Electronic Supplementary Material (ESI) for Analytical Methods.

Switching to a 'Turn-on' fluorescent probe for rapid detection of hydrazine in human breast cancer cell and test-strip

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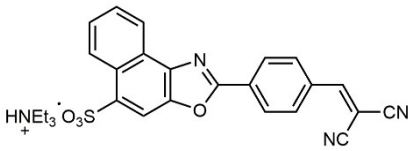
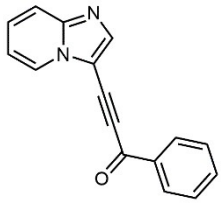
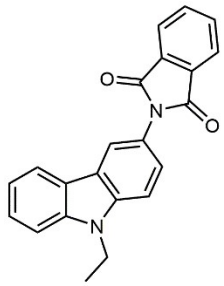
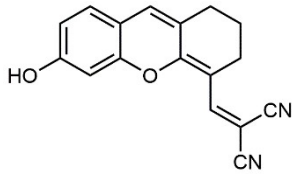
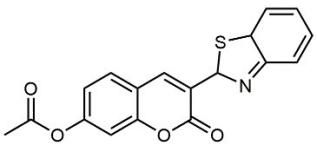
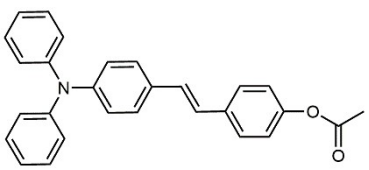
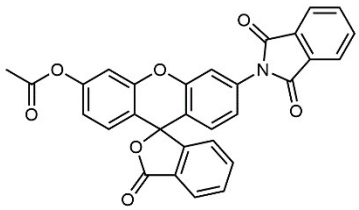
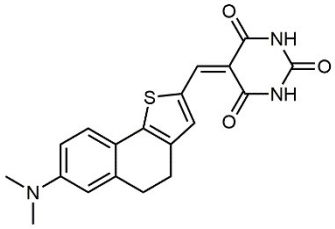
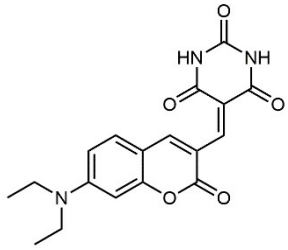
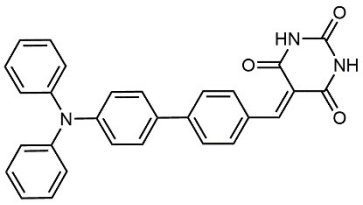
Table S1 Comparison between the previously reported hydrazine probe with our newly designed probe

Structure of the probe	Solvent	Mode of sensing	Application	LOD	Response time	Ref
	PBS buffer	Blocked ICT	Whatman paper strips	1.79×10^{-9} M	40 s	1
	Tris/HCl buffer			0.253 ppb	1 h	2
	CH_3CN–HEPES (1:1, v/v)		filter paper-based test strips	2.673×10^{-6} M	60 min	3
	DMSO/PBS buffer	Blocked ICT	real water samples, live cell imaging	0.09 μM	1 h	4
	PBS-DMSO (v/v = 1/1)	-	real water samples, live cell imaging	11.9 nM	3 min	5
	DMSO/PBS buffer (4/6, v/v) m	Blocked ICT	real water samples, live cell imaging	8.47 nM	180 s	6

	DMSO-HEPES (1/1, v/v)	ICT On, PET Off	live cell imaging	1.83 ppb	-	7
	DMSO/PBS	Blocked ICT	live cell imaging	5×10^{-8} M	10 min	8
	DMSO/PBS buffer (9/1, v/v) m	-	real water samples, live cell imaging	40.8 nM	30 min	9
	THF-H_2O (4:6, v/v, 10 mM HEPES buffer, pH = 7.4)	Blocked ICT	TLC based test strip, live cell imaging	1.22×10^{-7} M	50 s	Our work

2. Absorption and emission spectral response of TPBT in the presence of hydrazine

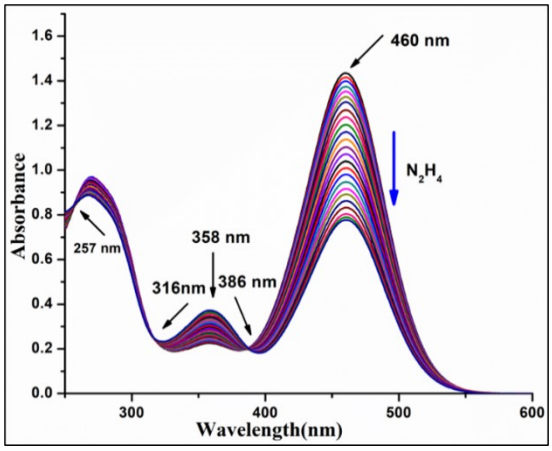


Figure S1. Absorption spectral data of the probe **TPBT** (4.0×10^{-5} M) in aqueous THF solution (THF/H_2O = 4:6 v/v, 10mM HEPES buffer, pH = 7.4), upon gradual addition of hydrazine (4.0×10^{-4} M).

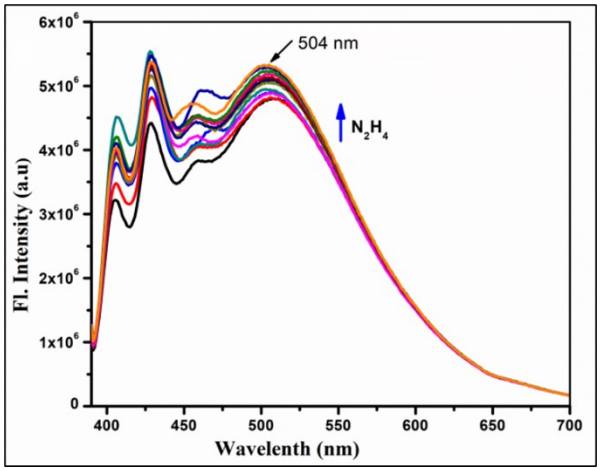


Figure S2. Emission spectral data of the probe **TPBT** (4.0×10^{-5} M) in aqueous THF solution (THF/H_2O =4:6 v/v, 10mM HEPES buffer, pH = 7.4) upon gradual addition of hydrazine (4.0×10^{-4} M), (Excitation= 380 nm).

3. Bar diagram

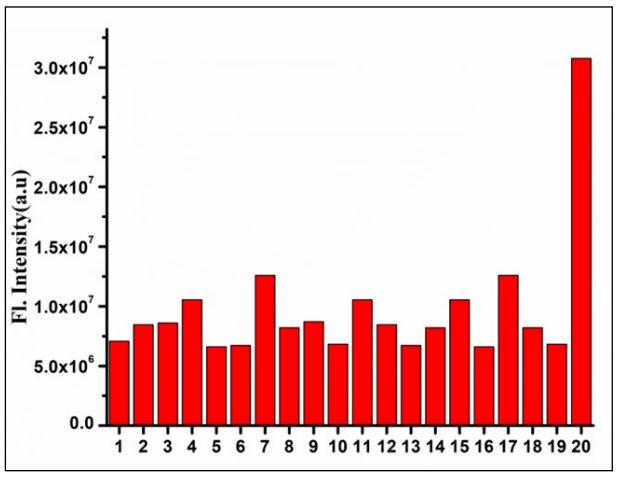


Figure S3. Change in emission in the selectivity study of the probe (40 μM) in the presence of different analytes (75 μM); where analytes are 1) Blank, 2) Cr^{3+}, 3) Fe^{2+}, 4) Na^{+}, 5) Al^{3+}, 6) Cu^{2+}, 7) Hg^{2+}, 8) Zn^{2+}, 9) F^{-}, 10) Cl^{-}, 10) SCN^{-}, 11) CN^{-}, 12) CH_3COO^{-}, 13) OCl^{-} 14) PA, 15) urea, 16) $PhNHNH_2$ 17) hydroxyl amine, 18) cysteine, 19) thiourea, 20) N_2H_4

4. Theoretical calculations:

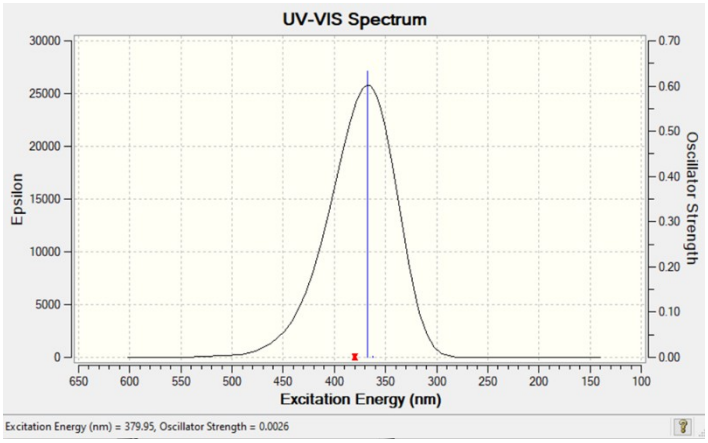


Figure S4. Absorption spectra of the Product (**TPBT-N_2H_4**)

Table S2 The vertical main orbital transition of the **TPBT-N_2H_4** calculated by TDDFT method

Energy (eV)	Wavelength (nm)	Osc. strength (f)	Transition
3.1531	393.21	0.8516	**HOMO→LUMO**
3.6081	343.62	0.0191	**HOMO→LUMO+1**
3.8266	324.00	0.2661	**HOMO→LUMO+2**

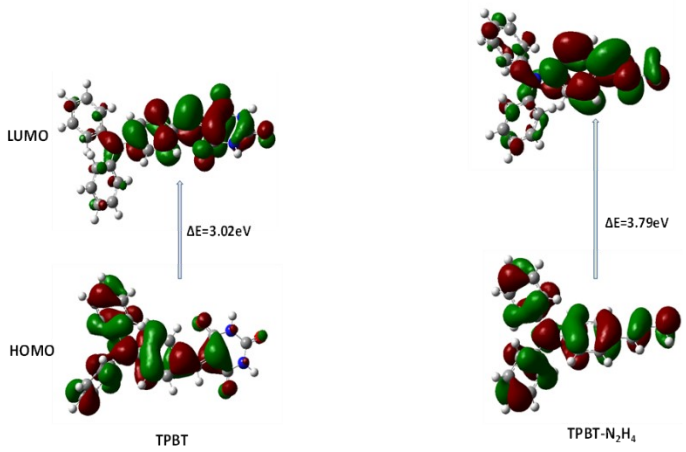


Figure S5. The frontier molecular orbitals of **TPBT** and **TPBT-N_2H_4** obtained at DFT level using B3LYP/6-31G+(d, p)basis set.

5. Determination of the response time of probe toward the product

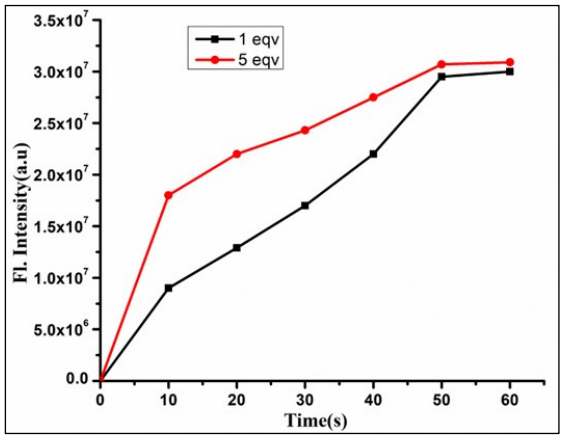


Figure S6. Time course (0–60 s) of fluorescence enhancement of **TPSBT** (10.0 μM) in THF-H_2O solution upon addition of two different concentrations of hydrazine (λex = 380 nm; λem = 511 nm).

6. Cell line study:

To envision the fluorescence ability of the ligand **TPSBT** in the presence of hydrazine fluorescence imaging was performed in cell line MDA-MB 231. Briefly, cells were grown in coverslips for 24 hrs. in a 37°C humidified incubator containing 5% CO_2 and then pre-treated the cells with 10 μM working concentration of hydrazine for the time period of 30 min and then washed with 1×PBS to remove any unbound hydrazine present within the medium followed by 10 μM working concentration **TPSBT** added and incubated for the time period of 15 min and 30 min in dark at 37ºC and then washed with 1×PBS two times to remove any unbound **TPSBT or hydrazine** and then they were mounted on a glass slide and detected under fluorescence microscope (Olympus).

Cytotoxicity assay:

MTT cell proliferation assay[10,11] was performed to assess the cytotoxic effect of the ligand **TPSBT** in both the cancer cell line MDA-MB-231 and normal cell line NKE. In brief, cells were first seeded in 96-well plates at a concentration of 1 × 10^4 cells per well for 24 h and exposed to the different working concentrations of ligand **TPSBT** (0 μM, 10 μM, 20 μM, 40 μM, 80 μM, 100 Mm) for 24 hrs. After incubation cells were washed with 1×PBS and MTT solution (0.5 mg/ml) were added to each well and incubated for 4 h and the resulting formazan crystals were dissolved in DMSO and the absorbance was measured at 570 nm by using a

microplate reader. Cell viability was expressed as a percentage of the control experimental setup.

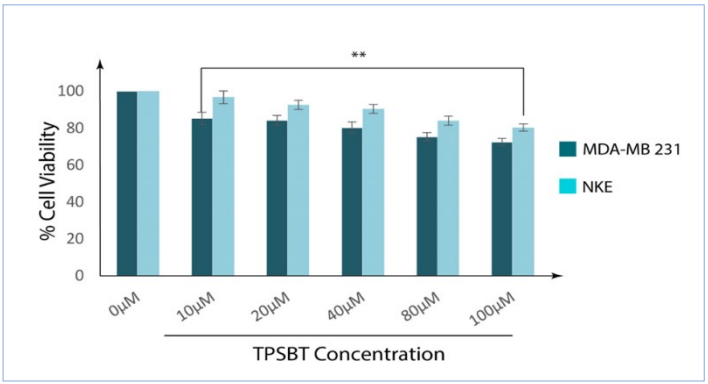


Figure S7: Cell survivability of MDA-MB 231 and NKE cells exposed to different ligand **TPSBT** concentrations. Data are representative of at least three independent experiments and bar graph shows mean ± SEM, $^{**}p < 0.001$ were interpreted as statistically significant, as compared with the control.

7. ESI-MS Spectra

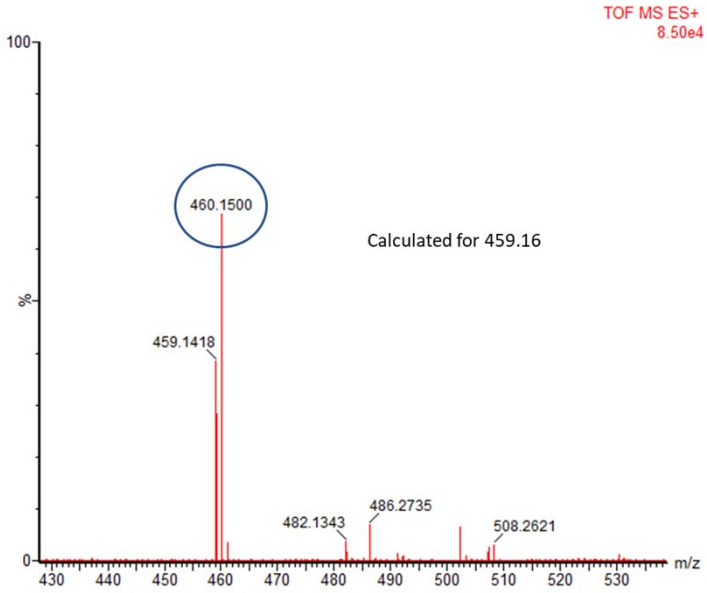


Figure S8: HRMS of the probe **TPSBT**

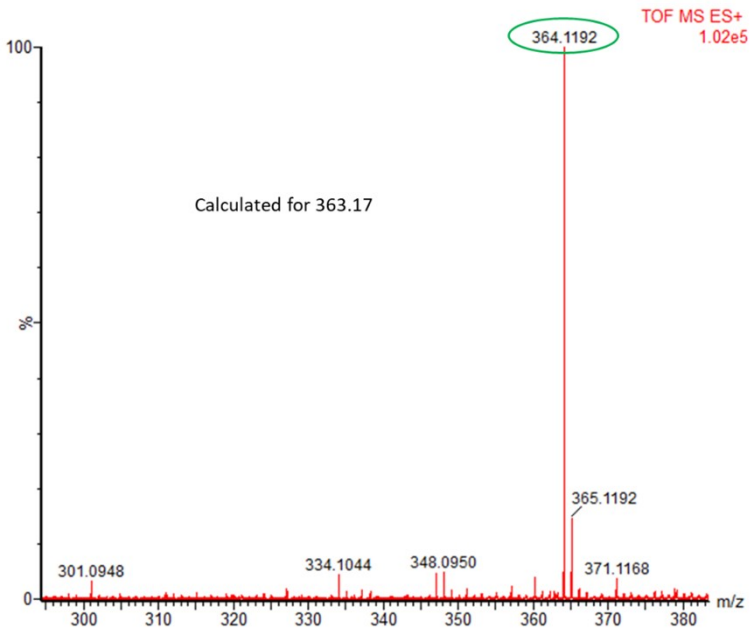


Figure S9: HRMS of the probe **TPSBT** +hydrazine adducts

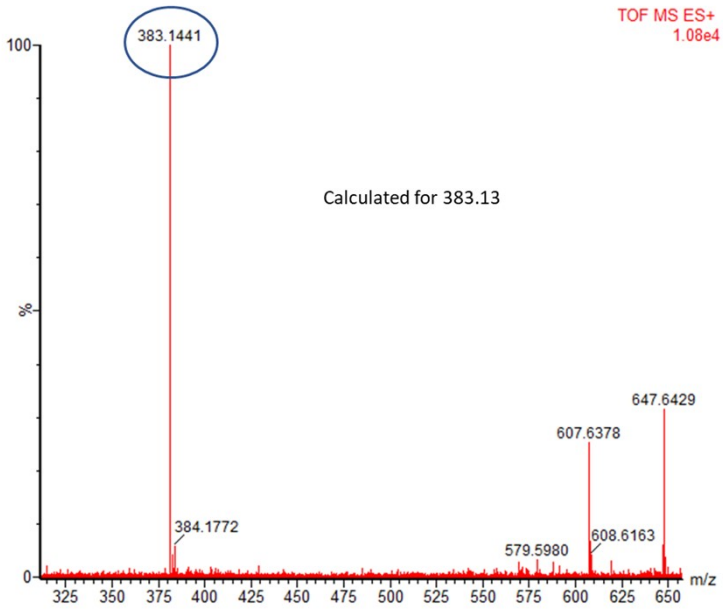


Figure S10: HRMS of the controlled compound **TPBT**

8. NMR Spectra: ^{1}H NMR, ^{13}C NMR

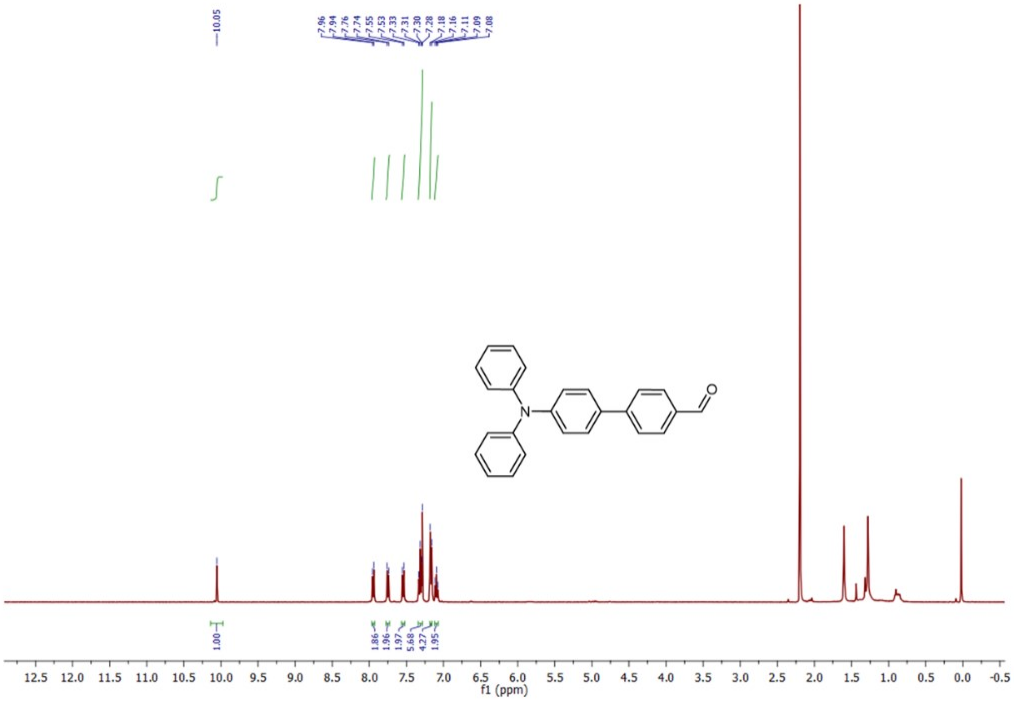


Figure S11: **^{1}H NMR** spectrum of the **Compound 2**

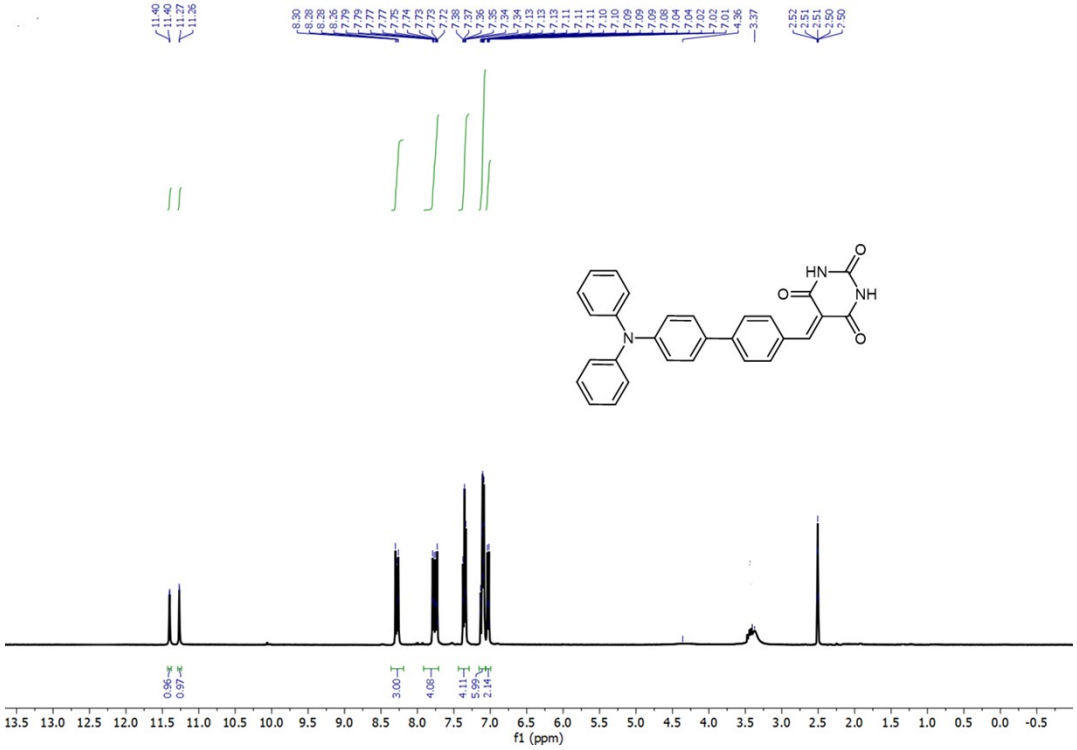


Figure S12: **^{1}H NMR** spectrum of the probe **TPSBT** in DMSO d_6

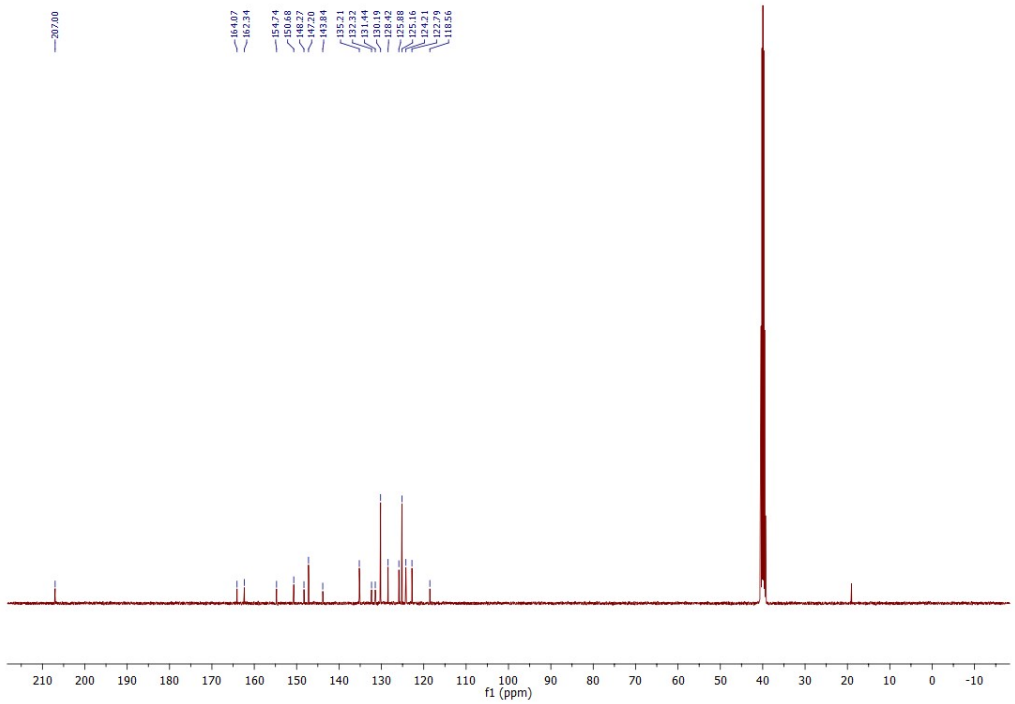


Figure S13: **^{13}C NMR** of the probe **TPSBT** in DMSO d_6

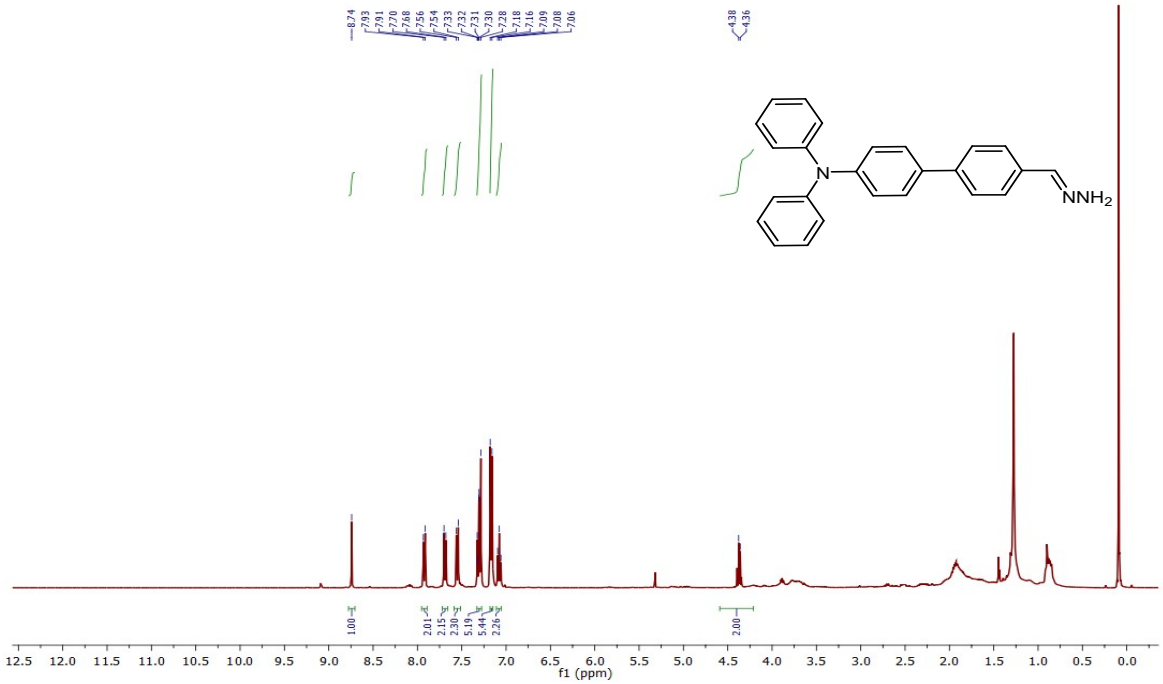


Figure S14: **^{1}H NMR** spectrum of the probe **TPSBT-N_2H_4** adduct in $CDCl_3$

9. Comparative fluorescence graph

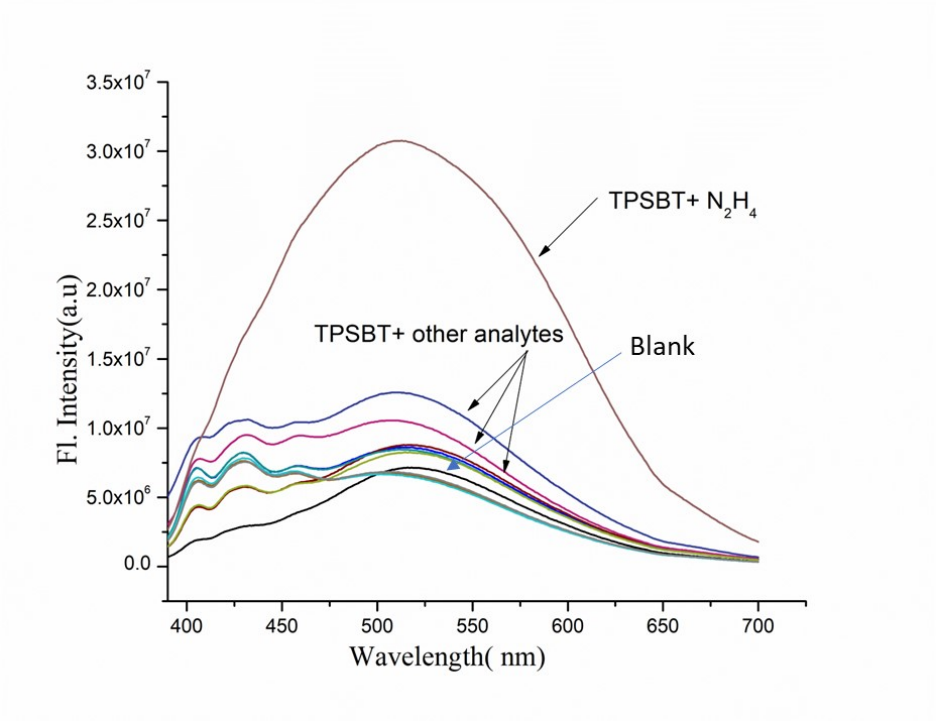


Figure S15: Comparative graph of change in fluorescence of **TPSBT** in presence of hydrazine and other analytes.

10. Effect of pH

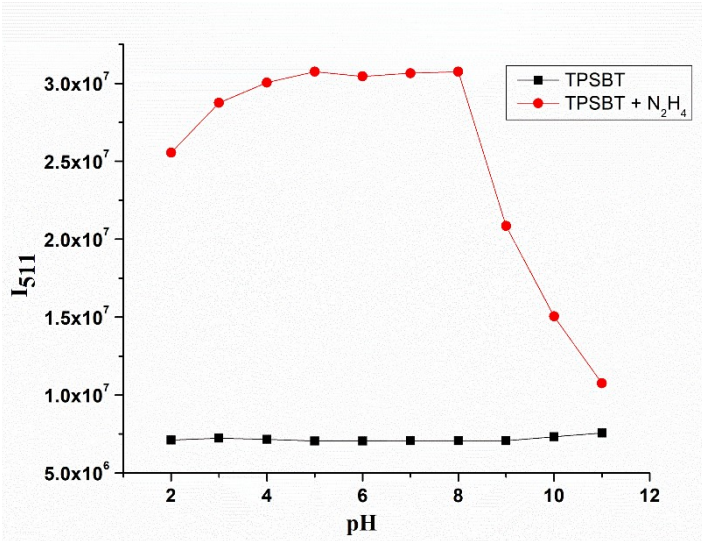


Figure S16: Effect of pH on fluorescence of TPSBT and TPSBT+ N_2H_4 in THF-H_2O at 511 nm

11. Plot of fluorescence change of TPSBT and concentration of N_2H_4

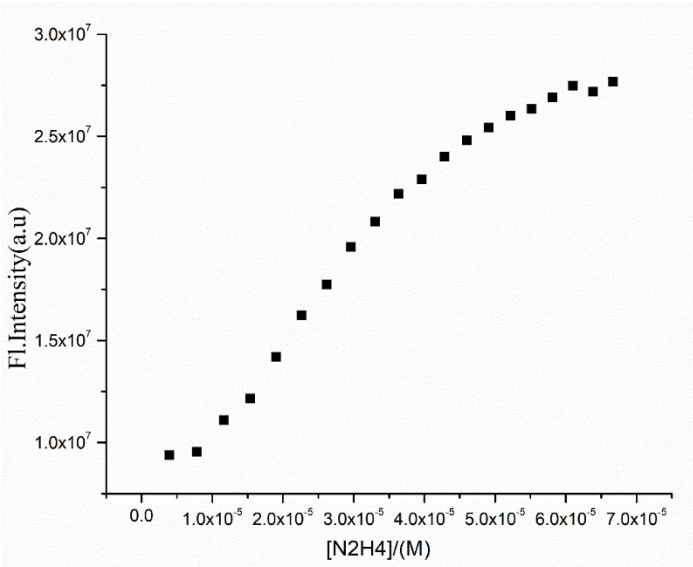


Figure S17: Plot of fluorescence change of TPSBT and concentration of N_2H_4

12. Calculation of detection Limit

The limit of detection (LOD) of TPSBT for hydrazine was calculated using the general equation LOD = K × δ/m

Where K = 2 or 3 (we take 2 in this case) and δ is the standard deviation of the blank solution and m is the slope of the calibration curve.

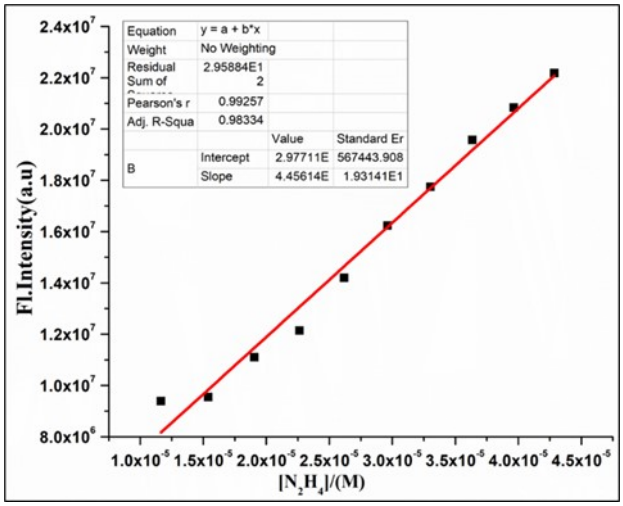


Figure S18: From the graph, we get slope (m) = 4.45×10^{11}, thus using the formula we obtained the detection limit of 1.22×10^{-7} M.

Table S3 Environmental water sample study

Samples	N_2H_4 spiked (M)	N_2H_4 recovered (M)	Recovery (%)
Tap water	0	not detected	—
	2×10^{-7}	$(1.97 \pm 0.05) \times 10^{-7}$	98.5
	2×10^{-6}	$(2.02 \pm 0.15) \times 10^{-6}$	101
	2×10^{-5}	$(1.94 \pm 0.12) \times 10^{-5}$	97
Ganges River water	0	not detected	—
	2×10^{-7}	$(1.91 \pm 0.14) \times 10^{-7}$	95.5
	2×10^{-6}	$(2.09 \pm 0.13) \times 10^{-6}$	104.5
	2×10^{-5}	$(1.98 \pm 0.10) \times 10^{-5}$	99
Lake water	0	not detected	—
	2×10^{-7}	$(2.08 \pm 0.16) \times 10^{-7}$	104
	2×10^{-6}	$(2.03 \pm 0.11) \times 10^{-6}$	101.5
	2×10^{-5}	$(1.99 \pm 0.11) \times 10^{-5}$	99.5

13. References

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