Check for updates

A selective fluorescent probe for an organophosphorous nerve agent mimic *via* an oxime-to-isoxazole cascade reaction

Muhammad Shar Jhahan Khan, [ID] [a] K. S. Al-Namshah,[b] Muhammad Saleem[c] and Ya-Wen Wang[d]

A class of chemical warfare agents and their facile use in terrorist attacks underscores the need to develop accurate system to detect these chemicals. For this paper, we synthesized and developed a new highly selective and sensitive fluorescence probe based on fluoresceinyloxime for the detection of a nerve agent mimic. The synthesized probe underwent an abnormal cascade reaction from oxime to isoxazole in DMF/HEPES buffer (1/1, v/v, pH 7.0). The probe responded by quenching the fluorescence intensity in very short time (within seconds) and gave a naked eye detection by changing the colour of the solution from dark orange to very light green with a detection limit of 0.18 μM. The probe not only shows sensitivity and selectivity in the liquid phase, but can also be applied for the detection of nerve agents in the vapour phase with a clear colour change easily observed by the naked eye.

Introduction

Recently, there has been a significant international threat posed by Chemical Warfare Agents (CWAs), as defined by the Organization for the Prohibition of Chemical Weapons, emphasizing the critical need for accurate and reliable detection methods.[1] Among the various classes of CWAs, nerve agents are identified as the most toxic.[2–5] These organophosphorous compounds, which include G-agents (*e.g.*, Sarin, Soman) and the more potent V-agents, exert their lethal effects by inhibiting the acetylcholinesterase enzyme in the central nervous system, leading to a dangerous accumulation of acetylcholine.[6,7] A key distinction is made between non-persistent G-type agents and persistent V-type agents, which are more stable in the environment.[8] Due to their ease of production, extreme lethality, and properties of being colourless and odourless, detecting nerve agents is a high priority.[9] While various methods exist for detection, such as surface acoustic wave devices, enzymatic assays, and gas chromatography-mass spectrometry,[10–14] these techniques often suffer from limitations including lack of portability, operational complexity, and false positives.

Consequently, we believe fluorometric and colorimetric chemosensors as a promising alternative. These optical methods offer advantages like simplicity, potential for naked-eye detection, and the use of low-cost instrumentation.[15] Although current colorimetric systems like military test strips have limitations, they set a standard for new methods. Stimulated by these concepts, the work provides a comprehensive overview of the strategies for designing probes to detect nerve agent simulant. Fig. S1 (SI) shows different types of nerve agents.

Experimental section

Materials

Commercial compounds such as diethyl chlorophosphate (DCP), dimethyl methylphosphonate (DMMP), diethyl cholorothiophosphate, triethyl phosphate and phosohoric acid used as received without any purification. Compounds such as probe **1** and probe TsCl (An alternate of DCP) adduct **2** were prepared according to published procedures and gave analytical data in agreement to the literature.[15–17] Details of the synthesis of fluorescein monoaldehyde, probe **1** and compound **2** (SI Schemes S1–S3) and characterization of all compounds are given in SI (Fig. S3–S13).

Methods

Using a range of specialized instruments under standard conditions, the study characterized the compounds through several analytical techniques: fluorescence properties were measured with a Hitachi F-7000 spectrophotometer, absorption spectra with a Cary 5000 UV-vis-NIR spectrophotometer, and structural data were obtained *via* NMR (Bruker 400 MHz) and single-crystal X-ray diffraction (Bruker SMART CCD). Additional characterization included ESI mass spectrometry, melting point

ᵃDepartment of Chemistry, Ghazi University, Pakistan. E-mail: seemab35usa@gmail.com
ᵇDepartment of Chemistry, Imam Mohammad bnI Saud Islamic University, Saudi Arabia. E-mail: KSNamshah@imamu.edu.sa
ᶜDepartment of Chemistry, Ghazi University, Pakistan. E-mail: msaleem@gudgk.edu.pk
ᵈSchool of Chemistry, Southwest Jiaotong University, China. E-mail: ywwang@swjtu.edu.cn

determination, and the calculation of fluorescence quantum yields using fluorescein as a standard.

Results and discussions

Designing of probe and sensing mechanism

Chromo-reactant approaches have been used before for the identification of CWA mimics using xenthenes based on photo-induced electron transfer process (PET), enzyme based sensors, cyclodextrins, supermolecular hydrogel based probes *etc.*[18–21] Previously we reported iminocoumarin benzothiazol derivatives **1** and **2** for the detection of nerve agent mimics.[22] Based on our previous experience with using probes[22–25] for the detection of various ions, amino acids, metals *etc.* here we are reporting fluorescein oxime **1** probe as an alternative, which is easily prepared by treating hydroxylamine on fluorescein mono-aldehyde according to modified literature procedure. And then, there is a conversion reaction of oxime **1** into isooxazole **2**, the mechanism is shown in the SI Scheme S4. The reaction mechanism involves in the rapid transformation proceeded by the activation of the oxime with the TsCl. The probe **1** was in good agreement with the reported spectral values. A test reaction of probe with DCP or TsCl gave adduct **2** in 52.7%. The formation of the product is clearly indicated in the ^{1}H NMR spectra (Fig. S2).

To gain insight into this unusual reaction mechanism, we monitored ^{1}H NMR spectra in DMSO-d_6. However these were too complicated to monitor because of slow kinetics and decomposition of highly reactive TsCl or DCP. Thus, we attempted to analyze probe **1** and **2** separately, as shown in Fig. S2. To compare NMR spectra disclosed the highly downfield shift of the imine proton (H^d) during the conversion of probe **1** and **2** (8.8–9.6 ppm) along with disappearance of resonance assisted hydrogen bonding (RAHB) proton (H^a)[26–28] with the addition of DCP, indicating the expected intramolecular cyclization reaction of a hydroxyl oxime **1** to afford isoxazole **2**. The mass spectral analysis showed corroborative evidence for the formation of isoxazole 2: m/z 358.2 $[M + H]^+$.

UV-visible and fluorescence response

Initial studies on possible sensing of DCP utilized UV-visible spectroscopy and we recorded the optical absorption spectra of probe (10.0 μM) and in the presence of diethyl chlorophosphate (DCP, 10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0). For probe maximum absorption band appeared at 504 nm and after the addition of DCP, there was hypochromically decreased due to plausible transformation of an electron donating phenyl group to a relatively less electron donating isoxazole group and the colour of the probe changed to very light green from dark orang within couple of seconds, as shown in the Fig. 1a. The results clearly indicate that recognition of DCP can be realized using UV-visible spectroscopy. Notably, the short time response observed here is not very common. Moreover, absorption spectra of **2** (10.0 μM) was obtained (Fig. 1b). These results show that the UV-visible spectrum of compound **2** was the same as the products of probe with DCP.
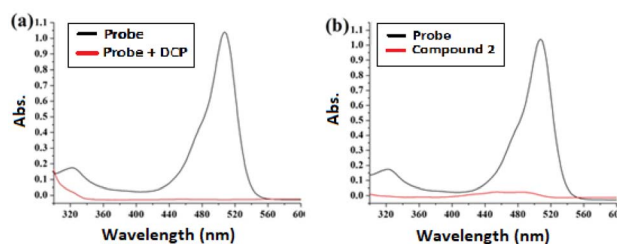

Fig. 1 UV spectra of (a) probe (10.0 μM) free and after addition of DCP (10.0 μM) in DMF/HEPES buffer (1/1, v/v, at pH 7.0) and (b) probe (10.0 μM) and compound 2 (10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0).

Next, to measure the use of oxime as fluorescence probe for the detection of nerve agent, fluorescence spectra were measured in DMF/HEPES buffer (0.10 M, v/v) at pH 7. The fluorescence spectrum of probe is shown in Fig. 2a. According to Fig. 2a, probe has maximum emission at 543 nm and after the addition of DCP, the fluorescence intensity of the probe quenched[29,30] remarkably and colour of the probe changed from dark orange to light green which could be observed with naked eye, as shown in the insets of Fig. 2a. And the probe became almost non fluorescent. This significant quenching upon DCP addition is due to its unique reactivity with the oxime-based probe, triggering an oxime-to-isoxazole conversion that introduces a strong electron-accepting group, leading to efficient photoinduced electron transfer (PET) and thus dramatic fluorescence quenching. Again, this very short response (within few seconds) makes probe an excellent detector of nerve agents. Moreover, the fluorescence quantum yield decreased from $\Phi = 0.10$ to $\Phi = 0.02$. The observed rapid optical changes are primarily due to a covalent reaction that triggers a Photoinduced Electron Transfer (PET) process, not FRET. This PET mechanism, supported by computational data, efficiently quenches fluorescence through a non-radiative pathway. The probe's structural transformation aligns with PET-based
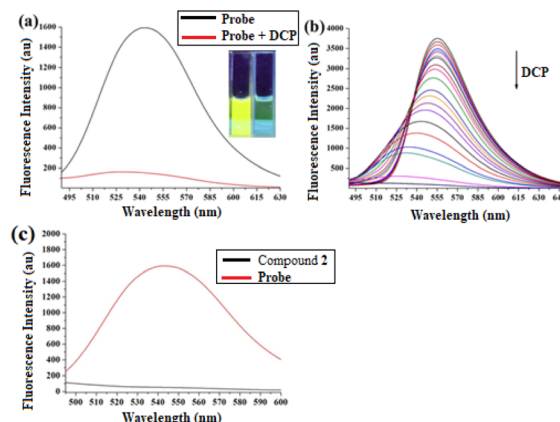

Fig. 2 Fluorescence spectra of (a) probe (10.0 μM) free and after addition of DCP (10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0) and (b) titration of probe (25.0 μM) and DCP (0.10 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0) (c) fluorescence spectra of probe (10.0 μM) and compound **2**(10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0). Inset shows image of probe free and after addition of DCP under UV light.
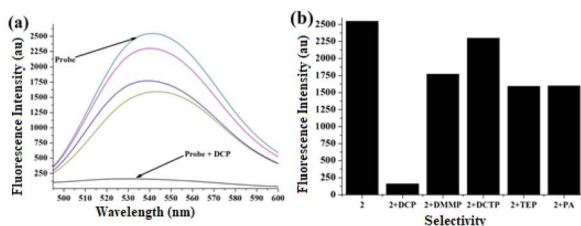
Fig. 3 Fluorescence selectivity comparison of (a and b) probe (10.0 μM) with DCP, DMMP, diethyl chlorothiophosphate, triethyl phosphate and phosphoric acid in DMF/HEPES buffer (1/1, v/v, pH 7.0).

quenching rather than distance-dependent energy transfer between separate chromophores.

After that, titration studies were carried out to further investigate the quenching phenomenon in detail. The fluorescence emission band at 543 nm of probe gradually decreased with the addition of DCP. As a result, 88% loss of fluorescence intensity of probe was observed, as shown in the Fig. 2b. These results suggested the high sensitivity of the probe for DCP and limit of detection of the probe was also determined from the titration studies. So, limit of detection (LOD) of the probe with DCP was measured as 0.18 μM, as shown in the Fig. S14. Detection sensitivity is limited to 0.18 μM due to the specific sensing mechanism employed, which relies on a covalent chemical reaction between the oxime-based probe and diethyl chlorophosphate (DCP) to form an isoxazole derivative. This design favours practical field application, emphasizing factors like rapid visual response and vapour detection. The achieved level remains functionally relevant for identifying real-world nerve agent threats. However, this value of LOD is lower than many of the reported work in this area of research. As for example Das, A. K. et al. reported[31] the LOD of their probe as 3.09 μM for the detection of DCNP (another nerve agent mimic). Similarly, the fluorescence response of adduct of probe 1 and DCP was also measured in the presence of 1, as shown in the Fig. 2c. From these studies, it was confirmed that the compound 2 has also the same fluorescence response as the probe with DCP and that the fluorescence of the probe is not affected by the presence of the analyte adduct.
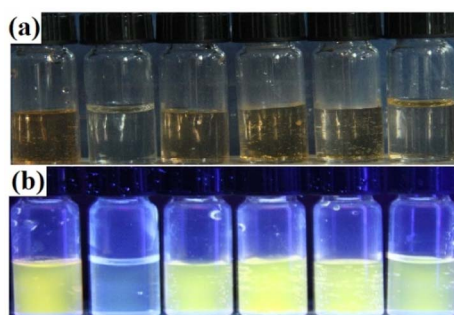

Fig. 4 Images of probe in the presence of organic phosphoric compounds (a) under normal light, while (b) is under UV light. From left to right: probe free, probe + DCP, probe + DMMP, probe + phosphoric acid, probe + triethyl phosphate and probe + diethyl chloro-thiophosphate, in DMF/HEPES buffer (1/1, v/v, pH 7.0).

Next, in order to investigate the selectivity of the probe, analogous experiments were performed with probe in the presence of other OPs, such as diethyl cholorophosphate, dimethyl methylphosphonate (DMMP), phosphoric acid, triethyl phosphate and diethyl chlorothiophosphate. A look at the fluorescence response of the probe after addition of several OPs clearly revealed the selectivity of the compounds. From these studies it was clear that the probe showed high selectivity for DCP as shown in Fig. 3a and b. Only upon addition of DCP was the fluorescence intensity of probes quenched significantly. Treatment of the probe with other OPs in solution only induced negligible fluorescence changes, but also gave no significant colour changes as shown in Fig. 4.

To quantitatively assess the probe's sensitivity, a titration experiment was performed by progressively adding DCP to a fixed concentration of the fluoresceinyl oxime probe in DMF/HEPES buffer. As depicted in Fig. S15, the fluorescence intensity at 543 nm decreased steadily with increasing DCP concentration, exhibiting a concentration-dependent quenching that reached an 88% reduction at saturation.

pH profile

To test chemical warfare agents under ambient and field conditions requires probes with sufficient stability, reactivity and tolerance of a wide range of pH and solvent conditions. Therefore, pH profile of probe was monitored in the presence and absence of DCP at a pH range 3–12. The fluorescence intensity of probe and DCP was greatly quenched at pH 7–10 relative to probe itself and maximum quenching was observed ate pH 8, as shown in Fig. S16. These results indicated that probe would be useful to detect the chemical warfare agent (nerve agents) in a real chemical warfare agent situation, such as in moisture and aerobic conditions.

HCl effect

Similarly, it is important to note that DCP is a compound which is hydrolyzed very fast and as a result, the presence of acid in the reaction mixture may influence the reaction of probe. To confirm the legitimacy of the reaction of probe with DCP, further UV and fluorescence investigations were carried out in the presence of HCl, as shown in Fig. S17 and S18 (SI). From these studies, it is proved that there is no significant influence of acid (HCl) on the reactivity of probe with DCP. Therefore, the UV and fluorescence responses of probe and DCP in the presence of HCl are in agreement with the responses in the absence of HCl.

Applications

Finally, to investigate the action of DCP vapours/fumes on probe, a flask was utilized in such a manner that fumes of DCP could be trapped inside the flask. The flask contained probe (10.0 μM) in DMF/HEPES buffer (1/1, v/v, pH 7.0). One small bottle containing DCP was placed in the flask, avoiding any contact of liquid DCP with the probe and the flask was sealed from air and kept at room temperature. As soon as the DCP

fumes came into contact with probe, the colour of test solution changes. Within 20 minutes the colour of probe changed from dark orange to light green (Fig. S19a and 19b).This is a clear indication of the utility of the probe **1** for use in practical sensing applications.[8,21,32,33]

Crystallographic and computational studies

The molecular structure of the reaction product was unequivocally confirmed by single-crystal X-ray diffraction (SC-XRD). Crystals of compound **2** were obtained by slow evaporation of an acetone solution. The ORTEP diagram (Fig. S20 SI) and associated crystallographic data (Table S1, SI) confirm that the crystal comprises the anticipated adduct formed between the original probe and DCP. The structural determination definitively verifies the core molecular framework and, most importantly, provides direct structural evidence for the key chemical transformation: the formation of a new N–O bond. The measured N–O bond distance of 1.45 Å is consistent with typical values for such single bonds, firmly corroborating the proposed mechanism in which the probe undergoes a specific covalent reaction with DCP to form this adduct.

The stark difference in fluorescence between probe **1** and its reaction product **2** was rationalized through density functional theory (DFT) calculations performed using the Gaussian 09 software package.[33] Studies revealed that the strong fluorescence of probe **1** stems from the extensive spatial overlap of its frontier molecular orbitals across the conjugated π-system, enabling efficient radiative decay As illustrated in Fig. S21. In contrast, its reaction product with DCP, compound **2**, undergoes a key structural change where a new N–O bond acts as a strong electron acceptor. This drastically separates the orbitals, localizing the HOMO on the xanthene donor and the LUMO on the N–O acceptor unit, which enables a photoinduced electron transfer process that provides a highly efficient non-radiative decay pathway, thereby completely quenching the fluorescence. Our new nerve agent detection probe was compared to existing optical sensors as shown in the Table S2. It shows strong performance with very low detection limits, rapid response, and works in both liquid and vapor.

Conclusions

In short, nerve agents are considered as the chemical weapons of mass destruction by the United Nations according to UN resolution 687. An accurate and reliable system for the detection of these compounds is a very hot topic since couple of decades in order to prevent terrorist attacks. So, we synthesized and designed an oxime based probe for the recognition of nerve agent mimic, which underwent an abnormal cascade reaction from oxime to isoxazole, which displayed highly selective and sensitive fluorescence response to a mimic of nerve agents by quenching the fluorescence intensity. Besides the clear and excellent fluorescence response of probe, it also gave naked eye detection of DCP by changing the colour of the probe from dark gold to light green. Moreover, probe has practical application as it can give response in the presence of nerve agent mimic's vapours.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the supplementary information (SI). Supplementary information: experimental procedures, synthesis of 1 and 2, experimental conditions for fluorescence sepectroscopy, UV-visible spectroscopy, NMR, MS analyses, crystalline structure and DFT details. See DOI: https://doi.org/10.1039/d5ra08944a.

Acknowledgements

Notes and references

1 D. R. Walt and D. R. Franz, *Anal. Chem.*, 2000, **72**, 738–746.
2 O. A. Sadik, W. H. Land and J. Wang, *Electroanalysis*, 2003, **15**, 1149–1159.
3 T. J. Dale and J. Rebek, *J. Am. Chem. Soc.*, 2006, **128**, 4500–4501.
4 Y. C. Cai, C. Lia and Q. H. Song, *J. Mater. Chem. C*, 2017, **5**, 7337–7343.
5 C. Chen, W. Zhang, Y. Ke, L. Jiang and X. Hu, *Anal. Methods*, 2024, **16**, 515–523.
6 S. M. Somani, *Chemical Warfare Agents*, Academic press, San Diego, 1992.
7 S. Royo, M. M. Raman, S. Felix, A. M. Costero, M. Parra and S. Gil, *Chem. Commun.*, 2007, 4839–4847.
8 H. H. Hill and S. J. Martin, *Pure Appl. Chem.*, 2002, **74**, 2281–2291.
9 L. M. Eubanks, T. J. Dickerson and K. D. Janda, *Chem. Soc. Rev.*, 2007, **36**, 458–470.
10 N. J. Ngwainbi, H. P. Foley, S. S. Kuan and G. G. Guilbault, *J. Am. Chem. Soc.*, 1986, **108**, 5444–5447.
11 M. Wheelis, *Pure Appl. Chem.*, 2002, **74**, 2247–2251.
12 Y. Zhou, B. Yu, E. Shiu and K. Levon, *Anal. Chem.*, 2004, **76**, 2689–2693.
13 H. Sohn, S. Letant, M. J. Sailor and W. C. Trongler, *J. Am. Chem. Soc.*, 2000, **122**, 5399–5400.
14 W. E. Steiner, S. J. Klopsch, W. A. English, B. H. Clowers and H. H. Hill, *J. Am. Chem. Soc.*, 2005, **77**, 4792–4799.
15 Z.-H. Fu, X. Han, Y. Shao, J. Fang, Z.-H. Zhang, Y.-W. Wang and Y. Peng, *Anal. Chem.*, 2017, **89**, 1937–1944.
16 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.
17 H. Lee and H. –J. Kim, *Trrtahedron*, 2014, **70**, 2966–2970.
18 A. B. Bon, A. M. Costero, S. Gil, F. Sancenon and R. M. Manez, *Chem. Commun.*, 2014, **50**, 13289–13291.

19 S. Royo, M. R. Martinez, F. Sancenon, A. M. Costero, M. Parra and S. Gil, *Chem. Commun.*, 2007, 4839–4847.

20 S. Royo, A. Costero, M. Parra, S. Gil, R. M. Manez and F. Sancenon, *Chem. – Eur. J.*, 2011, **17**, 6931–6934.

21 R. Gotor, S. Royo, A. M. Costero, M. Parra, S. Gil, R. M. Manez and F. Sancenon, *Tetrahedron*, 2012, **68**, 8612–8616.

22 M. S. J. Khan, Y. –W. Wang, M. O. Senge and Y. Peng, *J. Hazard. Mater.*, 2018, **342**, 10–19.

23 M. Dong, Y.-W. Wang and Y. Peng, *Org. Lett.*, 2010, **12**, 5310–5313.

24 Y. Peng, A.-J. Zhang, M. Dong and Y.-W. Wang, *Chem. Commun.*, 2011, **47**, 4505–4507.

25 Z.-H. Fu, X. Han, Y. Shao, J. Fang, Z.-H. Zhang, Y.-W. Wang and Y. Peng, *Anal. Chem.*, 2017, **89**, 1937–1944.

26 P. Gilli, V. Bertolasi, V. Ferretti and J. Gilli, *J. Am. Chem. Soc.*, 2000, **122**, 10405–10407.

27 H. –J. Kim, W. Kim, A. J. Lough, B. M. Kim and J. Chin, *J. Am. Chem. Soc.*, 2005, **127**, 16776–16777.

28 H. –J. Kim, H. Kim, G. Alhakimi, E. J. Jeong, N. Thavarajah, L. Studnicki, A. Koprianiuk, A. J. Lough, J. Suh and J. Chin, *J. Am. Chem. Soc.*, 2005, **127**, 16370–16371.

29 S. W. zhang and T. M. Swager, *J. Am. Chem. Soc.*, 2003, **125**, 3420–3421.

30 H. Zhang, Y. Xie, P. Wang, G. Chen, R. Liu, Y. –W. Lam, Y. Hu, Q. Zhu and H. Sun, *Talanta*, 2015, **135**, 149–154.

31 A. K. Das, S. Goswami, C. K. Quah and H. –K. Fun, *RSC Adv.*, 2016, **6**, 18711–18717.

32 M. R. Sambrook, J. R. Hiscock, A. Cook, A. C. Green, I. Holden, J. C. Vincent and P. A. Gale, *Chem. Commun.*, 2012, **48**, 5605–5607.

33 (*a*) R. Zhu, J. M. Azzarelli and T. M. Swager, *Angew. Chem., Int. Ed.*, 2016, **55**, 9662–9666; (*b*) M. J. Frisch, *Gaussian, Inc.*, Wallingford CT, 2009.