Electronic Supplementary Data

A Ratiometric Triazine-based Colorimetric and Fluorometric Sensor for the Recognition of Zn^{2+} Ions and its application in human lung cancer cells

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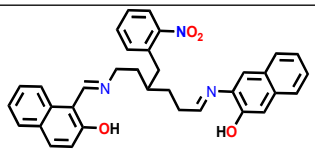
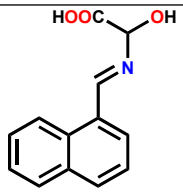
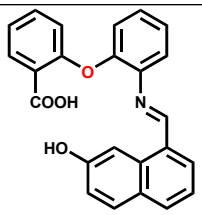
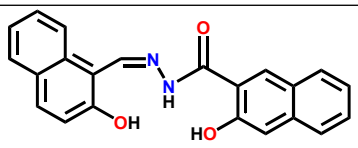
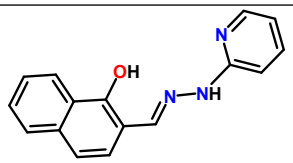
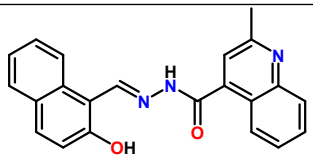
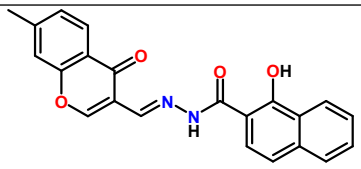
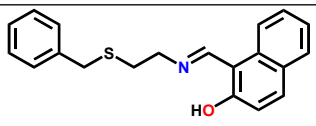
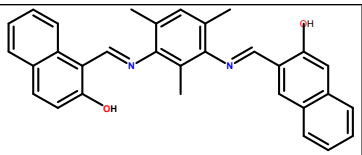
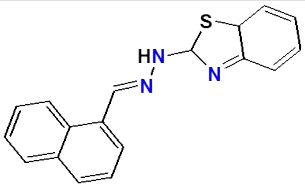
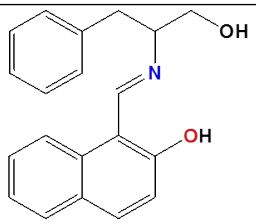
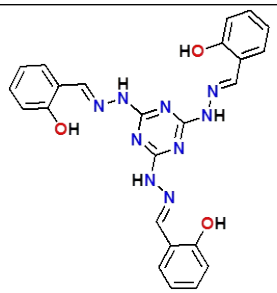
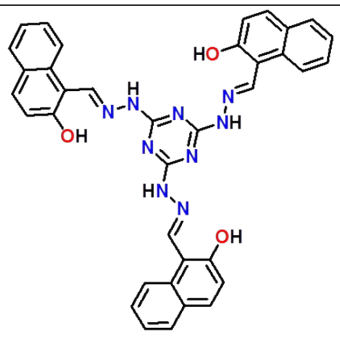
Table S1 Comparison between the previously reported zinc sensors with the current work.

Sl. No	Probe structure	LOD	Solvent	Mode of sensing	Application	Ref.
1		3.1×10^{-7} M	ACN:DMF	Inhibition of ESIPT and C=N isomerization	Logic gate	1
2		21×10^{-6} M	aqueous buffer Tris-HCl, (pH 7.5)	Inhibition of C=N isomerization and ICT	-	2

3		10×10^{-6} M	ACN	-	-	3
4		1.01×10^{-6} M	THF: H_2O	Inhibition of C=N isomerization and ICT	Cell imaging	4
5		0.17×10^{-6} M	aqueous buffer Tris-HCl, (Ph 7)	CHEF process	-	5
6		3.18×10^{-7} M	EtOH: H_2O	Inhibition of ESIPT and PET process	-	6
7		1.7×10^{-7} M	Ethanol	Inhibition of C=N isomerization and PET	-	7
8		5.03×10^{-7} M	EtOH/HEPES buffer	inhibition of PET and C=N isomerization	cell imaging	8

9		1.47×10^{-7} M	ACN:H_2O	Inhibition of ESIPT and C=N isomerization	cell imaging	9
10		6.5×10^{-5} M	MeOH/H_2O (7/3, V/V, pH 7.3)	-	-	10
11		10×10^{-6} M	aqueous buffer:CH_3OH	Inhibition of PET and CHEF	Logic gate	11
12		1.22×10^{-7} M	Ethanol-water (1:1)	Inhibit ESIPT	Cell imaging	Present-work
13		1.13×10^{-7} M	Ethanol	Inhibit ESIPT	-	Present-work

2. Experimental section

2.1. General information and chemicals

All the chemicals employed for synthesis were acquired from Sigma-Aldrich and utilized without further purification. Solvents used for the experimental rationale were all of analytical

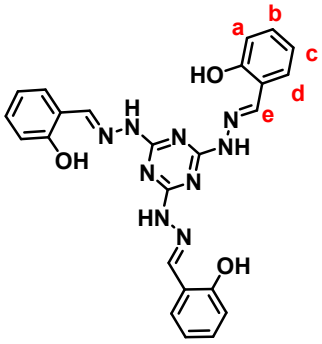
grade. Perchlorate salts of relevant cations were exploited for the preparation of the stock solutions. ^{1}H NMR spectra was recorded on a Brucker 500 MHz tool. For NMR spectra, $CDCl_3$ was exploited as solvent using TMS as an internal standard. Chemical shifts are expressed in d units and ^{1} H–^{1} H and ^{1} H–C coupling constants are in Hz. The following abbreviations were used to define NMR peak patterns: s = singlet, d = doublet, t = triplet, dd = doublet of doublets, ddd = doublet of doublets, td = triplet of doublets, br = broad, m= multiplet. The coupling constants are given in Hertz (Hz) and, wherever possible, assignment of protons is made. The carbons in the molecular skeletons were not necessarily numbered following the IUPAC nomenclature rules; numeration was entirely done for assigning NMR signals. UV-Vis titration experiments were executed on a JASCO UV-V530 spectrophotometer and fluorescence experiments were done using a PerkinElmer LS 55 fluorescence spectrophotometer using a fluorescence cell of 10 mm path length.

2.2. Preparation of the receptor

Synthesis of compound 1: Synthesis of 2,4,6-trihydazino-1,3,5-triazine (THDT)

The receptor THDT was produced via a simplistic and rapid single-step reaction (Scheme 1). 0.5 g (27.1 mmol) of cyanuric chloride was added to a 40.0 g (81.3 mmol) of hydrazine hydrate and the resulting mixture was stirred vigorously for 3 hrs at 115 °C. After 3 hrs, THDT was collected by means of filtration. To eliminate any unreacted hydrazine hydrate, the residue was washed several times with water to achieve pH = 7. A white powder was attained after vacuum drying at 40 °C.

2.2.1. Synthesis of compound R1



THDT (250 mg, 1.460 mmol) in dry DMF (20.0 ml) was taken in a round bottomed flask. Salicylaldehyde (4.38 mmol, 0.46 ml) is added dropwise into this solution for 15 minutes followed by 1 drops of acetic acid. Subsequently the whole reaction mixture was refluxed for 6 h. By verifying TLC, the completion of the reaction is monitored. After completion of the

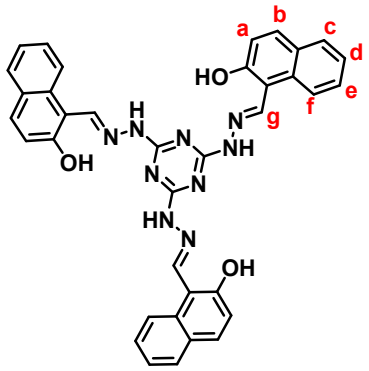
reaction, excess amount of water is added to get the precipitate. The crude residue was collected by filtration and then purified by column chromatography using 40% EtOAc in ether to obtain pure **R1** (solid, grey colour, 76% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.86-6.89 (m, 6H, [b,c]-H), 7.21-7.23 (m, 3H, d-H), 7.40-7.66 (m, 3H, a-H), 8.38 (s, 3H, e-H), 11.0-11.5 (brs, 6H, [OH, NH]-H), ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 116.8, 119.6, 120.3, 129.5, 130.9, 157.5, 162.8, 170.1 ppm.

HRMS: *m/z* (%) = 484.1935 (100) [**R1**+H]$^{+}$

2.2.2. Synthesis of compound (R2)



THDT (250 mg, 1.46 mmol) in dry DMF (20.0 ml) was taken in a round bottomed flask. 2-hydroxy naphthaldehyde (4.38 mmol, 0.46 ml) was inserted into the THDT solution followed by the addition of 1 drop of acetic acid. The entire reaction mixture was refluxed for 6 hrs. By monitoring TLC, the completion of the reaction was scrutinized. After the reaction was finished excess amount of water is added in the reaction mixture to obtain precipitate. The crude residue was collected by filtration and extracted the organic fraction from the inorganic fraction by using chloroform with the help of a separating funnel. Finally the product was purified by column chromatography by using 40% EtOAc in ether to acquire pure (**R2**) (solid, yellow color, 79% yield).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.24 (d, 3J = 8.8 Hz, 3H, b-H), 7.40 (t, 3J = 8.0 Hz, 3H, d-H), 7.61 (t, 3J = 8.0 Hz, 3H, e-H), 7.85-7.93 (m, 4H, [c,f]-H), 8.12 (brs, 3H, a-H), 9.37 (brs, 3H, g-H), 11.43 (brs, 3H, OH-H), 12.9 (brs, 3H, NH-H) ppm.

^{13}C NMR (100 MHz, DMSO-d_6): δ = 109.7, 119.8, 121.2, 124.2, 128.4, 128.6, 129.8, 132.3, 132.6, 143.7, 158.2, 164.6 ppm.

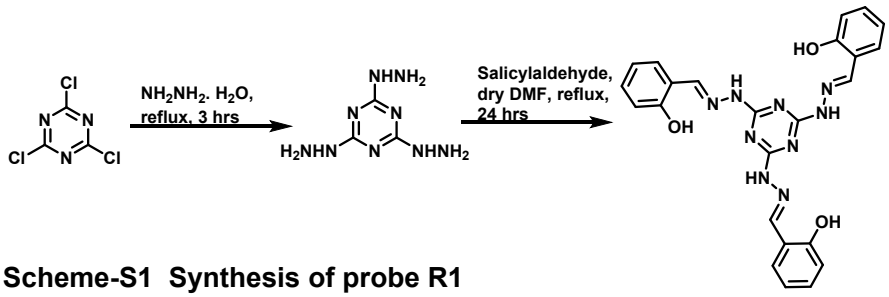
ESI-MS: *m/z* (%): 634.3 (100) [**R2**+H]$^+$

2.2.3. Synthesis of Zn-R1 complex

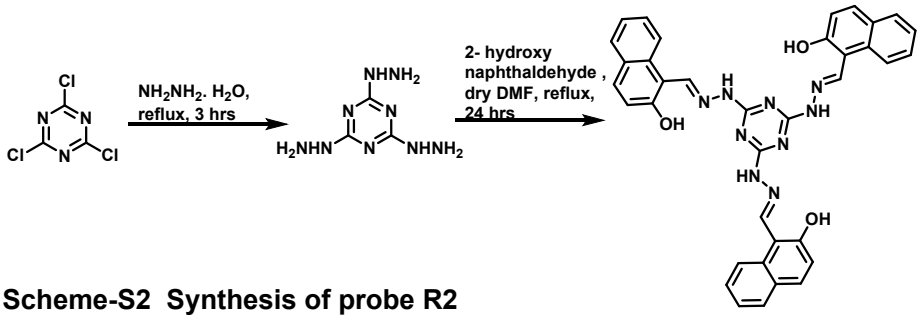
Zn^{2+} complex of sensor is synthesized by adding the sensor (100 mg, 0.206 mmol) into a ethanolic solution of $Zn(ClO_4)_2$ (231.06 mg, 0.620 mmol) and the entire mixture is refluxed for 1 h. The solvent is eradicated under vacuum and the entire mass is washed with diethyl ether several times. Ultimately a deep brown colour solid is obtained (70 mg, 64%) which is characterized by mass spectroscopy (ESI-MASS) and ^{1}HNMR.

2.2.4. Synthesis of Zn- R2 complex

Zn^{2+} complex of R2 was produced by adding the probe R2 (100 mg, 0.206 mmol) into an ethanolic solution of $Zn(ClO_4)_2$ (231.06 mg, 0.620 mmol) and the resulting mixture was refluxed for 1 hr. The solvent was eliminated under vacuum and the entire mass is washed with diethyl ether several times. Finally a deep yellow colour solid was accomplished (70 mg, 64% yield) which was consequently characterized by mass spectroscopy (ESI-MASS) and ^{1}HNMR spectroscopy.



Scheme-S1 Synthesis of probe R1



Scheme-S2 Synthesis of probe R2

3. General method of UV-Vis and fluorescence titrations

3.1. UV-Vis method: For UV-vis titrations, a stock solution of receptor R1 (1.07×10^{-5} M) and R2 (1.26×10^{-5} M) were arranged by dissolving 0.13 mg in 25 mL of mixed solvent EtOH-tris buffer (1/1, v/v) and 0.158 mg in 25.0 mL anhydrous ethanol solution respectively.

The solutions of the guest cations using their perchlorate salts were prepared in the order of (1.79×10^{-4} M) in HEPES buffer at pH = 7.2 in 10 ml of the identical solution for the receptor R1 and the order of (1.20×10^{-4} M) in 25 ml ethanol medium is prepared for the receptor R2. Analogous concentrations were taken for other metal ions to ascertain the potential interference. The spectra of these solutions were recorded by means of a UV-Vis method.

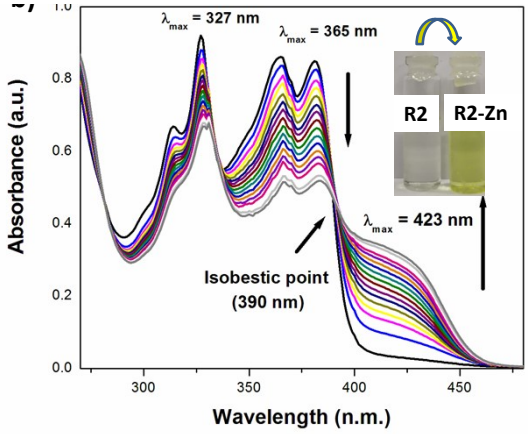


Figure S1: Absorption spectra of R2 (1.26×10^{-5} M) upon steady addition of Zn^{2+} (0 to 0.55 mL), Inset: Photograph illustrating the visible color change of R2 before and after the addition of Zn^{2+}.

3.2. Fluorescence method: For fluorescence titrations, the concentrations of the stock solutions of receptors and metal ions reserved the same as exploited for UV-Vis titration. Here as well the solutions of the guest cations using their perchlorate salts were prepared in the order of (1×10^{-4} M) in 10 ml. Equivalent concentrations were taken for other metal ions to establish the potential interference. The spectra of these solutions were recorded by means of a fluorescence technique.

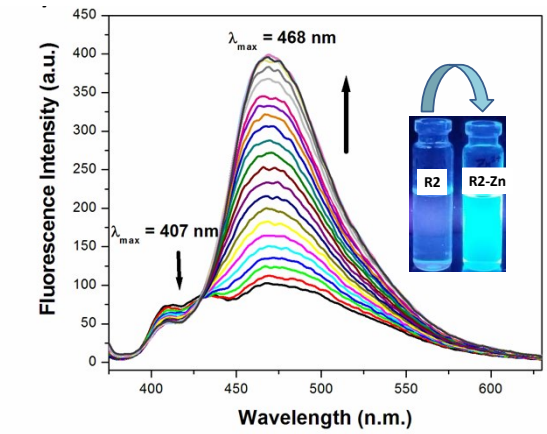


Figure S2: Emission spectra of **R2** (10 mM) upon steady addition of Zn^{2+} (0 to 0.55ml), Inset: photograph demonstrating the visible emission color change of R2 before and after Zn^{2+} (0.55ml) addition.

Bar diagram:

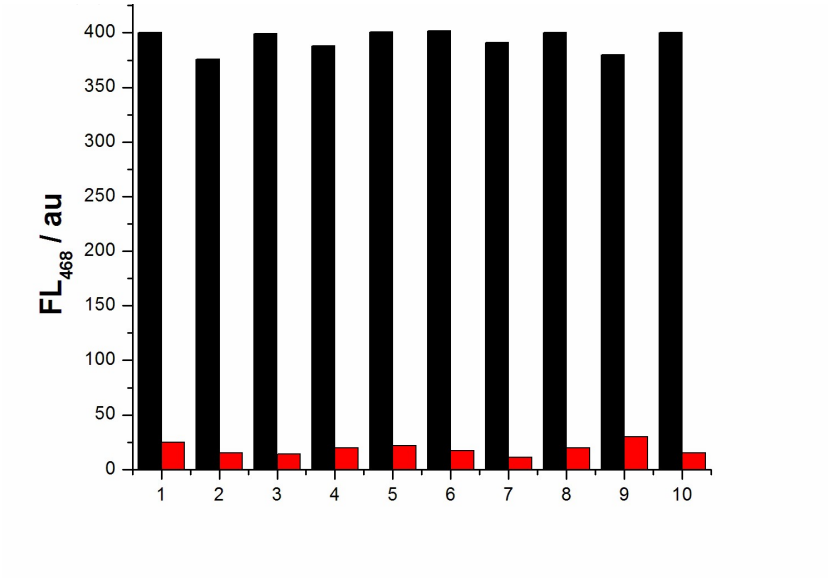
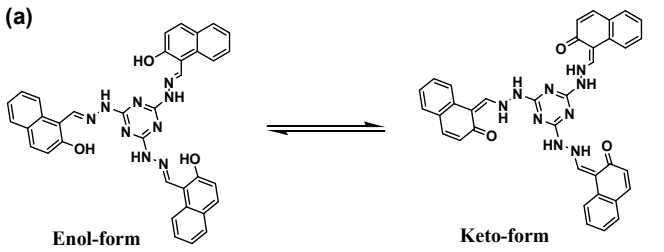
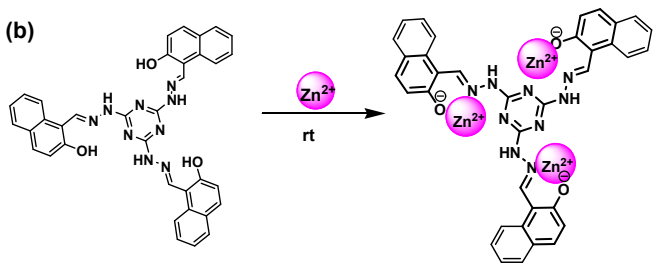


Figure S3: R2 (1.26×10^{-5} μM) upon addition of diverse species (1×10^{-4} μM); Red bars: Probe + other species; Black bars: Probe + other species + Zn^{2+}. Legend: 1) $Fe(ClO_4)_2$, 2) $Cu(ClO_4)_2$, 3) $AgClO_4$, 4) $Al(ClO_4)_3$, 5)$Cd(ClO_4)_2$ 6) $Ca(ClO_4)_2$, 7) $Cr(ClO_4)_3$ 8) $Mn(ClO_4)_2$ 9) $Mg(ClO_4)_2$ and 10) $Ni(ClO_4)_2$ (λ_{ex} = 375 nm; λ_{em} = 468 nm).

Mechanism of Zn^{2+} sensing of the probe R2:





Scheme S3: a) Probable ESIPT approach of the probe R2 from keto-enol tautomerism. b) Plausible binding mode of the R2 with Zn^{2+}.(ESIPT off – CHEF on)

4. Quantum chemical DFT method

For determination of the binding mode of the receptors R1 and R2 with Zn^{2+}, we executed quantum chemical DFT calculation using the Gaussian 09 program with the assist of the Gauss View visualization program. Both the receptors R1 and R2 were optimized using the B3LYP/6-311G+(d, p) basis set and for the complex B3LYP/LanL2MB basis set was utilized. Subsequently we performed the excitonic calculations by means of time-dependent density functional methods (TD-DFT).

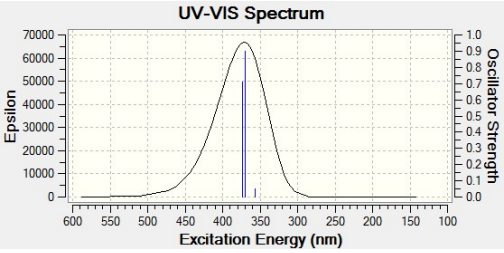
Table S2 The vertical main orbital transition of the receptor R1 calculated by TDDFT method.

Energy (eV)	**Wave length (nm)**	**Osc. strength (f)**	**Transition**
3.4398	360.45	0.0101	**HOMO→LUMO**
3.6455	340.10	0.2211	**HOMO→LUMO+1**
3.6751	337.36	3.6751	**HOMO-1→LUMO**

Table S3 The vertical main orbital transition of the receptor R2 calculated by TDDFT method.

Energy (eV)	**Wave length (nm)**	**Osc. strength (f)**	**Transition**
3.3203	373.41	0.7119	**HOMO→LUMO**

3.3514	369.95	0.9014	**HOMO→LUMO+1**
3.4822	356.06	0.0479	**HOMO-1→LUMO**



FigureS4: Absorption spectra of the probe R2.

5. Job's plot of the receptors (R1 and R2) for Zn^{2+}

Job's plots were drawn by plotting $\Delta F.X_{host}$ *vs* X_{host} (ΔF = change of intensity of the emission spectrum at 339 nm for R1 and 379 nm for R2 during titration and X_{host} is the mole fraction of the host in each case respectively).

a) b)

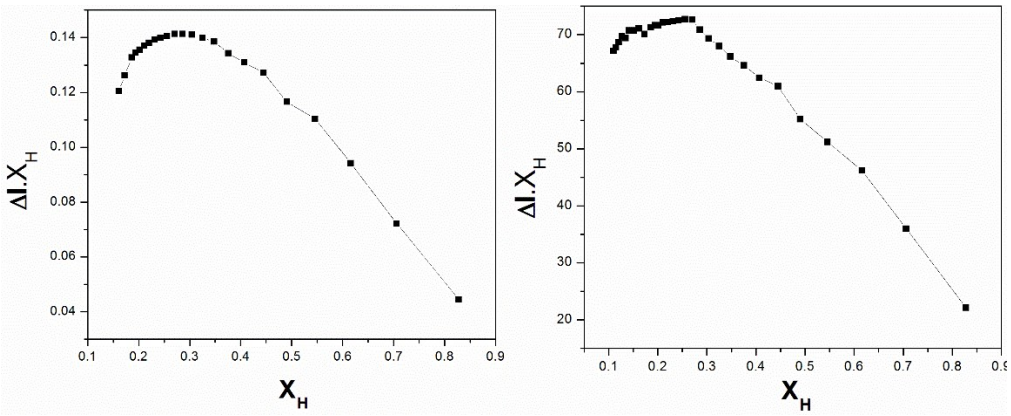


Figure S5 a) Job's plot of R1 with Zn^{2+} using UV-Vis data and b) Job's plot of R1 with Zn^{2+} using fluorescence data.

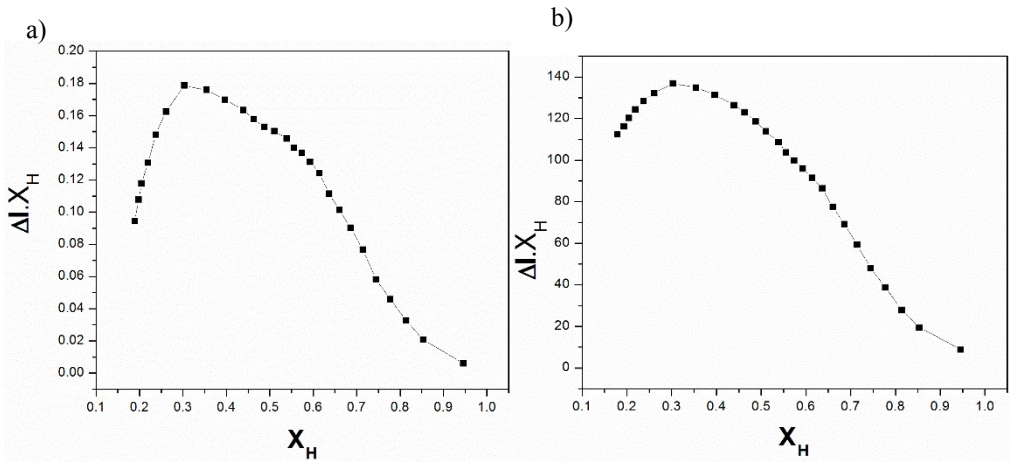


Figure S6 a) Job's plot of R2 with Zn^{2+}using UV-Vis data and b) Job's plot of R2 with Zn^{2+}using fluorescence data.

6. Determination of binding isotherm of metal co-ordinate complexes

Binding isotherms were drawn by plotting ΔI *vs* [G]/[H] [(ΔI = change of intensity of the emission spectrum at 339 nm for R1 and 379 nm for R2 throughout titration and [G] is the concentration of the metal ion and [H] is concentration of the receptors in each case respectively).

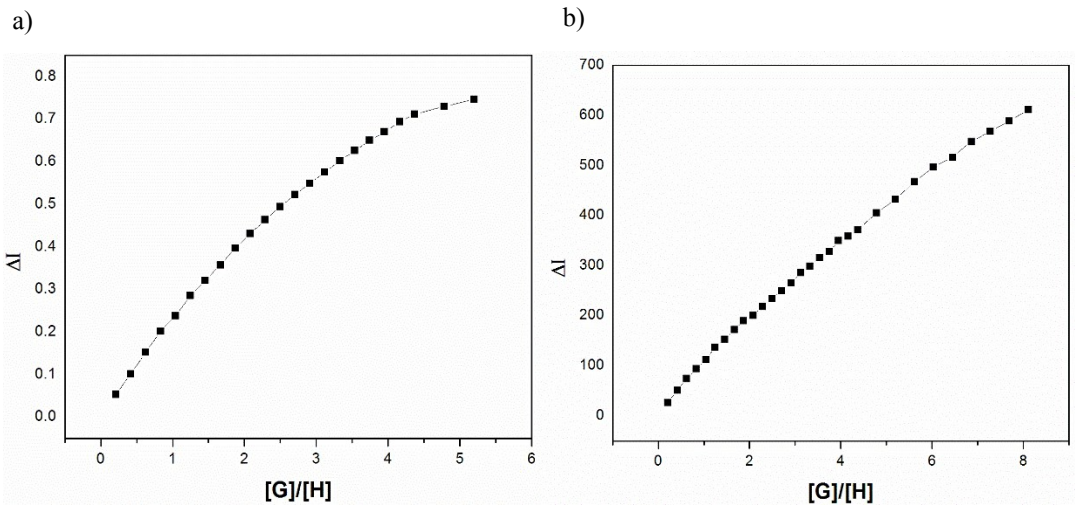


Figure S7 a) Binding isotherm of R1 with Zn^{2+} using UV-Vis data b) Binding isotherm of R1 with Zn^{2+} using FL data.

b)

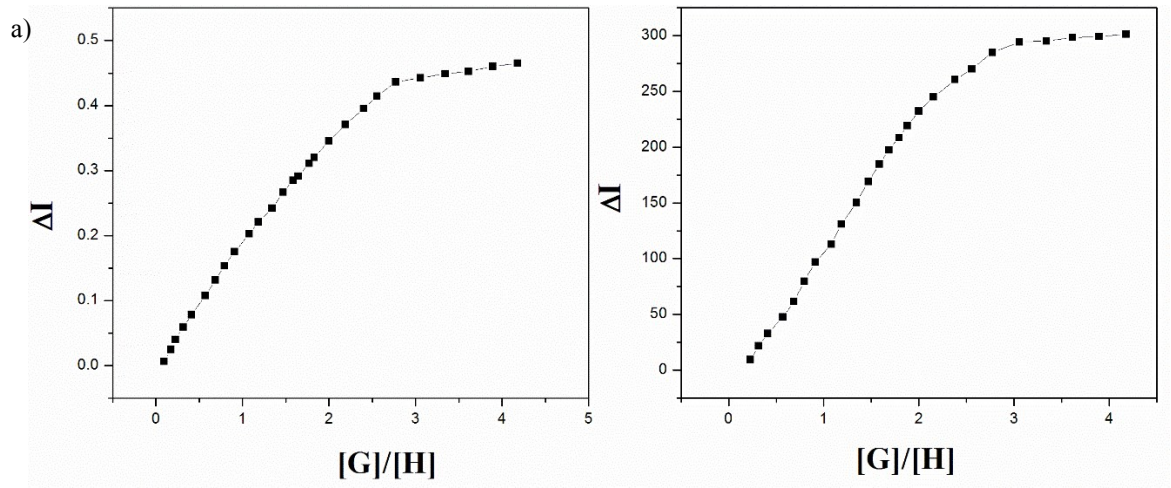


Figure S8a) Binding isotherm of R2 with Zn^{2+} using UV-Vis data b) Binding isotherm of R2 with Zn^{2+} using FL data.

7. Determination of binding constant value (K_a) using linear method of the receptors R1 and R2 for the recognition of Zn^{2+}.

Binding constant value (K_a) were calculated by plotting $1/\Delta I$ *vs* 1/[G] [(ΔI = change of intensity of the emission spectrum at 339 nm for **R1** and 379 nm for **R2** during titration and [G] is the concentration of the metal ion in each case respectively).

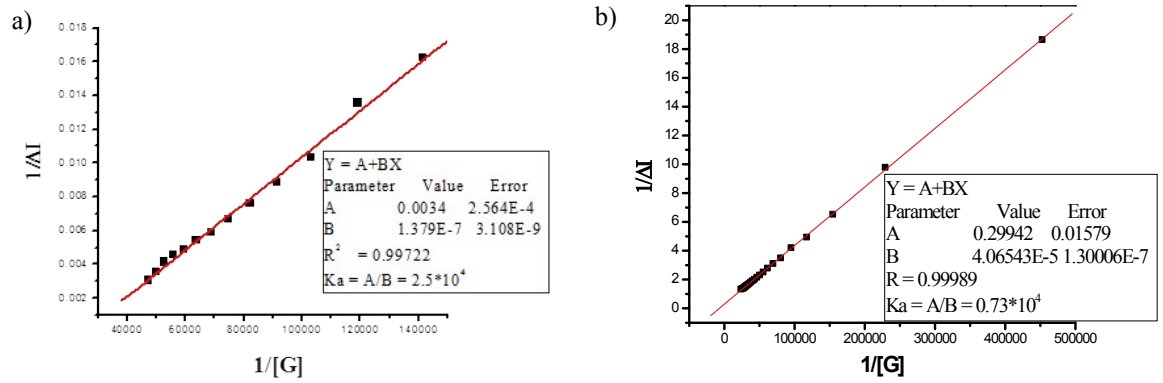


Figure S9 a) Binding constant value of **R2** with Zn^{2+} using FL data b) Binding constant value of **R1** with Zn^{2+} using FL data.

8. Determination of fluorescence quantum yields

The fluorescence quantum yield is calculated by the following equation:

$$\varphi_S = \varphi_A \times F_A/F_S \times A_A/A_S \times \eta_A^2/\eta_S^2$$

Where F_A and F_S are the integrated fluorescence intensity of the receptors (R1 and **R2**) and Perylene (reference) respectively. A_A and A_S are the absorbance of the receptors (R1 and **R2**)

and Perylene (reference) respectively at same excitation wavelength. η_A and η_S are the refractive indices of respective solvents employed as Acetonitrile and Ethanol.

The values obtained for quantum yield are 18% for **R1**and 12% for **R2.**

9. Determination of the Response time of probe towards the product

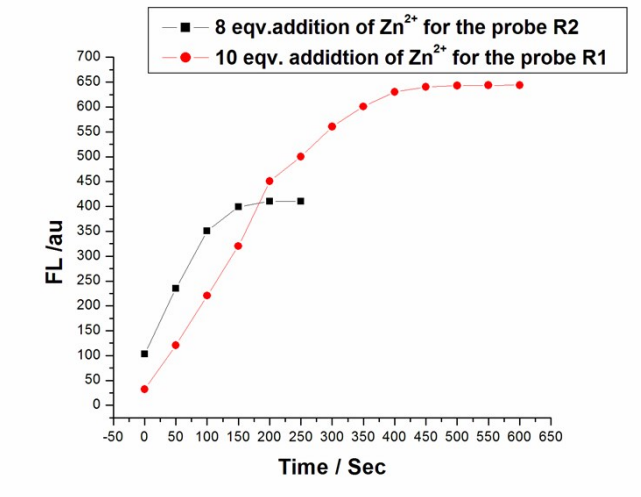


Figure S10 a) Time periods (0–130 Sec) of fluorescence enhancement of probe **R2** (1.26×10^{-5} M) in EtOH solution b) Time periods (0–550 Sec) of fluorescence enhancement of probe **R1** (1.07×10^{-5} M) in EtOH-trisbuffer (1/1, v/v) solution. (λex = 459 nm; for R1 and λex = 468 nm for R2).

10. NMR spectra: ^{1}H NMR, ^{13}C NMR

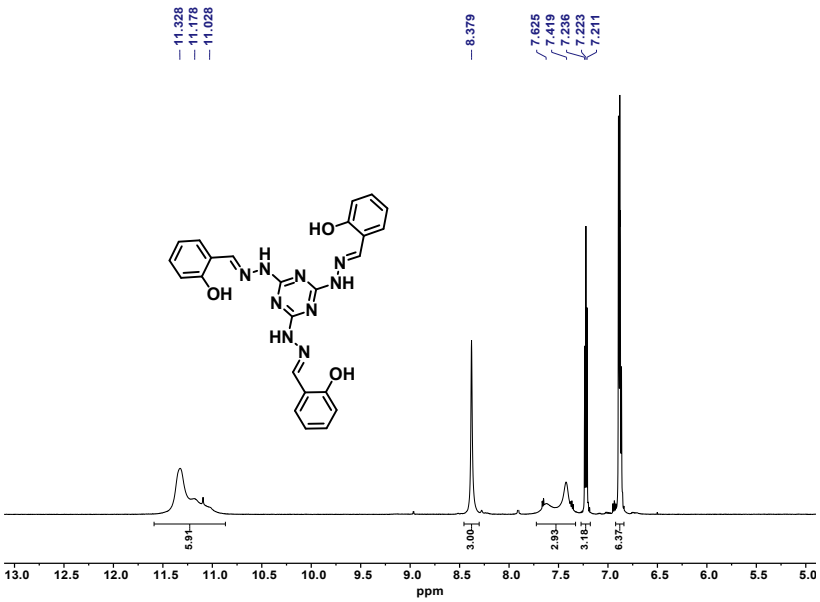


Figure S11: ^{1}H NMR spectrum of **R1** in DMSO-d_6 (400 MHz, 298 K).

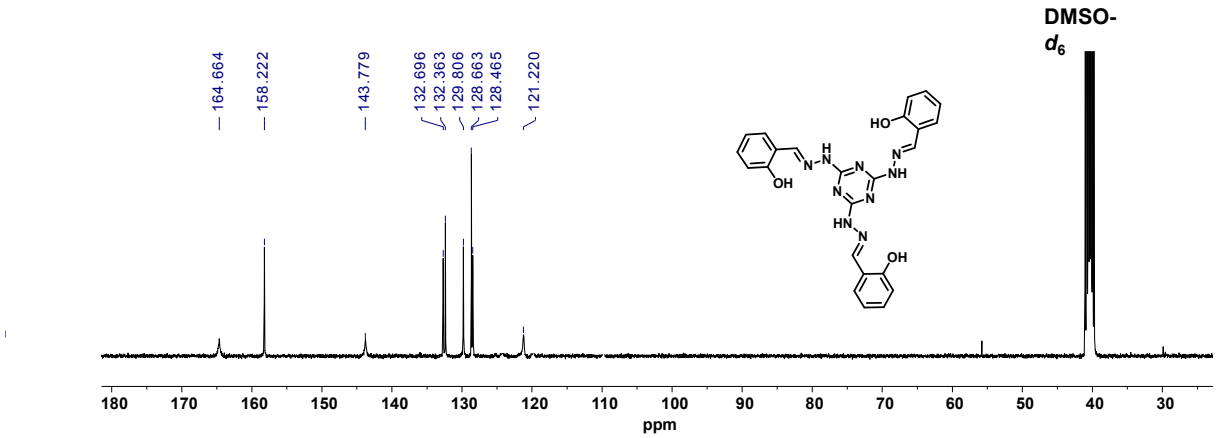


Figure S12: ^{13}C NMR spectrum of **R1** in DMSO-d_6 (100 MHz, 298 K).

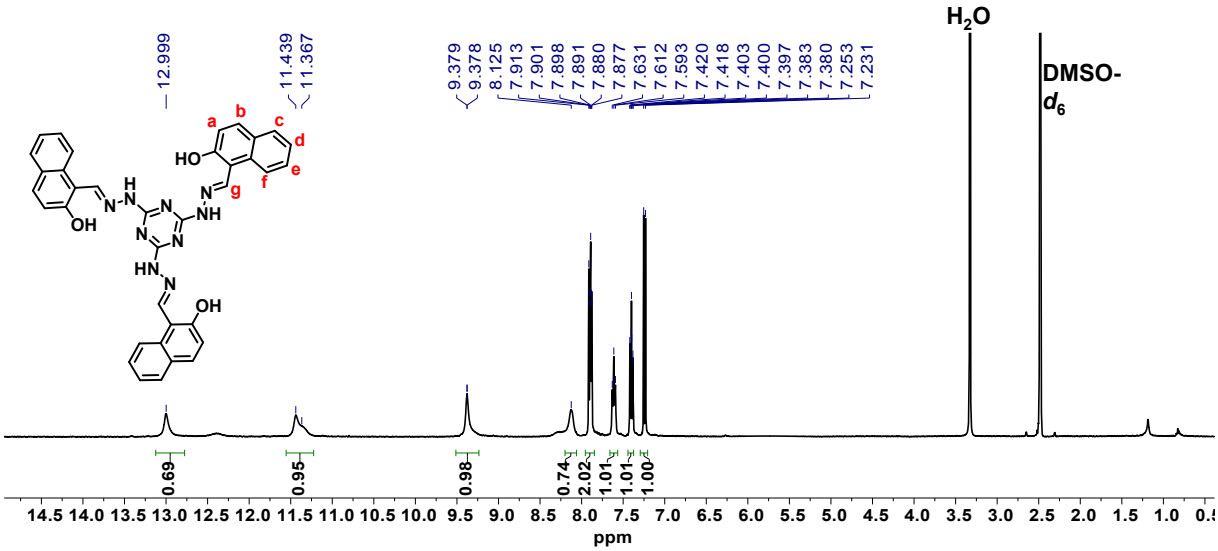


Figure S13: ^{1}H NMR spectrum of **R2** in DMSO-d_6 (400 MHz, 298 K).

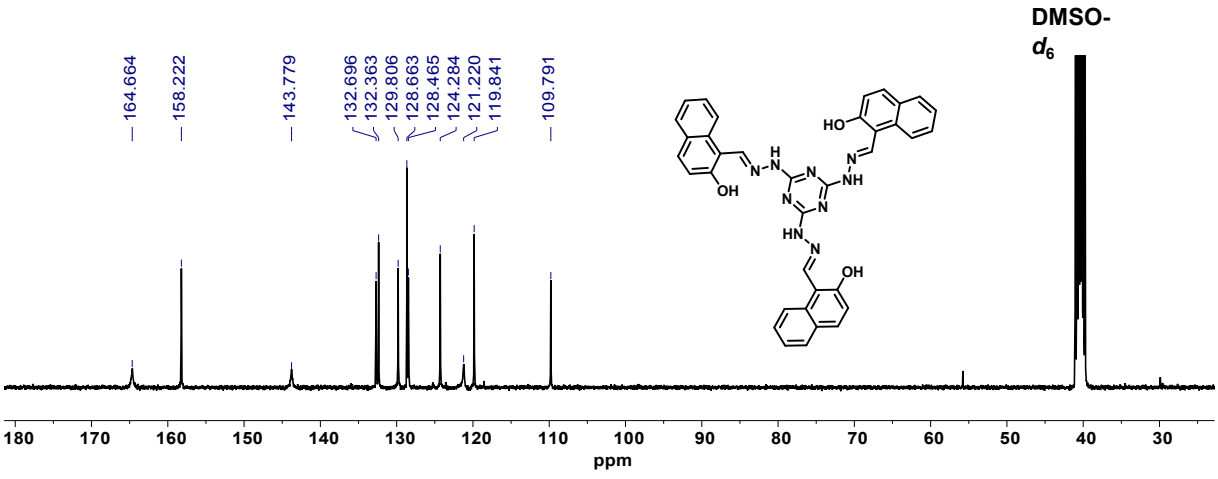


Figure S14: ^{13}C NMR spectrum of **R2** in DMSO-d_6 (100 MHz, 298 K).

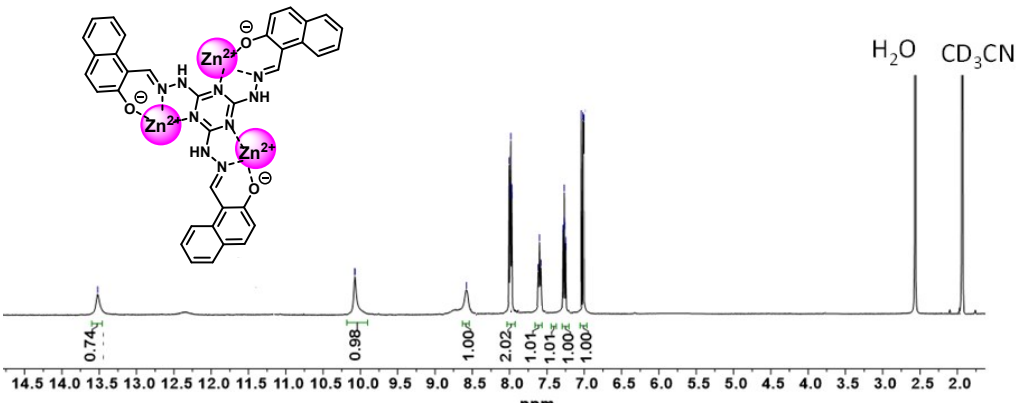


Figure S15: ^{1}H NMR spectrum of Zn_3**R2** in CD_3CN (400 MHz, 298 K).

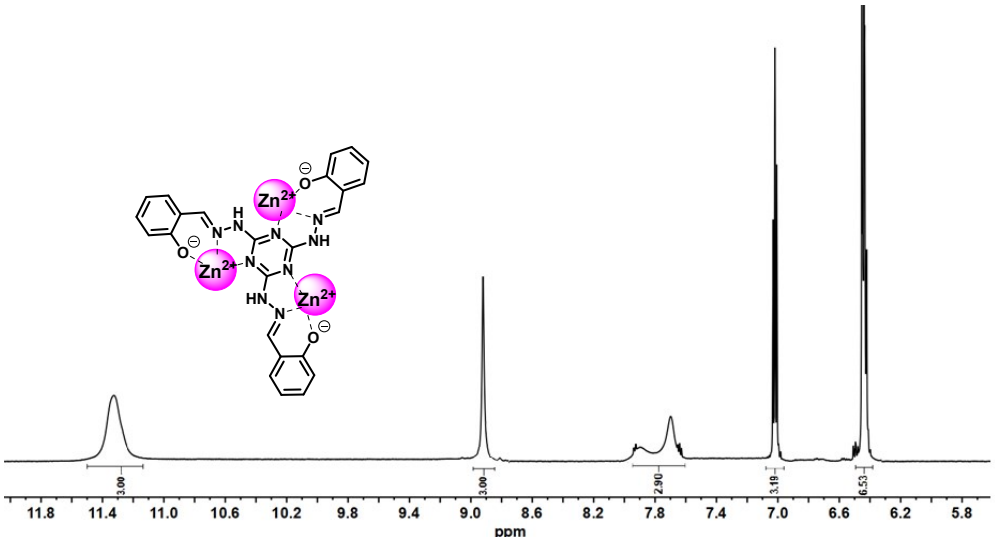


Figure S16: ^{1}H NMR spectrum of Zn_3**R1** in CD_3CN (400 MHz, 298 K).

11. ESI-MS Spectra

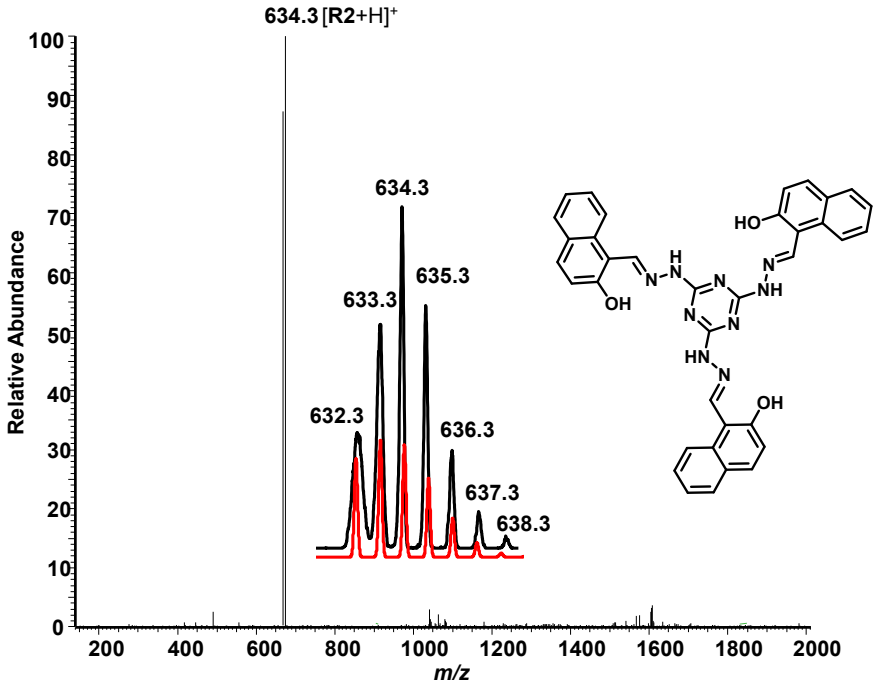


Figure S17: ESI-MS of **R2** after protonation.

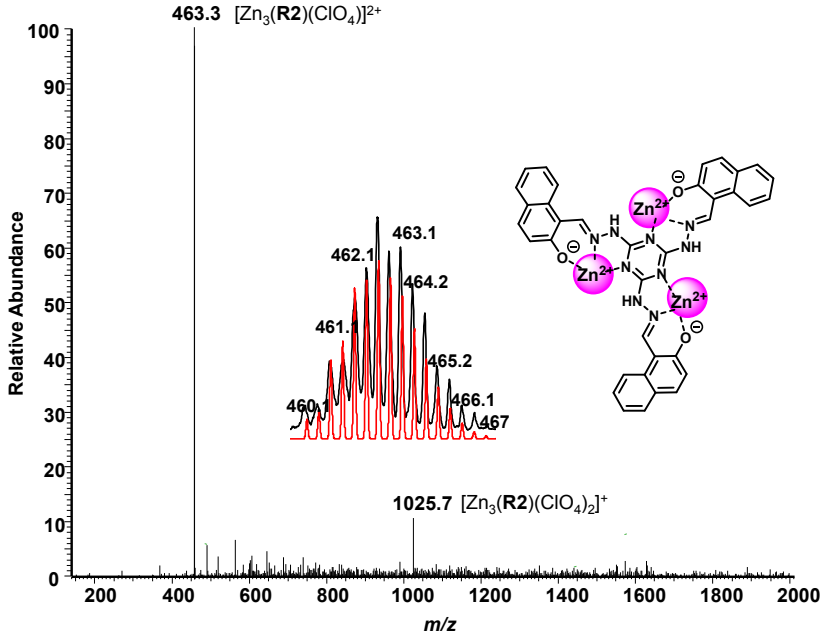
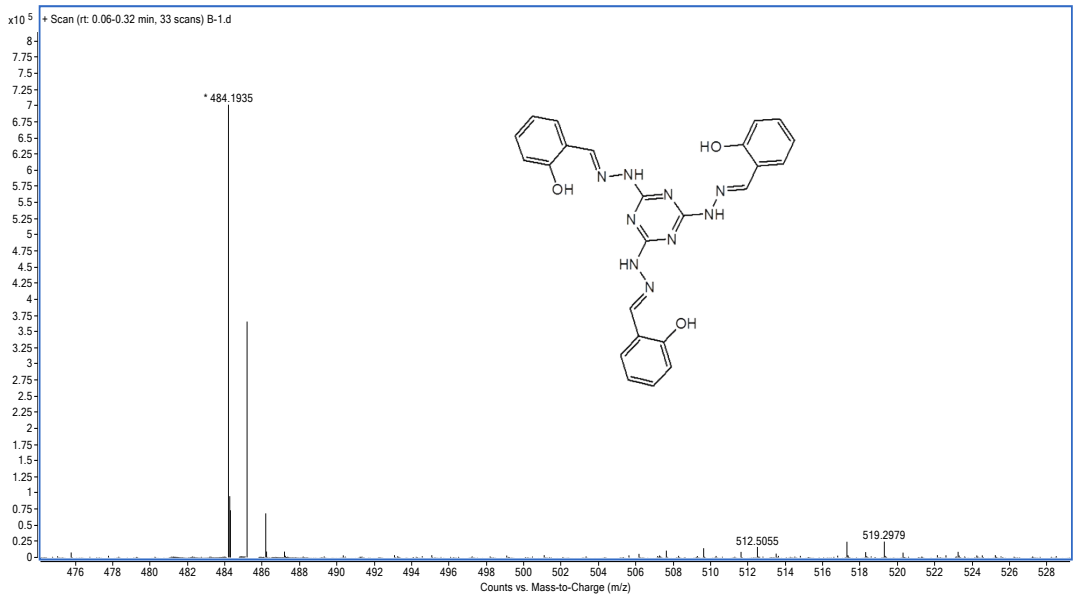


Figure S18: ESI-MS of Zn_3**R2**.



FigureS19: HRMS of **R1** after protonation.

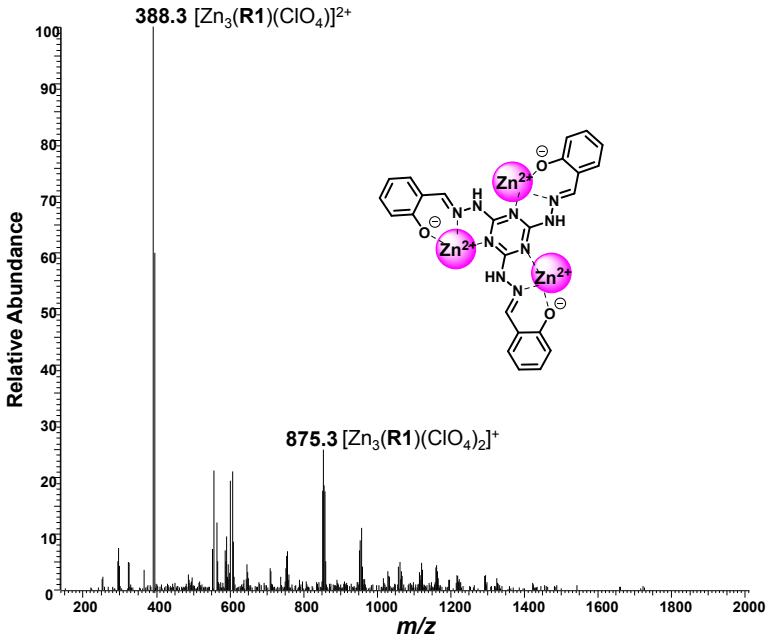


Figure S20: ESI-MS of Zn_3R1.

12. Calculation of Limit of Detection (LOD)

The limit of detection value was obtained from a plot of fluorescence intensity vs. function of Zn^{2+} concentration. The S/N ration was determined by the 10 times measurable emission intensity of the receptors (R1 and R2) without addition of Zn^{2+} and then standard deviation of blank measurements was calculated. The LOD value of probe for Zn^{2+} was determined by the following equation:

LOD = K × δ/m

Where K= 2 or 3 (We take 3 in this case)

δ is the standard deviation of the blank solution and m is the slope the calibration curve.

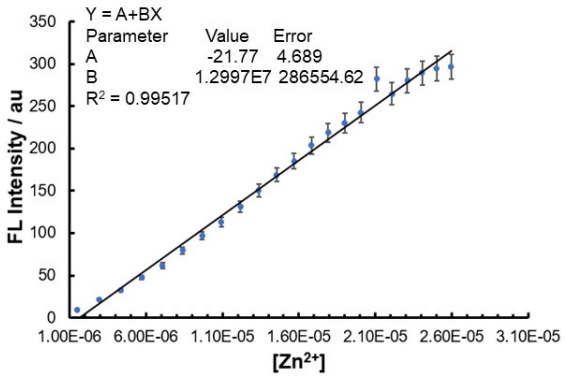


Figure S21 Calibration curve of R2 at 468 nm depending on Zn^{2+} concentration

13. Live cell imaging

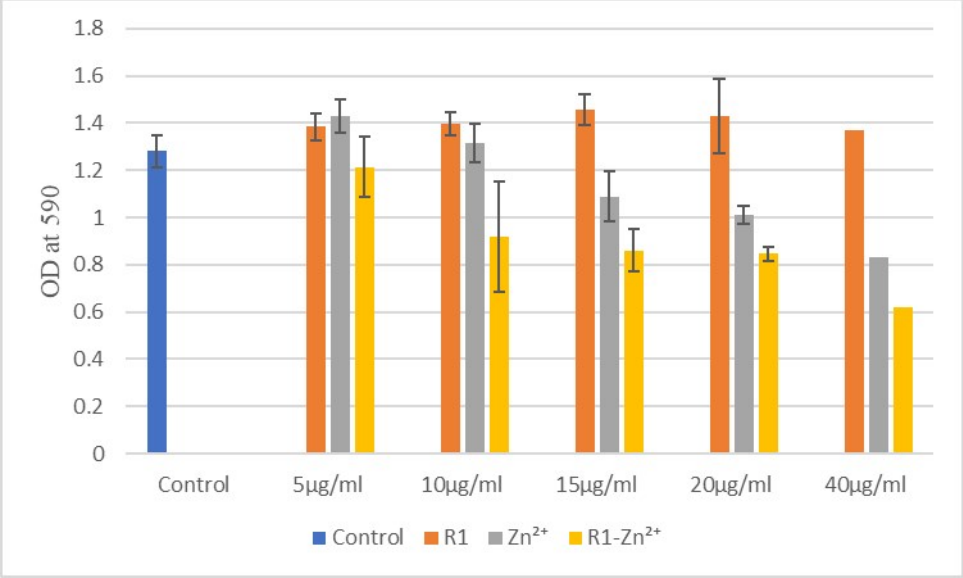


Figure S22: MTT assay to determine the cytotoxic effect of R1-Zn^{2+} complex on A549 cells (Human cancer cell A549, ATCC No CCL–185).

13.1 Cell culture: A549 cell (Human cell A549, ATCC No. CCL–185) lines were prepared from continuous culture in Dulbecco's Modified Eagle's Medium (DMEM, Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (100 µg/mL), and streptomycin (100 µg/mL). Cells were originally propagated in 75 cm^2 polystyrene, filter–capped tissue culture flask in an atmosphere of 5% CO_2 and 95% air at

37°C in CO_2 incubator. When the cells reached the logarithmic phase, the cell density was adjusted to 1.0 × 105 per/well in culture media. The cells were then used to inoculate in a glass bottom dish, with 1.0 mL (1.0 × 104 cells) of cell suspension in each dish. After cell adhesion, culture medium was removed. The cell layer was rinsed twice with phosphate buffered saline (PBS) (pH 7.0), and then treated according to the experimental necessitate.

Table S4: Real sample study for R1

Samples	**Spiked(µM)**	**Found(µM)**	**Actual concentration of Zn^{2+} in sample water (ppb)**
Tap Water	20	22.2	143
River Water	20	24.6	300

Table S5: Real sample study for R2

Samples	**Spiked(µM)**	**Found(µM)**	**Actual concentration of Zn^{2+} in sample water (ppb)**
Tap Water	20	22.3	150
River Water	20	24.7	309

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