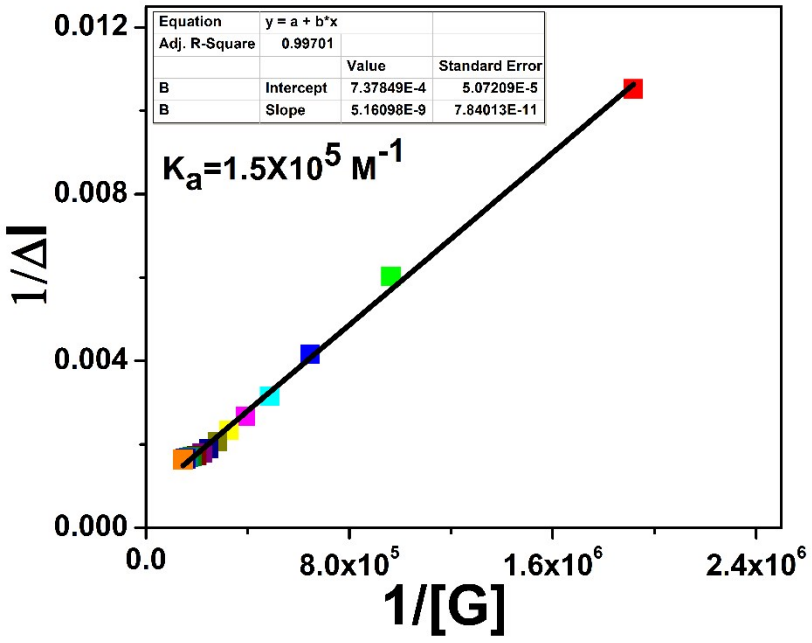


Fig. S12: Binding constant calculation was calculated using fluorescence spectra of complex R1:Ag^{+}

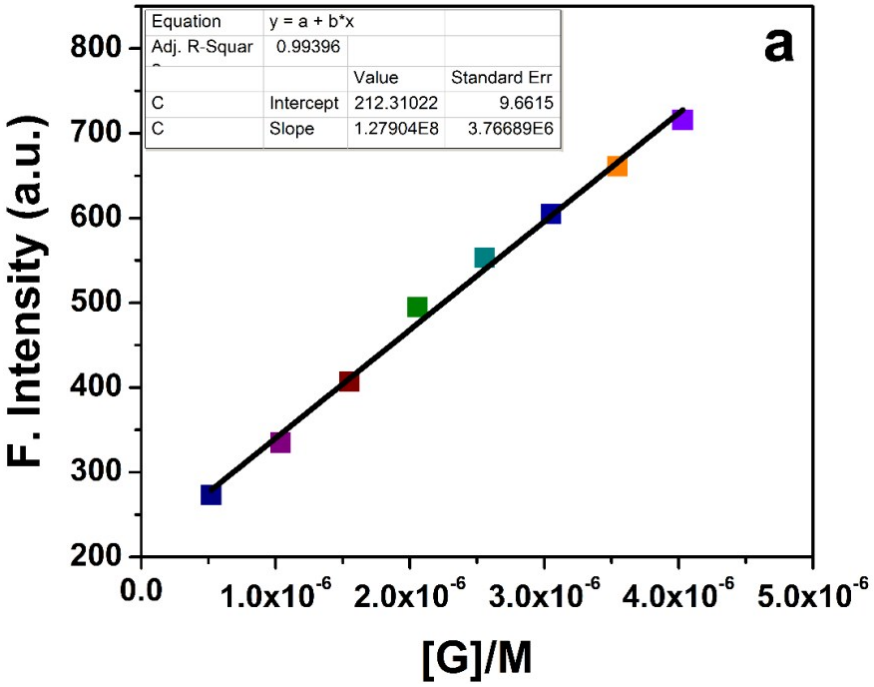
Calculation of limit of detection (LOD):

The detection limit of **R1** was calculated via fluorescence titration of R1:Cu^{2+} and R1:Ag^{+}. The standard deviation was calculated by taking 10 different values of fluorescence intensity of chemosensor **R1.** The limit of detection (LOD) was calculated using the following equation.

$$LOD = K \times SD/S$$

Where, K = 2 or 3 (we take 2 in this case); SD is the standard deviation (0.52) and S is the slope of the calibration curve.

From the linear fit graph slope of the complex R1:Cu^{2+} is 1.2×10^{8} and Complex R1:Ag^{+} is 2.3×10^{7}. After calculation of LOD using the abovementioned formula, and of the values were found as 8.1×10^{-9} M and 44.0×10^{-9} M for Cu^{2+} and Ag^{+} ions, respectively.



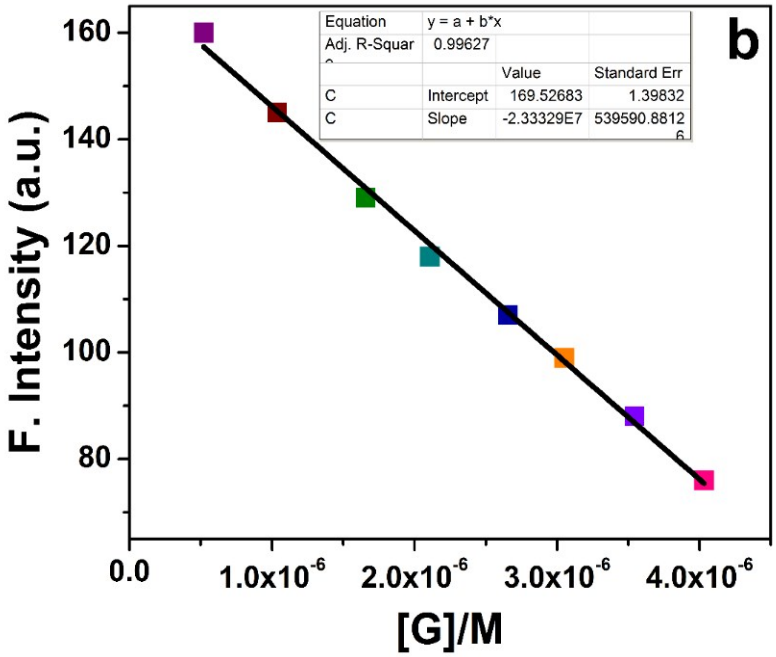


Fig. S13: Limit of Detection (LOD) of **R1** (a) against Cu^{2+} metal ions (b) against Ag^{+} ions.

Quantum yield calculation:

Fluorescence quantum yields (Φ) were calculated using the equation given below, using[1] quinoline sulfate ($\Phi_f = 0.55$ in water in 0.5 % H_2SO_4) as standard.

$$\Phi_u = \Phi_s \times \frac{I_u}{I_s} \times \frac{A_s}{A_u} \times \left(\frac{\eta_u}{\eta_s}\right)^2$$

Where, Φ_u and Φ_s are the fluorescence quantum yields of the sample and standard, I_u and I_s are the integrated emission intensities of the sample and standard, A_u and A_s are the absorbance of the sample and standard at the excitation wavelength (400 nm), and η_u and η_s are the refractive indices of the sample and standard solutions, respectively.

Bio-imagine analysis

The cell (U-2 OS) proliferation assay was carried out using different concentrations of the chemosensor **R1** in presence or absence of $Cu(ClO_4)_2$ and $AgNO_3$ (10, 25, 50, and 100μM) for 48h. The cells were grown in the presence of **R1** or $Cu(ClO_4)_2$ or $AgNO_3$ alone or in combination for 48h. Absence of any detectable loss in proliferation in U-2 OS cells indicates both probe and its metallation derivatives are tolerant to U-2 OS cell

growth in vitro. The cell proliferation remained unaffected by the compounds suggesting its cytocompatibility. Tumor cell proliferation reached >85% at a concentration 100µm indicating the compounds were safe for possible biological uses.

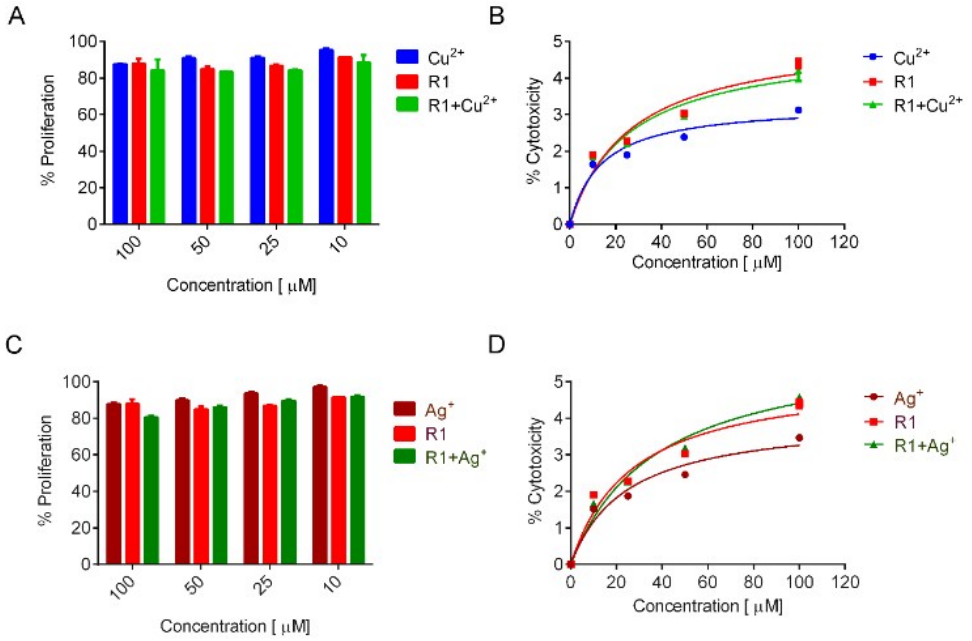


Fig. S14: Proliferation of U-2 OS cells in presence of Cu^{2+}(**A**) & Ag^{+} (**C**) contained Probe **R1**. Graphs show Cu^{+2} (**B**) & Ag^{+} (**D**) contained **R1** Probe on cytotoxicity of U-2 OS cells .

Direct cytotoxicity studies of the probe, $Cu(ClO_4)_2$ (**Fig.S14B**) or $AgNO_3$ (**Fig.S14D**) either alone or in combination against the U-2 OS cells were performed. The compounds are not cytotoxic to the cells at a concentration of 100µm. Percent cytotoxicity was less than 5% which is considered as non-significant. Taken together, these data suggest that the above compounds are safe and non-toxic to live cells (**Fig. S14B & D**).

The in vitro blood compatibility of the **R1** was determined by % hemolysis & % viability of lymphocytes & monocytes. Metallation of the coumarin based receptor (**R1**) was also found to be tolerant to peripheral blood mononuclear cells (lymphocytes and monocytes), which constitutes the major fraction of the mononuclear white blood cells (WBC), comprising T, B, NK cells as well as monocytes and dendritic cells. Cell viability data suggests that like U-2 OS cells, PBMC was also unaffected by the compounds with minimum loss of cell viability at highest concentration (100µm), which was found to be

not significant (**Fig. S15 A**). Like PBMC, human RBC remains unaffected by **R1**. The hemolysis experiment has been conducted at different concentrations (10, 25, 50, & 100 μM) of the samples and the % hemolysis caused by the **R1** probe was presented in (**Fig. S15 B**).

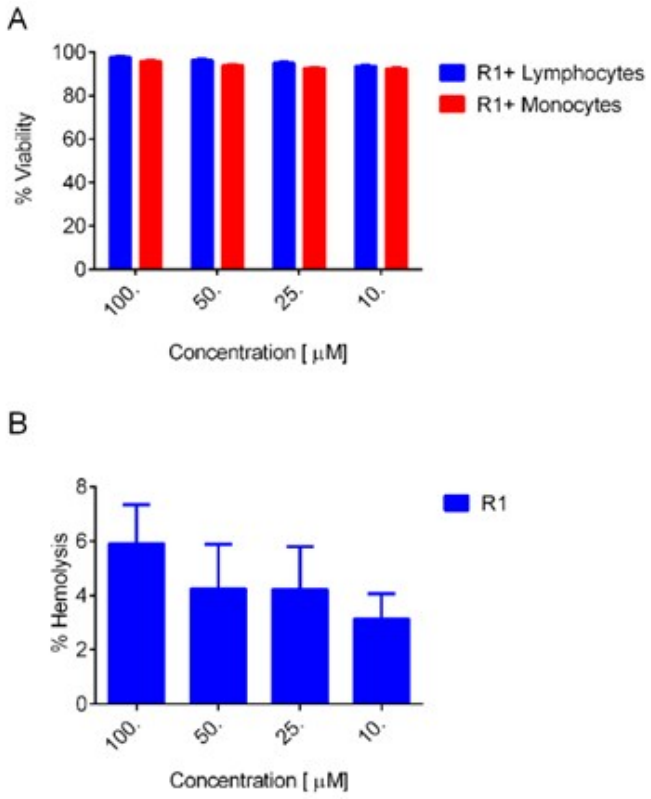


Fig. S15: Viability of human lymphocytes & Monocytes (A) and hemolysis of RBC (B) in presence of **R1**

References

1 D. Wu, W. Huang, C. Duan, Z. Lin and Q. Meng, *Inorg. Chem.,* 2007, **46**, 1538.