Small-Molecule Fluorogenic Probe for the Detection of Hypochlorite and Its Application in the Bio-imaging of Human Breast Cancer Cells

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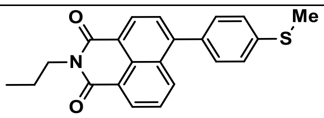
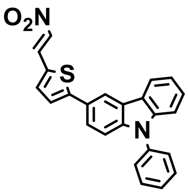
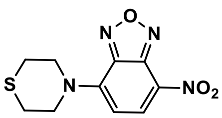
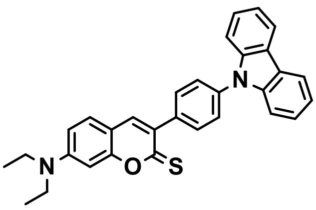
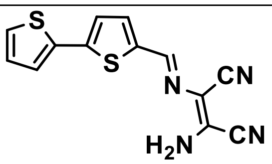
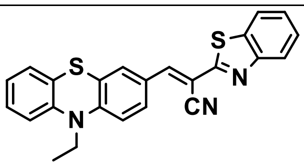
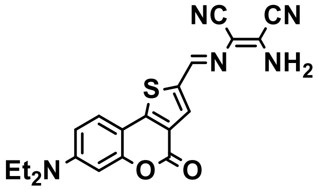
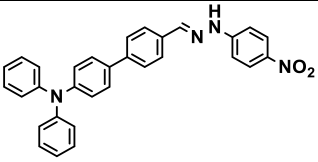
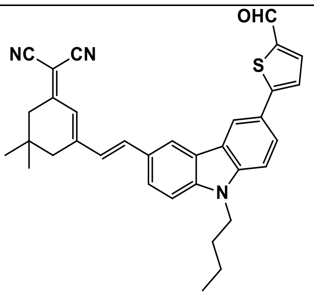
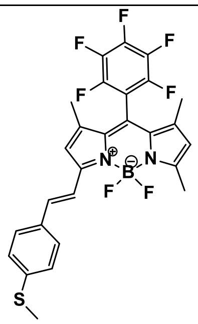
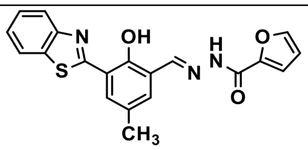
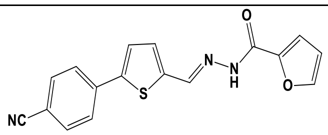
1. Table S1 Comparison between previously reported ClO^- sensors with the current work

Sl. No.	Probe structure	Solvent	Sensor type	LOD	Application	Reference
1.		DMF	Ratiometric	0.02μM	MCF-7 cells	Zhou et al., *Chem. Commun*., 2021, **57**, 11366
2.		DMSO (1:1)	Ratiometric	71.4 nM	HeLa cells	Li et al., *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2020, **241**, 118672
3.		Acetonitrile	Turn off	72nM	-	Podsiadły et al., *Dyes and Pigments*, 2021, **193**, 109563
4.		Acetonitrile	Turn on	0.17μM	HeLa cells	Yoon et al., *Sensors & Actuators: B. Chemical*, 2020, **317**, 128213
5.		~100% aqueous solution	Turn on	8.3nM	HeLa cells	Niu et al., *Journal of Molecular Liquids*, 2020, **320**, 114396
6.		DMSO	Turn off	0.64 μM	HepG-2 cells	Qu et al., *Ind. Eng. Chem. Res.* 2018, **57**, 7735−7741

7.		DMF	Ratiometric	94 nM	RAW264.7 cells	Shao et al., *New J. Chem.*, 2020, **44**, 6232
8.		DMSO	Turn on	0.36 µM	Zebrafish	Liu at al., *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2021,**258**, 119827
9		DMF	Ratiometric	1.4 × 10^{-7}M	HepG2	Li et al., Dyes and pigments, 2022, 208, 110879
10.		DMF	Ratiometric	59nM	Zebrafish and mice (in vivo)	Kim et al., Anal Chem, 2019, 91, 4172-4178
11.		DMSO (1:9)	Turn on	1.44 nM	HepG2 cells	Yan et al., *J Fluoresc*, 2021,**31**, 569–576
12.		THF/H_2O (2:8)	Turn On	53.8nM	Human breast cancer cells (MDA-MB 231)	This work

2. Solid state ClO^- sensing:

Small pieces of TLC sticks were dipped into 1×10^{-5}M probe solution followed by drying in open air. The sticks were further dipped into different concentrated solutions of NaOCl and dried for 5 minutes. After that photographs were taken to examine the sensing behaviour of the probe TPHZ.

3. Computational method:

Theoretical calculations:

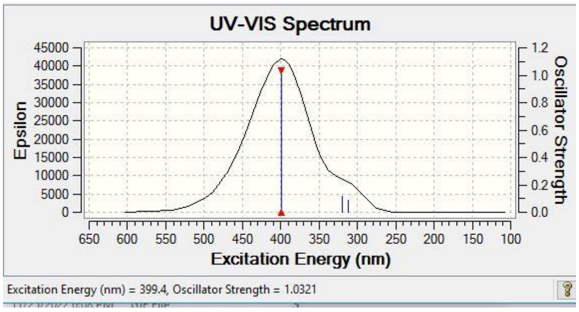


Figure S1. Absorption spectra of the Probe (**TPHZ**)

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

Energy (eV)	Wavelength (nm)	Osc. Strength (f)	Transition
3.1042	399.40	1.0321	**HOMO → LUMO**
3.8713	320.27	0.1164	**HOMO-1 → LUMO**
3.9800	311.52	0.0907	**HOMO →LUMO +1**

4. Live cell imaging study:

Cytotoxicity assay:

In the present study Human breast cancer cell line MDA-MB 231 and human normal lung fibroblast cell line WI-38 have been used and MTT cell proliferation assay[1] was performed to assess the cytotoxic effect of the ligand TPHZ. In brief, cells growing in a log phase were first seeded in 96-well plates at a concentration of 1×10^4 cells per well and were incubated overnight at 37 °C under 5% CO2. The cells were then exposed to the different working concentration of ligand TPHZ (0 µM, 10 µM, 20 µM, 40 µM, 80 µM, 100 µM) for 24 hrs.

Following incubation, 0.5 mg/ml of MTT solution were added to each well and incubated for 4h, and the cells were then rinsed with 1X PBS. The formazan crystals that formed were then dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as a percentage of the experimental design used as the control.

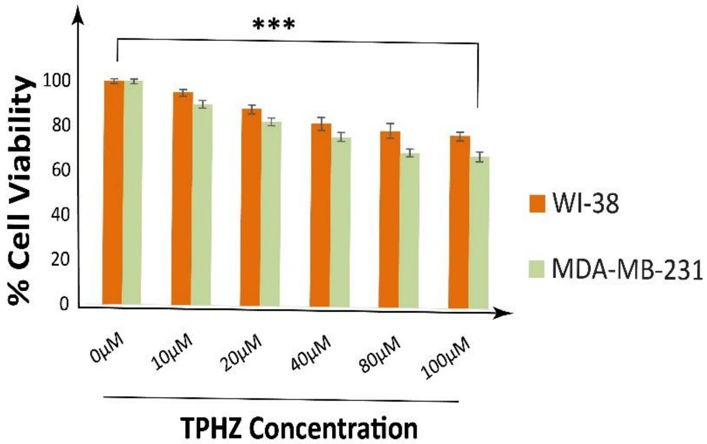


Figure S2. Cell survivability of MDA-MB 231 and WI-38 cells exposed to different ligand **TPHZ** concentration. Data are representative of at least three independent experiments and bar graph shows mean ± SEM, $*p < 0.0001$, $**p < 0.001$, $***p < 0.01$ were interpreted as statistically significant, as compared with the control.

5. Calculation of Limit of detection:

From the plot of fluorescence intensity I_{399} 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 blank solution (4484.72) and m is the slope of the calibration curve.

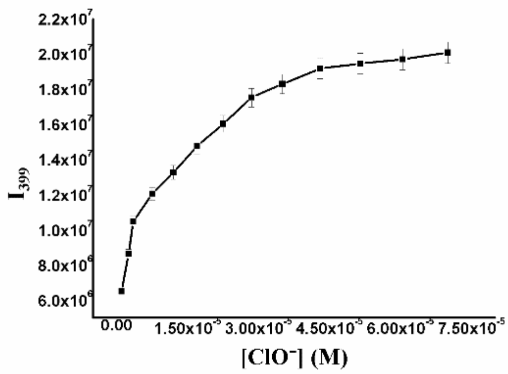


Figure S3. Plot of fluorescence intensity vs concentration of ClO^-

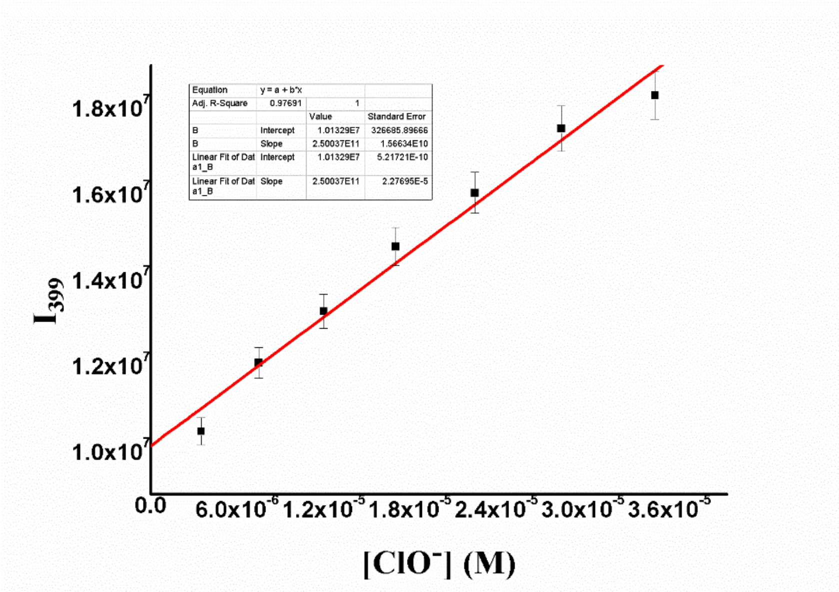


Figure S4. Calibration of the probe at an intensity I_{399} depending on ClO^- concentration.

LOD= 53.8 nM (R^2=0.977)

6.pH effect:

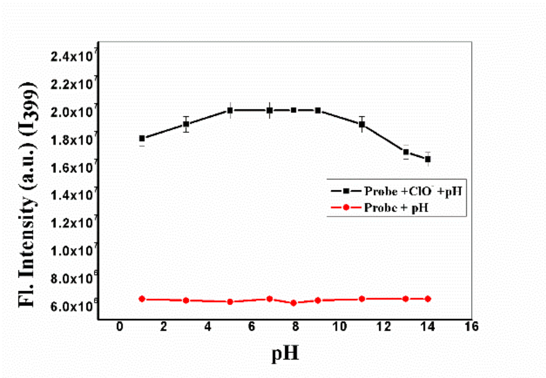


Figure S5. Effect of pH on fluorescence of TPHZ and TPHZ+ClO^- in THF/H_2O (λ_{ex}-339nm)

7. Application of the probe in commercial bleach and water samples:

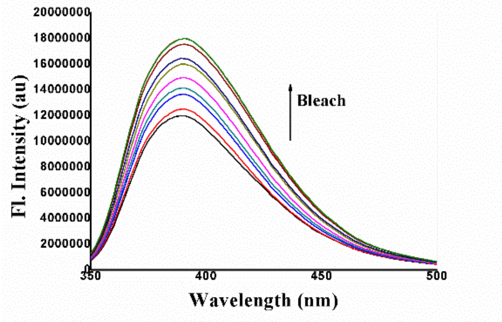


Figure S6: Fluorescence intensity changes of TPHZ upon gradual addition of bleach in **THF-water** (2:8 v/v) (λ_{em}=399nm)

Table S3: Water sample study for TPHZ

Water sample	Spiked (μM) ClO^-	Found (μM)	% Recovery	RSD (%)
Tap water	15	14.79 (± 0.33)	98.6	2.2
	30	29.82 (± 0.51)	99.4	1.7
River water	15	15.045(± 0.36)	100.3	2.4
	30	29.25 (±0.58)	97.5	2.0

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

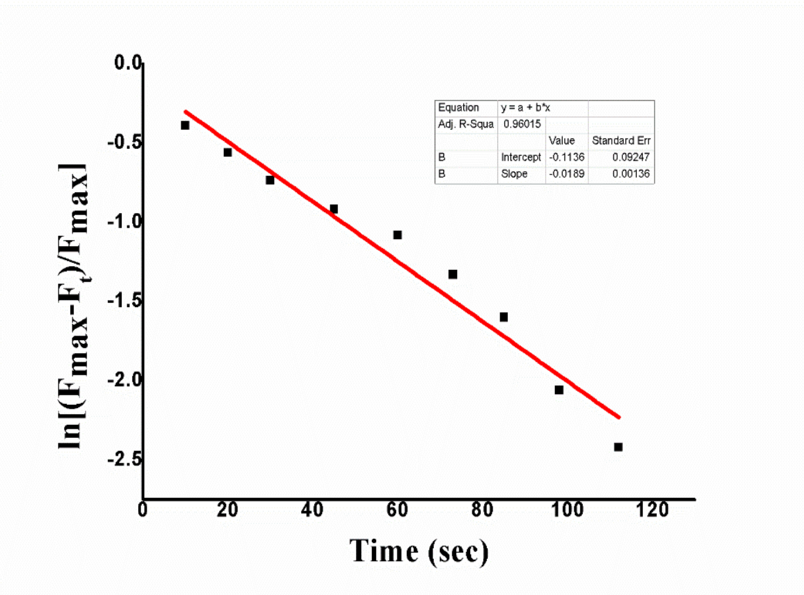


Figure S7. First order kinetic plot of probe (1×10^{-5}M) in the presence of 1×10^{-4}M ClO^- solution (λ_{em}=399nm)

First order rate constant k´= 0.0189 s^{-1}

9. Emission spectra of probe:

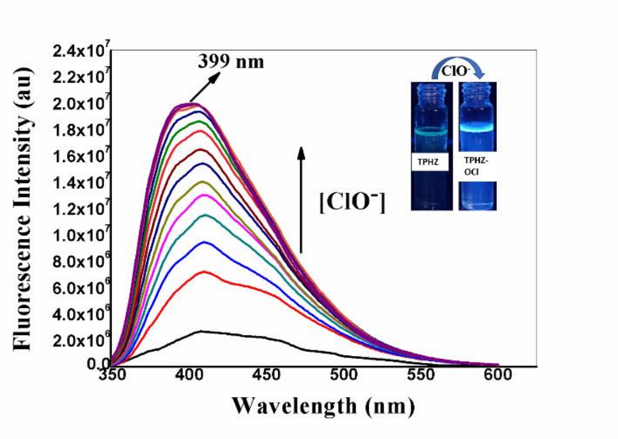


Figure S8. Fluorescence intensity changes of TPHZ (1×10^{-5}M) upon gradual addition of NaOCl in **THF-water** (2:8 v/v) (λ_{em}=399nm).

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

Job's plots were drawn by plotting ΔF.X(host) *vs* X(host) (ΔF = change of intensity of the emission spectrum [I_{399}] for TPHZ during titration and X(host) is the mole fraction of the host in each case respectively).

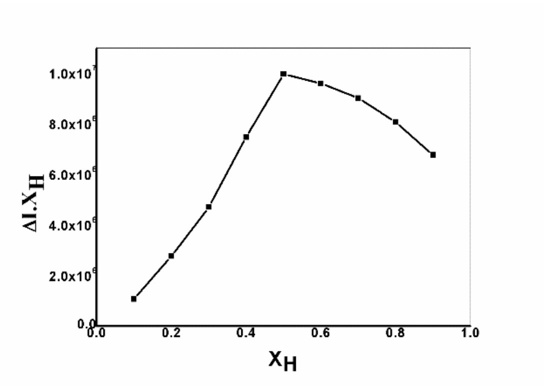
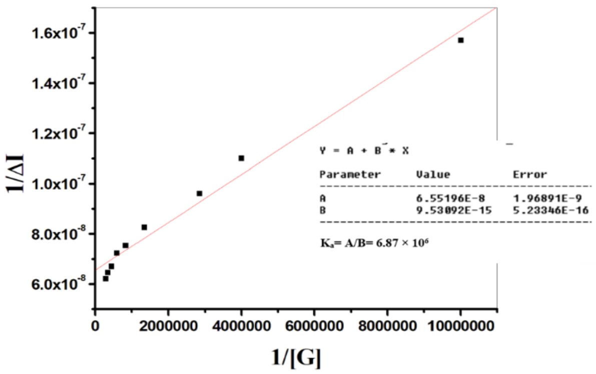


Figure S9. Job's plot of TPHZ with ClO^- using fluorescence data

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

Binding constant value (Ka) was calculated by plotting 1/ΔI *vs* 1/[G] [(ΔI = change of intensity of the emission spectrum at 399nm for TPHZ during titration and [G] is the concentration of ClO^- in each case respectively).



Binding constant K_a(A/B) = 6.87 ×10^{6}

Figure S10. Binding constant value of TPHZ with ClO^- using fluorescence data

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

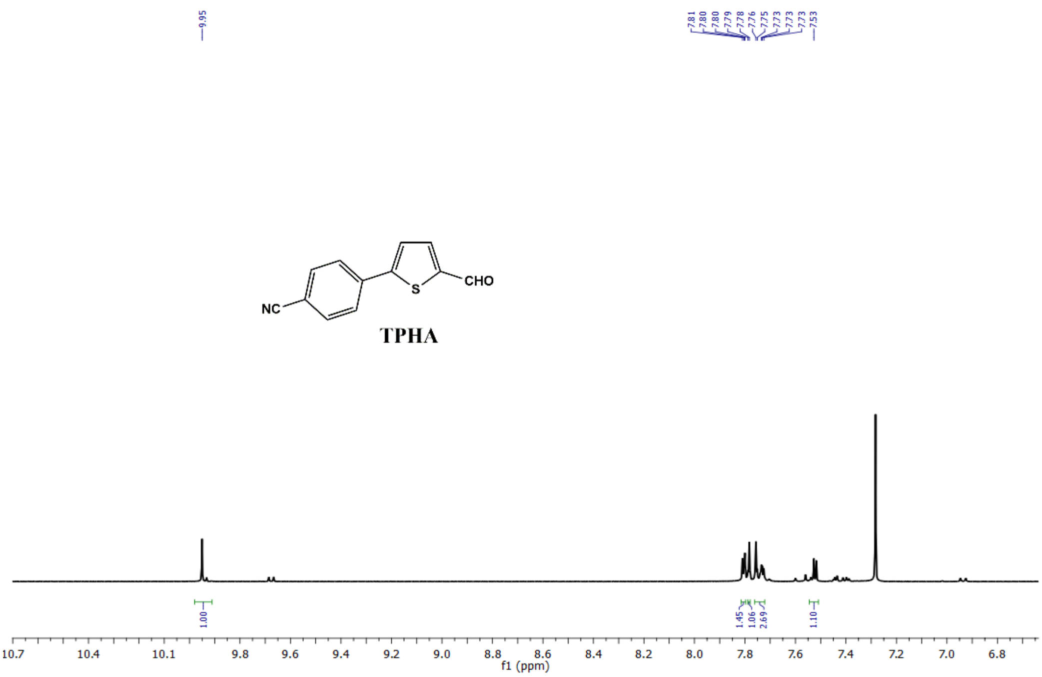


Figure S11: ^{1}H-NMR spectra of TPHA in $CDCl_3$

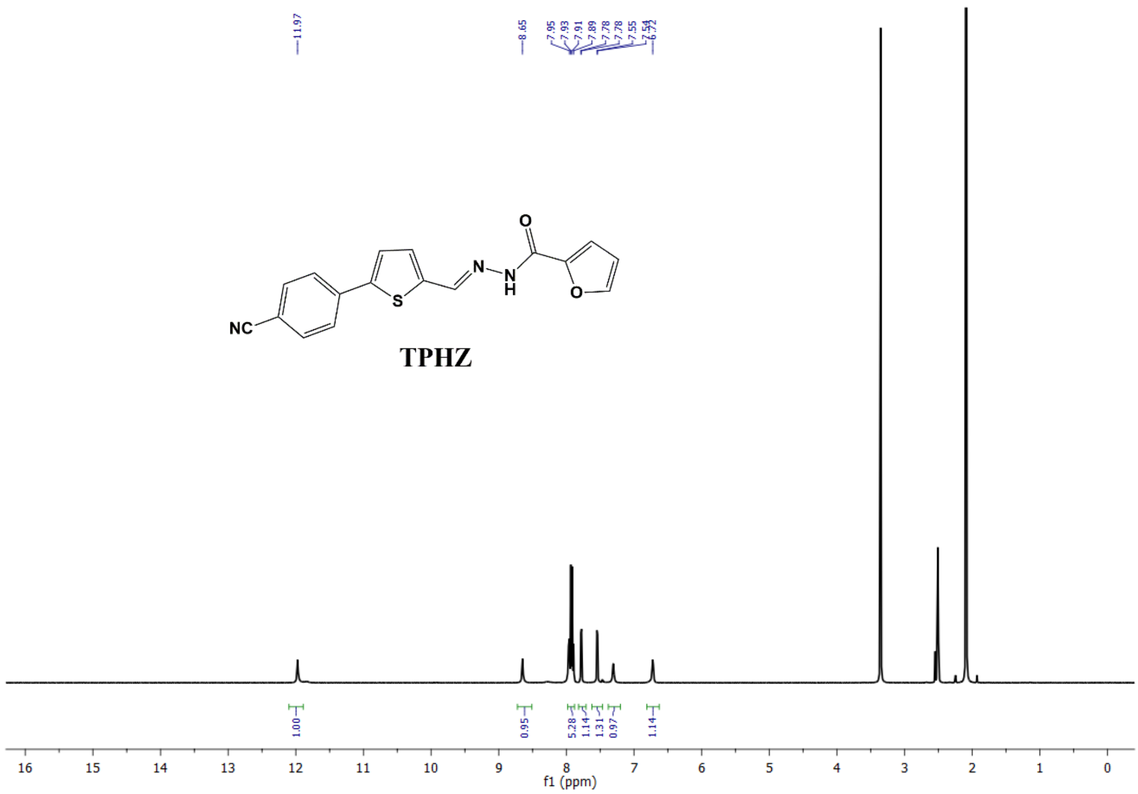


Figure S12: [1]H-NMR spectra of TPHZ in DMSO-d[6]

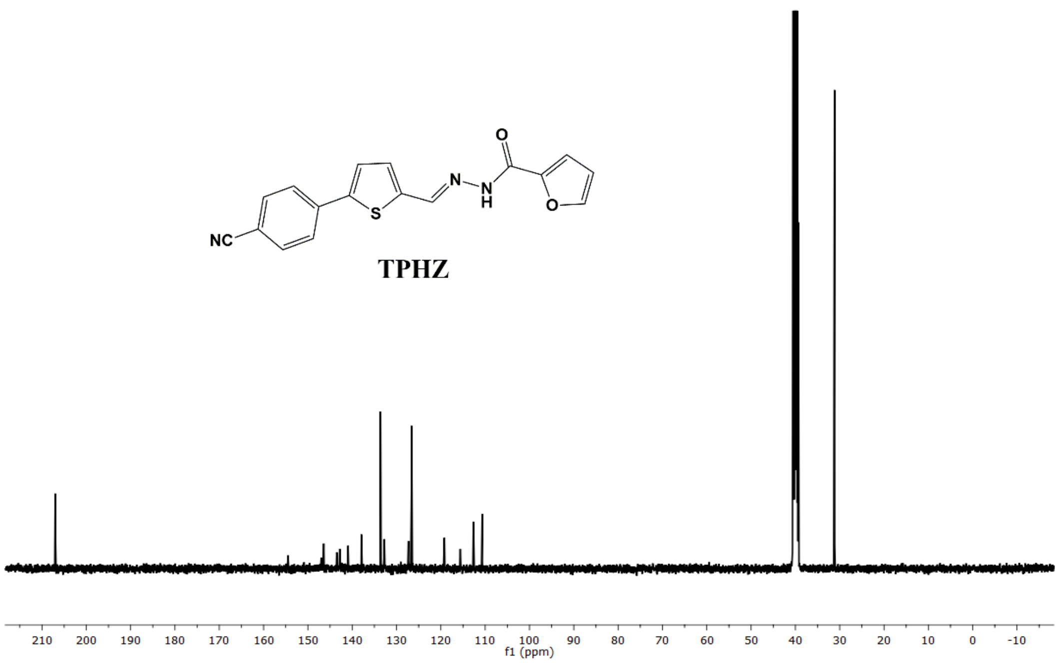


Figure S13 :^{13}C-NMR spectra of TPHZ in DMSO-d^6

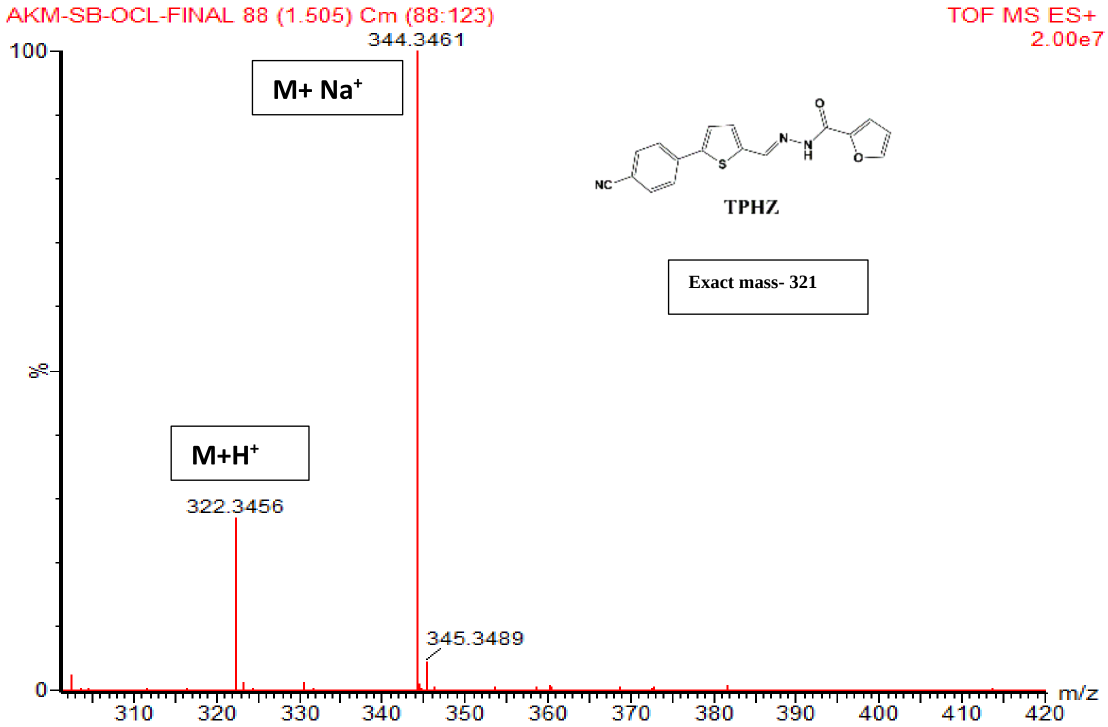


Figure S14 : ESI-MS of probe TPHZ

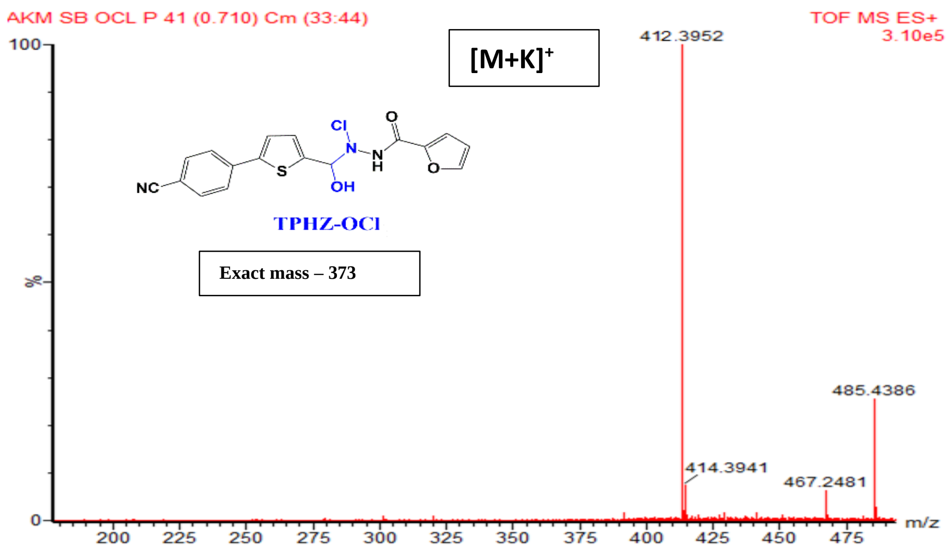


Figure S15: ESI-MS of product TPHZ-OCl

Reference:

1) P. R. Twentyman and M. Luscombe, *Br. J. Cancer*,1987, **56**, 279–285.