A fluorescent probe for a lewisite simulant†

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Herein, we report for the first time a highly sensitive fluorescent probe for the detection of an organoarsenic blister agent simulant, arsenic trichloride (AsCl₃). This probe showed high selectivity to AsCl₃, in the presence of other metal ions, even at low concentrations. Moreover, quantitative determination of AsCl₃ in soil was achieved with a detection limit of 61.2 μmol kg⁻¹ that is sufficiently lower than the reported LD₅₀ of lewisite.

Chemical warfare agents (CWAs) are classified as weapons of mass destruction,¹ because of their potential to cause indiscriminate damage with severe psychological and physiological effects. Although the military use of CWAs has been banned by the Chemical Weapons Convention,² its terroristic use is still a threat to the society.³⁴ Accordingly, various detection methods for CWAs have been developed based on ion-mobility spectrometry,⁵ mass spectrometry,⁶ electrochemistry,⁷ and other techniques.⁸⁻¹⁰ Of late, fluorescence-based detection methods are found to be attractive due to their simplicity, high sensitivity, cost-effectiveness, and fast response, thereby complementing the previous reports. Despite the recent advancements, fluorescence-based detection methods for arsenic compounds¹¹⁻¹³ and other sulfur/nitrogen mustard agents,¹⁴,¹⁵ in this context, there is renewed interest in other categories of CWAs such as organoarsenic compounds.

Although there are several methods for the detection of arsenic compounds,¹⁶ new methods for fast and simple tracking are still desired for environmental monitoring.¹⁷ Lewisite is an organoarsenic compound manufactured for use as a chemical weapon, and it acts as a vesicant and a lung irritant. Herein, we report for the first time a fluorescent probe which allows for a highly sensitive and selective detection of an organoarsenic blister agent, a lewisite simulant.

Lewisite (2-chloroethylarsonous dichloride) has an arsenic core that is a thiophilic metalloid.¹⁸ Thiol-containing biomolecules such as cysteine, glutathione, and lipoic acid are known to strongly bind arsenic,¹⁹ resulting in impaired gluconeogenesis and oxidative phosphorylation.²⁰ Lewisite exposure at low doses causes blisters and acute skin burns in humans. High-dose exposure can lead to severe irreversible issues such as liver necrosis, renal failure, and even the lethal “Lewisite shock”.²¹,²² During World War II, 2,3-dimercaptopropanol (British Anti-Lewisite, BAL) was exploited as an antidote for lewisite.²³ BAL contains two thiol groups that strongly bind to the arsenic core, which attenuates the toxicity. Inspired by the thiophilic characteristics of arsenic species, we have prepared a thiol containing 7-hydroxycoumarin for the fluorescence detection of a lewisite simulant, AsCl₃ (Scheme 1).²⁴

Probe 1 was synthesized in a four-step procedure with an overall yield of 7.3% (Scheme S1, ESI†). Compound 6 was prepared by the Duff reaction,²⁵ and was subjected to reductive amination with 7 to furnish compound 8 in 42% yield.²⁶ Removal of the trityl groups of 8 with a mixture of trifluoroacetic acid and triisopropylsilane was performed to provide compound 1. In addition, probe 2 (cyclic disulfide of 1) was prepared by the air oxidation of 1 for easy handling, because dithiols are relatively unstable and can spontaneously convert into the corresponding oxidized form.²⁷

The performance of probe 1 in response to AsCl₃ was confirmed by mass spectrometry. Probe 2 was pretreated with tris(2-carboxyethyl)phosphine (TCEP) to obtain its reduced form,
probe 1. The mass spectrum obtained upon the addition of TCEP to probe 2 showed a peak at $m/z$ 312.0 (calcd for $[\text{C}_{14}\text{H}_{18}\text{O}_{3}\text{NS}_{2}]^{+}$ 312.07), which indicated the presence of probe 1 (Fig. S7, ESI†). After AsCl$_3$ addition, a new peak appeared at $m/z$ 383.9, indicating the formation of complex 1−As (calcd for $[\text{C}_{14}\text{H}_{15}\text{O}_{3}\text{NS}_{2}\text{As}]^{+}$ 383.97), in a molar ratio of 1:1 (Fig. S8, ESI†).

The photophysical properties of probe 2 in aqueous solution (10 μM) upon the addition of AsCl$_3$ were monitored by UV-Vis and fluorescence spectroscopy in the presence of TCEP (12 μM). Earlier reports suggest that TCEP (12 μM) reduces probe 2 (10 μM) rapidly and stoichiometrically to generate probe 1 (10 μM).28 Probe 1 shows an absorption maximum centered at 320 nm. The addition of AsCl$_3$ induces a 7 nm hypochromic shift, showing the isosbestic point at 332 nm (Fig. 1a). The intrinsic fluorescence of probe 1 ($\lambda_{\text{max}} = 445$ nm, $\Phi_F = 0.62$)29 linearly decreased upon the addition of AsCl$_3$ (0−10 μM) and was completely quenched by 10 equiv. of AsCl$_3$ (Fig. 1b). However, there were no significant responses upon the addition of aqueous solution of various metal ions, Hg$^{2+}$, Ag$^+$, Cu$^{2+}$, Fe$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, and Cd$^{2+}$ (10 equiv. each), demonstrating selective sensing behaviour toward AsCl$_3$. It is noteworthy that probe 1 showed a selective response for AsCl$_3$ over other thiophilic metal ions such as Hg$^{2+}$, Cu$^{2+}$, and Ag$^+$, as these are the major competitors with As$^{3+}$ in real field samples. Subsequent addition of AsCl$_3$ to each metal ion solution resulted in a quenching factor ($F_0/F$) of 16.5−19.7, which was comparable with the value obtained for probe 1 in AsCl$_3$ solution ($F_0/F = 18.3$). Thus, the results obtained can be considered to indicate a competitive response toward AsCl$_3$, even in the presence of other metal ions (Fig. 2a). Furthermore, the quenching response of probe 1 towards AsCl$_3$ allows for the highly sensitive naked-eye detection, while there were no such noticeable changes with other metal ions (Fig. 2b). The limit of detection (LOD) estimated by the 3σ/slope was determined to be $1.34 \times 10^{-6}$ M (1.34 μmol kg$^{-1}$), showing a linear range upon addition of 0−10 μM AsCl$_3$, with an $R^2$ value of 0.981 (Fig. S2, ESI†). The LOD value demonstrates the sufficient sensitivity of probe 1 for practical applications, in accordance with the reported LD$_{50}$ of lewisite (30 mg kg$^{-1}$, 145 μmol kg$^{-1}$) (Table S1, ESI†). It is interesting to note that the fluorescence response toward AsCl$_3$ is visible over a broad pH range of 4−9 (Fig. S1, ESI†), and hence can be used for the detection of CWAs in battle fields under weakly acidic conditions (pH 4.8−5.8).31

From the observed results, we propose a fluorescence quenching mechanism of 1 toward AsCl$_3$. Thiophilic AsCl$_3$ approaches the dithiol group of probe 1, and the dithiol and hydroxyl groups simultaneously react with a nearby AsCl$_3$ to form a coordination complex, releasing 3 equiv. of HCl. The bound As$^{3+}$ triggers intersystem crossing of singlet excited electrons by the heavy atom effect, quenching the fluorescence of the probe.22 However, HCl, a byproduct of AsCl$_3$ detection, did not have a significant influence on the fluorescence change (Fig. S3, ESI†).

Interestingly, probe 2 in the absence of TCEP showed similar results to probe 1 in regard to AsCl$_3$ recognition (Fig. S5, ESI†). The 1,2,5-dithiazepane group on probe 2 would facilitate proximal arrangement of AsCl$_3$ as a result of the thiophilic characteristics. 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 (Fig. S6, ESI†).

$^1$H NMR experiments corroborate the role of 1,2,5-dithiazepane in AsCl$_3$ recognition. Upon the addition of increasing amounts of AsCl$_3$ to the solution of compound 3, the aromatic peaks (6.80−7.24 ppm) gradually shifted to the downfield region, while the $^1$H 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. These observations suggest that As$^{3+}$ is located around the 1,2,5-dithiazepane moiety of compound 3, binding at the phenolic oxygen (Fig. S4, ESI†).
Also, another plausible pathway would be as follows: As$^{3+}$ could be first positioned around the 1,2,5-dithiazeane moiety and reduce disulfide to dithiol,\textsuperscript{33} giving the As-ligated complex after binding of the dithiol with As$^{3+}$\textsuperscript{34}

Since lewisite, upon release, has deleterious effects on human health and the environment, its detection from samples such as soil is therefore very important.\textsuperscript{35} To demonstrate the in-field utility of probe 2, quenching experiments were conducted using AsCl$_3$-spiked soil after extraction with water.\textsuperscript{36} The amount of spiked AsCl$_3$ was quantitatively analyzed in the range of 0–3.5 mM through fluorescence. Most importantly, the LOD, estimated to be 6.12 $\times$ 10$^{-5}$ M (61.2 $\mu$mol kg$^{-1}$), was sufficiently low to detect doses below the LD$_{50}$ in soil.\textsuperscript{30} This demonstrates the utility of the present probes for actual field tests. The fluorescence intensity of probe 2 decreased upon increasing the amount of the spiked AsCl$_3$, and the corresponding intensity change could be detected by the naked eye (Fig. 3).

In summary, we have developed for the first time a fluorescence probe for the lewisite mimic, AsCl$_3$. The dithiol or 1,2,5-dithiazeane group on probe 1 or 2 drives proximal arrangement of the thiophilic AsCl$_3$, thereby resulting in binding of the probe with As$^{3+}$ and fluorescence quenching owing to the heavy atom effect. We have also demonstrated the selective and highly 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 been conducted only through methods requiring heavy equipment.

**Experimental section**

**Synthesis of compound 8**

To a solution of compound 6 (1.99 g, 10 mmol) in methanol/dichloromethane (100 mL/50 mL), compound 7 (6.22 g, 10 mmol) was added, and then a small amount of acetic acid was added. Sodium cyanoborohydride (0.63 g, 10 mmol) was added dropwise to the resulting ice-cooled solution under stirring. After the mixture was stirred for three days at room temperature, it was acidified by adding conc. HCl and the resulting solution was evaporated almost to dryness under reduced pressure. The residue was dissolved in a saturated aqueous solution of Na$_2$CO$_3$ and extracted with dichloromethane.\textsuperscript{26} Combined fractions were dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure to give an amber-colored oil. The residue was further purified on a silica-gel column with hexane and ethyl acetate to give compound 8 in 42.0% yield. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ = 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).

**Synthesis of compound 2**

Compound 8 (3.34 g, 4.2 mmol) was deprotected by treatment with dichloromethane: trifluoroacetic acid: triisopropylsilane (50:47.5:2.5, v/v/v, 400 mL) for 1 hour. Deprotection solution was evaporated under reduced pressure and residual trifluoroacetic acid was removed by co-evaporation with cyclohexane (3 $\times$ 100 mL) and dried in vacuo. The residue was extracted with dichloromethane and dried over anhydrous Na$_2$SO$_4$ and evaporated under reduced pressure to give an amber-coloured oil. The residue was air-oxidized in methanol/water with Na$_2$CO$_3$ prior to purification. The resulting residue was further purified on a silica-gel column with hexane and dichloromethane to give a white solid in 33% yield. $^1$H NMR (CDCl$_3$, 300 MHz) $\delta$ = 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) $\delta$ = 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 (TOF, MeOH): calculated for C$_{14}$H$_{16}$O$_3$NS$_2$ $^+$, [M + H$^+$]$^+$ 310.0572, found 310.0578.

**Fluorescence & UV-Vis experiments**

Probe 2 was dissolved in tetrahydrofuran to afford a stock solution (10 mM), which was then diluted to 10 $\mu$M in distilled water. After in situ reduction of 2 with TCEP (12 $\mu$M), analyte was added to the solution, and the photophysical properties were measured in real time.

**Acknowledgements**

This work was supported by the NRF grant (No. 2015R1A2A1A15055347) funded by the MSIP.
Notes and references

2 States that have neither signed nor acceded to the Chemical Weapons Convention: Angola, Egypt, North Korea, South Sudan; see: https://www.opcw.org/about-opcw/non-member-states, accessed 5/2015.
17 The currently accepted upper limit for arsenic in water is 10 ppb; United States Environmental Protection Agency Publication. EPA816-F-01-004, http://www.epa.gov/safewater/arsenic/compliance.html.
34 We thank an anonymous referee for valuable comments on a plausible mechanism.