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Engineering a bromophenol derivative for rapid detection of Hg^{2+}/CH_3Hg^+ in both environmental and biological samples through a unique activation process†

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Reaction-based sensory systems are always preferred for detecting Hg^{2+} and its organic form, CH_3Hg^+. A simple bromophenol derivative (**BDT**) has been developed as a cheap, small and ultrasensitive sensor for both Hg^{2+} and CH_3Hg^+ ions with rapid detection ability. The transformation of the 1,3-dithiolane segment to the formyl group has been utilized here as a key feature for the turn "off–on" fluorescence response due to the activation of the ESIPT process during the detection of the analytes. Most of the 1,3-dithiolane systems required a long response time for CH_3Hg^+, but **BDT** has been demonstrated as an excellent system that required a low response time to detect the organic form of mercury. Having a limit of detection (LOD) value of 3.8 nM and 0.8 μM towards Hg^{2+} and CH_3Hg^+ respectively in an aqueous medium, **BDT** served as an excellent detection probe with environmental detection capability. It has been supported by ICP-MS analysis of commercially available vermilion. **BDT** has been utilized to detect Hg^{2+} in a biological system with excellent efficiency along with ecological and real sample analysis. Furthermore, the desulfurization process triggered by Hg^{2+} has been proved using ^1H NMR analysis and spectroscopic fluorescence techniques. These observations encouraged us to claim BDT as an excellent cheap and easily synthesizable efficient tool for detecting inorganic and organic mercury in controlled, environmental, and biological systems.

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Sustainability spotlight

The bioaccumulation of Hg(II) and methylmercury (CH_3Hg^+), due to their solid binding tendency to thiols of different enzymes and proteins, caused several physiological issues such as kidney damage, central nervous system damage, motion disorder, and even death. Therefore, to avoid the hazardous effect of $Hg^{2+}$and CH_3Hg^+, an upper limit of Hg^{2+} has been set as 10 nM by the U.S. Environmental Protection Agency (EPA). By this virtue, the development of selective and sensitive detection strategies for mercury in environmental and living systems is an important issue to be addressed by researchers. However, traditional methods need expensive instrumentation and complicated experimental procedures despite high sensitivity and precision. In this respect, reaction-based probes are preferable due to their high selectivity and sensitivity. To the best of our knowledge, herein, we report the smallest 1,3-dithiolane-based probe BDT, where a diformyl system (BDA) has been modified using a 1,2-ethanethiol segment. BDT showed excellent selectivity and sensitivity towards Hg^{2+} and methylmercury ions in an aqueous environment and biological system comprehensively.

Introduction

The toxic effect of mercury is well known for Minamata disease and poisoning in Iraq.[1–3] Mercury contamination is mainly originated from the release of mercury from different chemical industries.[4,5] Uncontrolled release has increased the amount of mercury in surface water and heavily affects humankind throughout the world.[6] The bioaccumulation of Hg(II) and methylmercury (CH_3Hg^+) due to their solid binding tendency to thiols of different enzymes and proteins caused several physiological issues such as kidney damage, central nervous system damage, motion disorder, and even death.[7,8] On the other hand, organic species of mercury are known to be highly toxic.[9,10] Among different organic mercury species, CH_3Hg^+ is a commonly known toxicant that is significantly more harmful than the inorganic Hg^{2+} ions. It is frequently found in marine seafood. Compared to Hg^{2+}, CH_3Hg^+ can easily penetrate biological membranes and the blood–brain barrier and thus affect

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the nervous system.[11] To avoid the hazardous effect of Hg^{2+} and CH_3Hg^+, an upper limit of Hg^{2+} has been set as 10 nM by the U.S. Environmental Protection Agency (EPA).[12] By this virtue, the development of selective and sensitive detection strategies for mercury in environmental and living systems is an important issue to be addressed by researchers. Several traditional methods such as atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS), chromatographic techniques, and voltammetry studies are available for the detection of mercury with high sensitivity and accuracy.[10–20] Despite high sensitivity and precision, traditional methods need expensive instrumentation and complicated experimental procedures.[21–24] However, the spectroscopic fluorescence technique has emerged as a point of interest in recent times due to its comprehensive sensitivity, response, and easy sample preparation process.[25–29] In the last few years, most fluorescent probes for Hg^{2+} were designed using heteroatom-containing ligand systems. However, these kinds of probes suffer from low selectivity due to reversible complexation and interference from different ions.[30–32] In this respect, reaction-based probes are preferable due to their high selectivity and sensitivity, as an Hg-triggered reaction with a probe leads to a new fluorogenic or chromogenic substance formation with unique spectral properties.[33] The deprotection of thioacetals to aldehydes or ketones, promoted by Hg^{2+} is one of the most utilized reactions to design a chemodosimeter with high selectivity.[34–36] The formation of thioacetals can be achieved simply by reacting thiols with aldehyde or ketone segments of any molecule and can be deprotected explicitly by Hg^{2+} ions.[37] Besides, very few reaction-based probes have been reported for methylmercury, to date and most of the probes require a high concentration of CH_3Hg^+ and a considerable reaction time due to the minor thiophilic nature of methylmercury.[38–41]

Generally, three well-known fluorescence mechanisms, such as PET (Photoinduced Electron Transfer), FRET (Förster Resonance Energy Transfer), and ICT (Internal Charge Transfer), are explored vastly for the development of sensor molecules. However, excited-state intramolecular proton transfer (ESIPT) based probes have received great attention in terms of applications due to their unique photophysical properties such as large stokes shift, good photostability, and high quantum yield.[42,43]

By this virtue, to the best of our knowledge, herein, we report the smallest 1,3-dithiolane based probe **BDT**, where a diformyl system (**BDA**) has been modified using a 1,2-ethanethiol segment. **BDT** has been designed so that the Hg^{2+} triggered deprotection of the 1,3-dithiolane moiety to an aldehyde can turn on the ESIPT process to produce a unique emission signal. 2-(2′-Hydroxyphenyl) benzothiazole (HBT) is a well-known ESIPT active fluorophore used as the basic unit for developing different sensors throughout the years, and –OH and benzothiazole moieties are involved in the proton transfer process.[44,45] But in the case of **BDT**, Hg^{2+} and CH_3Hg^+ promoted thioacetal deprotection and produced a basic keto–enol tautomerizing unit that activated the ESIPT process by showing intense intensity green fluorescence as output. Moreover, a single benzene ring containing **BDT** showed excellent selectivity and sensitivity towards Hg^{2+} and methylmercury ions in an aqueous environment and biological system comprehensively.

Experimental section

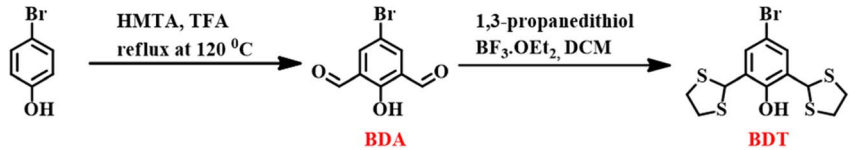
We have synthesized **BDT** in two steps starting from 4-bromophenol (Scheme 1, ESI†). Initially, 4-bromophenol has been converted to a dialdehyde system (**BDA**) using the Duff reaction where hexamethylenetetramine (HMTA) and TFA have been used as reagents. In the second step, **BDA** has been converted to **BDT** using 1,3-ethanedithiol. All these products have been successfully characterized using ^{1}H NMR, ^{13}C NMR, and ESI-MS techniques. The detailed synthetic methods and characterization of each compound have been discussed in the ESI (Fig. S1–S6†).

Results and discussion

Photophysical properties of BDT

The initial photophysical study of **BDT** showed absorbance maxima at 306 nm in a DMSO/PBS (4 : 1, v/v, pH 7.4) medium. However, the addition of Hg^{2+} resulted in the appearance of a new absorbance maximum at 350 nm (Fig. S7, ESI†). In terms of fluorescence emission, **BDT** showed an emission maximum of around 526 nm upon adding an Hg^{2+} ion, which was absent initially (Fig. S8, ESI†). The selectivity of **BDT** was thoroughly investigated using fluorescence spectroscopy for various metal ions such as Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}, Cu^{2+}, Zn^{2+}, Pb^{2+}, Co^{2+}, Fe^{2+}, Cd^{2+}, Sn^{2+}, Fe^{3+}, Cr^{3+} and Hg^{2+} in a DMSO/PBS (4 : 1, v/v, pH 7.4) medium, where the 526 nm emission band was observed only for the Hg^{2+} ion (Fig. 1A). Selectivity studies of **BDT** (Fig. 1B) established the precise nature of our probe towards Hg^{2+} ions, which is the beneficial aspect of reaction-based systems. Due to the activation of the ESIPT phenomenon, **BDT** showed green emissive nature in the presence of Hg^{2+} but remained non-emissive for other analytes, as depicted in Fig. 1C.

After a successful investigation of the selectivity of **BDT**, we have determined the response dynamics towards Hg^{2+} under the same experimental conditions used in the initial photophysical experiments. A rapid response time of 1 minute,



Scheme 1 Synthesis procedure of BDT.

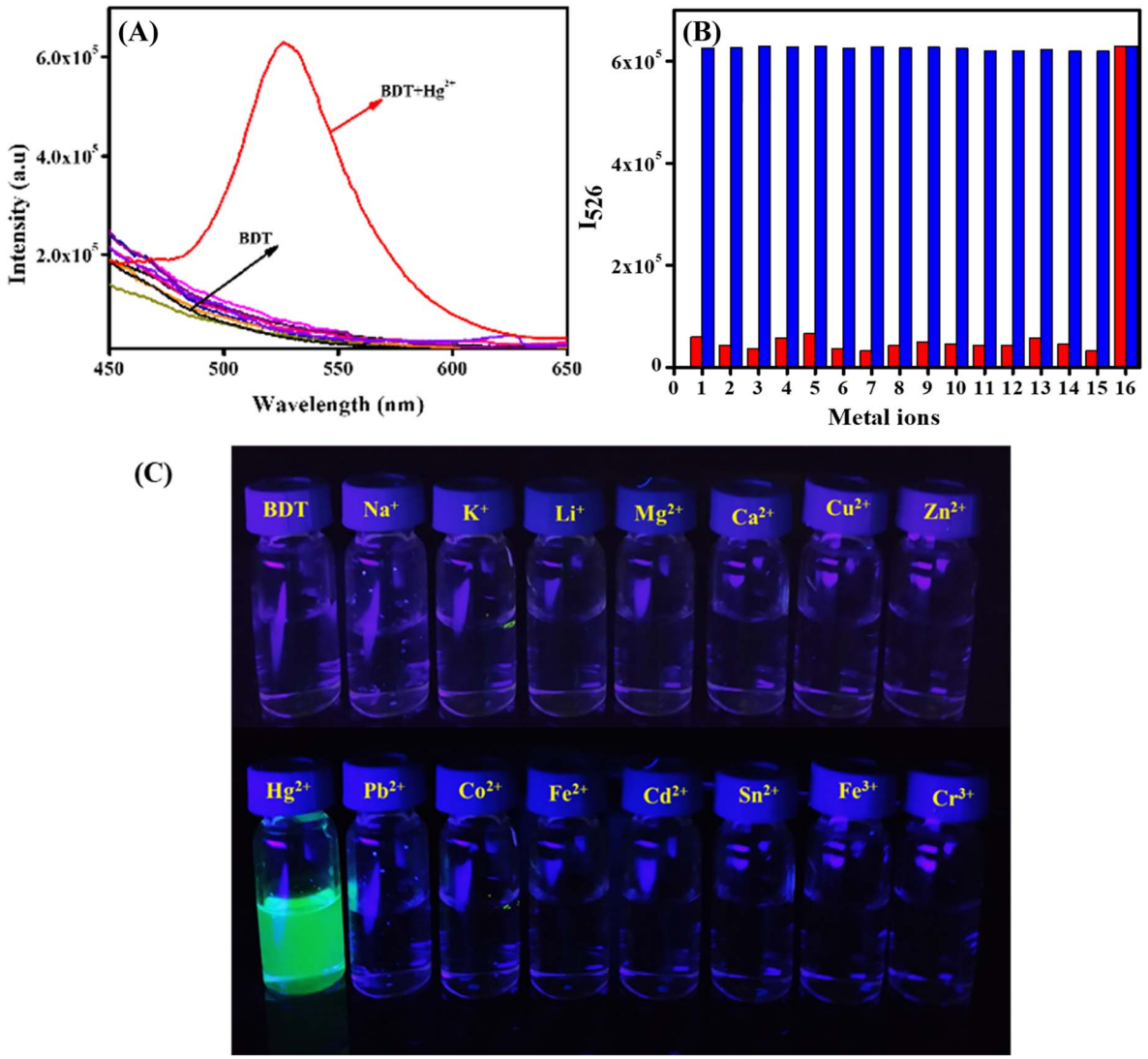


Fig. 1 (A) Fluorescence spectra of BDT (10 μM) and (B) corresponding comparative bar plot of $I_{526\ nm}$ in the presence of different metal ions in a DMSO/PBS buffer (4 : 1, v/v, pH 7.4) medium: (1) blank, (2) Na^+, (3) K^+, (4) Li^+, (5) Mg^{2+}, (6) Ca^{2+}, (7) Cu^{2+}, (8) Zn^{2+}, (9) Pb^{2+}, (10) Co^{2+}, (11) Fe^{2+}, (12) Cd^{2+}, (13) Sn^{2+}, (14) Fe^{3+}, (15) Cr^{3+}, and (16) Hg^{2+}; red columns = BDT + metal ions; blue columns = BDT + metal ions + Hg^{2+}. (C) Pictorial representation of BDT solutions in the presence of different analytes under a hand-held UV lamp (λ_{ex} = 350 nm).

required for a complete reaction between **BDT** and Hg^{2+}, was evident from the time-dependent fluorescence studies (Fig. S9, ESI†). This detection kinetics confirmed the 1 minute incubation time of Hg^{2+} with **BDT** for all future spectroscopic experiments. Reaction dynamics and selectivity studies confirmed the novelty of **BDT** towards detecting Hg^{2+} ions in terms of rapid response and high specificity. The quantum yield of **BDT** in the presence of Hg^{2+} has been evaluated using quinine sulfate as reference (eqn (1), ESI†). The value of quantum yield for **BDT** is calculated to be 0.11.

Sensitivity and detection parameters are essential for a probe to be an excellent candidate for sensing any analyte. Towards this, we have carried out a fluorescence titration study of **BDT** with varying concentrations of Hg^{2+} in a 4 : 1 DMSO/PBS (v/v, pH = 7.4) medium. As expected, the emission intensity of **BDT** at 526 nm increased gradually with the progressive addition of Hg^{2+} ions (0–60 μM) (Fig. 2A), with a change in emission from colourless to green (Fig. 2A, inset). A drastic 33-fold increase in emission intensity at 526 nm till two equivalent Hg^{2+} addition followed by plateau formation (Fig. S10, ESI†) convincingly suggested the gradual desulfurization process. The absorption studies of **BDT** with changing concentrations of Hg^{2+} (0–100 μM) (Fig. S11, ESI†) also exhibited a similar kind of spectroscopic change which again supported our claim of the desulfurization process.

From the titration study, a calibration plot has been constructed in 0–20 μM for **BDT**. The linear plot of intensity at 526 nm with the concentration of Hg^{2+} showed excellent linearity with an adjacent R^2 value of 0.99025. This linear plot has been utilized to calculate the theoretical lowest detection limit (LOD = $3\sigma/K$) of **BDT** with a values of 3.8 nM for Hg^{2+}, which is well comparable with other desulfurization based reported

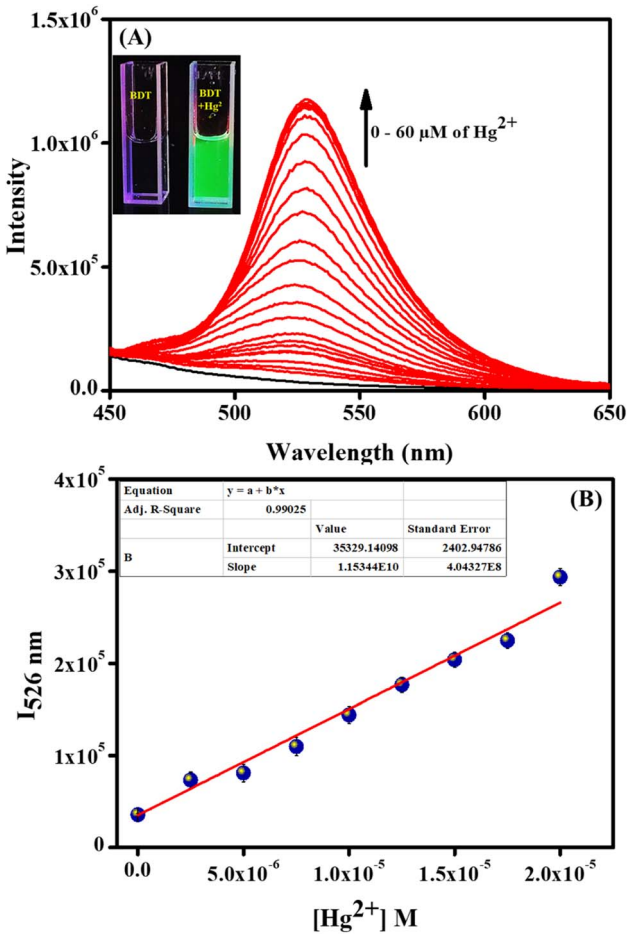


Fig. 2 Fluorescence spectra of **BDT** (20 μM), obtained (A) upon addition of Hg^{2+} (0–60 μM) in DMSO/PBS buffer (4 : 1, v/v, pH 7.4) solution (inset: picture of **BDT** solution before and after addition of Hg^{2+} ions under a hand-held UV lamp of 365 nm) and corresponding calibration plot of (B) $I_{526\ nm}$ *vs* concentration of Hg^{2+} ions (0–20 μM).

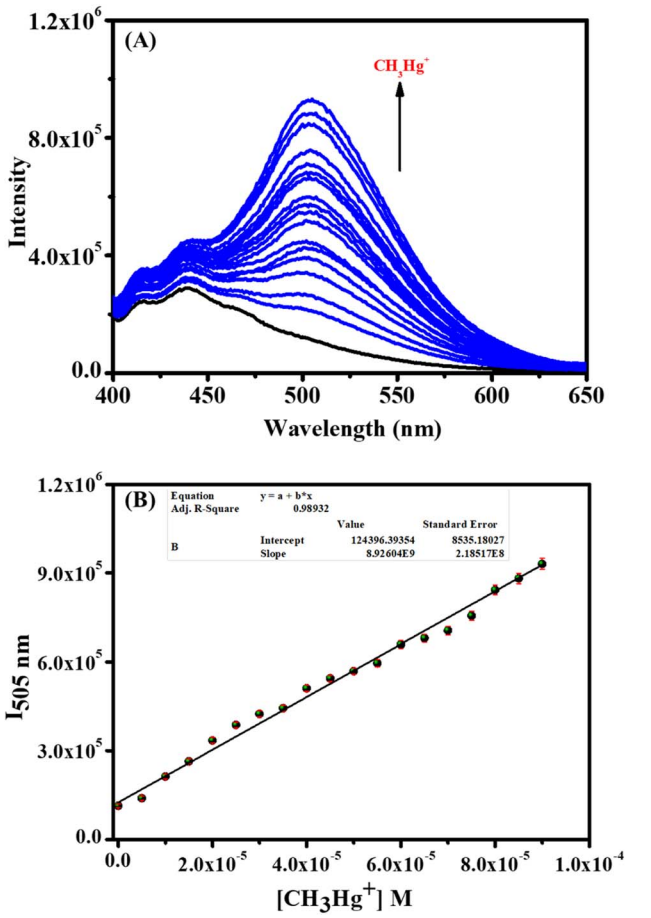


Fig. 3 Fluorescence spectra of **BDT**, 20 μM (A) CH_3Hg^+ (0–90 μM) in DMSO/PBS buffer (4 : 1, v/v, pH 7.4) solution and corresponding plot of (B) $I_{505\ nm}$ *vs* concentration of CH_3Hg^+ ions (0–90 μM) (λ_{ex} = 350 nm).

probes (Table S1, ESI†) and well below the permissible limit of 10 nM.

Detection of CH_3Hg^+ by BDT

Reaction-based probes of Hg^{2+} are also reactive towards CH_3Hg^+ in a similar mechanism. In this context, we carried out the sensing behavior studies of **BDT** towards the CH_3Hg^+ ion under identical experimental conditions. The initial time-dependent studies confirmed the rapid detection ability of **BDT** towards CH_3Hg^+ in DMSO/PBS (4 : 1, v/v, pH = 7.4) (Fig. S12, ESI†). Furthermore, a quick response time of **BDT** defined the excellent factor for detecting CH_3Hg^+ compared to other various reported probes (Table S1, ESI†). Furthermore, a gradual increase in the $I_{505\ nm}$ value was observed for **BDT** upon progressive addition of CH_3Hg^+ ions (Fig. 3A). Titrimetric results have been utilized to construct the calibration curve (Fig. 3B) in the concentration of 0–90 μM for methylmercury. This linear plot with an R^2 value of 0.98932 has been utilized to calculate the LOD value of 0.8 μM for **BDT** towards CH_3Hg^+ in a controlled experimental medium.

The obtained results of selectivity, response time, and LOD values suggested the superiority of the probe in comparison to various reaction-based reported fluorescent probes in recent time (Table S1, ESI†). Furthermore, all these results established the highly selective and sensitive sensing ability of the chemodosimeter **BDT** towards both Hg^{2+} and CH_3Hg^+ in aq. media, which can be utilized to detect toxic analytes with high efficiency.

Study of the sensing mechanism

To further validate the Hg^{2+} triggered desulfurization process of the 1,3-dithiolane moiety, we investigated the sensing mechanism by performing the ^1H NMR titration experiment (Fig. 4A) of **BDT** in the absence and presence of Hg^{2+} ions. Upon adding excess (3 equivalent) Hg^{2+} to **BDT**, the protons corresponding to the 1,3-dithiolane moiety, *i.e.*, Hb and Hc, had disappeared entirely, and a new peak around 10.1 ppm appeared for –CHO groups. As a consequence of this desulfurization reaction, aromatic signals are shifted downfield. The new spectrum is identical to the ^1H NMR spectrum of **BDA** (Fig. S1, ESI†), which firmly established the Hg^{2+} triggered desulfurization reaction of the 1,3-dithiolane moiety to form the formyl group, which activates the ESIPT process *via* keto–enol tautomerization (as

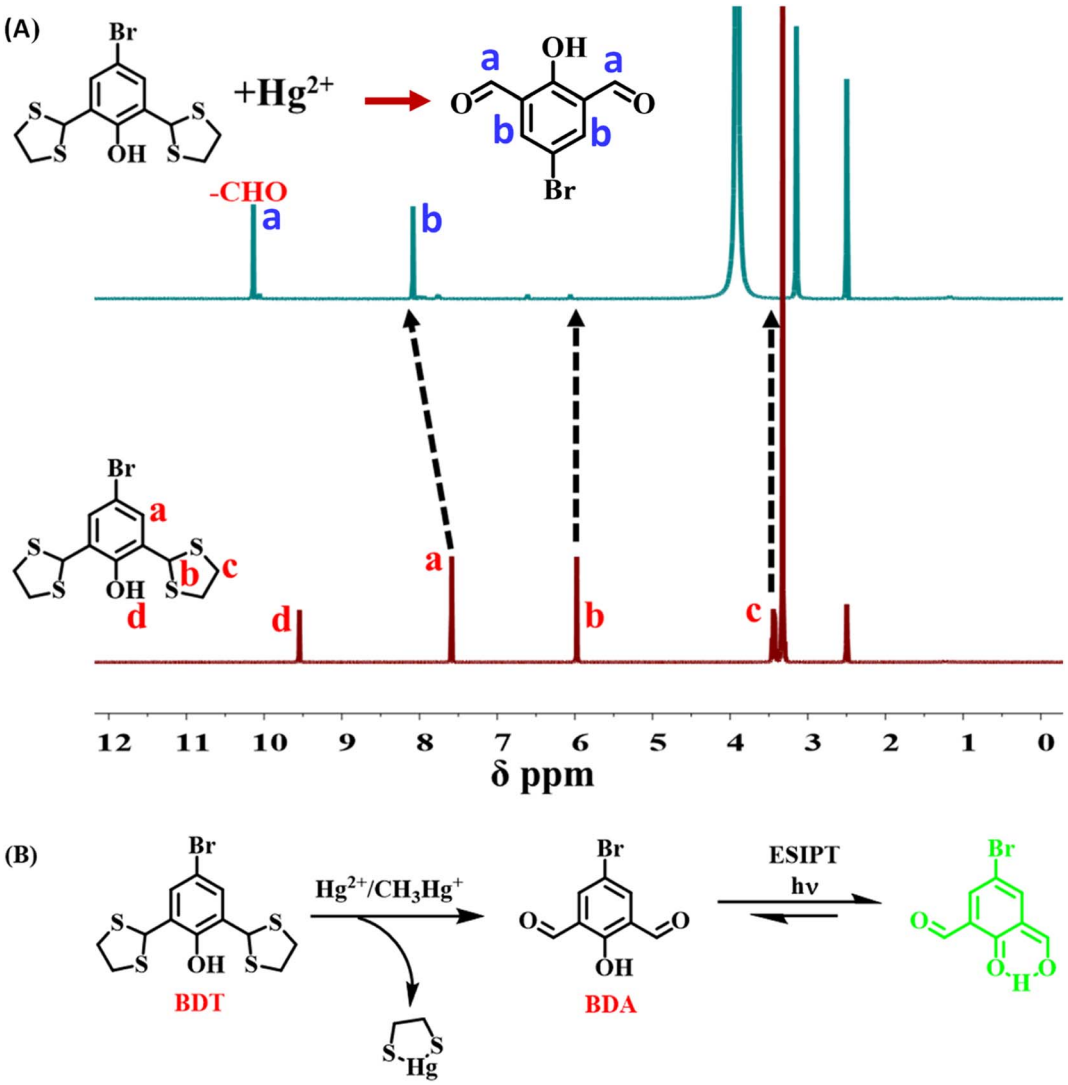


Fig. 4 (A) ^{1}H NMR spectra of **BDT** in the absence and presence of Hg^{2+} in DMSO-d_6. (B) Probable sensing mechanism of **BDT** for detecting Hg^{2+}/CH_3Hg^+.

shown in Fig. 4B). This conversion was further supported by the similar emission spectral nature of **BDA** and **BDT** + Hg^{2+} under identical experimental conditions (Fig. S13, ESI†). Therefore, a similar reaction process is also expected for CH_3Hg^+ towards **BDT** to show the changes in emission properties, as we proved in our previous work.[46] This mechanism has been further supported by the ESI-MS study of **BDT** in the presence of Hg^{2+}(Fig. S14, ESI†), where $m/z = 227.195$ represents the mass of **BDA** which confirms the desulfurization of **BDT**.

Practical application ability

For practical application purposes, environmental water samples were also investigated using **BDT**. For this, we have collected IISER Kolkata pond water to determine Hg^{2+} with the help of our probe **BDT**. However, real-life water samples contain several other competitive components, which can cause interference towards detecting Hg^{2+} by **BDT**. Therefore, we have carried out the estimation of the Hg^{2+} level with the help of the standard addition method.[47] Probe **BDT** could quantify the added Hg^{2+} precisely with excellent recovery (Table S2, ESI†), which entirely established the applicability of our probe towards the detection of Hg^{2+} in real-life samples. Furthermore, to further support the relevance of our probe towards real samples, we have checked the amount of Hg^{2+} present in commercially available powder vermilion using **BDT** and verified with ICP-MS (Table S2, ESI†). The obtained result suggested the excellent applicability of **BDT** towards the detection of Hg^{2+} in real samples.

Detection of Hg^{2+} in biological samples

After confirming the precise and sensitive detection ability of **BDT** for Hg^{2+} and CH_3Hg^+, it is essential to check the detection ability of our probe at the cellular level. Towards this, **BDT** has been utilized to detect Hg^{2+} in biological systems as an incredible turn-on fluorescent probe. The initial cell viability studies of **BDT** revealed its low cytotoxicity effects (Fig. S14,

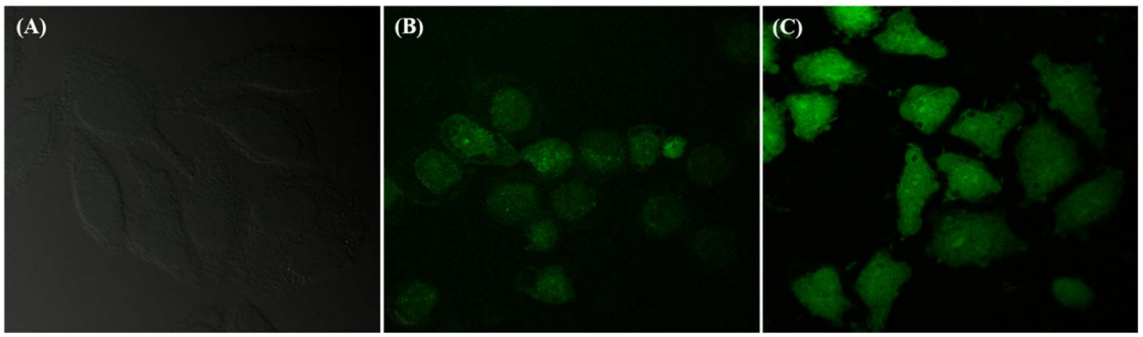


Fig. 5 Confocal laser scanning microscope images of HeLa cells pre-treated with 10 μM BDT in the presence of (A) 0 μM, (B) 10 μM, and (C) 20 μM Hg^{2+} ions. Excitation wavelength: 405 nm.

ESI†). Due to having the less toxic effect of BDT on cells, we carried out a cellular imaging study by varying the Hg^{2+} ion concentration. The fluorescence intensity change of **BDT** in immobilized HeLa cells was investigated by changing the concentration of Hg^{2+} ions. From Fig. 5A–C, it could be easily observed that with the increasing concentration of Hg^{2+}, the fluorescence intensity of **BDT** tagged HeLa cells increased. These results suggested that the reaction-based fluorescence probe **BDT** could monitor Hg^{2+} in cells with excellent specificity and efficiency.

Conclusion

We designed and synthesized one of the smallest, most specific, and economic reaction-based probes (BDT) to identify and quantify Hg^{2+} and CH_3Hg^+ in environmental and biological samples through the 'ESIPT' activation process. BDT was demonstrated as a precise and sensitive probe for detecting inorganic and organic mercury with a theoretical detection limit of 3.8 nM and 0.8 μM, respectively. The desulfurization reaction for the formation of dialdehyde systems was proved with the help of ^1H NMR spectroscopy and fluorescence spectroscopic studies. With the capability to detect the analyte at the cellular level, we believe that BDT will provide big advantages towards detecting Hg^{2+} in biosamples. Further improvement can be made using dialdehyde systems through increased π-conjugation for sensing purposes, and we think that BDT will initiate this progression.

Author contributions

T. S and N. D have equal contribution to this work. D. P and P. K performed all *in vitro* biology experiments. R. S. supervised throughout this project.

Conflicts of interest

There is no conflict of interest.

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