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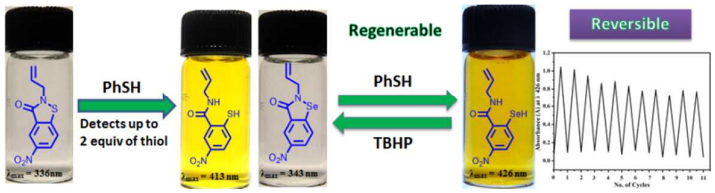

Abstract: Isothiazolone and Isoselenazolone based colorimetric probes for the detection of thiols have been reported. A regenerable probe for the detection of organothiols is developed from isoselenazolone. Both of these probes possess higher selectivity for aromatic thiols, cysteine and glutathione.

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Sensitive and Regenerable Organochalcogen Probes for the Colorimetric Detection of Thiols

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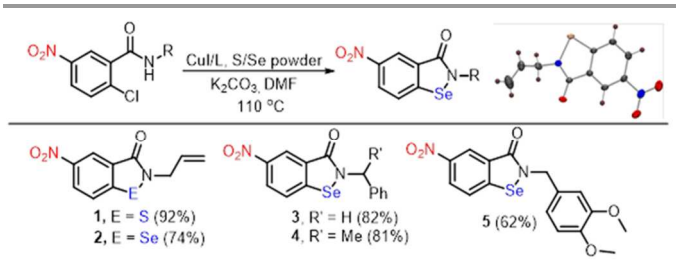
Isothiazolone and Isoselenazolone based colorimetric probes have been reported for the detection of thiols. Isothiazolone probe detected two equiv of thiols. A regenerable probe is developed from isoselenazolone. Both probes possess higher selectivity for aromatic thiols, cysteine and glutathione.

Rapid, sensitive and selective detection of biologically active and toxic molecules is of significant importance in the field of chemical, biological and environmental sciences.[1] Compounds with thiol (-SH) functionality are very important as low molecular weight aliphatic thiol containing amino acid (cysteine)[2a] and peptides (glutathione)[2b-c] play important roles in biological systems while aromatic thiols (thiophenols)[2d] are important reagents and possess broad synthetic utility. In spite of their application as the important building block in organic synthesis, aromatic thiols are considered as toxic and pollutant compounds.[3]

Recently, significant efforts have been made for the development of probes capable of detecting thiols such as cysteine (Cys), glutathione (GSH) and thiophenols.[4,5] Most of these probe molecules react with thiol functionality to form a covalent bond via irreversible reaction and possess probe: thiol detection ratio of 1:1. Therefore, a probe which can detect more than one equiv of thiol and a probe which can be easily recycled are highly desirable.

Isothiazolone and selenazolones are a class of organochalcogen compounds and possess many biological activities.[6] One of the isoselenazolone; ebselen is biologically non-toxic and decomposes hydroperoxides catalytically utilizing organothiol substrates. In continuation of our work on organochalcogen chemistry,[7] we report new and mechanistically different low molecular weight organochalcogen colorimetric probes with the following salient features; a) high specificity of both probes **1** and **2** for thiophenols, cysteine and glutathione, b) rapid response (colorless to bright yellow), c) detection of two equiv thiophenols by isothiazolone probe **1**, d) regenerability of isoselenazolone probe **2** for >10 cycles. Isothiazolone **1** and isoselenazolones **2-5** were synthesized from corresponding 2-chlorobenzamides by Cu-catalyzed thiolation/ selenation reaction.[6] The presence of S-N and Se-N bonds in **1-4** is also established by single crystal X-ray studies (Scheme 1).



Scheme 1 Synthesis of organochalcogen compounds used in the study

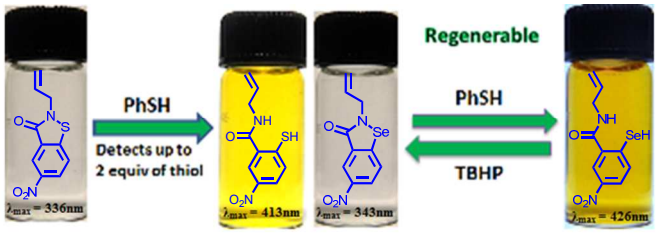


Fig. 1 Color change by the addition of thiol to organochalcogen probes

Isothiazolone **1** (100μM) absorbs at 336 nm (Figures 1-2). With increasing concentration of PhSH, peak at 336 nm due to **1** decreased gradually with appearance of a new peak at 413 nm and immediate yellow color appearance was noticed. The stoichiometric ratio between probe **1** and PhSH was observed as 1:2 based on the change of absorbance at 413 nm which suggest that the probe **1** can detect up to 2 equiv of PhSH. Next various aromatic and aliphatic thiols; ethane-, *n*-hexane-, *t*-butyl-thiols, benzyl thiol, 2-mercapto pyridine, 2-amino-, 2-methoxy-, 4-methoxy-, 4-methyl-, 4-chloro-thiophenols, biologically important organic molecules; glucose, L-proline, ascorbic acid, glycine, alanine, arginine, GSH, cysteine, N-acetyl-L-cysteine,

DTT^{red} and nucleophiles like aniline, 4-methoxy phenol were investigated to study selectivity of probe **1** towards these substrates

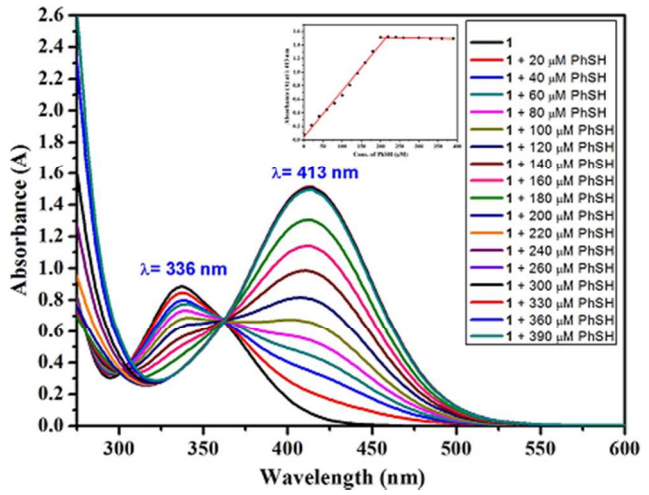


Fig. 2 Effect of [PhSH] on the absorption spectra of **1** (λ = 336 nm) was studied in PBS buffer (10mM, pH 7.4) with 25% acetonitrile as co-solvent. Significant red shift observed to λ = 413 nm upon addition of PhSH (upto 2 equiv). This shift in wavelength (Δλ = 77 nm) resulted into visual color change from colourless to bright yellow. Inset: Absorbance at 413 nm as a function of PhSH concentration indicates 1:2 ratio for probe **1** and PhSH.

(see Figure S39 in SI). Probe **1** exhibited excellent selectivity towards thiophenols, cysteine, glutathione and dithiothreitol compared to the remainder of the substrates. Next, the effect of various metal ions on the performance of probe **1** was studied by preparing solution containing probe **1** (100 μM) and the metal ion (200 μM). Salts of Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Pd^{2+}, Ba^{2+}, Ni^{2+}, Co^{2+}, Al^{3+} Cr^{3+}, Cd^{2+} didn't interfere in the analysis as the yellow color remained unaffected and also UV-Visible absorption spectra were unchanged. On the other hand, Cu^{2+}, Hg^{2+} and Ag^+ inhibited activity of probe **1** completely presumably due to their ability to form coordination complexes with PhSH. For these metals, equimolar (200 μM) solution of chelating EDTA ligand was added to shield them and probe **1** regained its ability to sense the thiol colorimetrically (page S50-S52 in SI).

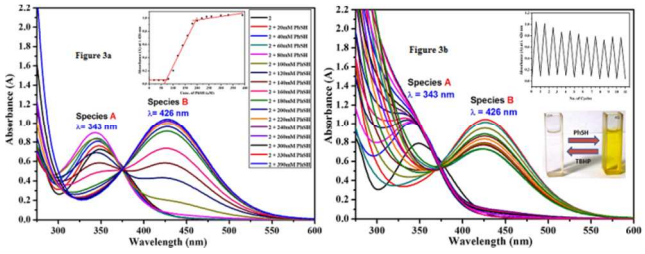
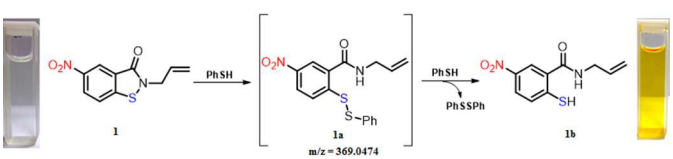


Fig. 3 (3a) Effect of [PhSH] on the absorption spectra of **2** (λ = 349 nm). Colourless species **A** (λ = 343 nm) formed upon addition of PhSH (0-1 equiv) while a significant red shift (Δλ = 83 nm) was observed upon gradual addition of PhSH (1-2 equiv) due to formation of species **B** (λ = 426 nm). (3b) Reversibility between colored species **B** and colorless species **A** by the addition of PhSH and *tert*-butyl hydroperoxide (TBHP) respectively. Inset: Reversibility of probe **2** was checked for >10 cycles by observing absorption spectrum.

Figure 3a describes the change in the UV spectrum of probe **2** (100 μM) when the PhSH solution is added to it in PBS buffer (10mM, pH 7.4): acetonitrile (75:25). Upon gradual addition of one equiv of PhSH, absorption peak of **2** at 349 nm is slightly blue shifted to 343 nm without any colour change. This peak at 343 nm is gradually decreased with an emergence of a new peak at 426 nm upon gradual addition of one more equivalent of PhSH. A rapid visual colour change is observed from colourless to bright yellow in solution. Absorbance at 426 nm as a function of PhSH concentration indicates 1:1 ratio for PhSH and probe **2** (Inset of Figure 3a). Probe **2** displayed similar selectivity towards aliphatic and aromatic thiols, biomolecules and also similar interference by metal salts as the case with probe **1**. However, in addition to Cu^{2+}, Hg^{2+} ions Ag^+, Cd^{2+} ion also inhibited the sensing property of isoselenazolone **2**. (see Figure S44, S45 in SI). Isoselenazolone functions as a catalyst using thiol substrates for the reduction of hydroperoxides. To see the reusability of probes in the sensing activity, yellow coloured solution of isothiazolone **1** and PhSH was treated with excess of TBHP. Unfortunately, regeneration of probe **1** was not noticed as absorption spectra of the solution remained unshifted. Next, isoselenazolones **2-5** were tested for regenerability. To the yellow solution of probe **2** and PhSH, excess of TBHP (5 equiv) was mixed and allowed to react for a minute. It resulted into colourless solution and UV spectrum was again blue shifted to 343 nm. Again PhSH (2 equiv) was added to this colourless solution and it resulted into red-shift to 426 nm in the absorption spectra. Reversibility of probe **2** was evaluated for 11 cycles by alternative addition of oxidant TBHP and reductant PhSH (Figure 3b). Other isoselenazolone derivatives **3-5** possessing structural similarities with probe **2** were also studied for regenerable thiol detection. Isoselenazolone **3-5** showed similar results as probe **2** but with poor regenerability as precipitation was observed after two cycles. Next we examined detection limits for probes **1** and **2** and they are found sensitive towards the detection of thiol up to 2μM and 10μM respectively (See Figure S50 in SI)

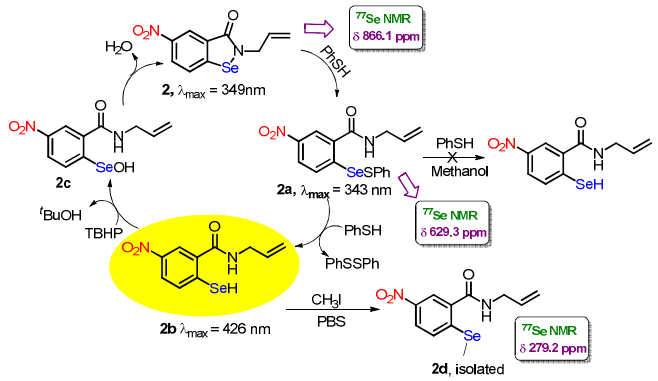
We were interested in detecting species responsible for the color change upon addition of PhSH to probe **1**. Therefore, a stoichiometric reaction was carried out between probe **1** and PhSH in PBS buffer: acetonitrile (75:25) at room temperature followed by isolation of compound, which confirmed to be **1b** after characterization (Scheme 2). UV spectrum on isolated **1b** (λ_{max} = 413 nm) provided further confirmation. We believe that probe **1** forms unsymmetrical disulfide **1a** with one equiv of PhSH. Disulfide **1a** seems to be transient species (detected by mass spectrometry only) and converts immediately into coloured thiol **1b**. This could correlate the unprecedented detection of two equiv of thiophenol.



Scheme 2 Isolation of **1b** which is responsible for colorimetric detection of PhSH

For mechanistic insight, probe **2** was reacted with one equiv of PhSH under identical experimental conditions and isolated product was confirmed as selenenylsulfide **2a**. UV absorption spectra of isolated **2a** gave λ_{max} at 343 nm. To the solution of **2a**, an equiv of PhSH was added, which resulted in the red shift to 426 nm with appearance of bright yellow colour. The sample was subjected for mass analysis and noticed to be selenol **2b** (*m/z* = 284.9). Worth

noticing, aqueous medium is important for the generation of **2b** from **2a** because **2a** failed to show any color change and also change in the UV absorption spectrum upon the addition of one equiv of PhSH in CH_3OH/ CH_3CN medium.



Scheme 3 Mechanistic pathway for reversible nature of probe **2** and trapping of key intermediate selenol **2b** by quenching it with CH_3I

Therefore, aqueous medium was necessary for generation of selenol **2b** which is responsible for sensing activity. The unprecedented solvent dependent generation of selenol **2b** from **2a** was further validated by reacting in situ generated **2b** with CH_3I. Complete conversion of selenol **2b** into methyl selenide **2d** was observed, which was isolated and characterized. Interestingly, reaction in CH_3OH failed to provide any **2d**. TBHP was added to in situ generated selenol **2b** to understand the intermediate involved in the regenerability and solution was analysed by mass spectrometry, which showed the molecular ion peak for selenenic acid **2c** (*m/z* = 300.9). Thus regenerable mode of probe **2** is presented in Scheme 3 based on mass analysis and control experiments.

Conclusions

In summary, we have developed two low molecular weight organochalcogen probes for the colorimetric detection of thiols which work well in an aqueous neutral (pH 7.4) medium with instantaneous response. Both probes showed selectivity for aromatic thiols, cysteine and glutathione. Isothiazolone probe efficiently detects two equiv of thiol following irreversible pathway, while isoselenazolone probe detected in a regenerable manner for >10 cycles. Important character of probe **2** is that the detection of thiols can be conducted in a reversible manner by simple visual inspection without the use of any expensive instrument. The species, responsible for the color change are also characterized. The formation of benzamide derived thiol and selenol in the aqueous medium is responsible for characteristic color change.

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Notes and references

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Electronic Supplementary Information (ESI) available: [Experimental procedures, spectroscopic data, crystal structures for **1-4** (CCDC no. 978032, 978031, 953727, 978033)]. See DOI: 10.1039/c000000x/

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