A Dual-Phase Coumarin Based Ratiometric Fluorescent Probe for Highly Sensitive Cyanide Sensing and Bioimaging Applications

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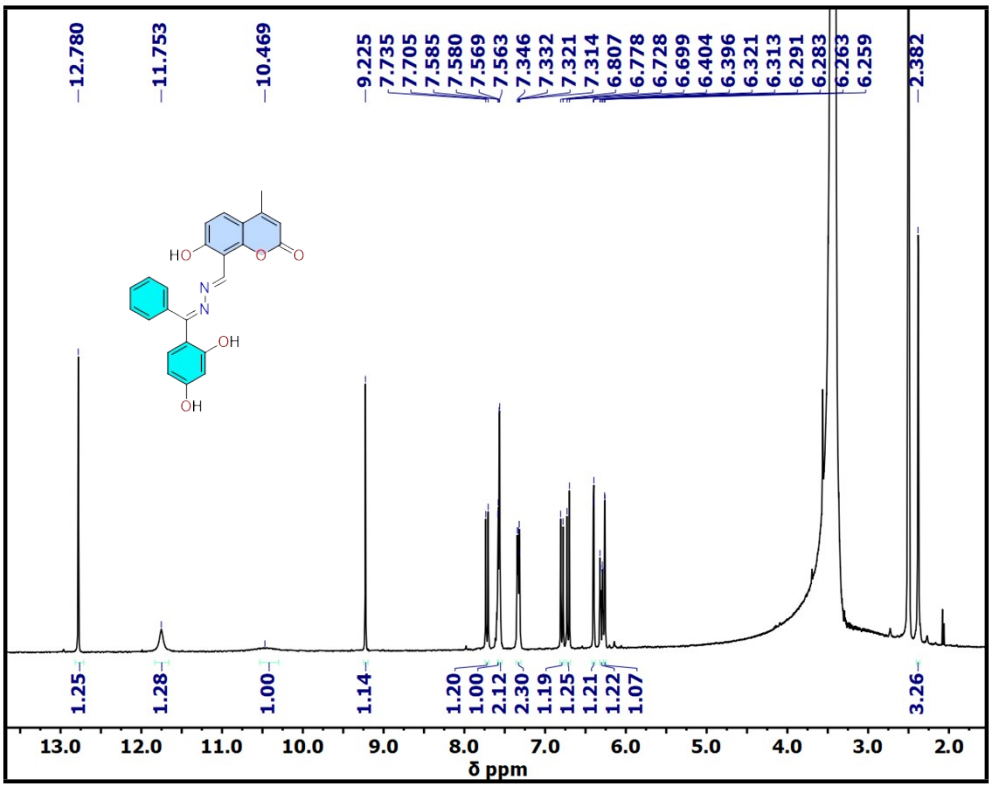


Figure S1: ^{1}H NMR (300 MHz) spectrum of the probe (DHMH) in DMSO-d_6

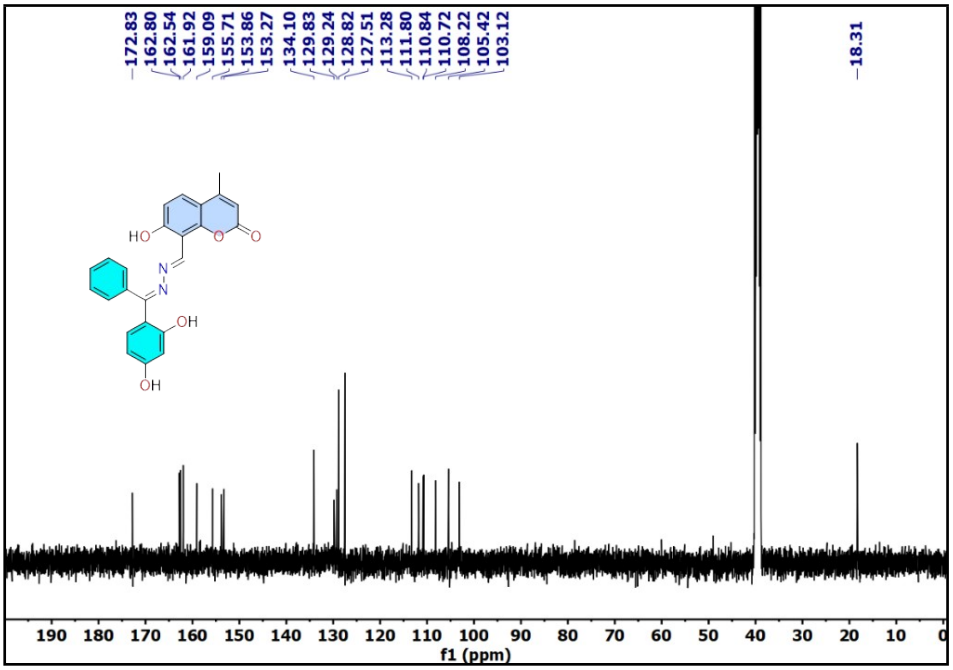


Figure S2: ^{13}C NMR (101 MHz) spectrum of the probe (DHMH) in DMSO-d_6

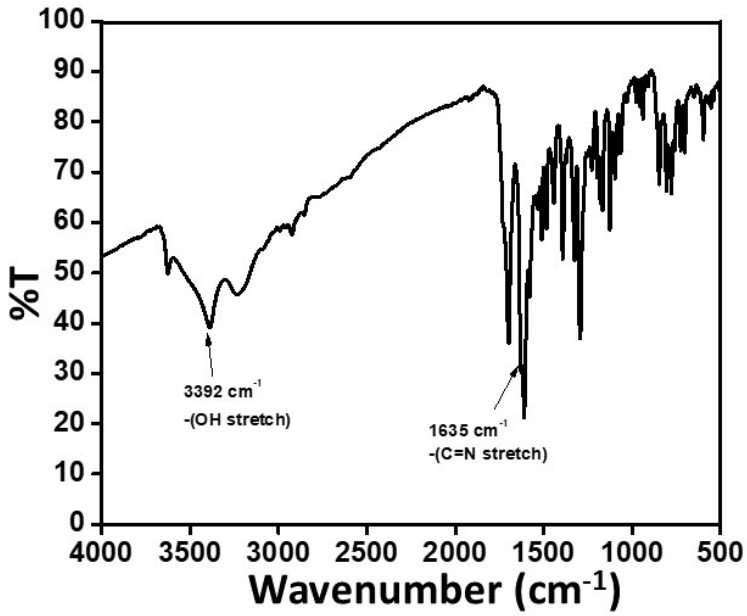


Figure S3: IR plot of the probe (DHMH)

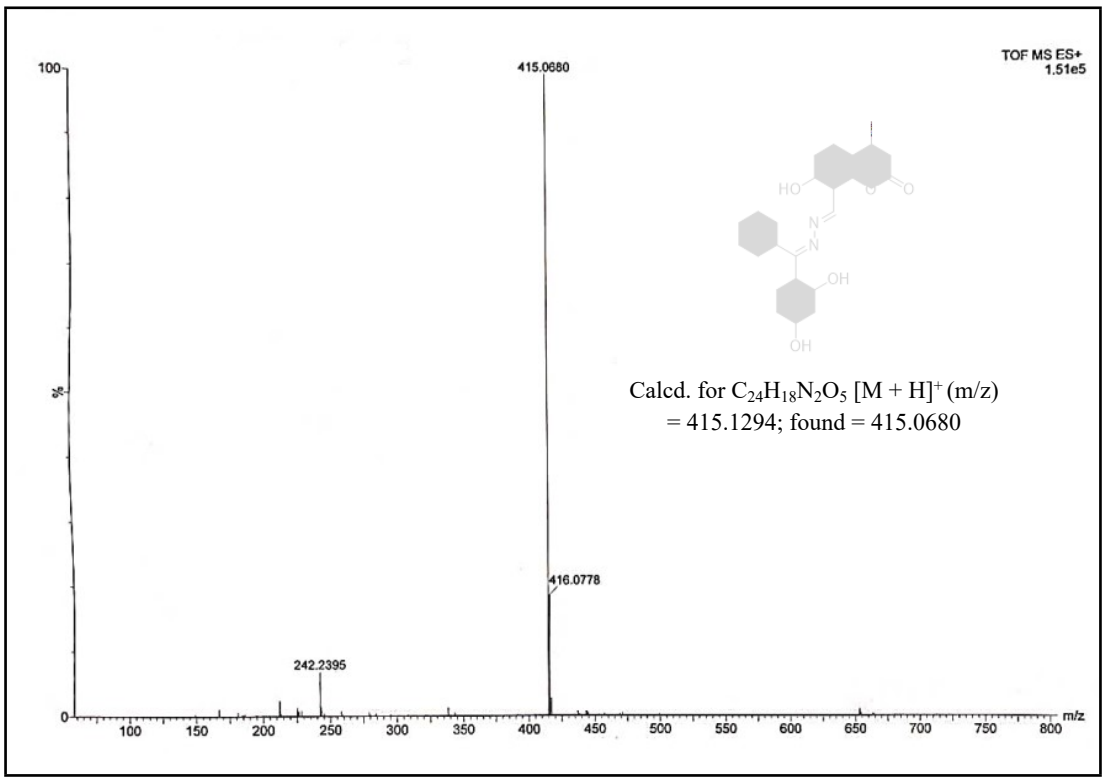


Figure S4: HRMS of the probe DHMH

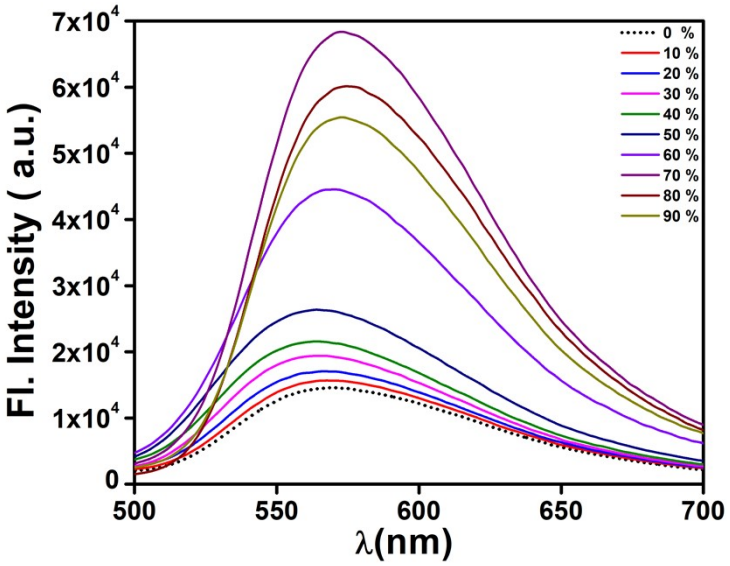


Figure S5: Fluorescence emission spectra of DHMH (10 μM) in DMSO-H_2O mixed solvents with increasing water fractions, excitation at 365 nm.

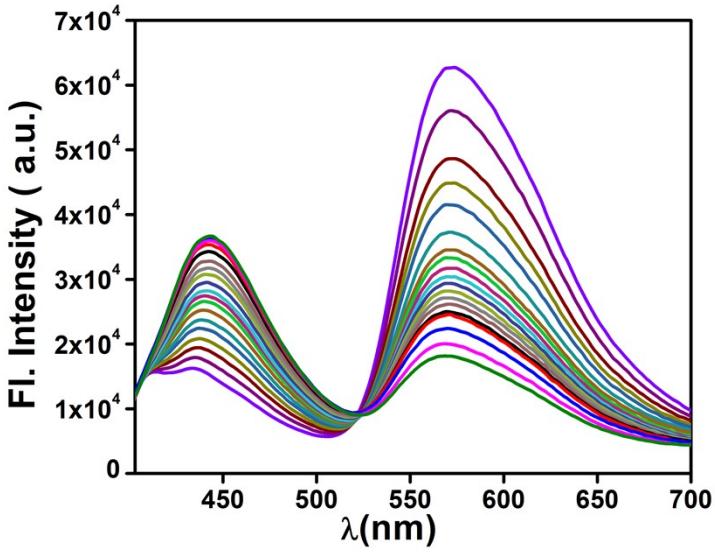


Figure S6: Change of emission spectra of DHMH (10 μM) after addition of CN^- (20 μM) in DMSO/H_2O (7/3, v/v) using HEPES buffered solution at pH=7.2. λ_{ex} = 365 nm.

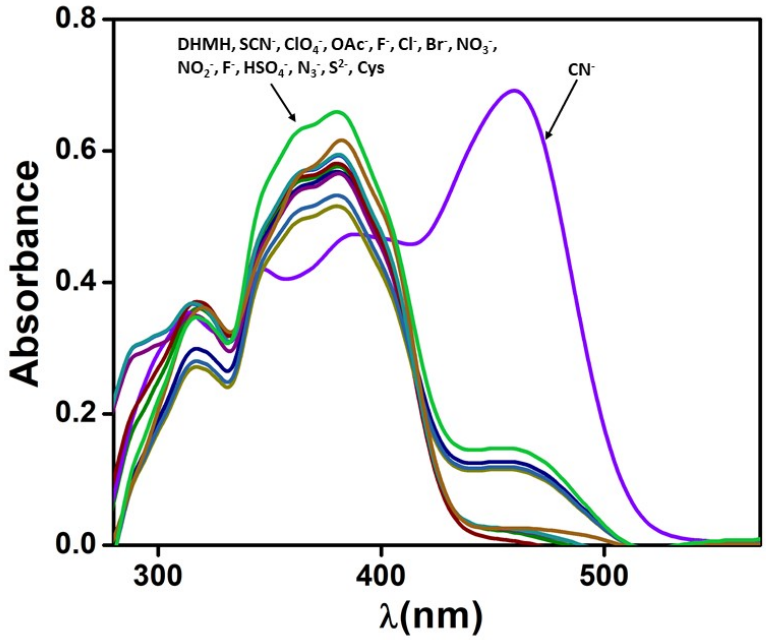


Figure S7: Absorption spectra of DHMH (10 μM) upon addition of various other anions (20 μM) in DMSO/H_2O (9/1, v/v) using HEPES buffered solution at pH=7.2.

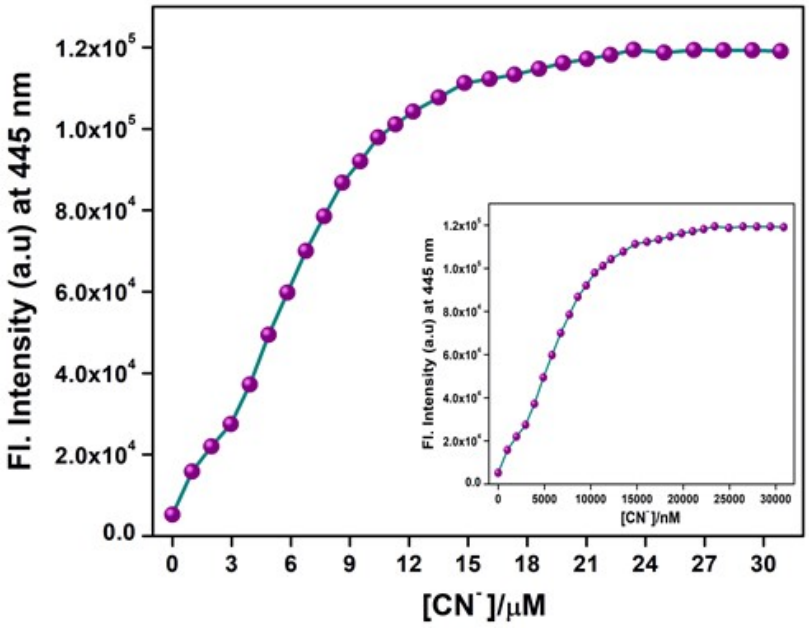


Figure S8: Mole ratio plot of DHMH (10 μM) for CN^- (20 μM) at 445 nm using fluorescence titration method (λ_{ex} = 365 nm).

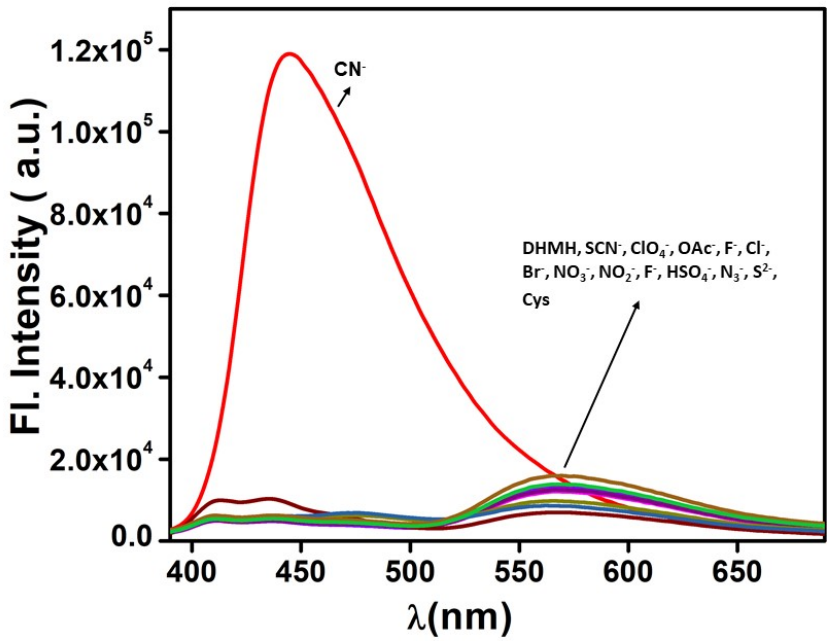


Figure S9: Change of emission spectra of DHMH (10 μM) after addition of other anions (20 μM) in DMSO/H_2O (9/1, v/v) using HEPES buffered solution at pH=7.2. λ_{ex} = 365 nm.

Determination of detection limit (LOD)

The limit of detection was determined based on the fluorescence titration. To determine the S/N ratio, the emission intensity of DHMH without CN^- was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit of DHMH for CN^- was determined from the following equation: DL = K × Sb_1/S, Where K = 2 or 3 (we take 3 in this case); Sb_1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

From the graph we get SD = 2158.79 and slope = 1.01093×10^{10}. Thus using the formula, we get the Detection Limit = $(0.64\pm0.13)\times10^{-6}$ M i.e., DHMH can detect CN^- in this minimum concentration by fluorescence techniques.

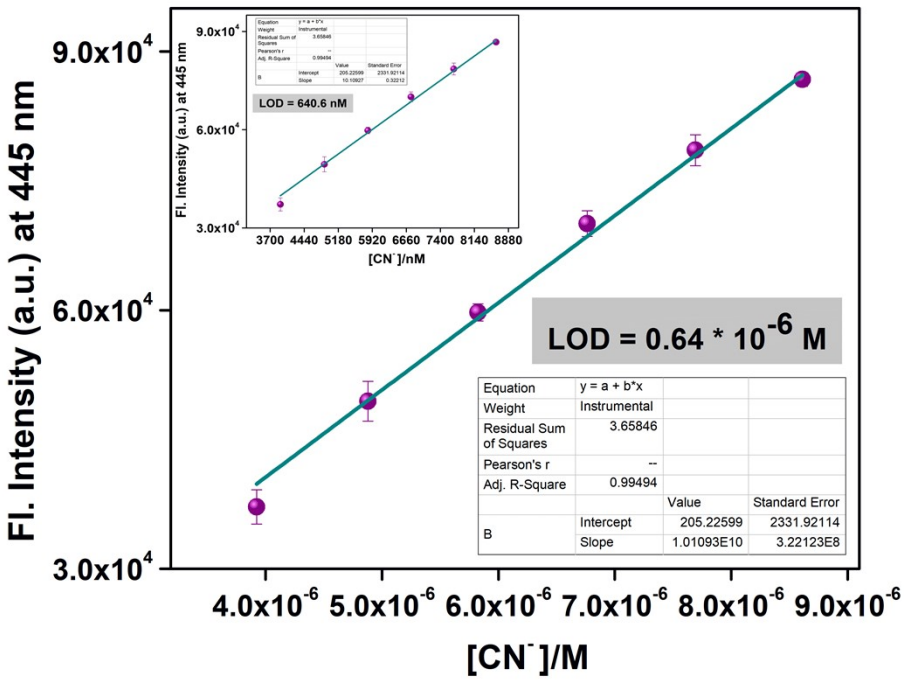
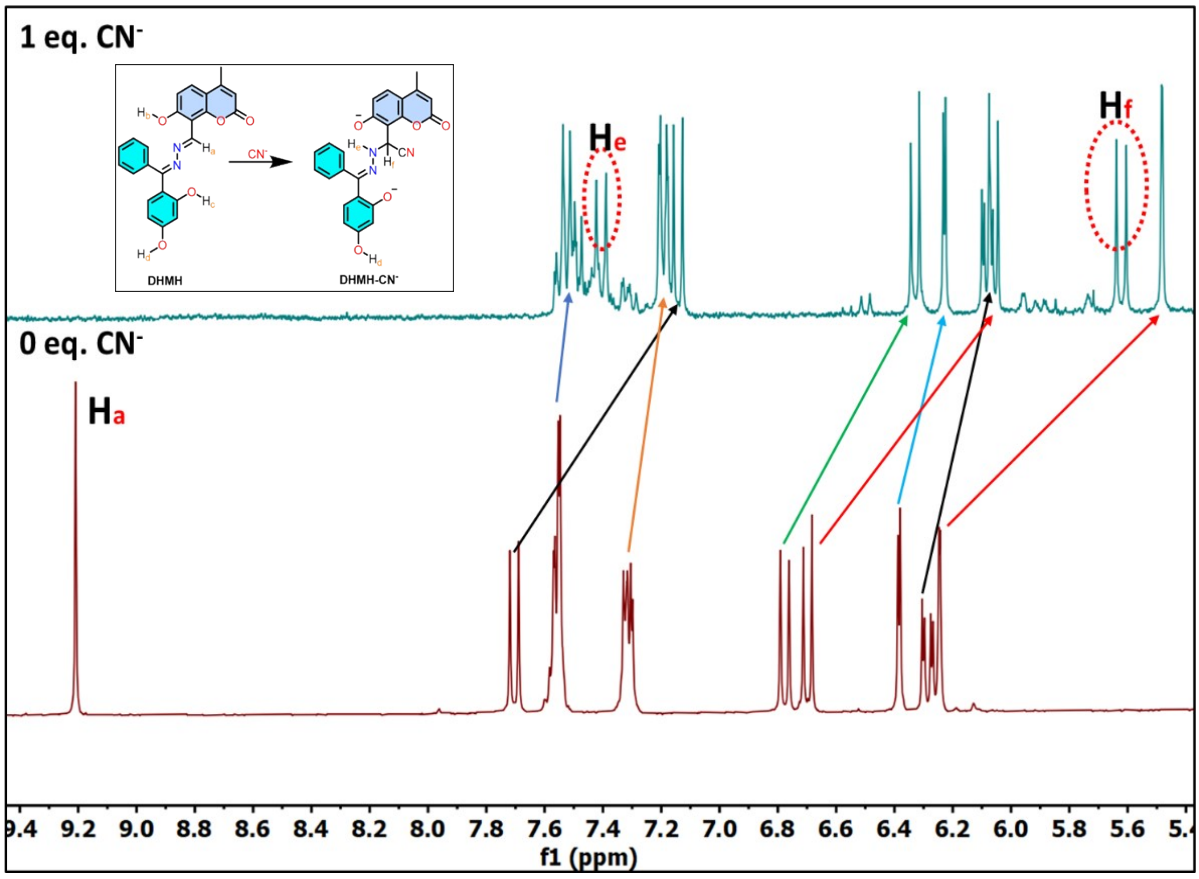


Figure S10: The linear response curve of at emission intensity at 445 nm of DHMH depending on CN^- concentration.



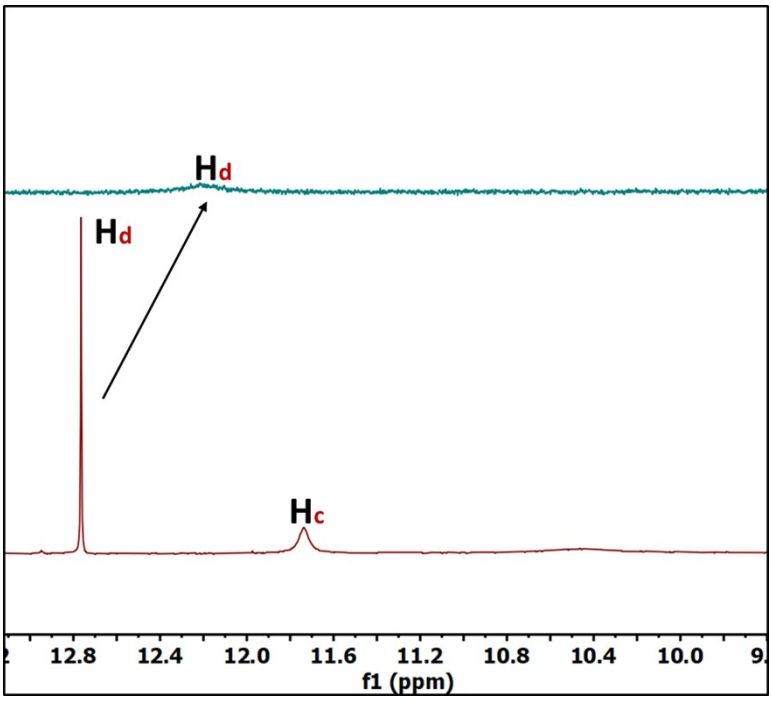


Figure S11: Enlarged ^{1}H NMR spectra of the probe before and after addition of 1.0 equiv. CN^{-} in DMSO-d_6.

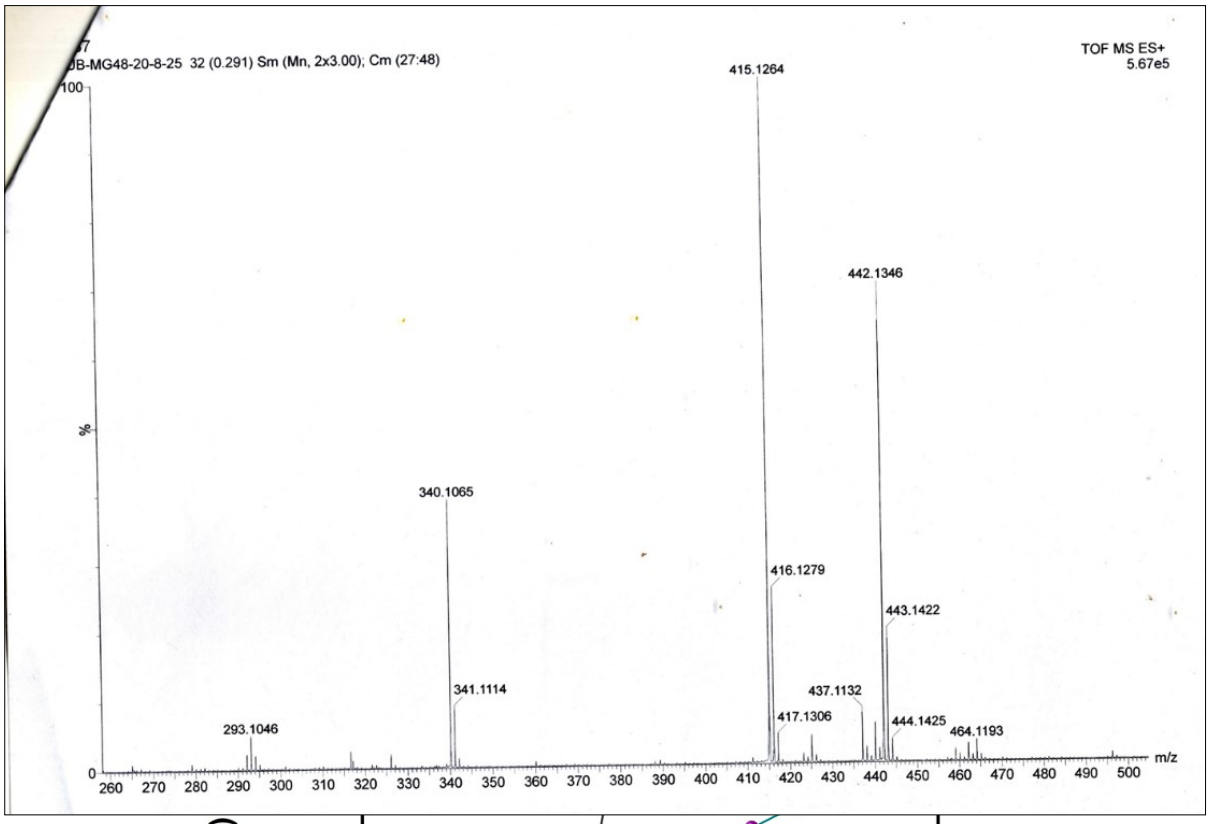


Figure S12: HRMS of DHMH+CN^{-}.

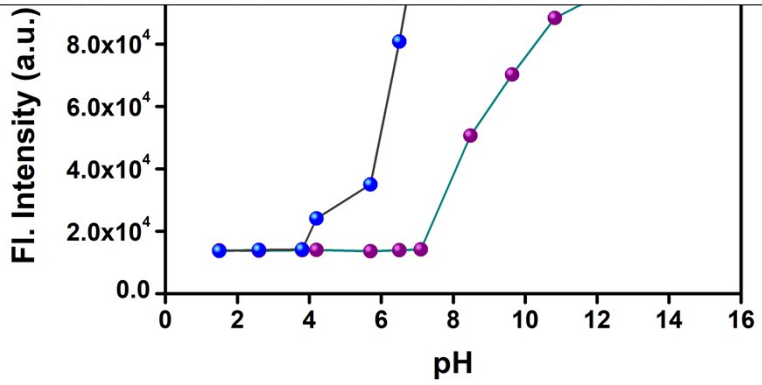


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Figure S13: pH titration plot of DHMH and DHMH-CN^-.

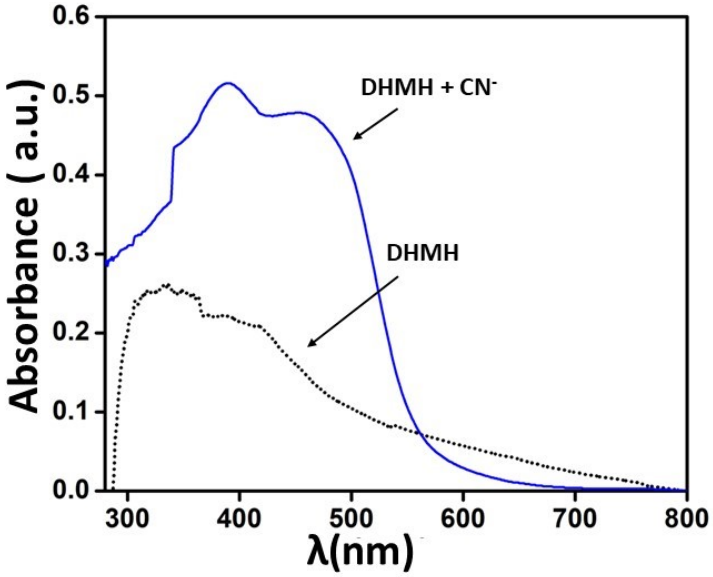


Figure S14: Solid state UV spectral Study of DHMH and DHMH-CN^- by drop-casting method.

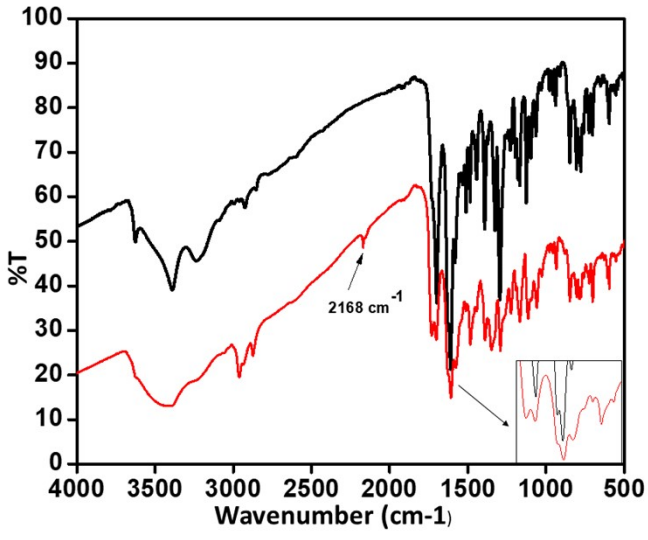


Figure S15: IR spectra of DHMH and DHMH-CN^-.

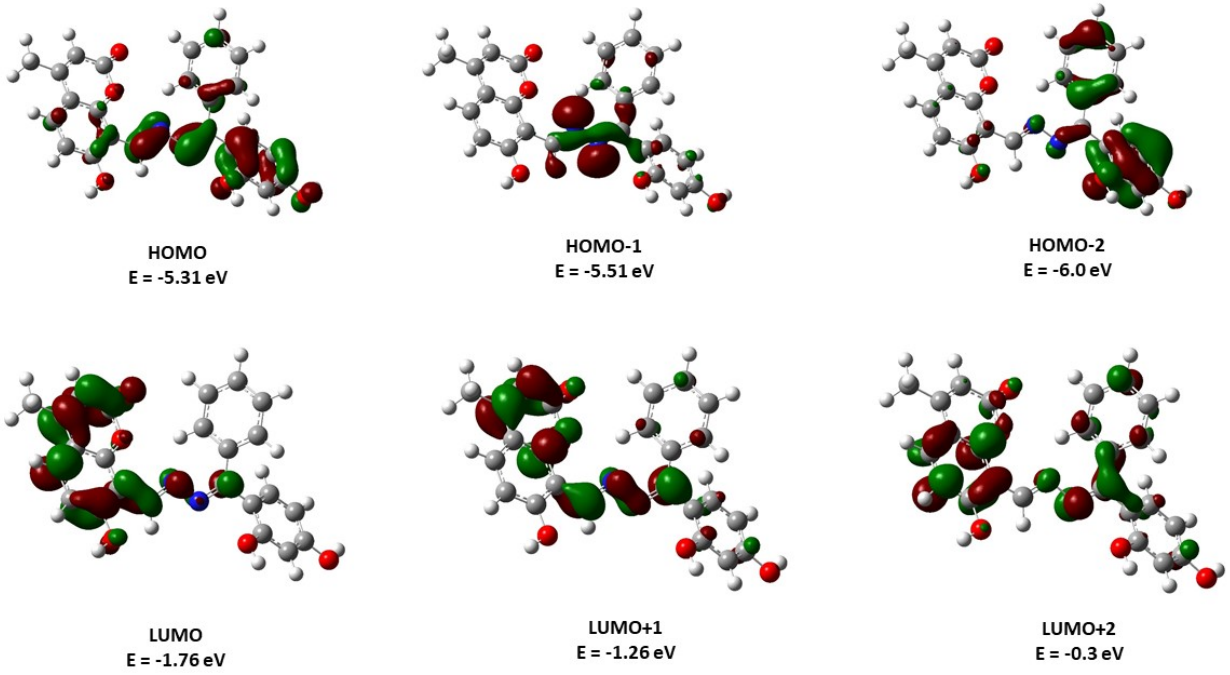


Figure S16. Contour plots of some selected molecular orbitals of DHMH.

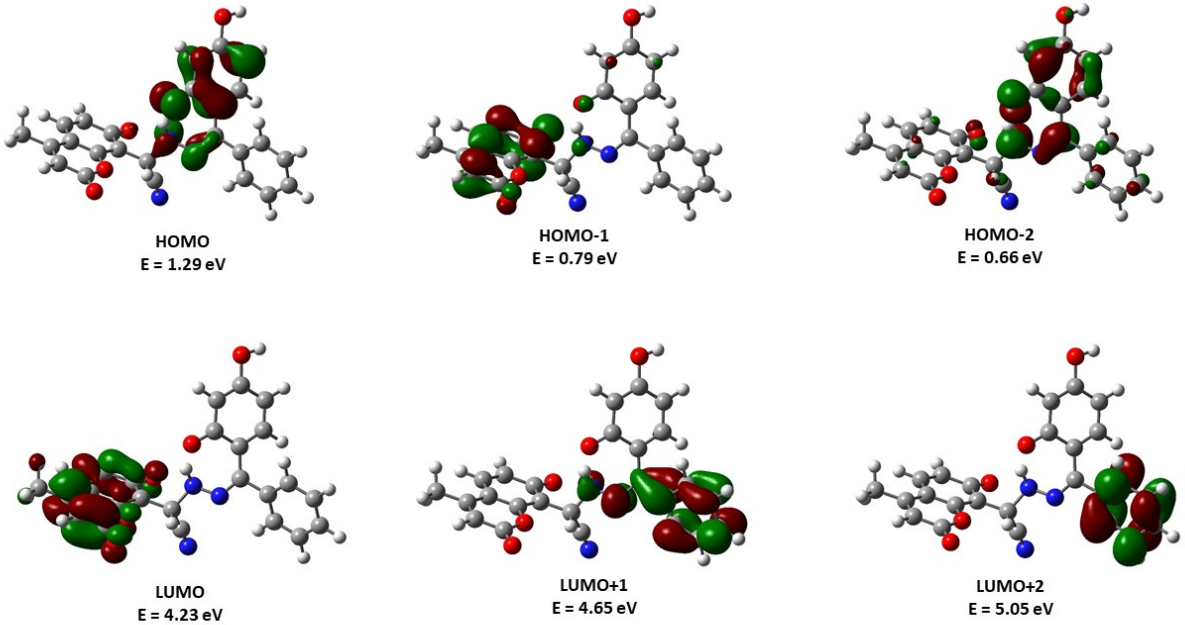


Figure S17. Contour plots of some selected molecular orbitals of DHMH-CN^-.

Determination of emission quantum yield (Φ) of DHMH and its complex with CN^-

For measurement of the quantum yields of DHMH and its product with CN^- (DHMH-CN^-), the absorbance of the compounds was recorded in DMSO solution. The emission spectra were then also recorded using the maximal excitation wavelengths and the integrated areas of the emission-corrected spectra were measured. The quantum yields were calculated by comparison with coumarin 153 (φ_s = 0.54 in 0.5M H_2SO_4) as reference using the following equation:

$$\Phi_x = \Phi_s \times \left(\frac{Ix}{Is}\right) \times \left(\frac{As}{Ax}\right) \times \left(\frac{nx}{ns}\right)^2$$

Where, 'x' & 's' designate the unknown and standard solution respectively, Φ is the quantum yield, 'I' refers to the integrated area under the fluorescence spectra, 'A' is the absorbance and 'n' is the refractive index of the solvent. Thus the quantum yields of DHMH and DHMH-CN^- were found out to be 0.009 and 0.286 respectively using the above equation.

Table S1. Vertical electronic transitions calculated by TDDFT/B3LYP/CPCM method for DHMH and DHMH-CN^- in DMSO

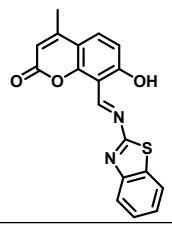
Compds.	Energy (eV)	Wavelength (nm)	Osc. strength (f)	Transition	Character
DHMH	3.2902	376.8	0.6113	(88%) HOMO→LUMO	$\pi(L) \rightarrow \pi^*(L)$
	3.6401	340.6	0.0994	(70%) HOMO→LUMO+1	$\pi(L) \rightarrow \pi^*(L)$
	3.0827	402.2	0.0370	(73%) HOMO-1→LUMO	$\pi(L) \rightarrow \pi^*(L)$

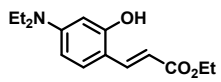
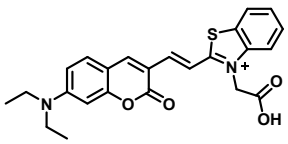
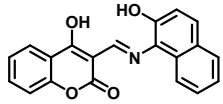
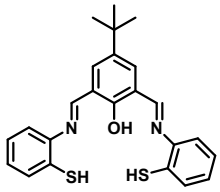
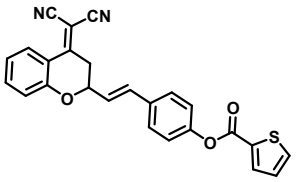
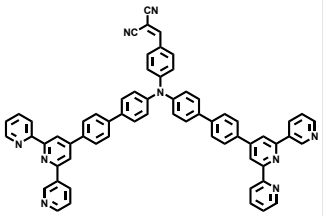
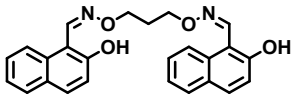
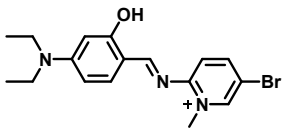
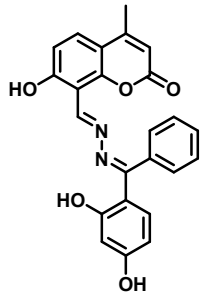
	3.8066	325.7	0.1478	(67%) HOMO-2→LUMO	$\pi(L)\rightarrow \pi^*(L)$
	3.8915	318.6	0.1160	(77%) HOMO-3→LUMO	$\pi(L)\rightarrow \pi^*(L)$
DHMH-CN^- (Solution)	2.9938	414.1	0.0064	(99%) HOMO→LUMO	$\pi(L)\rightarrow \pi^*(L)$
	3.2586	380.5	0.1078	(94%) HOMO→LUMO+1	$\pi(L)\rightarrow \pi^*(L)$
	3.4263	361.8	0.3671	(94%) HOMO-1→LUMO	$\pi(L)\rightarrow \pi^*(L)$
	3.5437	349.8	0.0242	(95%) HOMO-2→LUMO	$\pi(L)\rightarrow \pi^*(L)$
DHMH-CN^- (Solid)	2.6702	464.33	0.0234	(96%) HOMO→LUMO+4	$\pi(L)\rightarrow \pi^*(L)$
	3.1960	387.94	0.0417	(87%) HOMO→LUMO+5	$\pi(L)\rightarrow \pi^*(L)$
	3.6148	342.99	0.0346	(78%) HOMO-1→LUMO+1	$\pi(L)\rightarrow \pi^*(L)$

Table S2: Lifetime decay profile of DHMH and DHMH-CN^-

DMSO (Solvent)	Quantum Yield	τ (ns)	K_r (10^8 x S^{-1})	K_{nr} (10^8x S^{-1})
DHMH	0.009	3.58	0.025	2.793
DHMH-CN^-	0.286	2.18	1.311	3.276

Table S3: The comparison of the present probe (DHMH) with some previous probes for CN^-

Probe	Type of response	Response Time (min or sec)	Solvent System	Detection limit	Reference
	Fluorescence turn-on	-	DMSO/MeOH (1:40)	0.75 μM	[1]

	Fluorescence turn-on	Within 90 minutes	5% aqueous CH_3CN buffer	1.3 μM	[2]
	Ratiometric fluorescence change	-	CH_3CN/H_2O (9:1)	0.64 μM	[3]
	Fluorescence turn-on	< 1 min	DMSO	0.056 μM	[4]
	Ratiometric fluorescence change	-	DMSO-H_2O (1:4)	0.96 μM	[5]
	Fluorescence turn-on	180 min	DMSO/H_2O (9:1)	1.44 μM	[6]
	Fluorescence quenching	-	H_2O/THF, 9:1	3.8 μM	[7]
	Ratiometric fluorescence change	< 1 min	DMSO/H_2O (9:1)	0.128 μM	[8]
	Fluorescence turn-off	Within 2 minutes	DMSO/H_2O (7:3)	2.8 μM	[9]
	Fluorometric (ratiometric)	Approx. 2 minutes	DMSO/H_2O (9:1, v/v)	$(0.64\pm0.13)\times10^{-6}$ M	Present Work

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