Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Highly selective, sensitive and reversible fluorescence chemosensor for Zn^{2+} and its cell viability

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Content

Cytotoxicity studies:

In order to assess the toxicity of the ligand **H_2L** and **1**, two different cancer cell lines breast cancer (MCF-7), Skin melanoma cancer (A375) obtained from National Centre for Cell Science, Pune, were used.

Minimum essential medium (MEM, Himedia) supplemented with 10% fetal bovine serum (FBS, Life Technologies) and antibiotics, penicillin (100U/ml) and streptomycin (100µg/ml) was used to culture A375 and MCF7 cells. Both the cell lines were maintained at 37°C in a humidified atmosphere containing 5% CO_2 in CO_2 incubator. Cytotoxic nature of the **H_2L** and **1** was tested using standard 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.[1] To carry out the assay, all cells were seeded into 96 well plates in triplicates at a seeding density of 5000 cells/well in 100 µl of respective culture medium for 48 hours. **H_2L** and **1** dissolved in 1% DMSO were added to the wells in concentrations ranging from 10 µM-300 µM in such a way that the final concentration of DMSO was not exceeded 1% in any of the well. The treated cells were incubated for 24 hours. Further, the culture medium containing **H_2L** and **1**was replaced and fresh medium and 10 µl MTT (5 mg/ml in 1X PBS, pH 7.4) was added to each well and incubated at 37°C for 4 hours. Subsequently, MTT incubated media was removed from the wells and 100 µl DMSO was added to dissolve insoluble purple formazan crystals and incubated for 10 minutes. Absorbance for each well was taken at 570 nm using Biotekmicroplate reader. Control cells with 1% DMSO were used in the study. Cell viability was quantitated based on % cell viability using formula: % cell viability= $Abs_{(exp)}/Abs_{(control)}$ X 100 and plotted on bar graph using Origin 8.0.

Propidium Iodide and Hoechst 33342 Dual Staining: The cytotoxicity assessment using dual staining was performed based on the procedure reported elsevier.[2] A375 and MCF-7 cells were seeded into 12 well plates at a density of 50,000 cells per well. Cells were placed in a CO_2 incubator for 24 hours for allowing them to adhere to well surface. After being adherent, the cells were treated with 100 µM of **H_2L** and **1** for 24 hours. After treatment, the media containing **H_2L** and **1** was aspirated and replaced with Hoechst 33342 (5µg/mL in MEM) and incubated for 10 minutes. The cells were then washed with PBS and then stained with propidium iodide (50µg/mL in MEM) and incubated further for 10 minutes. The cells were then washed with PBS

and live cell imaging performed immediately using an inverted fluorescence microscope (Nikon Trinocular Inverted Tissue Culture Microscope TS100-F).

Table S1. Selected bond lengths (Å) and bond angles(º) of **1**

Bond Distances	**1**
Zn(1)-O(2)	1.918(3)
Zn(1)-O(1)	1.932(3)
Zn(1)-N(2)	1.991(3)
Zn(1)-N(1)	2.003(3)

Bond angles

O(2)-Zn(1)-O(1)	114.99(15)
O(2)-Zn(1)-N(2)	94.41(13)
O(1)-Zn(1)-N(2)	121.17(15)
O(2)-Zn(1)-N(1)	118.27(15)
O(1)-Zn(1)-N(1)	93.23(13)
N(2)-Zn(1)-N(1)	116.85(13)

Table S2. Hydrogen Bonding in **H_2L** and **1[**Å and (º)].

	D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
			H_2L		
1	C(17)-H(17)…C(14)#(1)	0.93(0)	2.791(1)	3.711(1)	180.0(0)
2	C(14)-H(14)…O(1)#(1)	0.960(.000)	2.311(.003)	3.257(.003)	168.40(0.07)
Equivalent positions: (1) -x,-y,-z					
			1		
1	C(17)-H(17)…O(1)#(1)	0.930(.000)	2.718(.000)	3.462(.000)	137.64(0.00)
Equivalent positions: (1) -x+1,-y+1,-z+2					

Table S3. Life time measurement of **1**

τ_1	τ_2	α_1	α_2	χ^2	$<\tau>$
0.646 ns	1.44 ns	0.1	0.9	1.23	1.36 ns

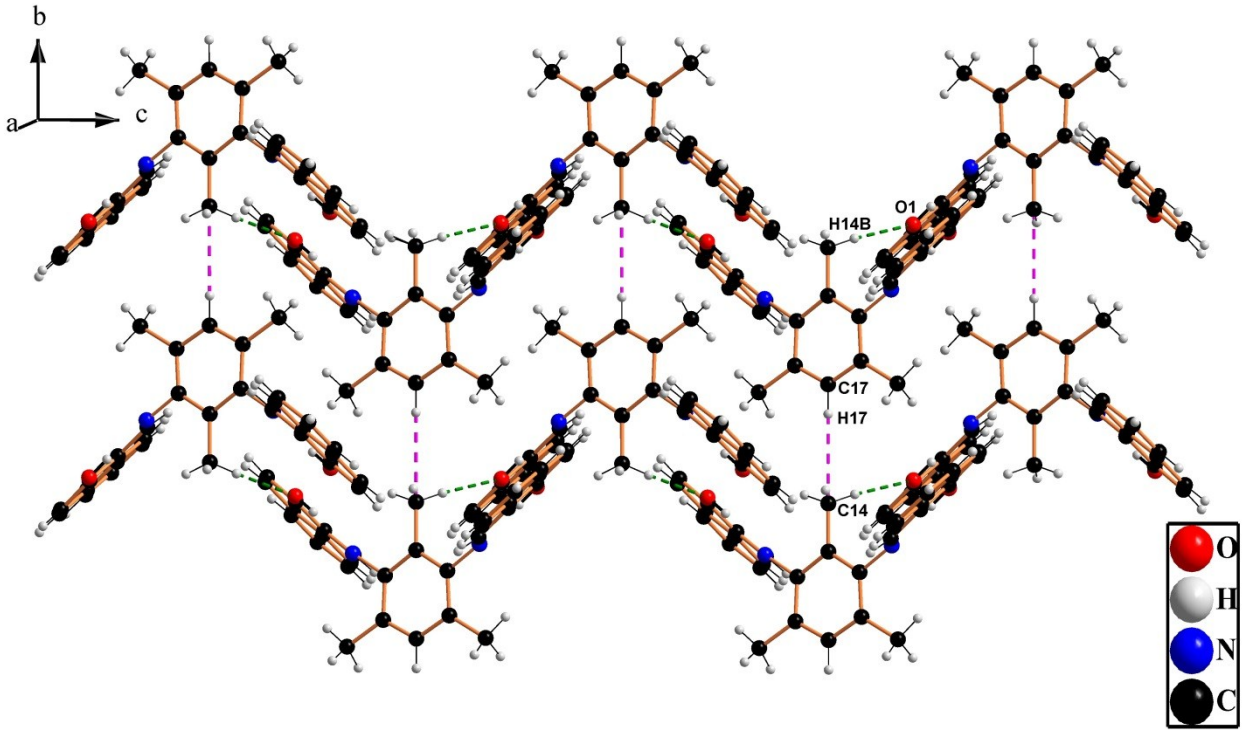

Figure S1. Intermolecular hydrogen bonding interaction(green line) and C−H···C interaction(pink line) 2-D layer in **H_2L.**

Association constant calculation graph (absorption method).

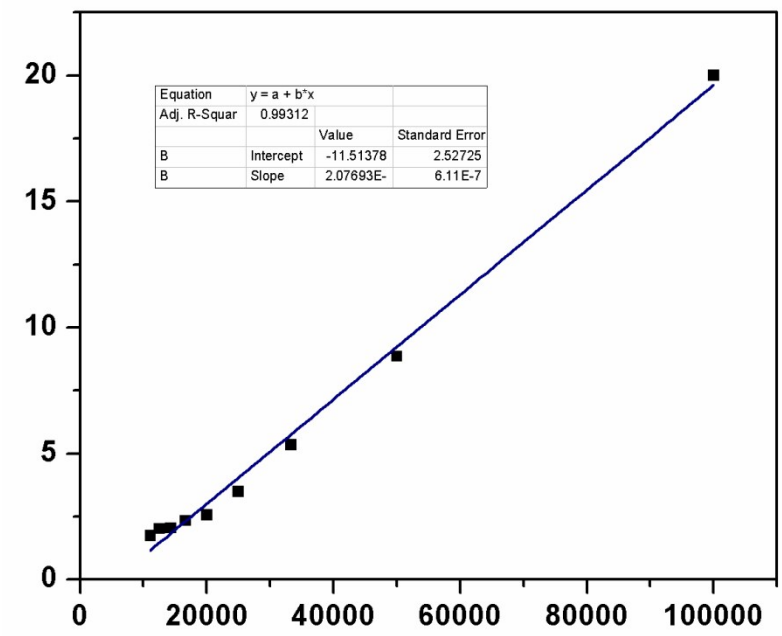

Figure S2. Bensei- Hildbrand plot obtained from absorption (at 370 nm excitation wavelength) studies. Binding constant ($1.13\times10^{5}M^{-1}$) curve of sensor $\mathbf{H_2L}$ with Zn^{2+} determined by UV-visible spectroscopy.

Association constant calculation graph (Fluorescence method).

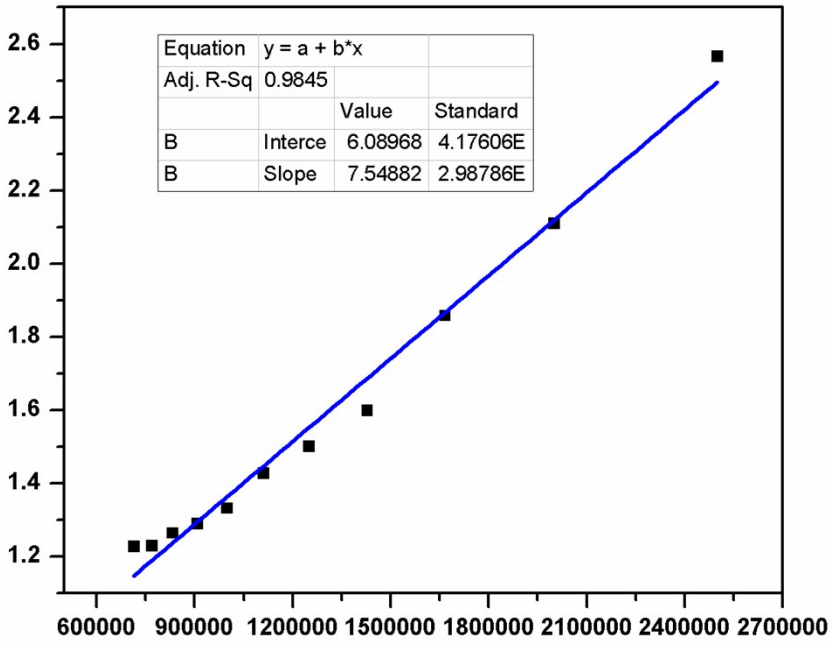

Figure S3. Bensei- Hildbrand plot obtained from Fluorescence (at 470 nm emission wavelength) studies. Binding constant ($1.13\times10^{5}M^{-1}$) curve of sensor $\mathbf{H_2L}$ with Zn^{2+} determined by fluorescence spectroscopy.

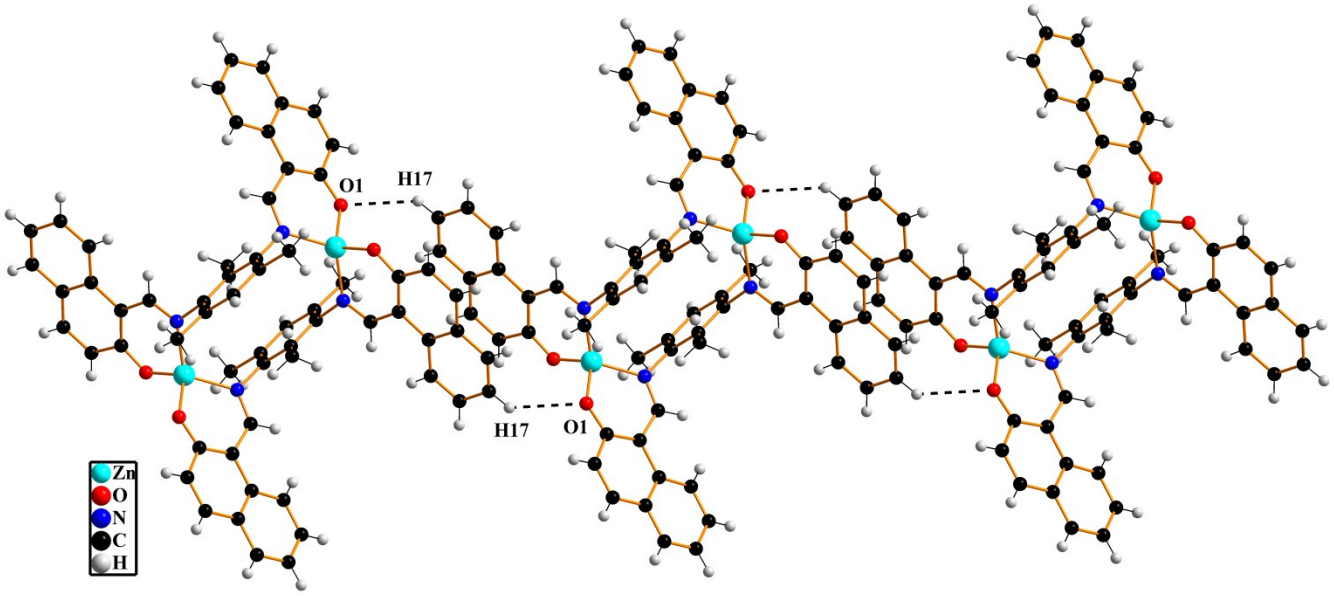

Figure S4. Intermolecular hydrogen bonding interaction 1-D layer in **1**.

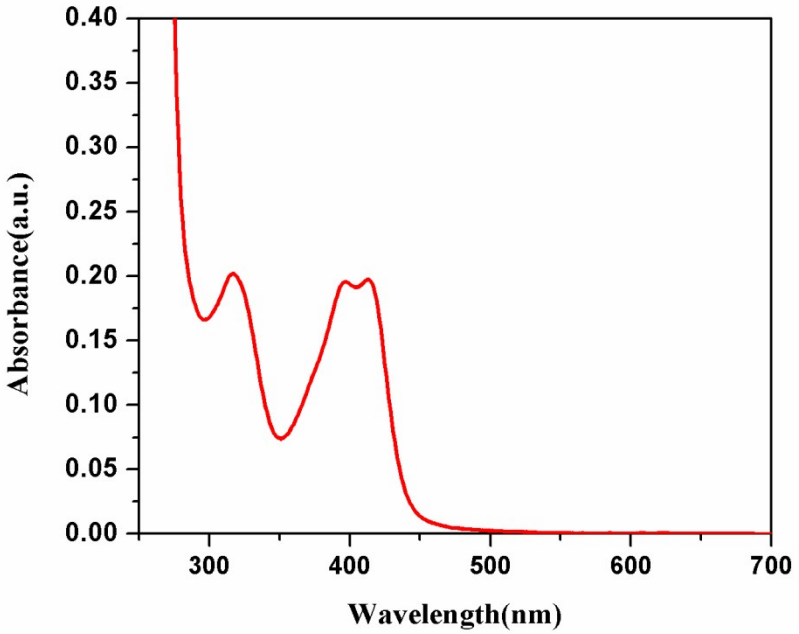

Figure S5. absorption spectra of **1** in CH_2Cl_2($c=1.0\times10^{-5}$M).

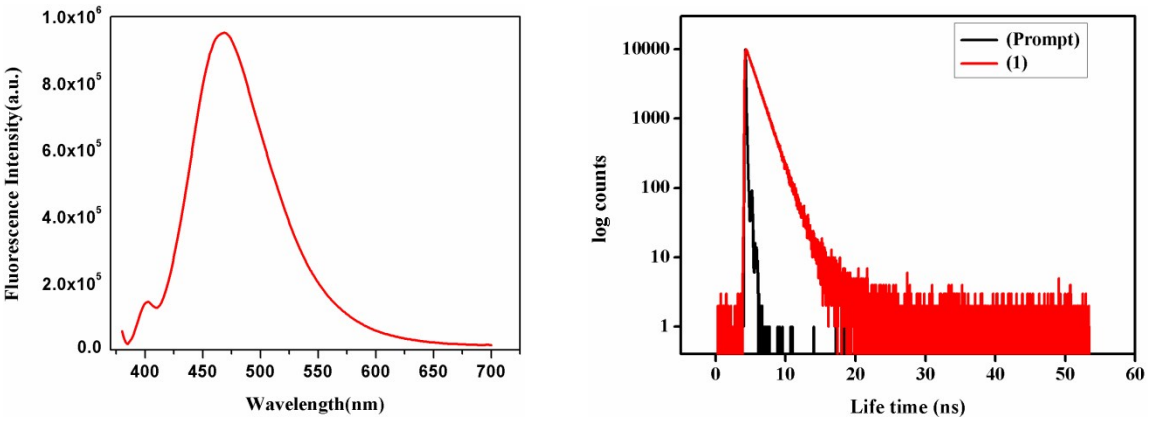

Figure S6. (a) Fluorescence spectra of **1** in CH_2Cl_2 (**b**) Average life time measurement of **1**in CH_2Cl_2.

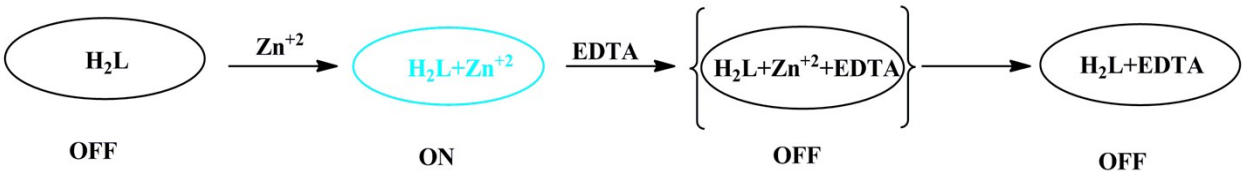

Figure S7. Schematic representation of Fluorescence Quenching.

References

1 J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna and J. B. Mitchell, *Cancer Res.*, 1987, **47**, 936-942.

2 S. M. McNeill, D. Preston, J. E. M. Lewis, A. Robert, K. Knerr-Rupp, D. O. Graham, J. R. Wright, G. I. Giles and J. D. Crowley, *Dalton Trans.,* 2015**, 44***,* 11129-11136