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Highly selective fluorometric sensing of Cu^{2+} and Hg^{2+} by using a benzothiazole based receptor in semi-aqueous medium and molecular docking studies

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Abstract

A new chemosensor (*Z*)-ethyl 2-((*Z*)-2-(benzo[d]thiazol-2-ylimino)-4-oxo-3-phenylthiazolidin-5ylidene)acetate (receptor **1)** is designed and synthesized in catalyst free condition. Receptor **1** is characterized by various spectroscopic techniques and finally solved by single crystal X-ray diffraction. Receptor **1** is building on the basis of internal charge transfer (ICT) mechanism with benzothiazole unit, and the results are complemented with density functional theory (DFT) calculations. In CH_3OH/H_2O (50:50, *v/v*) medium, the emission of receptor **1** is quenched significantly in the presence of Cu^{2+} and Hg^{2+} among the other tested metal ions. Receptor **1** formed complex in a 1:1 stoichiometry with Cu^{2+} and Hg^{2+} and the detection limit is found to be 0.36 μM and 2.49 μM, respectively. In addition to the sensing study, the nature of interactions of **1** with Aldose Reductase Inhibitor is also performed by molecular docking studies.

Introduction

The 2-iminothiazolidin-4-one core is a key advantage of heterocyclic scaffold[1] in numerous biologically active pharmacophores[2] and their synthesis has emerged as one of the availing topics in the field of heterocyclic chemistry.[3] The motifs carrying the 2-iminothiazolidin-4-one core reported to have a wide range of biological activities (Fig. 1) , which include anticancer,[4] anti-HIV,[5] antidiabetic,[6] potent antiproliferative,[7] antitumour,[8] cardiovascular,[9] antimicrobial,[10] antitubercular,[11] antihypersensative[12] etc. Organic fluorescent probes have significant submissions in a broad assortment of regions such as material sciences, biochemical and environmental processes.[13-14] The importance of the design and synthesis of new benzothiazole based receptor to recognize metal ions has been a considerable subject due to their fundamental role in biological, environmental, and chemical processes.[15] However, up till now, very limited molecular sensing systems have been reported to use in semi-aqueous media at physiological conditions.[16] Developments such as molecular recognition studies which often rendered as host guest chemistry or molecular gratitude of cations or anions can be deliberate by the assist of fluorescent systems.[17,18] Organic probes containing benzothiazole moiety have get attention due to the glowing possessions of this species. For example, non-cyclic structures containing the benzothiazole moiety have been found to bind with Al^{3+}.[19]

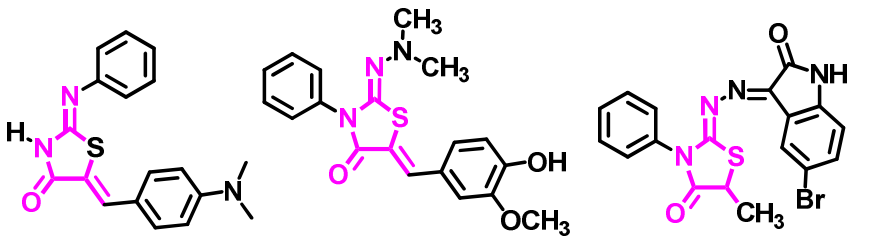
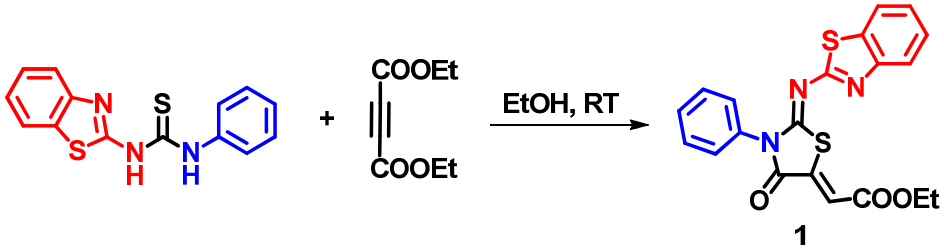


Fig. 1 Biologically active compound having iminothiazolidin-4-one unit

Our continuation exploration on heterocyclic molecules,[20] herein, envisioned the highly efficient, straight forward regioselective synthesis of (*Z*)-ethyl 2-((*Z*)-2-(benzo[d]thiazol-2-ylimino)-4-oxo-3-phenylthiazolidin-5ylidene)acetate **1** from benzothiazole based thiourea and diethyl acetylene dicarboxylates under catalyst free conditions in ethanol (**Scheme 1**). The cation recognition study revealed that the fluorescence of receptor **1** was selectively quenched in the presence of Cu^{2+} and Hg^{2+} over other tested cations. Spectroscopic studies confirmed the formation of receptor **1** and Cu^{2+}/Hg^{2+} ions complex in 1:1 binding stoichiometry. Various analytical parameters such as limit of detection (LOD), association constant (*Ka*), interference of other metal ions were obtained through spectroscopic methods. Docking studies were undertaken to gain insight into the binding continuum of the investigated receptor **1** in the active site of aldose reductase (ALR2). The predicted interactions elucidate the observed aldose reductase inhibitory activity.



Scheme 1 Synthesis route of receptor **1**

Experimental

All commercial grade chemicals and solvents were procured and used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Varian NMR mercury system 300 spectrometer operating at 400 and 100 MHz respectively in $CDCl_3$. The fluorescence and UV-visible spectra were recorded in CH_3OH/H_2O namely on fluoromax-4 spectrofluorometer and Shimadzu UV-24500 in the range of 200-600 nm respectively, at room temperature using quartz

cuvette of 1 cm path length. X-ray crystallographic data was measured on a Nonius Kappa CCD diffractometer at 150 K using an Oxford Cryosystems Cryostream Cooler. The data collection strategy was set up to measure an octant of reciprocal space with a redundancy factor of 4.6, which means that 90% of these reflections were measured at least 4.6 times. Phi and omega scans with a frame width of 0.5˚ were used. Data integration was done with Denzo and scaling and merging of the data was done with Scalepack. The structure was solved with direct methods procedure in SHELXS-97. Full-matrix least-squares refinements based on F^2 were performed in SHELXL-2013, as incorporated in the WinGX package. Neutral atom scattering factors were used and include terms for anomalous dispersion.

Spectroscopic studies

For spectroscopic study, all solutions were prepared in ultrapure water and HPLC grade methanol. Solutions of receptor **1** (*c*=1 mM and 0.01 mM) was prepared in CH_3OH/H_2O (50:50, *v/v*). Similarly, the stock solutions of all metal ions (*c*=1 mM) were prepared in CH_3OH/H_2O (50:50, *v/v*) followed by the corresponding working solutions (*c*=0.1 mM) were prepared by dilution. The cation binding assay was performed on a Fluoromax-4 spectrofluorometer towards different metal ions (Hg^{2+}, Zn^{2+}, Ag^{+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Al^{3+}, K^{+}, Cs^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Na^{+} and Co^{2+}) with receptor **1** in CH_3OH/H_2O (50:50, *v/v*) at room temperature. The fluorescence intensity was recorded at $\lambda_{ex}/\lambda_{em}$=365/405 nm alongside a reagent blank. The excitation and emission slits were set to 5.0 nm. The association constant (K_a) and limit of detection (LOD) was calculated from the titration experiments, which were performed between receptor **1** and Cu^{2+}/Hg^{2+}. The titrations were performed by successive incremental addition of metal salt solutions (*c* = 0.1 mM) to a fixed volume of receptor **1** solution (*c* = 0.01 mM) in 10 ml volumetric flask. The stoichiometry of the complex **1**-Cu^{2+}/Hg^{2+} was determined by mixing

the receptor **1** solution with respective cations (Cu^{2+} and Hg^{2+}) in the different ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The fluorescence intensity at λ_{em}=405 nm was used for further calculations.

Results and Discussion

Our initial investigation revealed that, as reaction proceed towards the formation of key receptor **1**, the color of the reaction mixture changes from white to yellow. Finally at the end of the reaction, we found the formation of a yellow precipitate. The obtained yellow precipitate was filtered, dried and after recrystalization subjected to spectroscopic analysis. For **1**, there was an ambiguity to conclude whether the product obtained was (*Z*)-ethyl 2-((*Z*)-2-(benzo[d]thiazol-2-ylimino)-4-oxo-3-phenylthiazolidin-5-ylidene)acetate or (*Z*)-ethyl 2-((*Z*)-3-(benzo[d]thiazol-2-yl)-4-oxo-2-(phenylimino)thiazolidin-5-ylidene)acetate, as both diastereomers have almost similar FT-IR, ^{1}H NMR, ^{13}C NMR and LC-MS (Fig. S1-4). Finally, **1** was crystallized out from a mixture of chloroform and hexane (1:9). X-ray crystallography of the synthesized key receptor unequivocally confirmed that benzothiazole unit on imino component in structure (**Fig. 2**). The crystallographic data are listed in (**Fig. S13**). The CIF file for receptor **1** was deposited in the Cambridge Structural Database with CCDC no. 999181. The structure was resolved by the direct methods procedure in SHELXS-2013/1.[21] Full-matrix least-squares refinements based on F^2 were performed in SHELXL-2014/1,[22] as incorporated in the WinGX package.

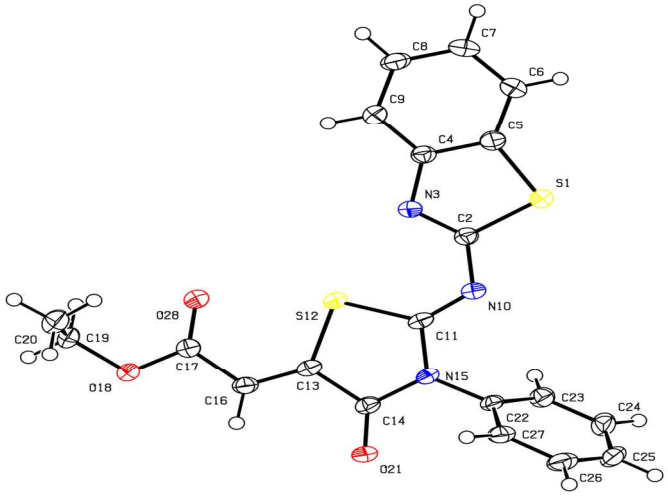
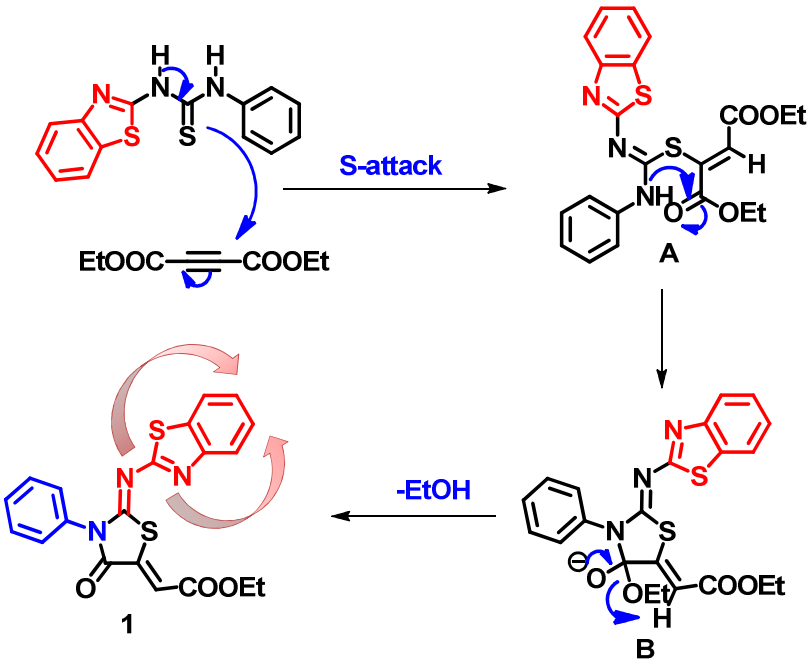


Fig. 2 A view of the receptor **1**, showing the atom-labelling scheme. Displacement ellipsoids are drawn at the 50% probability level and H atoms are represented by circles of arbitrary size.

A plausible mechanism for the **1** is outlined in **Scheme 2**. In concordance with reports from the literature,[23] we suggest that the sulfur atom (soft nucleophile) of the thiourea will preferentially attack activated alkynes (soft electrophile) giving **A** and NH (hard nucleophile) will then attack the carbonyl centre (hard electrophile) and finally the **1** obtained by elimination of ethanol from **B**. Interestingly, the enolization of thiourea takes place from the benzothiazole of the substrate due to the higher acidity of the NH proton flanked between benzothiazole and thiocarbonyl moiety.



Scheme 2 Reaction mechanism of receptor **1**

The cation recognition ability of the receptor **1** towards different cations (Hg^{2+}, Zn^{2+}, Ag^{+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Al^{3+}, K^{+}, Cs^{2+}, Cd^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Na^{+} and Co^{2+}) was investigated by fluorescence spectroscopy in CH_3OH/H_2O (50:50, *v/v*) medium. As shown in Fig. 3, the receptor **1** showed a broad fluorescence emission profile between 370-500 nm with maximum emission at 405 nm (λ_{exc}=365 nm). Upon subsequent addition of Cu^{2+} and Hg^{2+} to the solution of receptor **1**, the fluorescence intensity was remarkably quenched along with the red shift in emission band to 433 nm. However, there were no significant changes observed in the fluorescence emission profile of receptor **1** in the presence of other tested cations. The bar diagram of the fluorometric response of **1** towards the surveyed metal ions is shown in Fig. S5. The results clearly indicate a highly selective response of receptor **1** towards Cu^{2+} and Hg^{2+} ions as compared to the other surveyed metal ions. The fluorescence quenching of receptor **1** can be attributed due to the paramagnetic nature of Cu^{2+} ion[24,25] and the spin-orbit coupling effects in case of Hg^{2+}.[26]

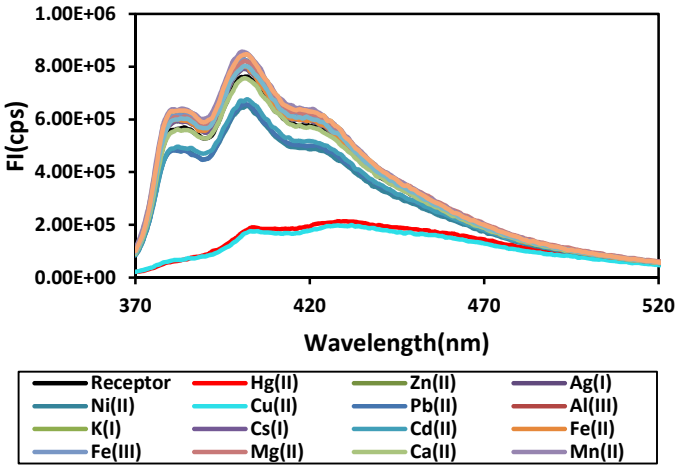


Fig. 3 Fluorescence emission spectra of receptor **1** (10 μM) in the presence of various cations (1 equiv) in CH_3OH/H_2O (50:50, *v/v*).

To understand the binding mechanism and to determine the detection limit, the spectrofluorimetric titrations of receptor **1** with Cu^{2+}/Hg^{2+} were performed in CH_3OH/H_2O (50:50, *v/v*) medium. The observation of decrease in the emission intensity of **1** at 405 nm clearly indicates the formation of a metal complex in solution as shown in Fig. 4 and 5. The calculated association constant for the **1**-Cu^{2+} and **1**-Hg^{2+} complexes was respectively 5.70 $\times 10^6$ M^{-1} and 1.73 $\times 10^5$ M^{-1} by applying the Stern-Volmer equation (Fig. S6-7).[27] The Stern-Volmer plot (F_o/F *vs* concentration of guest [G]) gives a straight line which confirmed the formation of a 1:1 equilibrium complex **1**-Cu^{2+}/Hg^{2+} (Fig. S8-9).[28] The binding stoichiometry was verified by the continuous variation method (Job's plot) [29] and showed that the **1**-Cu^{2+}/Hg^{2+} complexes formed with 1:1 stoichiometry (Figure S10-11). The detection limit based on the IUPAC definition (CDL= 3 Sb/m) was found to be 0.36 μM for Cu^{2+} and 2.49 μM for Hg^{2+}.[30] Further, the fluorescence quantum yield (ϕ) of receptor **1** (ϕ = 0.011), **1**-Hg^{2+} (ϕ = 0.0054), and **1**-Cu^{2+} (ϕ = 0.0044) were measured using quinine hemisulfate monohydrate salt as standard reference

material. It was noted that, upon complexation with cations, the corresponding quantum yield of **1** noticeably decreases, which is consistent with the fluorescence spectra attributed.

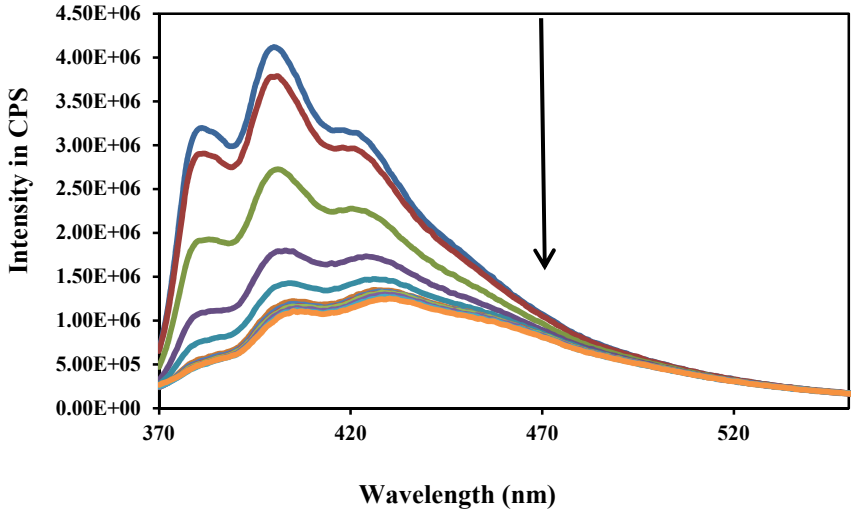


Fig. 4 Fluorescent titration of receptor **1** (10 μM) upon addition different concentrations of Cu^{2+}.

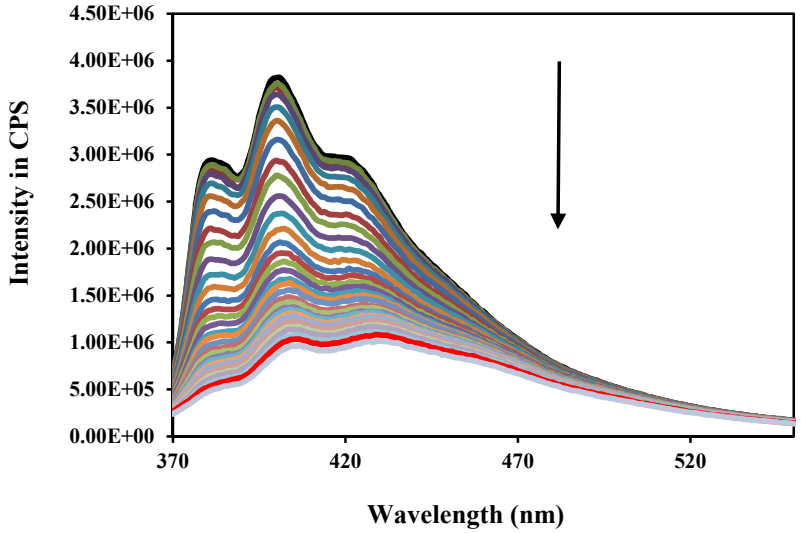


Fig. 5 Fluorescent titrations of receptor **1** (10 μM) upon addition different concentration of Hg^{2+}.

The competition experiments were conducted in the presence of 0.5 equiv. of Cu^{2+} and Hg^{2+} mixed with excess of other metal ions (2 equiv.). The fluorescence contour of receptor **1**

with Cu^{2+} and Hg^{2+} was not affected in the co-existence of other interfering metal ions in excess (**Fig. 6 and 7**). These results clearly delineated that the receptor **1** showed a good sensitivity and selectivity towards Cu^{2+} and Hg^{2+} over other competitive metal ions.

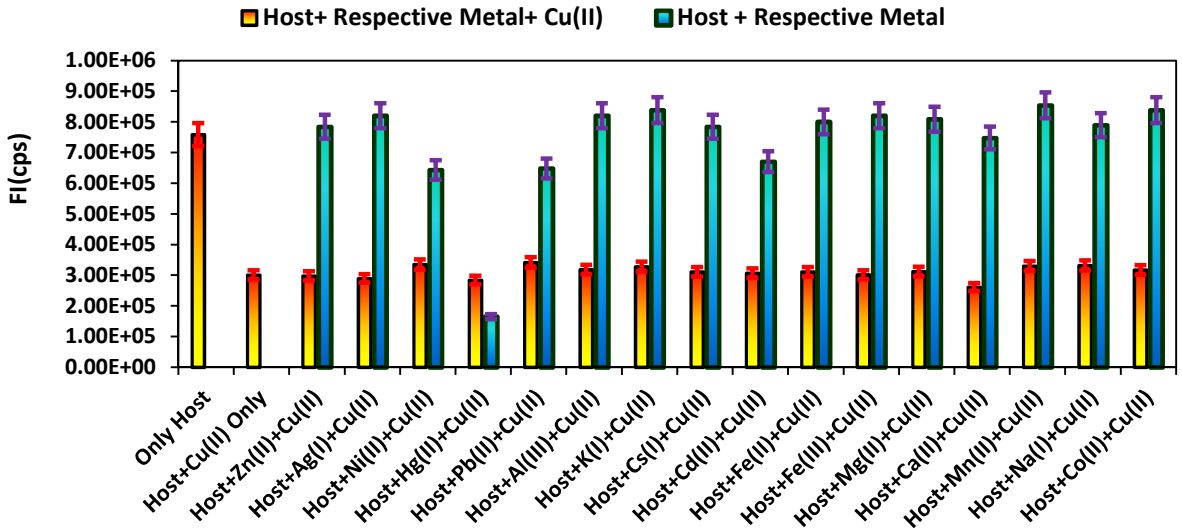


Fig. 6. Effect of competitive metal ions on the interaction between receptor **1** and Cu^{2+} ion.

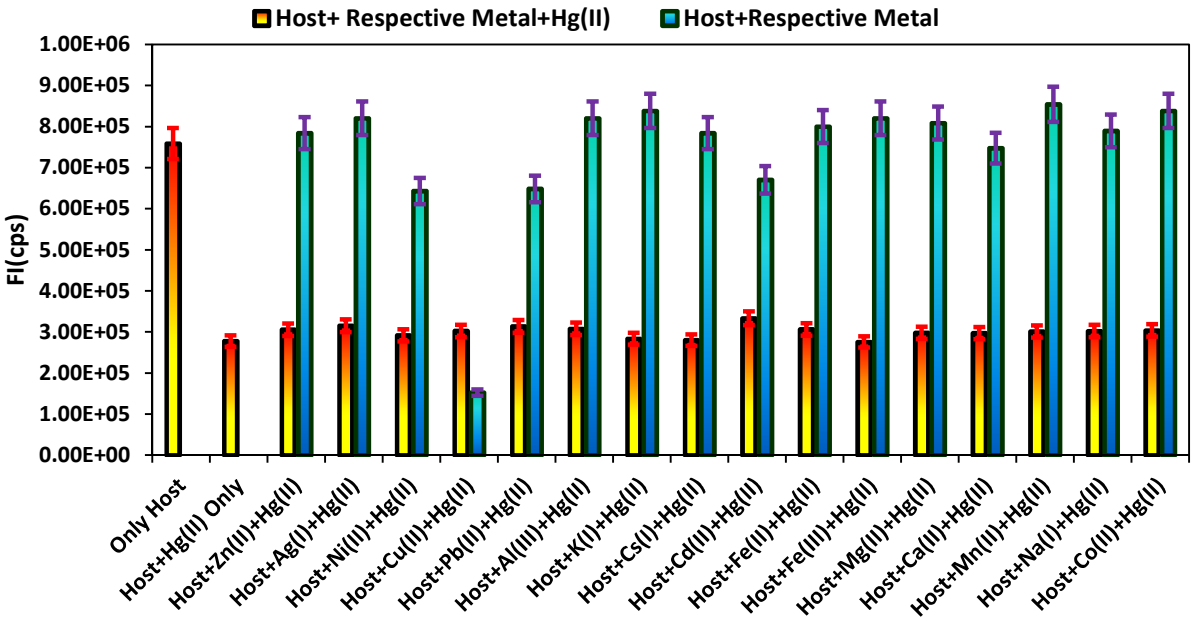


Fig. 7. Effect of competitive metal ions on the interaction between receptor **1** and Hg^{2+} ion.

Finally, the structure of the metal complexes, **1**-Cu^{2+}/Hg^{2+}, were predicted by using the density functional theory (DFT) method in order to get fuller understanding on the internal charge transfer (ICT) occurred between the receptor **1** and the respective metal ions during the complex formation (Fig. 8). The exchange-correlation functional B3LYP with the basis sets 6-31G(d,p) for S, O, C, N and H atoms whereas LANL2DZ for Cu^{2+} and Hg^{2+} was employed for the calculations by applying the computation code Gaussian 09W.[31] The computed structure of receptor **1** was found to be identical to the experimental structure, where the receptor provided a pseudo cavity with the three donor O, S and N atoms to form complex with Cu^{2+}/Hg^{2+} (Fig. 8a). Upon complexation of receptor **1** with Cu^{2+}/Hg^{2+}, there is an increase in the stability of the whole system which can be ascertained from the lowering in the interaction energy (E_{int} = $E_{complex}$-$E_{receptor}$-E_{Hg}^{2+}/Cu^{2+}) by -316.28 kcal/mol and -203.44 kcal/mol respectively. (Fig. S12) Also, the calculated E_{int} clearly delineated that the binding affinity of receptor **1** towards Cu^{2+} is higher than the Hg^{2+} that corroborates well with the experimentally calculated binding constants. Moreover, the band gap between HOMO-LUMO of **1** becomes lower for **1**-Cu^{2+}/Hg^{2+} complexes (Fig. 8b, 8c). The lowering of band gap was observed due to the possible charge transfer process occurred between the receptor **1** and the metal ions, which also responsible for the nonradiative decay of the excited state of the **1**-Cu^{2+}/Hg^{2+} complexes.

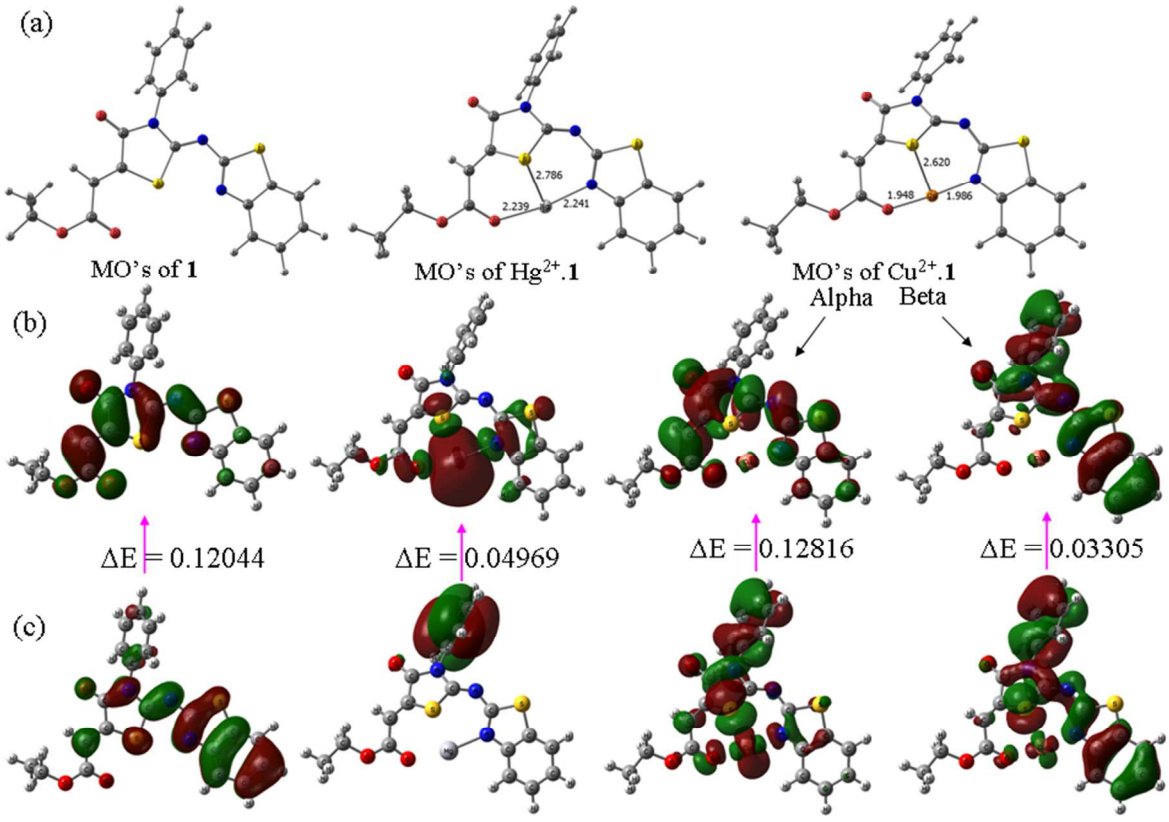


Fig. 8 DFT computed (a) optimized structure, (b) LUMO's and (c) HOMO's diagrams of the receptor **1** and its complexes with Hg^{2+} and Cu^{2+}.

Table 1 DFT computed some important electronic energy of **1** and its complexes with Cu^{2+} and Hg^{2+}.

Species	E_T (a.u)	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV) = E_{LUMO}-E_{HOMO}
Receptor **1**	-1957.50939074	-0.22187	-0.10143	0.12044
1-Cu^{2+}	-2153.07827308	-0.46740 (α) -0.45608 (β)	-0.33924 (α) -0.42303 (β)	0.12816 0.03305
1-Hg^{2+}	-1999.62706500	-0.43986	-0.39017	0.04969

In a recent studies, it is reported that iminothiazolidinone acetate derivatives play a crucial role as potent and selective inhibitors of aldose reductase (ALR2).[32] Therefore, the

additional biological importance of receptor **1** was examined by performing molecular docking (MD) simulations to examine the binding modes with the active site of ALR2. The molecular electrostatic potential (MEP) of **1** was first evaluated to understand the electrostatic contribution on the interaction with a receptor protein. In MEP diagram (Fig. 9), the most negative potential is assigned to be red and the most positive potential is assigned to be blue, and the color spectrum is mapped to all other values by linear interpolation. As shown in Fig. 9a, the receptor **1** has well defined negative and positive regions to favor non-covalent interactions in the active site of ALR2. Then, the MD simulation was performed on the ligand-docked structure of ALR2 inhibitor complex by using the computational code Hex.[33] (Fig. S12) The crystal structure of ALR2 (PDB code: 1US0) and the docking predicted conformation of the compound was prepared individually before carrying out MD simulations. The docking calculations suggested that the red regions in MEP (Fig. 9a) of docked **1** have displayed good interaction with ALR2. Also, the docking study of ALR2 with **1** has exhibited well established non-covalent bonds with amino acids in the ALR2 active pocket (Fig. 9b) and showed relatively good binding affinity (-392.72 kcal/mol). The multiple hydrogen bonds and electrostatic interactions with the various amino acids of ALR2 with **1** were shown in Fig. 9c, d.

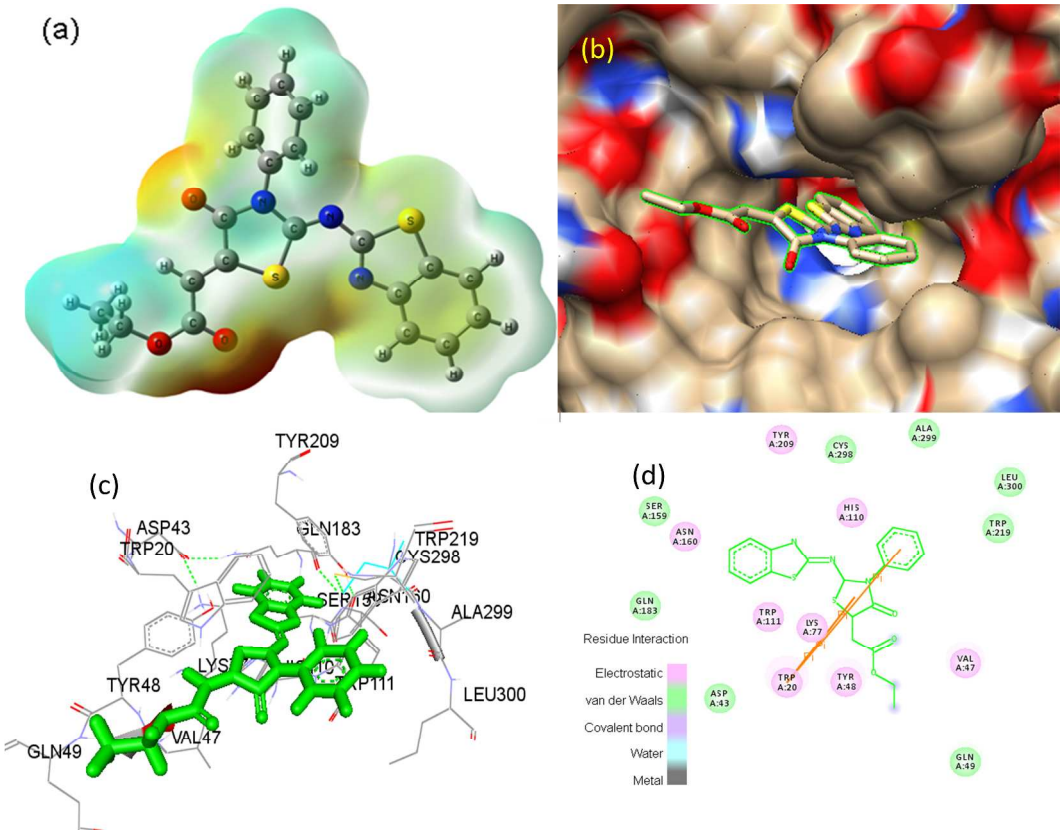


Fig. 9 (a) Molecular electrostatic potential (MEP) diagram of **1**, (b) molecular docking study of ALR2 (PDB code: 1US0) with **1**, and (c, d) the various non-covalent interactions between the **1** and ALR2.

In summary, we have synthesized an easy-to-make fluorescent receptor **1** containing benzothiazole core for the selective detection of Cu^{2+} and Hg^{2+} ions from many other tested metal ions in CH_3OH/H_2O (50:50, *v/v*). The receptor **1** showed a high selectivity and sensitivity towards Cu^{2+} and Hg^{2+} with the detection limit down to micromolar rage in semi aqueous medium. The cations recognition events were found in harmony for both experimental and DFT results. Therefore, it is revealed that the bioactive nature of **1** along with its ability to detect Cu^{2+} and Hg^{2+} from semi aqueous medium potentially leads to many more sensors using

benzothiazole unit as a core skeleton in future. The docking results provided potent and valuable information for the future fabrication of more useful aldose reductase inhibitors.

Supporting Information

Experimental procedures, spectroscopic data, analytical parameters, and X-ray structure data (CIF) for **1** (Fig. S1-S13). The materials are available free of charge via the Internet at http://pubs.rsc.org.

Acknowledgment

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Highly selective fluorometric sensing of Cu^{2+} and Hg^{2+} by using a benzothiazole based receptor in semi-aqueous medium and molecular docking studies

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Graphical Abstract