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

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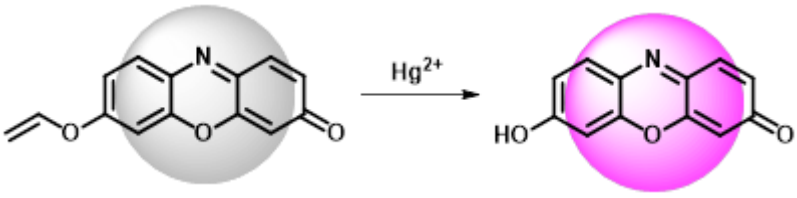
Abstract: Environmental pollution crisis, particularly mercury ions (Hg^{2+}) contamination, seriously threatens the health of all living organisms. Many studies have shown that even extremely low concentrations of Hg^{2+} can rigorously damage living organisms. Therefore, it is very much needed for real-time detection of Hg^{2+}. To tackle mercury contamination and for its detection, we herewith tried 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; Hg^{2+} ions; vinyl resorufin

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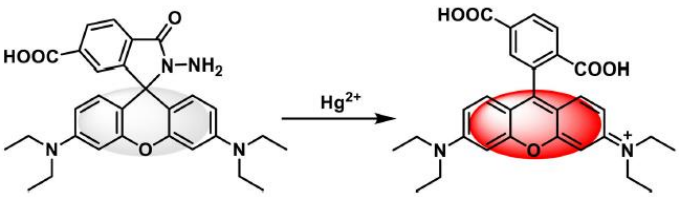
1. Introduction

Mercury is an extremely toxic and biological non-essential element that subsists naturally in the environment. Environmental pollution problems, particularly mercury ions (Hg^{2+}) pollution, seriously threatens 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 including plants, animals, even humans [4]. Many acute poisonings have occurred. For example, the famous Minamata Disease in Japan due to the pollution of mercury in the Agano Tributary and a striking epidemic in Iraq because the polluted seeds were used for bread [5]. The poisonousness of mercury depends on the form and the severity of the exposure. The forms include metallic mercury, inorganic mercury, and organic mercury. Among them, organic mercury is much more toxic than inorganic mercury due to strong fat solubility, they can easily enter 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, as the most famous one amongst 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 Hg^{2+} can severely damage organisms. Therefore, it is essential for real-time detection of Hg^{2+}. Fluorescent probe technology with great advantages has become the preferred technique for environmental detection and in vivo analysis of Hg^{2+}. In recent years, many contributions have been made to design and synthesize novel fluorescent probes for the detection of environmental pollutant Hg^{2+} analysis.

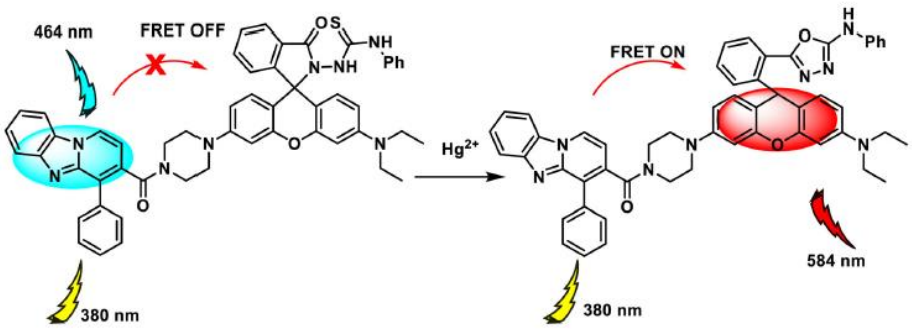
2. Previous Work

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



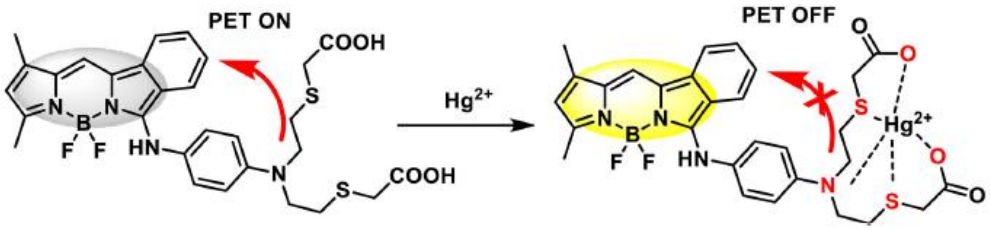
Li et al. [11] designed a simple Rhodamine derivative probe bearing a hydrophilic carboxylic acid group. The fluorescent probe selectively responded to Hg^{2+} in 100% aqueous solution with 42-fold fluorescence intensity enhancement within the pH range from 5.0 to 8.0. The fluorescent intensity change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

2.2. Fluorescent Probes for Hg^{2+} Analysis Based on Ring Opening Followed by Cyclization

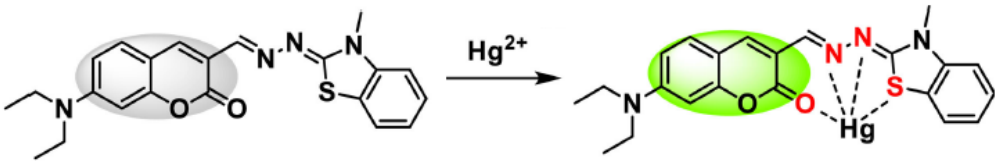


Ge et al. [12] designed a novel pyrido[1,2-a] benzimidazole- rhodamine-based ratiometric fluorescent probe for Hg^{2+}. The probe showed high sensitivity with the detection limit of 18.8 nM, and also exhibited satisfying selectivity with the maximum emission shifting from 464 nm to 584 nm. Remarkably,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

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

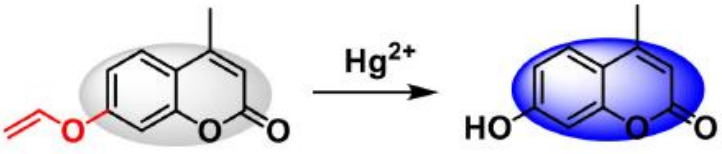


Zhou et al. [13] developed a BODIPY-based sensitive fluorescent probe which utilized the carboxyl-thiol metal bonding receptor to recognize the Hg^{2+} cations in neutral aqueous solution via PET mechanism. There was an about 630-fold fluorescence enhancement on the reaction of the probe with Hg^{2+}. Fluorescent probes showed selective response toward 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 in ppb level. Noticeably, the response time toward Hg^{2+} was below 30 s, which made the detection more convenient and avoided the time effects on probe performance and sample properties.



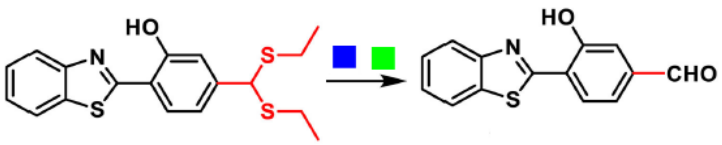
Jiao et al. [14] developed and synthesized a novel selective and sensitive fluorescent chemosensor, which was based on coumarin Schiff's base. They reported that the X-ray diffraction single-crystal structure analysis of the probe, which showed that the probe crystallizes in a monoclinic system and two aromatic groups of the compound were almost in the same plane, providing the explanation for the detection mechanism. The mercury ions form bonds with heteroatoms of the probe due to which 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 Mechanism



Wu et al. [15] designed and synthesized probe comprising 7-hydroxy-4-methylcoumarin as a fluorophore and a vinyl ether group as 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 25 was calculated as 0.12 μM and the response time was less than 10 min. Further, 7-hydroxy-4-methylcoumarin not only led to fluorescence rising but also was an important reaction site, which was the basic advantages of umbelliferone.

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 3-site which would be removed by Hg^{2+} to afford ratiometric fluorescent signal. The ratiometric signal indicated the change of the emission peak from 465 nm to 545 nm gradually when detecting Hg^{2+} without being affected by the microenvironment. This kind of ratiometric tools were more appreciative than intensity based fluorescent sensors. The emission intensity presented about 12-fold enhancement between 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 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 synthesized a new **VRF** probe comprising resorufin as a fluorophore and a vinyl ether group as 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 hypothesized a new approach involving masking and unmasking of 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 involving 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 delivers active fluorophore.

4. Materials and Instrumentations

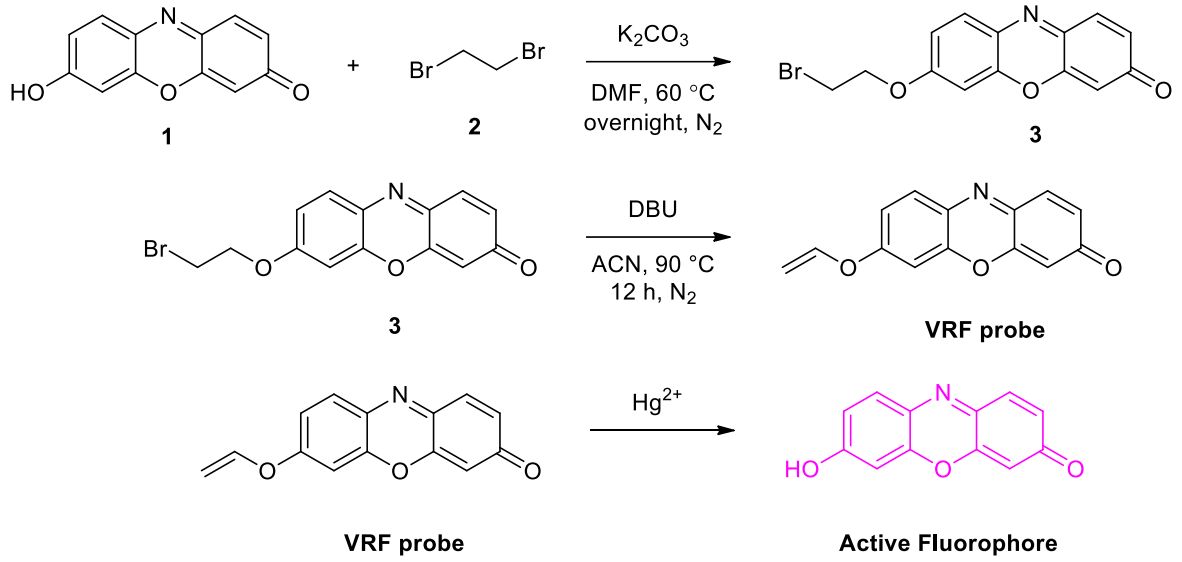
All chemicals were either borrowed or obtained from commercial suppliers and used as received without further purification. For performing all reactions, we have made use of oven-dried screw-cap vials with magnetic stirrers and nitrogen to maintain an inert atmosphere. Solvents, which were dried, as well 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. Further to confirm, the spots were exposed to $KMnO_4$ visualized after charring on a hot plate.

^1H 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 $CDCl_3$, TMS as internal standard). Data for ^1H NMR are reported as follows: chemical shift (ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet), integration, coupling constant (Hz). ESI mass spectra were carried out on an HPLC-MS spectrometer (Agilent 6100). Measurement of Fluorescence spectra were performed with a Perkin–Elmer 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 base catalysed dehydrohalogenation (Step-2) reaction offers desired probe which on mercury promoted hydrolysis selectively converts non-fluorescent molecule to fluorescent molecule.

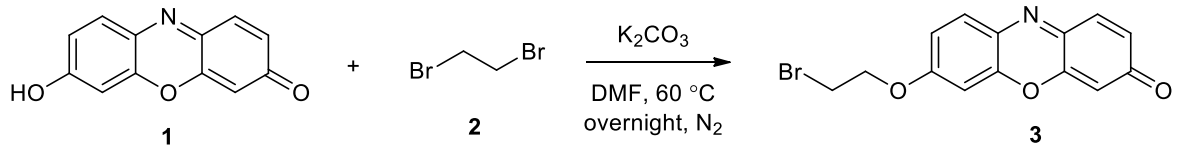
The overall scheme (Scheme 1) for the synthesis of **VRF** probe is as shown below.



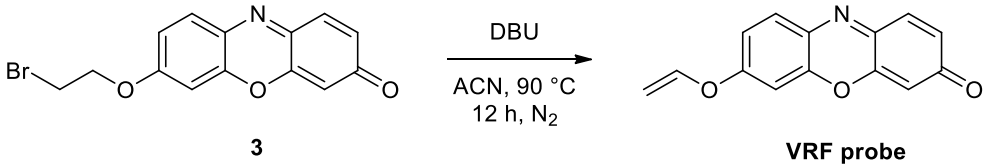
Scheme 1. The overall scheme for the synthesis of **VRF** probe and its mercury promoted cleavage to yield active fluorophore.

General Procedure for the Synthesis of VRF Probe

The VRF-Probe is prepared in two steps as following.



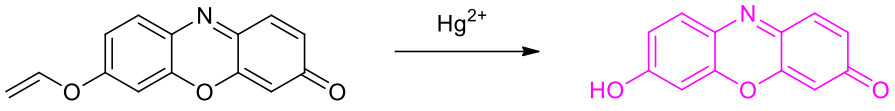
Step 1—Synthesis of 7-(2-bromoethoxy)-3Hphenoxazin- 3-one 3: To a solution of resorufin **1** (1 mmol) and K_2CO_3 (2 mmol) in DMF was added 1,2-dibromoethane **2** (1 mmol) in one portion under an inert atmosphere of N_2. The reaction mixture was stirred at 60 °C overnight. After evaporation of the 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 give the desired product as solid 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-(vinyloxy)-3h-phenoxazin-3- one (VRF probe): To a stirred solution of 7-(2-bromoethoxy)-3H-phenoxazin-3-one **3** (1 mmol) in CH_3CN (10 mL) was added DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) (2 mmol). The mixture was stirred at 90 °C for 12 h under N_2. 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_2SO_4 and evaporated under vacuum. The residue was purified by silica gel column chromatography (eluent: 20% ethyl acetate/hexanes) to give desired **VRF** probe as pale solid in 40% yield. ^{1}H NMR (500 MHz, $DMSOd_6$): δ 7.77 (d, J = 8.8 Hz, 1H), 7.46 (d, J = 9.9 Hz, 1H), 7.05 (dd, J = 8.8, 2.6 Hz, 1H), 6.95 (d, J = 2.6 Hz, 1H), 6.88 (dd, J = 9.8, 2.0 Hz, 1H), 6.71 (dd, J = 13.6, 5.9 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 5.03 (dd, J = 13.6, 2.0 Hz, 1H), 4.74 (dd, J = 6.0, 2.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 186.24, 153.57, 152.40, 149.26, 144.37, 135.24, 134.80, 131.31, 131.23, 130.65, 120.14, 118.54, 109.09, 107.33; HRMS ($C_{14}H_9NO_3$, $[M + Na]^+$): calcd 262.04, found 262.13.

6. Sensing Study

Mercury promoted cleavage of vinylic ether to yield active fluorophore.



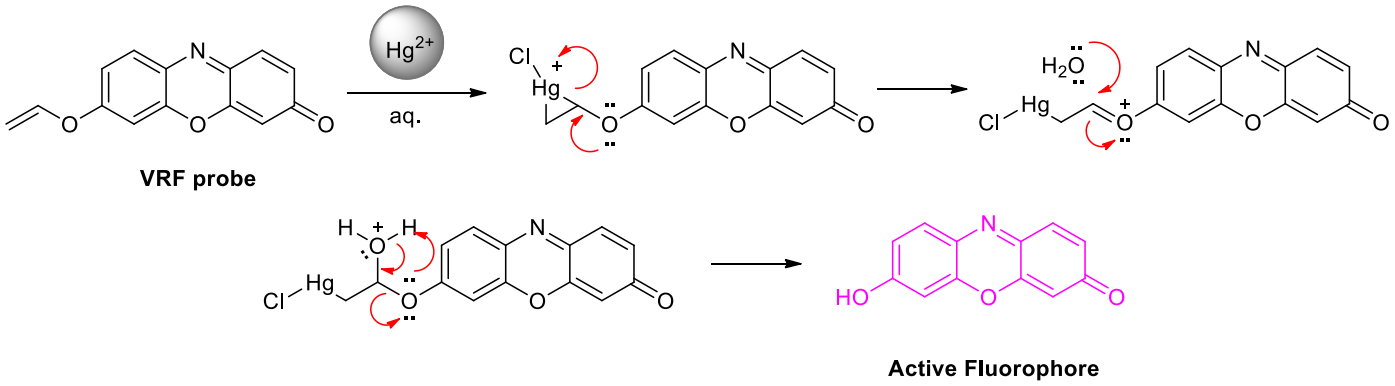
VRF probe

Resorufin anchored with vinyl ether

Active Fluorophore

Unanchored Fluorophore

Proposed Mechanistic route of vinyl ether deprotection using Hg^{2+} (Scheme 2):



Scheme 2. The possible reaction mechanism of **VRF** probe towards mercury (II).

7. Results and Discussion

Synthesis: The synthesis of **VRF** probe is quite straight forward, it was synthesized in two steps. The first step involved the reaction of the hydroxy group of resorufin **1** and 1,2-dibromoethane **2** in presence of base to give **3**. The second step involves the treatment of **3** with DBU, which undergoes 1-2 elimination to produce desired **VRF** probe in moderate yield (Scheme 1). The possible reaction mechanism of **VRF** toward mercury (II) is shown in Scheme 2. The obtained **VRF** probe was then characterized by NMR and mass spectroscopic techniques which showed its characteristic vinylic protons at 5.03 δ, 4.74 δ and 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 Hg^{2+} were studied. The absorption spectrum of **VRF** probe in the absence of Hg^{2+} exhibited a very weak broad absorption band at 480 nm (Figure 1). After treatment with Hg^{2+}, the absorption band maxima showed a bathochromic shift from 480 to 575 nm as well as a colour change from colourless to pink was observed. (Figure 1). The fluorescence spectrum of **VRF** probe in the absence of Hg^{2+} showed very weak emission at 582 nm, which is attributed to the strong quenching effect of the vinylic ether unit. However, the reaction of **VFR** 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 naked eyes under UV lamp (365 nm). The fluorescence spectrum of probe in presence of Hg^{2+} (100 μM) showed a remarkable more intense emission peak at 585 nm (Figure 2). The fluorescence enhancement of probe was attributed to the mercury-triggered cleavage reaction causing the release of free resorufin **1**. The changes of absorption and fluorescence spectra were observed due to the deprotection of the vinyl ether moiety and the formation of resorufin **1**.

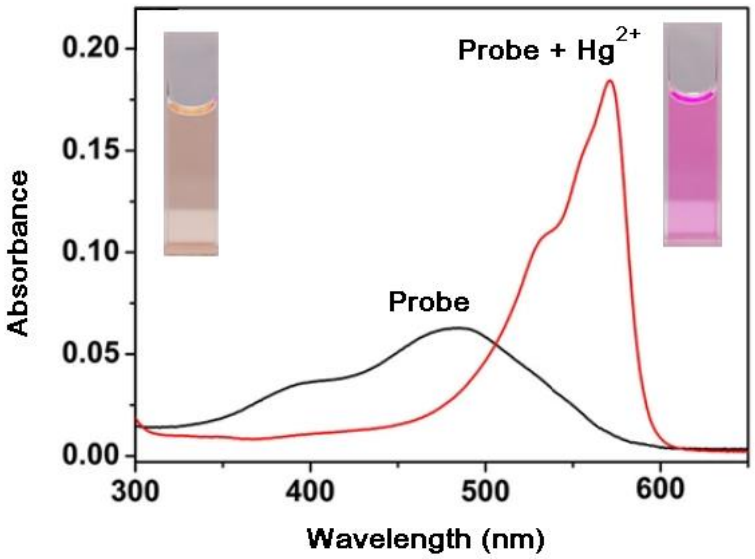


Figure 1. The absorption spectra of **VRF** (10 μM) in the absence and presence of Hg^{2+} (100 μM) in CH_3CN/HEPES buffer solution (1:1, *v*/*v*, 0.1 M, pH 7.4). Inset: the colour change of **VRF** in the absence and presence of Hg^{2+} (colourless to pink).

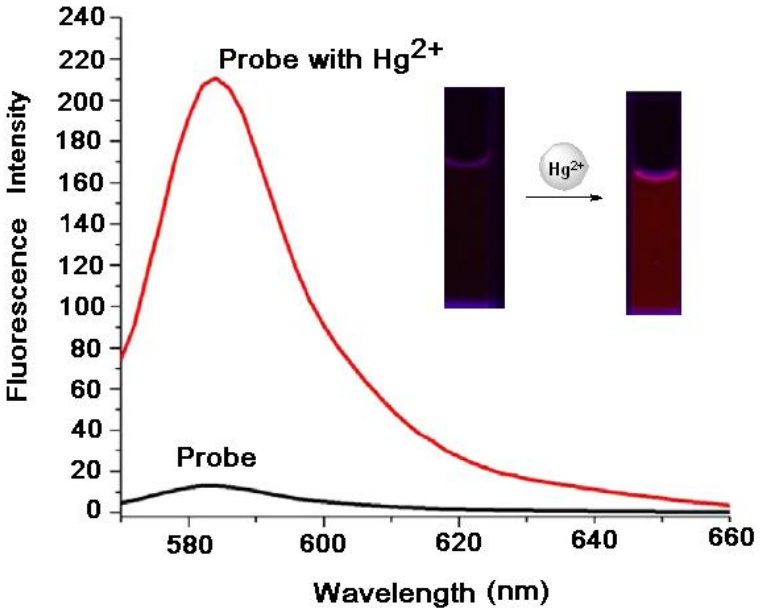


Figure 2. Fluorescence spectra of **VRF** probe (10 μM) in the absence and presence of Hg^{2+} (100 μM) in CH_3CN/HEPES buffer solution (1:1, *v*/*v*, 0.1 M, pH 7.4); excitation wavelength = 560 nm, the spectrum was acquired 60 min after $HgCl_2$ addition at 25 °C. Inset: fluorescence colour change observed under UV light at 365 nm.

The time dependent fluorescence response of **VRF** probe (10 μM) with Hg^{2+} (100 μM) in CH_3CN/HEPES buffer (1:1, 0.1 M, pH = 7.4) was measured at 25 °C (Figure 3). The fluorescence spectrum of **VRF** probe in the absence of Hg^{2+} showed a very weak emission at 582 nm (Figure 2). Then the fluorescence intensities of **VRF** probe in the presence of Hg^{2+} (100 μM) from 2–60 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 the free Resorufin **1**.

The concentration dependent fluorescence response for **VRF** probe (10 μM) upon addition of Hg^{2+} (10–100 μM) in CH_3CN:HEPES buffer (1:1, 0.1 M, pH = 7.4) was measured at 25 °C (Figure 4). The fluorescence spectrum of **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 **VRF** probe showed an excellent linearity with increase in concentration of Hg^{2+} from 10–100 μM. The fluorescence intensities were measured after every 60 min for each addition of Hg^{2+}. The highest fluorescence intensity was recorded at Hg^{2+} concentration of 100 μM.

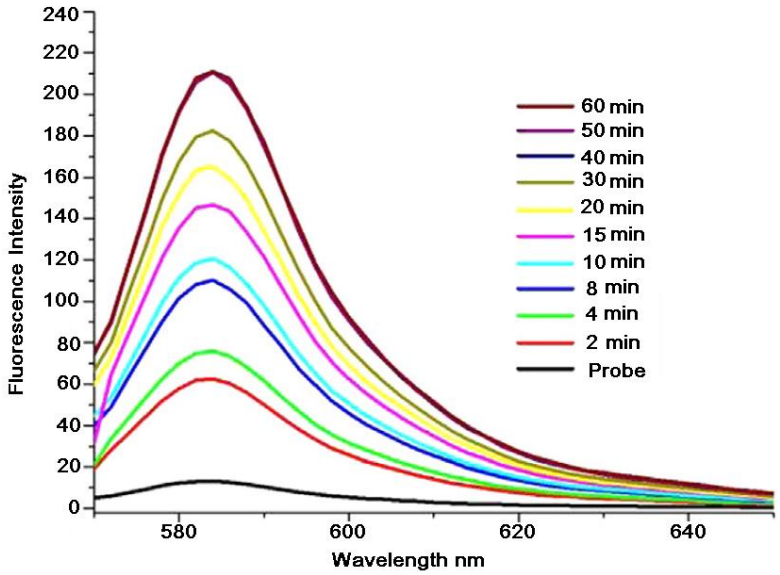


Figure 3. Time dependent fluorescence response of **VRF** probe (10 μM) with Hg^{2+} (100 μM) in CH_3CN/HEPES buffer (1:1, 0.1 M, pH = 7.4) at 25 °C; excitation wavelength = 560 nm, emission wavelength = 585 nm. The reaction was completed within one hour.

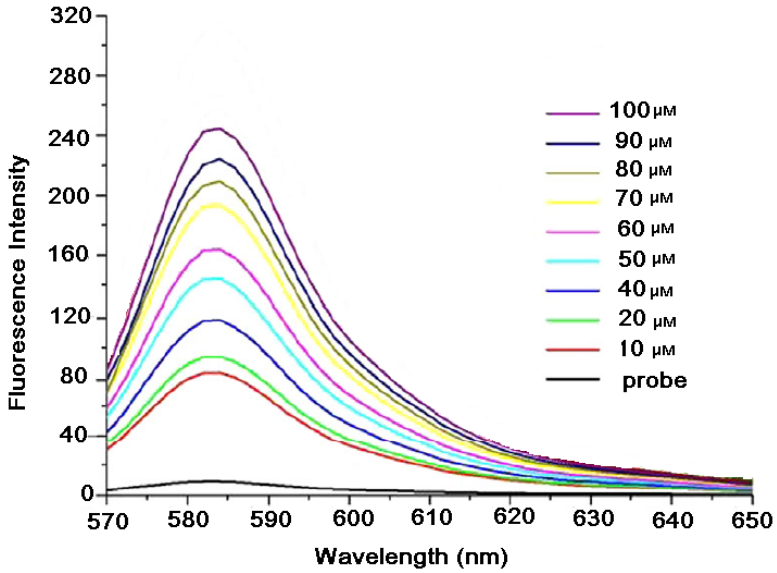


Figure 4. Concentration dependent fluorescence response for **VRF** probe (10 μM) upon addition of Hg^{2+} (10–100 μM) in CH_3CN:HEPES buffer (1:1, 0.1 M, pH = 7.4).at 25 °C; excitation wavelength = 560 nm, emission wavelength = 585 nm.

7.2. Response of VRF Probe to Mercury and Other Metal Ions

Selectivity is a very important parameter for evaluating the performance of probe. To investigate the selectivity of **VRF** probe the ions like, 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 **VRF** probe (10 μM) in the presence of Hg^{2+} and other metal ions (100 μM) in CH_3CN/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, here also the probe exhibited a very weak broad absorption band at 480 nm. The treatment of probe with metal ions other than Hg^{2+} did not cause in any noteworthy changes in the absorption profile. However, the absorption spectrum of 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 was observed (Figure 5).

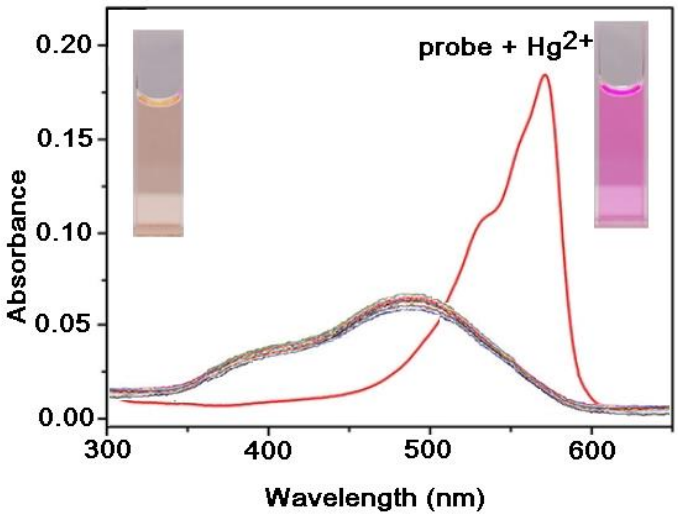


Figure 5. Absorption spectra of **VRF** probe (10 μM) in the presence of Hg^{2+} and other metal ions (100 μM) in CH_3CN/HEPES buffer solution (1:1, *v*/*v*, 0.1 M, pH = 7.4). Inset: the colour change of **VRF** in the absence and presence of Hg^{2+} (colourless to pink).

The emission spectra of **VRF** probe (10 μM) in the presence of Hg^{2+} and other metal ions (100 μM) in CH_3CN/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, here also the probe exhibited a very weak emission at 582 nm. This low background signal is extremely desirable for sensitive detection of metal ions. The treatment of **VRF** probe with metal ions other than Hg^{2+} did not cause in any noteworthy changes in the emission profile. However, the reaction of 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 naked eyes under UV lamp (365 nm). The fluorescence spectrum of probe in presence of Hg^{2+} (100 μM) showed a remarkable more intense emission peak at 585 nm (Figure 6). This observed distinct fluorescence colour change 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 **VRF** probe shows a high selectivity toward Hg^{2+}.

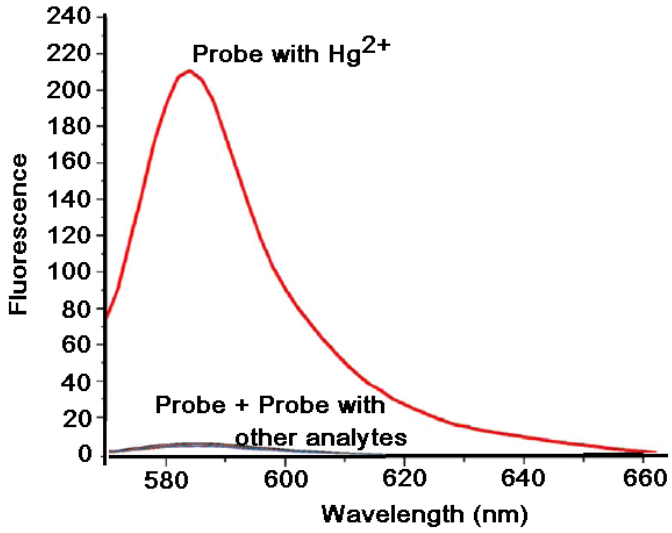


Figure 6. Fluorescence response of **VRF** probe (10 μM) in the presence of Hg^{2+} and other metals ions (100 μM) in CH_3CN/HEPES buffer solution (1:1, *v*/*v*, 0.1 M, pH = 7.4); excitation wavelength = 560 nm, emission wavelength = 585 nm.

8. Conclusions

In conclusion, we have proposed a new chromogenic and fluorescent probe based on protection and deprotection of fluorophore for the recognition of Hg(II), which now a days a widely spread contaminant in ecosystem. The present probe is highly sensitive and selective enough in determination of mercury. The probe displayed drastic changes in UV-vis absorption and fluorescence emission intensities selectively for Hg (II). Moreover, this "OFF-ON" fluorescent probe shows a noteworthy fluorescence increase. **VRF** probe displayed a substantial colour change from colourless to pink as well as noticeable fluorogenic signalling performance entirely toward Hg^{2+} ions. Selective Hg^{2+}- signalling by **VRF** probe was unaltered by the presence of other metal ions. We strongly believe that this approach of detection of notorious Hg (II) will definitely catch the attention scientific community and henceforth many probes based on this strategy may appear in near future. We expect that this probe shall be further useful in identification of Hg(II) at cellular level.

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References

1. Nendza, M.; Herbst, T.; Kussatz, C.; Gies, A. Potential for Secondary Poisoning and Biomagnification in Marine Organisms**.** *Chemosphere* **1997**, *35*, 1875–1885, doi:10.1016/ S0045-6535(97)00239-7.
2. Renzoni, A.; Zino, F.; Franchi, E. Mercury Levels along the Food Chain**.** *Environ. Pollut.* **1998**, *77*, 68–72**,** doi:10.1006/ enrs.1998.3832.
3. Mason, F.M.M.; Reinfelder, R.P.; Morel, J.R. Bioaccumulation of Mercury and Methylmercury. *Water Air Soil Pollut.* **1995**, *80*, 915–300, doi:10.1007/BF01189744.
4. Boening, D.W. Ecological Effects, Transport, and Fate of Mercury: A General Review. *Chemosphere* **2000**, *40*, 1335–1351, doi:10.1016/S0045-6535(99)00283-0.
5. Kim, K.H.; Kabir, E.; Jahan, S.A. A Review on the Distribution of Hg in the Environment and Its Human Health Impacts. *J. Hazard. Mater.* **2016**, *306*, 376–385, doi:10.1016/j. jhazmat.2015.11.031.
6. Kraepiel, A.M.L.; Keller, K.; Chin, H.B.; Malcolm, E.G.; Morel, F.M.M. Sources and Variations of Mercury in Tuna. *Environ. Sci. Technol.* **2003**, *37*, 5551–5558, doi:10.1021/ es0340679.
7. Burger, J.; Gochfeld, M. Mercury in Canned Tuna: White versus Light and Temporal Variation. *Environ. Res.* **2004**, *96*, 239–249, doi:10.1016/j.envres.2003.12.001.
8. Kuwabara, J.S.; Arai, Y.; Topping, B.R.; Pickering, I.J.; George, G.N. Mercury Speciation in Piscivorous Fish from Mining-Impacted Reservoirs. *Environ. Sci. Technol.* **2007**, *41*, 2745–2749, doi:10.1021/es0628856.
9. Kiefer, A.M.; Seney, C.S.; Boyd, E.A.; Smith, C.; Shivdat, D.S.; Matthews, E.; Hull, M.W.; Bridges, C.C.; Castleberry, A. Chemical Analysis of Hg0-Containing Hindu Religious Objects. *PLoS ONE* **2019**, *14*, e0226855, doi:10.1371/journal.pone. 0226855.
10. Guo, Q.; Zhang, Y.; Lin, Z.H.; Cao, Q.Y.; Chen, Y. Fluorescent Norbornene for Sequential Detection of Mercury and Biothiols. *Dye Pigment* **2020**, *172*, 107872, doi:10.1016/j. dyepig.2019.107872.
11. Li, D.; Li, C.Y.; Li, Y.F.; Li, Z.; Xu, F. Rhodamine-Based Chemodosimeter for Fluorescent Determination of $Hg^{(2+)}$ in 100% Aqueous Solution and in Living Cells. *Anal. Chim. Acta.* **2016**, *934*, 218–225, doi:10.1016/j.aca.2016.05.050.
12. Ge, Y.; Liu, A.; Ji, R.; Shen, S.; Cao, X. Detection of Hg2þ by a FRET Ratiometric Fluorescent Probe Based on a Novel Pyrido[1,2-a]Benzimidazole-Rhodamine System. *Sens. Actuator B Chem.* **2017**, *251*, 410–415, doi:10.1016/j.snb.2017.05.097.
13. Zhou, B.; Qin, S.; Chen, B.; Han, Y. A New BODIPY-Based Fluorescent "Turn-on" Probe for Highly Selective and Rapid Detection of Mercury Ions. *Tetrahedron Lett.* **2018**, *59*, 4359–4363, doi:10.1016/j.tetlet.2018.10.068.
14. Jiao, Y.; Zhou, L.; He, H.; Yin, J.; Duan, C. A New Fluorescent Chemosensor for Recognition of Hg^{2+} Ions Based on a Coumarin Derivative. *Talanta.* **2017**, *162*, 403–407, doi:10. 1016/j.talanta.2016.10.004.

15. Wu, C.; Wang, J.; Shen, J.; Bi, C.; Zhou, H. Coumarin-Based Hg2þ Fluorescent Probe: Synthesis and Turn-on Fluorescence Detection in Neat Aqueous Solution. *Sens. Actuator B Chem.* **2017**, *243*, 678–683, doi:10.1016/j.snb.2016.12.046.
16. Zhou, Y.; He, X.; Chen, H.; Wang, Y.; Xiao, S.; Zhang, N.; Li, D.; Zheng, K. An ESIPT/ICT Modulation Based Ratiometric Fluorescent Probe for Sensitive and Selective Sensing Hg^{2+}. *Sens. Actuator B Chem.* **2017**, *247*, 626–631, doi:10.1016/j. snb.2017.03.085.