Supporting Information (SI)

for

A Selective Fluorescent Probe for Organophosphorous Nerve Agents mimic via an Oxime-to-Isoxazole Cascade Reaction

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Keywords: Detection, Nerve Agents, Fluorescene Sensors, Chemical Warfare Agents, Oxime-Isoxazole, Limit of Detection, Sensitivity.

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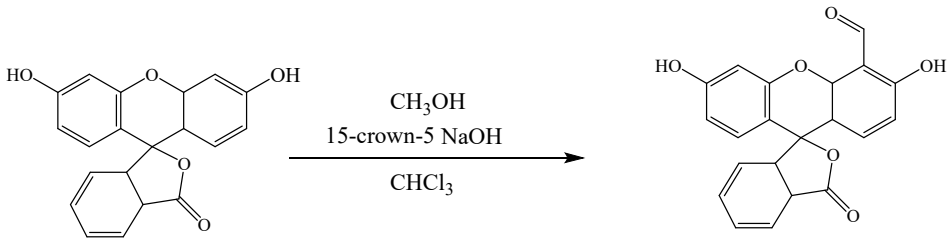
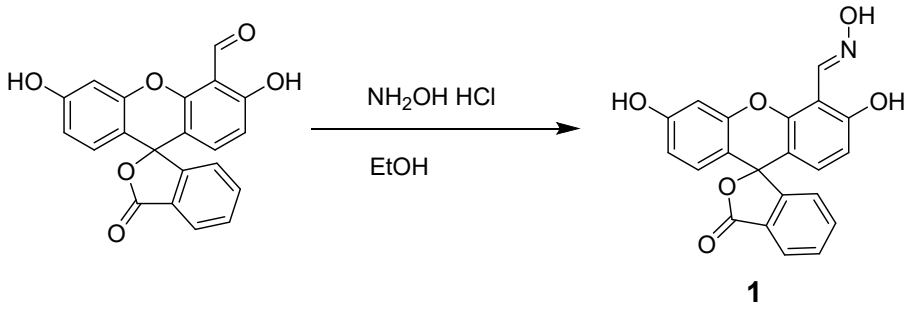
Synthesis of Fluorescein Monoaldehyde, 1 and 2

The synthesis procedure was adopted from known literature.[1] In a 500ml three neck flask, fluorescein (5.0g, 15.0mmol) was added along with 12ml methanol at room temperature. Then mixture was kept cold at 0.0 °C followed by the addition of NaOH (40.0g, 50%). 15-crown-5 (60 μL) within 5 minutes and stirred for 10 minutes at this temperature and after that mixture was allowed to warm gradually. CH_3Cl (20.0 ml) was added drop wise while the temperature of the mixture was kept 55 °C. After that reaction mixture was stirred for 10.0 hours at 55 °C and then allowed to cold at room temperature. Then the reaction mixture was acidified with H_2SO_4 (15ml, 10.0M). Purple-black precipitates were obtained which were separated by column chromatographic technique (using DCM/EtOAc 85:15). Finally, pale yellow precipitates were obtained which could be re-crystalized in acetone.



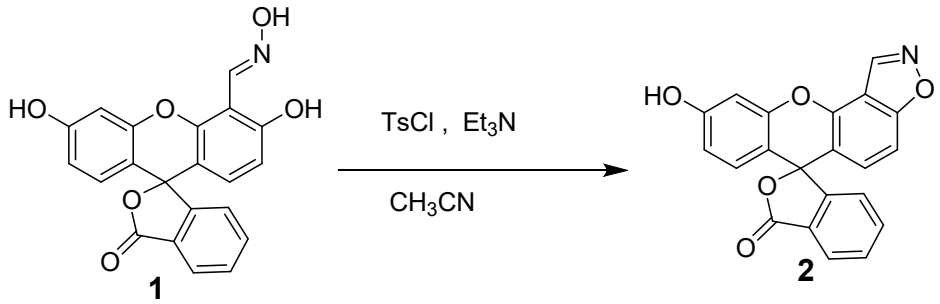
Scheme S1 Synthesis of fluorescein monoaldehyde.

The synthesis procedure was adopted from known literature.[2] Hydroxylamine hydrochloride (206 mg, 3 mmol) was added to 5 mL of ethanol and stirred at room temperature for 10 minutes. After dissolving, Fluorescein monoaldehydes (720 mg, 2 mmol) were dissolved in 10 mL of ethanol and added dropwise to the solution. After 5 minutes, add room temperature and stir for 22 hours. The reaction was stopped and the solvent was removed by steaming. The resulting crude product was separated by column chromatography using dichloromethane and ethyl acetate （DCM:EA=7:1,v:v） Make the eluent and collect the product point. DCM:EA=7:1, v:v, R_f =0.21; $CHCl_3$:MeOH=10:1, v:v, R_f =0.51），A yellow solid powder was obtained.（479.6 mg, 64 %）. Mp: 274-276 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 11.92 (s, 1H), 11.10 (s, 1H), 10.20 (s, 1H), 8.86 (s, 1H), 8.00 (d, J = 7.6 Hz, 1H), 7.80 (t, J = 6.8 Hz, 1H), 7.72(t, J = 7.2 Hz, 1H), 7.30 (d, J = 7.6 Hz, 1H), 6.86 (d, J = 2.0 Hz, 1H), 6.69 (d, J = 9.2 Hz, 1H), 6.64(d, J = 8.8 Hz, 1H), 6.61-6.56 (m, 2H) ppm. 13C NMR (75 MHz, DMSO-d6) d (ppm): 169.1, 160.0, 159.1, 152.7, 151.8, 149.5, 145.9, 136.1, 130.7, 130.3, 129.4, 126.2, 125.1, 124.5, 113.6, 113.1, 110.4, 109.8, 105.5, 103.1, 83.1. ESI−MS: *m/z* 375.4 $[M + H]^+$.



Scheme S2 Synthesis of probe.

The synthesis procedure was adopted from known literature.[3] 2: Synthesis of Compound **1** (187.5 mg, 0.5 mmol) and p-toluenesulfonyl chloride (143 mg, 0.75 mmol) were added to 3 mL of acetonitrile, After stirring at room temperature for 10 minutes, triethylamine (88.5 uL, 0.64 mmol) was added dropwise with a micro-syringe, After 2 min addition, the mixture was stirred at room temperature for 5.5 hours. The reaction was quenched and the solvent was removed by steaming. The resulting crude product was separated by column chromatography, the eluent was washed with dichloromethane and ethyl acetate (DCM: EA = 25: 1, v: v) to give a pale yellow solid. （94.1 mg， 52.7 %. Mp: 282-284. ^{1}H NMR (DMSO-d_6, 400 MHz) δ =10.33 (s, 1H), 9.64(s, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.80 (t, J = 7.6 Hz, 1H), 7.74 (t, J = 7.2 Hz, 1H), 7.50(d, J = 8.8 Hz, 1H), 7.32(d, J = 7.2 Hz, 1H), 7.02(d, J = 9.2 Hz, 1H), 6.83(d, J = 1.6 Hz, 1H), 6.68-6.63(m, 2H) ppm. 13C NMR (75 MHz, DMSO-d6) d (ppm): 169.0, 163.8, 160.1, 153.2, 151.2, 145.2, 136.3, 131.2, 130.8, 129.8, 126.1, 125.3, 124.6, 114.2, 113.3, 111.6, 109.7, 106.6, 102.8, 82.2. ESI−MS: *m/z* 358.2 $[M + H]^+$.



Scheme S3 Synthesis of compound **2**.

Types of Nerve Agents

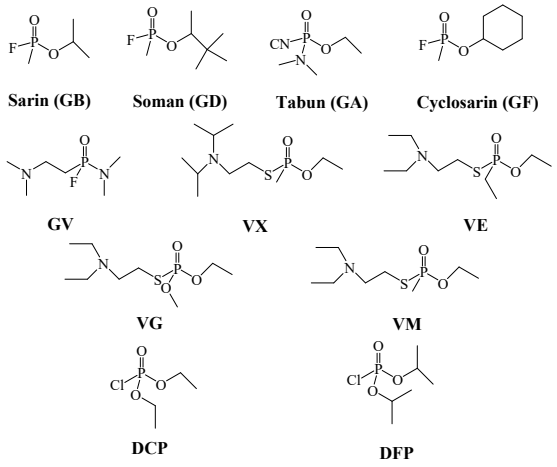
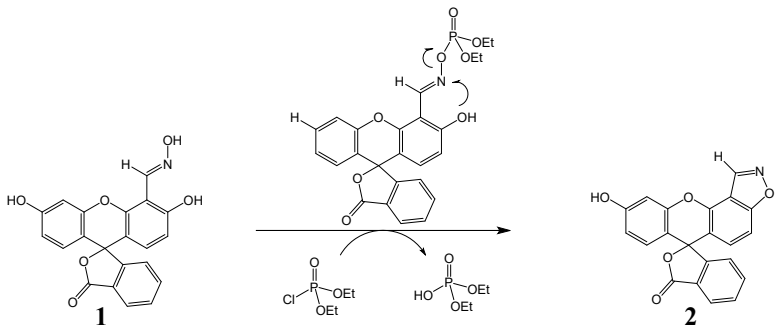


Figure S1 Chemical Warfare Agents and their Mimics

Mechanism



Scheme S4 Mechanism for the reaction of DCP with compound **1** to yield **2**.

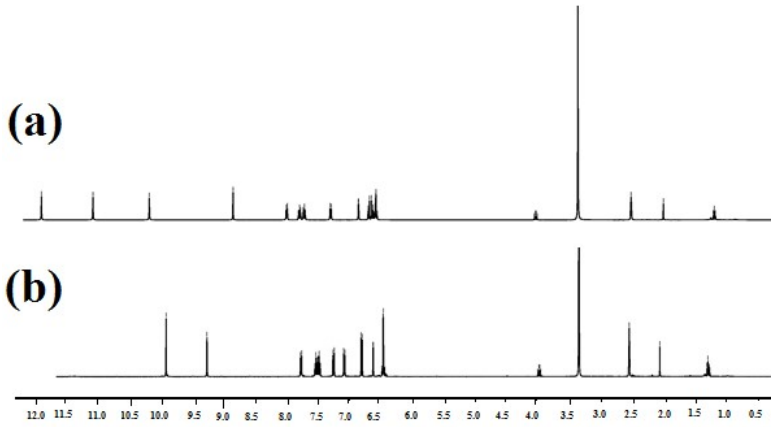


Figure S2 ^{1}H NMR spectra of (a) **1**, (b) **2**, in DMSO-d_6.

Characterization

^{1}H NMR, ^{13}C NMR, MS spectrum of monoaldehyde-functionalized fluorescein, **1** and **2**, Optimized structures of **1** and **2**.

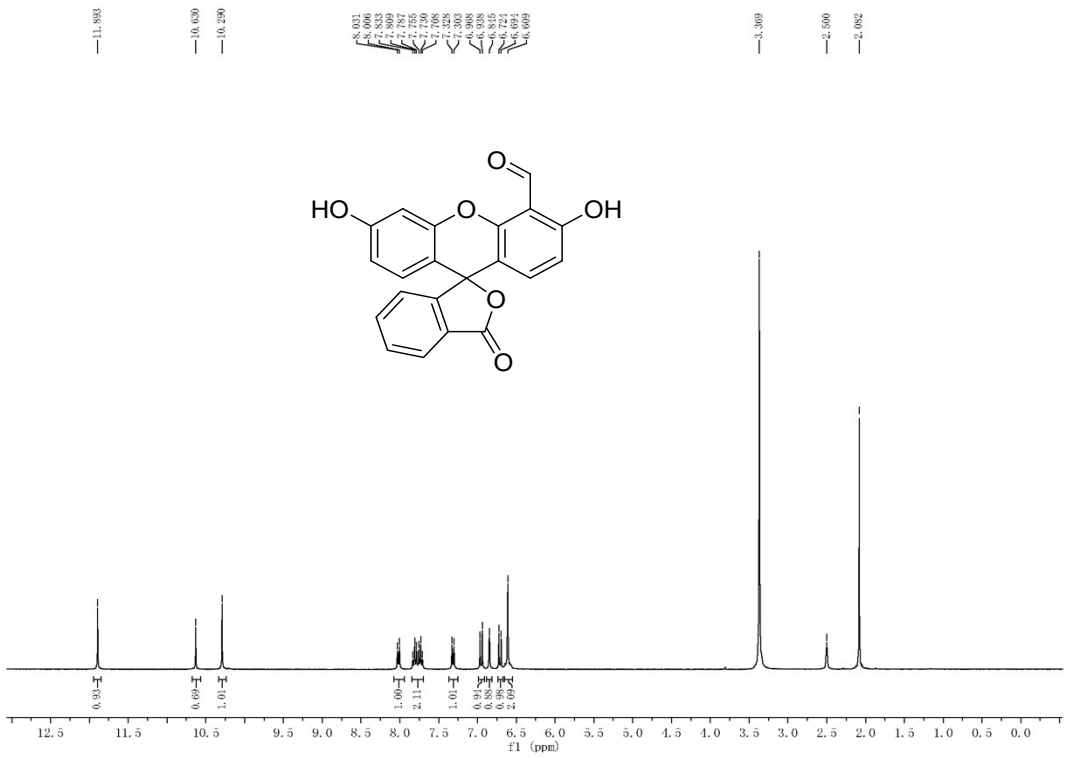


Figure S3 ^{1}H NMR spectrum (300 MHz, DMSO-d_6) of monoaldehyde-functionalized fluorescein.

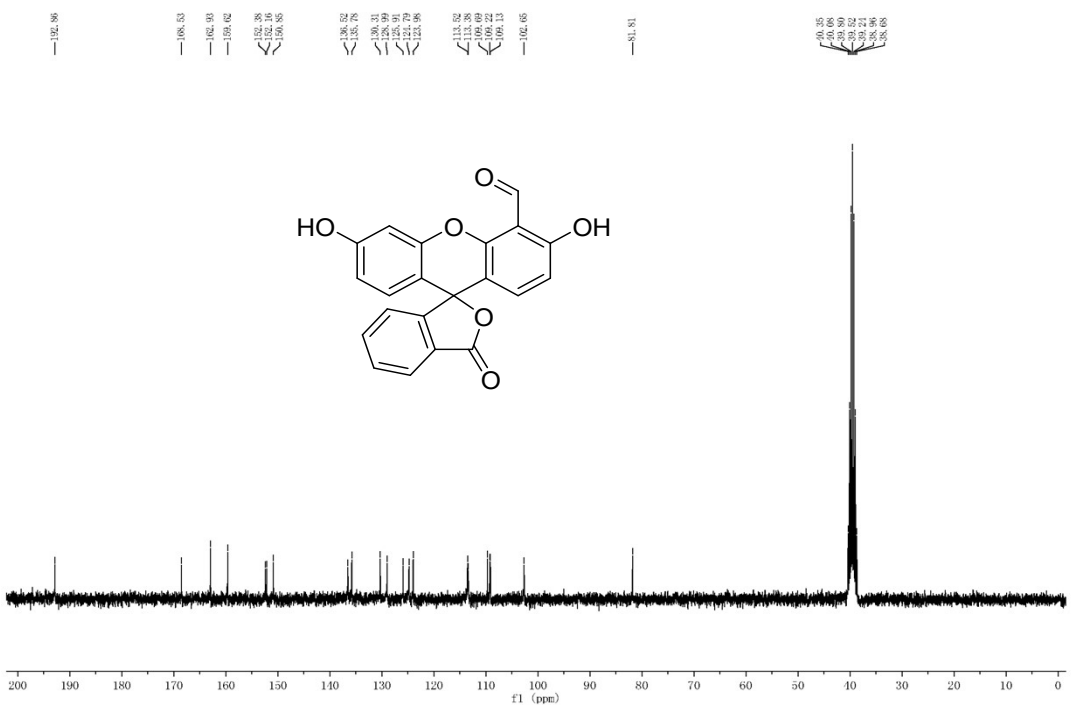


Figure S4 ^{13}C NMR spectrum (75 MHz, DMSO-d_6) of monoaldehyde-functionalized fluorescein.

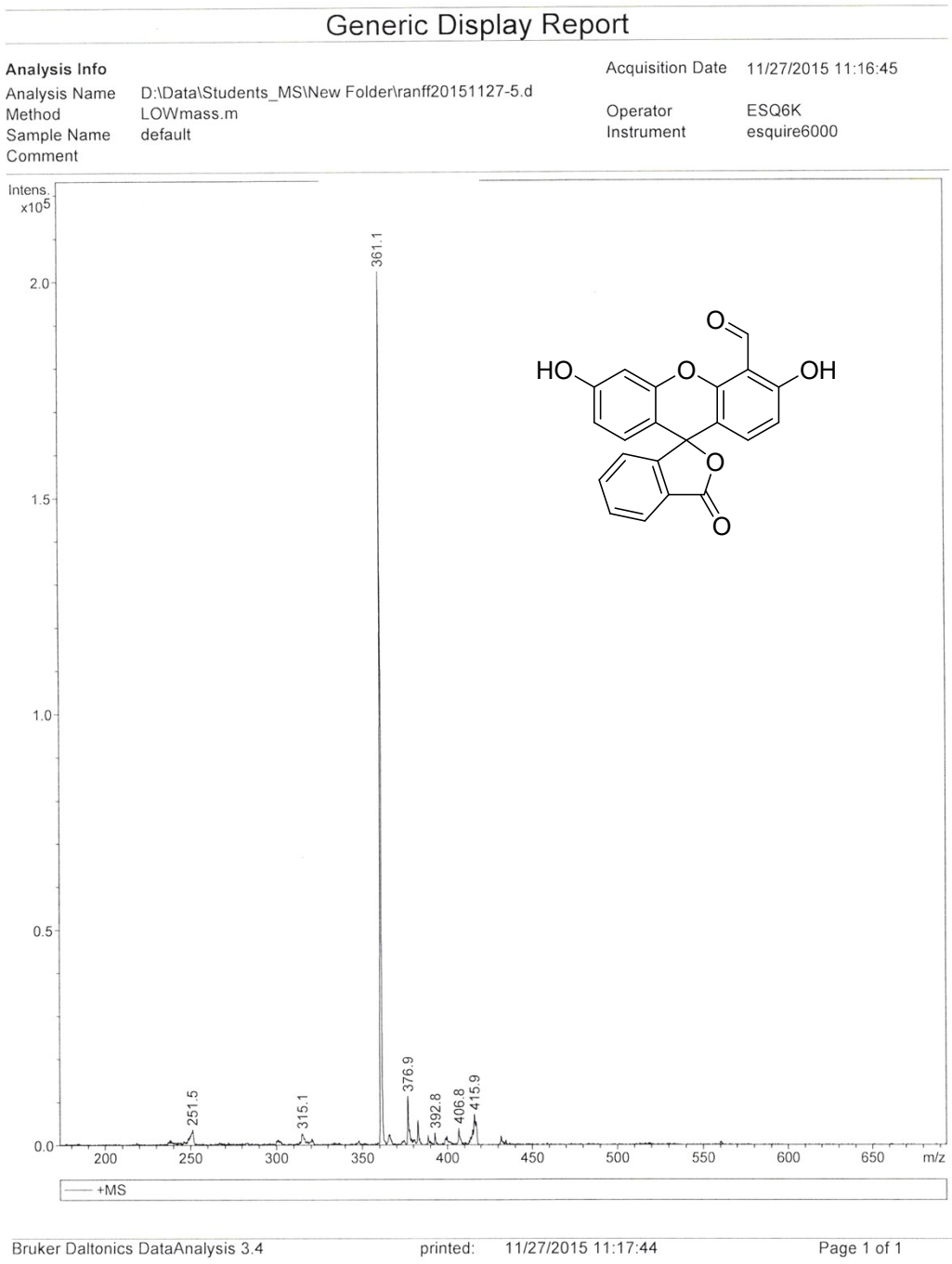


Figure S5 ESI-MS spectrum of monoaldehyde-functionalized fluorescein.

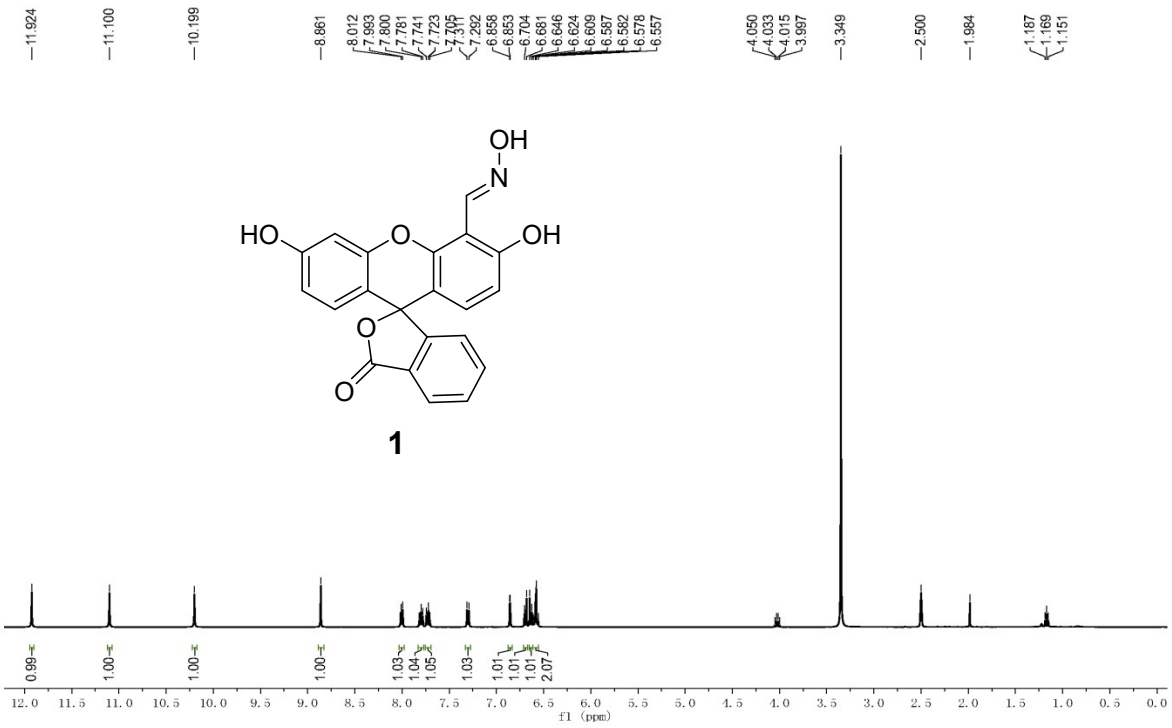


Figure S6 ^{1}H NMR spectrum (400MHz, DMSO-d_6) of **1**.

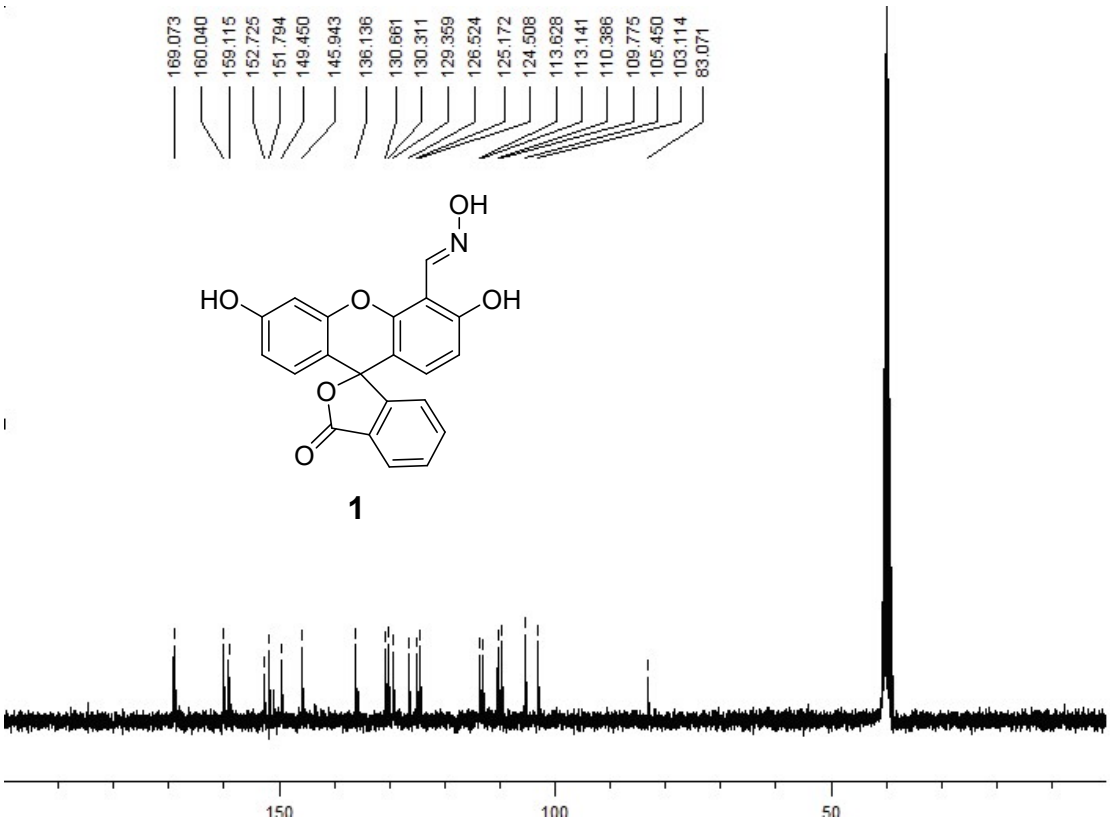


Figure S7 ^{13}C NMR spectrum (400MHz, DMSO-d_6) of **1**.

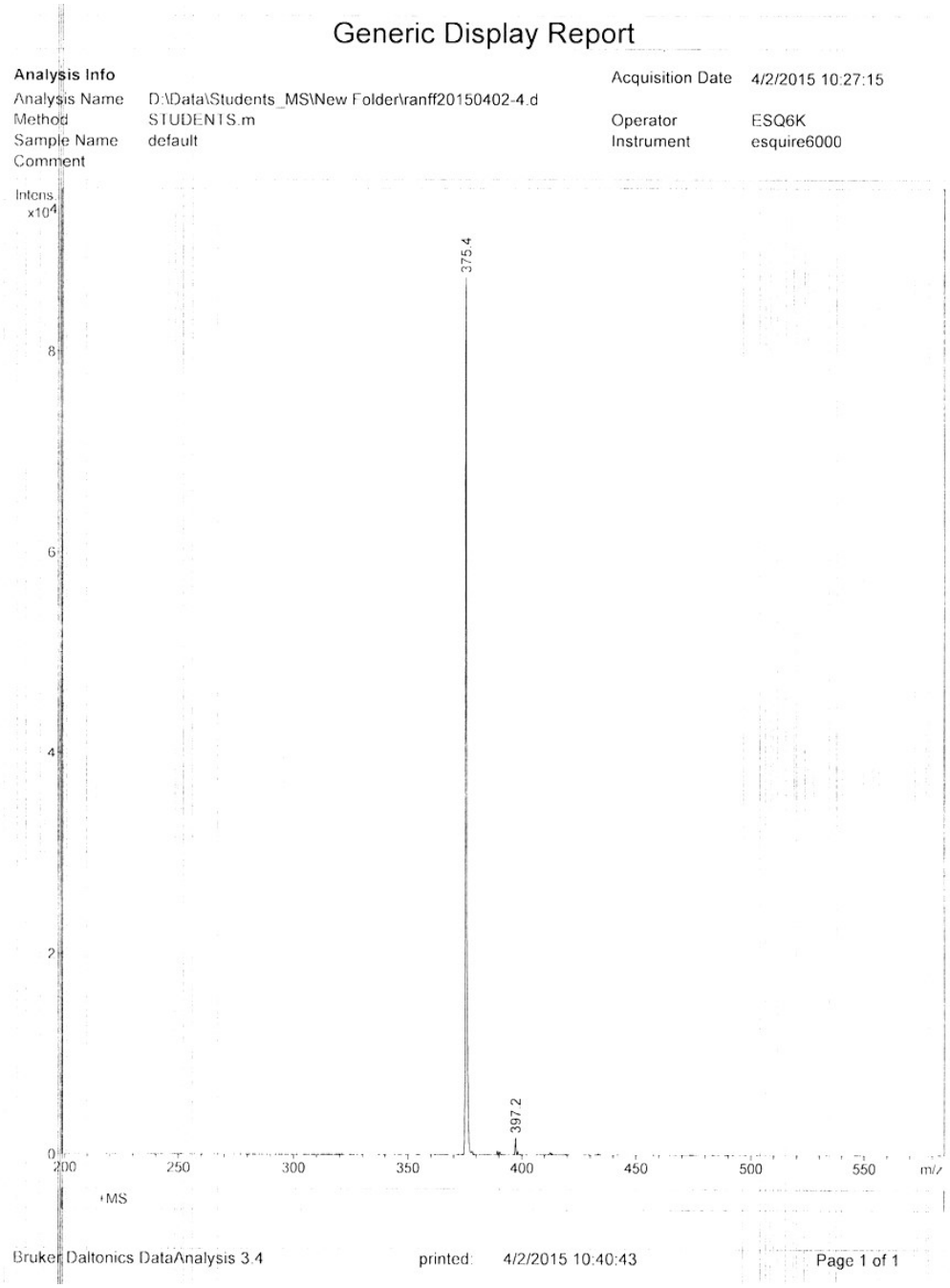


Figure S8 ESI-MS spectrum of **1**.

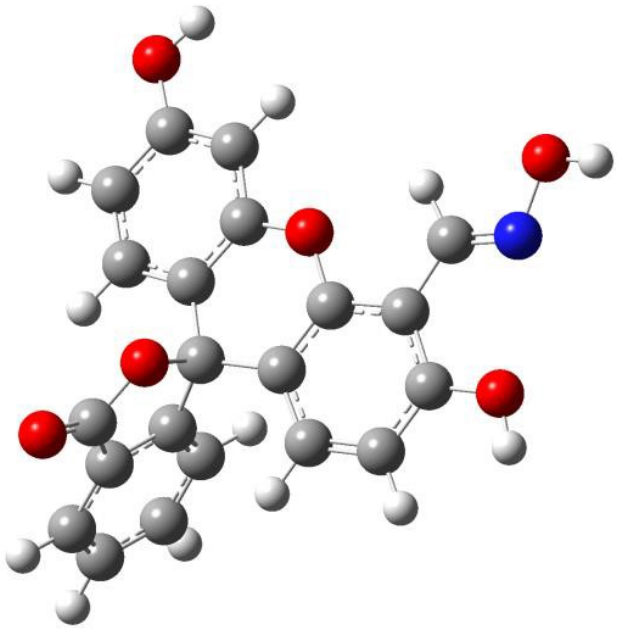


Figure S9 Optimized structure of **1**.

B3LYP/6-31++g(d,p) scrf=pcm geom=connectivity

Charge = 0 Multiplicity = 1

C	-3.7908	0.4126	1.3787
C	-3.7908	-0.9733	1.3787
C	-2.5906	-1.6664	1.3907
C	-1.3903	-0.9734	1.4027
C	-1.3903	0.4126	1.4027
C	-2.5906	1.1056	1.3907
C	-0.0566	-1.7434	1.4027
C	1.0647	-0.8293	1.9305
C	0.8059	0.5081	2.1862
O	-0.1692	1.1176	1.4027
C	2.3297	-1.3467	2.161
C	3.3358	-0.5267	2.6473
C	3.132	0.8681	2.6583
C	1.8395	1.3568	2.5501

O	-5.0119	1.1176	1.3787
O	4.2301	1.7425	2.5259
C	1.5497	2.8398	2.8471
O	2.2294	4.8686	3.4274
N	2.4771	3.6013	3.1736
O	-0.2115	-2.9529	2.1106
C	-0.0335	-4.2268	1.2263
C	0.291	-3.5741	-0.1303
C	0.2782	-2.1917	-0.0321
C	0.5852	-4.1801	-1.3416
C	0.8666	-3.4036	-2.4547
C	0.8034	-2.0209	-2.3684
C	0.5344	-1.4151	-1.1512
O	-0.0714	-5.4388	1.2335
H	-4.7623	-1.5343	1.3592
H	-2.5905	-2.7883	1.3809
H	-2.5905	2.2276	1.3809
H	2.5418	-2.4271	1.9449
H	4.2031	-0.9716	3.2029
H	-5.0649	1.6881	2.1885
H	5.0649	1.2143	2.4356
H	0.5004	3.2311	2.7783
H	2.7465	5.4388	2.8017
H	0.5862	-5.2991	-1.4235
H	1.136	-3.8936	-3.4274
H	0.9604	-1.3935	-3.2852
H	0.5145	-0.2959	-1.0738

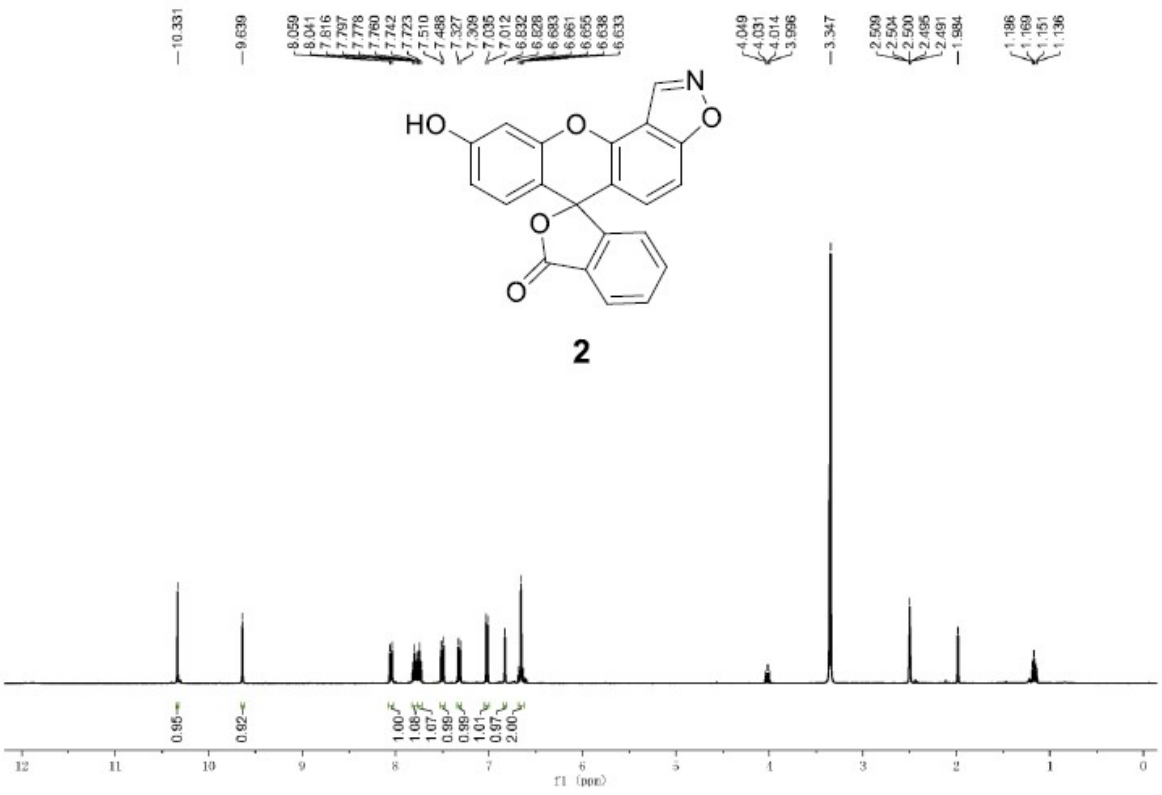


Figure S10 ^{1}H NMR spectrum (400MHz, DMSO-d_6) of **2**.

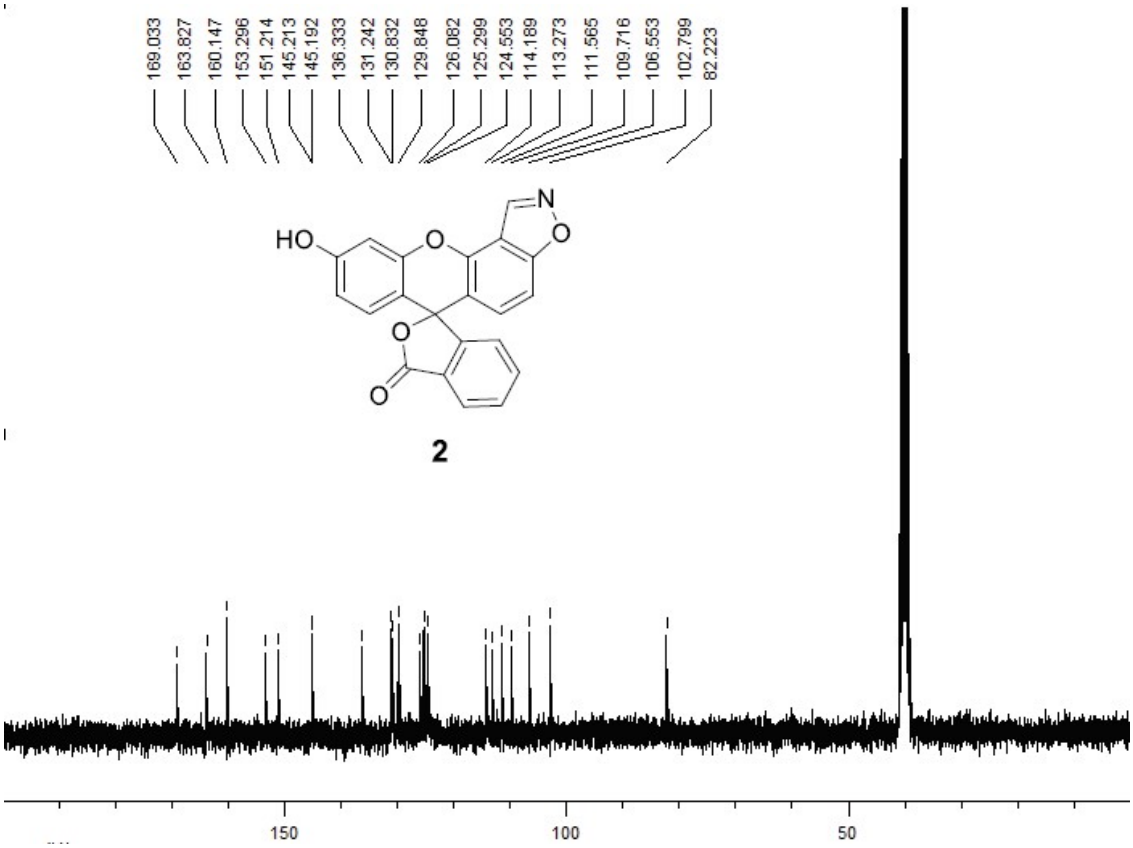


Figure S11 ^{13}C NMR spectrum (400MHz, DMSO-d_6) of **2**.

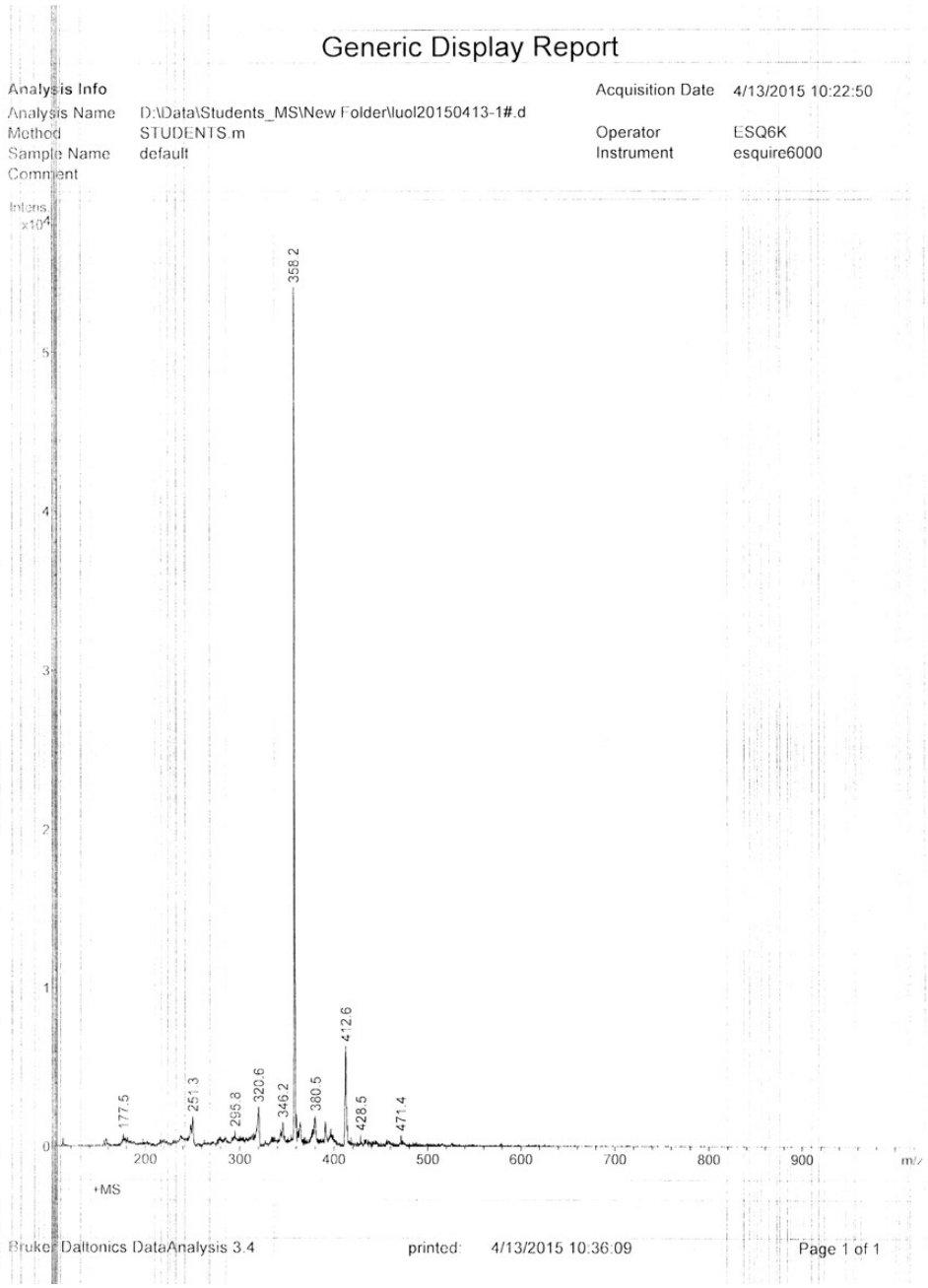


Figure S12 ESI-MS spectrum of **2**.

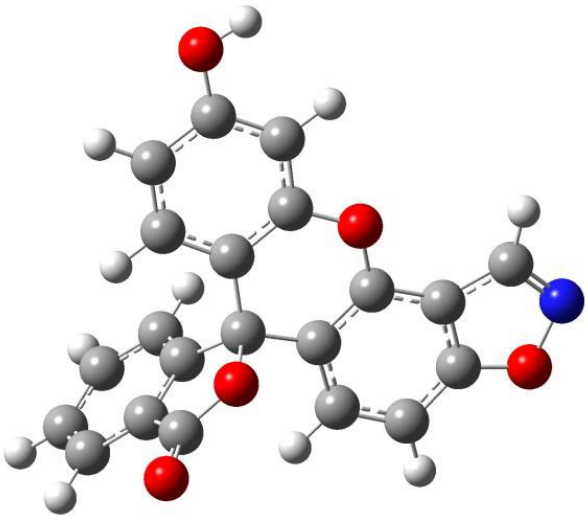


Figure S13 Optimized structure of **2**.

B3LYP/6-31++g(d,p) scrf=pcm geom=connectivity

Charge = 0, Multiplicity = 1

C	-1.06354	4.04332	-0.13211
C	-2.2045	3.22709	-0.1158
C	-2.05505	1.85041	-0.03072
C	-0.78745	1.24062	0.03675
C	0.32672	2.08765	0.0171
C	0.20872	3.47687	-0.05804
C	-0.62862	-0.24523	0.24164
C	0.80887	-0.68029	0.05421
C	1.82316	0.27258	0.02365
O	1.61403	1.61428	0.04046
C	1.15643	-2.05735	0.00758
C	2.46771	-2.50814	-0.0675
C	3.4458	-1.51336	-0.09515
C	3.16479	-0.15426	-0.05236
O	-1.26956	5.38457	-0.24295
O	4.79493	-1.69063	-0.1772

C	4.44942	0.44447	-0.1174
N	5.40698	-0.44902	-0.18786
O	-0.9812	-0.53675	1.68597
C	-2.02323	-1.39066	1.78111
C	-2.4592	-1.75679	0.41856
C	-1.64638	-1.09385	-0.49658
C	-3.49095	-2.6044	0.01116
C	-3.68435	-2.77516	-1.36068
C	-2.86126	-2.1103	-2.28708
C	-1.83124	-1.26056	-1.86633
O	-2.41551	-1.80624	2.85597
H	-3.18615	3.68508	-0.10789
H	-2.94155	1.22726	-0.01262
H	1.10586	4.0864	-0.04868
H	0.35615	-2.78741	0.034
H	2.71718	-3.56033	-0.10093
H	-0.43285	5.8681	-0.19135
H	4.71658	1.49105	-0.11268
H	-4.114	-3.11158	0.73951
H	-4.47775	-3.42235	-1.71721
H	-3.0251	-2.26119	-3.34848
H	-1.2039	-0.74962	-2.58858

Table S1 Crystal data and structure refinement parameters for **2**

Compound	Compound **2**
Empirical formula	$C_{24}H_{17}NO_6$
Formula weight	415.39
Temperature/K	173
Crystal system	monoclinic
Space group	P2/c
a/Å	24.892(2)
b/Å	7.5673(5)
c/Å	23.691(2)
α/°	90.00
β/°	118.281(12)
γ/°	90.00
Volume/Å^3	3930.0(6)
Z	8
ρ_{calc}g/cm^3	1.404
μ/mm^{-1}	0.102
F(000)	1728.0
Crystal size/mm^3	0.34 × 0.33 × 0.25
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.54 to 52.04
Index ranges	-30 ≤ h ≤ 30, -9 ≤ k ≤ 9, -29 ≤ l ≤ 29
Reflections collected	16011
Independent reflections	7627 [R_{int} = 0.0718, R_{sigma} =
Data/restraints/parameters	7627/0/565
Goodness-of-fit on F^2	1.050

Limit of Detection (LOD).

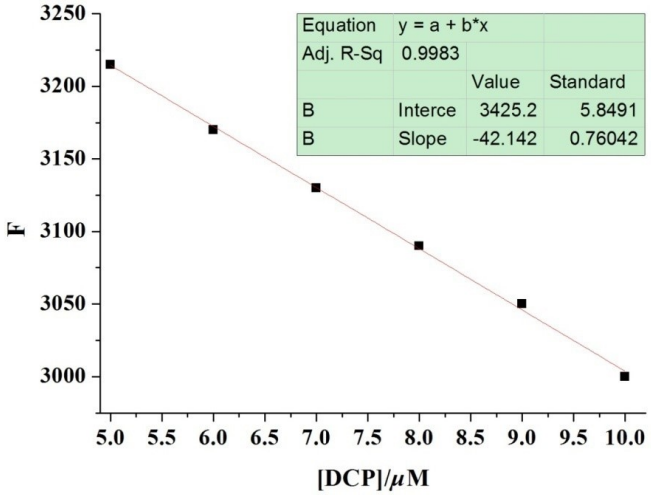


Figure S14 Fluorometric determination of limit of DCP to probe in DMF/HEPES buffer (1/1, v/v, pH 7).

Slope = 42 Standard Deviation = 2.51 K = 3

LOD = K × Standard Deviation / Slope = 3 × 2. 51 / 42 = 0.18 μM

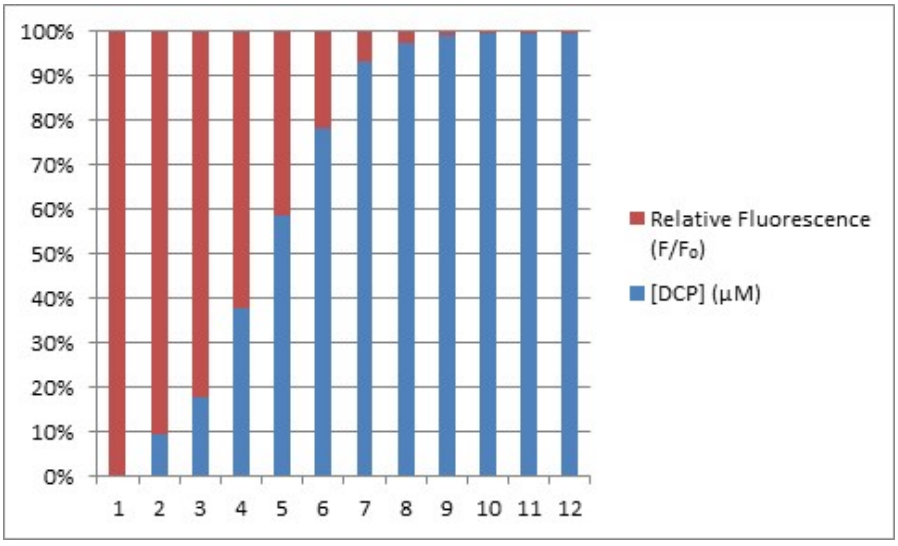


Fig. S15 Titration curve showing the fluorescence quenching of probe (10 μM) with increasing concentrations of DCP (0.1 μM – 50 μM) in DMF/HEPES buffer (1:1, v/v, pH 7.0).

pH Effect

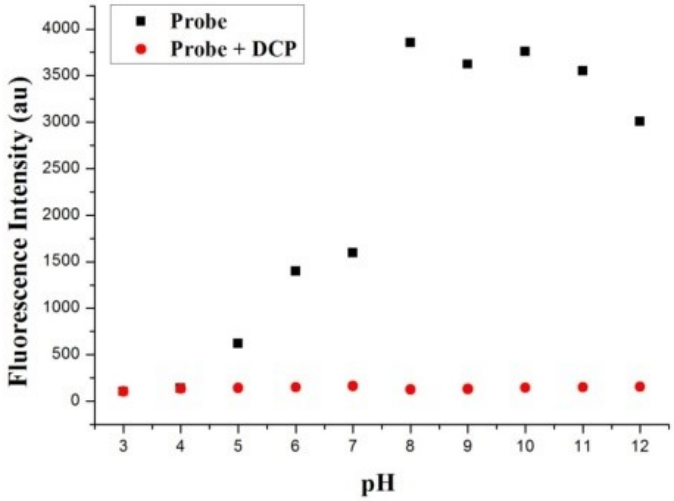


Figure S16 The fluorescence intensity of probe (10.0 μM) at 543 nm in the presence and absence of DCP (10.0μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0) under different pH (3.0-12.0).

Effect of HCl on UV/Fluorescence Spectra

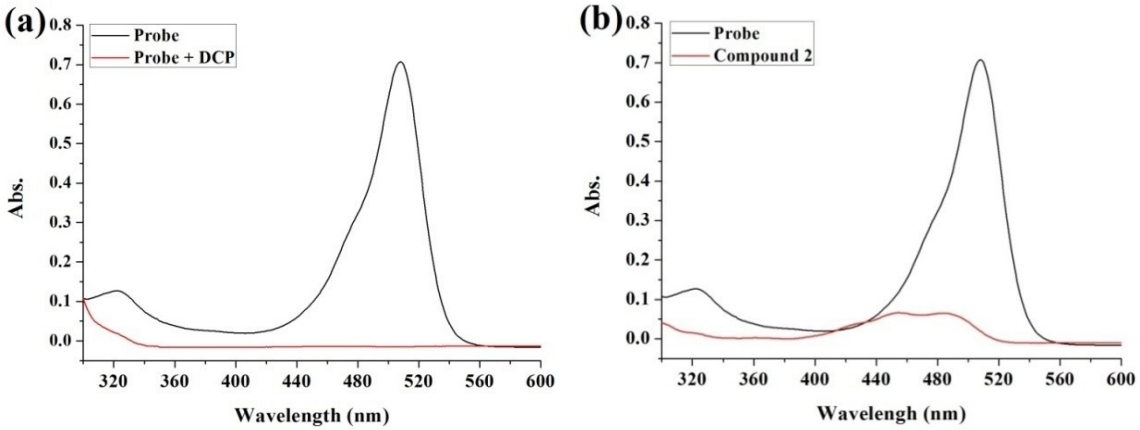


Figure S17 UV spectra in the presence of HCl, (a) Probe (10.0 μM) free and after addition of DCP (10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0) and **(**b**)** probe (10.0 μM) and compound **2** (10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0).

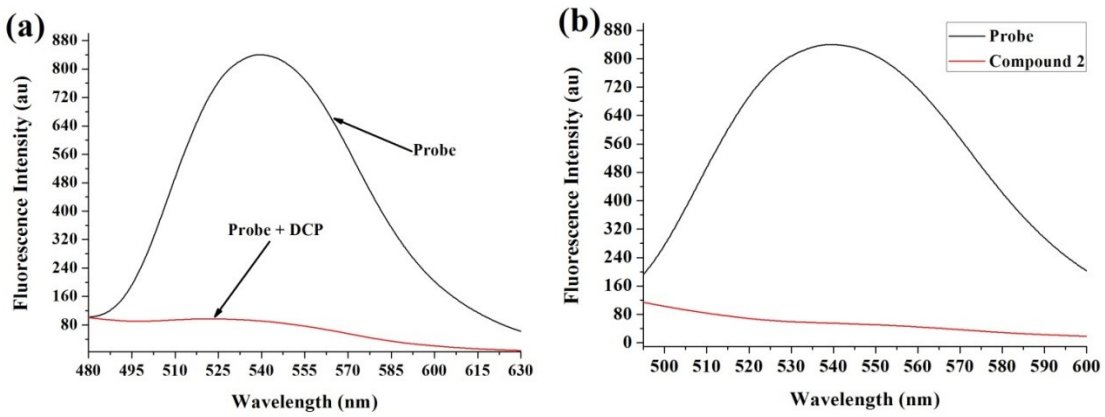


Figure S18 Fluorescence spectra in the presence of HCl, (a) Probe (10.0 μM) free and after addition of DCP (10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0) and **(b)** probe (10.0 μM) and compound **2** (10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0).

Applications

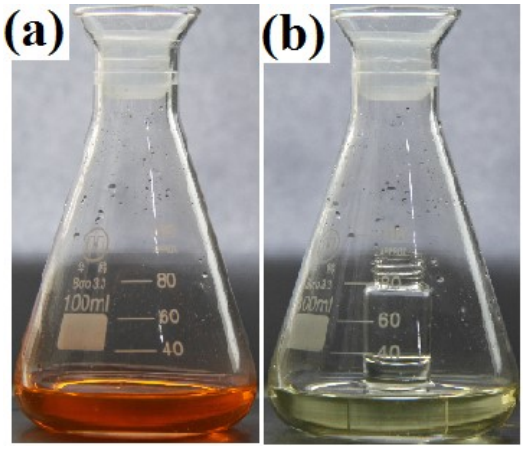


Figure S19 Images of (a) free probe (10.0 μM), and (b) after exposure to DCP (15.0 μM) vapors in DMF/HEPES buffer (1/1, v/v, pH 7.0).

Crystalline Structure

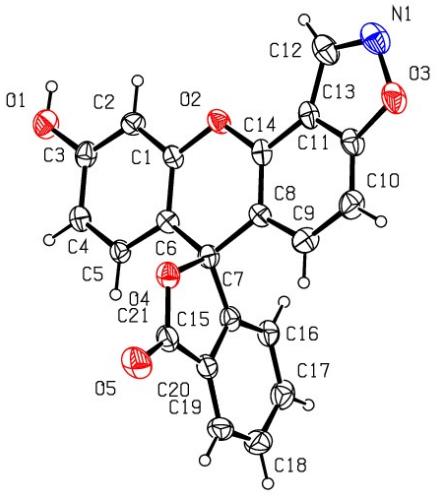


Figure S20 View of the molecular structure of **2.**

DFT Calculations

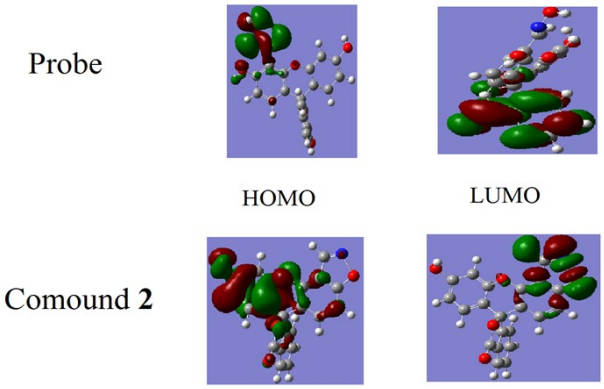


Figure S21 HOMO-LUMO orbital plots of **1** and **2**.

Table S2 A comparative analysis of critical performance metrics limit of detection (LOD), response rapidity, and selectivity for various fluorescent and colorimetric probes targeting nerve agent mimics.

Study	Detection Method	Target Agent(s)	LOD / Sensitivity	Response Time	Selectivity	Application Format
This Work	Fluorescence quenching + colorimetric	G-agent mimic	0.18 μM	Seconds (naked-eye color change in <30 s)	High for DCP over other OPs	Liquid & vapor phase
Termeau et al. [4]	Colorimetric	Novichok agents	~10–50 equiv. in liquid; visual detection	Minutes to 1 hour	Selective for Novichok over G/V agents	Glass fiber strips
Costero et al. [5]	Colorimetric	DCP, DFP, DCNP	~1×10^{-4} M	3.5–11.4 s (half-life)	Selective for phosphonate ester mimics	Silica gel support & solution
Urban et al.[6]	Colorimetric	Novichok & V-agents	~1% (v/v) liquid; ~4–9 mM for some agents	Immediate (color spots in <5 min)	Distinguishes A- vs. V-group; some cross-reactivity	Paper strips
Santonocito et al.[7]	Fluorescence	DMMP	0.1 ppm (gas)	1 hour exposure at 50°C	High via PCA fingerprinting	RP18 silica array

References

1. Z.-H. Fu, X. Han, Y. Shao, J. Fang, Z.-H. Zhang, Y.-W. Wang and Y. Peng, *Anal. Chem*. 2017, **89**, 1937–1944.
2. W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner and R. M. Strongin, J. Am. Chem. Soc. 2005, 127, 15949-15958.
3. H. Lee and H. –J. Kim, Trtrahedron, 2014, 70, 2966-2970.
4. L. Termeau, S. Penlou, and A. Carella, ACS Sensors, 2023, 8, 1510-1517.
5. M. Costero, S. Gil, M. Parra, P. M. E. Mancini, R. M. Manez, F. Sancenon and S. Royo, Chem. Commun., 2008, 6002–6004.
6. M. Urban, T. Dropa, M. Zelenkova, M. Dymak and V. Pitschmann, Chemosensors, 2025, 13, 77.
7. R.Santonocito, M. Spina, R. Puglisi, A. Pappalardo, N. Tuccitto and G. T. Sfrazzetto, Chemosensors, 2023, 11, 503.