Electronic Supplementary Information

A viscochromic, mechanochromic, unsymmetrical azine for selective detection of Al^{3+} and Cu^{2+} ions and its mitotracking studies

Richa Yadav,[a] Abhishek Rai,[a] Avinash Kumar Sonkar,[a] Vipin Rai,[b] Subash Chandra Gupta,[b] and Lallan Mishra*[a]

[a] *Department of Chemistry, Institute of Science, Banaras Hindu University, Varanasi - India (221005).*

[b] *Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi - India (221005).*

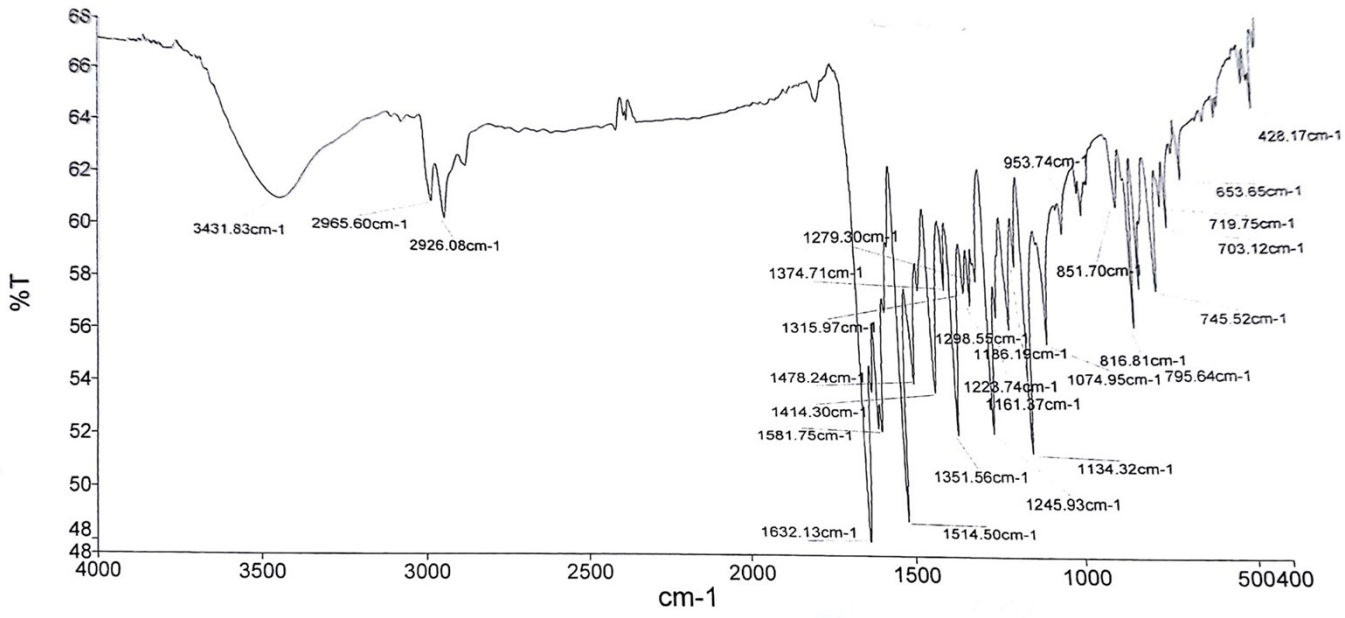

Fig. S1: IR spectrum of **NDEA** at room temperature.

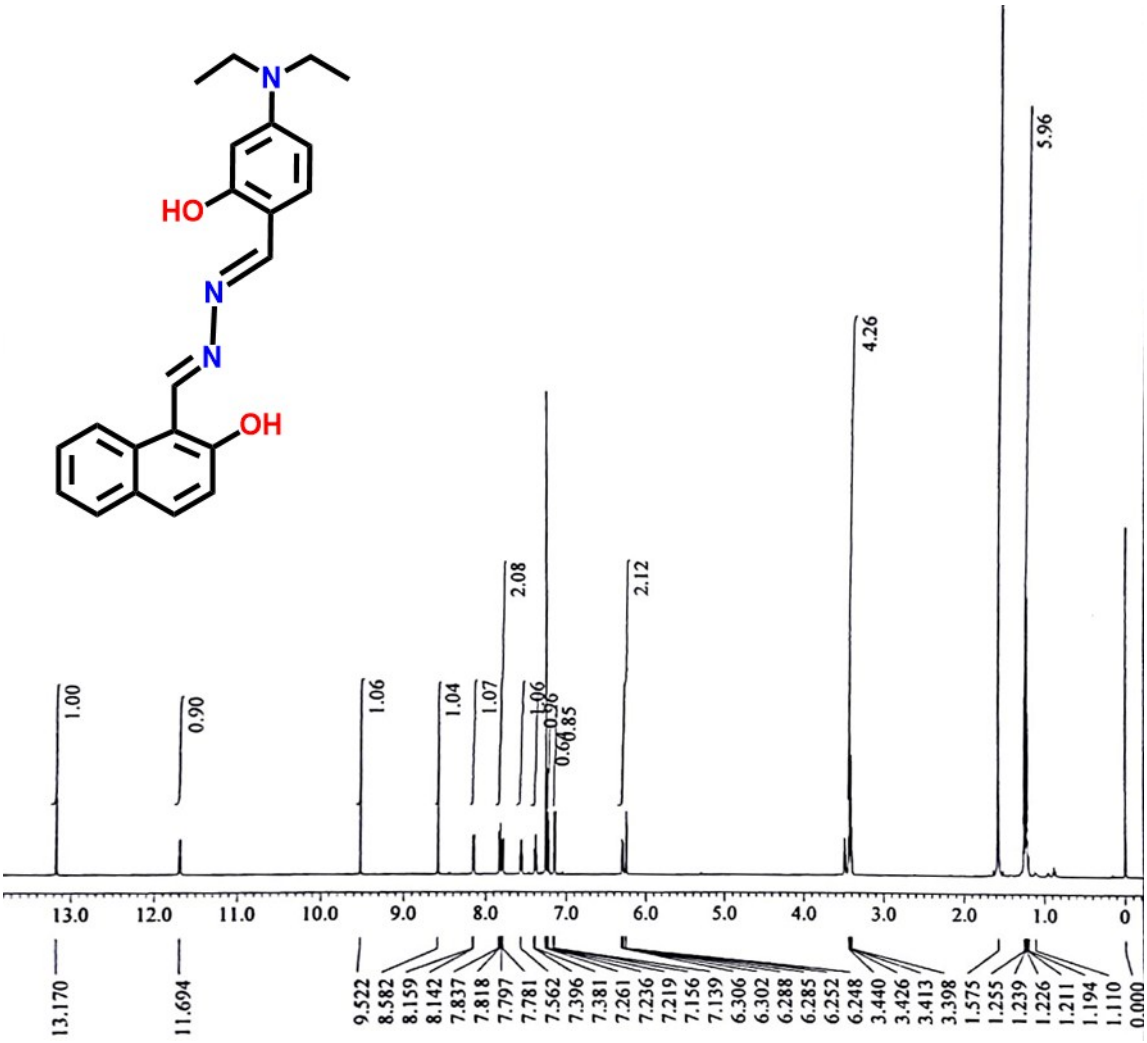

Fig. S2: ^{1}H-NMR spectrum of **NDEA** in $CDCl_3$ at room temperature.

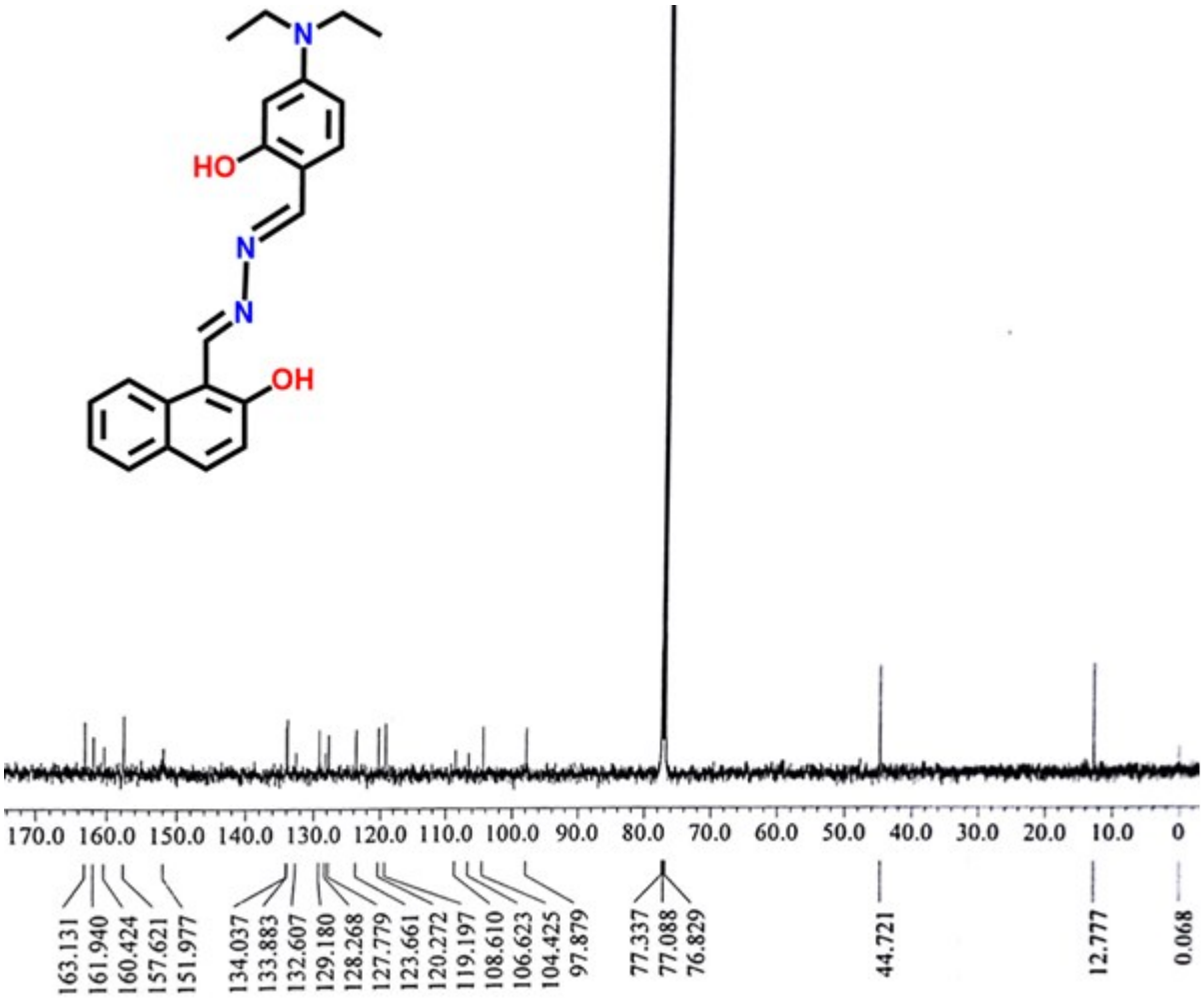

Fig. S3: ^{13}C-NMR spectrum of **NDEA** in $CDCl_3$ at room temperature.

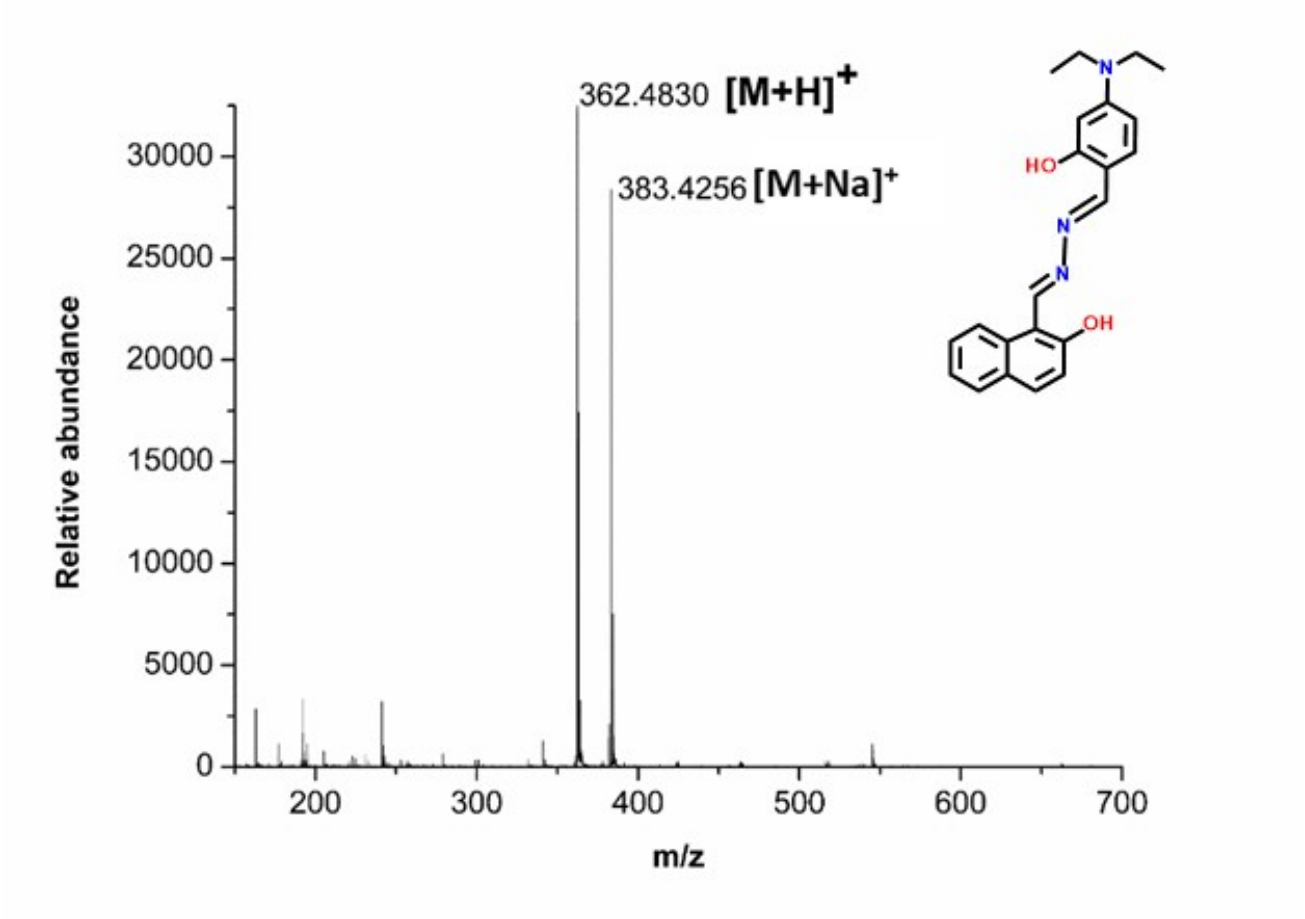

Fig. S4: ESI-MS spectrum of **NDEA**.

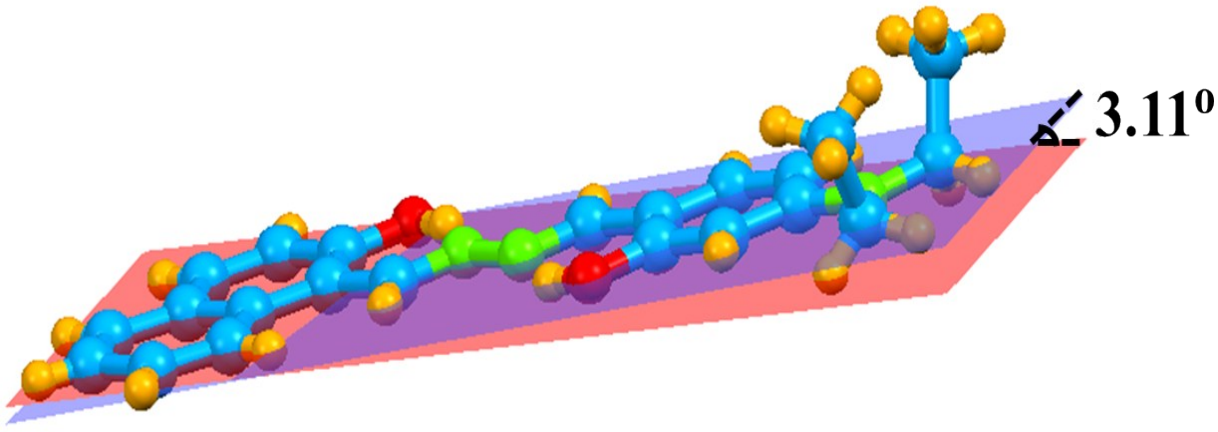

Fig. S5: Dihedral angle between two planes of **NDEA**.

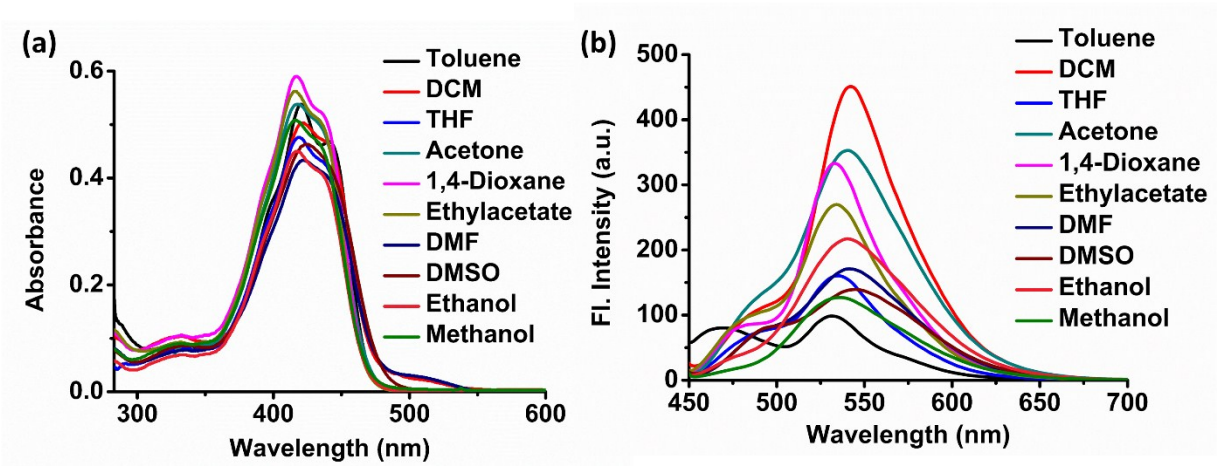

Fig. S6: (a) Absorbance and **(b)** Emission spectra of **NDEA** (1×10^{-5} M) in the solvents of varying polarity.

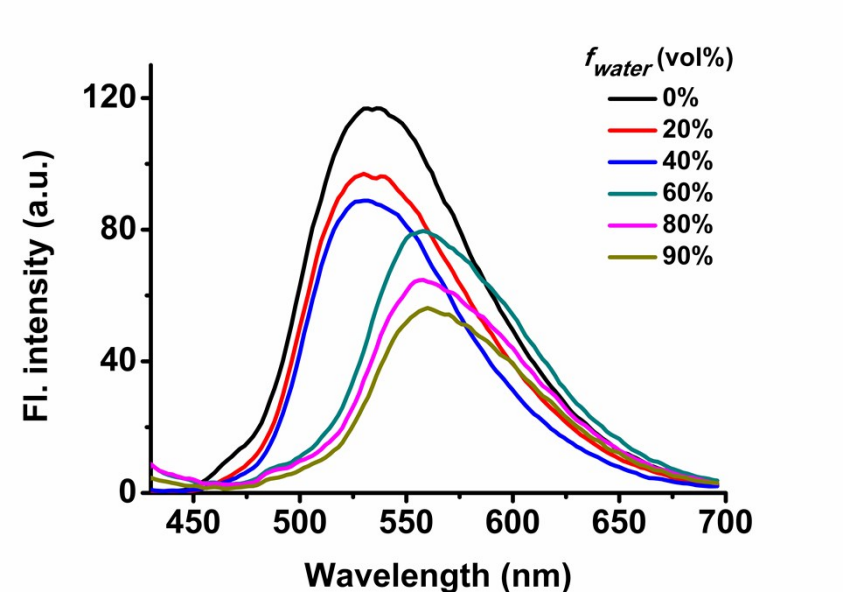

Fig. S7: Emission spectra of **NDEA** (1×10^{-5} M) in MeOH–H_2O with different f_{water} values.

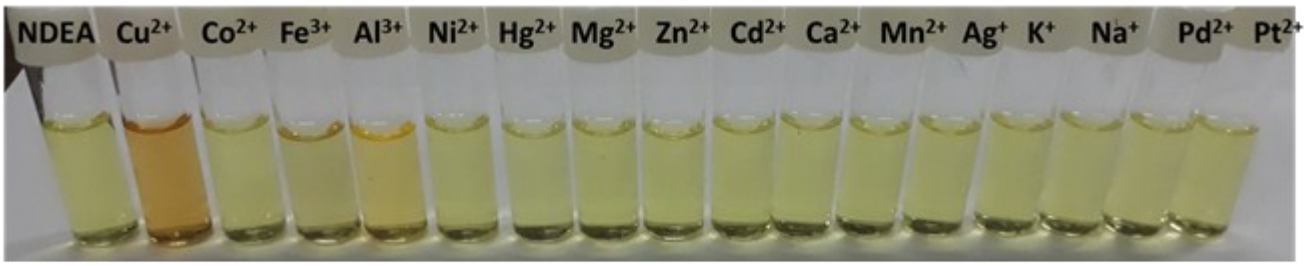

Fig. S8: Visual colour changes in the solution of **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) on addition of 10 equiv. of different cations in H_2O.

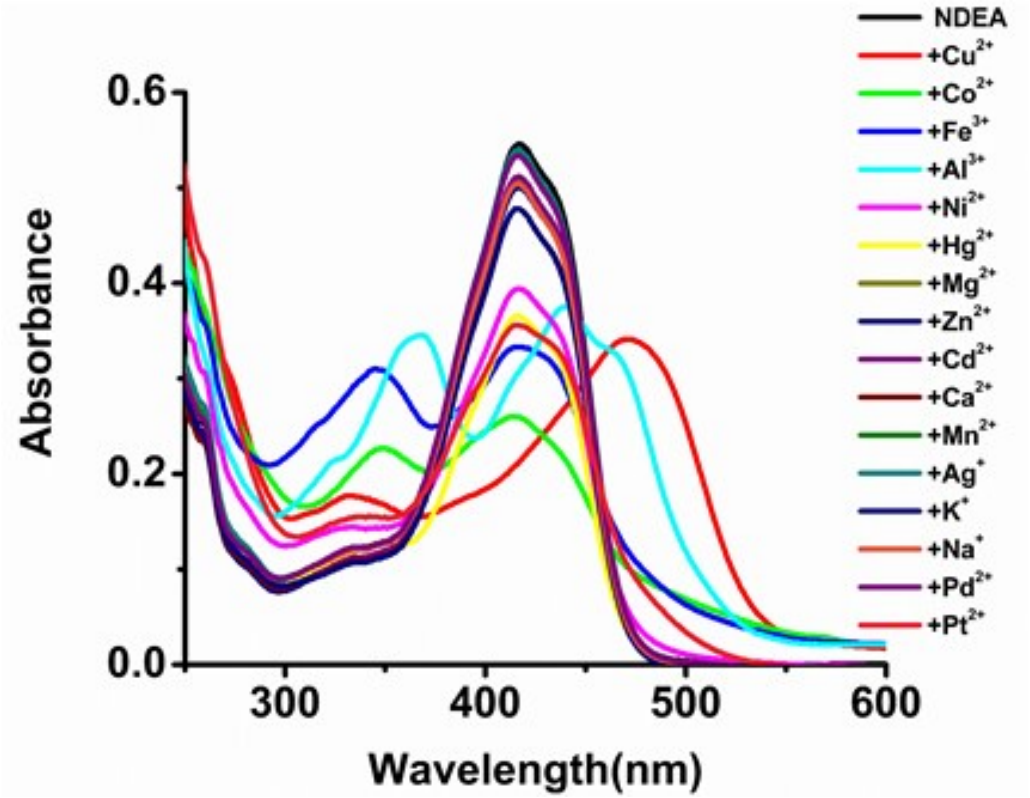

Fig. S9: Absorption spectra of **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) in the presence of various cations (10 equiv. each).

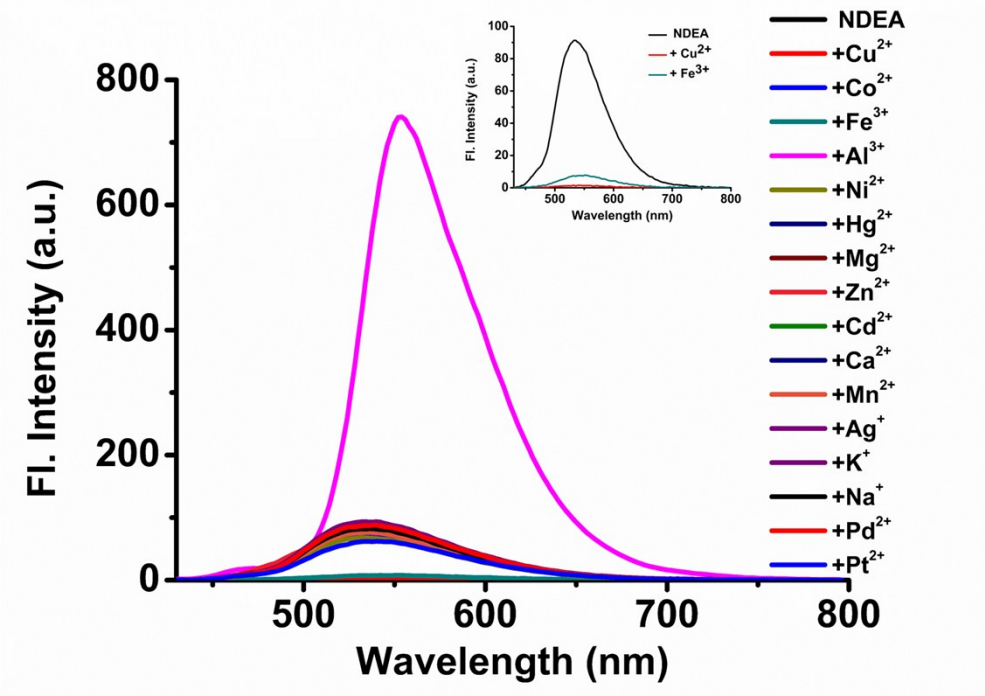

Fig. S10: Fluorescence spectra of **NDEA** (1×10^{-5}M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) in the presence of various cations (10 eq.), inset shows the quenching response of Cu^{2+} and Fe^{3+} ions.

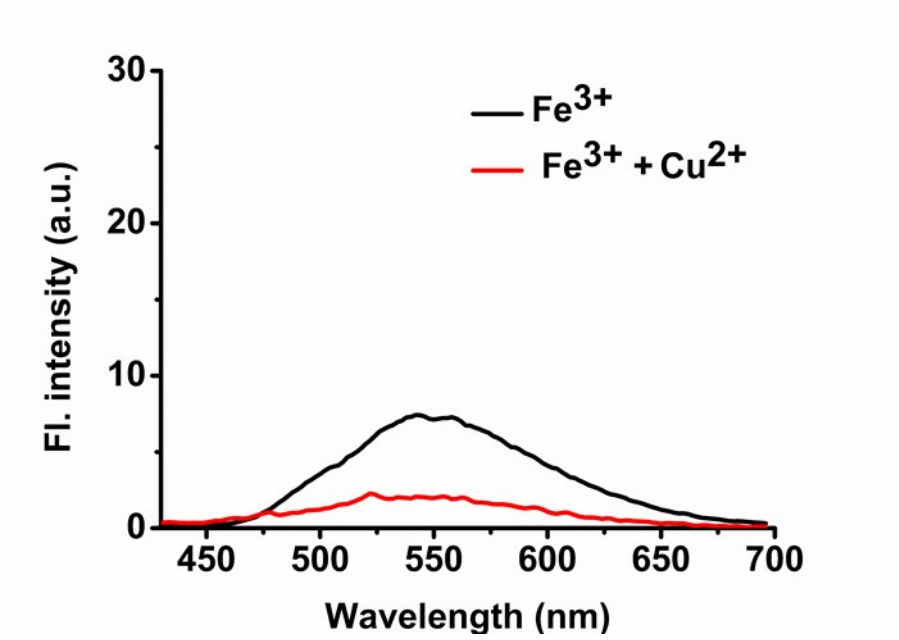

Fig.S11: Fluorescence spectra of **NDEA** (1×10^{-5}M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) showing quenching response of Cu^{2+} ions towards **NDEA+Fe^{3+}** ensemble.

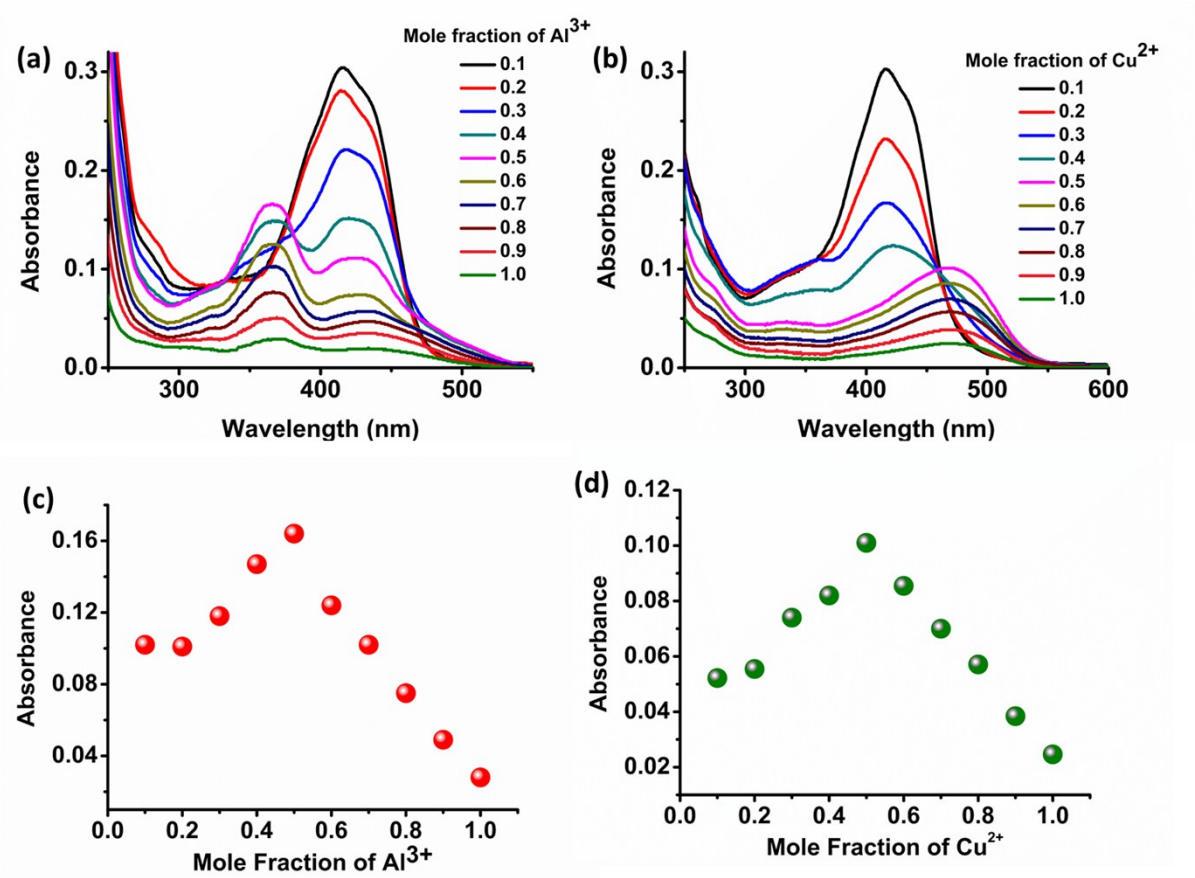

Fig.S12: Variation in the absorbance of **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) against the mole fraction of (a) Al^{3+} ions and (b) Cu^{2+}ions. Their corresponding Job's plots against mole fraction of (c) Al^{3+} ions at λ_{max} = 362 nm (1×10^{-5} M, H_2O) and (d) Cu^{2+} ions at λ_{max} = 471 nm (1×10^{-5} M) is shown.

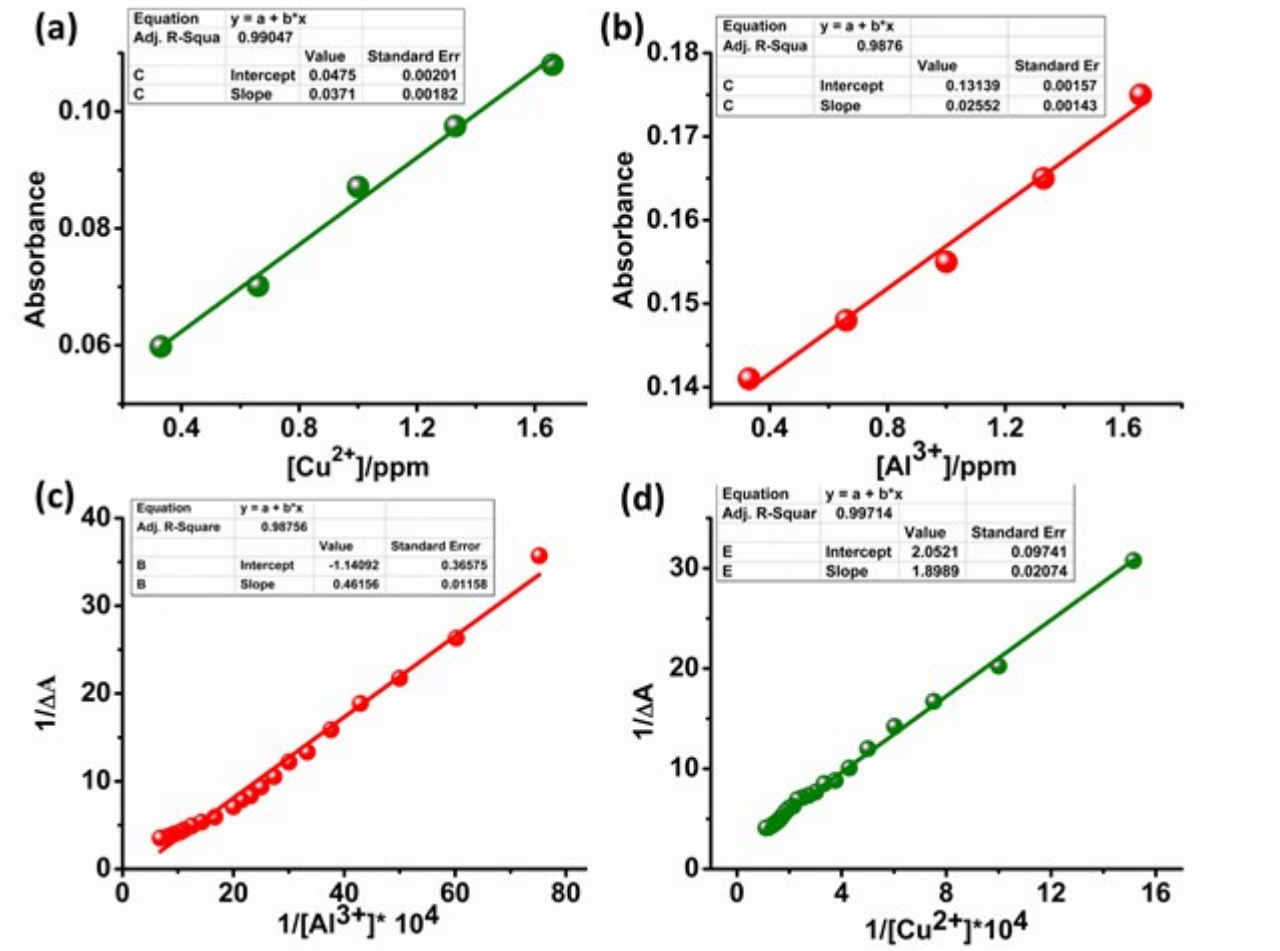

Fig. S13: (a) Calibration curves for determination of the detection limit of **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) for (a) Al^{3+} ions (b) Cu^{2+} ions, using absorption titrations data; graphs showing the variation of (c) $1/Al^{3+}$ vs $1/\Delta A$ of **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) ($R^2 = 0.987$) (d) $1/Cu^{2+}$ vs $1/\Delta A$ of **NDEA** (1×10^{-5}M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) ($R^2 = 0.997$), used for the determination of binding constants.

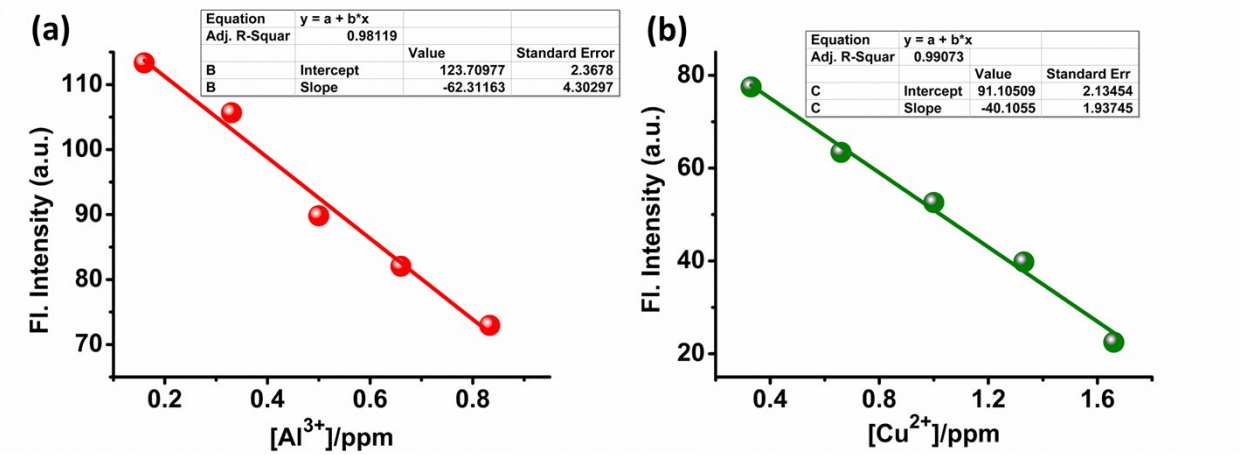

Fig. S14: Calibration curves for determination of the detection limit of **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) for (a) Al^{3+} ions (b) Cu^{2+} ions using fluorescence titrations data.

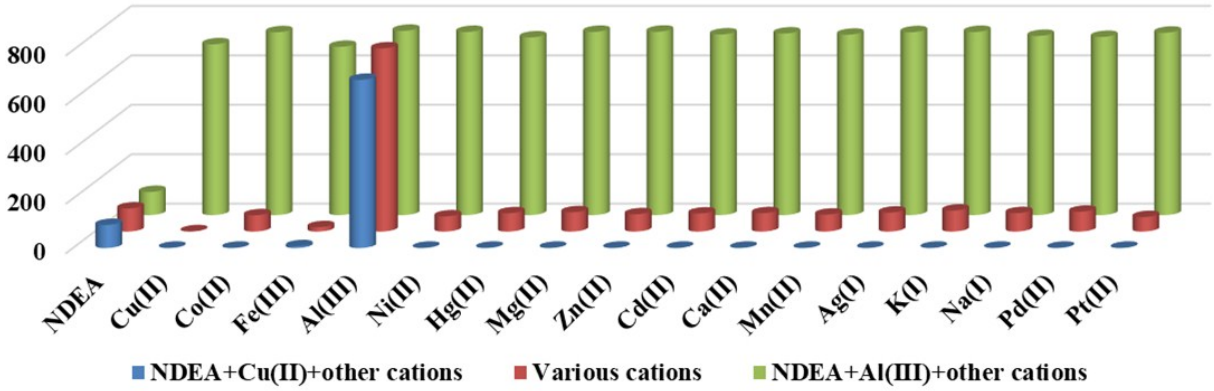

Fig. S15: Bar diagrams showing emission responses from **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) upon addition of 10 equiv. of various metal ions (red bars). The green and blue coloured bars depict interference test of 10 equiv. of Al^{3+} and Cu^{2+} at λ_{emm} = 552 and 534 nm respectively.

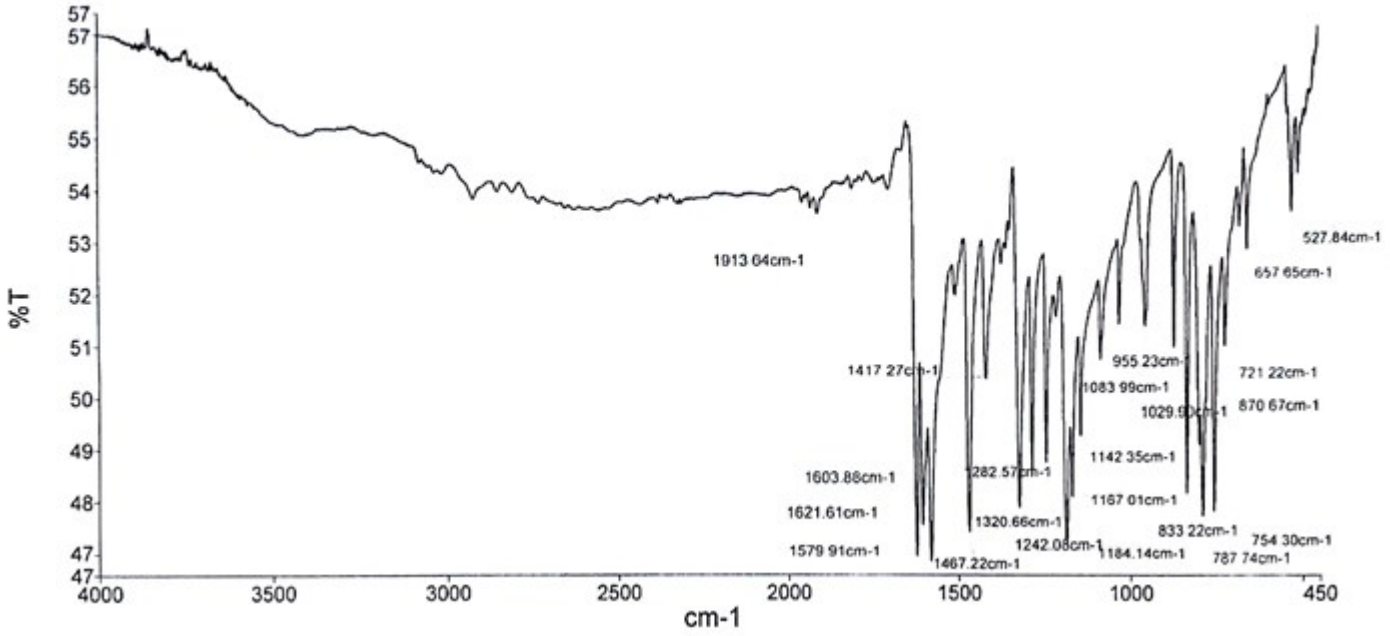

Fig. S16: FTIR spectra of **NDEA**+Al^{3+}.

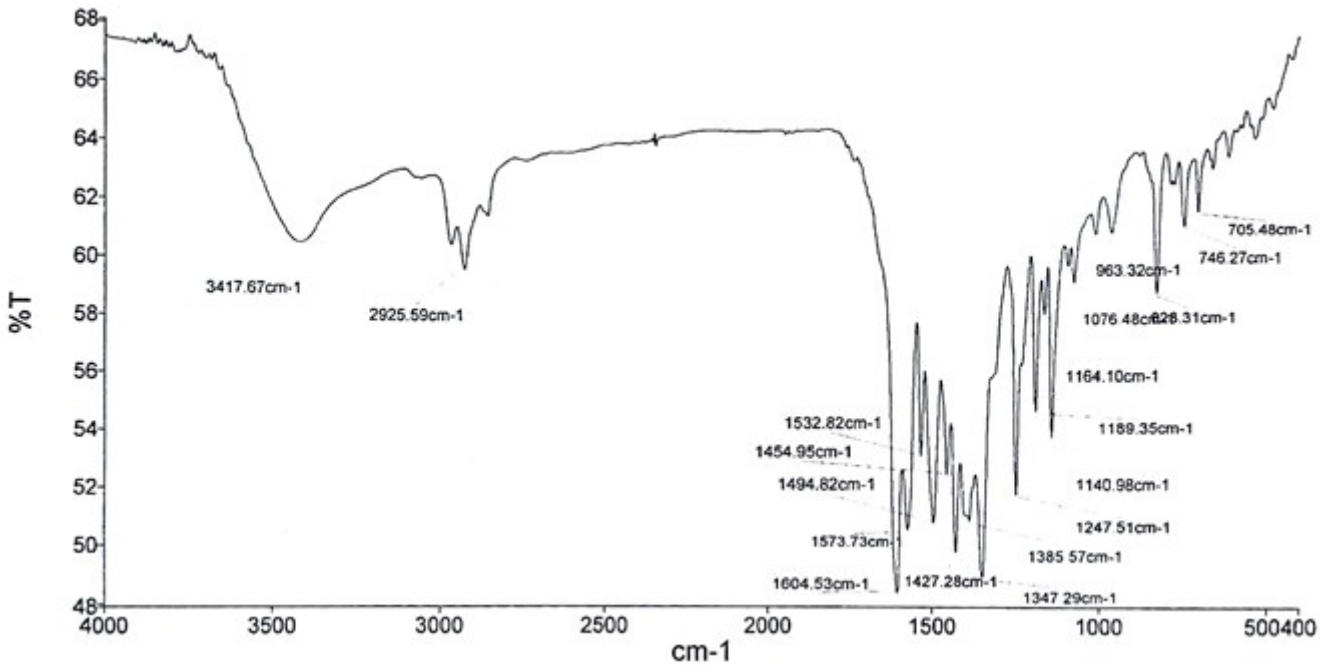

Fig. S17: FTIR spectra of **NDEA**+Cu^{2+}.

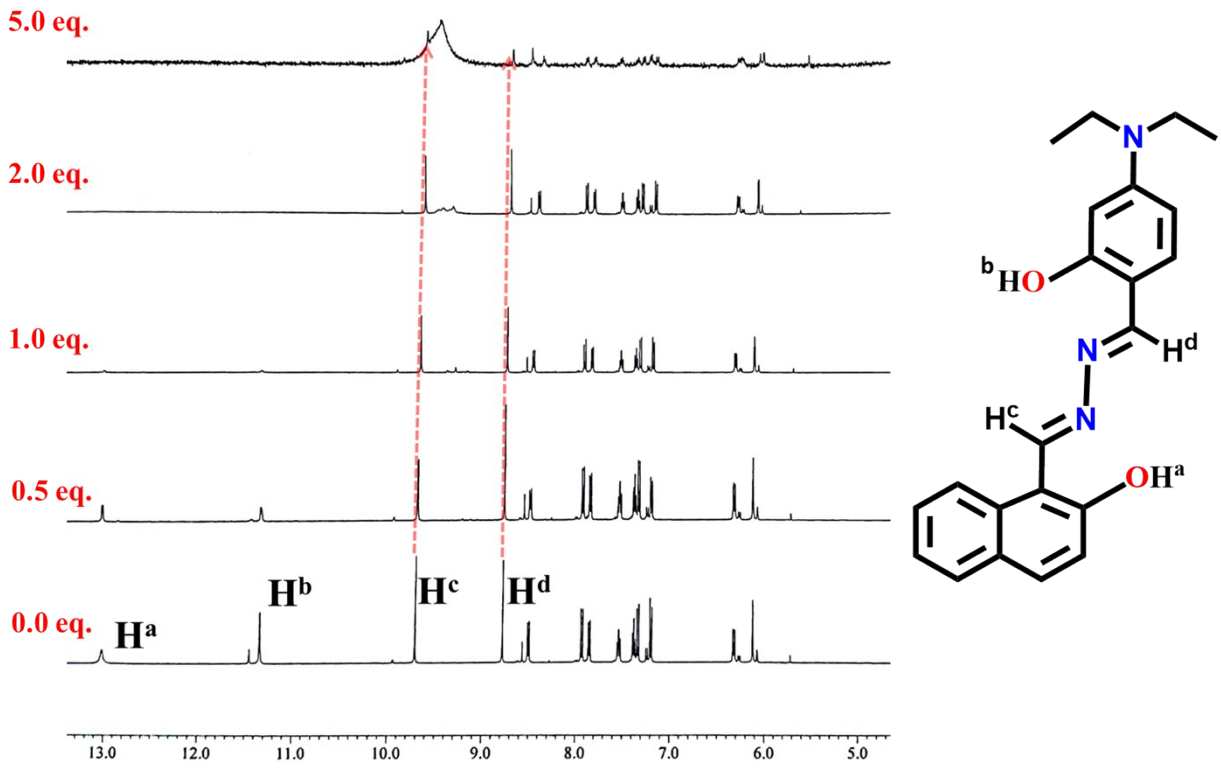

Fig. S18: Partial ^{1}H NMR spectral titrations of **NDEA** (in DMSO-d6) on incremental addition of Al^{3+} ions.

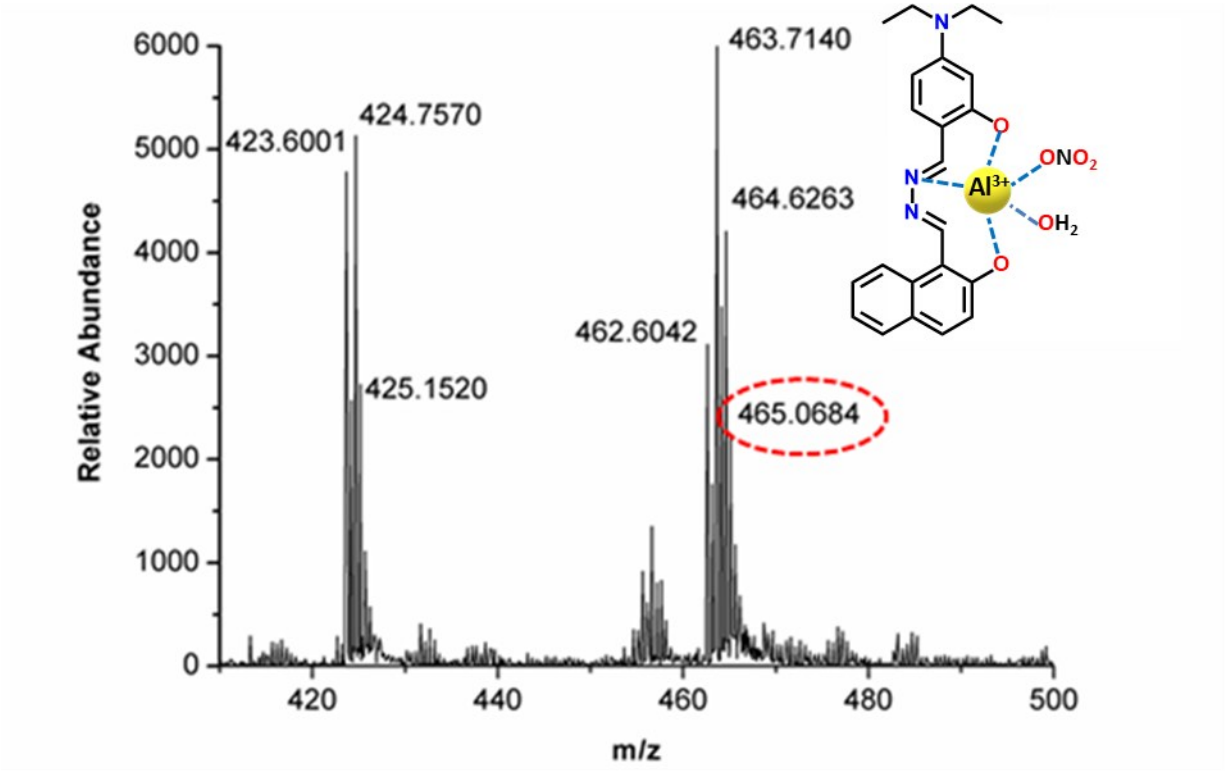

Fig. S19: ESI-MS spectrum of isolated solid (**NDEA**+Al^{3+}).

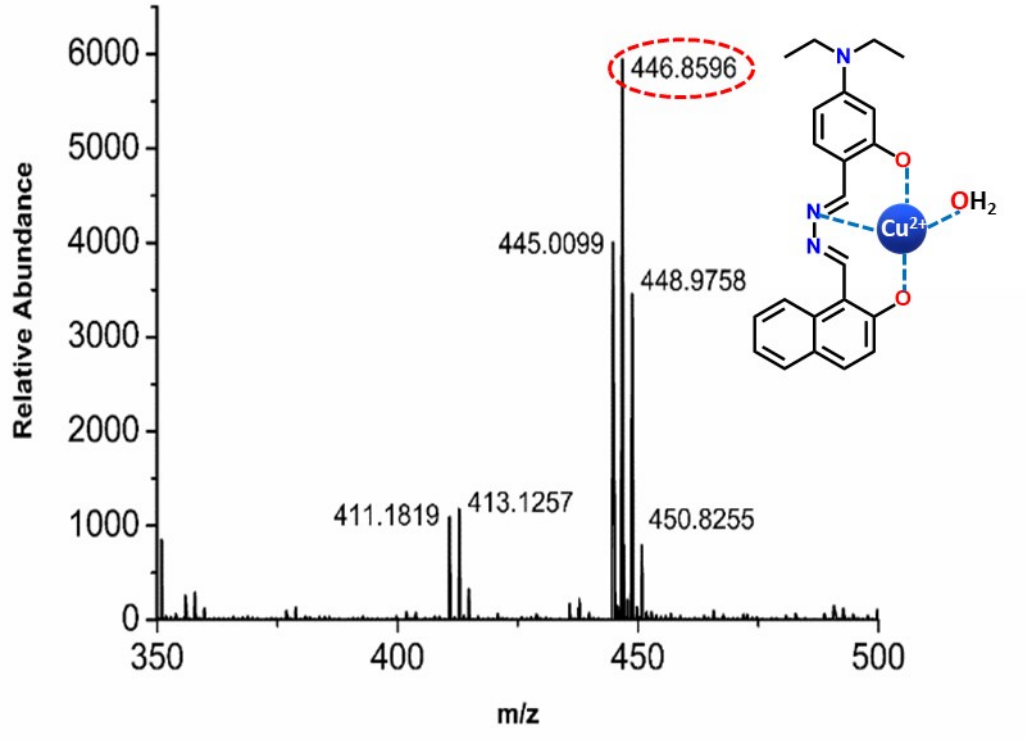

Fig. S20: ESI-MS spectrum of isolated solid (**NDEA**+Cu^{2+}).

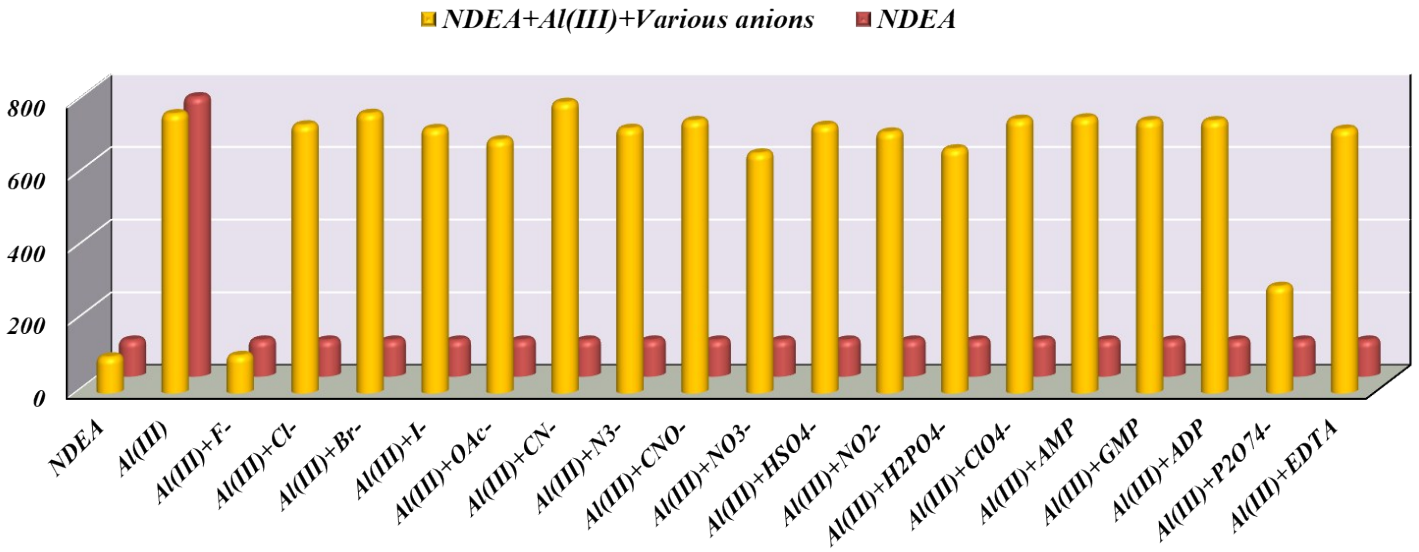

Fig. S21: Bar chart showing emission changes at λ_{max} = 552 nm on the addition of various anions (3.0 eq. each) in the solution of **NDEA**+Al^{3+} (1 × 10^{-5}M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4)**.**

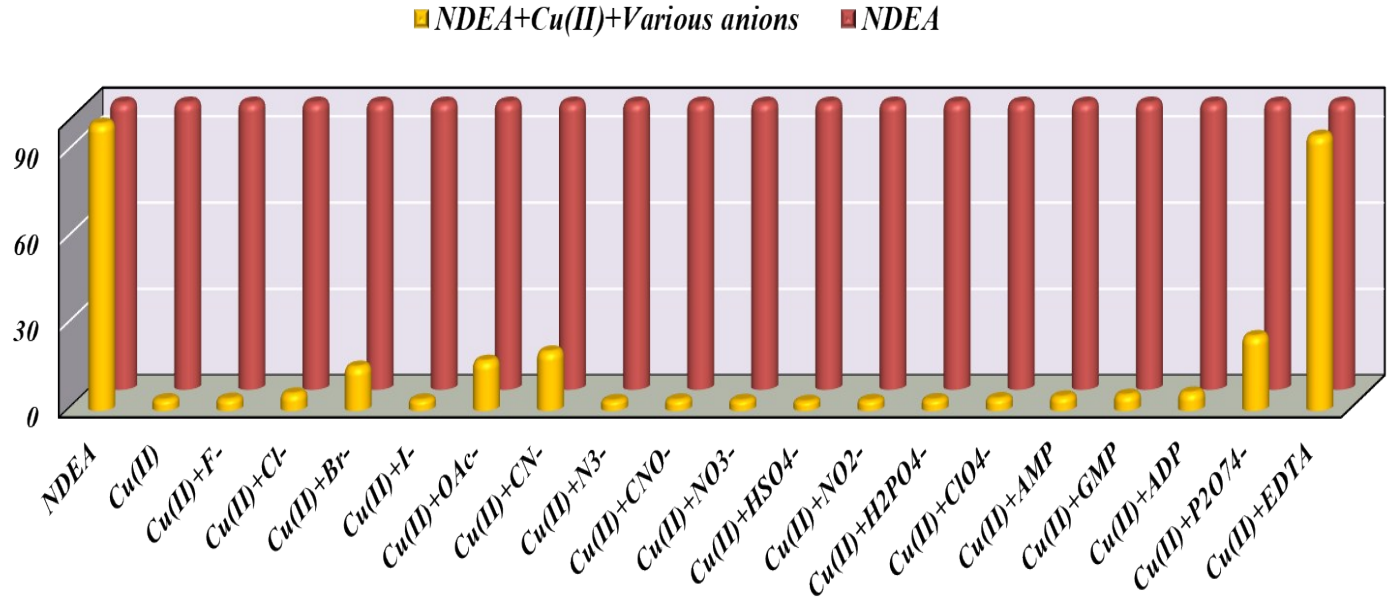

Fig. S22: Bar chart showing emission changes at λ_{max} = 534 nm on the addition of various anions (3.0 eq.) in the solution of **NDEA**+Cu^{2+} (1× 10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4)

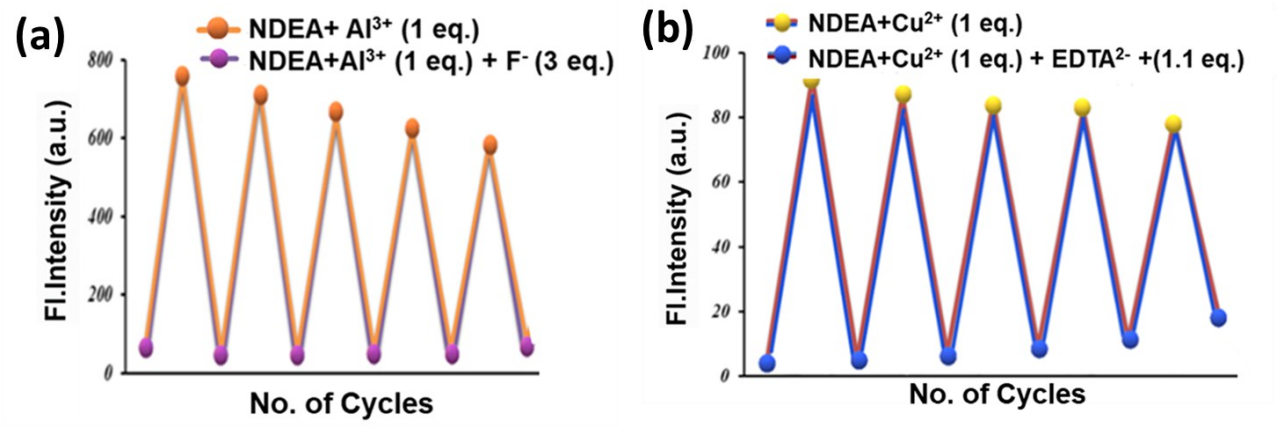

Fig. S23: Reversible cycles of **NDEA** (1×10^{-5} M, DMSO : H_2O : MeOH = 0.1 : 1.9 : 8.0, v/v, HEPES buffer, pH 7.4) (a) Al^{3+} with F^- and (b) Cu^{2+} with $EDTA^{2-}$ respectively.

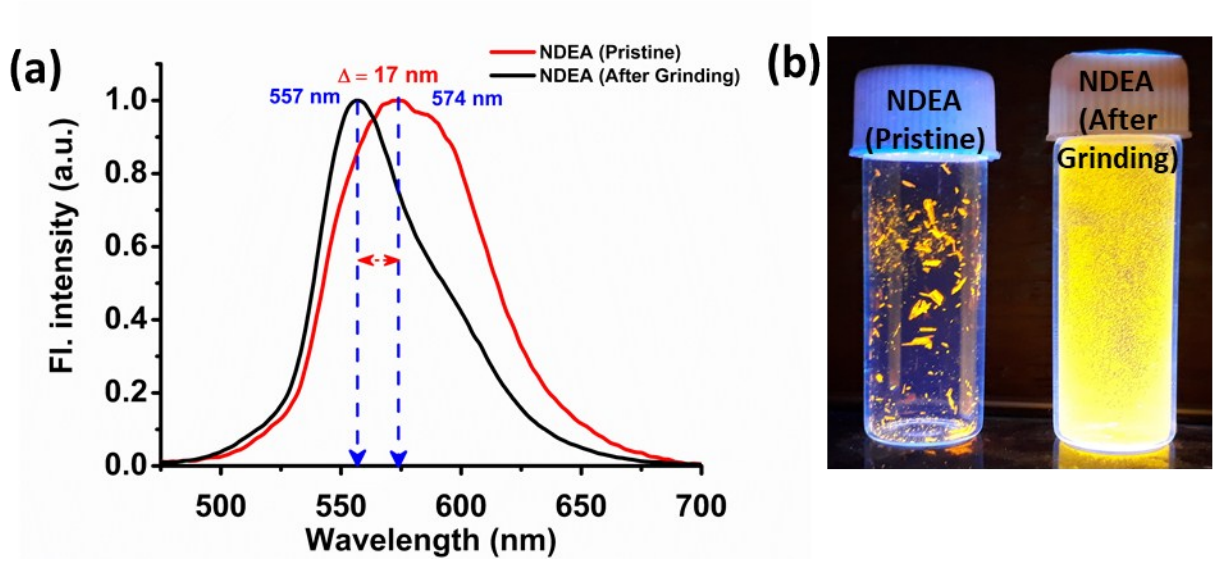

Fig. S24: (a) Normalized solid-state emission spectra of **NDEA** (pristine) and **NDEA** obtained after grinding and (b) the same as seen in UV lamp.

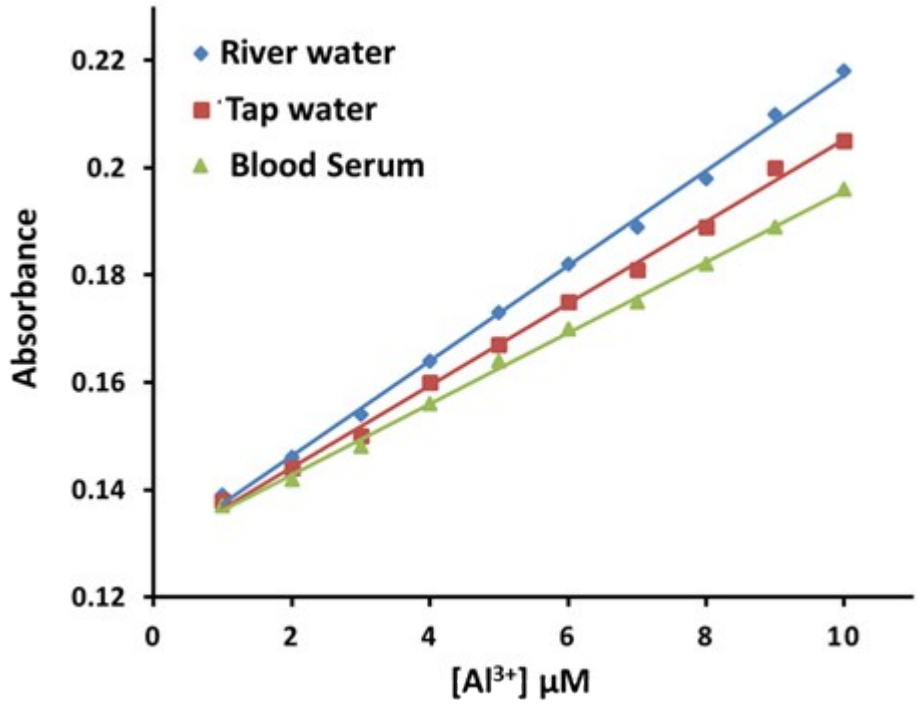

Fig. S25: Proof-of-concept experiments with **NDEA** (at λ_{max} = 362 nm) for determining Al^{3+} ions in real samples.

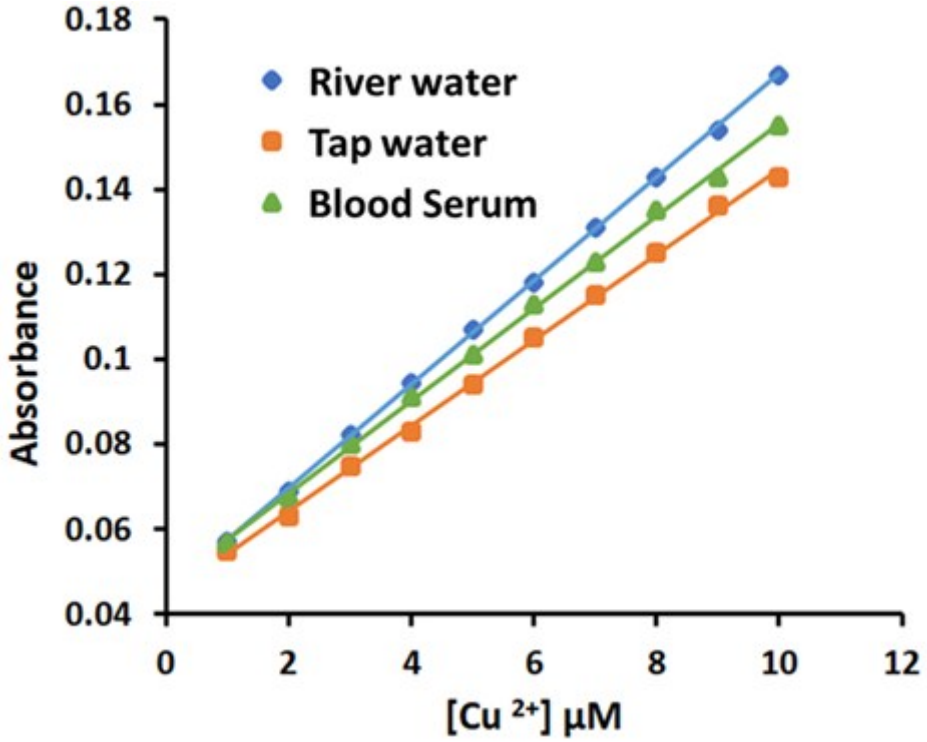

Fig. S26: Proof-of-concept experiments with **NDEA** (at λ_{max} = 471 nm) for determining Cu^{2+} ions in real samples.

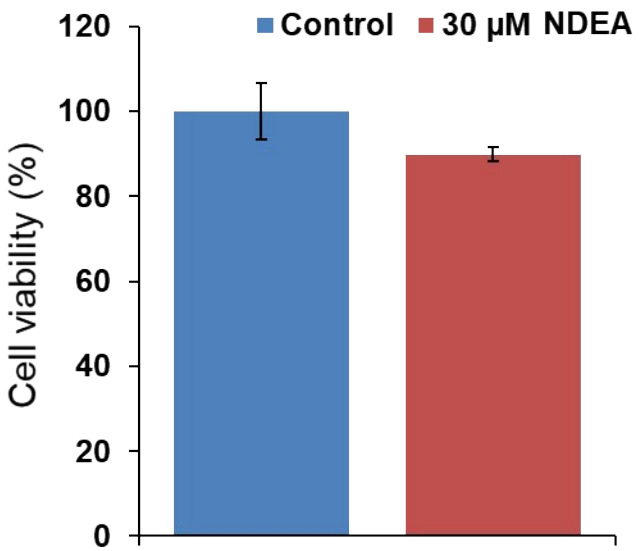

Fig. S27: The effect of **NDEA** on cell viability. C6 cells were treated with 30 µM **NDEA** for 1 hour and MTT assay was performed. The values indicate mean ± SE from 3 replicates.

Table S1. Selected Crystallographic Data of **NDEA**

Empirical Formula	$C_{22}H_{23}N_3O_2$
Formula Weight	361.43
Temperature	296 K
Space group	P-1
Crystal system	triclinic
a [Å]	5.7302(6)
b [Å]	11.9227(13)
c [Å]	14.2404(15)
α [deg]	105.609(3)
β [deg]	96.095(4)
γ [deg]	100.244(4)
ρ_{cald}[Mg m^{-3}]	1.319
V [$Å^3$]	909.82(17)
Z	2
F (000)	384.0
GOF on F^2	1.032
R_1; wR2 [I > 2σ(I)]	R1 = 0.0649 wR2 = 0.1402
R_1; wR2(all data)	R1 = 0.1164 wR2 = 0.1725
CCDC No.	1871351

Table S2. Selected Bond angles (°) and Bond lengths (Å) of **NDEA**

Bond angles (°)		**Bond lengths(Å)**	
O1 C1 C10	122.58(19)	C1 O1	1.352(2)
O1 C1 C2	116.30(18)	C11 N1	1.288(3)
N1 C11 C10	120.58(19)	C12 N2	1.291(3)
N1 C11 H11	119.7	C14 O2	1.354(2)
C10 C11 H11	119.7	C16 N3	1.367(3)
N2 C12 C13	123.25(19)	C19 N3	1.464(3)
N2 C12 H12	118.4	C21 N3	1.462(3)
O2 C14 C15	118.21(18)	N1 N2	1.393(2)
O2 C14 C13	120.68(19)	O1 H1O	0.92(3)
N3 C16 C15	121.68(18)	O2 H2O	0.86(3)
N3 C16 C17	120.93(18)		
N3 C19 C20	113.96(18)		
N3 C19 H19A	108.8		
N3 C19 H19B	108.8		
N3 C21 C22	114.16(18)		
N3 C21 H21A	108.7		
N3 C21 H21B	108.7		
C11 N1 N2	115.60(17)		
C12 N2 N1	112.29(17)		
C16 N3 C21	120.81(17)		
C16 N3 C19	121.70(17)		
C21 N3 C19	117.06(17)		
C1 O1 H1O	104.9(19)		
C14 O2 H2O	112(2)		

Table S3. Selected parameters for weak interactions in **NDEA**

D H···A	**D H(Å)**	**H···A(Å)**	**D···A(Å)**	**D H...A(°)**
Intra O1 H10...N1	0.92(3)	1.69(3)	2.538(2)	152(3)
Intra O2 H20...N2	0.86(3)	1.92(3)	2.655(2)	143(3)

Table S4: Fluorescence decay parameters of **NDEA** in different solvent mixture.

Entry	Relative Amplitude, A(%)	Lifetime, τ (ns)	Average lifetime, $\langle\tau\rangle$ (ns)
NDEA (water : methanol = 2:8)	3.11 (A_1) 96.89 (A_2)	0.0951592 (τ_1) 0.652912 (τ_2)	0.552138
NDEA-Al^{3+} (water : methanol = 2:8)	43.9 (A_1) 56.1 (A_2)	2.68342 (τ_1) 3.73071 (τ_2)	3.18501
NDEA (methanol : glycerol = 1:99)	57.68 (A_1) 48.32 (A_2)	0.680279 (τ_1) 01.57056 (τ_2)	0.936881

Table S5. Data for analysis of Al^{3+}ions in real sample by **NDEA.**

S.No.	**Samples**	**Al^{3+}added (µM)**	**Al^{3+}found (µM)**	**% Recovery**
1.	River Water	0.0	0.0	-
		2.0	1.97	98.6%
		4.0	3.98	99.5%
		6.0	5.97	99.5%
2.	Tap Water	0.0	0.0	-
		2.0	1.95	97.2%
		4.0	3.88	97.1%
		6.0	5.74	95.6%
3.	Blood Serum	0.0	0.0	-
		2.0	1.92	95.9%
		4.0	3.79	94.7%
		6.0	5.57	92.9%

Table S6. Data for analysis of Cu^{2+}ions in real sample by **NDEA.**

S. No.	Samples	Cu^{2+}added (µM)	Cu^{2+}found (µM)	% Recovery
1.	River Water	0.0	0.0	-
		2.0	1.96	98.3%
		4.0	3.86	96.7%
		6.0	5.84	97.4%
2.	Tap water	0.0	0.0	-
		2.0	1.79	89.7%
		4.0	3.40	85.0%
		6.0	5.21	86.8%
3.	Blood Serum	0.0	0.0	-
		2.0	1.92	96.0%
		4.0	3.73	93.4%
		6.0	5.58	93.1%

Table S7. Comparison Table with other reported probes for Al^{3+} and Cu^{2+} ions

Analyte	Solvent	LOD (M)	Reversibility	Mitotracker Co-localisation studies	Viscochromism And mechanochromism	References
Al^{3+} and Cu^{2+} ions	DMF: H_2O (2:8)	Al^{3+} ions (fluorescence) is **3.20×10^{-6}** and Cu^{2+} ions (absorbance) is **1.30×10^{-4}**	No	No	No	[1]
Al^{3+} and Cu^{2+} ions	DMSO: H_2O (9:1)	Al^{3+} ions (fluorescence) is **0.44×10^{-6}** and Cu^{2+} ions (absorbance) is **0.47×10^{-4}**	No	No	No	[2]
Al^{3+} and Cu^{2+} ions	DMSO: H_2O mixture (1:1)	Al^{3+} ions (fluorescence) is **0.020×10^{-6}** and Cu^{2+} ions (absorbance) is **0.27×10^{-6}**	No	No	No	[3]

Al^{3+} and Cu^{2+} ions	DMSO: MeOH	Al^{3+} ions (fluorescence) is **4.3695×10^{-6}** and Cu^{2+} ions (fluorescence) is **6.77×10^{-7}**	Yes	No	No	[4]
Al^{3+}, Zn^{2+}, Cu^{2+} and F^- ions	MeOH: H_2O (9:1) for Al^{3+}, Zn^{2+} and Cu^{2+} and ACN for F^- ions	Al^{3+} ions (fluorescence) is **6.86×10^{-7}** and Cu^{2+} ions (absorbance) is **4.64×10^{-6}**	No	No	No	[5]
Al^{3+} and Cu^{2+} ions	**MeOH : H_2O: DMSO (8.0 : 1.9 : 0.1) , v/v, HEPES buffer, pH 7.4**	**Al^{3+} ions is 1.76×10^{-7} (absorbance) and 1.65×10^{-7} (fluorescence). Cu^{2+} ions is 1.54×10^{-7} (absorbance) and 1.52×10^{-7} (fluorescence).**	**Al^{3+} with F^- and Cu^{2+} with EDTA**	**Yes**	**Yes**	**Present work**

References

[1] H. Kim, B. A. Rao, J.W. Jeong, S. Mallick, S-M. Kang, J.S. Choi, C-S. Lee, Y-A. Son, *Sensors and Actuators, B: Chemical* **2015,** *210*, 173–182.

[2] H-S. Kim, S. Angupillai, Y-A. Son, *Sens. Actuators, B: Chemical* **2016,** *222*, 447–458.

[3] A.S. Murugan, M. Pandit, J. Annaraj, *Chemistry Select* **2017**, *2*, 375–383.

[4] X. Zhang, P. Sun, F. Li, H. Li, H. Zhou, H. Wang, B. Zhang, Z. Pan, Y. Tian, X. Zhang, *Sensors and Actuators*, *B: Chemical* **2018,** *255*, 366–373.

[5] S. Samanta, U. Manna, T. Ray, G. Das, *Dalton Transactions* **2015**, *44*, 18902–18910.