Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A cyclization-induced emission enhancement (CIEE)-based ratiometric fluorogenic and chromogenic probe for the facile detection of a nerve agent simulant DCP

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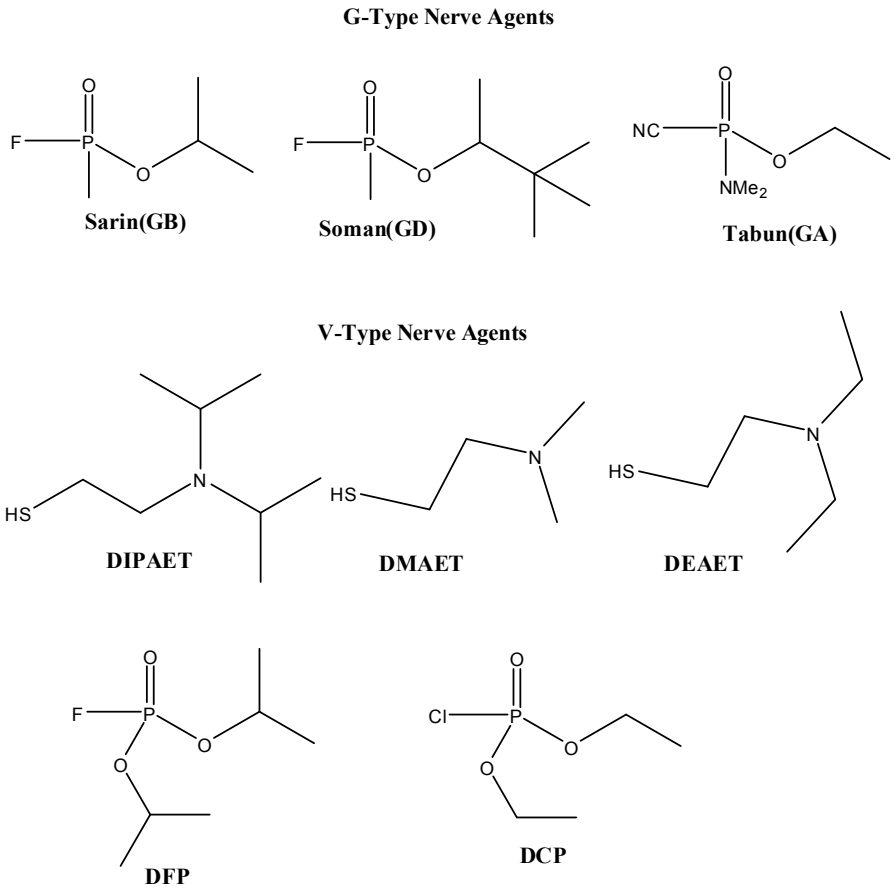
Experimental Section:

Materials and Methods:

Diethyl chlorophosphate was purchased from Sigma-Aldrich Pvt.Ltd. (India). Unless otherwise mentioned, materials were obtained from commercial suppliers and were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker 400 MHz instrument. For NMR spectra, DMSO-d_6 and $CDCl_3$ were used as solvent using TMS as an internal standard. Chemical shifts are expressed in δ ppm units and ^{1}H–^{1}H and ^{1}H–C coupling constants in Hz. Mass spectra were carried out using a Waters QTOF Micro YA 263 mass spectrometer. Fluorescence spectra were recorded on a Perkin Elmer Model LS 55 spectrophotometer. UV spectra were recorded on a JASCO V-530 spectrophotometer. Elemental analysis of the compounds was carried out on Perkin-Elmer 2400 series CHNS/O Analyzer.

Chemical structure of G and V type nerve agents and their stimulants DFP and DCP:



Plausible Mechanism of DCP binding:

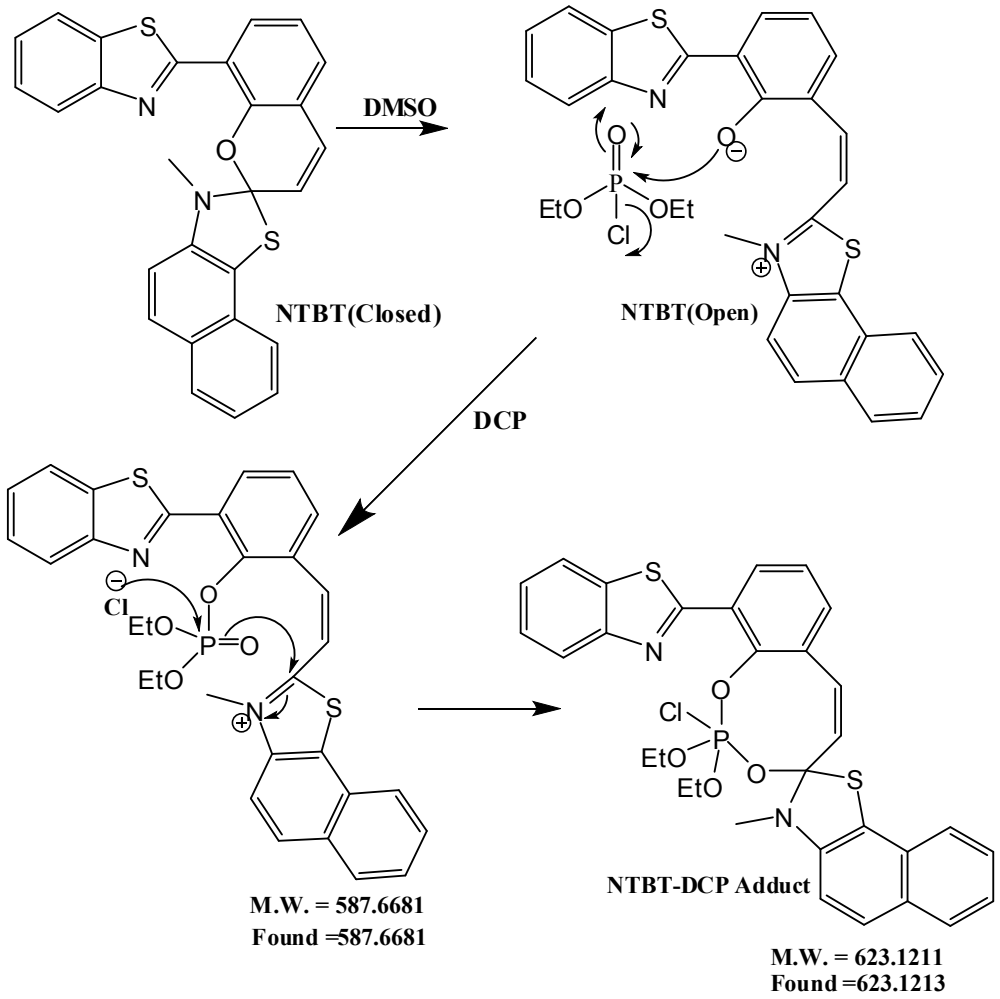


Figure S1: Mechanism of DCP binding with **NTBT**.

Synthetic Procedure:

Preparation of N, 2-dimethyl-alpha-napthothiazole :

2-methylnapthothiazole was dissolve in dry $CHCl_3$. Then methyl iodide drop wise added and stirred at room temperature for overnight. A pale green precipitate appeared. The precipitate was filtered, washed with $CHCl_3$ for several times and collected in Desiccators.

Preparation of 2-(benzo[d]thiazol-2-yl) phenol (1): Compound **1** was synthesized by using CdS nano- particle in the following way. A solution of 2-aminothiophenol (0.5 mL, 7.0 mmol) and salicylaldehyde (861.8 mg, 7.05 mmol) in MeOH (10 mL) and CdNS were stirred in the presence of light for 1 h. when a yellow precipitate appeared. The precipitate was filtered, washed with methanol, dried under vacuum. It was dissolved in $CHCl_3$ and purified by column chromatography (hexane-EtOAc = 9:1(v/v)) to afford the desired product as a white solid (1.1g, 90% yield).

Preparation of 3-Benzothiazole-2-yl-2-hydroxy-benzaldehyde (2):

2-(benzo[d]thiazol-2-yl) phenol (500mg, 2.2mmol) was dissolved in toluene (10ml) and acetic acid (10ml). Hexamethylenetetramine (350mg, 2.5mmol) was added in one portion and the solution was refluxed until all the starting material was consumed (TLC monitor, 16 h). Then the mixture was cooled to rt and poured into 6M HCl (20ml) and extracted with ethyl acetate. The combined organic extracts were washed with saturated brine. Next purification was done by column chromatography (Hexane-EtOAc = 8:2(v/v)) to get the pure product (100mg) as a yellow solid. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 13.35 (bs,1H), 9.93 (s,1H), 8.03 (d, 1H, J = 2.91 Hz), 7.92 (t, 2H,6.5 Hz), 7.55 (t, 1H, J = 5.3 Hz), 7.47 (d, 1H, 4.8 Hz), 7.45(d, 1H, J = 5.4 Hz), 7.22 (d, 1H,6.5 Hz); Anal. Calcd. C 65.85, H 3.55, N 5.49, S 12.57; found: C 65.59, H 3.59, N 5.62, S 12.55; MS (LCMS): (m/z, %): 255.9 [(2+H^+), 100 %].

Preparation of NTBT:

3-Benzothiazole-2-yl-2-hydroxy-benzaldehyde (0.39 mmol, 100mg) and N,2-dimethyl-alpha-napthothiazole (0.4 mmol, 85.6 mg) were refluxed in 10 ml ethanol solution for 5 h. Then the

solvent was stirred at room temperature for 1 hr. An orange solid compound was precipitated. The precipitate was filtered, washed with EtOH. The residue was recrystallized by acetic ether/hexane to get the product, orange-yellow solid (149 mg, 83%). M.P> 250°C.

^{1}H-NMR (DMSO-d6, 400 MHz): δ (ppm) 8.42(d, 3H, J=8.9 Hz), 8.34-8.25 (m, 4H), 8.23(d, 1H, J=4.6 Hz) 8.15 (t, 1H, J=8.0 Hz),7.90 (t, 1H, J=8.6 Hz), 7.82(t, 1H, J=8.7 Hz), 7.63 (t, 1H, J=8.7 Hz), 7.53 (t, 2H, J=8.6 Hz), 7.25 (t, 1H, J=8.8), 4.47(s, 3H,). Anal. Calcd. C 71.97, H 4.02, N 6.21, S 14.23; found: C 71.95, H 4.02, N 6.22, S 14.24; MS (ESI MS): (m/z, %): 451.5608 [(NTBT+H^{+}), 100 %]; Calculated for $C_{27}H_{18}ON_2S_2$: 450.5735.

^{13}C-NMR (DMSO-d6, 400 MHz): δ (ppm) 181.8, 167.9, 162.2, 157.4, 150.6, 146.2, 143.4, 141.8, 139.6 133.6, 132.4, 129.5, 129.0, 127.3, 126.3, 123.0, 122.9, 122.6, 122.1, 120.5, 115.3, 113.9, 108.1, 107.1, 52.1, 51.4, 34.6.

Preparation Procedure of NTBT-DCP Complex: In a DMSO solution of NTBT (0.221mmol, 100mg), DCP (0.471mmol, 0.1 ml) was drop wise added. The reaction mixture was stirred for 1 hour at room temperature till all the starting material was consumed (TLC monitor). After the completion of the reaction, DMSO was evaporated in vacuum. Next purification was done by column chromatography (Hexane-EtOAc = 9:1(v/v)) to get the pure product (100mg) as a dark red solid. M.P. > 250°C. MS (ESI MS): (m/z, %): 623.1213 [**NTBT**-DCP] $^{+}$, 100 %]; Calculated for $C_{31}H_{28}O_2N_2S_2PCl$: 623.1211. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.85(d, 3H, J=10.12 Hz), 7.76-7.47 (m, 5H), 7.34 (t, 1H, J=8.12 Hz),7.11 (t, 1H, J=8.2 Hz), 6.92(t, 1H, J=7.7 Hz), 6.73 (t, 1H, J=6.7 Hz), 6.68(t, 2H, J=8.5 Hz), 6.60(t, 1H, J=5.6 Hz), 4.01(s, 3H), 3.58(q, 2H), 1.61(t, 3H, J=4.50 Hz). ^{13}C-NMR (DMSO-d6, 400 MHz): δ (ppm) 170.3, 164.2, 162.6, 154.9, 151.3, 141.6, 141.5, 133.5, 133.4, 133.2, 132.1, 131.9, 130.1, 128.0, 127.0, 126.6, 123.3, 122.8, 121.3, 118.3, 115.5, 115.3, 108.1, 100.2, 96.6, 70.2, 46.6, 32.6, 20.3.

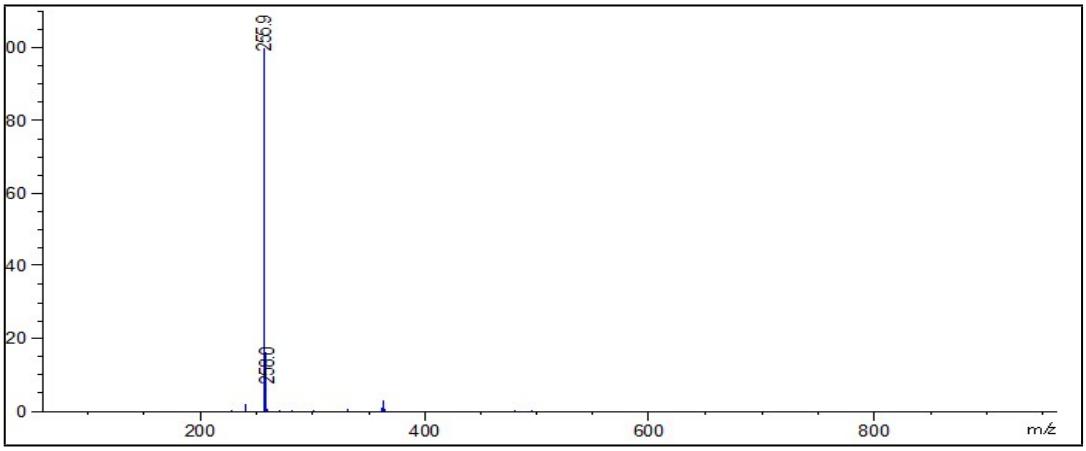


Figure S2: LC-MS of Compound **2**.

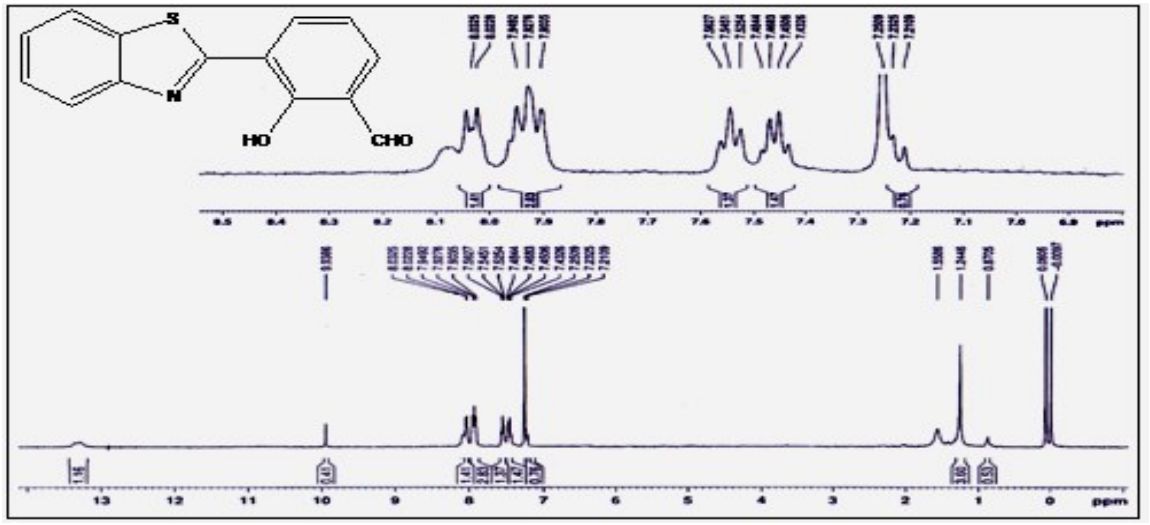


Figure S3: ^{1}H NMR of Compound **2** in $CDCl_3$.

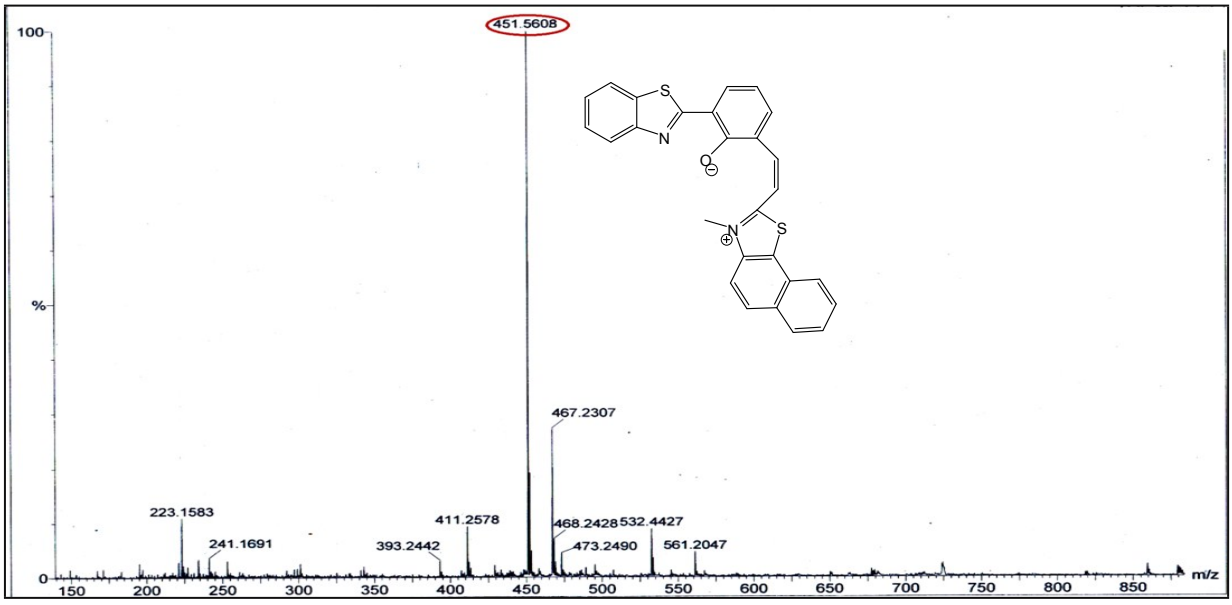


Figure S4: ESI-MS of **NTBT**.

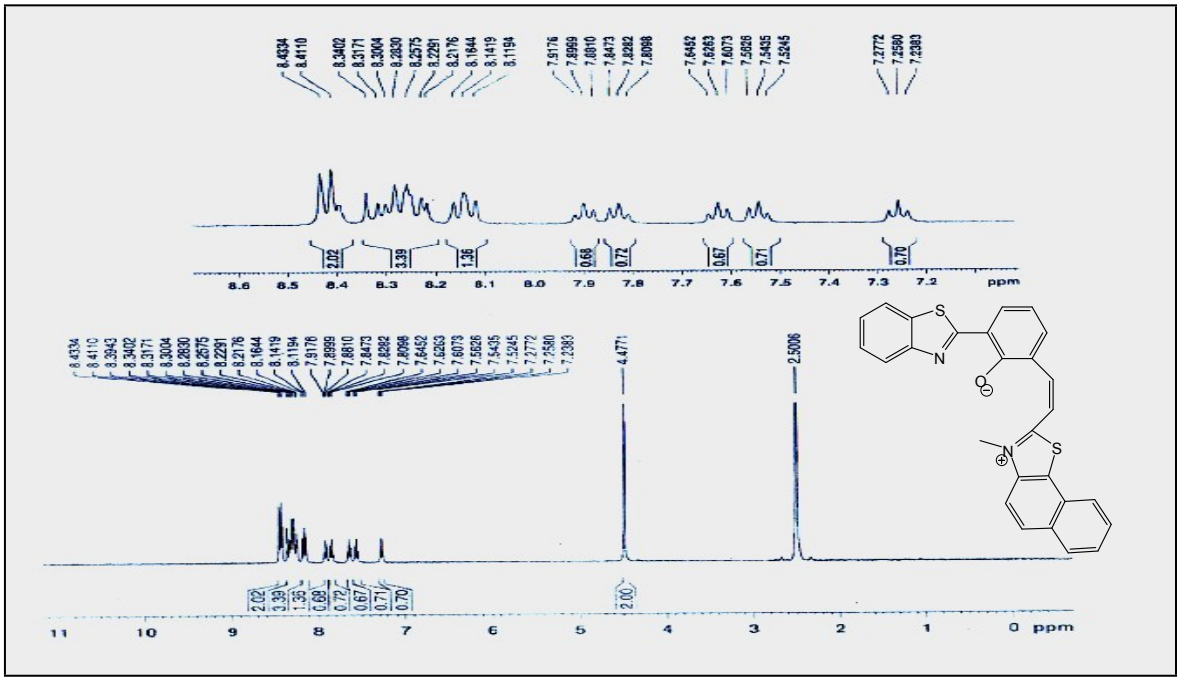


Figure S5: ^{1}H NMR of **NTBT** in (d_6-DMSO).

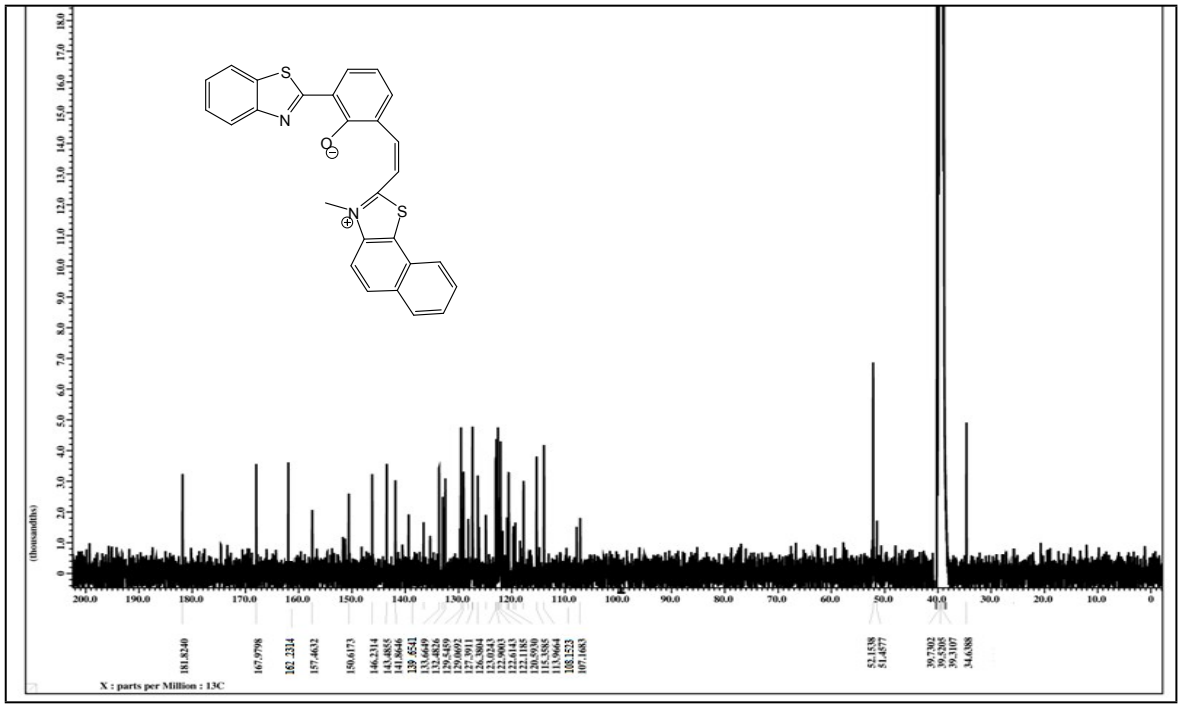


Figure S6: ^{13}C NMR of **NTBT** in (d_6-DMSO).

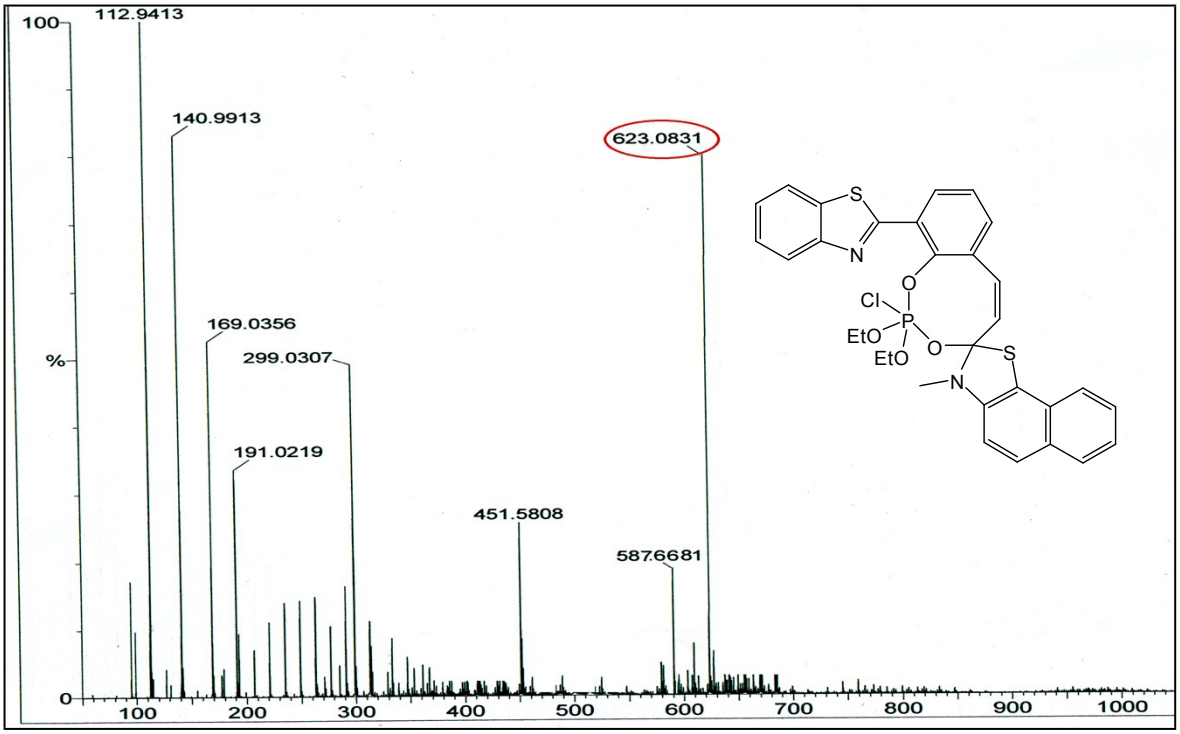


Figure S7: ESI-MS of **NTBT-DCP** adduct in reaction medium.

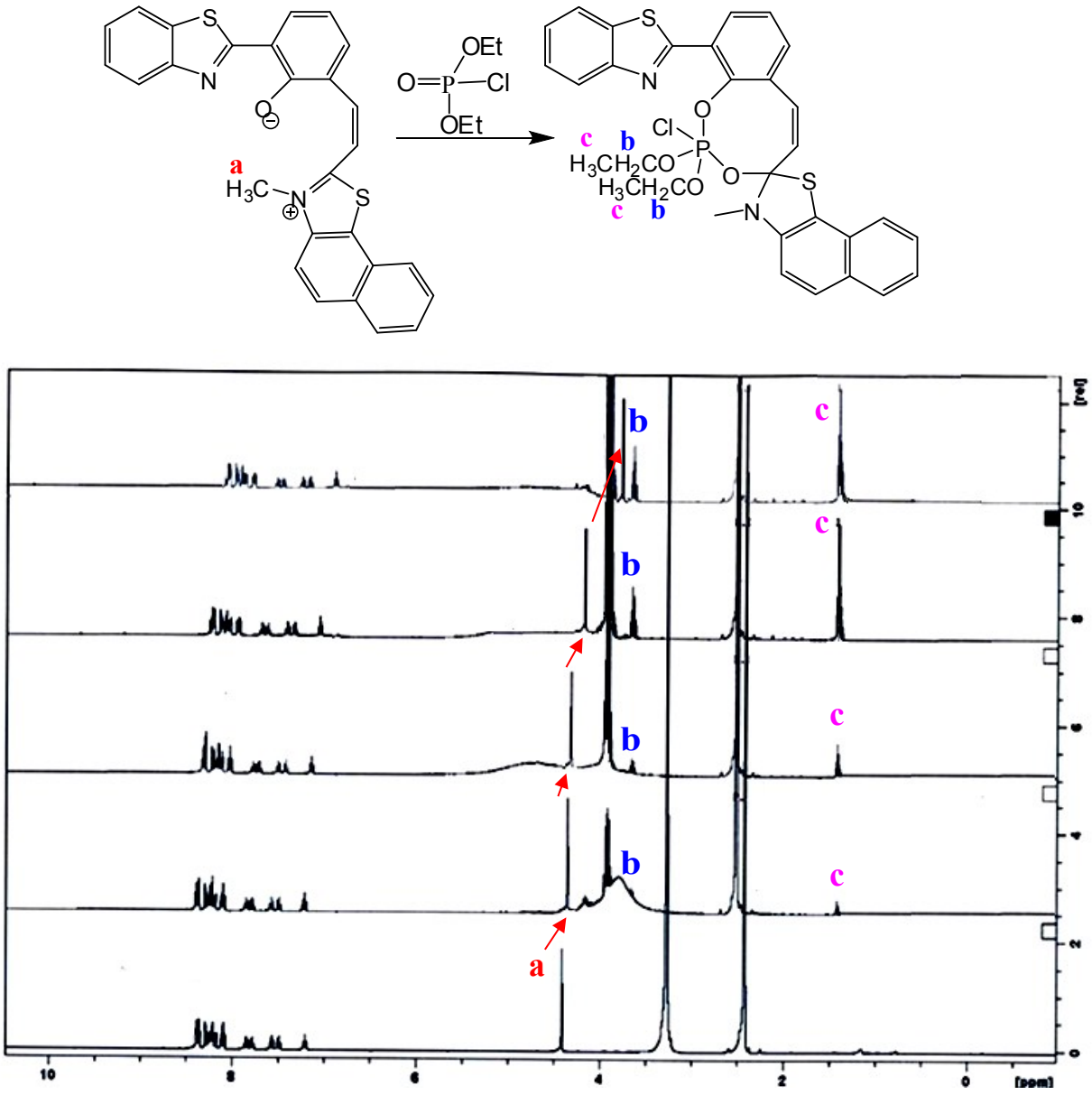


Figure S8: ^{1}H NMR titration chart of **NTBT** in d_6-DMSO with the gradual addition of DCP.

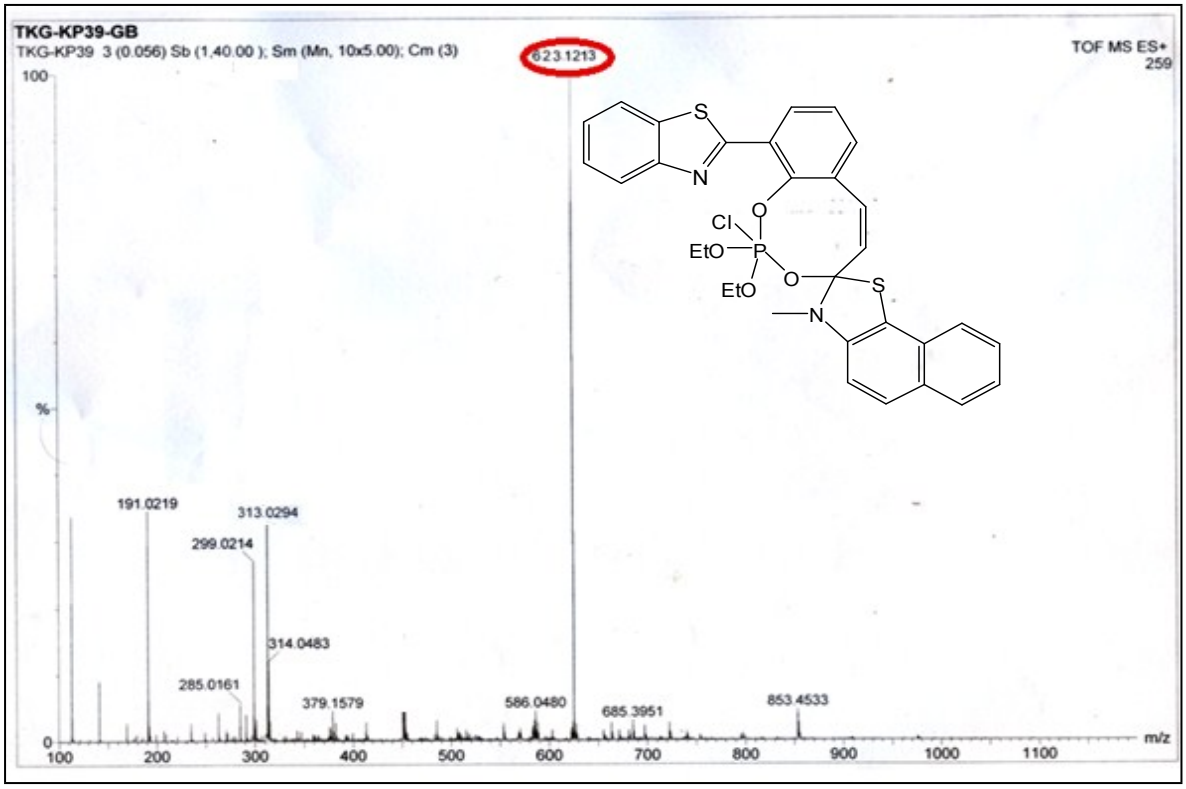


Figure S9 : HR-MS spectra of **NTBT**-DCP Complex after separation from reaction mixture.

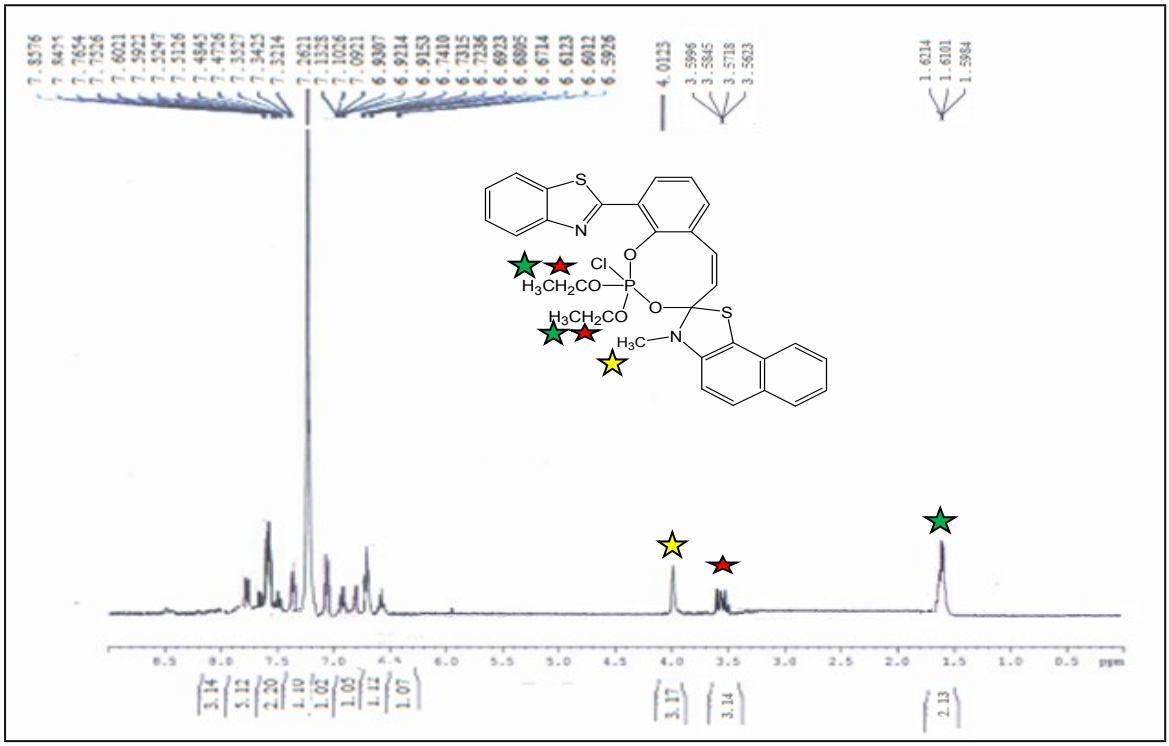


Figure S10 : [1]H NMR spectra of **NTBT**-DCP Complex after separation from reaction mixture

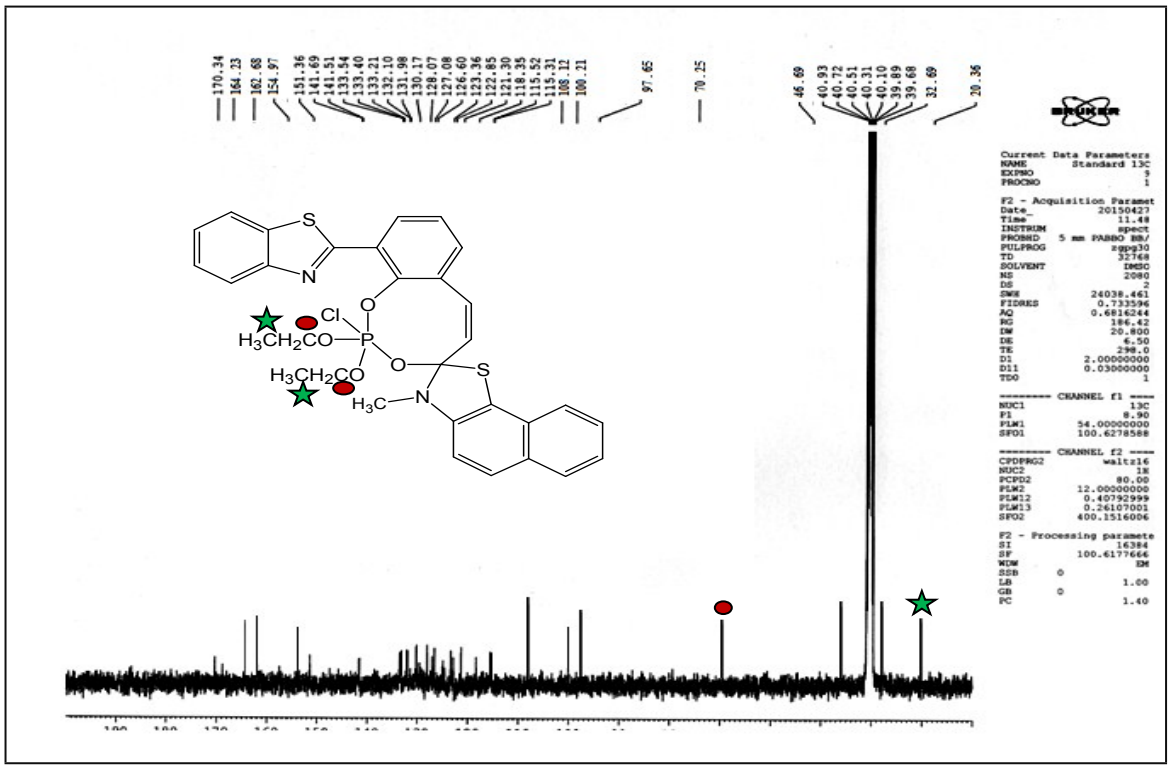


Figure S11: ^{13}C NMR spectra of **NTBT**-DCP Complex after separation from reaction mixture

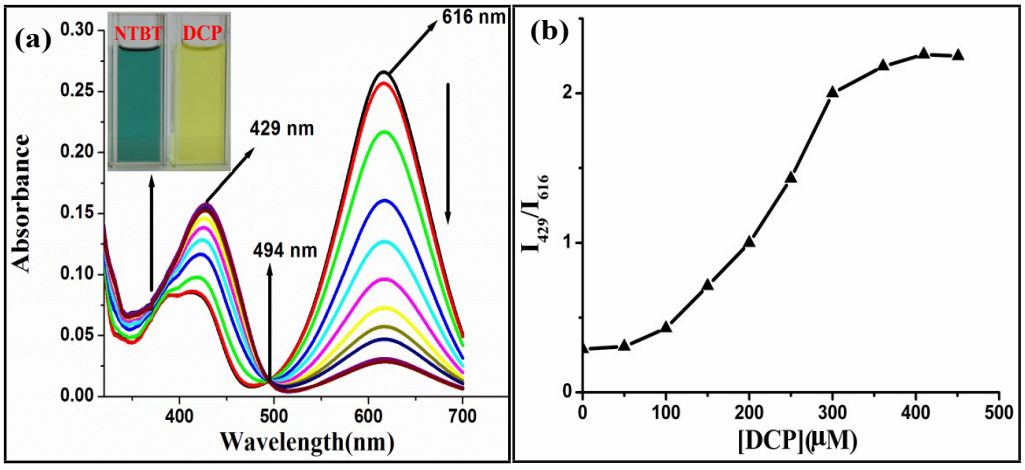


Figure S12: (a) Absorption spectra of **NTBT** (0.1 μM) upon addition of DCP (c = 2.0 μM). (b) Absorbance intensity ratio changes (I_{429}/I_{616}) of **NTBT** (0.1 μM) upon addition of various concentration of DCP.

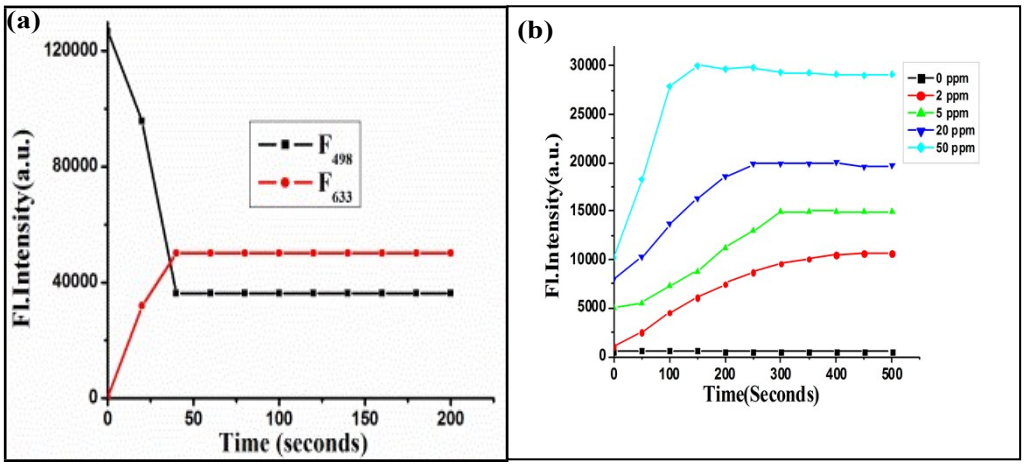


Figure S13: (a) The fluorescence intensities at 498 and 633 nm vary in a time range of 200 s. The excitation wavelength was set at 429 nm. (b) Kinetic profile of the reaction between **NTBT** (1.0 μM) and diethyl chlorophosphate in DMSO. The color formation was monitored by fluorescence emission intensity at 633 nm (Ex@429 nm).The concentrations of the analyte were used as indicated.

Kinetic Study:

The solution phase cyclization reactions of **NTBT** in DMSO were followed by measuring the fluorescence spectra after mixing **NTBT** and DCP in a cubic 4-sided quartz cell of 3 ml. The reaction was carried out at room temperature under the excess amount of DCP (initial concentration [**NTBT**] <<[DCP]) and the reaction was expected to reach 100% conversion of indicator **NTBT** to **NTBT-**DCP adduct**.** Separate solutions of different concentrations of **NTBT** and DCP in DMSO were prepared and mixed to investigate the kinetics. The excitation wavelength was 429 nm and in all cases the concentration was low enough to maintain a UV absorption that was < 0.1. The rate of the cyclization was determined by fitting the fluorescence intensities of the samples to the Pseudo-First Order Equation (1):

$Ln(F_{max} - F_t)/F_{max} = - kt$ ……(1)

Where F(t) and F(max) are the fluorescence intensities at the monitoring wavelengths at times t and the maxima values which are the last fluorescence intensities when the cyclization of **NTBT** reached the conversion of 100%. The k is the apparent rate constant.

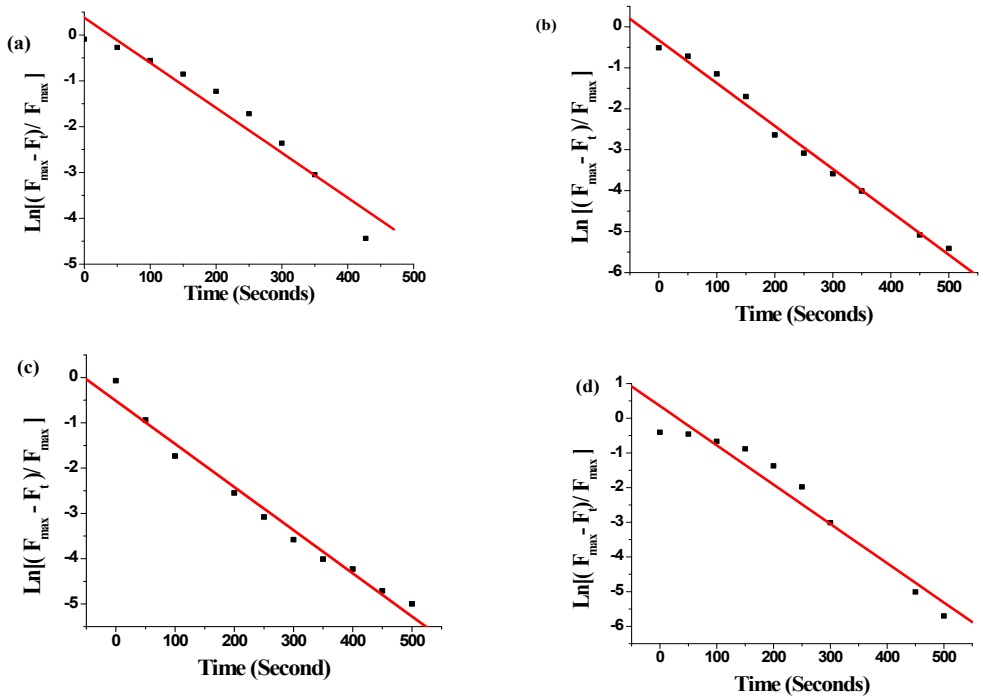


Figure S14: Pseudo first-order kinetic plot of reaction of **NTBT** (1.0µM) with (a) (1 x 10^{-4}M), (b) (2 x 10^{-4}M), (c) (5 x 10^{-4}M) and (d) (8 x 10^{-4}M), DCP in DMSO.

Figure S11 shows the fluorescent intensity at 633 nm of **NTBT**-DCP adducts vs. time at a variety of concentrations of DCP for a fixed concentration of **NTBT** (1.0µM). In all the cases slope = -0.010 Sec^{-1}. So the rate constant of the reaction at 25^0C, K= 0.010 Sec^{-1}

Binding Constant Calculation:

By UV-Vis Method:

The substrate binding interaction was calculated according to the Benesi-Hildebrand equation.

$$\frac{A_0}{A - A_0} = \left(\frac{\varepsilon_0}{\varepsilon_0 - \varepsilon}\right)^2 \left(\frac{1}{K_B[Substrate]^2} + 1\right) \quad \text{.....(i)}$$

Here A_o is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, ε_0 and ε are the corresponding molar absorption co-efficient and K_B represents the substrate binding interaction with guest.

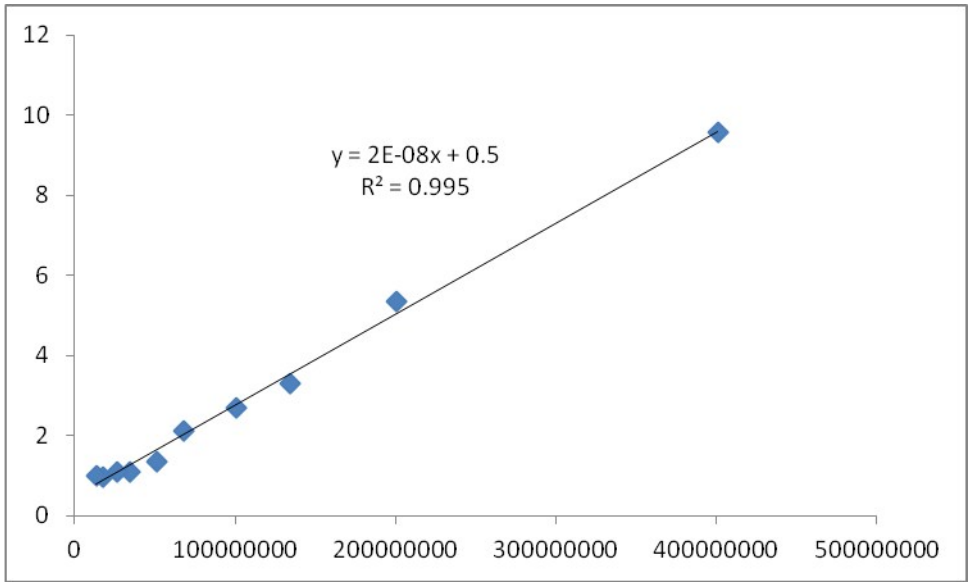


Figure S15: Binding constant (= 2.5×10^7M) of **NTBT** with DCP by UV-Vis method.

By Fluorescence Method:

Binding constant of the chemosensor **NTBT** also be calculated through emission method by using the following equation.

$$1/ (I - I_0) = 1/K(I_{max} - I_0)[G] + 1/(I_{max} - I_0) \quad \ldots\ldots\ldots\ldots\ldots\text{(ii)}$$

where I_0, I_{max}, and I represent the emission intensity of free **NTBT**, the maximum emission intensity observed in the presence of added DCP at 633 nm (λ_{ext} = 429 nm), [G] is the concentration of the guest DCP and the emission intensity at a certain concentration of the DCP, respectively.

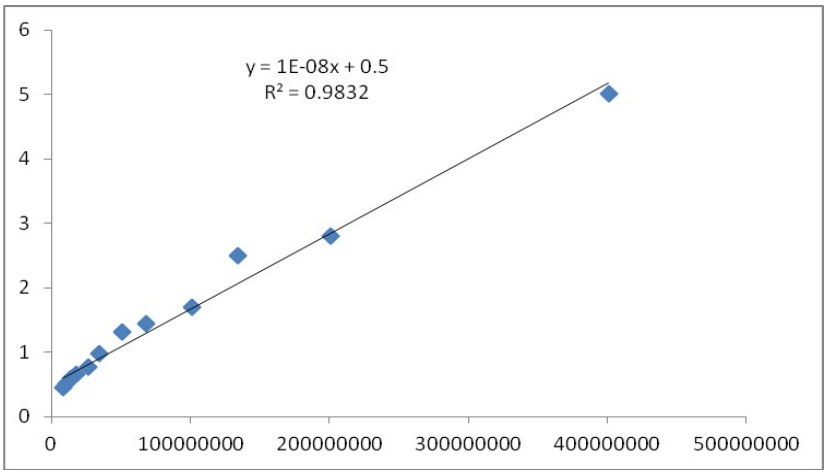


Figure S16: Binding constant(= 5×10^7M) of **NTBT** with DCP by UV-Vis method.

Calculation of Detection limit:

The detection limit (DL) of **NTBT** for DCP were determined from the following equation:

DL = K* Sb1/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.

By Uv-Vis Method:

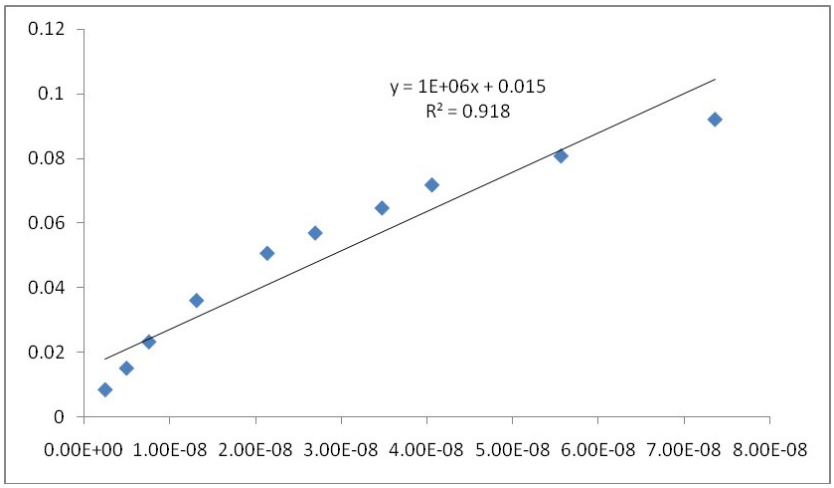


Figure S17: Calibration curve for UV-Vis titration of **NTBT** at 429 nm with DCP.

From the graph we get slope (S) = 1×10^{6}. Standard deviation (Sb1=0.0085)

Thus using the formula we get the detection limit=17 nM.

By Fluorescence Method:

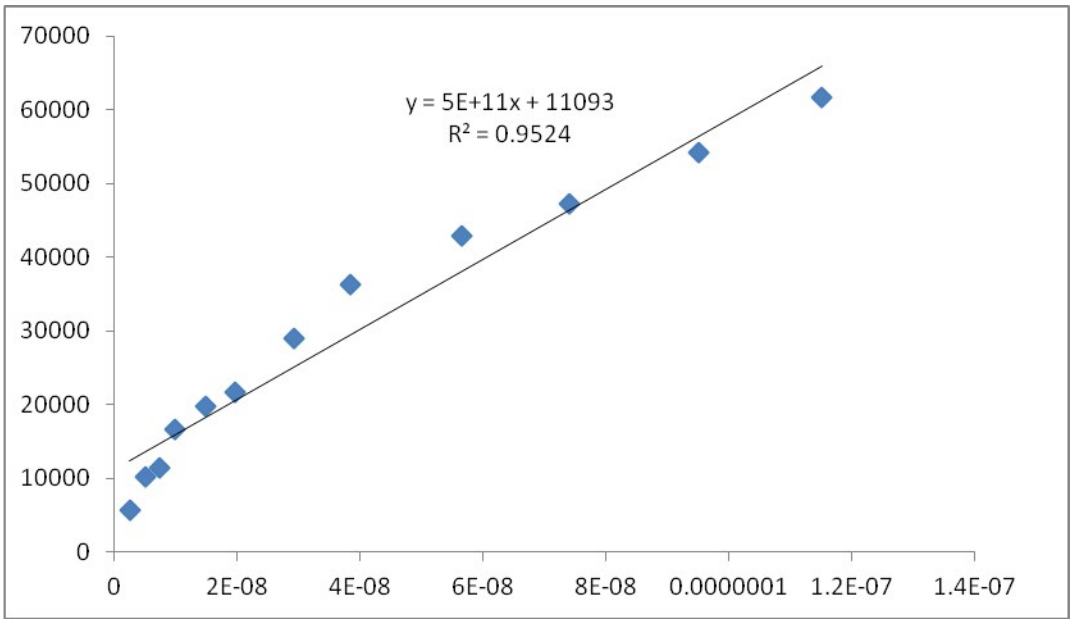


Figure S18: Calibration curve for Fluorescence titration of **NTBT** at 633 nm (Ex@429 nm).with DCP.

From the graph we get slope (S) = 5×10^{11}. Standard deviation (Sb1=4244.04588)

Thus using the formula we get the detection limit=17 nM

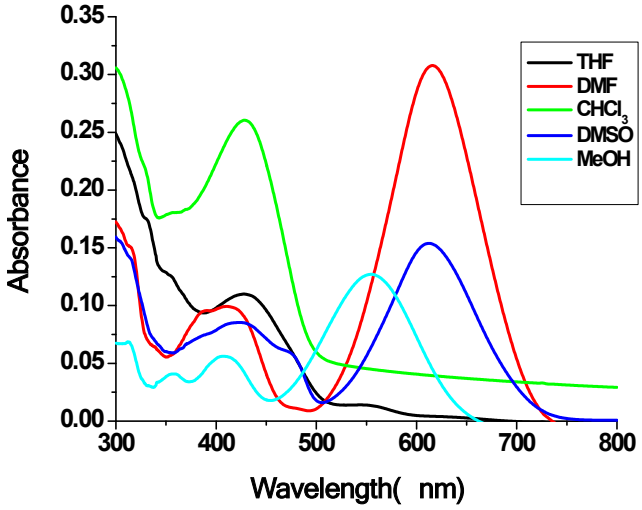


Figure S19: UV-Vis spectra of **NTBT** in different solvents.

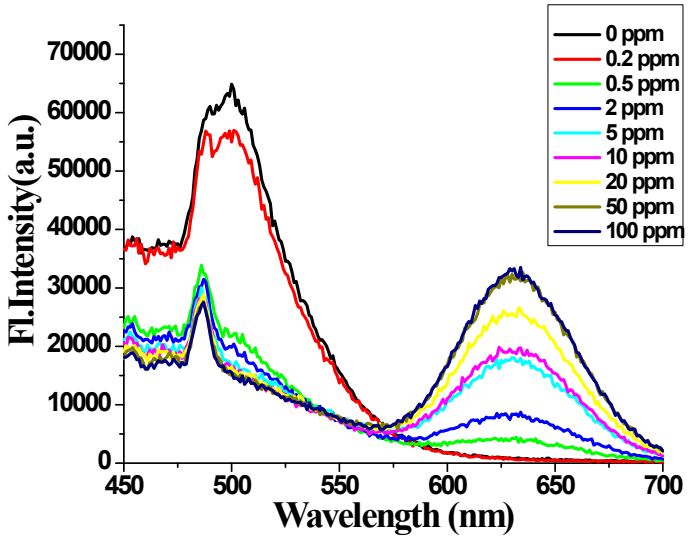


Figure S20: Change in fluorescence intensity of **NTBT (**0.1μM, DMSO-H_2O, 7:3 v/v, pH 7.4**)** with addition of increase DCP concentration.

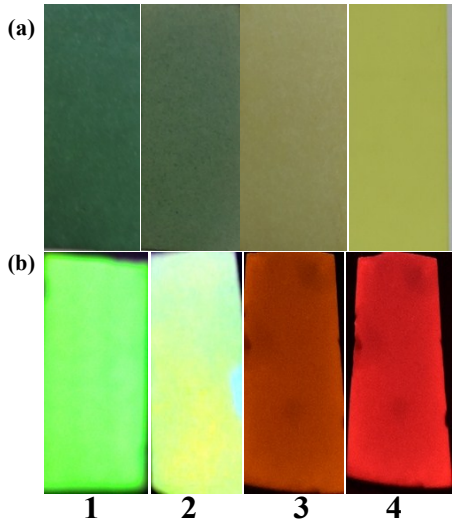


Figure S21: (a) Naked eye in room light and (b) Fluorescence color changes visualized on TLC plate strips of (1) **NTBT** (c = 1.0×10^{-2} M) and during addition of DCP at (2) 1.0×10^{-4} M; (3) 1.0×10^{-3} M; (4) 1.0×10^{-2} M in DMSO/H_2O = 70:30(v/v).

Table S1. Selected electronic excitation energies (eV), oscillator strengths (f), main configurations, and CI Coefficients of the low-lying excited states of CS1 and all the complexes. The data were calculated by TDDFT//B3LYP/6-31+G(*d,p*) based on the optimized ground state geometries.

Molecules	Electronic Transition	Excitation Energy[a]	f[b]	Composition[c]	(composition) %
NTBT(Closed)					
	$S_0 \rightarrow S_4$	3.7648 eV 329.32 nm	0.1838	H-1 → L	82.1
	$S_0 \rightarrow S_9$	4.2846 eV 289.37 nm	0.2069	H -3→ L H -1→ L +2	72.5
	$S_0 \rightarrow S_{23}$	5.0834 eV 243.90 nm	0.4449	H-5 → L H-4 → L +1	32.7
NTBT(Open)					
	$S_0 \rightarrow S_1$	2.1001 eV 590.58 nm	0.3053	H → L	96.0
	$S_0 \rightarrow S_4$	3.1110 eV 398.53 nm	0.1987	H-1 → L	72.8

	$S_0 \rightarrow S_7$	3.6796 eV 336.95 nm	0.2384	H → L+2	74.3
	$S_0 \rightarrow S_{28}$	5.0395 eV 246.02 nm	0.2254	H-3 → L+3 H-1 → L+3	35.0
NTBT-DCP					
	$S_0 \rightarrow S_1$	1.9823 eV 625.53 nm	0.2681	H → L	35.5
	$S_0 \rightarrow S_{19}$	2.8688 eV 432.23	0.1995	H-4 → L+2	76.4
	$S_0 \rightarrow S_{27}$	5.1798 eV 239.36 nm	0.2052	H-4 → L+3 H-3 → L+3	36.0

[a] Only selected excited states were considered. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength (only the f > 0.15 was considered). [c] H stands for HOMO and L stands for LUMO.

TABLE S2: HOMO-LUMO energy calculated for NTBT Closed, NTBT Open and NTBT-DCP complex.

Species	E(HOMO)	E(LUMO)	ΔE (Hartree)	ΔE(eV)
NTBT Closed	-0.18238	-0.05653	0.12585	3.4
NTBT Open	-0.17796	-0.10006	0.07790	2.11
NTBT-DCP	-0.17842	-0.10530	0.07312	1.98

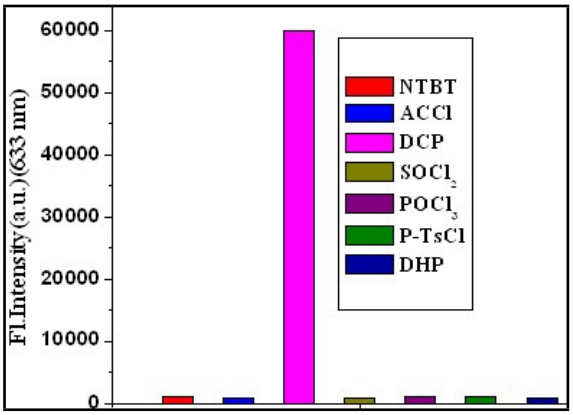


Figure S22: Competitive fluorescence spectra of **NTBT** with different reagents.

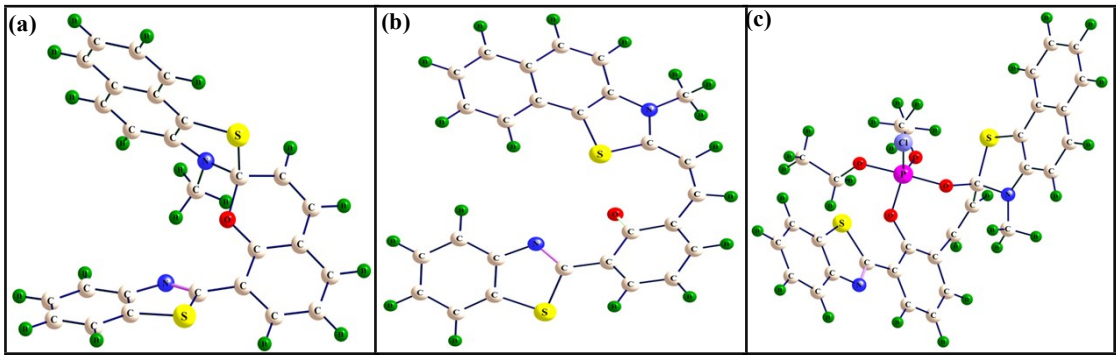


Figure S23: The energy optimized structures of (1) **NTBT** (Closed), (2) **NTBT** (Open) and (3) **NTBT-**DCP Complex.

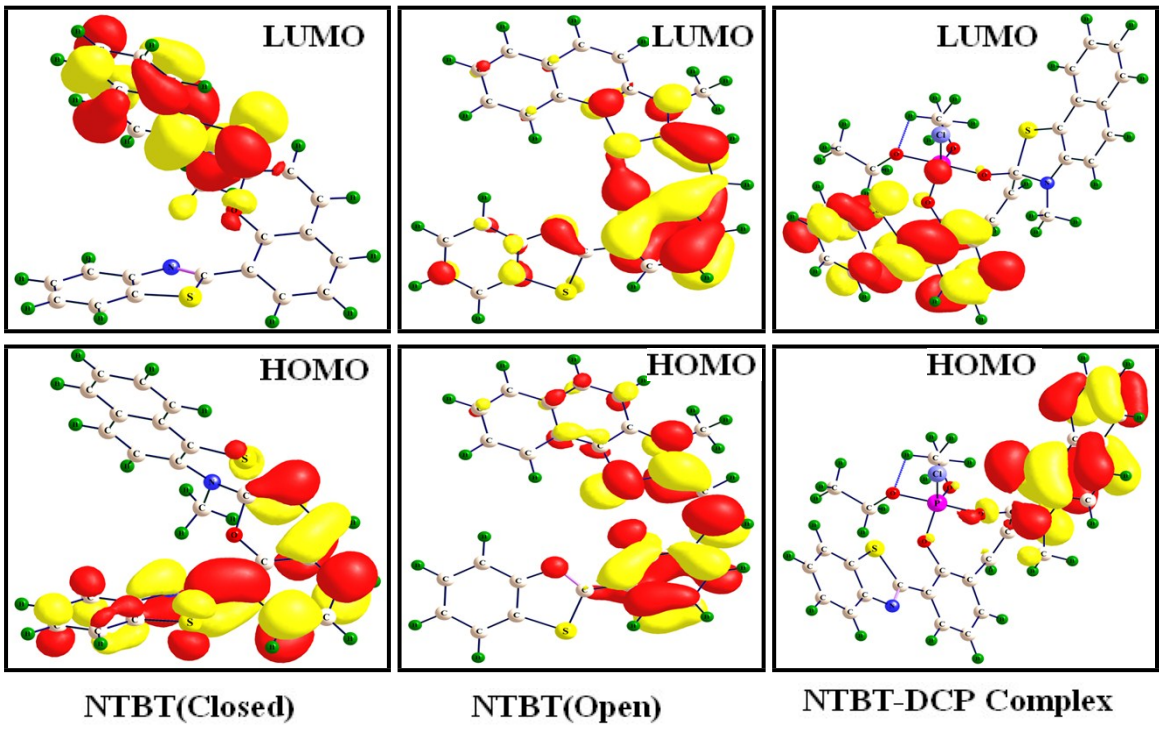


Figure S24: Molecular orbitals and electronic contribution of the relevant excitations of **NTBT**(Closed), **NTBT**(Open), **NTBT-**DCP complex.

Cell Imaging Study:

For fluorescence imaging studies RAW cells, 1.0 x 10^4 cells in 150 μL of medium, were seeded on sterile 35 mm μ-Dish, glass bottom culture dish (ibidi GmbH, Germany), and incubated at 37 °C in a CO_2 incubator for 24-30 hours. The next day, cells were washed three times with phosphate buffered saline (PBS) and fixed using 4% paraformaldehyde in PBS (pH 7.4) for 10 minutes at room temperature. Thereafter the cells were washed with PBS followed by permeabilization using 0.1% saponin for 10 min followed by incubation with 1.1 × 10^{-7} M **NTBT** dissolved in 100 μL DMEM at 37 °C for 1 h in a CO_2 incubator. Before microscopic imaging, all the solutions were aspirated and mounted on slides in a mounting medium containing DAPI (1 μg/mL) and stored in dark before microscopic images were acquired. The cells were observed under Andor spinning disk confocal microscope (SD-CM) with excitation at 488 nm monochromatic laser beam and the collected range of emission wavelength was between 510 and 560 nm (green channel). Cells were imaged live by SD-CM 63× oil-immersion objective. Images were acquired in z-stacks of 28 planes at 0.3-μm intervals with 400-ms exposure times every 20 seconds over a period of 30 minutes. In another dish new cells were again cultured in the same manner followed by treatment with DCP (1.1 x 10^{-6}). Thereafter the cells were washed thrice with PBS (pH 7.4) to remove any excess DCP and incubated in DMEM containing probe (**NTBT**) to a final concentration of 1.1 × 10^{-7} M. Finally the cells were washed again with PBS (pH 7.4) three times to remove excess probe outside the cells. The emissions were obtained at 580 - 630 nm (red channel).

Cytotoxic effect on Cells:

The cytotoxic effects of **NTBT**, DCP, and **NTBT**-DCP complex were determined by MTT assay following the manufacturer's instruction (MTT 2003, Sigma-Aldrich, MO). RAW cells were seeded onto 96-well plates (approximately 104 cells per well) for 24 h. Next day media was removed and various concentrations of probe NTBT, DCP (0, 15, 25, 50, 75, and 100 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. The plate was incubated for 3−4 h at 37°C. 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 nm.

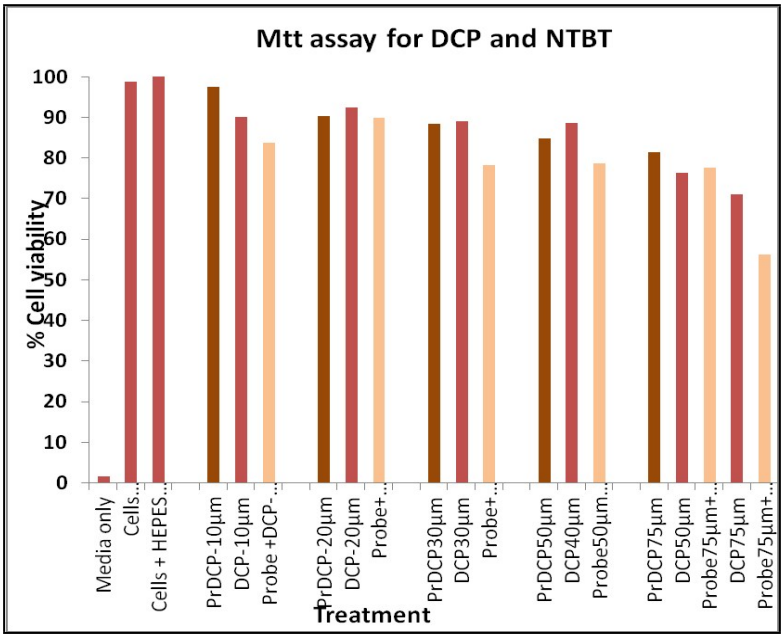


Figure S25: MTT assay to determine the cytotoxic effect of **NTBT** and **NTBT-**DCP complex on RAW cells.

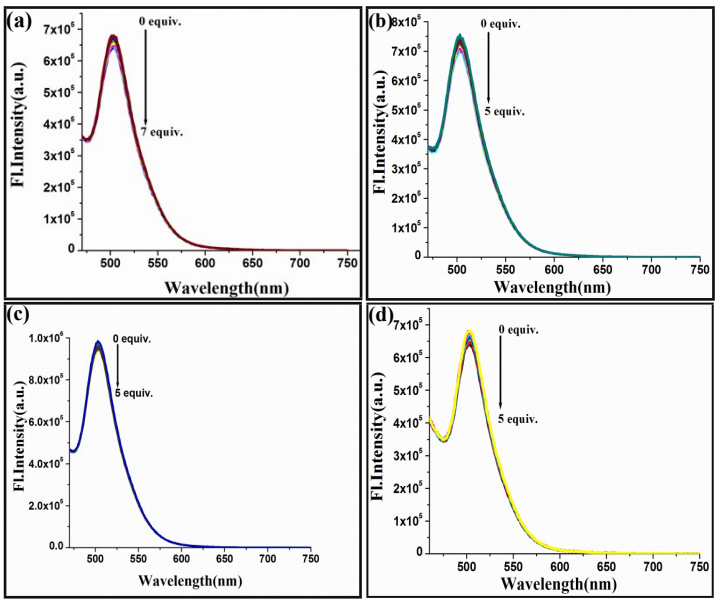


Figure S26 : Change in fluorescence (Ex@429 nm) intensity of **NTBT (**0.1µM, DMSO-H_2O, 7:3 v/v, pH 7.4**)** with addition of increase concentration of (a)(Tabun, 2.0 µM) , (b) (DEMP, 2.0 µM), (c)(DECP, 2.0 µM) (d)(DMMP, 2.0 µM)

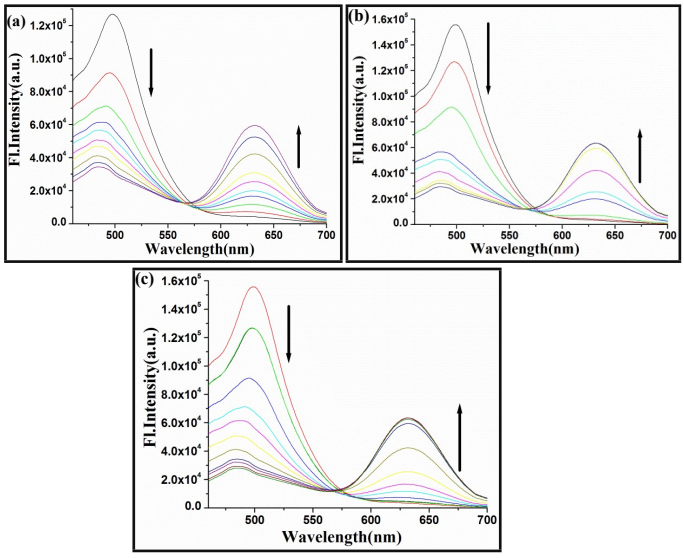


Figure S27 (a) Fluorescence spectra of **NTBT** (0.1 μM, λ_{ex} = 429 nm) in presence of (a)(H_2O_2, 100 μM), (b)(HOCl, 100 μM), (c)(HNO_2, 100 μM) upon addition of DCP (c = 2.0 μM).

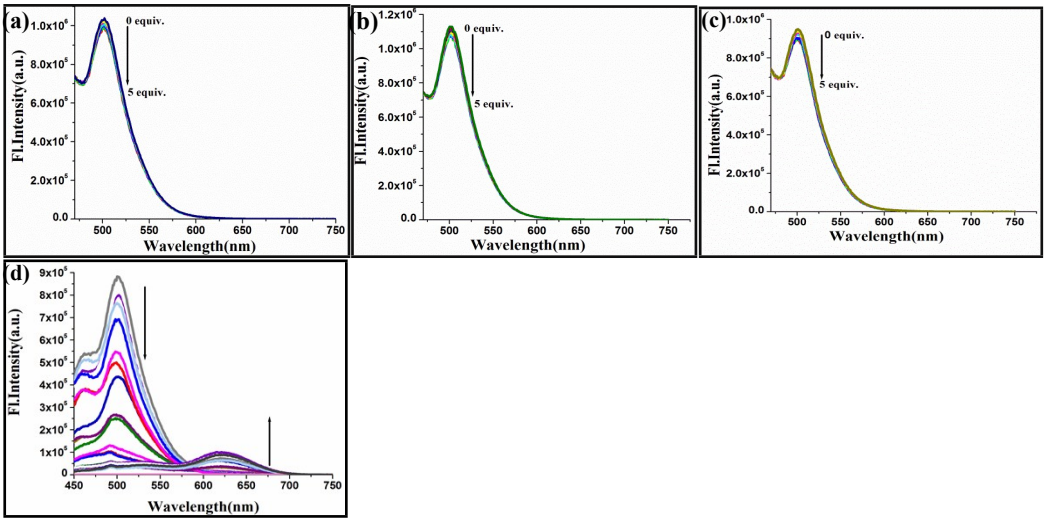


Figure S28: (a) Fluorescence spectra of **NTBT** (0.1 μM, λ_{ex} = 429 nm) in presence of (a)(pH=1.0, 100 μM), (b)(pH= 2.0, 100 μM), (c)(pH= 3.0, 100 μM), (d)(pH= 5.0, 100 μM) upon addition of DCP (c = 2.0 μM).

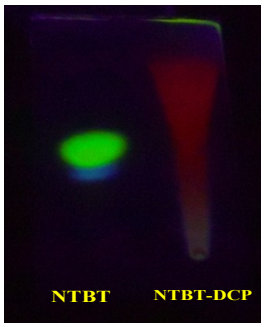


Figure S29: Change in fluorescence spot of NTBT alone and after reaction with DCP

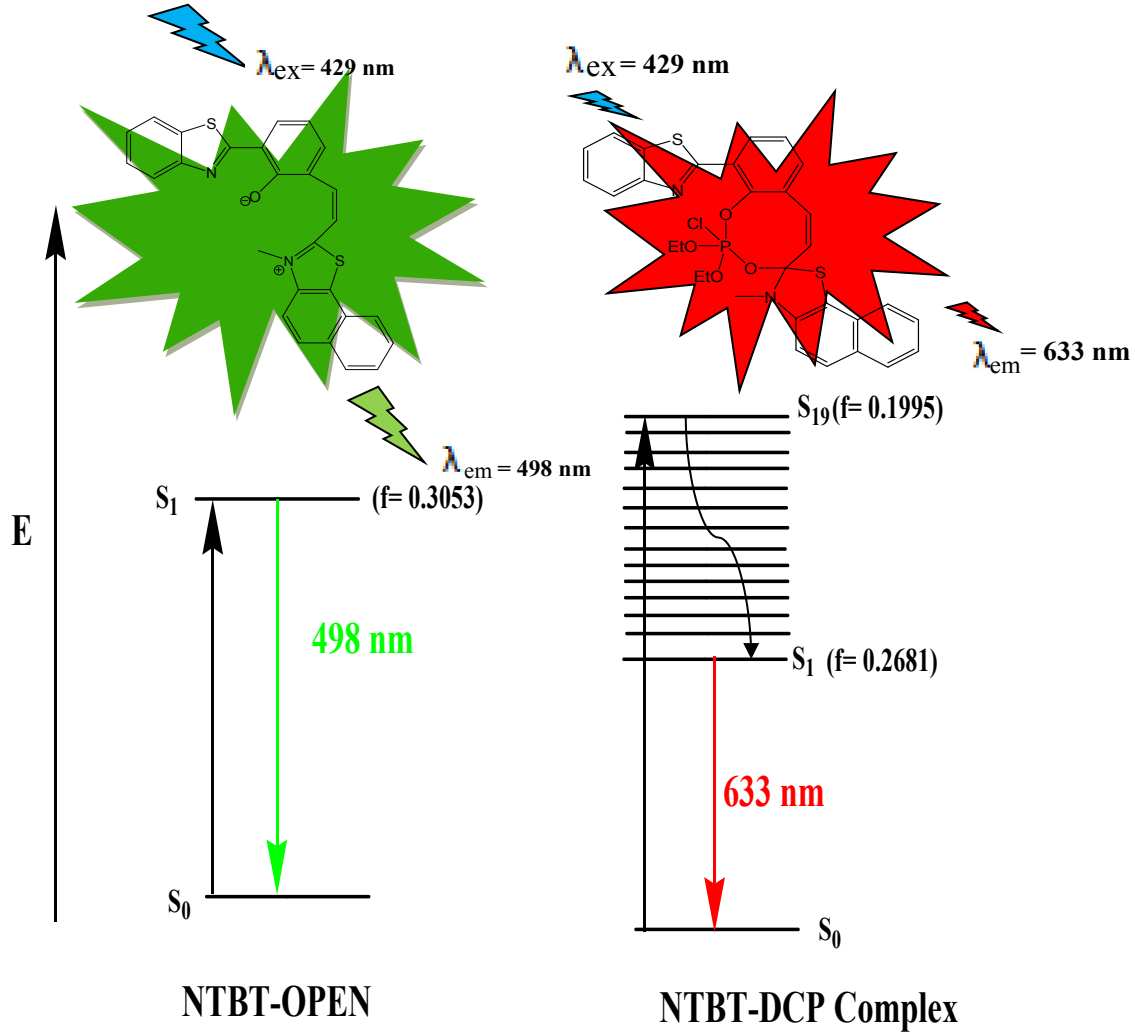
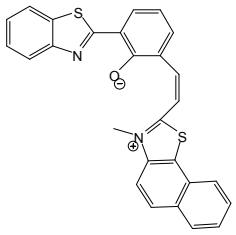
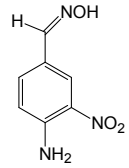
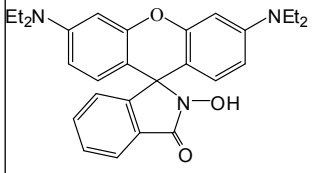
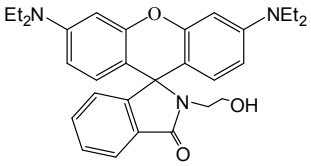
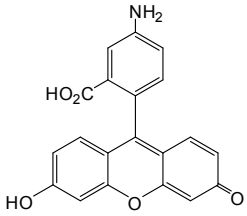
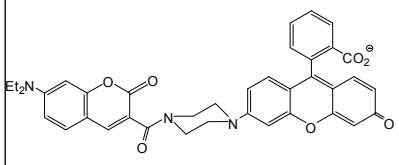
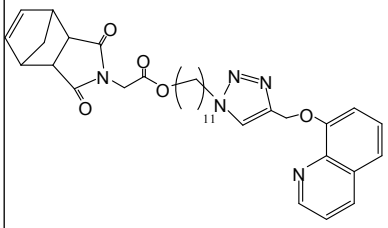


Figure S30: Selected Electronic transition.

Table S3: Summary of representative fluorescent probes for DCP

No	Chemical structure	Ratiometric	Detection-limit/ response time	If alkali used	Biological application	Vapor Sensing
1.	 **Our Compound (NTBT)**	Yes	17 nM/<40 seconds	No	Yes	Yes
2.		No	Not mentioned	Yes	Not mentioned	No
3.		No	25 ppm/20 minutes	Yes	Not mentioned	No
4.		No	< 25 ppm.	No	Not mentioned	No
5.		No	13ppm/120 min.	Not mentioned	Not mentioned	Yes
6.		Yes	0.17ppm/30 sec.	Yes	Not mentioned	Yes

7.		Yes	25ppb.	No	Not mentioned	Yes