### Supplementary Material for review only

# CHEF affected Fluorogenic Nanomolar detection of Al<sup>3+</sup> by an anthranilic acidnaphthalene hybrid: cell imaging and crystal structure

Arghyadeep Bhattacharyya, Subhash Chandra Makhal and Nikhil Guchhait $^{*}$ 

**Table of Contents:** 

- 1. FTIR spectrum of BBHAN
- 2. PMR spectrum of BBHAN
- 3. CMR spectrum of BBHAN
- 4. ESI-MS profile of BBHAN and isotopic modeling of BBHAN-Al complex ESI-MS
- 5. FTIR spectrum of BBHAN-Al<sup>3+</sup> complex
- 6. PMR spectrum of BBHAN-Al<sup>3+</sup> complex
- 7. Tables for important crystallographic information of BBHAN (Tables S1-S4)
- 8. ORTEP diagram of BBHAN
- 9. Unit Cell packing of BBHAN
- 10. UV-Vis response of BBHAN towards BBHAN and UV-Vis titration of BBHAN with Al<sup>3+</sup>
- 11. Interference test of cations on BBHAN and BBHAN-Al<sup>3+</sup> ensemble
- 12. Calibration curves for determining Limit of Detection.
- 13. Table of Comparison for Limit of Detection.
- 14. Table enlisting the shifts in PMR shifts during  $Al^{3+}$  titration.
- 15. MTT assay for cell survival.
- 16. Procedure for Cell Imaging

### 1. FTIR spectrum of BBHAN



Figure S1: FTIR spectrum of BBHAN.

### 2. PMR spectrum of BBHAN



Figure S2: PMR spectrum of BBHAN in DMSO-d<sub>6</sub>.

### 3. CMR spectrum of BBHAN



Figure S3: CMR spectrum of BBHAN in DMSO-d<sub>6</sub>.

### 4. ESI-MS profile of BBHAN and isotopic modeling of BBHAN-Al complex ESI-MS:





**Figure S4:** (top) ESI-MS profile of **BBHAN**, (bottom) Isotopic modeling of the Aluminium Complex.



# 5. FTIR spectrum of BBHAN-Al<sup>3+</sup> complex

Figure S5: FTIR spectrum of BBHAN-Al complex.

# 6. PMR spectrum of BBHAN-Al<sup>3+</sup> complex



**Figure S6:** PMR spectrum of **BBHAN-**Al<sup>3+</sup> complex in DMSO-d<sub>6</sub>.

Parameters	BBHAN
Formula	C27 H25 N3 O4 S
Formula Weight	409.14
Crystal System	Orthorhombic
Space group	P2(1)2(1)2(1)
a [Å]	5.7942(2)
b [Å]	16.1616(7)
c [Å]	25.9696(11)
α [°]	90
β [°]	90
γ[°]	90
V [Å <sup>3</sup> ]	2431.89(17)
h, k, l (max.)	7,20,32
Z	4
$D(calc) [g/cm^{-3}]$	1.332
F(000)	1020
Temperature (K)	296(2)
θ Min-Max [°]	2.52, 22.83
No. of unique data	4972
R(int)	0.031
Observed data $[I > 2.0 \sigma(I)]$	4204
R1, wR2	0.0547, 0.0920

 Table S1: General Crystallographic Information:

 Table S2: Bond lengths of H-bonding interactions within BBHAN and between BBHAN

 and DMSO:

Pairs	Distance(in Å)
N2-O4	2.797
N2-H9	0.911
Н9-О4	1.893
N3-O2	2.651
N3-H14	0.859
H14-O2	1.987
O1-N1	2.574
O1-H1	0.819
H1-N1	1.854

Table S3: Table for important bond lengths with various supramolecular interaction ofDMSO with BBHAN molecules:

Pairs	Distance(in Å)
O4-H8	2.395
C11-O4	3.132
С11-Н8	0.930
С8-Н25	2.853
О3-Н23	2.631
O3-C27	3.267
С25-Н20	2.877

 Table S4: Table for bond lengths of supramolecular interactions within three BBHAN

 units:

Pairs	Distance(in Å)
С7-Н6	0.931
Н6-О2	2.546
C7-O2	3.375
C2-C11	3.378
C7-O1	3.204

### 8. ORTEP diagram of BBHAN:



Figure S7: ORTEP diagram of BBHAN at 40% probability.

#### 9. Unit Cell packing of BBHAN:



Figure S8: Unit cell packing of BBHAN.

**10.** UV-Vis response of BBHAN towards BBHAN and UV-Vis titration of BBHAN with Al<sup>3+</sup>:



**Figure S9:** (left) UV-Vis response of **BBHAN** towards metal ions and (right) UV-Vis titration of BBHAN with Al<sup>3+</sup>.



## **11. Interference test of cations on BBHAN** and **BBHAN**-Al<sup>3+</sup> ensemble

**Figure S10:** (left) Individual Interference test of cations on **BBHAN** and **BBHAN**-Al<sup>3+</sup> ensemble





Figure S11: Calibration curve for determination of LOD.

### 13. Table of Comparison for Limit of Detection

#### Table S5

Probe as designated by	Detection Mode	Detection limit	Reference
authors		(M)	
Ру-L-СООН	Ratiometric Change in	0.29×10 <sup>-6</sup>	28
	Fluorescence		
H <sub>2</sub> bpet	Fluorescence Enhancement	10 <sup>-7</sup>	27
CN	Fluorescence Enhancement	7.1×10 <sup>-8</sup>	33
P-1	Fluorescence Enhancement	$8.65 \times 10^{-8}$	32
НМС	Fluorescence Enhancement	1.62×10 <sup>-6</sup>	26
BBHAN	Fluorescence Enhancement	5.49×10 <sup>-9</sup>	Current
			work

# 14. Table enlisting the shifts in PMR shifts during Al<sup>3+</sup> titration

The labeling has been done as per the assignment of protons in Fig. S2

#### Table S6

Proton	δ(ppm)/eqiv. of Al <sup>3+</sup>	δ(ppm)/eqiv. of Al <sup>3+</sup>	δ(ppm)/eqiv. of
Label	added	added	Al <sup>3+</sup> added
А	12.67/0.00	13.24/0.50	-/1.00
В	12.40/0.00	12.43/0.50	12.43/1.00
С	11.87/0.00	11.89/0.50	11.89/1.00
D	9.50/0.00	9.53/0.50	9.56/1.00

15. MTT assay for cell survival



Figure S12: MTT assay for Cell survival.

#### 16. Procedure for Cell Imaging:

Cellular imaging was studied by fluorescence microscope. In brief, **MDA-MB-468** cells were treated with **BBHAN** for 24 hrs. Then, the cells were washed three times with 1 X PBS. The cellular uptake of the **BBHAN** was observed under a fluorescence microscope (Leica) at different time intervals. From Fig. S12 it was obvious that more than 50% of cell population survived at concentration less than 40  $\mu$ M. Thus, cell imaging studies with Al<sup>3+</sup> were conducted at 15  $\mu$ M.