A Ratiometric Small-Molecule Fluorescent Probe for the Selective Detection of Hypochlorite by an Oxidative Cyclization Reaction: Application to Commercial Disinfectants and Live Cells

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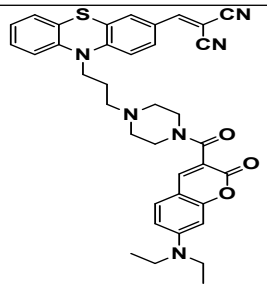
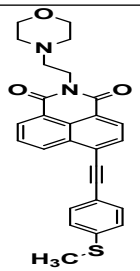
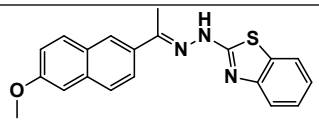
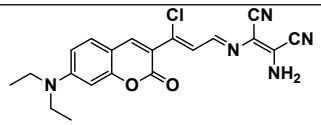
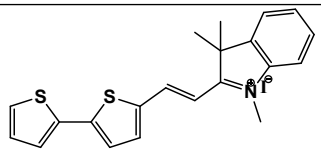
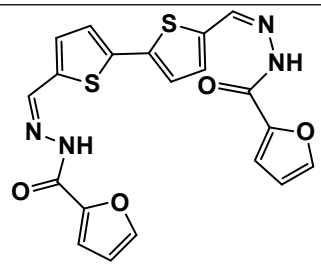
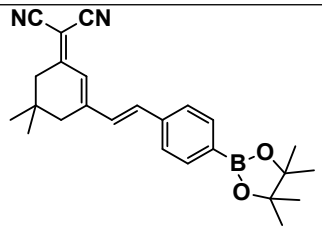
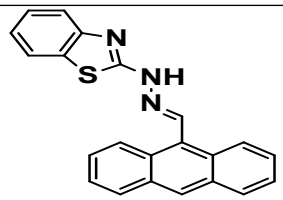
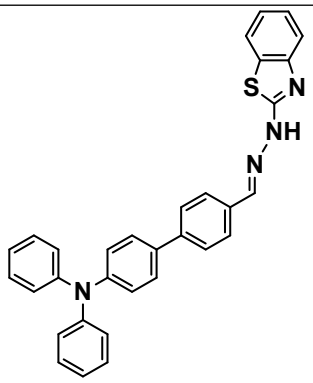
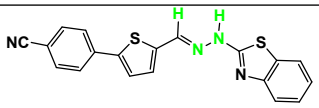
1. Table S1 Comparison between previously reported ClO^- sensors with the current work

Sl. No.	Probe structure	Solvent	Sensor type	LOD	Application	Reference
1.		DMSO	Ratiometric	0.321 μM	RAW264.7 macrophage cells	1
2.		DCM	Ratiometric	36 nM	HeLa cells	2
3.		Ethanol	Ratiometric	1.7 nM	A549 cells	3
4.		Ethanol	Turn on	0.015 μM	A549 cells	4
5.		Nearly 100% aqueous solution	Turn on	25 nM	Sprouts, Arabidopsis, Zebrafish	5
6.		DMF	Turn on	4.2 μM	Zebrafish	6

7.			Turn on	1.46 μM	Zebrafish	7
8.		DMF	Ratiometric	14 nM	Test strips	8
9.		THF	Turn on	8.05×10^{-7} M	Human breast cancer cell	9
10.		DMSO	Ratiometric	8.74 nM	Human breast cancer cell	This work

2. Solid state ClO^- sensing:

Small pieces of TLC sticks were coated with 1×10^{-5} M probe solution in aqueous DMSO followed by drying in an oven. The sticks were then dipped into different concentrated solutions of aqueous NaOCl and dried for 20 minutes. The sensing behaviour of the probe TPBN was examined and photographs were taken.

3. Computational method:

Theoretical calculations:

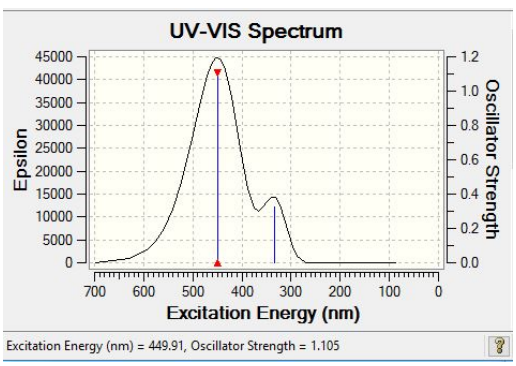


Figure S1. Absorption spectra of the Probe (**TPBN**)

Table S2 The vertical main orbital transition of the **TPBN** calculated by TDDFT method

Energy (eV)	Wavelength (nm)	Osc. Strength (f)	Transition
2.7557	449.91	1.1050	**HOMO ⟶ LUMO**
3.7043	334.71	0.3262	**HOMO ⟶LUMO +1**
3.7098	334.21	0.0267	**HOMO-1 ⟶ LUMO**

4. Live cell imaging study:

4.1 Cell line study:

To conduct this research, we obtained the NKE human normal kidney epithelial cell line and the MDA-MB 231 human breast cancer cell line from the National Center for Cell Science (NCCS) in Pune, India. The cell lines were cultured in T25 flasks using a medium composed of DMEM supplemented with 10% Fetal Bovine Serum (FBS), 1 mM sodium pyruvate, 2 mM L-glutamine, non-essential amino acids, 100 units/L penicillin, 100 mg/L streptomycin, and 50 mg/L gentamycin. These cells were maintained in a humidified incubator at 37 °C with 5% CO_2 to ensure their continued viability.

4.2 Cytotoxicity assay:

The cytotoxicity of the TPBN ligand was evaluated using the MTT cell proliferation assay[10,11] on both the MDA-MB-231 cancer cell line and the NKE normal cell line. In this experiment, cells were initially seeded in 96-well plates at a density of 1 × 104 cells per well and allowed to adhere for 24 hours before exposure to various concentrations of TPBN ligand (0 μM, 10 μM, 20 μM, 40 μM, 80 μM, 100 μM) for 24 hours. After the treatment period, the culture media was removed from each well, and the cells were washed with 1x PBS. Subsequently, each well was treated with 0.5 mg/ml of MTT solution and incubated for 4 hours, followed by a PBS rinse. The resulting formazan crystals were dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader. The measurement of cell viability was expressed as a percentage relative to the control experimental setup

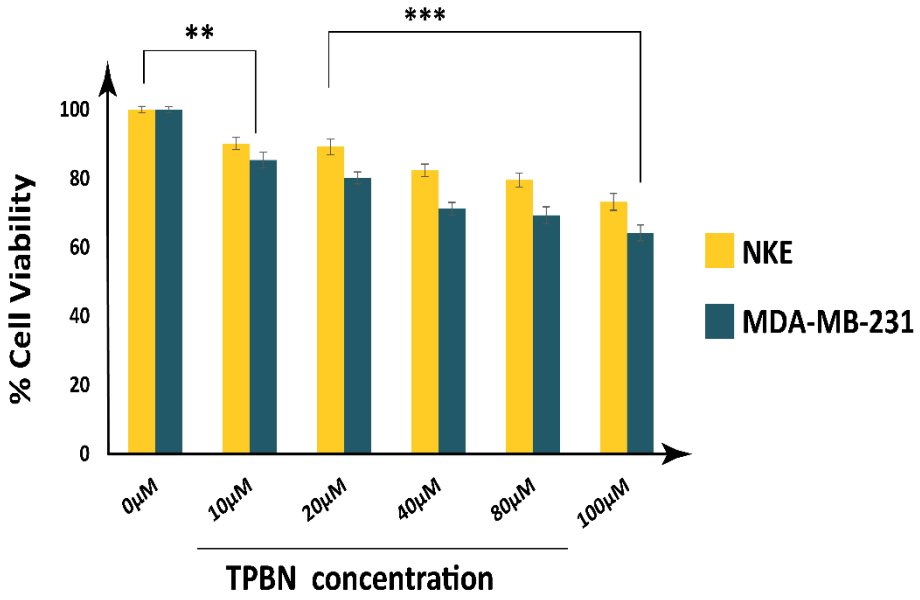


Figure S2. Cell survivability of MDA-MB 231 and NKE cells exposed to different ligand TPBN concentration. Data are representative of at least three independent experiments and bar graph shows mean ± SEM, ***$p < 0.0001$, **$p < 0.001$ were interpreted as statistically significant, as compared with the control.

5. Calculation of Limit of detection:

From the plot of fluorescence intensity ratio I_{434}/I_{527} vs concentration of ClO^- limit of detection was calculated by using the formula LOD= k ×δ/m where k= 3, δ is the standard deviation of the intensity ratio (I_{434}/I_{527}) of blank solution (0.003488) and m is the slope of the calibration curve (1.1974 ×10^6).

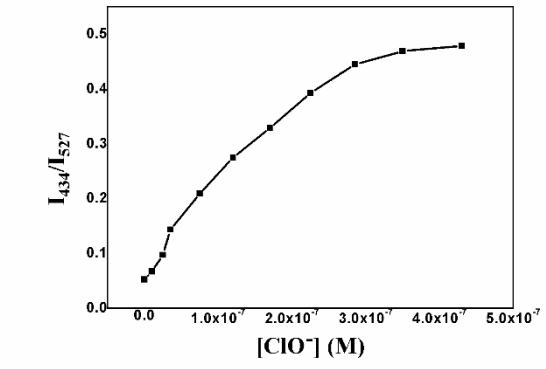


Figure S3. Plot of fluorescence intensity ratio vs concentration of ClO^-

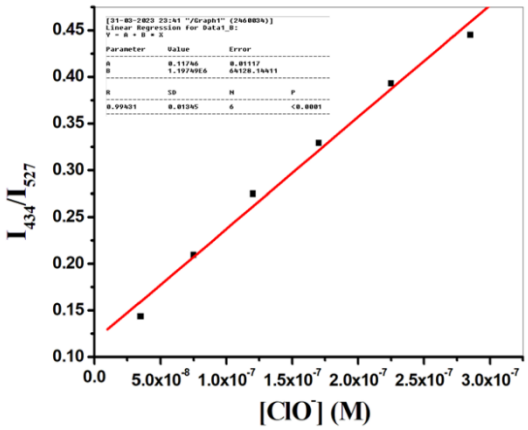


Figure S4. Calibration of the probe at an intensity ratio I_{434}/I_{527} depending on ClO^- concentration.

LOD= 8.74 nM (R^2=0.994)

6.pH effect:

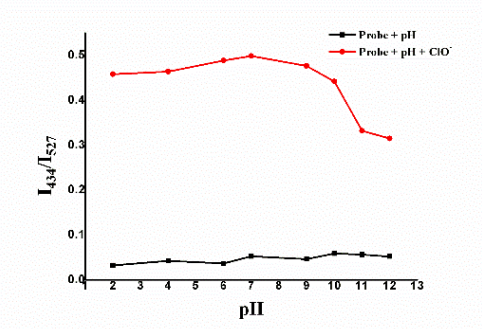


Figure S5. Variation of fluorescence intensity ratio (I_{434}/I_{527}) of TPBN with the change in pH in DMSO/H_2O (4:6 v/v) solution (λ_{ex}=411 nm).

7. Application of the probe in water samples:

Table S3: Water sample study for TPBN

Water sample	Spiked (µM) ClO^-	Found (µM)	% Recovery	RSD (%)
Tap water	20	19.69 (± 0.23)	98.45	1.16
	25	24.67 (± 0.41)	98.7	1.66
Pond water	20	19.08(± 0.26)	95.4	1.36
	25	24.12 (±0.38)	96.5	1.57

8. Calculation of first order rate constant (k′):

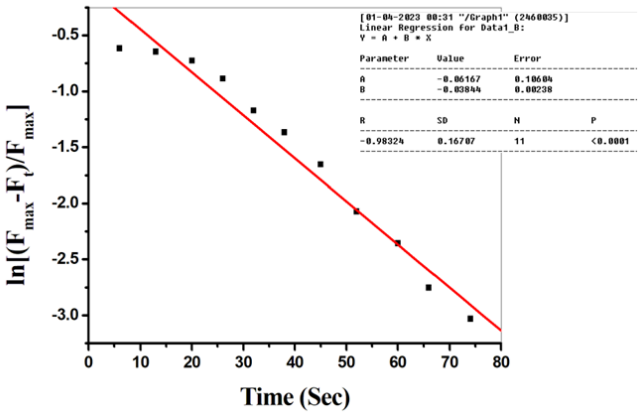


Figure S6. First order kinetic plot of probe (1×10^{-5}M) in the presence of 1×10^{-4}M ClO^- solution (λ_{ex}=411 nm)

First order rate constant k′= 0.0384 s^{-1}

9. Emission spectra of

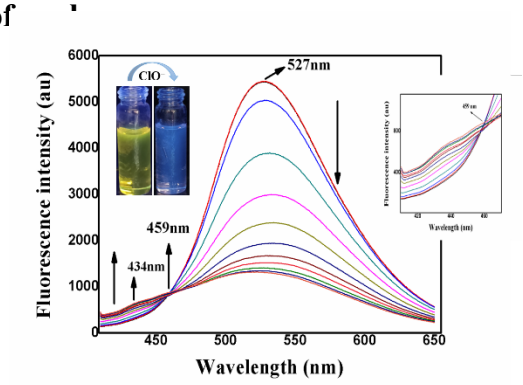


Figure S7. Fluorescence intensity changes of TPBN (1×10^{-5}M) upon gradual addition of NaOCl in **DMSO-water** (4:6 v/v) (λ_{ex}=411 nm).

10. Job's plot of the probe TPBN for ClO^-

Job's plots were drawn by plotting ΔI.X(host) *vs* X(host) (ΔI = change of intensity ratio of the emission spectrum [I_{434}/I_{527}] for TPBN during titration and X(host) is the mole fraction of the analyte in each case respectively).

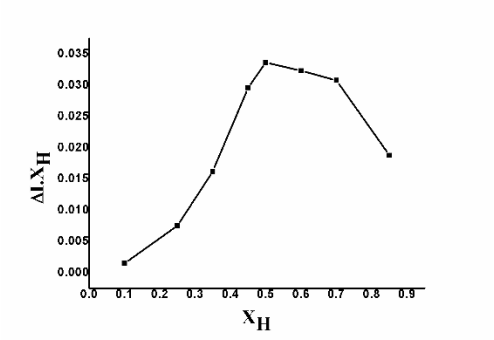
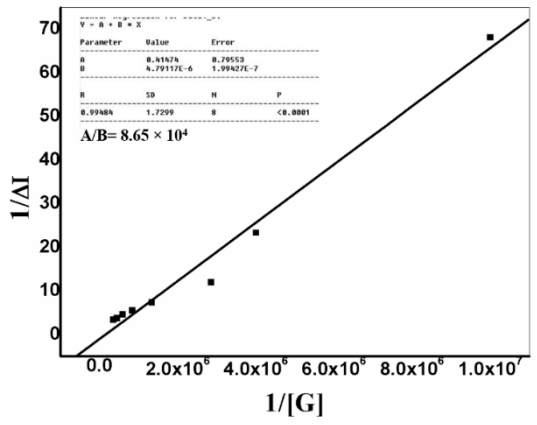


Figure S8. Job's plot of TPBN with ClO^- using fluorescence data

11. Determination of binding constant value (K_a) using linear method for TPBN

Binding constant value (K_a) was calculated by plotting 1/ΔI vs 1/[G] [ΔI= change of intensity ratio of the emission spectrum [I_{434}/I_{527}] for TPBN during titration and [G] is the concentration of ClO^- in each case respectively).



Binding constant $K_a(A/B) = 8.65 \times 10^4$

Figure S9. Binding constant value of TPBN with ClO^- using fluorescence data

12. NMR spectra: ^{1}H-NMR, ^{13}C-NMR

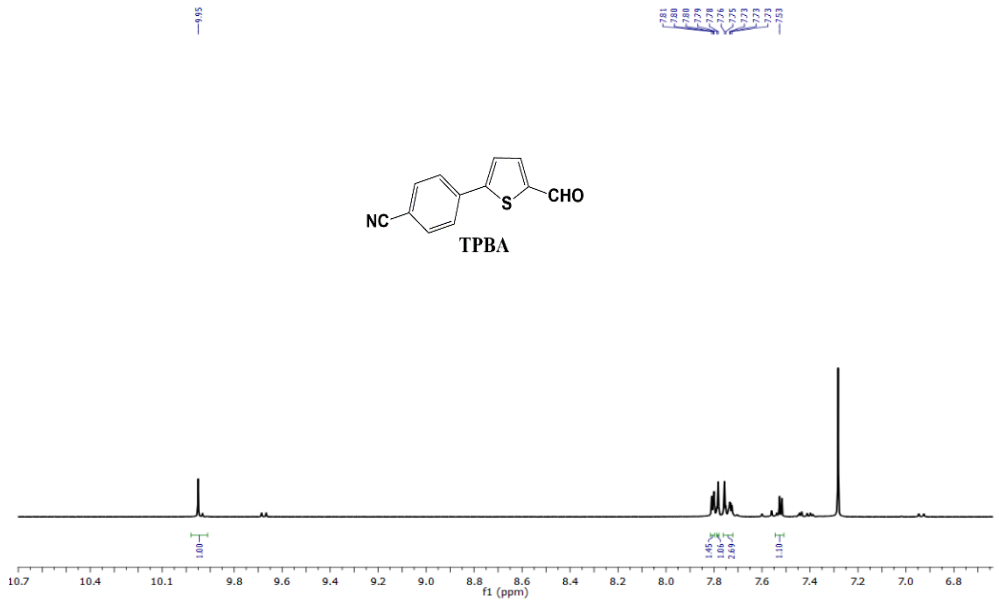


Figure S10: ^{1}H-NMR spectra of TPBA in $CDCl_3$

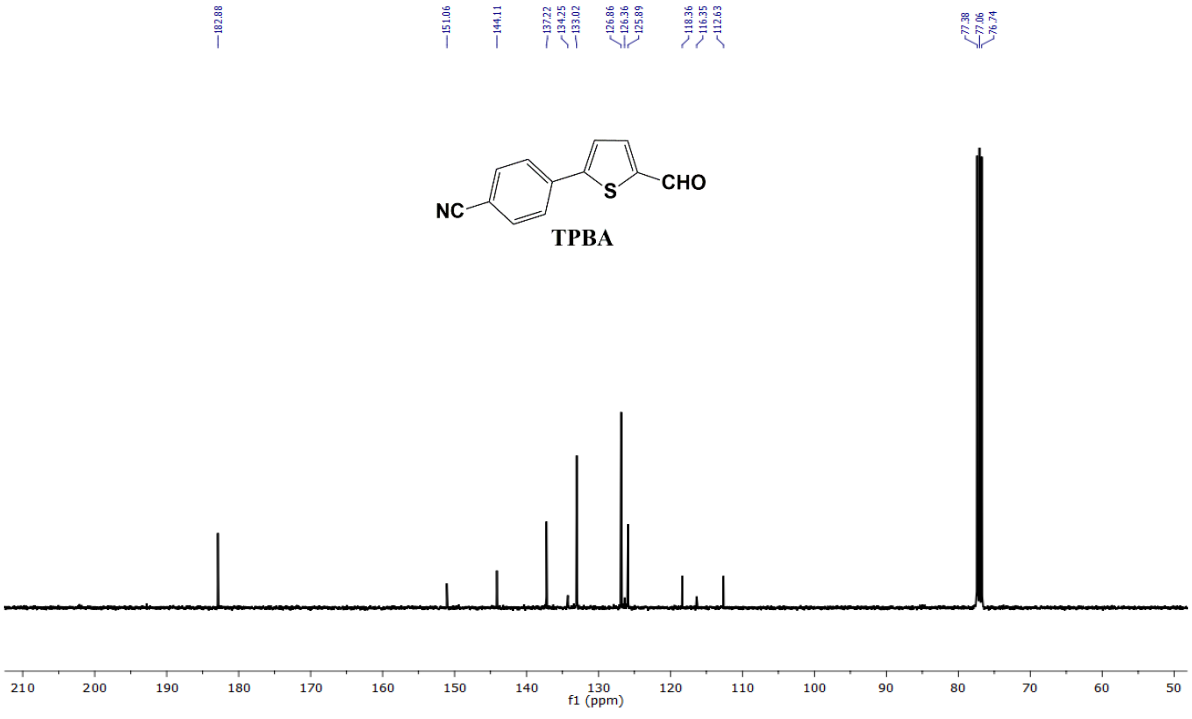


Figure S11: ^{13}C-NMR spectra of TPBA in $CDCl_3$

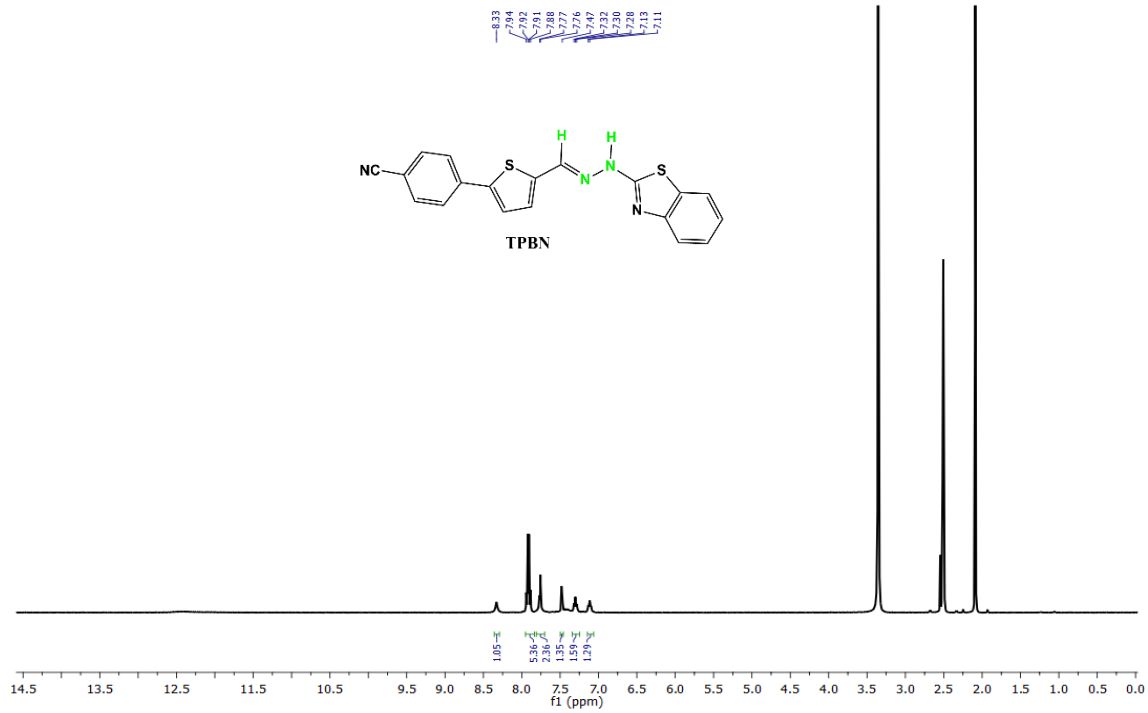


Figure S12: ^{1}H-NMR spectra of TPBN in DMSO-d_6

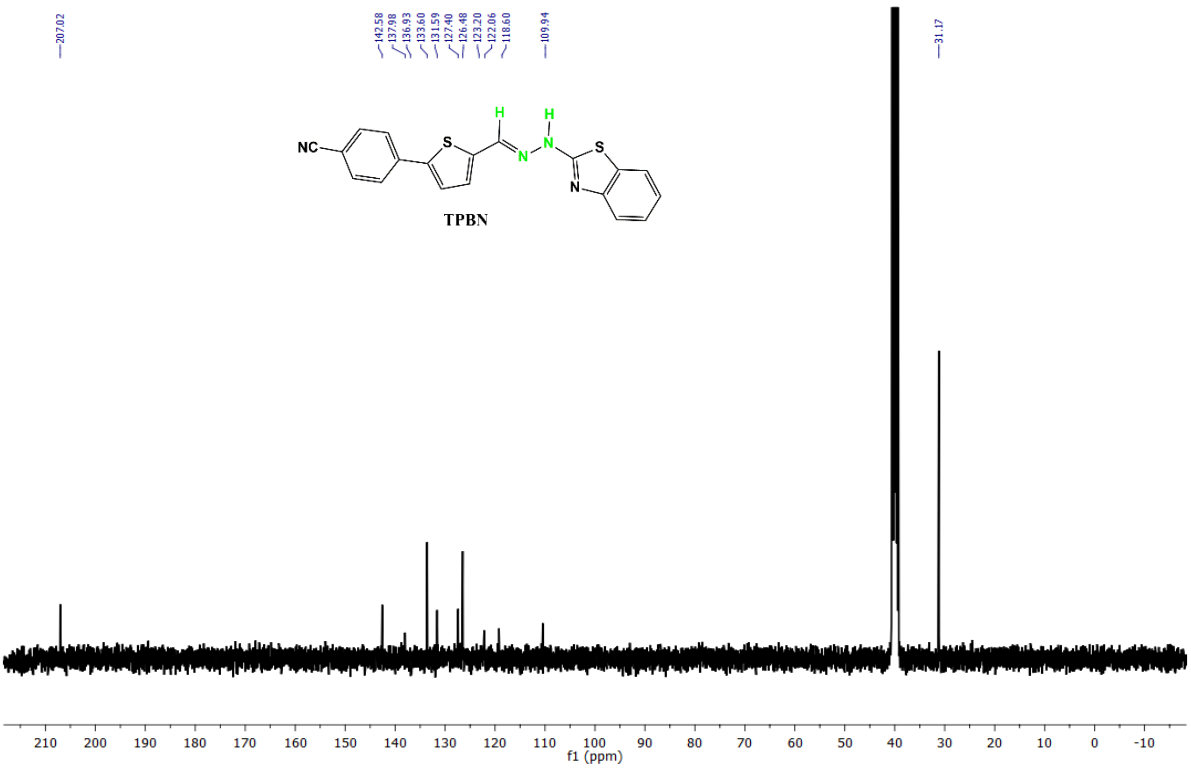


Figure S13 :^{13}C-NMR spectra of TPBN in DMSO-d_6

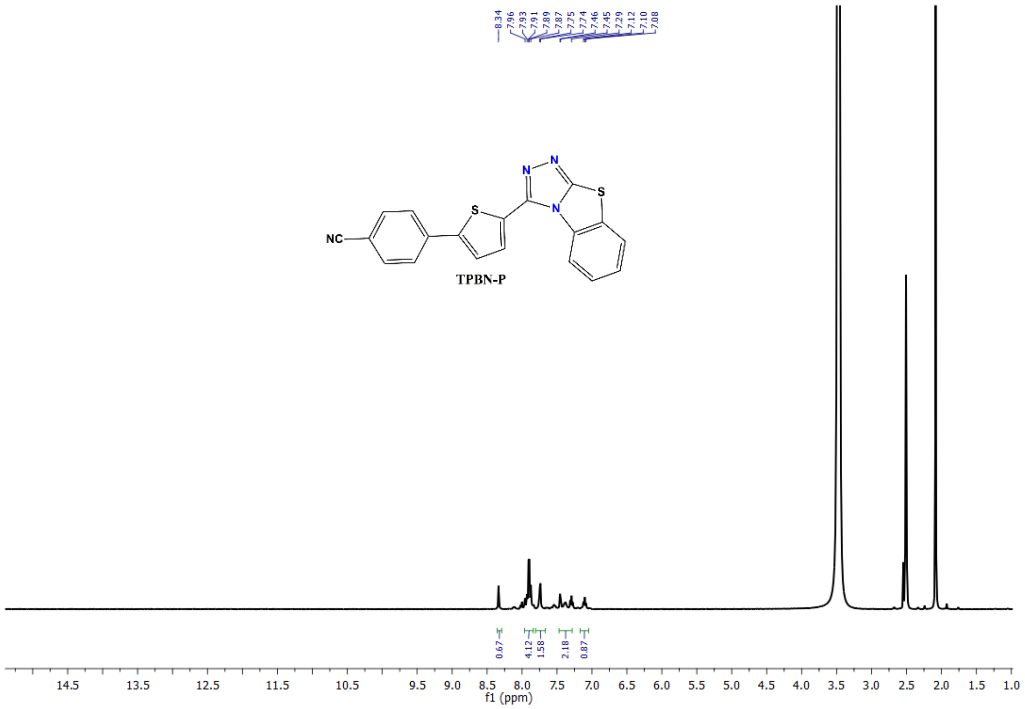


Figure S14: ^{1}H-NMR spectra of product TPBN-P in DMSO-d_6

13. Mass spectra

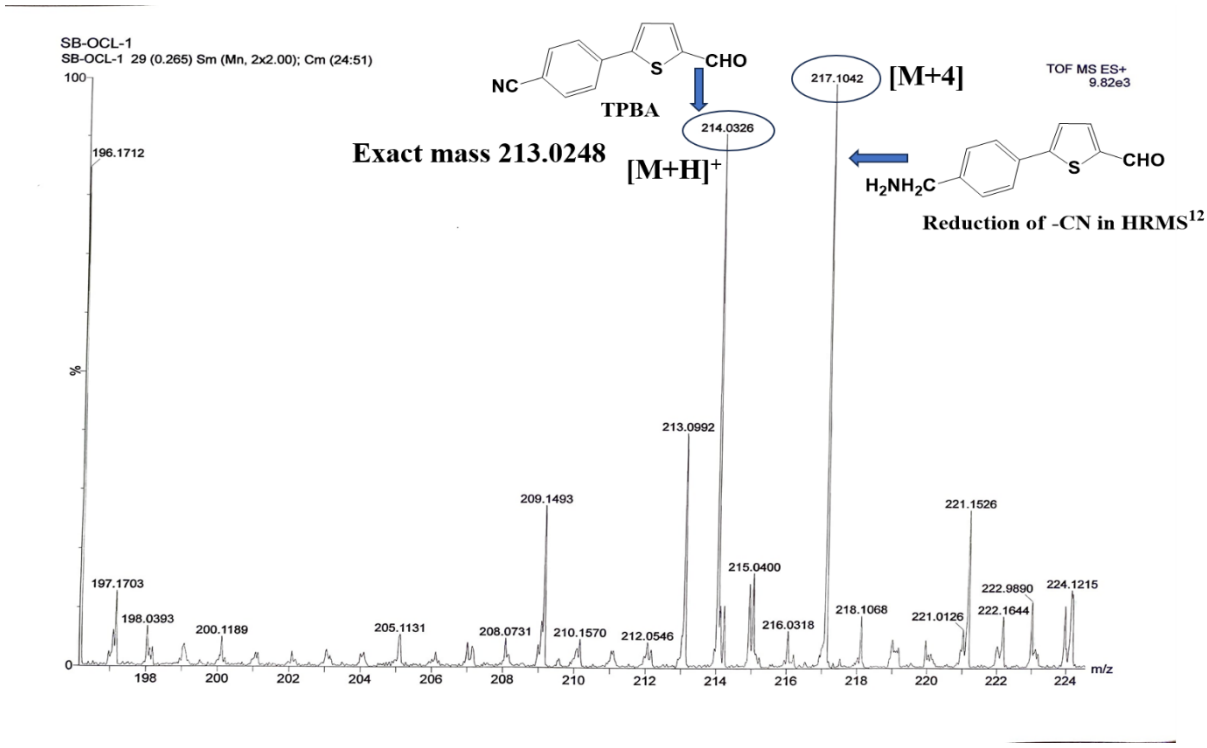


Figure S15: ESI-MS of TPBA

Reference for reduction of -CN in HRMS:

Z. Gu, J. Ma, X. Zhao, J. Wu, D. Zhang, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2969–2972

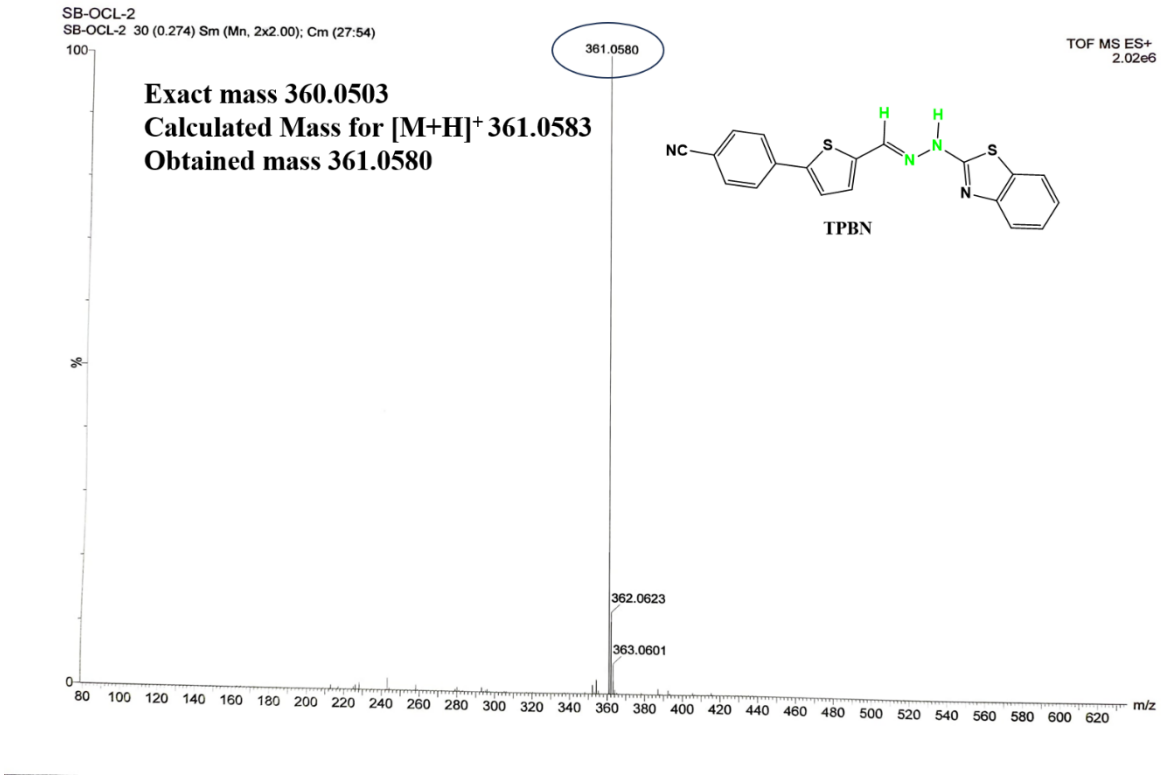


Figure S16: ESI-MS of probe TPBN

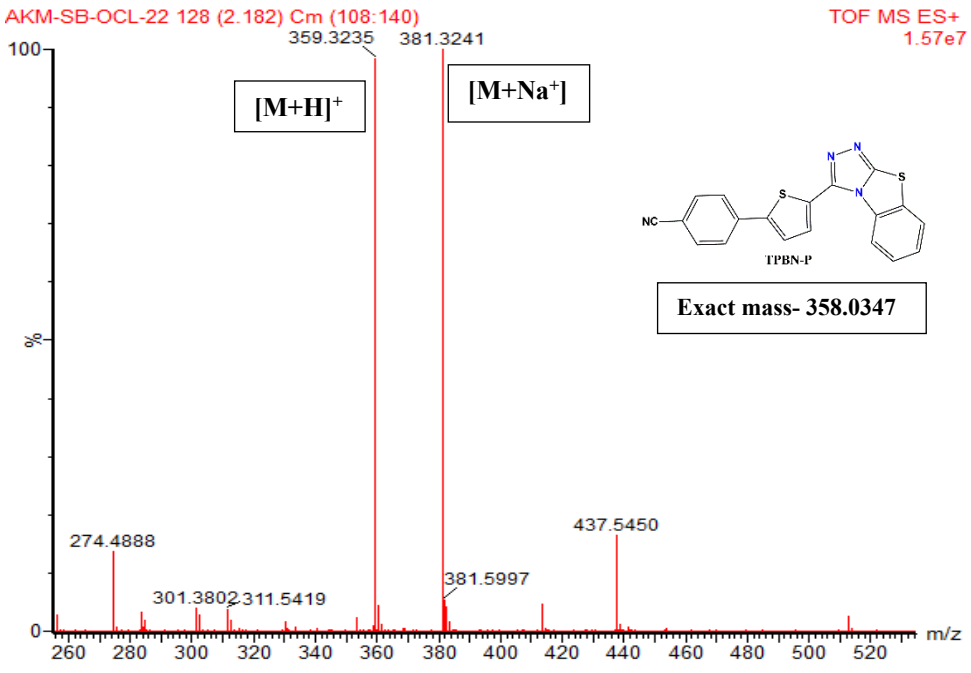


Figure S17: ESI-MS of product TPBN-P

14. References:

1. W. Wang, J.Y. Ning, J.T. Liu, J.Y. Miao and B.X. Zhao, *Dyes and Pigments*, 2019, **171**, 107708.
2. G. Zhou, S. Hou, N. Zhao, N. Finney and Y. wang, *Dyes and Pigments*, 2022, **204**, 110394.
3. N.N. Li, Y.E. Gao, X.Y. Xu, P. Qiu, Y. Gao, M. Yan, Q. Zhang, W.Y. Lin, Z.Y. Xing and Z.A. Zong, *Dyes and Pigments*, 2023, **210**,110965.
4. Q. Yang, X. Zhong, Y. Chen, J. Yang, C. Jin and Y. Jiang, *Analyst*, 2020, **145**, 3100.
5. R. Chen, S. Xing, T. Hu, Y. Li, J. Chen, Q. Niu and T. Li, *Analytica Chimica Acta*, 2023, **1237**, 340557.
6. P. A. Kim, D. Choe, H. So, S. Park, B. Suh, S. Jeong, K.T. Kim, C. Kim and R.G. Harrison, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2021, **261**, 120059.
7. L. Zhen, J. Lan, S. Zhang, L. Liu, R. Zeng, Y. Chen and Y. Ding, *Analytical Methods*, 2022, **14**, 2147-2152.
8. C. Jiang, Y. Yao, C. Kong, J. Du, J. Meng and C. Yao, *Analytical Methods*, 2019, **11**, 4157.
9. M. Mandal, U.N. Guria, S. Halder, A. Karak, D. Banik, K. Jana, A. Kar and A.K. Mahapatra, *Organic and Biomolecular Chemistry*, 2022, **20**, 4803.
10. P.R. Twentyman and M. Luscombe, *Br J Cancer*., 1987, **56**, 279-85.
11. S. Paul, S. Maity, S. Halder, B. Dutta, S. Jana, K. Jana and C. Sinha, *Dalton Trans*., 2022, **51**, 3198-3212.
12. Z. Gu, J. Ma, X. Zhao, J. Wu, D. Zhang, *Rapid Commun. Mass Spectrom.*, 2006, **20**, 2969–2972