Real time detection of the nerve agent simulant diethylchlorophosphate by non fluorophoric small molecules generating cyclization induced fluorogenic response

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Chemical structure of nerve agents and Simulants:

![Chemical structures]

Nerve Agents
- Sarin
- Soman
- Tabun

Simulants
- DCP
- DCNP
- DFP

Fig. S1 Plausible Mechanism of DCP binding
Fig. S2  LCMS of compound 2a

Fig. S3  LCMS of compound 2b
Fig. S4  LCMS of compound 2c

Fig. S5  LCMS of compound HNBM
Fig. S6  LCMS of compound **HBBM**

![LCMS of compound HBBM](image)

Fig. S7  LCMS of compound **HMBM**

![LCMS of compound HMBM](image)
Fig. S8 $^1$H NMR of HNBM in (d6-DMSO).

Fig. S9 $^1$H NMR of HBBM in (d6-DMSO).
Fig. S10 1H NMR of HMBM in (d6-DMSO).

Fig. S11 LCMS spectra of HNBM-DCP Complex.
Fig. S12 LCMS spectra of HBBM-DCP Complex.

Fig. S13 $^{13}$C NMR spectra of HNBM.
Fig. S14 $^{13}$C NMR spectra of HBBM.

Fig. S15 $^{13}$C NMR spectra of HMBM.
**Fig. S16** Absorption spectra of **HMBM** (2 μM) in acetonitrile–water (7:3) upon addition of increasing amount of DCP (40 μM).

**Fig. S17** Fluorescence spectra of **HMBM** (2 μM) in acetonitrile–water (7:3) upon addition of increasing amount of DCP (40 μM), $\lambda_{\text{exc}}$=345 nm.

**Calculation of Detection limit:**

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

$$DL = K \times \frac{Sb1}{S}$$

Where $K = 2$ or $3$ (we take $3$ in this case); $Sb1$ is the standard deviation of the blank solution; $S$ is the slope of the calibration curve.
Fig. S18 (a) LOD calculated from fluorescence data for HNBM with DCP in CH$_3$CN at $\lambda_{ex}$ = 373 nm, $\lambda_{em}$ = 503 nm. LOD is 0.10 µM (b) LOD calculated from fluorescence data for HBBM with DCP in CH$_3$CN at $\lambda_{ex}$ = 398 nm, $\lambda_{em}$ = 529 nm. LOD is 0.11 µM (c) LOD calculated from fluorescence data for HMBM with DCP in CH$_3$CN at $\lambda_{ex}$ = 345 nm, $\lambda_{em}$ = 483 nm. LOD is 0.20 µM.

**Job’s Plot:**

Fig. S19 Fluorescence Job’s plot of HNBM with DCP.
Kinetic Study:
The rate of the cyclization was determined by fitting the fluorescence intensities of the samples to the Pseudo-First Order Equation (1):

$$\ln\left(\frac{F_{max}-F_t}{F_{max}}\right) = -kt$$

(1)

Where $F(t)$ and $F(\text{max})$ 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.
Fig. S22 Time-dependent fluorescence intensity of HMBM (2 μM) at 483 nm with incremental (0-5 equiv) addition of DCP (40 μM) excitation at 345 nm.

Rate Constant:

Fig. S23 (a) Pseudo first-order kinetic plot of reaction of HNBM (0.2 μM) with various concentration of DCP a) 40 μM b) 80 μM c) 100 μM d) 150 μM in acetonitrile. Thus the Pseudo first-order rate constant of the reaction at 25°C, $K = -0.15071 \text{ Sec}^{-1}$. 
**Fig. S24** Plot of the observed $k'$ versus the concentration of DCP for the pseudo first-order reaction of HNBM (2 µM) with varying concentration of DCP (0-150 µM). **Slope** = 200.88 M⁻¹·Sec⁻¹.

**Fig. S25** Pseudo first-order kinetic plot of reaction of HBBM (2 µM) with DCP (40 µM). Rate constant of the reaction at 25°C, $K = -0.097$ Sec⁻¹

**Fig. S26** Pseudo first-order kinetic plot of reaction of HMBM (2 µM) with DCP (40 µM). Rate constant of the reaction at 25°C, $K = -0.087$ Sec⁻¹.
**Fig. S27** Fluorescence intensity response of HMBM (2 μM) to DCP (40 μM) and other interferents (100 μM). The emission intensity was measured at λem = 483 nm.

**Fig. S28** The visible color changes of HNBM in aq. CH3CN (CH3CN: H2O = 7:3 v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of various interferents.

**Fig. S29** The visible color (top) and fluorescence (bottom) changes of HBBM in aq. CH3CN (CH3CN: H2O = 7:3 v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of various interferents.

**Fig. S30** Fluorescence changes of HMBM in aq. CH3CN (CH3CN: H2O = 7:3 v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of various interferents.
**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. The numbers in parentheses are the excitation energy in wavelength. [b] Oscillator strength. [c] H stands for HOMO and L stands for LUMO.

![Energy Optimized structures of HNBM and HNBM-DCP](image)

**Fig. S31** Energy Optimized structures of HNBM and HNBM-DCP

**Table S1:** Selected electronic excitation energies (eV), oscillator strengths (f) and main configurations of HNBM and HNBM-DCP. The data were calculated by TDDFT/B3LYP/6-311+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.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Electronic Transition</th>
<th>Excitation Energy</th>
<th>f</th>
<th>Composition</th>
<th>(composition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNBM</td>
<td>$S_0 \rightarrow S_1$</td>
<td>3.1886 eV 346.09 nm</td>
<td>0.1275</td>
<td>H $\rightarrow$ L+1</td>
<td>74.8</td>
</tr>
<tr>
<td>HNBM</td>
<td>$S_0 \rightarrow S_6$</td>
<td>4.2434 eV 311.16 nm</td>
<td>0.2891</td>
<td>H-1 $\rightarrow$ L</td>
<td>68.6</td>
</tr>
<tr>
<td>HNBM-DCP</td>
<td>$S_0 \rightarrow S_1$</td>
<td>3.9418 eV 396.98 nm</td>
<td>0.2140</td>
<td>H $\rightarrow$ L</td>
<td>64.3</td>
</tr>
<tr>
<td>HNBM-DCP</td>
<td>$S_0 \rightarrow S_2$</td>
<td>4.2187 eV 369.86 nm</td>
<td>0.2504</td>
<td>H-1 $\rightarrow$ L</td>
<td>66.2</td>
</tr>
</tbody>
</table>
### Table S2: Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of HNBM and HNBM - DCP.

<table>
<thead>
<tr>
<th>Species</th>
<th>$E_{\text{HOMO}}$(a.u)</th>
<th>$E_{\text{LUMO}}$(a.u)</th>
<th>$\Delta E$(a.u)</th>
<th>$\Delta E$(eV)</th>
<th>$\Delta E$(kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNBM</td>
<td>-0.27131</td>
<td>-0.1147</td>
<td>0.1565</td>
<td>4.2593</td>
<td>98.2037</td>
</tr>
<tr>
<td>HNBM-DCP</td>
<td>-0.29688</td>
<td>-0.18415</td>
<td>0.11273</td>
<td>3.06749</td>
<td>70.7380</td>
</tr>
</tbody>
</table>

### Fig. S32 Energy Optimized structures of HBBM and HBBM-DCP

### Fig. S33 Energy difference in the respective HOMO and LUMO of HBBM and HBBM-DCP.
Table S3: Selected electronic excitation energies (eV), oscillator strengths (f) and main configurations of HBBM and HBBM-DCP. The data were calculated by TDDFT//B3LYP/6-311+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.

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Electronic Transition</th>
<th>Excitation Energy$^a$</th>
<th>f$^b$</th>
<th>Composition$^c$</th>
<th>(composition) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBBM</td>
<td>$S_0 \rightarrow S_1$</td>
<td>3.1702 eV, 349.09 nm</td>
<td>0.2075</td>
<td>H $\rightarrow$ L</td>
<td>93.8</td>
</tr>
<tr>
<td></td>
<td>$S_0 \rightarrow S_0$</td>
<td>4.0234 eV, 307.16 nm</td>
<td>0.5191</td>
<td>H-1 $\rightarrow$ L</td>
<td>93.6</td>
</tr>
<tr>
<td>HBBM-DCP</td>
<td>$S_0 \rightarrow S_1$</td>
<td>3.9018 eV, 389.98 nm</td>
<td>0.2140</td>
<td>H $\rightarrow$ L</td>
<td>56.3</td>
</tr>
<tr>
<td></td>
<td>$S_0 \rightarrow S_2$</td>
<td>4.8187 eV, 369.86 nm</td>
<td>0.2504</td>
<td>H-1 $\rightarrow$ L</td>
<td>51.2</td>
</tr>
</tbody>
</table>

Table S4: Energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) of HBBM and HBBM-DCP.

<table>
<thead>
<tr>
<th>Species</th>
<th>$E_{HOMO}$(a.u)</th>
<th>$E_{LUMO}$(a.u)</th>
<th>$\Delta E$(a.u)</th>
<th>$\Delta E$(eV)</th>
<th>$\Delta E$(kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBBM</td>
<td>-0.23596</td>
<td>-0.03986</td>
<td>0.1961</td>
<td>5.3360</td>
<td>122.05275</td>
</tr>
<tr>
<td>HBBM-DCP</td>
<td>-0.24668</td>
<td>-0.10415</td>
<td>0.1425</td>
<td>3.8783</td>
<td>89.36631</td>
</tr>
</tbody>
</table>

Quantum yield calculation:

Here, the quantum yield $\phi$ was measured by using the following equation:

$$\phi_x = \phi_s \left( \frac{F_x}{F_s} \right) \left( \frac{A_s}{A_x} \right) \left( \frac{n_x^2}{n_s^2} \right)$$

Where,

X & S indicate the unknown and standard solution respectively, $\phi$ = quantum yield,

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

n = index of refraction of the solvent. Here $\phi$ measurements were performed using Fluorescein in 0.1 M NaOH as standard ($\phi = 0.79$) and anthracene in ethanol as standard (0.27) were used. For standard (s) Fluorescein in 0.1 M NaOH and anthracene in ethanol the following values were determined:

$n_s = 1.3330$ (for 0.1 M NaOH); $n_s = 1.344$ (for CH$_3$CN); $\phi = 0.79$.

$n_x = 1.5948$ (ethanol); $n_x = 1.344$ (for CH$_3$CN); $\phi = 0.27$. 
Using the above equation, we calculated quantum yield of probes.

<table>
<thead>
<tr>
<th>Probes</th>
<th>Quantum yield ($\phi$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNBM</td>
<td>0.03</td>
</tr>
<tr>
<td>HNBM-DCP at 503 nm</td>
<td>0.17</td>
</tr>
<tr>
<td>HBBM</td>
<td>0.008</td>
</tr>
<tr>
<td>HBBM-DCP at 529 nm</td>
<td>0.02</td>
</tr>
<tr>
<td>HMBM</td>
<td>0.025</td>
</tr>
<tr>
<td>HNBCommon-DCP at 483 nm</td>
<td>0.135</td>
</tr>
</tbody>
</table>

**Table S5**: Quantum yield data

**MTT assay:**

*Fig.S34* Cell viability assay of Hep-2 cells to observe the cytotoxic effect of HNBM and DCP.
**Fig. S35** a) Time-dependent fluorescence intensity of **HNBM** (2 μM) at 503 nm with 6 equiv addition of DCP (40 μM) and 10 equiv addition of DFP, excitation at 373 nm. b) Time-dependent fluorescence intensity of **HNBM** (2 μM) at 503 nm with 6 equiv addition of DCP (40 μM) and 10 equiv addition of DCNP, excitation at 373 nm.

**Fig. S36** Fluorescence photos of **HNBM** soaked cellulose test papers have been exposed to various concentration (0-10 equiv) of HCl for 1 min.
**Fig. S37** Comparative fluorescence spectra of HNBM (2 μM) in acetonitrile-water (10 mM HEPES buffer, 7:3 V/V, pH 7.4, at 25°C) upon addition of DCP (40 μM), HCO₃⁻ (100 μM) and HCl (100 μM), $\lambda_{\text{exc}}$ = 373 nm.

**Fig. S38** LCMS of HNBM-DCNP.
Fig. S39 LCMS of HNBM-DFP.