# Electronic Supplementary Information(ESI†)

## Chromogenic and ratiometric fluorogenic probe for rapid detection of a nerve agent simulant DCP based on a hybrid hydroxynaphthalene–hemicyanine dye

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**General Information:**

**Finding the Detection Limit**. The detection limit was calculated on the basis of the fluorescence titration .The absorption and fluorescence spectrum of **CYD** were measured, and the standard deviation of blank measurement was achieved. To gain the slope, the ratio of the fluorescence intensity was plotted as a concentration of DCP. So the detection limit was calculated with the following equation.

$$\text{Detection limit} = 3Sb1/S \qquad (1)$$

where Sbl is the standard deviation of blank measurement and S is the slope of the calibration curve.

**Cytotoxicity Assay.** The cytotoxic effects of **CYD** and **[CYD + DCP]** were determined by MTT assay following the manufacturer's instruction (MTT 2003, Sigma-Aldrich, MO). **Hep-2** (**ATCC**: **CCL-23**) were seeded onto 96-well plates (approximately 104 cells per well) for 24 hrs. Next day media was removed and various concentrations of probe **CYD**, DCP (0, 0.625,1.25, 2.50 and 5.0 μM) made in DMEM were added to the cells and incubated for 24 h. Solvent control samples (cells treated with DMSO in DMEM), no cells and cells in DMEM without any treatment were also included in the study. Following incubation, the growth media was removed, and fresh DMEM containing MTT solution was added. Subsequently, the supernatant was removed, the insoluble colored formazan product was solubilized in DMSO, and its absorbance was measured in a microtiter plate reader (Perkin-Elmer) at 570. Flurochrome Filter, Qdot 525 for **CYD** & TRITC **for [CYD + DCP]** were used. The cell viability was calculated by the following formula: (mean OD in treated wells / mean OD in control wells) x 100.

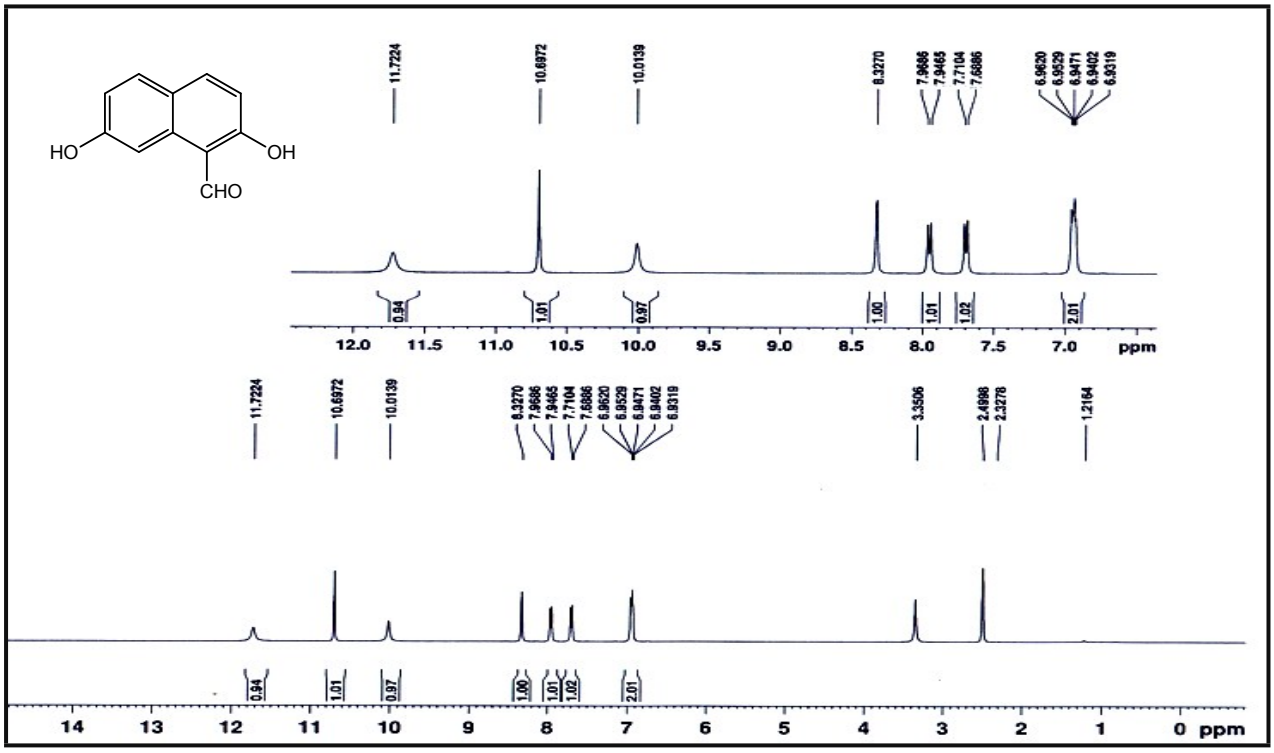


**Figure S1:** $^{1}$H NMR of Compound **1** in ($d_6$-DMSO).

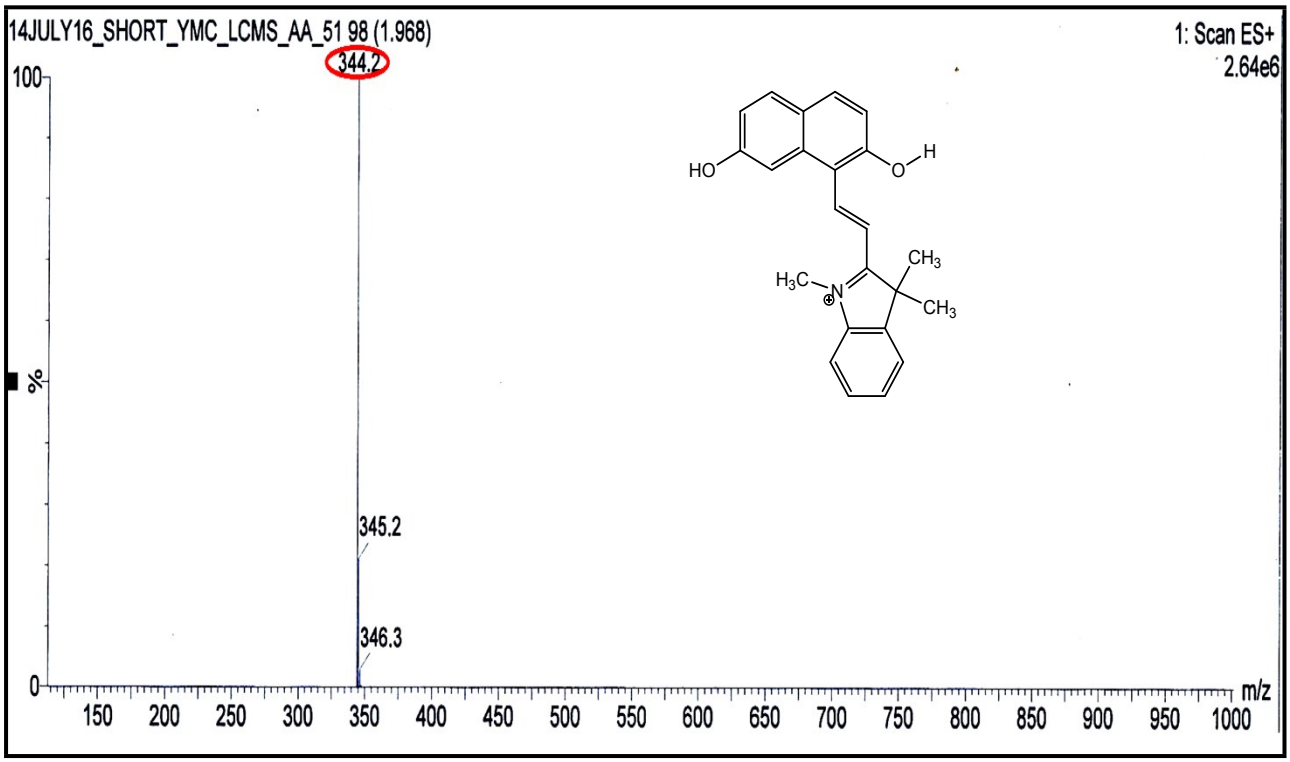


**Figure S2:** LCMS of Compound **CYD**.

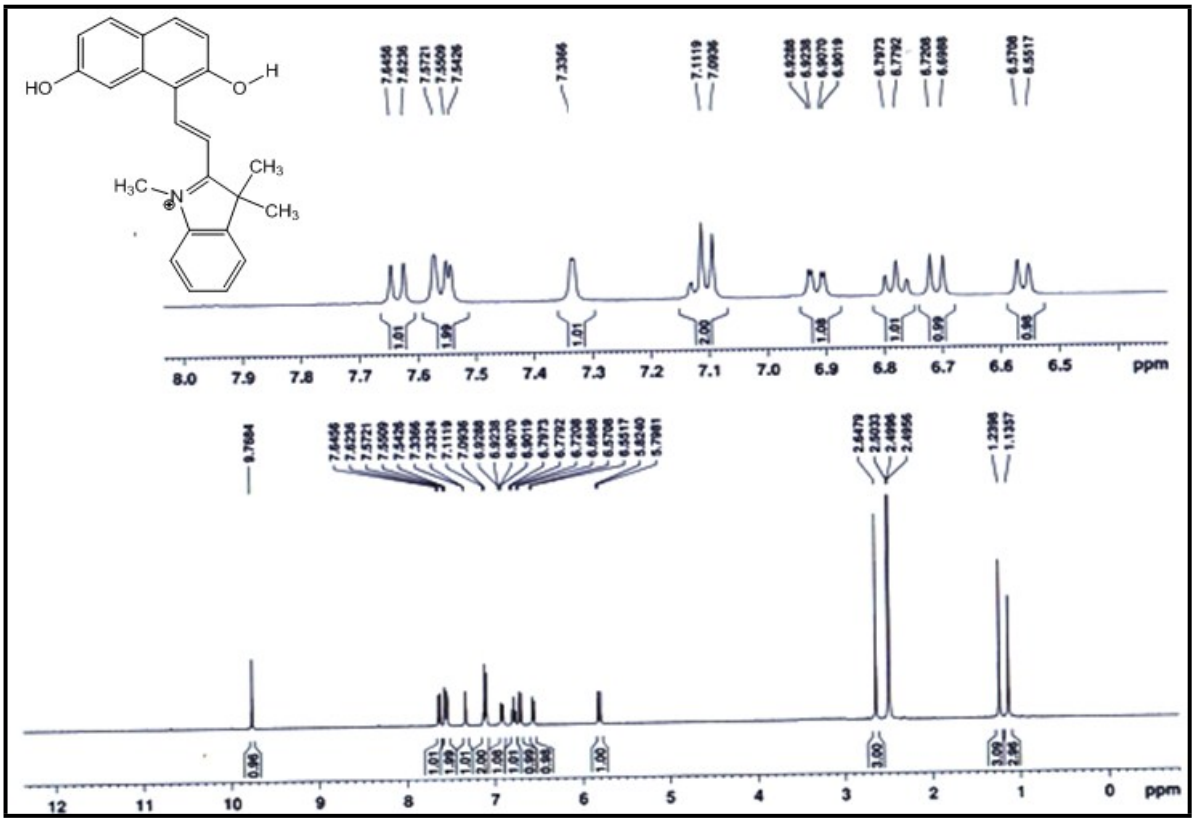

**Figure S3:** $^{1}$H NMR of Compound **CYD** in ($d_6$-DMSO).

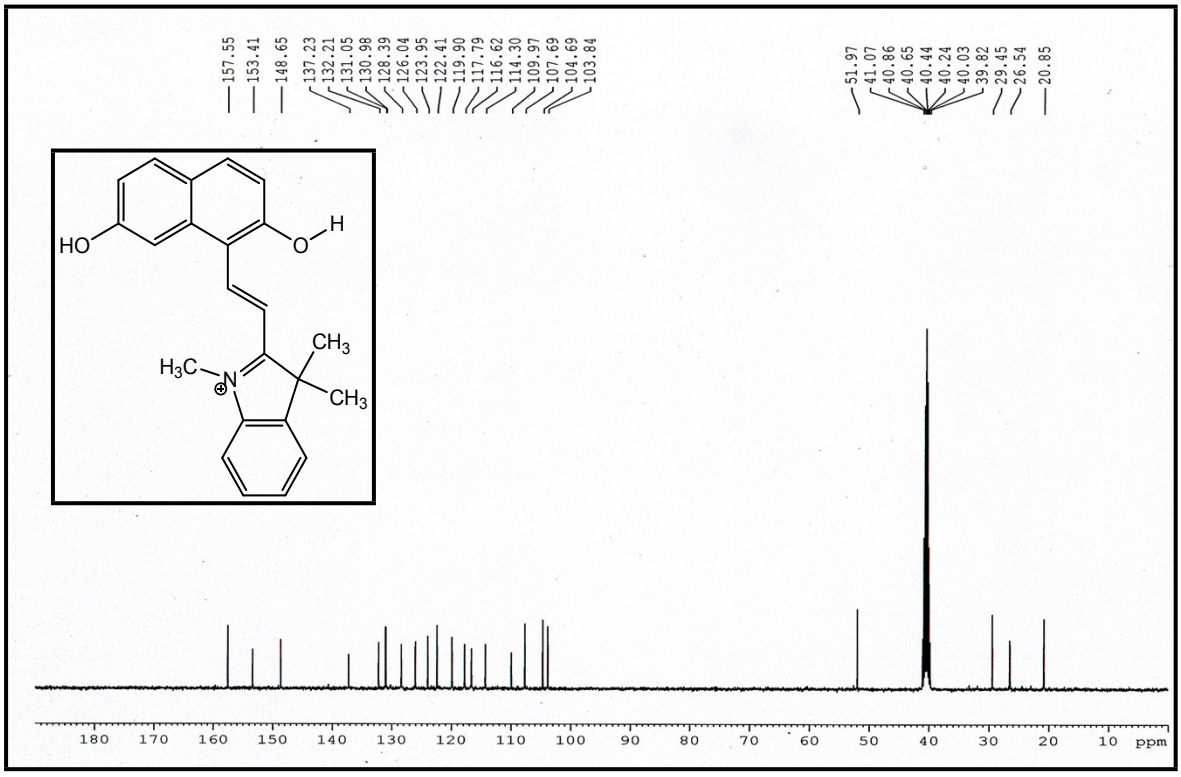

**Figure S4:** $^{13}$CNMR of Compound **CYD** in ($d_6$-DMSO).

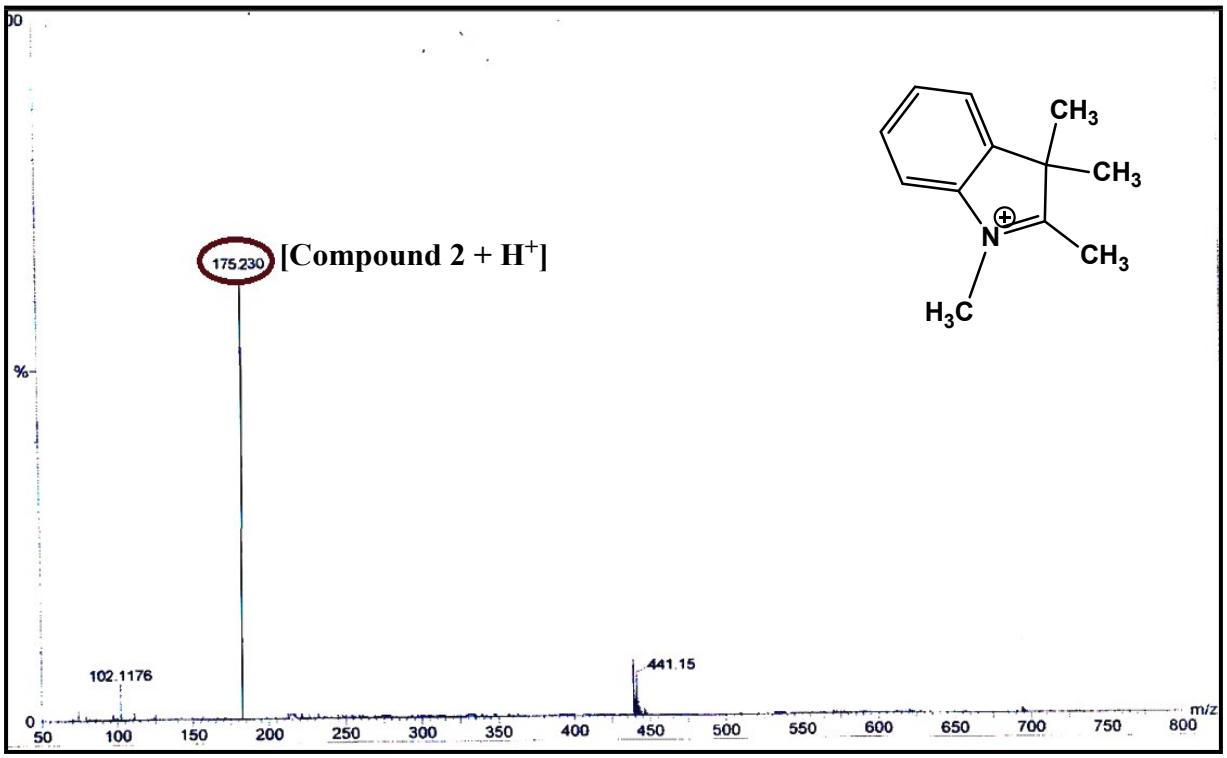


**Figure S5:** LCMS of Compound **2.**

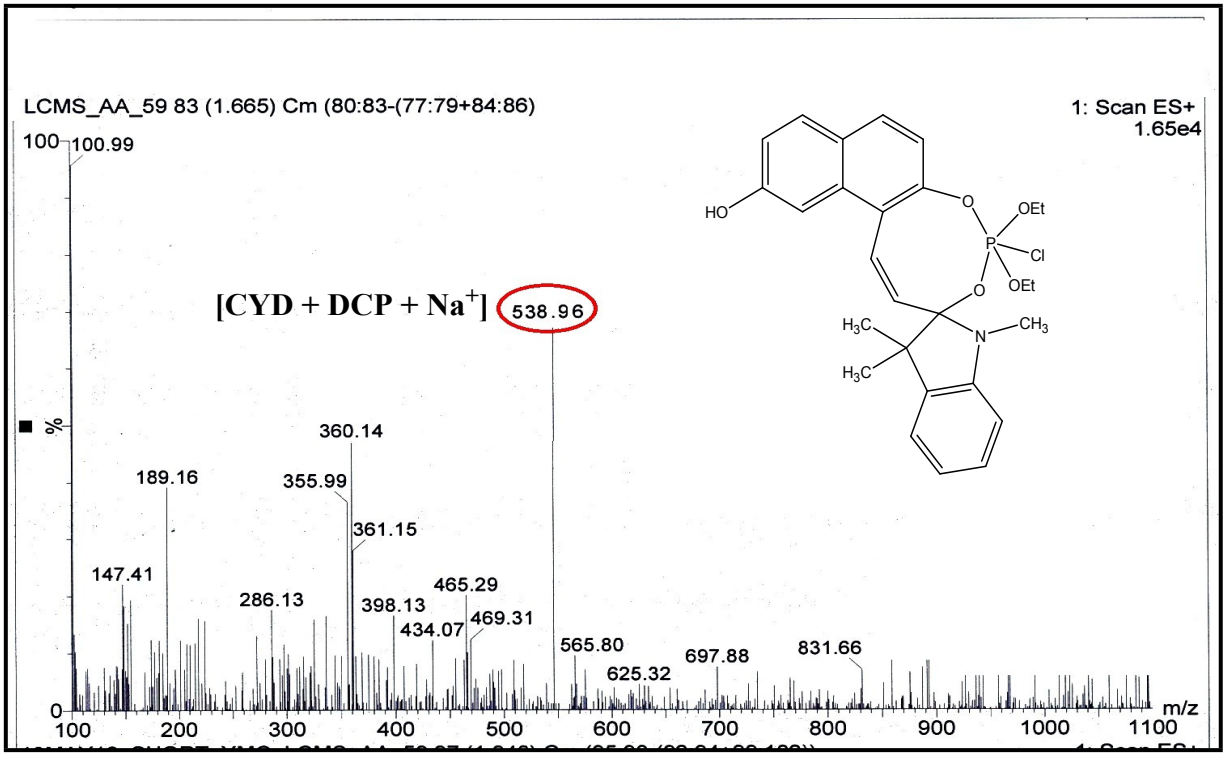


**Figure S6:** LCMS of Compound **(CYD + DCP)**.

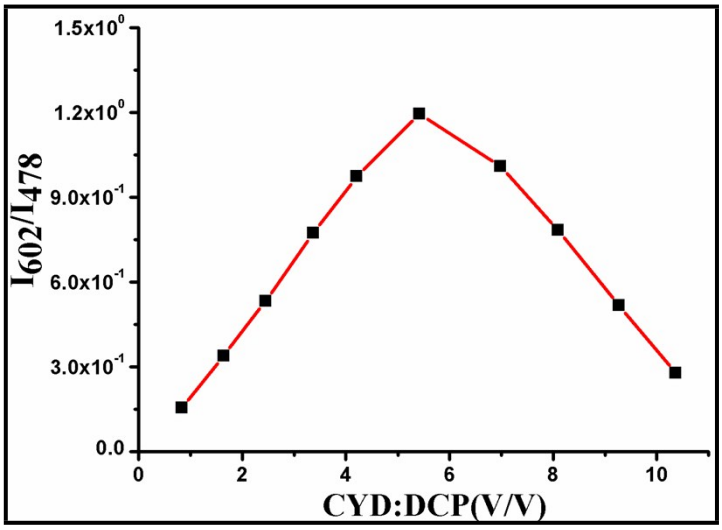


**Figure S7:** Fluorescence Job's plot of **CYD** with **DCP**.

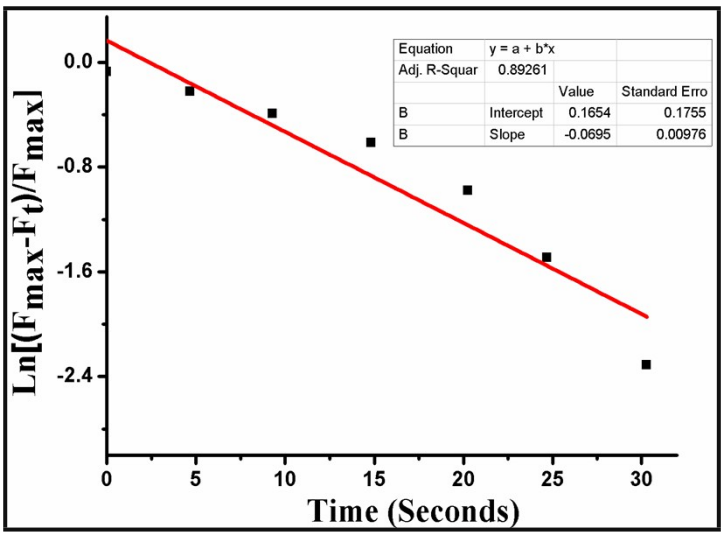


**Figure S8:** Pseudo first-order kinetic plot of reaction of **CYD** (0.4μM) with DCP (8.0 μM).

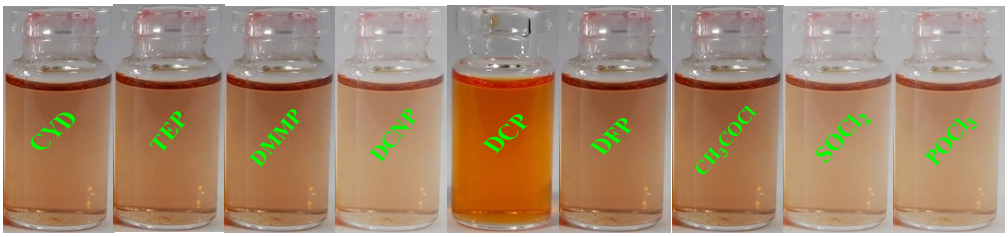


**Figure S9:** The visible color changes of receptor **CYD** in aq. $CH_3CN$ ($CH_3CN$: $H_2O$ = 1:1 v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of various analytes.

**Computational details**:

Geometries have been optimized using the B3LYP/6-31G (d, p) level of theory 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. All calculations have been carried out using Gaussian 09 program.

| Molecules | Electronic Transition | Excitation Energy[a] | f[b] | Composition[c] | (composition) % |
|---|---|---|---|---|---|
| **CYD** | $S_0 \rightarrow S_7$ | 4.7129 eV 351.08 nm | 0.1616 | H-1 → L+2<br>H-1 → L+3 | 63.2 |
| | $S_0 \rightarrow S_{16}$ | 5.4065 eV 229.32 nm | 0.1915 | H-5 → L<br>H-5 → L+1 | 22.1 |
| | $S_0 \rightarrow S_{17}$ | 5.4569 eV 227.21 nm | 0.1472 | H-3 → L+2 | 36.6 |
| | | | | | |
| **CYD-DCP** | $S_0 \rightarrow S_1$ | 2.4947 eV 485.98 nm | 0.5140 | H → L | 86.1 |
| | $S_0 \rightarrow S_2$ | 2.8187 eV 439.86 nm | 0.2504 | H-1 → L | 83.2 |
| | $S_0 \rightarrow S_{14}$ | 5.1394 eV 227.94 nm | 0.2190 | H-3 → L+1<br>H → L+4 | 38.5 |

**Table S1:** Selected electronic excitation energies (eV), oscillator strengths (f), main configurations, and CI Coefficients of all the complexes. The data were calculated by TDDFT//B3LYP/6-31+G (*d,p*) based on the optimized ground state geometries.[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.

| Species | $E_{HOMO}$ (a.u) | $E_{LUMO}$(a.u) | ΔE(a.u) | ΔE(eV) | ΔE(kcal/mol) |
|---|---|---|---|---|---|
| **CYD** | -0.19631 | -0.04515 | 0.15116 | 4.113305 | 94.85434 |
| **CYD-DCP** | -0.31101 | -0.20968 | 0.10133 | 2.757351 | 63.58554 |

**Table S2:** Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of **CYD** and **CYD - DCP.**

**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 0.1 M NaOH as standard [ϕ = 0.79] and Rhodamine 101 in Ethanol + 0.01 % HCl [ϕ = 1.00].

For standard (s) Fluorescein in 0.1 M NaOH the following values were determined:

$n_s$ = 1.3330 (for 0.1 M NaOH); $n_x$=1.344 (for $CH_3CN$); ϕ = 0.79.

For standard (s) Rhodamine 101 in Ethanol + 0.01 % HCl the following values were determined:

$n_s$ = 1.361; $n_x$=1.344 (for $CH_3CN$); ϕ = 1.00

Using the above equation, we calculated quantum yield of probes.

| **Probes** | **Quantum yield** (ϕ) |
|---|---|
| **CYD at 478 nm** | 0.56 |
| **CYD-DCP at 602 nm** | 0.21 |

**Table S3:** Quantum yield data

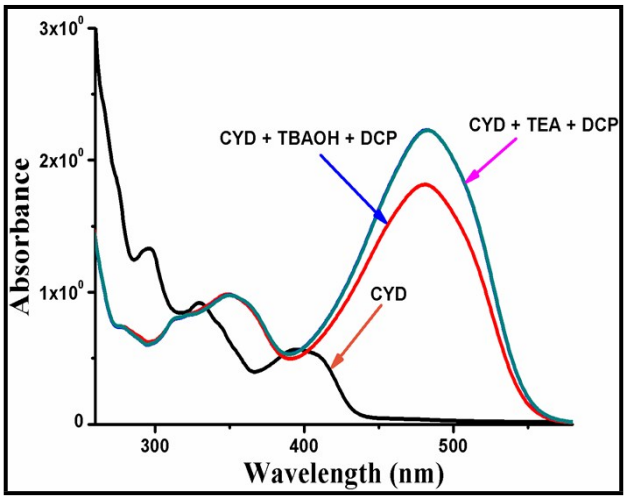


**Figure S10:** Absorption spectra of free **CYD**, **CYD + TBOH + DCP**, and **CYD + TEA + DCP**.

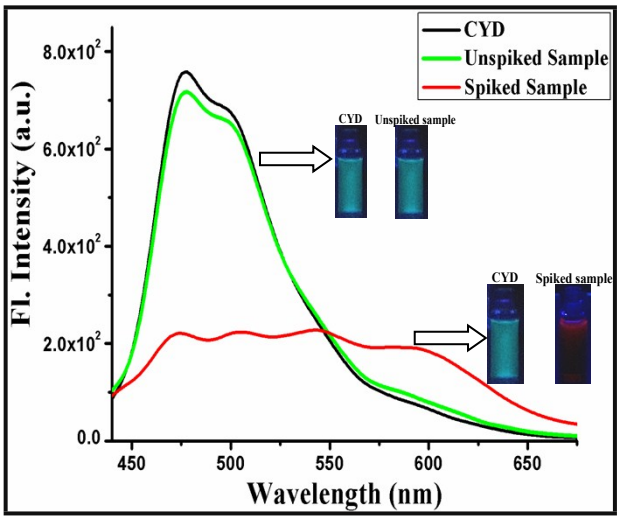


**Figure S11:** Fluorescence spectra of free **CYD**, Unspiked sample and Spiked sample.

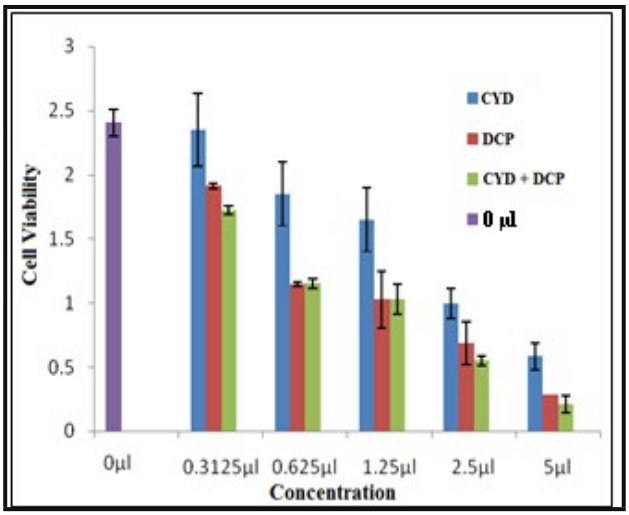


**Figure S12:** MTT assay to determine the cytotoxic effect of **CYD** on **Hep-2 (ATCC: CCL-23)** cell .

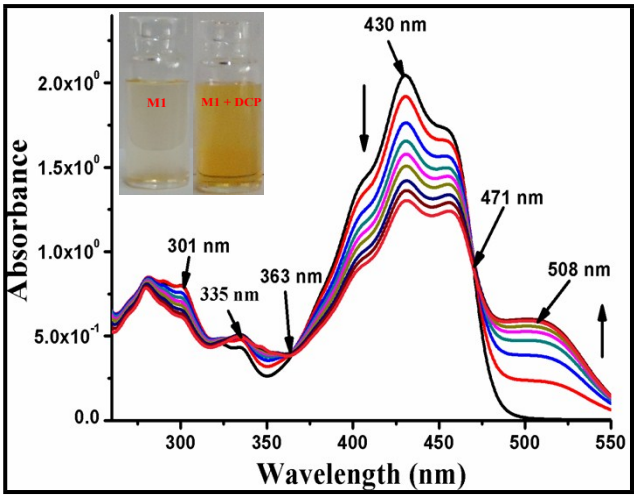


**Figure S13:** Absorption spectra of **M1** (0.4 µM) upon addition of DCP (8 µM) in $CH_3CN$-$H_2O$ (10 mM HEPES buffer, 1:1 V/V, pH 7.4, at 25°C).

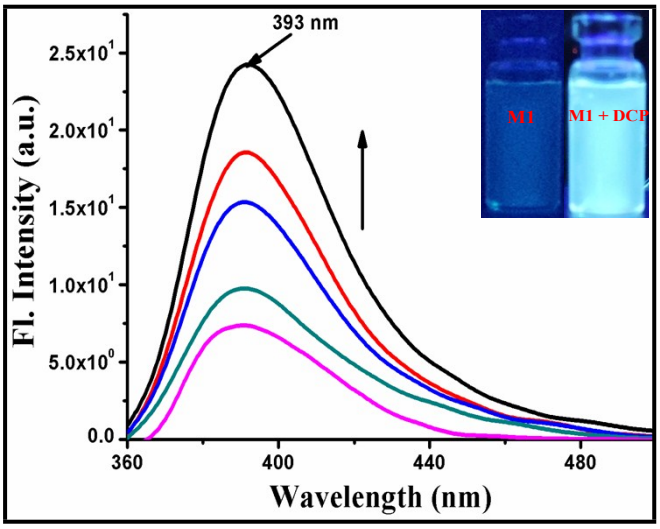


**Figure S14:** Fluorescence spectra of **M1** (0.4 μM) at λex = 335 nm, upon addition of DCP (8 μM) in $CH_3CN$-$H_2O$ (10 mM HEPES buffer, 1:1 V/V, pH 7.4, at 25°C).

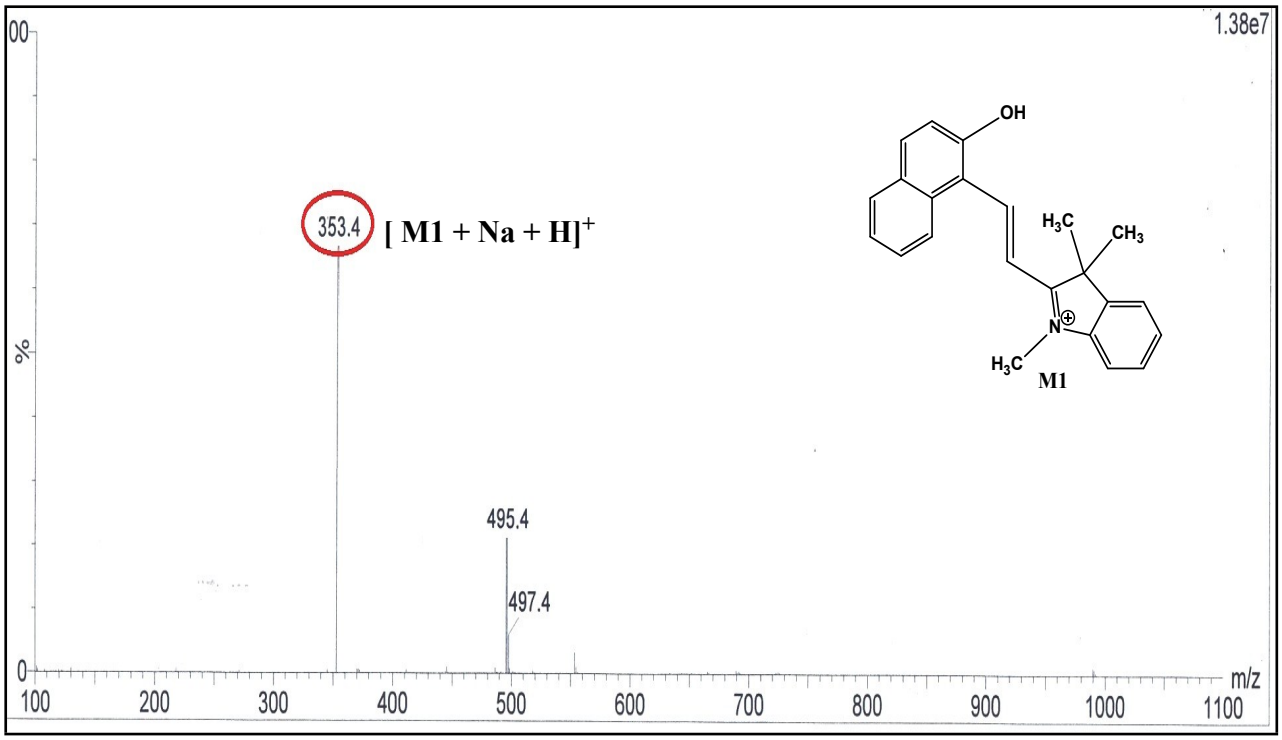


**Figure S15:** LCMS of control compound **M1.**

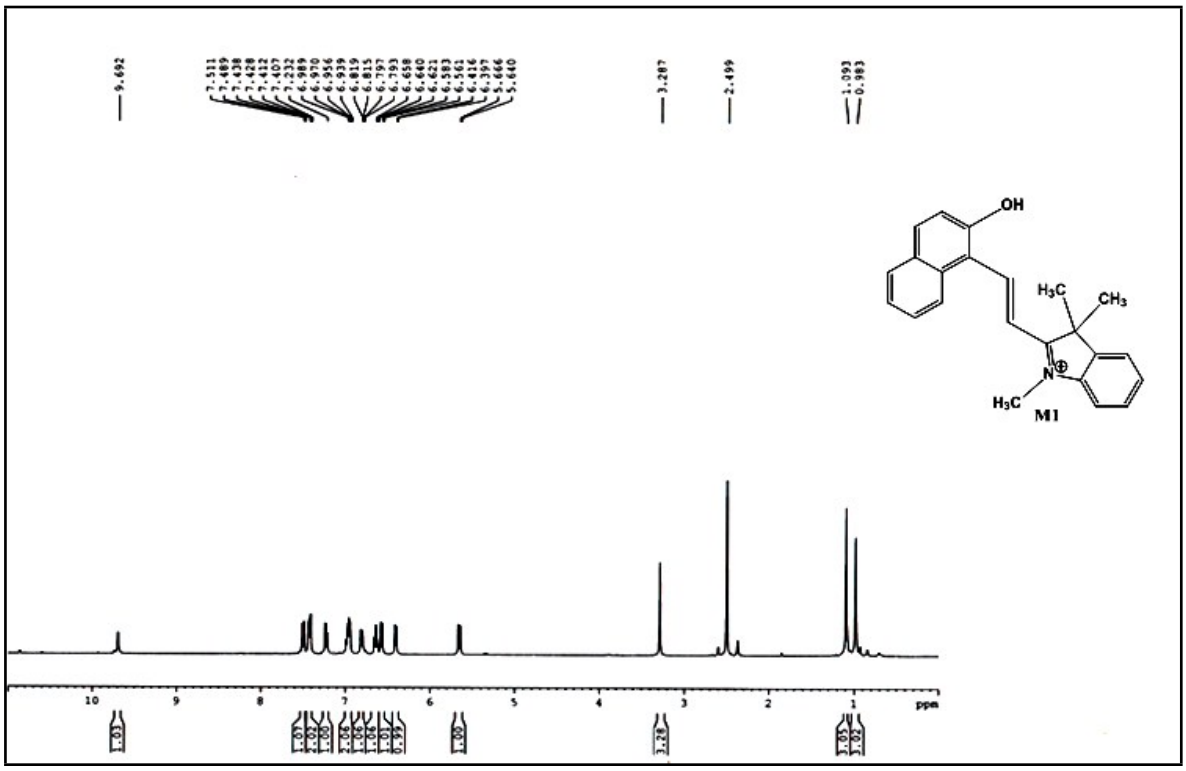


**Figure S16:** [1]HNMR of control compound **M1.**

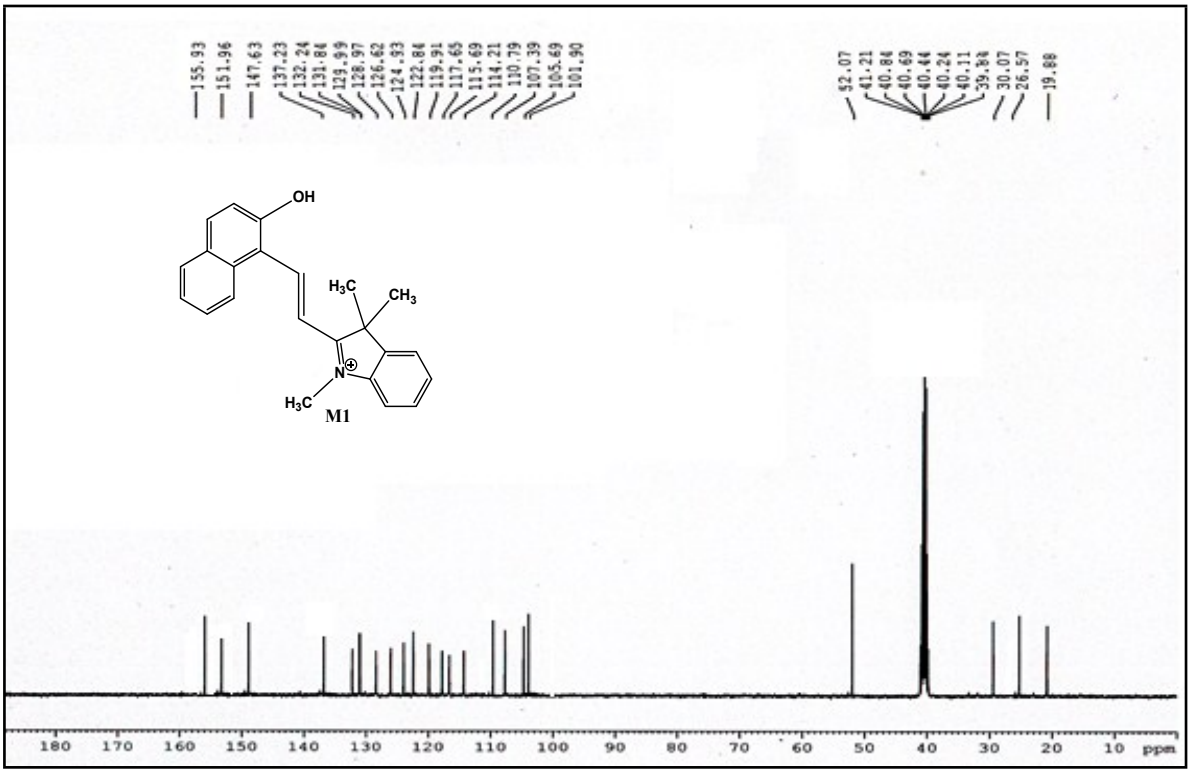


**Figure S17:** [13]CNMR of Compound **M1** in ($d_6$-DMSO).