Resorufin-Based Colorimetric and Fluorescent Probe for Selective Detection of Mercury (II) †

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Abstract: Environmental pollution crisis, particularly mercury ions (Hg²⁺) contamination, seriously threatens the health of all living organisms. Many studies have shown that even extremely low concentrations of Hg²⁺ can rigorously damage living organisms. Therefore, it is very much needed for real-time detection of Hg²⁺. To tackle mercury contamination and for its detection, we herewith proposed an intelligent design of a new fluorescent ‘turn-on’ probe, which was prepared based on the mercury-promoted hydrolysis of vinyl ether moiety. The probe rapidly reacted with mercury ions and showed good selectivity over other metal ions.

Keywords: fluorescent probe; Hg²⁺ ions; vinyl resorufin

1. Introduction

Mercury is an extremely toxic and biological non-essential element that subsists naturally in the environment. Environmental pollution problems, particularly mercury ions (Hg²⁺) pollution, seriously threaten the health of organisms. Currently, mercury is primarily used in the production of many chemical drugs and the manufacture of electronic or electrical appliances, resulting in the release of mercury-containing wastewater or waste [1–3]. Irrespective of the source and primary location of the deposit, eventually, mercury (II) mixes with freshwater and marine ecosystems, causing various environmental issues in aspects related to plants, animals, and even humans [4]. Many acute poisonings have occurred. For example, the famous Minamata disease in Japan occurred due to the pollution of mercury in the Agano Tributary, and a striking epidemic in Iraq occurred because polluted seeds were used in bread [5]. The poisonousness of mercury depends on the form and the severity of the exposure. The forms of mercury include metallic mercury, inorganic mercury, and organic mercury. Among them, organic mercury is much more toxic due to its strong fat solubility. Thus, it can easily penetrate the cell membrane and pass through the blood–brain barrier. When it gathers in the brain tissue, it will cause severe brain impairment. Methyl mercury, the most famous one among different types of organic mercury, is the most harmful to the human body [6–10]. Methyl mercury was the culprit in the watery disease epidemic that broke out in Japan in the 1950s. The US Environmental Protection Agency stipulates that the upper limit of mercury (II) in drinking water is 2 ppb (10 nM).

Many studies have shown that even extremely low concentrations of Hg²⁺ can severely damage organisms. Therefore, real-time detection of Hg²⁺ is essential. Fluorescent probe technology, with its great advantages, has become the preferred technique for environmental detection and in vivo analysis of Hg²⁺. In recent years, many contributions have been made to design and synthesise novel fluorescent probes for the detection of environmental pollutant Hg²⁺ analysis.
2. Previous Research

2.1. Fluorescent Probes for Hg\(^{2+}\) Analysis Based on Ring-Opening Reactions

Li et al. [11] designed a simple rhodamine derivative probe bearing a hydrophilic carboxylic acid group. The fluorescent probe selectively responded to Hg\(^{2+}\) in 100% aqueous solution with 42-fold fluorescence intensity enhancement within the pH range from 5.0 to 8.0. The fluorescent intensity change followed the concentration of Hg\(^{2+}\) in a linear range covering from 3.0 \(\times\) \(10^{-7}\) to 1.0 \(\times\) \(10^{-5}\) M, and the detection limit was found to be 9.7 \(\times\) \(10^{-8}\) M.

![Fluorescent probe for Hg\(^{2+}\)](image)

2.2. Fluorescent Probes for Hg\(^{2+}\) Analysis Based on Ring Opening, Followed by Cyclisation

Ge et al. [12] designed a novel pyrido[1,2-a] benzimidazole-rhodamine-based ratiometric fluorescent probe for Hg\(^{2+}\). The probe showed high sensitivity, with a detection limit of 18.8 nM, and also exhibited satisfying selectivity, with the maximum emission shifting from 464 nm to 584 nm. Remarkably, the ratiometric fluorescent probe presented an about 200 nm Stokes shift, and such a large Stokes shift could avoid auto-fluorescence interference, serious self-quenching, and fluorescence detection errors. With the addition of Hg\(^{2+}\), the molecular ring-opening reaction of the spironolactone resulted in a fluorescence resonance energy transfer (FRET) effect that brought about emission shifting and fluorescence changing from blue to red. Furthermore, the application of detecting Hg\(^{2+}\) in Glioma cells demonstrated the potential of this study.

![Fluorescent probe for Hg\(^{2+}\)](image)

2.3. Fluorescent Probes for Hg\(^{2+}\) Analysis Based on S-Atom Complexation

Zhou et al. [13] developed a BODIPY-based sensitive fluorescent probe that utilised the carboxyl-thiol metal bonding receptor to recognise the Hg\(^{2+}\) cations in a neutral aqueous solution via the PET mechanism. There was an about 630-fold fluorescence enhancement in the reaction of the probe with Hg\(^{2+}\). Fluorescent probes showed a selective response towards Hg\(^{2+}\) over other relevant competing metal ions. For sensitivity, the sensing limit of the probe was 5.7 nM, which met the detective requirement at the ppb level. Noticeably, the response time towards Hg\(^{2+}\) was below 30 s, which made the detection more convenient and avoided the time effects on probe performance and sample properties.
Jiao et al. [14] developed and synthesised a novel selective and sensitive fluorescent chemosensor, which was based on coumarin Schiff’s base. They reported that the X-ray diffraction single-crystal structure analysis of the probe showed that the probe crystallises in a monoclinic system, and two aromatic groups of the compound were almost in the same plane, providing the explanation for the detection mechanism. Mercury ions form bonds with heteroatoms of the probe due to the intramolecular charge transfer (ICT) effect was cut off, which resulted in the fluorescent intensity enhancement at 530 nm. The probe indicated a good selectivity over other common metal ions, and the lower limit of detection was calculated as 1 ppb.

2.4. Fluorescent Probes for Hg$^{2+}$ Analysis Based on Other Mechanisms

Wu et al. [15] designed and synthesised a probe comprising 7-hydroxy-4-methylcoumarin as a fluorophore and a vinyl ether group as a recognition unit. After treating with Hg$^{2+}$ for 10 min, the sensor revealed a 110-fold fluorescence enhancement at 450 nm, in HEPES buffer. The detection limit of probe was calculated as 0.12 μM, and the response time was less than 10 min.

2.5. Fluorescent Probes for Hg$^{2+}$ Analysis Based on Deprotection of Dithioacetals

Zhou et al. [16] reported a new ratiometric fluorescent probe with an electron-deficient dithioacetal group on the three-site, which would be removed by Hg$^{2+}$ to afford a ratiometric fluorescent signal. The ratiometric signal indicated a gradual change in the emission peak from 465 nm to 545 nm when exposed to Hg$^{2+}$, without being affected by the microenvironment. This kind of ratiometric tool is more appreciated than intensity-based fluorescent sensors. The emission intensity presented about a 12-fold enhancement in emission ratio, with good linearity, and the lower limit of detection was calculated to be 5.8 nM. Hg$^{2+}$-induced conversion of 1,3-dithiane to carbaldehyde is an efficient umpolung reaction, which can favour the formation of the intramolecular charge transfer (ICT) mechanism in compounds.
3. Hypothesis

With the above literature background, it was envisioned that an organic molecule with vinyl ether entity type would be activated by Hg$^{2+}$. Keeping this in mind, we designed and synthesised a new VRF probe comprising resorufin as a fluorophore and a vinyl ether group as a recognition unit, suggesting that the strong fluorescent appearance is attributed to the free form of resorufin, the Hg$^{2+}$-promoted hydrolysis reaction product, as depicted in the proposed mechanism. The reaction thus envisaged would liberate highly fluorescent fluorophore, and therefore the designed molecules would serve as a probe for sensing mercury. Thus, we hypothesised a new approach that involves masking and unmasking the fluorophore. It can be judged from the given mechanism that the fluorescence of fluorophore can be quenched or turned off by anchoring with the organic substrate. Once the mercury has been sensed, the probe would liberate highly fluorescent fluorophore with the formation of organic product. We herewith disclose a reaction-dependent strategy that involves the masking and unmasking of resorufin-engineered fluorophore for selective sensing of mercury. Considering the ability of Hg$^{2+}$ to activate alkene functionality, a dormant fluorophore was designed for the sensing of mercury, leading to cascade and delivery of active fluorophore.

4. Materials and Instrumentations

All chemicals were either borrowed or obtained from commercial suppliers and used as received without further purification. For performing all reactions, we used oven-dried screw-cap vials with magnetic stirrers and nitrogen, to maintain an inert atmosphere. Solvents, which were dried, as well as the liquid reagents, were transferred using sterile syringes or hypodermic syringes. Coated aluminium sheet silica plates (TLC) were used for monitoring and analysing the progress of the reactions. TLC plates plate were observed under the UV light to locate and analyse the position of sample spots. For further confirmation, the spots were exposed to KMnO$_4$ and visualised after charring on a hot plate.

$^1$H NMR and $^{13}$C NMR spectra were measured on a Bruker AV-400/500 spectrometer, with chemical shifts reported in ppm (in DMSO-d$_6$ or CDCl$_3$, with TMS as the internal standard). Data for $^1$H NMR are reported as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), integration, and coupling constant (Hz). ESI mass spectra were carried out on an HPLC–MS spectrometer (Agilent 6100). Measurement of Fluorescence spectra was performed with a PerkinElmer LAMBDA 950 UV–Vis Spectrophotometer and a Photon Technology International, quanta Master 400 Spectrofluorometer, respectively, in degassed spectral grade solvents.

5. Design and Synthesis of Probe

The probe design consists of alkylation of resorufin (Step-1), followed by a base-catalysed dehydrohalogenation (Step-2) reaction, offering the desired probe which on mercury promoted hydrolysis converts the non-fluorescent molecule to fluorescent molecule. The overall scheme (Scheme 1) for the synthesis of the VRF probe is shown below.
5. Design and Synthesis of Probe

The probe design consists of alkylation of resorufin 1 (1 mmol) and K$_2$CO$_3$ (2 mmol) in DMF, 1,2-dibromoethane 2 (1 mmol) was added in one portion, under an inert atmosphere of N$_2$. The reaction mixture was stirred at 60 °C overnight. After evaporation of the solvent, the resulting light brown material was dissolved in DCM (100 mL). After removal of insoluble materials by Celite filtration, the filtrate was concentrated in vacuo. The crude product was purified by silica-gel column chromatography (eluent: ethyl acetate/hexanes = 1:1) to obtain the desired product, in 60% yield. $^1$H NMR (CDCl$_3$, 500 MHz): δ 3.63 (2 H, t, J = 6.3 Hz), 3.72 (2 H, t, J = 5.7 Hz), 6.33 (1 H, d, J = 1.8 Hz), 6.82-6.86 (2 H, m), 6.95 (1 H, dd, J = 9.1, 1.8 Hz), 7.43 (1 H, d, J = 9.7 Hz), 7.72 (1 H, d, J = 9.1 Hz); $^{13}$C NMR (CDCl$_3$, 125 MHz): δ (ppm) = 29.6, 66.5, 100.9, 107.0, 114.1, 128.7, 131.8, 134.5, 134.9, 145.8, 145.9, 150.0, 162.8, 186.5; HRMS (C$_{14}$H$_{10}$BrNO$_3$, [M + Na]$^+$) calcd. 353.0, found 353.1.

Step 1—Synthesis of 7-(2-bromoethoxy)-3H-phenoxazin-3-one 3: To a solution of resorufin 1 (1 mmol) and K$_2$CO$_3$ (2 mmol) in DMF, 1,2-dibromoethane 2 (1 mmol) was added in one portion, under an inert atmosphere of N$_2$. The reaction mixture was stirred at 60 °C overnight. After evaporation of the solvent, the resulting light brown material was dissolved in DCM (100 mL). After removal of insoluble materials by Celite filtration, the filtrate was concentrated in vacuo. The crude product was purified by silica-gel column chromatography (eluent: ethyl acetate/hexanes = 1:1) to obtain the desired product, in 60% yield. $^1$H NMR (CDCl$_3$, 500 MHz): δ 3.63 (2 H, t, J = 6.3 Hz), 3.72 (2 H, t, J = 5.7 Hz), 6.33 (1 H, d, J = 1.8 Hz), 6.82-6.86 (2 H, m), 6.95 (1 H, dd, J = 9.1, 1.8 Hz), 7.43 (1 H, d, J = 9.7 Hz), 7.72 (1 H, d, J = 9.1 Hz); $^{13}$C NMR (CDCl$_3$, 125 MHz): δ (ppm) = 29.6, 66.5, 100.9, 107.0, 114.1, 128.7, 131.8, 134.5, 134.9, 145.8, 145.9, 150.0, 162.8, 186.5; HRMS (C$_{14}$H$_{10}$BrNO$_3$, [M + Na]$^+$) calcd. 353.0, found 353.1.

Step 2—Synthesis of 7-(vinloxy)-3h-phenoxazin-3-one (VRF probe): To a stirred solution of 7-(2-bromoethoxy)-3H-phenoxazin-3-one 3 (1 mmol) in CH$_3$CN (10 mL), DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) was added (2 mmol). The mixture was stirred at 90 °C for 12 h under inert atmosphere. The solvent was evaporated under vacuum. Water (20 mL) was added to the crude, and the solution was extracted thrice with DCM (20 mL). The combined organic layers were washed with brine solution, dried over Na$_2$SO$_4$, and evaporated under vacuum. The residue was purified by silica-gel column chromatography.

Scheme 1. The overall scheme for the synthesis of VRF probe and its mercury promoted cleavage to yield active fluorophore.

**General Procedure for the Synthesis of VRF Probe**

The VRF probe was prepared in two steps as follows:

1. **Step 1**—Synthesis of 7-(2-bromoethoxy)-3H-phenoxazin-3-one: To a solution of resorufin 1 (1 mmol) and K$_2$CO$_3$ (2 mmol) in DMF, 1,2-dibromoethane 2 (1 mmol) was added in one portion, under an inert atmosphere of N$_2$. The reaction mixture was stirred at 60 °C overnight. After evaporation of the solvent, the resulting light brown material was dissolved in DCM (100 mL). After removal of insoluble materials by Celite filtration, the filtrate was concentrated in vacuo. The crude product was purified by silica-gel column chromatography (eluent: ethyl acetate/hexanes = 1:1) to obtain the desired product, in 60% yield. $^1$H NMR (CDCl$_3$, 500 MHz): δ 3.63 (2 H, t, J = 6.3 Hz), 3.72 (2 H, t, J = 5.7 Hz), 6.33 (1 H, d, J = 1.8 Hz), 6.82-6.86 (2 H, m), 6.95 (1 H, dd, J = 9.1, 1.8 Hz), 7.43 (1 H, d, J = 9.7 Hz), 7.72 (1 H, d, J = 9.1 Hz); $^{13}$C NMR (CDCl$_3$, 125 MHz): δ (ppm) = 29.6, 66.5, 100.9, 107.0, 114.1, 128.7, 131.8, 134.5, 134.9, 145.8, 145.9, 150.0, 162.8, 186.5; HRMS (C$_{14}$H$_{10}$BrNO$_3$, [M + Na]$^+$) calcd. 353.0, found 353.1.

2. **Step 2**—Synthesis of 7-(vinloxy)-3h-phenoxazin-3-one (VRF probe): To a stirred solution of 7-(2-bromoethoxy)-3H-phenoxazin-3-one 3 (1 mmol) in CH$_3$CN (10 mL), DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) was added (2 mmol). The mixture was stirred at 90 °C for 12 h under inert atmosphere. The solvent was evaporated under vacuum. Water (20 mL) was added to the crude, and the solution was extracted thrice with DCM (20 mL). The combined organic layers were washed with brine solution, dried over Na$_2$SO$_4$, and evaporated under vacuum. The residue was purified by silica-gel column chromatography.
Step 2—Synthesis of 7-(vinyloxy)-3h-phenoxazin-3-one (VRF probe): To a stirred solution of 3 in presence of a base, to give 4. The possible reaction mechanism of VRF probe towards mercury (II) is shown below.

6. Sensing Study

Mercury promoted cleavage of vinylic ether to yield active fluorophore.

![Scheme 2. The possible reaction mechanism of VRF probe towards mercury (II).](image)

7. Results and Discussion

Synthesis: The synthesis of the VRF probe is relatively straightforward; it was synthesised in two steps. The first step involved the reaction of the hydroxy group of resorufin 1 and 1,2-dibromoethane 2 in presence of a base, to give 3. The second step involved the treatment of 3 with DBU, which underwent 1,2 elimination to produce desired VRF probe in moderate yield (Scheme 1). The possible reaction mechanism of VRF towards mercury (II) is shown in Scheme 2. The obtained VRF probe was then characterised by NMR and mass spectroscopic techniques, which showed its characteristic vinylic protons at 5.03 δ, 4.74 δ, and its mass peak at 262.23.

7.1. Effects of Mercury (II) on Absorption and Emission Spectroscopic Properties of VRF Probe

The absorption and emission spectroscopic properties of this probe in the absence and presence of Hg²⁺ were studied. The absorption spectrum of the VRF probe in the absence of Hg²⁺ exhibited a very weak broad absorption band at 480 nm (Figure 1). After treatment with Hg²⁺, the absorption band maxima showed a bathochromic shift from 480 to 575 nm, as well as a colour change from colourless to pink (Figure 1). The fluorescence spectrum of the VRF probe in the absence of Hg²⁺ showed a very weak emission at 582 nm, which is attributed to the strong quenching effect of the vinylic ether unit. However, the reaction of the VRF probe with Hg²⁺ resulted in a remarkable ‘turn-on’ fluorescence change from...
no fluorescence to strong reddish-brown fluorescence, which was directly observed by the naked eye under a UV lamp (365 nm). The fluorescence spectrum of the probe in presence of Hg$^{2+}$ (100 μM) showed a remarkable more intense emission peak at 585 nm (Figure 2). The fluorescence enhancement of the probe was attributed to the mercury-triggered cleavage reaction, causing the release of free resorufin 1. The changes in absorption and fluorescence spectra were observed due to the deprotection of the vinyl ether moiety and the formation of resorufin 1.

![Absorption spectra of VRF probe (10 μM) in the absence and presence of Hg$^{2+}$ (100 μM) in CH$_3$CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH 7.4). Inset: the change in the colour of VRF in the absence and presence of Hg$^{2+}$ (colourless to pink).](image1)

**Figure 1.** The absorption spectra of VRF (10 μM) in the absence and presence of Hg$^{2+}$ (100 μM) in CH$_3$CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH 7.4). Inset: the change in the colour of VRF in the absence and presence of Hg$^{2+}$ (colourless to pink).

![Fluorescence spectra of VRF probe (10 μM) in the absence and presence of Hg$^{2+}$ (100 μM) in CH$_3$CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH 7.4); excitation wavelength = 560 nm, the spectrum was acquired 60 min after HgCl$_2$ addition at 25 °C. Inset: fluorescence colour change observed under UV light at 365 nm.](image2)

**Figure 2.** Fluorescence spectra of VRF probe (10 μM) in the absence and presence of Hg$^{2+}$ (100 μM) in CH$_3$CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH 7.4); excitation wavelength = 560 nm, the spectrum was acquired 60 min after HgCl$_2$ addition at 25 °C. Inset: fluorescence colour change observed under UV light at 365 nm.

The time-dependent fluorescence response of the VRF probe (10 μM) with Hg$^{2+}$ (100 μM) in CH$_3$CN/HEPES buffer (1:1, 0.1 M, pH 7.4) was measured at 25 °C (Figure 3). The fluorescence spectrum of the VRF probe in the absence of Hg$^{2+}$ showed a very weak emission at 582 nm (Figure 2). Then, the fluorescence intensities of the VRF probe in the presence of Hg$^{2+}$ (100 μM) from 2 to 60 min were recorded. It was observed that
immediately after 2 min the fluorescence spectrum showed a notable increase in the intensity of fluorescence. From 2 to 60 min, there was a rapid increase in fluorescence intensity, and then it saturated. The highest fluorescence intensity was observed at 60 min. We also found that the proton NMR spectrum of the product of VRF + Hg\(^{2+}\) reaction was similar to that of free resorufin 1.

The concentration-dependent fluorescence response for the VRF probe (10 µM) upon addition of Hg\(^{2+}\) (10–100 µM) in CH\(_3\)CN/HEPES buffer (1:1, 0.1 M, pH = 7.4) was measured at 25 °C (Figure 4). The fluorescence spectrum of the VRF probe in the absence of Hg\(^{2+}\) (0 µM) showed very weak emission at 582 nm. However, with the addition of Hg\(^{2+}\) (10 µM), a notable enhancement in the fluorescence intensity at 585 nm was observed. The fluorescence response of the VRF probe showed excellent linearity with an increase in the concentration of Hg\(^{2+}\) from 10 to 100 µM. The fluorescence intensities were measured every 60 min for each addition of Hg\(^{2+}\). The highest fluorescence intensity was recorded at an Hg\(^{2+}\) concentration of 100 µM.

![Figure 3](image-url)  
**Figure 3.** Time-dependent fluorescence response of VRF probe (10 µM) with Hg\(^{2+}\) (100 µM) in CH\(_3\)CN/HEPES buffer (1:1, 0.1 M, pH = 7.4) at 25 °C; excitation wavelength = 560 nm, emission wavelength = 585 nm. The reaction was completed within one hour.

![Figure 4](image-url)  
**Figure 4.** Concentration-dependent fluorescence response for VRF probe (10 µM) upon addition of Hg\(^{2+}\) (10–100 µM) in CH\(_3\)CN:HEPES buffer (1:1, 0.1 M, pH = 7.4) at 25 °C; excitation wavelength = 560 nm, emission wavelength = 585 nm.
7.2. Response of VRF Probe to Mercury and Other Metal Ions

Selectivity is a very important parameter for evaluating the performance of the probe. To investigate the selectivity of the VRF probe, ions such as Hg$^{2+}$, Co$^{2+}$, Zn$^{2+}$, Fe$^{3+}$, Mg$^{2+}$, Mn$^{2+}$, K$^+$, Ni$^{2+}$, Ca$^{2+}$, Fe$^{2+}$, and Ag$^+$ were selected. The absorption spectra of the VRF probe (10 μM) in the presence of Hg$^{2+}$ and other metal ions (100 μM) in CH$_3$CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH = 7.4) were recorded (Figure 5). Similar to what we observed in Figure 1, the probe also exhibited a very weak broad absorption band here, at 480 nm. The treatment of the probe with metal ions other than Hg$^{2+}$ did not cause any noteworthy changes in the absorption profile. However, the absorption spectrum of the probe with Hg$^{2+}$ showed a remarkable enhancement in the absorption with absorption maxima at 575 nm, as well as a colour change from colourless to pink (Figure 5).

![Figure 5. Absorption spectra of VRF probe (10 μM) in the presence of Hg$^{2+}$ and other metal ions (100 μM) in CH$_3$CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH = 7.4). Inset: the change in the colour of VRF in the absence and presence of Hg$^{2+}$ (colourless to pink).](image_url)

The emission spectra of the VRF probe (10 μM) in the presence of Hg$^{2+}$ and other metal ions (100 μM) in CH$_3$CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH = 7.4) were also recorded (Figure 6). Similar to what we observed in Figure 2, the probe also exhibited a very weak emission here, at 582 nm. This low background signal is extremely desirable for the sensitive detection of metal ions. The treatment of the VRF probe with metal ions other than Hg$^{2+}$ did not cause any noteworthy changes in the emission profile. However, the reaction of the probe with Hg$^{2+}$ resulted in a remarkable ‘turn-on’ fluorescence change, from no fluorescence to strong reddish-brown fluorescence, which was directly observed by the naked eye under a UV lamp (365 nm). The fluorescence spectrum of the probe in the presence of Hg$^{2+}$ (100 μM) showed a remarkable, more intense emission peak at 585 nm (Figure 6). This observed and distinct change in fluorescence colour of the reaction system in the absence and presence of Hg$^{2+}$ is highly convenient for the rapid detection of Hg$^{2+}$ (Figure 2). These all results reveal that the VRF probe shows high selectivity towards Hg$^{2+}$.
Figure 6. Fluorescence response of VRF probe (10 μM) in the presence of Hg\textsuperscript{2+} and other metals ions (100 μM) in CH\textsubscript{3}CN/HEPES buffer solution (1:1, v/v, 0.1 M, pH = 7.4); excitation wavelength = 560 nm, emission wavelength = 585 nm.

8. Conclusions

In conclusion, we proposed a new chromogenic and fluorescent probe based on the protection and deprotection of fluorophore for the recognition of Hg (II), which is currently a widely spread contaminant in ecosystems. The present probe is highly sensitive and selective enough for the determination of mercury. The probe selectively displayed drastic changes in absorption and emission intensities for Hg (II). Moreover, this ‘OFF–ON’ fluorescent probe showed a noteworthy fluorescence increase. The VRF probe displayed a substantial colour change from colourless to pink, as well as noticeable fluorogenic signalling performance entirely towards Hg\textsuperscript{2+} ions. Selective Hg\textsuperscript{2+}- signalling by the VRF probe was unaltered by the presence of other metal ions. We strongly believe that this approach for the detection of notorious Hg (II) will definitely raise interest among the scientific community; thus, many probes based on this strategy may appear in the near future. We expect this probe to be further useful in the identification of Hg (II) at a cellular level.

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