Electronic Supplementary Material (ESI)
For
New Journal of Chemistry

<u>Benzilbis(2-hydroxyanil) – highly efficient ligand for sensing ferric (Fe^{3+}) ions</u>

Bony Pariyadan Joy [a], Sudeep Paul [a], Basavaraju Gari Manohar [a] and Vittal B. Gudimetla [a]*

[a] Department of Chemistry
School of Basic and Applied Sciences
Central University of Tamil Nadu
Thiruvarur – 610 005, Tamil Nadu, India
E-mail: vittalbabu@cutn.ac.in

Table of Contents

1. General Information

All the reagents and solvents used in the studies were obtained from Alfa-Aesar, SRL, Merck, Moly-Chem and Avra. Chloride, acetate, nitrate or sulphate salts of metal ions were used for the sensing studies. The metal salts used in the study are $CaCl_2$, $CdCl_2$, $CoCl_2.6H_2O$, $CuCl_2.2H_2O$, $FeCl_3$, $HgCl_2$, KCl, $LiCl$, $NaCl$, $NiCl_2$, $ZnCl_2$, $CrCl_3.6H_2O$, $Ba(OAc)_2$, $Mn(OAc)_2$, $FeSO_4.7H_2O$, $AgNO_3$ and $Pb(NO_3)_2$. HPLC grade water and methanol was used for the fluorescence analysis. Infrared (FT-IR) spectra were recorded on Shimadzu IR Affinity 1S WL spectrophotometers. NMR spectra were recorded Bruker Avance Neo-400 MHz FT-NMR spectrometer at 400 MHz (for ^1H NMR) and 100 MHz (for $\{^1H\}$ ^{13}C NMR). Chemical shifts for ^1H NMR were internally referenced to tetramethylsilane (δ = 0.00 ppm) or residual solvent signal $CDCl_3$ (δ = 7.26 ppm). $\{^1H\}^{13}C$ NMR spectra were internally referenced to the residual solvent signal $CDCl_3$ (δ = 77.0 ppm). The data of ^1H NMR are reported as follows: (chemical shift δ (ppm), multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet), integration, coupling constant (Hz)). High resolution mass spectrometry was performed using Thermo Fischer Scientific Exactive Plus Orbitrap mass spectrometer. Melting points were measured using Sigma industries melting point apparatus. The UV-visible absorption spectra were recorded using Shimadzu UV-2450 double beam spectrophotometer and fluorescence studies were performed using Jasco FP-8200 spectrofluorometer.

The cyclic voltammetry studies were performed using CH Instruments, Inc. CHI6035D potentiostat using a three electrode system. Glassy carbon, platinum wire were used as the working and counter electrode respectively and Ag/AgCl as the reference electrode. The CV studies were performed in a mixture of MeCN/Water (9:1). The solvents used for the CV studies were of HPLC grade and degassed prior to the experiment. Ferrocene was used for standardization of the electrochemical set up and tetrabutylammonium hexafluorophosphate was used as the supporting electrolyte. The glassy carbon electrode was polished prior to each experiment using 0.5 micron alumina on a microfiber cloth. The solvents were deoxygenated by bubbling the solvent with nitrogen for at least 10 minutes and the nitrogen environment above the solvent phase was maintained throughout the experiment.

2. Synthesis and characterisation of ligand (L)
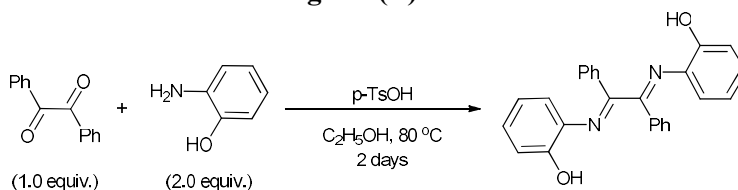
A 250 mL round bottom flask was charged with a magnetic stir bar, 2-aminophenol 2.18 g (20 mmol, 2.00 equiv.) and dissolved in 50 mL ethanol. Benzil 2.10 g (10 mmol, 1.00 equiv.) was added in small portion to the above solution over a period of 1hour. Thereafter, p-toluene sulfonic acid 0.95 g (5 mmol, 0.5 equiv.) was added to the above reaction mixture and it was refluxed for 48 hours. After completion of reaction time the solvent was removed using rotary evaporator, and column chromatography was performed using silica gel with ethyl acetate/hexane as eluent to isolate the desired product.

Yield: 0.5 g (13%); white powder; m.p: 210-215 °C; FT-IR (Neat, in cm⁻¹): 3361 (O-Hstr), 1604.77 (C=Nstr).^{1}H-NMR (400 MHz; CDCl₃): 7.27-7.24 (m, 2H), 7.10 (t, 4H, J = 8 Hz), 6.99-6.97 (m, 4H), 6.91-6.83 (m, 6H), 6.74-6.72 (m, 2H), 4.77 (s, 2H); ^{13}C{^{1}H} (100 MHz; CDCl₃): 144.2, 139.0, 129.5, 128.9, 127.7, 127.3, 121.3, 120.9, 115.6, 115.3, 85.9. HRMS: (ESI+): m/z calc. for [C₂₆H₂₁O₂N₂]⁺ ([M+H]⁺): 393.15975; found: 393.15889.
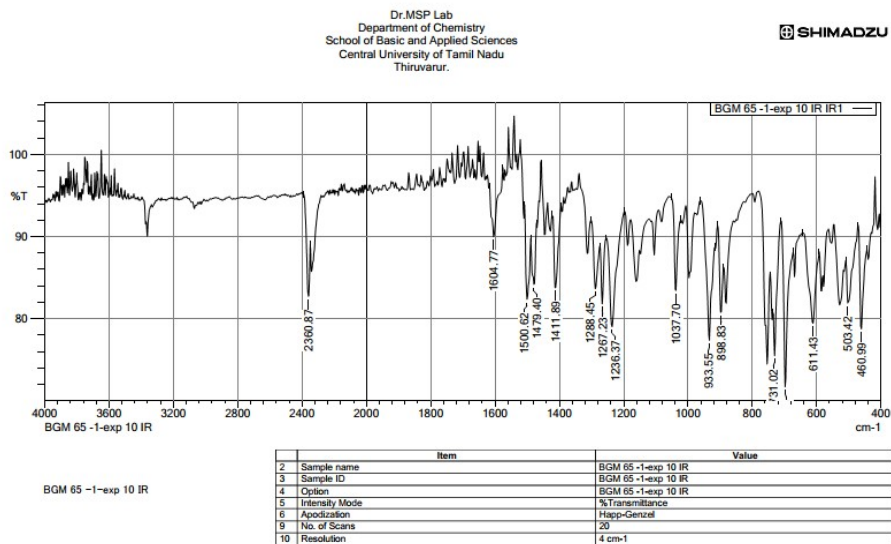
Figure S1: FT-IR spectra of ligand **L.**
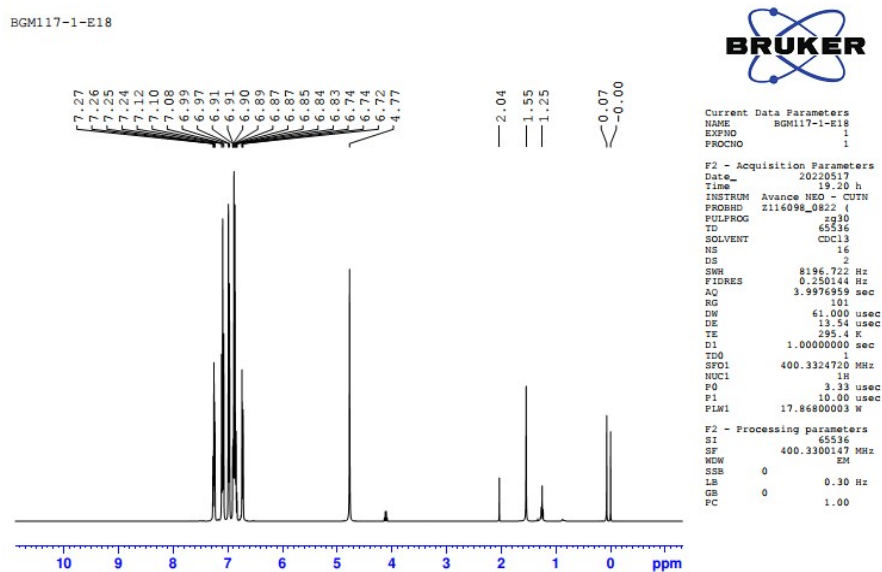
Figure S2: ^{1}H-NMR of ligand **L.**

4

Figure S3: ^1H {^{13}C} of ligand **L**.

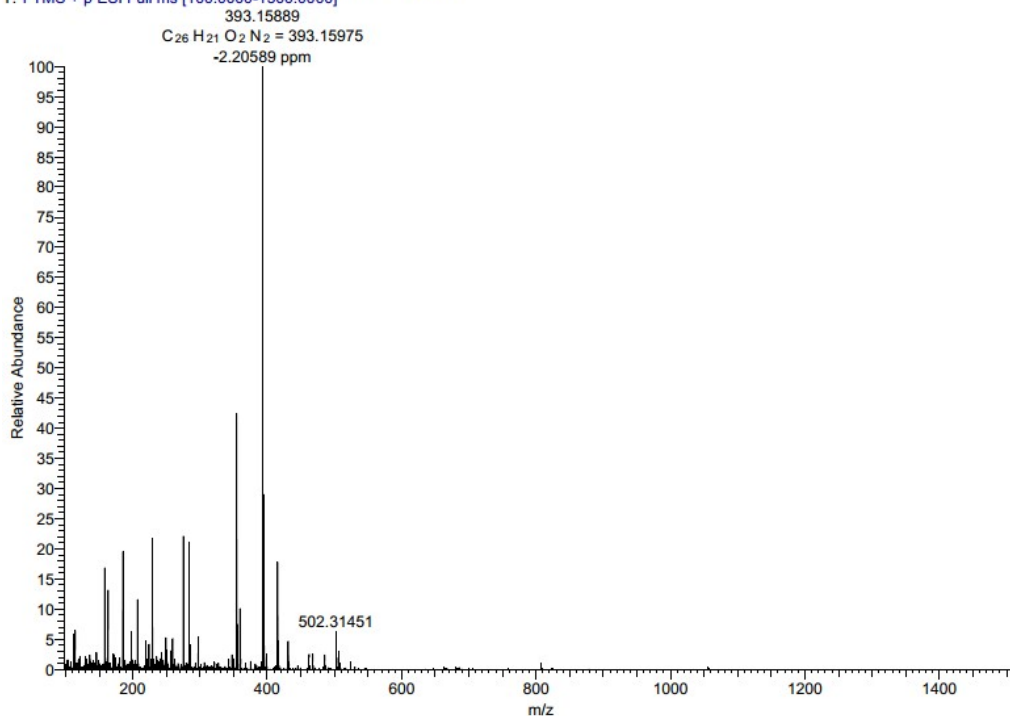**Figure S4:** HRMS data of **L**.

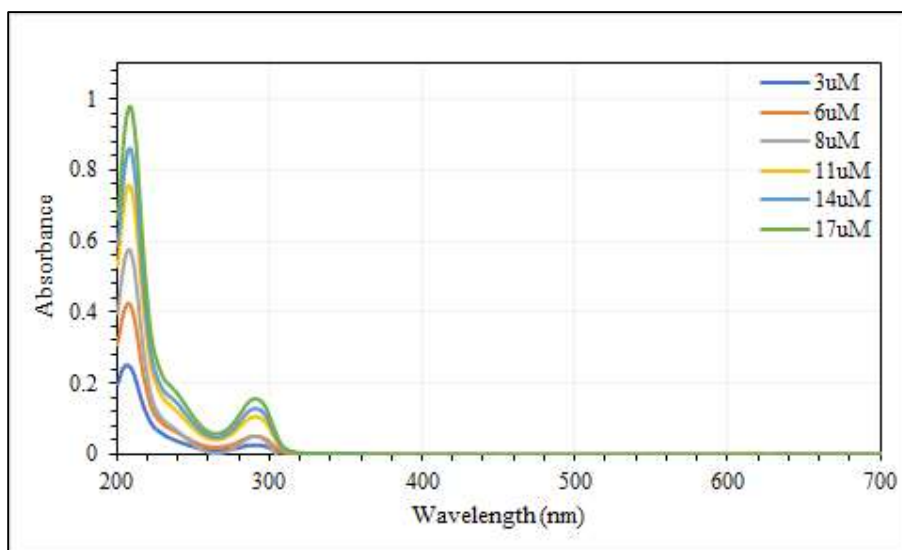### 3. Absorption spectra of L at different concentrations

Figure S5: UV-Visible absorption spectra of ligand **(L)** in MeOH at 3, 6, 8, 11, 14, 17 μM concentrations.

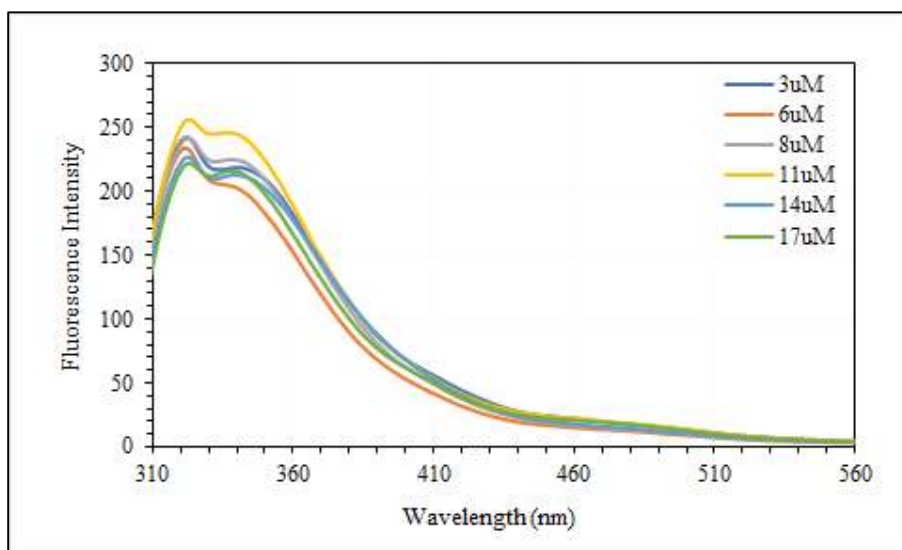### 4. Emission spectra of (L) at different concentrations

Figure S6: Fluorescence spectra of ligand **(L)** in MeOH at 3, 6, 8, 11, 14, 17 μM concentrations at λ_{ex} = 291 nm.

5. Experimental procedure for fluorescecne studies

Stock solution (2mM) of different metal salts were prepared in water and 50 µL of the above solution is added to 10 mL SMF containing 1.0 mL of ligand **L** solution (20µM) in methanol. The SMF is made up with water to give a solution with metal to ligand ratio of 1:5. This solution is used for the fluorescence analysis. The intensity at 354 nm is noted for excitation at 291 nm.
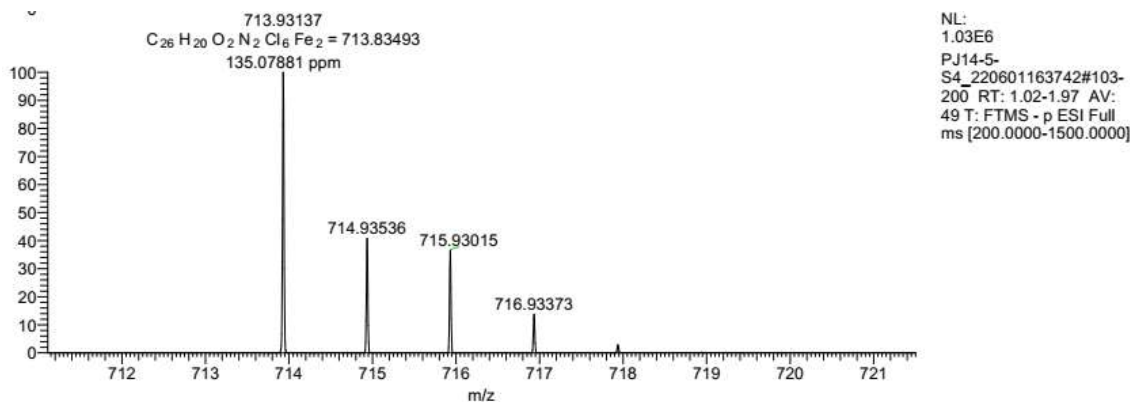
6. HRMS analysis

Figure S7: HRMS spectra of the analysis mixture (1L: 5M) used for fluorescence studies.
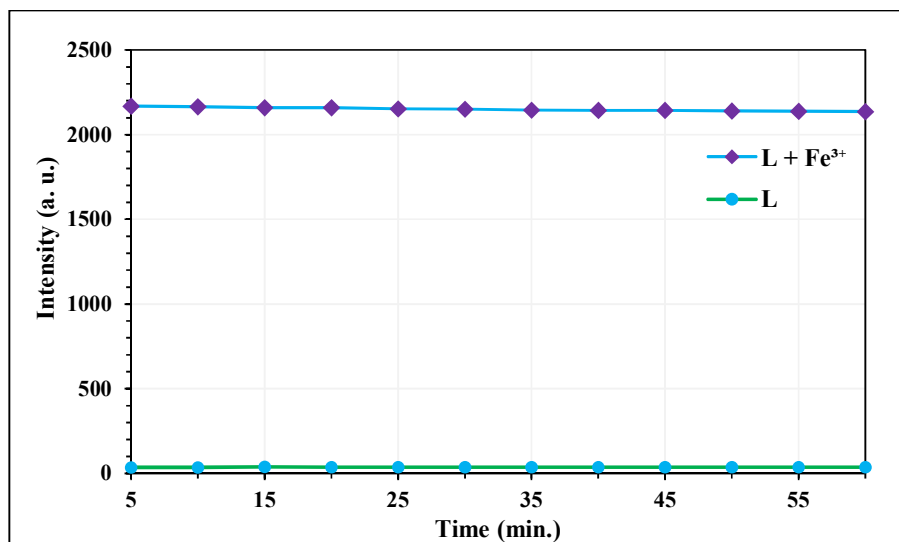
7. Time dependent fluorescence response

Figure S8: Time-dependent fluorescence response of **L** and **L+Fe³⁺** in MeOH/water (1:9 v/v) at 354 nm, excitation at 291 nm.

8. UV-Vis spectral changes of L in presence of Fe^{3+}
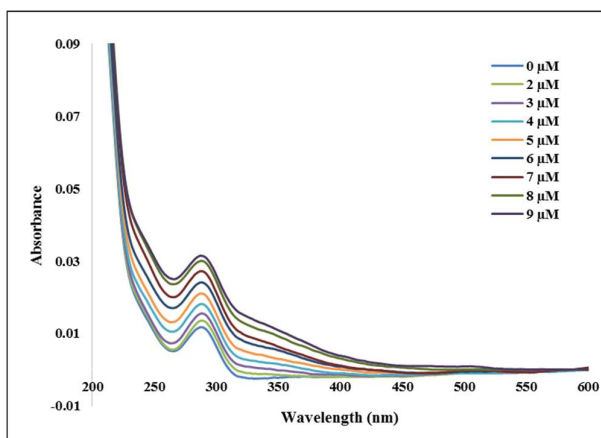

Figure S9: UV-Vis spectral changes of **L** in presence of Fe^{3+} (0-9 µM) in MeOH/water (1:9 v/v) solution.

9. Determination of binding constant (K_a)
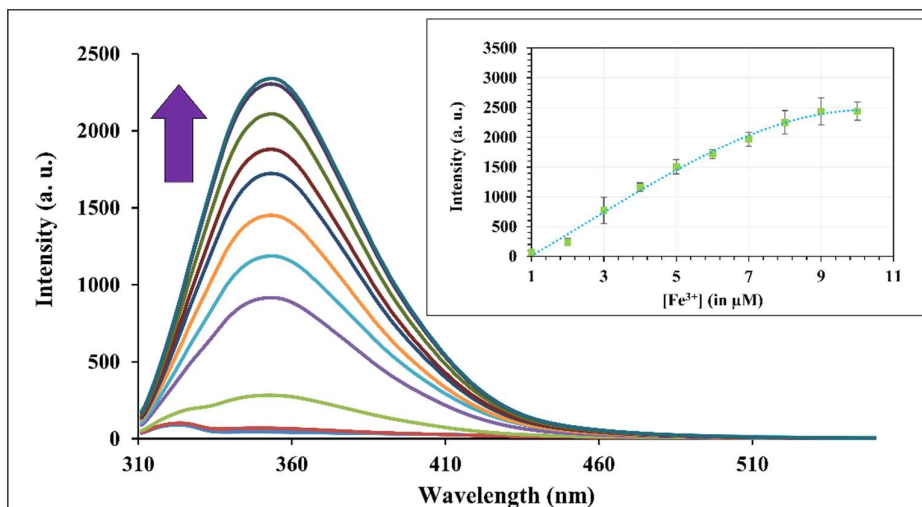
Figure S10: Fluorescence spectra of **L** (2µM) in MeOH/water (1:9 v/v) in the presence of different concentration of Fe^{3+} (1-10 µM). Inset: Fluorescence response of **L** (2 µM) as a function of Fe^{3+} (1-10 µM) at 354 nm, excitation at 291 nm.

The binding constant of **L** with Fe^{3+} was determined using the Hills equation as given below.

$$\log (Y/(1-Y)) = n\log[G] + \log K_a, \text{ where}$$

Y = fraction of ligand binding sites filled
$Y = (I-I_0)/(I_{max}-I_0)$
n = Hill constant
[G] = concentration of Fe^{3+}
K_a = association constant
From the equation of line obtained from $\log ((I-I_0)/(I_{max}-I))$ vs $\log[Fe^{3+}]$ plot, the $\log K_a$ was found to be 17.782 from which the K_a value was determined to be 6.05×10^{17} M^{-1}.

Figure S11: Hills plot of $\log((I-I_0)/(I_{max}-I))$ vs $\log[Fe^{3+}]$. (Values are obtained from the average of four independent experiments)

10. Determination of limit of detection (LOD)

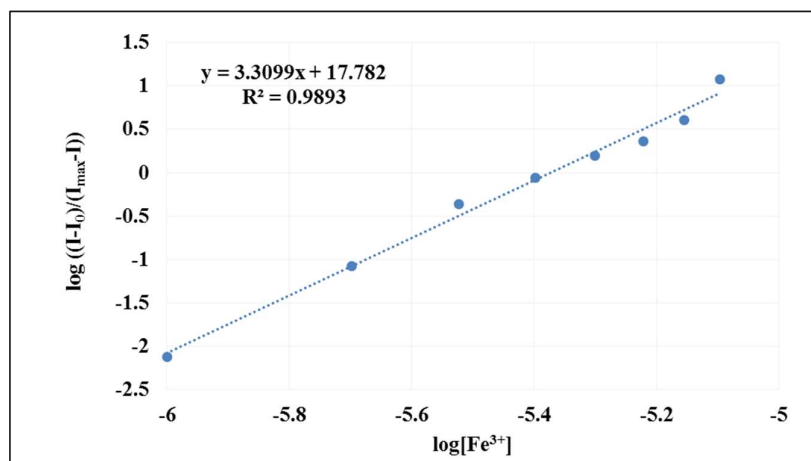The limit of detection (LOD) of ligand **(L)** was determined using the equation:

$$LOD = (3\sigma)/K,$$

where σ is the standard deviation of the blank measurement and K is the slope of the plot between fluorescence intensity and concentration of Fe^{3+} ion. The standard deviation of blank measurement was derived from four measurements ($\sigma = 14.65344$). The plot between fluorescence intensity and concentration of Fe^{3+} was linear in the range of 1µM to 8 µM and from those data the slope of the plot was found to be 322.76. Thus the LOD = $(3 \times 14.65344)/322.76 = 0.136$ µM or 136 nM.

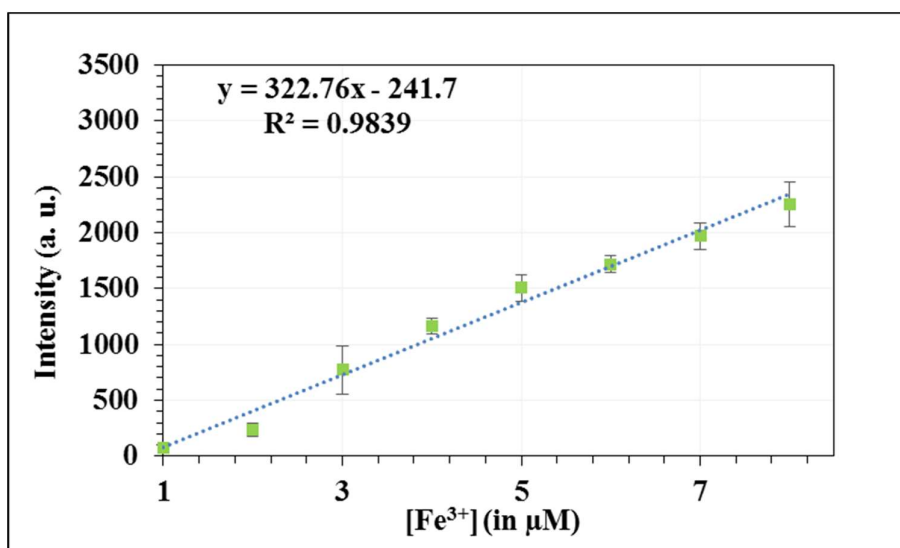**Figure S12:** Fluorescence response of **L** upon addition on 1.0-8.0 µM of Fe^{3+} in MeOH/Water (9:1) with $\lambda_{ex} = 291$ nm and $\lambda_{em} = 354$ nm. (Values are obtained from the average of four independent experiments)

9

11. Comparison of prior reports for Fe^{3+} ion sensing

Sl. No	LOD of Fe^{3+}	Solvent	Method	Reference
1.	0.136 µM	MeOH-H_2O (1:9)	Turn on	**This work**
2.	0.45 µM	CH_3CN	Turn on	*Sensors Actuators B Chem.*, 2016, **230**, 199–205[1]
3.	0.58 µM	MeOH-H_2O (1:1, Tris-HCl buffer, pH = 7.2)	Turn on	*Chem. Commun.*, 2014, **50**, 4631[2]
4.	50 nM	Tris HCl-CH_3CN (1:1) (pH=7.4)	Turn on	*Dalt. Trans.*, 2012, **41**, 11753[3]
5.	0.26 µM	CH_3CN-H_2O (1:1)	Turn on	*RSC Adv.*, 2016, **6**, 74389–74393[4]
6.	36.64 µM 14.33 µM	THF-H_2O (7:3)	Turn off	*ACS Omega*, 2022, **7**, 11114–11125[5]
7.	98.3 nM	CH_3CN-Tris	Turn on	*Dye. Pigment.*, 2021, **194**, 109637[6]
8.	97.3 nM	MeOH	Turn off	*ACS Omega*, 2019, **4**, 6597–6606[7]
9.	7.4 nM	DMSO-H_2O (3:7)	Turn on	*Tetrahedron Lett.*, 2016, **57**, 1852–1855[8]
10.	1 µM	H_2O	Turn on	*Dye. Pigment.*, 2019, **169**, 51–59[9]
11.	0.235 µM	H_2O	Turn on	*New J. Chem.*, 2019, **43**, 4867–4877[10]
12.	0.13 µM	H_2O-CH_3CN (9:1)	Turn on	*ACS Appl. Mater. Interfaces*, 2014, **6**, 18408–18412[11]
13.	6.93 µM	EtOH-H_2O (4:1)	Turn on	*Dalt. Trans.*, 2013, **42**, 10093[12]
14.	0.35 nM	H_2O-CH_3CN (7:3)	Turn on	*J. Mater. Chem. C*, 2014, **2**, 9077–9082[13]
15.	0.373 µM	THF-H_2O (6:4)	Turn on	*J. Photochem. Photobiol. A Chem.*, 2018, **358**, 157–166[14]
16.	0.29 µM	MeOH-H_2O (1:1)	Turn on	*New J. Chem.*, 2017, **41**, 8359–8369[15]

Table S1: Prior reports on Fe^{3+} ion sensing

12. References

1   T. Nandhini, P. Kaleeswaran and K. Pitchumani, *Sensors Actuators B Chem.*, 2016, **230**, 199–205.

2   L. Qiu, C. Zhu, H. Chen, M. Hu, W. He and Z. Guo, *Chem. Commun.*, 2014, **50**, 4631.

3   N. R. Chereddy, S. Thennarasu and A. B. Mandal, *Dalt. Trans.*, 2012, **41**, 11753.

4   S. Chan, Q. Li, H. Tse, A. W. M. Lee, N. K. Mak, H. L. Lung and W.-H. Chan, *RSC Adv.*, 2016, **6**, 74389–74393.

5   S. Sasan, T. Chopra, A. Gupta, D. Tsering, K. K. Kapoor and R. Parkesh, *ACS Omega*, 2022, **7**, 11114–11125.

6   B. Li, X. Gu, M. Wang, X. Liu and K. Xu, *Dye. Pigment.*, 2021, **194**, 109637.

7   Y. Liang, R. Wang, G. Liu and S. Pu, *ACS Omega*, 2019, **4**, 6597–6606.

8   Y. Gao, H. Liu, Q. Liu and W. Wang, *Tetrahedron Lett.*, 2016, **57**, 1852–1855.

9   B. Lim, B. Baek, K. Jang, N. K. Lee, J. H. Lee, Y. Lee, J. Kim, S. W. Kang, J. Park, S. Kim, N. W. Kang, S. Hong, D. D. Kim, I. Kim, H. Hwang and J. Lee, *Dye. Pigment.*, 2019, **169**, 51–59.

10   B. K. Kundu, R. Singh, R. Tiwari, D. Nayak and S. Mukhopadhyay, *New J. Chem.*, 2019, **43**, 4867–4877.

11   B. Sui, S. Tang, T. Liu, B. Kim and K. D. Belfield, *ACS Appl. Mater. Interfaces*, 2014, **6**, 18408–18412.

12   W. Chen, W. Gong, Z. Ye, Y. Lin and G. Ning, *Dalt. Trans.*, 2013, **42**, 10093.

13   C. Han, T. Huang, Q. Liu, H. Xu, Y. Zhuang, J. Li, J. Hu, A. Wang and K. Xu, *J. Mater. Chem. C*, 2014, **2**, 9077–9082.

14   S. K. Dwivedi, R. C. Gupta, R. Ali, S. S. Razi, S. K. Hira, P. P. Manna and A. Misra, *J. Photochem. Photobiol. A Chem.*, 2018, **358**, 157–166.

15   R. Alam, R. Bhowmick, A. S. M. Islam, A. Katarkar, K. Chaudhuri and M. Ali, *New J. Chem.*, 2017, **41**, 8359–8369.