Supporting Information

ESIPT and CHEF based highly sensitive and selective ratiometric sensor for Al^{3+} with imaging in human blood cell

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^{1}H NMR, ^{13}C NMR and HRMS spectra:

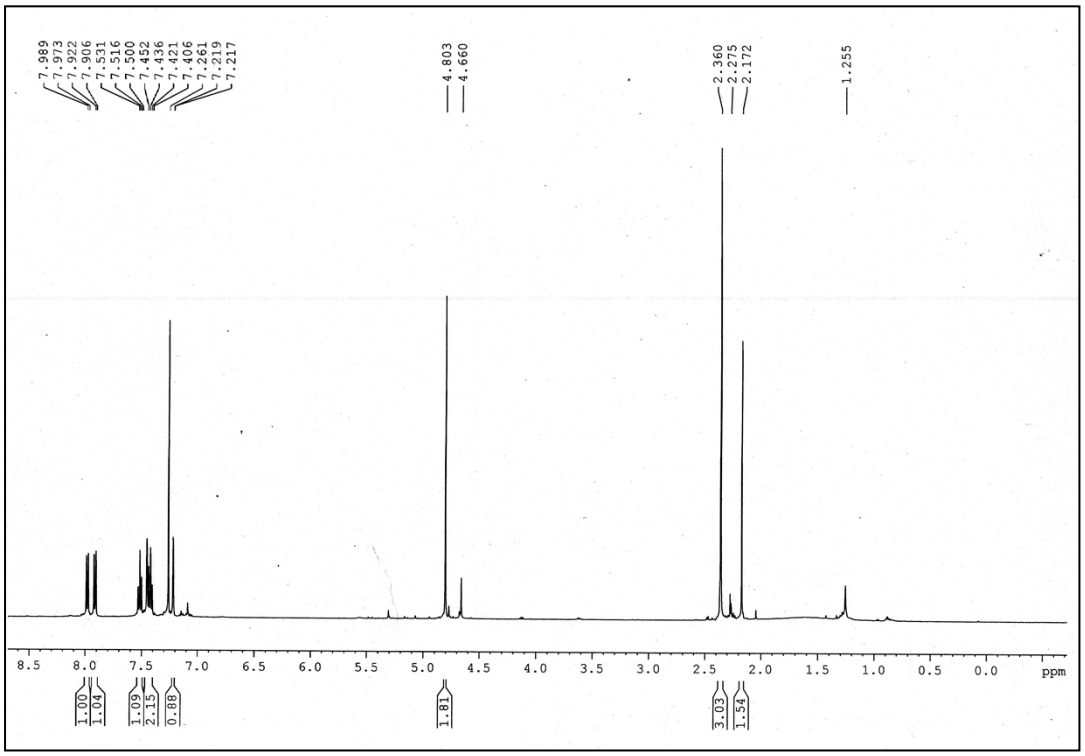

Figure S1: ^{1}H NMR (500 MHz) spectrum of compound 2 in $CDCl_3$.

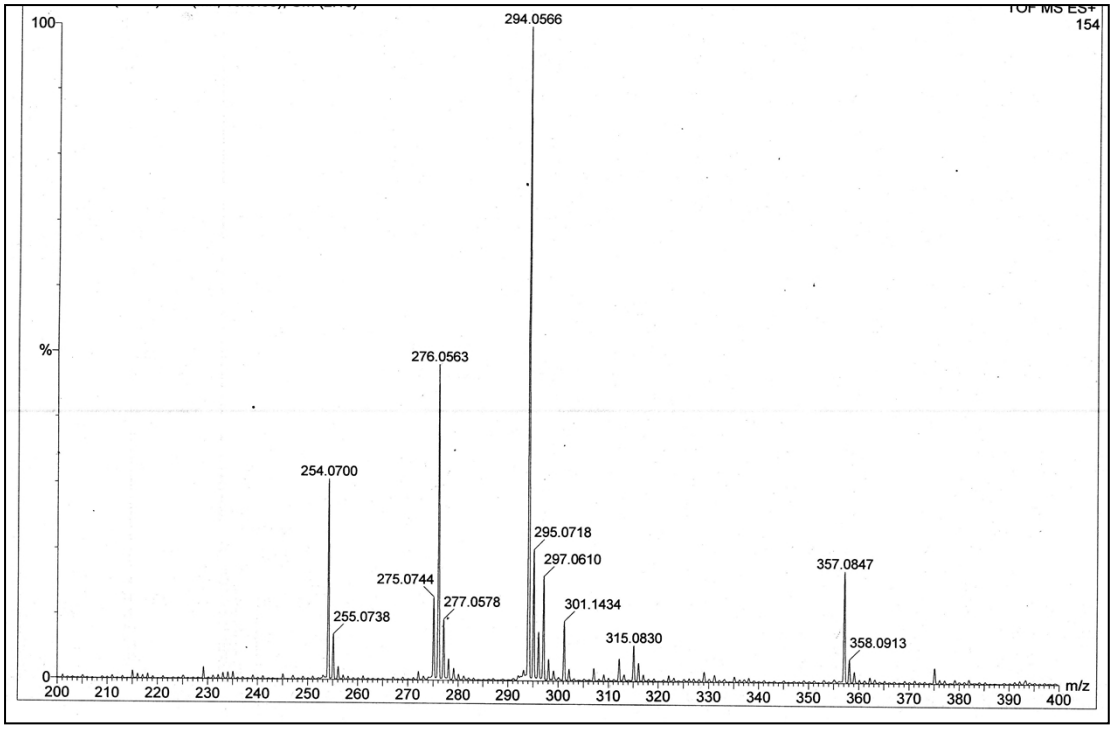

Figure S2: HRMS of compound 2.

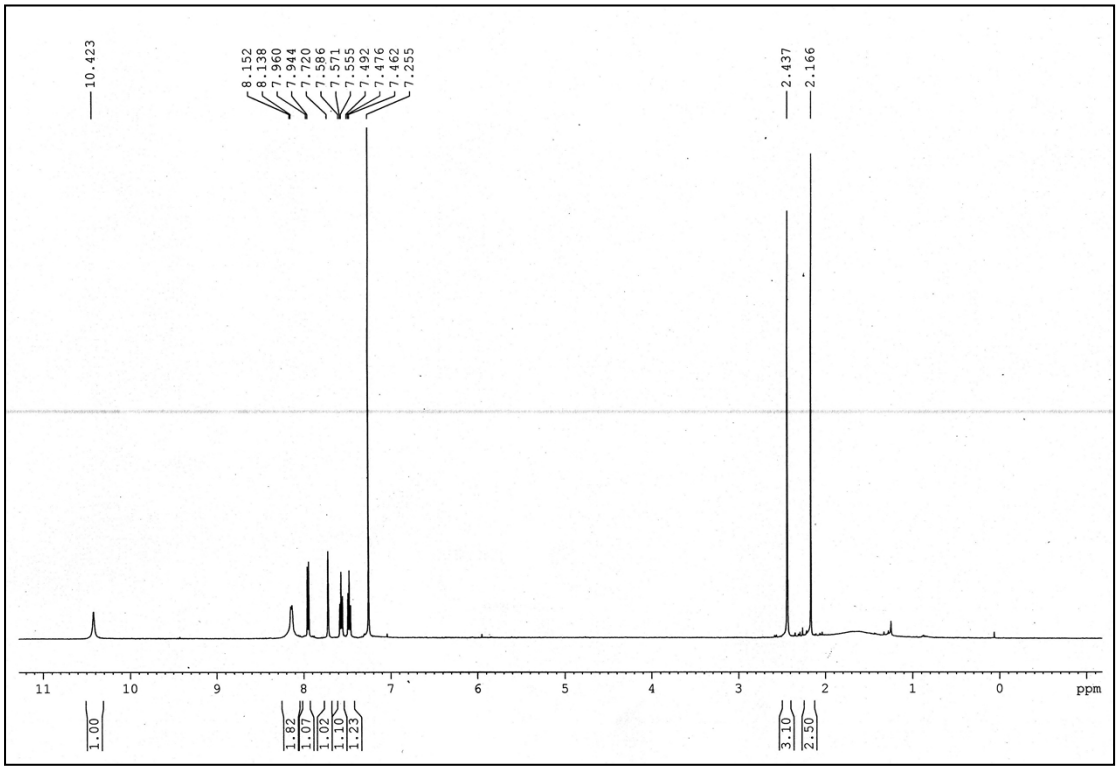

Figure S3: ^{1}H NMR (500 MHz) spectrum of compound 3 in $CDCl_3$.

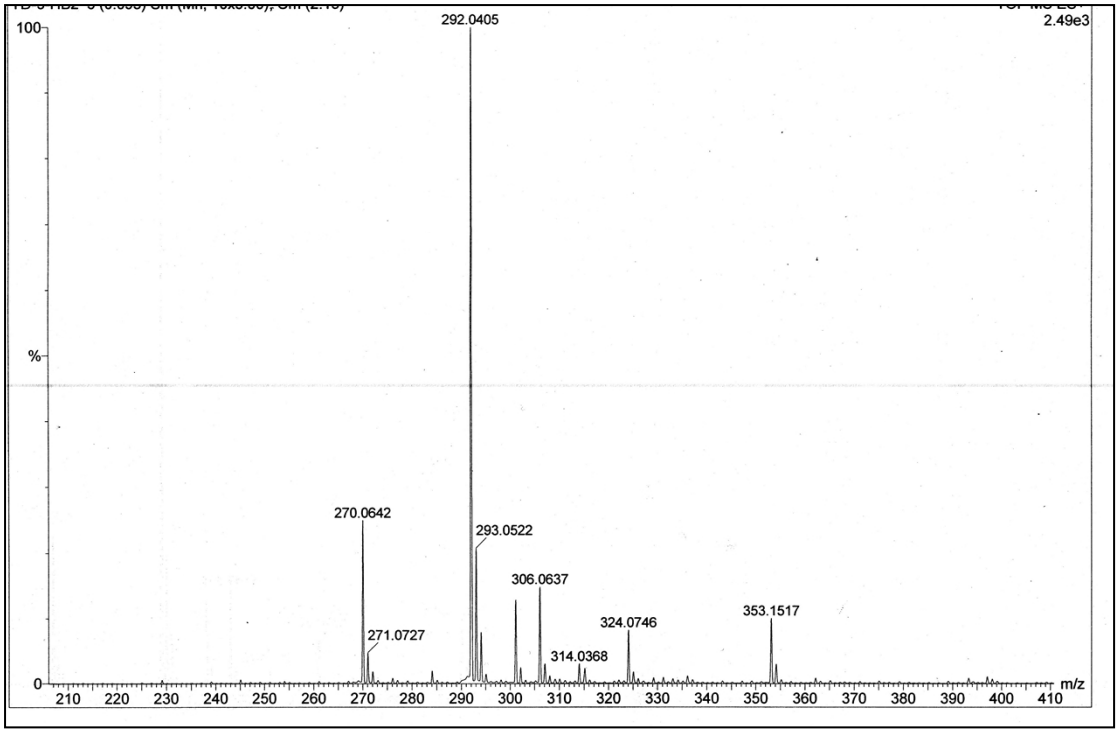

Figure S4: HRMS of compound 3

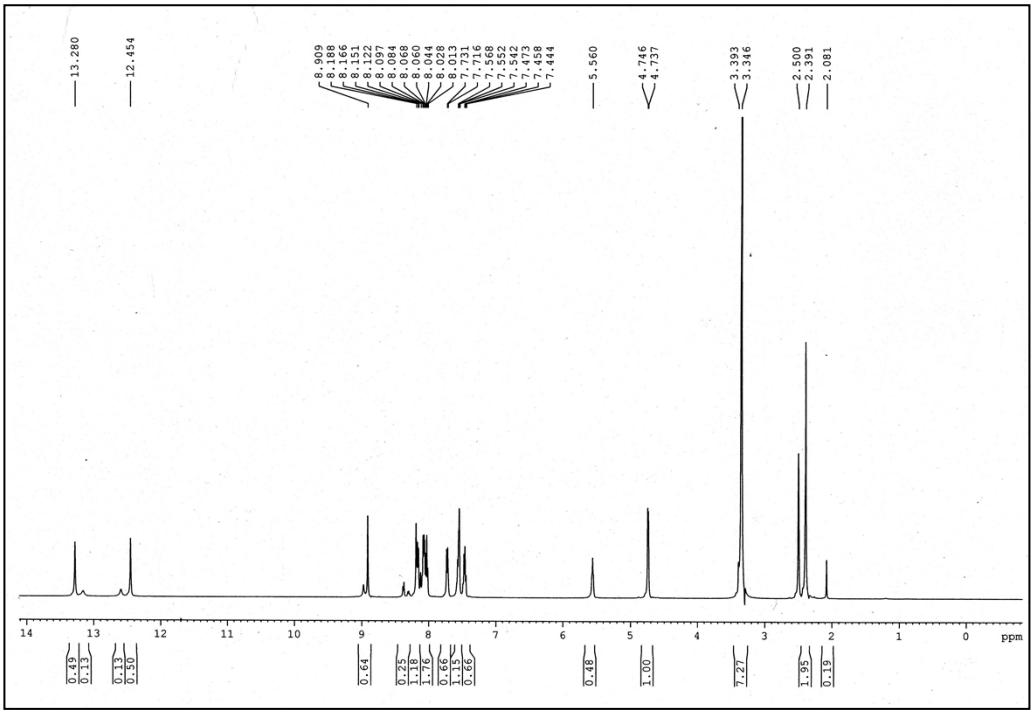

Figure S5: ^{1}H NMR (500 MHz) spectrum of HBTP in d_6-DMSO.

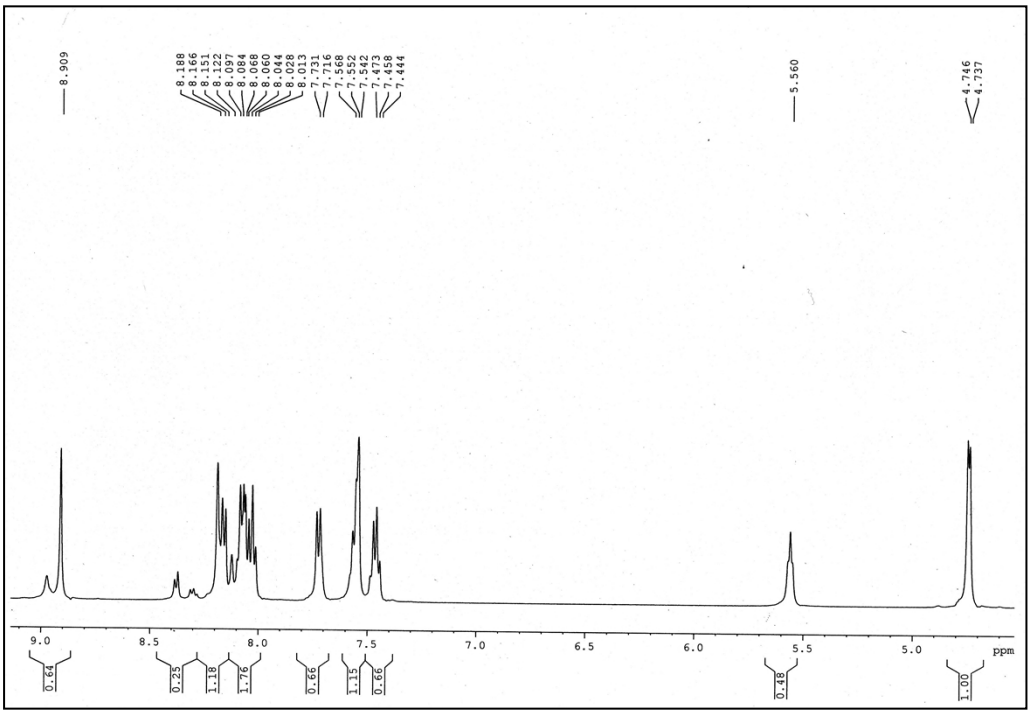

Figure S6: ^{1}H NMR (500 MHz) spectrum of HBTP in d_6-DMSO (expansion).

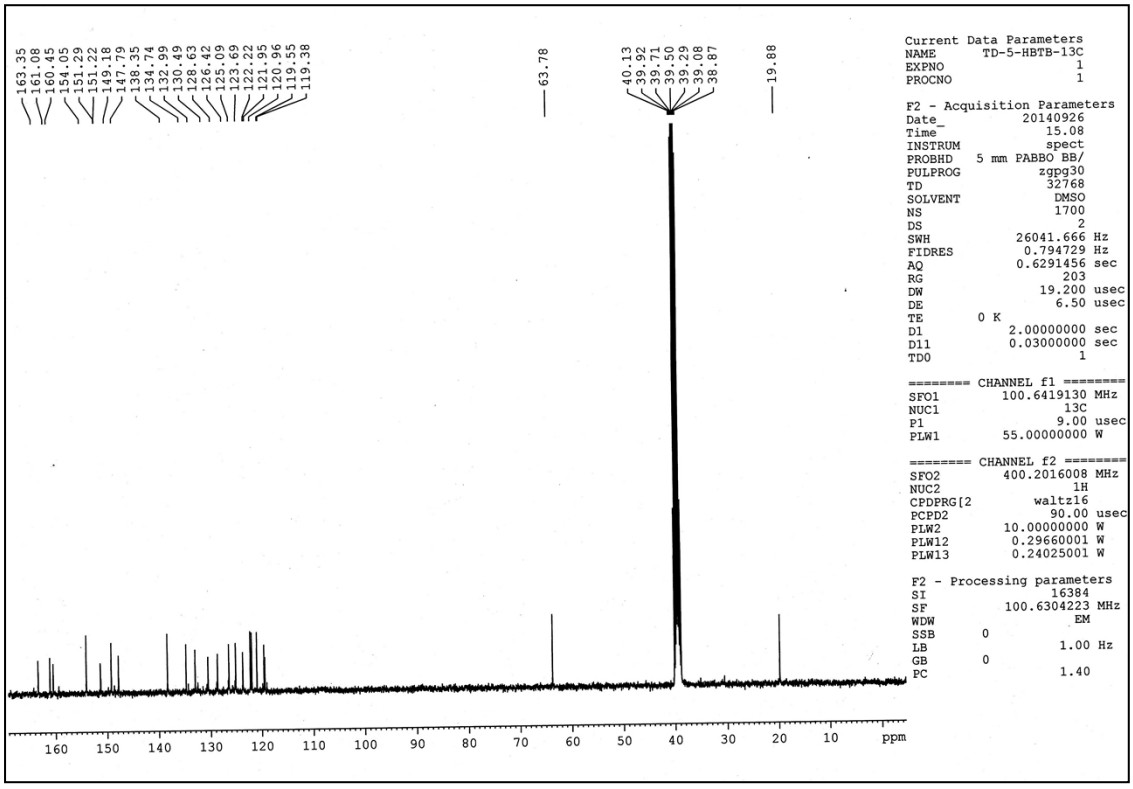

Figure S7: ^{13}C NMR (125 MHz) spectrum of HBTP in d_6-DMSO.

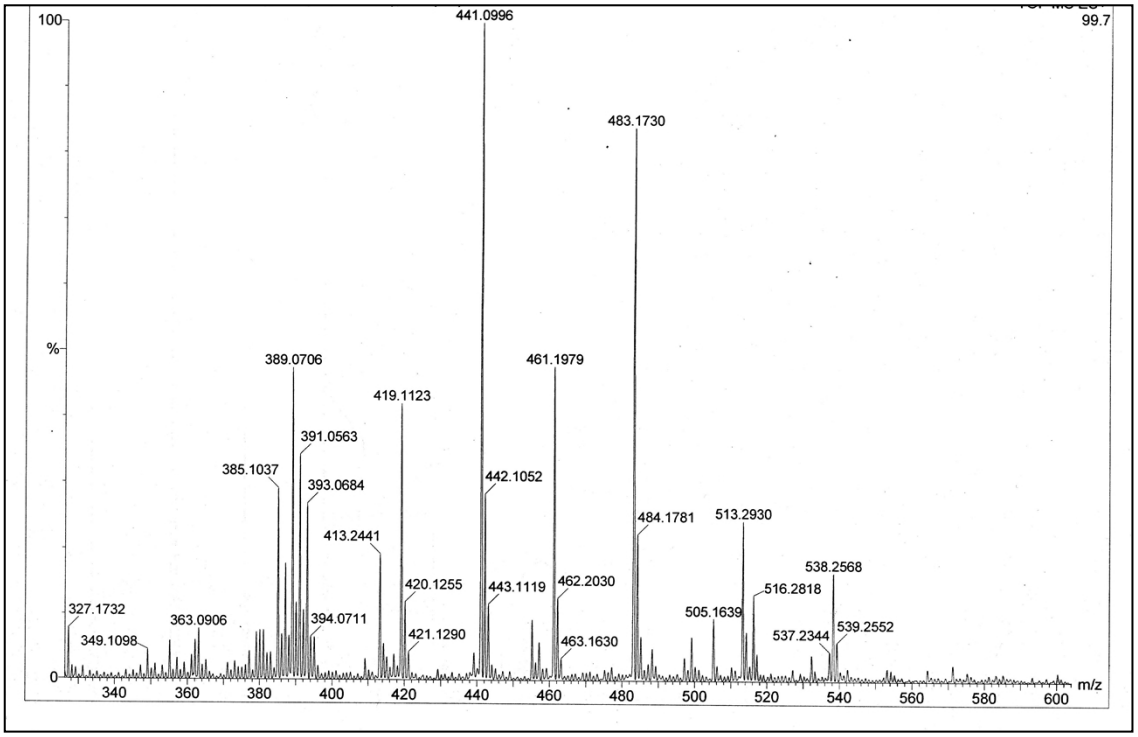

Figure S8: HRMS of HBTP

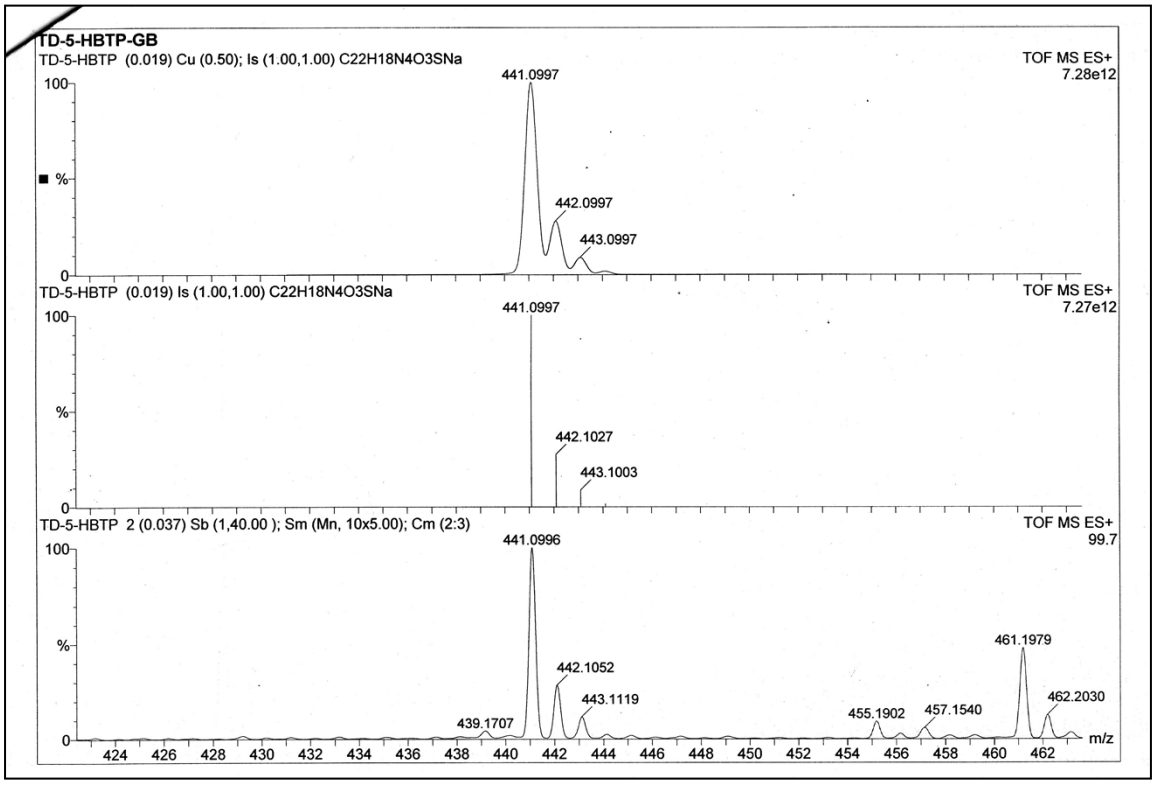

Figure S9: HRMS of HBTP (expansion)

X-ray crystal structure analysis:

A yellow, block shaped single crystal of the HBTP, with dimensions of 0.360 mm x 0.211 mm x 0.075 mm, was chosen and its X-ray analysis was done using Apex II Duo CCDC diffractometer with fine-focus sealed tube graphite-monochromated Mo *Kα* radiation (λ = 0.71073 Å) at room temperature. The data was processed with SAINT and corrected for absorption using SADABS [1]. The structure was solved by direct method using the program SHELXTL[2] and was refined by full-matrix least squares technique on F^2 using anisotropic displacement parameters for all non-hydrogen atoms. The non-hydrogen atoms were refined anisotropically. In **HBTP**, the N-bound and O-bound hydrogen atoms were located in a difference Fourier map and and were fixed to their parent atoms with U_{iso}(H) = 1.2 U_{eq}(N) or 1.5 U_{eq}(O) [N—H = 0.8535 Å; O—H = 0.8173 or 0.8250 Å]. The remaining C-bound H atoms were calculated geometrically with isotropic displacement parameters set to 1.2 (1.5 for methyl groups) times the equivalent isotropic *U* values of the parent carbon atoms [C—H = 0.93 or 0.96 Å]. A rotating group model was used for methyl groups. Crystallographic data has been deposited at the Cambridge Crystallographic Data Centre with CCDC 1045374 (**HBTP**). Copies of the data can be obtained free of charge on application to the CCDC, 12 Union Road, Cambridge CB2 IEZ, UK. Fax: +44-(0)1223-336033 or e-mail:deposit@ccdc.cam.ac.uk

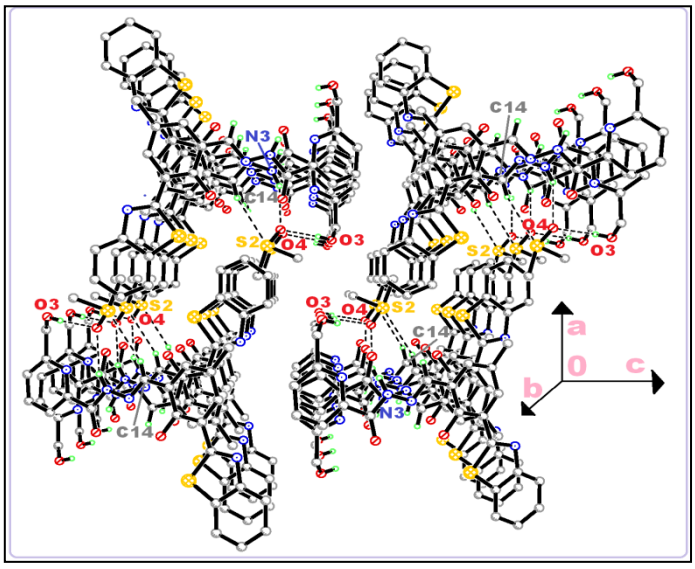

Figure S10: Crystal packing of **HBTP**, showing the molecules are stacked along the *b* axis. H atoms not involved in intermolecular interactions (dashed lines) have been omitted for clarity.

Table S1 Experimental details

Crystal data	
Compound	**HBTP** (CCDC 1045374)
Chemical formula	$C_{22}H_{18}N_4O_3S{\cdot}C_2H_6OS$
M_r	496.59
Crystal system, space group	Orthorhombic, *Pbca*
Temperature (K)	294
***a*, *b*, *c* (Å)**	23.177 (5), 8.5606 (17), 23.924 (5)
***V* (Å^3)**	4746.9 (17)
Z	8
Radiation type	Mo *K*α
μ (mm^{-1})	0.26
Crystal size (mm)	0.36 × 0.21 × 0.08
Data collection	
Diffractometer	Bruker *SMART APEX* II DUO CCD area-detector diffractometer
Absorption correction	Multi-scan (*SADABS*; Bruker, 2009)
T_{min}, T_{max}	0.911, 0.981
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	20862, 4898, 2126
R_{int}	0.107
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.628
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.062, 0.212, 0.96
No. of reflections	4898
No. of parameters	310
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.28, −0.23

Table S2 Hydrogen-bond geometry (Å, º)

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O1—H1*O*1···N2	0.82	1.83	2.575 (4)	152
O3—H1*O*3···O4	0.83	2.02	2.836 (5)	171
N3—H1*N*3···O4	0.85	2.31	3.106 (5)	155
C14—H14*A*···S2	0.93	2.83	3.693 (5)	155
C24—H24B ···Cg1^{i}	0.96	2.94	3.784(5)	148

Symmetry code: (i) *1/2+x,y,1/2-z*

* Cg1 is the centroid of benzene ring (C1-C6).

Determination of Association Constant (K_a):

By UV-vis method:

Association constant was calculated according to the Benesi-Hildebrand equation. K_a was calculated following the equation stated below.

$$1/(A-A_o) = 1/\{K(A_{max}-A_o)\,[M^{x+}]^n\} + 1/[A_{max}-A_o]$$

Here A_o is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, A_{max} is absorbance in presence of added $[M^{x+}]_{max}$ and K_a is the association constant, where $[M^{X+}]$ is $[Al^{3+}]$. The association constant (K_a) could be determined from the slope of the straight line of the plot of $1/(A-A_o)$ against $1/[Al^{3+}]$ and is found to be 1.24×10^5 M^{-1}.

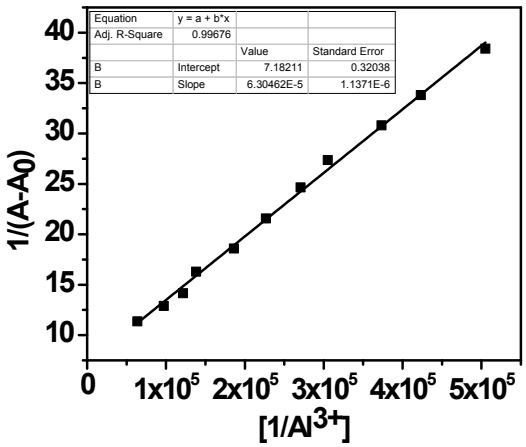

Figure S11: Benesi-Hildebrand plot from absorption titration data of receptor (10 μM) with Al^{3+}.

Determination of detection limit:

The detection limit (DL) of **HBTP** for Al^{3+} was determined from the following equation:

$$DL = K^* Sb_1/S$$

Where K = 2 or 3 (we take 3 in this case); Sb_1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

By fluorescence method:

From the graph, we get slope = 347248.167, and Sb_1 value is 0.00778

Thus using the formula we get the Detection Limit = 6.72 x 10^{-8} M i.e. HBTP can detect Al^{3+} in this minimum concentration through fluorescence method.

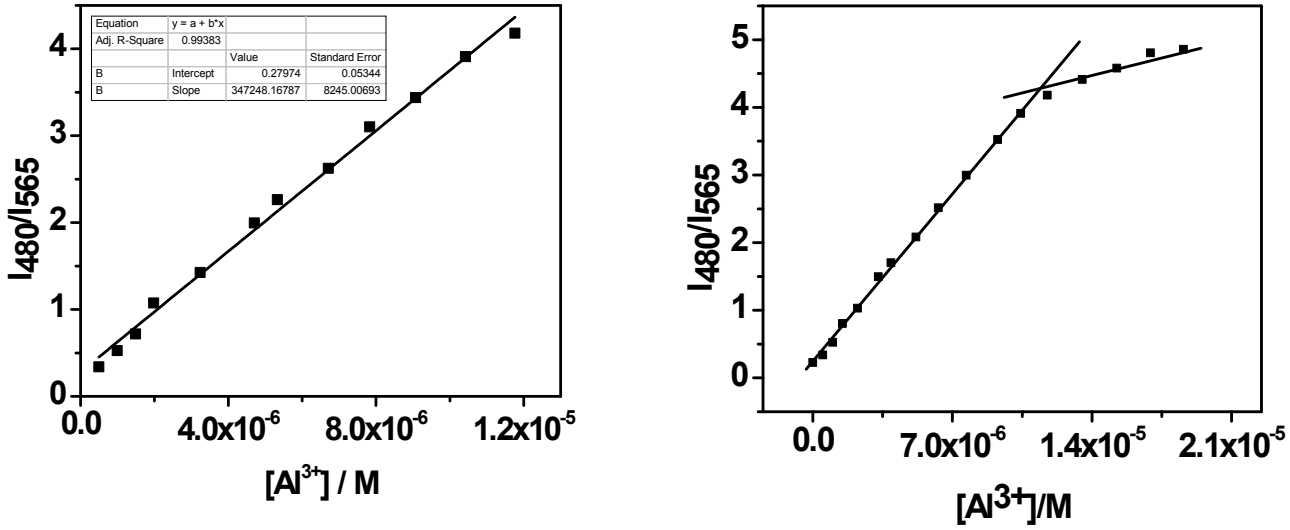

Figure S12: The linear response curve of emission ratio (I_{480}/I_{560}) depending on the Al^{3+} concentration.

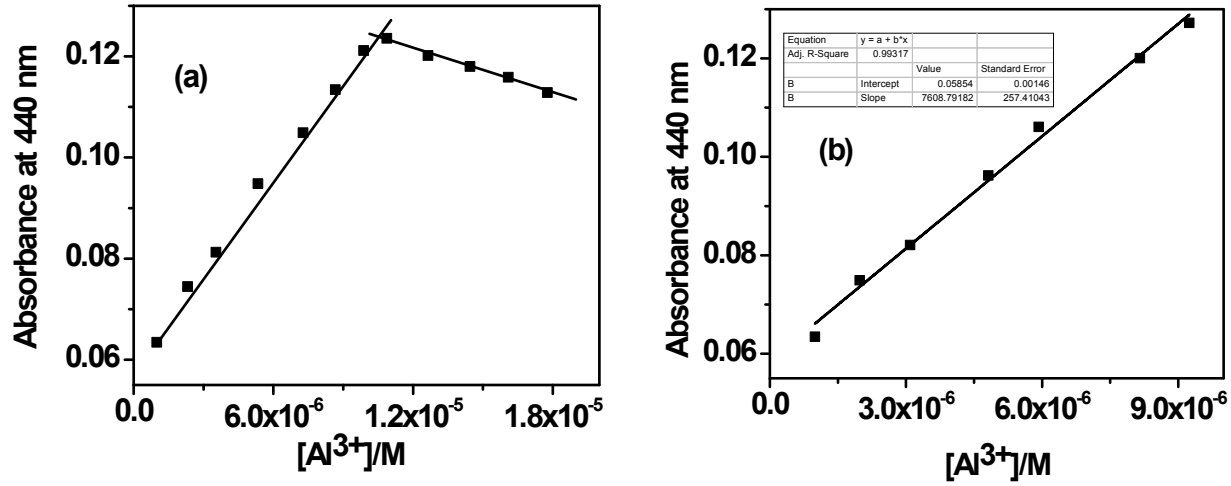

Figure S13: The linear response curve of HBTP with Al^{3+} concentration (440 nm).

General procedure for drawing Job's plot by fluorescence method:

Stock solution of same concentration of sensor and Al^{3+} were prepared in the order of 10 μM in [CH_3OH/ H_2O, 1/9, v/v] (at 25 °C) at pH 7.3 in PBS buffer. The emission spectrum in each case with different *host–guest* ratio but equal in volume was recorded. Job's plots were drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of intensity of the emission spectrum at 480 nm during titration and X_{host} is the mole fraction of the host in each case, respectively).

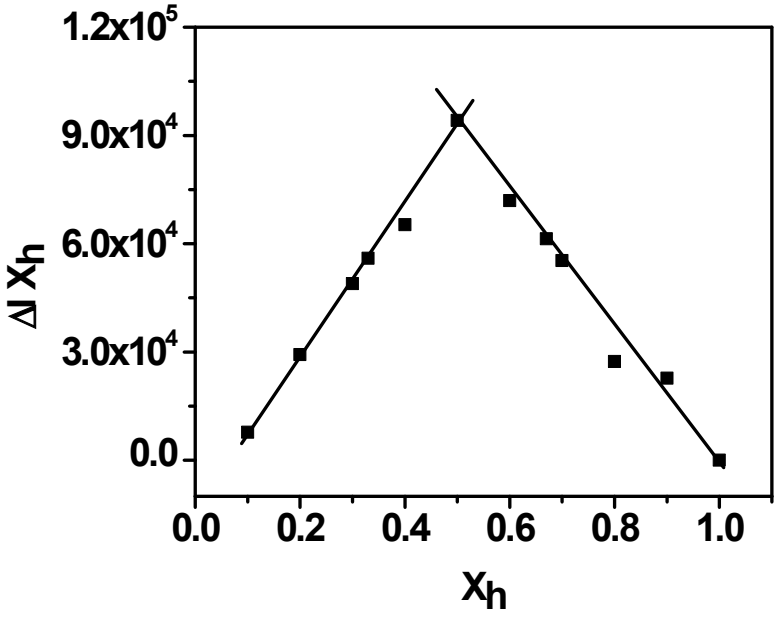

Figure S14: Job's plot diagram of receptor for Al^{3+} (where X_h is the mole fraction of the host and ΔI indicates the change of emission intensity at 480 nm)

Competition study:

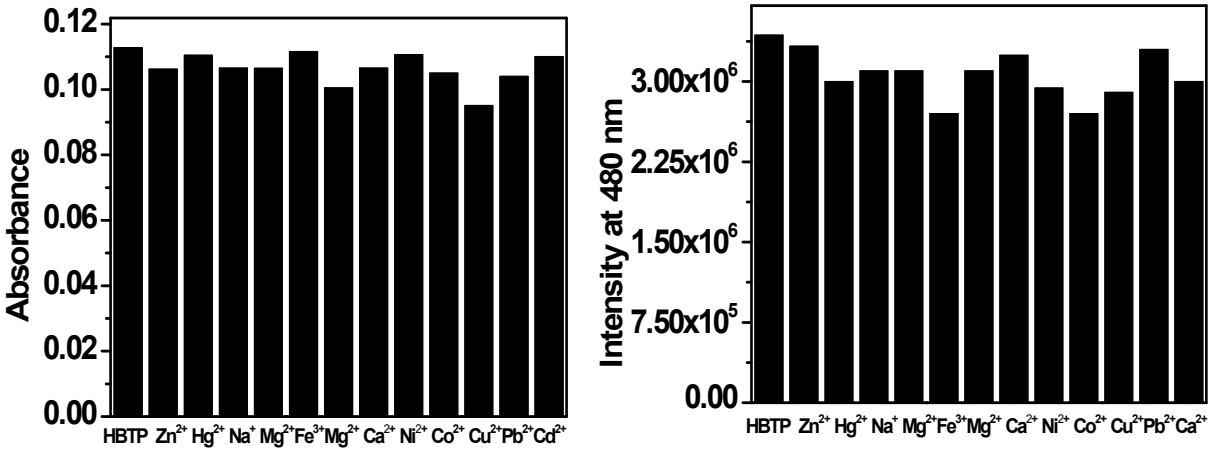

Figure S15: Competition study using (a) UV-vis and (b) Fluorescence method, after addition of different analytes (30 μM) in the solution of HBTP (10 μM) in presence of Al^{3+} (20 μM).

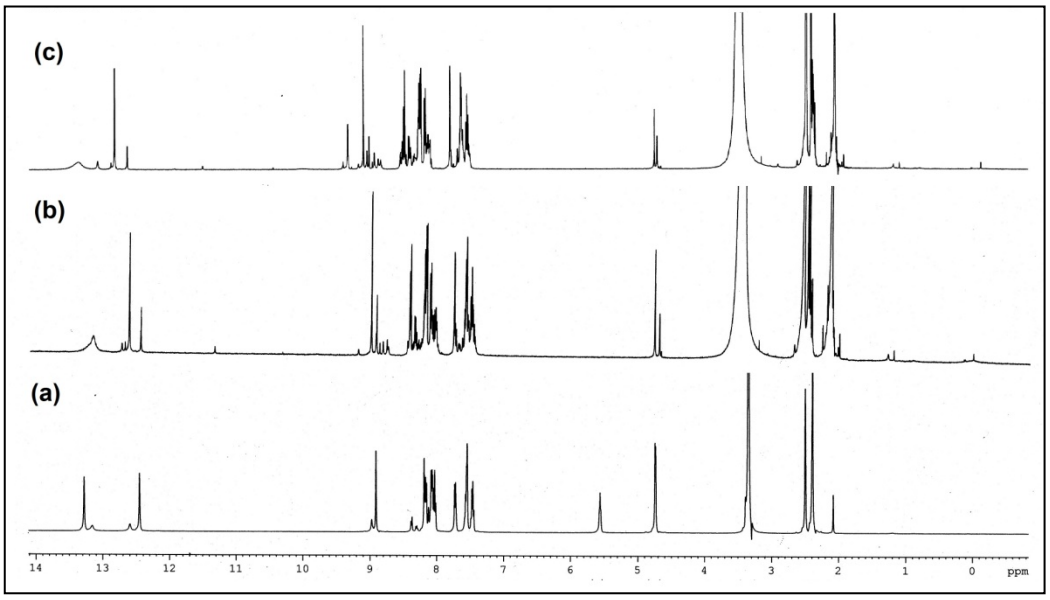

Figure S16. Partial ^{1}H NMR (400 MHz) spectra of (a) HBTP (4.7×10^{-3} M), (b) [HBTP+Al^{3+} (2.4×10^{-3} M)] and (c) [HBTP+Al^{3+} (4.8×10^{-3} M)] in d_6 DMSO.

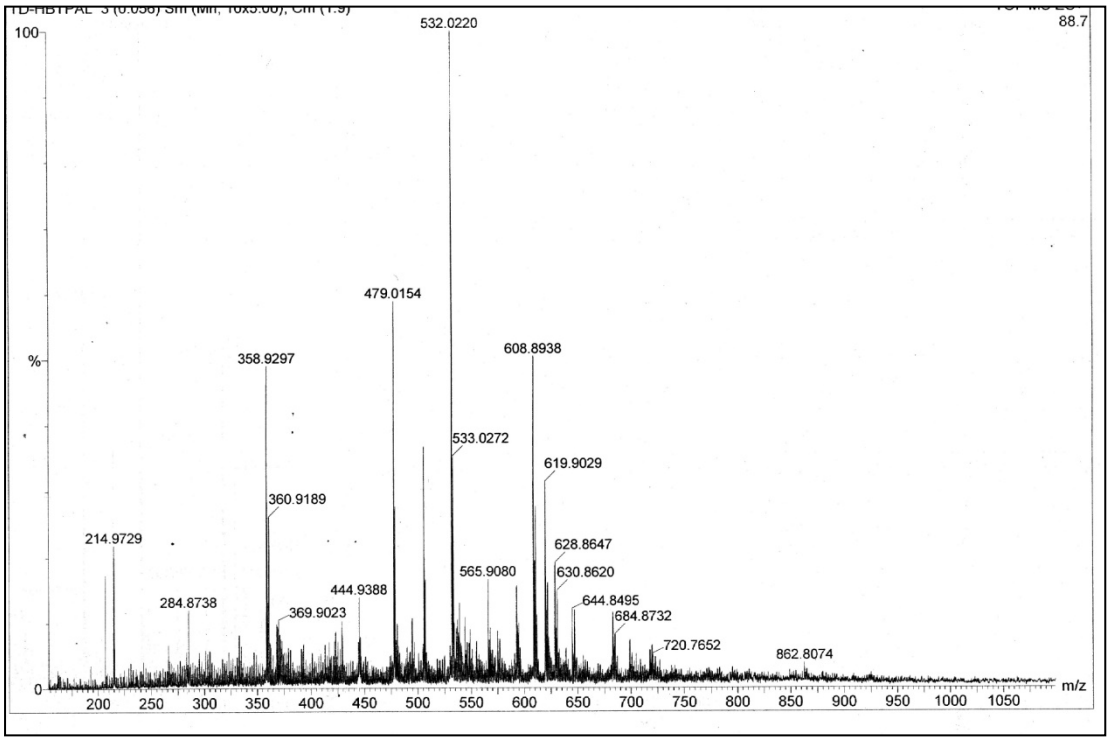

Figure S17: HRMS of HBTP-Al^{3+} complex.

pH study:

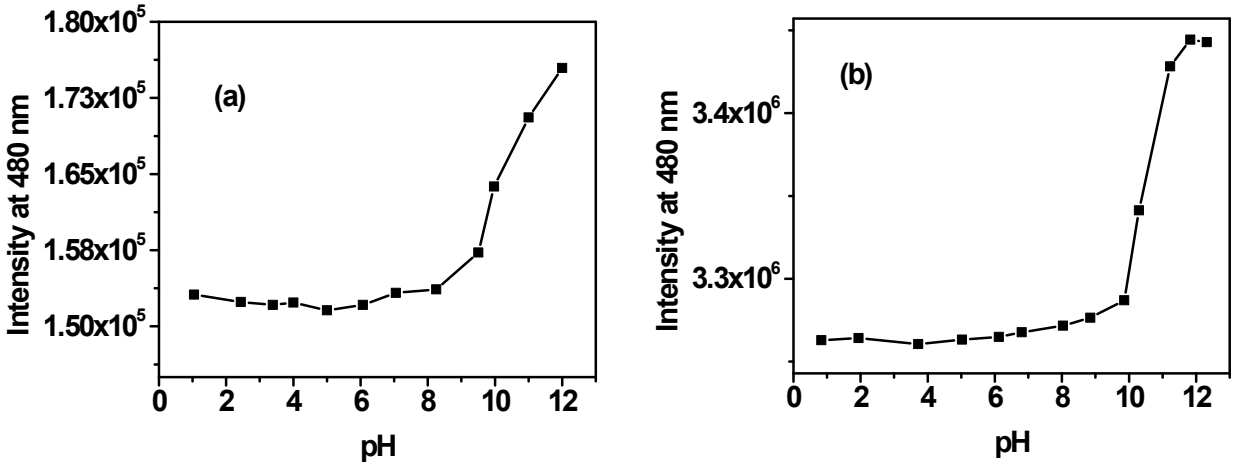

Figure S18: Fluorescence response of (a) HBTP and (b) HBTP-Al^{3+} at 480 nm (10 µM) as a function of pH in CH_3OH/ H_2O (1/ 9, *v/v*), pH is adjusted by using aqueous solutions of 1 M HCl or 1 M NaOH.

Details of live-cell imaging

Materials Methods

3 ml of venous blood was obtained from volunteer donor (age > 30 years) with his informed consent. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation by histopaque-1077 obtained by SIGMA. PBMCs were washed and suspended in PBS and were divided in two sets. In one set, 10 µM $Al(NO_3)_3$ solution was added as a source of Al^{3+}. Another set was devoid of any externally added Al^{3+}. HBTP samples were prepared in PBS containing 0.5% DMSO. Both the samples were incubated with 10 µM HBTP solutions for 15 minutes at 37^0C. Cells were observed under confocal fluorescence microscope (Olympus IX81 microscope) with fluorescence emissions at 480 nm and 560 nm, respectively.

Table S3: Average red/green fluorescence intensity in human PBMCs treated with HBTP with and without Al^{3+}

	Red fluorescence intensity	Green fluorescence intensity	Green : Red ratio
-ve Al^{3+}	4099	187	0.046
+ve Al^{3+}	581	3602	6.2

MTT assay:

To determine cell viability against HBTP, PBMCs were treated with different concentrations of HBTP solution (5-50 µM) with or without Al^{3+} (10 µM) for 1 hour at 37^0C against control cell suspension with no added HBTP. Cell density remains 10^6 cells per well in a 96- well plate. 100 µl of MTT solution (5 mg/ml) was added to each well including control and incubated for 4 hours at 37^0C. The purple colored formazan crystals were dissolved with 100 µl DMSO and the absorbance were measured at 570 nm. Cell viability was calculated using the following calculation:

$$\% \text{ of Cell Viability} = \frac{(\text{Absorbance of treatment group} - \text{blank})}{(\text{Absorbance of control group} - \text{blank})} \times 100$$

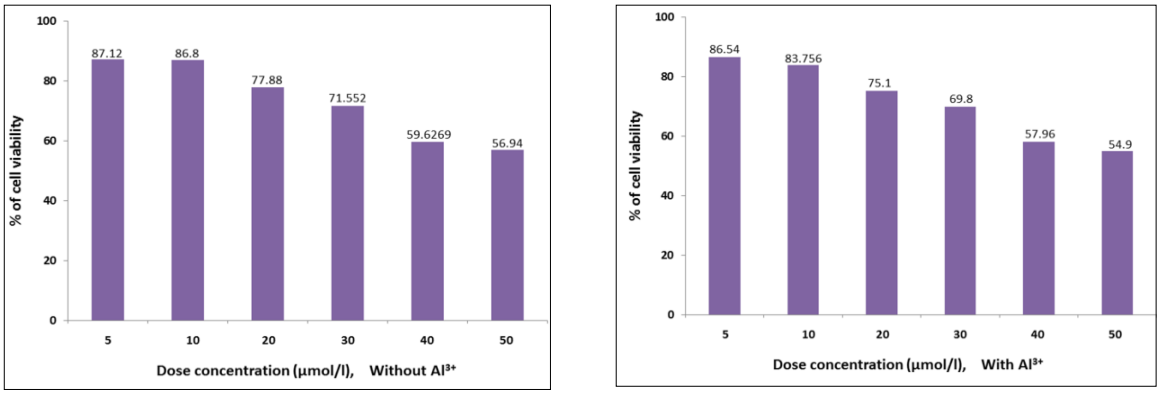

Figure S19: Percentage of viable cells over HBTP concentration range (5-50 µM) presence and absence of Al^{3+}.

Result:

Cell viability was represented in Figure S18, where up to 50 µmol/l concentrations of HBTP shows around 57% & 55% of viable cells respectively predicting it is a safe probe to use in a biological system. We have used 20 µmol/l HBTP solutions for imaging which shows fairly high number of viable cells (77.88% without Al^{3+} and 75.1% with Al^{3+}) concluding its nontoxic nature.

The emission change of HBTP after addition of Al^{3+} in DMSO solution:

The fluorescence tiration of HBTP (10 µM) in DMSO with increasing concentration of Al^{3+} showed similar charactersistic as that of in methanol/H_2O (1/9) solution. This observation indicates that the probe is similar effective in both the solution (aqueous solution and DMSO) to detect Al^{3+}.

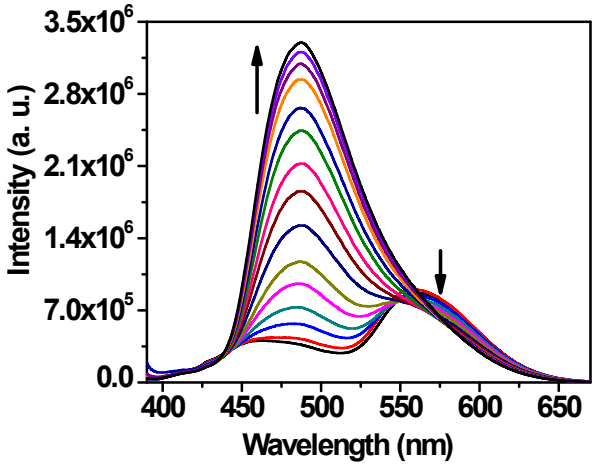

Figure S20: Fluorescence spectra of HBTP (10 μM) upon titration with Al^{3+} (0 to 3 equivalents) in DMSO solution.

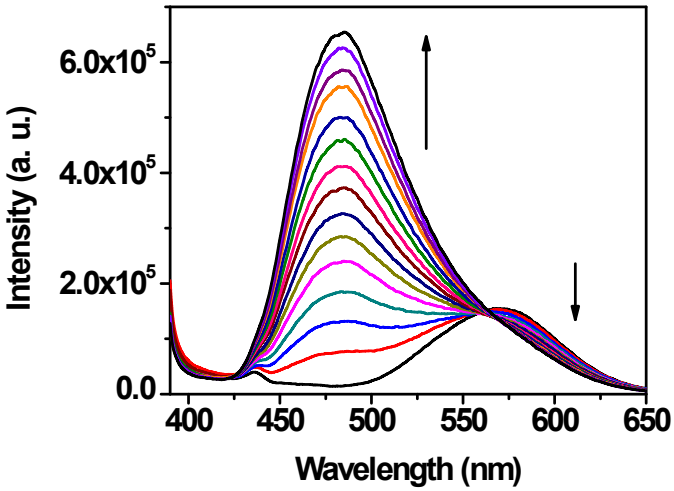

Figure S21: Fluorescence spectra of HBTP (10 μM) upon titration with Al^{3+} (0 to 3 equivalents) in 98% water (2% DMSO) solution.

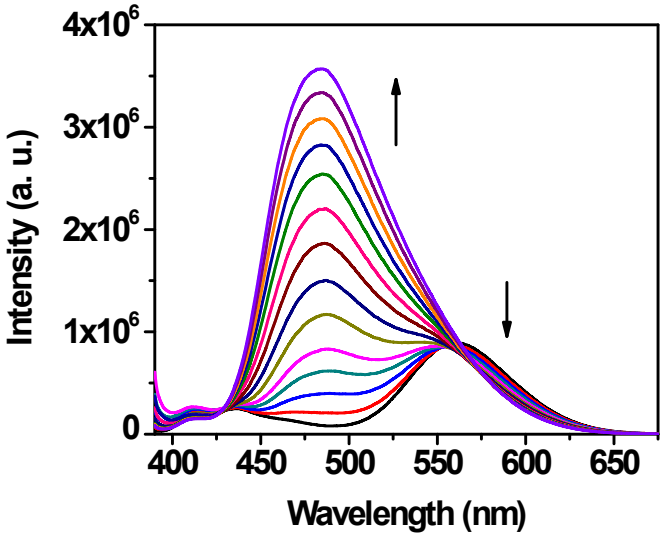

Figure S22: Fluorescence spectra of HBTP (10 μM) upon titration with Al^{3+} (0 to 3 equivalents) in 90% water (10% EtOH) solution.

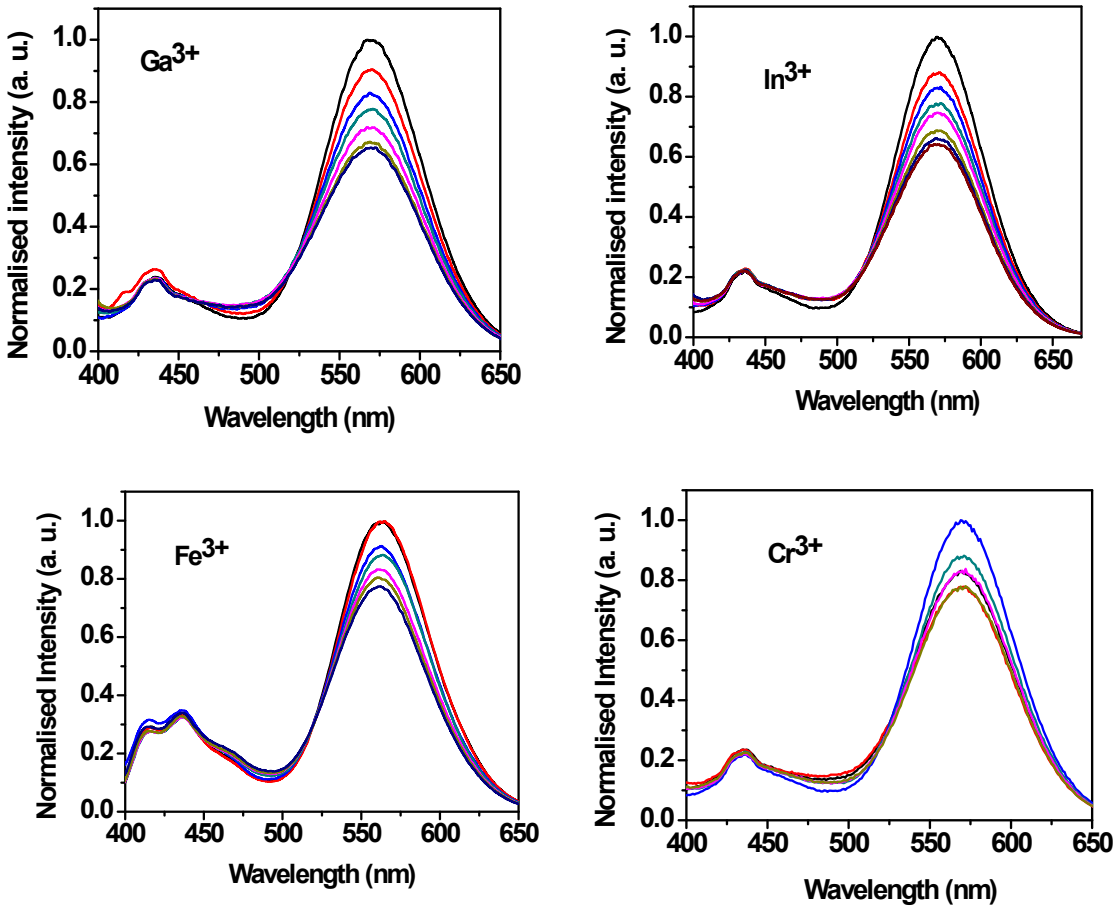

Figure S23: The change of fluorescence of HBTP in presence of different trivalent ions.

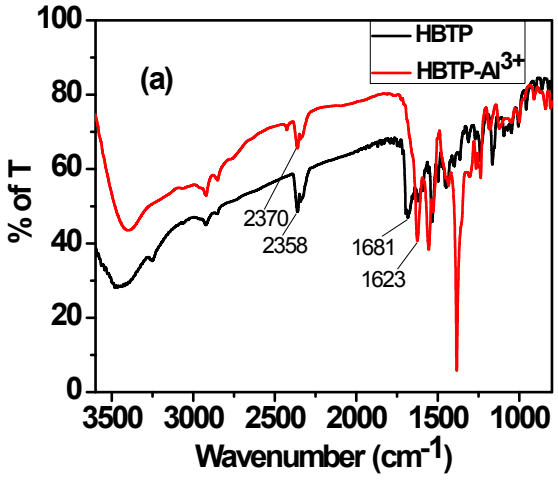

Figure S24: FT-IR spectrum of HBTP and HBTP-Al^{3+}.

Comparison of present probe with the existing probes:

Table S4: The comparison of the present probe with recently reported probes for Al^{3+} have been outlined in this table

Fluorophore used	Type of response	Selectivity	Detection limit	Living cell imaging	Reference
2-hydroxy naphthaldehyde	Colorimetric, fluorometric	Al^{3+}	3.0×10^{-7} M and 1.0×10^{-7} M in ethanol and 0.1 M HEPES buffer respectively	Yes	*Org. Biomol. Chem.*, 2011, **9**, 5523.
1,2-dihydroxyanthraquinone	Colorimetric, fluorometric	Al^{3+}	5.0×10^{-7} M	No	*Org. Lett.,* 2011, 13, 5274.
1-naphthylamine and benzaldehyde,	Colorimetric, fluorometric	Al^{3+}	5×10^{-5} M	Yes	*Dalton Trans.,* 2015, 44, 4576.
Naphthalene	Colorimetric, fluorometric	Al^{3+}	57 nM	No	*RSC Adv*., 2013, 3, 22572.
Naphthalene	Colorimetric, fluorometric	Al^{3+}	1×10^{-8} M	Yes	*Analyst*, 2012, 137, 2166.
Pyridyl-salicylimine Schiff base derivatives	Colorimetric, fluorometric	Zn^{2+}, Al^{3+} and OH^-	1.69×10^{-6} M , 1.42×10^{-6} M and 1.27×10^{-6} M (for Al^{3+})	No	*Analyst,* 2013, 138, 2931.
Salicylimine	Colorimetric, fluorometric	Al^{3+}	2.94×10^{-8} M	Yes	*Dalton Trans.,* 2015, 44, 11352.
Salicylaldehyde	Colorimetric, fluorometric	Zn^{2+} and Al^{3+}	2.4×10^{-7} M (for Al^{3+})	Yes	*Dalton Trans*., 2015, 44, 11797.
Hydroxybenzothiazole	Colorimetric, fluorometric	Al^{3+}	6.72×10^{-8} M	Yes	This work

References:

1. Bruker. APEX2, SAINT and SADABS. Bruker AXS Inc., Madison, Winconsin, USA.2009.
2. Sheldrick, G.M. A short history of SHELX. Acta Cryst.2008, A64, 112.