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A selective and sensitive chromogenic and fluorogenic detection of a sulfur mustard simulant†

Vinod Kumar‡ and Eric V. Anslyn*

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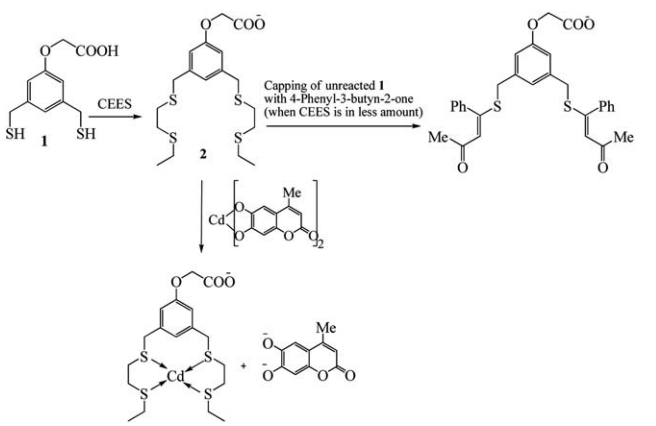
A simple and highly selective chromogenic and fluorogenic detection of sulfur mustard (SM) simulants is reported. Dithiol **1**, in the presence and absence of a mustard simulant behaves differently toward a squaraine dye (**SQ**), and thus provides a visual and spectroscopic signal for mustard gas. The sensor responds to the SM simulant, but not to the O-analog of mustard stimulant or nerve agent mimics and other electrophilic agents. The visual and fluorescent detection with less than 50 μM of SM simulant shows good sensitivity. The utility of the sensor was demonstrated by analysis of SM simulant on surfaces, in soil, and in the gas phase.

Introduction

Chemical warfare agents (CWAs) have been classified into two categories: nerve agents and vesicants. Sulfur mustard (SM), also known as Mustard gas or HD, is a strong vesicant, resulting in severe skin as well as eye blistering and lung lesions upon exposure, and in some cases may lead to death.[1] Mustard gas is also carcinogenic and mutagenic.[2] It is an alkylating agent of nucleobases in DNA.[3] The agent was used during World War I and II, and during the Iran–Iraq war in the 1980s.[4] In addition, American soldiers were exposed to Iraqi chemical agents when arms depots were destroyed during and after the 1991 Gulf War.[5] Apart from these incidents, there is always the threat of SM by terrorist groups, thus posing a concern for homeland security. Unfortunately, there are no effective antidotes or treatments for SM-induced injury.[6] Therefore, there is a need for the development of an "Ideal Detection" system for SM. Technologies that allow a rapid, selective, and sensitive optical detection of SM could be used for such analysis.

Over the past few years, many efforts have been made in the development of detection technologies against CWAs. A number of methods have been reported for the detection of nerve agents,[7] but very few for sulfur vesicants. So far, tandem mass spectrometry is the best detection technology for SM, but carries a correspondingly high price tag and lack of portability.[8] Chemically doped detection papers such as M8, M9 and DB-3 are used by the US military and are cheap, portable and easy to use, but are relatively insensitive, non-specific, and prone to false positives.[9] For on-site monitoring, many handheld chemical agent monitors are available for point detection of CWAs.[10] These detectors recognize the chemical agents on the basis of their weight, volatility, and presence of sulfur or phosphorus, but generally fail to give selective detection which lead to false positive alarms. Further, several new methodologies, such as molecularly imprinted polymers,[11] immunochemical,[12] quartz crystal microbalance,[13] and platinum(II) pincer complexes,[14] have been reported for the signaling of this chemical species.

Irrespective of the methods that exist, the preparation of a highly specific, facile, and cost-effective mustard gas sensor is still an important goal. To realize this, we took up a research program to develop a simple, selective and sensitive chromogenic and/or fluorogenic detection method for sulfur mustard with applicability in various matrixes. In this regard, we recently reported a fluorogenic sensor for mustard gas which allowed a selective detection at low millimolar levels.[15] The chemosensor



Scheme 1 Schematic presentation showing the detection of sulfur mustard simulant and the capping process.

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712, USA. E-mail: anslyn@austin.utexas.edu; Fax: +1-512-471-8696; Tel: +1-512-471-0068

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‡ Present Address: Process Technology Development Division, Defence R&D Establishment, Jhansi Road, Gwalior 474002, India, E-mail: vkpal77@yahoo.co.in, Fax: +91 751 2341148, Tel: +91-751-2390203.

was effective but required an intermediary step of capping the receptor before performing the measurement (Scheme 1). To improve upon this drawback associated with the previous method, we herein report an approach that eliminates the capping step. Hence, the purpose of this work is three fold: first, to develop a simpler chromogenic and fluorogenic sensor for mustard gas, second, to create a chemosensor that is selective over common interferents such as other electrophilic agents and even nerve agents, and third, to make a protocol that is applicable to common matrixes (solid, liquid, and gas).

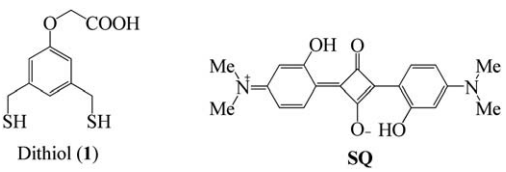
Result and discussion

Design criteria

It is well-known that chlorine atoms in mustard gas are reactive with good nucleophiles like thiols in basic medium,[16] and this property was exploited in our previous study.[15] With this knowledge, we envisioned the use of a dye which reacts reversibly with thiols resulting in a change of fluorescence as well as color. Squaraines are a particularly promising class of organic NIR dyes that exhibit unique photophysical properties, namely, a sharp and intense absorption band in the red to NIR region.[17] Squaraines are also known to react with nucleophiles.[18] In our approach, we have utilized the well-known chemistry of thiol–squaraine reactivity.[19] In absence of SM, a thiol, such as **1**, will react with **SQ** (Scheme 2) resulting in the bleaching of the dye. However, in the presence of analyte, the thiol reacts with SM resulting in the retention of the chromogenic and fluorogenic properties of **SQ** (Fig. 1). Furthermore, in this strategy, we sought out to perform the sensing protocols in basic methanolic solution in order to avoid interference from very reactive electrophiles such as acetyl chloride and nerve agents, which will rapidly hydrolyze in base.

The purpose of developing the reaction condition at 80 °C for SM simulant sensing protocol would be two fold: firstly, the dithiol reacts with **CEES** quickly (in one min.)[15] which is an important requirement of chemical sensor development of sulphur mustard. Secondly, interfering agents either decompose under these conditions or do not react with **1** *e.g.* CEEE.

2-Chloroethyl ethyl sulfide (**CEES**) is a simulant for sulfur mustard (Fig. 2). It mimics its physical and chemical properties, but with a relatively high LD_{50} (275 mg kg^{-1}),[20] and is an appropriate target analyte for studies within an academic setting. The LD_{50} values for SM are 2.4 mg kg^{-1} and 8.1–9.7 mg kg^{-1} for oral exposure in rats and mice, respectively. Percutaneously, these are found to be 3.4 mg kg^{-1} and 19.3 mg kg^{-1} in rats and mice respectively.[21]



Scheme 2 Schematic presentation of **1** and **SQ**.

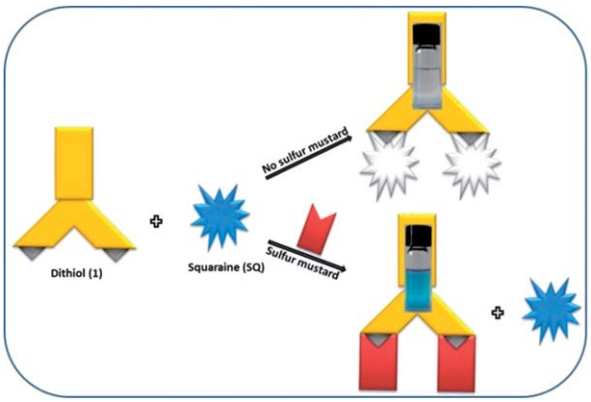


Fig. 1 Schematic illustration of chemical sensor for mustard gas.

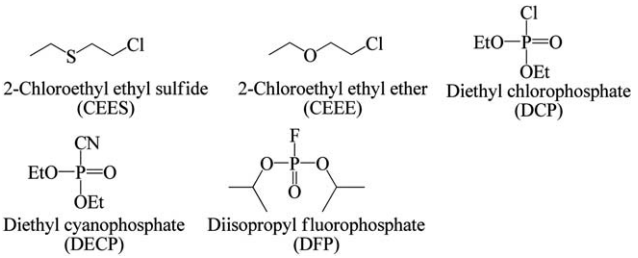


Fig. 2 Various chemical warfare agents mimics.

Reaction of dithiol with SQ

In validating the proposed hypothesis, the first aim was to demonstrate the chromogenic and fluorogenic responses of **SQ** with **1**. To investigate this, a solution of **SQ** in chloroform was prepared and then treated with a solution of **1** in methanol containing 3.0 equivalents of K_2CO_3, resulting in the bleaching of **SQ**. The electron-deficient central cyclobutene ring of squaraine adds to **1** which breaks the conjugation of the dye resulting in bleaching. To carry out the fluorescence study, a solution of **SQ** at 2.65 μM was titrated with **1** at 0.2 mM (Fig. 3). The addition of **1** to the solution of **SQ** gave a decrease in the intensity of fluorescence, which resulted in a nearly complete quenching of the dye. The binding isotherm (intensity *vs* [**1**], 652 nm) was fit with 1 : 1 binding equation[22] to afford the association constant (K_a) of 4.6×10^4.

Chromogenic and fluorogenic detection of mustard simulants

After determining the concentration of **1** that quenches the fluorescence properties of **SQ**, we went on to explore the detection of **CEES**. In this process, **1** in methanol containing 3.0 equivalents of K_2CO_3 was allowed to react with **CEES** (2.2 equiv.) at 80 °C for a minute to give podand-type compound **2** (Scheme 3). The formation of **2** was confirmed by mass spectrometry.[15] This solution was then mixed with **SQ**. The persistence of the blue color of **SQ** showed the presence of **CEES** (Fig. 4a). Hence, **1** had completely reacted with **CEES** and was no longer available to bleach **SQ**, thus the color persisted. In addition, a fluorescence titration was carried out for the quantitative determination of **CEES**. **1** (0.2 mM) was allowed to react

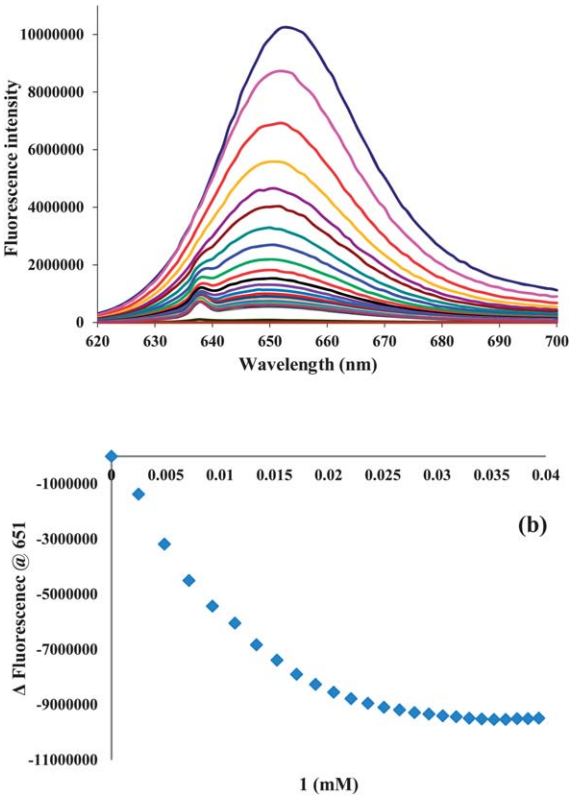
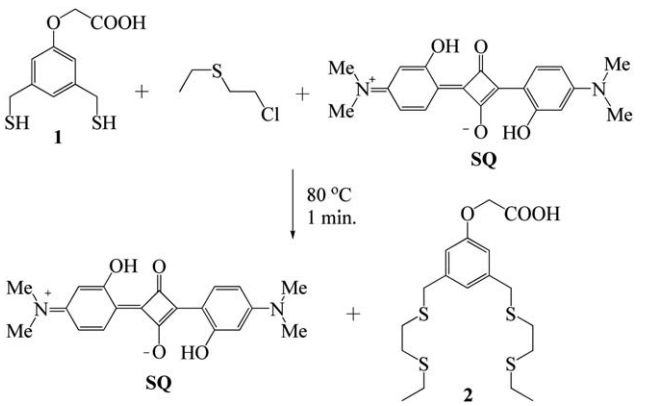


Fig. 3 Fluorescence intensity of **SQ** in $CHCl_3$ at 2.65 μM at 652 nm in the presence of increasing amounts of **1** (0.2 mM) in MeOH (Excitation wavelength at 638 nm). (b) Isotherm showing decrease in fluorescence intensity of **SQ** with the addition of **1**.



Scheme 3 Schematic presentation of the reaction between **1** and **CEES** in presence of **SQ**.

under above reaction conditions with **CEES** with varied concentrations from 25 μM to 250 μM and then each solution is mixed with **SQ** (2.65 μM). The fluorescence spectra of each solution (11 solutions), including that of solution which has no mustard (**1** only), was recorded. Fig. 5 shows intensity enhancement with an increase in the concentration of **CEES**, and becomes saturated with the addition of 200 μM of **CEES**. Thus, the magnitude of fluorescence intensity depends on the concentration of **CEES**. Moreover, using the calibration curve,

Fig. 4 (a) Colorimetric response of **SQ** in $CHCl_3$ at 14.0 μM, from left to right: **SQ** + **1** in MeOH (51 μM), **SQ** + [**1** (51 μM + **CEES** (112 μM))]. (b) Colorimetric response of **SQ** with subsequent addition of **CEES** (from 11–110 μM) from left to right.

concentration of two unknown samples of **CEES** (as prepared by the group members) was determined with less than 10% error.

Interference study

Interference of closely related analytes is always a major drawback in the development of a chemical sensor. This problem is

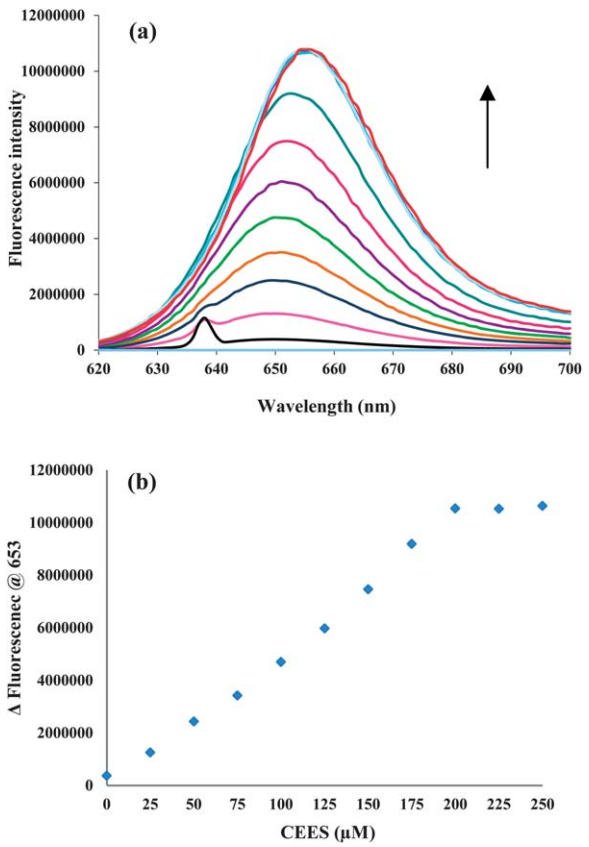


Fig. 5 Fluorescence intensity of **SQ** in $CHCl_3$ at 2.65 μM at 652 nm in the presence of increasing amounts of **1** (0.2 mM) in MeOH (excitation wavelength at 638 nm). (b) Isotherm showing decrease in fluorescence intensity of **SQ** with the addition of **1**.

inherent to previously developed sensors for CWAs, which are not sufficiently selective to many non-reactive and reactive simulants. In chemical warfare agent detection, interference from other electrophilic agents such acetyl chloride, 2-chloroethyl ethyl ether, and various nerve agents such as sarin, soman, and tabun, is a possible complication observed. To further demonstrate the specificity of this **SM** sensing protocol, we tested the interference of electrophilic agents and nerve agents. A naked-eye detection protocol was carried out with **SQ** and interferents such as 2-chloroethyl ethyl ether (CEEE), acetyl chloride, diethyl chlorophosphate (DCP), diethyl cyanophosphate (DECP), and diisopropyl fluorophosphate (DFP) following the identical analytical protocol. The 2.2 equivalents of these interfering agents were allowed to react with **1** (0.2 mM) in methanol containing 3.0 equivalents of K_2CO_3. This solution was then mixed with **SQ** (14.0 μM). Interestingly, we observed no response from these agents (Fig. 6). Because under these conditions, the interferents either do not react (as in case of CEEE) with **1** or they do not survive the reaction conditions (as in case of other than CEEE). Hence unreacted **1** bleaches **SQ**, while no response was found for the interferents. However, in some cases such as acetyl chloride, DFP and DCP, there was a slight formation of color but it disappeared by the addition of a small quantity of methanolic K_2CO_3. The color can be attributed to the protonation of the thiolate ions of **1** which occurs by the release of acid due to the reaction of the interferents and methanol. This makes **1** unreactive for **SQ**, and as a result, color persists. Addition of methanolic solution of K_2CO_3 further deprotonates **1**, and it regains nucleophilicity to bleach **SQ**.

Analytical applications

When deployed, sulfur mustard is sprayed using rockets, bombs or artillery shells. In spite of its high reactivity, SM exhibits enhanced environmental stability (at neutral pH).[23] Rapid detection of this environmentally persistent agent in various matrixes (solid, liquid and gas) is required in order to develop an early-warning system against chemical weapon attacks, and to help instigate countermeasures against the attack. To gain insight into the practical applications of our method, we sought to detect SM on surfaces, in soil, and in the gas phase. **CEES** was sprayed on a surface and absorbed by filter paper, and this paper was allowed to react with **1** at 80 °C for a minute. This solution was tested with **SQ** in a similar manner (*vide supra*). The persistent of color indicated the presence of **CEES**. To determine the presence of **CEES** in soil samples, soil was spiked using a solution of **CEES** (10 μL) in diethyl ether (2 mL). The solvent was evaporated to obtain dry soil spiked with **CEES**. This soil sample was treated with solution of **1** in a similar manner (*vide supra*). The persistence of blue color of the dye indicated the presence of the agent. Moreover, the soil sample without spiked **CEES** was also treated with **1**, which bleached the dye, showing the absence of **CEES** (Fig. 7a). Because the chemical agent can attack human beings in both liquid and gas phases, we sought to investigate the detection in the gas phase also. The chromogenic detection of **CEES** in the gas phase was achieved using a solution of **1** on a silica coated TLC plate. A solution of 200 μL of **1** (0.2 mM) in methanol containing 3.0 equivalents of K_2CO_3 was sprayed over the TLC plate, which was then allowed to dry. The **1** treated TLC plate was cut into two parts, one part was used for comparison, and another part was kept in gas generation chamber (Fig. S2, ESI†) by maintaining the surface temperature of the gas generation chamber at 80 °C. Because **CEES** was placed on the heated surface of the chamber, the vapor forms and reacts with **1** already present on the TLC plate. This TLC plate was removed and a drop of **SQ** was placed on the TLC plates. The TLC plate without the **CEES** shows no color while the color of **SQ** persists with **CEES** pretreated TLC, as shown in Fig. 7b.

Detection limit

Mustard gas is potentially deadly when a person is exposed to a large excess. However, the minimum quantity required to cause blisters on the skin is 0.2 mg. Our sensing protocol detects **SM** by both chromogenic and fluorogenic methods far below the level of toxicity that cause any health hazards. The detection limit of **CEES** was determined to be 50 μM and 10 μM by the visual (with good visibility) and fluorescence methods, respectively.

Fig. 6 Colorimetric response of **SQ** at 14.0 μM upon the addition of 2.2 equivalents of various agents in the solution of **1** (0.2 mM) in methanol containing 3.0 equivalents of K_2CO: from left to right (a) **CEES** (b) CEEE (c) DCP (d) DFP (e) DECP.

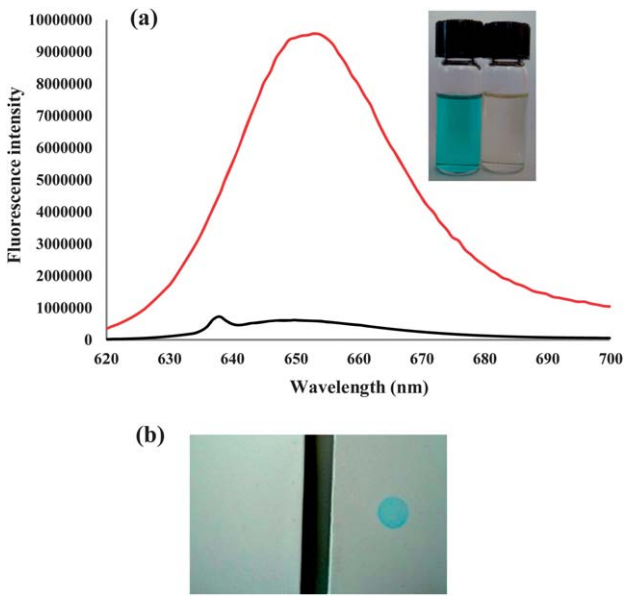
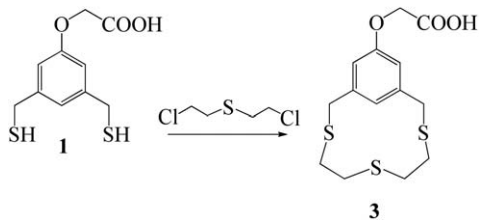


Fig. 7 (a) Colorimetric and fluorogenic responses of **SQ** with **CEES** spiked soil sample (left) and unspiked soil sample (right). (b) Detection of gaseous **CEES** with **SQ** dye absorbed on TLC plate exposed with **CEES** (right) and unexposed (left).



Scheme 4 Schematic presentation of proposed reaction between mustard gas and **1**.

The reason for using a dithiol over a monothiol

We are particularly interested in ultimately using the method with the real agent. This inspired us to develop the present protocol in such a way that it could be applicable in an actual scenario without much change in analytical parameters. Consequently, in this study, we chose to use a molecule with two thiols groups *i.e.* dithiol **1** rather than a monothiol. This is because of two reasons. First, **1** reacts with **CEES** in less than a minute at 80 °C as shown in our previous report.[15] Second, one molecule of mustard gas would be enough to give a 2,5,8-trithia[9]-*m*-cyclophane **3** (Scheme 4). This practice is not expected to change the sensitivity level while dealing with the real agent.

Conclusions

In summary, we report a chromogenic and fluorogenic chemodosimeter for mustard gas simulants. The presence of sulfur mustard simulant was successfully demonstrated by the appearance of the blue color of **SQ** from its colourless solution when there is no mustard. The detection was further confirmed by a fluorescence method. Both chromogenic and fluorogenic detection of **CEES** was achieved with high sensitivity (50 μM and 10 μM, respectively) which is a lower than reported for health hazards. This procedure can be applied to the detection of SM in several possible matrixes *i.e.* on sufaces, in soil, and in the gas phase. The investigation is particularly attractive as it was not reactive towards the oxygen analog of mustard gas, acetylating agents, and nerve agents. We anticipate that the developed protocols will be useful with little to no modification in the detection protocol when used with real agents. Furthermore, this could be an alternative and parallel chromogenic and fluorogenic detection for SM to our recently reported protocol.[15]

Acknowledgements

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