An Aurone-derived Low-molecular-weight Fluorescence Probe for the Detection of Hg^{2+} in Aqueous Solution and Living Cells

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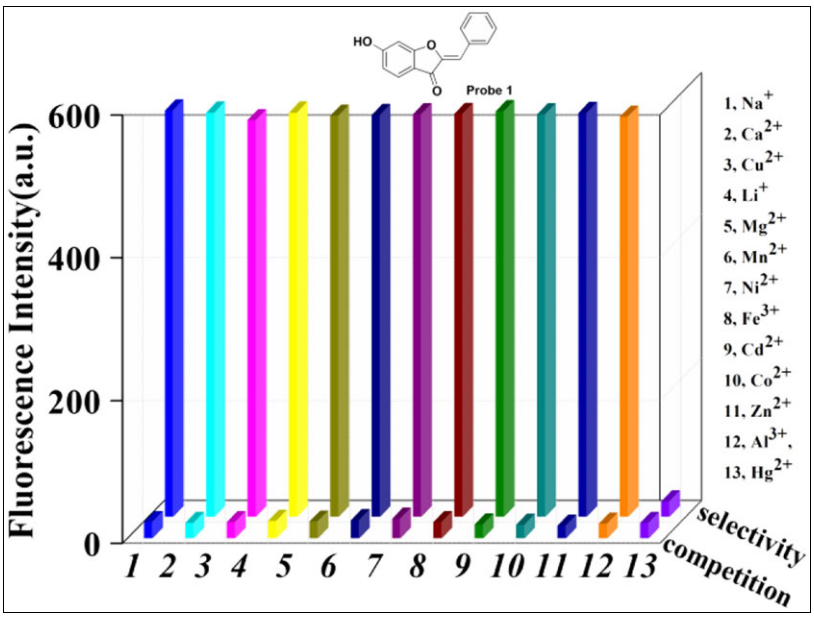

A low-molecular-weight fluorescent probe **1** (M.W. = 238.24) based on aurone was synthesized, and its application in fluorescent detection of Hg^{2+} in aqueous solution and living cells was reported. It exhibited an "on–off" fluorescent response toward Hg^{2+} in aqueous solution. Both the color and fluorescence changes of the probe were remarkably specific for Hg^{2+} in the presence of other common metal ions, satisfying the selective requirements for biomedical and environmental monitoring application. The probe has been applied in direct measurement of Hg^{2+} content in river water samples and imaging of Hg^{2+} in living cells, which further indicates the potential application values in environmental and biological systems.

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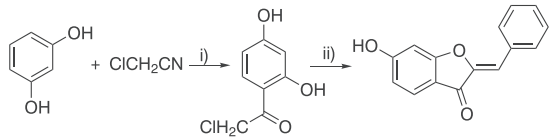
INTRODUCTION

Mercury(II) is a common contaminant in all environmental spheres. It can be bioaccumulation in the brain, kidneys, and developing fetus through food chain and atmospheric circulation [1–3] and cause severe damage to the nervous and reproductive development system of human beings [4]. Hence, the development of a rapid, selective, and sensitive detection method for Hg^{2+} is of significant importance.

Among numerous methods for detecting Hg^{2+}, fluorescent sensors have attracted considerable attention because of high selectivity and sensitivity, easy operation, rapid measurement, low cost, and real-time monitoring [5–7]. A variety of fluorescent probes for Hg^{2+} have been developed based on fluorescein [8–10], boron dipyrromethene [11–13], coumarin [14–16], rhodamine [5,17–21], cyanine [22–24], and naphthalimide [25–27]. Some have been applied in the analysis of Hg^{2+} in biological samples providing useful information of mercury biology. In order to monitor Hg^{2+} in living cells, fluorescence probes should have sufficient aqueous solubility and cell permeability. Most fluorescence probes for Hg^{2+} typically have large molecular weights owing to a fluorescent core and a Hg^{2+} binding moiety. Therefore, their solubility in water and cell permeability may be limited.

Aurones (2-benzylidenebenzofuran-3(2*H*)-ones), as isomers of flavones, are naturally occurring yellow pigments from plants. They demonstrate a great diversity of biological activities, including antimicrobial [28], anticancer [29,30], antiviral [31,32], antifungal [33,34], and herbicidal [35,36] activities. It was also reported that aurone derivatives could serve as fluorescent probes to detect CN^-[37–39] and biomacromolecules [40]. To the best of our knowledge, up to now, there are no

Scheme 1. Synthesis of compound **1**. Reagents and conditions: (i) $ZnCl_2$, HCl(g), ethyl ether, 0°C; H_2O, reflux; (ii) benzaldehyde, 10% NaOH, EtOH.



reports about fluorescent detection of Hg^{2+} based on aurone in the literature. Herein, a low-molecular-weight 6-hydroxyaurone **1** (M.W. = 238.24) (Scheme 1) with high sensitivity and selectivity for Hg^{2+} was synthesized, and the recognition properties to Hg^{2+} were investigated in aqueous solution and living cells.

RESULTS AND DISCUSSION

Synthesis of probe 1. The synthetic route for probe **1** was outlined in Scheme 1. 2-Chloro-1-(2,4-dihydroxyphenyl)ethanone (**2**) was prepared according to a reported procedure [41]. Condensation of compound **2** with benzaldehyde in the presence of NaOH afforded probe **1**. Its structure was well characterized by ^1H nuclear magnetic resonance (NMR), ^{13}C NMR, elemental analyses, and electrospray ionization-mass spectrometry (ESI-MS) spectroscopy.

Fluorescence spectra for Hg^{2+}. The fluorescence emission spectra of probe **1** were carried out in the mixture of 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid solution (Hepes) and *N*, *N*-dimethylformamide (DMF) (Hepes: DMF = 9:1, *v*/*v*) at room temperature. Firstly, pH effects on fluorescene of probe **1** were investigated in order to evaluate the potential applications of probe **1** in different environments. The results showed that free probe **1** displayed strong fluorescence at 430 nm in a wide pH range of 6–10 (Fig. 1). However, upon introduction of Hg^{2+}, the fluorescence intensity decreased significantly at the same pH range. These results indicated that probe **1** responded to Hg^{2+} in a pH-independent manner in the pH range from 6 to 10 and could conveniently detect Hg^{2+} in practical samples.

The fluorescence titration of Hg^{2+} was examined using a solution of 10 μM of compound **1** in Hepes: DMF (9:1, *v*/*v*, pH 7.0). As shown in Figure 2a, a strong emission peak was observed at 510 nm when excited at 430 nm. The original fluorescence intensity of **1** had a gradual decrease with the addition of Hg^{2+}. The fluorescence of **1** was almost fully quenched after addition of 8.0 equiv. of Hg^{2+} with a 26.8-fold decrease in fluorescence intensity. More importantly, the decrease of fluorescence intensity of **1** corresponded to the concentration of Hg^{2+} in a wide linear range from 0 to 80 μM (linearly dependent

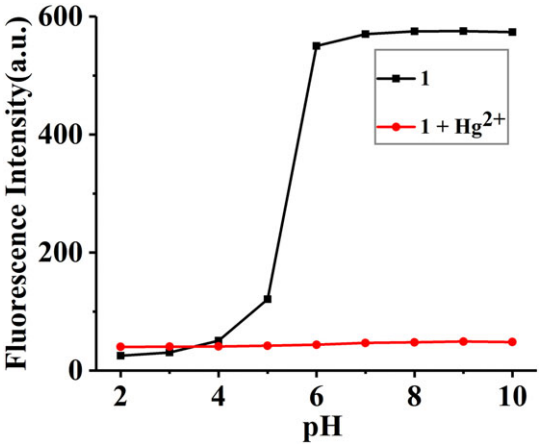


Figure 1. Fluorescence intensity of free **1** (10 μM) and in the presence of Hg^{2+} (70 μM) with different pH. [Color figure can be viewed at wileyonlinelibrary.com]

coefficient: R^2 = 0.9742) with a detection limit of 7.1 × 10^{-7} mol/L (based on 3 σ/s) (Fig. 2b). Furthermore, an obvious yellowish green color disappearance could be observed; while other metal ions such as Cu^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+} showed neither a color nor a fluorescence interfering effect after they were added to buffered solution of **1** (Fig. 3). So it is confirmed that compound **1** is able to show on–off fluorescence response to Hg^{2+} with 430 nm as the exciting wavelength.

Selectivity of probe 1 to Hg^{2+}. It is generally accepted that high selectivity is a necessary criterion for a desirable chemosensor. Thus, the influence of other metal ions on probe **1** was examined. As shown in Figure 4, the addition of 80 μM metal ions (Na^+, Ca^{2+}, Cu^{2+}, Li^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Fe^{3+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Al^{3+}, and Hg^{2+}) to the solution of probe **1** (10 μM) caused nearly no changes in the fluorescence intensity in aqueous solution (Hepes: DMF = 9:1, *v*/*v*, pH 7.0). The only prominent fluorescence quenching appeared when Hg^{2+} was introduced. Furthermore, the fluorescence response of probe **1** to Hg^{2+} in the presence of potentially competing metal ions was investigated. As displayed in Figure 4, most of metal ions did not induce visible effect on Hg^{2+} detection of probe **1**. In addition, the visual fluorescence responses of probe **1** to various metal ions (Fig. 3) demonstrated that the probe can be used conveniently as a "naked-eye" fluorescence indicator for Hg^{2+}. These results corroborated that probe **1** showed high selectivity toward Hg^{2+} over competitive metal ions.

Detection of Hg^{2+} in aqueous samples and living cells. The practical application of probe **1** was first evaluated by detection of Hg^{2+} in river water samples (obtained from different locations of Yudai River in Jiangsu University, with a concentration of Hg^{2+} ranging from 1.00 to 10.00 μM), and the results were compared with those

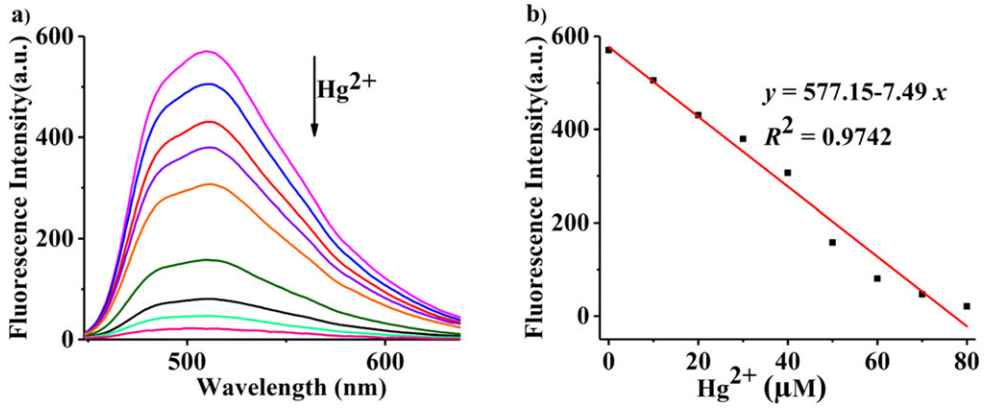


Figure 2. (a) Fluorescence spectra of compound **1** (10 μM) measured in Hepes-dimethylformamide (9: 1, *v*/*v*, pH 7.0) solution (λ_{ex} = 430 nm) with titration of Hg^{2+} (from top to bottom: 0, 10, 20, 30, 40, 50, 60, 70, and 80 μM). (b) Changes of the fluorescence intensity upon the titration at 510 nm. [Color figure can be viewed at wileyonlinelibrary.com]

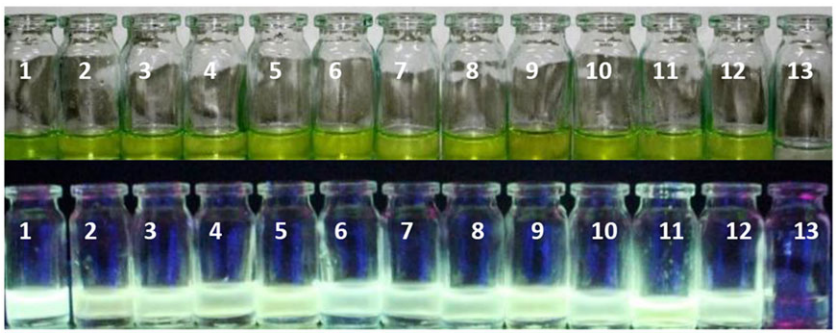


Figure 3. The colorimetric (top) and fluorometric (bottom) changes of **1** (10 μM) upon addition of different metal ions [80 μM of (1) Cu^{2+}, (2) Na^{+}, (3) Ca^{2+}, (4) Li^{+}, (5) Mg^{2+}, (6) Mn^{2+}, (7) Ni^{2+}, (8) Cd^{2+}, (9) Co^{2+}, (10) Zn^{2+}, (11) Al^{3+}, (12) Fe^{3+}, and (13) Hg^{2+}] in buffers (Hepes: dimethylformamide, 9:1, *v*/*v*, pH 7.0). The two photos were obtained in normal light (top) and upon at 365 nm using UV lamp (bottom), respectively. [Color figure can be viewed at wileyonlinelibrary.com]

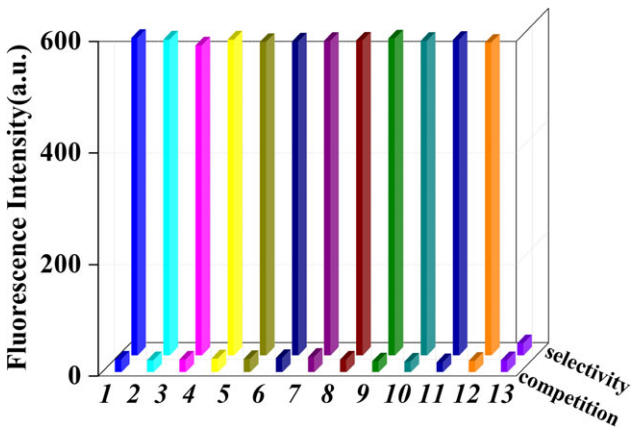


Figure 4. Histogram showing selectivity of probe **1** (10 μM) in Hepes-dimethylformamide (9: 1, *v*/*v*, pH 7.0) solution (λ_{ex} = 430 nm). The pillars in the back row from left indicate the change in the fluorescence intensity in the presence of metal ions [80 μM of (1) Na^{+}, (2) Ca^{2+}, (3) Cu^{2+}, (4) Li^{+}, (5) Mg^{2+}, (6) Mn^{2+}, (7) Ni^{2+}, (8) Fe^{3+}, (9) Cd^{2+}, (10) Co^{2+}, (11) Zn^{2+}, (12) Al^{3+}, and (13) Hg^{2+}]. The pillars in the front row from left represent the value of fluorescence intensity upon subsequent addition of Hg^{2+} (80 μM) to the solution containing probe **1** and the metal ions of interest. [Color figure can be viewed at wileyonlinelibrary.com]

given by the atomic absorption spectrometry reference method. All the samples were filtrated before use. Owing to pH having no obvious influence in the pH range of 6–10, **1** was directly added to water samples, and then its fluorescence intensity change was detected. As shown in Table 1, the results obtained with the proposed probe were in good agreement with those obtained by atomic absorption spectrometry with a relative error less than 4%, which confirmed that the proposed probe was applicable for practical Hg^{2+} detection [42]. These results suggested that probe **1** could meet the sensitivity and selectivity requirements for monitoring environmental water samples.

To further demonstrate the practical applicability of the probe in biological samples, fluorescence imaging experiments were carried out in living cells on an inverted fluorescence microscope. HepG-2 cells were incubated with **1** (10 μM) for 0.5 h at 37°C, then followed by the addition of Hg^{2+} (40 and 80 μM), and incubated for another 0.5 h. The cells were washed with phosphate-buffered saline (PBS) buffer solution three times, and their fluorescence images were recorded before

Table 1

Results of probe **1** detection Hg^{2+} in river water samples.

Samples	Concentration[a] (μM)	Concentration[b] (μM)	Relative error (%)	Recovery rate (%)
1	1.02 ± 0.04	1.01 ± 0.10	−1.00	101.0
2	2.50 ± 0.06	2.57 ± 0.53	2.80	102.8
3	5.08 ± 0.13	5.11 ± 0.54	0.50	102.2
4	7.47 ± 0.08	7.56 ± 0.52	1.20	100.8
5	10.10 ± 0.17	9.79 ± 0.55	−3.10	97.9

[a]Detected by atomic absorption spectrometry.
[b]Average of three detections found by the proposed method.

and after addition of Hg^{2+} (Fig. 5). In the absence of Hg^{2+}, a bright fluorescence was observed in living cells (Fig. 5b). After incubation with Hg^{2+} (40 μM), it was found that the fluorescence intensity decreased dramatically (Fig. 5c). However, almost no fluorescence signals were observed when 80 μM of Hg^{2+} was introduced (Fig. 5d). The results suggested that probe **1** could penetrate the cell membrane and be applied for *in vitro* imaging of Hg^{2+} in living cells.

In summary, we have developed a low-molecular fluorescent probe **1** based on aurone. It demonstrated fluorescence response toward Hg^{2+} in aqueous media with high sensitivity and selectivity. Most importantly, both the color and fluorescence changes of the probe are remarkably specific for Hg^{2+} in the presence of other common metal ions, satisfying the selective requirements for biomedical and environmental monitoring application. The living cell imaging experiments further indicated the possible potential application in biological systems.

EXPERIMENTAL

All analytical grade chemicals were used as received. Melting points were determined on an X-4 binocular microscope melting point apparatus (Beijing Tech

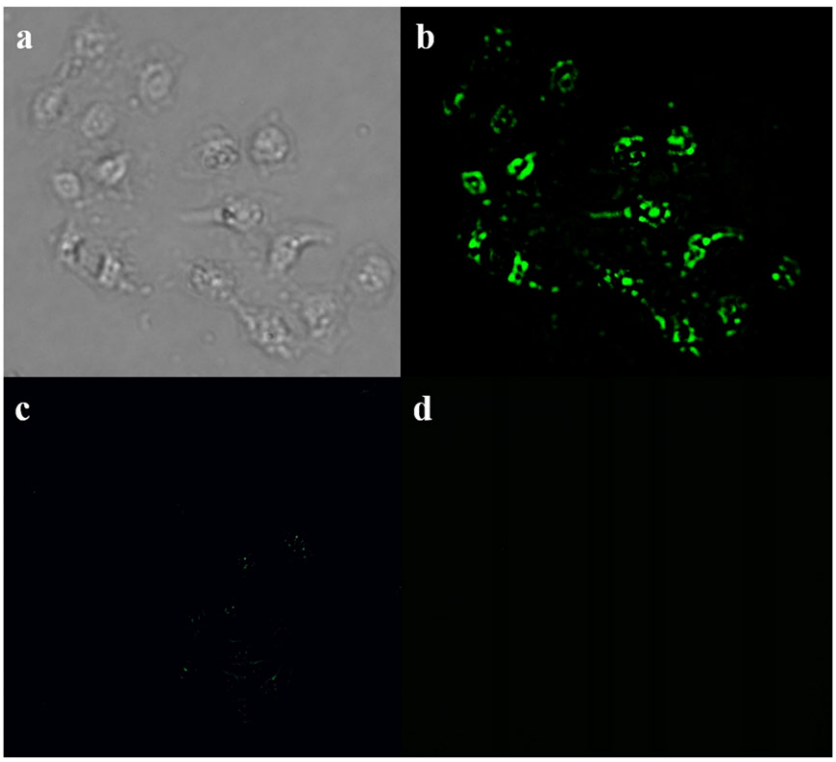


Figure 5. Fluorescence images of Hg^{2+} in HepG-2 cells with **1**. (a) Bright-field transmission image of HepG-2 cells incubated with **1** (10 μM) for 0.5 h. (b) Fluorescence image of HepG-2 cells incubated with **1** (10 μM) for 0.5 h (λ_{ex} = 430 nm). (c) Fluorescence image of HepG-2 cells incubated with **1** (10 μM) for 0.5 h, washed three times, and then further incubated with 40 μM Hg^{2+} for 0.5 h (λ_{ex} = 430 nm). (d) Fluorescence image of HepG-2 cells incubated with **1** (10 μM) for 0.5 h, washed three times, and then further incubated with 80 μM Hg^{2+} for 0.5 h (λ_{ex} = 430 nm). [Color figure can be viewed at wileyonlinelibrary.com]

Instruments Co., Beijing, China) and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCEII 400 MHz instrument in DMSO-d_6, and the chemical shifts are reported as parts per million. Mass spectra were obtained from a Thermo LXQ Liquid Chromatography-Ion Trap Mass Spectrometry. IR spectrum as KBr pellets and is reported in terms of frequency of absorption (cm^{-1}). Elemental analyses were carried out with a Perkin-Elmer 240C analyzer. Fluorescence spectra were measured with a Varian Cary Eclipse spectrophotometer. The cations, including Cu^{2+}, Na^+, Ca^{2+}, Li^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+}, Fe^{3+}, Al^{3+}, and Hg^{2+}, were obtained by using their sulfate, chloride, or nitrate.

Synthesis. The synthetic pathways for compounds **1** and **2** were outlined in Scheme 1, and the yields were not optimized.

Synthesis of 2-chloro-1-(2,4-dihydroxyphenyl)ethanone (2). To a mixture of resorcinol (2.00 g, 18.16 mmol) and chloroacetonitrile (1.30 mL, 2.10 mmol) in dry anhydrous ether (300 mL) was added dry $ZnCl_2$ (1.20 g, 9.20 mmol). The solution was cooled to 0°C, and dry HCl gas was bubbled through the reaction for 3 h. The solution was left in the refrigerator for 1 day, and HCl gas was bubbled again for 4 h at 0°C. Subsequently, the solution was left in the refrigerator for another 1 day. The precipitate was filtered off and washed three times with anhydrous ether, then followed by dissolving in 100 mL hot water and refluxing for 6 h. After cooling to room temperature, the solid was filtered off, washed three times with water, and recrystallized from ethanol to give 2-chloro-1-(2,4-dihydroxyphenyl)ethanone (**2**) as a yellow brown solid (1.72 g, 51.0%), m.p. 121–126°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.98 (s, 2H, CH_2Cl), 6.33 (d, J = 2.0 Hz, 1H, H-3), 6.39 (dd, J = 8.8, 2.1 Hz, 1H, H-5), 7.72 (d, J = 8.8 Hz, 1H, H-6), 10.70 (brs, 1H, OH), 11,67(brs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 47.3, 102.4, 108.4, 111.6, 132.8, 163.1, 164.8, 193.4. *Anal*. Calcd for $C_8H_7ClO_3$: C, 51.50; H, 3.78. Found: C, 51.78; H, 3.76. MS (ESI, *m/z*) 185.01(M^-).

Synthesis of (Z)-2-benzylidene-6-hydroxybenzofuran-3(2H)-one (1). To a mixture of **2** (0.50 g, 2.7 mmol), benzaldehyde (0.34 g, 3.2 mmol) and ethanol (2 mL) was added slowly 10% sodium hydroxide solution (5 mL, 12.5 mmol). The mixture was stirred at room temperature for 24 h. It was acidified to below pH 3 with 1.0 M HCl and was then placed at room temperature for 1 h. The precipitate was collected by filtration and washed with ice-cold water. The crude product was purified by recrystallization from dichloromethane and ethanol to yield (*Z*)-2-benzylidene-6-hydroxybenzofuran-3(2*H*)-one (**1**) as a yellow solid (0.44 g, 68.3%), m.p. 272–274°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.73 (dd, J = 8.4, 2.0 Hz, 1H, H-5), 6.79 (s, 1H, =CH), 6.82 (d, J = 1.6 Hz, 1H, H-7), 7.40–7.53 (m, 3H, ArH), 7.64 (d, J = 8.4 Hz, 1H, H-4), 7.92–7.97 (m, 2H, ArH), 11.06 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 98.6, 110.4, 112.8, 113.0, 126.0, 128.9(2C), 129.6, 131.0(2C), 132.1, 147.4, 166.4, 167.8, 181.5. IR (KBr) *v*: 3317, 2998, 2937, 2369, 1617, 1499, 1458, 1331, 1217, 1143 cm^{-1}. ESI-MS *m/z* (%): 239.27 (M + H^+, 100). *Anal*. Calcd for $C_{15}H_{10}O_3$: C, 75.62; H, 4.23. Found: C, 75.82; H, 4.27.

Preparation of the test solution. A 0.1-mM stock solution of probe **1** was prepared by dissolving in absolute DMF. Hg^{2+} was dissolved in deionized water to prepare 10-mM stock solution, which was further diluted to 0.8–0.1 mM stepwise. The stock solutions of other cations (0.8 mM) were also prepared using deionized water. The complex solution of cation/**1** was prepared by adding 1.0 mL of the stock solution of **1** and 1.0 mL of cation in a 10-mL volumetric flask, followed by diluting to 10 mL with Hepes buffer solution (10 mM, pH 7.0). The solution was protected from light and kept at 4°C for further use. For all measurements of fluorescence spectra, excitation was fixed at 430 nm with excitation slit set at 5.0 nm and emission at 5.0 nm.

Cell incubation and imaging. The living HepG-2 cells were provided by the School of The Environment and Safety Engineering of Jiangsu University (China). The cells were seeded into 96-well plates at a density of 4×10^3 cells per well and incubated for 24 h. The culture medium was discarded, and cells were washed three times with PBS, followed by incubating with 10 μM of compound **1** (dissolving in the culture medium containing 9.09% DMF) for 0.5 h at 37°C, and then washed three times with PBS to remove free **1**. Cell imaging of HepG-2 cells was observed with an inverted fluorescence microscope (Carl Zeiss, Oberkochen, Germany, Axio Observer A1). The brightness and fluorescence imaging of cells (Ex. 430 nm) were recorded. After that, a Hg^{2+} aqueous solution was diluted with culture medium, added to the cells, and incubated for another 0.5 h at 37°C, and the HepG-2 cells were rinsed with PBS three times to remove free Hg^{2+} ions. Fluorescence imaging of intracellular Hg^{2+} in HepG-2 cells was observed and recorded.

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SUPPORTING INFORMATION

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