Supporting Information

A bromophenol derivative for rapid detection of Hg^{2+}/CH_3Hg^+ in both environmental and biological samples through the activation of the ESIPT process

Tapendu Samanta,‡ Narayan Das,‡ Diptendu Patra, Pawan Kumar, Raja Shunmugam*

Corresponding Author:

*__Prof. Raja Shunmugam__-Polymer Research Centre, Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, India, West Bengal, 741246, email-sraja@iiserkol.ac.in

Authors:

‡ __Tapendu Smanta__-Polymer Research Centre, Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, India, West Bengal, 741246

‡ __Narayan Das__- Polymer Research Centre, Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, India, West Bengal, 741246

__Diptendu Patra__- Polymer Research Centre, Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, India, West Bengal, 741246

__Pawan Kumar__- Polymer Research Centre, Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, India, West Bengal, 741246

No of pages: 16 (S1-S14)

No of figures: 16 (S1-S16)

No of table: 2 (S1-S2)

Table of Contents

1. Experimental section

1.1 Materials:

4-Bromophenol, hexamethylenetetraamine (HMTA), Trifluoroacetic acid (TFA), 1,2-Ethanedithiol, Boron Trifluoride Diethyl Etherate, Dichloromethane (**DCM**) and **DMSO-d_6** were purchased from Aldrich, Acros, Merck and used as received. The stock solutions of metal salts used were NaCl, KF, LiCl, $MgSO_4.7H_2O$, $CdCl_2$, $CuSO_4.5H_2O$, $ZnCl_2$, $Pb(NO_3)_2$, $CoCl_2$, $FeCl_3$, $FeCl_2$, $SnCl_2$, $CaCl_2$, CrO_3, $Hg(NO_3)_2$. Deionised water was used to make the metal salt solutions.

1.2 Methods:

NMR Characterization:

NMR spectroscopy was carried out using DMSO-d_6 as a solvent on a Bruker 500 MHz spectrometer. NMR spectra of solutions in DMSO-d_6 were calibrated to Tetramethylsilane as internal standard (δH 0.00).

Fluorescence Measurements:

Fluorescence emission spectra were recorded on fluorescence spectrometerer (Horiba Jobin Yvon, Fluromax-4, 250-900 nm). Emission spectra for all solutions were measured with an excitation wavelength of 350 nm. Slit widths and scan rates were adjusted to allow adequate intensity if needed.

UV-Vis experiments:

UV-visible absorption measurements were carried out on Perkin-Elmer Lambda 35UV-Vis spectrometer, with a 240 nm/min scan rate. The absorption spectra for all solutions were measured in a quartz cell of 1 cm.

ESI-MS Analysis:

HRMS analyses were performed with Q-TOF YA263 high-resolution (Waters Corporation) instruments by (+)ve mode electrospray ionization.

Elemental analysis:

Elemental analysis of the compound was examined by the X-Max SN: 60499, Model: 51- XMX1004 of Oxford Instruments.

Confocal Microscopy

All the images for the biological samples were taken in CLSM-710 (Zeiss). We used blue laser (405 nm) for the excitation of the biological sample as our sample was showing fluorescence at 525 nm in presence of mercury.

Sample preparation for UV-Vis and fluorescence studies:

The stock solutions of **BDA**and **BDT**were initially prepared in DMSO medium, and further dilution was made by adding water as per requirement for spectroscopic studies. Then, a fixed concentration of BDT (10 μM) was treated with different metal ions of 50 μM concentration for the selectivity experiments, and the corresponding fluorescence spectra were measured.

Cell Culture:

HeLa cells were cultured in a DMEM medium containing 10% FBS and 1% antibiotic solution (penicillin and streptomycin). The cells were incubated at 37 °C and 5% CO_2 level inside the incubator. Trypsin-EDTA solution was used for Harvesting the cells.

MTT Assay:

5 $\times 10^3$ cells were seeded into each well of a 96-well plate. After 24 h, different concentrations of **BDT**(25-500 μM) were treated and incubated for the next 24 h in standard culture conditions. The treatment of MTT solution to each well was performed, and kept the 96-well plate inside the incubator at 37 °C for 4 h. The formation of formazan crystals was confirmed under a microscope. 100 μL of DMSO was added to each well of the well plate and allowed to solubilize the crystals for 5−10 min. The absorbance in each well was quantified in a multimode reader at a wavelength of 570 nm. The percentage viability of each treatment group was quantified compared to untreated control.

Confocal Laser Scanning Microscopy (CLSM) imaging: 5 × 10^4 HeLa cells were seeded into each well of a 24-well plate containing 13 mm coverslips. The cells were allowed to adhere firmly to the coverslips for 24 h. Then **BDT** (10 μM) was incubated in cell culture conditions for 12 h. The cells were then washed twice with PBS to remove cellular debris or dead cells. Cells were fixed by incubating in 4% PFA for 30 min. The incubated cells were treated with different concentrations of Hg^{2+} (10μM, 20 μM) ion for 60 minutes. The cells were then washed with PBS and treated with mounting media at RT, and placed on a sterile glass slide. The slides were then allowed to dry at RT for 24 h, and imaging was done under a CLSM microscope.

1.3 Synthesis and characterization

Synthesis of Compound BDA[17]**:**
In a 250 ml round bottom flask, 4-bromophenol (1.5 g, 8.7 mmol) was dissolved in trifluoroacetic acid (40 ml) followed by the addition of HMTA (4.9 g, 34.7 mmol). The resulted reaction mixture was refluxed overnight at 120 °C. After completion of the reaction, the reaction mixture was cooled and poured into 200 ml 2N HCl solution, and stirred at 80°C for one hour. The obtained yellow solid was filtered and washed by water (1 L). The obtained crude product was dried over a vacuum pump to get compound **BDA**as light-yellow powder **(**1.6 g, 80%).**^{1}H NMR (DMSO-d_6, 500 MHz), δ (ppm):**11.60 (bs, 1H), 10.19 (S, 2H), 8.12 (s, 2H). **^{13}C NMR (DMSO-d_6, 500 MHz), δ (ppm):** 190.9, 160.9, 138.5, 125.7, 111.3. ESI-MS: m/z: Calculated for $C_8H_4BrO_3^-$: 226.93 [M^-], found: 226.94

Synthesis of BDT:
In a 100 mL round bottom flask, **BDA** (0.7 g, 3.06 mmol) was dissolved in dry DCM (30 ml)by maintaining an inert atmosphere and $BF_3.OEt_2$(1.3 g, 9.18 mmol) was added by keeping the temperature at 0°C. After stirring the reaction mixture for 10 minutes, 1,2-ethanedithiol (0.720 g, 7.65 mmol) was added, and the reaction mixture was kept in a mixing condition at 0°C for overnight. After completing the reaction, the organic part was washed

with sodium bicarbonate, water, and brine solution. Organic layers were collected and dried over anhydrous sodium sulphate. The crude product was purified by flash column chromatography using hexane/ethyl acetate as eluent to obtain **BDT** as a white amorphous solid (0.95 g, 82%).

^{1}H NMR (DMSO-d$_6$, 500 MHz), δ (ppm):9.5 (s, 1H), 7.6 (s, 2H), 6.0 (s, 2H), 3.41-3.47 (m, 4H), 3.29-3.35 (m, 4H).**^{13}C NMR (DMSO-d$_6$, 500 MHz), δ (ppm)**: 150.4, 132.2, 129.9, 111.2, 48.2, 39.1. ESI-MS: m/z: Calculated for $C_{12}H_{12}BrOS_4^-$: 378.90 [M^-], found: 378.88

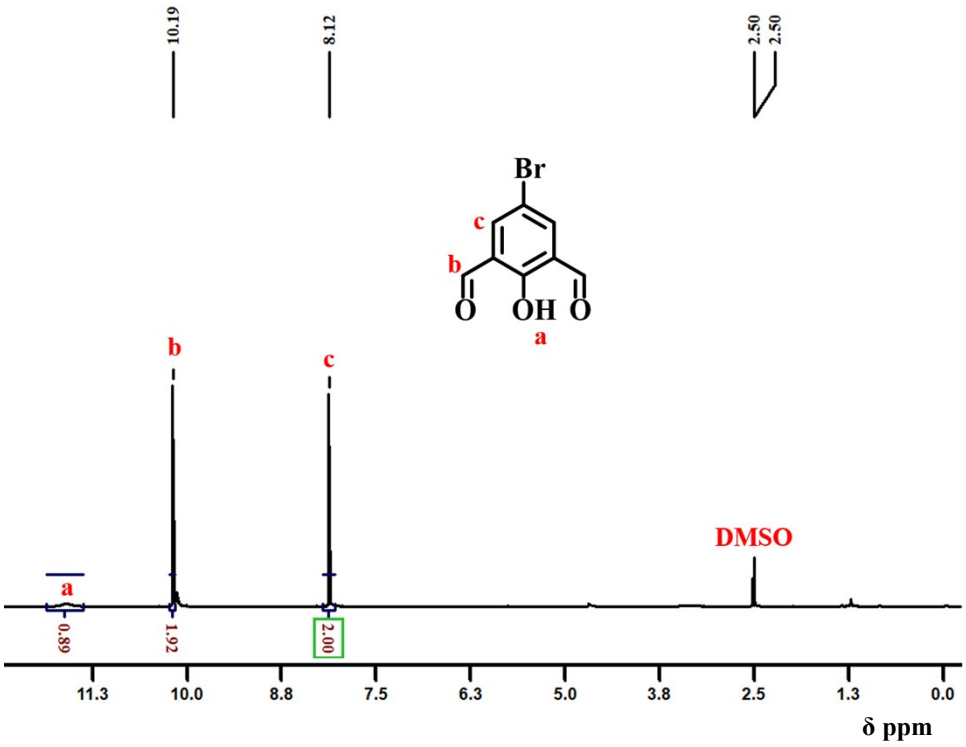


Figure S1: ^{1}HNMR spectrum of **Compound BDA**in **DMSO-d$_6$**

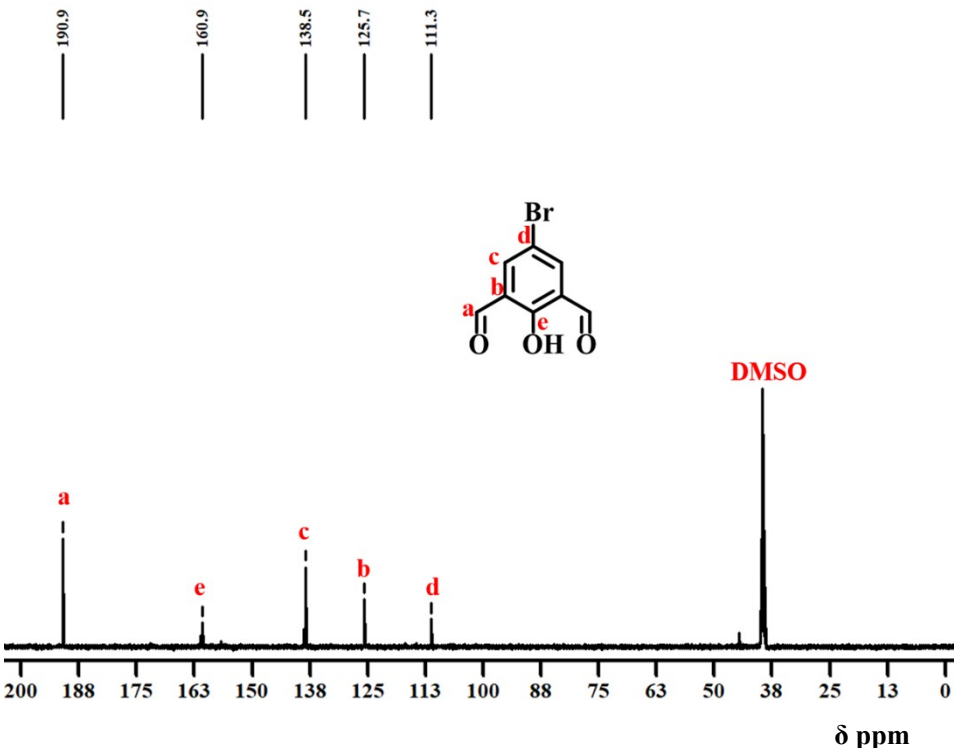


Figure S2: ^{13}C NMR spectrum of **Compound BDA** in **DMSO-d$_6$**

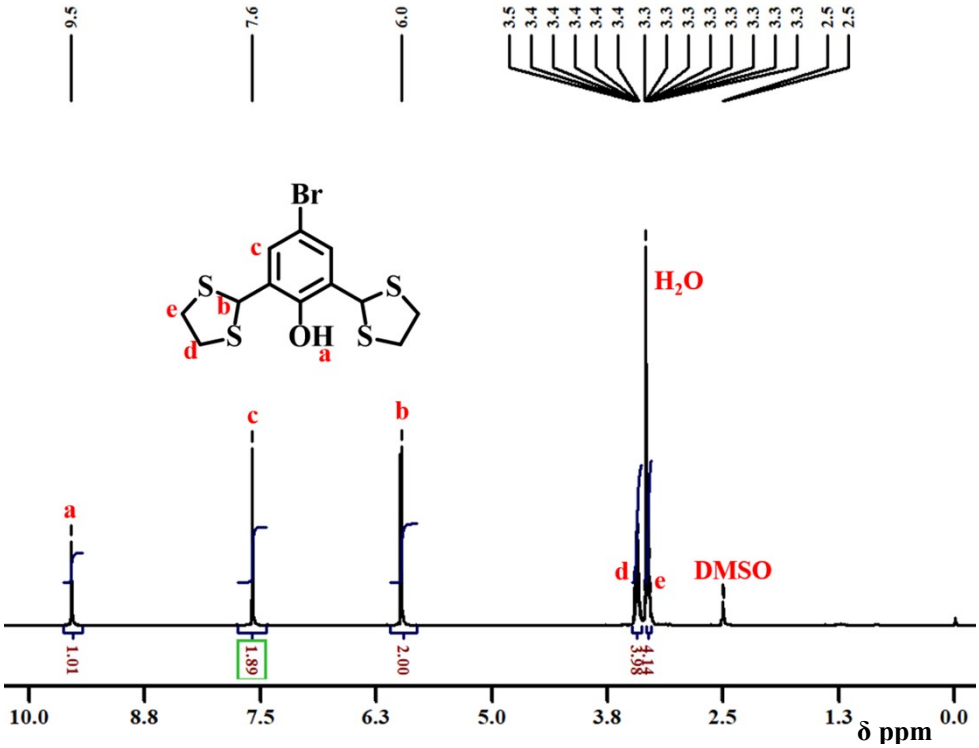


Figure S3: ^{1}HNMR spectrum of **Compound BDT** in **DMSO-d$_6$**

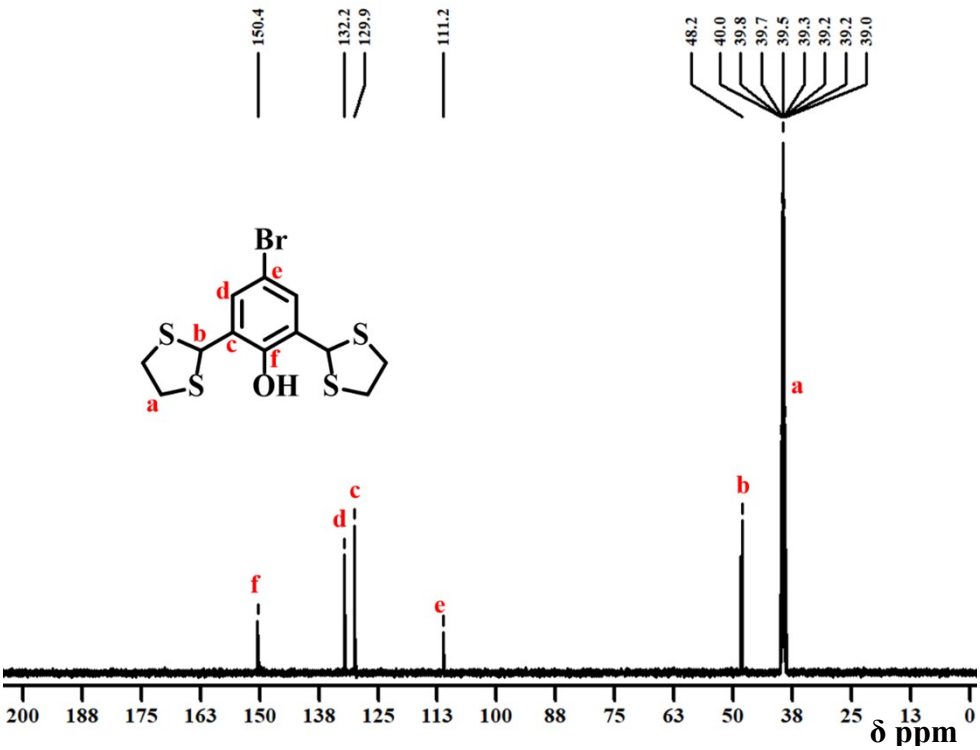


Figure S4: ^{13}C NMR spectrum of **Compound BDT** in **DMSO-d$_6$**

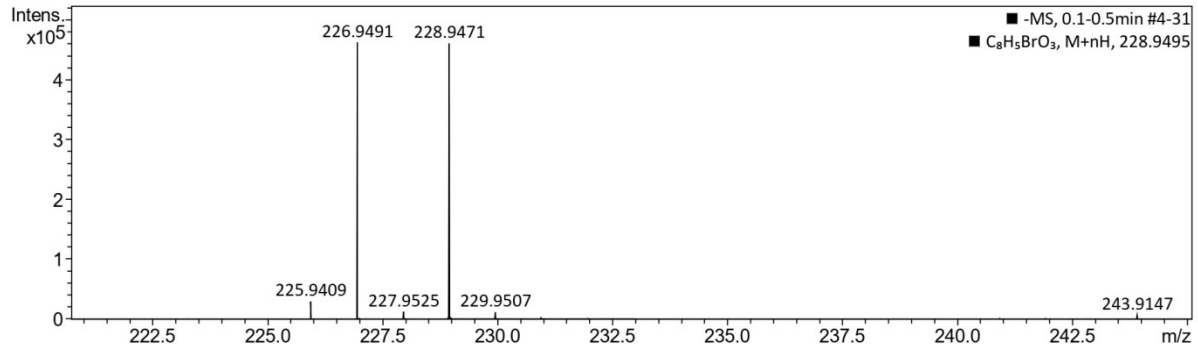


Figure S5: ESI-MS spectrum of **BDA**.

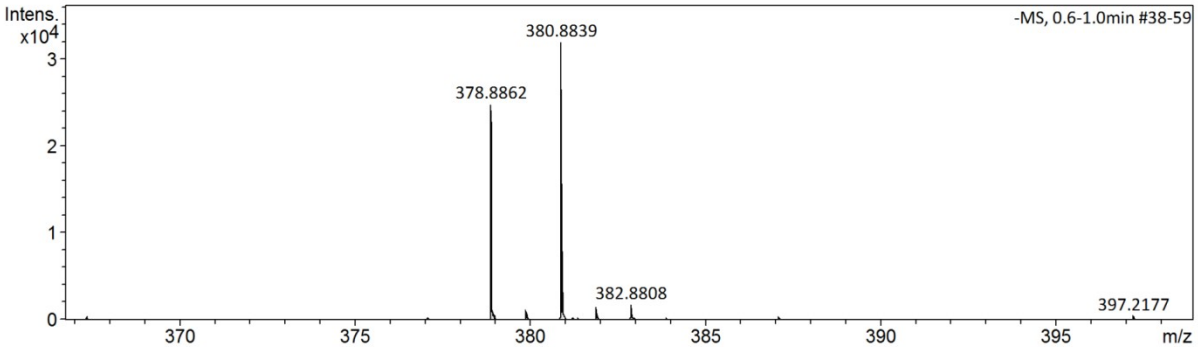


Figure S6: ESI-MS spectrum of **BDT.**

2. Photophysical studies of BDT

We have got a new peak at 350 nm of BDT in the presence of mercury. We have use 350 nm as the excitation wavelength for all the fluorescence study.

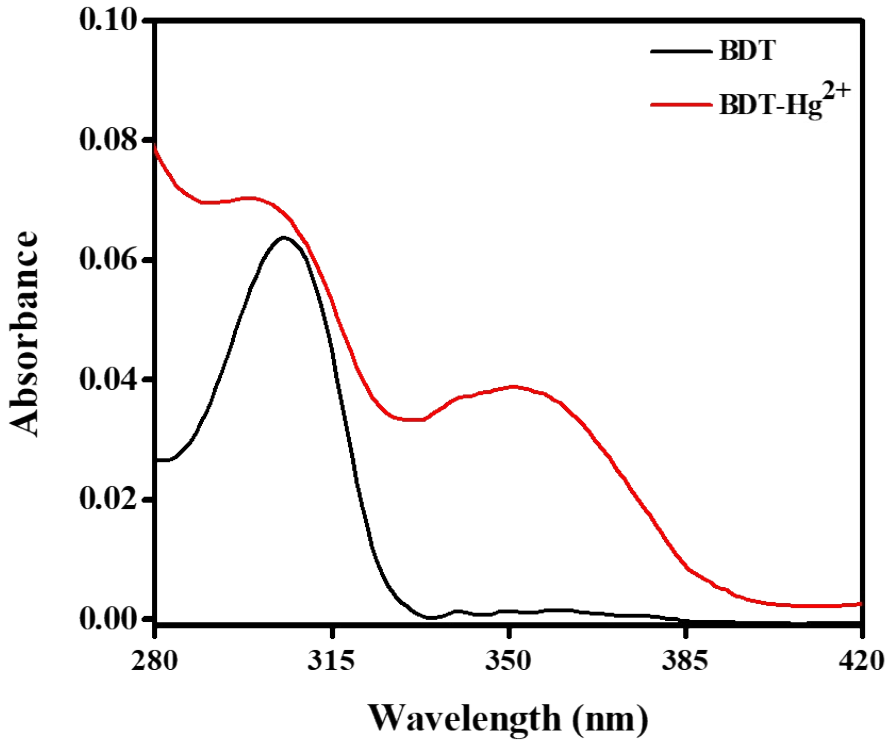


Figure S7:UV-vis spectra of **BDT** (15 μM) with and without Hg^{2+} (30 μM) in DMSO/PBS buffer (4:1, v/v, pH 7.4) medium.

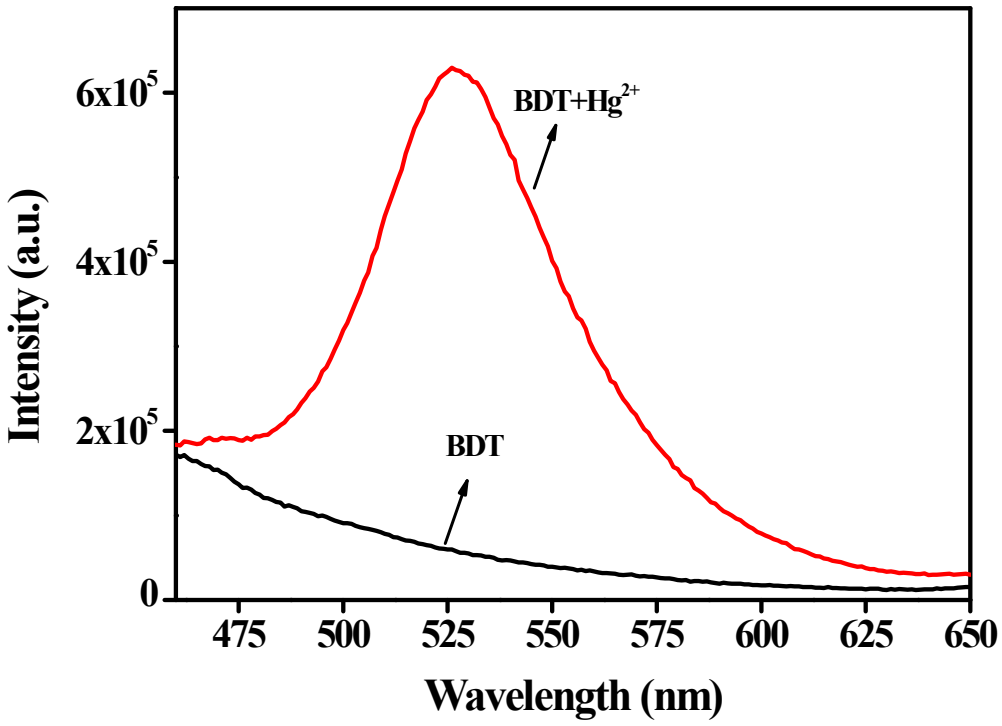


Figure S8: Fluorescence spectra of **BDT** (10 μM) in absence and presence of Hg^{2+} (50 μM) in DMSO/PBS buffer (4:1, v/v, Ph=7.4) medium.

3. Quantum yield (Q) calculation for BDT:

Quatum yield for **BDT** has been caluculated using the following equation,

$$Q_S = Q_R \times \frac{I_S}{I_R} \times \frac{A_R}{A_S} \times \frac{\eta_s^2}{\eta_R^2}$$ ----------Equation 1.

Q_s= quantum yield of the sample, Q_R=quantum yield of reference, I_s= area under the fluorescence curve of the sample, I_R= area under the fluorescence curve of reference, A_R = absorbance of the reference; A_S = absorbance of the sample; η_S = refractive index of sample; η_R = refractive index of reference.

Here Quinine sulfate (in 0.1 M H_2SO_4) has been used as reference to calculate the quatum yiled of BDT.

Quantum Yield of **BDT** (Q_S) = 0.11

Quantum Yield of **BDT** (%) = 11

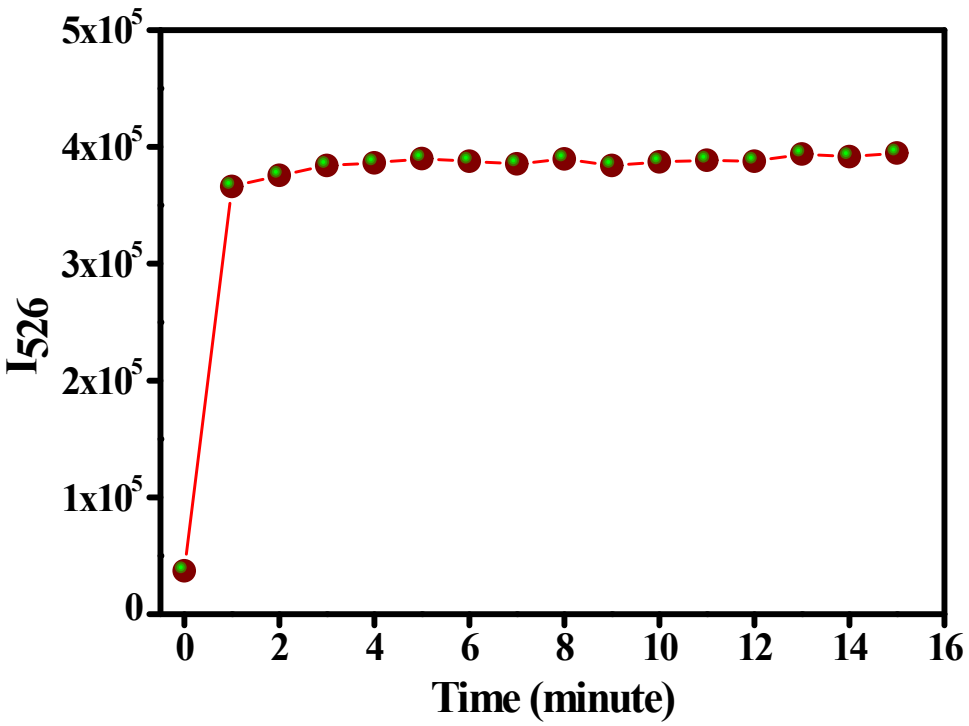


Figure S9: Time dependent fluorescence study of **BDT** (5 μM) in presence of Hg^{2+} (20 μM) in DMSO/PBS buffer (4:1, v/v, Ph=7.4) medium.

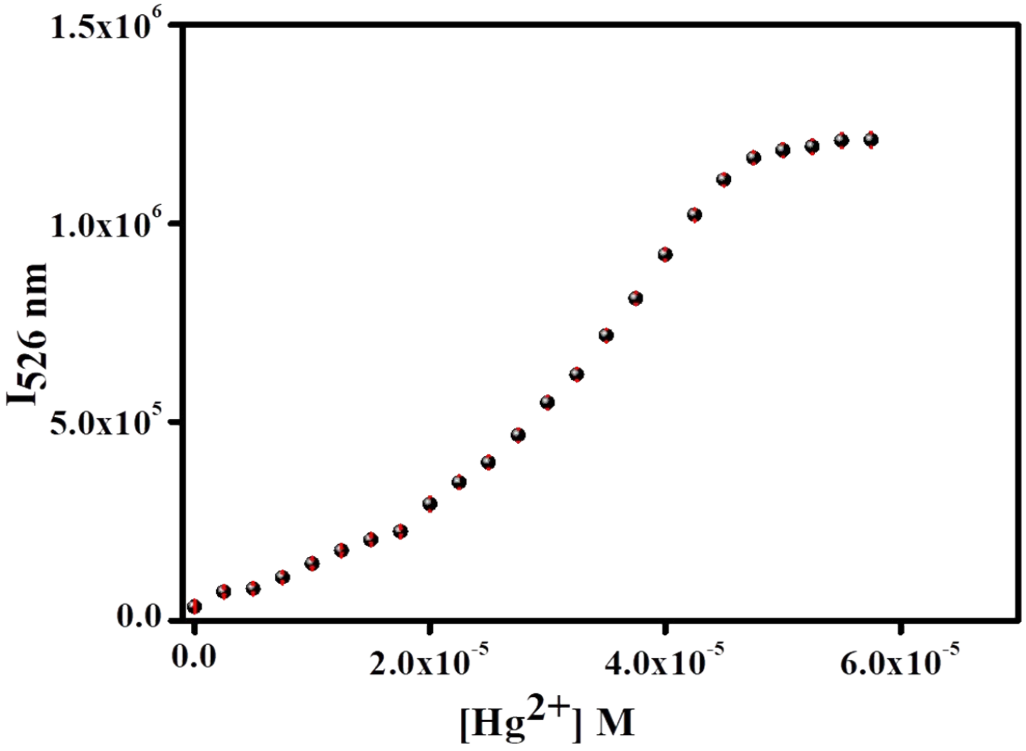


Figure S10: Plot of $I_{526\ nm}$ vs conc. of Hg^{2+} ion obtained from fluorescence titration studies of **BDT**.

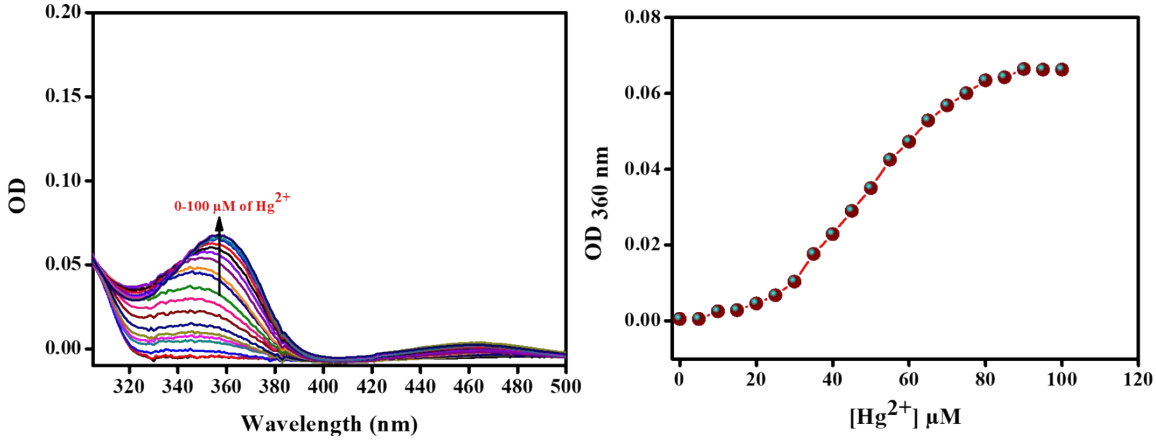


Figure S11: (A)UV-vis spectra of **BDT** (40 μM) with changing the concentration of Hg^{2+} (0-100 μM) in DMSO/PBS buffer (4:1, v/v, Ph=7.4) medium (B) Corresponding $OD_{360\ nm}$ vs. concentration of Hg^{2+} plot**.**

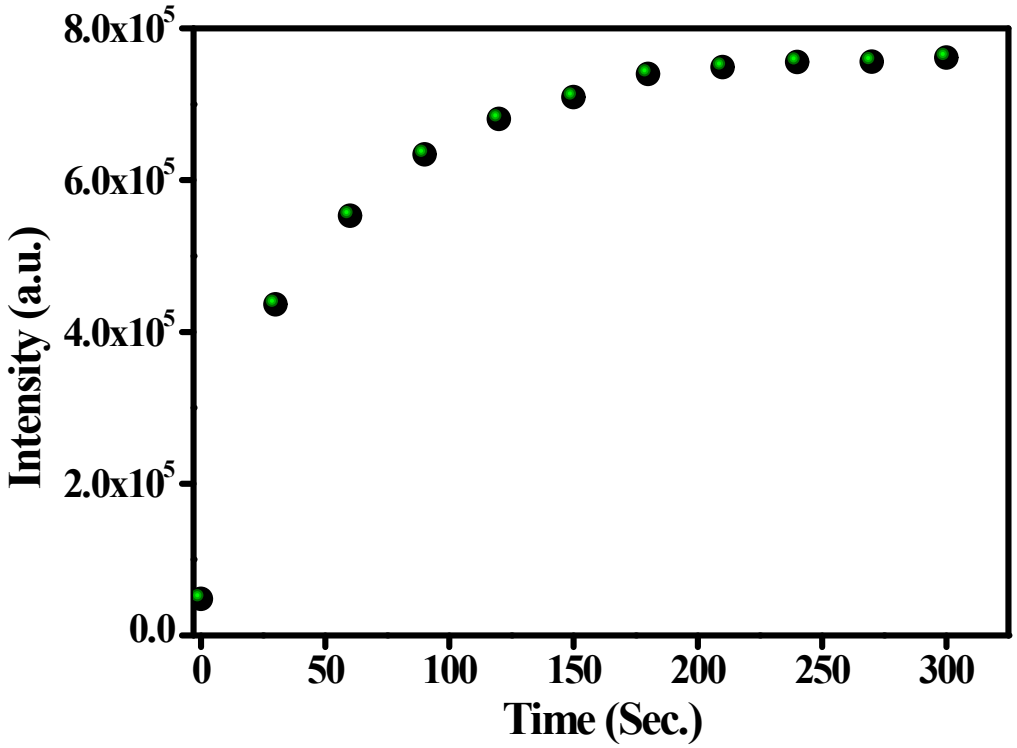


Figure S12: Time dependent fluorescence study of **BDT** (10 μM) in presence of CH_3Hg^+ (30 μM) ion in DMSO/PBS buffer (4:1, v/v, Ph=7.4) medium.

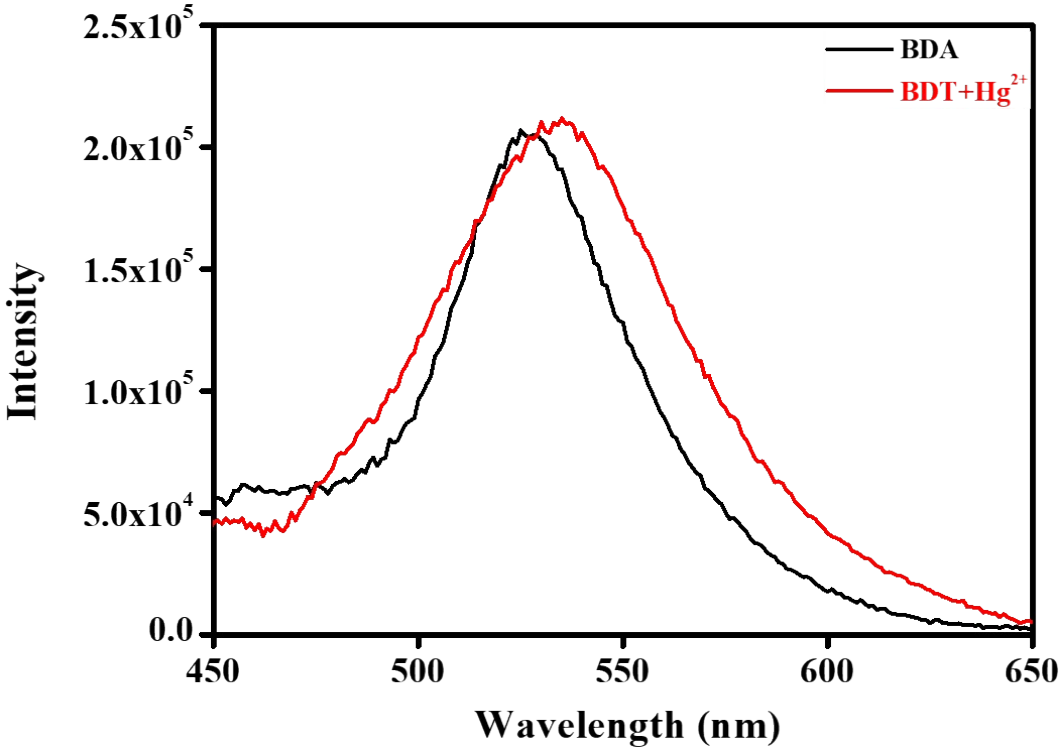


Figure S13: Fluorescence spectra of **BDA** (5 μM) and **BDT** (5 μM) in presence of Hg^{2+} (10 μM)in DMSO/PBS buffer (4:1, v/v, Ph=7.4) medium.

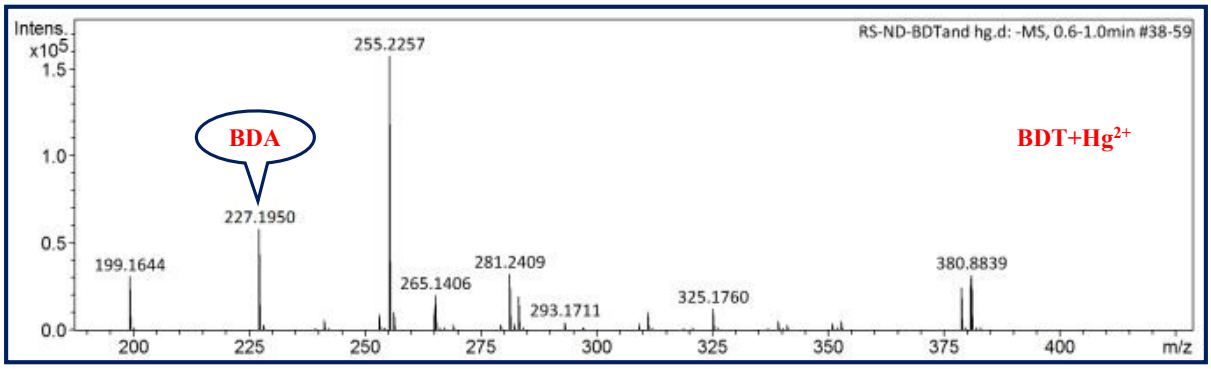


Figure S14: Mass Titration of BDT in the presence of Mercury

Table S1: Comparative table of various reported fluorescent sensors for Hg^{2+} and CH_3Hg^+ ions.

Sl. No	Analyte(s)	Medium	Detection time (min.)	LOD	Biological study	Ref.
1	Hg^{2+}	PBS buffer (2% DMSO)	35	7.6×10^{-9} M	Yes	**1**
2	Hg^{2+}	THF-water (1:1)	2	1.59×10^{-8} M	Yes	**2**
3	Hg^{2+}	EtOH-water (1:1)	1	1.03×10^{-8} M	No	**3**

4	Hg^{2+}	THF-water (1:1)	NA	3.1×10^{-7} M	No	**4**
5	Hg^{2+}	PBS-DMSO (5:5)	40	6.8×10^{-8} M	Yes	**5**
6	Hg^{2+}	HEPES buffer-EtOH (1:1)	10	5.8×10^{-9} M	No	**6**
7	Hg^{2+}	MeCN-water (1:1)	< 1	55×10^{-9} M	Yes	**7**
8	Hg^{2+}	99 % PBS buffer	15	19.3×10^{-9} M	Yes	**8**
9	Hg^{2+}	EtOH-water (2:8)	10	4×10^{-8} M	Yes	**9**
10	Hg^{2+}	THF	10	1×10^{-5} M	No	**10**
11	Hg^{2+}	PBS buffer	3	2.7×10^{-9} M	Yes	**11**
	CH_3Hg^+	PBS buffer	30	5.7×10^{-6} M		
12	Hg^{2+}	PBS buffer	60	5×10^{-9} M	Yes	**12**
	CH_3Hg^+		90	NA		
13	Hg^{2+}	buffered solution (10 mM HEPES, pH=7.4,1% CH3CN	<2	1.82×10^{-9} M	Yes	**13**
	CH_3Hg^+		<5	1×10^{-6} M		
14	Hg^{2+}	HEPES buffer (pH 7.4, 1% CH_3CN)	<10	20×10^{-9} M	Yes	**14**
	CH_3Hg^+		10	NA		
15	Hg^{2+}	DMSO-water (3:2)	< 1	8.2 × 10-9 M	Yes	**15**
	CH_3Hg^+		4	1.1 × 10-6 M		
16	Hg^{2+}	aqueous solution (HEPES, pH 7.4, 0.5% DMF)	5	9.1×10^{-9} M	Yes	**16**
	CH_3Hg^+		1	21.2×10^{-9} M		
17	Hg^{2+}	DMSO/PBS buffer (4:1, v/v, pH 7.4)	< 1	3.8×10^{-9} M	Yes	**This work**
	CH_3Hg^+		< 3	0.8×10^{-6} M		

Table S2: Quantification of Hg^{2+} in environmental samples using **BDT**.

Samples	**$[Hg^{2+}]$ in µM (Spiked)**	**$[Hg^{2+}]$ in µM (obtained)**	**% Of recovery**
Pond water in BDT	0	NA	NA
	2	1.926 ± 0.0308	96.3
	4	4.068 ± 0.0682	101.7

	6	5.746 ± 0.0894	95.7
	8	8.406 ± 0.126	105.0
	10	10.268 ± 0.242	102.6
Sample	**Obtained value of Hg^{2+} (μM) using BDT**	**Obtained value of Hg^{2+} (μM) using ICP-MS**	**% Of recovery**
Powdered Vermilion	0.534 μM	0.4985 μM	107

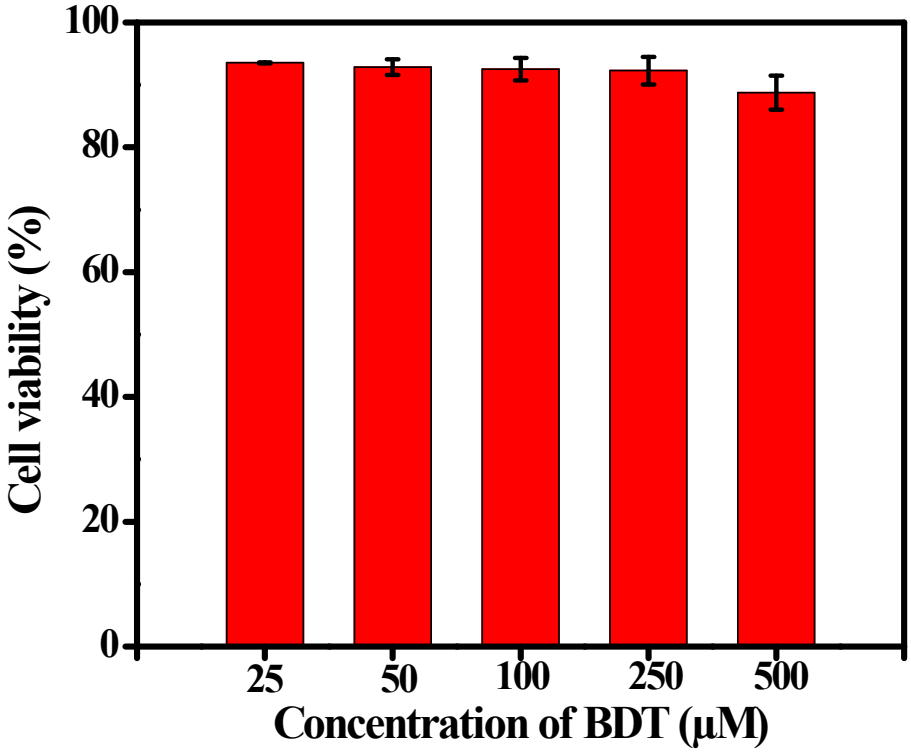
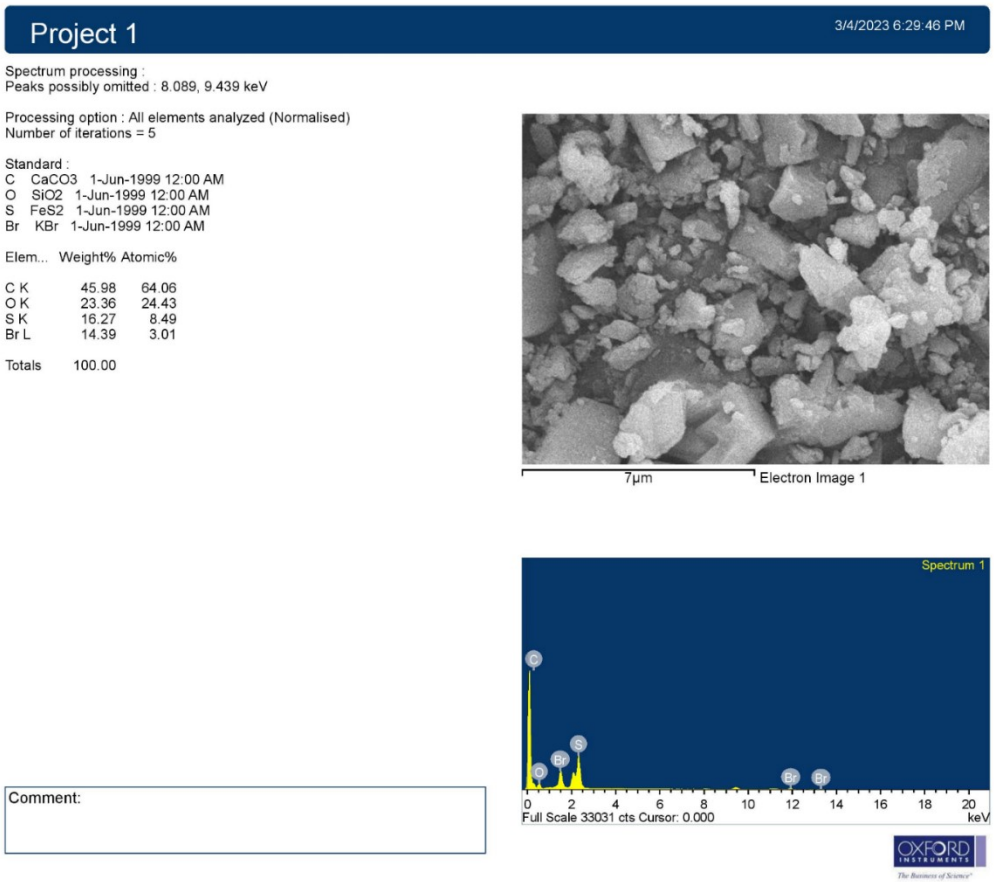


Figure S15: Cytotoxicity study of **BDT** in HeLa cells.



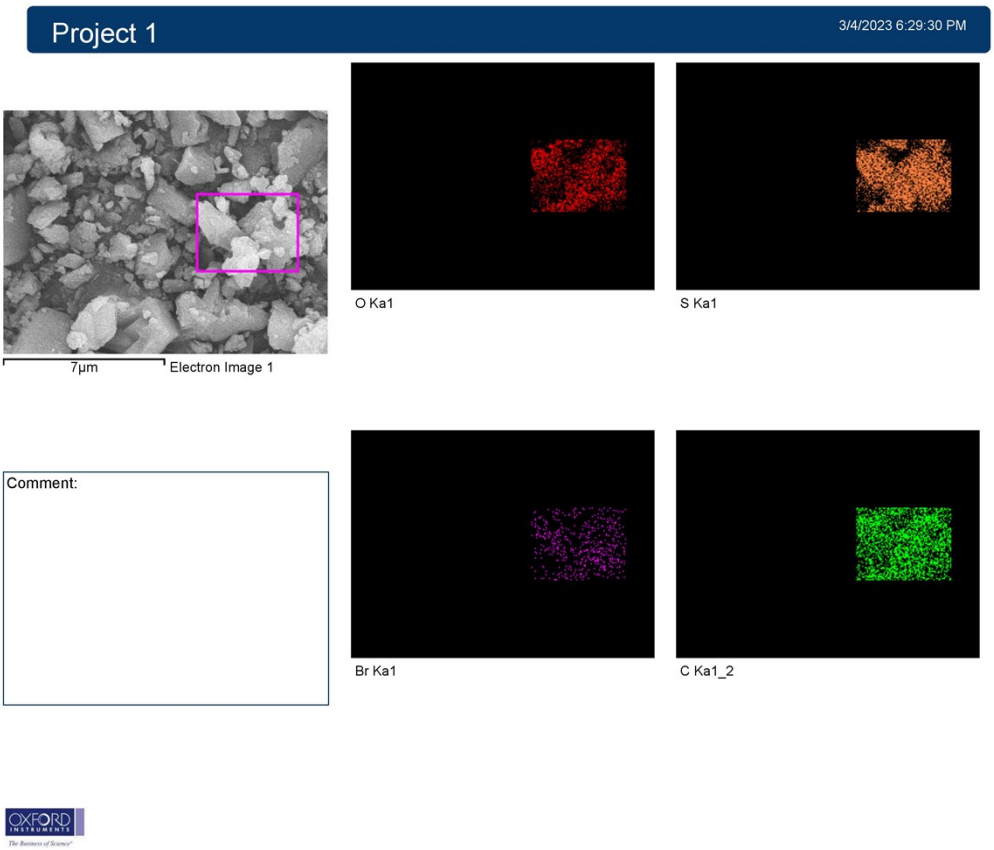


Figure S16: Elemental analysis of BDT.

4. References:

1. Gu, B.; Huang, L.; Su, W.; Duan, X.; Li H.; S.Yao, *Anal. Chim. Acta*, 2017, **954**, 97-104.
2. Xu, D.; Tang, L.; Tian, M.; He P.; X. Yan, *Tetrahedron Lett.,*2017, **58**, 3654-3657.
3. Lan, L.; Niu, Q.; T.Li, *Anal.Chim. Acta*, 2018, **1023**, 105-114.
4. Leng, T.; Ma, Y. ; Chen, G.*J. Photochem. Photobiol. A*, 2018, **353**,143-149.
5. Huang, L.; Yang, Z.; Zhou, Z.; Li, Y.; Tang, S.; Xiao, W.; Hu, M.; Peng, C.; Chen, Y.; Gu, B.; H.Li, *Dyes Pigm.* 2019, **163**, 118-125.
6. Zhou, Y.; He, X.; Chen, H.; Wang, Y.; Xiao, S.; N.; Zhang, Li, D.; K. Zheng, *Sens. Actuators B,* 2017, **247**, 626-631.
7. Y. Y.Liu, S.Naha, N.Thirumalaivasan, S.Velmathi and S. P.Wu, *Sens. Actuators B*, 2018, **277**, 673-678.
8. Wang, Z.; Zhang, Y.; Yin, J.; Yang, Y.; Luo, H.; Song, J.; Xu X.; Wang, S. *ACS Sustainable Chem. Eng.* 2020, **8**, 12348-12359.
9. Tian, M.; Wang, C.; Ma, Q.; Bai, Y.; Sun, J.; Ding, C.; *ACS Omega*, 2020, **5**, 18176-18184.
10. J. Ding, H. Li, Y. Xie, Q. Peng, Q. Li andZ.Li, *Polym. Chem.* 2017, **8**, 2221-2226.
11. Pan, S.L.; Li, K.; Li, L.L.; Li, M.Y.; Shi, L.; Liu, Y.H.; Yu, X.Q., *Chem. Commun.* 2018, **54**, 4955-4958.
12. Santra, M.; Ryu, D.; Chatterjee, A.; Ko, SK.; Shin, I.; Ahn, K. H. *Chem. Commun.,* 2009,**45**, 2115–2117.
13. Neupane, L.; Park, J.; Mehta, P.K.; Oh, ET.; Parkb, H. J.; Lee, KH. *Chem. Commun.,* 2020, **56**, 2941—2944.
14. Chen, X.; Baek, KH.; Kim, Y.; Kim, SJ.; Shin, I.; Yoon, J. *Tetrahedron*, 2010,**66**, 4016-402.
15. Samanta, T.; Das, N.; Patra, D.; Kumar, P.; Sharmistha B.; Shunmugam, R., ACS Sustainable Chem. Eng., 2021, 9, 5196–5203.
16. Oh, S.; Jeon, J.; Jeong, J.; Park, J.; Oh, E.T.; Park, H.J.; Lee, K.H., *Anal. Chem.***,** 2020, **92**, 4917-4925.
17. Reinhard, D.; Schöttner, L.; Brosius, V.; Rominger, F. and Mastalerz, M. Eur. J. Org. Chem., 2015: 3274-3285.