Electronic Supporting Information

L-tryptophan-based Pyrene Conjugate for Intracellular Zinc-guided Excimer Emission and Controlled Nano-assembly

Surabhi Asthana,[a,#] Tarkeshwar Maddeshiya,[a,#] Arpna Tamrakar,[a] Praveen Kumar, [b] Neha Garg [b] and Mrituanjay D. Pandey[a,*]

[a]*Department of Chemistry, Institute of Science, Banaras Hindu University, Varanasi- 221005 UP, India. Email: mdpandey.chem@bhu.ac.in*

[b]*Department of Medicinal Chemistry, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, 221005, India*

# Equal Contribution

Content:

Material and instrument: -

^{1}H-NMR spectra frequency at 500 MHz and ^{13}C-NMR spectra frequency at 126 MHz were performed on a JEOL AL 300 FT-NMR. Spectrometer operation at 500 MHz and 126 MHz in DMSO-d^{6} solutions respectively are given in parts per million (ppm) related to Tetramethyl silane (TMS, δ = 0.00ppm), and splitting pattern designated as s (singlet), d (doublet), t (triplet). FTIR and UV-visible spectra were recorded on an 8400S and Agilent Cary 60 single beam UV-visible spectrometer with serial no.-MY19329220, respectively, and fluorescence spectra were measured on a Fluoromax 4CP plus spectrofluorometer with a 10 mm quartz cell at 25^{o}C. The melting point was measured using an EZ-Melt (automated melting point apparatus). High-resolution mass spectra (ESI-HRMS) were recorded on the SCIEX X500R (TOF-MS) mass spectrometer. AFM measurements were performed on NT-MDT, with model no. Solver next. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) study was performed on Agilent 7800 ICP-MS mainframe from Agilent Technologies. Dynamic light scattering (DLS) study was performed on Zetasizer Ultra (ZSU5700).

All chemicals are purchased from a commercial supplier and used without further purification. Lithium Hydroxide and L-tryptophan were purchased from Spectrochem. 1-pyrenecarboxyaldehyde was purchased from Sigma-Aldrich, and sodium borohydride was purchased from S. d. fine. Metal salts $AgNO_3$, $Ca(NO_3)_2{\cdot}4H_2O$, $Cd(NO_3)_2{\cdot}4H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}3H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$, $Hg(NO_3)_2$, KNO_3, $NaNO_3$, $Ni(NO_3)_2{\cdot}6H_2O$, $Pb(NO_3)_2$, $Zn(NO_3)_2$ were purchased from Himedia.

Synthesis and characterization: -

The chemosensor denoted as **1** was synthesized via the condensation reaction between L-tryptophan and 1-pyrenecarboxyaldehyde. Comprehensive characterization was taken utilizing various analytical techniques, including FT-IR, UV-vis spectroscopy, ^{1}H-NMR, ^{13}C-NMR, HRMS (High-Resolution Mass Spectrometry) and atomic force microscopy (AFM). Our study is primarily focused on the strategic molecular design that aims to introduce an unoccupied binding site, specifically in the form of a carboxylic acid (-COOH) functional group. This molecular architecture offers the presence of vacant binding sites characterized by nitrogen (N) and oxygen (O) donor atoms. These sites exhibit the potential for multifunctional utility by enabling binding interactions with a wide range of cations, anions, and neutral species. This molecular framework holds promise for versatile applications across diverse scientific domains, particularly in the areas of supramolecular recognition and photophysical sensing.

General method for absorption and emission measurements: A stock solution of **1** (10 μM), was prepared in HEPES buffer. (DMSO: HEPES = 1:9 v/v, pH ~ 7.4; 10 mM) for the optical measurement at room temperature with quartz cuvette (4ml, path length, 1cm). Various metal ion (nitrate salts) solutions (100mM) including, Ag(I), Ca(II), Cd(II), Co(II), Cu(II), Fe(III), Hg(II), K(I), Na(I), Ni(II), Pb(II) and Zn(II) were prepared in doubly distilled water for metal ion selectivity measurement, metal ions solution added portion wise in 3.0ml solution of **1** in a quartz cuvette (4ml, path length, 1cm). The maximal excitation and emission wavelengths for fluorescence measurements were 343 nm and 374 nm, respectively, while the wavelength range for absorbance measurements was 200-800 nm.

The value of the Binding constant is calculated using the Benesi-Hildebrand equation for this 2:1 stoichiometry.

$1/I\text{-}I_0 = 1/ K_a [Zn(II)]^2 [I_{max} – I_0] + 1/ I_{max} \text{-}I_0$

I_o = Emission intensity of **1** when excited at 343 nm.

I = Emission intensity of **1**+Zn(II) at varying concentration of Zn(II)

I_{max} = Intensity of **1**+Zn(II) complex at the maximum concentration of analyte [Zn(II)]

K_a = Binding constant.

Water samples preparation for Zn(II) ion detection

The real water sample of Ganga water was collected from Assi Ghat of Varanasi, Lake water sample from IIT BHU, Pond water sample from the Agriculture Dept. of IIT BHU and Tap water from the Departmental tap of BHU. The samples obtained were filtered and boiled to remove any solid impurities and dissolved gases. In addition to this, spiked water samples were also analyzed by ICP-MS, according to the manufacturer's instructions.

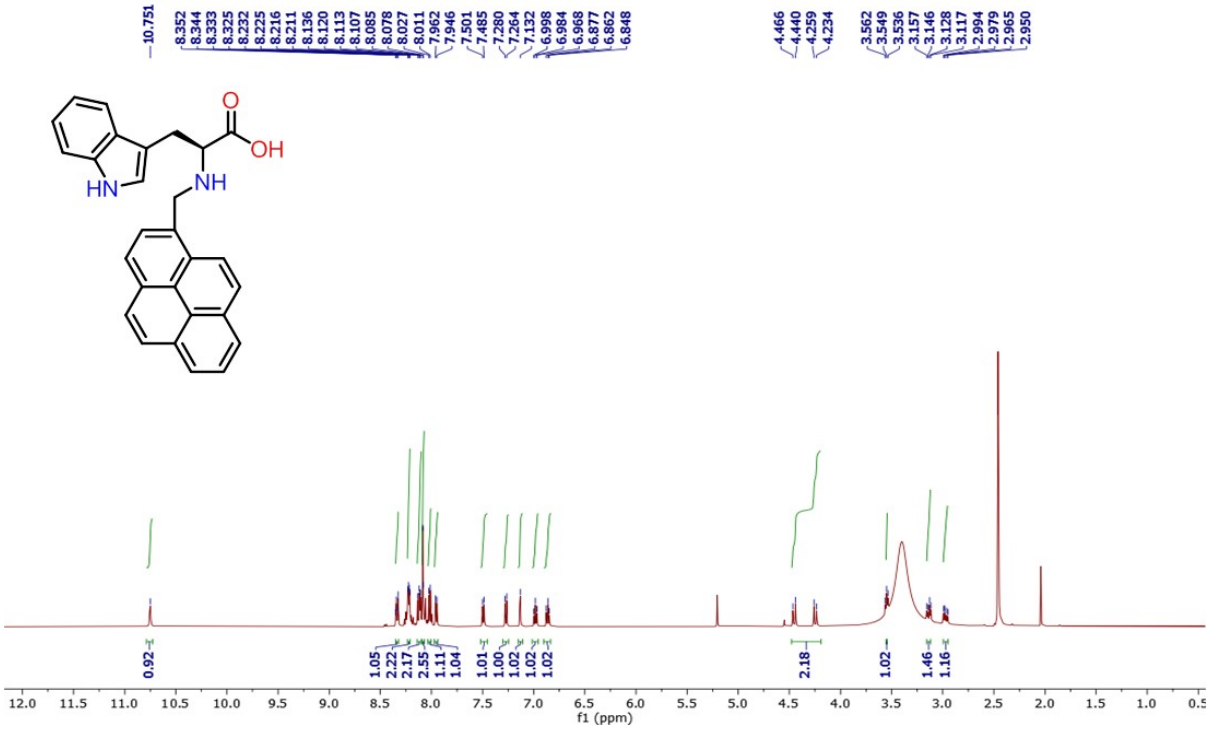


Figure S1. ^{1}H NMR of **1** (500 MHz, DMSO-d^6)

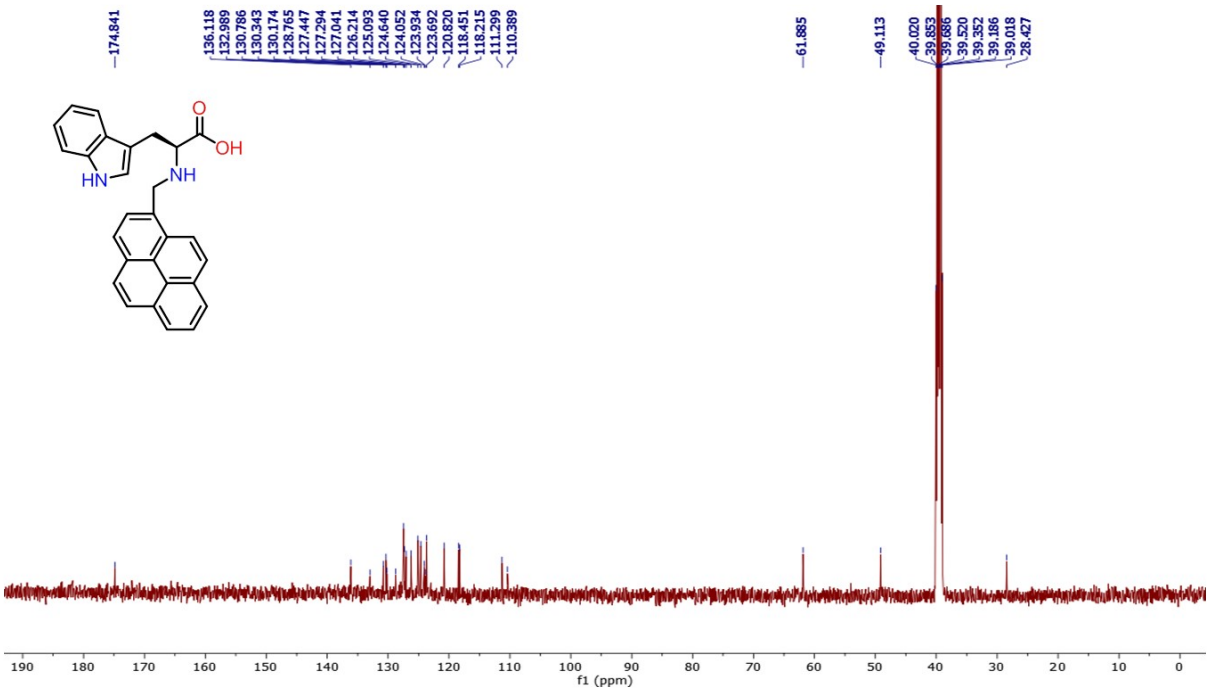


Figure S2. $^{13}C\{^{1}H\}$ NMR of **1**(126 MHz, DMSO-d^{6})

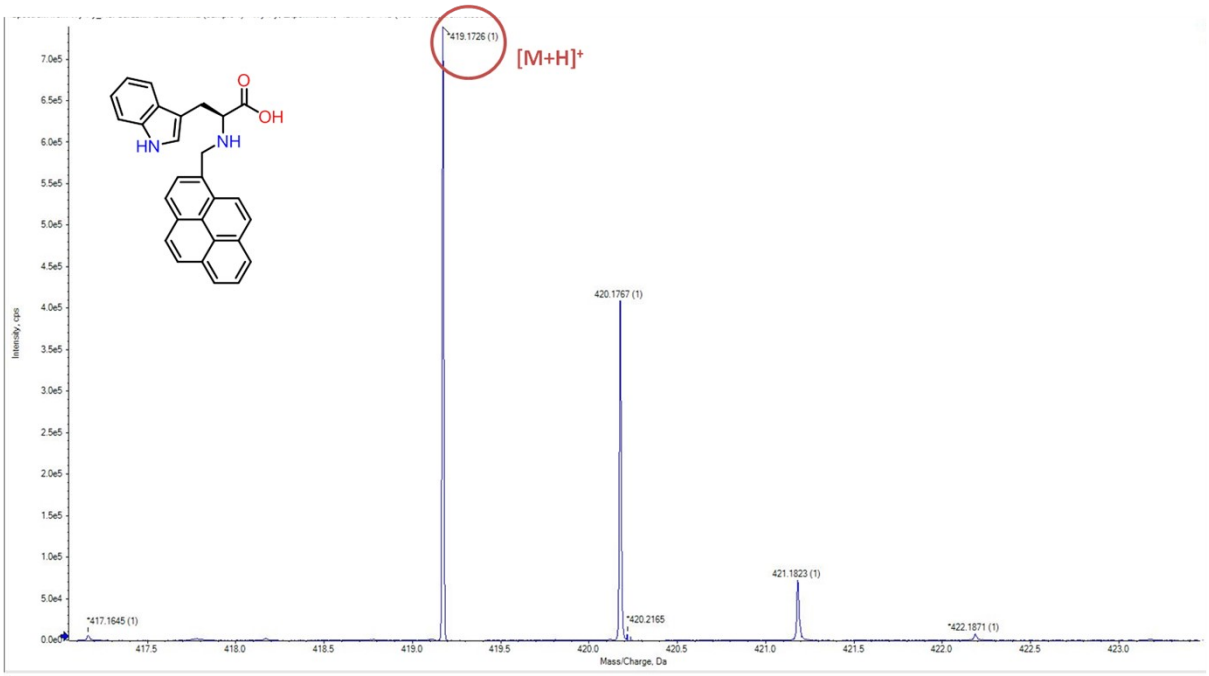


Figure S3. HRMS (ESI-TOF)$^{+}$ data of **1**

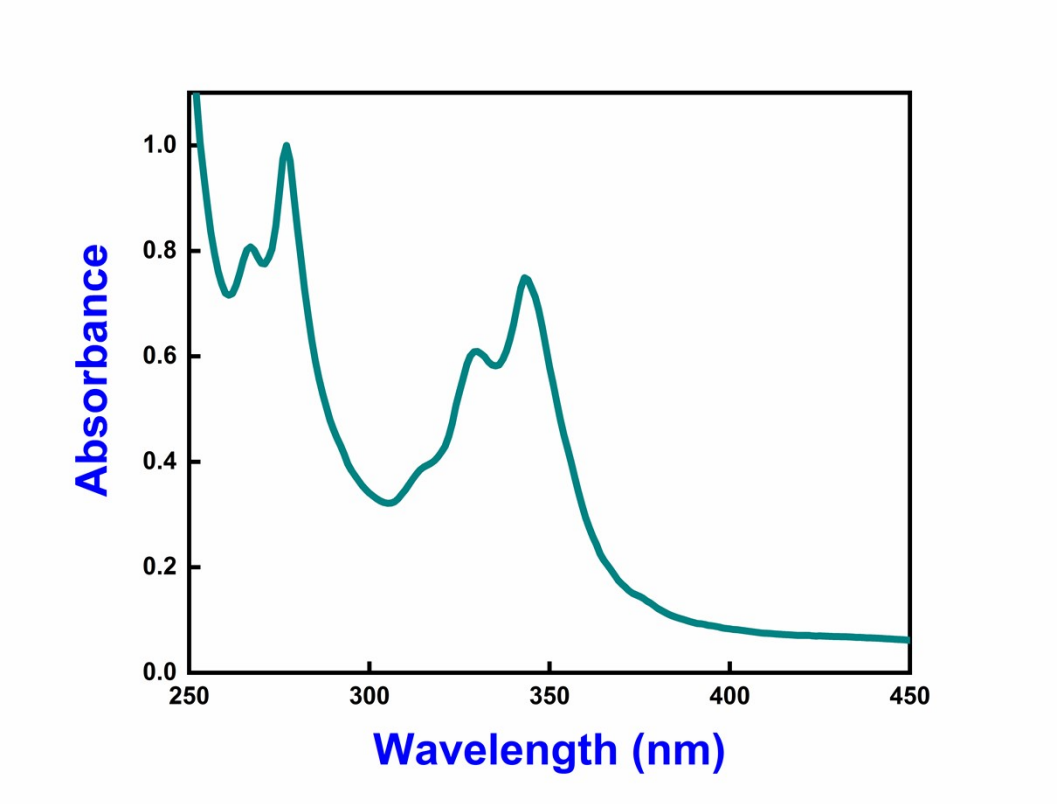


Figure S4. UV-Visible data of **1** (10 μM in HEPES:DMSO 9:1, v/v)

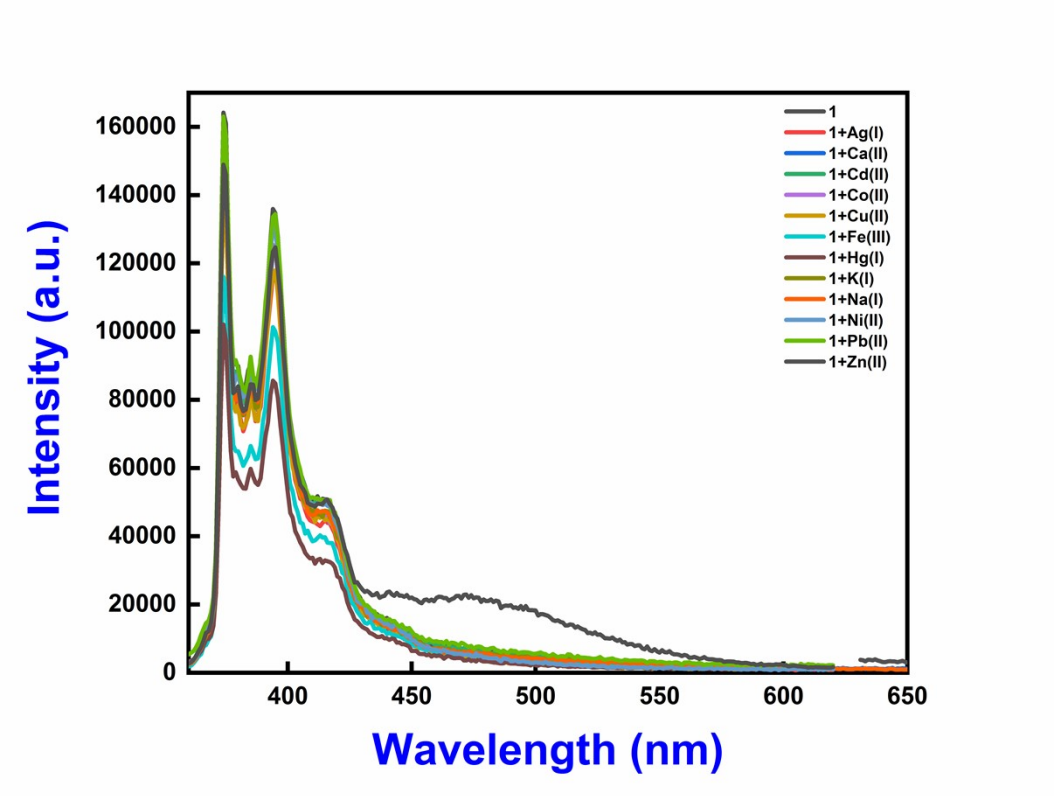


Figure S5. Emission spectra of **1** (10 μM in HEPES:DMSO 9:1, v/v)

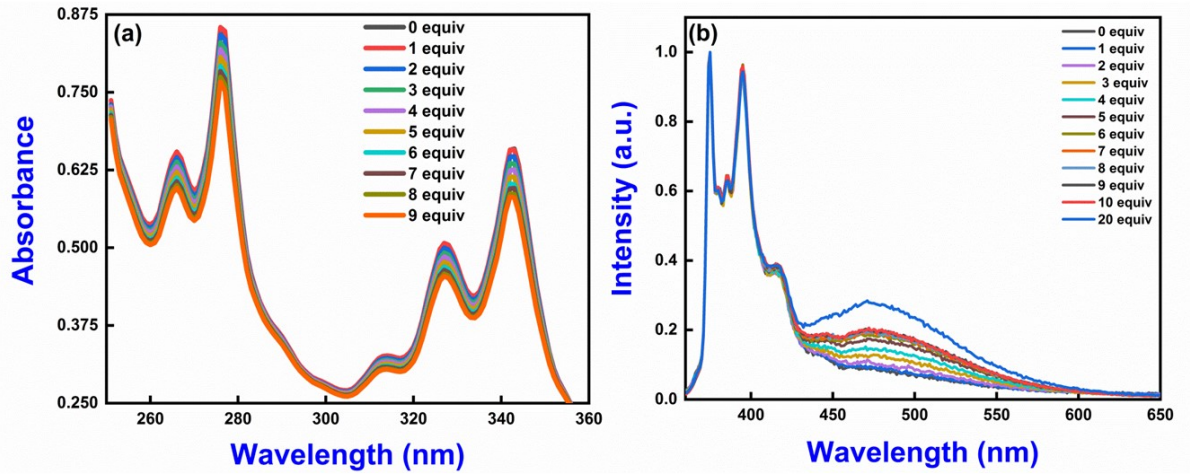


Figure S6. (a) Absorption titration spectra of **1** with Zn(II) (0-9 equiv.) **(b)** Normalised emission titration spectra of **1** with the continuous addition of Zn(II) (0-10 equiv., 10 mM) in HEPES buffer (DMSO:Water = 1:9, v/v, pH~7.4, at r.t., 10 μM) on excitation at 343 nm .

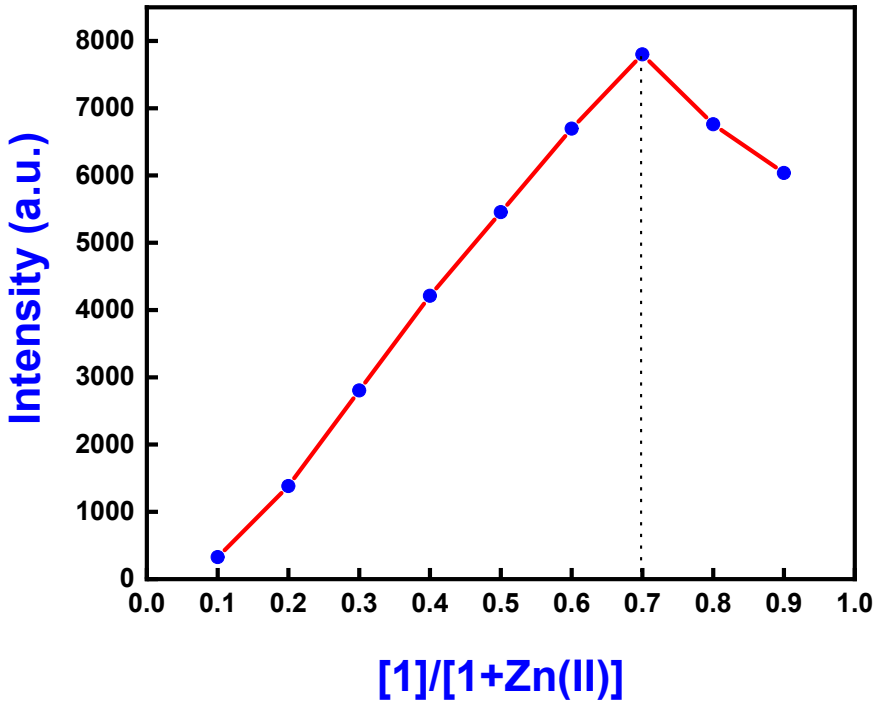


Figure S7. Jobs plot of **1**+Zn(II) complex

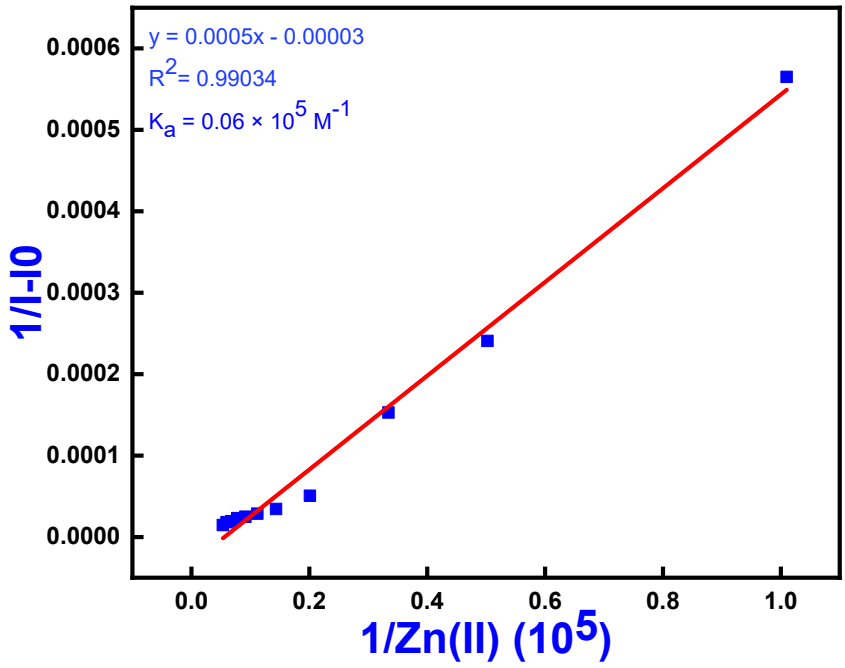


Figure S8. Benesi-Hildebrand plot of **1**+Zn(II)

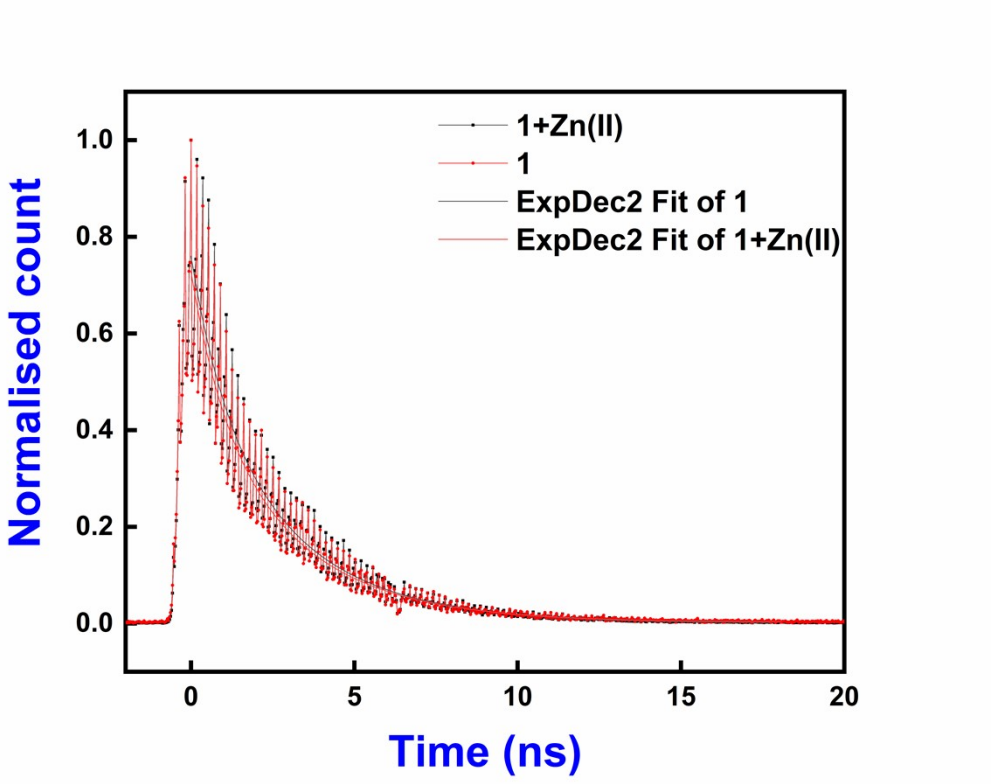


Figure S9. Fluorescence lifetime decay curve (biexponential) of **1** with Zn(II) ions.

Table S1 Fluorescence decay parameters at emission wavelength of 472 nm of **1** with and without Zn(II).

S.No.	A	τ(ns)	<τ>(ns)
1	0.2393(A1)	0.9658(τ_1)	2.3026
	0.4885(A2)	2.9575(τ_2)	
1+Zn(II)	0.2564(A1)	1.0338(τ_1)	2.3461
	0.5033(A2)	3.0146(τ_2)	

Dynamic parameters determined from A1 exp ($-x/\tau_1$) + A2 exp ($-x/\tau_2$) + y 0

The weighted mean lifetime <τ> was calculated by using following equation:

$\langle\tau\rangle = (A1\ \tau_1 + A2\ \tau_2) / (A1 + A2)$

Where, A1, A2 and τ_1, τ_2 are the fractions (A) and lifetimes (τ) respectively.

Solution concentration = 10 μM. λ = 472 nm.

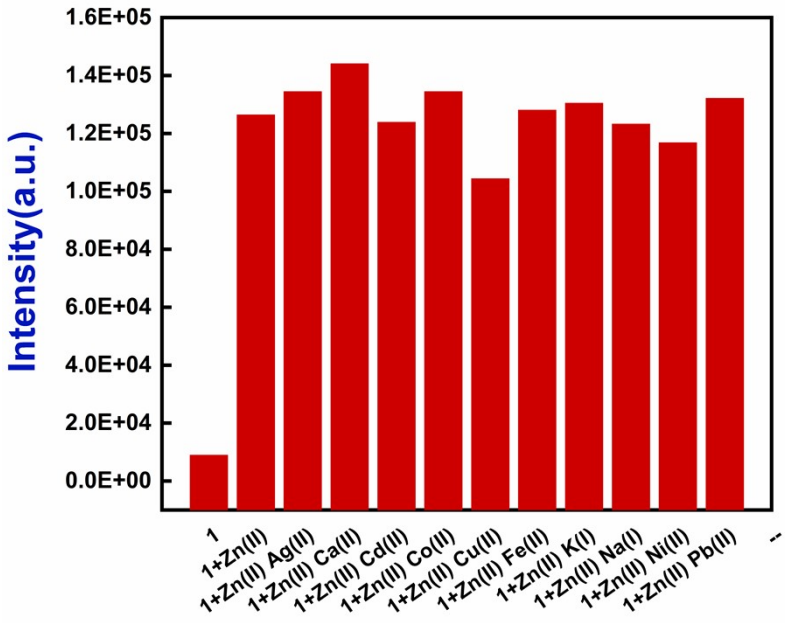


Figure S10. Interference experiment of **1**+Zn(II) complex with other competitive metal ions at room temperature.

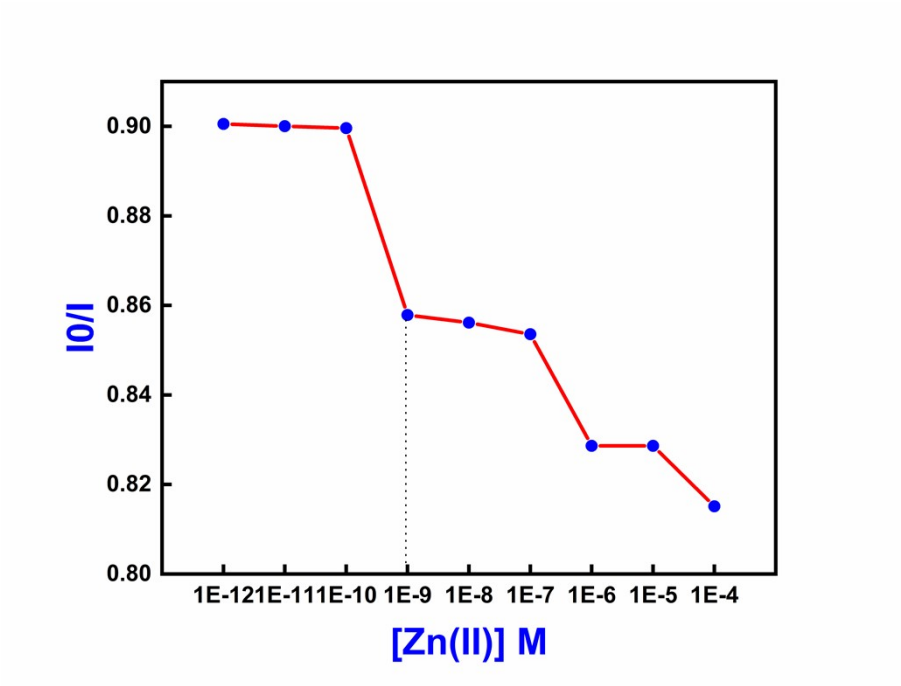


Figure S11. Sensitivity plots of **1** with different concentrations of Zn(II) ion (10^{-4} – 10^{-12} M) at room temperature.

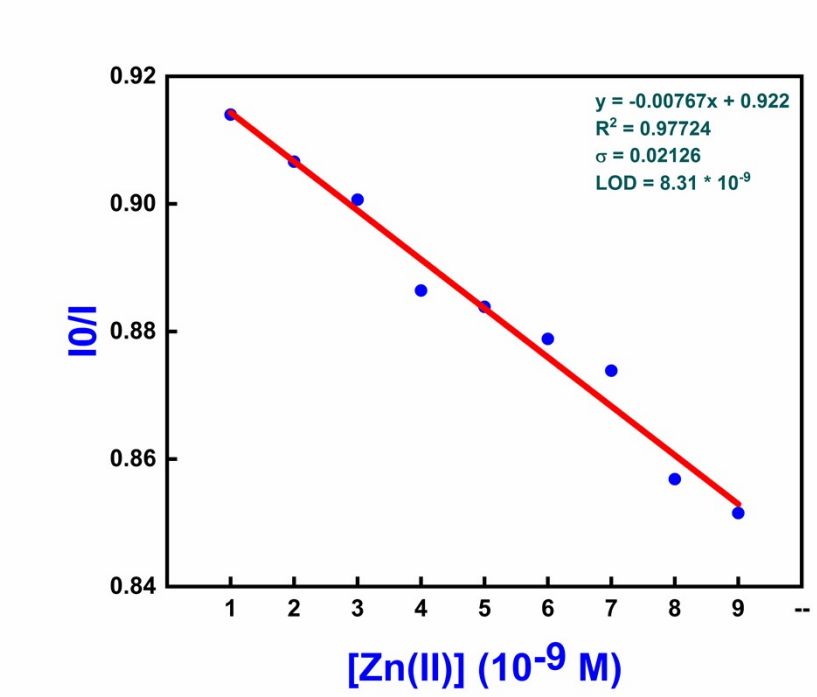


Figure S12. LOD plots of **1** with different concentration of Zn(II) ion. (1×10^{-9} M to 9×10^{-9} M via; fluorescence spectra).

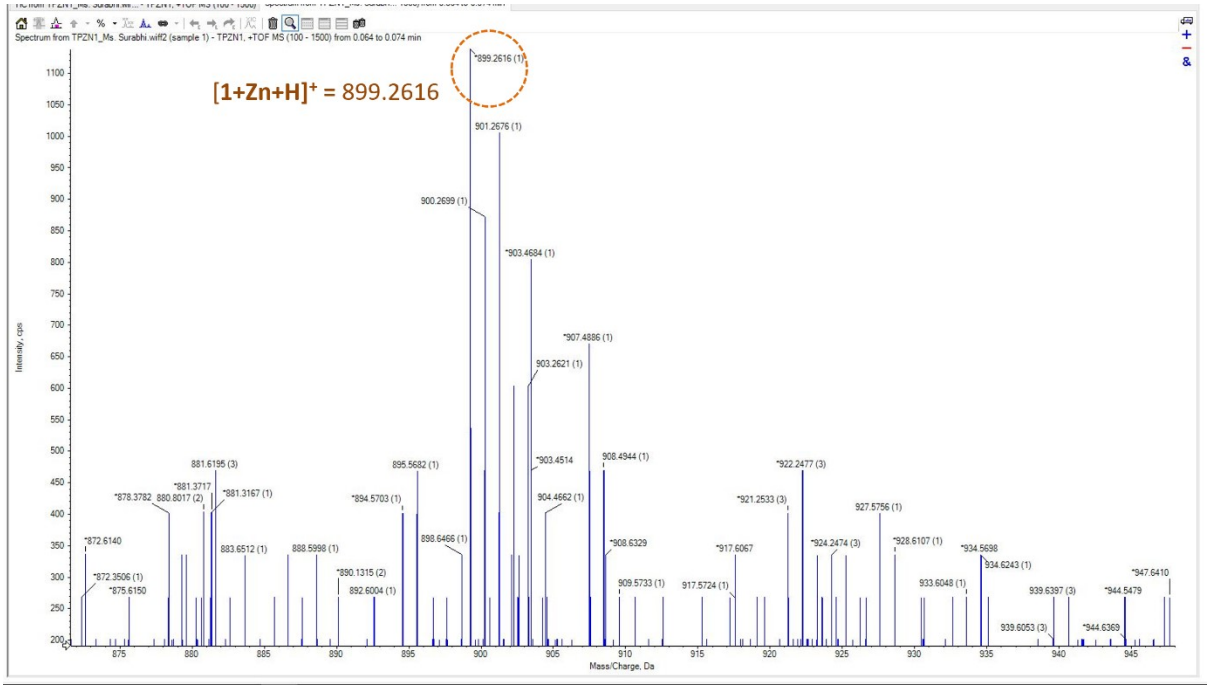


Figure S13. HRMS data of **1**+Zn(II).

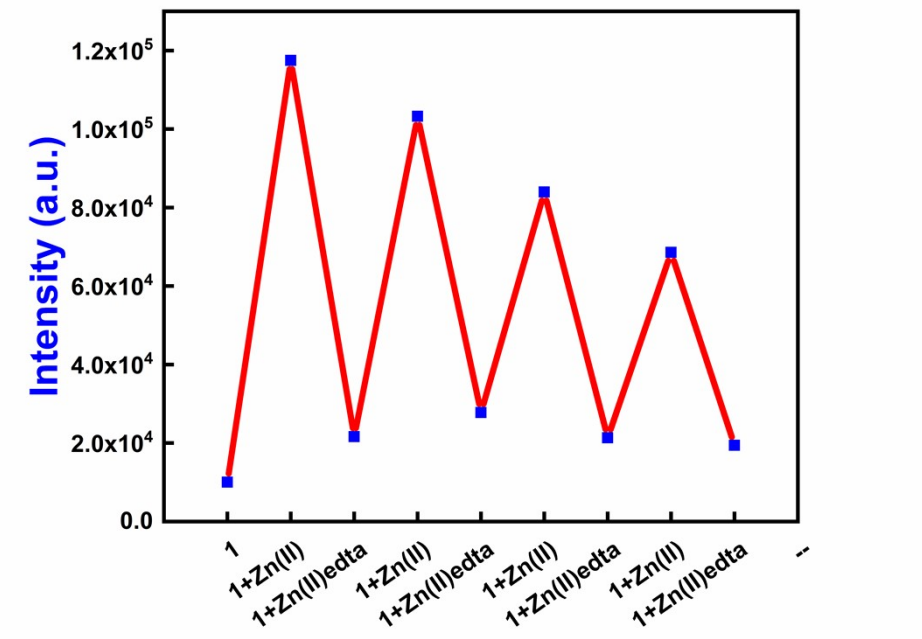


Figure S14. Reversibility study of **1**+Zn(II) with EDTA.

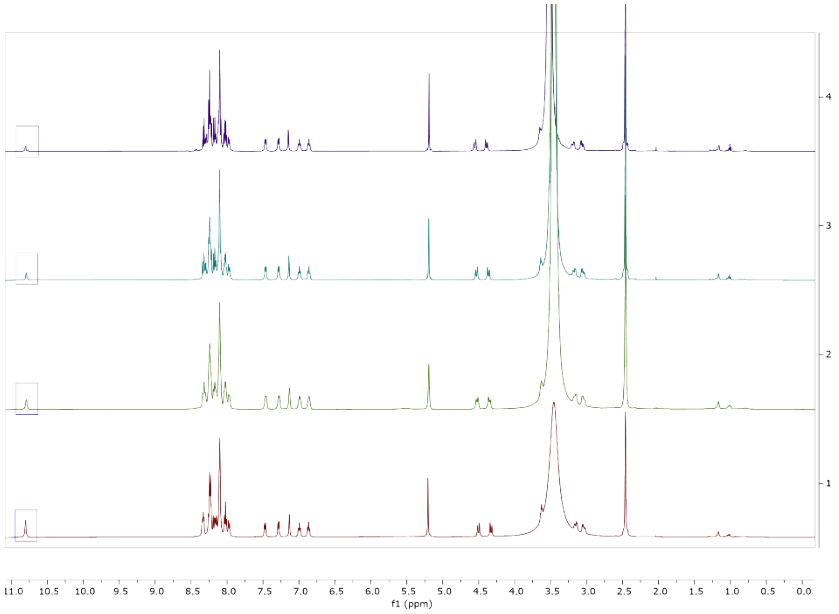


Figure S15. NMR titration experiment showing the effect of Zn(II) ion addition on NH bond of tryptophan.

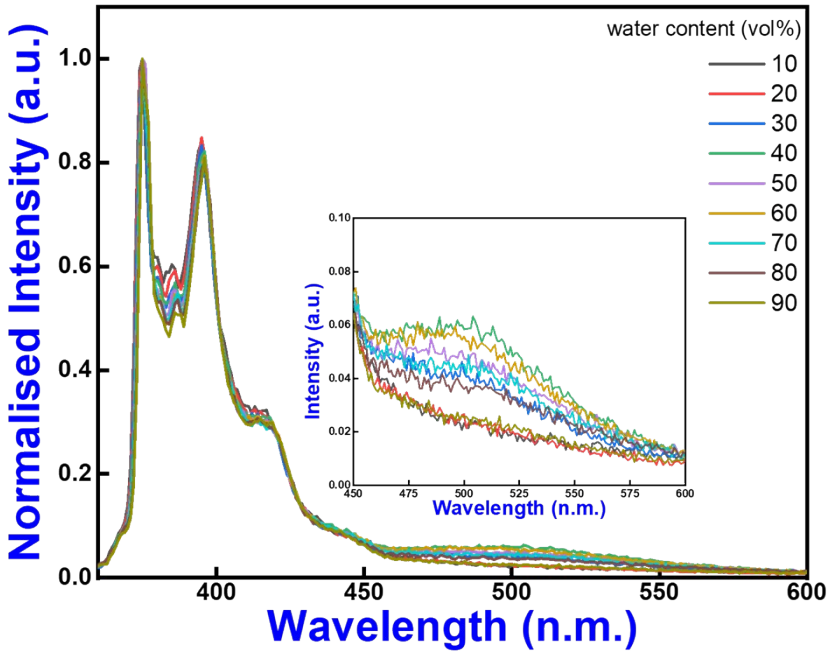


Figure S16: Fluorescence spectrum of changes in emission intensity of probe 1 in mixed solvent (DMSO and water) with increasing water fractions at 25 °C (λex = 343 nm). (Inset: zoomed image of the graph in the range 450-600 nm)

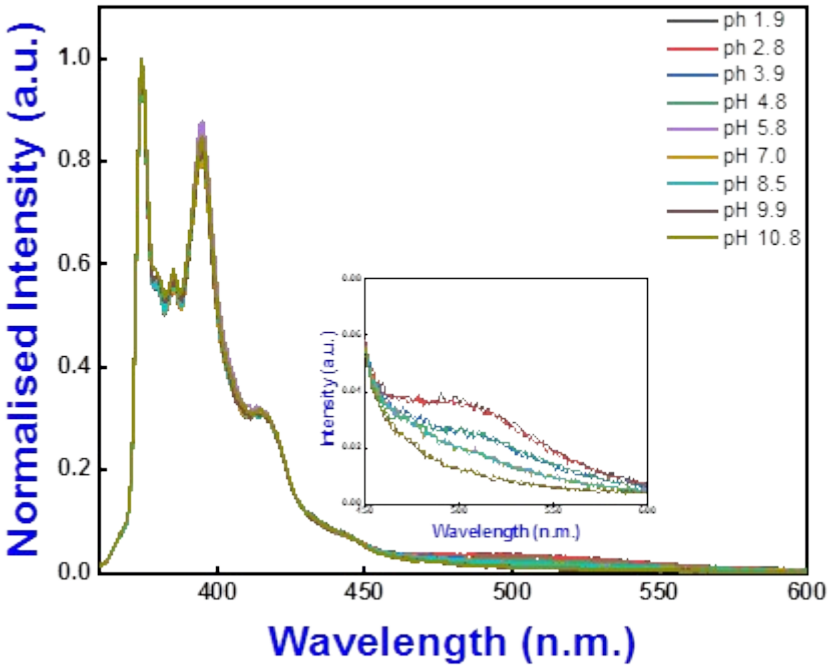


Figure S17: Fluorescence spectrum of changes in emission intensity of probe 1 in DMSO: Water (1:9) (10 μM) at different pH (λex = 343 nm). (Inset: zoomed image of the graph in the range 450-600 nm)

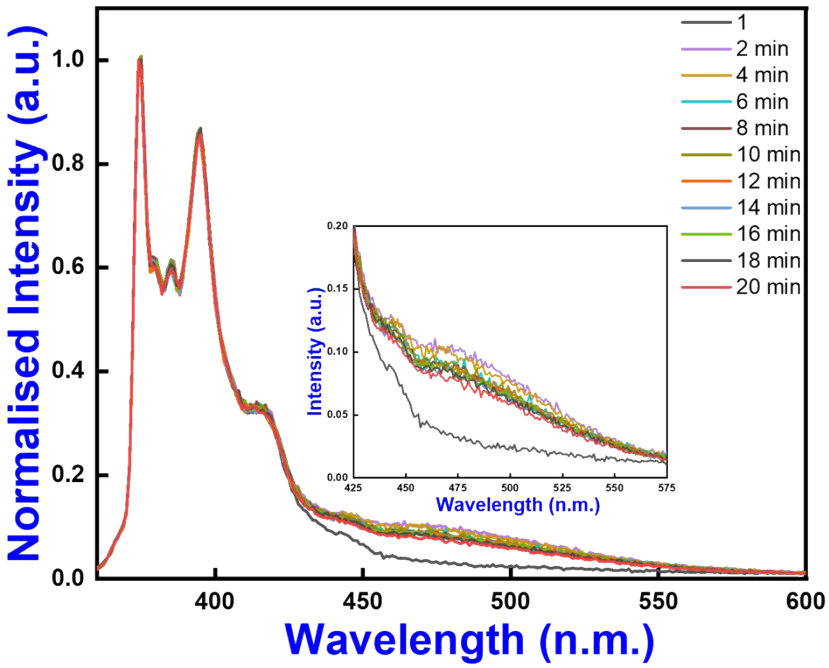


Figure S18: Fluorescence spectrum of changes in emission intensity of probe **1** in HEPES buffer (DMSO:Water = 1:9, v/v, pH~7.4, at r.t. 10 μM) at a time interval of 2 minutes (λex = 343 nm). (Inset: zoomed image of the graph in the range 425-575 nm)

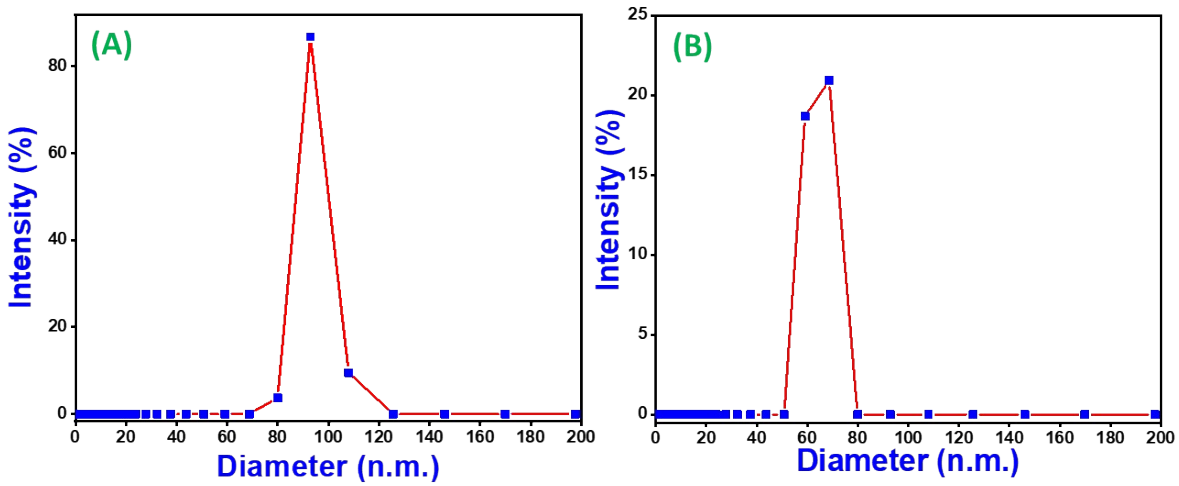


Figure S19. Dynamic light scattering (DLS) profiles of particles showing the average size distribution of (A) probe **1** (10 μM); (B) **1**+Zn(II) (10 μM).

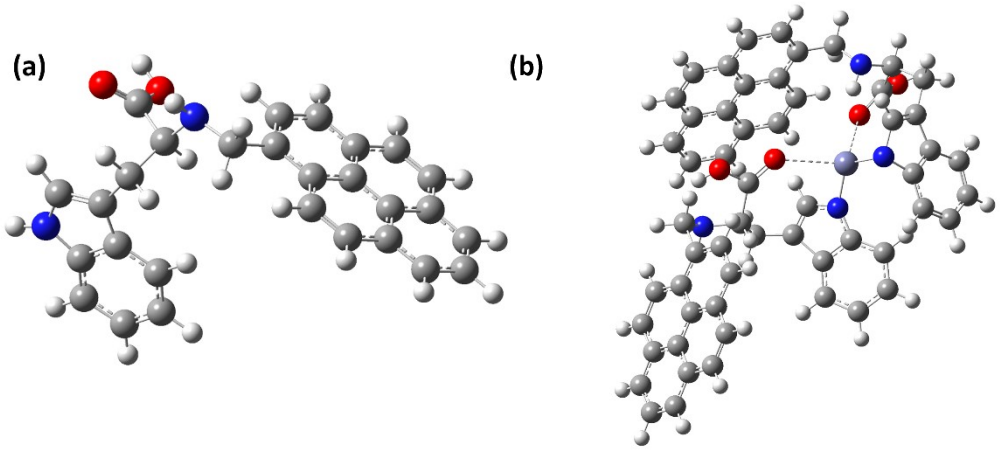


Figure S20. DFT –optimized ground state structure of **1**(a) and **1**+Zn(II) (b).

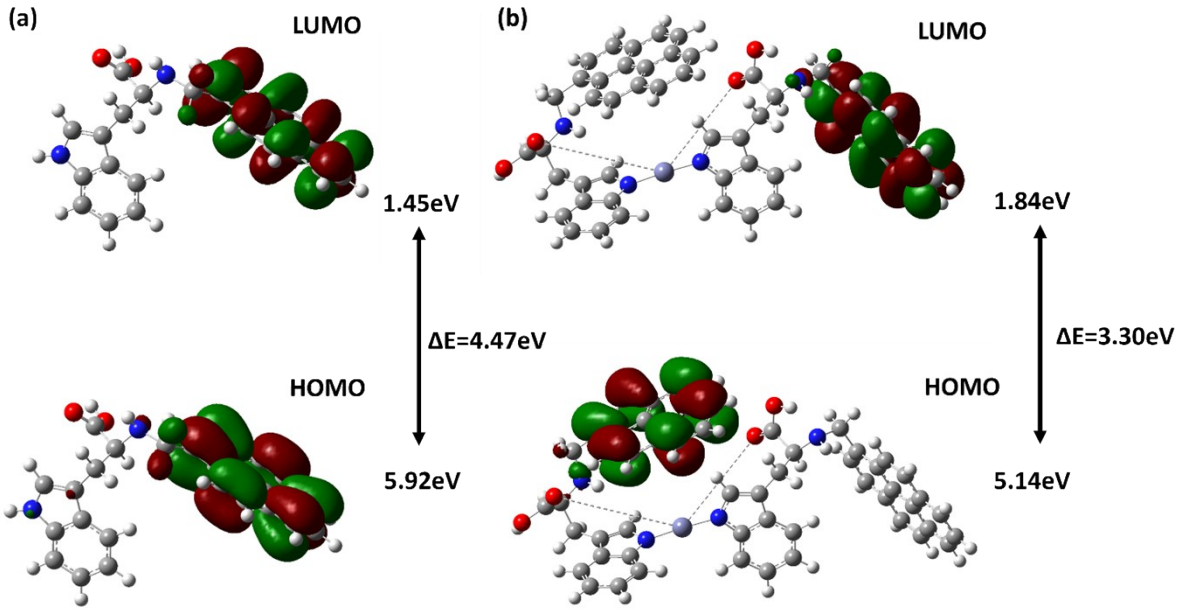


Figure S21. Energy gap between HOMO and LUMO of **1** and **1**+Zn(II).

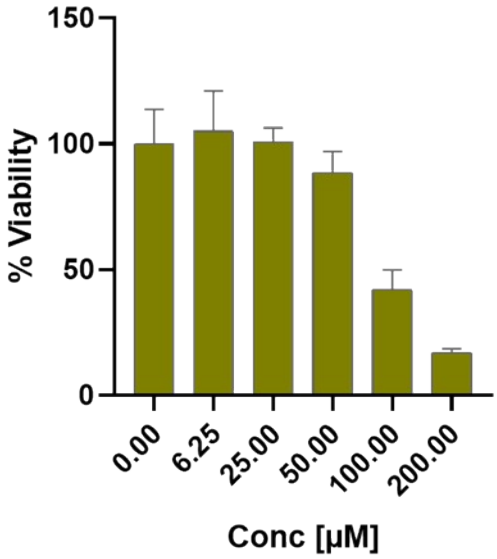


Figure S22. Cell viability determination of **1** in Raw 264.7 cells for 24 h of the treatment. IC 50 = 80.63 μM.

Methodology

Cell culture

Raw 264.7 cells were acquired from the National cell Line repository, National Center for Cell Science (NCCS) Pune, Maharashtra India. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)-high glucose, supplemented with 10% fetal bovine serum, 2mM glutamine, 1% antibiotics in a humidified 5% CO_2 environment at 37°C. Cells were passaged upon reaching 80% confluence.

Cell Viability

Raw 264.7 cells were seeded at a density of 5000 cells per well in 100 µl of DMEM in a 96 well plate. Cells were allowed to grow overnight in the CO_2 incubator. After 12h, cells were incubated with various concentration of the **1** (0, 6.25, 12.5, 25, 50, 100 and 200 µM) for 24h. Cell viability in various concentrations of **1** was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Briefly, cells were incubated with 10 µL of the MTT (5 mg/ml stock) for 2h in a CO_2 incubator at 37°C. Media was discarded and formazan crystals formed after conversion from MTT inside the cells were dissolved in 100 µL of DMSO. Absorbance was measured at 570 nm on microplate reader from Erba.

Live cell imaging microscopy

1×10^5 cells per well were seeded in a 6-well plate. Cells were allowed to grow overnight. Cells were treated with either (i) "**1**[(50 µM)]" or (ii) "**1**+ Zn(II) [**1**(50 µM) with Zn(II) (20 µM)" for 4 hours. Cells were washed thrice with warm 1X PBS followed by imaging using Leica Super-resolution SP8 microscope for live imaging (Ex 343 and Em 472 nm) at SATHI facility at Banaras Hindu University.

Data analysis

Images were processed using 'Las X' software from Leica and cell viability data were analysed using Microsoft Excel and GraphPad software was used to calculate IC-50.

Determination of the quantum yield.

Standard used: Quinine sulfate salt (QS); QS in 0.5M sulfuric acid has $\phi_f = 0.546$ (at 25˚C)

A 1×10^{-5} M solution of Quinine sulfate is prepared in 0.5M H_2SO_4, the absorbance maximum of the solution at 345 nm, Abs = 0.305. The emission spectrum is also recorded with $\lambda_{exc.}$= 345nm, fluorescence emission is integrated into the range 365-627 nm range: 6.14×10^7 a.u.

A 1×10^{-5}M solution of **1** and **1**+Zn(II) is prepared in HEPES:DMSO (9:1, v/v), and the absorbance maximum of the solution at 343 shows Abs.= 0.36248 for **1** and Abs.= 0.30418 for **1**+Zn(II). The emission spectrum is also recorded with $\lambda_{exc.}$= 343 nm for **1** and **1**+Zn(II). Fluorescence emission of **1** was integrated into the range 360-481 nm range: 4.96013×10^6 a.u. and Fluorescence emission of **1** on the addition of Zn(II) was integrated into the range 360-581 nm range: 1.68228×10^7 a.u.

Formula used for calculating quantum yield

$\Phi_s = \phi_{rf}. I_s/I_{rf}. A_{rf}/A_s$

Where Φ_s is the fluorescence quantum yield of the sample, ϕ_{rf} is the fluorescence quantum yield of the standard reference, I_s and I_{rf} are the integrated emission intensities of the sample and the standard reference respectively, A_{rf} and A_s are the absorbance of the standard reference and the sample at the excitation wavelength, respectively, and n_s and n_{rf} are the refractive indexes of the corresponding solution of sample and reference.

The refractive index of the solvent η_s/η_{rf} were considered one.

So,

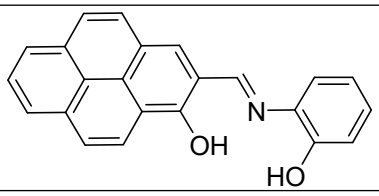
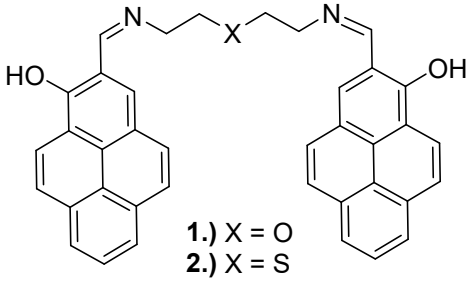
Quantum yield of neat 1 (ϕ) = 0.0136

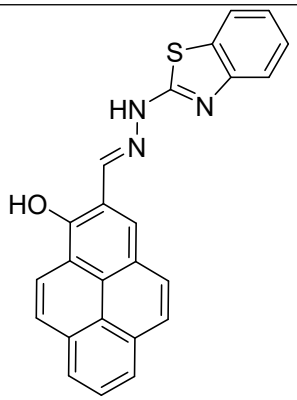
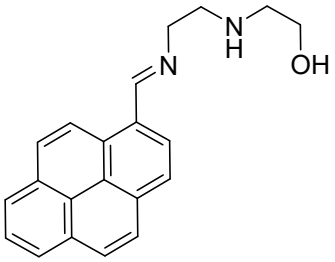
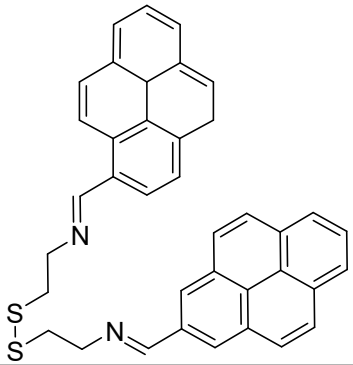
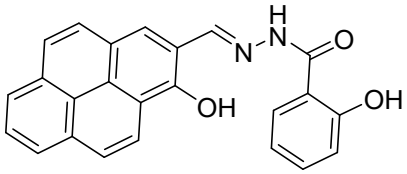
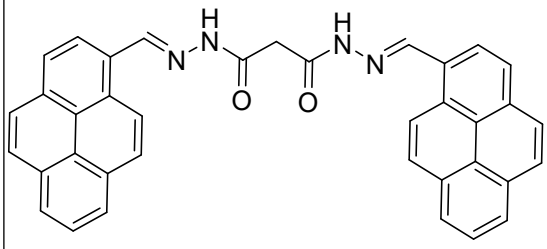
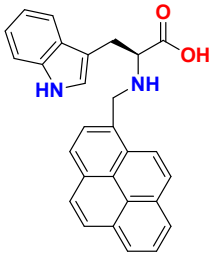
Quantum yield of 1+Zn(II) (ϕ) = 0.0548

Table S2: Quantitative determination of quantity of Zn(II) in water samples.

S.No.	Sample	Zn(II) added (µM)	Zn(II) found (µM)	% Recovery	%RSD (n=3)	Zn(II) added (µM) (from ICP-MS)	Zn(II) found (µM) (from ICP-MS)	% Recovery (from ICP-MS)
1	Ganga Water	20	20.9	104.5	0.876472	30.5810	31.0092	101.4
2	Lake Water	20	24.51	122.55	5.183332	30.5810	31.7125	103.7
3	Pond Water	20	25.58	127.9	9.041003	30.5810	32.4617	106.15
4	Tap Water	20	30.41	152.05	3.657323	30.5810	33.1193	108.3

Table S3: Comparison of applicability of **1** and previously reported pyrene-based zinc sensors.

S.No.	Structures of sensors	Method	LOD	Applications	Ref.
1		Fluorescence	3.29×10^{-7} M	NA	1
2	 **1.)** X = O **2.)** X = S	Fluorescence	**1.)** 1.24 mM **2.)** 0.85 mM	biology studies	2

3		Fluorescence	2.58×10^{-7} M	biology studies	3
4		Fluorescence	4.82×10^{-7} M	biology studies	4
5		Fluorescence	8.548×10^{-8} M	NA	5
6		Fluorescence	1.38×10^{-6} M	NA	6
7		Fluorescence	5.1 nm	Biology studies	7
8		Fluorescence	8.31×10^{-9} M	Biology studies, Real water analysis	This work

References:

1. J. Y. Choi, D. Kim and J. Yoon. A highly selective “turn-on” fluorescent chemosensor based on hydroxy pyrene–hydrazone derivative for Zn^{2+}, *Dyes Pigm.*, 2013, **96**, 176-179. https://doi.org/10.1016/j.dyepig.2012.08.009
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