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A highly selective fluorescent 'turn-on' chemosensor for Zn^{2+} based on a benzothiozole conjugate, their applicable in live cell imaging and resultant complex as secondary sensor of CN^-

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Abstract

Benzothiozole derivative linked "off-on" multi-responsive and selective chemosensor **3** has been synthesized and evaluated for cation recognition properties. The receptor **3** shows a high sensitivity and selectivity for Zn^{2+} through a turn-on fluorescence response over the other tested cations with the detection limit down to 0.40 μM. The receptor **3** was successfully applied for the detection of Zn^{2+} in live HeLa cells. Then, the Zn^{2+} complex of the receptor **3** was also used for cyanide detection and recognition.

Keywords: Fluorescent 'turn-on' sensor, Zn^{2+}, live cells imaging, secondary sensor, CN^-.

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Introduction

There is bourgeoning needs of the development of cost effective analytical techniques for the selective detection of bioactive analytes such as cations and anions due to their active roles in diversified biological and environmental processes.[1-3] Accordingly, materials constructive for chemosensing of cations and anions based on optical responses (UV-Vis and fluorescence) are extensively investigated. The pitch of fluorescence sensing due to its high sensitivity and selectivity, simplicity and reliability are self-assurance to contribute in solving the long-standing problem of cations and anions sensing. However, the development of chemosensors for the detection of cations and anions from pure aqueous medium are still known to be a complex assignment. In addition, the selective recognition of a target anion is tedious task because of the lower charge to radius ratio, highly solvated, pH sensitive and they come in wide range of different geometries.[4-7]

Zinc, the second most abundant transition metal after iron in mammals known to play an important role in various physiological processes such as catalytic cofactor for a variety of metalloenzymes, stabilization of protein structure, neurotransmission, signal transduction, cellular apoptosis and modulation of interactions between macromolecules.[8-10] However, excessive Zn^{2+} ion will cause imbalance in cellular processes, resulting in neurodegenerative diseases such as Menkes and Wilson diseases, Alzheimer's disease, Parkinson's disease, prostate cancer and diabetes.[11-13] In modern years, the design and development of fluorescent sensors for Zn^{2+} has become an extremely vigorous district in the field of supramolecular chemistry. However, the spectroscopically silent nature of Zn^{2+} ($3d^{10}$) and the interfering effect of Cd^{2+} (due to the similar coordination behaviour of Zn^{2+} and Cd^{2+}) make the research very challenging to develop highly selective sensing system for Zn^{2+}. Herein, we have developed a new fluorescent chemosensing system **3** for the selective detection of Zn^{2+} in DMSO/H_2O (90:10, *v/v*). Further, the complex **3.**Zn^{2+} was utilized for the selective

recognition of cyanide ions by dual-modes optical (chromogenic and fluorogenic) responses in DMSO/H_2O (90:10, *v/v*).

Experimental

All chemical and reagents were obtained from commercial suppliers and used without further purification. Solvents used were purified and dried by standard methods. ^{1}H NMR were measured on a Bruker ARX 400 spectrometer and the chemical shifts were reported in ppm by using TMS as an internal standard. Fluorescence spectra were recorded with a Horiba fluorescence spectrofluorometer at room temperature. Absorption spectra were carried out on a Perkin Elmer spectrophotometer.

Synthesis of receptor 3

The solution of 2,4-diisocyanato-1-methylbenzene (0.17 gm, 1 mmol) in acetone (25 mL) was mixed with the solution of benzo[*d*]thiazol-2-amine (0.30 gm, 2 mmol) in acetone (25 mL) at room temperature. The reaction mixture was stirred and refluxed for 4 hrs. Then, the mixture was cooled and the white colored precipitate was filtered followed by washed with cold ethanol and dried under vacuum. Yield: 82 %; FTIR (Nujol mull, cm^{-1}): 3342, 3051, 2924, 2854, 1637, 1574, 1463, 1377, 1343, 1245,1136, 778,722, 678, 559; ^{1}H-NMR (400 MHz, δ, ppm, DMSO-d_6): 2.36 (s, 3H, -CH_3), 6.1 (s, 4H, NH), 7.04 (d, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.56-8.14 (m, 8H, Ar-H), 7.95 (s, 1H, Ar-H); ^{13}C-NMR (100 MHz, δ, ppm, DMSO-d_6): 111.30, 113.92, 121.31, 122.03, 122.74, 122.86, 125.80, 130.49, 131.32, 136.54, 136.69, 159.43. LC-MS (M+1): Calculated 475.10 found 475.20.

Fluorescence spectral measurements

The metal ions Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+}, Ba^{2+}, Cs^+, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, Cr^{3+}, Zr^{4+} and were added as their nitrate salts where as Sr^{2+} and Mn^{2+} were added as their chloride salts for the fluorescence spectroscopic experiments. Stock solutions of metal ions (1×10^{-3} M) and the receptor **3** (1×10^{-4} M) were prepared in

DMSO/H_2O (90:10, *v/v*). These stock solutions were used for different spectroscopic experiments after appropriate dilution. For the absorbance and fluorescence measurements 1 cm width and 3.5 cm height quartz cells were used. The excitation was carried out at 300 nm for receptor **3** with 5 nm emission slit widths in fluorometer.

Cellular imaging study

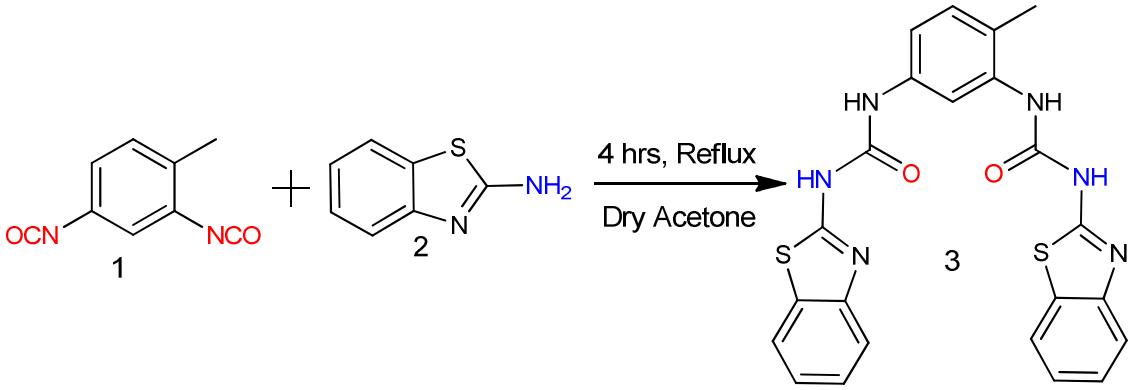
HeLa cells were procured from NCCS, Pune and grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 1% L-glutamine-penicillin *streptomycin*. The cells were maintained at 37°C in a humidified atmosphere provided with 5% CO_2. Cells after reaching 60-70% confluence, were trypsinized and seeded on coverslips placed in 12-well plate and allowed to adhere for overnight. At the time of experiment, complete media was replaced with serum free medium. The cells were incubated with the receptor **3** (1.39 μM) for 2 hours. After 2 hours of incubation with **3**, the cells were then incubated with $ZnSO_4$ (2.5 μM) for further 1 hour. Then, the cells were washed twice with Phosphate Buffer Saline (1X PBS) followed by fixed with 100% methanol for 5 minutes and again washed with 1X PBS for 10 minutes. The cover slip was then mounted on a glass slide using glycerol and then the images were taken under fluorescence microscope Leica DMI 6000B using 20X objective under UV filter. The fluorescence images of cells were captured through an attached CCD camera using LAS software.

Results and Discussion

Chemosensing of Zn^{2+}

The synthesis of receptor **3** was shown in **Scheme 1**. Receptor **1** was obtained by refluxing 2,4-diisocyanato-1-methylbenzene with benzo[*d*]thiazol-2-amine in acetone. The receptor **3** was obtained with quantitative yield and characterized by several techniques such as IR, $^{1}H/^{13}C$-NMR, and mass spectra (**Figure S5-S8,** Supporting information). The spectral

investigation gave consistent data for the molecular structure of **3**. Then, the cations sensing ability of **3** was investigated by fluorescence spectroscopy in DMSO/H_2O (90:10, *v/v*).



Scheme 1. Synthesis of receptor **3**.

The emission spectrum of **3** was obtained in the range 300–600 nm, on excitation at 323 nm. The maximum intensity of fluorescence was observed at 360 nm. This fluorescent receptor **3** using benzothioazole derivative was investigated for the highly selective and sensitive recognition towards Zn^{2+} over to the assessor metal ions such as Na^{+}, K^{+}, Mg^{2+}, Al^{3+}, Cs^{+}, Ba^{2+},Ca^{2+}, Sr^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Zr^{4+} in aqueous media. The change of fluorescence emission spectra of **3** after the addition of different cations is shown in **Figure 1**. Upon addition of Zn^{2+} to the solution of **3**, the weak fluorescence of **3** was selectively and remarkably enhanced with a red shift from 360 nm to 385 nm ($\Delta\lambda$ = 25 nm). In favour of the discriminating enhancement with red shifting of wavelength upon addition of Zn^{2+} ion chelation with receptor **3**, photoinduced electron transfer (PET) from nitrogen of benzothiazole to the phenyl fluorophore of receptor **3** may be responsible for the preliminary feeble fluorescence of receptor **3.**[21] This enhancement of fluorescence intensity was attributed to the formation of a strong complex between **3** and Zn^{2+} which inhibited the conformational isomerization of **3** at the excited state.[14-16.] Then, the metal ions selectivity profile of receptor **3** is shown by a bar diagram (**Figure S1, Supporting information**). The bar diagram clearly delineated that the receptor **3** acts as a selective fluorescent sensor for Zn^{2+} over all the other tested metal ions.

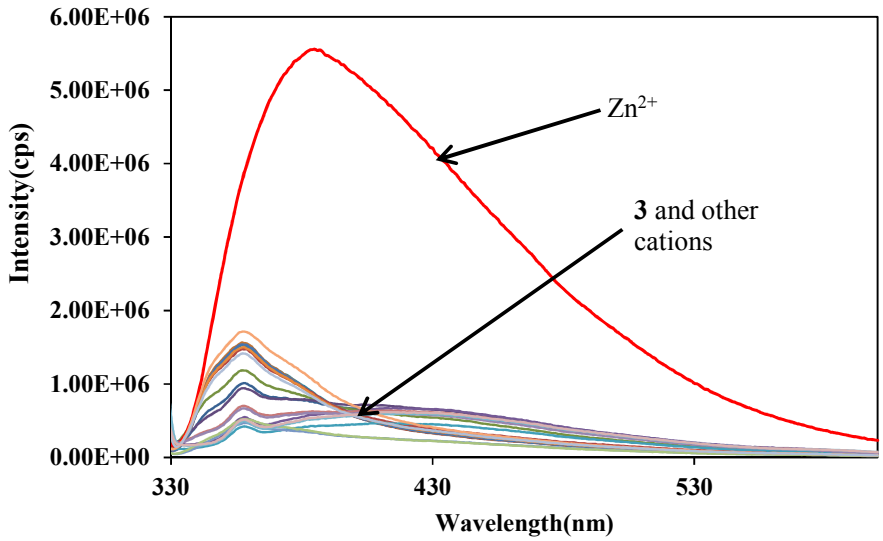


Figure 1. Fluorescence emission spectra of **3** (10 μM) in the absence and presence of 5 equivalents of various metal ions in DMSO/H_2O (9:1, *v/v*) solution.

To check the practical ability of **3** as a fluorescence sensor for Zn^{2+}, the competition experiments were conducted in the presence of Zn^{2+} mixed with other relevant metal ions, such as Na^{+}, K^{+}, Mg^{2+}, Al^{3+}, Cs^{+}, Ba^{2+},Ca^{2+}, Sr^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Zr^{4+} When the receptor **3** was treated with 5 equivalents of Zn^{2+} in the presence of the same concentration of other metal ions (**Figure 2**), the coexistent metal ion had a small and negligible effect on the emission intensity. These results indicate the high specificity of receptor **3** for the selective and sensitive detection of Zn^{2+} in the presence of other interfering metal ions.

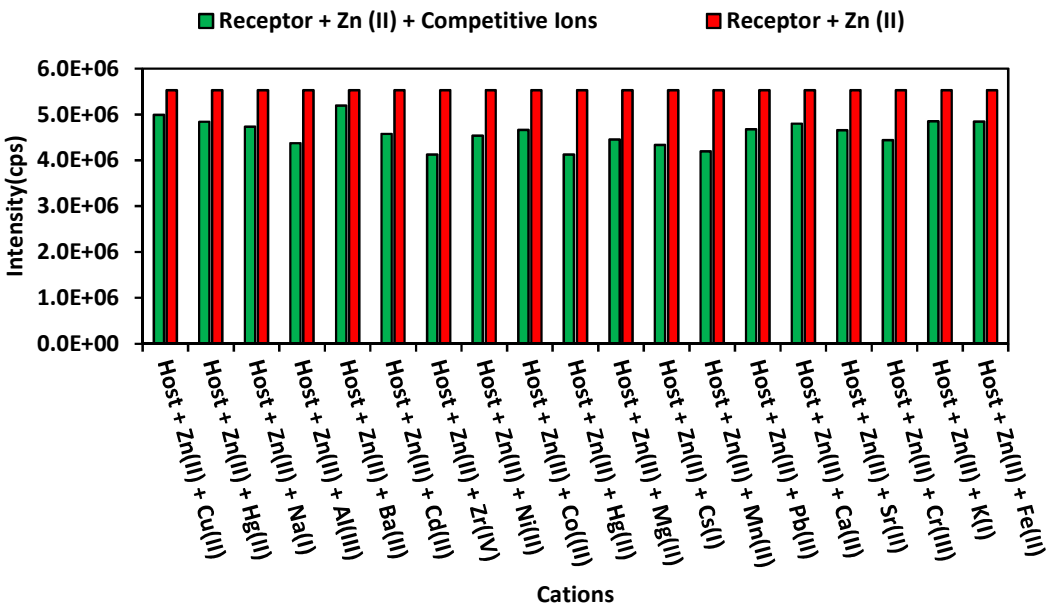


Figure 2. Effect of competitive metal ions on the interaction between receptor **3** and Zn^{2+} ion.

Next, the Zn^{2+} concentrations dependent fluorescence measurements of **3** were carried out to determine the binding ability and limit of detection (**Figure 3**). According to linear Benesie Hildebrand expression, [17] the measured intensity [1/(ΔF)] where $\Delta F=F-F_0$ at 385 nm for receptor **3** was varied as a function of $1/[Zn^{2+}]$ in a linear relationship (R = 0.9938) (**Figure S2**), which indicates the formation of 1:1 stoichiometry between Zn^{2+} and receptor **3**. (Binding constant is 1×10^5 M^{-1}). The 1:1 binding ratio between Zn^{2+} and receptor **3** was also supported by the Job's plot[18] (**Figure S3**). Then, the proposition is further augmented by the peak in the ESI-mass spectrum at m/z (M+1) 539 corresponding to **3**.Zn^{2+} complex.

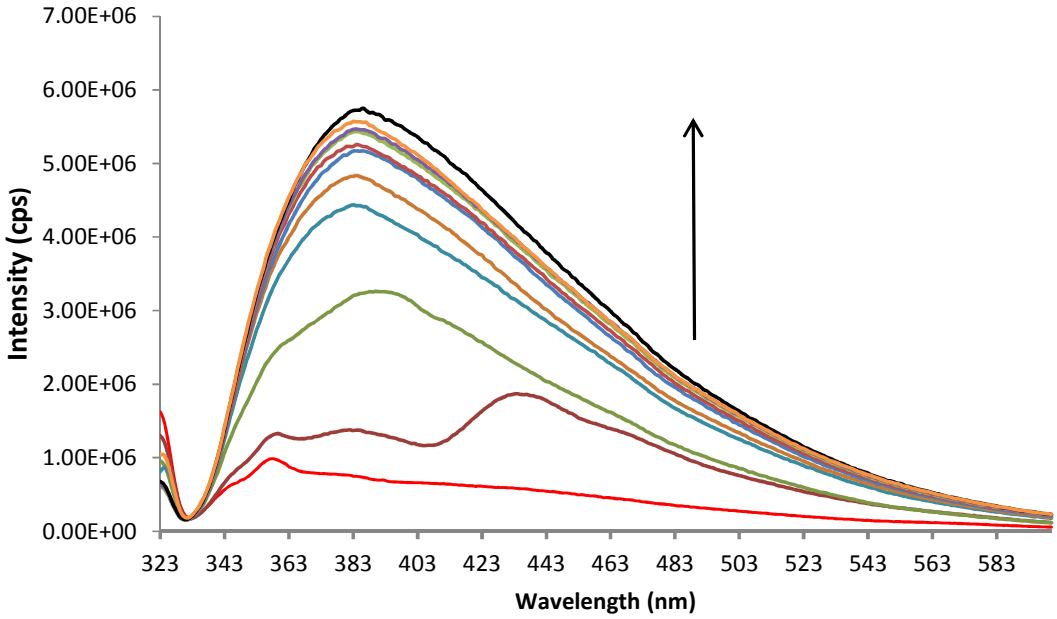


Figure 3. Fluorescence spectra of **3** (1×10^{-4} M) upon incremental addition of Zn^{2+} (1×10^{-3} M) in DMSO: H_2O (9:1, *v/v*).

To determine the detection limit of this receptor for Zn^{2+}, the receptor **3** was treated with different concentrations of Zn^{2+} and then the relative fluorescence intensity at 385 nm was plotted as a function of the Zn^{2+} concentration. Using the calibration curve (**Figure S4**), the detection limit based on the IUPAC definition (CDL= 3 Sb/m) [19] was found to be 0.40 μM from 15 blank solutions. Further, to show the biological usefulness of the receptor **3**, it was utilized for the sensing of Zn^{2+} in living HeLa cells (**Figure 4**). The cells showed no detectable fluorescence upon incubated only with Zn^{2+} (2.5 μM) and a very weak fluorescence when incubated only with **3**. However, an increase in the brightness of the fluorescence was observed when the cells were incubated with **3** (1.39 μM) for 2 hours. Thus, the receptor **3** showed the potential to detect Zn^{2+} *in vitro* cellular system.

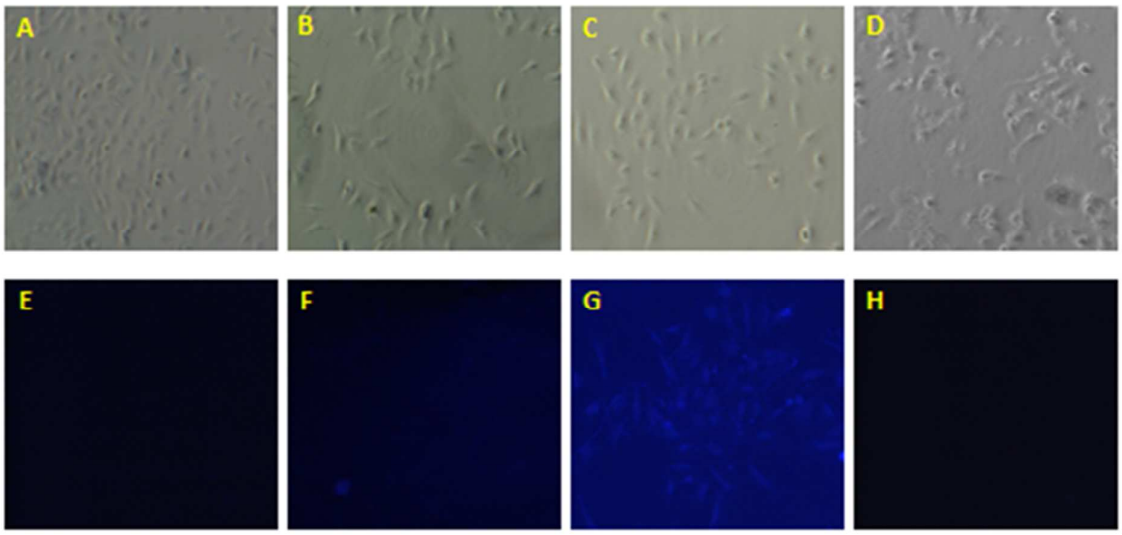


Figure 4. A) Phase contrast image of the control cells, B) Phase contrast image of the cells treated with **3** only, C) Phase contrast image of the cells treated with both **3** and Zn^{2+} (2.5 μM), D) Phase contrast image of the cells treated with Zn^{2+} (2.5 μM) only. E) Fluorescence image of the control cells under UV filter; F) Fluorescence image of the cells treated with **3** only, G) Fluorescence image of the cells treated with **3** for 2 hours, H) Fluorescence image of the cells treated with Zn^{2+} (2.5μM) only.

Chemosensing of CN^-

The zinc complex of receptor **3** was synthesized by refluxing the aqueous solution of zinc nitrate with a solution of **3** in ethanol. The **3.**Zn^{2+} complex was isolated and characterized by ESI Mass, IR and compared through fluorescence spectroscopy. The mass spectrum showed *m/z* at 539, which correspond to $[M+1]^+$ where M is [**3**.Zn^{2+}] (**Figure S9, SI**). The IR spectra of **3.**Zn^{2+} complex indicates that the C=N stretching band of **3** on complexation with Zn^{2+} was shifted to a higher frequency by 10 cm^{-1} confirming that C=N linkages of receptor **3** are coordinating with Zn^{2+} (**Figure S10, SI**). Then, the effect of anions on the fluorescence profile of **3.**Zn^{2+} was examined in DMSO/H_2O (9:1, *v/v*) solvent system. The UV and fluorescence profile of **3.**Zn^{2+} complex showed no considerable change in the presence of any of F^-, C^-, Br^-, I^-, NO_3^-, $H_2PO_4^-$,CH_3COO^- and HSO_4^-. However, addition of CN^- to the **3.**Zn^{2+} complex leads to a red shifting in UV from 277 nm to 326 nm and

enhancement in the fluorescence band at 340 nm. Out of the tested anions, maximum red shifting and enhancement were observed in case of CN^- (**Figure 5** and **6**) presumably due to the intermolecular charge transfer (ICT). Also, the four NH moieties of **3**.Zn^{2+} complex of the amide groups were selectively interacted with CN^- by forming multiple hydrogen bonding interactions. Hence a significant enhancement is observed upon addition of CN^- to the 3.Zn^{2+} complex [**Figure S11**]. The detection limit of **3.**Zn^{2+} complex as a fluorescent sensor for the analysis of CN^- was concluded from a plot of fluorescence intensity as a function of the concentration of the added different amounts of CN^-. It was found that **3.**Zn^{2+} has a detection limit of 18.2 μM for CN^- [**Figure S12**]**.** The association constant *Ka* of **3.**Zn^{2+} for CN^- was calculated on the basis of the Benesi-Hildebrand plot [17] [**Figure S13**] and it was found to be 3.33×10^3 M^{-1}.

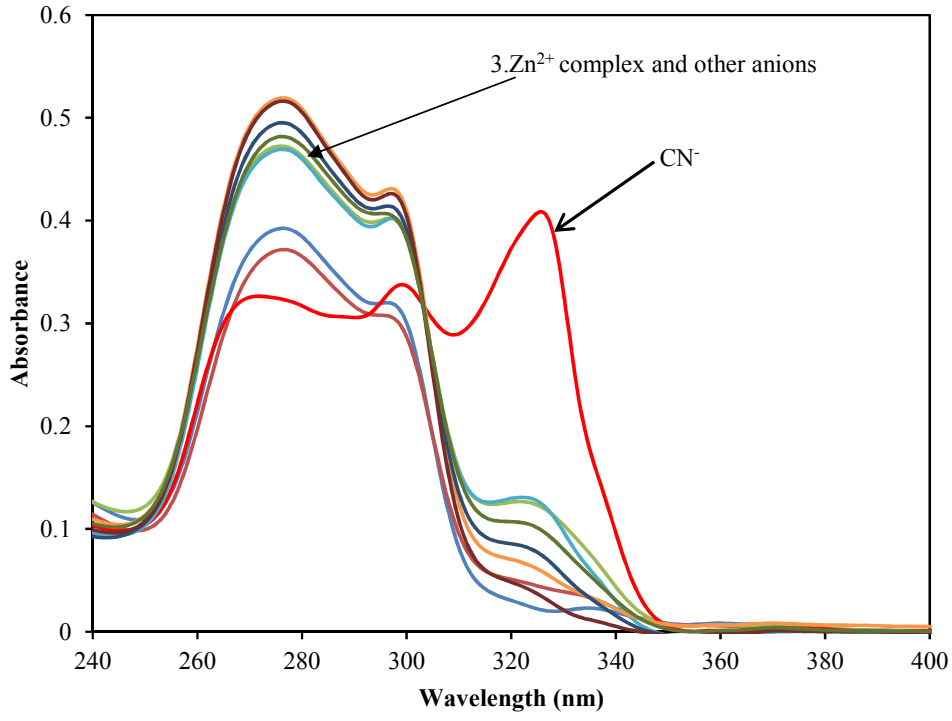


Figure 5. UV-visible spectra of **3.**Zn^{2+} (10 μM) upon addition of 20 μM of tetrabutyl ammonium salts of different anions in DMSOF/H_2O (9:1, v/v) solvent system.

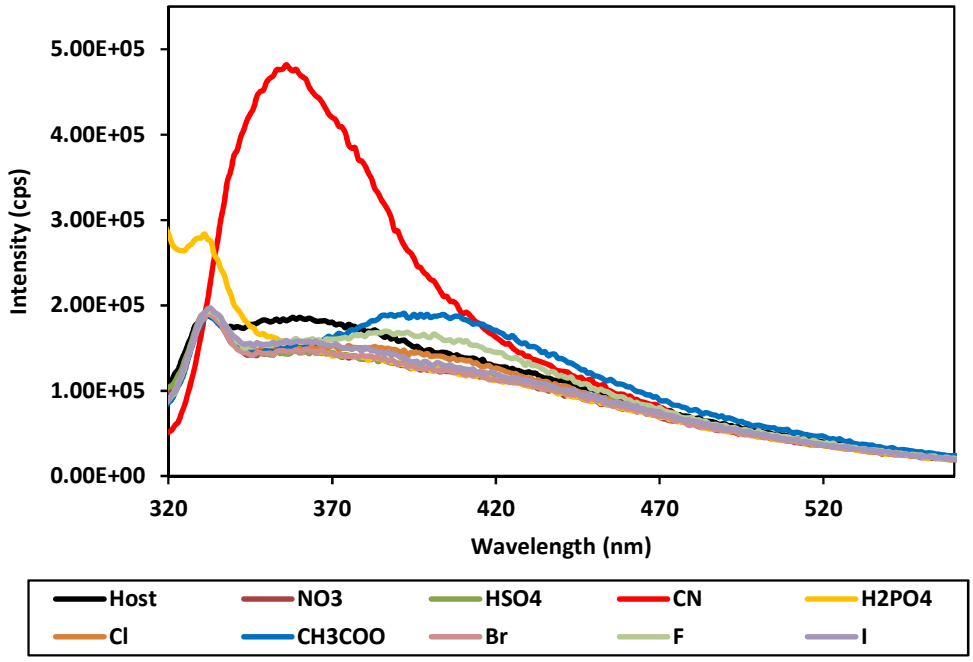


Figure 6. Changes in fluorescence intensity of **3.**Zn^{2+} (10 μM) upon addition of 20 μM of tetrabutylammonium salts of different anions in DMSO/H_2O (9:1, v/v) solvent system.

The structural optimization of **3** and its **3.**Zn^{2+} complex was performed in the gas phase by applying the DFT method (B3LYP/6-31G**/LANL2DZ) coded in the computational program Gaussian 09W.[20] The basis set LANL2DZ was considered only for Zn atom whereas 6-31G** for remaining atoms (C, H, N and S). As shown in **Figure 7**, the optimized structure of **3** was stabilized by the presence of multiple hydrogen bonds and was not properly preorganized to encapsulate both Zn^{2+} and cyanide ions. However, on interaction with Zn^{2+}, the orientation of two urea groups of **3** was found suitable for to encapsulate anions through intermolecular hydrogen bonds. Further, the frontier molecular orbitals (FMOs) plots (**Figure 8**) of **3** and its **3.**Zn^{2+}.CN complex was analyzed which indicate the intramolecular charge transfer occurred in the receptor upon interaction with cyanide ions. Also, the band gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of **3** was lowered on formation of **3.**Zn^{2+}.CN complex.

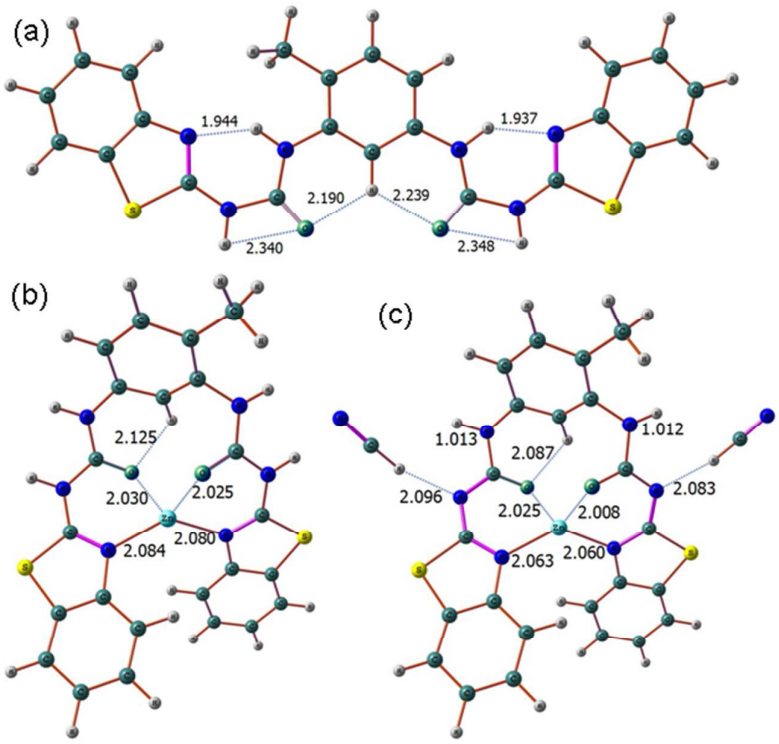


Figure 7. DFT computed optimized structure of **3, 3.**Zn^{2+} and **3.**Zn^{2+}.CN.

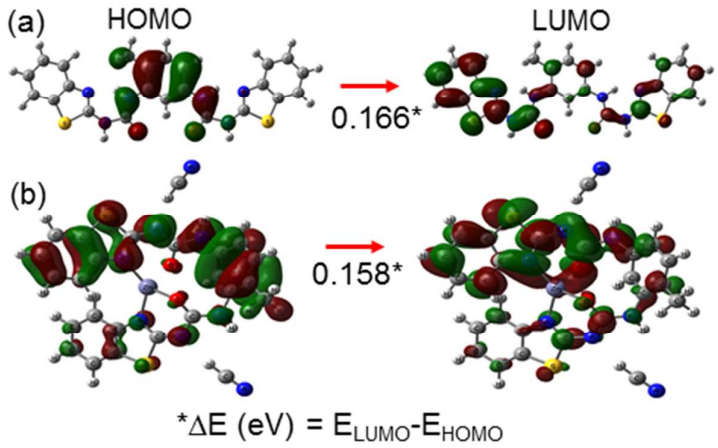


Figure 8. DFT computed HOMO and LUMO diagrams of (a) **3** and (b) **3.**Zn^{2+}.CN.

Conclusion

In conclusion, we have developed an "off-on" type chemosensor **3** for Zn^{2+}. The receptor showed a high sensitivity and selectivity for Zn^{2+} detection at concentrations ranging from 0 to 1 μM with the detection limit of 0.40 μM. The receptor **3** showed the potential to detect Zn^{2+} *in vitro* cellular system. Furthermore, the changes in the fluorescence signature of the **3.**Zn^{2+} complex in the presence of cyanide anion are significantly promising. Also, the cation dislocation approaches utilize a metal-complex for anion recognition permits proposing innovative receptors for sensitive anion detection in aqueous solutions.

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