Supporting Information

A new "turn-on" molecular switch for idiosyncratic detection of Al^{3+} ion along with its application in live cell imaging

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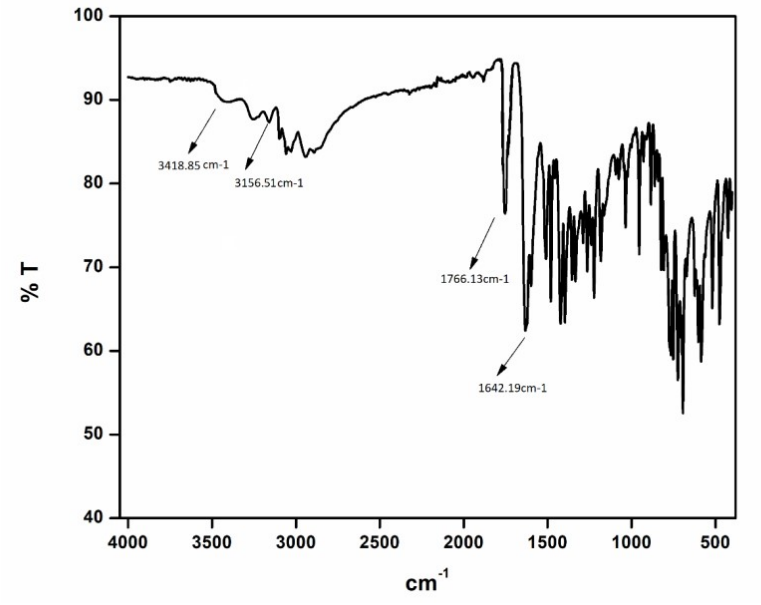


Fig. S1: IR spectra of the probe HBTC

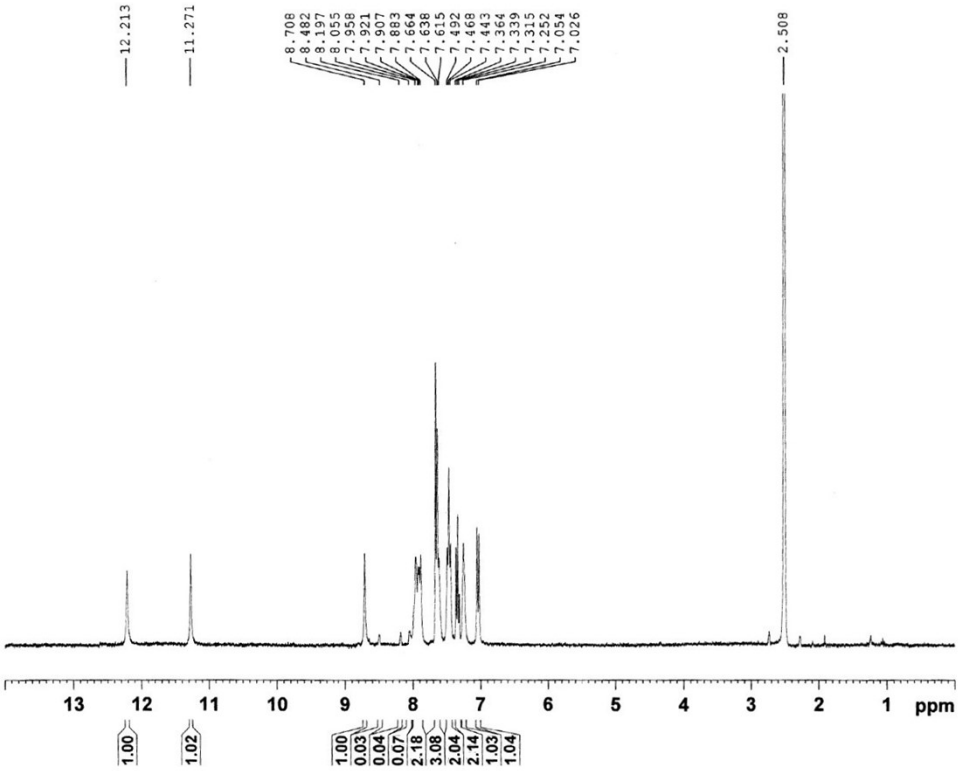


Fig. S2: ^{1}H NMR (300 MHz) spectrum of HBTC in DMSO-d_6

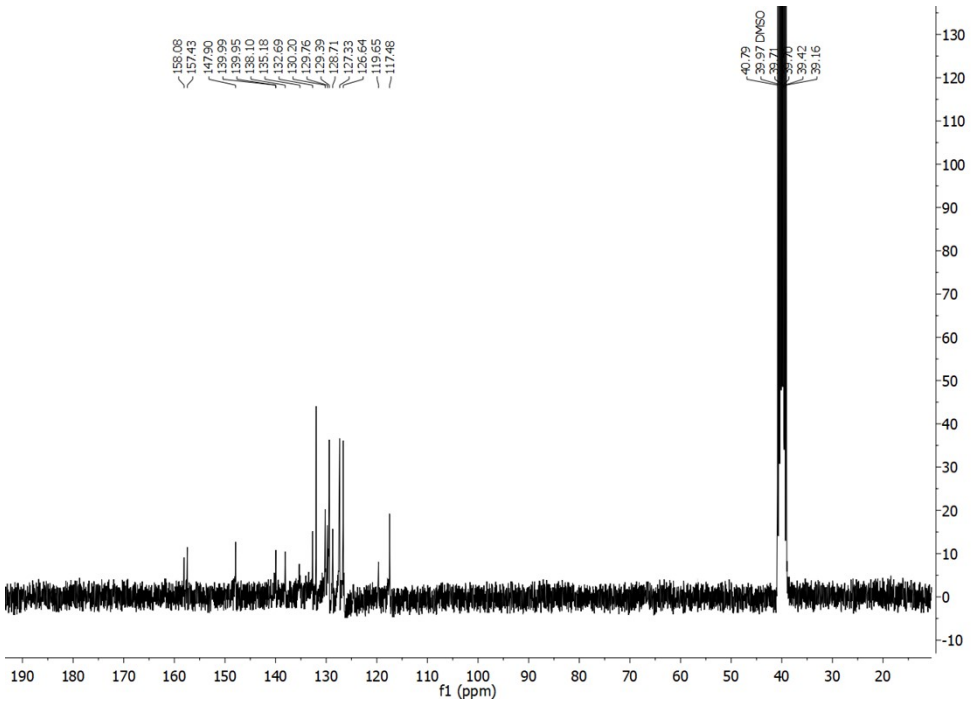


Fig. S3: ^{13}C NMR spectrum of HBTC in DMSO-d_6

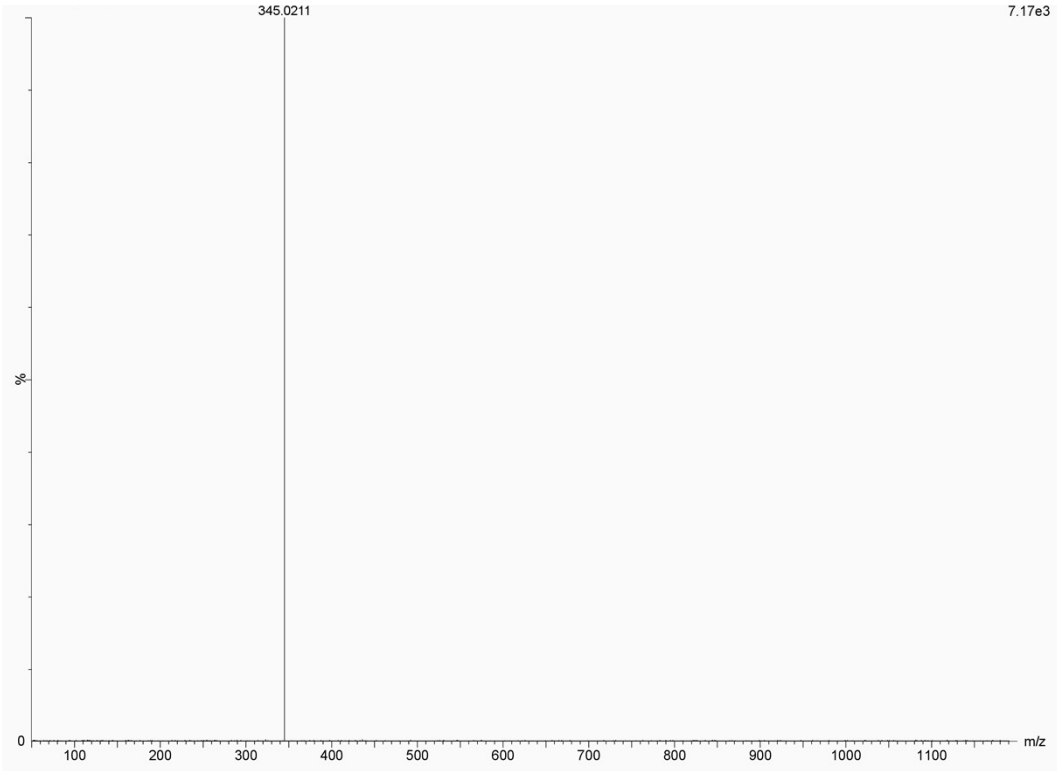


Fig. S4: HRMS of probe HBTC

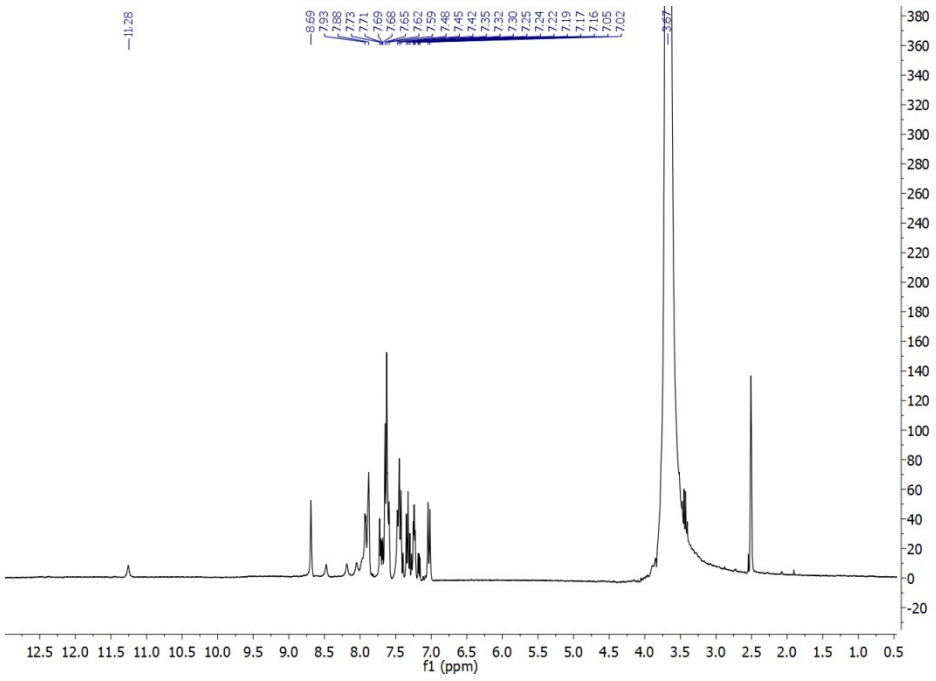


Fig. S5: ^{1}H NMR spectrum of the HBTC with Al^{3+} in DMSO-d_6

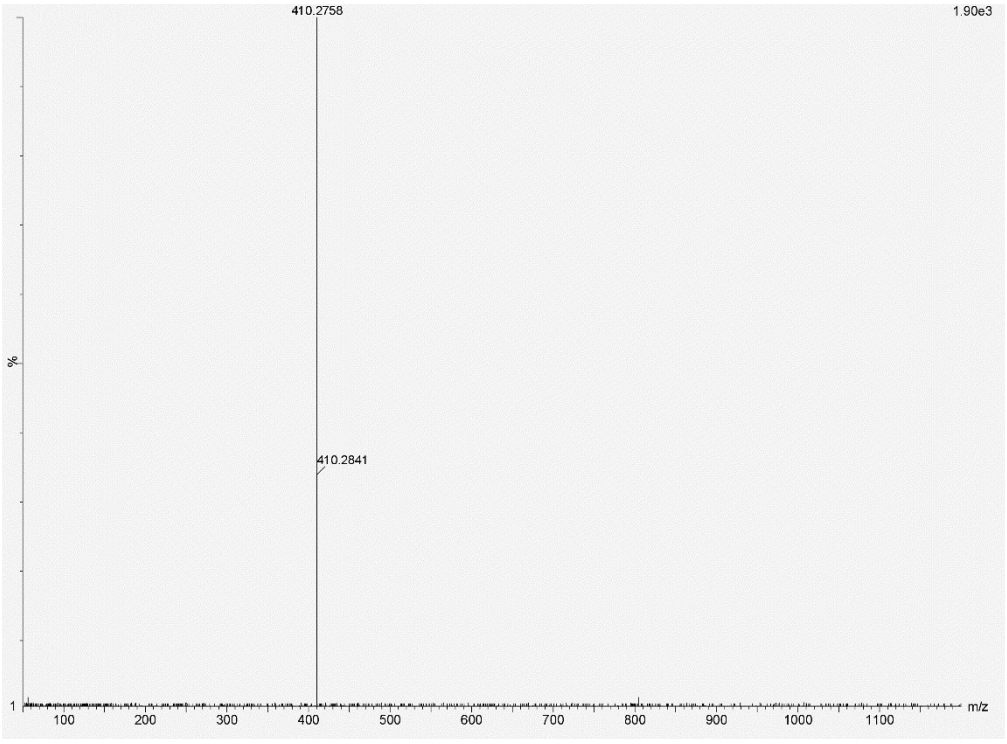


Fig. S6: HRMS spectrum of HBTC-Al^{3+} complex

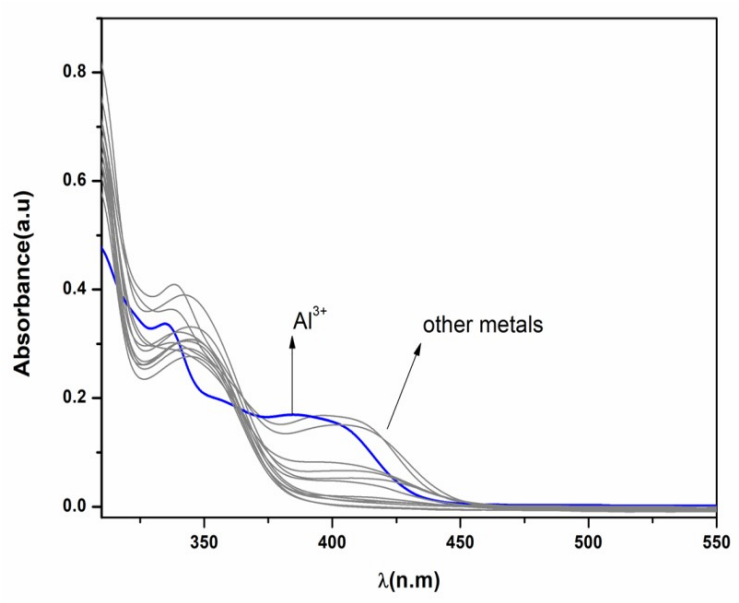


Fig. S7: UV-Vis spectra of HBTC (20 μM) in presence of 40 μM various metal ions i.e. Ca^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} and Hg^{2+} in ACN/H_2O (4/1, v/v, pH = 7.2) solution

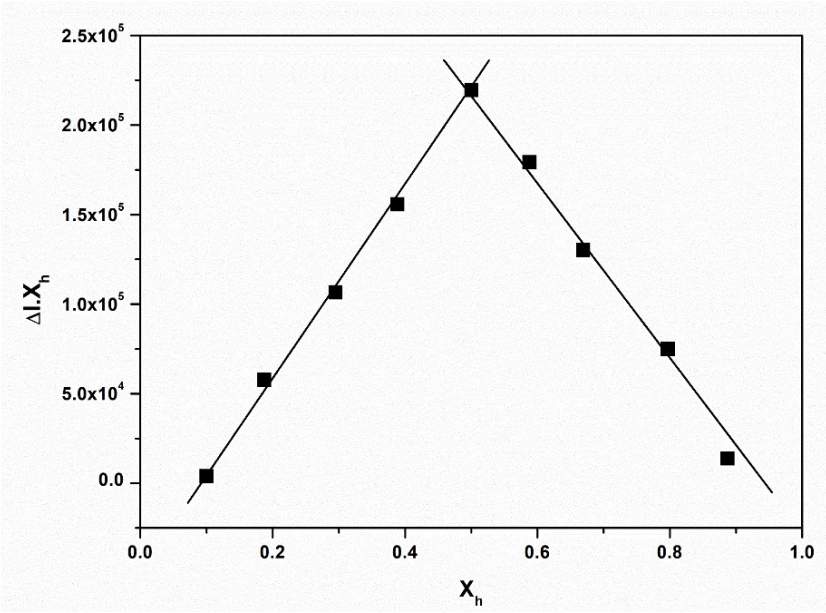


Fig. S8: Job's plot for HBTC (10 μM) with Al^{3+} in 10 mM HEPES buffer solution (pH = 7.2) (λ_{ex} = 384 nm); where X_h is the mole fraction of the host and ΔI indicates the change of emission intensity at 480 nm

Determination of association constant:

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

$$1/(F-F_o) = 1/\{K_a(F_{max}–F_o)\ [M^{n+}]^x\} + 1/[F_{max}-F_o]$$

Here F_0, F and F_{max} indicate the emission in absence of, at intermediate and at infinite concentration of metal ion respectively.

Plot of $1/\ [F-F_0]$ vs. $1/[Al^{3+}]$ gives a straight line indicating 1:1 complexation between HBTC and Al^{3+} where K_a is found to be $3.04\times10^4\ M^{-1}$ for HBTC.

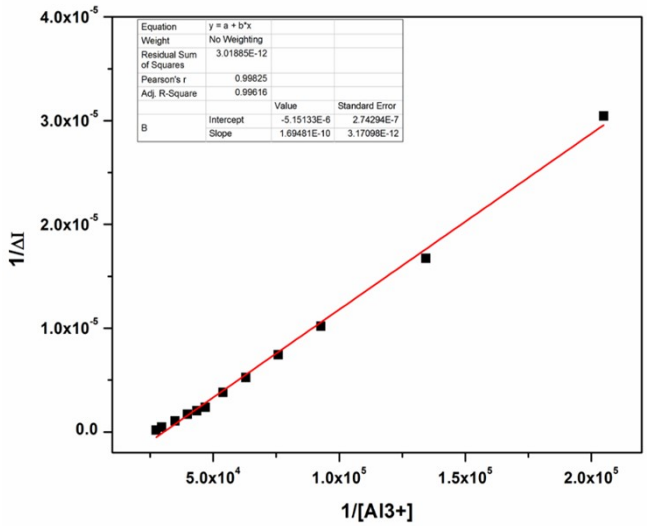


Fig. S9: Benesi–Hildebrand plot from fluorescence titration data of HBTC (20 μM) with Al^{3+}

Determination of limit of detection:

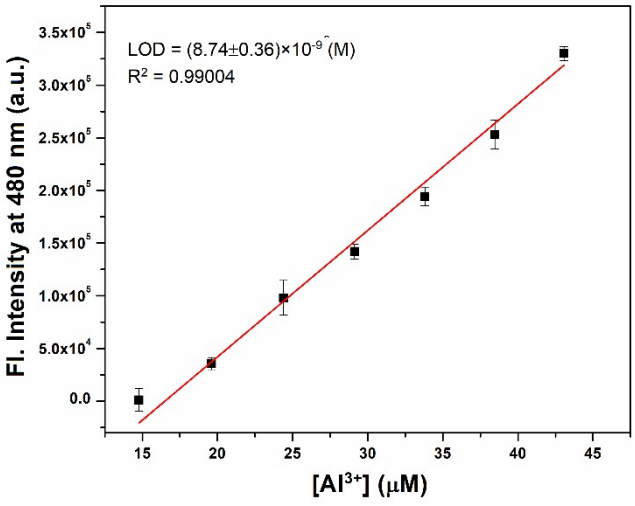


Fig. S10: Linear response curve of HBTC at 480 nm depending on the Al^{3+} concentration

Table S1: Fluorescence lifetime data

Compds.	Quantum yield *(φ)*	τ (ns)	k_r ($10^8 \times s^{-1}$)	k_{nr} ($10^8 \times s^{-1}$)
HBTC	0.001	1.42	0.008	7.04
HBTC-Al^{3+}	0.196	5.14	0.381	1.564

Radiative rate constant K_r and total non-radiative rate constant K_{nr} have been calculated using the equation $\tau^{-1} = K_r + K_{nr}$ and $K_r = \varphi_f / \tau$

Determination of fluorescence quantum yield:

The luminescence quantum yield was determined using coumarin 153 as reference dye. The compounds and the reference dye were excited at the same wavelength, maintaining nearly equal absorbance (~0.1), and the emission spectra were recorded. The area of the emission spectrum was integrated using the software available in the instrument and the quantum yield is calculated according to the following equation:

$$\phi_S/\phi_R = [A_S / A_R] \times [(Abs)_R / (Abs)_S] \times [n_S^2/n_R^2]$$

where, ϕ_S and ϕ_R are the luminescence quantum yields of the sample and reference, respectively. A_S and A_R are the area under the emission spectra of the sample and the reference respectively, $(Abs)_S$ and $(Abs)_R$ are the respective optical densities of the sample and the reference solution at the wavelength of excitation, and n_S and n_R are the values of refractive index for the respective solvent used for the sample and reference.

We calculated the quantum yields of HBTC and HBTC-Al^{3+} using the abovementioned equation; the values are found 0.001 and 0.196 respectively.

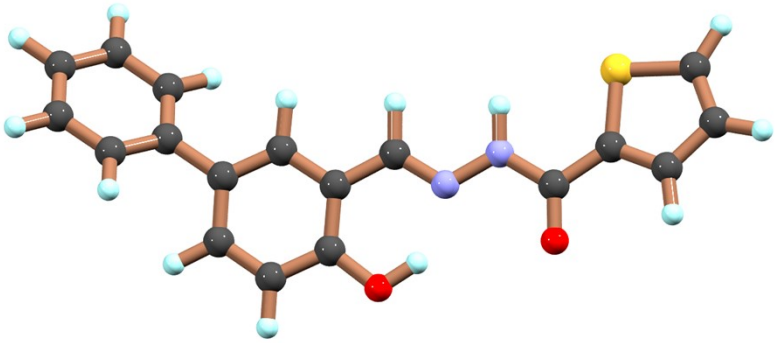


Fig. S11: Optimized structure of HBTC by DFT/B3LYP/6-31+G(d) method

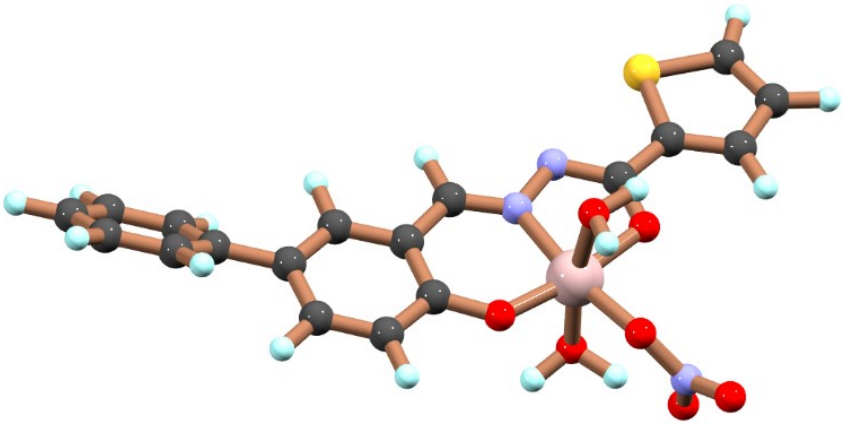


Fig. S12: Optimized structure of HBTC-Al^{3+} complex by DFT/B3LYP method

Table S2: Energy and compositions of some selected molecular orbitals of HBTC-Al^{3+} complex.

MO	Energy	% of composition	
		HBTC	Al
LUMO+5	0.46	99	1
LUMO+4	-0.07	100	0
LUMO+3	-0.61	100	0
LUMO+2	-0.84	100	0
LUMO+1	-1.38	98	2
LUMO	-2.01	100	0
HOMO	-5.52	99	1
HOMO-1	-6.06	100	0
HOMO-2	-6.83	100	0
HOMO-3	-6.91	100	0
HOMO-4	-6.97	100	0
HOMO-5	-7.05	100	0
HOMO-6	-7.32	98	2
HOMO-7	-7.75	99	1
HOMO-8	-7.76	100	0
HOMO-9	-8.16	97	3
HOMO-10	-8.6	100	0

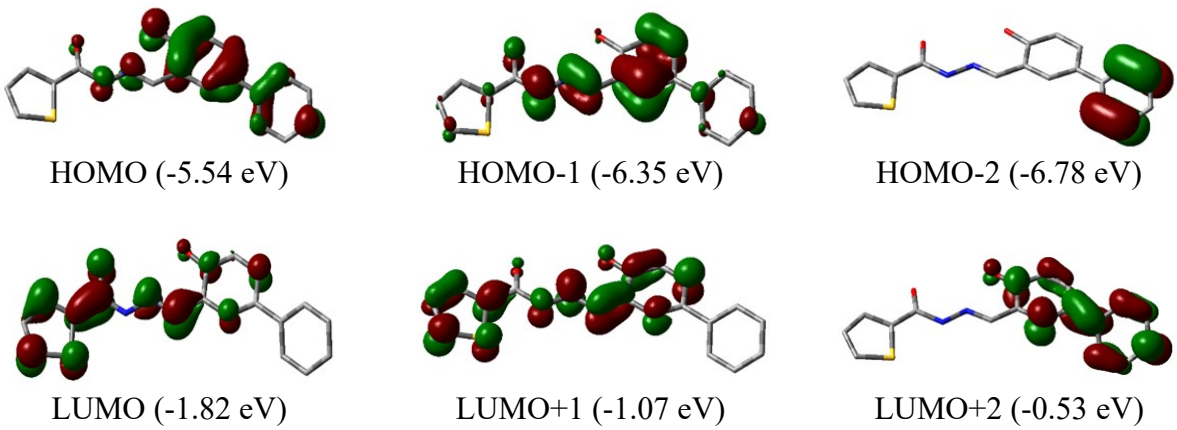


Fig. S13: Contour plot of some selected molecular orbitals of HBTC

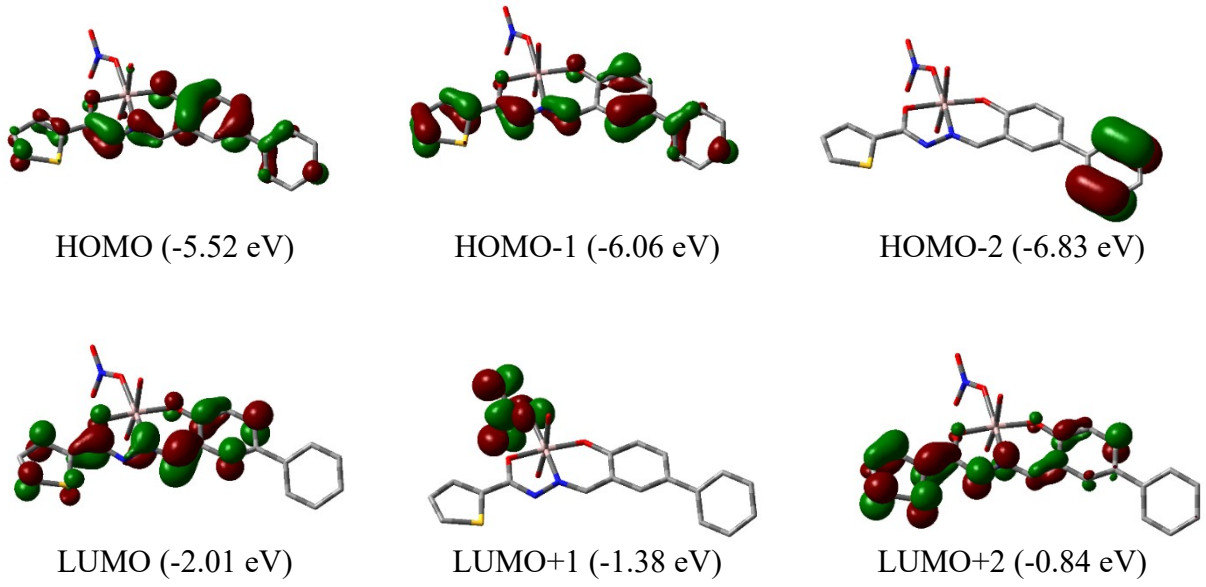


Fig. S14: Contour plot of some selected molecular orbitals of HBTC-Al^{3+}

Table S3: Vertical electronic excitations of HBTC and HBTC-Al^{3+} calculated by TDDFT/CPCM method

Compds.	**E (eV)**	**λ (nm)**	**Osc. Strength (f)**	**Transition**	**Character**
HBTC	4.0500	306.13	0.6054	(88%) HOMO-1→LUMO	$\pi \rightarrow \pi^*$
	3.4153	363.03	0.3381	(97%)HOMO→LUMO	$\pi \rightarrow \pi^*$
	4.5817	270.61	0.3756	(80%) HOMO→LUMO+2	$\pi \rightarrow \pi^*$
	4.9552	250.21	0.4244	(65%)HOMO-1→LUMO+1	$\pi \rightarrow \pi^*$
	4.2420	292.28	0.1656	(82%) HOMO→LUMO+1	$\pi \rightarrow \pi^*$
HBTC-Al^{3+}	3.0712	403.70	0.5377	(98%) HOMO→LUMO	L(π) → L(π^*)
	3.6314	341.42	0.6558	(97%) HOMO-1→LUMO	L(π) → L(π^*)
	4.8482	255.73	0.2353	(67%) HOMO-1→LUMO+2	L(π) → L(π^*)
	4.4416	279.14	0.2155	(58%) HOMO→LUMO+3	L(π) → L(π^*)
	5.6347	220.04	0.1212	(57%) HOMO-3→LUMO+2	L(π) → L(π^*)

Cell-bio imaging:

MTT assay

Triple negative breast cancer cell lines MDA MB-231 were evaluated for cytotoxicity with HBTC and HBTC-Al^{3+} complex. MB-231 cells were seeded in 96 well plate at a density of 5 × 10^3 cells per well followed by incubation at 37°C for 24 h at a 5% CO_2 atmosphere. The cells were separately treated with increasing doses of HBTC and HBTC-Al^{3+} complex concentrations (0, 1, 12.5, 25, 50, 100, 150, 200) μM, along with control for 24 h. Al^{3+} was treated in aqueous medium while the receptor HBTC was dissolved in DMSO but final concentration of DMSO was maintained below 1%. After 24h, methyl tetrazolium dye (MTT) (5 mg/mL) solution was added to each well (10 μl/well). The plates were incubated under dark condition in CO_2 atmosphere at 37°C for 3 h. Then 100 μL DMSO was added to each well to solubilize the formazan crystals and the plates were shaken briefly before quantification at 570 nm with the help of a multi-mode reader (SpectraMax i3x, Molecular devices). IC_{50} value of HBTC was determined by plotting a non-linear regression curve between the log of concentration of HBTC and O.D value at 570 nm. Untreated cells were served as 100% viable.

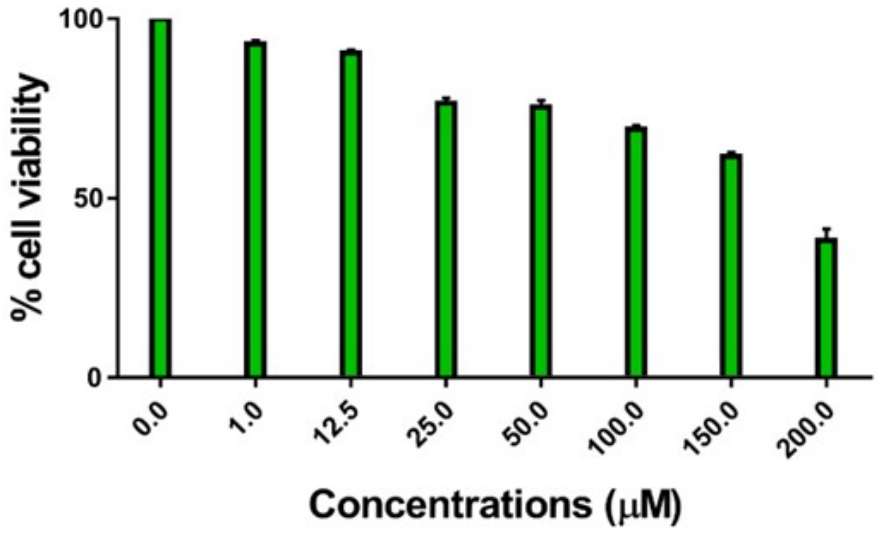


Fig. S15: MTT assay of HBTC on breast cancer cell lines (MDA-MB231)

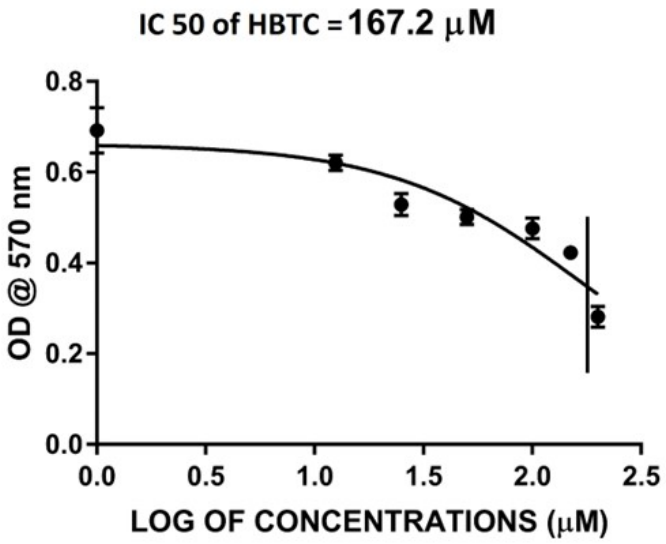
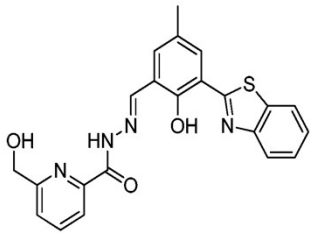
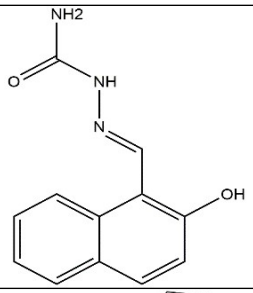
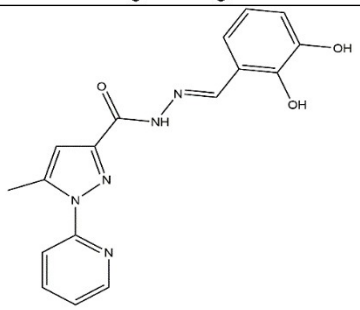
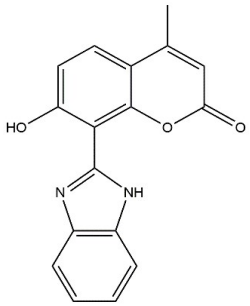
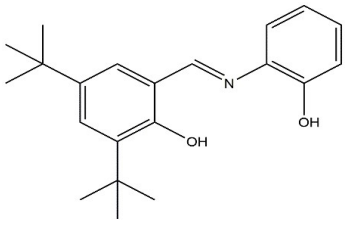
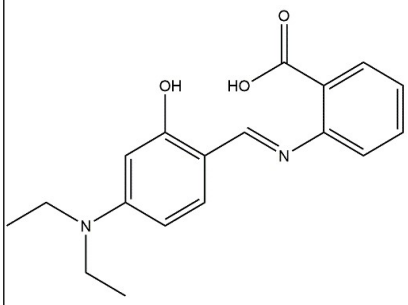
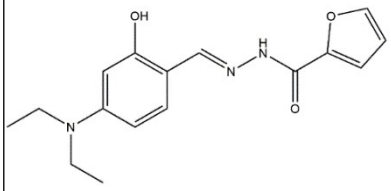
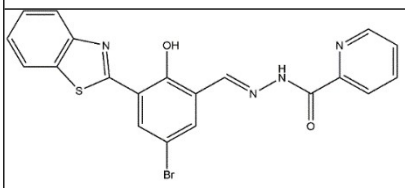
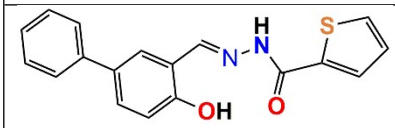


Fig. S16: IC_{50} dose of the receptor HBTC in breast cancer cell lines (MDA-MB231)

Cell bio imaging. In a six well plate containing 22×22 mm glass cover slips placed at the bottom of the well, the MDA MB 231 cells were seeded and allowed to adherer overnight. 15 μM of the HBTC as well as HBTC-Al^{3+} was added to the respective well containing cells along with a separate control. Then fixation of the cells was done with methanol and washed with 0.5% phosphate buffer saline tween (PBST) twice and then with 1 × PBS thrice. The cover slips were then mounted on a glass slide by glycerol and were observed under a fluorescence microscope (Leica DM4000 B, Germany) at 40x magnification.

Table S4: Comparison of solvent systems and limit of detection (LOD) of the receptor (HBTC) with some recently reported fluorescence organic probes for the detection of Al^{3+}

Chemosensors	Solvent system	LOD	References
	CH_3OH/H_2O (1/9, v/v, pH = 7.3, 25 °C)	6.72×10^{-8} M	*New J. Chem.*, 2015, **39**, 8582-8587
	$EtOH/H_2O$ (1:9, v/v, pH = 5.3)	6.75×10^{-8} M	*RSC Adv.*, 2020, **10**, 21629-21635
	MeOH–H_2O (9:1, v/v)	4.78 μM	*Anal. Methods*, 2021, **13**, 4266-4279
	Acetonitrile-water (2:1, v/v) of pH, 7.2 (HEPES buffer)	0.62 μM	*J. Mol. Struct.*, 2022, **1250**, 131870
	HEPES buffer (1% EtOH, pH = 7.04).	2.94×10^{-8} M	*Dalton Trans.,* 2015, **44**, 11352-11359

	Water:CH_3OH =1:1 (v/v) pH 7.0	2.9×10^{-7} M	*New J. Chem.*, 2016, **40**, 171-178
	bis–Trisbuffer solution, pH 7.0	0.19 mM	*New J. Chem.,* 2017, **41**, 15590-15600
	MeOH/water (v/v,1:1, pH 6.0).	31.2 nM	*Anal. Methods*, 2019, **11**, 5598-5606.
	Aetonitrile/H_2O (4/1, v/v, pH = 7.2)	0.5×10^{-9} M	*Present work*