Electronic Supplementary Information

Near-Infrared Fluorescent Turn-On Probe for Hydrazine Detection: Environmental Samples and live cell imaging

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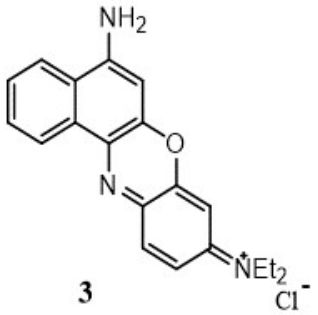
Table of Contents

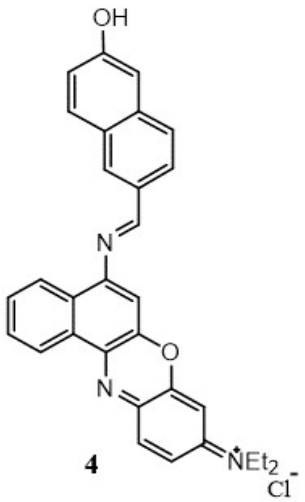
Structure and NMR characterization data of compounds 3,4:



3

^{1}H NMR (400 MHz, MeOH-d$_4$): δ 0.63 (t, J=8.0Hz, 6H), 1.25(q,J=8.0Hz, 4H), 3.71 (s,2H), 4.75 (s,1H), 5.33 (d,J=8.0Hz,2H), 6.43 (s,1H), 7.30 (t, J=8.0Hz,2H), 7.87 (d,J=8.0Hz,1H), 7.96 (d,J=8.0Hz,1H).

^{13}C NMR (400 MHz, MeOH-d$_4$): δ 11.40 (2C), 44.20 (2C), 90.50 (1C), 111.79 (1C), 120.21 (1C), 120.50 (1C),121.20(1C), 122.21 (1C), 122.70 (1C), 123.69 (1C), 123.96 (1C), 124.07 (2C), 125.30 (1C), 132.06 (1C), 140.07 (1C), 145.96 (1C), 148.77 (1C).



4

^{1}H NMR (400 MHz, DMSO-d$_6$): δ1.26 (t,J=8.0Hz,6H), 3.08 (s,1H), 3.46 (q,J=8.0Hz,4H), 5.39 (d,J=8.0Hz,2H), 6.19 (s,1H), 7.01 (d,J=8.0Hz, 2H), 7.03 (s,1H),7.05 (d,J=8.0Hz, 2H), 7.54 (t,J=8.0Hz,2H), 7.96 (d,J=8.0Hz, 2H), 7.99 (d, J=8.0Hz,1H), 9.50 (s,1H), 11.0 (s, 1H).

^{13}C NMR (400 MHz, DMSO-d$_6$): δ 11.43 (2C), 44.70 (2C), 90.82 (1C), 99.38 (1C), 112.40 (1C), 120.21 (1C), 120.55 (1C),121.24 (6C), 122.60 (1C), 122.77 (2C), 123.69 (2C), 123.71 (1C), 123.74 (2C), 126.06 (2C), 130.70 (1C), 132.45 (1C), 142.52 (1C), 149.31 (1C), 160.82(1C), 162.08 (1C).

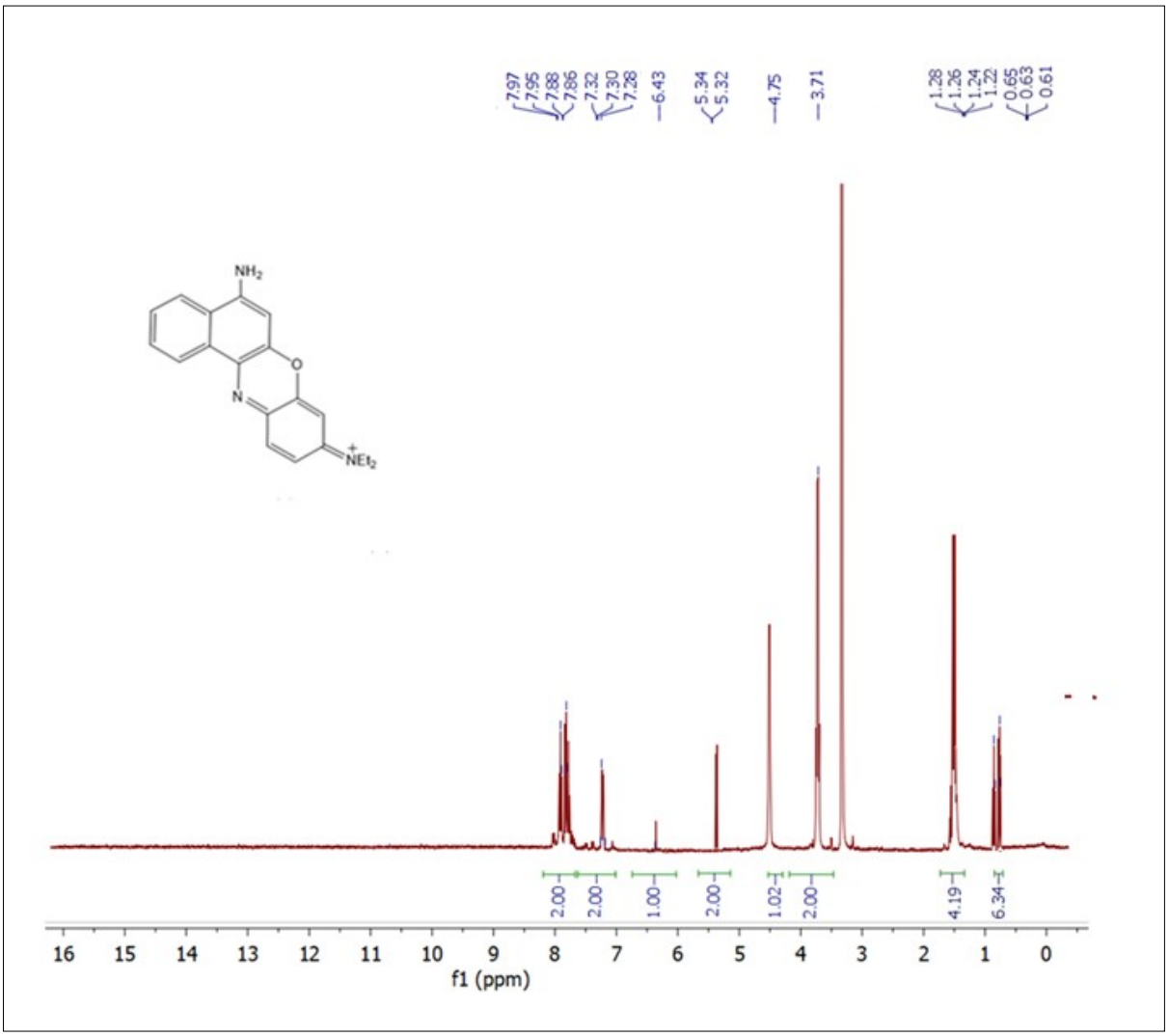


Figure S1: ^{1}HNMR of compound **3** in MeOH-d$_4$

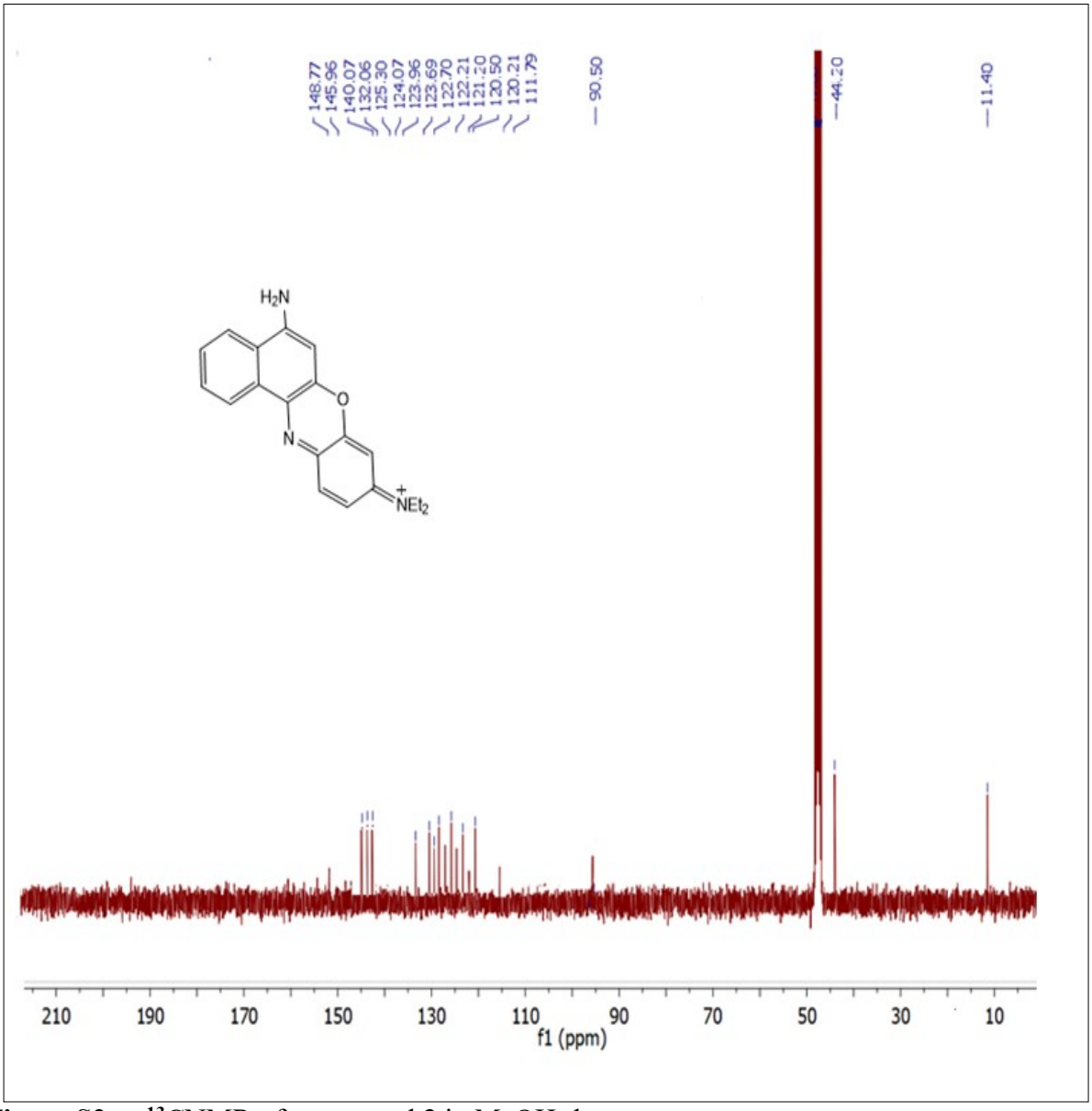


Figure S2: ^{13}CNMR of compound **3** in MeOH-d_4

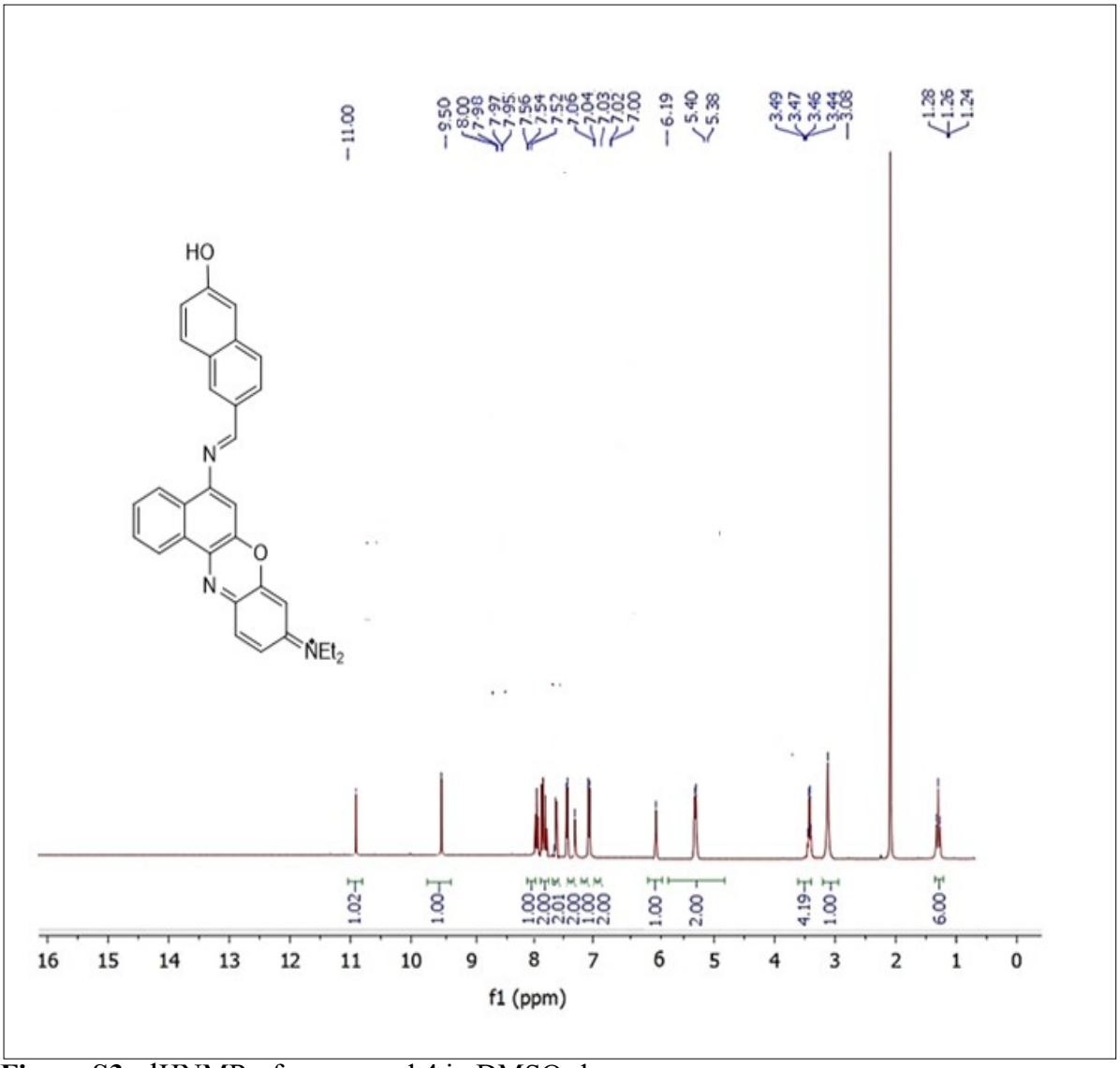


Figure S3: ^{1}HNMR of compound **4** in DMSO-d_6

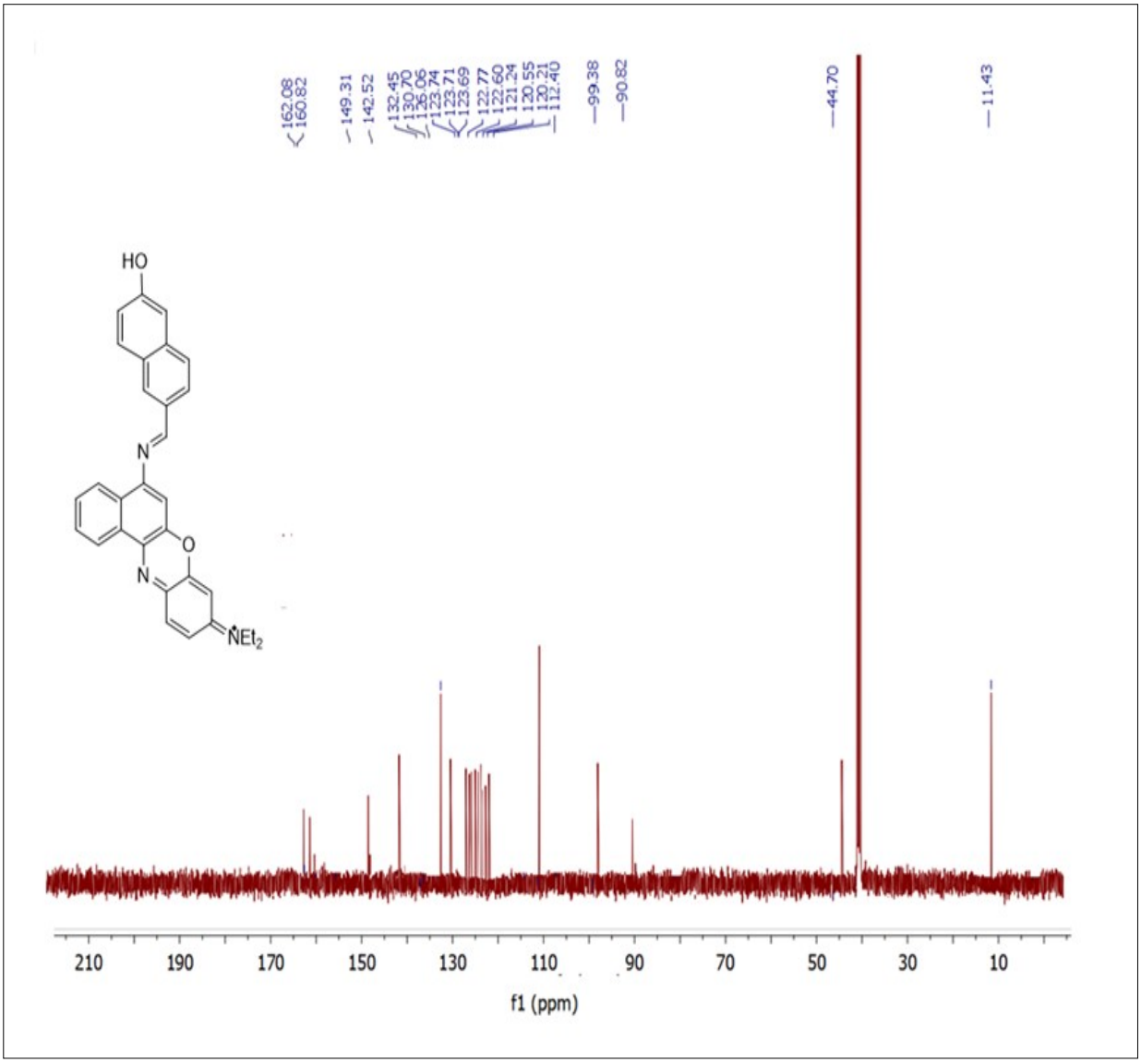


Figure S4: ^{13}CNMR of compound **4** in DMSO-d_6

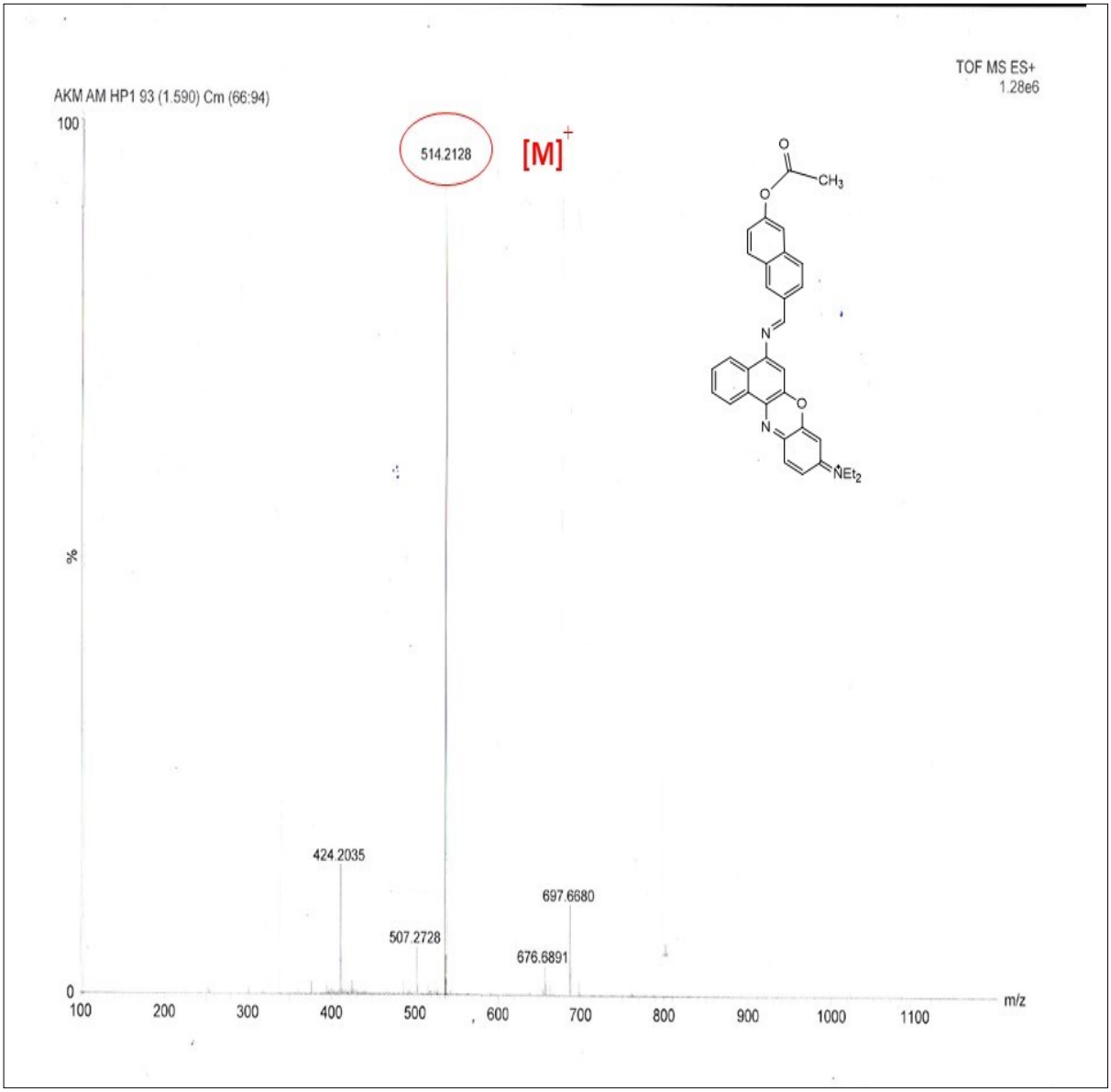


Figure S5: HRMS of probe **BPN**

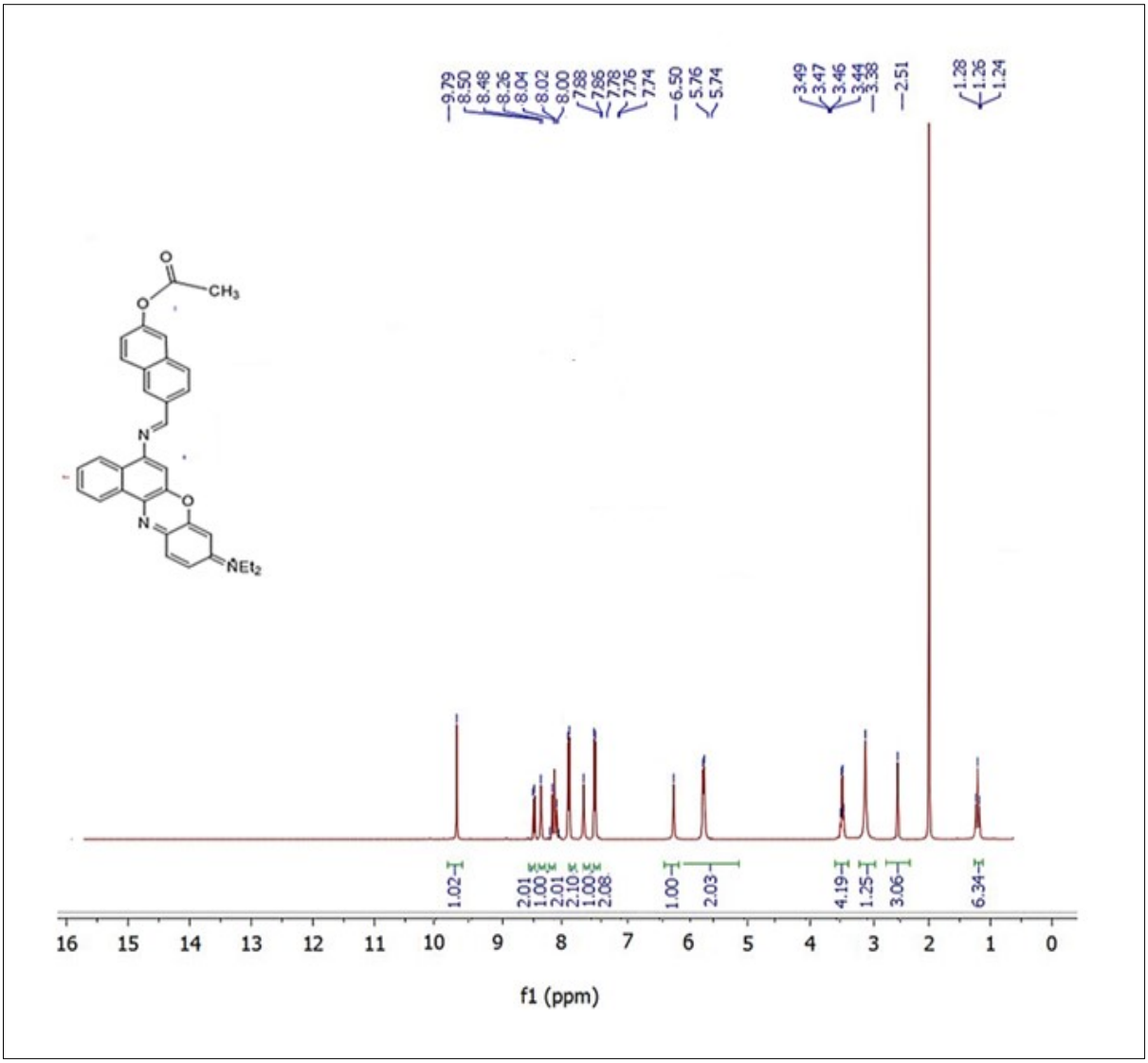


Figure S6: ^{1}HNMR of probe **BPN** in DMSO-d_6

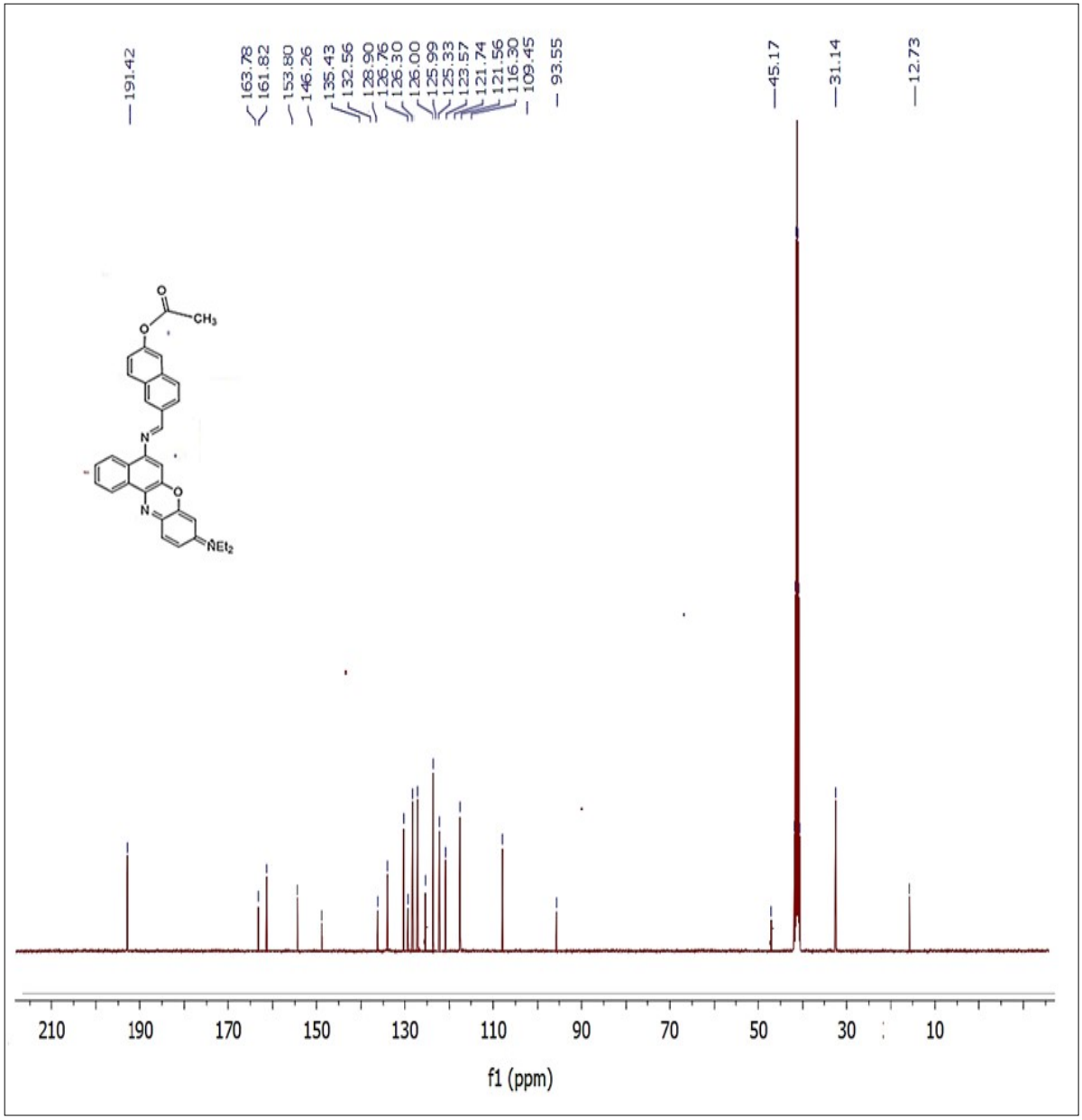
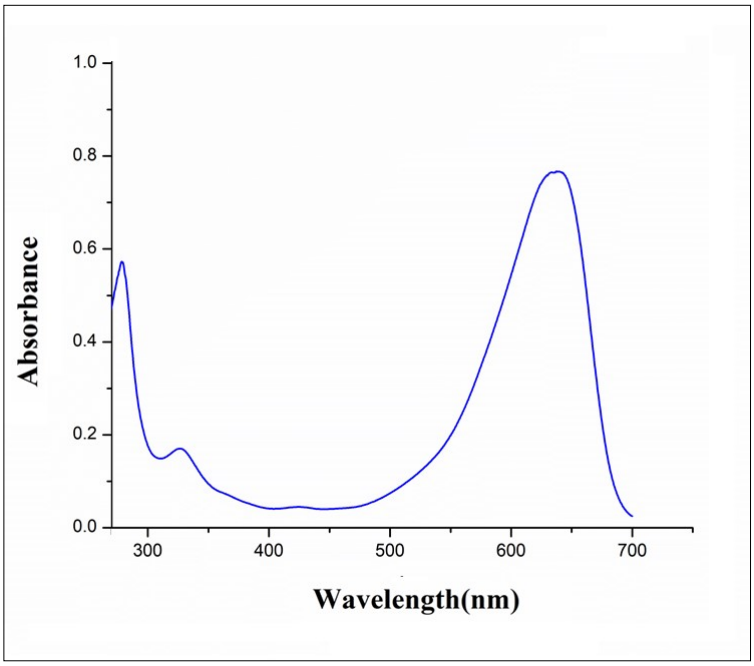


Figure S7: ^{13}C NMR of probe **BPN** in DMSO-d_6



FigureS8:UV−vis absorbance of compound 3 (c = 4×10^{-5} M) in aq. DMSO (DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4).

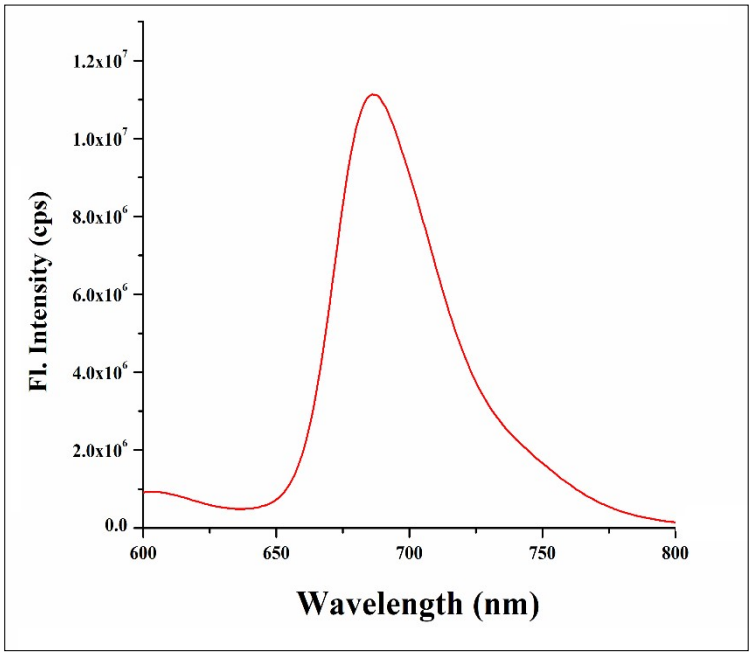


Figure S9: Fluorescence spectrum of compound 3 (c = 4×10^{-5} M) in aq. DMS(DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4).

The quantum yield of compound 3 is 0.28 using Rhodamine B in ethanol as standard.

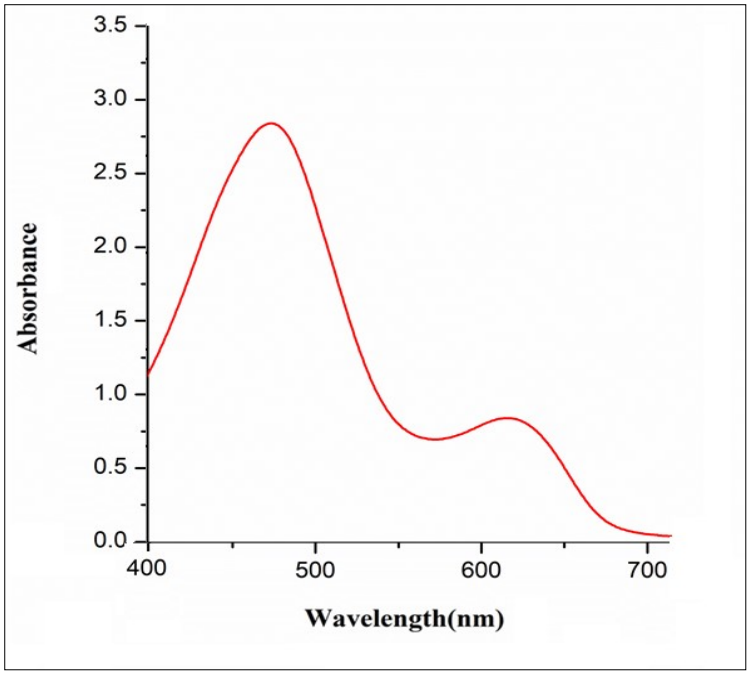


Figure S10: UV−vis absorbance of compound 4 (c = 4×10^{-5} M) in aq. DMSO (DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4).

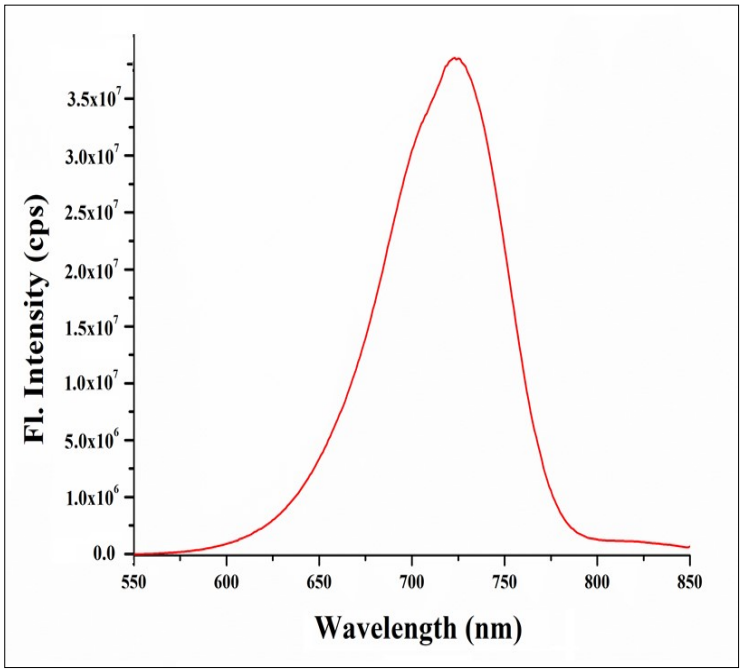


Figure S11: Fluorescence spectrum of compound 4 (c = 4×10^{-5} M) in aq. DMSO (DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4).

The quantum yield of compound 4 is 0.62 using Rhodamine B in ethanol as standard.

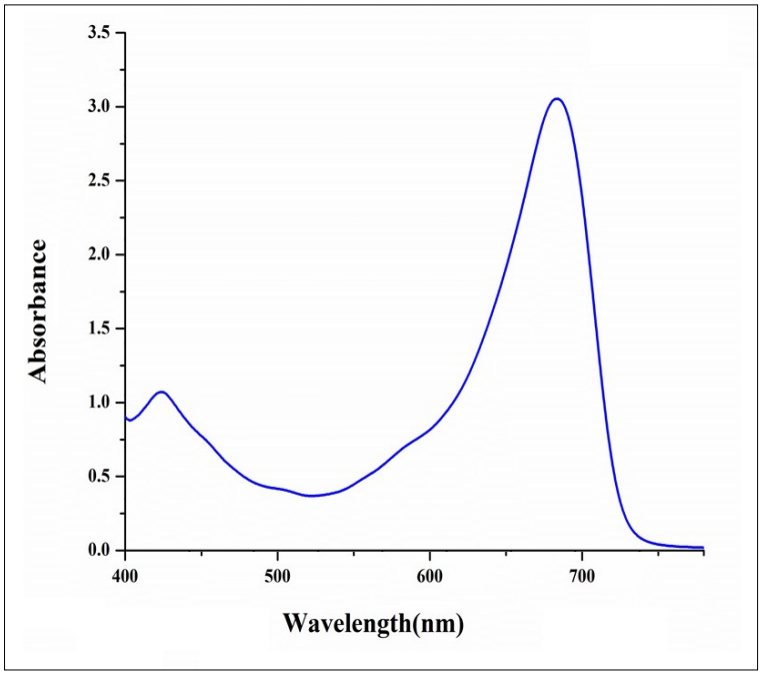


Figure S12: UV−vis absorbance of probe BPN (c = 4×10^{-5} M) in aq. DMSO (DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4).

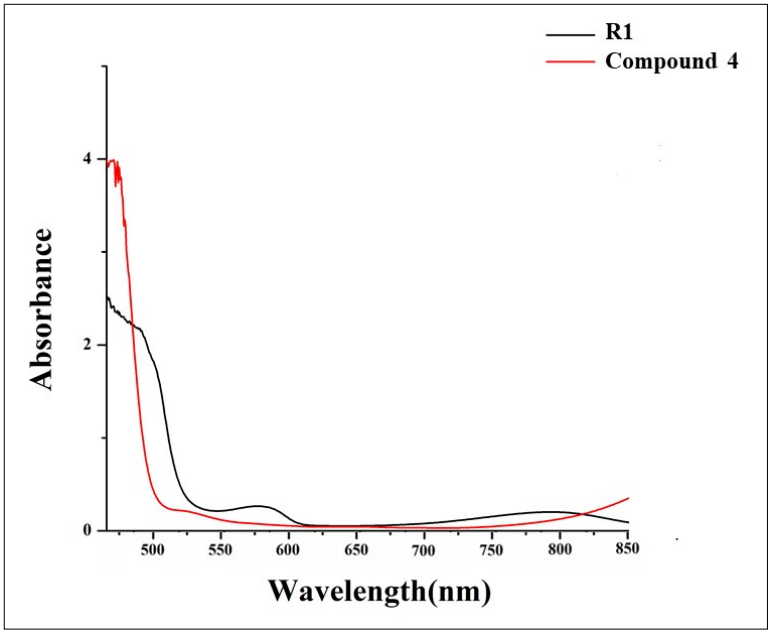


Figure S13: ComparativeUV−vis absorption spectrum of compound 4 and R1 (c = 4×10^{-5} M) in aq. DMSO (DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4)

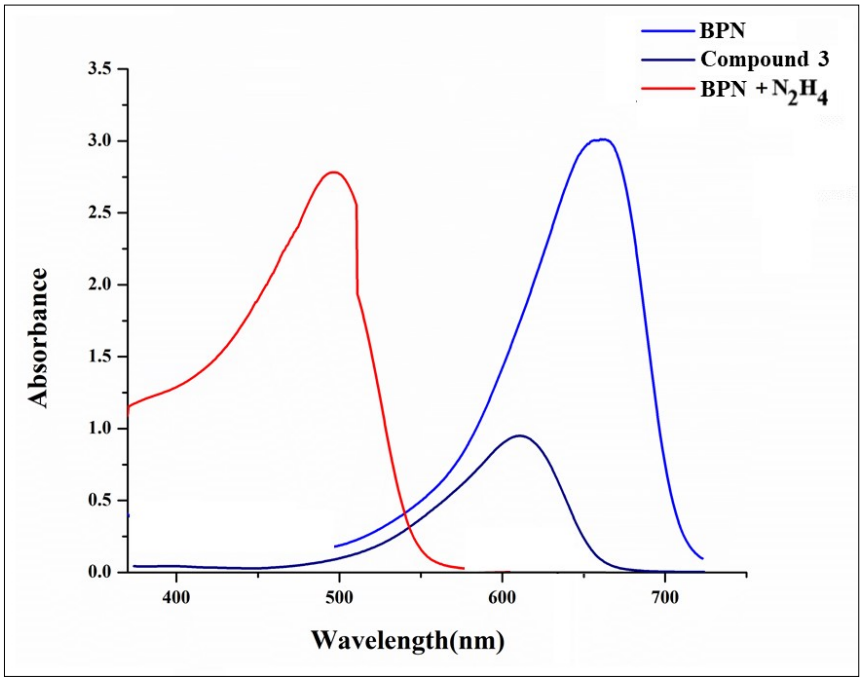


Figure S14: Comparative UV−vis absorption spectrum of probe BPN, Compound 3 and BPN treated with N_2H_4(c = 4×10^{-5} M) in aq. DMSO (DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4).

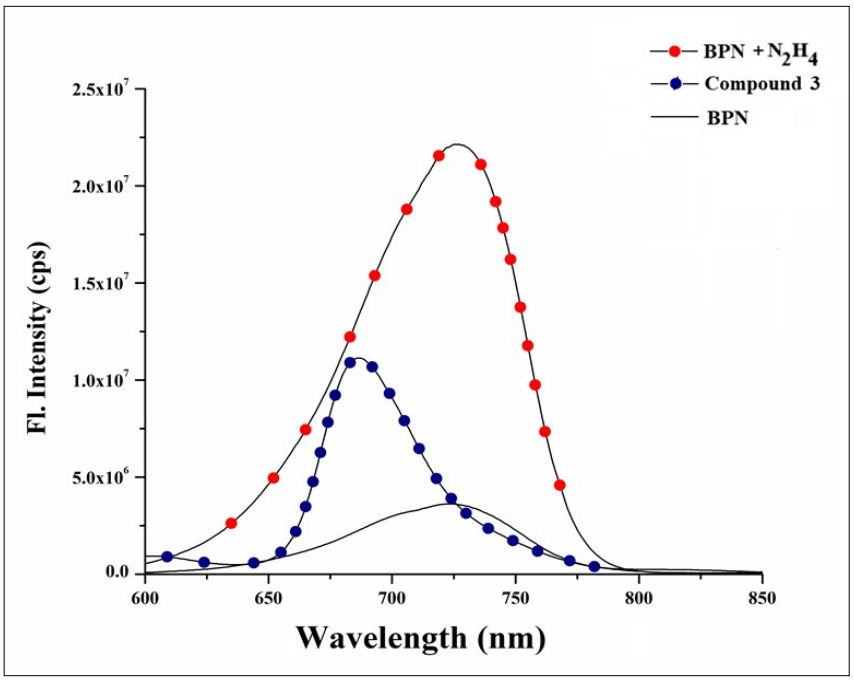


Figure S15: ComparativeFluorescence spectrum of probe BPN, Compound 3 and BPN treated with N_2H_4(c = 4×10^{-5} M) in aq. DMSO (DMSO/H_2O = 1:9 v/v, 10 mM PBS buffer, pH = 7.4).

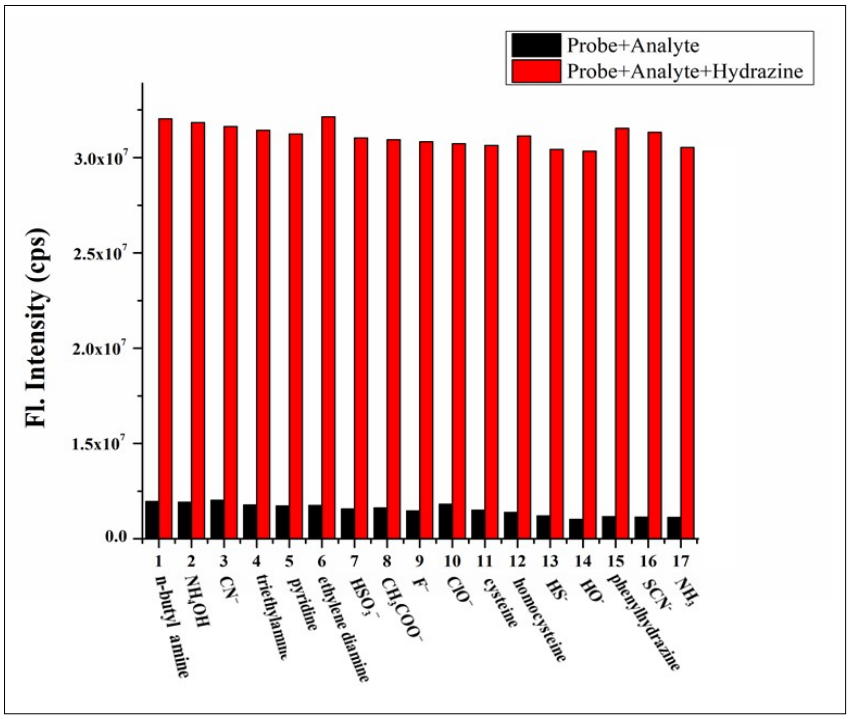


Figure S16: Change of fluorescence emission intensities of **BPN**(c = 4×10^{-5} M)upon additionof other interfering analytes (20 equivalents) and then hydrazine(10 equivalents).

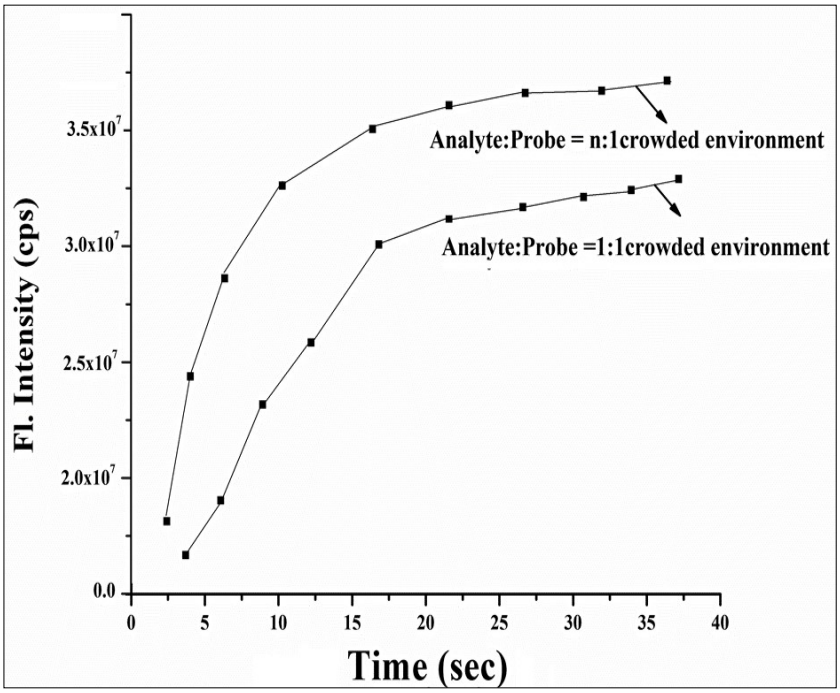
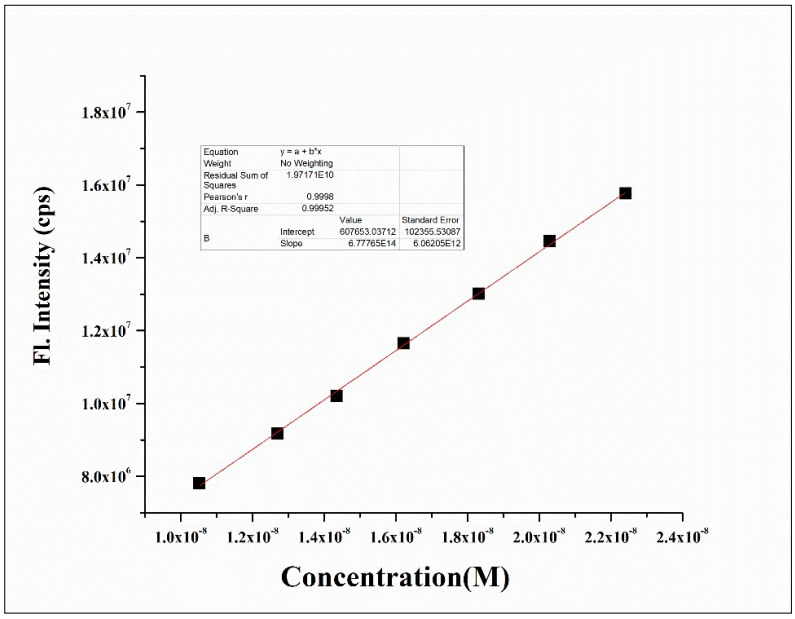


Figure S17: Change of fluorescence emission intensities of **BPN** in presence of hydrazine and other interfering analytes both in 1:1 and n:1 crowded environment.

Calculation of Detection limit:

By using the following equation DL = K*Sb1/S;the detection limit (DL) of probe**BPN**for N_2H_4 wascalculated,where K= 2 or 3 (we take 2 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.



FigureS18: From the graph we get slope (S)=6.77765×10^{14}, Standard deviation (Sb1=102355.53087).Thus, using the formula, we get the detection limit = 4.5×10^{-10} M

Kinetic study of probe BPN:

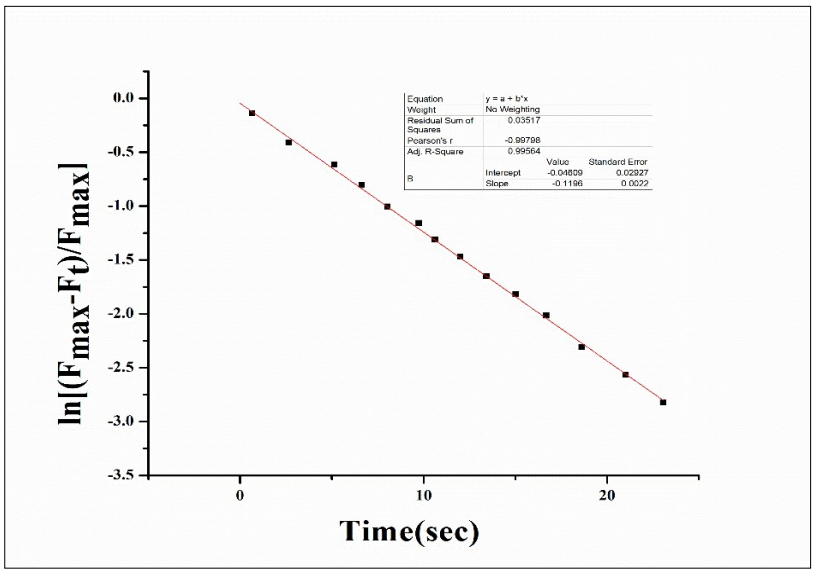


Figure S19: Pseudo first order kinetic diagram of probe **BPN**(1×10^{-5} M) with $\mathbf{N_2H_4}$ (1×10^{-4} M) in DMSO-H_2O.

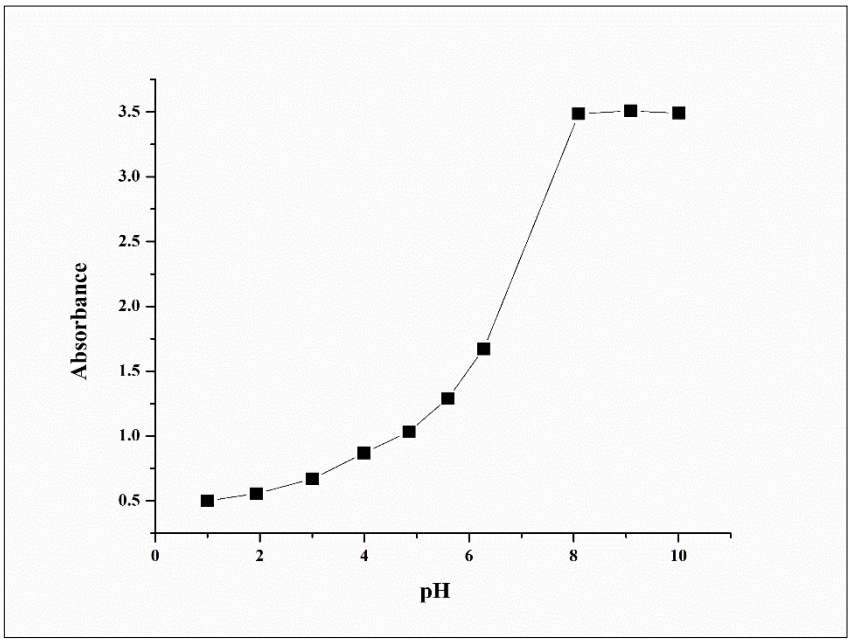


Figure S20: pH mediated absorbance change of probe **BPN** in presence of hydrazine.

Calculation of fluorescence quantum yield of BPN and BPN-N_2H_4 adduct:

Here, the fluorescence quantum yield Φ was calculated by using the following equation:

$\Phi_x = \Phi_s (F_x/F_s) (A_s /A_x) (\eta_x^2 / \eta_s^2)$

Where,

X and S indicate the unknown and standard solution respectively, Φ = quantum yield

F = Area under the emission curve, A= Absorbance at the excitation wavelength,

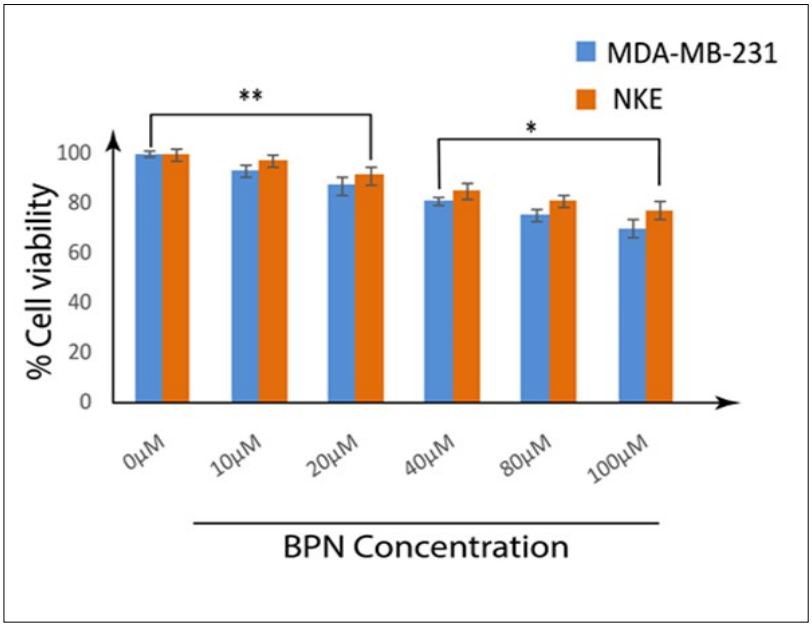
η= Refractive index of solvent. Here Φ measurements were performed using Rhodamine B in ethanol as standard [Φ = 0.49]

The fluorescence quantum yield of **BPN** and **BPN-N_2H_4** product was calculated by taking Rhodamine B (Φ = 0.49 in ethanol) as standard.

η_s = 1.3614 (for ethanol); η_x = 1.479 (for DMSO)

The quantum yield of **BPN** was calculated using the above equation and the value is 0.0075.

The quantum yield of **BPN-N_2H_4**adduct was calculated using the above equation and the value is 0.6193.



FigureS21: Cell survivability of MDA-MB 231 and NKE cells exposed to different probe**BPN** concentration. Data are representative of at least three independent experiments and bar graph shows mean ± SEM, $^{**}p < 0.001$, $^{*}p < 0.01$ were interpreted as statistically significant, as compared with the control.

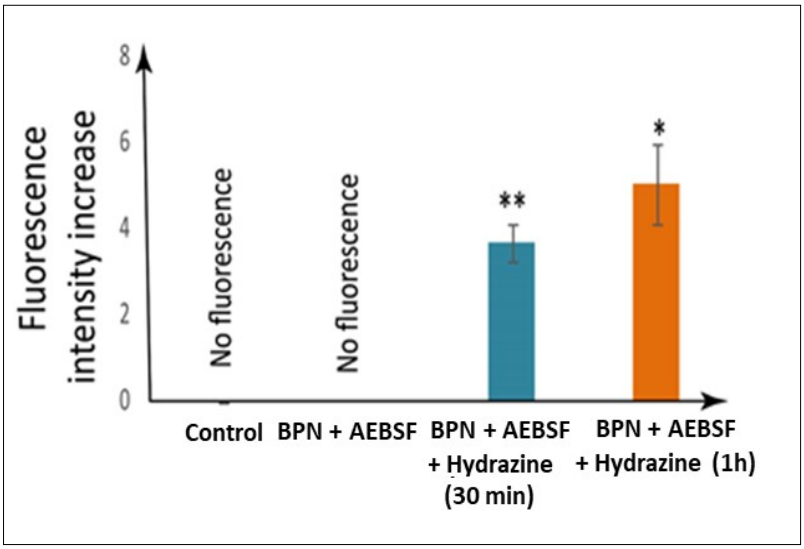


Figure S22: Pictorial fluorescence intensity variation ofuntreated MDA-MB 231 cells (Control), cells treated with probe **BPN** (10μM) + AEBSF(1 mM), probe**BPN** (10μM) + AEBSF (1 mM) + Hydrazine together after 30 min and 1h, incubation period.

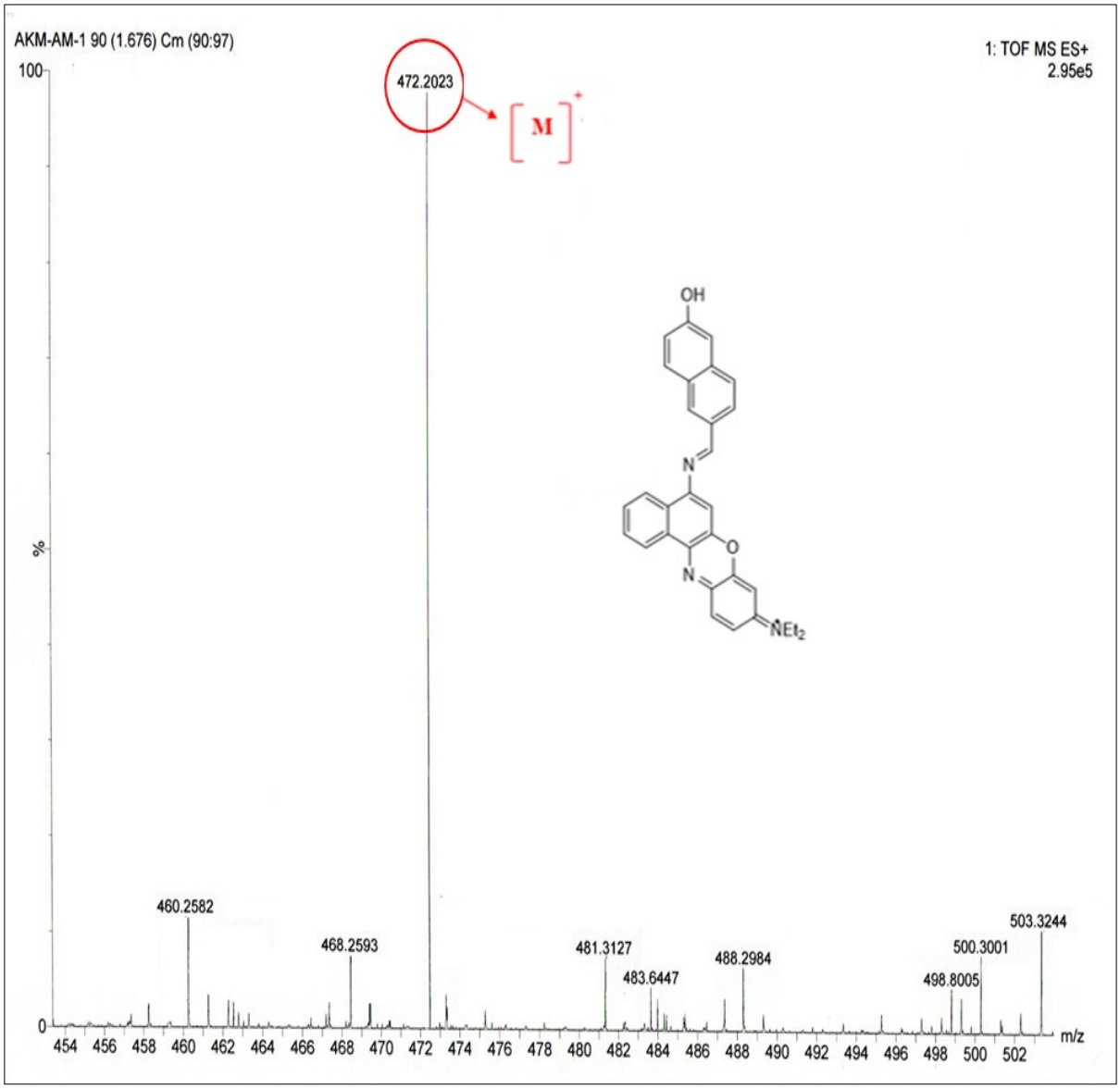


Figure S23: HRMS of BPN-N_2H_4 adduct.

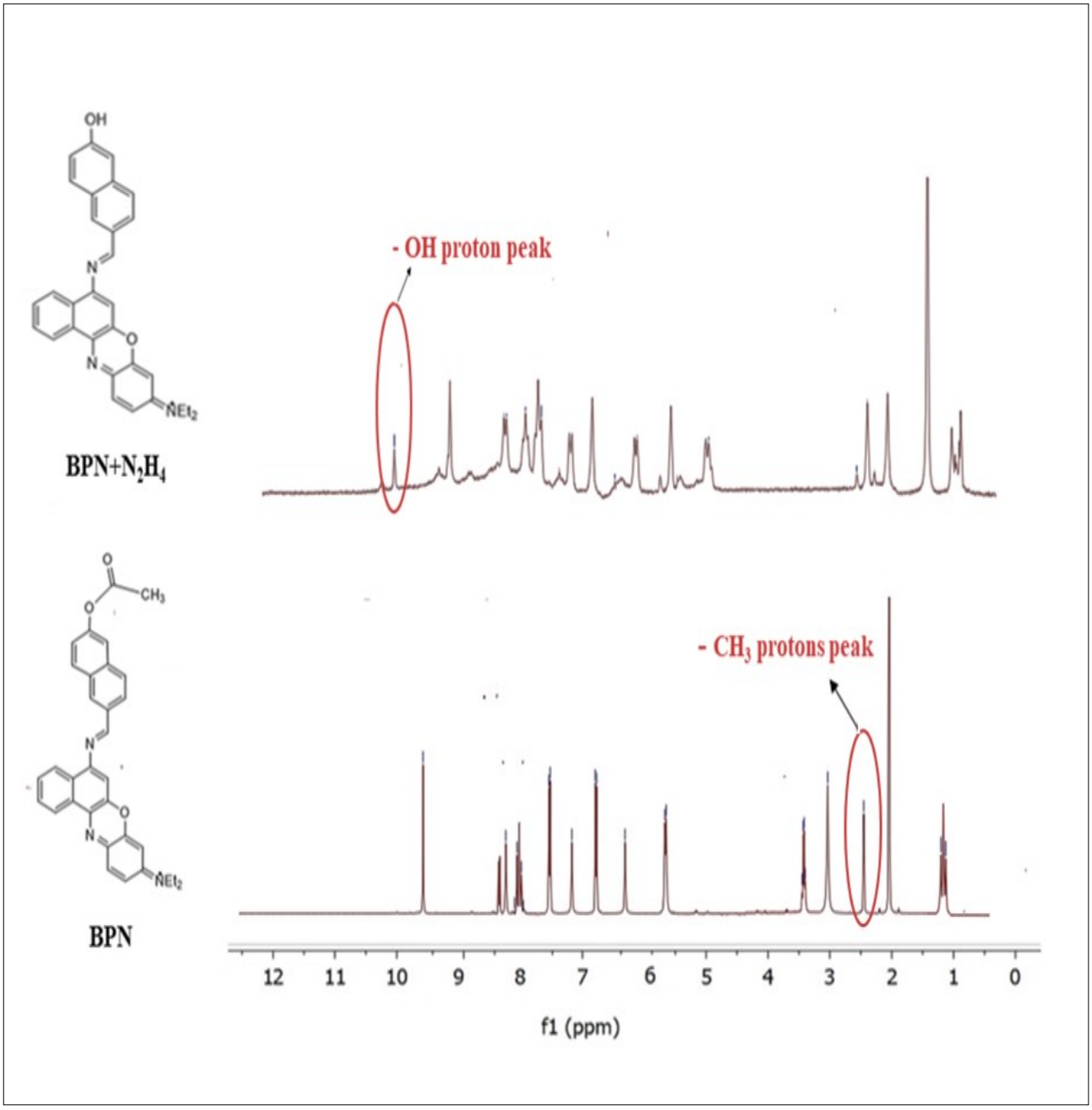
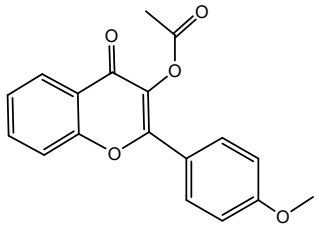
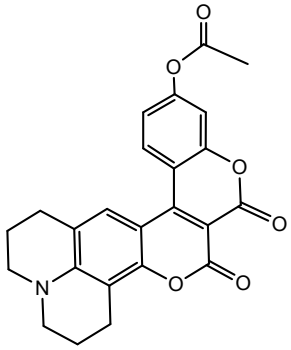
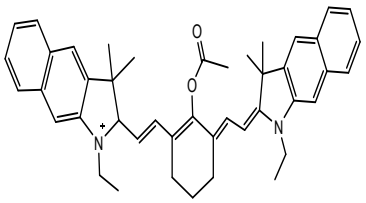
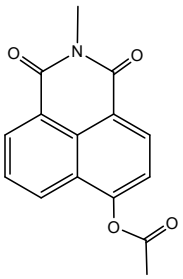
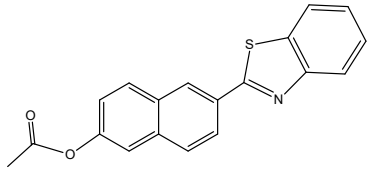
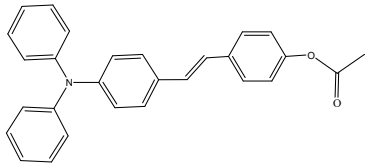
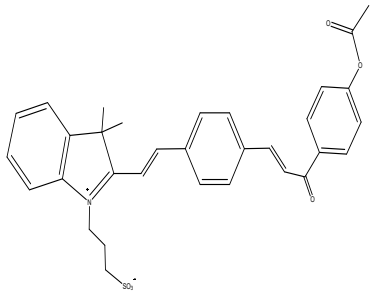
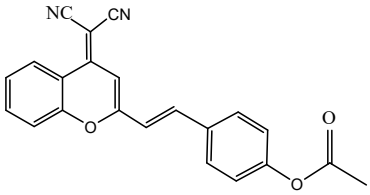
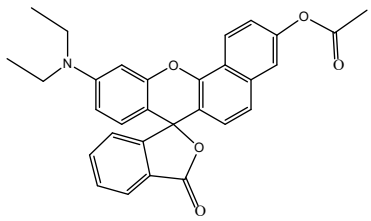
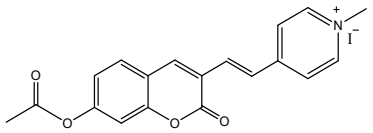
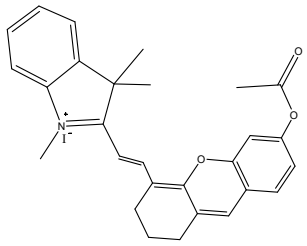
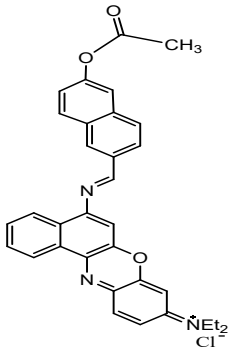


Figure S24: ^{1}H NMR titration of probe **BPN** with hydrazine.

Table S2: Comparison data of previously reported N_2H_4 sensors with current data

Sl. No.	Probe structure	Excitation	Emissioon in presence of hydrazine	Detection limit	Response time	Application	Reference
1.		370 nm	415 nm (N* form) and 540 nm (T* form)	10 µM	60 min	Live stem cell and *invivo*zebrafish imaging	[1]
2.		480 nm	542nm	5.4 ppb	10 min	Live HeLa cell and *invivo*zebrafish imaging	[2]
3.		540 nm and 730 nm	662 nm to825nm	2.56 ppb	7 min	Livecell, kidney tissue and *invivo* mouse body imaging	[3]
4.		385 nm	445 nm to545nm	9.40 nM	5 min	Livecell and *invivo*zebrafish imaging	[4]

5.		-	390 nm to508 nm	0.96 µM	60 min	Live HeLa cell imaging	[5]
6.		350 nm	440 nm	8.47 nM	180s	Live cancer cell imaging	[6]
7.		430 nm	532 nm	1.72 ppb	-	Livecell, kidney tissue and *invivo*mous e body imaging	[7]
8.		560 nm	680 nm	57 nM	1 min	(a)Detection in living cells (MCF-7 cells); (b) Detection of hydrazine in solution by test kits.	[8]
9.		592 nm	654 nm	3.4 ppb	-	Live HeLa cell imaging.	[9]

10.		510 nm	559 nm	-	60 min	Live cell imaging	[10]
11.		675 nm	706 nm	5.4 ppb	10 min	Livecell, kidney and *invivo* mouse body imaging	[11]
12.		590 nm	725 nm	4.5×10^{-10} M	4s	Livecell imaging and vapor phase detection by test strips.	Our Work

References:

[1] B. Liu, Q. Liu, M. Shah, J. Wang, G. Zhang and Y. Pang, *Sens. Actuators, B*, 2014, **202**,194–200.

[2]Q. Fang, L. Yang, H. Xiong, S. Han, Y. Zhang, J. Wang, W. Chen and X. Song, *Chinese Chem. Lett*., 2019, 2–5.

[3] Y. Song, G. Chen, X. Han, J. You and F. Yu, *Sens. Actuators, B*, 2019, **286**, 69–76.

[4] X. Xia, F. Zeng, P. Zhang, J. Lyu, Y. Huang and S. Wu, *Sens. Actuators, B*, 2016, **227**, 411-418.

[5] C. Liu, K. Liu, M. Tian and W. Lin, *Spectrochim. Acta A,*2019, **212**, 42–47.

[6]X.Wang, G. Ding, Y. Wang, S. Mao, K. Wang, Z. Ge, Y. Zhang, X. Li and C. H. Hung, *Tetrahedron Lett.,* 2020,**76**,131726.

[7]T. Li, J. Liu, L. Song, Z. Li, Q. Qi and W. Huang,*J. Mater. Chem. B*.**2019**, 7, 3197–3200.

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