Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

A fluorescent probe bearing two reactive groups discriminates between fluoride-containing G series and sulfur-containing V series nerve agents

Wu Weihui*, Sui Shaohui, Li jian, Zong Liang, Li Dan, Xiao Yanhua, Wang Lianyuan, Zhu Haiyan, Shen Yonglin, Jiang Zhigang*

General methods

Most of the chemicals and organic solvents are A.R. grade. The purities of nerve agent GB and VX are greater than 95%.

Column chromatography was performed on silica gel (300-400 mesh).

Proton nuclear magnetic resonance (1H NMR) and carbon nuclear magnetic resonance (13C NMR) were recorded on a JEOL JNM-ECS 400 MHz spectrometer.

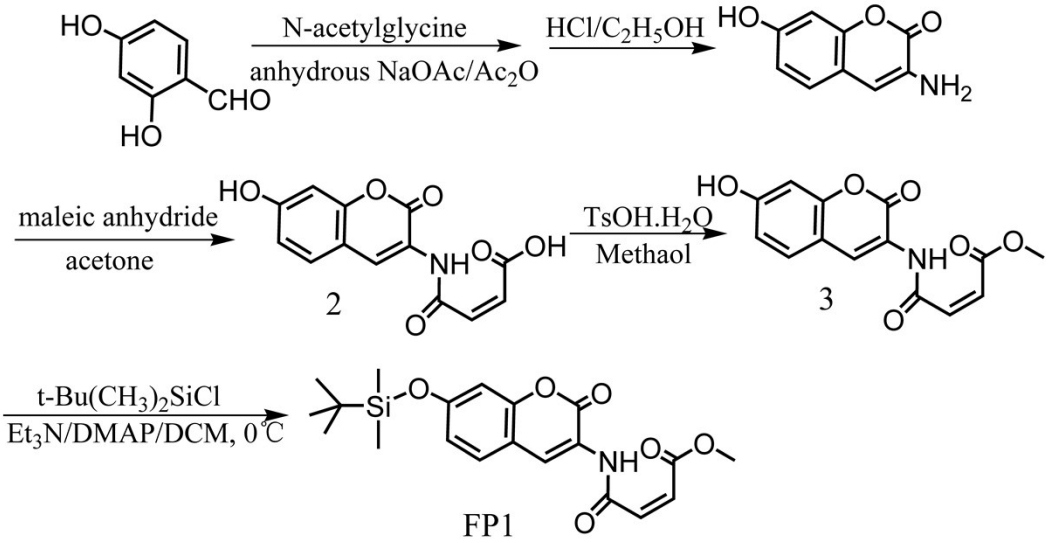
Electrospray ionization mass spectra (ESI-MS) were recorded on an Angilent 6460 mass spectrometer.

The fluorescent spectra measurements were investigated by Hitachi F-4600 fluorescence at RT using a 1-cm pathlength quartz cell.

GB and VX alcoholysates were prepared by adding 10 mM GB and VX to 5% NaOH/EtOH solutions and then stirring for 10 and 30 min respectively.

For the fluorescence measurements for thiols and VX alcoholysate, the excitation wavelength was 330 nm with excitation and emission slit widths of 5 nm, respectively. And for the fluorescence measurements for F^- and GB alcoholysate with exogenous thiols, the excitation wavelength was 376 nm with excitation and emission slit widths of 5 nm, respectively.

Synthesis procedure



Scheme S1 The synthetic routes of fluorescent probe FP1.

Preparation of 3-amino-7-hydroxy-coumarin.

2,4-dihydroxy benzaldehyde (2.76 g, 20 mmol), N-acetylglycine (2.34 g, 20 mmol), 60 mmol anhydrous sodium acetate (4.92 g, 60 mmol) and acetic anhydride (100 mL) were added to a three-necked round-bottomed flask and refluxed under N_2 atmosphere

for 8 h. After cooling, the reaction mixture was poured into ice to give a yellow precipitate. After filtration and washing with water for three time, the solid was refluxed in a solution of concentration HCl and ethanol (2:1) for 2 h. After cooling, the resultant solution was poured into ice (100 mL), then the solution was adjusted to pH 5~6 with NaOH aqueous solution. The solution was concentrated to 30 mL under vacuo and placed in a refrigerator for 12 hrs and the crude product was collected and recrystallized from EtOH to give 3-amino-7-hydroxy-coumarin.

Preparation of 2.

3-amino-7-hydroxy-coumarin (0.142 g, 0.8 mmol) and maleic anhydride (0.094 g, 0.96 mmol) were resolved in 5 mL acetone and the mixture was stirred at room temperature overnight. An orange solid was formed during stirring. After filtration, the solid was washed by acetone to give product **2**.

Preparation of 3.

The compound **2** (0.196 g, 0.71 mmol) and $TsOH \cdot H_2O$ (0.010 g, 0.05 mmol) were added to 30 ml methanol and the mixture was refluxed for 12 h. After evaporation of the solvent, the residue was purified by silica gel chromatography (dichloromethane /ethyl acetate, 1:5) to give 3.

Preparation of FP1.

A mixture of compound 3 (0.145 g, 0.5 mmol), 4-dimethylamino pyridine (DMAP, 5 mg, 0.04 mmol) and triethylamine (Et_3N, 57.6 mg, 0.57 mmol) in dichloromethane (DCM, 5 mL) was stirred at 0 ◦C for 10 min under an argon atmosphere. Then tert-butyldimethylsilyl chloride (0.086 g, 0.57 mmol) in DCM (1 mL) was added dropwise to the mixture at same temperature. The mixture was stirred at room temperature overnight under the argon atmosphere. After the reaction was completed, the mixture was treated with a saturated $NaHCO_3$ solution (10 mL). The aqueous layer was extracted with DCM (10 mL × 3). Organic extracts were combined and washed with water and brine. The resultant solution was dried with Na_2SO_4 overnight. The solvent was further evaporated under vacuo. The obtained residue was purified by silica gel chromatography (petroleum ether/ethyl acetate, 5:1) to give FP1 as a yellow solid. Yield: 0.140 g (69.5%).

^1H NMR ($CDCl_3$, 400 MHz): δ 9.93 (s, 1H), 8.70-8.74 (d, J=14.2 Hz, 1H), 7.34-7.36 (d, J=9.2 Hz, 1 H), 6.78-6.80 (d, J=7.3 Hz, 2 H), 6.40-6.43 (d, J=12.8 Hz, 1H), 6.24-6.27 (d, J=12.4 Hz, 1H) , 3.84 (s, 3 H), 0.97 (s, 9H), 0.20 (s,6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ:166.23, 163.04, 158.73, 157.95, 151.62, 136.24, 128.80, 127.08, 125.50, 121.80, 118.26, 113.74, 107.54, 52.80, 25.65, 18.33, -4.33; ESI-MS ($C_{20}H_{26}NO_5Si^+$): calculated $(M+H)^+$: 404.2, found: 404.2.

FP1 probe shows no fluorescence with excitation wavelengths of 330 and 376 nm.

In our study, FP1 is non-fluorescence and stable molecule in detecting solutions of EtOH/HEPES (1:1). There is no clear fluorescence change with the detecting excitation wavelengths of 330 and 376 nm (Figure S1 and Figure S2).

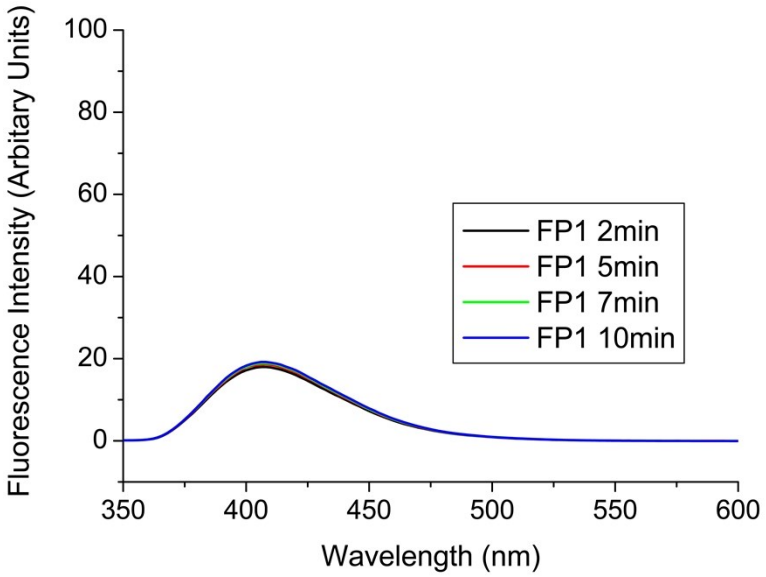


Figure S1 Fluorescence spectra of FP1 probe (20 μM) in EtOH/HEPES solutions with excitation wavelength of 330 nm within 10 mins.

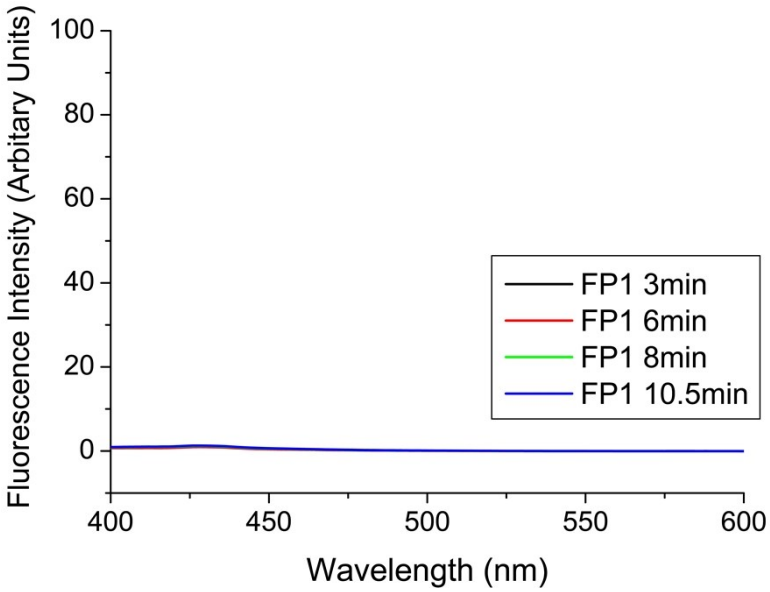


Figure S2 Fluorescence spectra of FP1 probe (20 μM) in EtOH/HEPES solutions with excitation wavelength of 376 nm within 10.5 mins.

FP1 probe reacting with F^- shows no fluorescence with excitation wavelengths of 330 and 376 nm.

In our study, the solution of FP1 reacting with F^- also shows non-fluorescence. There is no clear fluorescence change with the detecting excitation wavelengths of 330 and 376 nm (Figure S3 and Figure S4).

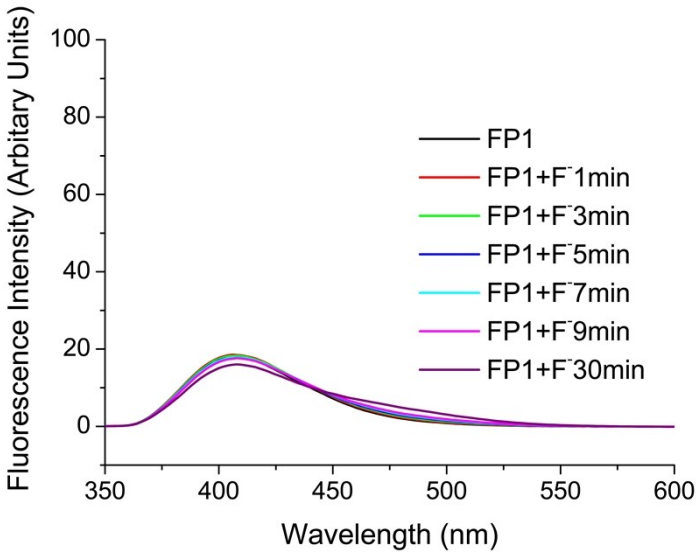


Figure S3 Fluorescence spectra of FP1 probe (20 μM) with F^- (200 μM) in EtOH/HEPES solutions with excitation wavelength of 330 nm.

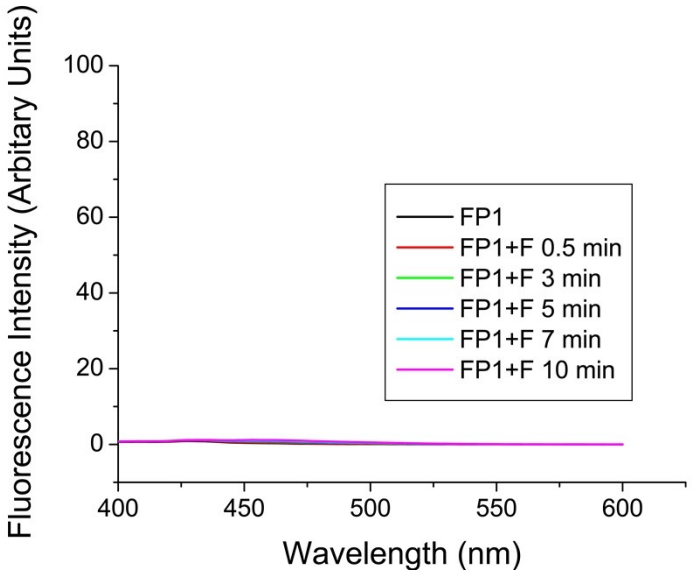


Figure S4 Fluorescence spectra of FP1 probe (20 μM) with F^- (200 μM) in EtOH/HEPES solutions with excitation wavelength of 376 nm.

FP1 probe reacting with thiol shows no clear fluorescence with excitation wavelength of 376 nm.

In our study, FP1 detecting thiol system measures fluorescence signals with the emission peak of 404 nm by using excitation wavelength of 330 nm (see the article). There is no clear fluorescence peak of emission with excitation wavelength of 376 nm (Figure S5).

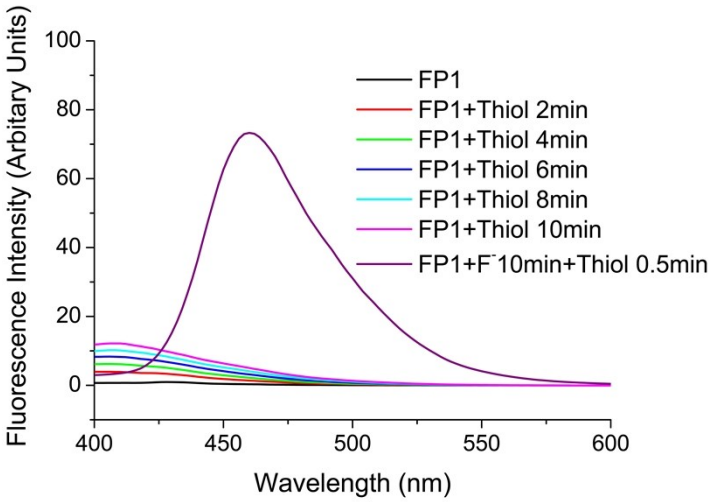


Figure S5 Fluorescence spectra of FP1 probe (20 μM) with the thiol (200 μM) in EtOH/HEPES solutions with excitation wavelength of 376 nm.

FP1 probe detecting F^- system with exogenous thiols shows a fluorescence peak of 460 nm with excitation wavelength of 330 nm.

In our study, FP1 detecting F^- system measures fluorescence spectra with the emission peak of 460 nm by using excitation wavelength of 376 nm (see the article). When the excitation wavelength is adopted at 330 nm, there is two fluorescence peaks of emission at 404 nm and 460 nm. The generation of the peak at 404nm is due to the probe directly reacting with thiols (Figure S6), while the peak at 460 nm is due to the probe reacting with thiol and F^- together. However, the clarity of the peak of 460 nm depends on the pre-incubating time of FP1 and F^- before adding exogenous thiols (Figure S6, S7) or the concentration of F^- (Figure S8).

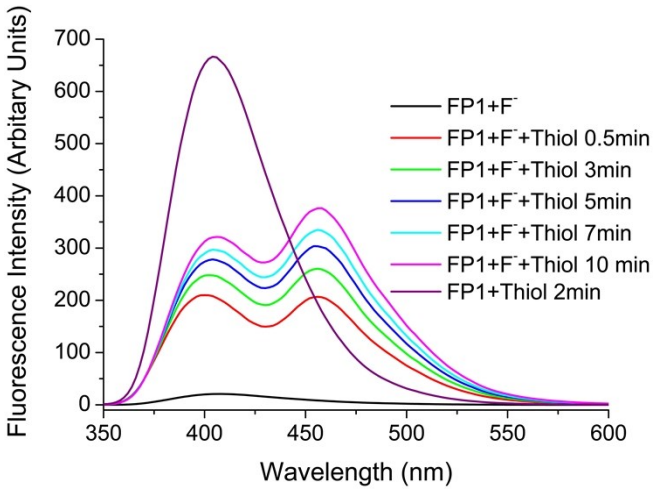


Figure S6 Fluorescence spectra of FP1 probe (20 μM) incubating with F^- (100 μM) in EtOH/HEPES solutions for 10 mins and then exogenous thiols (Ex 330nm).

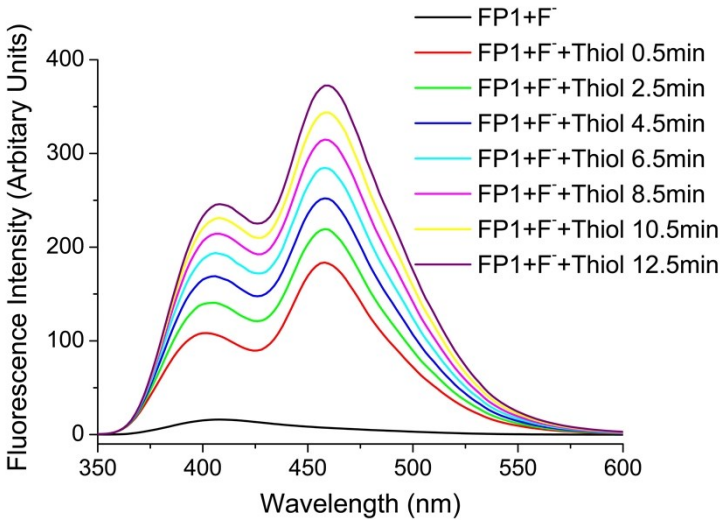


Figure S7 Fluorescence spectra of FP1 probe (20 μM) incubating with F^- (200 μM) in EtOH/HEPES solutions for 30 mins and then exogenous thiols (100 μM) (Ex 330nm).

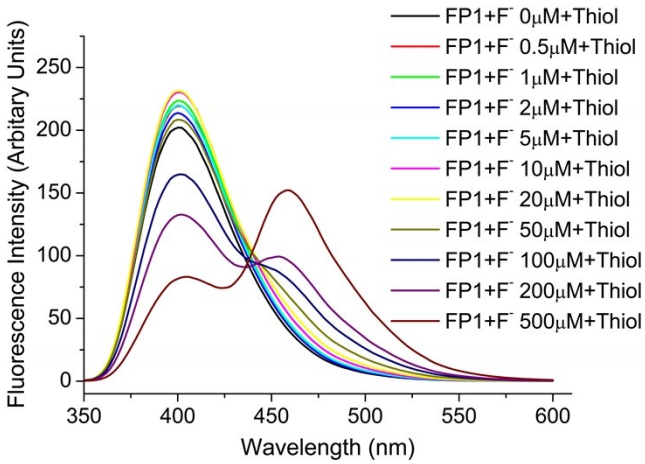


Figure S8 Fluorescence spectra of FP1 with F^- in EtOH-HEPES solutions with the exogenous thiols (Ex: 376 nm). FP1 (20 μM) was pre-incubated with F- at different concentrations (from top to bottom:500, 200, 100, 50, 20, 10, 5, 2, 1, 0.5, and 0 μM) for 10 minutes and then incubated for another 2mins with exogenous thiols. The data were obtained at room temperature.

MS spectra analyzed the samples of FP1 probe detecting thiol system and FP1 probe detecting F^- system with exogenous thiols.

In the sample of FP1 detecting thiols, a peak located at 510.10 was identified in positive ion mode (Figure S9a), which is consistent with thiol-maleimide conjugate adduct of FP1 (TM-FP1) (the theoretical molecular weight is 510.16, $C_{23}H_{31}NO_8SSi^+$), thus indicating that conjugate addition of FP1 with thiols accounts for fluorescence generation in the FP1 detecting thiol solutions. In contrast, in the sample of FP1 detecting F- solution with exogenous thiols, there is a peak locating at 394.00 (Figure S9c), which well matches with the desilysation and conjugate addition product of FP1 (DC-FP1) (the theoretical molecular weight is 394.07, $C_{17}H_{16}NO_8S^-$). So, DC-FP1 is responsible for fluorescence changes for the F- detecting system, while DS-FP1 (Figure S9b, the observed peak is 288.10, the theoretical molecular weight is 288.06, $C_{14}H_{10}NO_6^-$) from the sample of FP1 reacting with F^- without thiols doesn't generate any fluorescence.

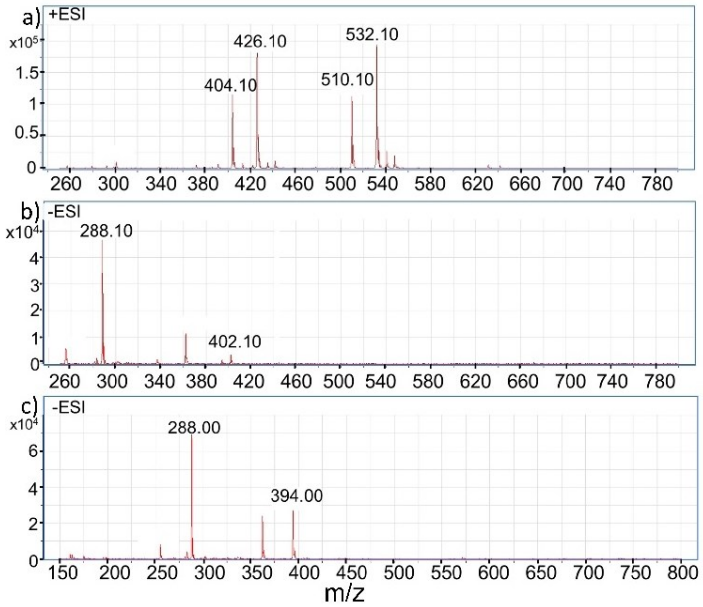


Figure S9 MS analysis of FP1 with Thiols (a), F^- (b) and F^- with the exogenous thiols (c) in EtOH-HEPES solutions.

FP1 probe detecting and discriminating GB and VX shows a good selectivity to prevent from the interference of other organophosphates.

The selectivity of FP1 was investigated by evaluating possible interference from other organophosphates. Four organophosphorus compounds, triethyl phosphate (TEP), tributyl phosphate (TBP), dimethyl methanephosphonate (DMMP) and diethyl chlorophosphite (DCP) were chosen to the selectivity experiment.

The four organophosphates, GB and VX were each subjected to 5% $NaOH/C_2H_5OH$ for alcoholysis. Each of the six resultant alcoholysis solutions was added to the FP1 detecting systems. As we expected, a clear peak of fluorescence emission at 404 nm (Ex: 330nm) was observed (Figure S10a) within a few seconds after alcoholysate of VX were added to FP1/EtOH-HEPES solutions at room temperature, while no obvious fluorescence emission (Ex: 330nm) appeared after the alcoholysates of GB, TEP, TBP, DMMP and DCP were added to FP1/EtOH-HEPES solutions separately.

Meanwhile, the sample of FP1 mixing with GB alcoholysate in EtOH-HEPES with exogenous thiols (Methyl mercaptoacetate) gave an obvious fluorescence peak at 460 nm (Ex:376 nm) (Figure S10b). In contrast, each sample of FP1 with VX, TEP, TBP, DMMP and DCP in EtOH-HEPES with the exogenous thiols didn't show a clear fluorescence emission peak (Ex:376 nm).

Moreover, FP1/EtOH-HEPES detecting solution with VX alcoholysate showed a violet color under the UV light (360nm) while the other FP1/EtOH-HEPES detecting solutions with GB, TEP, TBP, DMMP and DCP was colorless under the UV light (360nm) (Figure S10c). In the other hand, the sample of FP1 mixing with GB alcoholysate in EtOH-HEPES with exogenous thiols has a blue color under the UV light (360nm) and the samples of FP1 with VX, TEP, TBP, DMMP and DCP alcoholysates in EtOH-HEPES with the exogenous thiols show violet colors under the UV light (360nm) ((Figure S10d)).

The results indicated that FP1 shows a good selectivity to prevent from the interference of other organophosphates when it detects and discriminates VX and GB.

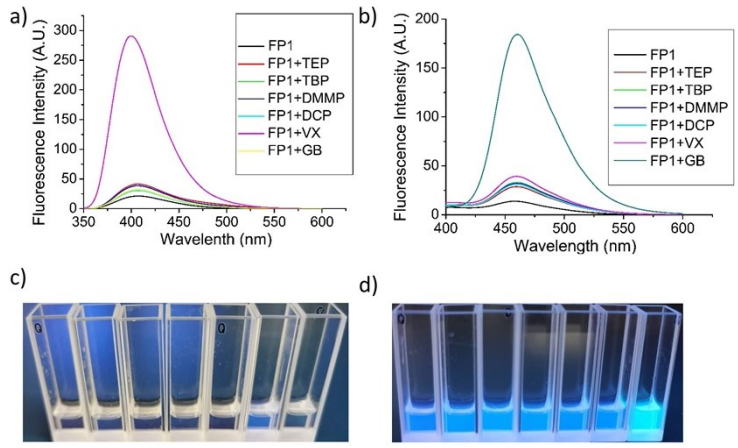


Figure S10 FP1 probe shows a good selectivity to prevent from the interference of other organophosphates. a) Fluorescence changes of FP1 (20 μM) with alcoholysates (200 μM) of VX, GB and four organophosphates (Ex:330 nm); b) Fluorescence changes of FP1 (20 μM) with alcoholysates (200 μM) of VX, GB and four organophosphates with the exogenous thiols (Ex:376 nm); c) Fluorescence color changes of FP1 detecting VX, GB and four organophosphates (from left to right: blank, TEP, TBP, DMMP, DCP, VX and GB alcoholysates) under UV light; d) Fluorescence color changes of FP1 detecting VX, GB and four organophosphates with the exogenous thiols (from left to right: blank, TEP, TBP, DMMP, DCP, VX and GB alcoholysates) under UV light.