

Full Paper

Diphenylmaleimide derivatives and their efficiency in off-on Hg²⁺ fluorometric sensing

Chantana Wainiphithapong¹, Oranual Hanmeng¹, Vannajan Sanghiran Lee², Kate Grudpan³ and Nantanit Wanichacheva^{1,*}

¹Department of Chemistry, Faculty of Science, Silpakorn University, Nakorn Pathom, 73000, Thailand

²Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, 50603, Malaysia

³Department of Chemistry, Centre for Innovation in Chemistry, Faculty of Science, and Centre of Excellence in Innovation for Analytical Science and Technology, Chiang Mai University, Chiang Mai 50200, Thailand

* Corresponding author, e-mail: wanichacheva.nantanit@gmail.com, nantanit@su.ac.th

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Abstract: Two novel fluoroionophores (Sensors **1** and **2**) possessing one and two units of diphenylmaleimide fluorophore covalently bound to 2-[3-(2-aminoethylsulfanyl)propylsulfanyl] ethanamine were prepared for the selective detection of Hg²⁺ ions. The binding ability with Hg²⁺ was investigated by fluorescence spectroscopy. Sensor **1** exhibited highly sensitive and selective off-on fluorescence enhancement at 500 nm upon binding to Hg²⁺ and was shown to discriminate various competing metal ions, particularly Cu²⁺ and Pb²⁺, as well as Li⁺, Na⁺, Mg²⁺, Cd²⁺, K⁺, Al³⁺, Fe³⁺, Ca²⁺, Ba²⁺, Co²⁺, Mn²⁺ and Zn²⁺ with a detection limit of 6.72 x 10⁻⁷ M. On the other hand, Sensor **2** was found to be inferior fluoroionophore to Sensor **1** in terms of selectivity in the presence of competitive ions such as Mn²⁺ and Al³⁺.

Keywords: mercury sensor, fluoroionophores, diphenylmaleimide, Hg²⁺-selectivity

INTRODUCTION

Mercury is one of the most highly toxic and hazardous pollutants with recognised accumulative characters in the environment and biota [1-3]. Mercury can cause serious human health problems since it can easily pass through the skin, respiratory and cell membrane, leading to DNA damage, mitosis impairment and permanent damages of the central nervous system, including Minamata disease [4-7].

Recent techniques for the determination of Hg^{2+} , including atomic absorption spectroscopy [8], inductively coupled plasma mass spectrometry [9] and electrochemistry [10], often require a large amount of samples, high cost and sophisticated instrumentation, which pose serious limitations on the detection of Hg^{2+} in biological samples and the tracking of Hg^{2+} for environmental monitoring. Alternatively, fluorometric sensing for the detection of Hg^{2+} offers many advantages since it is highly sensitive and allows nondestructive, prompt determination and real time tracking for the detection of Hg^{2+} .

While many fluorescent sensors have been designed for Hg^{2+} -sensing, many lack the suitability for practical uses due to multi-step syntheses, synthetic difficulty, high costs of starting materials or high detection limits for the determination of Hg^{2+} [11-21]. In addition, they often suffer from cross-sensitivity towards other ions, particularly potential competitors such as copper (Cu^{2+}) and lead (Pb^{2+}), due to their similar chemical behaviours to Hg^{2+} [15-16, 18-19, 22-25]. Notably, most of the reported Hg^{2+} fluorescent chemosensors reveal a fluorescent quenching “turn-off” mechanism due to the quenching characteristic of Hg^{2+} ions, which limits the number of fluorescent enhancement “turn-on” Hg^{2+} sensors reported so far [11, 14, 26].

In the present study, the major motivation is the design and synthesis of new fluorescent enhancement “turn-on” Hg^{2+} sensors which are expected to provide high sensitivity and selectivity to Hg^{2+} , but with a significantly reduced synthetic effort.

These novel sensors were fabricated from the structure of 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine, which consists of two sulphur and nitrogen atoms into the platform. It is expected that the sensor can provide appropriately located sulphur and nitrogen atoms as donor atoms that can self-assemble around the Hg^{2+} ions due to the favorable electrostatic interactions [26-28]. This study also focused on the effect of utilising diphenylmaleimide as a fluorophore to increase the sensitivity of the sensor system due to its photostability, relatively high fluorescence quantum yield and long emission wavelengths (~ 500 nm) in the visible region [29-31]. Although many modified structures of diphenylmaleimide fluorophores have been utilised for optoelectronic applications such as organic light emitting diodes [32, 33] and fluorescence photopatterned images materials [34], there are no known reports of utilisation of diphenylmaleimide fluorophores for Hg^{2+} fluorometric sensing applications.

MATERIALS AND METHODS

All reagents and solvents were purchased from Fluka Chemical Corporation and were used as received. All of the metal salts used in this study were perchlorate salts and were purchased from Strem Chemicals, Inc. NMR spectra were obtained with a Bruker Avance 300 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C . All NMR spectra were obtained in CDCl_3 solutions with TMS as internal standard. Mass spectra were performed by a ThermoElectron LCQ-DECA-XP, electrospray ionisation trap mass spectrometer. Fluorescence measurements were performed on a Perkin Elmer Luminescence spectrometer LS 50B. Samples were measured in a 1x1 cm quartz cuvette. The excitation and emission slit widths were 5.0 nm. The scan rate was 300 nm/min. Molecular modelling was performed with the Discovery Studio 2.5 program package.

Synthesis: 2-[3-(2-Aminoethylsulfanyl)propylsulfanyl]ethanamine

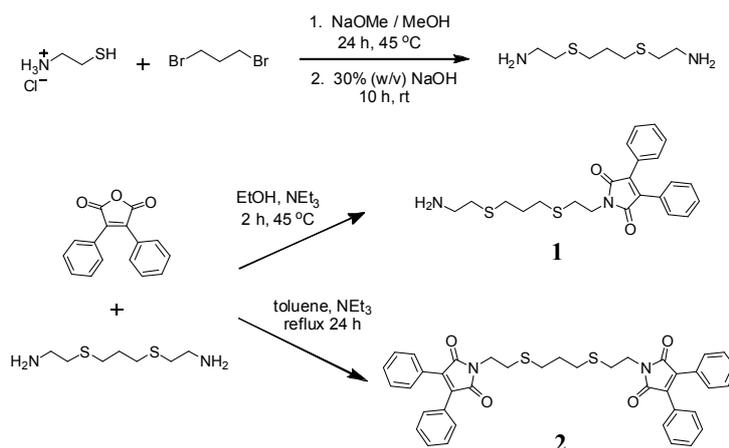
The synthesis of the titled compound was performed in the same manner as described previously [28] by alkylation of cysteamine hydrochloride with 1,3-dibromopropane and the synthetic steps are outlined in Scheme 1.

Synthesis of Sensor 1

Sensor **1** was prepared according to the synthetic pathway in Scheme 1. In a round bottom flask, 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine (0.155 g, 0.80 mmol) and triethylamine (0.20 mL, 3.4 mmol) were dissolved in dry ethanol (15.0 mL) under an argon atmosphere. Diphenylmaleic anhydride (0.100 g, 0.40 mmol) was added and the mixture was stirred for 2 hr at 45°C. The solvent was then removed under vacuum to give a yellow oil. The crude product was purified by preparative thin layer chromatography using silica gel as a stationary phase and 10% methanol in dichloromethane as mobile phase ($R_f = 0.63$) to yield 39 mg of a yellow oil (23%). ^1H NMR (300 MHz, CDCl_3): δ 1.84-1.94 (m, 2H), 1.95 (s, NH_2), 2.59-2.63 (m, 4H), 2.69-2.75 (m, 2H), 2.80-2.89 (m, 4H), 3.87 (t, $J = 7.5$, 2H), 7.32-7.49 (m, 10H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ 29.2 (CH_2), 30.0 (CH_2), 30.4 (CH_3), 30.5 (CH_2), 36.0 (CH_2), 37.5 (CH), 42.5 (CH_2), 128.5 (2CH), 129.8 (8CH), 136.2 (4C), 170.5 (2C) ppm. HRMS calculated for $\text{C}_{23}\text{H}_{27}\text{N}_2\text{O}_2\text{S}_2^+$ ($\text{M}+\text{H}$) $^+$ 427.1508 (found 427.1487).

Synthesis of Sensor 2

Sensor **2** was obtained according to the synthetic pathway in Scheme 1. In a round bottom flask, 2-(3-(2-aminoethylsulfanyl)propylsulfanyl)ethanamine (0.078 g, 0.40 mmol) and triethylamine (0.20 mL, 3.4 mmol) were dissolved in dry toluene (8.0 mL) under an argon atmosphere. Diphenylmaleic anhydride (0.200 g, 0.80 mmol) was added and the mixture was refluxed for 24 hr. The solvent was then removed under vacuum to give a yellow oil. The crude product was purified by preparative thin layer chromatography using silica gel as a stationary phase and 15% ethyl acetate in hexane as mobile phase ($R_f = 0.42$) to yield 123 mg of a yellow oil (47%). ^1H NMR (300 MHz, CDCl_3): δ 1.94 (quintet, $J = 7.2$, 2H), 2.74 (t, $J = 7.2$, 4H), 2.84 (t, $J = 6.9$, 4H), 3.87 (t, $J = 7.5$, 4H), 7.33-7.51 (m, 20H) ppm. ^{13}C NMR (75 MHz, CDCl_3): δ 28.9 (2 CH_2), 29.9 (CH_2), 30.4 (2 CH_2), 37.5 (2 CH_2), 128.6 (4CH), 129.9 (4C), 130.0 (16CH), 136.2 (4C), 170.5 (4C) ppm. HRMS calculated for $\text{C}_{39}\text{H}_{34}\text{N}_2\text{O}_4\text{S}_2\text{Na}^+$ ($\text{M}+\text{Na}$) $^+$ 681.1852 (found 681.1773).



Scheme 1. Syntheses of Sensors **1** and **2**

RESULTS AND DISCUSSION

The target sensors were prepared using a conventional two-step synthesis (Scheme 1). Sensors **1** and **2** contain two sulphur atoms and two nitrogen atoms for the binding sites which are covalently bound to diphenylmaleimide fluorophore(s). The selective binding was expected to take place through favorable electrostatic interactions between the carbonyl carbon as well as sulphur and nitrogen atoms of the sensors and Hg^{2+} ions.

Sensitivity Studies of Sensors **1** and **2**

The sensing properties of Sensors **1** and **2** were investigated in order to elucidate the quantitative binding affinity of the sensors to Hg^{2+} . Herein, the sensitivity studies of Sensors **1** and **2** were examined by measuring the fluorescence signals in the presence of various concentrations of Hg^{2+} ions. Figures 1 and 2 show the fluorescence spectra of Sensors **1** and **2** respectively, in the presence and absence of different concentrations of Hg^{2+} , which exhibited fluorescence emission maximum at 500 nm when excited at 367 nm.

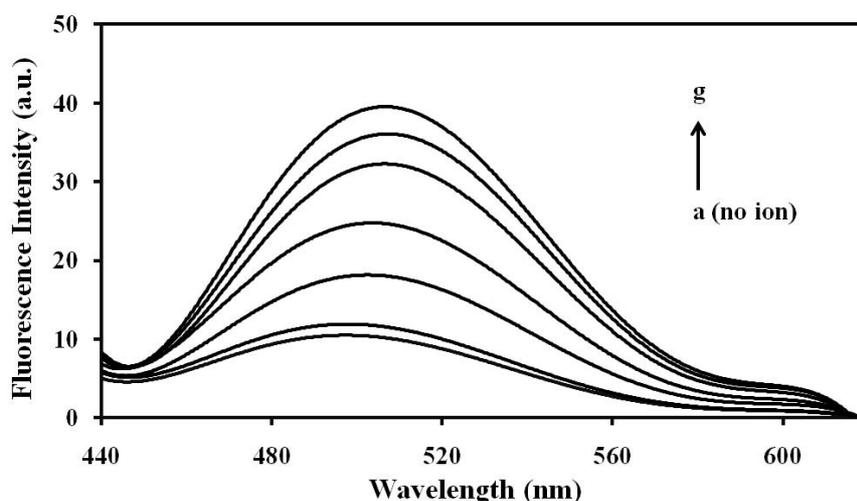


Figure 1. Fluorescence emission spectra ($\lambda_{\text{ex}}=367$ nm) of Sensor **1** ($1.0 \mu\text{M}$) in dichloromethane as a function of $[\text{Hg}^{2+}]$. a: $0 \mu\text{M}$, b: $0.7 \mu\text{M}$, c: $1.0 \mu\text{M}$, d: $1.3 \mu\text{M}$, e: $1.7 \mu\text{M}$, f: $2.0 \mu\text{M}$, g: $2.7 \mu\text{M}$

When an ion-complexation was operative, the fluorescence behaviour of Sensor **1** clearly demonstrated the off-on switching mechanism that occurred in response to Hg^{2+} ion complexation, as demonstrated in Figure 1. In the absence of Hg^{2+} ions, the fluorescence response was at a minimum and the fluorescence “turn on” as the Hg^{2+} concentration was increased. When the added mercury perchlorate attained a concentration 2.7 times higher than that of Sensor **1**, the fluorescence response reached a maximum point and reached a plateau. The fluorescence quantum yield (ϕ_f) of Sensor **1** with 6.7 equiv. of Hg^{2+} was determined to be 0.021, using quinine sulphate standard with a ϕ_f of 0.54 in 0.1 M H_2SO_4 as a reference [35]. The association constant, K_{assoc} , was obtained by Benesi-Hildebrand plot of the signal changes in the fluorescence titration results [14, 36] and was found to be $3.27 \times 10^5 \text{ M}^{-1}$ and the 1:1 complex formation of **1**- Hg^{2+} was suggested. The 1:1 complex formation was consistent with molecular modelling studies. The detection limit of Sensor **1** for the analysis of Hg^{2+} was determined from the plot of fluorescence intensity as a function of the Hg^{2+} concentrations [37]. It was found that Sensor **1** had a detection limit of $6.72 \times 10^{-7} \text{ M}$ for Hg^{2+} ions, which is sufficient for the detection of sub-micromolar concentrations of Hg^{2+} ions found in many environmental systems such as edible fish [38]. In addition, Sensor **1** offered long-wavelength emission and the change in fluorescence signals in the visible regions, which could be employed to fabricate an economical Hg^{2+} testing tool.

In a similar study, the fluorescence titrations of Sensor **2** with Hg^{2+} were carried out and Sensor **2** acted as an off-on fluorescence switch upon Hg^{2+} binding as illustrated in Figure 2. The sensor showed a high Hg^{2+} -sensitivity and the emission intensity of Sensor **2** was effectively enhanced upon the addition of Hg^{2+} ions. However, Sensor **2** was found to be a comparable sensor to Sensor **1** in terms of sensitivity. It was found that Sensor **2** provided a detection limit of $6.67 \times 10^{-7} \text{ M}$ for Hg^{2+} ions. The fluorescence quantum yield (ϕ_f) of Sensor **2** with 6.7 equiv. of Hg^{2+} was found to be 0.06, based on quinine sulphate standard. The association constant was found to be $5.81 \times 10^5 \text{ M}^{-1}$ and the 1:1 complex formation of Sensor **2**- Hg^{2+} was suggested.

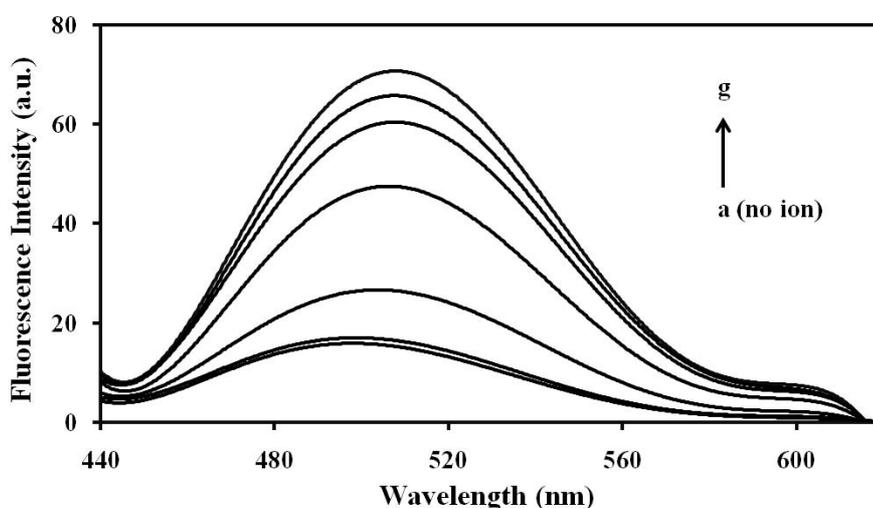


Figure 2. Fluorescence emission spectra ($\lambda_{\text{ex}}=367 \text{ nm}$) of Sensor **2** ($1.0 \mu\text{M}$) in dichloromethane as a function of $[\text{Hg}^{2+}]$. a: $0 \mu\text{M}$, b: $0.7 \mu\text{M}$, c: $1.0 \mu\text{M}$, d: $1.3 \mu\text{M}$, e: $1.7 \mu\text{M}$, f: $2.0 \mu\text{M}$, g: $2.7 \mu\text{M}$

Binding Modes of the Sensors

To explain the coordination geometry of Sensors **1** and **2** and Hg^{2+} upon binding, molecular modelling was performed using the Discovery Studio 2.5 program. The structures of Sensors **1** and **2** were initially modified from the X-ray crystal structure of N,N'-(3,7-diazanonylene)-bis-naphthalimide in the protein databank PDB ID = 1CX3 and diphenylmaleic anhydride from PubChem compound (CID 78530), and optimised using density functional theory with local density approximation (LDA) of local functional PWC [39]. Then, the initial structures were optimised using CHARMM force field. The complexation energy of the host-guest structure was calculated from the Energy of complex – Energy of compound – Energy of Hg^{2+} using density functional theory with LDA of local functional PWC with implicit distance-dependent dielectrics.

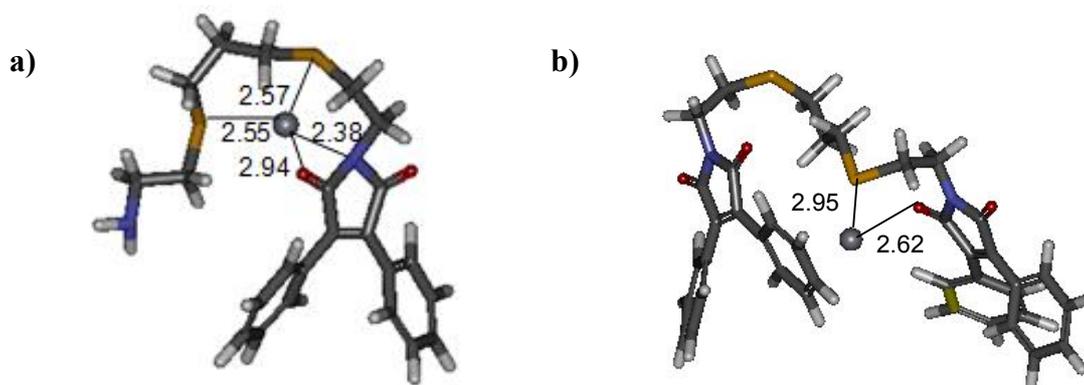


Figure 3. Optimised structures of 1:1 complexes of (a) **1**- Hg^{2+} and (b) **2**- Hg^{2+} from molecular dynamic with LDA of local functional PWC. The Hg^{2+} ions were shown in ball model. The C, N, O, S, H were in grey, blue, red, yellow and white respectively. The distances are shown in Angstrom

The optimised structures of the host-guest complexes are shown in Figure 3, indicating that ion-recognition of the sensors originated from self-assembly processes of the sensors and Hg^{2+} from the favorable electrostatic interactions (ion-dipole interactions) of the carbonyl carbon as well as the sulphur and nitrogen atoms to Hg^{2+} to form a wrapping structure. The distances to indicate the binding sites of Hg^{2+} bound to Sensors **1** and **2** are illustrated in Figure 3. For Sensor **1**, Hg^{2+} was coordinated to the carbonyl oxygen, two sulphur atoms and nitrogen atom with the distances of 2.94, 2.55, 2.57 and 2.38 Å respectively. As to Sensor **2**, Hg^{2+} was bound by carbonyl carbon and sulphur atom with the distances of 2.62 Å and 2.95 Å respectively.

Selectivity Studies of Sensors **1** and **2**

The selectivity studies of Sensors **1** and **2** were performed in dichloromethane solutions by recording the fluorescence spectra of the solutions before and after the addition of each representative metal ion. In this study, the selectivity studies were obtained by a similar method to the separate solution method (SSM) used in ion-selective electrode applications [40]. This method involves the measurement of a series of separate solutions, with each solution containing only a salt of the determined ion. Figure 4 represents the dependence of the fluorescence intensity of Sensors **1** and **2** as a function of cation concentrations of Hg^{2+} , Cu^{2+} , Pb^{2+} , Li^+ , Na^+ , Mg^{2+} , Cd^{2+} , K^+ , Al^{3+} , Fe^{3+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Mn^{2+} and Zn^{2+} .

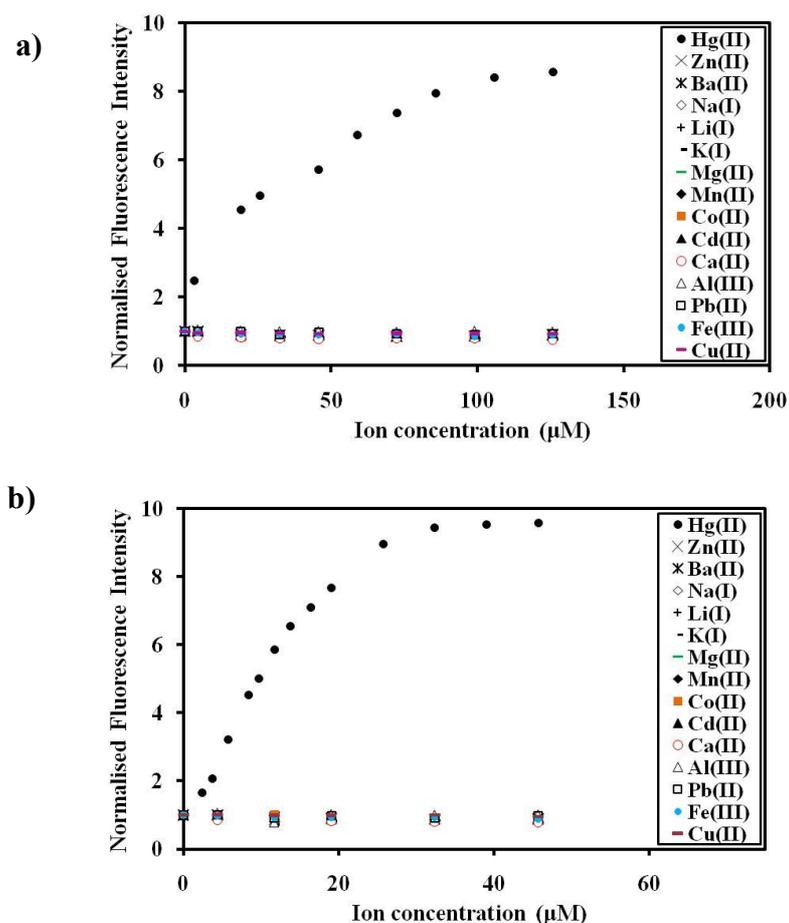


Figure 4. a) Normalised fluorescence intensity of Sensor 1 (1.0 μM) and b) normalised fluorescence intensity of Sensor 2 (1.0 μM) at 500 nm versus the concentrations of various metal ions, i.e. Hg^{2+} , Cu^{2+} , Li^+ , Na^+ , Mg^{2+} , Cd^{2+} , K^+ , Al^{3+} , Fe^{3+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Mn^{2+} , Zn^{2+} and Pb^{2+}

The values in the plots were normalised to the fluorescence intensity at 500 nm. The selectivity studies clearly exhibited the excellent selectivity of Sensors 1 and 2 to Hg^{2+} ions in comparison with other metal ions. The results showed that the fluorescence responses at 500 nm increased as a function of added Hg^{2+} until it reached the maximum points. On the other hand, the responses of Sensors 1 and 2 did not cause any significant changes after the addition of Cu^{2+} , Pb^{2+} , Li^+ , Na^+ , Mg^{2+} , Cd^{2+} , K^+ , Al^{3+} , Fe^{3+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Mn^{2+} and Zn^{2+} under identical conditions.

To explore further utilisation of 1 and 2 as Hg^{2+} -selective sensors, competitive studies of Sensors 1 and 2 were performed. Figures 5 and 6 demonstrated the competitive signalling behaviours of Sensors 1 and 2 respectively, with Hg^{2+} in the presence of environmentally important metal ions (Cu^{2+} , Pb^{2+} , Li^+ , Na^+ , Mg^{2+} , Cd^{2+} , K^+ , Al^{3+} , Fe^{3+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Mn^{2+} and Zn^{2+}).

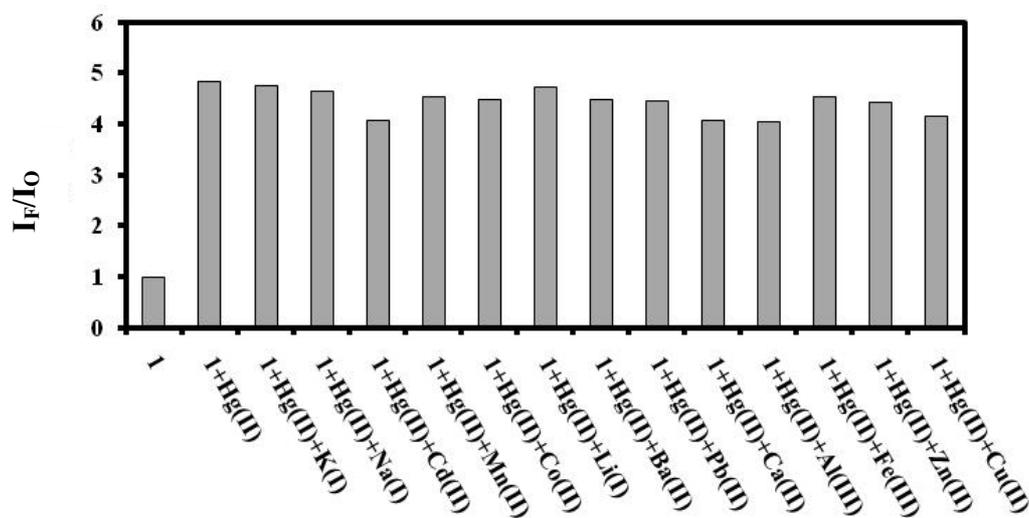


Figure 5. Competitive experiment in the **1**-Hg²⁺ system with common foreign metal ions: [**1**] = 1.0 μ M, [Hg²⁺] = [Mⁿ⁺] = 1.0 μ M in dichloromethane solutions (λ_{ex} 367 nm)

The bars in the Figure represent the final fluorescence emission response (I_F) over the initial fluorescence emission response (I_0) at 500 nm. I_F was the fluorescence emission of Sensor **1** in the presence of competitive cations (1.0 μ M each of Cu²⁺, Li⁺, Na⁺, Mg²⁺, Cd²⁺, K⁺, Al³⁺, Fe³⁺, Ca²⁺, Ba²⁺, Co²⁺, Mn²⁺, Zn²⁺ and Pb²⁺) and Hg²⁺ (1.0 μ M). I_F/I_0 (where I_F was the fluorescence intensity of Sensor **1** in the presence of Hg²⁺ only) was used as a reference and the I_F/I_0 reference value was equal to 4.8. The I_F/I_0 values were found to lie between 4.1-4.8, indicating that a relatively consistent Hg²⁺-induced fluorescence enhancement was observed in the presence of equimolar amounts of competing ions. It should be noted that the sensing ability of Sensor **1** showed the sensitivity for Hg²⁺ in the presence of Cu²⁺ and Pb²⁺, which are potential competitors. The observed selectivity for Hg²⁺ was remarkable compared to many multidentate thioether-containing ligands, i.e. calixarenes, cyclams and cyclens, in previous reports [15, 16, 18, 19, 22-25].

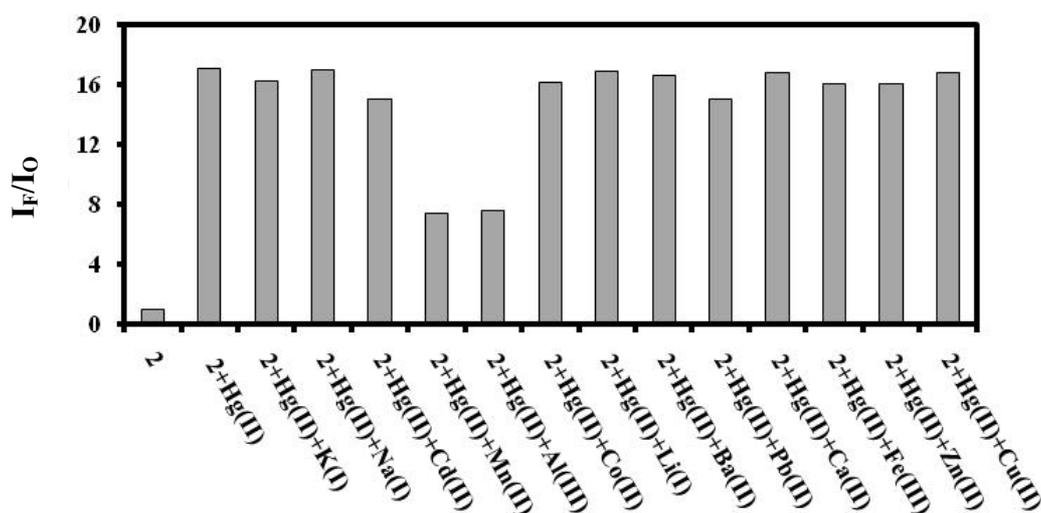


Figure 6. Competitive experiment in the **2**-Hg²⁺ system with common foreign metal ions: [**2**] = 1.0 μ M, [Hg²⁺] = [Mⁿ⁺] = 1.0 μ M in dichloromethane solutions (λ_{ex} 367 nm)

Figure 6 shows the competitive signalling behaviours of Sensor 2 with Hg^{2+} in the presence of equimolar amounts of competing ions. Sensor 2 was found to be inferior to Sensor 1 in terms of selectivity in the presence of competitive ions since the sensing ability of Sensor 2 showed poor selectivity for Hg^{2+} in the presence of equimolar amounts of Mn^{2+} and Al^{3+} . The lower selectivity of Sensor 2 might be due to the steric effect from two diphenylmaleimide fluorophores upon ion binding. From the computational data, these two bulky groups give rise to a larger binding site from the self-assemble process. Therefore, in a competitive experiment, the Hg^{2+} binding could be interfered and replaced by some other ions such as Mn^{2+} and Al^{3+} in this case.

CONCLUSIONS

The first use of diphenylmaleimide fluorophore as new fluorescent enhancement “turn-on” Hg^{2+} sensors was successfully demonstrated. Two new sensors based on the 2-[3-(2-aminoethylsulfanyl)propylsulfanyl]ethanamine ligand covalently bound to one and two units of the diphenylmaleimide fluorophore, Sensors 1 and 2, were prepared by a conventional two-step synthesis. Especially, Sensor 1 showed highly sensitive and selective fluorescence “turn-on” behaviour toward Hg^{2+} in solutions and was shown to discriminate various foreign ions, i.e. Cu^{2+} , Pb^{2+} , Li^+ , Na^+ , Mg^{2+} , Cd^{2+} , K^+ , Al^{3+} , Fe^{3+} , Ca^{2+} , Ba^{2+} , Co^{2+} , Mn^{2+} and Zn^{2+} . The molecular design presented could serve as an alternative mercury fluorometric sensor due to the advantages of synthetic simplicity, cost-efficient synthetic route, high sensitivity to Hg^{2+} by “turn-on” fluorescence response in the visible region and high selectivity with particular discrimination of the potential competitors including Cu^{2+} and Pb^{2+} . The new off-on type fluorescence enhancement sensor based on diphenylmaleimide fluorophore could serve as a new potential design for future development of sensor systems.

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