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Fluorescent Sensor for Organoarsenic Blister Agent Lewisite

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Fluorescent Sensor for Organoarsenic Blister Agent Lewisite

by

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Abstract

Chemical warfare agents (CWAs) are dangerous due to their potential of causing indiscriminate damage with severe chronic aftereffects. Growing international concern over terrorist attacks using CWAs call for affordable, quick and specific methods of detecting them. CWAs are typically divided into two categories, nerve agent and blister agent. There have been various methods for detection of nerve agents and mustard blister agent, but sensing methods for arsenic-based blistering warfare agents have been very scarce. Lewisite, an organoarsenic compound, is a blister agent which causes severe skin, eye and mucosal pain and irritation upon exposure. Herein, we report two fluorescence-based chemosensors, a dithiol-containing 7-hydroxycoumarin and cysteine-modified pyrene, for a lewisite simulant, arsenic trichloride. Fluorescence signals of 7-hydroxycoumarin and pyrene are changed respectively upon treatment with $AsCl_3$, and calculated limit of detection (LOD) values were in the range of ppm.

Keywrods : chemical warfare agent (CWA), lewisite, fluorescent sensor, arsenic, British anti-lewisite (BAL), cysteine, coumarin, pyrene

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A. Background

A.1. Chemical Warfare Agent

A.1.1. Historical Background

Among the Weapons of Mass Destruction (WMD), chemical warfare agent (CWA) is one of the most brutal weapons. Often called the "poor man's atomic bomb",[1] CWAs are inexpensive and easy to manufacture yet possess destructive power often compared to that of the atomic bomb. Thus, CWAs have historically been widely used, and currently, several countries and terrorist organizations are known to possess them.

Modern CWAs made the first appearance in World War I in the form of tear gas, mustard gas, chlorine, and phosgene.[2] Even though many countries declared not to use any more CWAs in the signing of the Geneva Protocol in 1925, the research and development of lethal agents have continued.

During World War II, German scientists developed several nerve agents which have been called G-series gas, and they were capable of causing neural paralysis and death in minutes.[3] In the 1950s, several countries including the Soviet Union and the United Kingdom developed similar nerve agents known as the V-series.[4, 5]

After that, proliferation and weaponization of various CWAs have raised global alertness. During the Iran-Iraq War of the 1980s, Iraq used the mustard gas and G-series agents (GA) to fight against Iranian and the Kurdish forces in the northern Iraq.[6] The Aum Shinrikyo group, a Japanese religious cult, used a G-series agent (GB) in two separate attacks in Matsumoto city in 1994 and in the Tokyo subway system in 1995 against civilian populations.[7] From 2013 to present, various CWAs including GB, mustard agent, chlorine gas, and tear gas were used in the Syrian Civil War.[8, 9] Notably, the recent abuse of CWAs have not been limited to the soldiers and are being used to attack civilians indiscriminately.. Furthermore, the threat of terrorist attacks on domestic soil are emerging as a major concern worldwide.

A.1.2. Classification of CWAs

CWAs can be classified into several categories based on their effects on humans; nervous damage, blister formation, pulmonary damage, blood damage, vomiting, tear agents, and others (Table 1).[10] Among these agents, nerve and blister agents are regarded the most dangerous. The rest are used for the purpose of incapacitation rather than destruction.

Agent Type	Effect	Examples
Nerve Agent	Binds to acetylcholinesterase inhibiting vital enzymes' normal biological activities in nervous system.	GA, GB, GD, GF and VX
Blister Agent	Produces blistering and irritant the eyes, respiratory tract, and skin.	H, HD, HN and L
Pulmonary Agent	Damages respiratory track and lungs.	CG, DP, PS and Chlorine
Blood Agent	Interferes in transferring oxygen into the bloodstream.	AC, CK and SA
Vomiting Agent	Causes acute pain and vomiting.	DM
Tear Agent	Induces tearing causing irritation to the eyes and skin.	CS, CR and CN

Table 1. Types of CWAs

A.1.3. Blister Agent

Blister agents are toxic chemical compounds that produce skin, eyes and respiratory pain and irritation. As the name suggests, they causes severe chemical burns, resulting in large fluid blisters on the bodies of those affected. Blister agents are heavier than air, and are classified as persistent agents. There are two types of blister agents, mustard agents and arsenicals. The effects of mustard agents are typically delayed when exposure to vapors becomes evident in 4 to 6 hours, and skin exposure in 2 to 48 hours, while the effects of arsenicals are immediate.

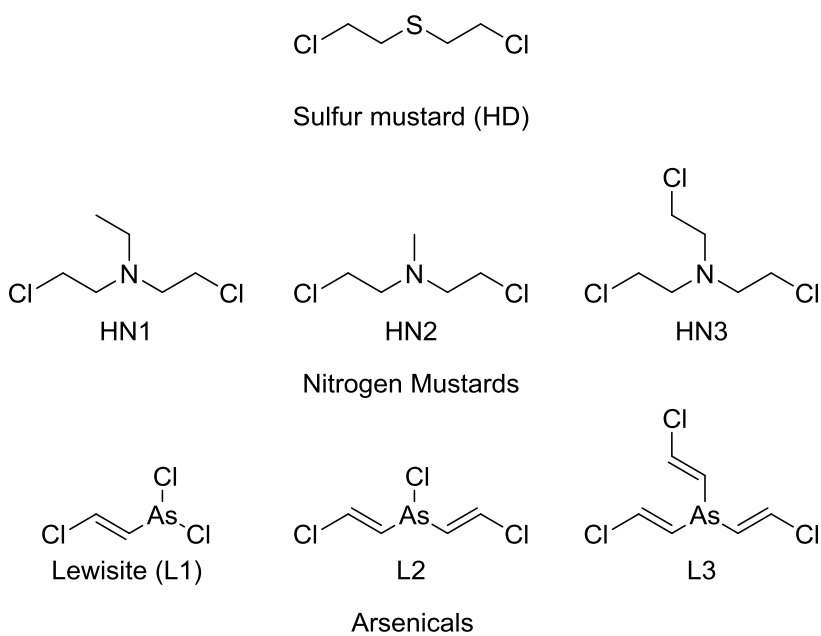
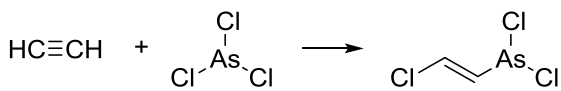


Figure 1. Chemical structures of blister agents

A.1.4. Lewisite

Lewisite (2-chlorovinyldichloroarsine) is an extremely toxic organoarsenic compound. It was manufactured for use as a CWA in World War I and is known to act as a vesicant and a lung irritant. Exposure to more than a certain levels can be fatal. Also, both the liquid and vapor lewisite causes severing burning pain and irritation immediately upon exposure, and it is difficult to detect the agent by odor.

The compound is prepared by the addition of arsenic trichloride to acetylene by in the presence of a suitable catalyst.



Lewisite's systemic toxicity and skin corruption result from its arsenic content. Arsenic is a thiophilic metalloid,[12] and the trivalent arsenic presumably reacts with thiol groups (-SH) in proteins. Lewisite is highly lipid-soluble, and it inhibits several enzymes including the co-enzyme lipoic acid, which is necessary for energy production in the cell (Figure 2). Similarly, other thiol-containing compounds such as a glutathione cysteine are also known to bind arsenic strongly.[13]

2,3-dimercaptopropanol (British Anti-Lewisite, BAL) was manufactured as an antidote for lewisite in World War II. BAL contains two thiol groups that can strongly bind to the arsenic of lewisite, which reduces the toxicity. BAL is given by intramuscular injection as a treatment for internal health effects of lewisite but has no effect on local blisters of the skin or eyes.

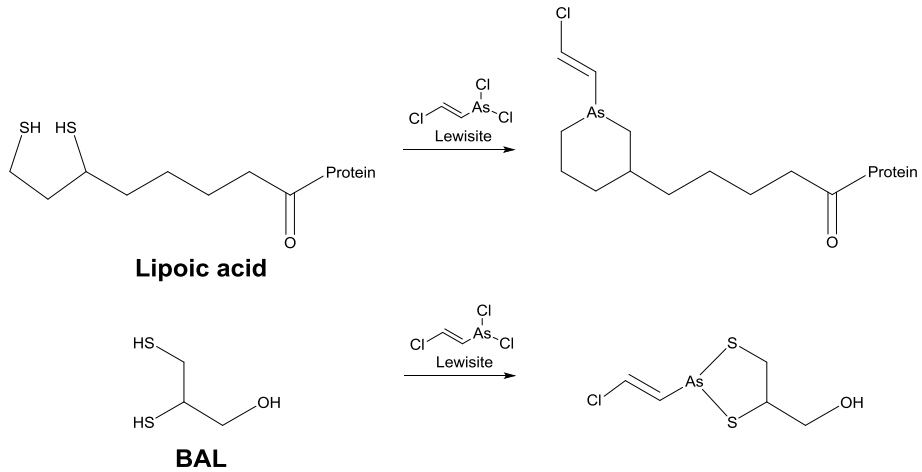


Figure 2. Lewisite is considered to bind with a lipoic acid interfering with energy production. BAL forms a stable chelate with lewisite, thus attenuating its toxicity.

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A.2. Fluorescence

A.2.1. Principles of Fluorescence

Fluorescence is an emission of light by a substance during exposure to external light or electromagnetic radiation. An electron in the excited singlet state orbital is paired to a second electron of opposite spin, in the ground state orbital. When the electron in excited state falls back to the ground state, it is a spin-allowed process and rapidly leads to the emission of an energy, which, in the case of fluorescence is a photon. Such an emission of photons for which the average lifetime of the excited atoms and molecules is near about 10^{-8} seconds is known as fluorescence.[1]

During the short excitation period, some of the energy is dissipated by molecular collisions or transferred to a proximal molecule, and then the remaining energy is emitted. The emitted light, therefore, usually carries less energy and has a longer wavelength than the absorbed light (Figure 1). Absorption of light by a conjugated organic molecule can give rise to the promotion an electron to the lowest unoccupied molecular orbital (LUMO). The energy levels are broadened by vibration and rapid vibronic relaxation to the bottom of the LUMO is followed by radiatvie decay (k_r) to the ground state. The radiative decay process involves a transition between states of the same multiplicity and is called fluorescence. And there are various non-radiative decays such as intersystem crossing and photo-induced electron transfer, the fluorescence quantum yields (Φ) are decreased.

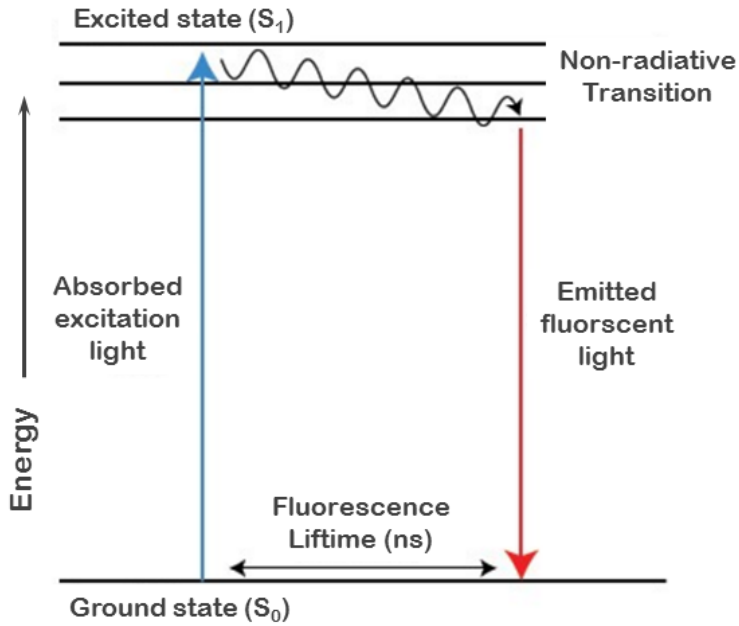

Figure 1. Jablonski energy diagram of fluorescence.

A.2.2. Heavy Atom Effect

The heavy atom effect is defined by the International Union of Pure and Applied Chemistry as following.

> *"The enhancement of the rate of a spin-forbidden process by the presence of an atom of high atomic number, which is either part of, or external to, the excited molecular entity. Mechanistically, it responds to a spin-orbit coupling enhancement produced by a heavy atom."*[2]

The presence of a heavy atom activates intersystem crossing by spin-orbit coupling by mixing its large, unfilled d orbitals with those of the excited molecule.

In firmly bound complexes where intersystem crossing (k_{isc}) is one of the non-radiative decay (k_{nr}) factors, binding or chelating of heavier ions leads to increased spin–orbit interaction, so intersystem crossing and fluorescence quenching increases. This produces both, reduced fluorescence quantum yields and lifetimes (τ).[3]

A.2.3. Monomer-Excimer Formation

An excimer is a short-lived dimeric molecule formed from two identical fluorophores which are present within a certain distance, with one being in an excited state and the other in ground state.[1] It is a dimer which is associated in the electronically excited state and dissociative in the ground state. It emits at longer wavelength than the monomer, so the emission spectrum of the excimer is red-shifted compared to the monomer. Electronically excited state of the collision complex is more strongly bound than the ground state due to stabilization by association (Figure 2).

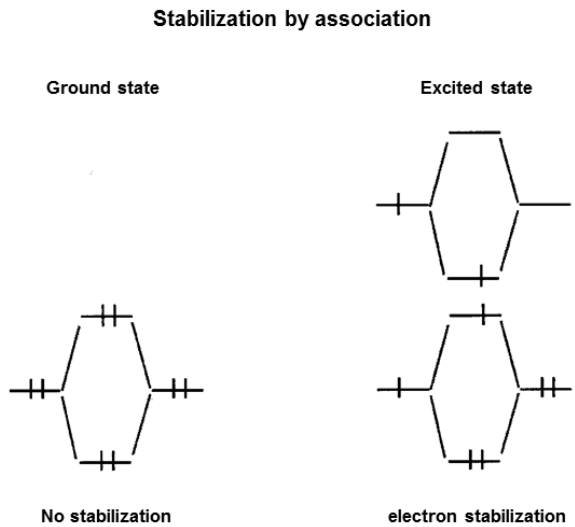

Figure 2. Diagrams of dimeric association

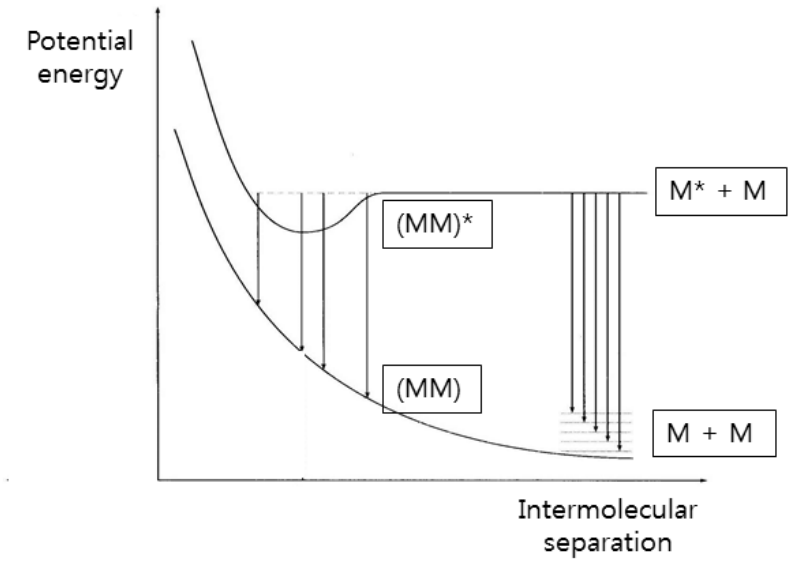

Figure 3. Energe state of excimer emission.

A.2.4. References and Notes

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B. Applications

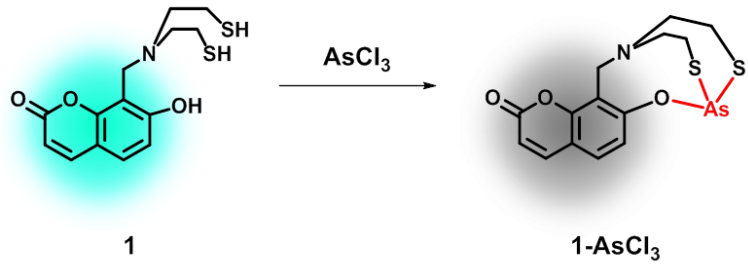
B.1. Fluorescent Probe for Lewisite Simulant

Lewisite, an organoarsenic compound, is a potent chemical warfare agent which causes liver necrosis, renal failure, and lethal shock. Herein, we report for the first time fluorescent probe for detection of lewisite simulant, arsenic trichloride ($AsCl_3$). The probe shows high sensitivity and selectivity toward $AsCl_3$. Moreover, quantitative determination of $AsCl_3$ in soil was achieved with a detection limit of 61.2 μmol/kg that is sufficiently low concentration to detect dose below the reported LD_{50} of lewisite.

B.1.1. Introduction

Chemical warfare agents (CWAs) are classified as weapons of mass destruction (WMD)[1] due to their potential of indiscriminate damage and severe chronic consequences. Although the military use of CWAs has been banned by the Chemical Weapon Convention (CWC),[2] a terroristic use remains as a real threat today.[3, 4] Accordingly, varied detection methods for CWAs have been developed based on ion-mobility spectroscopy,[5] mass spectrometry,[6] electrochemistry,[7] and others.[8-10] Recently, fluorescence-based detection methods have been applied extensively due to their simplicity, sensitivity, cost-effectiveness and fast response. In spite of the recent advances, fluorescence-based detection methods for CWAs have been focused on nerve agents[11-13] and sulfur/nitrogen mustard agent.[14, 15] Herein, we report for the first time a fluorescent probe which allows for a highly sensitive and selective detection of organoarsenic blister agent, lewisite.

Lewisite (2-chlorovinyldichloroarsine) has an arsenic core which is a thiophilic metalloid.[16] Thiol-containing biomolecules such as cysteine, glutathione, and lipoic acid are known to strongly bind arsenic,[17] resulting in impaired gluconeogenesis and oxidative phosphorylation.[18] Lewisite exposure at lose doses causes blister formation and deep skin burns in humans. High-dose exposure can lead to severe irreversible damages such as liver necrosis, renal failure, and even lethal "Lewisite shock".[19, 20] During World War II, 2,3-dimercaptopropanol (British Anti-Lewisite, BAL) was exploited as an antidote for lewisite.[21] BAL contains two thiol groups that chelates to arsenic, attenuating its toxicity. Inspired from the thiophilic characteristics of arsenic species, we have prepared thiol containing 7-hydroxycoumiarn for the fluorescent detection of lewisite simulant, $AsCl_3$ (Scheme 1).[22]



Scheme 1. Proposed mechanism of lewisite simulant detection.

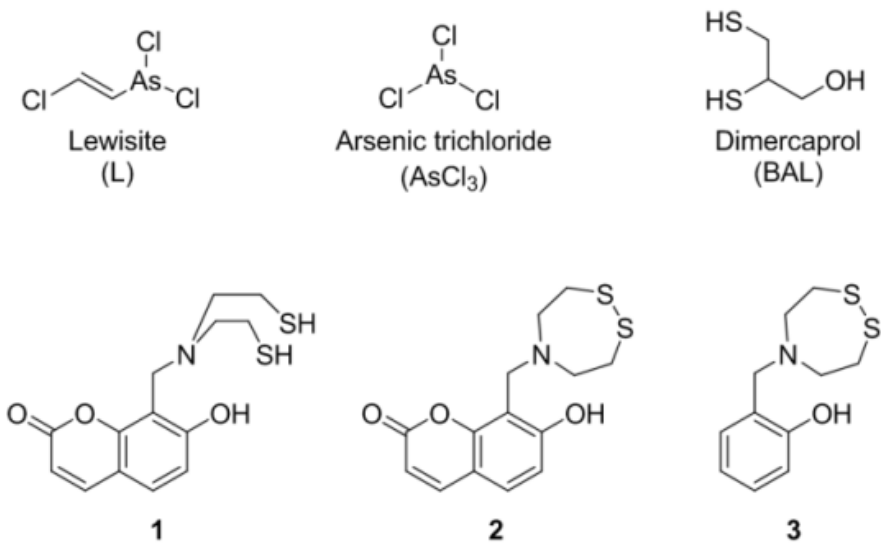
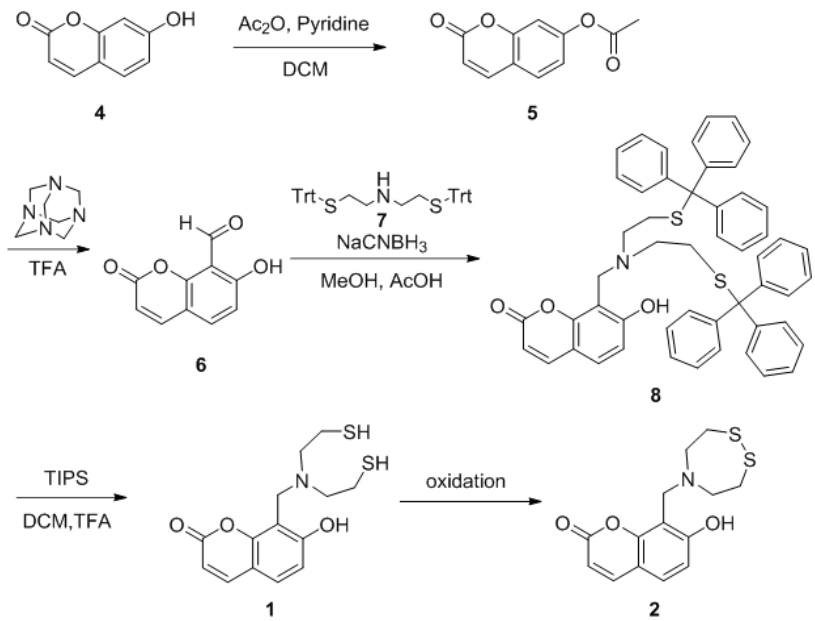


Chart 1. Chemical structures of probes, BAL, lewisite, and lewisite simulant.

B.1.2. Result and Discussion

The probe **1** was synthesized via a simple four-step procedure with an overall yield of 7.3%. Compound **6** was prepared by Duff reaction,[23] and subjected to reductive amination with **7** to yield compound **8**. Deprotection of trityl group presented probe **1**. Additionally, probe **2** was prepared from air-oxidation of **1** for easy handling due since the dithiol moiety in **1** was prone to air-oxidation (Scheme 2).[24]



Scheme 2. Synthetic procedure of probes.

The performance of probe **1** in response to $AsCl_3$ was confirmed by mass spectrometery. Probe **2** was pretreated with tris(2-carboxyethyl)phosphine (TCEP) to afford its reduced form, probe **1**.[25] The mass spectrum of probe **2** upon addition of TCEP showed m/z 312.0 (calcd for $[C_{14}H_{18}O_3NS_2]^+$ 312.07), confirming the formation of probe **1**. After $AsCl_3$ addition, a heavier peak featuring an m/z of 383.9 emerged, which corresponded to complex **1**-As (calcd for $[C_{14}H_{15}O_3NS_2As]^+$ 383.97) (Figure 1).

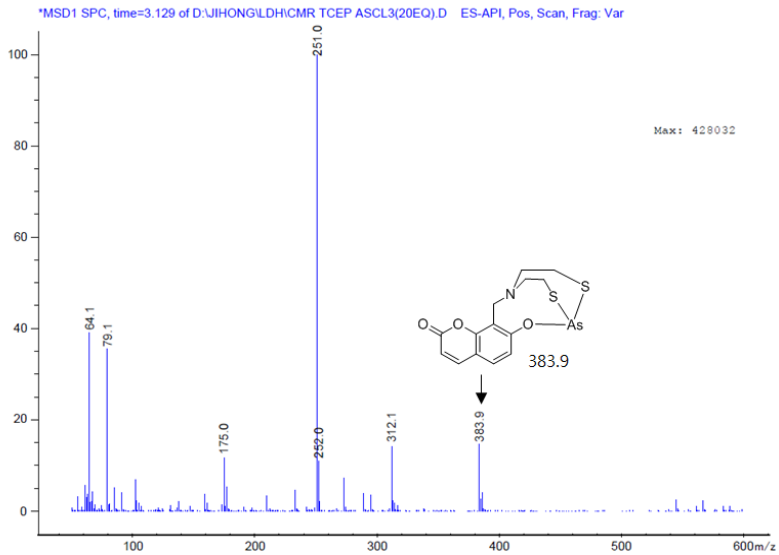


Figure 1. Mass spectrum of **1** upon presence of $AsCl_3$ (**2**+TCEP+$AsCl_3$)

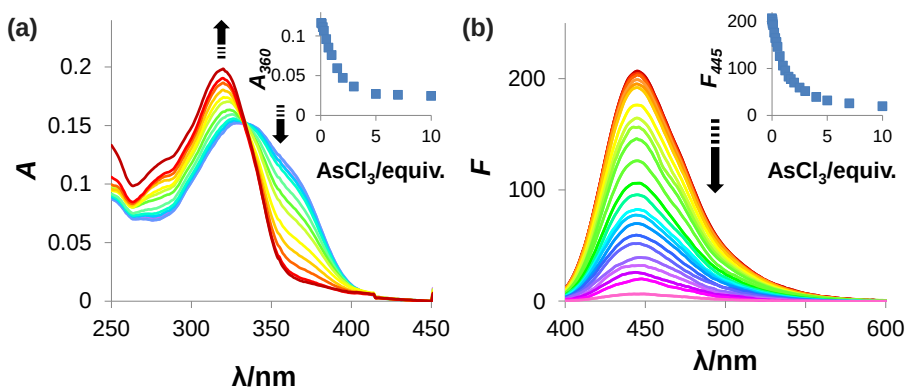


Figure 2. UV−Vis absorbance (a) and fluorescence spectra (b) of **1** (10 μM) upon addition of $AsCl_3$ (0−10 equiv.) in the co-presence of TCEP (12 μM) in water. The inset of panels shows absorption (λ_{abs} = 360 nm) (a) and emission (λ_{em} = 445 nm) (b) intensity of **1** (10 μM) decreasing upon $AsCl_3$ addition (0−10 equiv.). The fluorescent emission was monitored by excitation at 370 nm.

Photo-physical properties of probe **2** (10 μM) upon addition of $AsCl_3$ was monitored by UV−Vis absorbance and fluorescence spectroscopy in the presence of TCEP (12 μM). According to the previous literature,[25] it is presumed that probe **2** (10 μM) in the presence of TCEP (12 μM) rapidly and irreversibly converts probe **1** (10 μM). The addition of $AsCl_3$ induces 7 nm hypsochromic shift showing isosbestic

point at 332 nm (Figure 2a). The intrinsic fluorescence of probe **1** (λ_{max} = 445 nm, Φ_F = 0.62)[26] linearly decreases upon the addition of $AsCl_3$ (0−10 μM) and is completely quenched by 10 equiv. of $AsCl_3$ (Figure 2b). However, there were no significant responses upon the addition of varied metal ions, Hg^{2+}, Ag^{+}, Cu^{2+}, Fe^{2+}, Zn^{2+}, Pb^{2+}, and Cd^{2+} (10 equiv. each) in water, which demonstrates selective sensing behaviour toward $AsCl_3$. It is noteworthy that the probe showed the selective response for $AsCl_3$ over other thiophilic metals such as Hg^{2+}, Cu^{2+}, and Ag^{+}, which are major competitors in real field samples. Subsequent addition of $AsCl_3$ into each metal ion solution led to quenching, with a quenching factor (F_0/F = 16.5−19.7) comparable with the quenching factor of the probe **1** in $AsCl_3$ solution (F_0/F= 18.3), demonstrating the competitive response toward $AsCl_3$ even in the presence of other metal ions (Figure 3a). Furthermore, the quenching response of probe **1** towards $AsCl_3$ allows for naked eye detection, while there are no such significant changes with other metals (Figure 3b). The limit of detection (LOD), estimated by 3σ/slope, was determined to be 1.34 × 10^{-6} M (1.34 μmol/kg water) showing a linear range upon 0−10 μM of $AsCl_3$ addition with an R^2 value of 0.981 (Figure 4). The LOD value demonstrates sufficient sensitivity of probe **1** for practical application and is orders of magnitude below the LD_{50} of lewisite (30 mg/kg, 145 μmol/kg) (Table 2).[27] The fluorescent response toward $AsCl_3$ was appeared in broad pH range (pH 4–9) (Figure 5), which is crucial for CWAs detection because an active battle field is known to possess weakly acidic conditions (pH 4.8−5.8).[28]

Blister agent	LCt_{50} Inhalation mg·min/m^3	LD_{50} Skin mg/kg
Lewisite (L)	1400	30
Sulfur mustard (HD)	1500	100
Nitrogen mustard (HN-1)	1500	No data available

Table 2. Lethal concentration and time (LCt_{50}) and lethal dose (LD_{50}) of lewisite, sulfur/nitrogen mustard

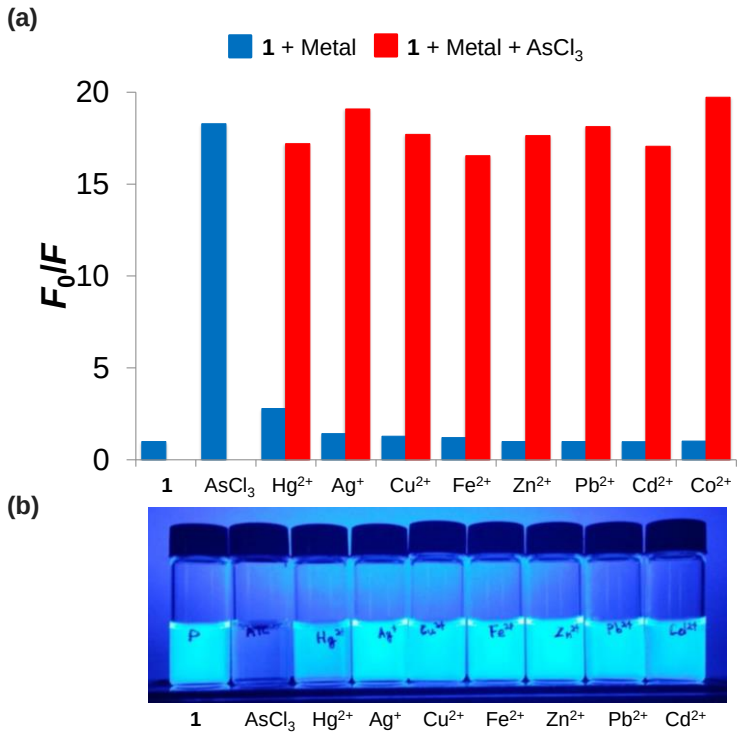


Figure 3. (a) Fluorescence intensity changes of **1** (10 μM) upon addition of varied metal ions (10 equiv.) in water (blue bar) and competitive response upon subsequent addition of $AsCl_3$ (10 equiv.) into the each metal solution (red bar). The fluorescence intensity was obtained by excitation at 370 nm. (b) Their corresponding photograph under UV hand lamp irradiation at 365 nm showing fluorescence quenching upon $AsCl_3$ addition.

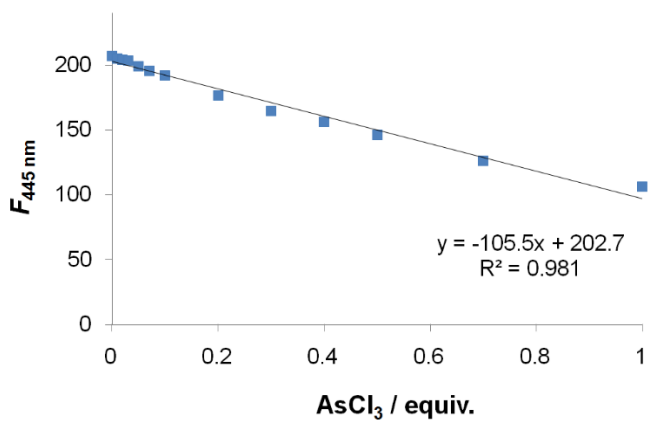


Figure 4. Linear range of fluorescence quenching of **1** (10 μM) upon addition of $AsCl_3$ (0−1.0 equiv.) in co-existence of TCEP (12 μM) in water. The fluorescence intensity was obtained by excitation at 370 nm.

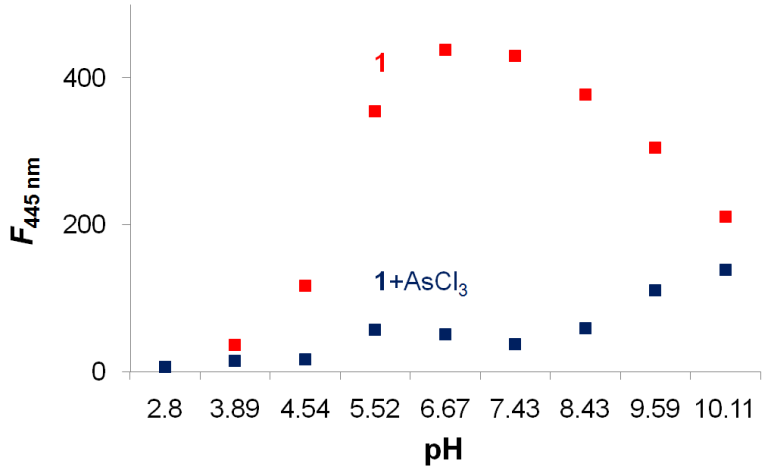


Figure 5. Fluorescence intensity changes (445 nm) of **1** (10 μM) (red square) upon addition of 10 equiv. of $AsCl_3$ (blue square) in co-existence of TCEP (12 μM) in water (pH 2.8−10.11). The fluorescence intensity was obtained by excitation at 370 nm.

From the observations, we propose that the chelation of As^{3+} to probe **1** induces derives intersystem crossing of excited electrons by heavy atom effect, quenching the fluorescence of the probe.[29]

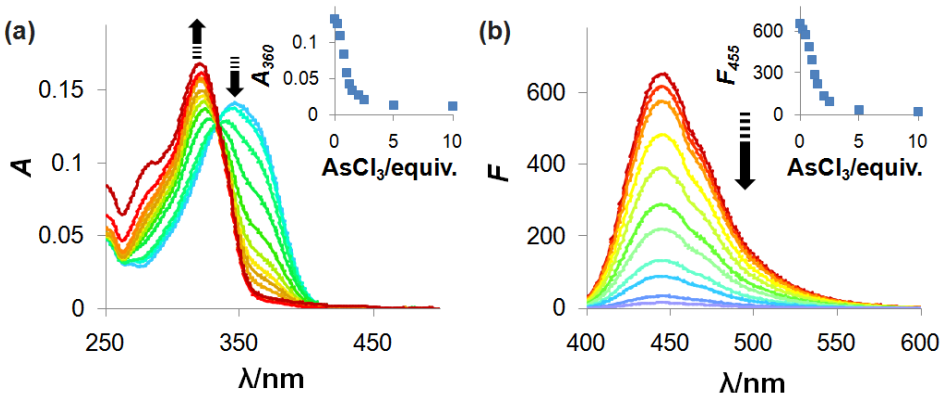


Figure 6. UV−Vis absorbance (a) and fluorescence spectra (b) of **2** (10 μM) upon addition of $AsCl_3$ (0−10 equiv.) in water. The inset of panels shows absorption (λ_{abs} = 360 nm) (a) and emission (λ_{em} = 445 nm) (b) intensity of **2** (10 μM) decreasing upon $AsCl_3$ addition (0−10 equiv.). The fluorescent emission was monitored by excitation at 370 nm.

Interestingly, probe **2** in the absence of TCEP showed similar results for $AsCl_3$ recognition (Figure 6). 7-hydroxycoumarin, a control fluorophore lacking any sulfur-containing functional group, shows negligible fluorescence changes upon treatment with $AsCl_3$, which demonstrates that 1,2,5-dithiazepane plays an active role in $AsCl_3$ detection (Figure 7).

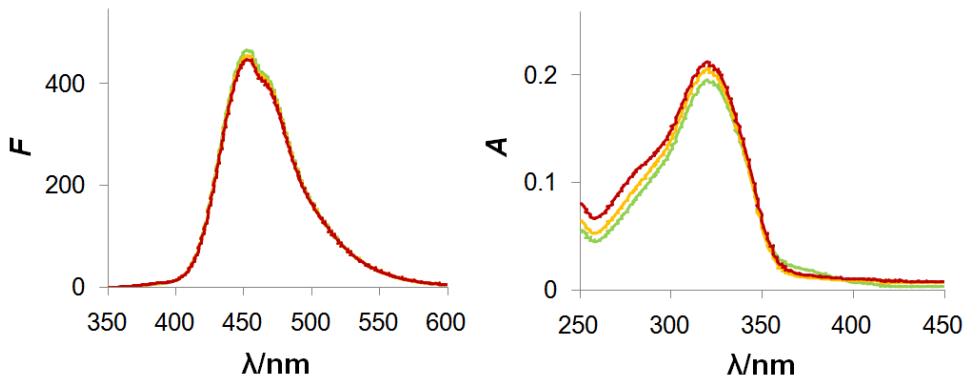


Figure 7. Fluorescence (a) and UV−Vis absorption spectra (b) of **4** (10 μM) upon addition of $AsCl_3$ (0−10 equiv.) in water. The fluorescent emission was monitored by excitation at 340 nm.

^1H NMR experiment corroborates the role of 1,2,5-dithiazepane for $AsCl_3$ recognition. Upon the addition of increasing amount of $AsCl_3$ into the solution of compound **3**, the aromatic peaks (6.80−7.24 ppm) gradually shifted downfield, while the ^1H resonance from the 7-hydroxyl group at 10.4 ppm disappeared. The aliphatic proton resonances on 1,2,5-dithiazepane also shifted from 2.93 and 3.27 ppm to 3.24 and 3.85 ppm accompanied by broadening of the peaks. The results suggest that As^{3+} binds to the 1,2,5-dithiazepane and the phenolic oxygen of compound **3** (Figure 8).

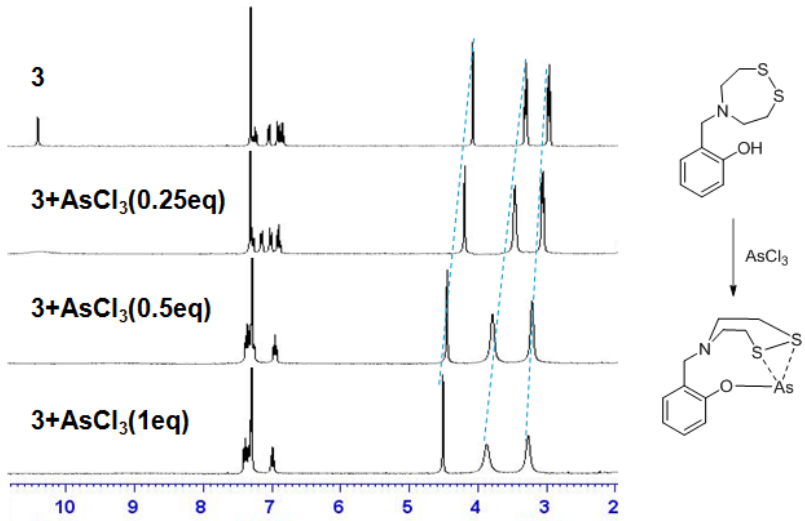


Figure 8. ^{1}H NMR spectra of **3** upon the addition of $AsCl_3$ (0−1.0 equiv.) in $CDCl_3$.

Lewisite, upon release to environment, is known to be highly persistent and its detection from samples such as soil is therefore very impotant.[30] To demonstrate on-field utility of probe **2**, quenching experiments were conducted using $AsCl_3$–spiked soil was after extraction with water. The amount of spiked $AsCl_3$ is quantitatively analysed in the range of 0−3.5 mM through fluorescence. Importantly, the LOD is estimated to be 6.12×10^{-5} M (61.2 μmol/kg) an order of magnitude lower than the LD_{50} of $AsCl_3$,[27] which demonstrates the utility of the probes for real field tests. The fluorescence intensity of probe **2** was decreased upon increasing amount of the spiked $AsCl_3$, and the corresponding intensity change could be detected by through naked eye (Figure 9).

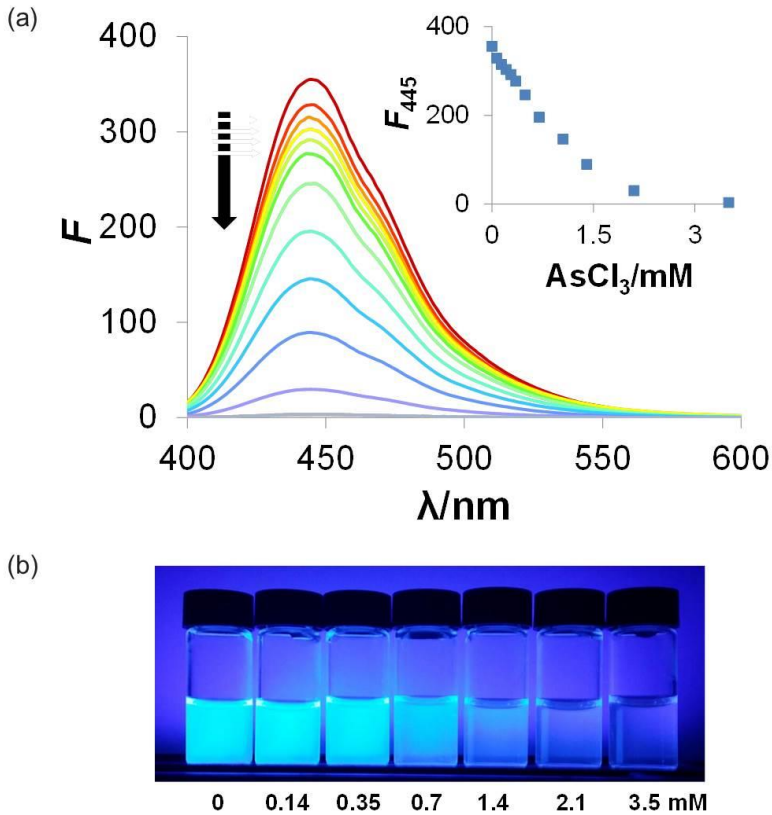


Figure 9. (a) Fluorescence change of **2** (10 μM) in response to the spiked concentration of $AsCl_3$ (0−3.5 mM) into soil. The spiked $AsCl_3$ was extracted to aqueous layer following by mixing with probe **2**, and the fluorescence intensity of **2** was monitored by excitation at 370 nm. Inset shows the fluorescence intensity (λ_{em} = 445 nm) of **2** (10 μM) decreasing in response to the spiked $AsCl_3$. (b) Photograph under UV hand lamp (λ_{ex} = 365 nm) shows fluorescence quenching of **2** in response to the spiked concentration of $AsCl_3$ (0−3.5 mM).

In conclusion we have developed for the first time the fluorescent probe for lewisite mimic, $AsCl_3$. The dithiol or 1,2,5-dithiazepane group on probe **1** or **2** seems to induce binding of $AsCl_3$ to the coumarin core, leading to fluorescence quenching through the heavy atom effect. We have demonstrated the selective and sensitive sensing behaviour of the probes toward $AsCl_3$. Moreover, quantitative determination of $AsCl_3$ in soil has been performed at low concentrations, suggesting the potential utility of our system in real-world applications. The studies herein constitute a fluorescence-based detection for a class of CWAs which have previously only been conducted through methods requiring heavy equipments.

B.1.3. Experimental

B.1.3.1. General

Materials

7-hydroxycoumarin, acetic anhydride, 1,3,5,7-tetraazaadamantane, triphenylmethanethiol, bis(2-chloroethyl)amine, sodium cyanotrihydroborate, trifluoroacetic acid (TFA), triisopropylsilane (TIPS), dichloromethane (DCM), methanol (MeOH), cyclohexane, hexane, ethyl acetate, tetrahydrofuran (THF) and $CDCl_3$ were purchased as reagent grade from Aldrich, Acros, Samchun, TCI and used as received. The used metal salts are $Hg(OAc)_2$, $Zn(ClO_4)_2$, $AgNO_3$, $Cd(ClO_4)_2$, $Cu(ClO_4)_2$, $Fe(ClO_4)_2$, and $Pb(ClO_4)_2$.

Instruments

NMR characterization: ^1H and ^{13}C NMR spectra were recorded by Advance 300 and 75 MHz Bruker spectrometer in chloroform-d_3. Chemical shifts were expressed in parts per million (δ) and reported as s (singlet), d (doublet), t (triplet) and m (multiplet).

Fluorescence & UV−Vis experiment: Probe **2** was dissolved in THF to afford a concentration of 10 mM stock solution, which was diluted to 10 μM with distilled water up. Analyte was added into 10 μM of **2** in co-existence of TCEP (12 μM), and photo-physical property of **2** was measured in real time. Fluorescence and UV−Vis absorbance were recorded on Jasco FP-6500 and Beckman DU 800 spectrophotometer, respectively.

B.1.3.2. Synthesis of Probe

8-((bis(2-(tritylthio)ethyl)amino)methyl)-7-hydroxy-2H-chromen-2-one (8)

To solution of compound **6** (1.99 g, 10 mmol) in methanol/DCM (100mL/50mL) were added compound **7** (6.22 g, 10 mmol), and then small amount of acetic acid was further added. Sodium cyanotrihydroborate (0.63 g, 10 mmol) was added

dropwise to the ice-cooled resulting solution under stirring. After the solution was stirred for three days at room temperature, it was acidified by adding conc. HCl and then evaporated almost to dryness under reduced pressure. The residue was dissolved in saturated Na_2CO_3 and extracted to DCM layer. The factions were combined, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to give amber oil. The residue was further purified on a silica-gel column with hexane and ethyl acetate with 42.0% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.13 (t, 4H), 2.26 (t, 4H), 3.61 (s, 2H), 6.17 (d, 1H), 6.70 (d, 1H), 7.20 (m, 31H), 7.62 (d, 1H).

8-((1,2,5-dithiazepan-5-yl)methyl)-7-hydroxy-2H-chromen-2-one (2)

Compound **8** (3.34 g, 4.2 mmol) was deprotected by treatment with DCM:TFA:TIPS (50:47.5:2.5, v/v/v, 400 mL) during 1 hour. Deprotection solution was evaporated under reduced pressure and residual TFA was removed by co-evaporation with cyclohexane (3 × 100 mL) and drying *in vacuo*. The residue was extracted to DCM layer and dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give amber oil. The residue was air-oxidized in methanol/water with Na_2CO_3 prior to the purification. The resulting residue was further purified on a silica-gel column with hexane and DCM to give white solid with 33% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.95 (t, 4H), 3.30 (t, 4H), 4.35 (s, 2H), 6.20 (d, 1H), 6.81 (d, 1H), 7.31 (d, 1H), 7.62 (d, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 162.7, 161.0, 153.0, 144.3, 128.4, 113.9, 111.7, 111.5, 108.3, 56.6, 53.3, 38.0. HRMS: calculated for $C_{14}H_{16}O_3NS_2$ $[M+H^+]^+$ 310.0572; found 310.0581.

B.1.4. References and Notes

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B.2. Pyrene Excimer-based Chemosensor for Lewisite

A pyrene excimer-based probe has been developed for the detection of lewisite simulant, arsenic trichloride ($AsCl_3$). The design principle of the probe involves the use of pyrene-bearing cystein which leads to excimer formation upon binding arsenic. The probe exhibited high sensitivity and selectivity toward $AsCl_3$ along with a very low detection limit (LOD), well below the reported LD_{50}.

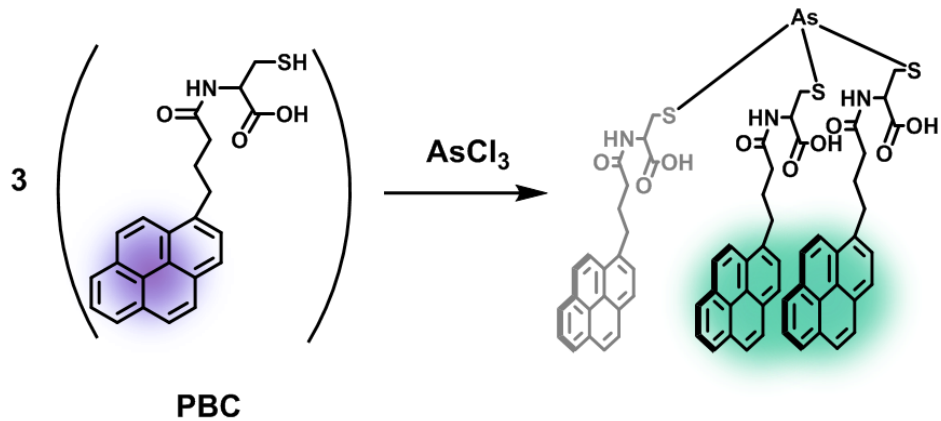
B.2.1. Introduction

Chemical Chemical warfare agents (CWAs) are typically classified into two categories, nerve agents and blister agents. Thus far, there various methods for the detection of nerve agents, such as ionmobility spectrometry, mass spectrometry, fluorogenic and chromogenic sensors, and gelator sensor, have been reproted.[1-4] Reports on the detection for blister agents, however, have been relatively scarce. More specifically, sensing methods for arsenic-containing blister agents have largely been lacking. Lewisite (2-chloroethenylarsonous dichloride) is a representative arsenic-based blister agent used as a CWA, and is also known as the "Dew of Death".[5] Lewisite produces immediate health effects within seconds to minutes after exposure, causing severe skin, eye, and respiratory damage. Blister formation and deep skin burns are known to ensure approximately 12 hours after exposure, and this is more severe compared to mustard series blister agents. High-dose exposure may also result in hepatic necrosis and renal failure.[6]

The effects of lewisite are attributed to its chemical structure. Arsenic, at the center of lewisite, is thiophilic,[7] and easily binds to thiol-containing compounds in tissues such as a glutathione, cysteine and lipoic acid.[8] Such properties lead to impaired gluconeogenesis and oxidative phosphorylation in living systems. Arsenic is also known to inhibit the enzyme pyruvate dehydrogenase by binding to its sulfhydryl groups.[9] Although lewisite is highly hazardous, it can be easily mass-produced through simple chemical processes, rendering it to be one of the most inexpensive of known CWAs. Thus, an effective methods of detecting arsenic-based blister agents is very important.

Herein, we report a fluorescent probe for detection of lewisite simulant, arsenic trichloride ($AsCl_3$). The probe, (4-(pyren-1-yl)butanoyl)cysteine (**PBC**), features a simple design involving a signaling group, pyrene, connected to an arsenic binding group, cysteine. It was envisioned that the binding of the probe to arsenic would bring up to three pyrene moieties in close proximity, leading to excimer[10] emissions

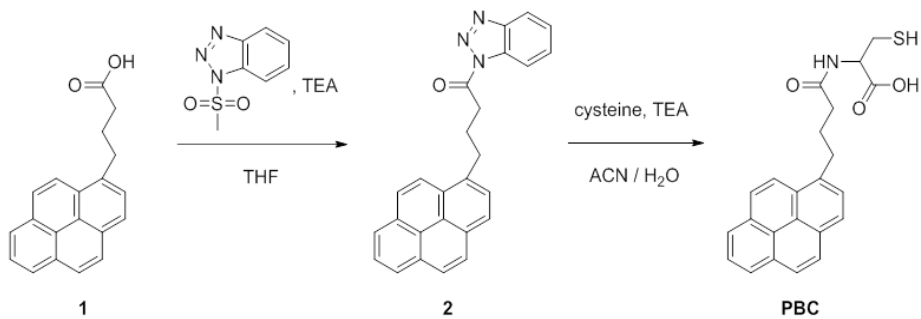
(Scheme 1). This binding between one As^{3+} and three cysteine moieties has been reported previously.[11] The probe exhibited ratiometric changes between monomer and excimer, allowing for a highly sensitive detection of $AsCl_3$.[12]



Scheme 1. Proposed mechanism of lewisite simulant detection.

B.2.2. Result and Discussion

PBC was synthesized through a very simple, two-step procedure (Scheme 2) involving carboxylic acid activation and substitution.



Scheme 2. Synthetic procedure of probe.

Solution of **PBC** exhibits a strong monomer emission with a maximum at 378 nm along with a very weak excimer emission at 475 nm (λ_{ex} = 345 nm) (Figure 1). Upon treatment of this solution with 10 equivalents of $AsCl_3$, a gradual decrease in monomer emission accompanied by an increase in excimer emission was observed with an explicit isoemissive point at 429 nm (Figure 1). The ratio of fluorescence intensities at 415 nm and 378 nm showed near-linear relationship over time (Figure 1 inset). These two signals were similar to other pyrene dyes.[13, 14]

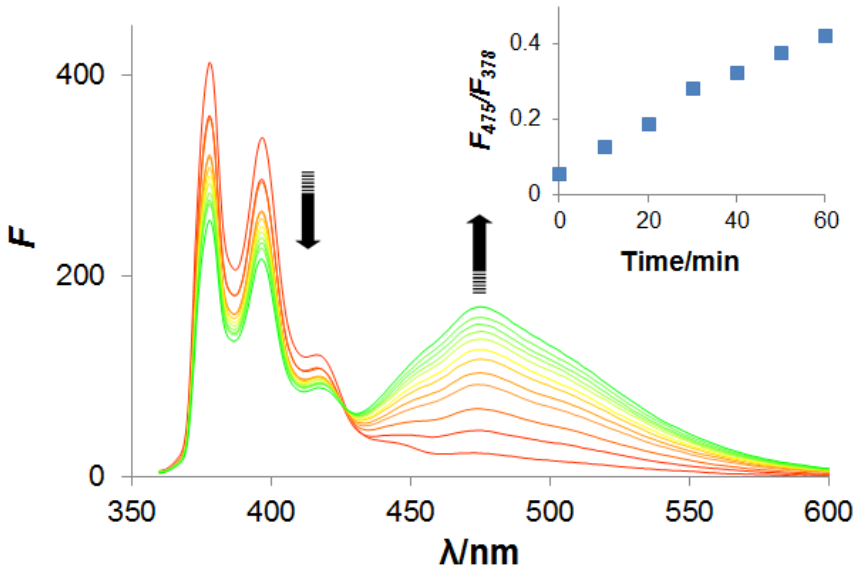


Figure 1. Time dependent fluorescence spectra (λ_{ex} = 345 nm) of solution of PBC (10 μM) in acetonitrile in response to addition of $AsCl_3$ (10 equiv.).

When identical experiments were performed using other metal halides instead of $AsCl_3$, negligible excimer emission changes were observed with Cu^{2+}, Hg^{2+}, Pb^{2+}, Fe^{2+}, Ag^{+}, and Co^{2+}. When Cd^{2+} or Zn^{2+} was used, however, noticeable alterations in fluorescence spectrum of **PBC** were observed, with Cd^{2+} leading to more dramatic changes than As^{3+}.

The response of **PBC** toward Cd^{2+} featured some noticeable differences compared to the response toward As^{3+}. First, the emergence of excimer emission was nearly instantaneous. Upon addition of Cd^{2+} to a solution of **PBC**, we observed variations of monomer and excimer emission intensities immediately, while with $AsCl_3$, at least 30 minutes were required for an intensity ratio between 475 nm and 378 nm (F_{475}/F_{378}) of above 0.2. Furthermore, in the sole case of Cd^{2+}, a strong quenching of monomer fluorescence was observed, leading to F_{475}/F_{378} values which were orders of magnitude larger than those from other metal ions (Figure 2). The quenching response and excimer formation could also be conducted in water-containing systems (water:EtOH, 20:80, v/v) with Cd^{2+}.

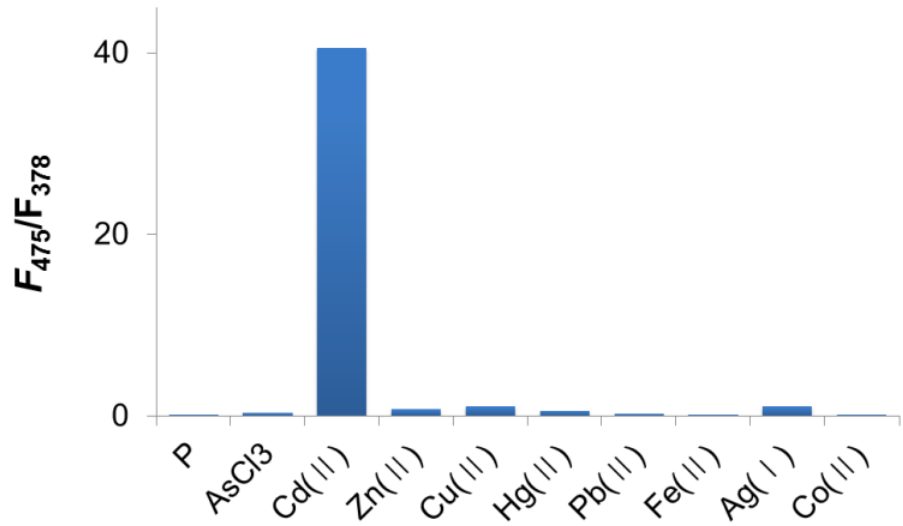


Figure 2. The ratio of excimer to monomer emission of **PBC** (10 μM) upon addition of various metal ions (10 equiv.) at once in acetonitrile (λ_{ex} = 345 nm).

Subsequently, a fluorescence emission analysis on the response characteristics of **PBC** toward Cd^{2+} was carried out in water and ethanol mixture. The maximum excitation wavelength of **PBC** (10 μM) was at 345 nm with a monomer peak emission at 378 nm. Upon the addition of Cd^{2+}, the monomer emission intensity was significantly reduced along with an emergence of excimer peak at 475 nm with an isoemissive point at 432 nm. The limit of detection (LOD) of **PBC** for Cd^{2+} was 7.49 × 10^{-7} M (3σ/slope) in a linear range of 0−5 μM Cd^{2+} with R^2 value of 0.986, which suggested that the probe had a good sensitivity towards Cd^{2+} (Figure 3)

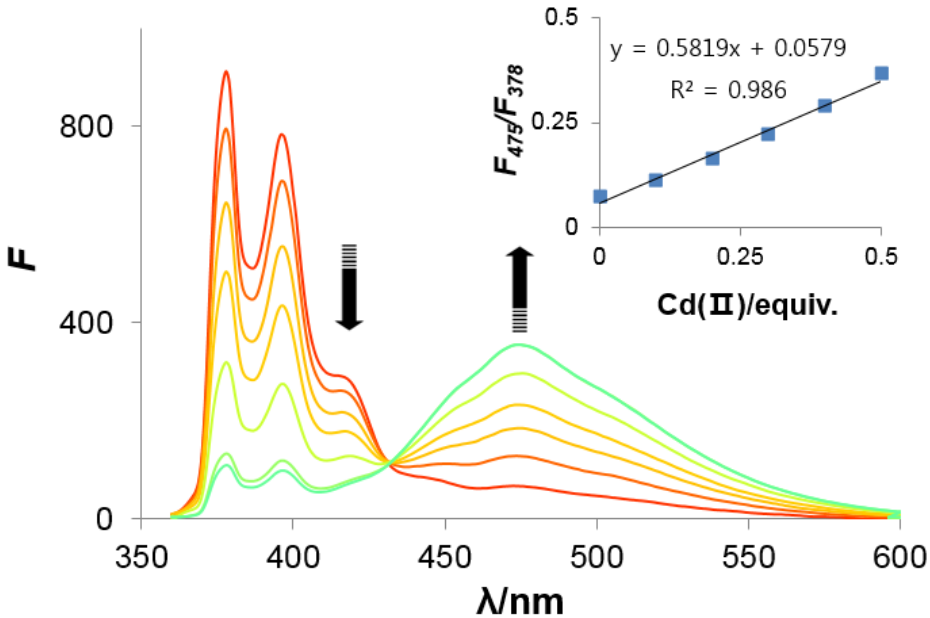


Figure 3. Fluorescence spectra (λ_{ex} = 345 nm) of **PBC** (10 μM) in H_2O:EtOH (20:80, v/v) upon addition of increasing amounts of Cd^{2+} (0-2 equiv.).

Competition test for Cd^{2+} with various metal ions was conducted. 5 equiv. of Cd^{2+} was added to the solutions which contained PBC (10 μM) and metal ions (20 equiv.). But, there were no changes except with Zn^{2+} and Mg^{2+} (Figure 4). The observations were attributed to the possibility of zinc and magnesium forming chelates in the presence of cadmium.

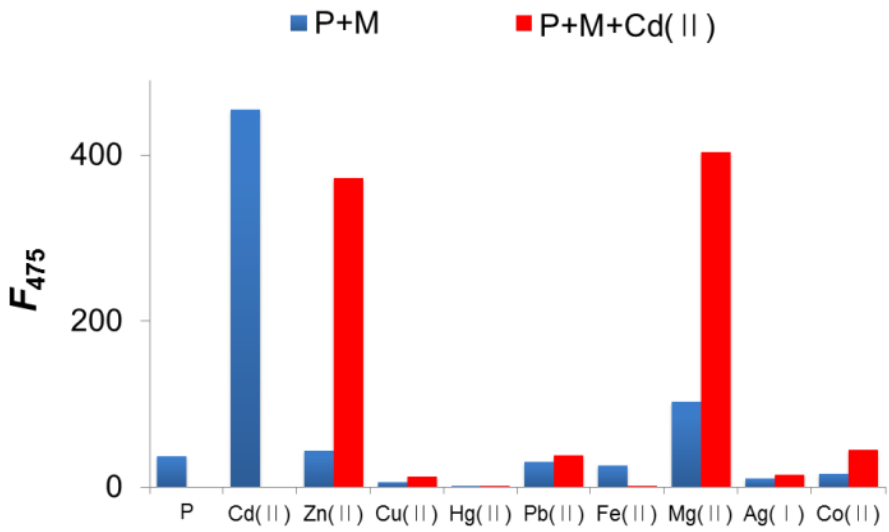


Figure 4. Blue bars represent fluorescence intensity changes of **PBC** (10 μM) at 475 nm upon addition of Cd^{2+} (5 equiv.) and various metal ions (20 equiv.) respectively in H_2O:EtOH (20:80, v/v). Red bars represent the addition of competitive response upon subsequent addition of Cd^{2+} (5 equiv.) into the each metal solution (λ_{ex} = 345 nm).

Just then we came up with a bright idea to overcome these problems. As shown in Figure 4, if one of Pb^{2+}, Fe^{2+}, Ag^{+} and various metal ions chelated with **PBC**, other metal ions could not chelated with the probe. But because the affinity between arsenic and thiol group is known for being great, it could be worked to metal and PBC complex by $AsCl_3$. To carry out fluorescence titrations, the solution of **PBC** (10 μM) in acetonitrile was performed with several metal ions including $AsCl_3$ in the presence of Pb^{2+}, Fe^{2+}, and Ag^{+} separately. Among them, the case of containing Fe^{2+} was the most well-defined result for $AsCl_3$. So we planned for the lewisite simulant sensor with iron ion containing probe.

To selective detect only for $AsCl_3$, a solution of **PBC** (10 μM) in acetonitrile was titrated with 10 equiv. of various metal ions respectively in the presence of Fe^{2+} (2 equiv.). When we measured the fluorescence titration after one hour, it exhibited a significant result. As mentioned prior, Fe^{2+} in the solution of **PBC** already chelated cysteine moieties of the probe so other metal ions couldn't change the photophysical

properties of PBC because other metal ions couldn't chelate with the probe. But arsenic was a thiophilic metalloid so the tripodal complex was made through arsenic binding with three thiols. This indicated $AsCl_3$ was bound with **PBC** with or without Fe^{2+}. The results of selectivity titration and competition test supported the opinion (Figure 5). Experimentally, the excimer emission intensity of **PBC** (10 μM) containing Fe^{2+} (2 equiv.) was largely enhanced by adding $AsCl_3$ (10 equiv.) after 1 hour, differentiated from other metal ions (10 equiv.), indicative of its selectivity to $AsCl_3$ in acetonitrile. Next, $AsCl_3$ was allowed to added to the solution **PBC** containing Fe^{2+} in the presence of various metal ions such as Cd^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Mg^{2+}, Ag^{+}, and Co^{2+}. After 1 hour, the excimer emission increased in the presence of other metal ions except Hg^{2+}. In the case of mercury, it is known for good affinity with thiol, so arsenic was probably not able to bind with cysteine moieties due to thiols blocked by mercury. Because silver ion also somewhat blocked the thiols as thiophilic metal, the rate of increase of excimer emission was less than other metal ions.[15] Anyway Hg^{2+} was able to discriminate between $AsCl_3$ and Hg^{2+}, owing to no enhancement of excimer peak in presence of Hg^{2+}.

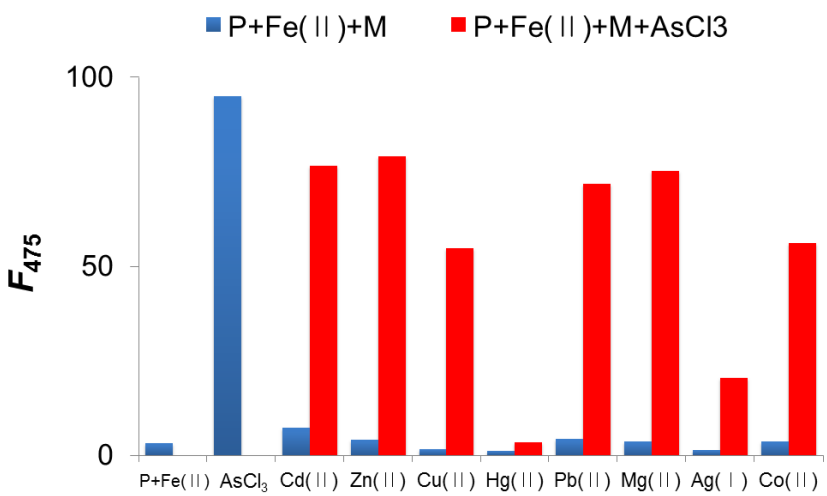


Figure 5. Blue bars represent fluorescence intensity changes of **PBC** (10 μM) at 475 nm upon addition of various metal ions (10 equiv.) respectively in presence of Fe^{2+} (2 equiv.) in acetonitrile. Red bars represent the addition of competitive response upon subsequent addition of $AsCl_3$ (10 equiv.) into the each metal solution (λ_{ex} = 345 nm).

To implement fluorescence titrations, a solution of **PBC** (10 μM) in acetonitrile was titrated with $AsCl_3$ (0-20 equiv.) in the presence of Fe^{2+} (2 equiv.). The addition of $AsCl_3$ to the solution of **PBC** showed an increase of excimer (475 nm) and a decrease of monomer (378 nm) in the intensity of fluorescence after 1 hour (Figure 6a). Also the ratio of excimer to monomer emission increased well. The limit of detection (LOD) was estimated to be 2.28×10^{-7} M (3σ/slope) in a linear range of 0−1 μM $AsCl_3$ with R^2 value of 0.968 (Figure 6b). The LOD is lower than the LD_{50} of lewisite (1.45×10^{-4} M, 30 ppm),[16] indicating sufficient sensitivity of probe **1** for practical application (Table 1).

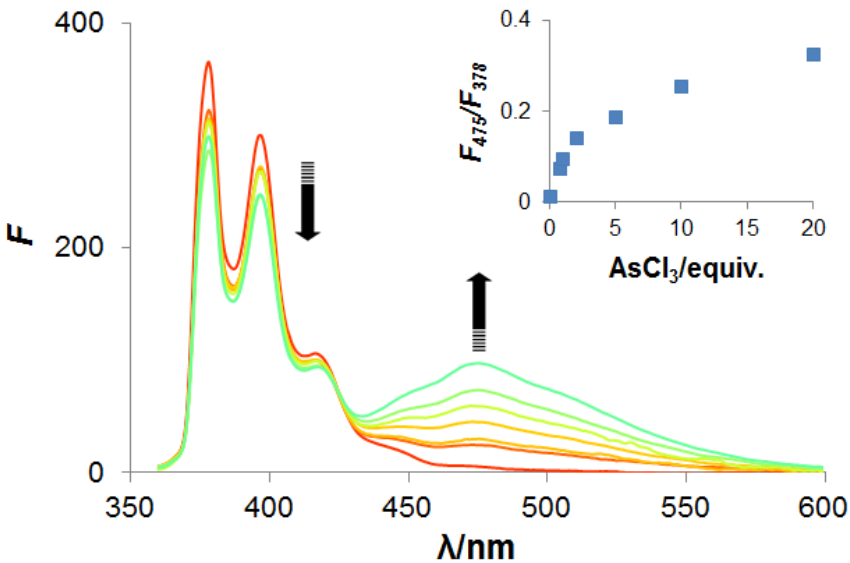


Figure 6. Fluorescence spectra (λ_{ex} = 345 nm) of **PBC** (10 μM) in acetonitrile upon addition of increasing amounts of $AsCl_3$ (0-20 equiv.).

Blister agent	LCt_{50} Inhalation mg·min/m^3	LD_{50} Skin mg/kg
Lewisite (L)	1400	30
Sulfur mustard (HD)	1500	100
Nitrogen mustard (HN-1)	1500	No data available

Table 1. Lethal concentration and time (LCt_{50}) and lethal dose (LD_{50}) of lewisite, sulfur/nitrogen mustard

We tested time-dependence fluorescence titrations, upon addition of $AsCl_3$ (10 equiv.) to a solution of **PBC** (10 μM) and Fe^{2+} (2 equiv.). Measured every 10 minutes, the excimer emission intensity (475 nm) was gradual enhanced when the monomer emission intensity (378 nm) was diminished with an explicit isoemissive point at 429 nm as before the experiment without Fe^{2+} (Figure 7). In the one instance when only **PBC** existed in acetonitrile, disulfide bonds were formed between thiol groups of the probe via auto-oxidaion. The ratio of excimer to monomer emission increased slightly, but the extent was not considerable compared to changes by $AsCl_3$.

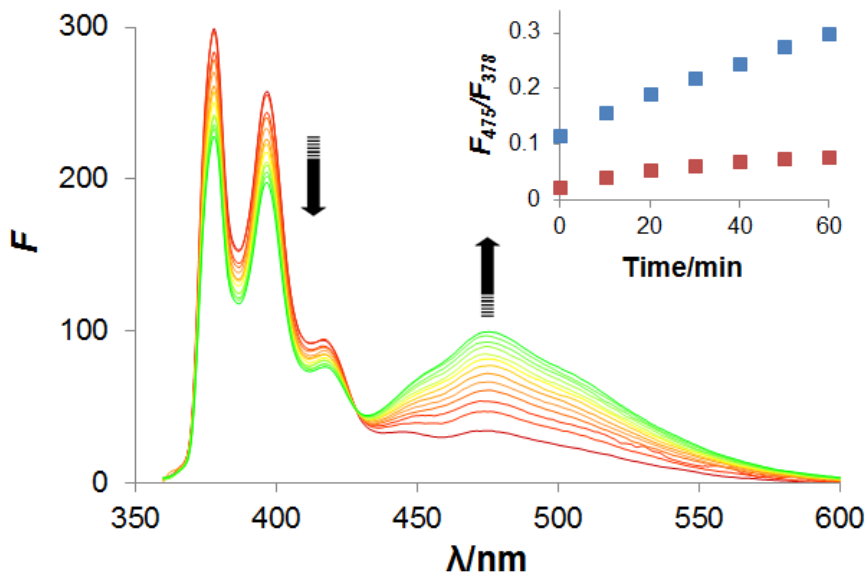


Figure 7. Time dependent fluorescence spectra (λ_{ex} = 345 nm) of solution of **PBC** (10 μM) in acetonitrile in response to addition of $AsCl_3$ (10 equiv.) in presence of Fe^{2+} (2 equiv.). Inset shows time dependent ratio of excimer to monomer emission changes. Blue dots represent the addition of $AsCl_3$ into solution of **PBC** in presence of Fe^{2+} and red dots only **PBC** in acetonitrile.

PBC exhibited a distinct monomer emission at 378 nm along with an obvious excimer emission at 475 nm. The ratio of the excimer to monomer fluorescence intensity (F_E/F_M) is highly sensitive to the distance changes between pyrene monomers. Compared to various other metal ions, the ratio is unique for lewisite simulant. Also the changes of these two different signals, monomer and excimer emission, increase the information content which enable to efficient detect for

lewisite simulant. Both of the two signals are dissimilar to other metal ions. In particular, the monomer emission of **PBC** was almost quenched by other thiophilic metal ions Hg^{2+}, Cu^{2+}, and Ag^{+}. And only the excimer emission intensity of $AsCl_3$ increased along the time-dependence titration. These results allow of discriminating lewisite simulant noticeably from other metal ions.

In conclusion, a new pyrene excimer-based fluorescent probe has been prepared to detect organo-arsenic blister agent lewisite. The mechanism of detection occurred via 1:3 binding between As^{3+} and thiol moieties of three probes. As a result, the ratio of excimer to monomer emission was changed with ratiometry. To selective detect lewisite simulant, Fe^{2+} was added to a solution of **PBC** in acetonitrile to block that cysteine moiety of the probe was chleated with other metal ions. Also outcome of sensitivity test exhibited the detection limit (LOD) is lower than the reported LD_{50}. Thus, this study is worthwhile to detect lewisite simulant efficiently.

B.2.3. Experimental

B.2.3.1. General

Materials

N-(1-methanesulfonyl)benzotriazole, 1-pyrenebutyric acid, L-cysteine, triethylamine (TEA), trifluoroacetic acid (TFA), acetonitrile, dichloromethane (DCM), methanol (MeOH), ethanol (EtOH), chloroform, tetrahydrofuran (THF) and $CDCl_3$ were purchased as reagent grade from Aldrich, Acros, Samchun, TCI and used as received. The used metal salts are $Hg(ClO_4)_2$, $Zn(ClO_4)_2$, $AgNO_3$, $Cd(ClO_4)_2$, $Cu(ClO_4)_2$, $Fe(ClO_4)_2$, $Pb(ClO_4)_2$, $Mg(ClO_4)_2$, $Co(ClO_4)_2$.

Instruments

NMR characterization: ^{1}H and ^{13}C NMR spectra were recorded by Advance 300 and 75 MHz Bruker spectrometer in chloroform-d_3. Chemical shifts were expressed

in parts per million (δ) and reported as s (singlet), d (doublet), t (triplet) and m (multiplet).

Fluorescence experiment: PBC was dissolved in THF to afford a concentration of 10 mM stock solution, which was diluted to 10 μM with acetonitrile. Analyte was added into 10 μM of **PBC**, and photo-physical property of **PBC** was measured after 1 hour. Fluorescence was recorded on Jasco FP-6500.

B.2.3.2. Synthesis of Probe

1-(1H-benzotriazol-1-yl)-4-(pyren-1-yl)butan-1-one (2)

To solution of 1-pyrenebutyric acid (2.88 g, 10 mmol), N-(1-methanesulfonyl)benzotriazole (1.97 g, 10 mmol) in THF (50 mL) was added TEA (2 mL, 14 mmol), and the solution was stirred for 18 hour at 65 ℃. The room temperature-cooled resulting solution was evaporated in vacuo, and the residue was dissolved in $CHCl_3$. The organic layer was washed with water, dried over Na_2SO_4, and evaporated in vacuo. The residue was further purified on a silica-gel column with DCM and MeOH to give a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.49 (m, 2H), 3.55 (t, 2H), 3.58 (t, 2H), 7.47 (t, 1H), 7.60 (t, 1H), 7.90 (d, 1H), 7.96 (d, 1H), 8.00 (d, 2H), 8.10 (m, 3H), 8.13 (d, 1H), 8.15 (d, 1H), 8.22 (d, 1H), 8.33 (d, 1H).

(4-(pyren-1-yl)butanoyl)cysteine (PBC)

Cysteine (93.3 mg, 0.77 mmol) was dissolved in water (1.25 mL), and then TEA (110 μL, 0.77 mmol), acetonitrile (4 mL) were added subsequently. To the solution was added **2** and small amount of THF slowly, and the solution was stirred for 1 hour. The resulting solution was acidified by adding conc. HCl and then evaporated almost to dryness under reduced pressure. The residue was dissolved in saturated Na_2CO_3 and extracted to DCM layer. The factions were combined, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was further purified on

a silica-gel column with DCM and MeOH with 72.0% yield. ^{1}H NMR (DMSO, 300 MHz) δ = 1.18 (t, 1H), 2.03 (m, 2H), 2.34 (t, 2H), 2.76 (d, 1H), 2.87 (d, 1H), 3.33 (t, 2H), 4.45 (m, 1H), 7.95 (d, 1H), 8.06 (d, 1H), 8.14 (d, 2H), 8.20 (d, 1H), 8.23 (d, 1H), 8.26 (d, 1H), 8.28 (d, 1H), 8.39 (d, 1H). ^{13}C NMR (DMSO, 75 MHz) δ = 26.0, 28.0, 32.6, 35.2, 54.9, 124.0, 124.6, 124.7, 125.2, 125.3, 126.5, 126.9, 127.6, 127.9, 128.0, 128.6, 129.7, 130.8, 131.3, 137.0, 172.3, 172.7. HRMS: calculated for $C_{23}H_{22}O_3NS$ $[M+H^+]^+$ 392.1320; found 392.1472.

B.2.4. References and Notes

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국문초록

화학작용제는 심각한 만성후유증을 동반한 무차별적 손상을 일으키므로 위험하다. 화학작용제를 사용하는 테러 공격에 대한 문제의 증가는 저렴하면서도 빠르고 정확한 탐지 방법을 필요로 한다. 화학작용제는 일반적으로 신경작용제와 수포작용제로 구분된다. 신경작용제와 겨자계열 수포작용제에 대한 탐지 방법은 여러가지가 연구되었지만, 비소계열 수포작용제에 대한 탐지방법은 연구된 바가 거의 없다. 유기비소 화합물인 루이사이트는 노출 시 피부와 눈, 점막의 심각한 통증과 염증을 일으키는 수포작용제이다. 여기에, 우리는 루이사이트의 모사체인 삼염화 비소를 탐지하는 화학센서인 다이싸이올기 (dithiol)를 포함하는 7-하이드록시쿠마린 (7-hydroxycoumarin)과 시스테인을 수정한 파이렌 (cysteine-modified pyrene)을 보고한다. 7-하이드록시쿠마린과 파이렌의 형광 신호는 각각 삼염화 비소에 대해 변화되며, 검출한계값은 ppm 범위로 계산된다.

주요어 : 화학작용제, 루이사이트, 형광센서, 비소, 발 (BAL), 시스테인, 쿠마린, 파이렌