Supplementary Information (SI) for Analytical Methods.

A distinctive and proficient fluorescent switch for ratiometric recognition of the menacing cyanide ion: Biological studies on MDA-MB-231 cells

Amitav Biswas[a], Saswati Gharami*[a], Atanu Maji[a], Subhabrata Guha[b], Gaurav Das[b], Rahul Naskar[a] and Tapan Kumar Mondal*[a]

[a]Department of Chemistry, Jadavpur University, Kolkata-700 032, India.

E-mail: tapank.mondal@jadavpuruniversity.in ; saswatiju91@gmail.com

[b]Department of Signal Transduction and Biogenis Amines (STBA), Chittaranjan National Cancer Institute, Kolkata- 700026, India.

CONTENTS

1. ^{1}H NMR spectrum of BOHB

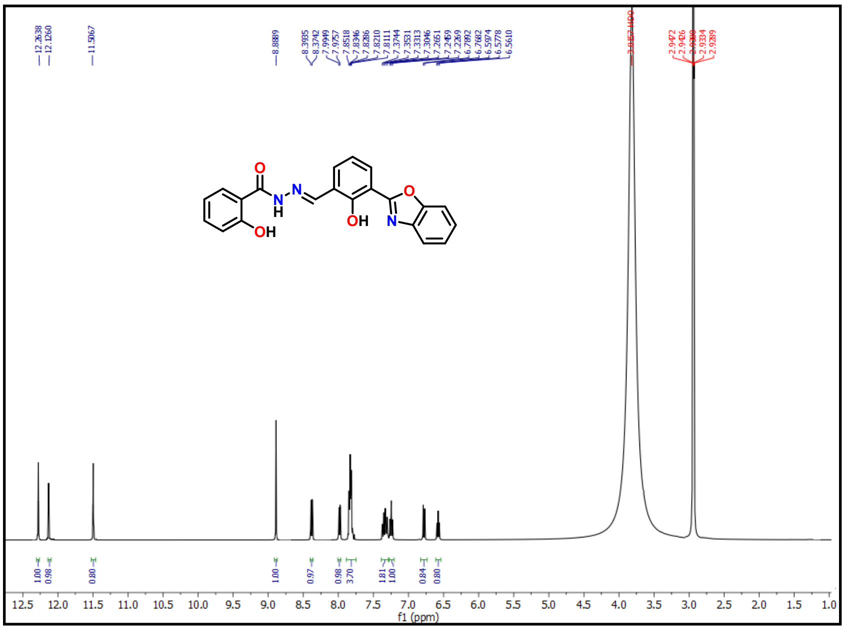
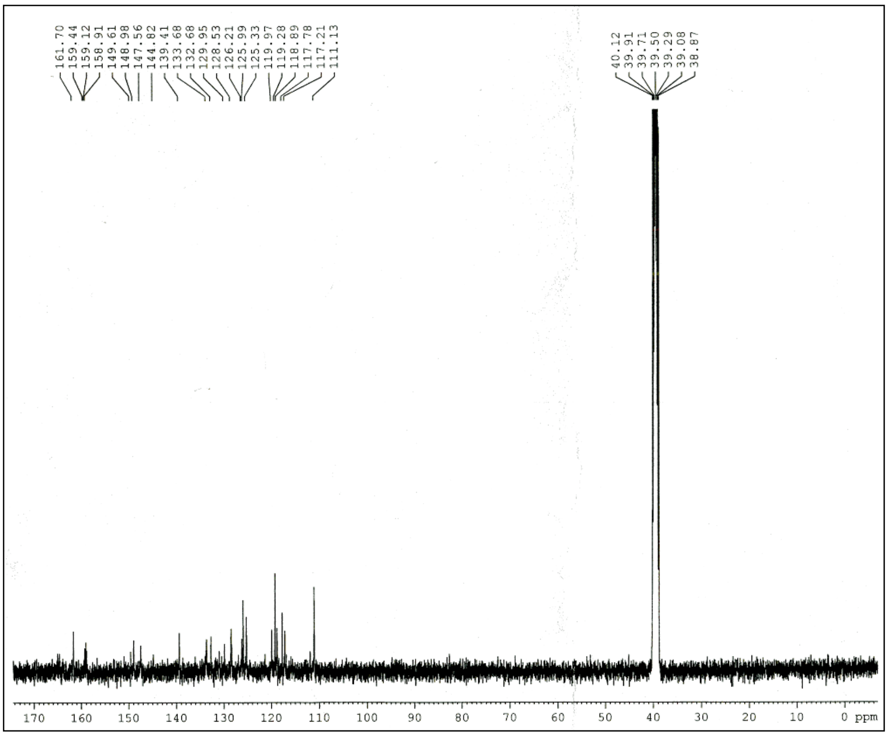


Figure S1: ^{1}H NMR (400 MHz) spectrum of the probe (BOHB) in DMSO-d_6

2. ^{13}C NMR spectrum of BOHB



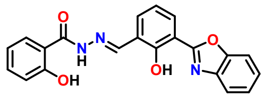


Figure S2: ^{13}C NMR (100 MHz) spectrum of the probe (BOHB) in DMSO-d_6

3. **IR plot of BOHB**

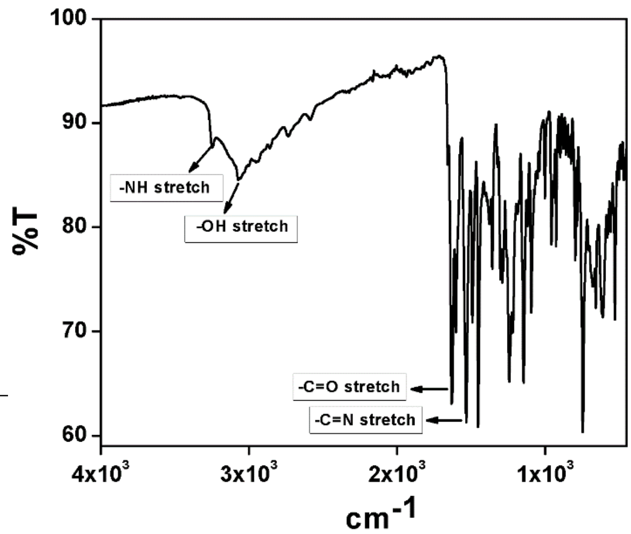
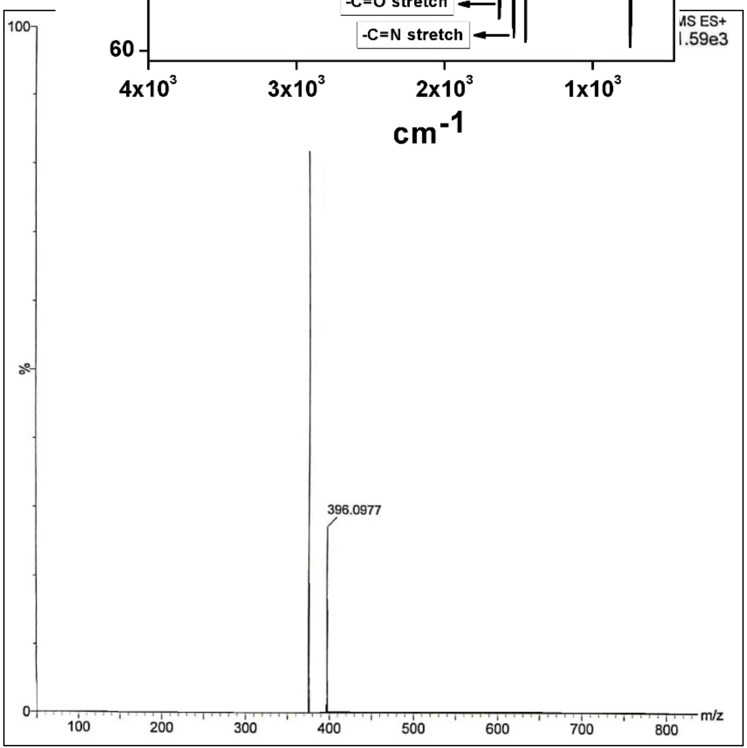
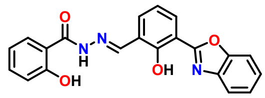


Figure S3: IR plot of the probe (BOHB)

4. **Mass spectrum (HRMS) of BOHB**





Calcd. for $C_{21}H_{15}N_{3}O_{4}$ $[M + H]^{+}$
(m/z) = 373.1063; found = 374.1131

Figure S4: HRMS of the probe (BOHB)

5. ^{1}H NMR spectrum of BOHB-CN^{-}

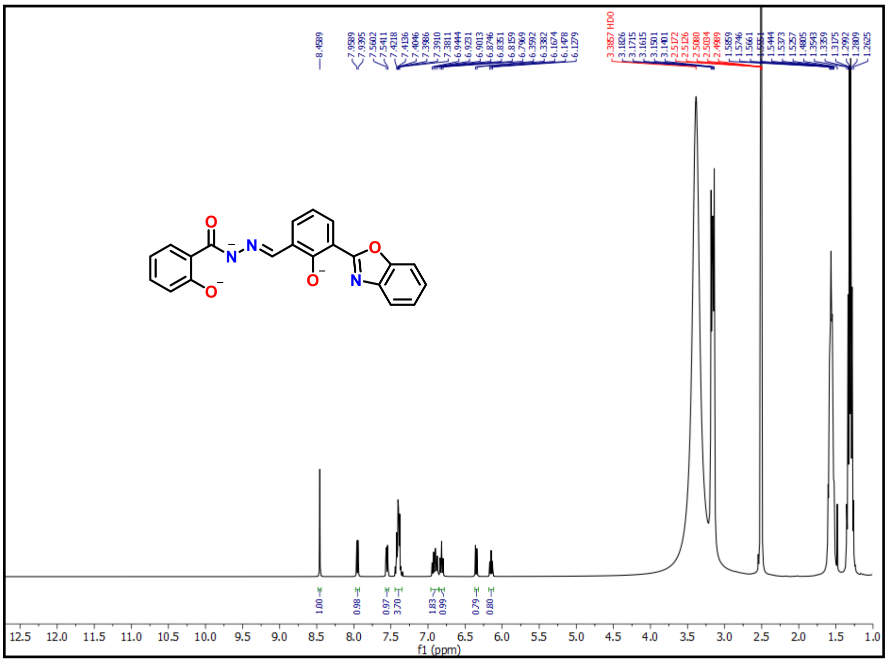


Figure S5: ^{1}H NMR (400 MHz) spectrum of BOHB-CN^{-} in DMSO-d_6

6. UV Study

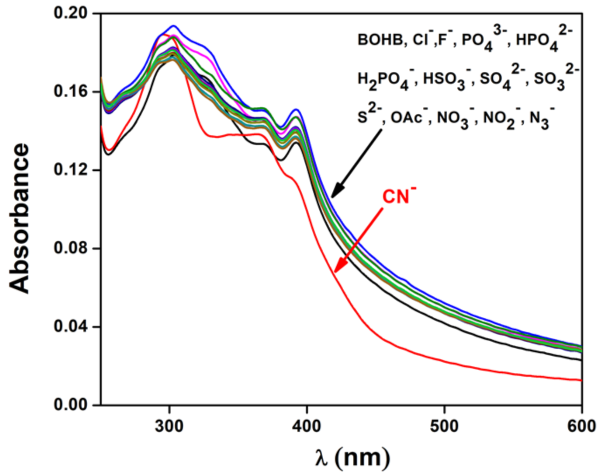


Figure S6: Absorption spectra of BOHB (10 μM) upon addition of various other anions (20 μM) in MeOH/H_2O (1/1, v/v) using HEPES buffered solution at pH=7.2

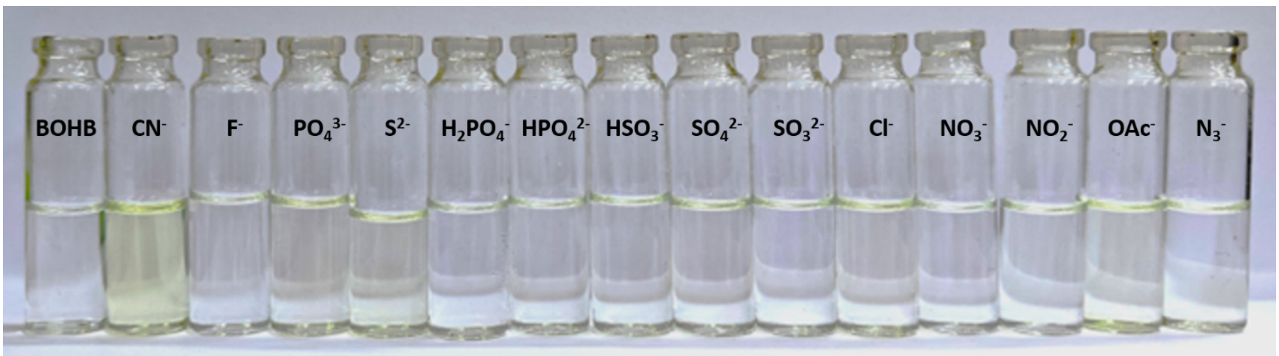


Figure S7: Color variations upon addition of different anions such as CN^-, Cl^-, F^-, PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}, HSO_3^-, SO_4^{2-}, SO_3^{2-}, S^{2-}, OAc^-, NO_3^-, NO_2^- and N_3^- into the probe solution in MeOH/H_2O (1/1, v/v) in naked eye.

7. Emission Study

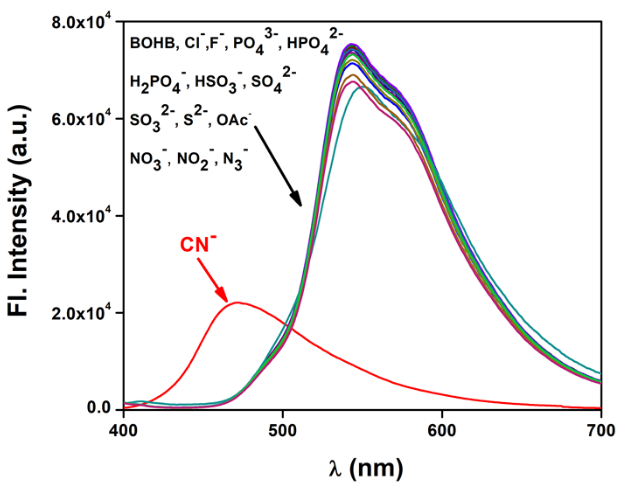


Figure S8: Change of emission spectra of BOHB (10 μM) after addition of other anions (20 μM) in MeOH/H_2O (1/1, v/v) using HEPES buffered solution at pH=7.2. λ_{ex} = 368 nm.

8. Mole ratio plot

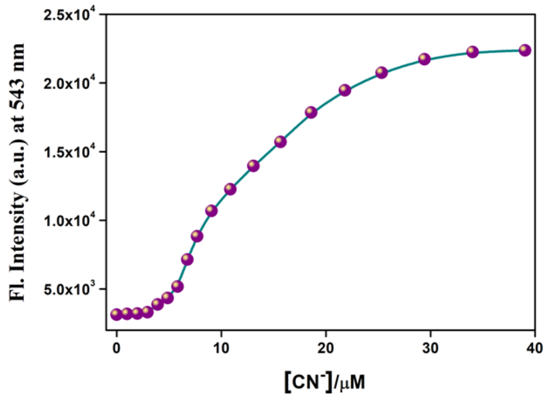


Figure S9: Plot of emission intensity at 543 nm of BOHB (10 μM) depending upon the CN^- concentration.

9. Determination of detection limit (LOD)

The limit of detection was determined based on the fluorescence titration. To determine the S/N ratio, the emission intensity of BOHB without CN^- was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit (DL) of BOHB for CN^- was determined from the following equation: DL = K × Sb_1/S, Where K = 2 or 3 (we take 3 in this case); Sb_1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

We get the value of Sb_1 as 0.531. Thus using the formula, we get the Detection Limit = (22.1±0.89) μM i.e. BOHB can detect CN^- in this minimum concentration by fluorescence techniques.

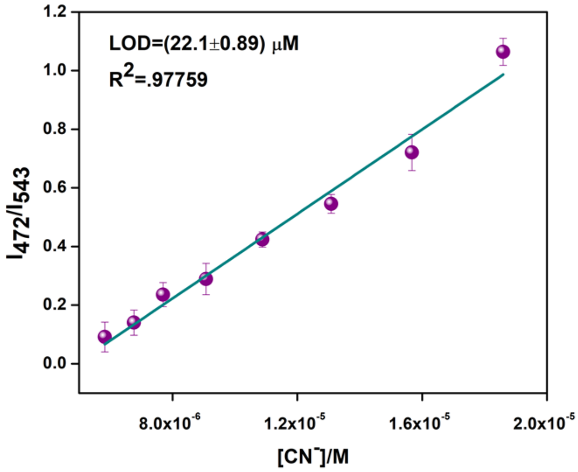


Figure S10: The linear response curve of emission intensity at I_{472}/I_{543} of BOHB depending on CN^- concentration.

10. pH study of BOHB and BOHB-CN^-

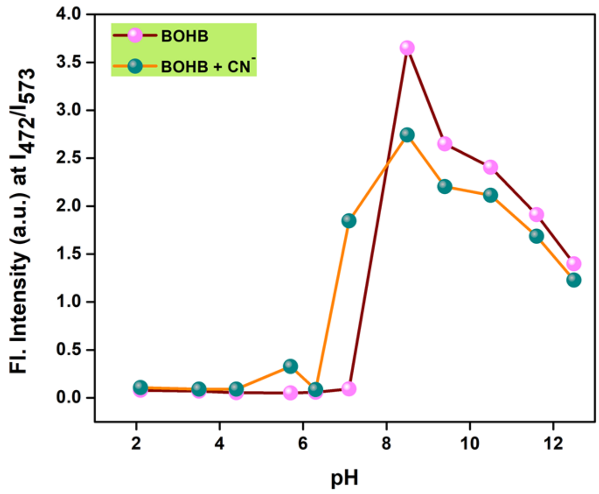


Figure S11: pH titration plot of BOHB and BOHB-CN^-.

11. Stability study of BOHB and BOHB-CN^-

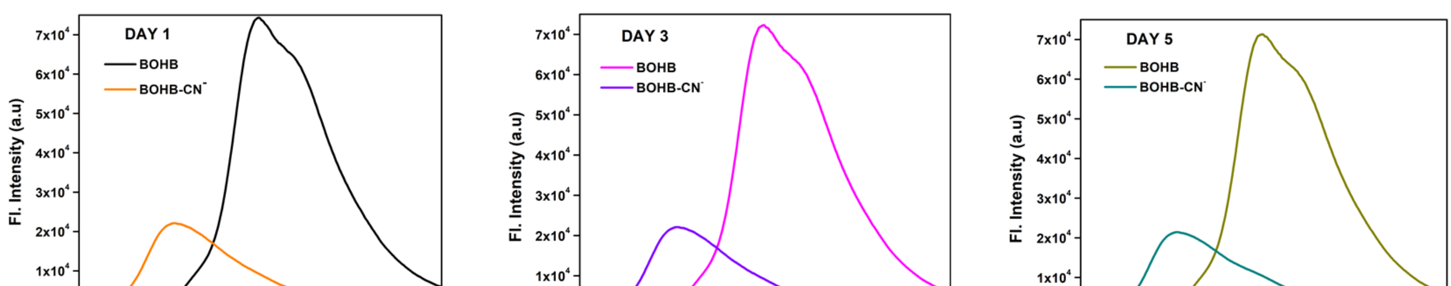


Figure S12: Fluorescence response of BOHB and BOHB-CN^- over different days.

12. Determination of Quantum yield of BOHB and BOHB-CN^-

For measurement of the quantum yields of BOHB and its reaction product with (BOHB-CN^-), we recorded the absorbance of the compounds in DMSO solution. The emission spectra were recorded using the maximal excitation wavelengths and the integrated areas of the emission-corrected spectra were measured. The quantum yields were then calculated by comparison with quinine sulfate ($\varphi_s = 0.54$ in 0.5M H_2SO_4) as reference using the following equation:

$$\Phi_x = \Phi_s \times \left(\frac{Ix}{Is}\right) \times \left(\frac{As}{Ax}\right) \times \left(\frac{nx}{ns}\right)^2$$

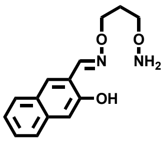
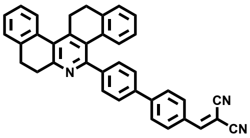
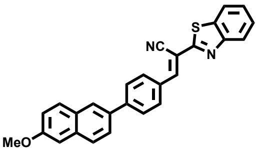
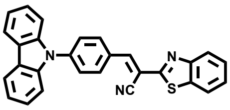
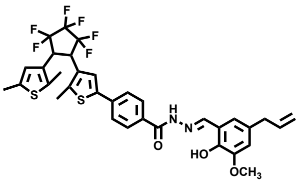
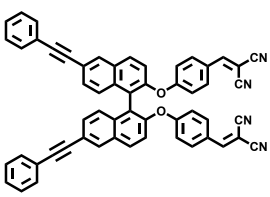
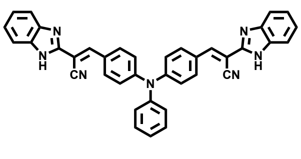
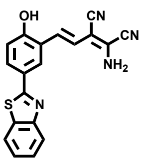
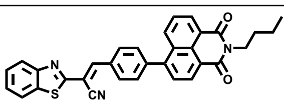
Where, x & s indicate the unknown and standard solution respectively, Φ is the quantum yield, I is the integrated area under the fluorescence spectra, A is the absorbance and n is the refractive index of the solvent. We calculated the quantum yields of BOHB and BOHB-CN^- using the above equation and the values are 0.245 and 0.098 respectively.

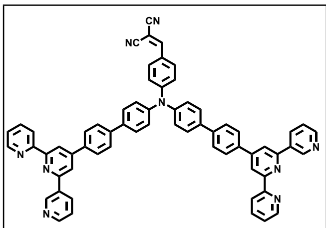
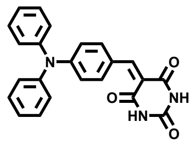
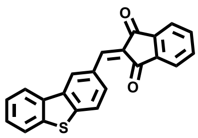
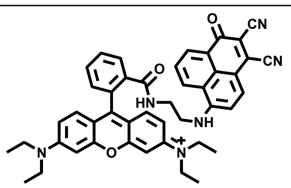
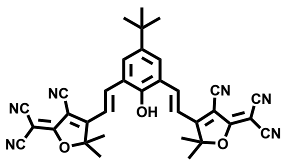
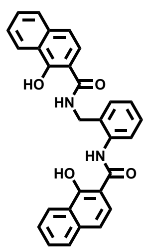
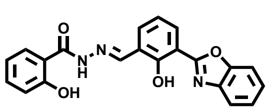
Table S1: Lifetime decay profile of BOHB and BOHB-CN^-

Methanol (Solvent)	**Quantum Yield**	**τ (ns)**	**K_r (10^8 x S^{-1})**	**K_{nr} (10^8x S^{-1})**
BOHB	0.245	2.33	1.051	3.241
BOHB-CN^-	0.098	0.92	1.065	0.021

Table S2: The comparison of the present probe (BOHB) with some previous probes for CN^-

Probe	**Type of response**	**Response Time (min or**	**Solvent System**	**Detection limit**	**Referen ce**

		sec)			
	colorimetric and fluorescent ratiometric turn-on	< 3 seconds	DMSO/H_2O, 9/1	2.16 $\times 10^{-7}$ M	[1]
	Fluorescence turn-off	Within 20 seconds	Acetonitrile	3.93 $\times 10^{-8}$ M	[2]
	Ratiometric fluorescence change	Within 2 minutes	DMSO/H_2O, 1/1	2.1($\pm$0.0022) $\times$ 10^{-8} M	[3]
	Ratiometric fluorescence change	Within 22 seconds	DMSO/H_2O, 40/60	(7.68 $\pm$ 0.29) $\times$ 10^{-8} M	[4]
	Fluorescence turn-on	-	Acetonitrile	1 $\times 10^{-7}$ M	[5]
	Ratiometric fluorescence (blue shift)	Within 1 second	CH_3CN/HEPES in water, 7/3	1.89$\times 10^{-7}$ M	[6]
	Ratiometric fluorescence change	Within 20 seconds	DMSO	(6.56 $\pm$ 0.26) $\times 10^{-8}$ M	[7]
	Fluorescence turn-on	-	DMSO/H_2O, 7/3	1.4$\times 10^{-7}$ M	[8]
	Fluorescence turn-on	-	THF	3.4$\times 10^{-8}$ M	[9]

	Fluorescence quenching	-	H_2O/THF, 9:1	3.8×10^{-6} M	[10]
	Fluorescence quenching	-	DMSO/H_2O, 1/99	2.95×10^{-8} M	[11]
	Fluorometric turn-off	15 seconds	DMSO/H_2O, 1/99	2.26×10^{-7} M	[12]
	Fluorometric (turn-off)	-	MeOH/H_2O, 4/1	1.3×10^{-7} M	[13]
	Fluorometric (turn-on)	-	DMSO	7.0×10^{-8} M	[14]
	Fluorometric (turn-on)	Within 8 minutes	DMSO/H_2O, 8/2	2.65×10^{-7} M	[15]
	Fluorometric (ratiometric)	Almost 15 seconds	MeOH/H_2O, 1/1	(22.1±0.89) µM	Present Work

References:

1. Z.-Z. Chen, R.-Y. Li, W.-Z. Zhang, Y. Zhang and W.-K. Dong, *New J. Chem.*, 2020, **44**, 21038.
2. S. Manickam and S. Kulathu Iyer, *RSC Adv.*, 2020, **10**, 11791.
3. S. Banerjee, M. Mandal, S. Halder, A. Karak, D. Banik, K. Jana and A. K. Mahapatra, *Anal. Methods*, 2022, **14**, 3209.

4. A. Maji, A. Biswas, A. Das, S. Gharami, K. Aich and T. K. Mondal, *New J. Chem.*, 2023, **47**, 11557.
5. W. Luo, Z. Yuwen, H. Li and S. Pu, *New J. Chem.*, 2022, **46**, 2411.
6. S. Munusamy, S. Swaminathan, D. Jothi, V. P. Muralidharan and S. K. Iyer, *RSC Adv.*, 2021, **11**, 15656.
7. A. Biswas, R. Mukherjee, A. Maji, R. Naskar, K. Aich, N. Murmu and T. K. Mondal, *Sens. Diagn.*, 2024, **3**, 1201.
8. S. Malkondu, S. Erdemirand and S. Karakurt, *Dyes Pigm.*, 2020,**174**,108019.
9. T. S. Reddy, H. Moon and M.-S. Choi, *Spectrochim Acta A.*, 2021, **252**, 119535.
10. Y. Li, Z. Gu, T. He, X. Yuan, Y. Zhang, Z. Xu, H. Qiu, Q. Zhang and S. Yin, *Dyes Pigm.*, 2020, **173**, 107969.
11. B. Zuo, L. Liu, X. Feng, D. Li, W. Li, M. Li, M. Huang and Q. Deng, *Dyes Pigm.*, 2021, **193**, 109534.
12. Q. Zou, J. Du, C. Gu, D. Zhang, F. Tao and Y. Cui, *J. Photochem. Photobiol. A. Chem.*, 2021, **405**, 112993.
13. R. Mehta and V. Luxami, *ChemistrySelect*, 2020, **5**, 13429.
14. K. Satheeshkumar, P. Saravanakumar, A. Kalavathi, K. N. Vennila, K. P. Elango, *Spectrochim Acta A.*, 2023, **302**, 123054.
15. B. Tavakoli, S. Meghdadi, Z. Salarvand, K. Eskandari, A. Amiri and M. Amirnasr, *J. Photochem. Photobiol. A. Chem.*, 2023, **440**, 114661.