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A dual responsive probe based on bromo substituted salicylhydrazone moiety for the colorimetric detection of Cd^{2+} ions and fluorometric detection of F^- ions: Applications in live cell imaging

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ABSTRACT

A new fluorimetric and colorimetric dual-mode probe, 4-bromo-2-(hydrazonomethyl) phenol (**BHP**) has been synthesized and successfully utilized for the recognition of Cd^{2+}/F^- ions in DMSO/H_2O (9:1, v/v) system. The probe displays dual channel of detection via fluorescence enhancement and colorimetric changes upon binding with F^- and Cd^{2+} ions respectively. The Job's plot analysis, ESI-MS studies, Density Functional Theoretical (DFT) calculations, ^1H NMR and ^{19}F NMR titration results were confirmed and highly supported the 1:1 binding stoichiometry of the probe was complexed with Cd^{2+}/F^- ions. Furthermore, intracellular detection of F^- ions in HeLa cells and fluorescence imaging analysis in Zebrafish embryos results of the probe **BHP** might be used to reveal their potential applications in a biological living system.

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1. Determination of Binding constant from Benesi-Hildebrand method

The binding constant of Cd^{2+}/F^- with **BHP** has been calculated by using UV-visible and Fluorescence spectrometer respectively. The fixed concentration of **BHP** was used throughout the titration and any given concentration of F^- with **BHP** gives good linear relationship. The binding constant value of F^- with **BHP** was determined by using Benesi-Hildebrand eqn[1].

$$\mathbf{1/ (A-A_o) = 1/ \{K (A_{max}-A_o) [F^-]\} + 1/ [A_{max}-A_o]}$$

Here, A_o is the absorbance of **BHP** without F^- ions, A is the absorbance of **BHP** with F^- ions (at given concentration), A_{max} is the absorbance of **BHP** with F^- ions (in saturated concentration). K is the association constant (M^{-1}). The association constant (K) could be determined from the slope of plot 1/ $(A-A_o)$ VS 1/ $[F^-]$.

Further, the binding constant values of F^- with **BHP** have been calculated by using a fluorescence method. The concentration of **BHP** was kept constant throughout the titration and varying the concentration of the F^- gives good linear relationship. The binding constant value of F^- with **BHP** was calculated from by using modified Benesi - Hildebrand equation[2].

$$\mathbf{1/I-I_{min} = 1/ I_{max}-I_{min} + (1/K[C]) (1/ I_{max}-I_{min})}$$

Here, I_{min} is the emission intensity of **BHP** without F^- , I is the emission intensity of **BHP** with any given concentration of F^-, I_{max} is the emission intensity of **BHP** at a concentration of complete saturation, K is the binding constant, [C] is the concentration of **BHP**. The value of K has been determined from the slope of the plot $(I_{max}-I_{min}) / (I-I_{min})$ VS 1/[C] for **BHP**- F^-.

2. Determination of Limit of Detection (LOD)

The limit of detection was calculated using this equation[3].

DL = CL× CT

where **CL** is the Conc. of Ligand, **CT** is the Conc. of Titrant at which changes are observed.

The LOD values of F^- ions; DL = 5×10^{-6} M× 0.1×10^{-5}M = 0 .05 nM.

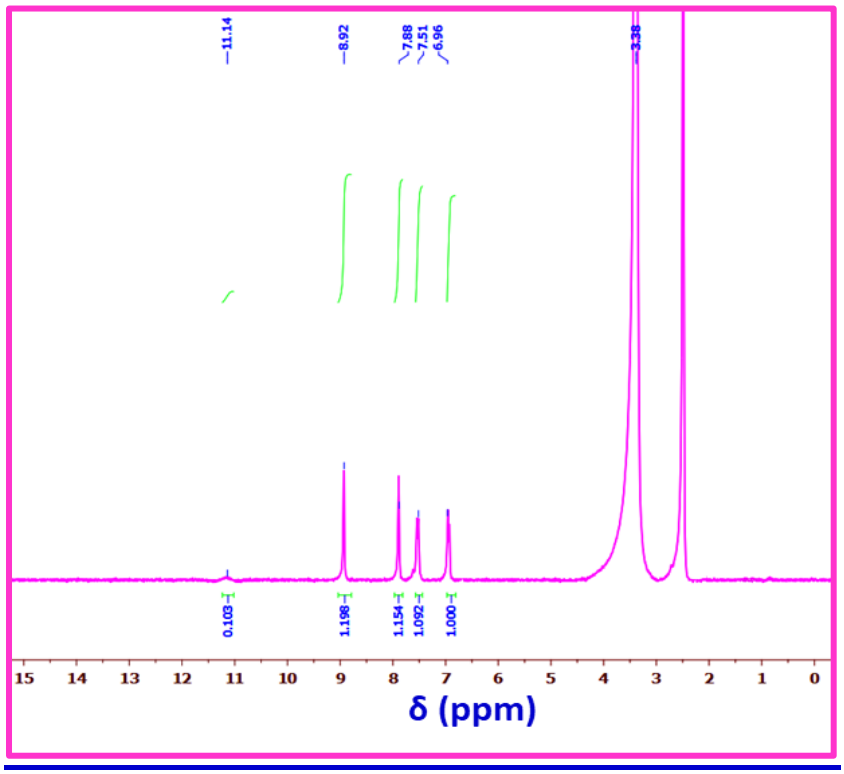

Figure S1. ^{1}H NMR spectrum of BHP

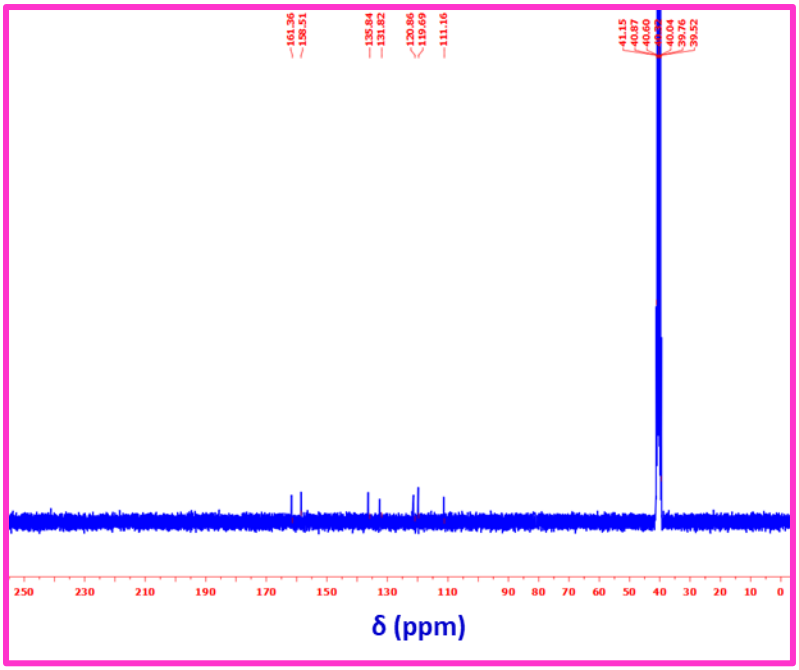

Figure S2. ^{13}C NMR spectrum of BHP

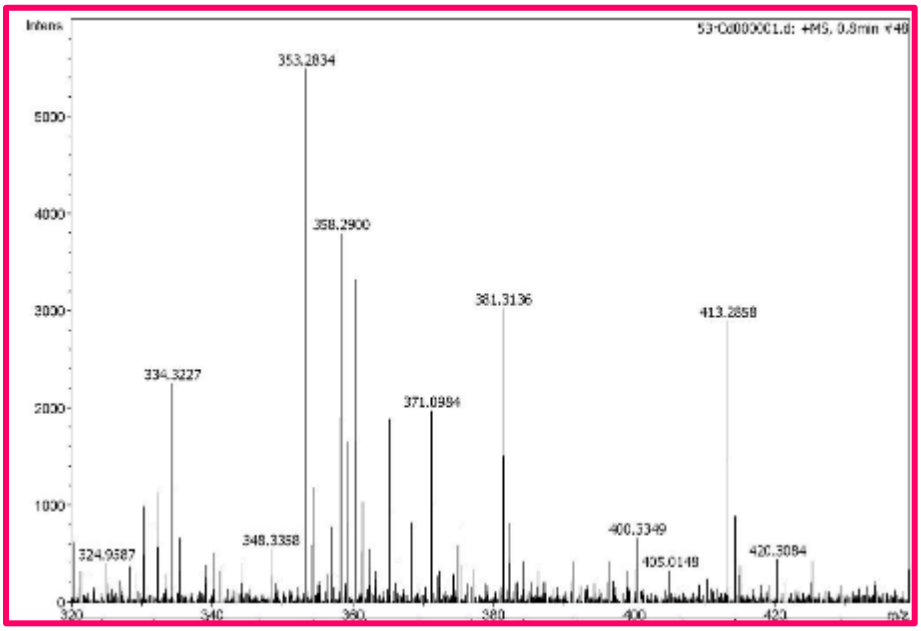

Figure S3. ESI-MS spectrum of BHP-Cd^{2+}

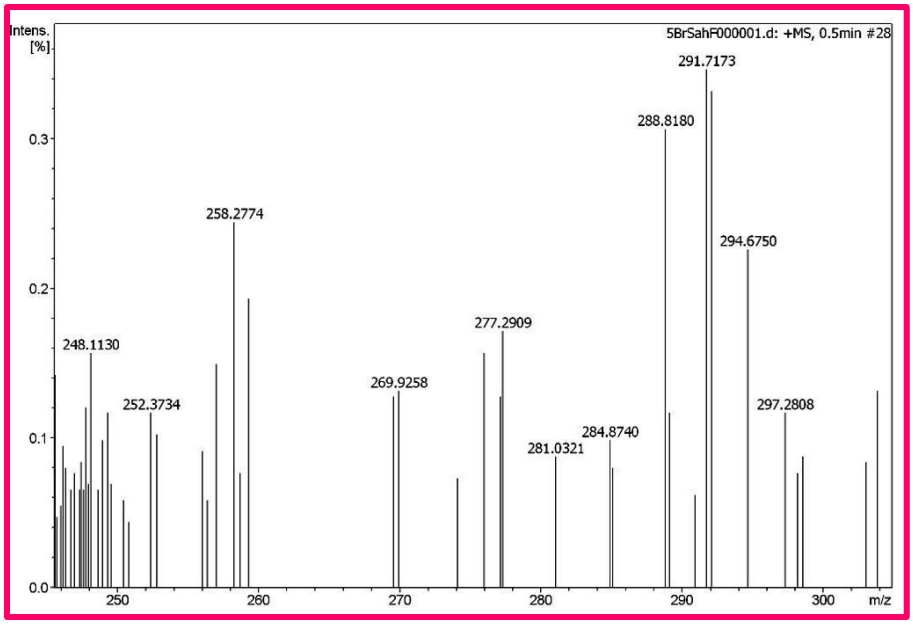

Figure S4. ESI-MS spectrum of BHP-F^{-}

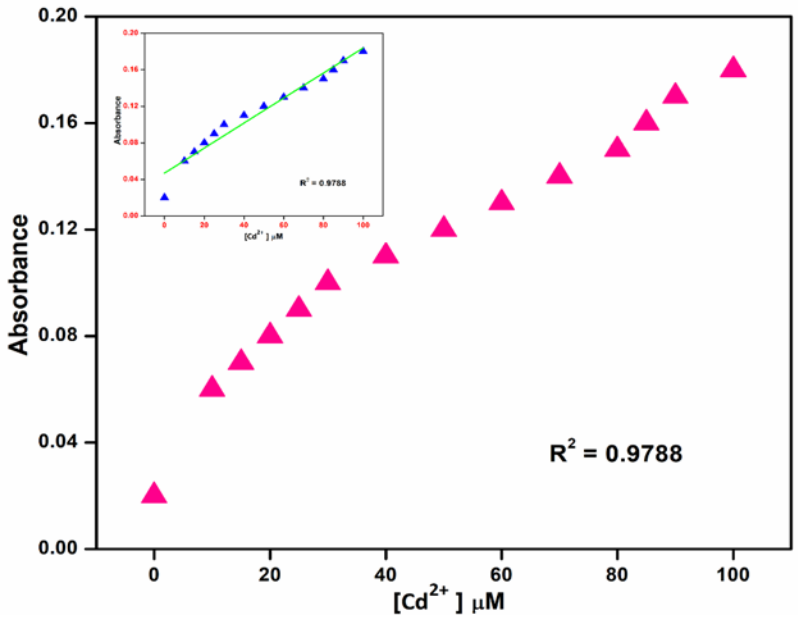

Figure S5. Linear fit analysis of probe BHP vs Cd^{2+} ion by UV-visible spectroscopy (Insert Figure is fitted linear plot). Absorbance measured at 472 nm.

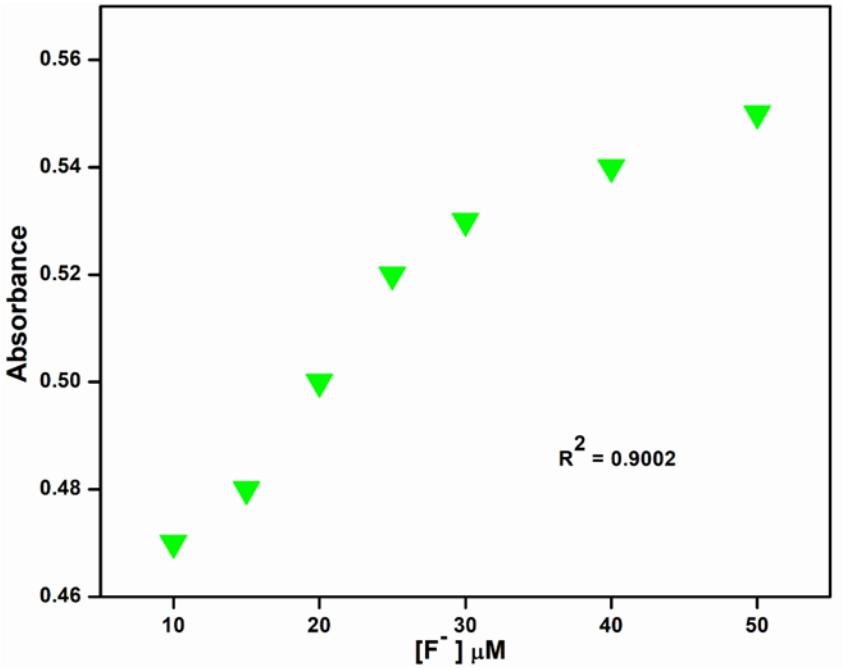

Figure S6. Linear fit analysis of probe BHP vs F^- ion by UV-visible spectroscopy (Insert Figure is fitted linear plot). Absorbance measured at 482 nm.

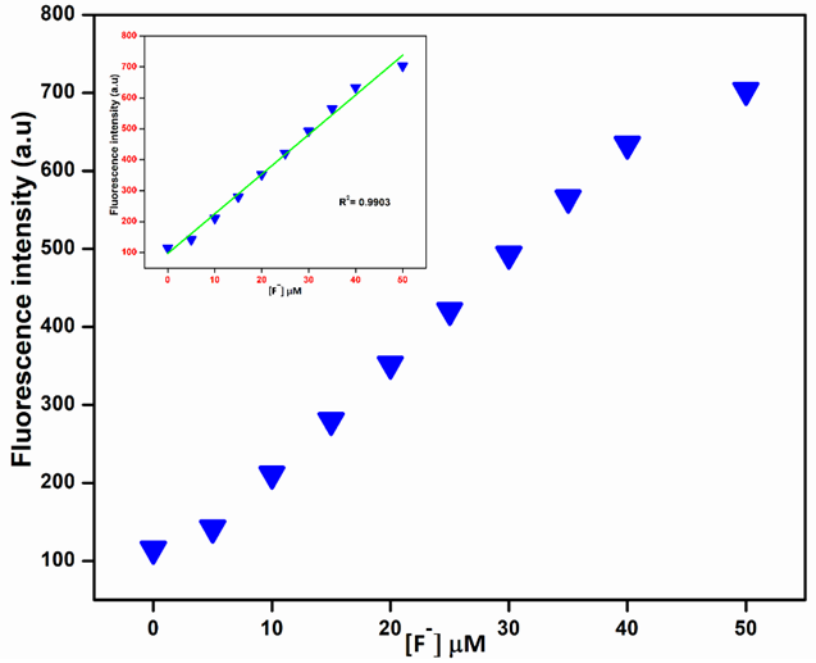

Figure S7. Linear fit analysis of probe BHP vs F^- ion by Fluorescence spectroscopy (Insert Figure is fitted linear plot). Fluorescence measured at 603 nm.

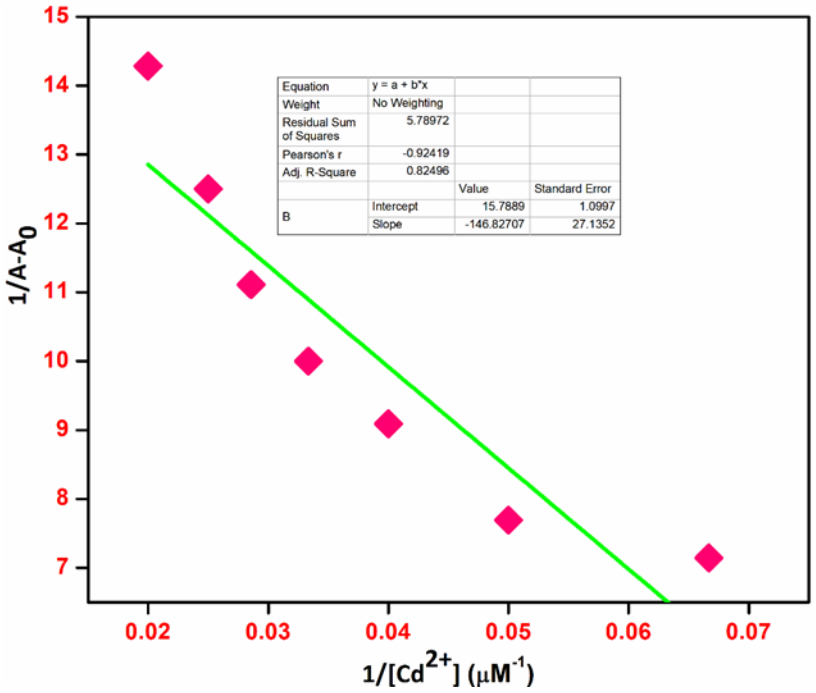

Figure S8. Gauging of binding constant value of Cd^{2+} ion with BHP by B-H plot from UV-visible titration profile. Absorbance measured at 472 nm.

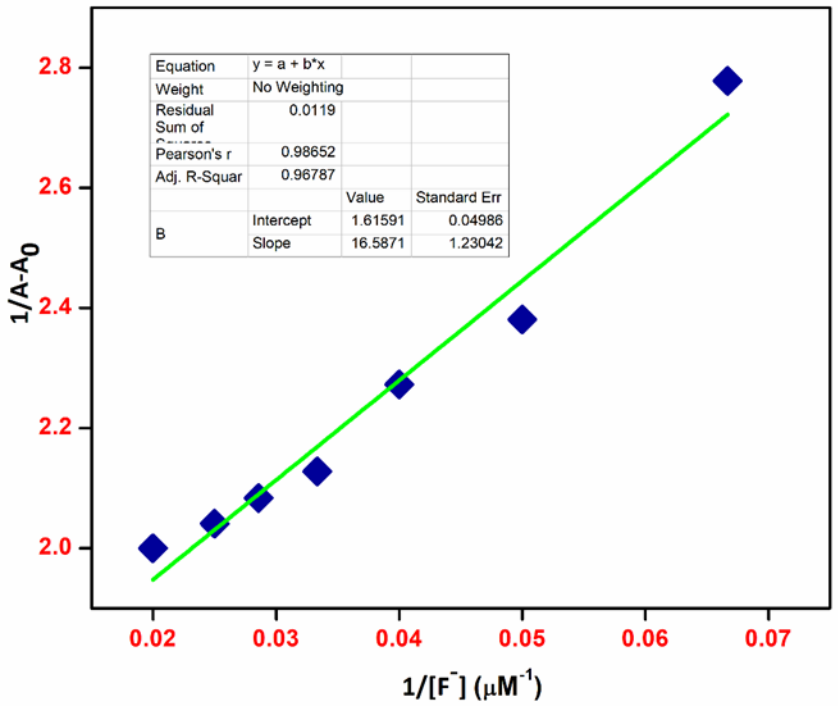

Figure S9. Gauging of binding constant value of F^- ion with BHP by B-H plot from UV-visible titration profile. Absorbance measured at 482 nm.

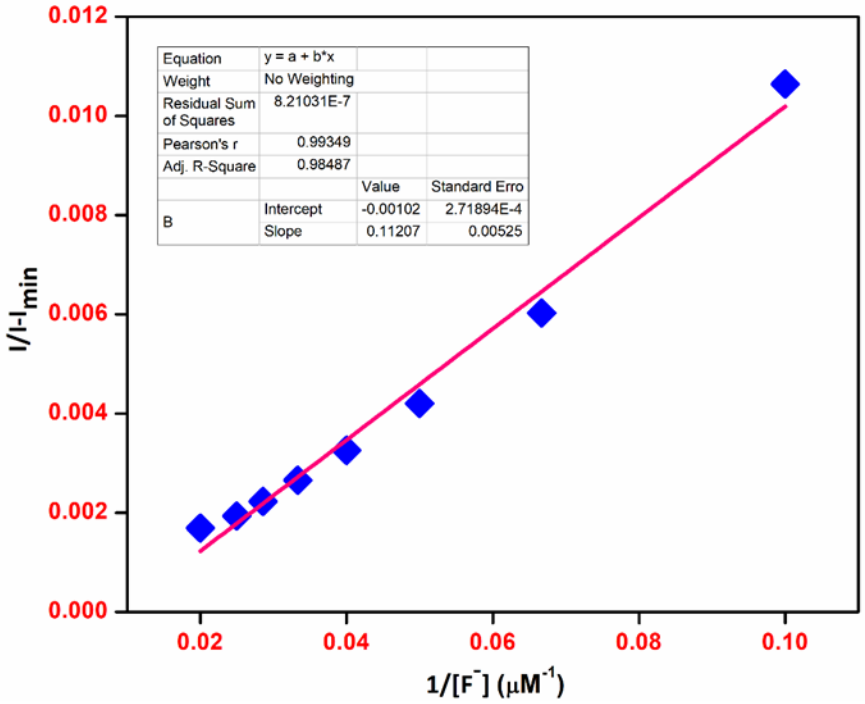

Figure S10. Gauging of binding constant value of F^- ion with BHP by B-H plot from fluorescence titration profile. Fluorescence measured at 603 nm.

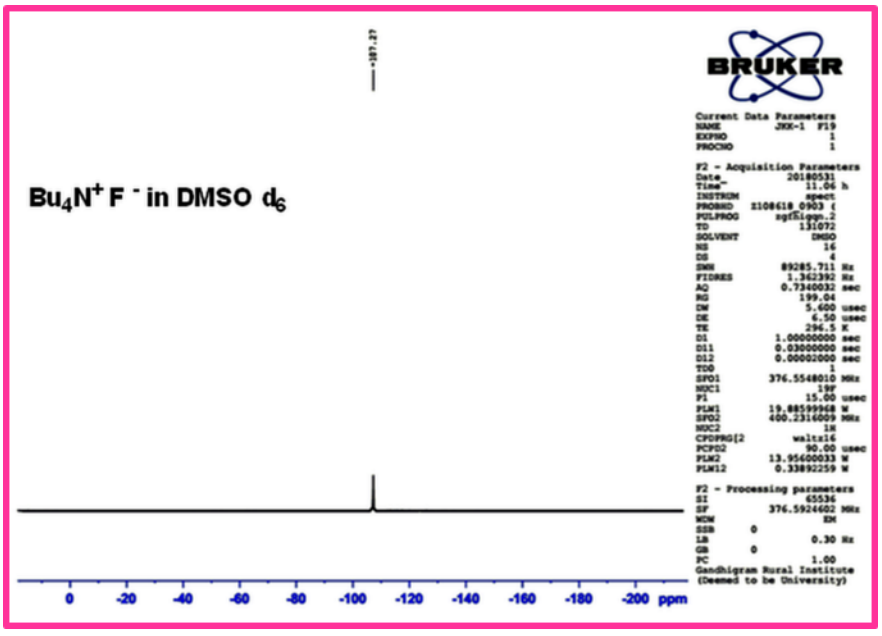

Figure S11. ^{9}F NMR spectrum of Tetrabutylammonium fluoride (TBAF) in DMSO-*d*$_6$

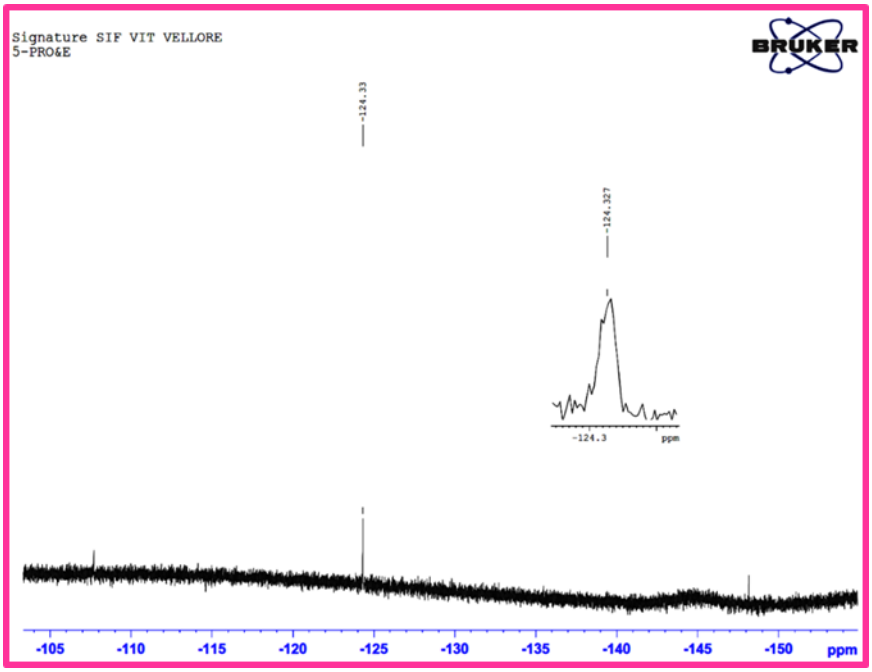

Figure S12. ^{19}F NMR spectrum of BHP-F^- complex in DMSO-d_6

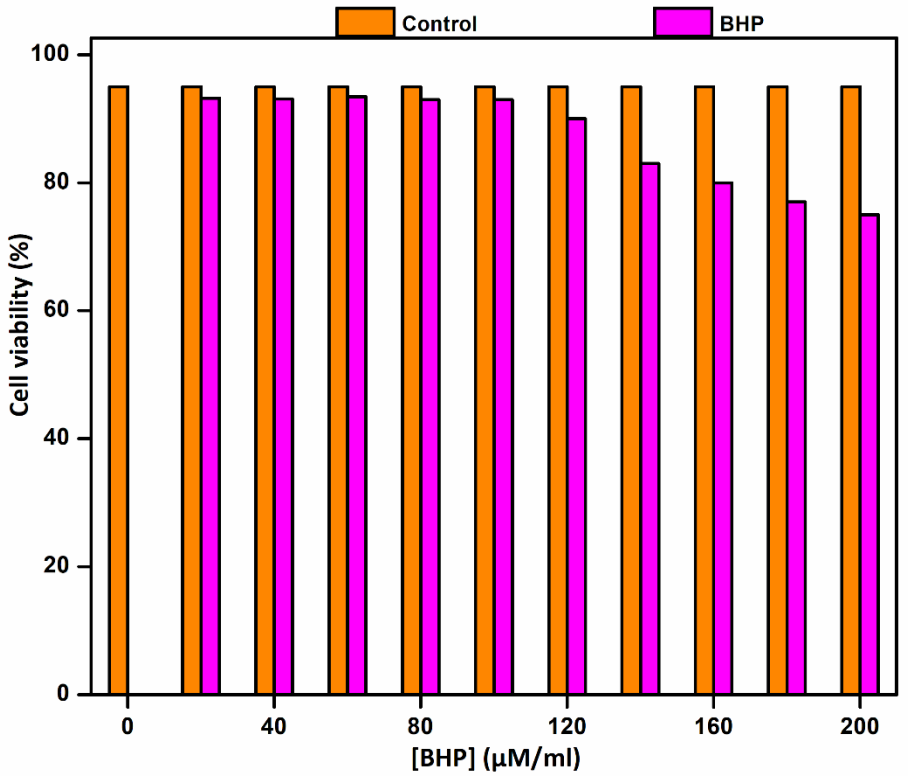

Figure S13. Cytotoxicity analysis of BHP VS HeLa cells

Table S1: Previous reports of chemoreceptors for sensing F^- ion and their LOD

S.No	Chemosensors	Fluorescence Responses	LOD	Application	References
1.	Acridinedione derivative	Ratiometric	9.29×10^{-5} M	-	Iqbal, N.et al, *RSC Adv*, **2018**, *8*, 1993.
2.	Indole derivatives	Turn-off	1.8×10^{-6} M	Cell and Zebrafish Imaging	Naha, S. et al, *Chemistry Select*, **2019**, *4*, 2912.
3.	Ferrocene-triazole-derivatives	Turn-on	2.98×10^{-6} M	-	Hosseinzadeh, R. et al, *Chemistry Select*, **2019**, *4*, 3914.
4.	Fluorenone derivatives	Colorimetric	2.31×10^{-6} M	-	Mohar, M, et al, *Chemistry Select*, **2019**, *4*, 8061.
5.	Pyrrole derivatives	Turn-on	1.58×10^{-5}M	-	Tao, T. et al, *Dyes Pigm*, **2019**, *170*, 107638.
6.	Boronic acid derivatives	Colorimetric	3.45×10^{-7} M	-	Wu, H., et al, *Spectrohim. Acta, Part A*, **2019**, *214*, 393.
7.	Imidazole derivatives	Turn-on	0.02 mg/ L or 20 ppb	-	A. Das, et al, *Spectrochim. Acta, Part A*, **2019**, *220*,117099.
8.	Acylhydrazone derivatives	Turn-on	8.31×10^{-7} M	-	B.Bai, et al, *Soft Matter*, **2019**, *15*, 6690.
9.	**BHP**	Colorimetric and Turn-on	0.05 nM	HeLa Cell/ Zebrafish Imaging	Present work

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