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Fluorescent Discrimination between Traces of Chemical Warfare Agents and Their Mimics

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Supporting Information

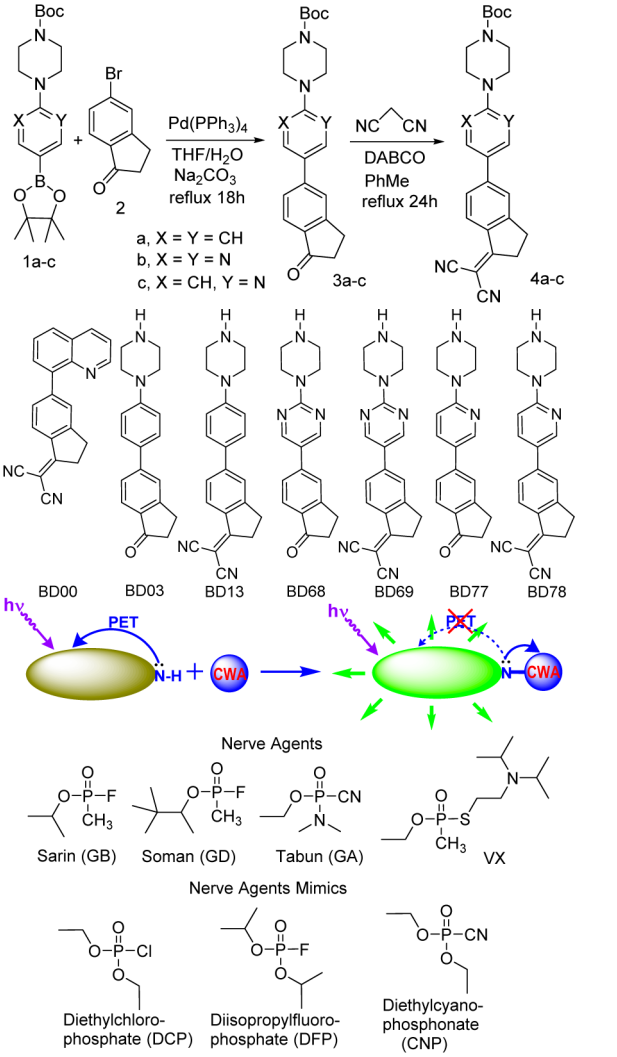
ABSTRACT: An array of fluorogenic probes is able to discriminate between nerve agents, sarin, soman, tabun, VX and their mimics, in water or organic solvent, by qualitative fluorescence patterns and quantitative multivariate analysis, thus making the system suitable for the in-the-field detection of traces of chemical warfare agents as well as to differentiate between the real nerve agents and other related compounds.

Nerve agents are highly toxic volatile liquids that irreversibly block the enzyme acetylcolinesterase in the neuronal synapsis, thus disrupting nerve impulse transmission and causing death through the paralysis of respiratory muscles.[1] They are used as chemical warfare agents (CWA) for dirty war in undeveloped countries, causing hundreds of victims, although their use as chemical weapons is prohibited.[2] Their quick detection can be achieved by hand-held instruments that are costly and prone to false positives[3] so the availability of safe and easy to use portable devices is most sought-after. More importantly, the investigation of chemical weapons allegations is a very slow process that implies unequivocal detection of CWA residuals in water and organic samples,[4] with the risk of long delays in the environment of worrying war scenarios.[5] Colorimetric[6] or fluorimetric[7] reactive dyes in solution or as arrays,[8] as well as supported in nanomaterials,[9] have been used for fast detection of CWA as good alternatives to classic methods, but most of these methods are implemented for nerve agents mimics, and so there is no clear proof that they will work for real CWA.[10] To complement the existing methodologies, we have developed a series of new highly solvatochromic fluorescent indicators for phosphorylating reagents capable of developing large differences in fluorescence. In this paper, we report our findings upon the selective fluorescent discrimination of real nerve agents from their mimics.

We have previously prepared some charge-transfer fluorogenic probes, bearing conjugated donor and acceptor groups in their structure, that were useful for the detection of significant analytes.[11] For our current purpose we have designed new fluorescent probes (Scheme 1).

In this case, they have a secondary donor group that was not involved in the charge-transfer process. Thus, the Suzuki

Scheme 1. Synthesis of Fluorescent Probes and Their Action



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reaction of aryl boronates **1a−c** and 5-bromoindanone **2** catalyzed by $Pd(PPh_3)_4$ in tetrahydrofuran/water in the presence of Na_2CO_3 gave arylindanones **3a−c** in 85−95% yields. Knoevenagel reaction of **3a−c** and malononitrile in the presence of DABCO in toluene at reflux for 24 h gave arylindanes **4a−c** in 55−68% yields. *N*-Boc deprotection with trifluoroacetic acid for 15 min from **3a−c** and **4a−c** gave the unprotected amine derivatives BD03, BD13, BD68, BD69, BD77 and BD78, in which the initial fluorescence of **3a−c** and **4a−c** is quenched in some extension by a photoinduced electron transfer from the free amine group. Subsequent acylation or phosphorylation of the amine group should therefore increase fluorescence of these compounds, thus making these compounds suitable for phosphorylating agents detection (Scheme 1). Fluorescence of these compounds can be also affected by protic acids, therefore we added to the series a fluorogenic dye, BD00,[12] which is not fluorescent but develops a blue fluorescence in the presence of common protic acids. In this way, false positives are prevented. We next tested 10^{-4} M solutions of the seven fluorescent probes in dimethylsulfoxide (DMSO) or acetonitrile (MeCN) with 1 equiv of 5×10^{-3} M solutions of nerve agent simulants (DCP, DFP, CNP) (Scheme 1) and phosgene[13] (Cl_2CO) in MeCN or water and recorded all changes that the fluorescent probes underwent with every analyte under a common TLC-UV light, λ = 366 nm, by qualitative (photographs) and quantitative measurements such as initial and final λ_{max}^{abs} and λ_{max}^{fluo}, variations in the relative intensity of fluorescence and kinetics of processes. The qualitative measurements gave clear and distinct fingerprints of every nerve agent mimic used for testing the probes, undoubtedly discriminating between them. The quantitative measurements were subjected to hierarchical cluster analysis (HCA).[8] HCA dendrogram obtained from fluorescent measures showed a clear clustering for all the nerve agent simulants, blank and phosgene, giving a good separation of every analyte (Figure S65b). Absorbance or mixed data from absorbance and fluorescence afforded a poor separation between some analytes (Figures S64 and S65a), therefore establishing that discrimination between analytes is best obtained by fluorescence measurements. Likewise, principal components analysis (PCA)[14] of the same data afforded good discrimination between each one of the CWA mimics as well as phosgene (Figure S66), therefore probing that the array of fluorescent dyes is able to discriminate between closely related phosphorylating or acylating reagents by both their fingerprints, HCA or PCA. The next step was testing the system with real nerve agents, but because of the extreme toxicity we performed the tests at the laboratories of the FOI CBRN Defense and Security (Umeå, Sweden), where handling of nerve agents was performed under appropriate conditions. Again, the seven different fluorescent probes were mixed with a series of nerve agents, Soman, Sarin, Tabun, and VX and chemically similar substances diethylchlorophosphate (DCP) and diethylcyanophosphonate (CNP), in the same conditions used for CWA mimics. The acquired samples of mixtures were then subjected to light (300−500 nm) in which they fluoresced with different colors. Light intensities were registered with a spectrofluorometer and photographs were taken for a chart of visible colors of all the test samples. The probes and CWA were solved in two different solvents, DMSO and MeCN for the probes, and MeCN and water for the CWA/CWA-simulants. The probes were also tested without CWA or simulant. The acquired mixtures were named as in the following example: Sarin solved in water mixed with probe DM13 solved in DMSO was called GB_W13D. For the mixtures with only probes the name begins with NaN. To photograph the samples they were placed under a 366 nm UV-lamp in a dark room. A color reference sheet illuminated with white light was placed nearby (Figure S89). Copies of the RAW-files were edited, all in the same way (batch process), before being converted to JPG for extraction of the colors as RGB-values. Both the colors from the edited images and from the original images were analyzed. As an example, a photograph of Soman samples is seen in Figure 1. f1

Figure 1. Samples contained Soman in MeCN mixed with each of the seven probes in DMSO. From left to right the samples contained probes BD00 to BD78.

Since there were three images of every set of seven samples, the mean values in R, G, and B had to be computed. A table of these colors in the form of colored squares was then created as seen in Figure 2. Looking at the tables of observed colors it was f2

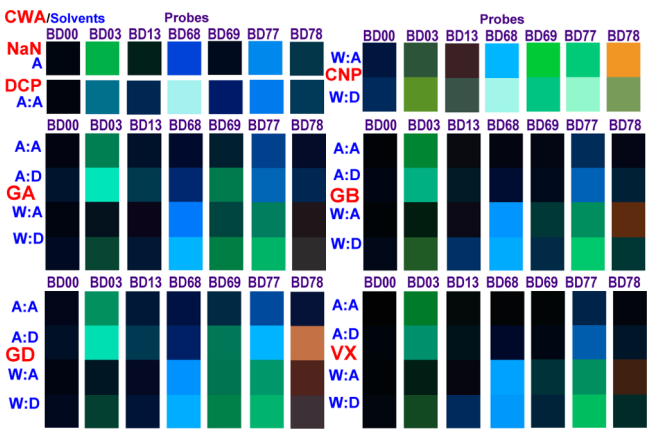

Figure 2. Observable colors in 366 nm excitation light. CWA: Sarin (GB), Soman (GD), Tabun (GA), VX (VX). CWA-simulants: CNP and DCP. CWA/simulants solvent: water (W) and acetonitrile (A). Probes: BD00, BD03, BD13, BD68, BD69, BD77, BD78. Probe solvents: dimethylsulfoxide (D) and acetonitrile (A).

clear that several probes could be used to guarantee the absence of Sarin, Soman, Tabun, and VX. For some choices of solvents there was a possibility to make the distinction between Soman and the other CWA. If a sample with probe BD69 in acetonitrile fluoresced very weakly (as in probe BD69 in acetonitrile, known as NaN A in the table of observed colors), then the risk of there being Sarin, Soman, Tabun, or VX in the samples is low since the corresponding CWA samples, with nerve agent in water and probe in acetonitrile, all fluoresced green. Probe BD78 acts in a similar way, but here the CWA-samples fluoresced in orange, while for probe BD03 it is the other way around. The NaN A sample with probe BD03 fluoresced in bright green, while there is barely any fluorescence from the corresponding CWA samples. Probe BD77 also gave valuable information but in a different way. For this probe both the probe and the CWA samples fluoresced clearly, but the

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probe samples did so in blue, while the CWA samples all fluoresced in green. It was probe BD78 that indicated that there was a possibility to distinguish Soman from the rest of the CWA. It is visible in the Figure 2 of observed colors that the mixture of Soman in MeCN and probe in DMSO fluoresced in a clear orange color, while the rest of the CWA samples with the same solvents fluoresced with weak obscure blue color. A table with the colors from the unedited images can also be found in Figure S90. The colors from preliminary experiments with only simulants have been included in a similar table in Figure S92. For quantitative measurements we used a calibrated spectrofluorometer. In the analysis of the spectral data a multivariate data analysis with Simca[15] software was used. To analyze the data we used a couple of approaches. Some of the basic analysis was made just by looking at the plots of the spectroscopy data. We were able to see that some of the mixtures just gave fluctuations in the data, while other gave clear tops. We found that the probes BD03, BD68, and BD77 were the probes that gave the highest number of clear tops, while the other only gave a few clear tops. In Figure 3 six

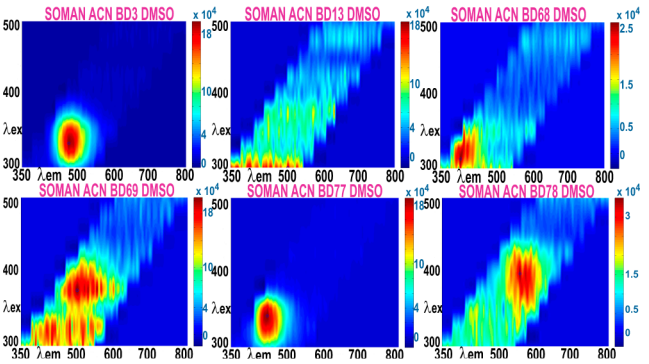

Figure 3. Plots of the spectrofluorometer data for CWA Soman (GD) solved in MeCN with the probes BD03 to BD78 in DMSO. In the plots we see how probe BD13 (top middle) causes fluctuations over the whole spectra while the probes BD03 and BD77 (top left and bottom middle) have very clear spectra. One of the few tops of probe BD78 (bottom right) can be seen and it is also this clear orange color that makes Soman stand out from the other CWA in the photographs.

spectra for Soman are plotted, showing all types of spectra that occurred, along with the distinct spectra for probe BD78 that distinguished Soman from the other probes in the spectra as well as in the photographs.

By use of multivariate data analysis we found that we were able to detect in which solvent the CWA were solved. We were also able to see a clear difference between the probes that gave clear tops in the spectrofluorometer data, and those that did not. In the analysis of the spectrofluorometer data the measured values were emission (λ_{em}) and excitation (λ_{ex}) wavelengths, and intensity of the maximum (fl_max) in each of the produced two-dimensional spectra. We also calculated the area in the spectra with intensities of 50% and 75% or more of the maximum (area50 and area75, respectively). We were able to see a clear difference between the simulants and the CWA when performing a multivariate analysis on agent-probe combinations (Figure 4). After our analysis we can conclude that there is a large probability that the probes are able to detect the most important CWA from their mimics. In the analysis only probes BD03, BD68, and BD77 were used to avoid the fluctuations, as variables we used area50, λ_{ex}, λ_{em}, and fl_max for

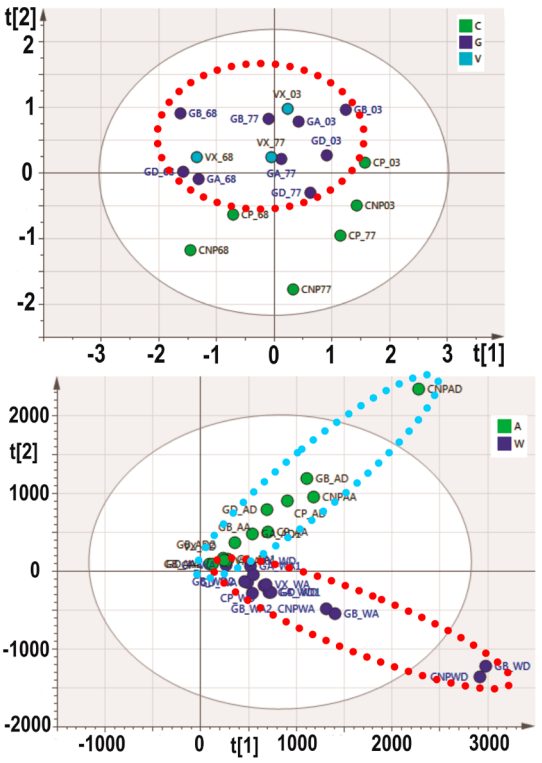

Figure 4. (Upper) A plot of the first two principal axes when running a multivariate analysis over the agent-probe combinations. In the plot we see a clear separation of the CWA (G and V) and the simulants (C). In this plot only the probes BD03, BD68, and BD77 were used, i.e., the probes that did not have a large tendency to fluctuate. (Down) A plot of the first two principal axes when running a multivariate analysis over the agent-solvent combinations. In the plot we can see a clear separation of the agents solved in water and those solved in acetonitrile.

each of the four solvent combinations. Before the PCA analysis was run all data were transformed logarithmically and grouped in blocks of $\lambda_{em}/\lambda_{ex}$, fl_max, and area50 before unit variance was run. From the load vectors for the analysis of the agent-probe combinations (Figure S72) we could see how the areas and florescence were the main parameters for the second component. In addition, the simulants generally had a bit higher fluorescence and for some a bit smaller areas, therefore they tended toward the lower values on the second component. When studying the combinations of agent and solvents against probes we were able to see a clear separation between the agents that were solved in water and those that were solved in acetonitrile. This can clearly be seen in Figure 4, the main reason behind this separation seems to be that the intensity of probe BD68 becomes higher for those agents solved in water (Figure S88).

In summary, we have synthesized a new series of fluorogenic probes that are able to discriminate between traces of CWA and their mimics, in water or organic solvent. Discrimination is achieved by means of the different fluorogenic response triggered by CWA or their mimics on the fluorogenic probes in different solvent combinations of CWA and probes.

The different response given by the series of fluorogenic probes is charted as a fingerprint of the fluorescent response of every CWA/probe/solvent combination under a common 366 nm UV light, thus permitting a fast visual differentiation between CWA and their mimics. More accurate discrimination

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is achieved by multivariate analysis by using quantitative measurements in fluorescence spectroscopy. In this way we have obtained a complete differentiation between CWA and their mimics, so the system is suitable for the accurate in-the-field detection of traces of CWA. We have seen that the response given by CWA mimics is very different to the response given by the real CWA, because of the slightly different chemical functionality of CWA and their mimics. Since most of the chromogenic and fluorogenic probes hitherto studied for the detection of CWA are based in the study of the response given by their mimics, there is no guarantee that previously known probes for CWA mimics will work with real CWA samples. Our work clearly shows that the response can be very different. In addition, the synthesis of the reported fluorogenic probes is simple and straightforward, therefore these fluorescent probes are suitable for the development of upcoming practical methodology.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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