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A Quinoline-thiooxorhodamine Conjugate for Fluorescent Hg^{2+} Recognition in Aqueous Media and Living Cells

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A quinoline-thiooxorhodamine conjugate fluorescent sensor (**1**) has been synthesized. Sensor **1** exhibits high selectivity and sensitivity to Hg^{2+} in H_2O/DMSO (95/5, v/v, HEPES 20 mM, pH = 7.4) solution with fluorescence detection. Other tested metal ions do not induce any significant fluorescence intensity changes. Sensor **1** interacts with Hg^{2+} through a 1:1 binding stoichiometry with a good anti-inference ability. In addition, fluorescent imaging of Hg^{2+} in Hela cells is also successfully demonstrated.

Key Words : Fluorescence, Hg(II) recognition, Rhodamine, Cell imaging

Introduction

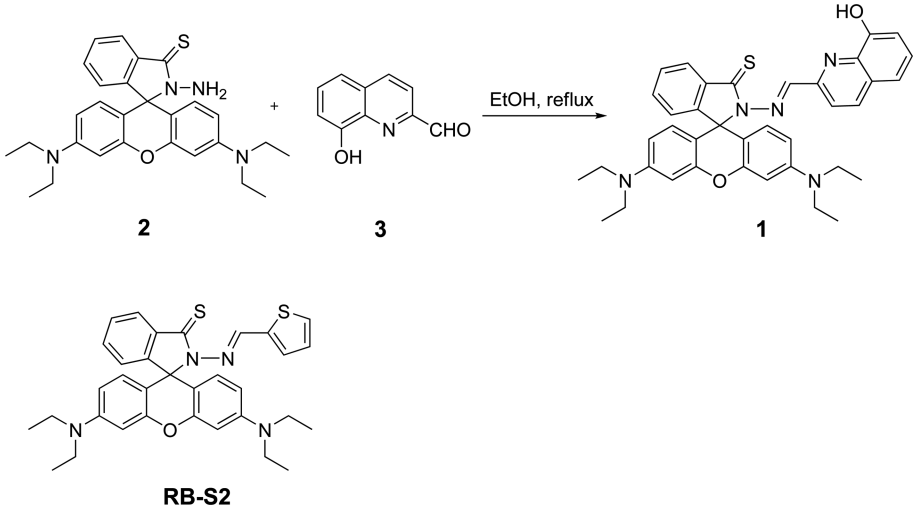
Highly selective and sensitive detection of transition and heavy metal ions by artificial chemosensors has received considerable attention.[1] Hg^{2+} is an extremely toxic metal ion to environment and human health, its accumulation in the human body can lead to the dysfunction of the brain,[2] kidney,[3] and central nervous systems.[4] Therefore, great efforts have been focused on the development of chemosensors for Hg^{2+} detection.[5] Among the various detection methods available, fluorescence spectroscopy still remains the most frequently used detection method due to its high sensitivity and easy operational use.[5b,6]

Rhodamines are the ideal platforms for development of chemoprobes for specific heavy and transition metal ions due to their excellent spectroscopic properties such as long-wavelength emission, high fluorescence quantum yield, and large molar extinction coefficient.[7] The rhodamine spirolactam form is basically colorless and non-fluorescent, while its metal induced ring-opened amide form has strong absorption within the visible range and usually displays strong fluorescence emission. Recently, utilization of rhodamine spirolactam ring-opening process for the detection of metal ions has been well documented.[8]

Herein, we report the Hg^{2+} recognition properties of a quinoline-thiooxorhodamine conjugated sensor (**1**) (Scheme 1). Sensor **1** exhibits highly selective recognition to Hg^{2+} *via* colorimetric and fluorescence turn-on responses, sensor **1** was also applied to living cell imaging.

Experimental

Materials and Instruments. Unless otherwise stated, solvents and reagents were purchased as analytical grade and used without further purification. Ethanol was freshly distilled from Magnesium powder and Iodine grain. Doubly distilled water was used for spectral detection. Compounds **2**[9] and **3**[10] were prepared according to literature methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent 400-MR spectrometer. High-resolution mass spectroscopy (HRMS)



Scheme 1. Synthesis of sensor **1** and the structure of **RB-S2**.

was measured on a Bruker microTOF-Q mass spectrometer (Bruker Daltonik, Bremen, Germany). Fluorescence measurements were performed on a 970-CRT spectrofluorometer (Shanghai Sanco instruments Co., Ltd., China). UV-vis absorption spectra were measured on a SP-1900 spectrophotometer (Shanghai Spectrum instruments Co., Ltd., China). The pH measurements were made with a PHS-25B meter (Shanghai Dapu instruments Co., Ltd., China).

The salts used in stock solutions of metal ions are $Ni(NO_3)_2{\cdot}6H_2O$, $Hg(NO_3)_2$, $Ba(NO_3)_2$, $Mg(NO_3)_2{\cdot}6H_2O$, $AgNO_3$, $FeSO_4{\cdot}7H_2O$, KNO_3, NaCl, $CaCl_2{\cdot}6H_2O$, $Al(NO_3)_3{\cdot}9H_2O$, $Mn(NO_3)_2$, $Pb(NO_3)_2$, $Sr(NO_3)_2$, $Cu(NO_3)_2{\cdot}3H_2O$, $Co(NO_3)_2{\cdot}6H_2O$, $Zn(NO_3)_2{\cdot}6H_2O$, $Cd(NO_3)_2$, $CrCl_3{\cdot}6H_2O$, $Fe(NO_3)_3{\cdot}9H_2O$, respectively.

Synthesis of Sensor 1. Compound **2** (0.2 g, 0.42 mmol) and **3** (0.1 g, 0.58 mmol) were dissolved in absolute ethanol (50 mL) and heated to reflux about 3 h. After removal of ethanol, the residue was purified by flash column chromatography with petroleum ether and ethyl acetate as eluent (8:1, v/v) to afford **1** as yellow solids. Yield: 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.24-8.19 (m, 3H), 8.09 (d, J = 8.8 Hz, 1H), 7.49-7.44 (m, 3H), 7.31 (d, J = 8.4 Hz, 1H), 7.19-7.16 (m, 2H), 6.79 (d, J = 8.4 Hz, 2H), 6.34-6.31 (m, 4H), 3.33 (q, J = 6.8 Hz, 8H), 1.16 (t, J = 6.8 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.3, 158.6, 155.5, 152.4, 151.9, 151.7, 148.3, 137.9, 136.1, 135.1, 132.7, 130.9, 130.1, 128.8, 128.7, 128.0, 127.1, 122.6, 119.6, 117.8, 110.3, 110.0, 108.2, 97.4, 65.6, 44.4, 12.6. HRMS (ESI+), calcd for $C_{38}H_{38}N_5O_2S$ $[M+H]^+$ 628.2746, found 628.2741.

Details for Cell Imaging Experiments. The Hela cells (human cell line derived from cervical cancer) were cultured in Dulbecco's modified eagle's medium (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (Hyclone). Before imaging, the cells were seeded in 6-well flat-bottomed plates for one day. Then the cells were incubated with 5 μM sensor **1** for 0.5 h at 37 °C under 5% CO_2 and then washed with phosphate-buffered saline (PBS) three times before incubating with 10 μM Hg^{2+} for another 0.5 h, cells were rinsed with PBS three times again, then the fluorescence imaging of intracellular Hg^{2+} was observed under inverted fluorescence microscope with a 20× objective lens (excited with green light). The Hela cells only incubated with 5 μM **1** for 0.5 h at 37 °C under 5% CO_2 was as a control. For all images, the microscope settings, such as brightness, contrast, and exposure time were held constant to compare the relative intensity of intracellular Hg^{2+} fluorescence.

Results and Discussion

Compound **1**[11] has been synthesized as an intermediate for the preparation of a bifunctional fluoroionphore-ionic liquid hybrid that suitable for Hg^{2+} extraction. It is noteworthy that **1** itself is also a promising Hg^{2+} sensing probe owing to the structural similarity of **1** to some known rhodamine-based metal ion sensors, such as the thiophen-thiooxorhodamine conjugate fluorescent Hg^{2+} sensor **RB-S2** (Scheme 1) reported by Yao *et al*..[9] It is rational to envision that replace the thiophen moiety in **RB-S2** with 8-hydroxy-2-quinolinecarboxaldehyde unit could supply an additional metal ion coordination site (OH group), which is usually favorable to the metal ion selectivity of a sensor. Bearing this idea in mind, we prepared compound **1** (for ^{1}H NMR, ^{13}C NMR and HRMS spetra, see supplementary data) and investigated its metal ion sensing properties. For its potential application in some real samples, co-solvent H_2O-DMSO (95/5, v/v, HEPES 20 mM, pH = 7.4) was selected as the working moiety in the spectroscopic investigations. Figure 1 shows the fluorescence responses of **1** solution (5 μM) to various metal ions. Upon excitation at 530 nm, **1** solution displayed ignorable fluorescence emission, suggesting that **1** exists mainly in its ring-closed spirolactam form.[12] On addition 40 equiv. of Hg^{2+}, **1** solution exhibited a strong emission band at 599 nm. The fluorescence color changes from dark to yellow orange is naked eye observable (Fig. 1, inset). Addition of other interested metal ions such as Ag^+, Pb^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+},

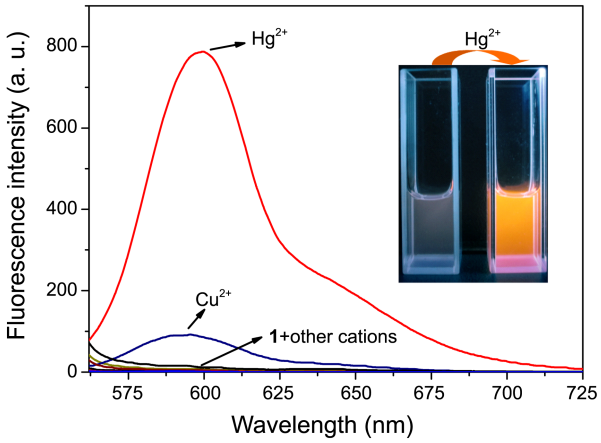


Figure 1. Fluorescence spectrum changes of **1** (5 μM) in H_2O/DMSO (95/5, v/v, HEPES 20 mM, pH = 7.4) solution upon addition of 40 equiv. of different metal ions. λ_{ex} = 530 nm. Inset: Fluorescence color changes of **1** solution before and after addition of Hg^{2+} under a portable UV lamp at 365 nm.

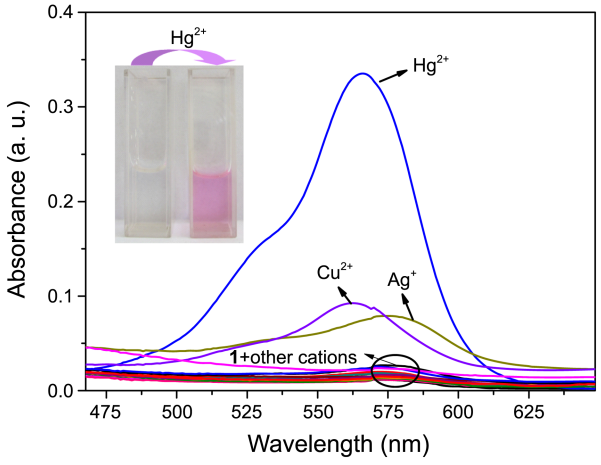
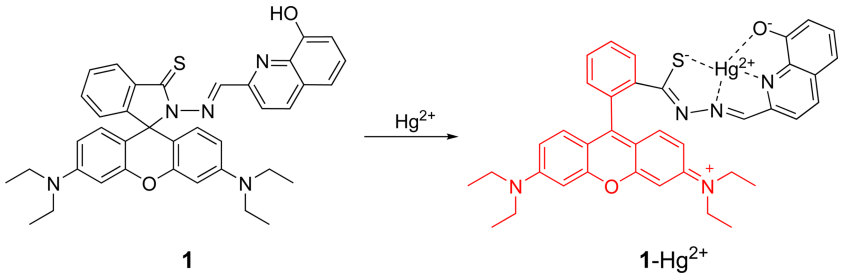


Figure 2. UV-vis absorption spectrum changes of **1** (5 μM) in H_2O/DMSO (95/5, v/v, HEPES 20 mM, pH = 7.4) solution upon addition of 40 equiv. of different metal ions. Inset: Color changes of **1** solution before and after addition of Hg^{2+}.



Scheme 2. Proposed binding mode of sensor **1** and Hg^{2+}.

Fe^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, Mg^{2+}, Cr^{3+}, Al^{3+}, Ni^{+}, Co^{2+}, Zn^{2+}, K^{+} and Na^{+} did not cause any significant fluorescence emission changes, except that Cu^{2+} induced a slight emission enhancement. This result is quite similar to that of sensor **RB-S2**. The UV-vis spectra of **1** solution in the presence of different metal ions were also checked. Free sensor **1** solution showed no absorption band in the visible range, however, addition of Hg^{2+} elicited a strong absorption band at 565 nm (Fig. 2), indicative the formation of ring-opened form of **1**. Among the metal ions tested, Cu^{2+} and Ag^{+} could induce the appearance of slight absorption band at 565 nm and 577 nm, respectively. Other metal ions did not produce any evitable color changes. These results show that sensor **1** has a good selectivity to Hg^{2+}. The proposed binding mode of **1** with Hg^{2+} was illustrated in Scheme 2.

The time-dependent response of **1** to Hg^{2+} was assayed by monitoring the UV-vis absorption changes (Fig. 3). Upon addition of 40 equiv. of Hg^{2+}, the solution color changed from colorless to pink imediately, with prolonging the interaction time, the absorbance at 565 nm increased continuously and reached stable after 1 h. Although this equilibrium time is rather long compared to its analogue **RB-S2** (within 1 min), the initial Hg^{2+} induced color change is quick enough for naked eye detection. For easy comparision and accurate calculations, all of the follwing spectroscopic studies were performed after 1 h of Hg^{2+} addition.

To evaluate the sensing property of **1** to Hg^{2+}, fluorescence titration experiments were then conducted. On incremental addition of Hg^{2+}, the newly appeared emission band gradually increased, which reached stable when 40 equiv. of Hg^{2+} was added (Fig. 4). The association constant (K_a) of **1** and Hg^{2+} was calculated using the Benesi-Hildebrand plot based on a 1:1 binding equation.[13] Fitting the titration data results in a nice linear line (coorelation coefficient is over 0.99) (Fig. 4 inset), which supports the 1:1 binding stoichiometry, and the K_a is calculated to be 2.2×10^3 M^{-1}. This binding constant is much smaller than that of **RB-S2** with Hg^{2+} in

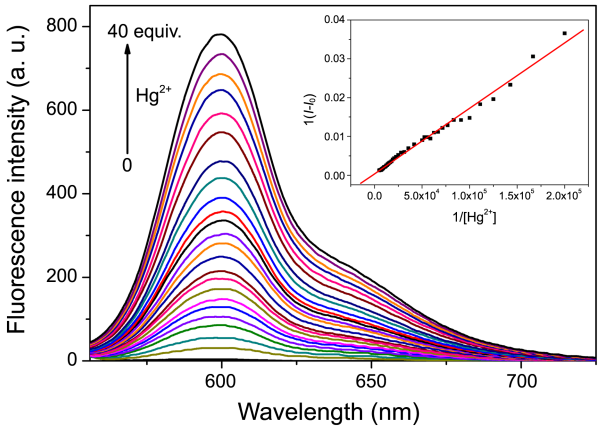


Figure 4. Fluorescence spectra of sensor **1** solution (5 μM) in H_2O/DMSO (95/5, v/v, HEPES 20 mM, pH = 7.4) on addition of different amounts of Hg^{2+}. λ_{ex} = 530 nm. Inset: Fluorescence intensity changes on incremental addition of Hg^{2+} (0 to 10 μM).

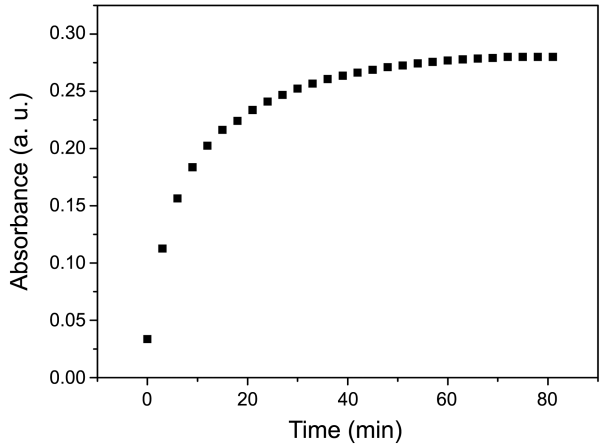


Figure 3. Time-dependent UV-vis absorption changes (λ_{ab} = 565 nm) of **1** solution.

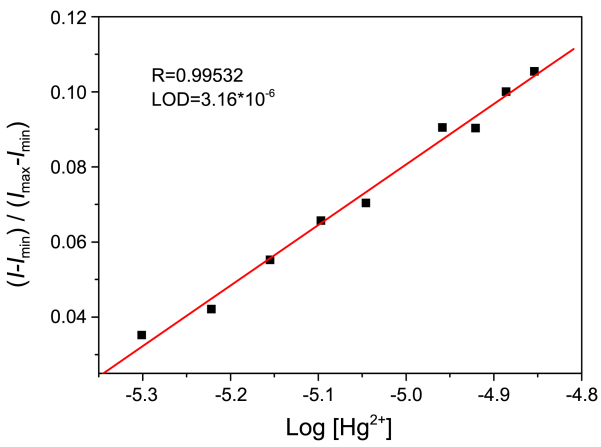


Figure 5. Normalized response of fluorescence intensity (at 599 nm) of **1** solution (5 μM) to Log[Hg^{2+}].

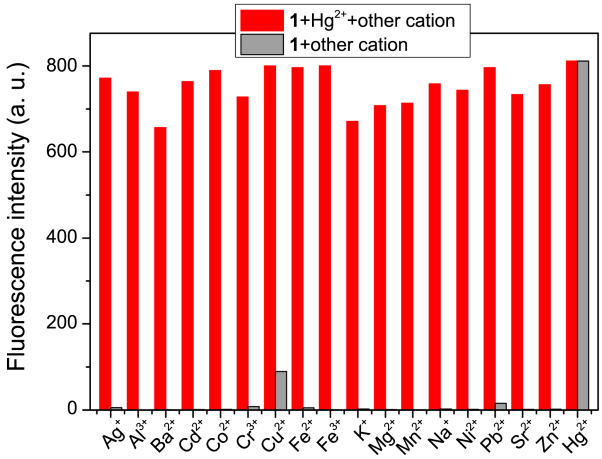


Figure 6. Fluorescence intensity changes (at 599 nm) of **1** solution (5 μM) with various metal ions. The gray bars represent the fluorescence intensity ratio of **1** solution in the presence of 40 equiv. of miscellaneous metal ions; the red bars represent the fluorescence intensity ratio of the above solution upon further addition of 40 equiv. of Hg^{2+}.

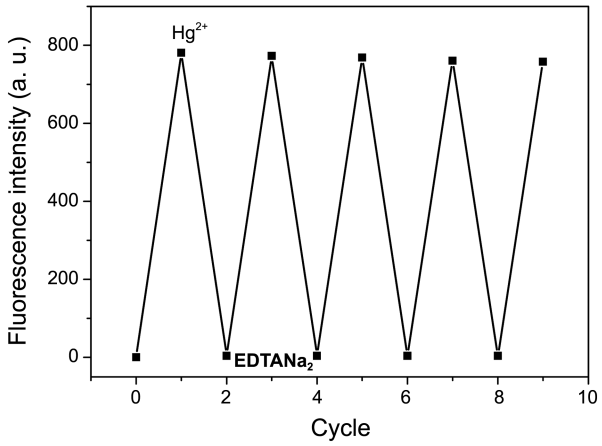


Figure 7. Fluorescence intensity (at 599 nm) changes of **1** solution (5 μM) upon alternating addition of Hg^{2+} and $EDTANa_2$.

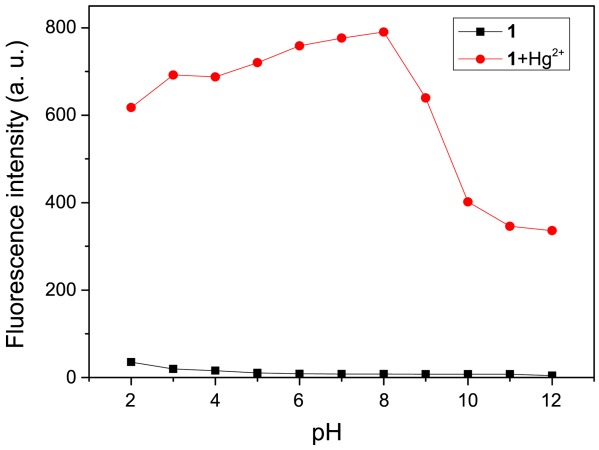


Figure 8. pH-dependent fluorescence intensity (at 599 nm) changes of **1** solution in the absence and presence of Hg^{2+}.

aqueous ethanol (pH 7.0, 50:50,v/v). Ploting the normalized fluorescence intensity against Log[Hg^{2+}] result in a nice linear line (Fig. 5), and the detection limit of **1** to Hg^{2+} was estimated as 3.2×10^{-6} M.[14] Although the detection limit of **1** for Hg^{2+} is much higher than that of **RB-S2** (nano-molar level) as well as some reported chemsensors, sensor **1** still has potential applicability in Hg^{2+} detection owing to its micro-molar level detection limit.

As a good metal ion selective fluorescent probe, the interference from other metal ions should be taken into account. Thus, the competition experiments were then carried out. Firstly, different metal ions were respectively added to **1** solution, and none of them can induce dramatic fluorescence enhancement. Further addition of Hg^{2+} to the above metal ion containg solutions, all these samples showed significant fluorescence emissions (Fig. 6). These results demonstrate that the Hg^{2+} recognition event is hardly hamped by other metal ions.

Subsequently, the recognition reversibility of **1** toward Hg^{2+} was verified by alternating addition of Hg^{2+} and $EDTANa_2$ to **1** solution. The fluorescence intensity of the tested solution displayed alternative enhancing and quenching processes (Fig. 7), indicating that the Hg^{2+} recognition event is reversible.

For potential biological applications, the pH effects on the fluorescence intensity of probe **1** in the absence and presence of Hg^{2+} were also explored (Fig. 8). In the absence of Hg^{2+}, probe **1** showed almost no emission between pH 3 and 11. However, significant enhanced fluorescence intensity at

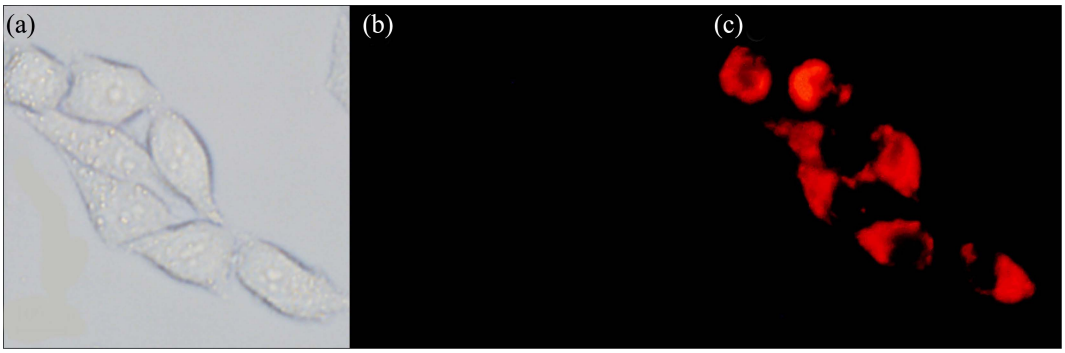


Figure 9. Bright-field transmission image (a) and fluorescence image (b) of Hela cells incubated with 5 μM of sensor **1** for 30 min at 37 °C, and fluorescence image of the above cells after further incubated with 5 μM of Hg^{2+} for 30 min at 37 °C (c).

599 nm were observed in the presence of Hg^{2+}, especially within the near neutral pH conditions. These phenomena demonstrate that probe **1** is applicable for Hg^{2+} detection in a wide pH range and is more suitable under physiological conditions.

To assay the potential biological application of **1**, the sensing performances of **1** to Hg^{2+} in Hela cells was monitored by fluorescence microscopy (Figure 9). Incubation of Hela cells with 5 μM of sensor **1** in PBS buffer for 30 min at 37 ºC displays no fluorescence in the bright-field transmission image (Fig. 9(a)) and fluorescence image (Fig. 9(b)). The cells were then washed three times with PBS buffer, and incubated with Hg^{2+} (10 μM) for another 30 min at 37 ºC, a red fluorescence from the intracellular area was observed (excited with green light) (Fig. 9(c)), providing a visual evidence of sensor **1** entering cells and the intracellular existence of Hg^{2+}. These results demonstrate that the probe is permeable to cells, bind to intracellular Hg^{2+} and emits strong fluorescence upon binding to Hg^{2+}. These features make it have a potential applicability for detecting unicellular Hg^{2+}.

Conclusions

In conclusion, we have taught a known compound a new trick as a fluorescence off-on sensor for Hg^{2+} recognition. Sensor **1** displays highly selective and sensitive recognition to Hg^{2+} in an aqueous solution. Sensor **1** interacts with Hg^{2+} through a 1:1 stoichiometry with a low detection limit. Moreover, sensor **1** is cell permeable and suitable for the detection of Hg^{2+} in living cell.

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