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Research paper

A dual-response fluorescent probe for Al^{3+} and Zn^{2+} in aqueous medium based on benzothiazole and its application in living cells

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ABSTRACT

A new benzothiazole-based fluorescent chemosensor (**BZDM**), namely 2-amino-3-(((E)-3-(benzo[*d*]thiazol-2-yl)-2-hydroxy-5-methylbenzylidene)amino)maleonitrile, has been designed and synthesized for detection of Al^{3+}/Zn^{2+} in aqueous medium. The sensing behavior displayed that **BZDM** possessed an excellent selectivity towards Al^{3+}/Zn^{2+} with a distinct fluorescence enhancement in DMSO/Tris-HCl buffer solution. The binding stoichiometry was determined to be 1: 1 between **BZDM** and Al^{3+}/Zn^{2+} according to Job's plot method and NMR analysis. The sensing of Al^{3+}/Zn^{2+} was almost free from interference of other relevant metal ions. Under optimal conditions, the LOD value for Al^{3+} and Zn^{2+} was found to be 7.06 μM and 2.98 μM, separately. Notably, the probe was found to have good biological compatibility and was employed to detect Al^{3+}/Zn^{2+} in living Hela cells.

1. Introduction

Aluminum is one of the abundant elements that make up the lithosphere and soil minerals, which exists as insoluble silicate or alumina. It is widely used in aerospace, metallurgical and chemical industry, automobile industry, agricultural production and our daily life [1–5]. Nevertheless, aluminum has been listed as one of the food pollution sources by World Health Organization (WHO) and the limited concentration of Al^{3+} is 7.41 μM (200 μg L^{-1}) in drinking water. Excessive ingestion of Al^{3+} in the human body can be toxic, which causes various diseases like Alzheimers' disease, Parkinson's disease, osteomalacia and breast cancer [6–9]. However, the sensing of Al^{3+} is still challenging on account of its poor coordination ability and strong hydration ability as well as the lack of spectroscopic characteristics. Hence, it is of great significance to detect Al^{3+} selectively and sensitively in human body.

On the other hand, zinc is one of the most abundant microelement in human body, most of which binds to proteins and plays a crucial role in cell division, immune regulation, signal transmission and gene transcription as cofactor or structural unit [10–13]. Epidemiological studies have shown that zinc deficiency is closely related to specific skin disorders, breast and ovarian cancer, alzheimer's disease and other diseases [14–17]. Meanwhile, selective detection of Zn^{2+} is challenging as it is easily interfered by other transition metal ions like Cd^{2+}. Hence, it is essential to detect Zn^{2+} selectively and sensitively in a physiological environment.

The development of a single chemosensor for multiple analytes has aroused great interest in environmental and biological system. The one-to-more strategy has advantages like low cost, simple sample preparation and easy operation. Nevertheless, the reported probes suffered from a range of problems such as complicated synthesis [18,19], interfered by other transition metal ions such as Cd^{2+} and Fe^{3+} [20,21] and only applied in pure organic solvent [22–25], et al. Hence, the development of novel probes for detecting Al^{3+}/Zn^{2+} in aqueous medium is desirable.

Schiff base derivatives are routinely used as organic ligand to complex metal ions like Al^{3+}/Zn^{2+} [26–29]. Herein, we report a new fluorescent probe **BZDM** based on benzothiazole for sensing Al^{3+}/Zn^{2+} in aqueous medium, which was synthesized as depicted in Fig. 1. We have observed the excellent selectivity of probe **BZDM** towards Al^{3+}/Zn^{2+} in DMSO buffer solution. The LOD value was estimated to be 7.06 μM and 2.98 μM respectively, which was below the permissible limit of Al^{3+}/Zn^{2+} in drinking water defined by WHO. Notably, probe **BZDM** has good biocompatibility and is applied to detect Al^{3+}/Zn^{2+} in Hela cells.

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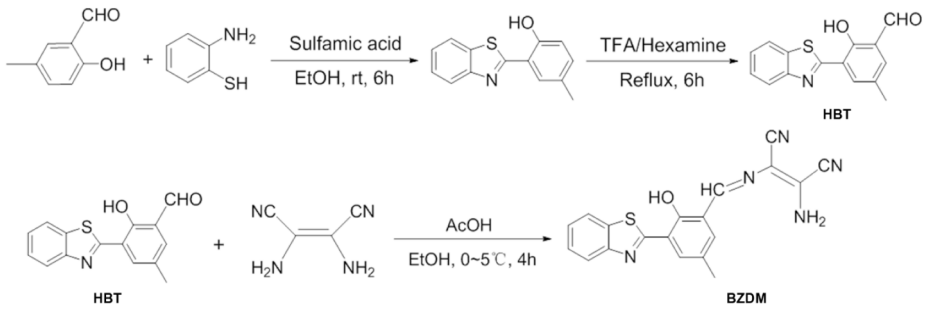

Fig. 1. The synthetic route for probe **BZDM**.

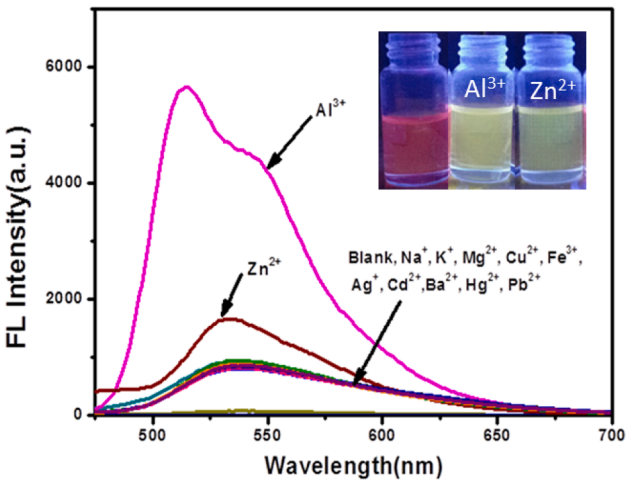

Fig. 2. Emission spectra of probe **BZDM** with or without 50.0 equiv various cations in DMSO/Tris-HCl (pH = 7.2, 8:2, v: v, 10 mM) buffer solution under UV radiation. Insert: images of the **BZDM** solution with 50.0 equiv Al^{3+}/Zn^{2+}.

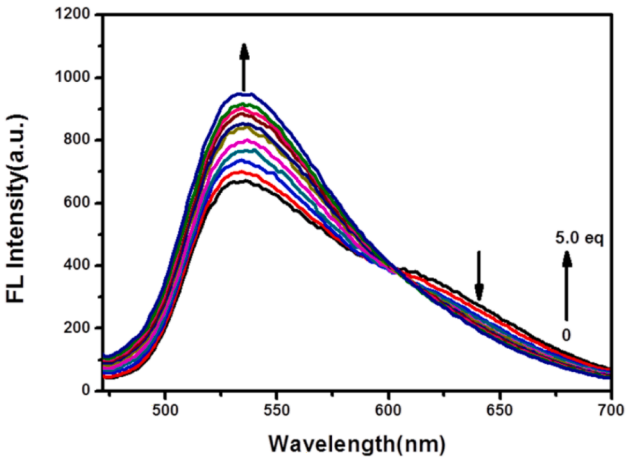

Fig. 3. Emission spectra of probe **BZDM** upon gradient addition of Al^{3+}(0 ~ 5.0 equiv) in DMSO buffer solution at 25 °C.

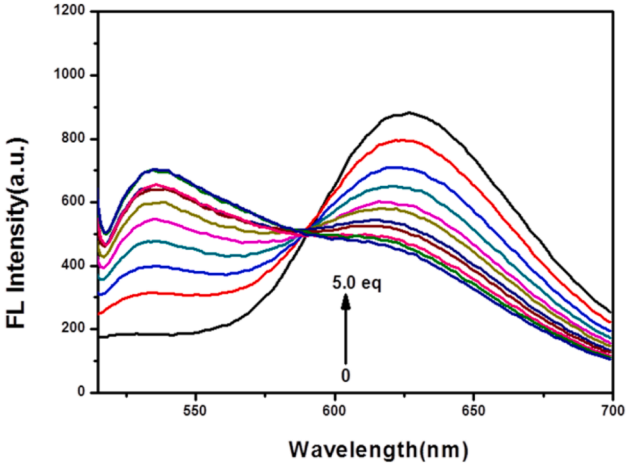

Fig. 4. Emission spectra of probe **BZDM** upon gradient addition of Zn^{2+}(0 ~ 5.0 equiv) in DMSO buffer solution.

2. Experimental

2.1. Materials and methods

All reagents were obtained from Sigma Aldrich without further purification. Dimethyl sulfoxide used was spectral grade. The solutions of various metal ions in their perchlorates (0.01 M) were prepared in deionized water. 3-(benzo[d]- thiazol-2-yl)-2-hydroxy-5-methylbenzaldehyde (HBT) was prepared in the light of previous literature [30].

Fusion point was measured on an X-4 smelting point instrument. FT-IR spectrometry was recorded on a Schimadzu Prestige-21apparatus in the range of 400–4000 cm^{-1}. Fluorescence emission spectra were performed on a Hitachi F-4600 fluorometer and the excitation and emission slit widths were both 5.0 nm. NMR spectra were performed on a Bruker Ultrashield 400 MHz spectrometer at 298 K using tetramethylsilane as internal standard. Mass spectrum was determined on a Shimadzu LCMS-IT-TOF apparatus. Cell imaging was obtained on an Olympus BX50 microscope.

2.2. Synthesis of the probe BZDM

3-(benzo[*d*]thiazol-2-yl)-2-hydroxy-5-methylbenzaldehyde (3.23 g, 12 mmol) and diaminomaleonitrile (1.30 g, 12 mmol) were mixed into 30 mL anhydrous methanol. Three drops of acetic acid were added into it as a catalyst. The solution was heated to reflux and kept for 3 h. The mixture was cooled down and yellow precipitates was obtained after filtration. Finally, the precipitate was dried under vacuum to gain yellow powder. Yield: 74%, m.p. 284 ~ 285℃. FT-IR(KBr, cm^{-1}): 3422, 3309 (–NH_2), 2924, 2854(–CH_3), 1611(Ar, C═C), 1460(C═N) (Fig. S1).^{1}H NMR (400 MHz, DMSO) δ 12.73 (s, 1H), 8.67 (s, 1H), 8.20 (d, J = 7.9 Hz, 1H), 8.15 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.06 (s, 2H), 7.95 (d, J = 1.4

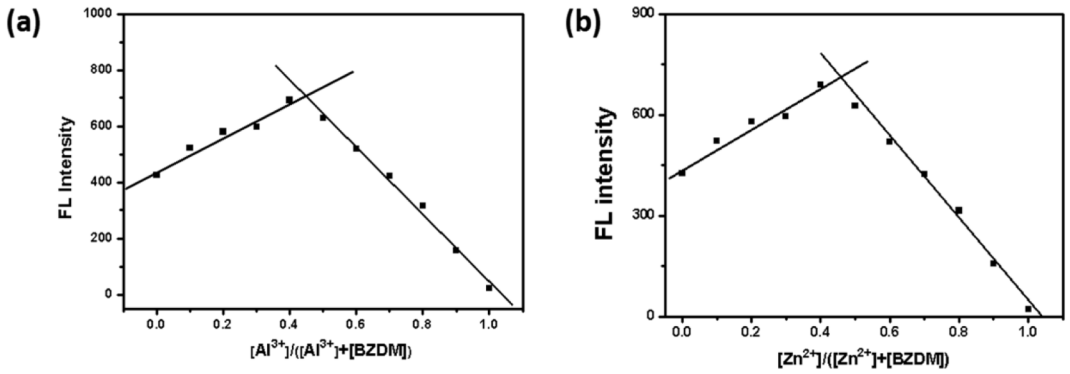

Fig. 5. Job's plot for (a) **BZDM** and Al^{3+}; (b) **BZDM** and Zn^{2+} in DMSO buffer solution.

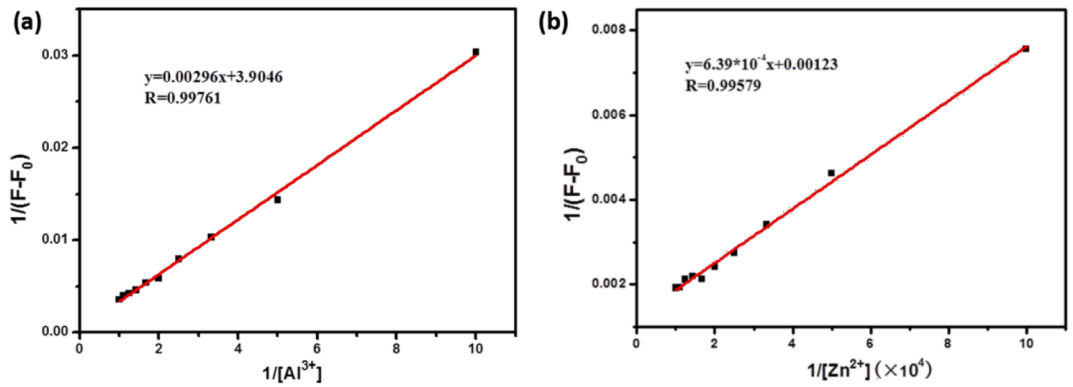

Fig. 6. B-H plot of (a) **BZDM** and Al^{3+}; (b) **BZDM** and Zn^{2+} in DMSO buffer solution.

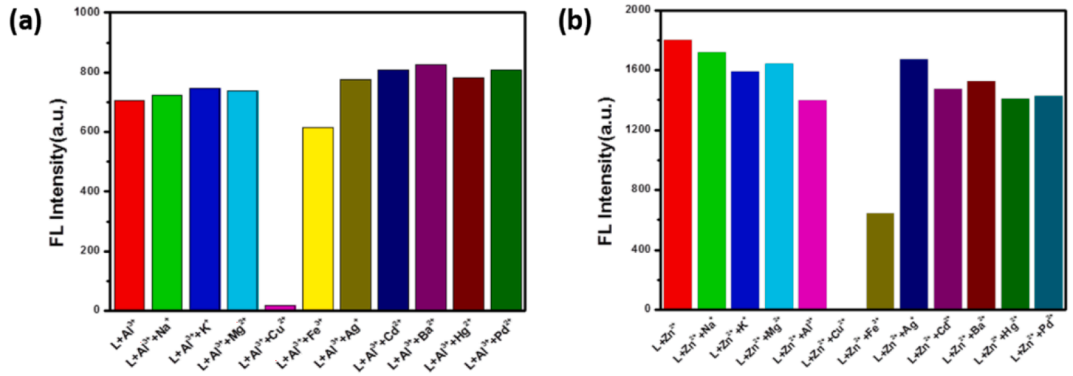

Fig. 7. Effect of coexisting ions on fluorescence emission intensity of (a) **BZDM** + Al^{3+} complex; (b) **BZDM** + Zn^{2+} complex in DMSO buffer solution.

Hz, 1H), 7.60 (t, J = 7.7 Hz, 1H), 7.51 (t, J = 7.2 Hz, 1H), 2.39 (s, 3H) (Fig. S2). ^{13}C NMR (101 MHz, DMSO) δ 167.18 (s), 155.38 (s), 151.71 (s), 151.41 (s), 133.45 (s), 132.66 (s), 132.37 (s),129.42 (s), 127.38 (s), 127.07 (s), 126.24 (s), 123.07 (s), 122.61 (s), 122.50 (s), 118.17 (s), 114.88 (s), 114.19 (s), 103.66 (s), 20.37 (s) (Fig. S3). HRMS: m/z calcd for $C_{19}H_{13}N_5OS$: $[M+H]^+$ 360.0914; found: 360.0918 (Fig. S4).

2.3. Spectra experiments

Stock solutions of different metal ions were made up in deionised water. Stock solutions of probe **BZDM** was prepared in dimethyl sulfoxide and then was diluted with dimethyl sulfoxide and Tris-HCl buffer solution. Subsequently, the selectivity experiments were conducted by adding 50.0 equiv different perchlorates into the above stock solutions. Fluorescence titration spectra was performed by incremental addition of 5.0 equiv Al^{3+}/Zn^{2+} into the above solution. Competitive test was carried out by mixing 1.0 equiv **BZDM** solution and 5.0 equiv Al^{3+}/Zn^{2+} as well as other metal ions.

2.4. Cell imaging

Hela cells was applied in cell imaging assay. Hela cells were cultured in a Roswell Park Memorial Institute 1640 medium at 37℃ with 5% CO_2. The cells were cultivated with **BZDM** and **BZDM** + Al^{3+}/Zn^{2+} for

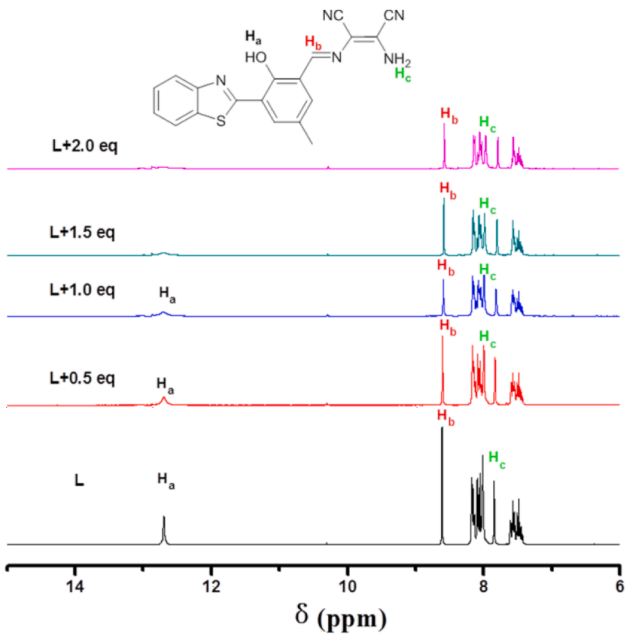

Fig. 8. ^{1}H NMR spectra of **BZDM** with and without various Al^{3+} in DMSO-d_6.

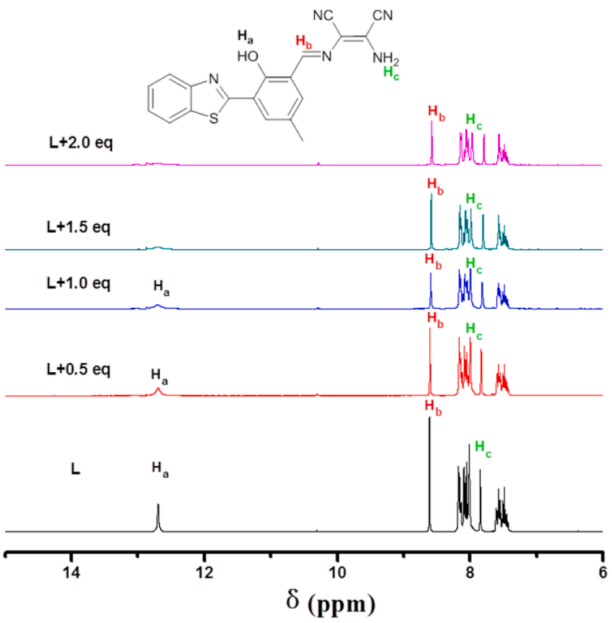

Fig. 9. ^{1}H NMR spectra of **BZDM** with and without various Zn^{2+} in DMSO-d_6.

40 min at 25 °C. Upon incubation, the cells were rinsed with phosphate buffer saline solution 3 times to remove the residual probe. Finally, the bright-field and its respective fluorescence images were obtained under an Olympus BX50 microscope.

3. Results and discussion

3.1. Fluorescence spectra studies

Selective behavior for the analyte is an essential parameter for a chemosensor. Therefore, to investigate the recognition performance of probe **BZDM** for Al^{3+}/Zn^{2+} among surveyed metal ions, fluorescence spectra studies were performed in DMSO/Tris-HCl (pH = 7.2, 8: 2, v/v, 10 mM) solution. As depicted in Fig. 2, free **BZDM** exhibited weak red fluorescence in DMSO/Tris-HCl buffer solution. Upon addition of 50.0 equiv various perchlorates, other ions caused negligible fluorescence changes as well as fluorescence intensity except Al^{3+}/Zn^{2+} when excited at 456 nm. Intriguingly, after addition of 50 eq Al^{3+}, the maximum emission was hypochromatic shifted by 20 nm and an obvious fluorescence intensity enhancement (5.1 fold) at 515 nm accompanied with a shoulder peak at 540 nm was observed, which may be ascribed to the chelation enhanced fluorescence (CHEF) effect [31,32]. Meanwhile, a distinct change in fluorescence color from red to yellow was easily observed by the naked eye when exposed to UV light, which was due to the formation of **BZDM**-Al^{3+} complex. Analogously, the addition of Zn^{2+} induced a relatively small fluorescence enhancement at 536 nm (1.9 fold) and the color of **BZDM** solution also changed like the former. Notably, Cd^{2+}, which had a strong coordination ability towards schiff base, did not show distinct fluorescence change when it was added into the solution of **BZDM**. Hence, these results demonstrated that probe **BZDM** can be employed to detect Al^{3+}/Zn^{2+} selectively in aqueous medium.

In order to study the quantitative sensing characteristics of **BZDM** for Al^{3+}/Zn^{2+}, fluorescence titration experiments were performed in DMSO/Tris-HCl buffer solution. As depicted in Figs. 3 and 4, free **BZDM** displayed the maximum emission at 622 nm. Upon gradient addition of

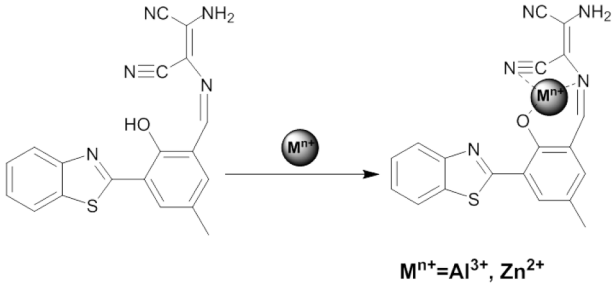

Fig. 10. The plausible coordination mode between probe **BZDM** and Al^{3+}/Zn^{2+} in DMSO buffer solution.

5.0 equiv Al^{3+}/Zn^{2+}, the peak at 622 nm decreased gradually and a new peak appeared at 536 nm accompanied with the fluorescent color change from red to yellow. An obvious isosbestic point was observed at 604 nm and 590 nm for Al^{3+} and Zn^{2+} separately, which indicated a clean conversion of probe **BZDM** into the **BZDM**-Al^{3+}/Zn^{2+} complex [33].

3.2. Stoichiometric ratio

To confirm the binding mode between **BZDM** and Al^{3+}/Zn^{2+}, Job's plot analysis was performed. As depicted in Fig. 5, the emission intensity approached the maximum when the molar fraction of [Al^{3+}]/([Al^{3+}] + [**BZDM**]) and [Zn^{2+}]/([Zn^{2+}] + [**BZDM**]) was 0.45 and 0.46 separately, demonstrating a binding ratio of 1: 1 between **BZDM** and Al^{3+}/Zn^{2+}. Based on fluorescence titration results, the plot of $1/(F–F_0)$ versus 1/[Al^{3+}] or 1/[Zn^{2+}] gave a good linear relationship (R^2 = 0.99761 and 0.99147, respectively) (Fig. 6), which supported the 1:1 binding ratio [34]. The association constant (K_a) of the **BZDM**-Al^{3+}/Zn^{2+} complex was calculated to be 1.32 × 10^7 M^{-1} and 1.92 × 10^4 M^{-1} separately based on Benesi-Hildebrand equation [35], which is within the range of 10^3-10^{14} M^{-1} for the reported Al^{3+}-chemosensors and 10^4–10^{12} M^{-1} for

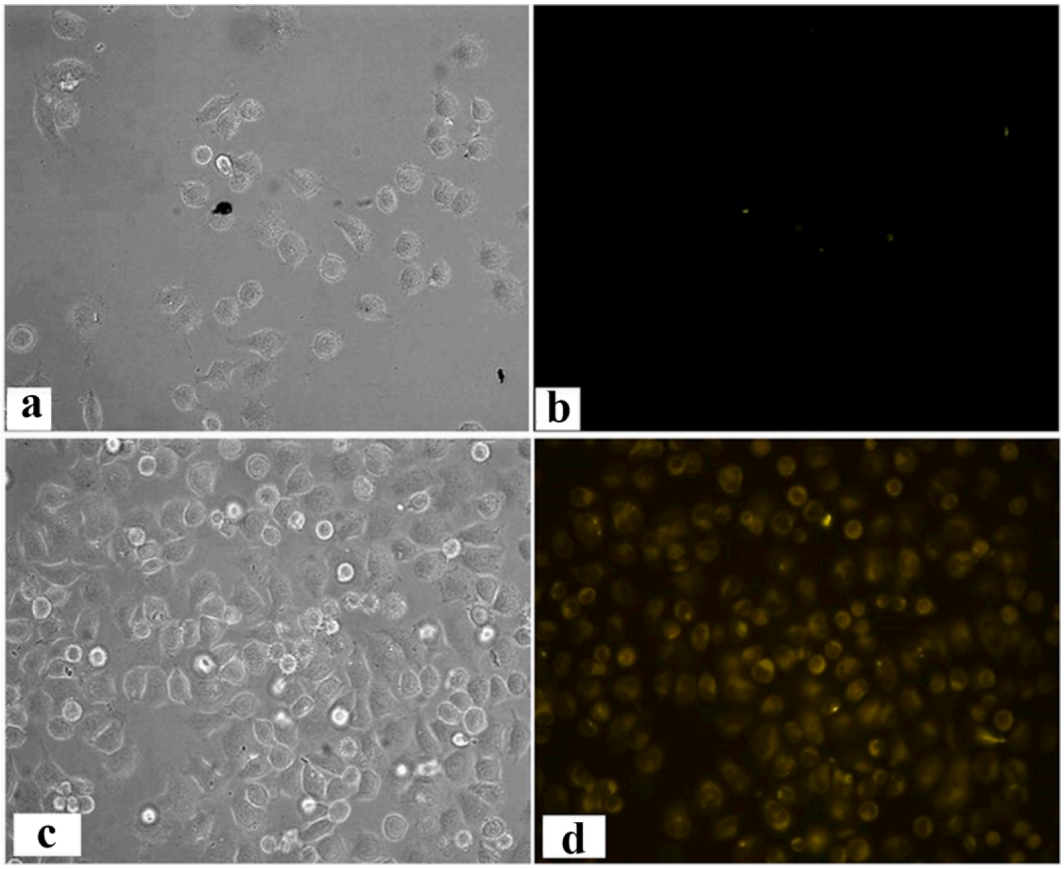

Fig. 11. Bright-field image and its respective fluorescence image of living Hela cells with free **BZDM** (a,b) and with **BZDM** + Al^{3+}(c,d).

the reported Zn^{2+}-chemosensors [36–39]. From the results of fluorescence titration, the limit of detection (LOD, 3σ/K) of probe **BZDM** towards Al^{3+}/Zn^{2+} was estimated to be 7.06 μM and 2.98 μM respectively [40,41] (Figs. S5 and S6), which was below the permissible limit of 7.41 μM for Al^{3+} and 76 μM for Zn^{2+} in drinking water defined by WHO [42,43].

3.3. Competitive test

To evaluate the practical applicability of probe **BZDM** as Al^{3+}/Zn^{2+}-specific sensors, competitive test was performed in DMSO buffer solution. Firstly, 1.0 equiv **BZDM** solution and 5.0 equiv Al^{3+}/Zn^{2+} solution was mixed to prepare mother liquor.

Subsequently, 5.0 equiv other perchlorates was added into the mixture. Finally, fluorescence emission spectra were obtained on a molecular fluorescence spectrometer. The results demonstrated that most metal ions had negligible impact on the detection of Al^{3+}/Zn^{2+} except Cu^{2+} which had an obvious fluorescence quenching effect on account of the paramagnetic effect [44,45] (Fig. 7). In addition, Fe^{3+} also has fluorescence quenching effect in some extent on the detection of Zn^{2+}. Accordingly, probe **BZDM** would not be interfered by most other relevant metal ions, indicating that the probe can be employed to detect Al^{3+}/Zn^{2+} in environmental and biological conditions.

3.4. Sensing mechanism

To shed light on the sensing mechanism of probe **BZDM** towards Al^{3+}/Zn^{2}, ^{1}H NMR titration experiment was conducted in DMSO-d_6. Before addition of Al^{3+}, the signal appeared at 12.73 ppm which was due to the proton of hydroxyl (H_a). The signal at 8.67 ppm was attributed to the proton of imine (H_b). Upon the addition of Al^{3+}, the peak of H_a diminished gradually and disappeared completely as depicted in Fig. 8 when 1.0 equiv Al^{3+} was added, implying the 1: 1 stoichiometry between **BZDM** and Al^{3+}. Meanwhile, the signal of H_b was shifted upfield by 0.02 ppm and a new peak at 9.19 ppm occurred, indicating that nitrogen atom of imine participated in the coordination with Al^{3+} [46]. Meanwhile, the peak of amino (H_c) was shifted upfield by 0.01 ppm, which was ascribed to the coordination of nitrogen atom of cyan to Al^{3+}. Similarly, the ^{1}H NMR titration spectra of Zn^{2+} was almost the same with that of Al^{3+} (Fig. 9). Consequently, a plausible coordination mode was put forward as shown in Fig. 10.

3.5. Cell imaging

To further explore the utility of probe **BZDM** in living cells, cell imaging was conducted on living Hela cells. As depicted in Figs. 11 and 12, no obvious fluorescence was observed before coculturing with Al^{3+}/Zn^{2+}. The cell morphology remains intact upon incubation with Al^{3+}/Zn^{2+}, indicating probe **BZDM** has negligible effect *in vivo* toxicity on Hela cells [47,48]. Notably, upon addition of Al^{3+}/Zn^{2+}, the bright-field and its respective fluorescence image revealed that probe **BZDM** can penetrate the cell membrane and image Al^{3+}/Zn^{2+} with a turn-on yellow fluorescence. The results manifested that probe **BZDM** has the potential to be an effective tool to image Al^{3+}/Zn^{2+} in living cells.

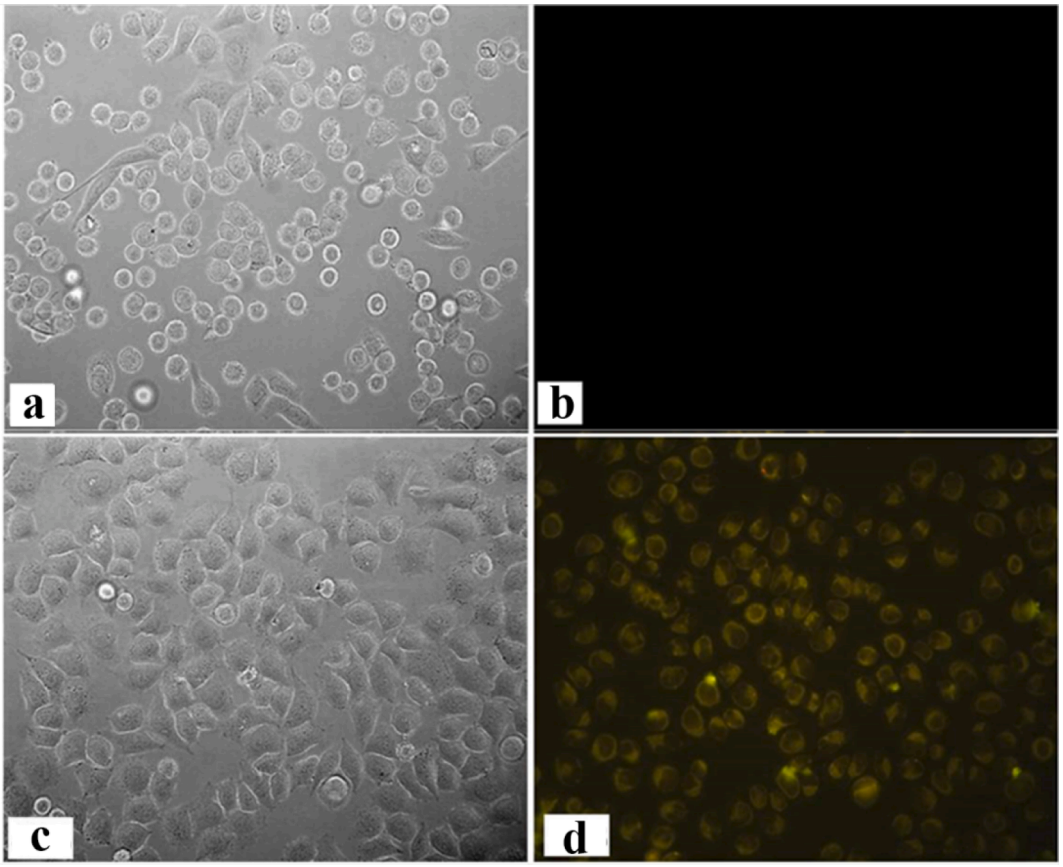

Fig. 12. Bright-field image and its respective fluorescence image of living Hela cells with free **BZDM** (a,b) and with **BZDM** + Zn^{2+}(c,d).

4. Conclusion

To conclude, a new benzothiazole-based fluorescent probe **BZDM** showing dual-response for Al^{3+} and Zn^{2+} have been developed. It displayed a dual-response towards Al^{3+}/Zn^{2+} in aqueous medium with fluorescence color changing from red to yellow. The sensing of Al^{3+}/Zn^{2+} was almost free from interference from other important biological species. The LOD value was calculated to be 7.06 μM and 2.98 μM respectively, which was below the allowable limit of Al^{3+}/Zn^{2+} in drinking water defined by WHO. Furthermore, probe **BZDM** is biocompatible and it can function as a useful tool to detect Al^{3+}/Zn^{2+} in living Hela cells, which will provide guidance for the design of novel Al^{3+}/Zn^{2+}-specfic fluorescent chemosensors in future.

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.

Acknowledgments

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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.2020.120147.

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