Electronic Supplementary Information(ESI†)

Benzopyrylium-phenothiazine-conjugate of flavylium derivative as fluorescent chemosensor for cyanide in aqueous media and its bioimaging

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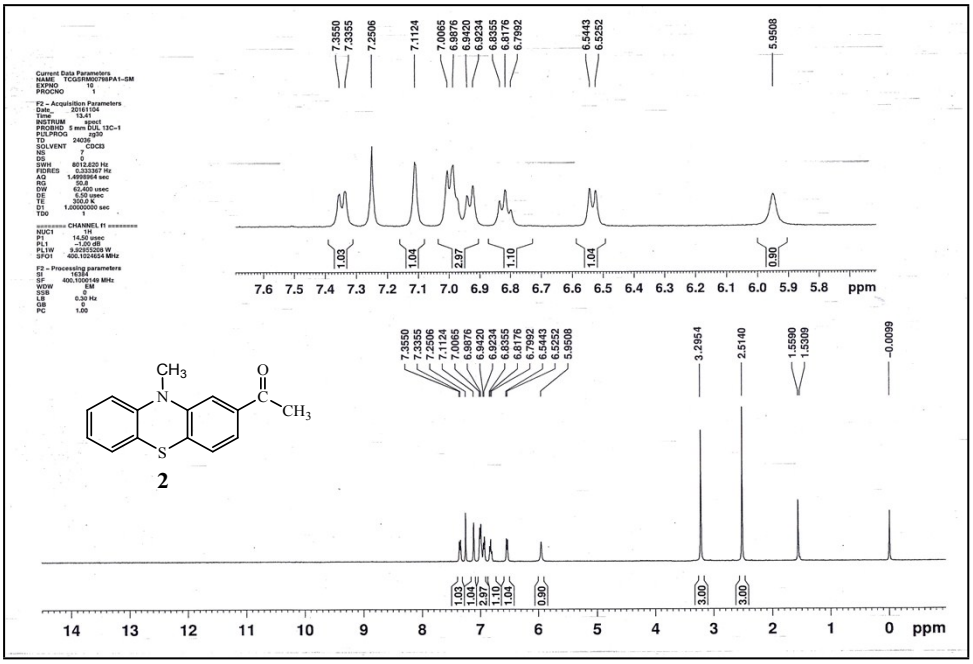

Figure S1: ^{1}H NMR of compound **2** in $CDCl_3$.

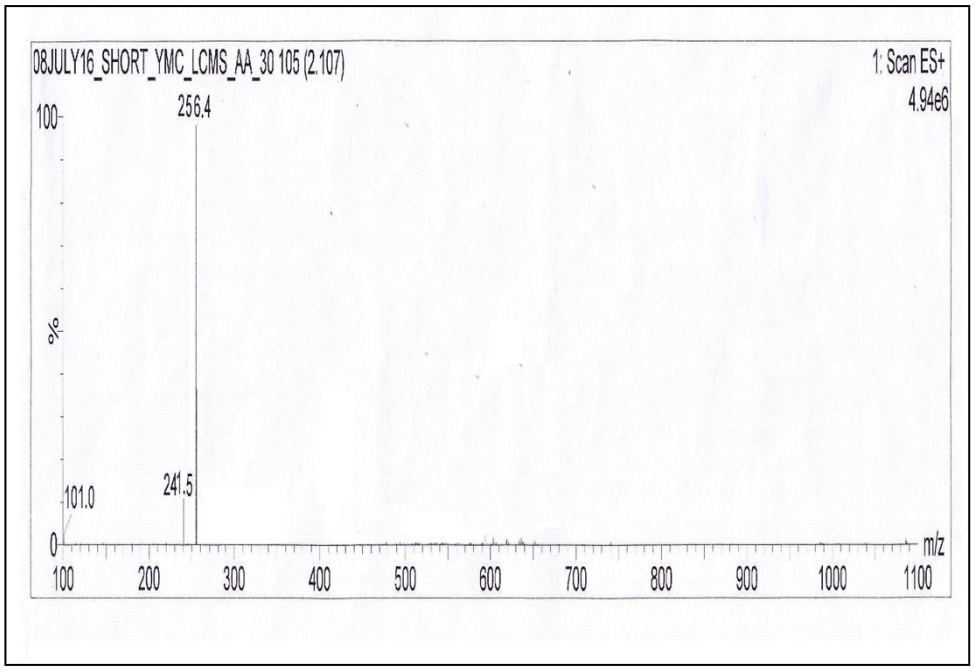

Figure S2. ESI-MS mass spectrum of **2**.

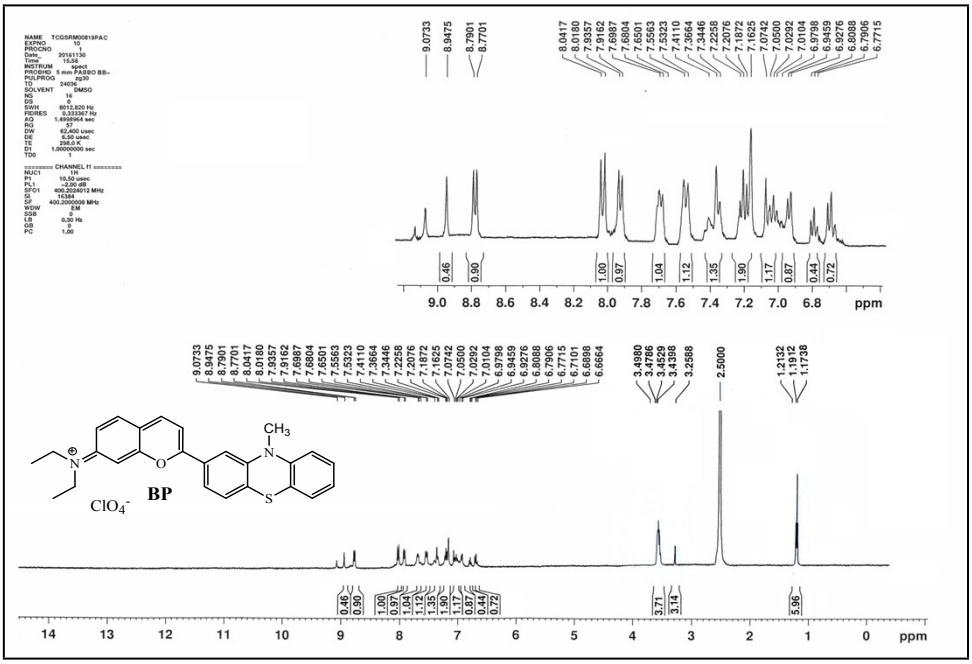

Figure S3: ^{1}H NMR spectrum of **BP** in DMSO-d_6.

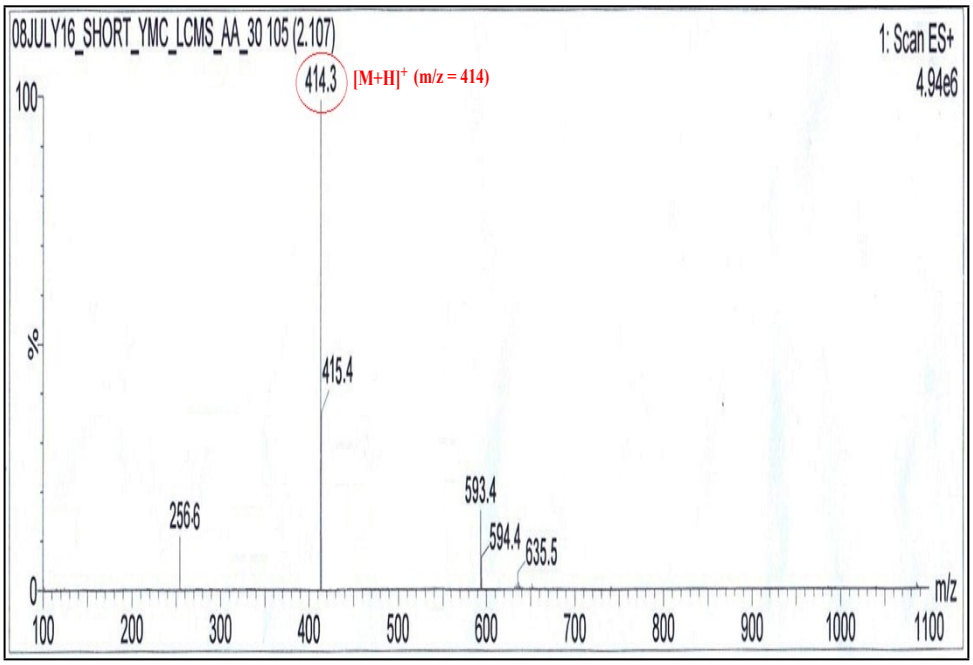

Figure S4: ESI-MS mass spectrum of **BP**.

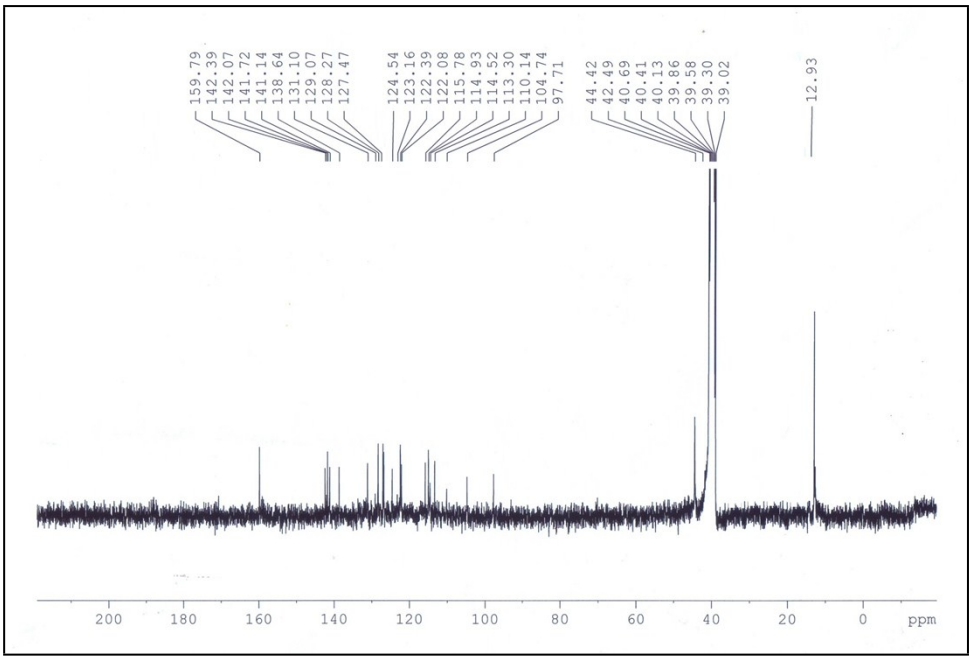

Figure S5: ^{13}C NMR spectrum of **BP** in DMSO-d_6 (75 MHz).

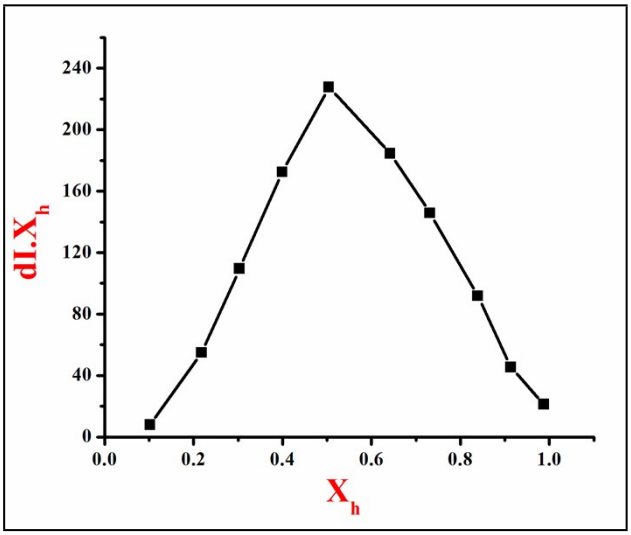

Figure S6: Job's plot of sensor **BP** (1×10^{-5} M) with CN^- (1×10^{-5} M) in CH_3CN–H_2O (7:3 v/v; 20 mM, HEPES buffer, pH = 7.4) by fluorescence method, that indicates 1:1 stoichiometry.

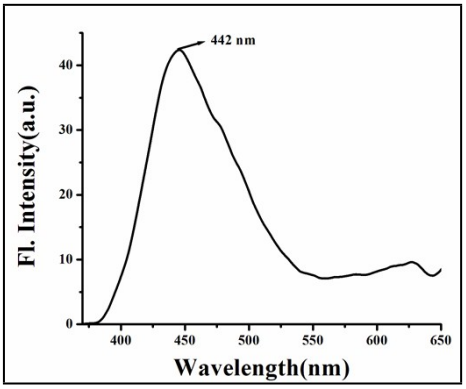

Figure S7: The fluorescence response of probe **BP** (1×10^{-5} M) in aq. CH_3CN (H_2O/CH_3CN = 3:7 v/v, 20 mM HEPES buffer, pH = 7.4).

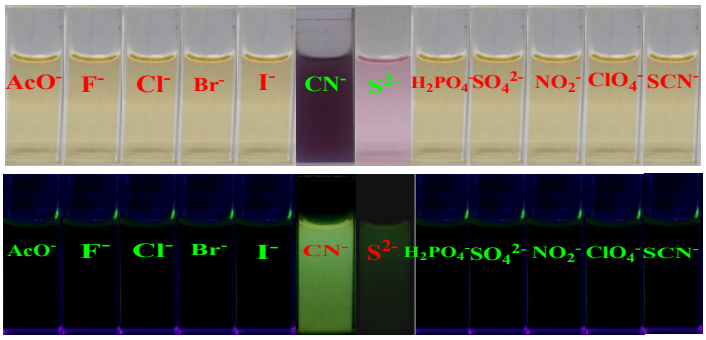

Figure S8: The Visible color (top) and fluorescence changes (buttom) of receptor **BP** in aq. CH_3CN solution (H_2O/ CH_3CN = 3 : 7, v/v, 20 mM HEPES buffer, pH = 7.4) upon addition of various anions.

Detection limit calculation:

The detection limit (DL) of **BP** for CN^- was determined from the following equation:

DL = K* Sbl /S, 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.

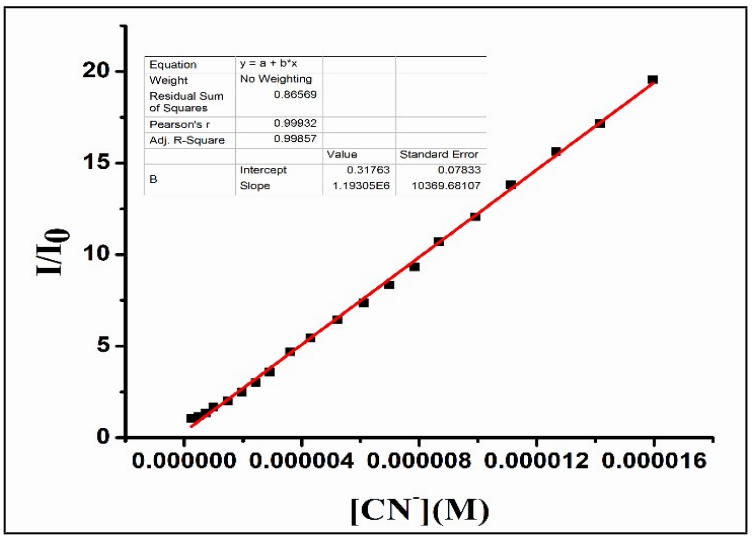

Figure S9: Calibration curve for fluorescence titration of **BP** with CN^-.

From the graph we get slope (S) = 1.19×10^6 Standard deviation (Sb1 = 0.07833)

Thus using the formula we get the detection limit = 1.3×10^{-7} M =0.13 μM.

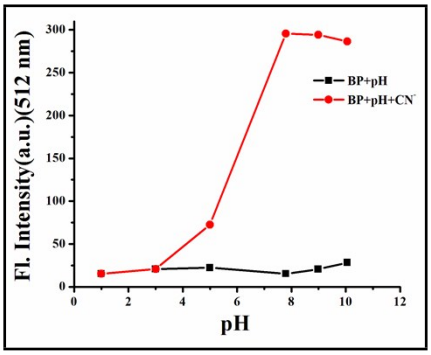

Figure S10: Fluorescence pH titration of **BP** (1.0×10^{-5} M) in presence (red) and absence (black) of CN^- (1.0×10^{-4} M) at 512 nm in CH_3CN-H_2O (7:3, V/V).

Computational method:

Selected electronic excitation energies (eV), oscillator strengths (f), main configurations, and CI Coefficients of the low-lying excited states of **BP** and **BP-CN** complex. The data were calculated by TDDFT//B3LYP/6-31G(*d,p*) + solv(PCM) based on the optimized ground state geometries.

Table S1

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c]	(composition) %
BP	$S_0 \rightarrow S_1$	1.8700 eV 663.02 nm	0.1838	H → L	99.1
	$S_0 \rightarrow S_2$	2.5759 eV 481.32 nm	0.6590	H-1 → L	97.2
	$S_0 \rightarrow S_5$	3.5995 eV 344.44 nm	0.1843	H-3 → L	88.2
	$S_0 \rightarrow S_8$	3.9832 eV 311.26 nm	0.1801	H → L+2	66.1
	$S_0 \rightarrow S_{12}$	4.5552 eV 272.18 nm	0.2258	H-2 → L+1	71.5
	$S_0 \rightarrow S_{22}$	5.2918 eV 234.30 nm	0.2220	H-2 → L+2	24.1
	$S_0 \rightarrow S_{38}$	6.1619 eV 201.21 nm	0.2661	H-3 → L+2	57.7
BP-CN	$S_0 \rightarrow S_5$	4.3059 eV 287.94 nm	0.1944	H → L+2	51.0
	$S_0 \rightarrow S_8$	4.5443 eV 272.83 nm	0.3358	H-3 → L	57.8
	$S_0 \rightarrow S_9$	4.6484 eV 266.72 nm	0.2062	H → L+3	31.0
	$S_0 \rightarrow S_{12}$	4.8427 eV 256.02 nm	0.3306	H-1 → L+4	60.0
	$S_0 \rightarrow S_{15}$	5.0813 eV 244.00 nm	0.2375	H → L+5	28.5
	$S_0 \rightarrow S_{17}$	5.1281 eV 241.78 nm	0.2415	H-4 → L	35.1
	$S_0 \rightarrow S_{21}$	5.3624 eV 231.21 nm	0.2765	H-2 → L+4	46.5

[a] Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength. [c] H stands for HOMO and L stands for LUMO.

Table S2

Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO)

Species	E_{HOMO} (a.u)	E_{LUMO}(a.u)	ΔE(a.u)	ΔE(eV)	ΔE(kcal/mol)
BP	-0.2002	-0.11681	0.08339	2.269175324	52.32802
BP-CN	-0.19361	-0.04583	0.14778	4.021330248	92.73335

Fukui Function Calculation: We have calculated fukui function using the following equation.

$f_k^+ = [q_k(N+1) - q_k(N)]$ for nucleophilic attack

The $q_k(N)$, and $q_k(N+1)$ are the electron population for the atom k in the N and (N+1) electron containing system respectively.

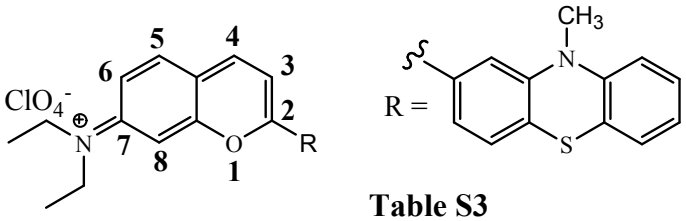

Table S3

Reactive carbon centres of BP	**Electrophilic Fukui function (au)**
C2	3.0741
C4	4.4663
C5	1.3922

Geometries have been optimized using the B3LYP/6-31G(d,p) level of theory in presence of solvent water. Solvent effects were incorporated using PCM solvent model implemented in Gaussian 09. The Fukui function has been calculated using the NBO charge. The geometries are verified as proper minima by frequency calculations. Time-dependent density functional theory calculation has also been performed at the same level of theory.

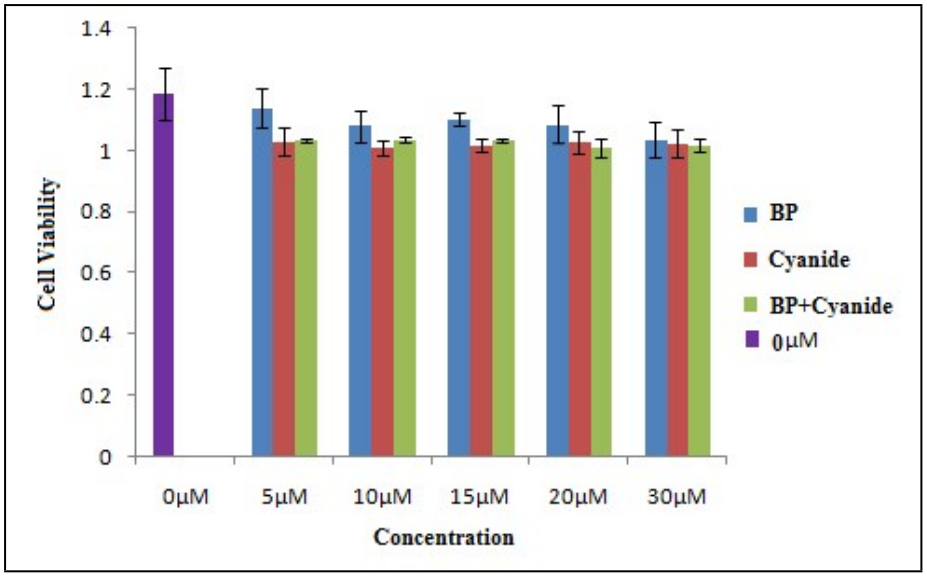

Figure S11: MTT assay to determine the cytotoxic effect of **BP** on A549 human cells (ATCC:CCL-185).

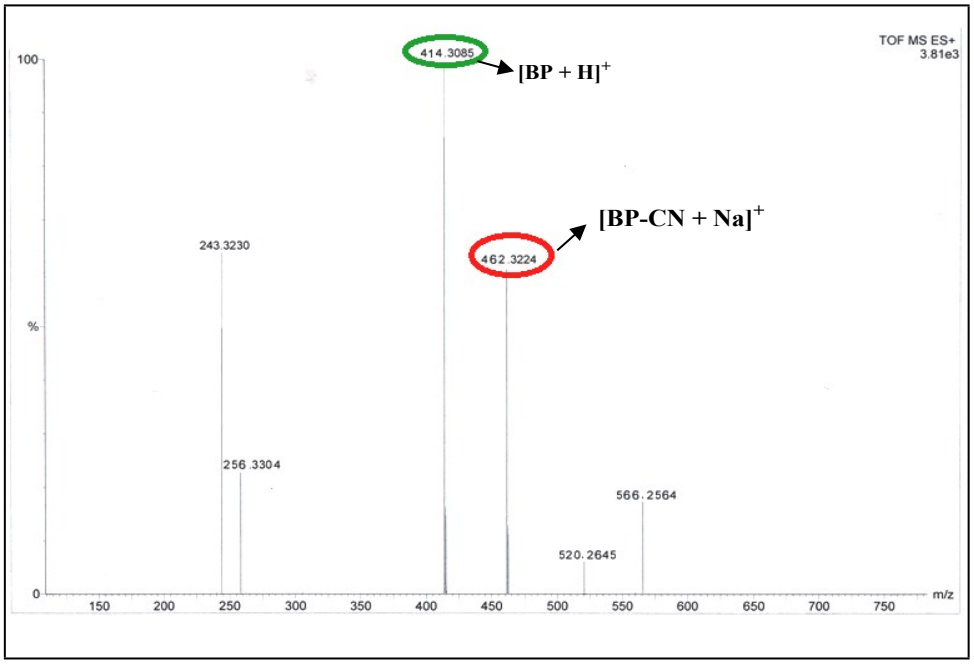

Figure S12. HRMS of **BP-CN** complex in assay.

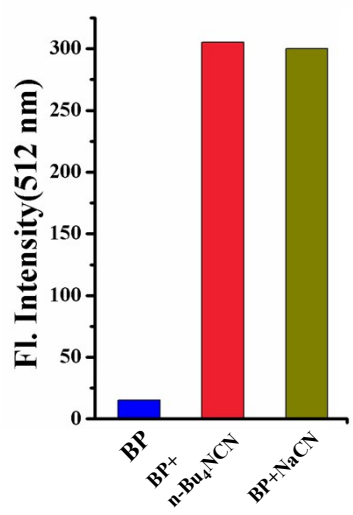

Figure S13. Comparative graph of **BP** alone, in presence of n-Bu_4NCN and NaCN.

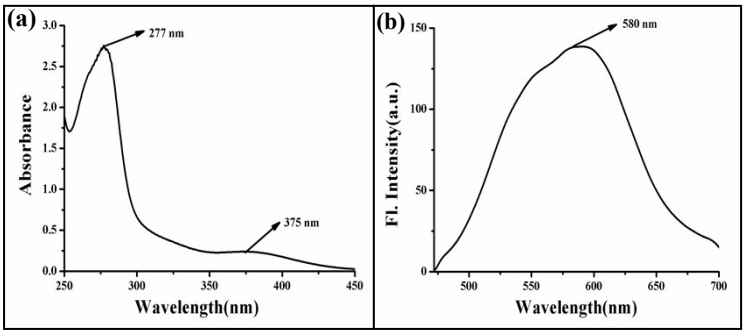

Figure S14. (a) The UV-visible response of N-methyl phenothiazine (1×10^{-5} M) in aq. CH_3CN (H_2O/CH_3CN = 3:7 v/v, 20 mM HEPES buffer, pH = 7.4). (b) The fluorescence response of N-methyl phenothiazine (1×10^{-5} M) in aq. CH_3CN (H_2O/CH_3CN = 3:7 v/v, 20 mM HEPES buffer, pH = 7.4).

Quantum yield calculation:

Here, the quantum yield ϕ was measured by using the following equation:

$\phi_x = \phi_s (F_x / F_s)(A_s / A_x)(n_x^2/ n_s^2)$

Where,

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

F = area under the emission curve, A = absorbance at the excitation wave length,

n = index of refraction of the solvent. Here ϕ measurements were performed using fluorescein in ethanol as standard [ϕ = 0.79]

For standard (s) fluorescein in ethanol the following values were determined:

n_s = 1.3614 (for ethanol); n_x=1.3441 (for acetonitrile); ϕ_s = 0.79.

We calculated the quantum yield of **BP** using the above equation and the value is 0.011.

We calculated the quantum yield of **BP-CN** complex using the above equation and the value is 0.12.