Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

SUPPORTING INFORMATION

Fabrication of luminescent chemosensor for selective detection of Al^{3+} used as an adjuvant in pharmaceutical drugs

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1. NMR Studies:

^{1}H NMR of NAN in DMSO-d_6 :

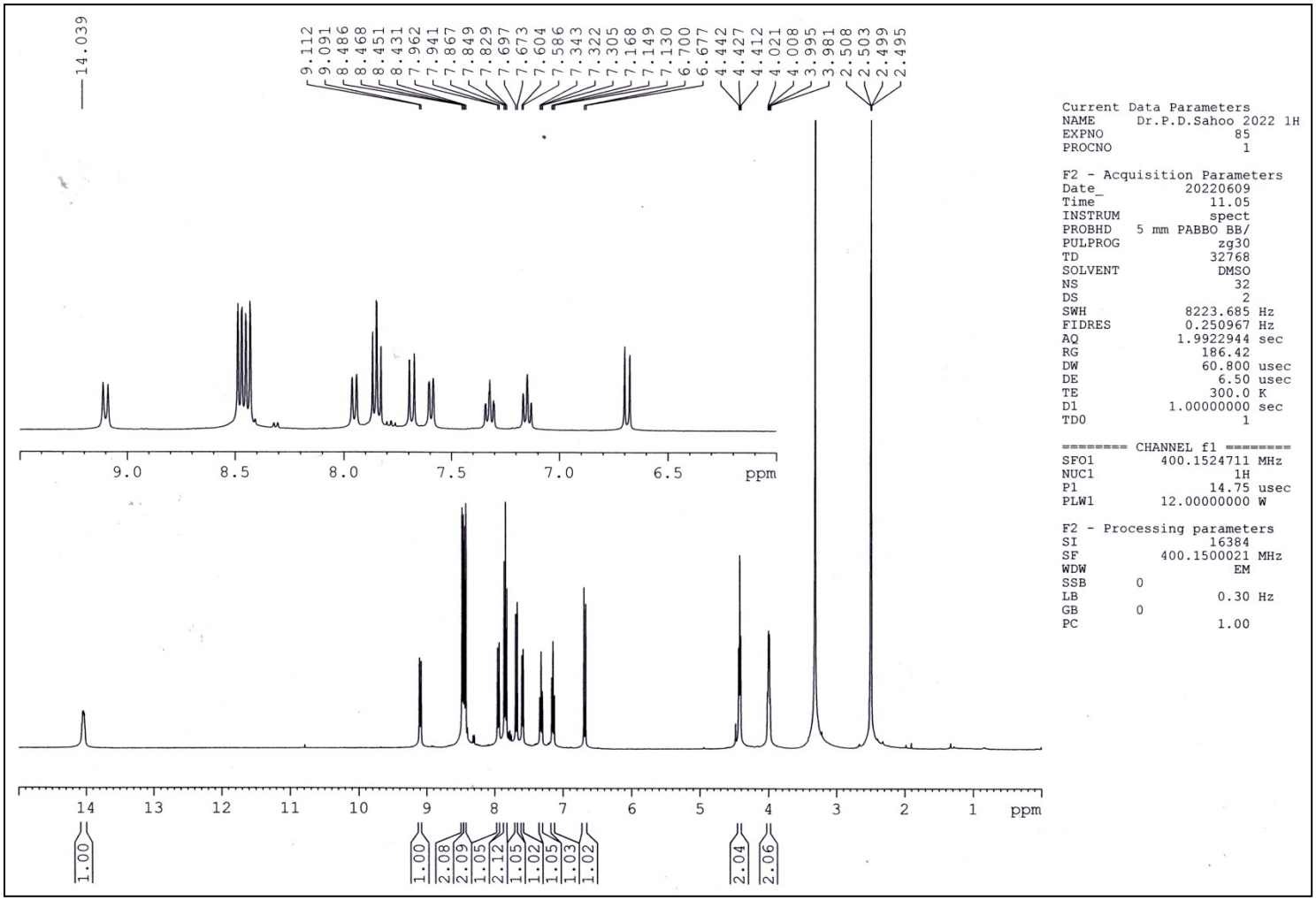

Fig. S1. ^{1}H NMR of **NAN** in DMSO-d_6 (400 MHz).

^{13}C NMR of NAN in DMSO-d$_6$:

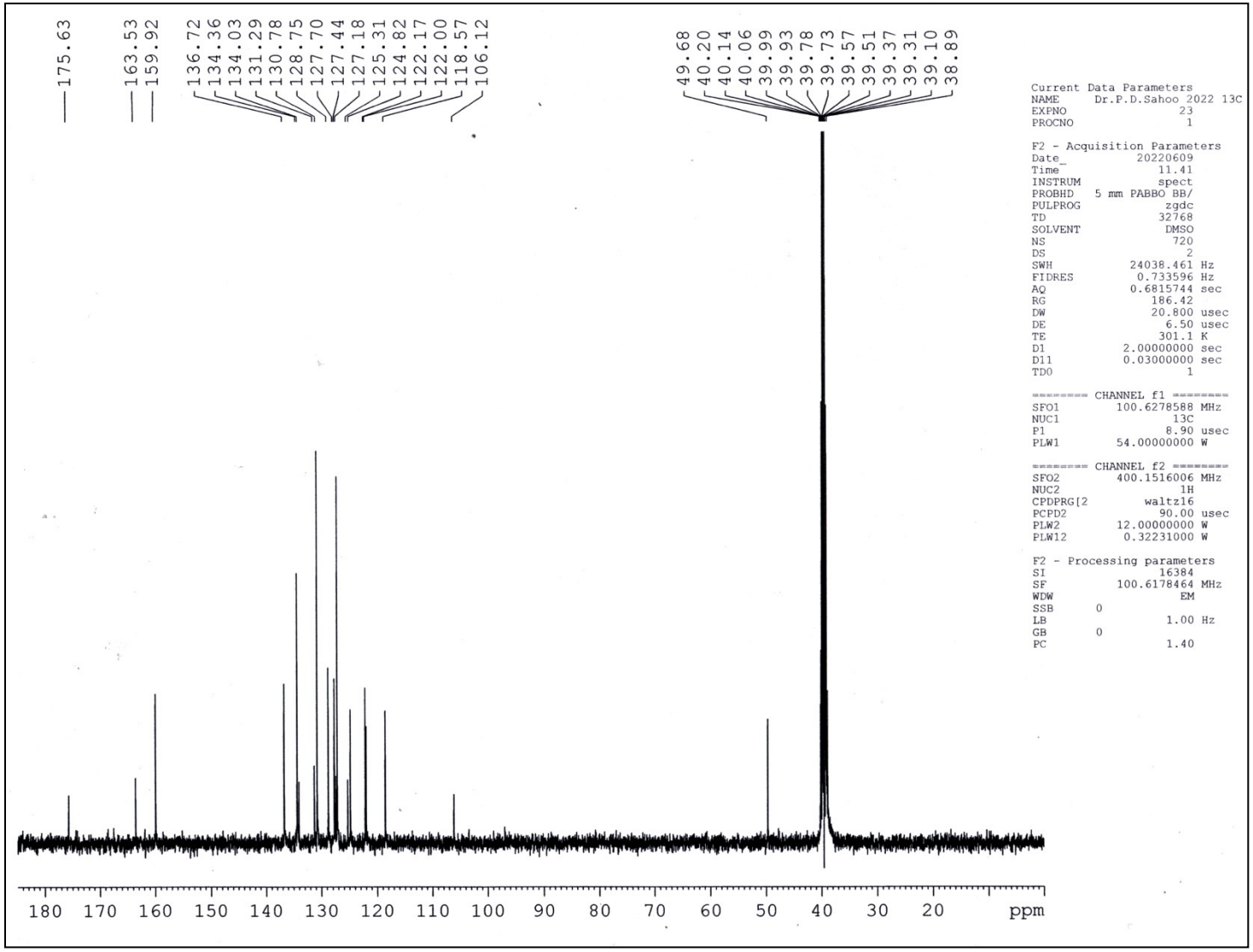

Fig. S2. ^{13}C NMR of **NAN** in DMSO-d$_6$ (100 MHz).

2. Materials and Instruments

2-hydroxynaphthaldehyde, 1,8-naphthalic anhydride, methanol, DMSO and all the reagents were purchased from Sigma-Aldrich Pvt.Ltd. Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. Solvents were dried according to standard procedures. Elix Millipore water was used throughout all experiments. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz instrument. For NMR spectra, DMSO-d_6 is used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. The following abbreviations are used to describe spinmultiplicities in ^{1}H NMR spectra: s = singlet; d = doublet; t = triplet; m = multiplet.The mass spectrum (HRMS) was carried out using a micromass Q-TOF Micro™ instrument by using Acetonitrile as a solvent. Fluorescence spectra were recorded on a Fluorescence spectrophotometer Hitachi 7100. UV spectra were recorded on a Hitachi U-2910 spectrophotometer. Elemental analysis of NAN was carried out on CHNS/O analyzer.

3. UV-vis and fluorescence titration. A stock solution of **NAN** (1 μM) was prepared in water-dimethylsulphoxide (1:1, v/v). Al^{3+} solution of concentration 10 μM was prepared in Millipore water. All experiments were carried out in aqueous medium at neutral pH. During the titration, each time a 1 μM solution of **NAN** was filled in a quartz optical cell of 1 cm optical path length and Al^{3+} stock solution was added into the quartz optical cell gradually by using a micropipette. Spectral data were recorded at 1 min after the addition of Al^{3+}.

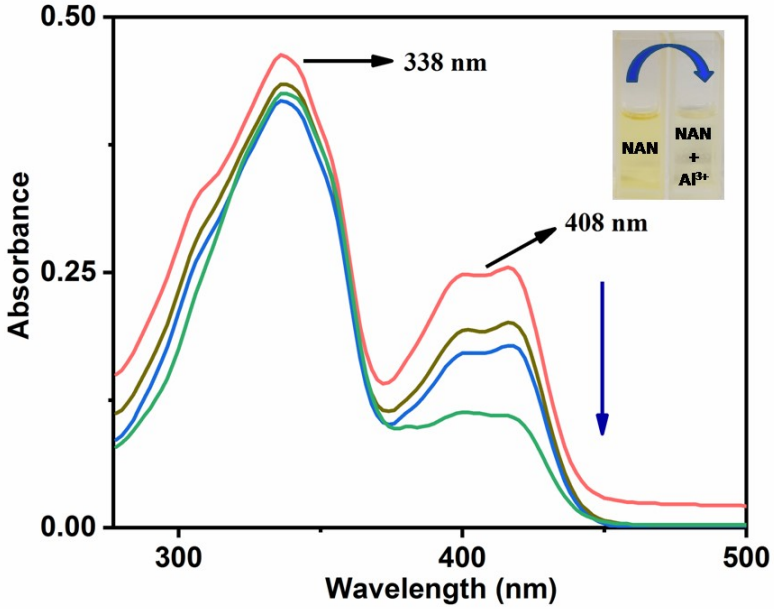

Fig. S3. UV−vis absorption spectra of **NAN** (1 μM) upon gradual addition of Al^{3+} up to 1 μM in H_2O:DMSO (1:1, v/v) at pH 7.0 (10 mM phosphate buffer) [inset: naked eye colour change of **NAN** upon interaction with Al^{3+}]

4. Evaluation of the association constants for the formation of NAN-Al^{3+} complex:

By Fluorescence Method:

Binding constant of the chemosensor **NAN** was calculated through emission method by using the following equation:

$$1/ (I – I_0) = 1/K(I_{max}– I_0) [G] + 1/(I_{max}– I_0) \quad ……..(ii)$$

Where I_0, I_{max}, and I represent the emission intensity of free **NAN**, the maximum emission intensity observed in the presence of added Al^{3+}at 445 nm (λ_{ex}= 340 nm),the emission intensity at a certain concentration of the Al^{3+} respectively and [G] is the concentration of the guest Al^{3+}**.**

Binding constant calculation graph (Fluorescence method):

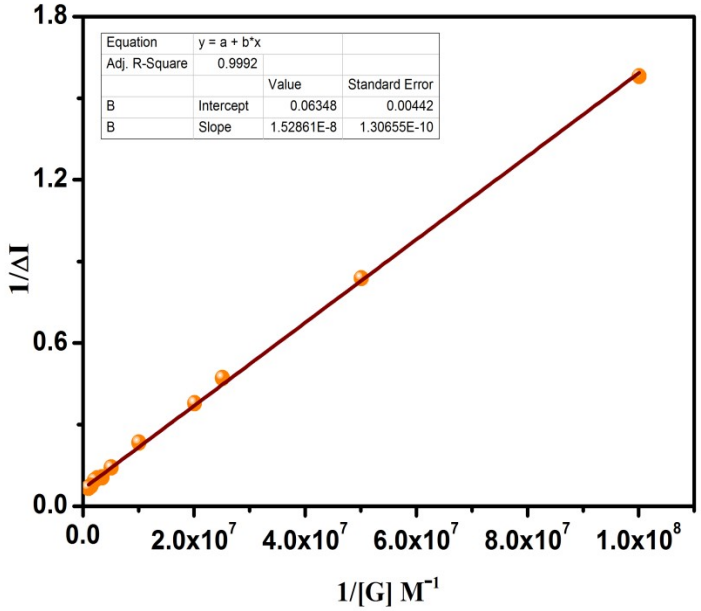

Fig. S4. Linear regression analysis for the calculation of association constant value by fluorescence titration method

The association const. (K_a) of **NAN** for sensing Al^{3+} was determined from the equation:

K_a = intercept/slope. From the linear fit graph, we get intercept = 0.06348, slope = 1.52861×10^{-8}. Thus, we get, $\mathbf{K_a}$= (0.06348) / (1.52861×10^{-8}) = $\mathbf{4.15 \times 10^6\ M^{-1}}$.

5. Calculation of limit of detection (LOD) of NAN with Al^{3+}:

The detection limit of the chemosensor **NAN** for Al^{3+} was calculated on the basis of fluorescence titration. To determine the standard deviation for the fluorescence intensity, the emission intensity of four individual receptors without Al^{3+} was measured by 10 times and the standard deviation of blank measurements was calculated.

The limit of detection (LOD) of **NAN** for sensing Al^{3+} was determined from the following equation.

$$LOD = K \times SD/S$$

Where K = 2 or 3 (we take 3 in this case); SD is the standard deviation of the blank receptor solution; S is the slope of the calibration curve.

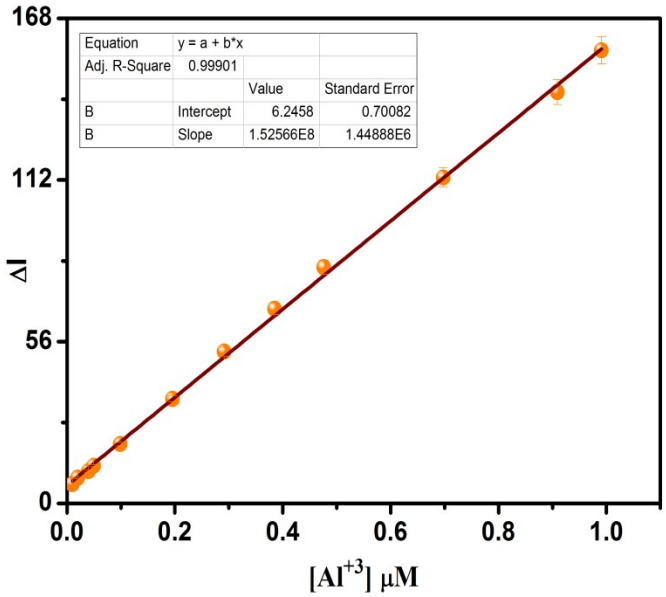

Fig. S5. Linear fit curve of **NAN** at 445 nm with respect to Al^{3+} concentration. Standard deviations are represented by error bar (n=3).

For **NAN** with Al^{3+}**:**

From the linear fit graph, we get, slope = 1.52566×10^{8}, and SD value is 1.69116

Thus, using the above formula we get, the Limit of Detection = 3.3254×10^{-8} M, i.e 33 nM. Therefore, **NAN** can detect Al^{3+} up to this very lower concentration by fluorescence technique.

6. Job's plot for determining the stoichiometry of binding by fluorescence method:

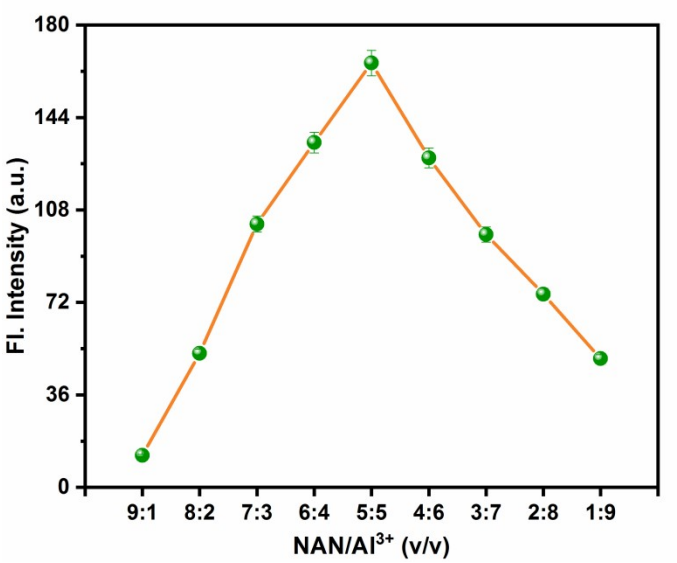

Fig. S6. Job's plot of **NAN** (1μM) with Al^{3+} (1μM) in DMSO-H_2O (1:1, v/v), at neutral pH =7.0 (10 mM phosphate buffer), by fluorescence method ($\lambda_{ex.}$=340 nm), which indicate 1:1 stoichiometry for **NAN** with Al^{3+} ion. Standard deviations are represented by error bar (n=3).

7. Selectivity studies

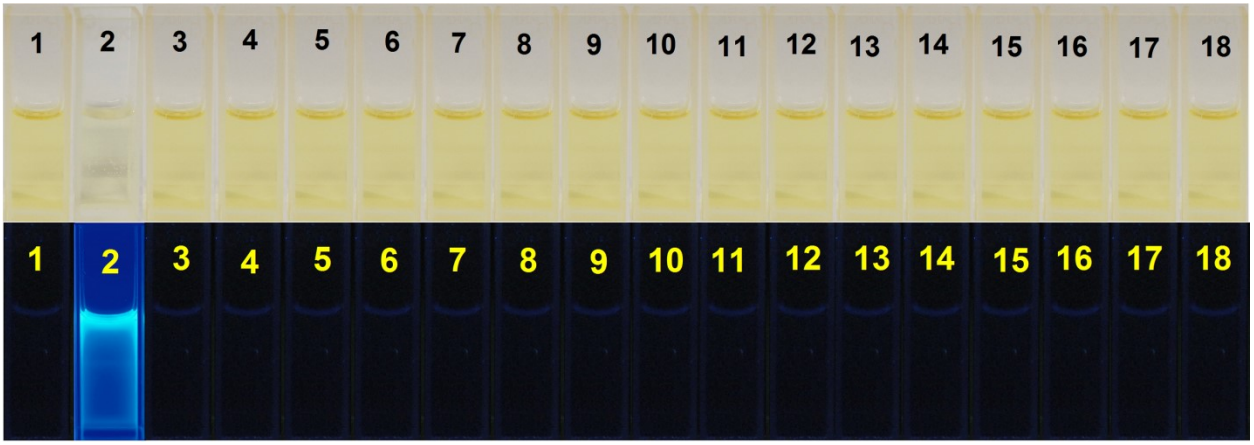

Fig. S7. Photograph of colorimetric changes (upper panel) and fluorescence responses (lower panel) of **NAN** (1 μM) to various bio-relevant cations [From left to right: 1) Only **NAN**, **NAN** with 2) Al^{3+} 3) Sn^{2+}, 4) Zn^{2+}, 5) Cu^{2+}, 6) Hg^{2+}, 7) Cd^{2+}, 8) Mg^{2+}, 9) Pb^{2+}, 10) Fe^{2+}, 11) Fe^{3+}, 12)

Ni^{2+}, 13) Co^{2+}, 14) Mn^{2+}, 15) Ca^{2+}, 16) Cr^{2+}, 17) Na^{+} and 18) K^{+} in H_2O–DMSO (1:1 v/v, pH 7.0, 10 mM phosphate buffer) solution].

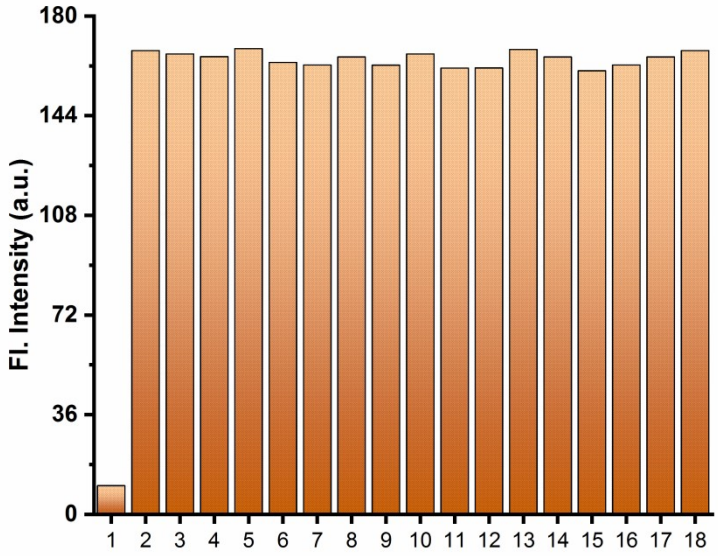

Fig. S8. Histogram representing competitive fluorescence spectra of **NAN** with different bio relevant cations at 445 nm (λ_{ex}= 340 nm) in DMSO-H_2O (1:1, v/v), at neutral pH.[From left to right: 1) Only **NAN**, **NAN** with 2) Al^{3+}, 3) Al^{3+}+ Sn^{2+} 4) Al^{3+}+ Zn^{2+}, 5) Al^{3+}+ Cu^{2+}, 6) Al^{3+}+ Hg^{2+}, 7) Al^{3+}+ Cd^{2+}, 8) Al^{3+}+ Mg^{2+}, 9) Al^{3+}+ Pb^{2+}, 10) Al^{3+}+ Fe^{2+}, 11) Al^{3+}+ Fe^{3+}, 12) Al^{3+}+ Ni^{2+}, 13) Al^{3+}+Co^{2+},14) Al^{3+}+ Mn^{2+}, 15) Al^{3+}+ Ca^{2+}, 16) Al^{3+}+ Cr^{2+}, 17) Al^{3+}+ Na^{+} and 18) Al^{3+}+K^{+}].

8. pH titration

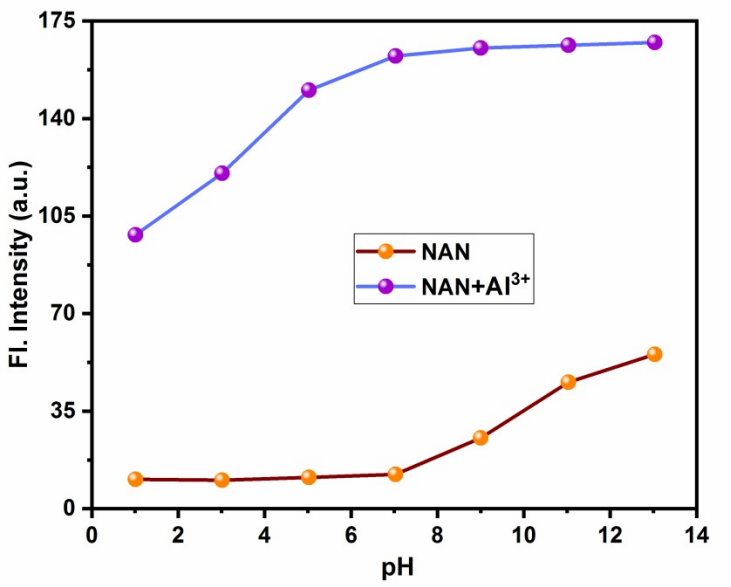

Fig. S9. Fluorescence responses of probe **NAN** (yellow) and **NAN-Al^{3+} complex** (purple) in different pH conditions in H_2O-DMSO (1:1, v/v) (λ_{ex}= 340 nm).

9. ^{1}H NMR titration studies

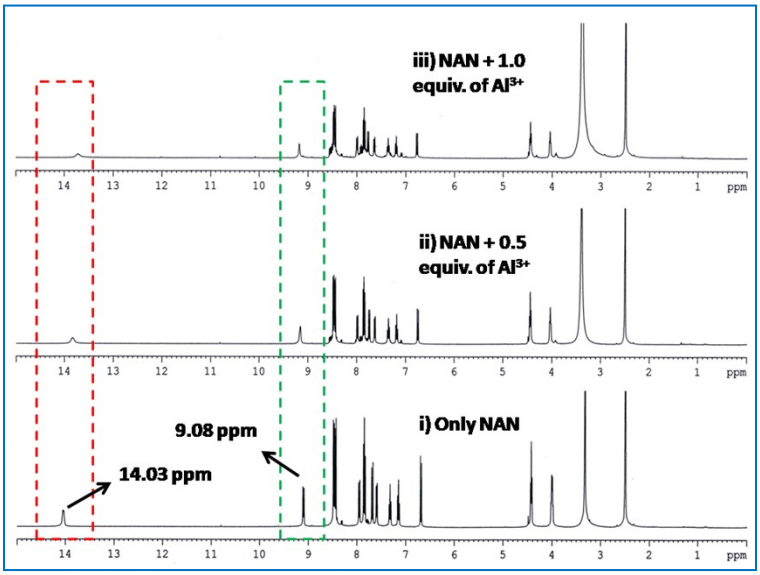

Fig. S10. ^{1}H NMR titration [400MHz] of **NAN** in DMSO-d_6 at 25^{0}C and the corresponding changes after the addition of 1 equiv. of Al^{3+} from (i) only **NAN**, (ii) **NAN** + 0.5 equiv. of Al^{3+} (iii) **NAN** + 1.0 equiv. of Al^{3+}.

^{13}C NMR titration studies

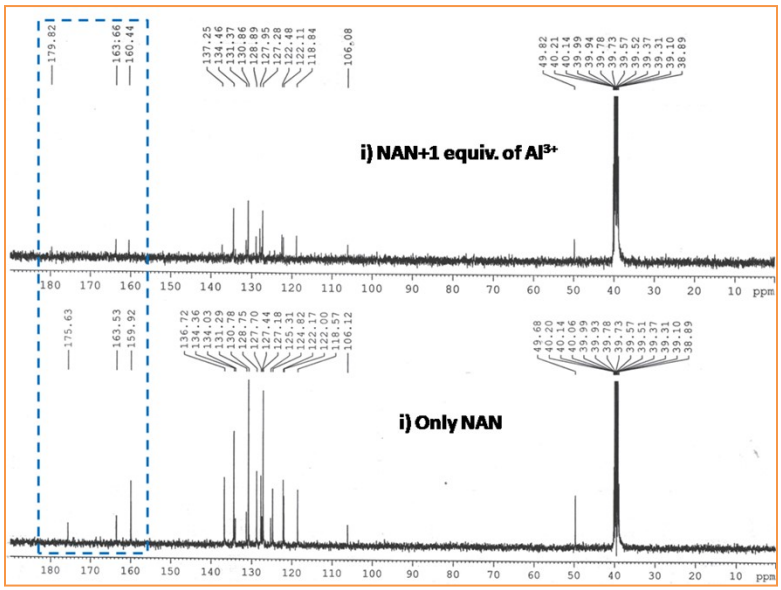

Fig. S11. ^{13}C NMR titration [100 MHz] of **NAN** in DMSO-d_6 at 25^{0}C and the corresponding changes after the addition of 1 equiv. of Al^{3+} from (i) only **NAN**, (ii) **NAN** + 1 equiv. of Al^{3+}.

10.Table S1 Details of the geometry optimization in Gaussian 09 program.

Details	**NAN**	**NAN-Al^{3+}complex**
Calculation method	B3LYP	B3LYP
Basis set	6-311G(d, p)	6-311G (d, p) /LANL2DZ
E(CAM-B3LYP) (a.u.)	-1299.0787	-1657.9893
Charge, Multiplicity	0, 1	+2, 1
Solvent (CPCM)	Water	Water

11. Energy minimized structures of NAN and NAN-Al^{3+} complex

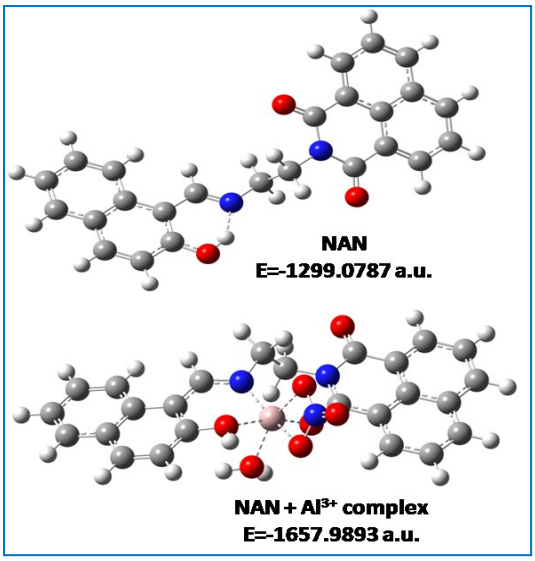

Fig. S12. Energy minimized structures of **NAN** and **NAN-Al^{3+}complex** from B3LYP level.

12. TDDFT calculations

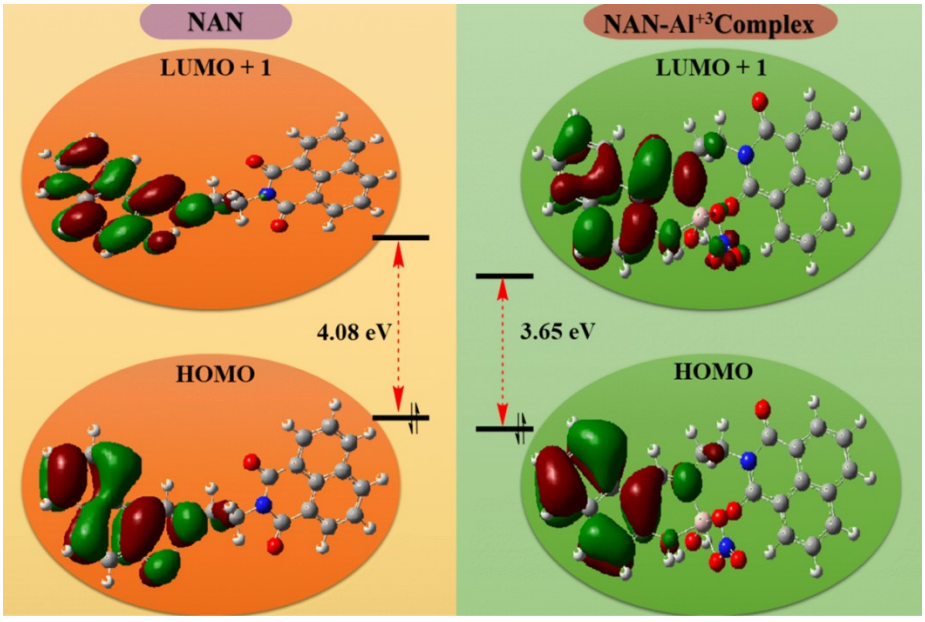

Fig. S13. DFT optimized charge densities and the HOMO-LUMO energy gap of **NAN** and **NAN-Al^{3+}** complex.

13.Table S2. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations of the low-lying excited states of all the molecules and complexes. The data were calculated by TDDFT//B3LYP/6-311G(d,p)/LANL2DZ based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c](%)
NAN	$S_0 \rightarrow S_2$	3.6245eV 342.07 nm	0.5260	H → L+1 (59%)
	$S_0 \rightarrow S_7$	4.1398eV 299.50 nm	0.1032	H-1 → L+1 (64%)
NAN-Al^{3+}complex	$S_0 \rightarrow S_2$	3.1674 eV 391.43 nm	0.2880	H → L+1 (67%)
	$S_0 \rightarrow S_3$	3.3448 eV 370.68 nm	0.2454	H-1 → L (68%)

[a]Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength.[b]Oscillator strength. [c]H stands for HOMO and L stands for LUMO.

14. Table S3. Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

Species	**E_{HOMO} (a.u)**	**E_{LUMO} (a.u)**	**ΔE(a.u)**	**ΔE(eV)**	**ΔE(kcal/mol)**
NAN	-0.21954	-0.06973	0.14981	4.08	94.08
NAN-Al^{3+}complex	-0.25749	-0.12306	0.13443	3.65	84.17

15. Table S4 Determination of Al^{3+} in different drug samples under UV-lamp and by standard HPLC method

Sample Name	Conc. of NAN (µM)	Amount of sample solution taken (µl)	Conc. of Al^{3+} (µM)	Avg conc. of Al^{3+} (µM)	HPLC Method (µM)
Bplex Forte	1	200	0.21	0.22	0.20
	1	200	0.20		
	1	200	0.22		
Enteroquinol	1	200	0.23	0.25	0.26
	1	200	0.25		
	1	200	0.24		
Dolokind	1	200	0.35	0.35	0.33
	1	200	0.36		
	1	200	0.34		
Deplatt CV	1	200	0.36	0.37	0.38
	1	200	0.37		
	1	200	0.38		
Diagene	1	200	0.56	0.56	0.53
	1	200	0.57		
	1	200	0.58		
Disprin	1	200	0.65	0.64	0.63
	1	200	0.63		
	1	200	0.64		
Aspirin	1	200	0.81	0.81	0.80
	1	200	0.82		
	1	200	0.81		
Telma	1	200	0.89	0.89	0.87
	1	200	0.90		
	1	200	0.90		