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Research paper

Fluorescent chemosensor for Al(III) based on chelation-induced fluorescence enhancement and its application in live cells imaging

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ABSTRACT

A tridentate Schiff base receptor BPB was synthesized by condensation of 2-(benzo[*b*]thiophen-2-yl)benzenamine with salicylaldehyde in ethanolic medium and characterized by various spectral (FT-IR, ^{1}H NMR, ^{13}C NMR and mass) data. The methanolic solution of BPB was applied for the fluorescent sensing of metal ions dissolved in aqueous medium. The selectivity experiment revealed that the receptor BPB showed significant fluorescence enhancement at 430 nm in the presence of A(III) due to the chelation-induced fluorescence enhancement (CHEF) mechanism. Receptor BPB formed complex with Al(III) in 2:1 binding ratio with the estimated binding constant of $K = 3.02 \times 10^{8}\ M^{-2}$. Without any interference from other tested metal ions, the receptor BPB can detect the concentration of Al(III) down to 256 nM. The receptor BPB showed good cell permeability and was applied for the qualitative and quantitative detection of intracellular Al(III) in A549 cell line (adenocarcinomic human alveolar basal epithelial cells) by using a confocal imaging technique.

1. Introduction

Aluminum, one of the most abundant metallic element in the earth's crust has diverse applications in chemical industries, aluminum based pharmaceuticals, food additives, water purification, paper making, cosmetic and aerospace industries etc. [1–6]. Although aluminum offers various applications on the day to day life, but excessive use of aluminum is hazardous to the environment and human life. Aluminum components in the earth's atmosphere constitutes to nearly 8% [7]. Apart from this, acid rain contributes to the enrichment of Al(III) ion concentration by leaching from the soil, which may be deleterious to the plant growth and hazardous to the organisms [8]. The excessive accumulation of Al(III) in brain tissues can cause severe damage to central nervous system and increase the risk of neurological diseases like Alzheimer and Parkinson [9,10]. Beside this, toxicity of Al(III) ions can also cause glucose intolerance, kidney failure, cardiac arrest and osteoporosis [11–13]. Because of the health hazards to human life due to excessive exposure or intake of Al(III) ions, the World Health Organization (WHO) has recommended dietary limit of aluminum intake should be controlled up to 7 $mgKg^{-1}$ per week [14], whereas the permissible limit of Al(III) concentration in drinking water is 7.41 μM. Therefore, the monitoring of Al(III) ions concentration is considered to be important owing to its potential impact on the environment and human health.

Analytical approaches like inductively coupled plasma atomic emission spectroscopy (ICP-AES), atomic absorption spectrometry (AAS) and inductively coupled plasma-mass spectroscopy (ICP-MS), voltammetry, ion-selective membrane and liquid chromatography-mass spectrometry techniques etc. are available for the monitoring of Al(III) ion concentration [15–21]. But all these methods are relatively expensive and suffer from issues like tedious pretreatment procedure, need of skilled person and sophisticated laboratory facilities. In comparison, the methods based on colorimetric and fluorescence approaches are proved to be of much interest to scientist due to its simplicity, cost-effectiveness and high sensitivity for the monitoring of target analyte [22–24]. Therefore, there is expedite growth in the

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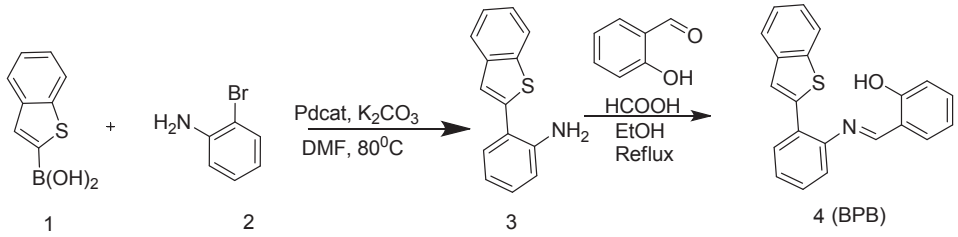

Scheme 1. Synthesis of receptor BPB.

development of optically active chromogenic and fluorogenic chemosensors for the detection of various toxic analytes including Al(III) ion [25–30]. The Schiff bases with suitable binding sites are extensively investigated for developing chemosensors for metal ions [5,31–44], because of the efficient synthetic approaches and fascinating coordination behavior towards metal ions. Recently, the multidentate Schiff bases with the mechanisms like chelation-enhanced fluorescence (CHEF), aggregation induced emission (AIE), C═N isomerization, intramolecular charge transfer (ICT), excited state intramolecular proton transfer (ESIPT), photoinduced charge transfer (PET) and excimer/exciplex formation etc. are commonly employed for the designing of fluorescent sensors for Al(III) [40–44].

In this manuscript, a new 2-(benzo[*b*]thiophen-2-yl)benzenamine derived tridentate Schiff base receptor BPB was synthesized (Scheme 1) and applied for the fluorescent sensing of Al(III) ion. The receptor BPB showed a selective fluorescence enhancement in the presence of A(III) due to the CHEF mechanism, and can be applied for the monitoring of intracellular Al(III) ions concentration in live A549 cells (adenocarcinomic human alveolar basal epithelial cells).

2. Experimental

2.1. Materials and instruments

All analytical grade reagents used in research were procured from commercial suppliers. Salicylaldehyde, 2-bromoaniline, thiophen-2-ylboronic acid and other chemicals used for the synthesis of BPB were purchased from Sigma-Aldrich. All metal salts used in the sensing experiments were procured from Renkem Pvt. Ltd., India. Thin layer chromatography (TLC) was used as an analysis tool to control and monitor the progress of chemical reaction. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Brukar 400 MHz instrument, where the chemical shifts are given in ppm downfield from TMS as an internal standard. The HRMS and infrared spectra were recorded on a maXis impact and Perkin-Elmer FTIR RX1 spectrometer, respectively. All fluorescence spectra were recorded on an Agilent Technologies (Cary Eclipse Fluorescence Spectrophotometer).

2.2. Synthesis of BPB

Synthesis of BPB was achieved by the condensation of 2-(benzo[*b*]thiophen-2-yl) benzenamine (3) with salicylaldehyde in ethanolic medium in the presence of catalytic amount of acid (Scheme 1). The reported intermediate compound 2-(benzo[*b*]thiophen-2-yl)benzenamine [45] was synthesized by Suzuki coupling between thiophen-2-ylboronic acid and 2-bromoaniline in the presence of $PdCl_2(PPh_3)_2$ and potassium carbonate in DMF by following the reported procedure [46].

2.2.1. Synthesis of 2-(benzo[b]thiophen-2-yl)benzenamine (3)

The reaction was carried out in a 100 mL two-necked reaction flask equipped with a reflux condenser, and nitrogen flushing provision. Thiophen-2-ylboronic acid (2.01 g, 11.33 mmol), $PdCl_2(PPh_3)_2$ (611 mg, 0.871 mmol) and potassium carbonate (1.245 g, 8.71 mmol) were placed in reactor assembly, and the flask was flushed with nitrogen. 50 mL DMF, 9 mL water and 2-bromoaniline (1.5 g, 8.7 mmol) were added, and the resulting solution was stirred at 80 °C for 24 hr. After cooling, the reaction mixture was diluted with ethyl acetate and the aqueous layer was saturated with $NaHCO_3$ and filtered off through a short pad of celite. The filtrate was extracted with ethyl acetate and the combined organic layer was dried over sodium sulfate. The solvent was evaporated using a rotary evaporator followed by silica gel column purification with hexane/ethyl acetate (5:1, v/v) gave 2-(benzo[*b*]thiophen-2-yl)benzenamine (1.0 g, 4.43 mmol) in 63.5% yield.

2.2.2. Synthesis of BPB

2-(benzo[*b*]thiophen-2-yl)benzenamine (0.5 g, 2.21 mmol), salicylaldehyde (0.271 g, 2.21 mmol) and catalytic amount of formic acid in 15 mL ethanol were placed in 100 mL single necked reaction flask equipped with a reflux condenser, and the resulting solution was stirred at 80 °C for 16 hrs. After completion of the reaction, the excess solvent was removed under reduced pressure. The crude product was washed with H_2O and dried, gave BPB as yellow solid (0.5 g, 1.51 mmol) in 65% yield.

FT-IR (KBr discs, cm^{-1}): 3250, 3057, 2904, 1609, 1587, 1576, 1515, 1493, 1455, 1479, 1395, 1370, 1308, 1274, 1249; **^{1}H NMR (400 MHz, DMSO-d_6, δ ppm)**: 12.20 (s, 1H), 8.94 (s, 1H), 7.96–7.95 (dd, 1H), 7.85–7.77 (m, 3H), 7.71 (s, 1H), 7.53–7.49 (m, 1H), 7.44–7.34 (m, 5H), 7.00–6.99 (m, 1H), 6.94–6.93 (m,1H); **^{13}C NMR (100 MHz, DMSO-d_6, δ ppm):** 163.63, 159.76, 146.93, 140.11, 140.033, 139.45, 133.67, 132.16, 129.82, 129.58, 127.89, 126.92, 124.57, 124.50, 123.76, 123.44, 122.12, 120.12, 119.98, 116.62; **M/Z (M + H)$^{+}$:** Expt. 330.06, Calc. 329.09.

2.3. Spectroscopic study

Due to the insolubility of the receptor BPB in water, the stock solution of BPB was prepared in CH_3OH. All cations (1.0×10^{-3} M) solutions were prepared in water. These solutions are used for various spectroscopic studies after appropriate dilution. For the fluorescence titration, the required amount of the diluted receptor BPB (2 mL, 1×10^{-5} M, in CH_3OH) was taken directly into the cuvette and the spectra were recorded after each aliquot (10 μL) addition of Al(III) (1×10^{-3} M, H_2O) by using a micropipette. Using the fluorescence titration data, the calibration curve between the emission intensity at 430 nm was plotted against the added concentration of Al(III). Using the IUPAC approved equation, the limit of detection (LOD) of the receptor BPB was calculated. The LOD was calculated using the relationship, LOD = (3 × standard deviation)/slope of the calibration curve. To calculate the relative standard deviation, the fluorescence spectra of ten blank samples were recorded. Using the fluorescence titration data, the binding constant (K) of the complex formed between BPB and Al(III) was determined by applying the Benesi-Hildebrand equation:

$$\frac{1}{F-F0} = \frac{1}{K(F\max - F0)[Al(III)]^2} + \frac{1}{Fmax-F0}$$

where F and F0 are the emission intensity of BPB solution in the

presence and absence of Al(III) ions, Fmax is the saturated emission intensity of BPB in the presence of excess amounts of Al(III), [Al(III)] is the concentration of added Al(III) ions (mol L^{-1}).

2.4. Living cells imaging

The A549 cells were incubated with DMEM supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, USA) at 37 °C under a humidified atmosphere containing 5% CO_2. A549 cells were placed on Petri-dishes (Φ = 20 mm) and allowed to adhere for 24 hrs before the treatments. The fluorescence images of A549 cells were acquired on a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (oil, × 60). The excitation wavelength was 405 nm, and the collected wavelength was 410–440 nm. The cell imaging was carried out by adding receptor BPB of 10 μM to glass bottom cell culture Petri-dishes.

3. Results and discussion

3.1. Preliminary selectivity study of BPB

The receptor BPB was synthesized by reacting equimolar amount of 2-(benzo[*b*]thiophen-2-yl)benzenamine with salicylaldehyde in ethanolic medium in the presence of catalytic amount of formic acid. The receptor BPB was characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS data (Figs. S1–S4). The recognition ability of BPB (2 mL, 1 × 10^{-5} M, in CH_3OH) was examined by observing naked-eye detectable colour changes under day and UV light after adding different metal ions (100 μL, 1 × 10^{-3} M, in H_2O), such as Al(III), Ag(I), Ca(II), Cd(II), Co (II), Cu(II), Fe(II), Hg(II), K(I), Li(I), Mg(II), Mn(II), Ni(II), Pd(II) and Zn (II). In the naked-eye experiments, the BPB solutions showed no-obvious visual color changes under day light in the presence of tested metal ions (Fig. S5). However, the receptor BPB illustrated a selective "turn-on" fluorescence response in the presence of Al(III) ions over other tested metal ions under UV light irradiated at 365 nm (Fig. 1). The observed distinct fluorescent colour change prompted us to develop the receptor BPB for the fluorescent sensing of Al(III).

3.2. Emission spectroscopic study of BPB

The metal ions sensing ability of BPB was investigated by fluorescence spectroscopy. The receptor BPB (2 mL, 1 × 10^{-5} M, in CH_3OH) was non-fluorescent, when excited at 320 nm. The non-fluorescent nature of BPB may be due to the conformational flexibility that allows non-radiative decay from the excited state along with the photo-induced electron transfer (PET) [47–49]. When the BPB was interacted with different metal ions (100 μL, 1 × 10^{-3} M, in H_2O), addition of Al (III) caused a significant fluorescence enhancement at 430 nm (Fig. 2). The fluorescence enhancement can be explained due to the chelation-induced fluorescence enhancement (CHEF) and the inhibition of PET [47–49]. The selective complexation between BPB and Al(III) restricts the conformational flexibility at the excited state upon inhibition of C=N isomerization resulted significant fluorescence enhancement. Other tested metal ions failed to perturb the fluorescence profile of BPB, which supported the high selective towards Al(III).

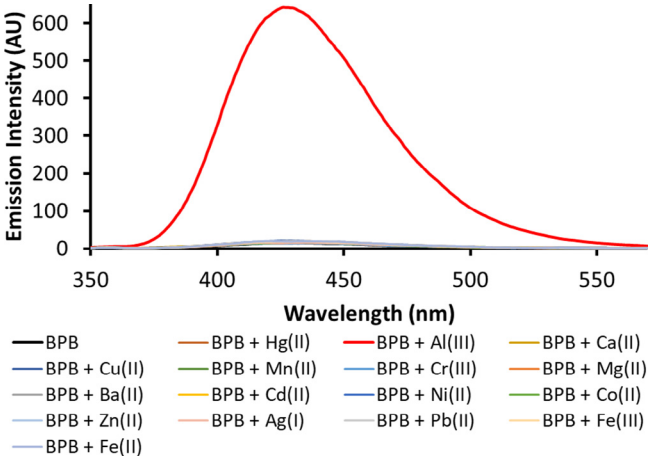

Fig. 2. Fluorescence emission changes of BPB (2 mL, 1 × 10^{-5} M, CH_3OH) upon the addition of ~5 equivalents (4.76 × 10^{-5} M) of different metal ions (100 μL, 1 × 10^{-3} M, in H_2O).

The interference of coexisting metal ions on the detection of Al(III) by BPB was investigated by performing competitive experiments, where the fluorescence spectra of BPB (2 mL, 1 × 10^{-5} M, in CH_3OH) were recorded in the presence of Al^{3+} (60 μL, 1 × 10^{-3} M, in H_2O) and equimolar amounts of other interfering metal ions (60 μL, 1 × 10^{-3} M, in H_2O). The bar representation of change in fluorescence intensity of BPB at 430 nm revealed that the detection of Al(III) is not interfered in the coexistence of tested interfering metal ions (Fig. 3). Therefore, the receptor BPB can be explored for the highly selective fluorescent "turn-on" sensing of Al(III) ions.

The fluorescence titration experiment was carried out to determine the sensitivity of the receptor BPB, where the fluorescence spectra of BPB (2 mL, 1 × 10^{-5} M, in CH_3OH) were recorded after each aliquot (10 μL) addition of Al(III) (0–100 μL, 1 × 10^{-3}, in H_2O). The fluorescence intensity of BPB was increased at 430 nm with the successive incremental addition of Al(III) (Fig. 4). The increase in fluorescence intensity at 430 nm of BPB was plotted against the added concentration of Al(III). From the calibration curve (Fig. S6), the LOD of receptor BPB was estimated as 256 nM Al(III). The estimated LOD is far better than the permissible limit of Al(III) concentration in drinking water. Also, the LOD is comparable/superior than the recently reported Al(III) fluorescent sensors summarized in Table S1.

The fluorescence spectral changes of BPB clearly supported the formation of new species in solution after interaction with Al(III). To investigate the binding stoichiometry of new species formed between BPB and Al(III) in solution, the job's plot analysis was carried out that showed the fluorescence maxima at the mole fraction of 0.3. The job's plot analysis indicates the formation of a host–guest BPB-Al(III) complex in 2:1 binding stoichiometry (Fig. 5). Analyzing fluorescence titration data with Benesi-Hildebrand equation, the binding constant (K) of 3.02 × 10^{8} M^{-2} was estimated for the BPB-Al(III) complex species formed in solution (Fig. S7). The obtained binding constant revealed the high affinity of the receptor BPB to form complex with Al(III),

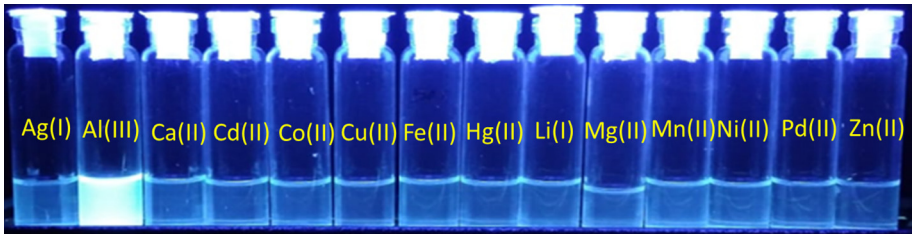

Fig 1. Vials irradiated with UV light at 365 nm containing BPB (2 mL, 1 × 10^{-5} M, in CH_3OH) in presence of ~5 equivalents (4.76 × 10^{-5} M) of different tested metal ions (100 μL, 1 × 10^{-3} M, in H_2O).

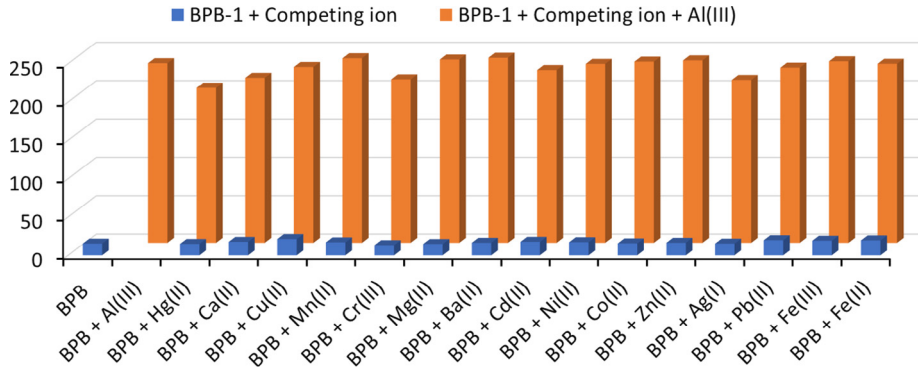

Fig. 3. Competitive fluorescence study of BPB (2 mL, 1×10^{-5} M, in CH_3OH) upon the addition of Al(III) (60 μL, 1×10^{-3} M, in H_2O) and other interfering metal ions (60 μL, 1×10^{-3} M, in H_2O).

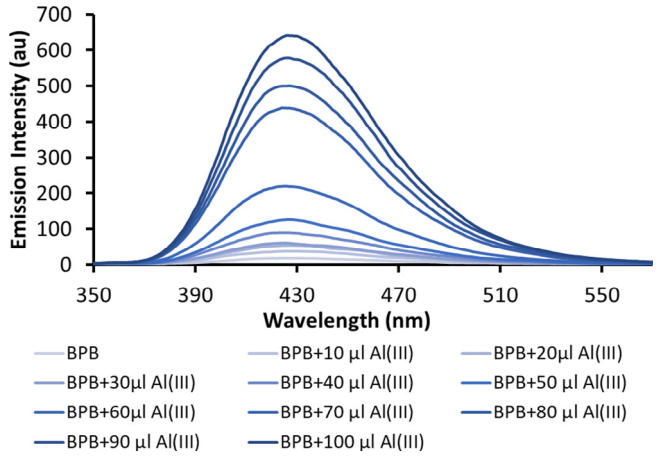

Fig. 4. Fluorescence spectral changes of BPB (2 mL, 1×10^{-5} M, in CH_3OH) upon the incremental addition of Al(III) (0–100 μL, 1×10^{-3} M, in H_2O).

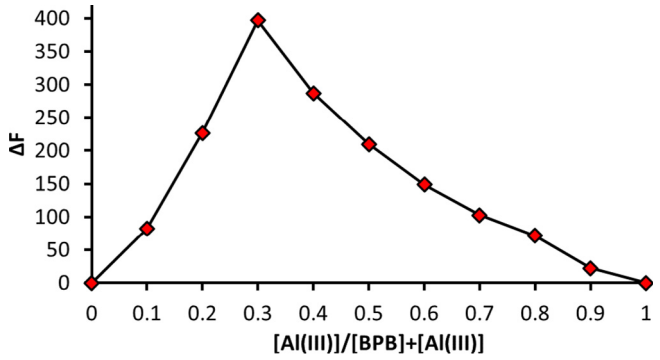

Fig. 5. Job,s plot for determining 2:1 binding stoichiometry of BPB (1×10^{-5} M, in CH_3OH) and Al(III) ions (1×10^{-5} M, in H_2O).

where the receptor is expected to provide three donor sites, i.e., phenolate-O, imine-N and thiol-S. Further, the formation of a complex in 2:1 binding ratio was confirmed by recording the LC-MS spectra of BPB in the presence of Al(III) in MeOH (Fig. S4). The MS spectra showed a peak at m/z = 805.2 matching to the complex $[(2BPB\text{-}2H) + Al^{3+} + (ClO_4^-) + Na]^+$ (Fig. S8).

The formation of complex species between BPB and Al(III) was complemented by performing ^1H NMR titration experiment (Fig. S9). The ^1H NMR spectra of BPB were recorded after adding different equivalents (0.5, 1 and 2) of Al(III) in DMSO-d_6. The titration experiment revealed that the peak at 12.20 ppm corresponding to the phenolic-OH proton gets disappeared with the successive addition of Al(III) ions to the solution of **BPB**. Also, the imine proton peak of BPB at 8.94 ppm was shifted slightly towards upfield region. These observations confirm the participation of the donor atoms phenolate-O, imine-N and thiol-S of BPB in complex formation with Al(III). Based on the experimental evidences, the 3D structure of the receptor BPB and its complex with Al(III) was calculated by applying the semi-empirical PM6 method at the gas phase [50,51]. The record BPB is planar in structure and undergoes some apparent conformational adjustment to form pseudo-octahedral complex with Al(III) (Fig. 6).

3.3. Live cells imaging study of BPB

The fluorescent 'turn-on' response from the receptor BPB upon addition of Al(III) prompted us to examine its ability to detect intracellular Al(III) concentration both qualitatively and quantitatively in A549 cell lines. The A549 cells were incubated with DMEM supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco, USA) at 37 °C under a humidified atmosphere containing 5% CO_2. The A549 cells were planted on petri-dishes (Φ = 20 mm) and allowed to adhere for 24 hrs before the treatments. After being incubated with BPB (10 μM) in DMEM for 10 min, the cells were imaged by a confocal fluorescence microscope. The results show very weak emission from the cells treated with the BPB alone (Fig. 7a). Subsequently, the cells containing BPB were treated with different concentrations of Al(III), i.e. 20 μM, 30 μM and 40 μM of Al(III) and incubated for 30 min (Fig. 7b–d). There is a significant intracellular fluorescence increase compared with the control cells revealed the good cell membrane permeability of the receptor BPB and also its ability to detect intracellular Al(III) ions. Further, the confocal fluorescence images became gradually brighter as the concentration of Al(III) increased from 20 μM to 40 μM, which indicate the potential of BPB for quantitative monitoring of intracellular Al(III) concentrations.

4. Conclusions

In conclusion, a new fluorescent turn-on chemosensor was added into the library of Al(III) selective chemosensors. This easy-to-prepare tridentate Schiff base receptor BPB detects Al(III) in the aqueous methanolic medium by giving significant fluorescent enhancement at 430 nm due to the CHEF mechanism. The receptor BPB and Al(III) formed complex species in 2:1 binding stoichiometry. The limit of detection of receptor BPB for Al(III) is much better than the permissible limit of Al(III) concentration in drinking water. More importantly, the receptor BPB showed good cell permeability and detects intracellular Al (III) concentration both qualitatively and quantitatively in living A549 cells.

5. Author's contribution

Nilesh Kshirsagar, Prashant Patil, Jitendra Nandre and Pathan

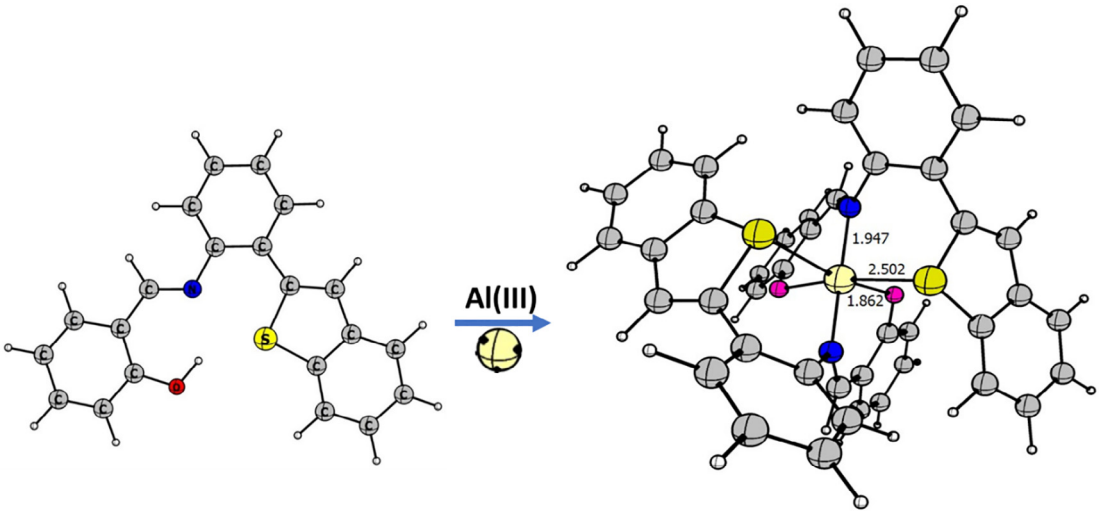

Fig. 6. The computed structure of receptor BPB and its complex with Al(III) by applying the semi-empirical PM6 method.

Sultan: Synthesized the receptor, performed the sensing studies, interpretation of data and drafting of manuscript.

Suman Sehlangia and Chullikkattil P. Pradeep: Recorded the spectra of NMR and Mass

Yue Wang and Lingxin Chen: performed the live cells imaging experiments

Ratnamala Sonawane: Drafting of the manuscript.

Suban K. Sahoo: Conception and design of study, drafting and editing of the manuscript.

Declaration of Competing Interest

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.

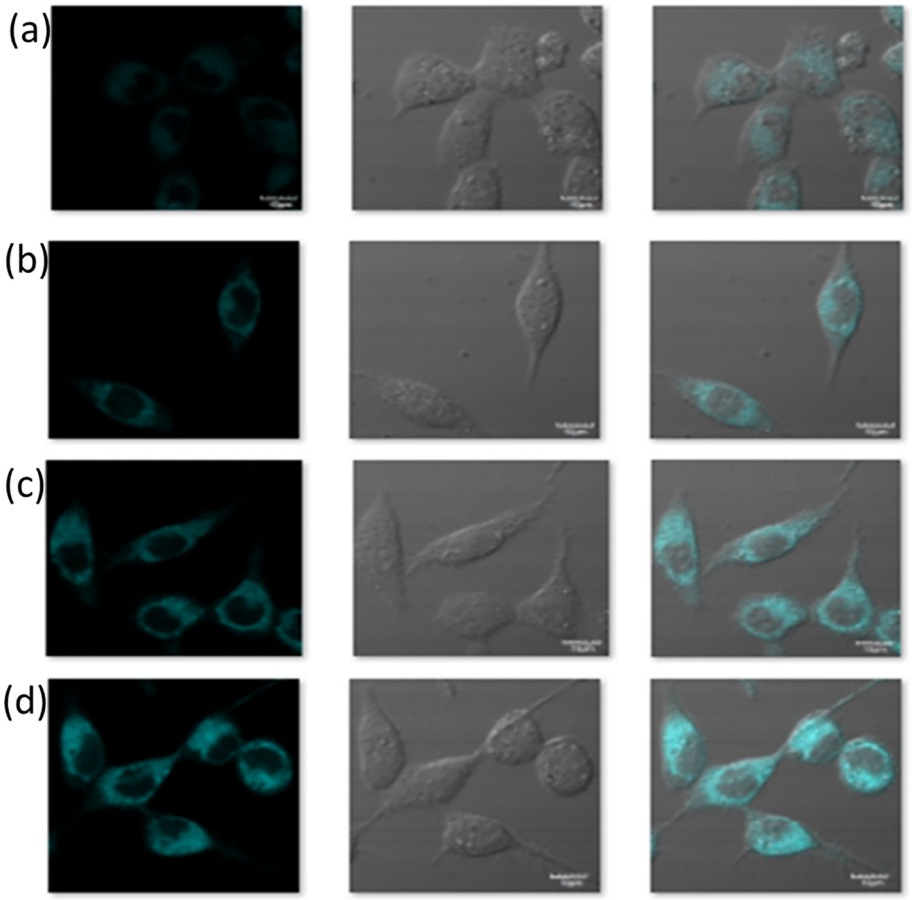

Fig. 7. Fluorescence confocal microscopic images of living A549 cells (left: fluorescence imaging; middle: bright field; right: merged images of fluorescence imaging and bright field): (a) cells loaded with 10 μM of BPB, and cells loaded with both 10 μM of BPB with Al(III) 20 μM (b), 30 μM (c) and 40 μM (d).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ica.2020.119805.

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