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Original article

A colorimetric chemosensor based on new water-soluble PODIPY dye for Hg^{2+} detection

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ABSTRACT

The phosphorus-containing PODIPY **1** as a chemosensor can detect Hg^{2+} by a color change from pink to violet red without the use of any instrumentation. PODIPY **1** was selective to Hg^{2+} with a remarkable absorption change, and addition of other relevant metal ions caused almost no absorption change. The new PODIPY dye **1** was sensitive to various concentrations of Hg^{2+}. The energy gap between the HOMO and LUMO of the metal complex **1**–Hg^{2+} is smaller than that of chemosensor **1**, which is in good agreement with the red shift in the absorption observed upon treatment of **1** with Hg^{2+}. The **1**-based test strips were easily fabricated and low-cost, useful in practical and efficient Hg^{2+} test kits.

1. Introduction

Mercury pollution is a global problem, and a major source of human exposure stems from contaminated waters [1,2]. The terrific toxicity of mercury results from its high affinity for thiol groups in proteins and enzymes, leading to the dysfunction of cells and consequently causing health problems [3]. Inorganic mercury, including Hg(0) and Hg(II), is released into the environment through a wide variety of ways. Industrial sources of mercury include coal and gold mining, solid waste incineration, wood pulping, fossil fuel combustion, and chemical manufacturing. As the results of numerous applications in industry and agriculture, mercury has badly contaminated rivers and soil and accumulated in agricultural products and aquatic products, which causes serious environmental and health problems directly or indirectly [4]. The U.S. Environmental Protection Agency (EPA) standard for the maximum allowable level of inorganic mercury in drinking water is 2 ppb [5]. Moreover, a significant problem stemming from the ecological oxidation chemistry is that bacteria living in the sediments of aqueous environments transform inorganic Hg^{2+} into methylmercury, a potent neurotoxin that concentrates through the food chain in the tissues of fish and marine mammals [6]. Subsequent ingestion of methylmercury by humans from seafood and other dietary and environmental sources is connected to serious sensory, motor, and cognitive disorders. Therefore, in the interests of safety the detection of Hg^{2+} is still of great importance.

Among the available techniques to detect and quantify Hg^{2+}, colorimetric chemosensors, so-called "naked eye chemosensors", have been developed and are attracting increasing interest [7,8]. This sensing approach has clear potential application in the development of commercial Hg^{2+} indicators, such as paper test strips that can be evaluated visually without illumination [7,8]. Water solubility is arguably a less important criterion for colorimetric chemosensors than for fluorescent ones, which are better suited for in vivo work [9,10]. As long as the colorimetric indicator responds to Hg^{2+} in the presence of water and can be absorbed onto a paper strip or into a membrane or film, it has potential. Therefore, we herein communicate our studies on colorimetric Hg^{2+} detection, based on the new water-soluble PODIPY dye.

Our recent research interest lies in the novel BODIPY/aza-BODIPY family of fluorescent dyes and their application [11–13], and very recently we developed a type of PODIPY dye [14]. The new PODIPY **1** (Fig. 1) can be successfully synthesized by the reaction of dipyrromethene and azadipyrromethene with $POCl_3$ in the presence of Et_3N. The new PODIPY had photophysical properties similar to related PODIPYs/aza-PODIPYs [15–21], with a large

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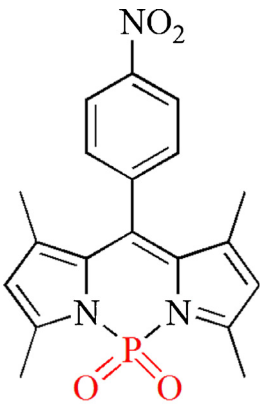

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Fig. 1. Structure of PODIPY dye **1**.

Stokes shift and moderate fluorescence quantum yield. The dye **1** is more polar than BODIPYs, increasing its water solubility. Moreover, the interaction between the p-orbital of the phosphorus atom and the lone pair electrons of the nitrogen atom, and many resonators of the limit structure of charge separation in dye **1**, may also enhance the stability in water. Based on the photophysical properties of **1**, we made further efforts for the modifications in PODIPY as potential chemosensors and probes. Because of the low fluorescence quantum yield ($\Phi_f = 0.17$) of PODIPY **1** [14] and the additional fluorescence quenching by detecting Hg^{2+}, the investigation of emission spectra becomes meaningless and was abandoned. Delightedly, we found that PODIPY **1** can be used as a chemosensor for the colorimetric Hg^{2+} detection.

2. Experimental

2.1. *General methods*

^1H NMR spectra were recorded on a Bruker AVANCE III 500 MHz spectrometer. ^1H NMR chemical shifts (δ) are given in ppm downfield from Me_4Si, determined by chloroform ($\delta = 7.26$ ppm). ^{13}C NMR spectra were recorded on a Bruker AVANCE III 125 MHz spectrometer. ^{13}C NMR chemical shifts (δ) are reported in ppm with the internal $CDCl_3$ at δ 77.0 ppm as standard. ESI was measured by LCQ Deca XP. Tetrahydrofuran (THF) was freshly distilled from Na/benzophenone, *n*-hexane was distilled over Na, and other solvents were distilled over CaH_2. Merck silica gel 60 was used for the column chromatography. Fluorescence spectra were recorded on an F-4600 spectrophotometer. UV/Vis spectra were recorded on a UV-2550 spectrophotometer at room temperature. The pH measurement was performed with a PHS-3E pH meter. The refractive index of the medium was measured by 2 W Abbe's refractometer at 20 °C.

2.2. *Synthesis of PODIPY* **1**

2.4-Dimethylpyrrole (0.51 mL, 4.9 mmol) and paranitrobenzaldehyde (300 mg, 1.9 mmol) were dissolved in 20 mL of absolute CH_2Cl_2 under a N_2 atmosphere. One drop of trifluoroacetic acid (TFA) was added and the solution was stirred at room temperature overnight. When TLC monitoring (silica; CH_2Cl_2) showed complete consumption of the benzaldehyde, a solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (900 mg) in CH_2Cl_2 (10 ml) was added, and stirring was continued for 1 h. The reaction mixture was washed with water, dried over $MgSO_4$, filtered, and evaporated. The crude compound was purified by column chromatography on aluminum (CH_2Cl_2/*n*-hexane, 1:1). This solid and triethylamine (2 mL) were dissolved in 50 mL of CH_2Cl_2 under air and the solution was stirred at room temperature for 10 min. $POCl_3$ (4 mL) was added, and stirring was continued for 1 h. The reaction was slowly quenched with crushed ice, extracted with CH_2Cl_2, and purified by recrystallization from CH_2Cl_2/*n*-hexane to afford 1 (171.2 mg, 23.5%) as red solids. ^1H NMR (500 MHz, $CDCl_3$): δ 8.37 (d, 2H, $J = 8.0$ Hz), 7.56 (d, 2H, $J = 8.0$ Hz), 6.24 (s, 2H), 2.56 (s, 6H), 1.39 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.5, 153.2, 142.6, 133.9, 131.5, 129.5, 123.8, 120.8, 99.3, 13.8, 13.7. ^{31}P NMR (202 MHz, $CDCl_3$): δ −50.011. FTMS-MALDI (*m/z*): calcd. for $C_{19}H_{19}N_3O_4P$: 384.1113 $[M+H]^+$, found: 384.1085; calcd. for $C_{19}H_{19}N_3O_2$: 322.1511 $[M–PO_2+H]^+$; found: 322.1525.

2.3. *Preparation of metal ion titration solutions*

Stock solutions (4×10^{-4} mol/L) of the salts of $HgCl_2$, $Al(NO_3)_3$, $AgNO_3$, $CoCl_2$, $MnCl_2$, $PbCl_2$, $CuCl_2$, $MgCl_2$, $NiCl_2$, $FeCl_3$, $ZnCl_2$, $CaCl_2$, $PdCl_2$, $CrCl_3$, KCl, and NaCl in H_2O were prepared. PODIPY **1** (1×10^{-4} mol/L) was also prepared in CH_3CN. Test solutions were prepared by placing 40 µL of the sensor stock solution into a test tube, then adding an appropriate aliquot of each metal stock (0 − 1.0 mL) and diluting the solution to 4 mL with CH_3CN/H_2O (1/9, v/v).

2.4. *MO calculation*

Frontier molecular orbitals have been performed at the Becke3LYP (B3LYP) level of the density functional theory. The SDD basis set are used to describe Hg and P and 6-31G(d) basis set was used for all the other atoms (see Supporting information).

3. Results and discussion

The sensitivity of phosphorus-containing PODIPY **1** ($\lambda_{abs} = 507$ nm) was first studied by the UV–visible absorption response toward various concentrations of Hg^{2+} in CH_3CN/H_2O at pH 7.2 (Fig. 2) [22]. As can be observed, a distinct response of 1 µmol/L PODIPY **1** to Hg^{2+} in the concentration range of 0–20 µmol/L was discovered. When 10 equiv. of Hg^{2+} (10 µmol/L) was added, the reduction of the absorption intensity ($\lambda_{abs} = 550$ nm) was minimal, and a further increase in of Hg^{2+} concentration did not provide

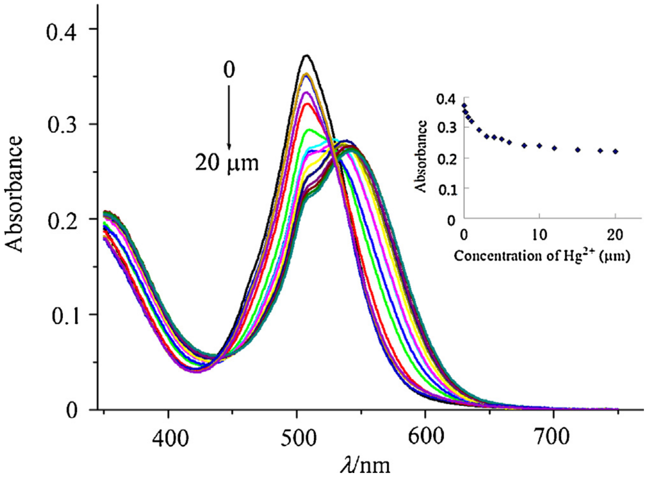

Fig. 2. Absorption change of 1 µmol/L PODIPY **1** after the addition of increasing amounts of Hg^{2+} (0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18 and 20 µmol/L) in CH_3CN/H_2O (1:9, v/v) at room temperature. The inner panel displays the absorption intensity ($\lambda_{abs} = 507$ nm) of 1 µmol/L PODIPY **1** toward Hg^{2+} at 0, 0.1, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15,18 and 20 µmol/L.

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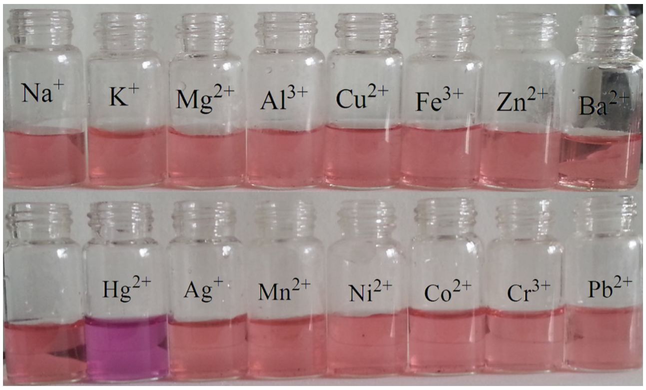

Fig. 3. Digital picture of PODIPY **1** (1 μmol/L) in CH_3CN/H_2O (1:9, v/v). From left to right: Na^+, K^+, Mg^{2+}, Al^{3+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, Ba^{2+}; no-metal, Hg^{2+}, Ag^+, Mn^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Pb^{2+}, equivalent amounts of all the cations and Hg^{2+}. The cation total concentration was 10 μmol/L.

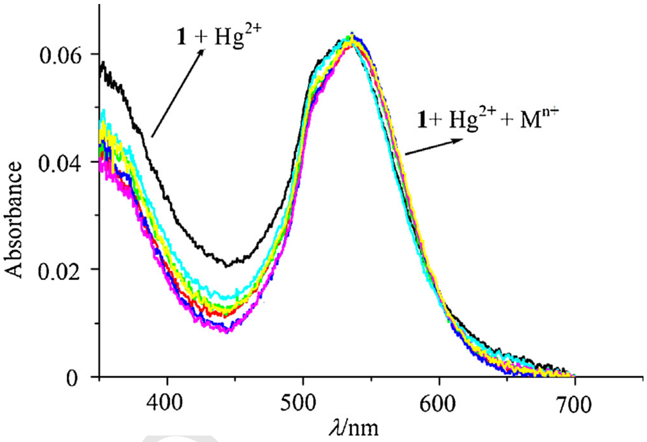

Fig. 5. The relative absorption of PODIPY **1** and its complexation with Hg^{2+} in the presence of various metal ions. PODIPY **1** + Hg^{2+}; PODIPY **1** + Hg^{2+} + M^{n+}, where M^{n+} = Zn^{2+}, Ag^+, Fe^{3+}, Ni^{2+}, Cr^{3+}, and Pb^{2+} ions. Conditions: 0.3 μmol/L of PODIPY **1**, 5 μmol/L of Hg^{2+} in the presence of 5 μmol/L of other metal ions.

further reduction (Fig. 2). Based on a linear range for Hg^{2+} covering from 0.1 μmol/L to 3 μmol/L (inner panel of Fig. 2), the detection limit ($3\sigma/k$) was found to be 0.5 μmol/L [23].

In fact, the presence of Hg^{2+}, can be detected without the use of any instrumentation as illustrated in Fig. 3, with the color change from pink to violet red. The selectivity toward mercury has been demonstrated even in the presence of similar amounts of other metal ions, such as Na^+, K^+, Mg^{2+}, Al^{3+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, Ba^{2+}, Ag^+, Mn^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Pb^{2+} and no metal (Fig. 3).

Furthermore, the selectivity of the chemosensor **1** toward other metal ions was investigated with UV–visible spectroscopy. As shown in Fig. 4, PODIPY **1** was highly selective to Hg^{2+} with a remarkable absorption change. In contrast, addition of other relevant metal ions, including Na^+, K^+, Mg^{2+}, Al^{3+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, Ba^{2+}, Hg^{2+}, Ag^+, Mn^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, and Pb^{2+}, caused almost no absorption change.

Some ions, for example, Zn^{2+}, Ag^+, Fe^{3+}, Ni^{2+}, Cr^{3+}, or Pb^{2+}, often interfere with the selectivity of Hg^{2+} [24,25]. Therefore, to investigate the selectivity for Hg^{2+} in a complex background of potentially competing species, the absorption change of PODIPY **1** with Hg^{2+} was examined in the presence of other metal ions. From Fig. 5, we observed that there was no interference in the detection of Hg^{2+} in the presence of Zn^{2+}, Ag^+, Fe^{3+}, Ni^{2+}, Cr^{3+}, or Pb^{2+}, and the absorption spectra were almost identical to those obtained in the presence of Hg^{2+} alone.

The recognition mechanism of the chemosensor **1** with Hg^{2+} was investigated by FT-IR spectra (Fig. 6a). 1 equiv. of Hg^{2+} with PODIPY **1** (red curve) intensified and widened the peak at the characteristic stretching frequency (1346.2/cm) [26] of the P=O bond of POPDIPY **1** alone (black curve). This indicated a strong polarization of the P=O bond upon efficient binding to the Hg^{2+} ion (Fig. 6b).

To gain insight into the response of chemosensor **1** to Hg^{2+}, chemosensor **1** and the corresponding metal complex species **1**–Hg^{2+} were examined by density function theory (DFT) at the Becke3LYP (B3LYP) level of the Gaussian 03 program. The SDD

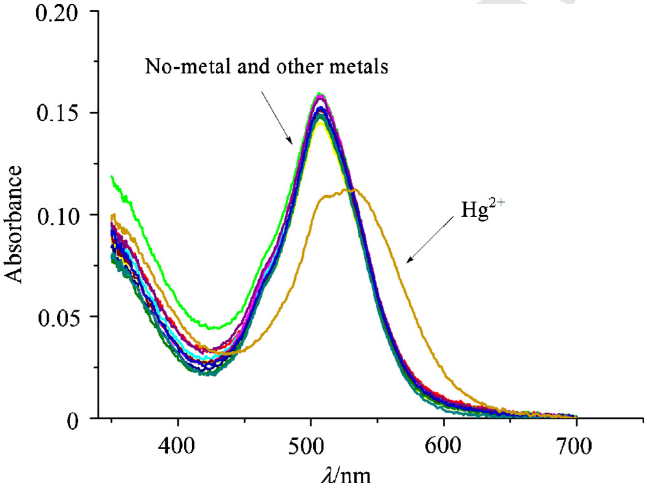

Fig. 4. Absorption spectra of PODIPY **1** (0.5 μmol/L) before and after the addition of metal salts (10 μmol/L) of Na^+, K^+, Mg^{2+}, Al^{3+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, Ba^{2+}, Hg^{2+}, Ag^+, Mn^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Pb^{2+} in CH_3CN/H_2O (1:9, v/v).

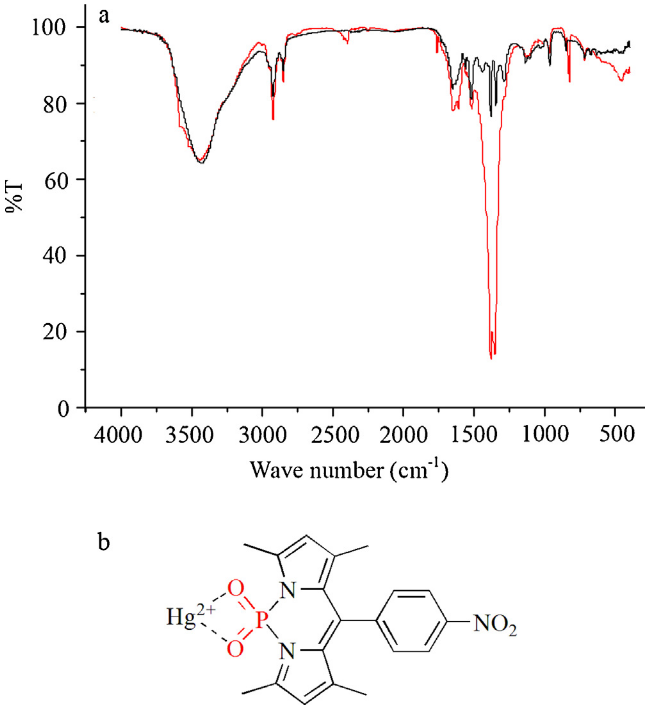

Fig. 6. (a) IR spectra of compound PODIPY **1** (black curve) and PODIPY **1**–Hg^{2+} complex (red curve) in KBr disks. (b) Structure of **1**–Hg^{2+}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) Q4

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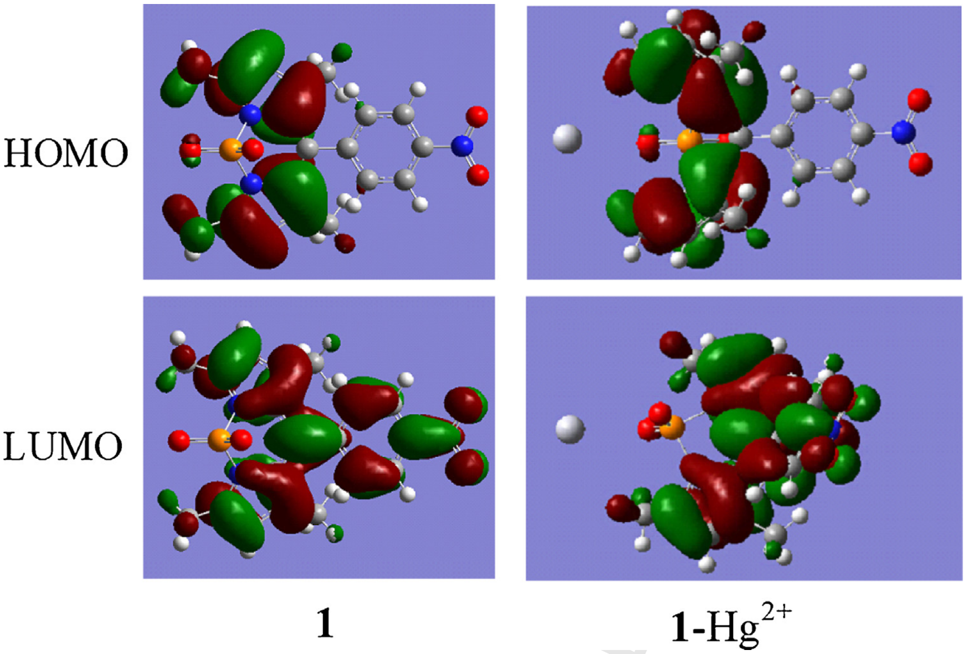

Fig. 7. Frontier molecular orbitals of **1** and **1**–Hg^{2+} at the Becke3LYP (B3LYP) level of the density functional theory with Gaussian 03. The SDD basis set is used to describe Hg and P, and 6-31G(d) basis set was used for all the other atoms. HOMO/LUMO (eV) = –5.96/–3.78 for **1**; HOMO/LUMO (eV) = –5.84/–3.80 for **1**–Hg^{2+}.

basis set are used to describe Hg and P, and 6-31G(d) basis set was used for all the other atoms. As shown in Fig. 7, for chemosensor **1**, the HOMO are distributed at the PODIPY core, and the LUMO are localized on the whole PODIPY structure including the *p*-nitrophenyl group in the neutral compounds. In addition, the planar BODIPY fragment is almost coplanar to the adjacent *p*-nitrophenyl part with a small dihedral angle (4°). While for the metal complex species **1**–Hg^{2+}, the HOMO is distributed in the left BODIPY core, and the LUMO is mostly located at the right BODIPY unit. Furthermore, the energy gap between the HOMO and LUMO of the metal complex species **1**–Hg^{2+} (2.04 eV) is smaller than that of chemosensor **1** (2.18 eV) (Fig. 7), which is in good agreement with the red shift in the absorption observed upon treatment of chemosensor **1** with Hg^{2+}.

Motivated by the obvious color change of the system in metal ions, test strips were prepared by immersing filter papers in the CH_3CN/H_2O solution of **1** (1.0×10^{-5} mol/L) and then drying them in air. When each drop of 5.0×10^{-5} mol/L different metal ions was dripped into the **1**-based test strips, a clear color change from pink to brown was observed in the presence of Hg^{2+}, comparing to noncolor change for the other metal ions, such as Al^{3+}, Cr^{3+} Ba^{2+}, Pb^{2+} Ni^{2+} Mn^{2+},Cu^{2+}, Ag^{+} and free (Fig. 8). Therefore, the **1**-based test strips can conveniently detect Hg^{2+} in solutions without any additional equipment. The **1**-based test strips were easily fabricated and low-cost, useful in practical and efficient Hg^{2+} test kits.

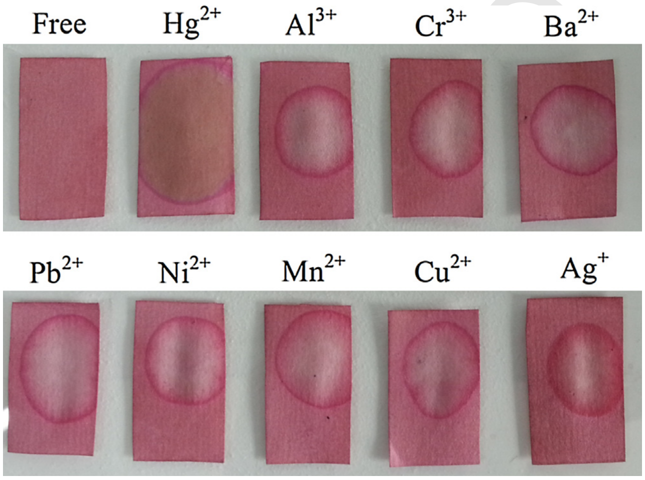

Fig. 8. Photographs of test strips **1** to one drop of different metal ions. Test strips were prepared by immersing filter papers in the CH_3CN/H_2O (1/1, v/v) solution of **1** (1.0×10^{-5} mol/L) and then drying them in air.

4. Conclusion

PODIPY **1** can detect Hg^{2+} by the color change from pink to violet red without the use of any instrumentation. PODIPY **1** was selective to Hg^{2+} with remarkable absorption change, and the addition of other relevant metal ions caused almost no absorption change. The phosphorus-containing PODIPY **1** was sensitive to various concentrations of Hg^{2+}. From the changes in Hg^{2+}-dependent absorption intensity, the detection limit was found to be 0.5 μmol/L. Using PODIPY **1** there was no interference in the detection of Hg^{2+} in the presence of other ions. FT-IR indicated a strong polarization of the $P{=}O$ bond in PODIPY **1** upon efficient binding to Hg^{2+}. The energy gap between the HOMO and LUMO of the metal complex **1**–Hg^{2+} (2.04 eV) is smaller than that of chemosensor **1** (2.18 eV), which is in good agreement with the red shift in the absorption observed upon treatment of sensor **1** with Hg^{2+}. The **1**-based test strips were easily fabricated and low-cost, useful in practical and efficient Hg^{2+} test kits. Further efforts for development of probes based on PODIPY/aza-PODIPY in biotechnology are ongoing in our lab.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2015.07.002.

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