

NOVEL “TURN-ON” FLUORESCENT CHEMOSENSOR FOR THE SELECTIVE DETECTION OF Hg²⁺ AND THEIR LIVE CELL IMAGING

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Abstract

We have designed, synthesized, and evaluated a novel 2-hydroxynaphthalene-1,4-dione derivative based “turn on” fluorescent chemosensor (L1, L2) for the detection of Hg²⁺ ions in THF:H₂O. The fluorescent spectra changes observed upon addition of various metal ions show that L1, L2 is highly selective for Hg²⁺ over other metal ions. An important feature for the new chemosensor probe displayed an apparent colour change, which could be observed by under a UV lamp. Experimental results have been verified with DFT and TDDFT calculation. Confocal fluorescence microscopy imaging using cells showed that (L1, L2) could be used as an effective fluorescent probe for detecting Hg²⁺ in living cells.

Introduction

Mercury and compounds containing mercury are widely used in industry, although they have inherent toxicity. Several diseases have been known to be associated with mercury contamination [1–6]. Therefore, design and development of new sensitive and selective Hg(II) sensors have received a lot of attention. In 1992, Chea and Czarnik [7] reported a chemodosimeter that showed significant fluorescence alteration after reaction with Hg(II) ion. Fluorescent chemosensors for Hg(II) ion have also been described. We have recently found that 4-[2-(9-anthryloxy) ethyl]thio-1,3- dithiole-2-thione also displays similar fluorescence change upon reaction with Hg(II) ion [8]. However, most of the reported chemosensors for Hg(II) ion belong to fluorescence quenching chemosensors, since Hg(II) ion like other HTM (heavy transition metal) ions is known as fluorescence quenchers [9–16]. Compared with fluorescence quenching, fluorescence enhancement is more favorable for sensitive detection and wide use. By now, only a few Hg(II) ion chemosensors with fluorescence enhancement (“turn on” fluorescent chemosensor) have been described [17–21]. In addition, it is desirable that the chemosensors can be easily prepared since complicated or time-consuming synthesis may limit their applications. Herein we describe a sensitive and selective “turn on” chemosensor for Hg(II) ion based on a new naphthoquinone derivatives. The design rationale for this new Hg(II) ion chemosensor is illustrated in scheme 1. We want to take the advantage of the fact that a molecule with two pyrene units shows strong excimer emission around 470 nm [22–26]. If a quinone unit is linked to a ligand that can coordinate with Hg(II) ion, such a coordination compound with two pyrene units would show strong excimer emission of pyrazole. In order to achieve high sensitivity and selectivity, the candidate ligand should display very high affinity to Hg(II) ion. During our studies of thymine derivatives we unexpectedly found that quinine scaffold and its pyrazole can target Hg(II) ion very selectively. The nitrogen or oxygen atoms of the carbonyl group of pyrazole are possibly the coordination sites. Ono and To gashi reported a highly selective oligonucleotide-based sensor for Hg(II) ion [27].

In this present work we have synthesized a novel fluorescent sensor (L₁, L₂) based on fluorescein and 2-hydroxynaphthalene-1,4-dione as the receptor and highly selective, sensitive colorimetric fluorescent sensor for Hg²⁺ ions. The photo physical properties and recognition behaviours of the chemo sensor have been investigated in detail through UV–vis absorption spectra, fluorescence spectra, Mass, theoretical calculations and cell imaging were captured by Olympus FV-1000 laser scanning confocal fluorescence microscope

Experimental section

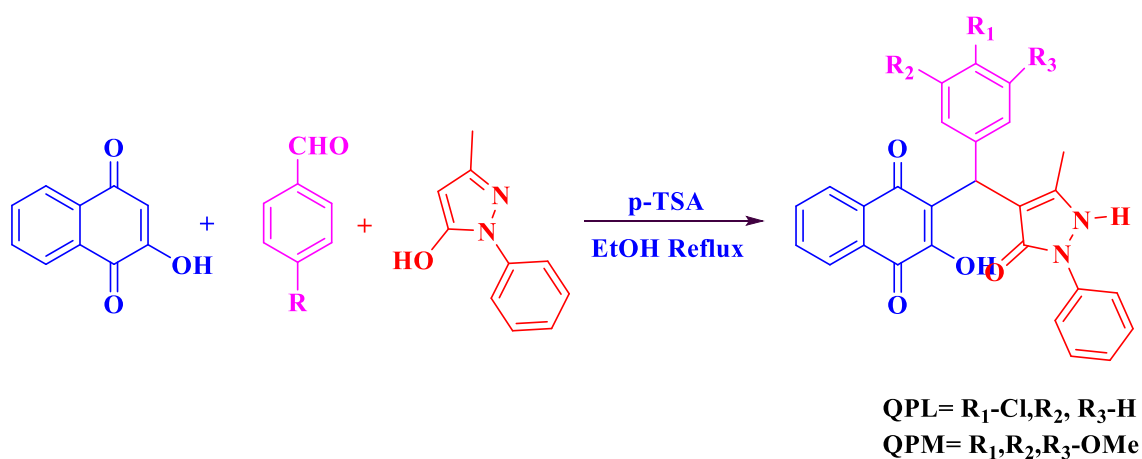
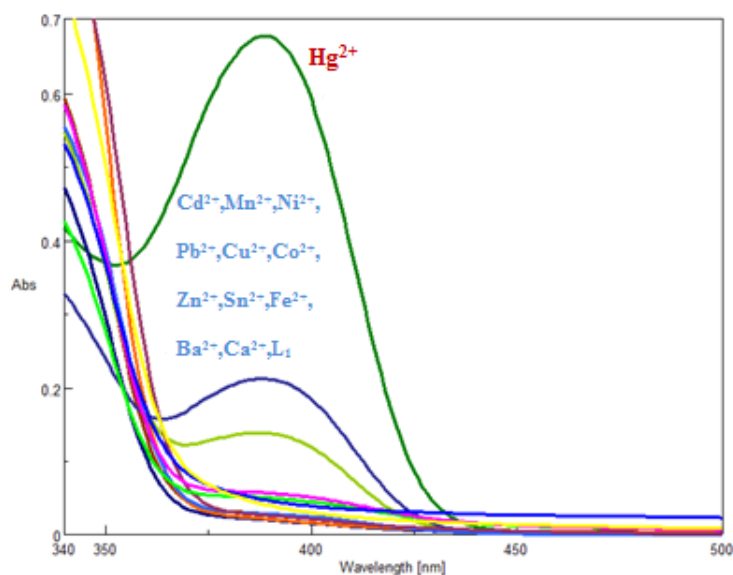
Melting points were measured in open capillary tubes and are uncorrected. The ^1H -NMR and ^{13}C -NMR were recorded on a Bruker (Advance) 300 MHz NMR instrument using TMS as an internal standard and DMSO as a solvent. Standard Bruker software was used throughout. Chemical shifts are given in parts per million (δ -scale) and the coupling constants are given in Hertz. Silica gel-G plates (Merck) were used for TLC analysis with a mixture of petroleum ether (60-80 .C) and ethyl acetate as the eluent. Absorption measurements were carried out using a JASCO V-530 UV-vis spectrophotometer. Fluorescence spectra were recorded on a F-4500 Hitachi fluorescence spectrophotometer. The slit width was 5 nm for both excitation and emission. Elemental analyses were performed on a Perkin Elmer 2400 Series II Elemental CHNS analyzer. ESI mass was recorded using a Thermo Fleet-LC mass instrument. Fluorescence microscopic images were taken with a Nikon fluorescence microscope using a filter.

Synthesis of chemosensor QPL

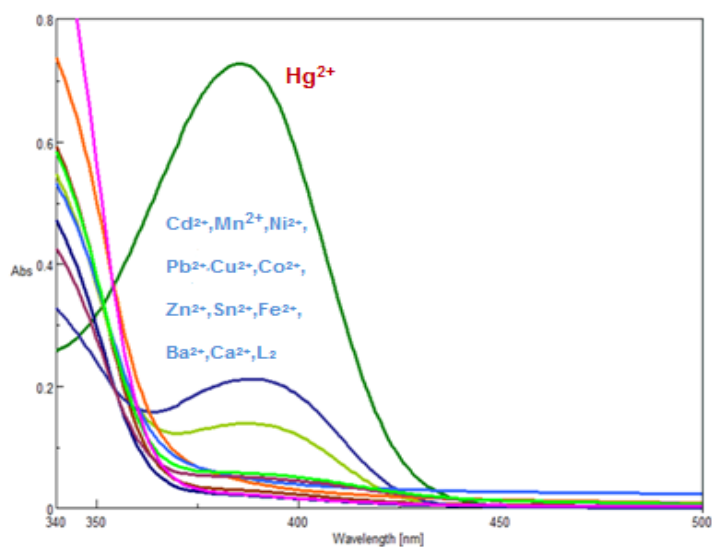
To a mixture of 10 mol% p-TSA and 4 ml ethanol, hydrazine **1** (1 mmol), ethyl acetoacetate **2** (1 mmol), aromatic aldehyde **3** (1 mmol) and 2-hydroxy naphthoquinone **4** (1 mmol) were added and heated to reflux. The resulting clear solution, that gradually became turbid, was stirred for the stipulated time mentioned in table 1. After completion of the reaction (indicated by TLC), the free flowing solid was filtered and washed with ethanol (10 ml) to afford the desired products as pale yellow solids. The product thus obtained was recrystallized from ethanol to get pure compounds as yellow crystals. The isolated compounds were well characterized by IR, ^1H NMR, ^{13}C NMR, MASS, elemental analysis. NMR data of compound **QPL** ^1H NMR (300 MHz, DMSO) δ 7.99 (dd, J = 18.9, 7.5 Hz, 2H), 7.89 – 7.73 (m, 2H), 7.68 (d, J = 8.2 Hz, 2H), 7.49 (t, J = 7.9 Hz, 2H), 7.27 (dd, J = 20.2, 8.5 Hz, 5H), 5.81 (s, 1H), 2.27 (s, 3H). compound **QPM** ^1H NMR (300 MHz, DMSO) δ 8.06 – 7.92 (m, 2H), 7.88 – 7.73 (m, 2H), 7.68 (d, J = 8.0 Hz, 2H), 7.49 (t, J = 7.9 Hz, 2H), 7.29 (t, J = 7.4 Hz, 1H), 6.35 (d, J = 4.6 Hz, 2H), 5.74 (s, 1H), 3.65 (s, 9H), 2.25 (s, 3H).

Result and Discussion

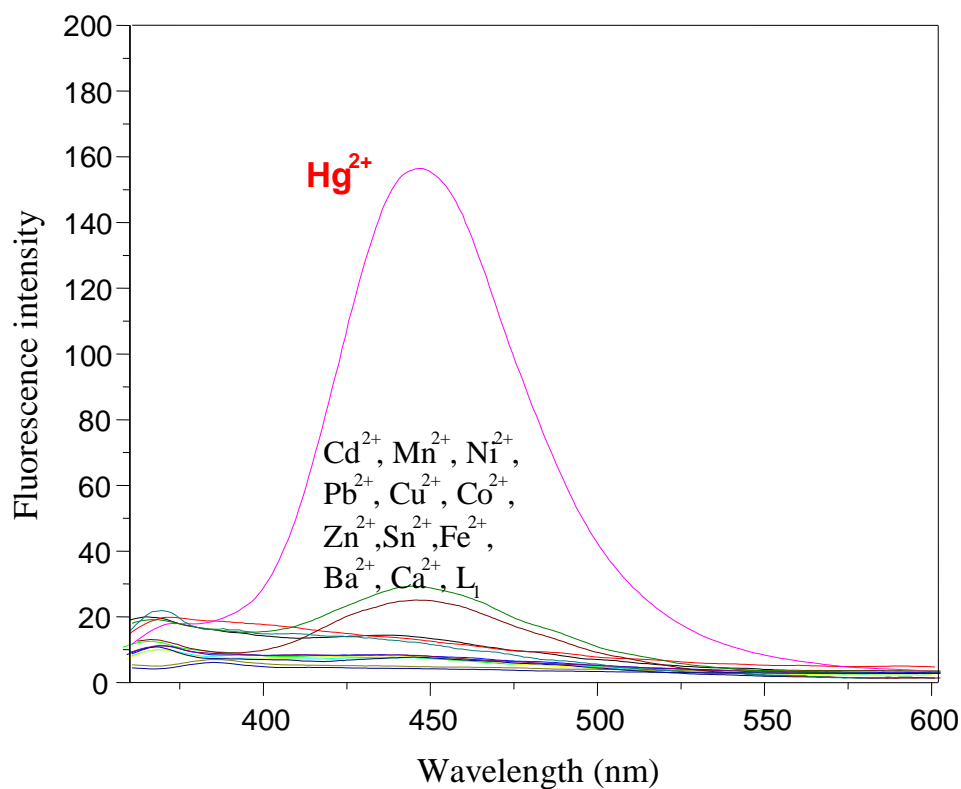
Chemosensor QPL and QPM was synthesised by a one step condensation of 2-hydroxynaphthoquinone, Aldehyde, and Pyrazolones moiety [2,3-dihydro-3-methyl-1-phenyl-1-pyrazol-5-ol], derivatives in good yield (89%). L1, L2 was characterized by ^1H -NMR, ^{13}C -NMR and ESI-MS, (L1, L2) forms colorless and non-fluorescent solutions in organic solvent mixtures. The spectral changes of probe L1, L2 upon binding of various metal ions were studied by UV-vis absorption spectra. Among the competitive metal ions such as K^+ , Na^+ , Ag^+ , Mn^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Zn^{2+} , Pb^{2+} , Cu^{2+} , Sn^{2+} , Cd^{2+} , Ni^{2+} and Co^{2+} tested in 1:1 (v/v) THF/ H_2O , only Hg^{2+} responds to probe. It shows a characteristic UV-vis absorbance band centered at 342 nm. In presence of Hg^{2+} , this band is blue-shifted to 330 nm (Fig. 1,2) Upon addition of various other metal ions (1.0×10^{-5} mol L^{-1}) to a solution of L1, L2, no significant change is observed the photophysical properties of the probe. Likewise, the fluorescence behavior of probe L1, L2 is also investigated in 1:1 (v/v) THF/ H_2O medium, which shows a characteristic emission band at 496 nm. In presence of Hg^{2+} (1.0×10^{-5} mol L^{-1}), probe shows a remarkable fluorescence quenching and the emission band at 496 nm is also red-shifted to 514 nm (Fig.4, 5). The increase absorbance and fluorescence intensity of the probe effected by the addition of Hg^{2+} ions not interfered with the other competing metal ions (fig.6,7). Increase the addition of Hg^{2+} ions also leads to increase in fluorescence maximum.

Scheme1. Synthesis of chemo sensors **QPL** and **QPM**

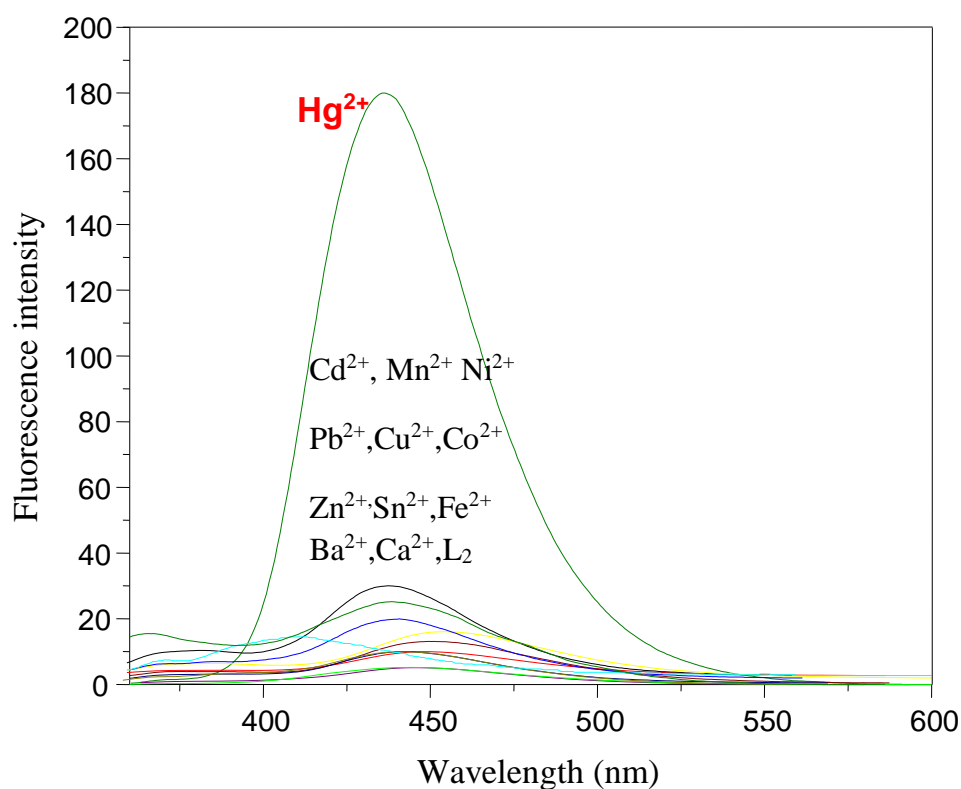
UV-visible absorption spectra of compound L₁ (10 μM) in the presence of various metals perchlorate salts of Cd²⁺, Ni²⁺, Zn²⁺, Pb²⁺, Cu²⁺, Mn²⁺, Co²⁺, Sn²⁺, Ca²⁺, Ba²⁺, Fe²⁺ and Hg²⁺ (1 x 10⁻⁵ M) in (THF/H₂O).



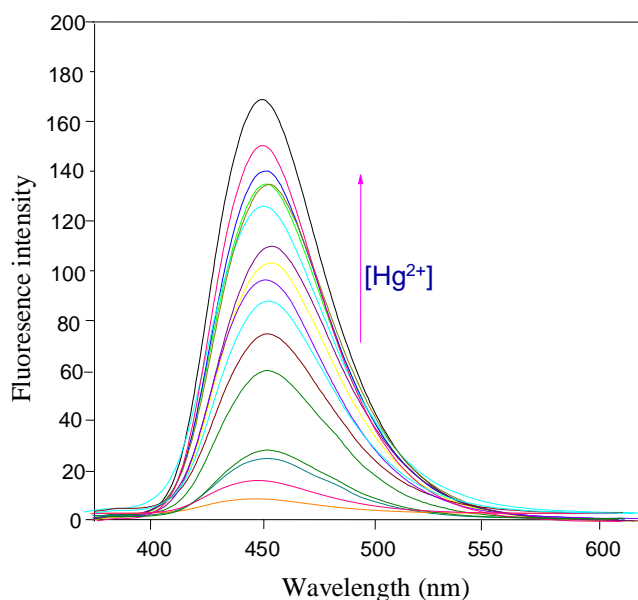
UV-visible absorption spectra of compound L_2 ($10\ \mu\text{M}$) in the presence of various metals perchlorate salts of Cd^{2+} , Ni^{2+} , Zn^{2+} , Pb^{2+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Sn^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} and Hg^{2+} ($1 \times 10^{-5}\text{M}$) in (THF/ H_2O).



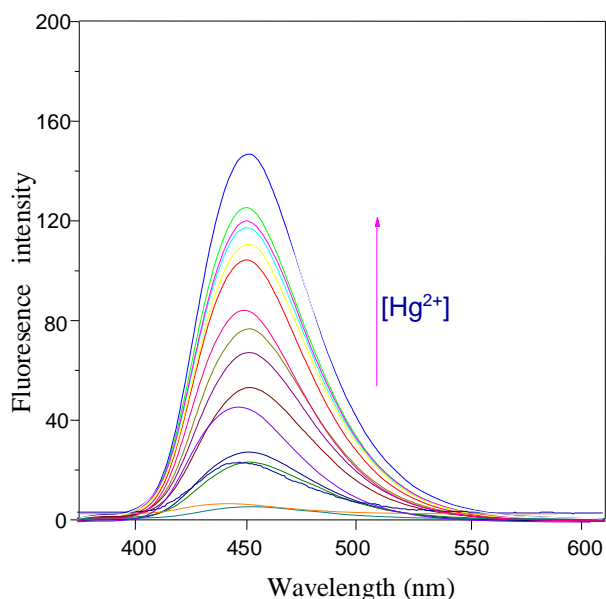
Fluorescence emission profile of compound QPM ($1.0 \times 10^{-6}\text{M}$) in the presence of various metals perchlorate salts of Cd^{2+} , Ni^{2+} , Hg^{2+} , Pb^{2+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Sn^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , Zn^{2+} QPM and Hg^{2+} (1.0 equiv) in (THF/ H_2O). Excitation wavelength was 330 nm with 5nm slit width.



Fluorescence emission profile of compound QPL ($1.0 \times 10^{-6} \text{M}$) in the presence of various metals perchlorate salts of Cd^{2+} , Ni^{2+} , Hg^{2+} , Pb^{2+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Sn^{2+} , Ca^{2+} , Ba^{2+} , Fe^{2+} , QPM and Hg^{2+} (1.0 equiv) in (THF/ H_2O). Excitation wavelength was 330 nm with 5nm slit width.



Concentration-dependent fluorescence spectra of L1 ($10 \mu\text{M}$) on the addition of various amounts of Hg^{2+} ($1.0 \times 10^{-5} \text{M}$) 0-1 equivalents (THF/ H_2O). Excitation wavelength was 330 nm with 5cm slit width



Concentration-dependent fluorescence spectra of L_2 (10 μ M) on the addition of various amounts of Hg^{2+} (1.0×10^{-5} M) 0-1 equivalents (THF/ H_2O). Excitation wavelength was 330 nm with 5cm slit width.

Conclusion

In summary, by combining the high affinity of thymine towards $Hg(II)$ ion with the excimer emission of pyrene units, $L1$, which can be easily synthesized in two steps, was designed as a potential “turn on” chemosensor for $Hg(II)$ ion with good sensitivity and high selectivity, which was demonstrated by the fluorescent spectral studies of $L1$ under different conditions. Further studies will address its real application to real samples.

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