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SHORT COMMUNICATION

Luminescent Benzothiazole-Based Fluorophore of Anisidine Scaffoldings: a “Turn-On” Fluorescent Probe for Al^{3+} and Hg^{2+} Ions

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Abstract A new anisidine possessing benzothiaozle-based chemosensor (**1**) has been designed and synthesized. The chemosensor **1** was designed to provide hard base environment for ratiometric detection of comparatively less studied Al^{3+} ions. In CH_3CN, the fluorescence spectra of chemosensor **1** red shifted from 368 to 430 nm with addition of Al^{3+} and Hg^{2+} ions; while Cu^{2+} ions caused quenching of emission intensity of **1**. These differential changes observed with Al^{3+} and Cu^{2+} ions addition enabled chemosensor **1** to construct “NOR” and “TRANSFER” logic gates.

Keywords Benzothiazole · Fluorescent chemosensor · Ratiometric · Metal ion probe · Molecular logic gates

Introduction

Trivalent metal ions have vital biological properties and are in a straight way involved in the cell function where there is a serious control of M^{3+} levels [1]. For example, Al^{3+} have injurious effects on human health, as an excessive amount of Al^{3+} in the brain is believed to cause neurodementia, including neurological disorders such as Alzheimer’s disease, Parkinson’s disease and dialysis encephalopathy [2–4]. Thus, the monitoring of Al^{3+} is imperative to control the concentration levels in the biosphere and diminish direct effects on human health [5]. Similarly, the development of fluorescent probes for heavy transition metal ions such as Hg^{2+} and Cu^{2+} is also of particular interest, due to their biological and environmental consequences like prenatal brain damage, serious cognitive disorders and Minamata diseases [6–9]. For these reasons, substantial efforts have been made thus far to establish fluorescent chemosensors of different architectures for the selective detection of Al^{3+}, Hg^{2+} and Cu^{2+} ions [10, 11]. However, compared with other metal ions, the detection of Al^{3+} has always been challenging because of its poor coordination ability, strong hydration ability and the lack of spectroscopic characteristics [12]. Hitherto, a small number of fluorescent chemosensors for Al^{3+} had been developed [13–16]. Nitrogen and oxygen-rich coordination surroundings can offer a hard base environment for the hard acid Al^{3+} [17]. Therefore, the design and synthesis of Al^{3+} selective fluorescent probes have fetched considerable attention [18, 19]. In relation to other methods [20, 21], the fluorescence method can provide a simple and cost-effective detection with high sensitivity, short response time, good selectivity and real-time monitoring [22, 23]. Moreover, Benzothiazoles are renowned to be good ligands for metal ions such as Hg^{2+} and Cu^{2+} due to the presence of N and S heteroatoms [24]. Also, the development of single molecular sensors capable of detecting multiple metal analytes with different spectral responses is still a great challenge [25]. So, chemosensor **1** has been designed keeping in mind the hard base environment provided by methoxy of anisidine ring and nitrogen of benzothiazole ring which will also serve the purpose for multi analyte detection.

In recent times, the advances in chemosensing field have attracted huge attention in the construction of photonic devices [26] that function as molecular level logic gates [27, 28] based on the optical sensing of particular analytes. The customized chemosensors can be sighted as computational

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devices that use physical or chemical inputs to generate outputs based on a set of logical operators. In the molecular recognition field, the objective is often selectivity, that is, a specific analyte persuade specific change in color or fluorescence. In contrast, molecular logic gates looks for more complex systems, which usually enclose multiple binding sites and show certain exclusive response patterns, demonstrating the desired logic operation in the presence of either or both inputs [29]. The chemically encoded information was transformed into the optical changes as the output, which outcomes in development of molecular logic gates such as OR, NOR, AND, INHIBIT, YES, NOT, XOR, and XNOR logic gates. The integration of these molecular logic gates at molecular stage is allied to the integration and processing of binary data of conventional microprocessor systems [30]. The rapidity of the conventional computers can be improved by miniaturizing electronic components to a very tiny micron-size scale so that those electrons have to travel only very short distances within a very short time [31].

Experimental Section

Reagents and Apparatus

All reagents and chemicals were purchased from Aldrich and used without further purification. Solvents used for spectroscopic studies were purified by standard procedures before use. ^{1}H NMR spectra were recorded on a BrukerAvance II 400 at 400 MHz spectrometer with TMS (trimethylsilane) as internal standard, and the chemical shifts (δ) were expressed in ppm and coupling constants (*J*) in Hertz. Absorption spectra were recorded on Shimadzu UV-240 spectrophotometer. Fluorescence spectra of solutions were recorded on Hitachi F–7000 equipped with 220–240 V Xe lamp.

Synthesis of 2-(2-methoxy-phenyl)benzothiazole (1)

2-Aminothiophenol (250 mg, 3.5 mmol) and 2-methoxyaldehyde (275 mg, 3.5 mmol) were mixed thoroughly and taken in a round bottom flask (RBF, 50 mL) placed in an alumina bath inside a microwave oven and irradiated under 640 W for 10 min with an interval of 2 min between two halves of the duration [32]. After completion of reaction (TLC monitoring), the crude product was directly recrystallized from ethyl acetate to yield light brown colored product **1**. Yield (%) 82; m.p. (°C) 106–110, FT-IR (cm^{-1}) 2923 m (ν Ar-H str.), 2029 m ($\nu_{\mathrm{Ar-C=C\ str}}$.$\nu_{\mathrm{Ar-H\ str}}$.), 1620s ($\nu_{\mathrm{C=N}}$), 1494 m ($\nu_{\mathrm{C-N}}$); ^{1}H NMR (CDCl$_3$, 300 MHz) δ (ppm): 3.95 (s, 3H, - OCH$_3$), 6.93 (d, 1H, $J=6.0$ Hz, Ar-H), 7.04 (t, 1H, $J_1=J_2=6.0$ Hz, Ar-H), 7.24– 7.41 (m, 3H, Ar-H),7.82 (d, 1H, $J=6.0$ Hz, Ar-H), 7.99 (d, 1H, $J=6.0$ Hz, Ar-H),8.47 (d, 1H, $J=6.0$ Hz, Ar-H); **^{13}C NMR** (CDCl$_3$, 75 MHz) δ (ppm): 162.7, 157.2, 152.2, 136.2, 131.6, 129.7, 125.8, 124.5, 122.9, 122.5, 121.2, 121.1, 111.5, 55.5; **ESI-MS**: *m/z* (relative abundance (%), assignment) = 242.1 [100, (M + 1)$^+$]. (Found C, 69.59; H, 4.62; N, 5.76%. $C_{14}H_{11}NOS$ requires C, 69.68; H. 4.59, N, 5.80%).

General Procedure for Spectroscopic Analysis

Preparation of Stock Solutions

Stock solutions of metal ions (10^{-1} M) were prepared in DMSO using their perchlorate salts. Stock solutions of the receptor **1** (10^{-2} M) was prepared in DMSO, which were further diluted with CH$_3$CN (99:1, CH$_3$CN:DMSO, *v*/*v*) and used further for different spectroscopic experiments.

Fluorescence and UV–vis Absorption Selectivity Experiments

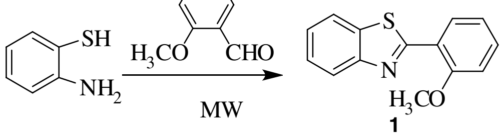
For the different absorption and fluorescence experiments, all the metal ions (Na$^+$, K$^+$, Mg^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}) were added as their perchlorate salts. Aliquots of ions under investigation were then injected into the sample solution through a rubber septum in the cap. The solutions were allowed to get stabilized after each addition, and were then scanned. Excitation wavelength was fixed at 300 nm in case of **1**.

Results and Discussion

Scheme 1 outlines the synthesis of the chemosensor **1** and the desired product was explicitly characterized by NMR spectra (^{1}H NMR, ^{13}C NMR), FT-IR and Mass spectrum (Fig. S1–S2).

The photophysical properties of **1** (10 μM) was investigated in CH$_3$CN solution by recording both UV–vis. and fluorescence spectra. The UV–vis spectra of **1** showed an absorption maximum at 321 nm, which remained unperturbed upon addition of different cations such as Na$^+$, K$^+$, Mg^{2+}, Al^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+} (Fig. S3).

On the other hand, the fluorescence spectra of **1** (10 μM; λ_{ex} 300 nm) displayed emission maximum at 368 nm.



Scheme 1 Synthetic pathway of chemosensor **1**

Addition of Hg^{2+} and Al^{3+} ions to solution of **1** resulted in new red shifted emission band at 430 nm; whereas Cu^{2+} addition resulted in quenching of fluorescence intensity of **1** (Fig. 1a). Moreover, presence of other metal ions did not perturb the fluorescence emission spectra of **1**. The fluorescence changes observed with addition of Al^{3+} / Hg^{2+} / Cu^{2+} ions were also associated with fluorescent color changes under illumination with UV lamp at 365 nm (Fig. 1b).

For quantitative analysis of interaction of **1** with different metal ions, fluorescence titrations of **1** with Al^{3+}/ Hg^{2+}/ Cu^{2+} ions were carried out. Upon gradual addition of Al^{3+} metal ion (0–50 equiv.) to the solution of **1** (10 μM; λ_{ex}300 nm), the emission spectra showed ratiometric behavior in which the band centered at 368 nm decreased, while the intensity of a new band at 430 nm enhanced concomitantly (Fig. 2a). The appearance of isoemissive point at 400 nm clearly supported the existence of more than one species in the medium. Similar type of ratiometric change has been observed upon addition of Hg^{2+} ions to solution of **1** (Fig. 2b). The observed significant red shift in the photophysical behavior of **1** upon interaction with Al^{3+}/Hg^{2+} ions has attributed to the transformation from the flexible structure of **1** to a rigid one upon metal complexation, which was most likely caused by increased intramolecular charge transfer (ICT) transition within the whole molecule arising due to Al^{3+} / Hg^{2+} ion binding as well as the inhibition of photoinduced electron transfer (PET) from nitrogen donors [33]. The Job's plot [34] suggested a 1:1 stoichiometry for a **1**.M^{n+} ($M^{n+} = Al^{3+}$ or Hg^{2+}) interaction (Fig. S4).The binding constants have been estimated through the Benesi–Hildebrand (B–H) method [35] and were found to be $K_a = 2.9 \times 10^6$ and $8.8 \times 10^5\ M^{-1}$, respectively. The limit of detection (LOD) of **1** for $Al^{3+}$and Hg^{2+} ions was then evaluated based on the titration profiles using the equation LOD = 3σ/slope and was estimated to be 31 and 40 μM, respectively [36].

Figure 3a shows the emission titration spectra of **1** with incremental addition (0–60 equiv.) of Cu^{2+} ions. The decrease in fluorescence (~ 70%) intensity was observed at 368 nm because Cu^{2+} ions typically have a pronounced quenching effect on fluorophores by mechanisms inherent

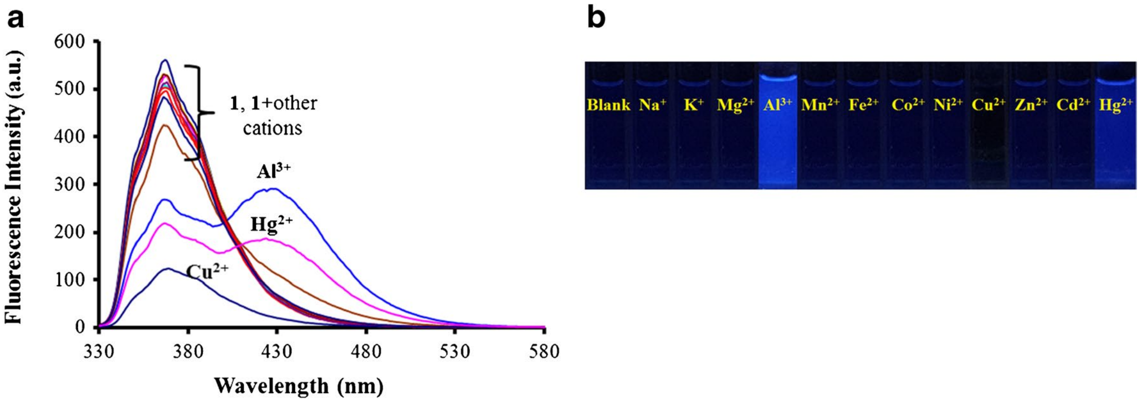


Fig. 1 **a** Fluorescence spectra and **b** Fluorescence color changes of **1** (10 μM; λ_{ex}300 nm) upon addition of 100 equiv. of various metal ions as their perchlorate salts in CH_3CN and under illumination with UV lamp at 365 nm

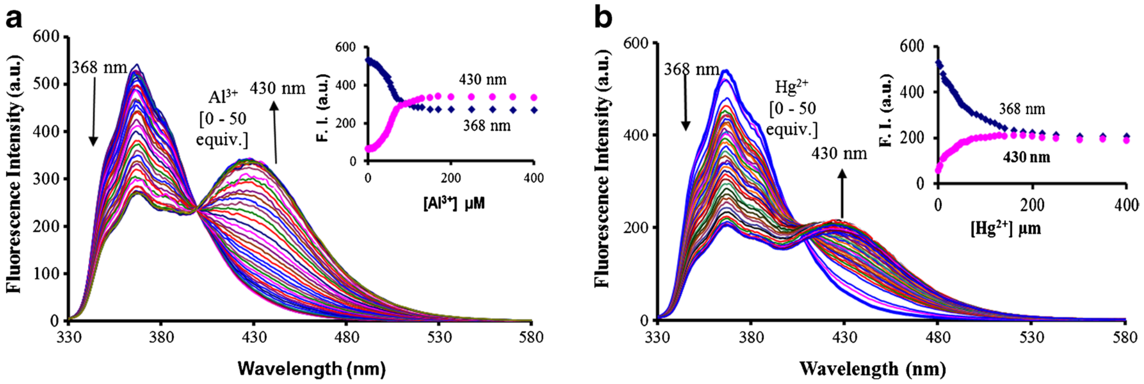


Fig. 2 Fluorescence spectra of **1** (10 μM; λ_{ex}300 nm) upon addition of (**a**) Al^{3+} and (**b**) Hg^{2+} ions; Inset: Binding isotherms at 368 and 430 nm in CH_3CN

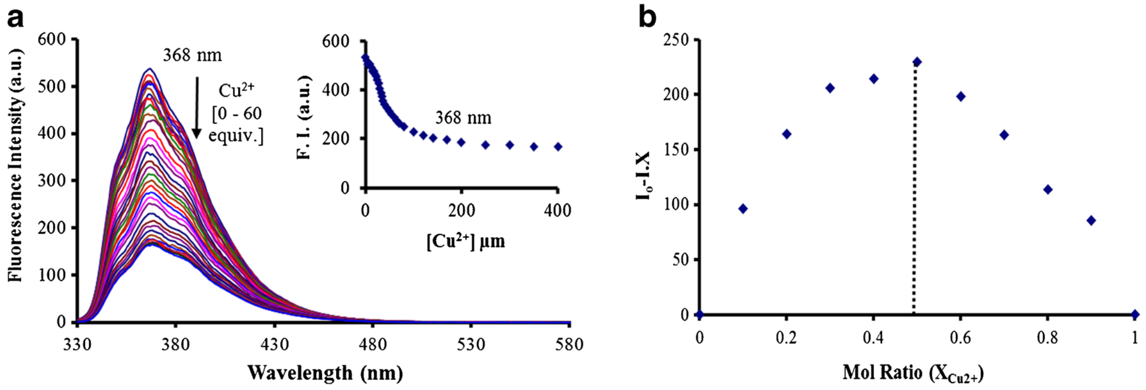


Fig. 3 **a** Fluorescence spectra of **1** (10 µM; λ_{ex} 300 nm) upon addition of Cu^{2+}ions; Inset: Binding isotherms at 368 nm and **b** Job's plot of **1** for Cu^{2+} ions in CH_3CN

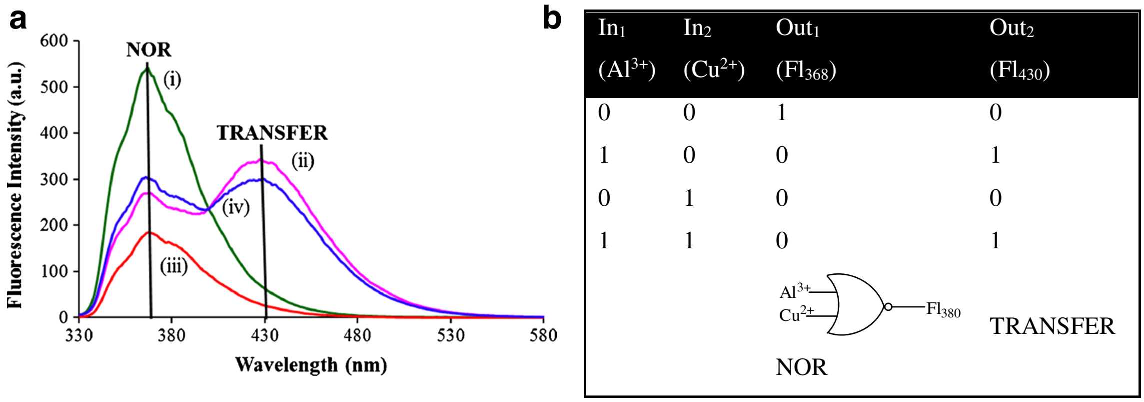
to the paramagnetic species [37]. The stoichiometry of the fluorescence quenching interaction between receptor **1** and Cu^{2+} was further evaluated by Job's plot method and found to be in the ratio of 1:1 (Fig. 3b). The binding constant and LOD were also determined to be 2.4×10^5 M^{-1} and 28 µM, respectively.

The differential behavior of chemosensor **1** towards Al^{3+} and Cu^{2+} ions enabled chemosensor **1** to elaborate molecular logic gates i.e. NOR and TRANSFER at two different wavelengths. Now a days, unconventional computing based on chemical reactions has stimulated interest in the development of molecular logic gates and devices at the molecular level. Molecular logic gates convert input stimulations into output signals with intrinsic protocols. It follows that the principles of binary logic can be applied to the signal transduction operated by molecular switches. For the analysis of their logic behaviors, first of all, threshold values are assigned and then logic convention is used for their inputs and outputs signals [29].

The molecular switching behavior of chemosensor **1** with two chemical inputs (20 equiv. of Al^{3+} and Cu^{2+}) can be demonstrated with the help of binary logic. The input can take values of 0 and 1 corresponding to the absence or the presence of input. The output, the fluorescence intensity at a given wavelength, can be of values 0 and 1 depending on the fluorescence intensity value below or above a certain threshold.

There are two input signals, viz. In_1 (Al^{3+}) and In_2 (Cu^{2+}), whereas the output signals are, Out_1 ($Fl_{368\ nm}$) and out_2 ($Fl_{430\ nm}$). Figure 4a shows different fluorescence spectra observed with addition of two inputs. Monitoring the absorption changes at 368 and 430 nm i.e. the addition of Al^{3+}, Cu^{2+} and an equimolar mixture of Al^{3+} and Cu^{2+}, lead to "NOR" and "TRANSFER" logic gates, respectively as represented in truth table (Fig. 4b).

"NOR" gate is a combination of OR gate followed by an inverter. The output of NOR gate is high when both inputs are low [38]. The fluorescence intensity at 368 nm was decreased with addition of either Al^{3+} / Cu^{2+}/ both of the



In_1 (Al^{3+})	In_2 (Cu^{2+})	Out_1 (Fl_{368})	Out_2 (Fl_{430})
0	0	1	0
1	0	0	1
0	1	0	0
1	1	0	1

Fig. 4 **a** Molecular scale implementation of different logic gates; (*i*).blank; (*ii*).**1** + Al^{3+}; (*iii*).**1** + Cu^{2+}; (*iv*).**1** + Al^{3+}+Cu^{2+} and **b** Truth table for "NOR" and "TRANSFER" gates

ions i.e. the output was read as “0” in the presence of Al^{3+} / Cu^{2+} ions (Fig. 4).

Moreover, by changing the observation wavelength from 368 to 430 nm, the spectral changes led to “TRANSFER” logic gate [39]. The output of TRANSFER gate is high when either one of the inputs or both of the inputs are high (Fig. 4).

Conclusion

In conclusion, we have designed and synthesized simple tailor made benzothiazole chemosensor possessing anisidine ring (**1**) for providing hard base environment for ratiometric detection of Al^{3+} ions. Apparently, **1** could be used as single molecular multianalyte fluorescent probe for the determination of Al^{3+} and Hg^{2+} ions via fluorescent enhancement; while Cu^{2+} ions via fluorescence quenching in a wide concentration range along with different color changes. These differential absorption changes were responsible for construction of “NOR” and “TRANSFER” logic gates.

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