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Benzimidazole-based ratiometric fluorescent receptor exhibiting molecular logic gate for Cu^{2+} and Fe^{3+}

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ABSTRACT

We synthesized a novel fluorescent receptor based upon a benzimidazole moiety in a dipodal framework. The receptor exhibited a dual fluorescence emission which is quenched upon addition of Cu^{2+} or Fe^{3+}. Interestingly, the receptor offers a ratiometric property and an 'OR' logic gate property to Cu^{2+} and Fe^{3+}.

Supramolecular chemistry has attracted considerable attention in the area of molecular electronics[1] and probes in biological systems.[2] Fluorescent techniques are preferred because they are quite sensitive and well suited to meet the need for in vivo applications such as mapping the spatial and temporal distribution of the biological analytes.[3] Nowadays, fluorescent probes are used for constructing molecular devices such as wires,[4] switches,[5] and diodes.[6] Extensive studies have been conducted on molecular logic gates such as 'AND', 'NOT', 'OR', and their combinational logic circuits that generally produce light signals in response to a variety of inputs.[7] A typical 'OR' logic gate has two input ports and one output port. A positive input signal on either one of the input ports or both input ports activates the output signal of the gate.[8] On the basis of this principle, several anticancer pro-drugs with a molecular logic gate have been designed for selective activation in malignant tissues by a specific enzyme which generates the input signal.[9] The gate activation leads to signal translation for bond cleavages that release the active drug molecule to the target.

Recent interest has been focused on the development of a new chemical system involving multiple fluorescent output modes.[10] Therefore, providing more functional materials with excellent properties is an exigent challenge. As part of our ongoing studies on the development of benzimidazole-based chemosensors,[11] we herein evaluate a new dipodal receptor developed for ratiometric fluorescence determination and molecular logic gate applications for Cu^{2+} and Fe^{3+}—the most abundant transition metal ions in biological systems that are found to function in proximity to several biological processes.[12]

Receptor **2** was prepared through a series of steps as shown in Scheme 1. For the synthesis of compound **1**, 2-aminobenzimidazole was treated with α,α′-dibromo-*m*-xylene in acetone in the presence of KOH at room temperature. Compound **2** was synthesized by a condensation reaction of compound **1** with salicylaldehyde in MeOH in the presence of a catalytic amount of $Zn(ClO_4)_2$, followed by reduction with $NaBH_4$.[13] The final product was characterized by using the spectroscopic methods, which were fully interpreted and found to be in accord with the formula of compound **2**.

Upon excitation at 285 nm, receptor **2** in a HEPES buffered (10 mM, pH 7.0) aqueous acetonitrile solution (CH_3CN/H_2O, 8:2, v/v) exhibited a dual fluorescence emission that peaked at 320 nm and 420 nm. The receptors with a dual emission are better fluorescence sensors than those which offer the measurement of fluorescence changes on a single wavelength because the dual emission minimizes the measurement errors expected due to such factors as phototransformation, receptor concentrations, and environmental effects.[14]

To obtain a quantitative insight into the binding affinity of receptor **2** with metal ions, the fluorescence intensity changes were measured upon adding various metal ions (Figs. 1 and 2). Upon addition of Cu^{2+} or Fe^{3+}, the fluorescence spectrum underwent dramatic changes. The fluorescence emissions both at 320 nm (originated from local excited state) and at 420 nm (attributed to charge transfer state) were quenched, while a new band appeared at 490 nm. The charge transfer band assignment for

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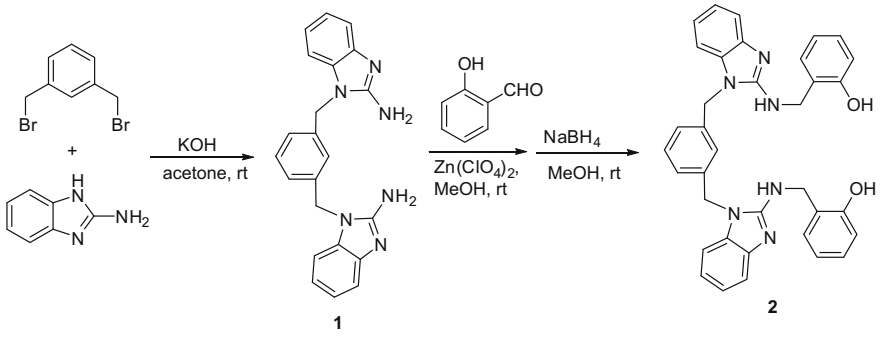


Scheme 1. Synthesis of receptor **2**.

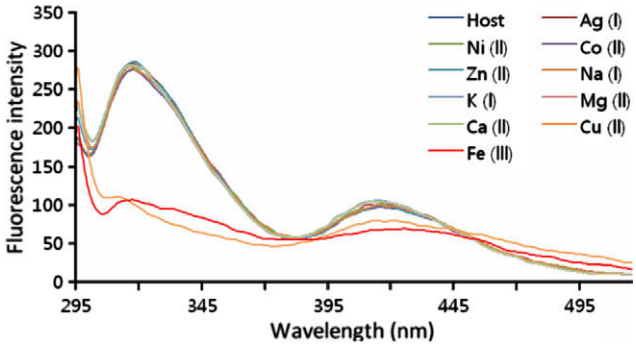


Figure 1. Changes in fluorescence intensity of receptor **2** (10 μM) upon addition of a particular metal salt (40 μM) in a HEPES (10 mM, pH 7.0) buffered CH_3CN/H_2O (8:2, v/v) solution (λ_{ex} = 285 nm).

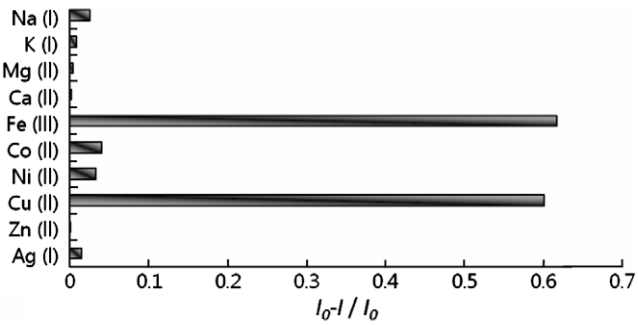
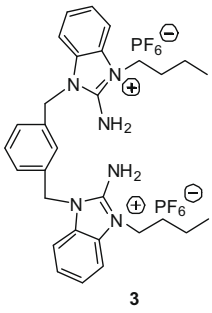


Figure 2. Fluorescence response of receptor **2** (10 μM) at 320 nm upon addition of a particular metal salt (40 μM) in a HEPES (10 mM, pH 7.0) buffered CH_3CN/H_2O (8:2, v/v) solution (λ_{ex} = 285 nm).

420 nm is based upon our previous reported research work, where we found the shift in fluorescence band upon anion binding.[11f] This reported pure receptor **3** showed a band at 443 nm, but upon anion binding this band was shifted, thus the original band was due to charge transfer transition (Scheme 2). Receptor **2** has the close structural similarity with receptor **3**. This is a sufficient reason to believe that due to charge transfer the pure receptor **2** shows emission at 420 nm. One can expect that metal binding can quench the intensity of band at 420 nm, and a new band must appear. This expectation can be due to the fact that metal binding may lead to CT band shifting on accounts of change in energy gap. But in present case, the band at 420 nm is sandwiched by two other bands, which limit to see this new band development. The new band that appeared at 490 nm is attributed to the stacking between two benzimidazole units (stacking in fluorophores). In free receptor, the two pods are oriented freely. However, when a



Scheme 2. Structure of receptor **3**.

complex formation takes place, two fluorophores come close and consequently undergo stacking. This point is supported by the structures calculated with molecular modeling. Finally, the band at 320 nm is originated from quenching local excited state with metal binding, causing reduction in fluorescence intensity by energy/electron transfer mechanisms. Under the same conditions, the effect of other metal ions such as alkali, alkaline earth, and transition metal ions on receptor **2** was investigated. The data clearly demonstrated that only Cu^{2+} and Fe^{3+} modulate the fluorescence intensity at three wavelengths, and no such significant effects are observed with other metal ions. The changes in the fate of fluorescence intensity at three wavelengths can offer advantages in studying ratiometric fluorescence recognition and molecular logic functions.

To study the relative effect of different equivalents of metal ions on the fluorescence intensity of receptor **2** at these three wavelengths, titrations were performed on Cu^{2+} and Fe^{3+} ions (Figs. 3 and 4). These titrations were performed by increasing the amounts

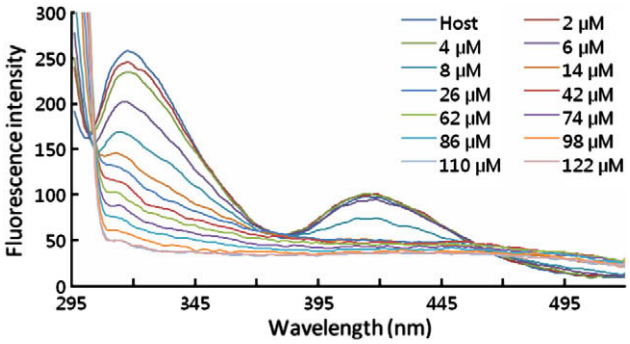


Figure 3. Changes in fluorescence spectrum of receptor **2** (10 μM) upon addition of $Fe(NO_3)_2$ (0–122 μM) in a HEPES (10 mM, pH 7.0) buffered CH_3CN/H_2O (8:2, v/v) solution (λ_{ex} = 285 nm).

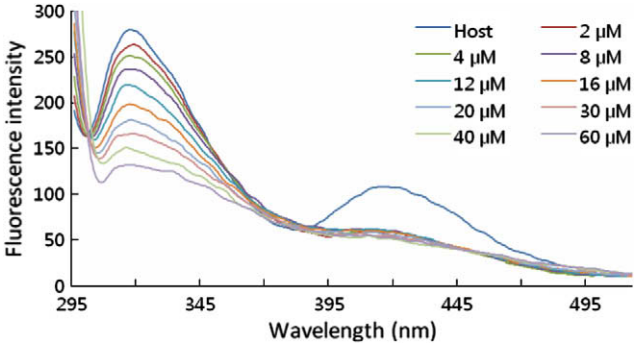


Figure 4. Changes in fluorescence spectrum of receptor **2** (10 μM) upon addition of $Cu(NO_3)_2$ (0–60 μM) in a HEPES (10 mM, pH 7.0) buffered CH_3CN/H_2O (8:2, v/v) solution (λ_{ex} = 285 nm).

of metal ions in the 10 μM solution of receptor **2**. After each addition of metal ions, the solutions were shaken with a mechanical stirrer to make the solution homogenous and attain equilibrium before measuring the fluorescence intensity. The titrations showed three common features upon continuous addition of metal ions: (i) The fluorescence intensity was quenched at 320 nm in a regular order. (ii) The relative extent of intensity quenching was very fast at 420 nm upon additions of first few equivalents of metal ions, but after that the changes at this wavelength were relatively small. Thus, the successive additions of metal ions do not follow the regular trend of quenching at this wavelength. (iii) Small but regular fluorescence enhancement was observed at 490 nm.

In the cases of Cu^{2+} and Fe^{3+}, upon metal binding, the receptor showed regular fluorescence quenching at 320 nm and a fluorescence enhancement at 490 nm. Thus, the receptor can be used for ratiometric sensing of Cu^{2+} and Fe^{3+}. The fluorescence ratiometric response of receptor **2** to a selected metal ion is displayed in Figure 5. The figure shows that Cu^{2+} can be analyzed ratiometrically in the concentration range of 2–40 μM, while the receptor shows a ratiometric response to Fe^{3+} in the concentration range of 8–122 μM.

From the viewpoint of logic gate, receptor **2** satisfies the Boolean algebra 'OR' gate if the two inputs are defined to be Cu^{2+} and Fe^{3+}, and if the output is defined to be the intensity of fluorescence emission. Receptor **2** is selective in its response (quenching) to Cu^{2+} and Fe^{3+} when either of these two metal ions is present. No changes were observed in the fluorescence intensity of receptor **2** (no quenching) upon addition of any other investigated metal ions. When both Cu^{2+} and Fe^{3+} were present together in a solution, the fluorescence intensity was low (quenching). Fluorescence emission spectra showing 'OR' logic gate with receptor **2** are displayed in Figure 6, and the truth table is summarized in Table 1.

The Job plots revealed that Cu^{2+} and Fe^{3+} ions form a 1:1 complex with receptor **2** (Figure S1 and S2).[15] Binding constants of receptor **2** with metal ions were calculated by using the Benesi–Hildebrand plots and were found to be $(3.9 \pm 0.1) \times 10^3\ M^{-1}$ for Cu^{2+} and $(5.1 \pm 0.2) \times 10^3\ M^{-1}$ for Fe^{3+}, respectively (Figure S3 and S4).[16]

The quest for the structure of complexes formed between receptor **2** and metal ions (Cu^{2+} or Fe^{3+}) was determined by energy minimization studies with the MacroModel v 9.0 using MM-2* force field (Fig. 7).[17] The structures calculated with the MacroModel revealed that a metal ion binds in the cavity of receptor **2** through both nitrogen and –OH donor sites. A similar type of binding modes for Cu^{2+} and Fe^{3+} authenticates that the receptor sites are equally available for both metal ions, and thus, the receptor shows the 'OR' logic gate behavior. All our attempts failed to obtain a single crystal of the complexes suitable for determination of the crystal structure. On the other hand, the paramagnetic nature of Cu^{2+} and Fe^{3+} limited us to use the NMR spectroscopy for the structure determination of these complexes.

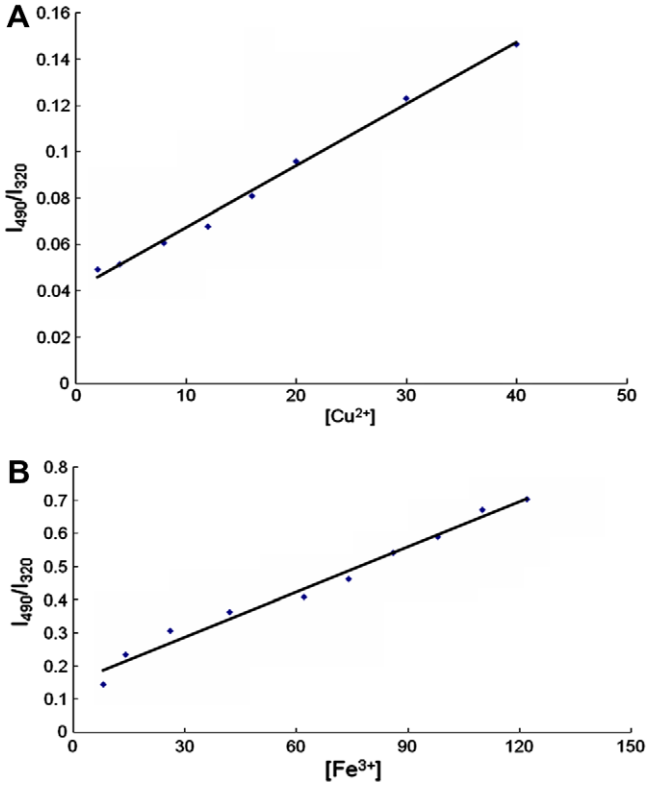


Figure 5. Plots of ratiometric fluorescence intensity (I_{490}/I_{320}) of receptor **2** against metal ion concentration for: (A) Cu^{2+} (2–40 μM) and (B) Fe^{3+} (8–122 μM) in a HEPES (10 mM, pH 7.0) buffered CH_3CN/H_2O (8:2, v/v) solution (λ_{ex} = 285 nm).

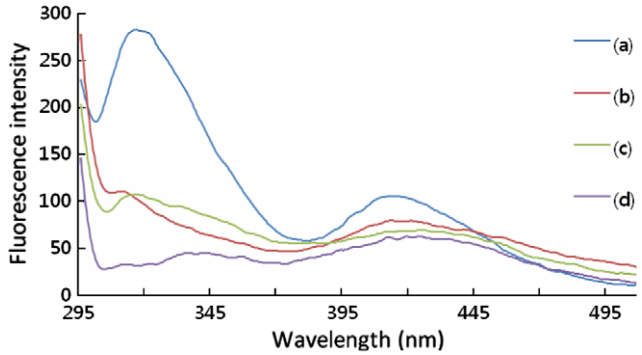


Figure 6. Fluorescence emission spectra showing 'OR' logic gate with receptor **2** in a HEPES (10 mM, pH 7.0) buffered CH_3CN/H_2O (8:2, v/v) solution (λ_{ex} = 285 nm) under four experimental conditions: (a) receptor **2** only, (b) receptor **2** with Cu^{2+}, (c) receptor **2** with Fe^{3+}, and (d) receptor **2** with both Cu^{2+} and Fe^{3+}.

Table 1
Truth table for the operation of receptor **2** with Cu^{2+} and Fe^{3+}

IN_1 (Cu^{2+})	IN_2 (Fe^{3+})	OUTPUT (Flu_{320nm})
0	0	0 (High)
1	0	1 (Low)
0	1	1 (Low)
1	1	1 (Low)

We synthesized a novel fluorescent receptor on the basis of a benzimidazole moiety in a dipodal framework. The binding

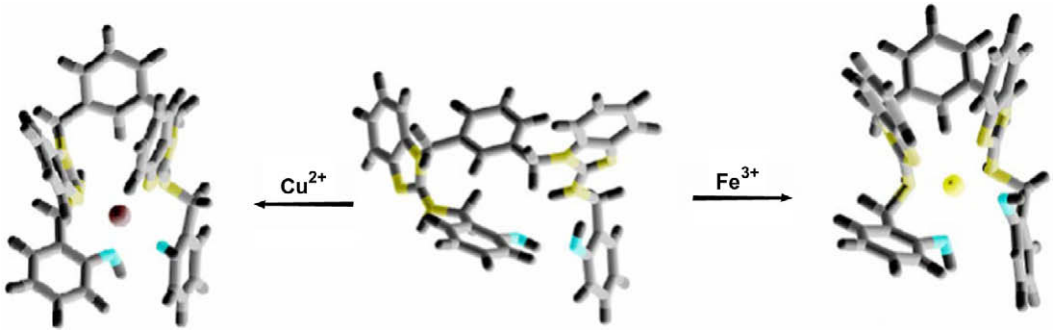


Figure 7. Energy minimized structure of receptor **2** and its complexes with Cu^{2+} or Fe^{3+} as obtained by the MacroModel calculation.

investigations were performed in aqueous acetonitrile, and the receptor exhibited a dual fluorescence emission which is quenched upon addition of Cu^{2+} or Fe^{3+}. The receptor acted as a ratiometric fluorescent probe over a wide concentration range of Cu^{2+} and Fe^{3+}. The receptor offered an interesting property of molecular 'OR' logic gate.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.07.083.

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13. *Synthesis of compound* **1**: A solution of 2-aminobenzimidazole (100 mg, 0.76 mmol), α,α′-dibromo-*m*-xylene (100 mg, 0.38 mmol), and KOH (21 mg, 0.38 mmol) in acetone (30 mL) was stirred at room temperature for 24 h. Upon completion of the reaction, cold water was added to the reaction mixture, and then solid was separated out. The solid material was filtered and washed with diethylether affording a white solid (120 mg, 86%); mp 284–285 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.22 (s, 4H, $-CH_2$), 6.55 (s, 4H, $-NH_2$), 6.77–6.81 (m, 2H, Ar), 6.90–6.97 (m, 6H, Ar), 7.12–7.14 (m, 2H, Ar), 7.20–7.24 (m, 1H, Ar), 7.28 (s, 1H, Ar); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 107.9, 114.7, 118.1, 120.5, 125.8, 126.1, 128.8, 134.1, 137.5, 142.9, 155.0. Anal. Calcd for $C_{32}H_{20}N_6$: C, 71.72; H, 5.47; N, 22.81. Found: C, 71.71; H, 5.45; N, 22.60.
Synthesis of compound **2**: A solution of compound **1** (108 mg, 0.29 mmol) and salicylaldehyde (127 mg, 0.73 mmol) along with a catalytic amount of $Zn(ClO_4)_2$ in MeOH (30 mL) was stirred at room temperature for 12 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the reaction mixture was treated with $NaBH_4$ (219 mg, 5.8 mmol) at room temperature for 4 h. The solvent was evaporated, and water was poured into the reaction mixture. After neutralization with 1 M HCl, the organic material was extracted with CH_3Cl. The organic layer was dried over anhydrous $MgSO_4$. After filtration and evaporation, the residue was purified by column chromatography on silica gel (hexanes/EtOAc, 6: 4) to give a white solid (122 mg, 72%); mp 253–255 °C; ^{1}H NMR (CD_3CN, 400 MHz) δ 4.39 (d, 4H, $-CH_2$, J = 6.4 Hz), 5.01 (s, 4H, $-CH_2$), 6.14 (br, 2H, –NH), 6.79–6.83 (m, 4H, Ar), 6.95–7.00 (m, 5H, Ar), 7.04–7.10 (m, 4H, Ar), 7.12–7.14 (m, 2H, Ar), 7.15–7.19 (m, 3H, Ar), 7.33–7.35 (m, 2H, Ar), 12.38 (br, 2H, –OH); ^{13}C NMR (CD_3CN, 100 MHz) δ 44.0, 46.5, 109.7, 116.3, 120.0, 120.9, 121.5, 123.0, 126.8, 127.7, 128.2, 130.6, 130.8, 133.0, 135.7, 138.2, 141.5, 156.3, 158.2. Anal. Calcd for $C_{36}H_{32}N_6O_2$: C, 74.46; H, 5.55; N, 14.47. Found: C, 74.42; H, 5.57; N, 14.46.
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