Supporting Informations

Terpyridine derivative as "turn-on" fluorescent chemosensor for the selective and sensitive detection of Zn^{2+} ions in solution as well as in live cells

Tripti Mandal,[a] Anowar Hossain,[a] Anamika Dhara,[a] Abdulla Al Masum,[b] Saugata Konar,[c] Saikat Kumar Manna,[*d] Saikat Kumar Seth,[e] Sudipta Pathak[*d] and Subrata Mukhopadhyay [a]

[a]Department of Chemistry, Jadavpur University, Jadavpur, Kolkata, West Bengal, India.

[b]Department of Life science & Bio-technology, Jadavpur University, Jadavpur, Kolkata, West Bengal, India.

[c]Department of Chemistry, Bhawanipur Education Society College, Bhowanipore, Kolkata, West Bengal, India.

[d]Department of Chemistry, Haldia Government College, Debhog, Purba Medinipur, West Bengal, India.

[e]Department of Physics, Jadavpur University, Jadavpur, Kolkata, West Bengal, India.

*Corresponding Author, Tel: (03224) 252278; Fax: (03224)255873.

E-mail: sudiptachemster@gmail.com; saikat.manna.chem@gmail.com

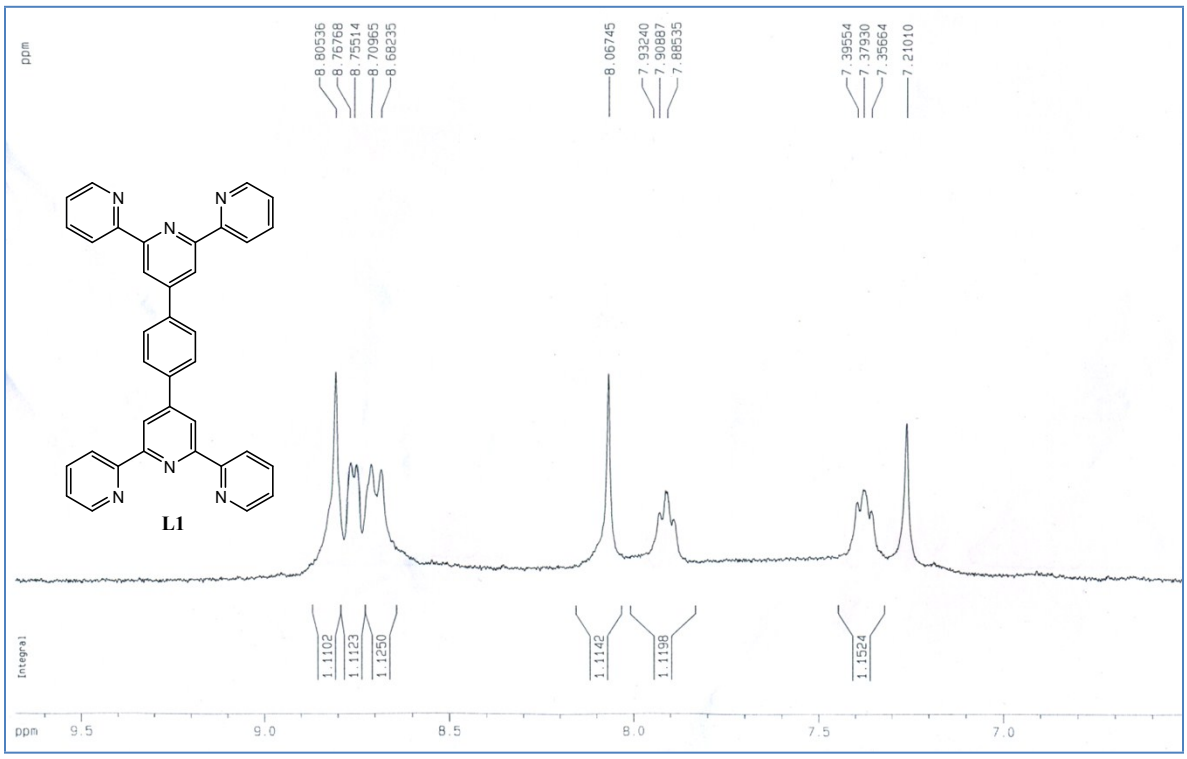


Fig. S1 ^{1}H NMR spectrum of sensor **L1** in $CDCl_3$.

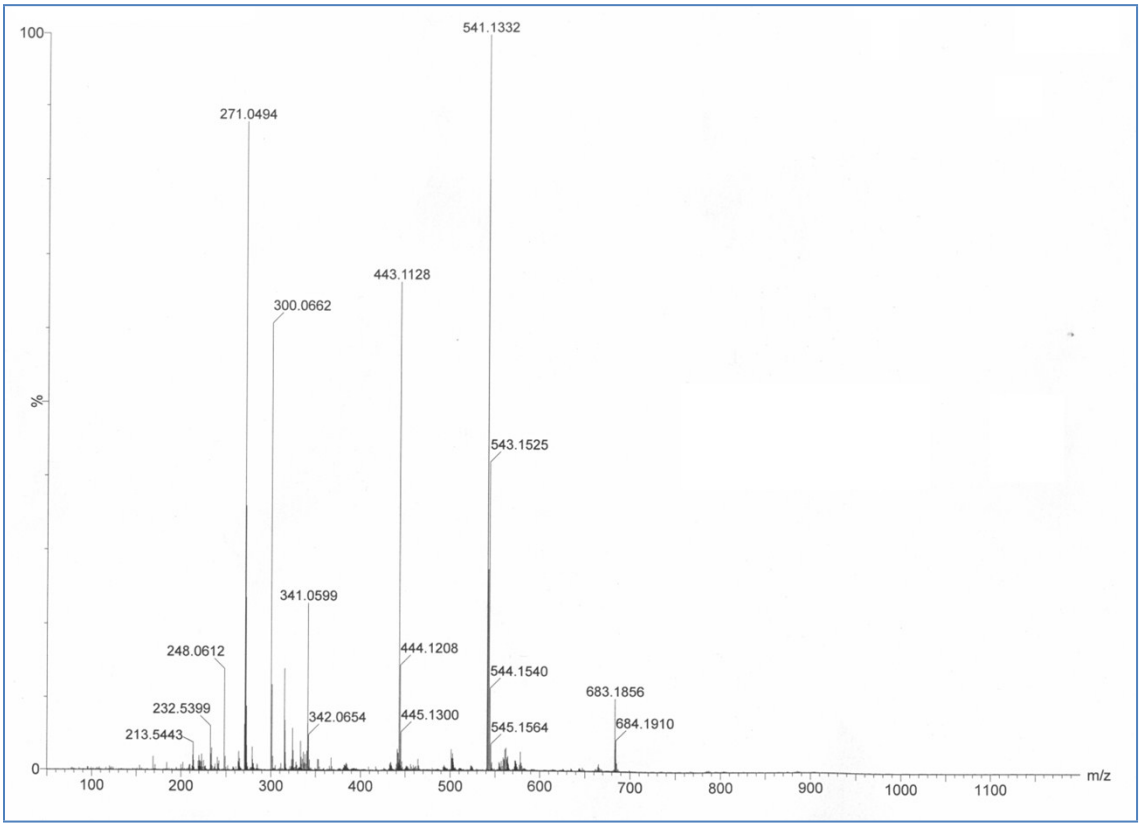


Fig. S2 Mass spectrum of sensor **L1**.

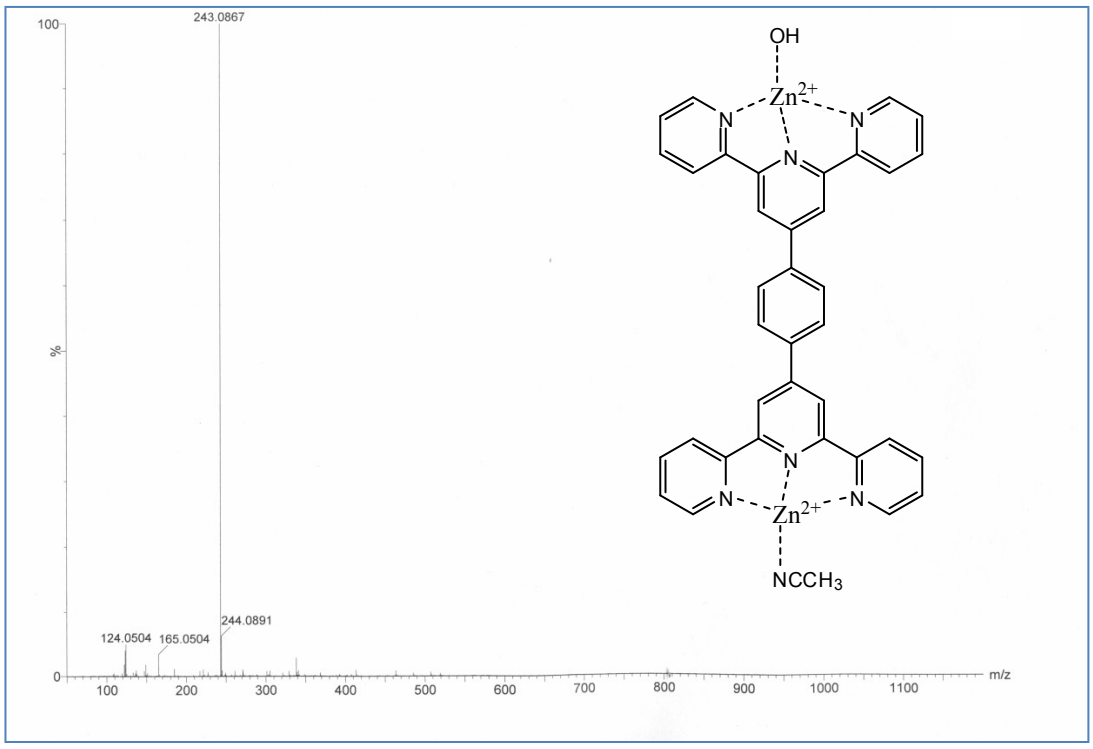


Fig. S3 Mass spectrum of sensor **L1**-2Zn^{2+} complex.

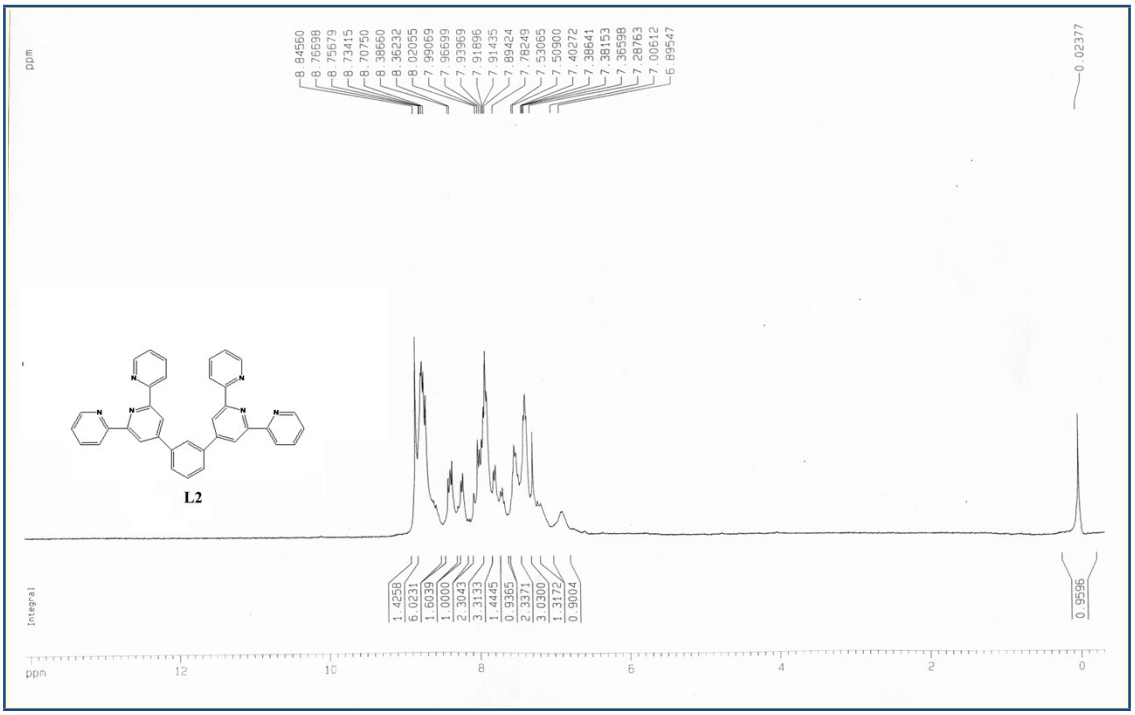


Fig. S4 ^{1}H NMR spectrum of sensor **L2** in $CDCl_3$.

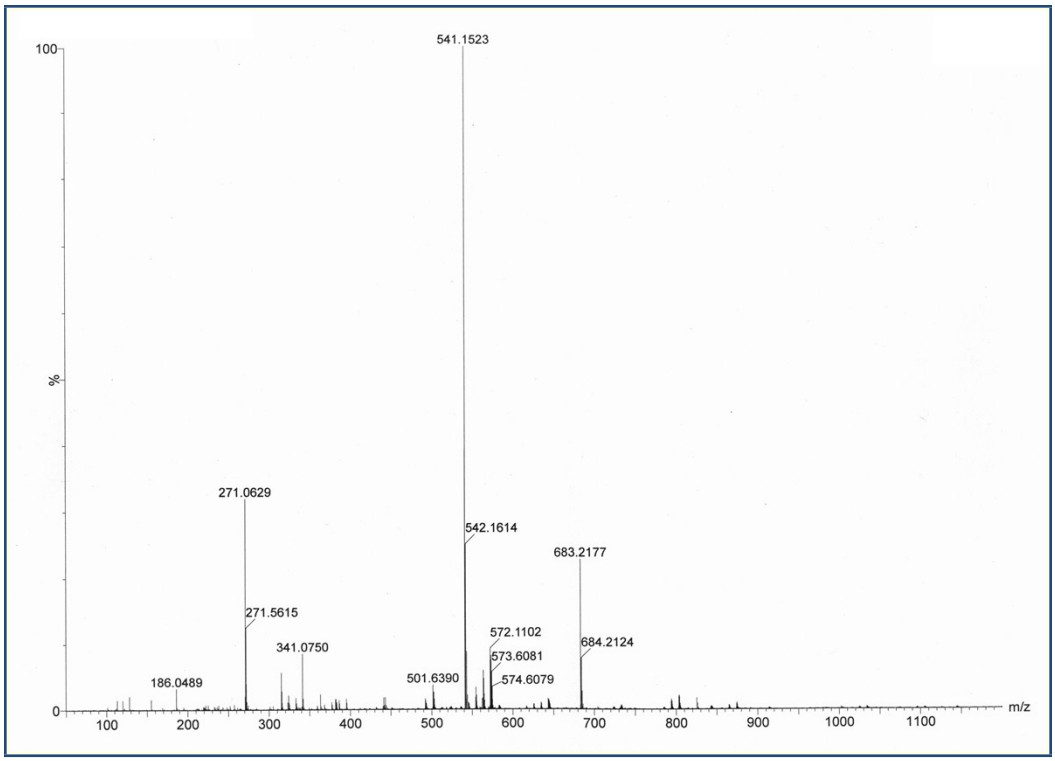


Fig. S5 Mass spectrum of sensor **L2**.

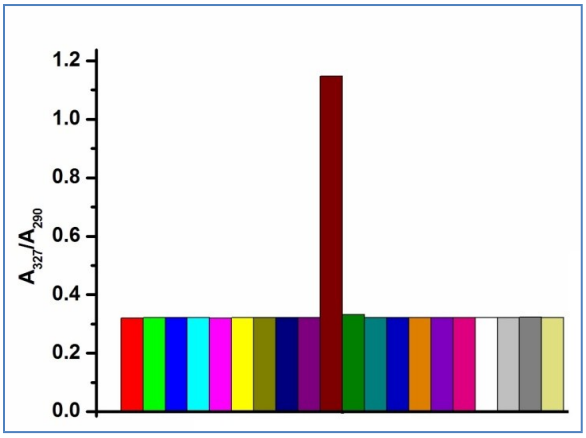


Fig. S6 Change in the absorption spectrum of receptor **L1** [c = 4× 10^{-5} M, CH_3CN/H_2O = 1 :1, v/v, 10 mM HEPES buffer, pH = 7.4) with respective metal cations (c = 4× 10^{-4} M, left to right- **L**, Li^{+}, Na^{+}, K^{+}, Ca^{2+} , Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Zn^{2+}, Al^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, Pd^{2+}, and Ag^{+}).

Binding constants calculation using fluorescence titration data:

The association constant and stoichiometry for the formation of the representative complexes were evaluated using the Benesi−Hildebrand (B−H) plot (eq 1).[1]

$$1/(I - I_0) = 1/K(I_{max} - I_0)[M^{n+}] + 1/(I_{max} - I_0) \quad (1)$$

where I_0, I_{max}, and I represent the emission intensity of free **L1**, the maximum emission intensity observed in the presence of added metal ion at 370 nm for Zn^{2+} (λ_{ext} = 312 nm), and the emission intensity at a certain concentration of the metal ion added, respectively.

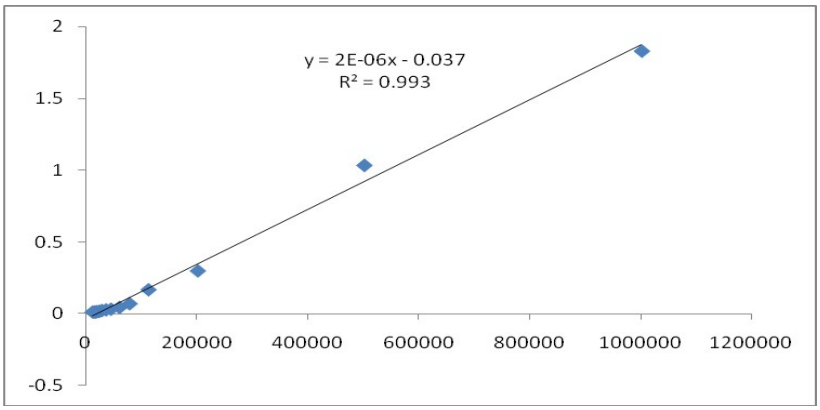


Fig. S7 Benesi-Hildebrand plot obtained from the Fluorescence titration data of **L1** (4 x 10^{-5} M) with Zn^{2+} (4 x 10^{-4} M).

Determination of Detection Limit:

The detection limit of **L1** for Zn^{2+} was determined from the following equation[2]:

Detection limit= K* Sb1/S Where K = 2 or 3 (we take 2 in this case); (2)

Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve

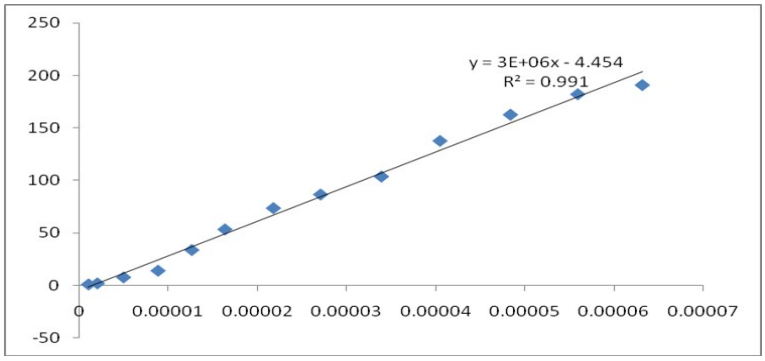


Fig. S8 Calibration curve for fluorescence titration of **L1** with Zn^{2+}.

From the graph **(Fig. S8)** we get slope = 3×10^6, and Sb1 value is 14.64524

Thus using the formula we get the Detection Limit = 9.76×10^{-6} M = 9.76 μM

Job's plot:

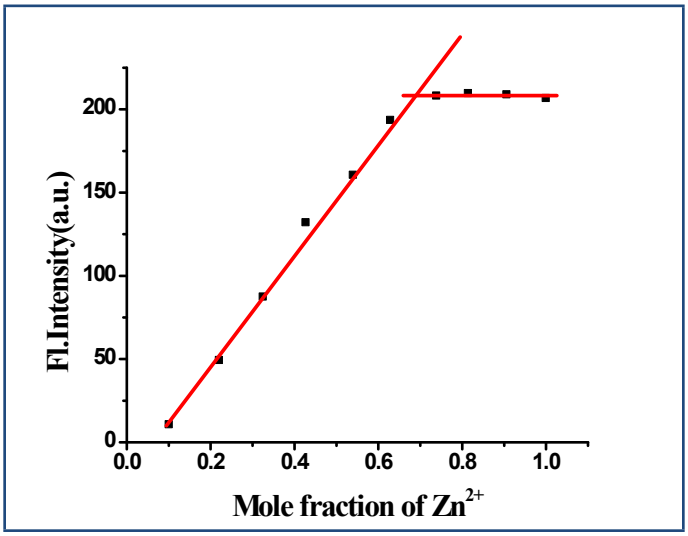


Fig. S9 Fluorescence Job's plot for **L1** with Zn^{2+} in CH_3CN/H_2O solution (9:1, v/v, 10 mM HEPES buffer, pH 7.4). ([H] = [G] = 4×10^{-5} M).

Theoretical study:

Table S1. HOMO-LUMO energy calculated for **L1** and **L1**-$2Zn^{2+}$ complex using [(B3LYP/6311G(d,p)] level of theory)

Species	E(HOMO)	E(LUMO)	ΔE(Hartree	ΔE(eV)	ΔE(kcal/mol)
L1	-0.24382	-0.07043	0.17339	4.718	108.804
L1-Zn^{2+}	-0.58153	-0.43667	0.14486	3.942	90.901

1 Hartree = 27.2116 eV, 1 Hartree = 627.5095 kcal mol^{-1}

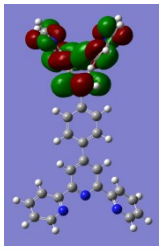
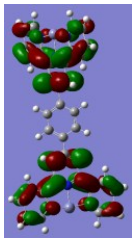
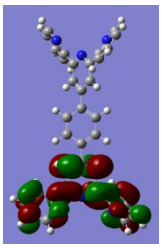
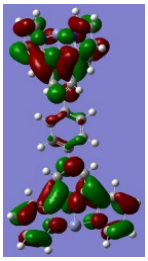
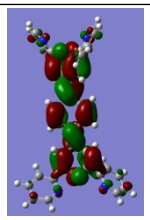
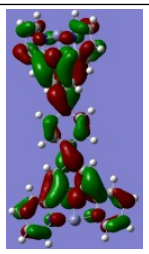
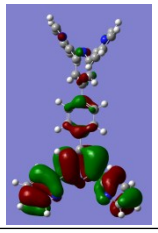
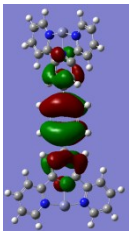
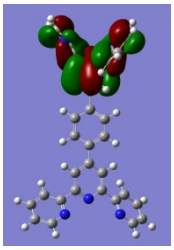
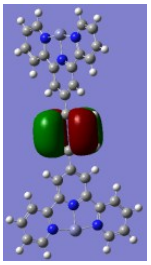
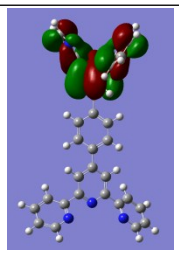
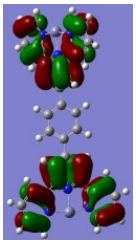
LUMO+2		
LUMO+1		
LUMO		
HOMO		
HOM O-1		
HOMO-2		

Fig. S10 Molecular orbital plots of **L1** and **L1**–2Zn^{2+}.

Table S2. Selected electronic excitation energies (eV) and oscillator strengths (f) of **L1** and **L1**-$2Zn^{2+}$. The data were calculated by TDDFT//B3LYP/6-311+G(d,p) based on the optimized ground state geometries.

Molecules	Excitation Energy[a]	f[b]	Composition[c]	(composition) %
L1	4.1620 eV 297.89 nm	0.4316	H → L H-1 → L+3	63.6 3.49
	4.1908 eV 295.85 nm	0.1616	H-2 → L H-2 → L+3	80.97 6.69
	4.1991 eV 295.26 nm	1.0404	H → L H-1→ L H-1→ L+1	28 62.89 2.92
L1-$2Zn^{2+}$	3.7806 eV 337.95 nm	0.9158	H → L	97.38
	3.7806 eV 327.95 nm	0.2146	H -1→ L H → L+3	67.74 11.58
	4.0723 eV 309.46 nm	0.0840	H → L+2 H-2 → L	86.22 10.89

[a] Only selected excited states were considered. [b] Oscillator strength. [c] H stands for HOMO and L stands for LUMO.

Cell imaging experiments:

Minimum Inhibitory Concentration (**MIC**): We have treated probe **L1** with both gram positive and gram negative bacteria. After 24 hrs of treatment probe **L1** shows no effect on gram negative bacteria but it showed some effect on gram positive bacteria. Probe **L1** showed

bacteriostatic activity on 150 µM. The chances of survivability of bacteria decrease near about 80%.

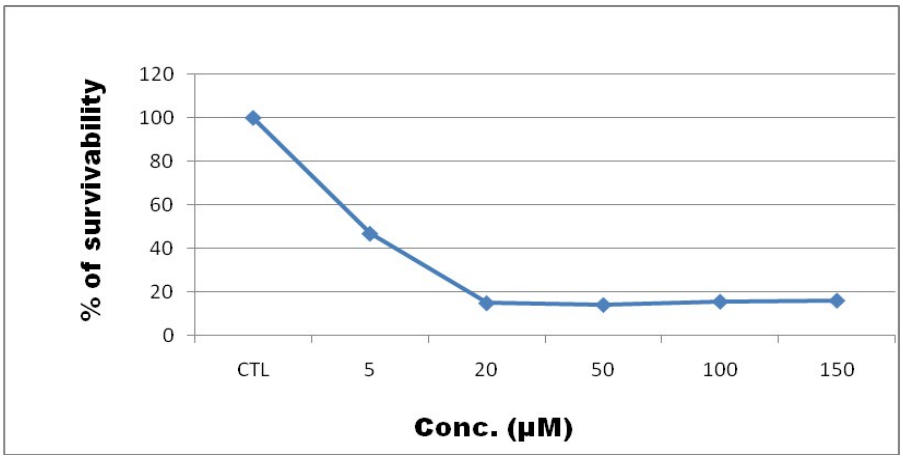


Fig. S11 Activity of the probe **L1** on gram positive bacteria with increasing concentrations of compound.

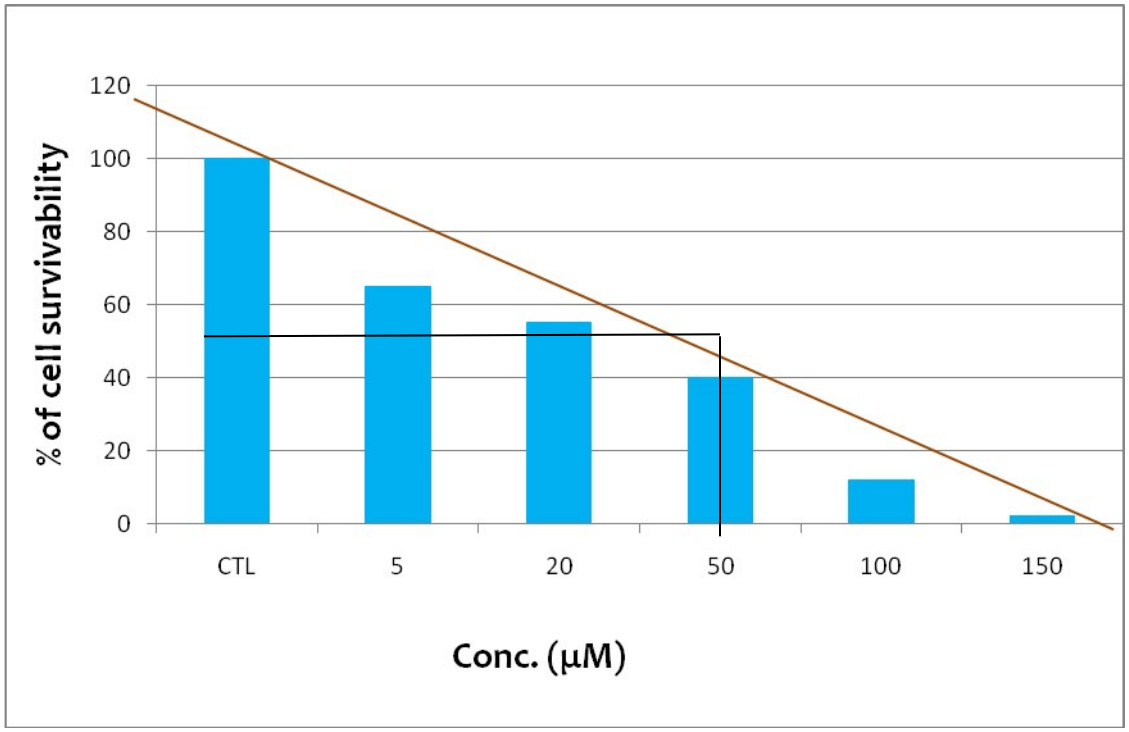
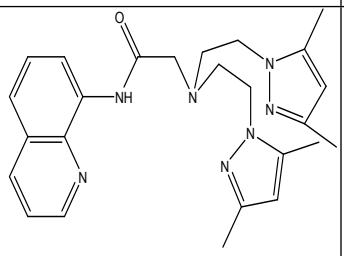
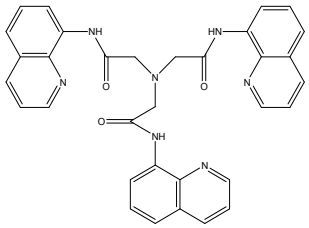
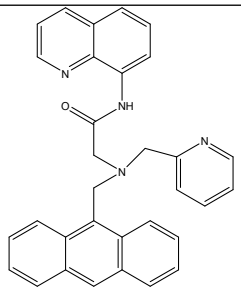
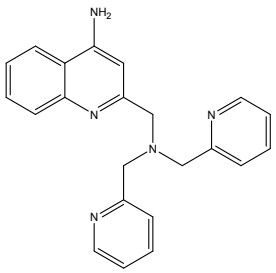
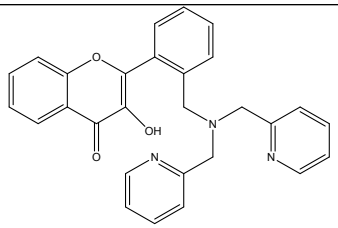
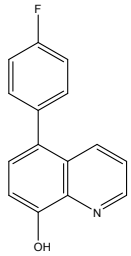
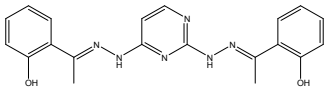
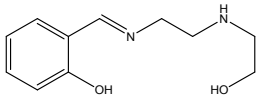
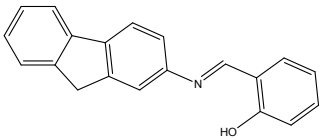
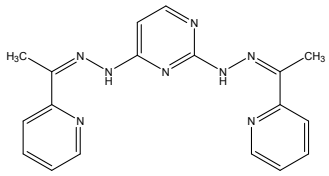
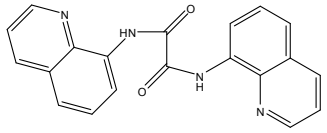


Fig. S12 Cytotoxic effect of probe **L1** on MDA-MB-468 cells. Cells were incubated with increasing concentrations in compound and their survivability was assessed by MTT assay.

Table S3. Summary of representative fluorescent probes for Zn^{2+}

Paper	Zn^{2+} detection limit	Interferences (cation/ anion)	Fluorescence probe structure	Any other remark	Our work
1. Selective zinc sensor based on pyrazoles and quinoline used to image cells. (Dyes and Pigments, 113, 2015, 723–729)	30 nM	-		Starting materials are not readily available.	Starting materials are commercially available.
2. Ratiometric and absolute water-soluble fluorescent tripodal zinc sensor and its application in killing human lung cancer cells. (Analyst, 2013, 138, 4593–4598)	3.2 μM	-		Starting materials are not readily available. Multistep reaction.	Starting materials are commercially available. Comparable detection limit (9.76μM)
3. A highly sensitive and selective ratiometric fluorescent sensor for Zn^{2+} ion based on ICT and FRET (Dyes and Pigments, 102, 2014, 301–307)	33.6 nM	-		Starting materials are not readily available. Multistep reaction.	Starting materials are commercially available. One pot synthesis.
4. A small molecular fluorescent sensor for highly selectivity of zinc ion. (Sensors and Actuators B, 176, 2013, 775– 781)	0.198 μM	-		Starting materials are not readily available. Multistep reaction.	Starting materials are commercially available.

5. A novel 3-Hydroxychromone fluorescence sensor for intracellular $Zn^{2+}$and its application in the recognition of prostate cancer cells. (Sensors and Actuators B, 245, 2017, 129–136)	-	-		Starting materials are not readily available. Multistep reaction.	Starting materials are commercially available. Detection limit: 9.76μM
6. Spectrofluorimetric determination of Zn^{2+} ions in aqueous medium using 5-(4-flourophenyl)-quinolin- 8-ol. (Journal of the Association of Arab Universities for Basic and Applied Sciences , 2017, 24, 66–73)	3 ppb.	Fe^{3+} and Cu^{2+}		Starting materials are not readily available. Multistep reaction. Interferences are present.	Starting materials are commercially available. No interference.
7. Pyrimidine-based fluorescent zinc sensor: Photophysicalcharacteristics, quantum chemical interpretation and applicationin real samples (Sensors and Actuators B, 201 2014, 204–212)	0.69 μM			Starting materials are not readily available. 23 fold fluorescence enhancement	Starting materials are very common. 51 fold fluorescence enhancement.
8. An Easy and Accessible Water-soluble Sensor for the Distinctive Fluorescence Detection of Zn^{2+} and Al^{3+} ions (RSC Adv., 2015, 5, 76939–76942)	0.643 μM	Al^{3+} (0.611 μM)		Interference present.	No interference.
9. Selective fluorometric detection of F and Zn(II) ions by a N, O coordinating sensor and naked eye detection of Cu(II) ions in mixed-	-	F^- & Cu^{2+}		Interference present.	No interference.

aqueous solution. (RSC Adv., 2015, 5, 44764–44777)					
10. An efficient, Schiff-base derivative for selective fluorescence sensing of Zn^{2+} ions: quantum chemical calculation appended by real sample application and cell imaging study. (Org. Biomol. Chem., 2014, 12, 6447–6456)	0.141 μM	-		Starting materials are not readily available. 45 fold fluorescence enhancement .	Starting materials are very common. 51 fold fluorescence enhancement.
11. A Ratiomeric Fluorescent Sensor for Zn^{2+} Based on N,N′-Di(quinolin-8-yl)oxalamide. (J Fluoresc, 2017, 27, 723–728)	2.4 μM	-		Effective at pH 8.66. No biological application.	pH 7.4. Our sensor can detect intracellular Zn^{2+} in living cells.

Quantum yield calculation:

Here, the quantum yield ϕ was measured by using the following equation:

$$\phi_x = \phi_s (F_x / F_s) (A_s / A_x) (n_x{}^2 / n_s{}^2)$$

Where, X & S indicate the unknown and standard solution respectively, ϕ = quantum yield, F = area under the emission curve, A = absorbance at the excitation wave length, n = index of refraction of the solvent. Here ϕ measurements were performed using anthracene in ethanol as standard [ϕ = 0.28] for standard (s) anthracene in ethanol the following values were determined: n_s = 1.36 (for ethanol); n_x=1.34 (for acetonitrile); ϕ_s = 0.28.

Using the above equation, we calculated quantum yield of **L1** and **L1**-2Zn^{2+}. Quantum yield data are given in table S4.

Table S4. Quantum yield data

Molecules	Quantum Yield (ϕ)
L1	0.0005
L1-2Zn^{2+}	0.0254

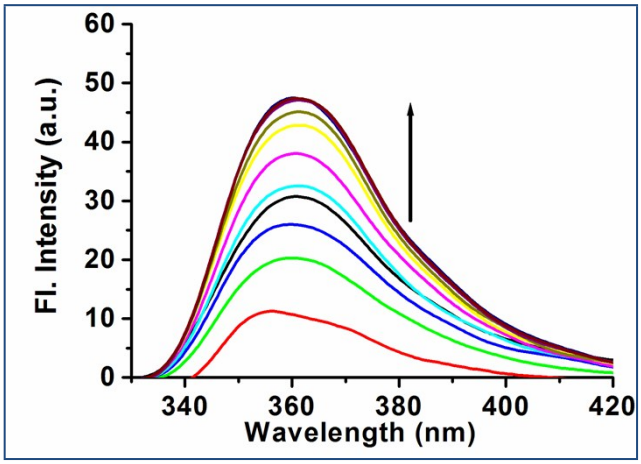


Fig. S13 Fluorescence emission changes of **L2** (4×10^{-5} M) with the continuous addition of Zn^{2+} ($c = 4 \times 10^{-4}$ M) in CH_3CN/H_2O solution (9:1, v/v, 10 mM HEPES buffer, pH 7.4). λ_{ex} = 300 nm.

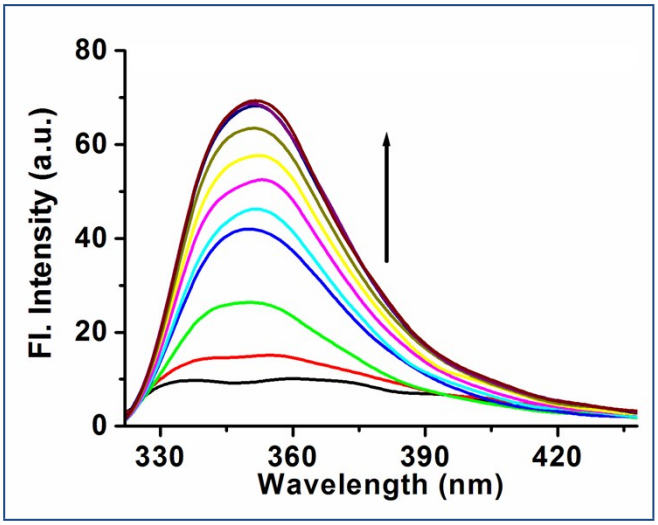


Fig. S14 Fluorescence emission changes of **L3** (4×10^{-5} M) with the continuous addition of Zn^{2+} ($c = 4 \times 10^{-4}$ M) in CH_3CN/H_2O solution (9:1, v/v, 10 mM HEPES buffer, pH 7.4). λ_{ex} = 290 nm.