Supporting Information

A novel AIE fluorescent probe for the monitor of aluminum ion in living cells and zebrafish

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I. Experimental Section

Cell culture

BEL-7402 were obtained from China Center for Type Culture Collection (Wuhan, China). BEL-7402 cells were cultured in minimal essential medium(MEM) with 10% (V/V) heat-inactivated fetal bovine serum (FBS) (Gibco BRL, Grand Island, NY, USA), streptomycin/penicillin (100 μg/mL) in a humidified incubator of 5% CO_2 and 95% air at 37 °C.

MTT assay

Cell cytotoxicity was evaluated by MTT assay. Cells were cultivated in a 96-well plate until 50-70% confluence, and then incubated with different concentrations of probe BTD (0-5 μM) for 24 h. Then 20 μL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ mL) was added for 4 h at 37 °C. After removing MTT, 150 μL DMSO was added. Absorbance was measured at 570 nm with a Varioskan Flash (Thermo Fisher Scientific, Waltham, MA, USA). All experiments were repeated three times, and the data were presented as the percentage of control cells.

Quantum yield measurements

Quinine sulfate (Φ = 0.54 in 0.1 M H_2SO_4) was used as quantum yield reference. The QY_s were determined by comparing the integrated fluorescence intensity and the absorbance value of the probe solution samples with those of the references. The absorbances (less than 0.05 at the excitation wavelength) at maximal absorbance for probe BTD-Al^{3+} and Fluorescein were recorded. The slope method was used to calculate the QY_s using the equation:

$QY_u = QY_s (m_u/m_s) (n_u/n_s)^2$.

Where QY is the quantum yield, m is the slope determined by the curves. And n is the refractive index (1.33 for 0.1 M H_2SO_4 aqueous solution at room temperature). The subscript "s" refers to the standards and "u" refers to the unknown samples. A series of concentrations for the references and the required samples were measured to obtain the slopes.

$QY_{Al^{3+}} = 0.157$

Calculation of the detection limit (LOD)

$$LOD = 3\sigma/S$$

$$\sigma = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{n-1}}$$

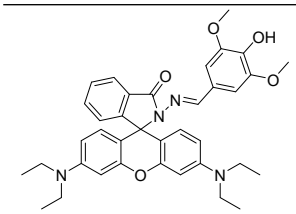
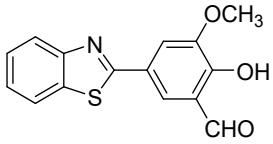
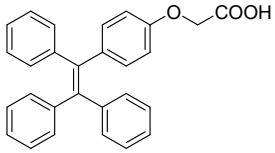
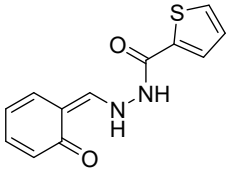
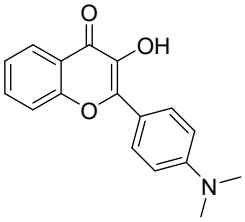
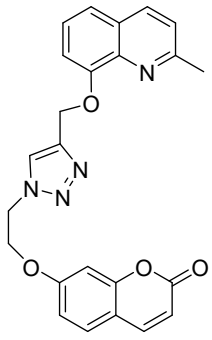
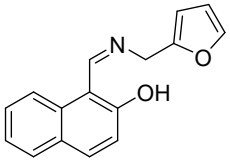
σ: the standard deviation of the blank solution.

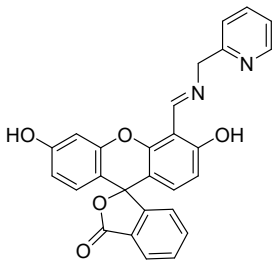
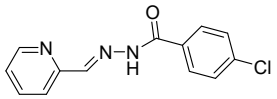
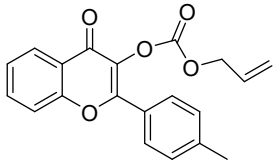
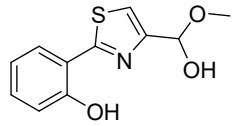
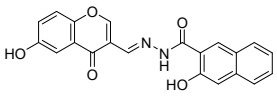
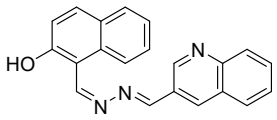
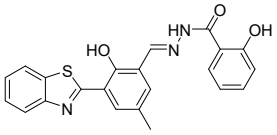
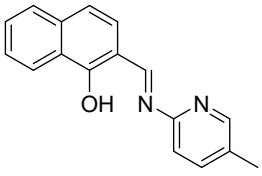
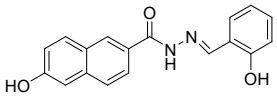
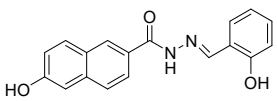
$\bar{x}$ is the mean of the blank measures; x_i is the values of blank measures; n is the number of tested blank measure (n = 10)

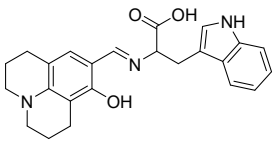
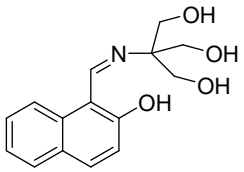
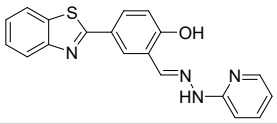
S: the slope of the linear calibration plot between the fluorescence emission intensity and the concentration of Al^{3+} respectively.

Table S1. Summary of the properties of representative fluorescent probes for selective detection of Al^{3+}

probe	Tested Media	DL	Linear	Quantum	Recovery	Ref.

			range	yield		
	EtOH/HEPES (1/5)	10.6 nM	1.0-10.0 μM	---	F^-	1
	DMF/H_2O (1:1)	99 nM	0-12 μM	---	---	2
	H_2O	21.6 nM	0.1-5 μM	---	EDTA	3
	PBS/DMSO (3:1)	42.6 nM	0-40 μM	0.27	---	4
	H_2O	50 nM	0-10 μM	0.10-0.35	---	5
	EtOH/H_2O (2:3)	24 nM	0.2-10 μM	0.41	---	6
	DMSO/H_2O (1:100)	603 nM	0-8 μM	0.64	---	7

	Tris-HCl aqueous buffer	92 nM	0-10 μM	0.09	F^-	[8]
	EtOH	200 nM	1-15 μM	---	---	[9]
	CH_3CN/H_2O (1:3)	750 nM	0-20 μM	---	---	[10]
	MeOH/H_2O (19:1)	924 nM	0-30 μM	0.1974	F^-	[11][11]
	EtOH/H_2O (3:1)	10 nM	5.0–50 mM	---	F^-	[12]
	MeOH/HEPES (8:2)	3.10 μM	1-10 μM	---	---	[13]
	DMF/H_2O (1:1)	14.2 μM	0-5 μM	---	EDTA	[14]
	DMSO/H_2O (8:2)	430 nM	0-10 μM	---	---	[15]
	C_2H_5OH	75.5 nM	3-6.5 μM	0.3246	EDTA	[16]
	MeOH/H_2O (8:2)	2.73 nM	0.04-0.14 μM	---	---	[17]

	bis-tris buffer solution	640 nM	0-250 μM	---	---	[18]
	C_2H_5OH/H_2O (1:1)	11.9 nM	0-40 μM	---	---	[19]
	H_2O	3.25 nM	1.0-10.0 μM	0.157	Lys	This Work

II. Supplementary Spectra

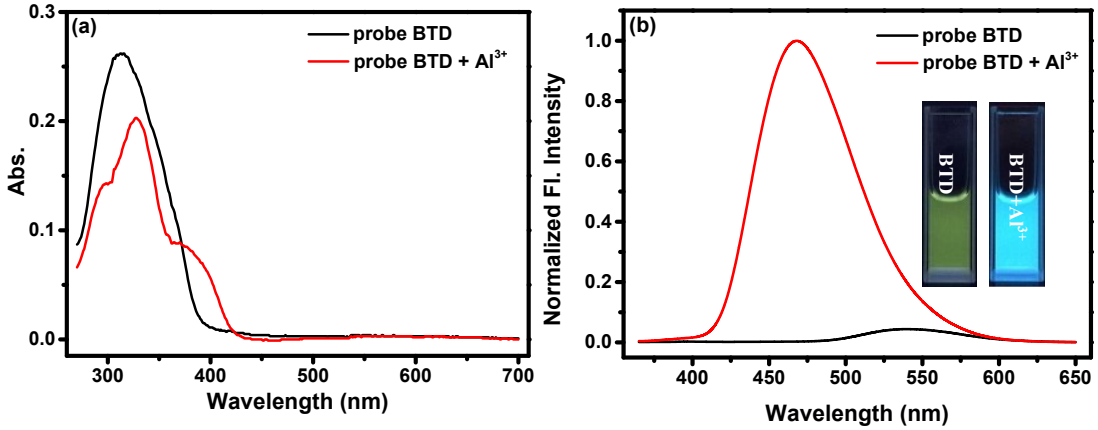


Fig. S1 (a) Absorption spectra of probe BTD (10 μM) in the absence and presence of Al^{3+} (100 μM) in pure water (containing 1% DMSO). (b) The corresponding normalized fluorescence spectra. λ_{ex} = 345 nm, slit (nm): 2.5/2.5. The inset photos show the color changes of probe BTD (10 μM) in the absence and presence of 10 equiv. of Al^{3+} under UV lamp at 365 nm, respectively.

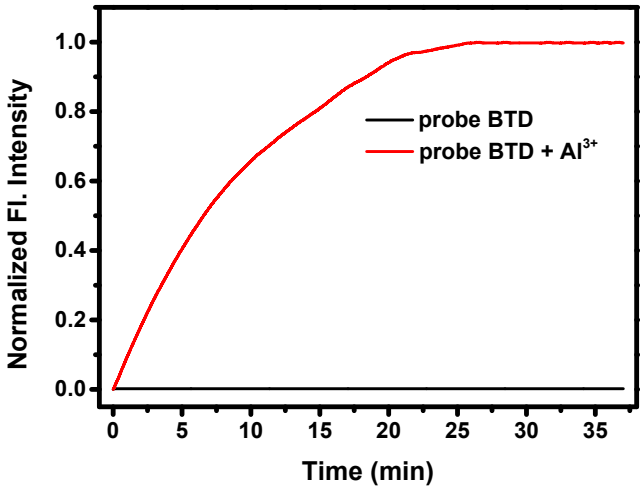


Fig. S2 Time-dependent normalized fluorescence intensity changes at 470 nm of probe BTD (10 μM) in the absence and presence of Al^{3+} (100 μM) in pure water (containing 1% DMSO). λ_{ex} = 345 nm, slit (nm): 2.5/2.5.

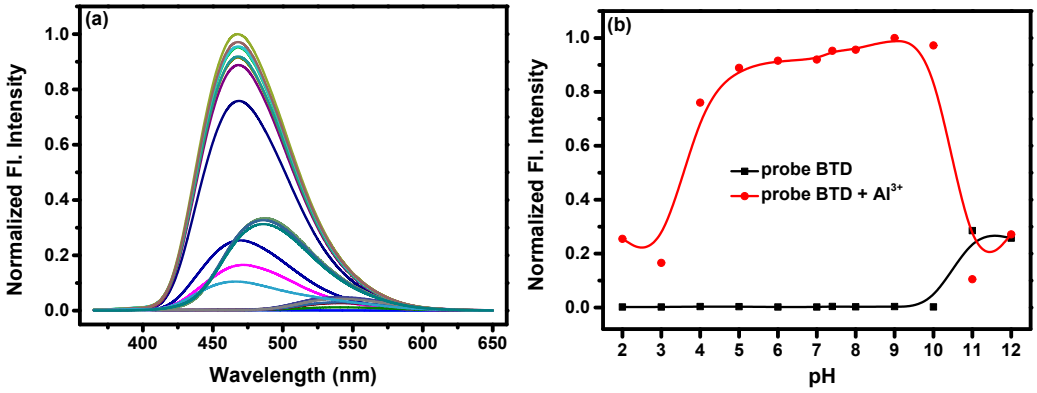


Fig. S3 (a) Normalized fluorescence spectra of probe BTD (10 μM) in the absence and presence of Al^{3+} in pure water (containing 1% DMSO) under different pH. (b) The corresponding normalized fluorescence intensity changes at 470 nm. λ_{ex} = 345 nm, slit (nm): 2.5/2.5.

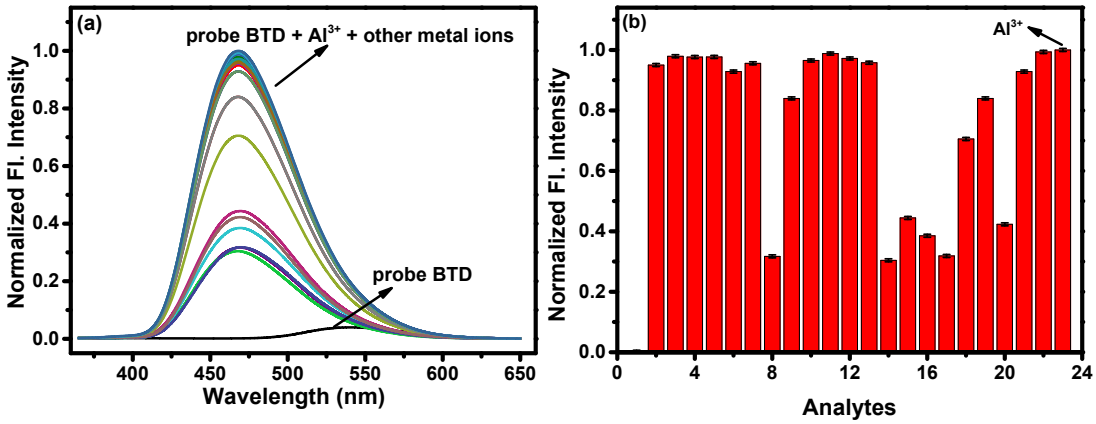


Fig. S4 (a) Normalized fluorescence spectra of the probe BTD (10 μM) to other metal ions (100 μM) and Al^{3+} (100 μM) in pure water (containing 1% DMSO). (b) The corresponding normalized fluorescence intensity changes at 470 nm. Other metal ions including: 1. None; 2. Na^{+}; 3. K^{+}; 4. Li^{+}; 5. Ag^{+}; 6. Ba^{2+}; 7. Ca^{2+}; 8. Co^{2+}; 9. Zn^{2+}; 10. Mg^{2+}; 11. Mn^{2+}; 12. Pb^{2+}; 13. Cd^{2+}; 14. Cu^{2+}; 15. Sn^{2+}; 16. Fe^{2+}; 17. Fe^{3+}; 18. Cr^{3+}; 19. Bi^{3+}; 20. Ni^{2+}; 21. Zr^{4+}; 22. Ge^{4+}; 23. Al^{3+}. λ_{ex} = 345 nm, slit (nm): 2.5/2.5.

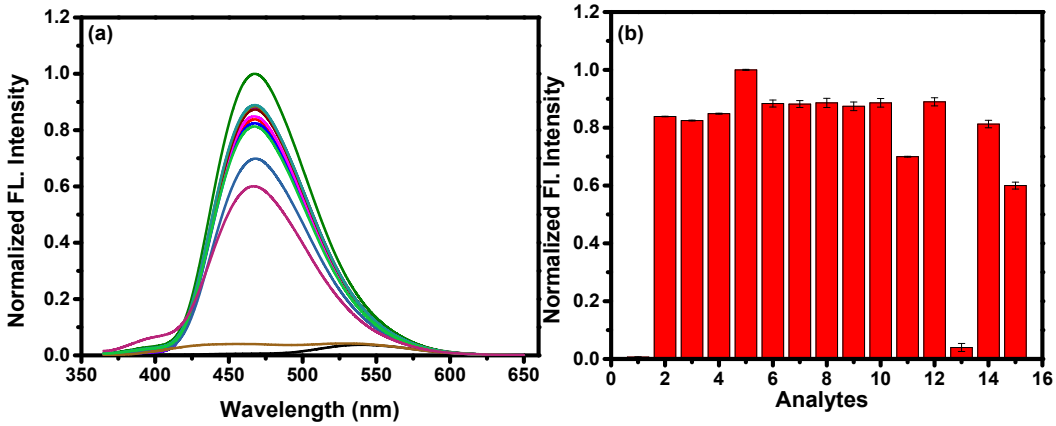


Fig. S5 (a) Normalized fluorescence spectra of probe BTD (10 μM)-Al^{3+} (100 μM) upon addition of various analytes (200 μM) in pure water (containing 1% DMSO) at 37 °C. (b) The corresponding normalized fluorescence intensity changes at 470 nm. Analytes: 1. probe BTD; 2. probe BTD and Al^{3+}; 3. Cys; 4. Hcy; 5. GSH; 6. Na_2S; 7. Na_2SO_3; 8. Gly; 9. Glu; 10. Met; 11. Asp; 12. Thr; 13. Lys; 14. Try; 15. His. λ_{ex} = 345 nm, slit(nm): 2.5/2.5.

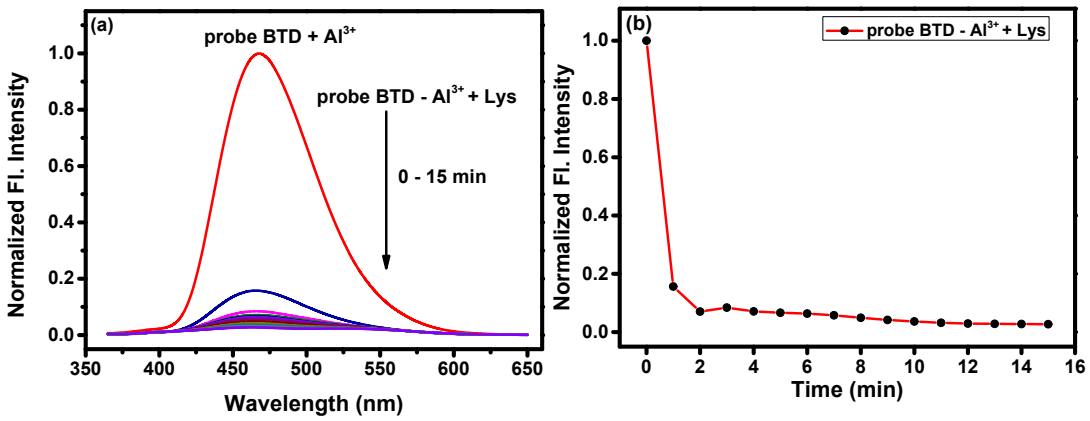


Fig. S6 (a) Time dependent normalized fluorescence intensity spectra of probe BTD (10 μM) and Al^{3+} (100 μM) in the presence of Lys (500 μM) in pure water (containing 1% DMSO). (b) The corresponding normalized time-dependent fluorescence intensity changes at 470 nm. λ_{ex} = 345 nm, slit (nm): 2.5/2.5.

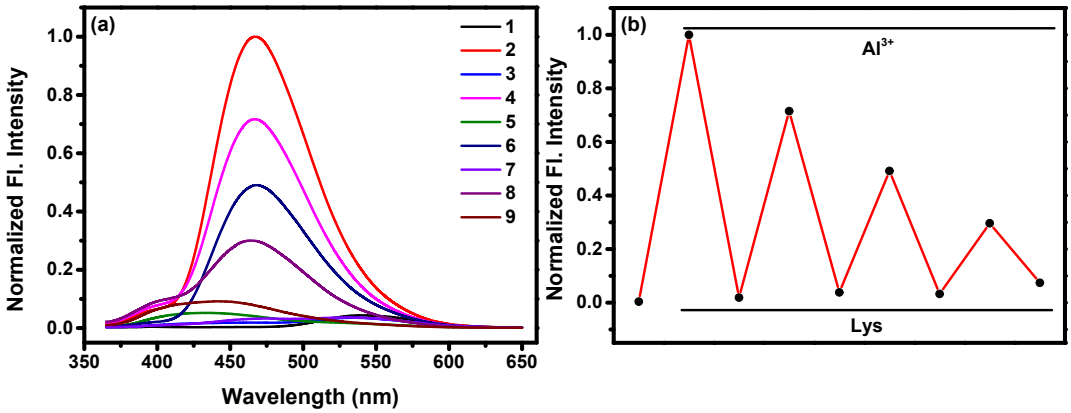


Fig. S7 (a) Change of normalized fluorescence of probe BTD (10 μM) after alternate addition of Al^{3+} (50 μM) and Lys (100 μM) in pure water (containing 1% DMSO) at 37 °C. Line 1 (probe BTD), line 2 (probe BTD + Al^{3+}), line 3 (probe BTD + Al^{3+} + Lys), line 4 (probe BTD + Al^{3+} + Lys + Al^{3+}), line 5 (probe BTD + Al^{3+} + Lys + Al^{3+} + Lys), line 6 (probe BTD + Al^{3+} + Lys + Al^{3+} + Lys + Al^{3+}), line 7 (probe BTD + Al^{3+} + Lys + Al^{3+} + Lys + Al^{3+} + Lys), line 8 (probe BTD + Al^{3+} + Lys + Al^{3+} + Lys + Al^{3+} + Lys + Al^{3+}), line 9 (probe BTD + Al^{3+} + Lys + Al^{3+} + Lys + Al^{3+} + Lys + Al^{3+} + Lys). (b) Reversibility of the reactions of the probe with Al^{3+} and Lys. Each spectrum was recorded after addition of the Al^{3+}/Lys and shaked well. λ_{ex} = 345 nm, λ_{em} = 470 nm, slit (nm): 2.5/2.5.

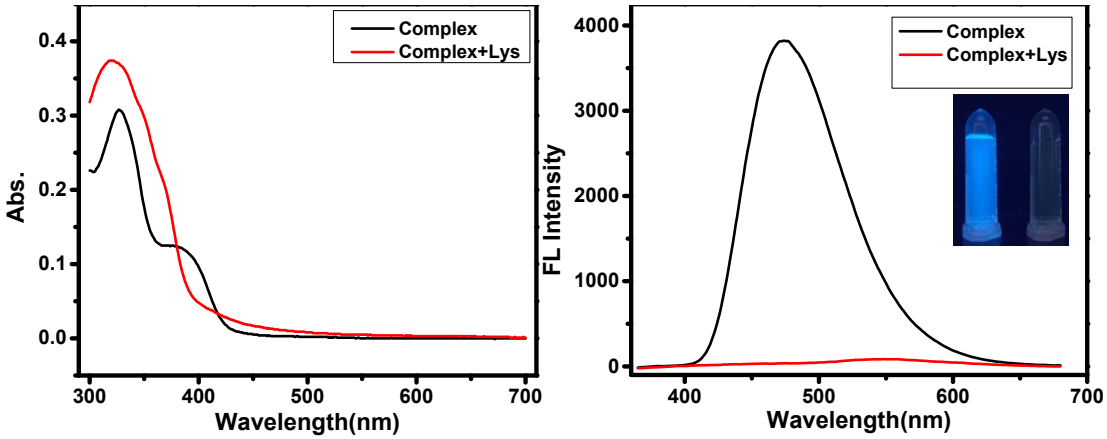


Fig. S8 (a) Absorption spectra of complex in the absence and presence of Lys in pure water (containing 1% DMSO). (b) The corresponding fluorescence spectra. λ_{ex} = 345 nm, slit (nm): 2.5/2.5. The inset photos show the color changes of complex in the absence and presence of 10 equiv. of Lys under UV lamp at 365 nm.

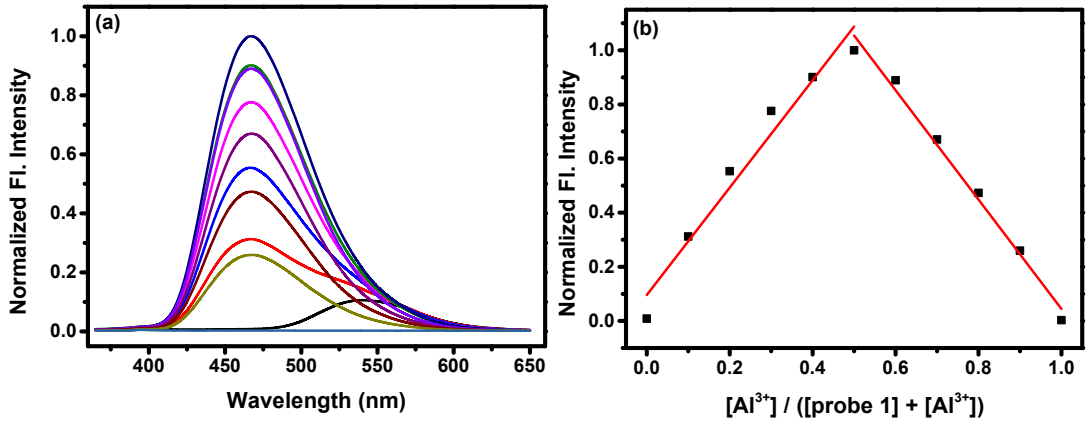


Fig. S9 (a) Normalized fluorescence intensity spectra for the binding of probe BTD with Al^{3+} in pure water (containing 1% DMSO). (b) The corresponding normalized fluorescence intensity at 470 nm was plotted as a function of the molar ratio of [Al^{3+}] / ([probe BTD] + [Al^{3+}]). λ_{ex} = 345 nm, slit (nm): 2.5/2.5.

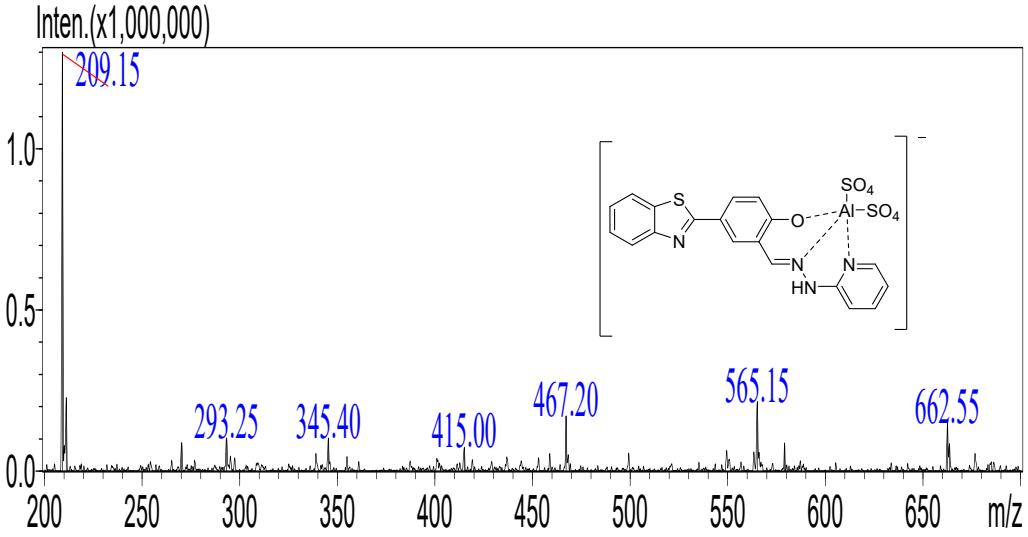


Fig. S10 Mass spectra of probe BTD after treated with Al^{3+}.

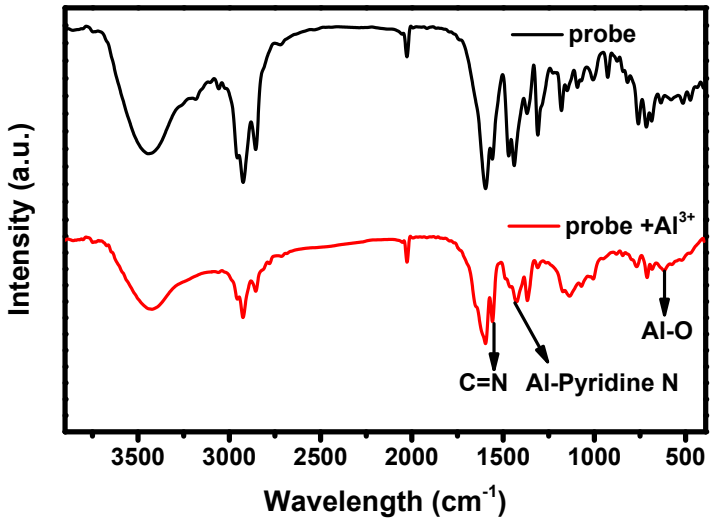


Fig. S11 FT-IR spectra of probe BTD and probe BTD with Al^{3+}.

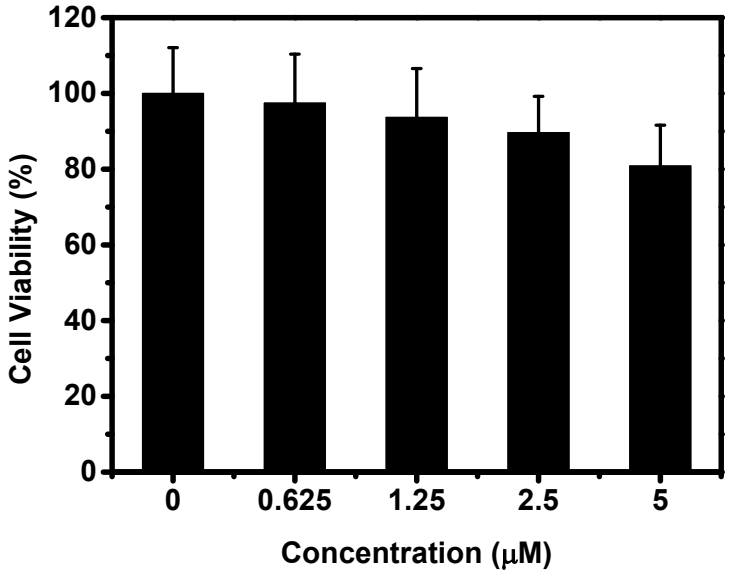


Fig. S12 MTT assay for the survival rate of living BEL-7402 cells treated with various concentrations of probe BTD for 24 h.

III. ^{1}H NMR and ^{13}C NMR

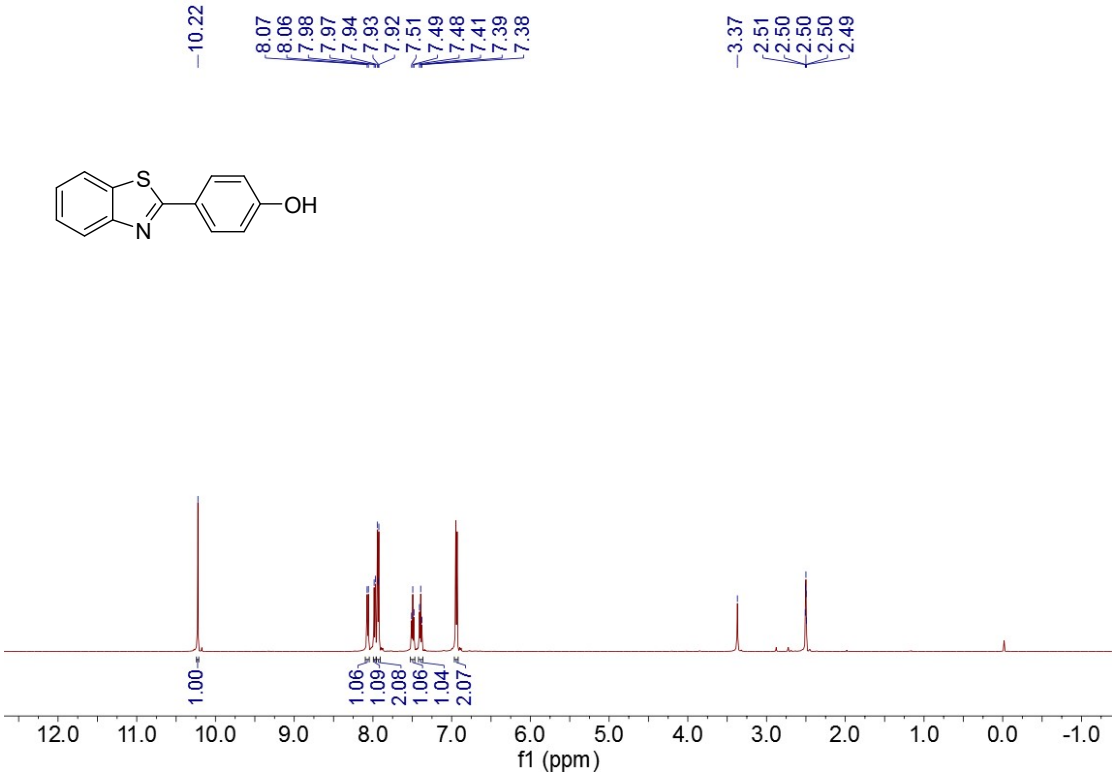


Fig. S13 ^{1}H NMR spectrum of compound 1 in DMSO-d_6.

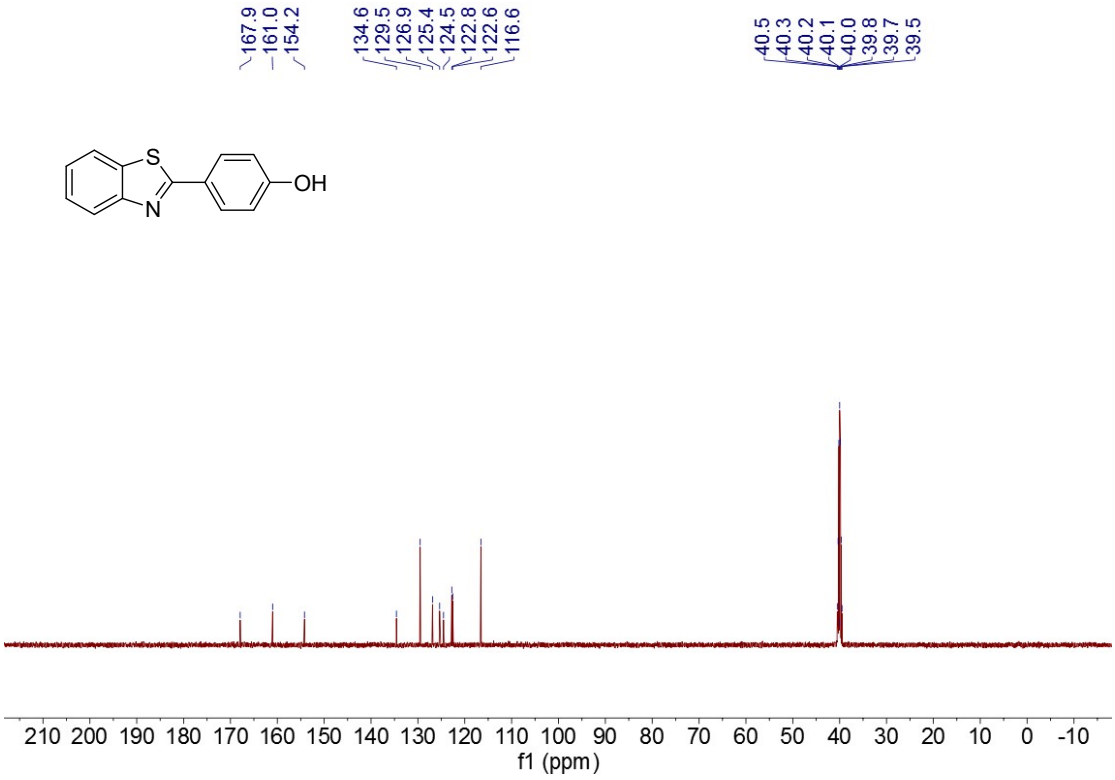


Fig. S14 ^{13}C NMR spectrum of compound 1 in DMSO-d_6.

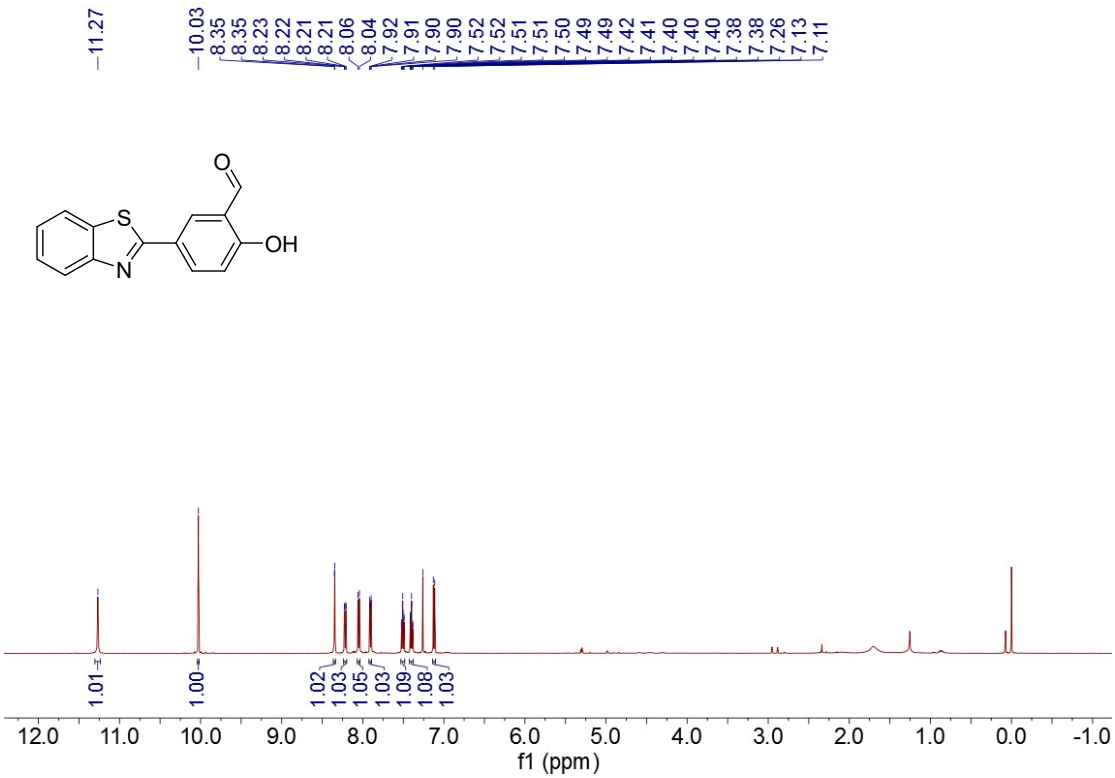


Fig. S15 ^{1}H NMR spectrum of compound 2 in $CDCl_3$.

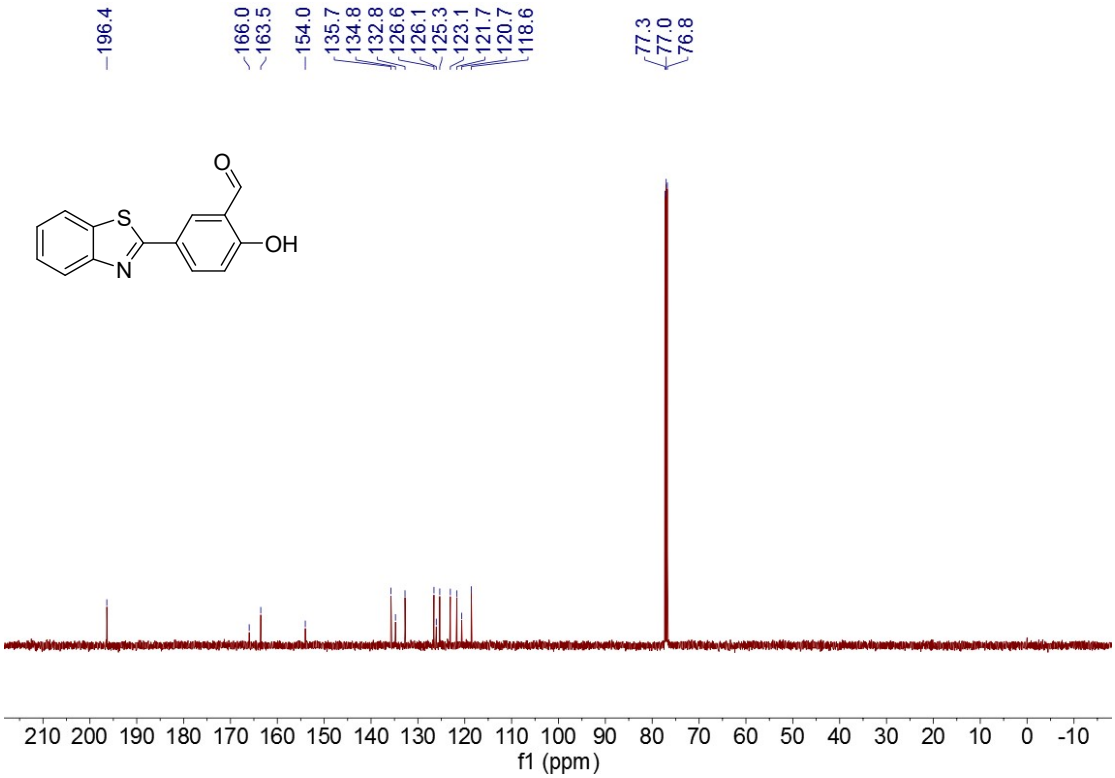


Fig. S16 ^{13}C NMR spectrum of compound 2 in $CDCl_3$.

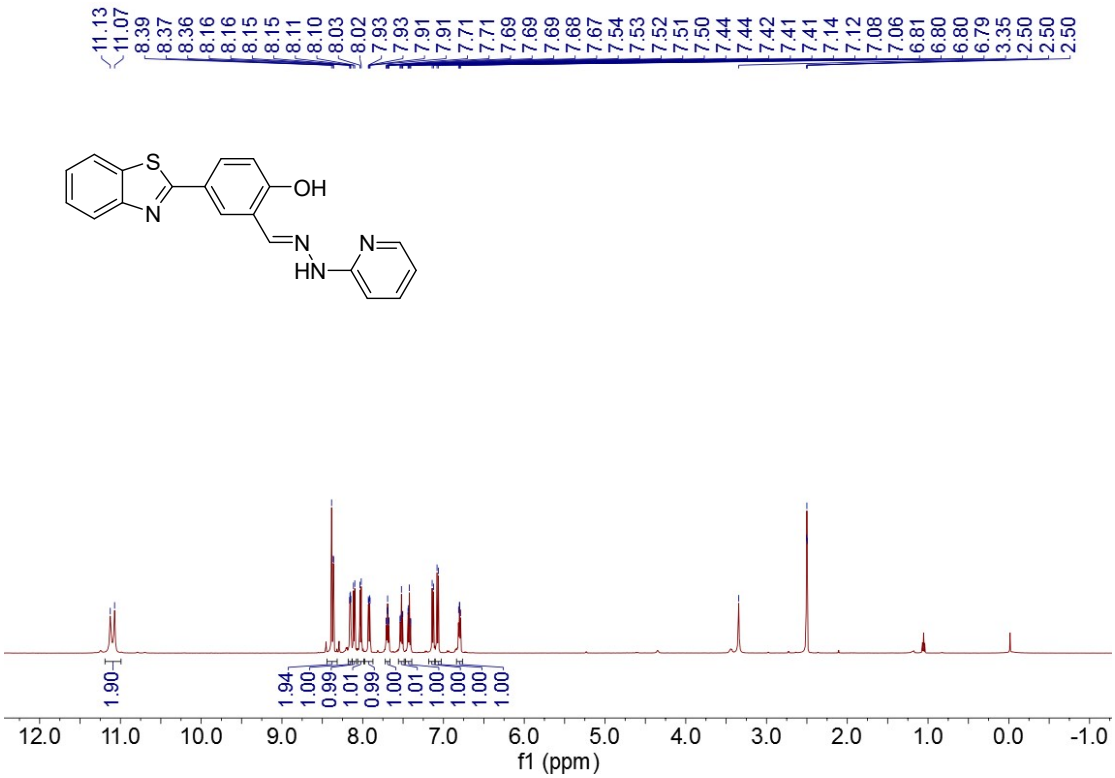


Fig. S17 ^{1}H NMR spectrum of probe BTD in DMSO-d_6.

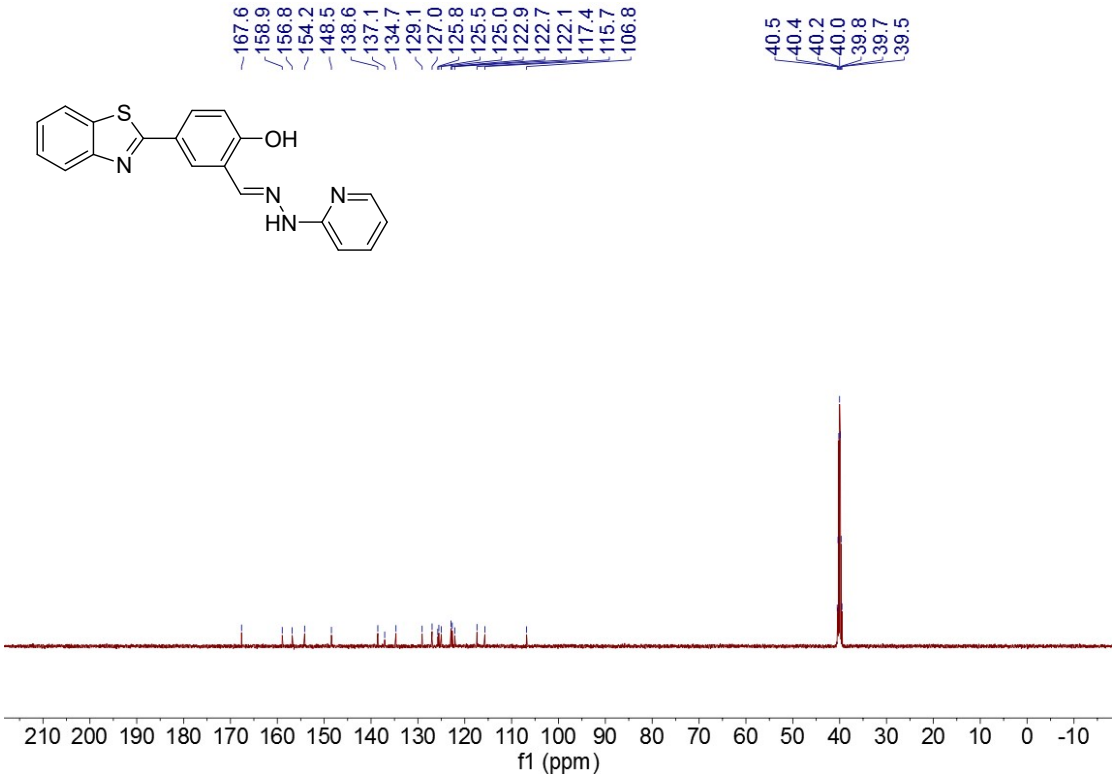


Fig. S18 ^{13}C NMR spectrum of probe BTD in DMSO-d_6.

IV. Reference

1. T. Liu, X. Wan and Y. Yao, *Sens. Actuators B Chem.*, 2018, 254, 1094-1100.
2. Z.-N. Tian, D.-Q. Wu, X.-J. Sun, T.-T. Liu and Z.-Y. Xing, *Int. J. Mol. Sci.*, 2019, 20.
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