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# A bisphenol based fluorescence chemosensor for the selective detection of Zn<sup>2+</sup> and PPi ions and its bioluminescence imaging

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#### Abstract

A bisphenol based fluorescence "turn-on" chemosensor 4,4'-(propane-2,2-diyl)bis(2-((E)-(2-(benzo[d]thiazol-2-yl)hydrazineyldene)methyl)phenol) (BHMP) has been synthesized and its sensing behavior was tested towards various ionic species. The chemo-sensing behavior of BHMP has been established through absorption, fluorescence, NMR, and mass spectroscopic techniques. The probe BHMP selectively detects zinc ions over other metal ions and the resulting BHMP+Zn<sup>2+</sup> ensemble serves as a secondary probe for the detection of pyrophosphate (PPi) anion specifically over other anions. The spectroscopic studies reveal the fluorescence enhancement of BHMP in association with  $Zn^{2+}$  ions was quenched in the presence of pyrophosphate (PPi) anions. A probable mechanism of this selective sensing behavior was described on the basis of "OFF-ON-OFF" strategy for detection of both cations and anions. Moreover, the biological applicability of the chemosensor BHMP was examined via cell imaging studies.

Key words: Fluorescence probe, zinc ions, pyrophosphate anions, live cell imaging.

#### **1. Introduction**

There is a growing interest in the design and syntheses of organic molecules to recognize environmentally and biologically important species and it became a necessary research topic in life science area [1-6]. Among different recognizing techniques, photosensitive measurements using fluorescent chemosensors become suitable methodology for the detection of various analytes due to the fact that they are sensitive, cheap, convenient, non-destructive and are having high selectivity towards detection of analytes [7-10]. Generally, fluorescent sensors were composed of subunits (fluorophore and ionophore) in their structure and the subunits were connected through a linker/spacer.

The metal cations and anions serve essential roles in environment and are generally important to lead the metabolic processes in biological systems. But, their presence in both excess and deficient level may leads to severe problems in ecological and biological systems [11-14]. Among the essential metal cations, the trace quantity of the zinc metal ions in human body involved many physiological processes such like gene transcription, enzyme regulation and neural signal transmission. Moreover, the zinc metal is having an excellent potential activity towards therapeutic drug designing and was extensively investigated during nowadays. However, the action of zinc metal cation towards the biological and therapeutic mechanisms are not yet well understood due to the hindrance of  $d^{10}$  configuration of  $Zn^{2+}$  ion by usual spectroscopic techniques [15-18]. Furthermore, due to modern industrial growth, there is a vast usage and release of  $Zn^{2+}$  metal ions to the environment, which in turn leads to toxicity by causing severe problems to soil as well as plants by inhibiting their growth and biological systems by causing severe diseases like Alzheimer's disease, ischemic stroke and epilepsy [19-25]. Therefore, due to the above mentioned toxicity, the detection of zinc ions even in trace level with high reliability and effective response became essential. In this sense, the development of fluorescent chemosensors for the detection of zinc ions constitutes a very active field of research, because of its simplicity, sensitivity and selectivity.

On the other hand, among the anions which are playing vital roles in both biotic and natural systems [26,27], pyrophosphate (PPi) anion is significant due to its essential role in various metabolic processes such as DNA, RNA polymerizations, transduction in organisms and

cellular ATP hydrolysis [28,29]. The presence of PPi in excess or lower level in biological system may leads to several diseases including chondrocalcinosis, calcium pyrophosphate deposition disease (CPPD) and rheumatologic disorder [30-37]. So, the development of chemosensors to detect PPi anions is also gets importance.

Up-to-date, countless numbers of chemosensors bearing different types of fluorophores to detect zinc ions have been reported including fluorescein [38], rhodamine [39], BODIPY [40], coumarin [41] and PPi anions [42]. But nowadays, the design and synthesis of new molecular probes for sequential detection of two analytes becomes an emerging area. Hence, a new probe is synthesized and utilized to detect  $Zn^{2+}$  ions and the resulting ensemble is applied to sense PPi anions since PPi having strong binding affinity towards the zinc complexes.

In this work, bisphenol A was chosen as fluorophore with hydrazinobenzothazole ionophores units in the design of new chemosensor 4,4'-(propane-2,2-diyl)bis(2-((E)-(2-(benzo[d]thiazol-2-yl)hydrazineyldene)methyl)phenol) (BHMP) to detect metal ions. Initially, the probe was non fluorescent because of excited-state intramolecular proton transfer (ESIPT) involving efficient photoelectron transfer (PET) process. However, on complexation with zinc metal ions, the fluorescence property enhances strongly because of the restriction in the C=N bond rotation and suppression of PET process. The BHMP+Zn<sup>2+</sup> complex selectively detects PPi anions with quenching in the fluorescence intensity.

#### 2. Experimental section

#### 2.1. Materials and methods

All chemicals taken for the syntheses were analar pure grade and are brought from Sigma-Aldrich. The solvents were delivered from commercial suppliers and are used without any further purification. The double distilled water was used all through the experiments. Technico micro heating table was used to measure melting points in an open capillary tube and was uncorrected. Vario EL III Elemental analyzer was used to acquire the analytical data. UV-Visible spectra and fluorescence spectra were recorded on Shimadzu 1800 UV-Visible spectrophotometer and JASCO FP-8200 spectrophotometer respectively at room temperature using a  $1 \text{ cm}^2$  quartz cuvette (made of pure silica) at pH 7.4. Bruker AMX-500 spectrometer was used to obtain <sup>1</sup>H NMR spectra in dimethyl sulfoxide-d<sub>6</sub> or duterated chloroform.

Tetramethylsilane (TMS) is used as a reference to record the chemical shifts ( $\delta$ ) in ppm. By using a high voltage electro spray ionization probe (aerosol) in an advanced Q-TOF micro<sup>TM</sup> mass spectrometer, the mass spectrum was recorded. By using fluorescence microscope (Japan, Eclipse Ti-U, Nikon,) imaging studies were carried out. The bisphenol A dialdehyde (1) was synthesized according to the method reported in the previous work [43].

#### 2.2. Synthesis of probe BHMP

The probe BHMP was synthesized from bisphenol A dialdehyde (0.28 ml, 1 mmol) on reaction with hydrazinobenzothiozole (0.3304 g, 2 mmol) in acetonitrile (25 ml) for about 4 h. After completion of the reaction confirmed by Thin Layer Chromotography (TLC), the yellow color precipitate formed was separated out, washed with ethanol and dried to afford the probe BHMP.

Yield: 78%, 0.46 g; M.pt: 170-173 °C. Anal. Calc. for  $C_{31}H_{26}N_6O_2S_2$ : C, 64.34; H, 4.53; N, 14.52; S, 11.08 %. Found: C, 63.84; H, 4.03; N, 14. 02; S, 10.58 %. UV-vis (DMSO,  $\lambda_{max}$ , nm): 335, 370. <sup>1</sup>H NMR (DMSO- $d_6$ , ppm): 12.22 (s, 2H) 8.52 (s, 2H) 7.69-7.61 (t, Ar 4H) 8.08-8.02 (d, Ar 4H) 7.31-8.28 (d, Ar 2H) 6.98-6.96 (d, Ar 4 H) 4.49 (s, NH 2H) 1.80 (s, CH<sub>3</sub> 6H). <sup>13</sup>C NMR (DMSO- $d_6$ , ppm): 168.47, 157.08 (C-OH), 132.50 (Ar), 127.18 (Ar), 126.21 (Ar), 124.42 (Ar), 123.53 (Ar), 122.61 (Ar), 119.52 (Ar), 114.3(Ar), 56.20 (Ar), 33.67 (CH<sub>3</sub>). (Fig. S1 and S2). MS (ESI, *m/z*) 579.5 [M+H]<sup>+</sup> (Fig. S3).

#### 2.3. Synthesis of BHMP-Zn complex

The probe BHMP (0.578 g, 1 mmol) was dissolved in ethanol-chloroform mixture (25 ml, 1:1 v/v) and zinc acetate (0.438 g, 2 mmol) was added and stirred for 3 h at 68 °C. The greenish yellow color precipitate formed was separated and washed with cold ethanol.

Yield: 82%, 0.73 g; M.pt: 250-254 °C; Anal. Calc. for  $C_{34}H_{29}N_6O_6S_2Zn_2$ : C, 50.26; H, 3.60; N, 10.34; S, 7.77 %. Found: C, 50.76; H, 4.10; N, 10.84; S, 7.27 %. UV-vis (DMSO,  $\lambda$ max, nm): 338, 385, 440. <sup>1</sup>H NMR (DMSO- $d_6$ , ppm): 8.87 (s, 2H) 7.69-7.61 (t, Ar 4H) 8.08-8.02 (d, Ar 4H) 7.31-8.28 (d, Ar 2H) 6.98-6.96 (d, Ar 4 H) 4.47 (s, NH 2H) 1.80 (s, CH<sub>3</sub> 6H) (Fig. S4) MS (ESI, *m*/*z*) 826.2 [M+H]<sup>+</sup> (Fig. S5).

#### 3. Results and discussion

#### 3.1 Synthesis and spectral characterization

The ligand was synthesized by two step reaction sequences and it was displayed in scheme 1. The formation of the ligand was confirmed by <sup>1</sup>H & <sup>13</sup>C NMR and mass spectroscopic analysis. The <sup>1</sup>H NMR spectrum shows some important signals of the ligand at 12.22, 8.52, and 4.49 due to the presence of OH, azomethane and NH protons respectively. The ESI-MS spectrum of BHMP displays molecular ion peak with m/z 579.5 ( $[M + H]^+$ ) in accordance with calculated molecular masses 578.7.



Scheme 1. Synthesis of probe BHMP

#### 3.2. Absorption spectroscopic studies

To study the sensing behavior of probe as a preliminary study, the absorption properties of the probe BHMP alone and with various metal ions like K<sup>+</sup>, Na<sup>+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup>, Cd<sup>2+</sup>, Mg<sup>2+</sup>, Hg<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Ag<sup>+</sup>, Mn<sup>2+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup> were examined in DMSO-H<sub>2</sub>O (1:9 v/v) in HEPES buffer at pH 7.4 and the spectra were shown in Fig. 1. The probe BHMP shows peak at 335 and 370 nm. Upon the addition of Zn<sup>2+</sup> ions to the probe BHMP, the peaks show slight red shift and appeared in the region 338 and 385 nm. Moreover, a new peak was also appeared at 440 nm due to the inhibition of ESIPT (excited state intramolecular proton transfer) and PET process [44]. The addition of other ions do not show any characteristic change in the absorption spectrum.

#### < Insert Fig. 1>

In addition, the sensing ability of the probe with  $Zn^{2+}$  ions was studied by UV-vis titration experiments (Fig. 2). Upon incremental addition of  $Zn^{2+}$  ions (0-100  $\mu$ M) to BHMP

solution, the absorption band intensity at 370 nm gets decreased and at 440 nm the intensity of the absorption band increases gradually. The appearance of isosbestic points at 405 nm shows the formation of new product.

< Insert Fig. 2>

#### 3.3. Emission spectroscopic studies

The emission spectra were carried out to confirm selectivity of the probe BHMP with the various metal ions in DMSO-H<sub>2</sub>O (1:9 v/v) in HEPES buffer at pH 7.4. The probe BHMP shows weak emission at 515 nm, after the addition of  $Zn^{2+}$  ions the fluorescence emission intensity was suddenly increased. However, the other analytes such as Pb<sup>2+</sup>, Cr<sup>3+</sup>, Cu<sup>2+</sup>, K<sup>+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, Cu<sup>2+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, Hg<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup> do not induce any change in the fluorescence emission intensity (Fig. 3). The emission results indicate that the probe BHMP selectively detects the Zn<sup>2+</sup> ions over other metal ions.

## < Insert Fig. 3>

After confirming the selectivity of the probe, fluorescence titrations experiments were carried out to analyze the binding strength and detection limit of BHMP towards Zn<sup>2+</sup> ions. The addition of increasing concentration of  $Zn^{2+}$  ions (0 - 100  $\mu$ M) to the probe (BHMP) solution, the fluorescence intensity was gradually increased at 515 nm and reached the saturation when 100  $\mu$ M of Zn<sup>2+</sup> ions was added (Fig. 4). Fig. S6 shows the result of fluorescence intensity vs concentration of  $Zn^{2+}$  ions (1–100  $\mu$ M), and the spectra exhibits the good linear relationship. The detection limit was calculated as 0.28 nM using the equation  $3\sigma/K$  ( $\sigma$  is the standard deviation of the blank measurement and k is the slope between the intensity versus  $Zn^{2+}$  ions concentration). The high association constant value and low detection limit reveal that the probe BHMP can be an effective fluorescence probe for the detection of zinc metal ions. The binding constant was calculated by using Benesi–Hildebrand equation [45] and it was found to be  $9.3 \times 10^7 \text{ M}^{-1}$  (Fig. S7). The quantum yields ( $\Phi$ ) of BHMP and BHMP+Zn<sup>2+</sup> complex were found to be 0.016 and 0.713, respectively. Moreover, the color change occurs while addition of  $Zn^{2+}$  ions (100  $\mu$ M) to the probe solution can be seen through naked eye under day light and UV lamp (Fig. S8). But, addition of other ions such as  $K^+$ ,  $Na^+$ ,  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $Mg^{2+}$ ,  $Hg^{2+}$ ,  $Ag^+$ ,  $Mn^{2+}$ ,  $Fe^{3+}$  and  $Al^{3+}$  do not exhibit any notable color change.

#### < Insert Fig. 4>

In addition, the binding stoichiometry between the probe BHMP and  $Zn^{2+}$  ions was established by the Job's experiment, <sup>1</sup>H NMR titration and mass spectral analysis. Job's fluorescence emission experiment was performed with various mole fractions of probe BHMP and  $Zn^{2+}$  ions and the results denote the formation of 1:2 binding between the BHMP with the  $Zn^{2+}$  ions (Fig. S9). The <sup>1</sup>H NMR spectrum (Fig. 5) of BHMP displays peaks at 12.22, 4.49 and 8.52 ppm due to the presence of OH, NH and imine protons respectively. The addition of 1 equivalent  $Zn^{2+}$  ions to the probe, the intensity of the OH peak decreased and the imine proton peak was shifted to downfield. While, addition of 2 equivalent  $Zn^{2+}$  ions, the OH peak completely disappeared and the imine proton peak was shifted to downfield further. This confirms the 1:2 binding ratio between the BHMP with the  $Zn^{2+}$  ions. Moreover, the ESI mass spectrum of BHMP +  $Zn^{2+}$  complex displays molecular ion peak at m/z 826.2 which corresponds to 1:2 stoichiometry.

# < Insert Fig. 5>

In order to evaluate the specificity of BHMP, competitive experiments were performed. In the competitive experiments,  $Zn^{2+}$  ions mixed with other interfering cations including  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Fe^{3+}$ ,  $Co^{2+}$ ,  $Li^+$ ,  $Ni^{2+}$ ,  $Na^+$ ,  $Cu^{2+}$ ,  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $K^+$ ,  $Hg^{2+}$  and  $Ag^+$  ions (2 equiv.  $Zn^{2+}$  and 2 equiv. other metal ions) and the emission spectra were recorded in presence of the probe BHMP. As shown in Fig. S10, addition of  $Zn^{2+}$  ions into BHMP lead to effective enhancement in the fluorescence intensity. The presence of other competitive metal ions along with  $Zn^{2+}$  do not interfere the emission spectra. The above-mentioned results reveal that the probe BHMP possesses suitable selectivity for the system containing  $Zn^{2+}$  ions over other analytes.

#### 3.4. Anion sensing studies

After confirming the selectivity of the probe for the recognition of  $Zn^{2+}$  ions, the detection ability of the BHMP +  $Zn^{2+}$  complex has been analyzed for the selective detection of anions through displacement approach. Initially absorption spectra were recorded for BHMP +  $Zn^{2+}$  complex alone and with various anions using DMSO-H<sub>2</sub>O (1:9 v/v) in HEPES buffer at pH 7.4. From the Fig. 6, it is observed that the addition of PPi to the BHMP+ $Zn^{2+}$  ensemble retrieve

spectral pattern of free probe whereas other anions ( $F^-$ ,  $Cl^-$ ,  $\Gamma$ ,  $S^{2-}$ ,  $NO^{3-}$ ,  $ClO^{3-}$ ,  $SO_4^{2-}$ ,  $HSO^{3-}$  and  $Br^-$ ) do not change any spectral pattern.

The sensing property of the BHMP+  $Zn^{2+}$  ensemble was further analyzed by emission spectra. The emission spectra were recorded for BHMP +  $Zn^{2+}$  complex with various anions such as F<sup>-</sup>, Cl<sup>-</sup>, CN<sup>-</sup>,  $\Gamma$ , S<sup>2-</sup>, NO<sup>3-</sup>, ClO<sup>3-</sup>, ClO<sup>4-</sup>, SO<sub>4</sub><sup>2-</sup>, HSO<sup>3-</sup>, PPi and CH<sub>3</sub>COO<sup>-</sup> in DMSO-H<sub>2</sub>O (1:9 v/v) in HEPES buffer at pH 7.4. Fig. S11 shows that the above mentioned anions do not alter the fluorescence intensity except PPi anion. The addition of PPi anions quenches the fluorescence intensity of BHMP+Zn<sup>2+</sup> complex. To further extend, photoluminescence titrations were carried out for the BHMP+Zn<sup>2+</sup> complex solution (1 equiv) with 0-100 µM concentration of PPi anions (Fig. 7). The stepwise addition of the PPi anions to the BHMP+Zn<sup>2+</sup> complex solution, the emission intensity of the BHMP + Zn<sup>2+</sup> ensemble decreases gradually at 515 nm and it remains stable when it gets saturated. The Zn<sup>2+</sup> ions on interaction with the PPi anion, more stable precipitate was formed with release of free probe BHMP. The LOD (calculated using formula  $3\sigma/K$ ) for PPi ions was found to be 0.87 nM (Fig. S12). In addition, the 1:2 binding stoichiometry for BHMP + Zn<sup>2+</sup> and PPi anions was found from Job's plot (Fig. S13).

# < Insert Fig. 7>

Moreover, competitive experiments were done to evaluate the response of BHMP+Zn<sup>2+</sup> ensemble with other anions in addition to PPi anions using the emission spectra. The results show that the PPi anions quench the emission intensity even in presence of other anions (Fig. S14). Therefore, it is concluded that the BHMP + Zn<sup>2+</sup> ensemble shows good selectivity towards PPi anions. Besides, color change of the BHMP + Zn<sup>2+</sup> ensemble for addition of PPi ions (100  $\mu$ M) was examined which describe that it can be seen through nacked eye under day light and UV lamp. But, addition of other ions such as F<sup>-</sup>, Cl<sup>-</sup>,  $\Gamma$ , S<sup>2-</sup>, NO<sup>3-</sup>, ClO<sup>4-</sup>, SO<sub>4</sub><sup>2-</sup>, Br<sup>-</sup>, PPi and CH<sub>3</sub>COO<sup>-</sup> do not exhibit any notable color change (Fig. S15).

#### 3.5. pH study

In order to explore the application of probe in physiological medium, the fluorescence responses of BHMP, BHMP+ $Zn^{2+}$  and BHMP+ $Zn^{2+}$ +PPi were examined under different pH

conditions (2-14) (Fig. S16). The results reveal that the probe can be applicable to detect  $Zn^{2+}$  and PPi ions in the environment and biotic system with the pH range of 5-10.

#### 3.6. Time resolved fluorescence study

The fluorescence lifetime decay experiment was done and the lifetime of the BHMP was found to be 1.56 ns (at  $\lambda_{em}$ = 440 nm). There is an increase in the life time from 1.56 to 1.98 was observed on adding Zn<sup>2+</sup> ions to the probe BHMP solution. Due to the suppression of PET and ESIPT process the average life gets enhanced. While subsequent addition of PPi anions to the BHMP+Zn<sup>2+</sup> ensemble, there is a significant quenching in the lifetime to 1.61 ns (Fig. 8).

#### < Insert Fig. 8>

Based on the above spectroscopic results, a mechanism was proposed for detection of  $Zn^{2+}$  cations and PPi anions in scheme 2.



Scheme 2. Plausible mechanism for the interaction of  $Zn^{2+}$  and PPi ions with probe BHMP.

#### 3.7. Application in Bio imaging

Before entering the bio imaging studies, the probe BHMP,  $BHMP+Zn^{2+}$  and  $BHMP+Zn^{2+}$  with PPi were tested their cytotoxicity against A549 cells using MTT assay. For

this assay, primarily the cells were incubated with different concentrations (0, 5, 10, 15, 20, 25, 30, 40 and 50  $\mu$ M) of the BHMP, BHMP+Zn<sup>2+</sup> and BHMP+Zn<sup>2+</sup>+PPi for 48 h at 37 <sup>o</sup>C. The results show that the probe BHMP has no toxicity against the tested cells upto 50  $\mu$ M. The BHMP+Zn<sup>2+</sup> and BHMP+Zn<sup>2+</sup>+PPi also do not show toxicity upto 20  $\mu$ M (Fig. S17). The toxicity results reveal that the probe BHMP, BHMP+Zn<sup>2+</sup> and BHMP+Zn<sup>2+</sup>+PPi can have the capability to use in live cell imaging. In order to prove the detection capacity of the probe BHMP towards Zn<sup>2+</sup> and PPi ions in the live A549 cell, the cells were incubated with 10  $\mu$ M of BHMP solution and examined with fluorescence microscopy. Initially no fluorescence was observed but on adding 20  $\mu$ M of Zn<sup>2+</sup> ions solution to the cells incubated with BHMP solution, fluorescence enhancement takes place. After that, the 20  $\mu$ M of PPi ions solution was added to the cells were shown in Fig. 9. From this result, it is proved that the OFF-ON-OFF mechanism was taking place and the observation from the imaging studies shows that the probe BHMP possesses cell permeability and excellent selectivity to detect both Zn<sup>2+</sup> and PPi ions.

## < Insert Fig. 9>

The present sensor BHMP was compared with the already reported chemosensors towards zinc ions detection (Table S1). Although the reported chemosensors have some advantages such as easy synthesis and sensitivity of detection, the present chemosensor BHMP possesses merits like high binding constant, low detection limit and naked eye detection. Therefore, the reported chemosensor BHMP has the greater ability to monitor  $Zn^{2+}$  and PPi ions in both quantitatively and qualitatively.

#### 4. Conclusions

We have successfully synthesized the luminescent chemosensor BHMP which shows high selective and sensitive response for the sequential detection of  $Zn^{2+}$  and PPi ions in DMSO-H<sub>2</sub>O (1:9 v/v) in HEPES buffer at pH 7.4. The probe BHMP has low detection limit (LOD = 0.28 nM) and high binding affinity (9.3×10<sup>7</sup>) towards  $Zn^{2+}$  ions. In addition, the resultant BHMP+Zn<sup>2+</sup> complex detect the PPi anions over the other anions in the similar physiological condition with detection limit of 0.87 nM. The fluorescent enhancement occurs due to inhibition of photoelectron transfer (PET) process after formation of BHMP+Zn<sup>2+</sup> complex. While the addition of PPi anions into the solution of BHMP+Zn<sup>2+</sup> quenches the fluorescence intensity due to restoration of PET process in the free BHMP. Moreover, the probe BHMP was effectively applied for the detection of  $Zn^{2+}$  and PPi ions in live cells.

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**Fig. 1.** UV-Vis spectra of probe BHMP (10  $\mu$ M) in DMSO/H<sub>2</sub>O (1:9 (v/v), 50 mM HEPES, pH=7.4) with 100  $\mu$ M of metal ions (Na<sup>+</sup>, Cd<sup>2+</sup>, Mg<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Cu<sup>2+</sup>, Hg<sup>2+</sup>, Pb<sup>2+</sup>, Fe<sup>3+</sup>, K<sup>+</sup>, Ni<sup>2+</sup>, and Al<sup>3+</sup>).

Solution



Fig. 2. UV-Vis titration spectra of the probe BHMP (10  $\mu$ M) with 0-100  $\mu$ M of Zn<sup>2+</sup> ions.

Southor



Fig. 3. Fluorescence response of probe BHMP (10  $\mu$ M) in DMSO/H<sub>2</sub>O (1:9 (v/v), HEPES=50mM, pH=7.4) with 100  $\mu$ M of metal ions (K<sup>+</sup>, Na<sup>+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, Cd<sup>2+</sup>, Cu<sup>2+</sup>, Mg<sup>2+</sup>, Hg<sup>2+</sup>, Co<sup>2+</sup>, Fe<sup>3+</sup> and Al<sup>3+</sup>) ( $\lambda_{ex}$ =440 nm).



Fig. 4. Fluorescence titration spectra of the probe BHMP (10  $\mu$ M) with 0 - 100  $\mu$ M of Zn<sup>2+</sup>.



**Fig. 5.** <sup>1</sup>H NMR titration spectra of BHMP with  $Zn^{2+}$  ions.



**Fig. 6.** UV-Vis spectra of probe BHMP+Zn<sup>2+</sup> (10  $\mu$ M) in DMSO/H<sub>2</sub>O (1:9 (v/v), 50 mM HEPES, pH=7.4) with 100  $\mu$ M of varies anions (F<sup>-</sup>, Cl<sup>-</sup>, PPi, I, S<sup>2-</sup>, NO<sup>3-</sup>, ClO<sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, HSO<sup>3-</sup> and Br<sup>-</sup>).



Fig. 7. Fluorescence response of the probe BHMP +  $Zn^{2+}$  (10  $\mu$ M) with 0 - 100  $\mu$ M of PPi anion.



**Fig. 8.** Fluorescence lifetime decay profiles of probe BHMP,  $BHMP+Zn^{2+}$  and  $BHMP+Zn^{2+}+PPi$ .



**Fig. 9.** Fluorescence cell imaging studies of cells (A549) treated with probe BHMP (left column), BHMP +  $Zn^{2+}$  (middle column) and BHMP +  $Zn^{2+}$  + PPi (right column).

#### Credit author statement

Rajasekaran Dhivya: Conceptualization Investigation, Writing - Original Draft.

Venkatachalam Kavitha: Verification, whether as a part of the activity or separate, of the overall replication/ reproducibility of results/experiments and other research outputs.

Periasamy Viswanathamurthi: Methodology, Validation, Supervision, Project administration.

Journal Reading

#### **Declaration of interests**

 $\boxtimes$  The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:







A bisphenol based fluorescence "turn-on" chemosensor (BHMP) has been synthesized and its sensing behavior was tested towards various ionic species. The probe BHMP selectively detects zinc ions over other metal ions and the resulting  $Zn^{2+}$ -BHMP ensemble serves as a secondary probe for the detection of PPi anion. The biological applicability of the chemosensor BHMP was examined via cell imaging studies.

Solution

## Highlights

- The probe BHMP selectively detects zinc ions over other metal ions and the resulting Zn<sup>2+</sup>-BHMP ensemble serves as a secondary probe for the detection of pyrophosphate (PPi) through displacement approach.
- The probe shows an excellent selectivity towards zinc and PPi ions.
- The probe detects  $Zn^{2+}$  and PPi ions with low detection limit of 0.28 and 0.87 nM.
- The probe BHMP was successfully applied to detect  $Zn^{2+}$  and PPi ions in live cells.