

Indole-based Fluorescent Sensors for Selective Detection of Hg^{2+}

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Abstract A series of indole-based fluorescent chemosensors **1–4** were prepared and investigated characteristic features with transition metal ions. Sensors **1** and **2** were selective for Hg^{2+} ion among a series of metal ions in H_2O –DMSO with association constants of 4.60×10^4 and $5.90 \times 10^4 \text{M}^{-1}$ and detection limits of 140 and 101.6 μM , respectively.

Keywords Chemosensor · Indole · Fluorescence · Hg^{2+} ion

Introduction

The design and synthesis of new chemosensors for efficient detection of trace metal ions is among the most important research topics in environmental chemistry and biology [1, 2]. Indole is a versatile heterocyclic compound with biological activities can be found in many organic compounds like the amino acid tryptophan and in tryptophan-containing protein, in alkaloids, and in pigments. Other indolic compounds include the plant hormone Auxin (indolyl-3-acetic acid, IAA), the anti-inflammatory drug indomethacin, and the betablocker pindolol. Consequently, indole derivatives remains an attention area of organic and medicinal chemists today.

Indole-based receptor systems have recently attracted attention [3–9] because an inherent fluorescent property of the indole chromophore is sensitive to change in the local

environment. The receptor strongly bind anions because they contain a single hydrogen bond donor group. To the best of our knowledge, few indole derivatives have been developed as potential fluorescent chemosensors for cations, such as Mercuric ion (Hg^{2+}) [10, 11]. Mercuric ion is a significant environmental pollutant that accumulates in plants, soil, and water. In the marine environment, Hg^{2+} ion is converted by bacteria into toxic methylmercury, a highly potent neurotoxin, and is passed up the food chain to accumulate in humans [12]. Therefore, it is very important to develop highly sensitive assays for detecting Hg^{2+} ion [13, 14]. In recent years, many sensors specific for Hg^{2+} have been developed [15–19], however, some of them are insoluble in aqueous solutions. Thus, for practical applications, it is necessary to develop water soluble Hg^{2+} sensors that are easily prepared, and possess selective and sensitive signaling mechanisms. In this study, we synthesize four indole-based fluorescent chemosensors **1–4** for metal-ion screening studies. Among of the indole-based chemosensors, **1** and **2** exhibited selectivity and sensitivity for Hg^{2+} ion compared to other transition and heavy metal ions.

Experimental

Apparatus

The ^1H and ^{13}C NMR spectra were recorded with Bruker AM 300 (300 MHz) spectrometers. Chemical shifts are expressed in ppm with residual DMSO, CD_3OD or CDCl_3 as reference. Low- and high-resolution mass spectra were recorded under fast atom bombardment (FAB) conditions. UV–vis spectra were recorded by using HP-8453 spectrophotometer with a diode array detector, and there solution was set at 1 nm. Fluorescence spectra were recorded on a Cary Eclipse Fluorescence spectrophotometer.

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Synthesis

Synthesis of Compound 1

To a stirred solution of 1H-indole-2-carbonyl chloride (0.45 g, 2.50 mmol) in dry CHCl_3 (10 mL) was added (6-(aminomethyl)pyridin-2-yl)methanamine (0.165 g, 1.20 mmol), K_2CO_3 (0.24 g, 1.74 mmol). The reaction mixture was stirred overnight at room temperature. After completion of reaction, the reaction mixture was added three drops of H_2O to quench the reaction. The mixture was extracted with chloroform (5 mL \times 3). The chloroform layer was dried over MgSO_4 and the solvent was removed under reduced pressure to give the solid crude product. The resulting residue was purified by silica column chromatography (EtOAc) to give white yellow solid **2** (0.18 g, 36 %). $R_f = 0.6$ (EtOAc); $^1\text{H NMR}$ (300 MHz, DMSO) δ : 9.37 (s, 2H), 7.73 (t, 2H, $J = 7.8$ Hz), 7.61 (d, 2H, $J = 7.8$ Hz), 7.45 (d, 2H, $J = 8.1$ Hz), 7.22 (m, 8H), 7.03 (t, 2H, $J = 7.2$ Hz), 4.61 (s, 4H); $^{13}\text{C NMR}$ (75 MHz, DMSO) δ : 161.40, 158.35, 137.48, 136.59, 131.63, 127.14, 123.45, 121.61, 119.81, 119.16, 112.40, 103.22, 44.31, FABMS m/z Calcd for $\text{C}_{25}\text{H}_{21}\text{N}_5\text{O}_2$ $[\text{M}+\text{H}]^+$, 423.1695, found 423.1701.

Synthesis of Compound 2

To a stirred solution of 2-(1H-indol-3-yl)acetic acid (1.763 g, 10.06 mmol) in dry DMF was added (6-(aminomethyl)pyridin-2-yl)methanamine (0.631 g, 4.60 mmol), TEA (1.94 mL, 13.80 mmol) and DEPC (1.54 mL, 10.12 mmol). The reaction mixture was stirred overnight at room temperature. After completion of reaction, filtrate and remove the solvent by vacuum. The resulting residue was purified by column chromatography (SiO_2 ; 1:18 MeOH/ CH_2Cl_2) and get white solid **3** (0.52 g, 1.15 mmol, 25 %). $R_f = 0.2$ (1:18 MeOH/ CH_2Cl_2); $^1\text{H NMR}$ (300 MHz, CD_3OD) δ : 7.37 (d, 2H, $J = 7.2$ Hz), 7.23 (d, 2H, $J = 8.4$ Hz), 7.05 (s, 2H), 6.97 (t, 2H, $J = 6.9$ Hz), 6.86 (t, 4H, $J = 6.9$ Hz), 4.18 (s, 4H), 3.59 (s, 4H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ : 175.05, 158.27, 138.78, 138.15, 128.45, 125.18, 122.66, 120.69, 120.01, 119.41, 112.41, 109.19, 45.33, 34.01; FABMS m/z Calcd for $\text{C}_{27}\text{H}_{25}\text{N}_5\text{O}_2$ $[\text{M}+\text{H}]^+$, 451.2008, found 452.2089.

Synthesis of Compound 3

To a stirred solution of (6-(aminomethyl)pyridin-2-yl)methanamine (0.54 g, 3.93 mmol) in MeOH was added 1H-indole-3-carbaldehyde (1.14 g, 7.87 mmole). The reaction mixture was reflux overnight. After completion of reaction, the solvent was evaporated and further purified by column chromatography (SiO_2 ; 1:2 MeOH/EtOAc) to get the imine product. To a stirred solution of the imine

product in MeOH was slowly added NaBH_4 (0.74 g, 19.6 mmole). The reaction mixture was stirred 2 h and then evaporated the solvent. The resulting residue was purified by column chromatography (SiO_2 ; 1: 2 MeOH/EtOAc) and get white solid **5** (0.31 g, 20 %). $R_f = 0.2$ (MeOH); $^1\text{H NMR}$ (300 MHz, $\text{CD}_3\text{OD}:\text{CD}_3\text{Cl}$ 2:1) δ : 7.620 (t, 2H, $J = 7.8$ Hz), 7.48 (d, 2H, $J = 7.8$ Hz), 7.33 (d, 2H, $J = 8.1$ Hz), 7.15 (d, 2H, $J = 7.8$ Hz), 7.13 (s, 2H), 7.08 (t, 2H, $J = 6.9$ Hz), 3.95 (s, 4H), 3.88 (s, 4H); $^{13}\text{C NMR}$ (75 MHz, $\text{CD}_3\text{OD}:\text{CD}_3\text{Cl}$ 2:1) δ : 156.71, 136.23, 135.45, 125.74, 122.57, 120.27, 119.97, 117.71, 116.78, 110.64, 110.13, 51.78, 42.19; FABMS m/z Calcd for $\text{C}_{25}\text{H}_{26}\text{N}_5$ $[\text{M}+\text{H}]^+$, 396.2188, found 396.2184.

Synthesis of Compound 4

To a stirred solution of compound **3** (0.66 g, 1.46 mmol) in dry THF was added $\text{Me}_2\text{S}\cdot\text{BH}_3$ (1 M, 1.8 mL, 1.8 mmol). The reaction mixture was reflux 6 h, and then 1 M HCl was added and reflux for another 3 h. After completion of reaction,

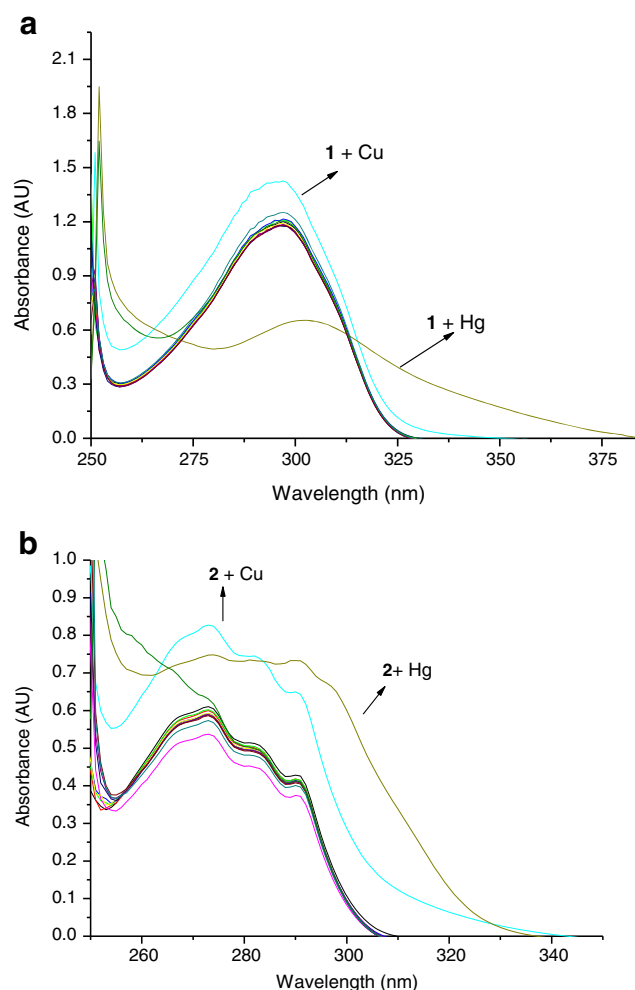


Fig. 1 UV-vis spectra of (a) **1** (14.6 μM) (b) **2** (10.7 μM) with 3 equiv of metal ion (18.5 μM) in DMSO/ H_2O (v/v , 7 : 3)

neutralize with NaHCO_3 , the mixture was extracted with CH_2Cl_2 (5 mL \times 3), the organic layer was dried with MgSO_4 . Further purified by column chromatography (SiO_2 ; 1:2 MeOH/EtOAc) and get white yellow solid **4** (0.073 g, 0.174 mmol, 26 %); ^1H NMR (300 MHz, CD_3OD) δ : 7.41 (t, 1H, $J = 7.2$ Hz), 7.29 (d, 2H, $J = 7.8$ Hz), 7.17 (d, 2H, $J = 7.8$ Hz), 3.53 (s, 4H), 2.77 (t, 4H, $J = 6.3$ Hz), 2.67 (t, 4H, $J = 6.3$ Hz); ^{13}C NMR (75 MHz, CDCl_3) δ : 157.51, 136.47, 136.21, 126.62, 121.55, 120.35, 120.06, 117.54, 111.32, 110.30, 52.66, 48.05, 24.19.

Results and Discussion

The chemosensor behavior of receptors **1–4** with the following 12 metal ions (as perchlorate salts): Li^+ , Na^+ , K^+ , Ca^{2+} , Mn^{2+} , Hg^{2+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Pb^{2+} , Cd and Zn^{2+} in H_2O – DMSO (3:7 v/v), was investigated by UV–vis and fluorescence measurements. We found that the UV–vis spectra of **1** (Fig. 1a) caused a small red shift (6 nm) with

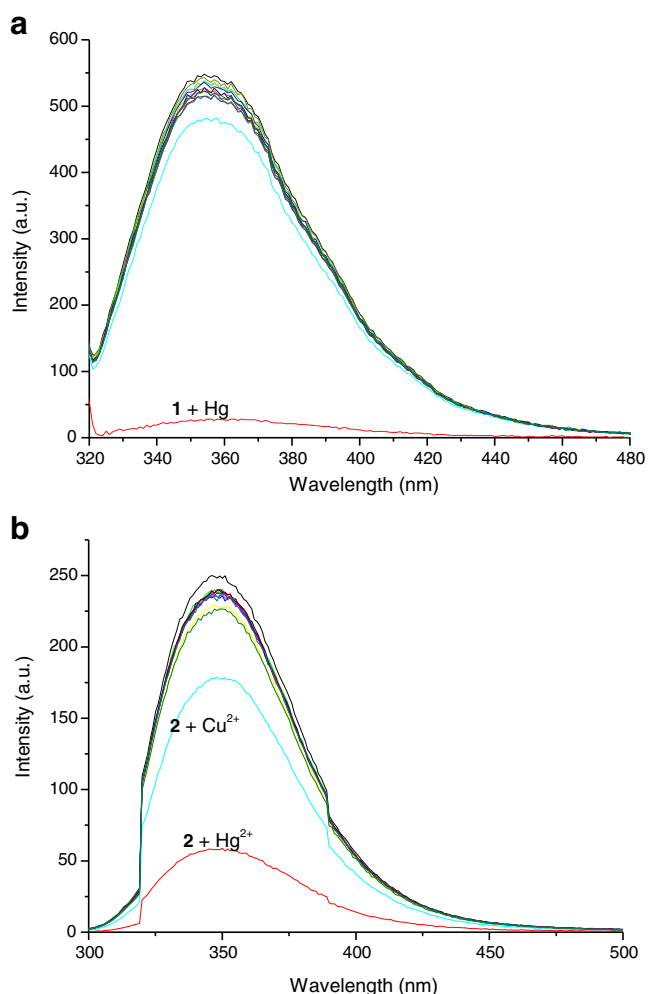


Fig. 2 Fluorescence spectra of (a) **1** (14.6 μM) (b) **2** (10.7 μM) with 3 equiv of Hg^{2+} in $\text{DMSO}/\text{H}_2\text{O}$ (v/v, 7 : 3)

decrease in absorption only in the presence of the Hg^{2+} ion. However, a Hyperchromic effect was observed with the addition of Cu^{2+} ion. For receptor **2**, a red shift (20 nm) with increase in absorption was observed only in the presence of the Hg^{2+} ion. (Fig. 1b) and a Hyperchromic effect was also observed with the addition of Cu^{2+} ion. Similarity, for receptor **3** or **4**, a Hyperchromic effect was also observed in the presence of Hg^{2+} or Cu^{2+} ions (Fig. S9 and S10).

Further investigate the chemosensing properties of receptors **1–4**, the results indicated that the fluorescence intensity of receptor **1** strongly quenched in the presence of the Hg^{2+} ion in H_2O – DMSO (3:7 v/v) (Fig. 2a), where presence of other metal ions did not affect the fluorescence intensity of receptor **1** significantly. Similar, selective quenching by Hg^{2+} ion was also observed for receptor **2** (Fig. 2b). For receptor **1**, the observed quenching efficiency ($(I-I_0)/I_0 \times 100\%$) at 365 nm was nearly 97 % where the other metal ions caused small quenching in fluorescence

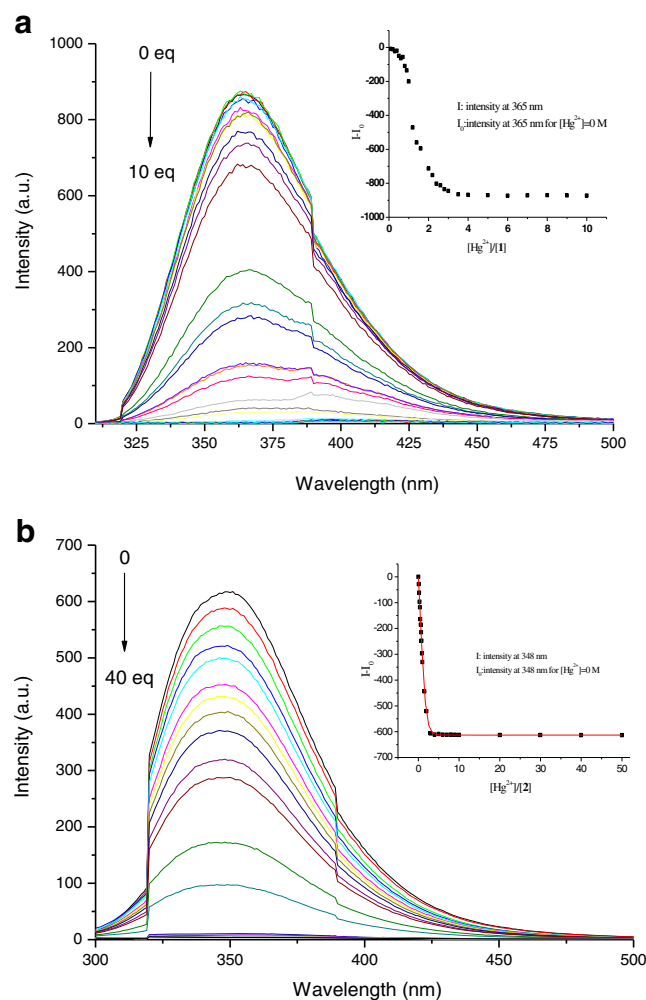
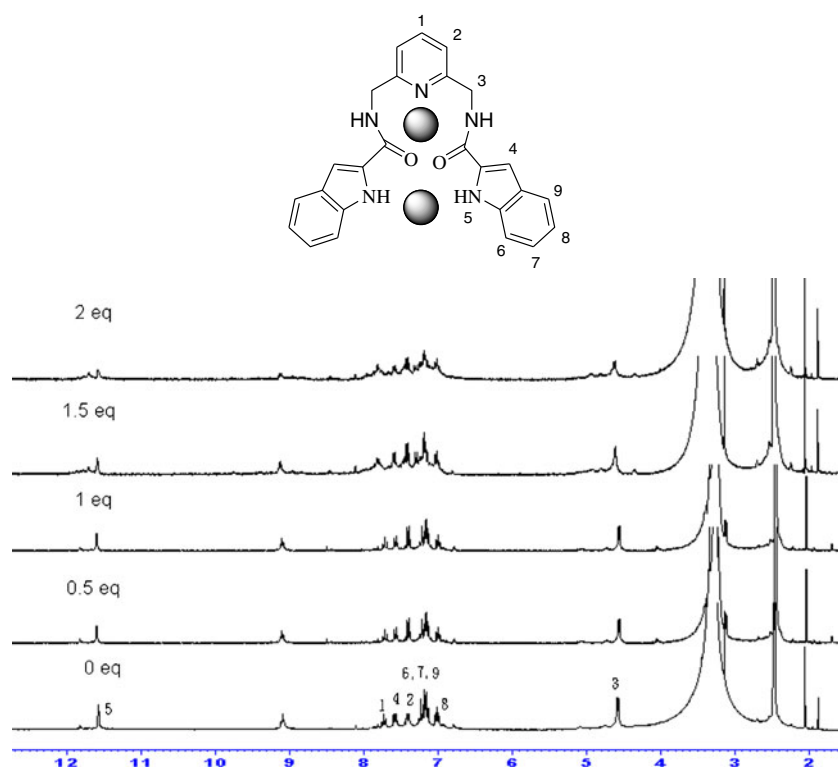


Fig. 3 Fluorescence spectra of (a) **1** ($1.46 \times 10^{-5}\text{M}$) (b) **2** (11.2 μM) in $\text{DMSO}/\text{H}_2\text{O}$ (v/v, 7 : 3) upon addition of increasing concentrations Hg^{2+} ; Inset is a plot of intensity change vs equiv. of Hg^{2+} added

Fig. 4 ^1H NMR spectra of **1** (4.6 mM) in the presence of 0.5–2.0 equiv of Hg^{2+} in DMSO

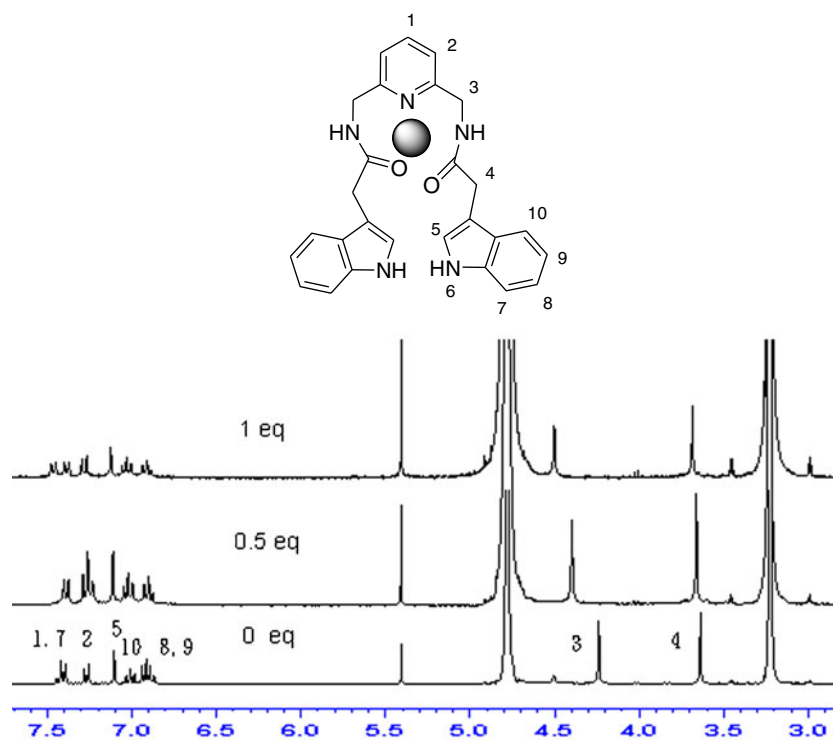


intensity. For receptor **2**, the quenching efficiency by Hg^{2+} ion was 77 % where the Cu^{2+} ions also caused small quenching in fluorescence intensity. For receptors **3** and **4**, did not exhibit highly selective recognition towards Hg^{2+} ion among a series of tested metal ions (Fig. S11 and S12). In contrast, receptor **1** or **2** had extremely high selectivity for

Hg^{2+} ion. The results indicated that **1** or **2** react with Hg^{2+} and showed that the length of the linker in receptors **1–4** would affect the recognition of the Hg^{2+} ion.

Since pH value in H_2O –DMSO (3:7 v/v) affects the charge distribution of **1** or **2** and may change its indigenous fluorescence properties, we also evaluated the effect of pH

Fig. 5 ^1H NMR spectra of **2** (4.1 mM) in the presence of 0.5–1.0 equiv of Hg^{2+} in DMSO



on the emission bands of receptors **1** and **2** in H₂O–DMSO (3:7 v/v), respectively. The fluorescence intensity of receptor **1** or **2** in H₂O–DMSO (3:7 v/v) maintained a constant level and no emission band shifts occurred in the pH range 3–12 (Fig. S13 and S14). Therefore, the pH change causing from exogenous addition of cation was eliminated as a possible factor contributing to the observed fluorescent intensity changes. Thus, the sensing behavior of receptor **1** or **2** for Hg²⁺ ion was suitable in the pH range 3–12.

The fluorescence titrations of receptors **1** (14.6 μM) and **2** (11.2 μM) was performed in the presence of different concentrations of Hg²⁺ ion in H₂O–DMSO (3:7 v/v). Figure 3a and b show the gradual reductions in fluorescence intensity for receptors **1** and **2** upon addition of increasing concentrations of Hg²⁺. From the fluorescence titration profiles, the association constant for **1***Hg²⁺ and **2***Hg²⁺ were found to be 4.60×10⁴ and 5.90×10⁴M⁻¹, respectively, according to the Stern-Volmer plot (Fig. S15 and S16).

By using above-mentioned fluorescence titration results, the detection limits for Hg²⁺ ion were 140 and 101.6 μM for receptors **1** and **2**, respectively. These detection limits were sufficiently low to detect submillimolar concentrations of the Hg²⁺ ion, which belongs the range found in many chemical and biological systems. In the Job's plot (Fig. S17), a maximum fluorescence change was observed with a 0.35 molar fraction of ionophore to Hg²⁺ ion for receptor **1**, which indicated the forming of a 1:2 complex. In addition, the formation of 1:2 complex between **1** and Hg²⁺ was further confirmed by the appearance of a peak at m/z 824, assignable to [**1** + 2Hg²⁺] in the ESI/MS (Fig. S18). For receptor **2**, the Job plot show that receptor **2** formed a 1:1 complex with Hg²⁺ ion (Fig. S19). The formation of 1:1 complex between **2** and Hg²⁺ was also confirmed by the appearance of a peak at m/z 653, assignable to [**2** + Hg²⁺ + 1H⁺] in the ESI/MS (Fig. S20).

Selectivity for the Hg²⁺ ion was further ascertained with competition experiments (Fig. S21 and S22). We found that the fluorescence intensity of receptors **1** and **2** in the presence of 10 equivalents of Hg²⁺ ion was unaffected by the addition of 11 equivalents of competing metal ions (Li⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺, Co²⁺, Ni²⁺, Cu²⁺, Pb²⁺, Cd²⁺, or Zn²⁺). This indicates that **1** or **2** is selective towards the recognition of Hg²⁺ ion over other metal ions.

To further elucidate the binding mode of receptor **1** or **2** with Hg²⁺ ion, the ¹H NMR titration experiments were carried out in DMSO. The spectral differences are depicted in Figs. 4 and 5. The results showed that down-field shifts were observed for most of the protons of receptors **1** and **2**. For receptor **1**, in the presence of 2.0 equivalents of Hg²⁺ ion, the protons of methylene were shifted down-field by 0.07 ppm. For receptor **2**, in the presence of 1.0 equivalents of Hg²⁺ ion, the protons of two methylene were shifted down-field by 0.26 and 0.04 ppm, respectively. There was no

further change after addition of more than 1.0 equiv of Hg²⁺, which further confirmed the 1:1 stoichiometry. The results suggested the binding of receptors **1** or **2** to Hg²⁺ forms a rigid conjugation system through interactions with amide proton and the carbonyl group.

Conclusion

In summary four aqueous indole-based receptors **1–4** were designed and synthesized. Among of them, receptors **1** and **2** showed selectivity for Hg²⁺ ion over other metal ions in H₂O–DMSO (v/v 3:7). These results indicated that the carbonyl group and length of linker could influence the binding of Hg²⁺ to the receptor and subsequently the ability of Hg²⁺ ion to quench the receptor fluorescence.

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