

<Research Paper>

Rhodamine 6G Based New Fluorophore Chemosensor Toward Hg²⁺

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Abstract: Rhodamine dyes belong to xanthene family has excellent photostability and photophysical properties. In rhodamine dyes, Rhodamine 6G and its precursors also have xanthene chromophore and it shows high fluorescent quantum yield. Rhodamine 6G derivatives are simple to synthesis and its high sensitivity and water solubility are suitable as good chemosensor. In this regard, Rhodamine 6G derivatives which have selectivity to specific metal cation can be used to detect various heavy metal ions. In this study, rhodamine 6G derivatives were synthesized by reaction of rhodamine 6G hydrazide and glyoxal and 4-phenyl thiosemicarbazide and it showed colorimetric and fluorescence sensing toward Hg²⁺ ion. This novel chemosensor was analyzed and measured on UV-Vis and fluorescence spectrophotometer. HOMO/LUMO values were also calculated by computational calculation.

Keywords: rhodamine 6G, chemosensor, absorbance, fluorescence, heavy metal ions, HOMO/LUMO

1. Introduction

In environment, mercury is the one of famous toxic heavy metal which could cause various diseases for living organisms. For this reason the many fluorescent chemosensor was studied for sensing mercury^{1,2)}. Rhodamine was first synthesized by Noeltling and Dzienwonsky in 1905 and has been widely used in many research fields, including the lasing medium in dye lasers and fluorescent markers in biological studies³⁾. The rhodamine based dyes are functional substance to sense metals. So many previous reports, showing rhodamine derivatives are the high-quality to sense metal ions like Hg²⁺, Cu²⁺, Fe³⁺, Zn²⁺, Pb²⁺, Pd²⁺, Cr³⁺, Ba²⁺ etc.⁴⁻⁶⁾.

Sensing principle of rhodamine, spiroactam or spiro-lactone derivatives are nonfluorescent and colorless, but ring-opening of the corresponding spiroactam /lactone gives rise to strong fluorescence emission.

In general, rhodamine derivative displays a red color change and strong fluorescence in acidic solutions by activation of a carbonyl group in a spiroactone or spiroactam moiety.

In a similar way, an appropriate ligand on a spiro-lactam ring can induce a color change as well as a fluorescence change upon addition of metal ions⁷⁻⁹⁾.

In this study, we reported a novel class of chemosensor involved in rhodamine 6G based on 4-phenyl thiosemicarbazide. This chemosensor can detect Hg²⁺ ion, which release red fluorescence emission compared with other heavy metal ions like Hg²⁺, Zn²⁺, Ag²⁺, Ba²⁺, Pb²⁺, Fe³⁺. Its properties of Hg²⁺ detection were investigated and analyzed in details. Structural analysis of this novel chemosensor dye was identified by Proton-NMR, Mass. Its absorption and fluorescence emission measured by job's plot method.

Last, electron distribution and energy level calculation such as HOMO and LUMO was computationally simulated using DMol³ program of *Material Studio 4.3* package.

2. Experimental

2.1 Measurement

All the reagents and solvents, used for synthesis of rhodamine 6G based dye sensor, were purchased from Aldrich and used without further purification.

Absorption and fluorescence spectra of the prepared

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dye sensor were measured with an Agilent 8453 spectrophotometer and a Shimadzu RF-5301PC fluorescent spectrophotometer, respectively. ¹H NMR spectra and elemental analyses were recorded AVANCE III 600 spectrometer operated at 600 MHz NMR respectively. Mass spectra were recorded on a JEOL MStation [JMS-DX303].

HOMO/LUMO calculation and modeling simulation proceed with DMol³ of *Material Studio 4.3*.

2.2 Synthesis

Rhodamine 6G hydrazide (compound 1) was synthesized in a high yield from rhodamine 6G according to the literature procedure (compound 1)^{10,11}.

Rhodamine 6G hydrazide reacted with glyoxal gives result as compound 2 according to the literature¹². 4-phenyl thiosemicarbazide 0.167 g (1 mmol) and compound 2 0.469 g (1 mmol) were dissolved in 40ml of acetic acid and refluxed for 8 h, then cooled to room temperature. The red precipitate was collected by filtration and washed with acetic acid and dried in vacuum (Scheme 1).

The solid was purified by chromatography on SiO₂ eluted with 10% ethyl acetate in CHCl₃ to give Chemosensor 1 as 0.208g (37%)^{13,14}.

3. Result and Discussion

3.1 Analysis data of chemosensor 1

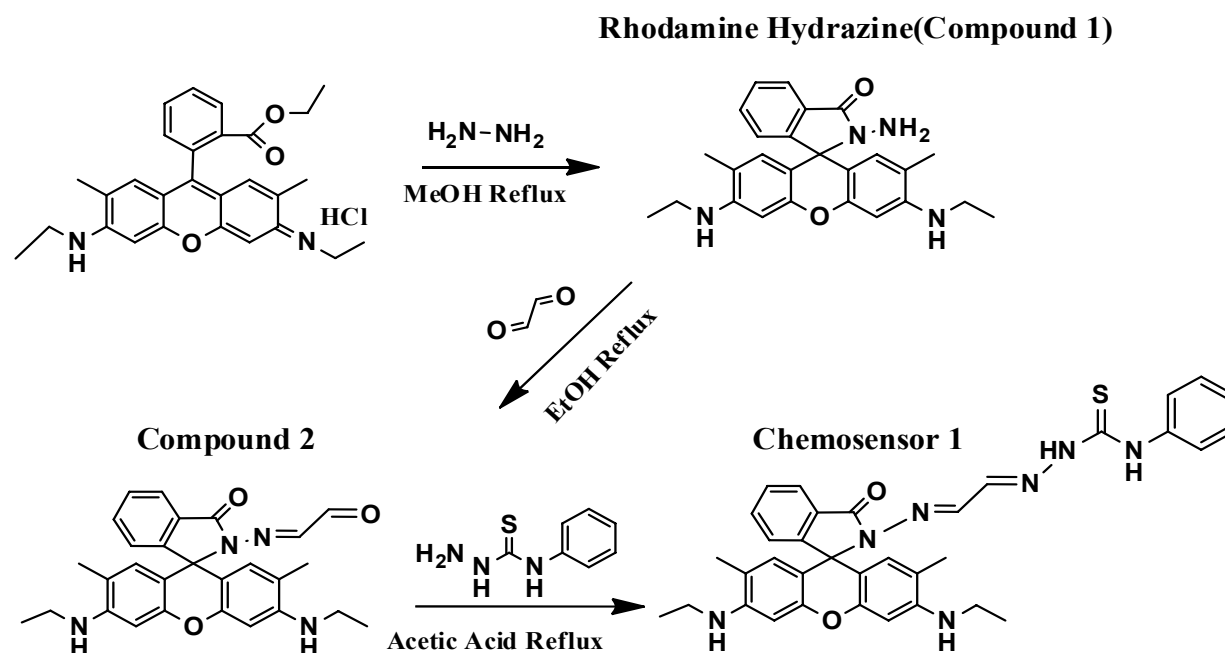
¹H-NMR (600MHz, DMSO₄) : 11.88(s, 1H), 10.041(s, 1H), 8.44-8.42(d, 2H), 7.88-7.87(d, 1H), 7.59-7.44(m, 2H), 7.32-7.31(d, 1H), 7.29-7.16(d, 1H), 6.95-6.94(d, 1H), 6.28-6.17(d, 4H), 5.14-5.04(m, 3H), 3.18-3.02(m, 4H), 2.07-1.83(m, 6H), 1.22-1.09(m, 6H) (Figure 1). Anal. Calcd: for C₃₅H₃₅N₇O₂S: C, 68.05; H, 5.71; N, 15.87; Found : C, 71.67; H, 7.30; N, 9.47. MS m/z: 617.7 (M⁺). M.P : 202.6°C.

3.2 Sensing of Hg²⁺ in acetonitrile

Photograph image for proving the characteristic of chemosensor 1, showed chemosensor 1 has sensing ability toward Hg²⁺ ((a) in Figure 2).

And image which use fluorescence showed that chemosensor 1 has sensitivity toward Hg²⁺ than other metal ions ((b) in Figure 2).

Chemosensor 1 has nonfluorescent and colorless, but ring-opening of the corresponding spirolactam gives rise to strong fluorescence emission. This mechanism of these reactions, colorimetric and fluorescence, are followed by the literature reference⁷.



Scheme 1. Synthesis of rhodamine 6G based new chemosensor (chemosensor 1).

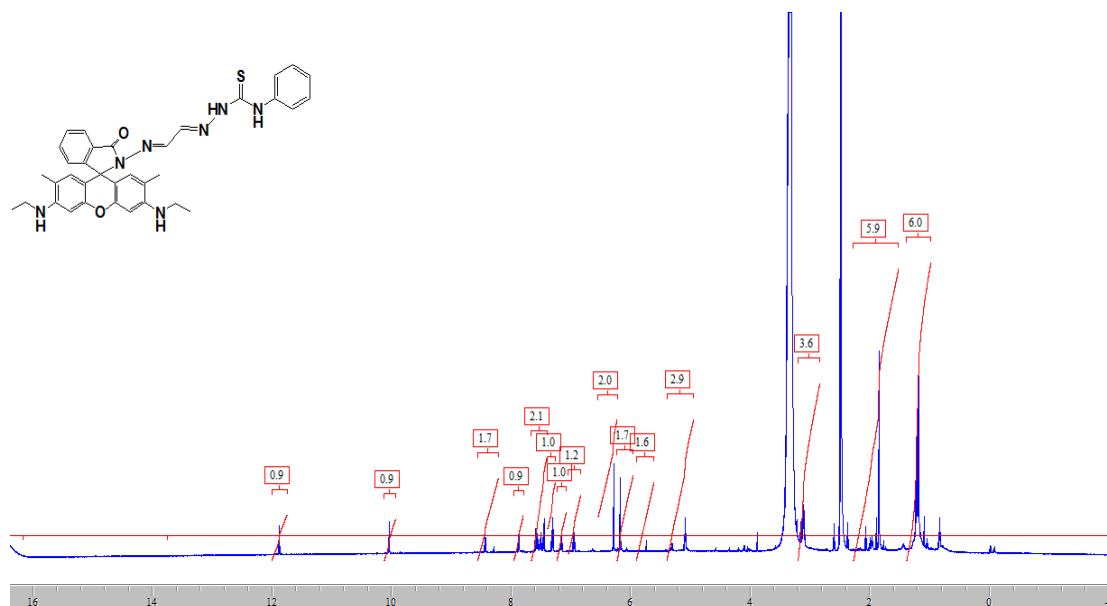


Figure 1. H-NMR data of Chemosensor 1.

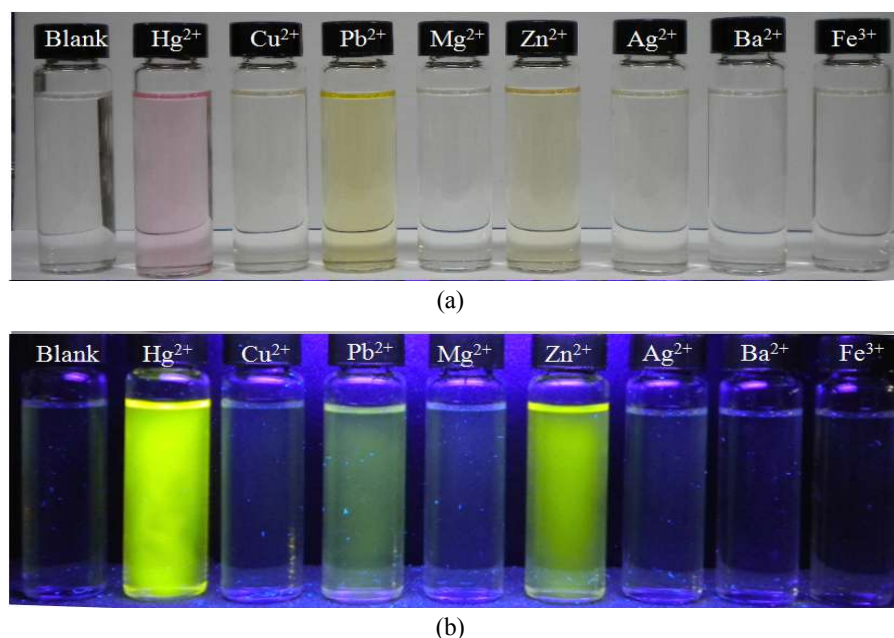


Figure 2. Photographs of absorption (a) and emission (b) site for chemosensor 1.

(a) Various colorimetric absorption of chemosensor 1 and metal ions in acetonitrile.

(b) Various fluorescence emission of chemosensor 1 and metal ions in acetonitrile.

3.3 UV Visible spectra and fluorescence of chemosensor 1

To investigate the selectivity of chemosensor 1 with Hg^{2+} the absorption response between chemosensor 1 and Hg^{2+} ($1 \times 10^{-5} \text{ mol}$) was monitored by UV-Visible absorption in acetonitrile as shown in Figure 3.

A strong absorption peak at 523nm and color of

chemosensor 1 was changed from colorless to red with Hg^{2+} .

Besides in attendance observed be color changes between chemosensor 1 and Zn^{2+} , between chemosensor 1 and Pb^{2+} .

In addition, the fluorescence intensity of chemosensor 1, the fluorescence peak appeared at 553nm when mixed

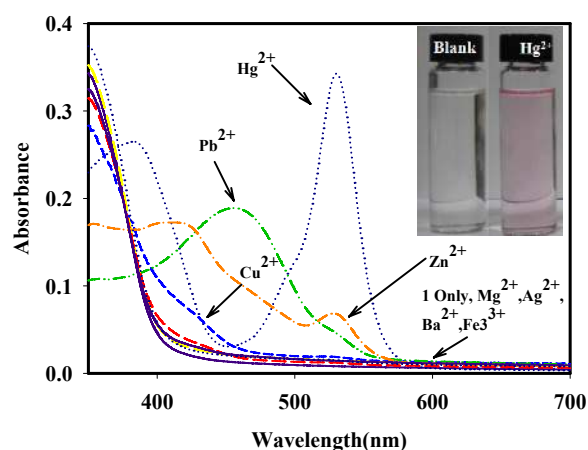


Figure 3. The absorption spectra of chemosensor 1 (1×10^{-5} mol) when it mixed with various metal ions (1×10^{-5} mol) in acetonitrile. Inset: showing the change of color before and addition of Hg²⁺.

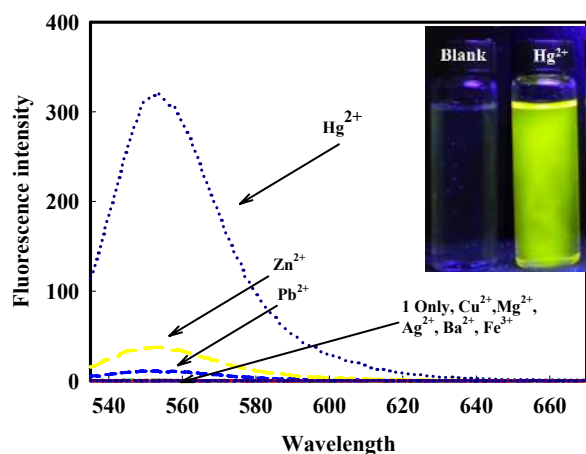


Figure 4. The fluorescence intensity of chemosensor 1 (1×10^{-5} mol) when it mixed with various metal ions (1×10^{-5} mol) in acetonitrile. Inset: showing the change of fluorescence color before and addition of Hg²⁺.

with Hg²⁺ (Figure 4). Although the fluorescences of Zn²⁺, Pb²⁺, were occurred at 553nm when mixed with chemosensor 1, the peaks are very weak fluorescence intensity compared with Hg²⁺.

3.4 Metal competition of chemosensor 1

Furthermore, to prove the selectivity of chemosensor 1, with other metal ion was added in mixture of chemosensor 1 and Hg²⁺. As shown in Figure 5 we can prove that Cu²⁺, Fe³⁺ have little disturb competition toward Hg²⁺. Although effect of Cu²⁺, Fe³⁺, it is confirmed that chemosensor 1 has selectivity toward Hg²⁺ when use fluorescence.

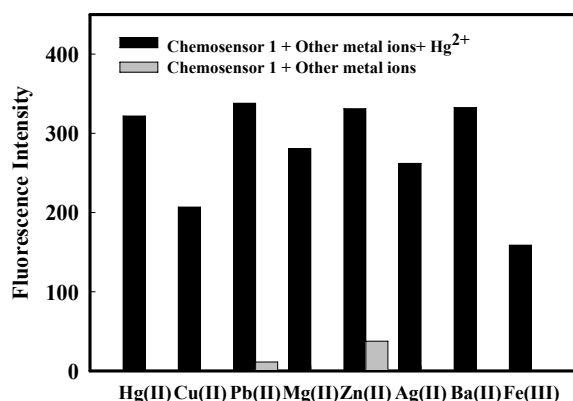


Figure 5. The fluorescence intensity of chemosensor 1 (1×10^{-5} mol) with Hg²⁺ (1×10^{-5} mol) when it complete with other metal ion 1×10^{-5} mol) in acetonitrile.

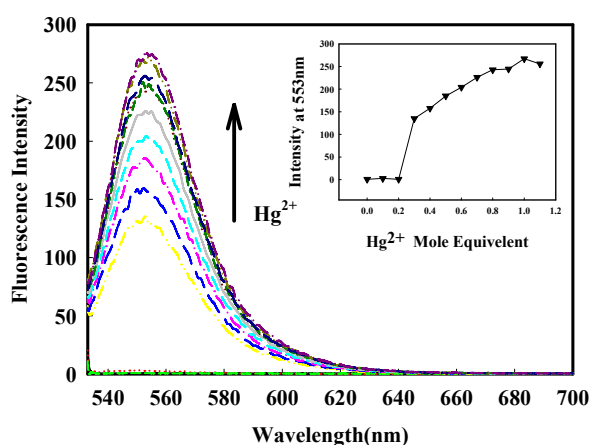


Figure 6. Fluorescence intensity changes of chemosensor 1 depend on the addition of Hg²⁺. Inset: showing fluorescence intensity addition of Hg²⁺ mole equivalent.

3.5 UV Visible spectra and fluorescence intensity of Hg²⁺ titration

To prove the interaction of chemosensor 1 and Hg²⁺, fluorescence titration of chemosensor 1 and Hg²⁺ was performed. When Hg²⁺ added, the fluorescence peak was enhanced at 553nm (Figure 6). The fluorescence emission color was changed from colorless to greenish yellow with addition of Hg²⁺.

In addition UV-Visible titration of chemosensor 1 and Hg²⁺ was also proved for the interaction (Figure 7). UV-Visible absorbance peak pointed high intensity at 523nm.

The solution color was changed from colorless to red with addition of Hg²⁺ indicated by changes near 320nm absorption peak intensity was decreased when

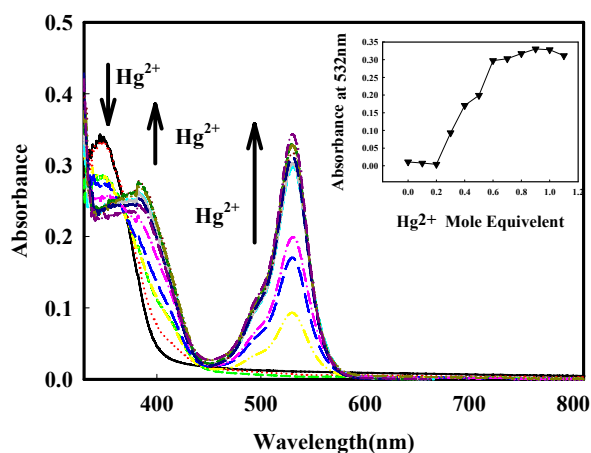


Figure 7. UV-vis absorbance changes of chemosensor 1 depend on the addition of Hg^{2+} . Inset: showing UV-Visible absorbance addition of Hg^{2+} mole equivalent.

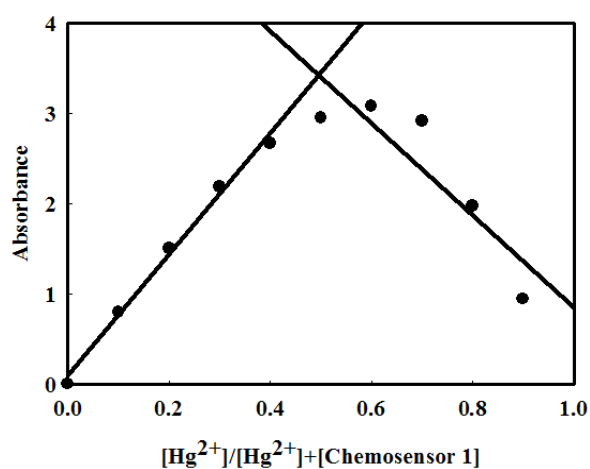


Figure 8. To use Job's plot, chemosensor 1 and Hg^{2+} ion mixture was monitored at 523nm wavelength: the total concentration of $[\text{Hg}^{2+}] + [\text{chemosensor 1}]$ was 5.0×10^{-5} mol.

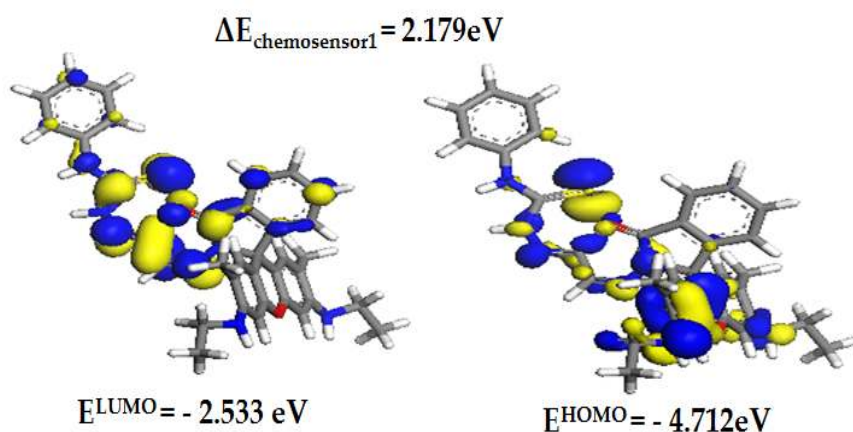


Figure 9. Electron distributions and HOMO/LUMO energy levels of chemosensor 1.

chemosensor 1 contain more Hg^{2+} , and also another two peaks intensity increased at near 400nm and 523nm as shown in Figure 7.

3.6 Jobs method of chemosensor 1 toward Hg^{2+}

We investigated the binding ratio between chemosensor 1 and Hg^{2+} using the Job's method^{15,16}.

For the measurement of Job's plot method, various molar ratios between chemosensor 1 and Hg^{2+} metal ions (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) in MeCN were prepared. The relationship between maximum absorption peaks versus various mole fractions is shown in Figure 8.

The result take the 1:1 binding ratio of chemosensor 1 toward Hg^{2+} with total concentration of $[\text{Hg}^{2+}] + [\text{chemosensor 1}]$ at 5.0×10^{-5} mol.

The absorbance made average lines from molar fraction of Hg^{2+} 0 to 0.4 and from molar fraction of Hg^{2+} 0.5 to 0.9, two lines met at 0.5 molar fraction.

3.7 Electron distributions of chemosensor 1

We computationally calculated to investigate electron distributions and HOMO/LUMO energy levels of chemosensor 1. It has been simulated with *Material Studio 4.3* program which is the quantum mechanical code using density functional theory.

Perdew-Burke-Ernzerhof (PBE) function of generalized gradient approximation (GGA) level with double numeric polarization basis set was used to calculate the energy level of the frontier molecular orbitals¹⁷⁻¹⁹. As shown in Figure 9, chemosensor 1 was calculated electron distributions and its HOMO/LUMO energy levels of chemo-

sensor 1.

HOMO and LUMO values were calculated respective -4.712eV and -2.533eV. In this regard, the ΔE value was finally calculated -2.179eV.

HOMO and LUMO states were showed that electron distributions moved from xanthene part to 4-phenyl thiosemicarbazide parts in structure of chemosensor 1. This indicates that chemosensor 1 has interesting property of intra molecular charge transfer system.

4. Conclusion

In this study, we synthesized rhodamine 6G based dye which characteristic is proved by various metal ions(Hg²⁺, Zn²⁺, Ag²⁺, Ba²⁺, Pb²⁺, Mg²⁺, Fe³⁺). It was proved by H-NMR, E.A, and Mass spectrum.

The result showed that chemosensor 1 changed from colorless to red color absorption and colorless to yellowish green color fluorescence intensity with Hg²⁺. This sensor's reaction ratio with Hg²⁺ revealed 1:1 from job's plot method. HOMO/LUMO energy potential was invested by Dmol³ program. In addition, chemosensor 1 also has interesting property of intra-molecular charge transfer (ICT) system.

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