Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

A FRET-based ratiometric fluorescent and colorimetric probe for the facile detection of organophosphonates nerve agent mimic DCP

Weimin Xuan,[a] Yanting Cao,[a] Jiahong Zhou,[a,b]* and Wei Wang[a,c]*

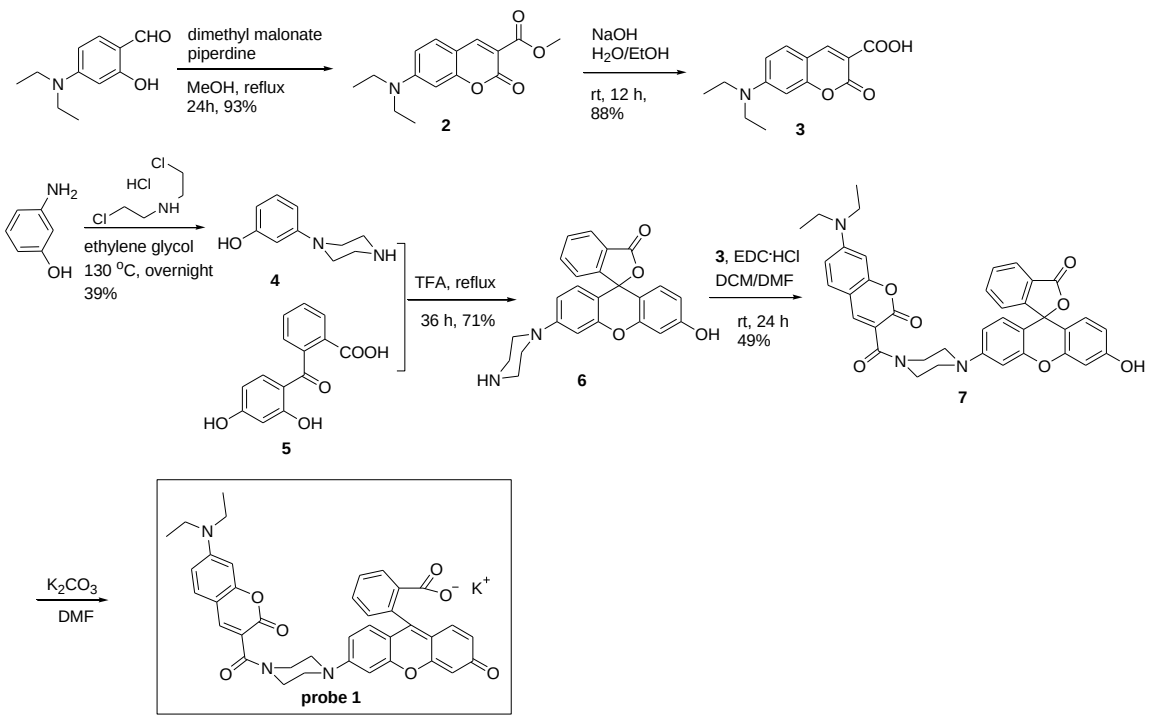
[a] *Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, NM, 87131-0001, USA*
[b] *Analysis & Detecting Center, Nanjing Normal University, 1 Wen-Yuan Road, Nanjing, Jiangsu 210046, China*
[c] *School of Pharmacy, East China University of Science & Technology, 130 Meilong Road, Shanghai 200237, China*

Contents

General Information: Commercial reagents were used as received, unless otherwise stated. Merck 60 silica gel was used for chromatography, and Whatman silica gel plates with fluorescence F_{254} were used for thin-layer chromatography (TLC) analysis. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 500 and Bruker tardis (sb300). Data for ^{1}H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad). Data for ^{13}C NMR are reported as ppm.

Spectroscopic materials and methods: Fluorescence emission spectra were obtained on a SHIMADZU spectrofluorophotometer RF-5301pc. The UV absorption spectra were obtained on a SHIMADZU UV-1800.

1. Synthesis probe 1



Methyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (2): To a solution of 4-diethylaminosalicyl aldehyde (200 mg, 1.03 mmol) in 20 mL of methanol was added dimethyl malonate (356 μL, 3.11 mmol) and piperidine (20 μL) at room temperature. The mixture was heated to reflux, and stirred for 24 h. The solvent was evaporated *in vacuo*. The crude product was purified by silica gel chromatography to afford **2** as yellow liquid (264 mg, 93%). ^{1}H NMR

(500 MHz, $CDCl_3$): δ 8.46 (s, 1H), 7.36 (d, 1H, *J* = 9.0 Hz), 6.60 (d, 1H, *J* = 8.5 Hz), 6.46 (s, 1H), 3.91 (s, 3H), 3.44 (q, 4H, *J* = 7.0 Hz), 1.23 (t, 6H, *J* = 7.0 Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 165.02, 158.54, 158.33, 152.96, 149.66, 131.11, 109.58, 108.54, 107.72, 96.70, 52.33, 45.12, 12.41.

7-(Diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (3): To a solution of **2** (48 mg, 0.17 mmol) in 5 mL of ethanol was added 3 mL of 0.5M aqueous NaOH solution. The mixture was stirred at room temperature for 12 h. The solvent was evaporated *in vacuo*. H_2O (5 mL) was added to dissolve the residue, then pH was adjusted to acidic by 1.0 M aqueous HCl solution. The mixture was extracted by CH_2Cl_2 (10 mL × 3). The combined layer was dried with sodium sulfate, filtered and evaporated *in vacuo* to afford **3** as orange red solid (40 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.65 (s, 1H), 7.45 (d, 1H, *J* = 9.0 Hz), 6.70 (dd, 1H, *J* = 1.8 Hz, 8.7 Hz), 6.52 (d, 1H, *J* = 1.5 Hz), 3.49 (q, 4H, *J* = 7.1 Hz), 1.26 (t, 6H, *J* = 7.1 Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 165.39, 164.30, 157.88, 153.65, 150.05, 131.80, 110.82, 108.37, 105.28, 96.65, 45.22, 12.25.

3-(Piperazin-1-yl)phenol (4): To a solution of 3-aminophenol (1.0 g, 9.16 mM) in 20 mL of ethylene glycol was added *bis*(2-chloroethyl)amine hydrochloride (1.63 g, 9.13 mM), then the solution was heated to 130 °C and stirred under the atmosphere of Ar overnight. After the solution was cooled to room temperature, 40 mL of H_2O was added, and the pH was adjusted to 10 by 1 M NaOH. The product was extracted by EtOAc (100 Ml × 5), dried over Na_2SO_4. The EtOAc was removed by rotavapor, and the crude product was further washed with 5 mL of CH_2Cl_2. The final product was obtained as a white solid (640 mg, 39%). ^{1}H NMR (300 MHz, MeOD): δ 7.03 (t, 1H, *J* = 8.1 Hz), 6.45 (dd, 1H, *J* = 1.8 Hz, 8.4 Hz), 6.39 (t, 1H, *J* = 2.1 Hz), 6.30 (dd, 1H, *J* = 2.1 Hz, 8.4 Hz), 3.07(t, 4H, *J* = 2.7 Hz), 2.95 (t, 4H, *J* = 2.7 Hz); ^{13}C NMR (300 MHz, MeOD): δ 159.22, 154.63, 130.75, 109.21, 108.40, 104.69, 51.30, 46.48.

2-(2,4-Dihydroxybenzoyl)benzoic acid (5) was synthesized according to the reported method.[1]

3'-Hydroxy-6'-(piperazin-1-yl)-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (6): To compound **5** (1.74 g, 6.74 mM) in 20 mL of TFA was added compound **4** (1g, 5.61 mM), then

heated to reflux and stirred for 36 h. The solvent was removed by rotavapor, and the left residue was dissolved in 30 mL of H_2O. The crude product was extracted with EtOAc (40 mL × 3), and dried with Na_2SO_4. The product was further purified by column chromatograph, and obtained as a red solid (1.6 g, 71%). ^{1}H NMR (300 MHz, d_6-acetone): δ 7.97 (dd, 1H, *J* = 1.2, 7.1 Hz), 7.77-7.65 (m 2H), 7.20 (dd, 1H, *J* = 1.2 Hz, 7.5 Hz), 6.83-6.73(m, 3H), 6.66-6.61 (m, 3H), 3.64-3.60 (m, 4H), 3.49-3.45 (m, 4H); ^{13}C NMR (300 MHz, d_6-acetone): δ 169.65, 161.96, 161.51, 153.65, 153.31, 153.19, 152.75, 135.95, 130.63, 129.87, 129.58, 127.69, 125.32, 124.81, 119.77, 115.88, 113.36, 113.09, 111.26, 111.13, 103.35, 103.21, 84.34, 63.92, 54.86, 45.97, 43.85.

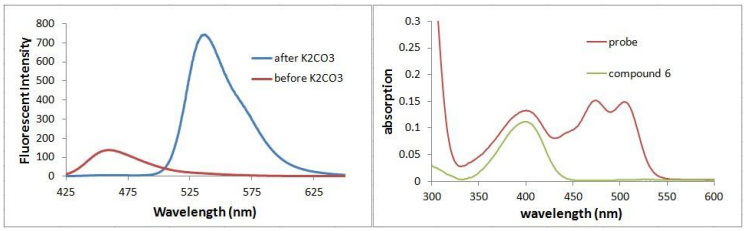
3'-(4-(7-(Diethylamino)-2-oxo-2H-chromene-3-carbonyl)piperazin-1-yl)-6'-hydroxy-3H-spiro[isobenzofuran-1,9'-xanthen]-3-one (7): To a solution of compound **6** (980 mg, 2.45 mM) in 30 mL of DCM/DMF (5:1) was added compound **3** (640 mg, 2.45 mM) and EDC•HCl (563 mg, 2.94 mM), then stirred at room temperature for 24 h. The solvent was removed by rotavapor, then the product was purified through column chromatograph and obtain as a red solid (780 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (dd, 1H, *J* = 1.2, 6.6 Hz), 7.88(s, 1H), 7.65-7.54 (m, 2H), 7.27 (d, 1H, *J* = 8.7 Hz), 7.11 (d, 1H, *J* = 6.9 Hz), 6.74 (d, 1H, *J* = 1.8 Hz), 6.65-6.52 (m, 6H), 6.45 (d, 1H, 2.1Hz), 3.87 (br, 2H), 3.55 (br, 2H), 3.41 (q, 4H, *J* = 7.2 Hz), 3.29 (br, 4H), 1.2 (t, 6H, *J* = 7.2 Hz); ^{13}C NMR (300 MHz, $CDCl_3$): δ 169.95, 165.44, 159.40, 157.36, 152.80, 152.70, 152.54, 151.95, 145.81, 134.80, 130.10, 129.59, 129.24, 128.91, 127.30, 125.10, 124.26, 115.21, 112.87, 112.26, 110.75, 109.97, 109.61, 107.75, 103.16, 102.39, 96.88, 48.47, 48.00, 46.94, 44.99, 42.14, 12.42.

Probe 1: Compound of **7** was dissolved in DMF to make a 5 μM solution, than excessive K_2CO_3 was added and stirred for 1 h. The conversion was monitored by fluorescence shift and UV absorption change. When the fluorescence and UV spectra stayed the same for a while, the solid K_2CO_3 was filtered out to give a 5 μM probe solution in DMF.

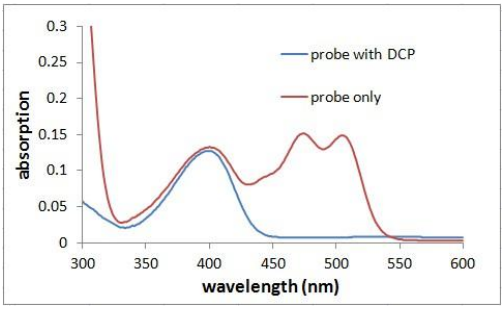
Reference

(1) Inouye, M.; Tsuchiya, K.; Kitao, T. *Angew. Chem., Int. Ed.* **1992**, *31*, 204.

2. **Figure S1. The fluorescence and UV comparison of compound 7 and probe 1 at 5 μM respectively.**

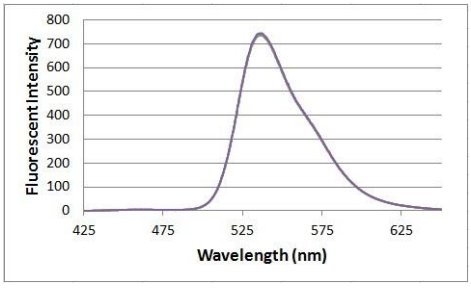


3. **Figure S2. UV absorption change when probe was treated with DCP.**



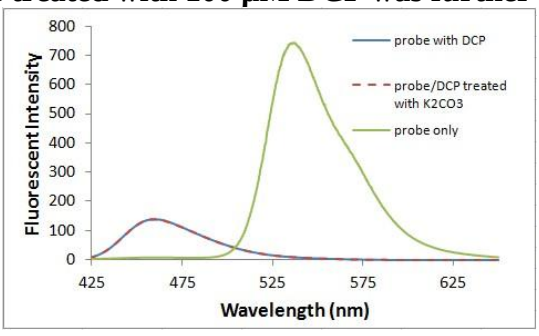
The UV absorption of 5 μM probe and 5 μM probe treated with 100 μM DCP.

4. **Figure S3. Stability investigation**

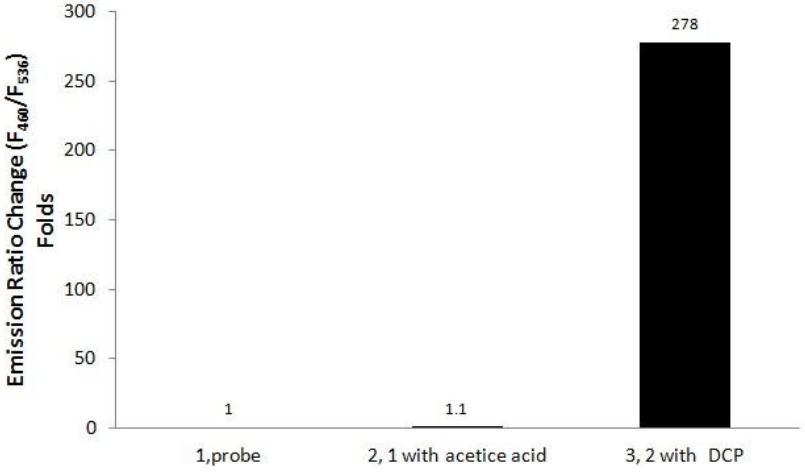


The fluorescence of 5 μM probe in DMF was recorded in 3 hours, and no observable change can be seen.

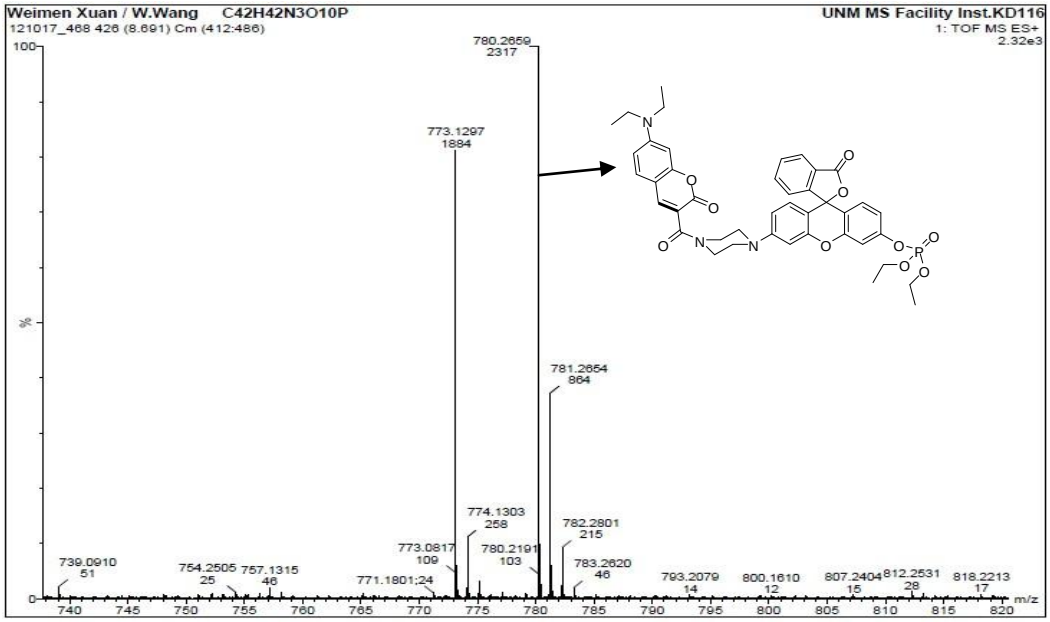
5. **Figure S4. 5 μM probe treated with 100 μM DCP was further added K_2CO_3.**



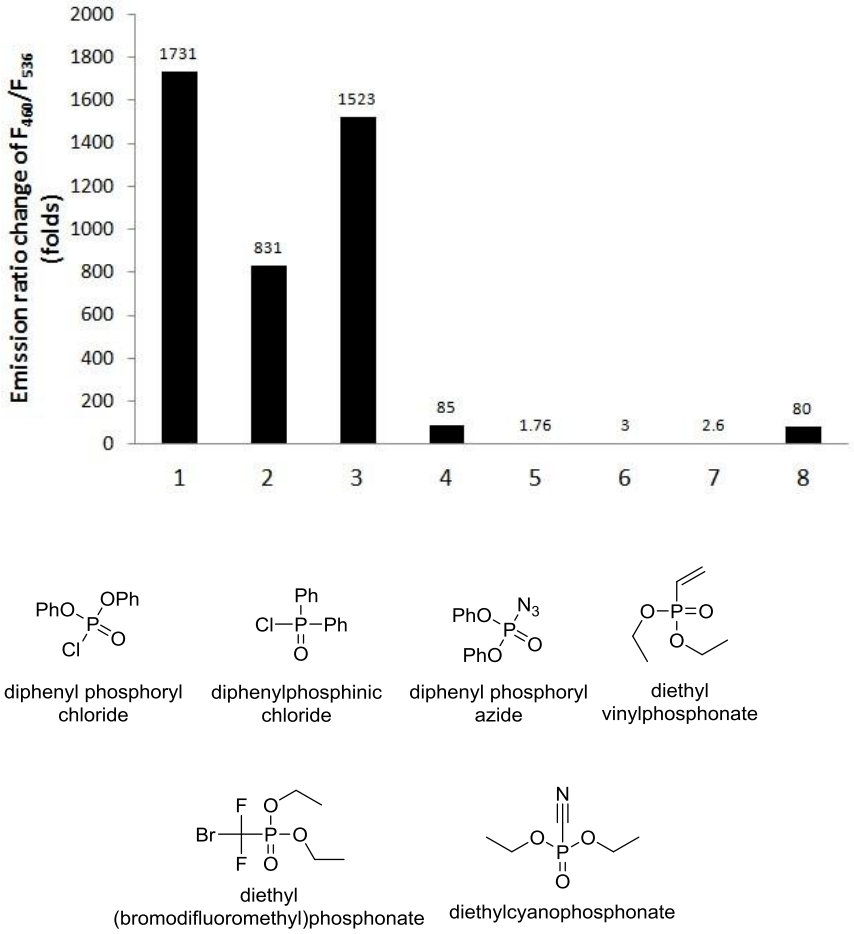
6. **Figure S5. DCP induced the fluorescence change in the presence of acetic acid (40 equiv.) with excessive K_2CO_3.**



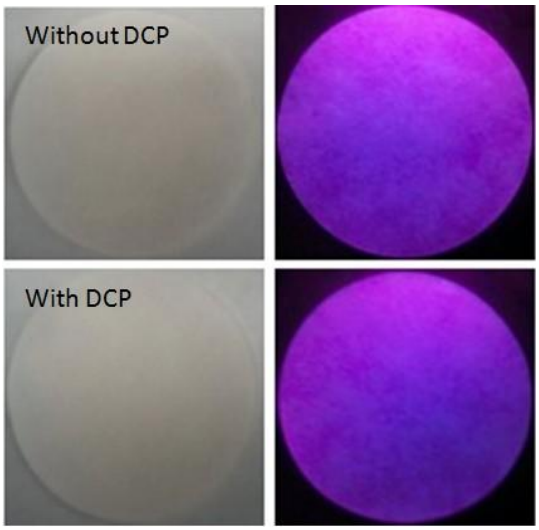
7. **Figure S6. The confirmation of the proposed detection product by Mass spectra.**



8. **Figure S7.** 1) DCP, 100 μM; 2) diphenyl phosphoryl chloride, 100 μM; 3) Diphenylphosphinic chloride, 100 μM; 4) diphenyl phosphoryl azide, 100 μM; 5) diethyl vinylphosphonate, 200 μM; 6) diethyl(bromodifluoromethyl)phosphonate, 200 μM; 7) phosphoric acid, 200 μM; 8) DCNP, 100 μM. All detection data were obtained in DMF in the presence of excessive K_2CO_3 in 5 min.



9. **Figure S8.** Control experiment of DCP vapor detection with probe-loaded filter paper.



10. Original ^{1}H and ^{13}C NMR spectra.