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Selective Reduction of Peptide Isothiazolidin-3-ones

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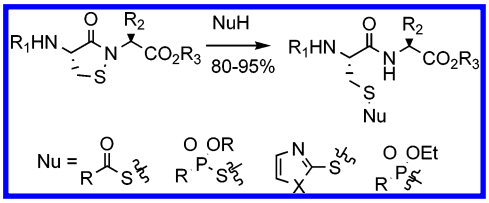
ABSTRACT

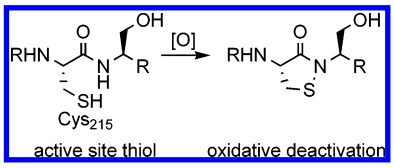


Isothiazolidinones are a rare but potentially important chemical moiety in biochemistry. We report the identification of several thiol, phosphinate, and carbon nucleophiles that form covalent adducts by addition to the sulfenamide sulfur. This reduction is selective for isothiazolidinones over similar peptide disulfides. We synthesized a coumarin-based thioacid nucleophile which shows a marked fluorescence increase after addition to an isothiazolidinone sulfenamide bond.

PTP-1B is an archetypical protein tyrosine phosphatase (PTP)[1] and has been implicated in a wide variety of diseases including type II diabetes[2] and obesity.[3] Attempts to develop cell-permeable active site[4] inhibitors have met with limited success due to the anionic nature of most inhibitors. Although researchers have discovered inhibitors that target an allosteric site distal to the active site[5] as well as a covalent modifier that targets Cys121,[6] a second allosteric site, alternative strategies of inhibition are needed to realize the therapeutic potential of this target.

Recently, a new oxidized form of PTP-1B was observed crystallographically, in which the active site cysteine forms an isothiazolidin-3-one (also referred to as an isothiazolidinone or sulfenamide) (Scheme 1).[7] PTP-1B undergoes

Scheme 1. Isothiazolidin-3-one Form of PTP-1B



reversible oxidation of the active site cysteine upon exposure to reactive oxygen species both in biochemical[8] and cellular[9] models, and direct evidence for a PTP-1B sulfenic acid had

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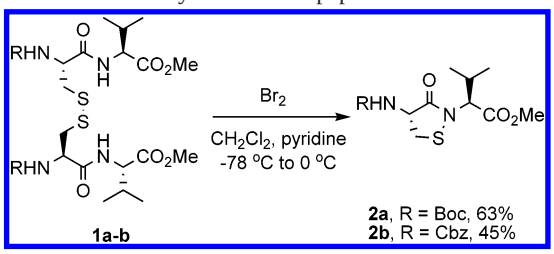
been previously reported.[10] However, it remains unclear how the protein protects itself against irreversible oxidation to the sulfinic and sulfonic acid forms. The isothiazolidin-3-one provides a plausible mechanism to prevent overoxidation.

Because this isothiazolidinone represents an unusual chemical moiety in biochemistry, we sought to develop a set of chemical probes that could be used to detect the presence of the PTP-1B sulfenamide inside the cell or as potential "warheads" for inhibitors. We first needed a chemical model of the isothiazolidinone.

Although an isothiazolidinone-containing small molecule has been previously reported as a chemical model of oxidized PTP-1B,[11] this model is not based on a peptide backbone.

We turned to a class of molecules first reported by Morin and colleagues[12] as a potentially more biologically relevant model of the PTP-1B sulfenamide. Cystinyl peptide disulfides **1a**,**b** can be oxidized with bromine to give the corresponding dipeptide isothiazolidinones **2a**,**b** (Scheme 2).

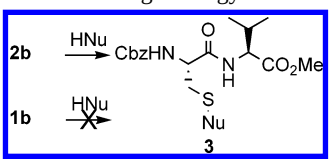
Scheme 2. Synthesis of Dipeptide Sulfenamides



Although the reaction is very sensitive to stoichiometry, the best results were generally obtained with a significant excess of bromine, slow warming, and reductive workup with aqueous $Na_2S_2O_3$.

Because isothiazolidinones are rare in biochemistry, we sought to identify nucleophiles that would specifically react with the isothiazolidinone under physiological conditions. Our strategy depended on the electrophilicity of the isothiazolidinone, focusing on nucleophiles that were too weak to react with common physiological electrophiles such as disulfides (Scheme 3).

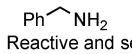
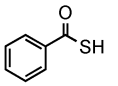
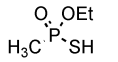
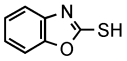
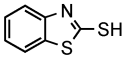
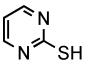
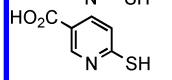
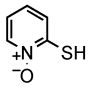
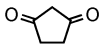
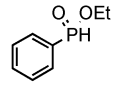
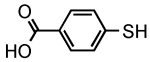
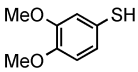
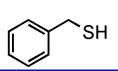
Scheme 3. Screening Strategy for Nucleophiles



Isothiazolidinone **2b** in ethanolic solution was treated with a variety of sulfur-based, phosphorus-based, nitrogen-based, and oxygen-based nucleophiles with alkyl, aromatic, and acyl variants of each. In general, we found that oxygen- and nitrogen-based nucleophiles were too weak, whereas sulfur- and phosphorus-based nucleophiles were sufficient to break the sulfenamide bond. The regiochemistry of these compounds was verified by ^{1}H NMR, ^{13}C NMR, and IR spectra (see Supporting Information).

Next, we sought to eliminate those nucleophiles that were so potent that they would react with disulfides as well as with sulfenamide bonds. By cross-screening the reactive nucleophiles with the disulfide **1b**, we were able to determine which nucleophiles were chemoselective for the sulfenamide moiety. The results are shown in Table 1.

Table 1. Cross-Screening of **1b** and **2b** with Nucleophiles

HNu	reaction with **1b**	reaction with **2b**
Non-reactive:		
	no reaction	no reaction
Reactive and selective:		
	no reaction	product **3a**
	no reaction	product **3b**
	no reaction	product **3c**
	no reaction	product **3d**
	no reaction	product **3e**
	no reaction	product **3f**
	no reaction	product **3g**
	no reaction	product **3h**
	no reaction	product **3i**
Reactive but non-selective:		
	product **3j**	product **3j**
	product **3k**	product **3k**
	product **3l**	product **3l**

Interestingly, all thiophenols, even electron-deficient ones, failed to achieve any selectivity.

Because fluorescence spectroscopy is one of the most sensitive and straightforward detection systems, we sought to generate a fluorescent probe in which nucleophile addition to a sulfenamide would alter the chromophore.

Coumarin-3-carboxylic acids possess an intramolecular hydrogen bond between the acid functionality and the

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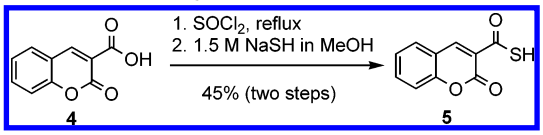
2-carbonyl.[13] Because the internal ester is intimately linked to the fluorescent properties of the molecule, we reasoned that coversion of a coumarin-3-thioacid to a disulfide would alter the fluorescent properties of the coumarin.

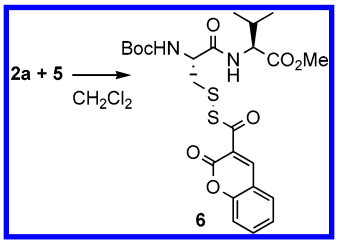
Coumarin-3-thioacid (**5**) was synthesized in two steps from commercially available coumarin-3-carboxylic acid. Formation of the acid chloride in neat thionyl chloride followed by reaction with concentrated NaSH yielded the thioacid **5** in 45% overall yield (Scheme 4).

Scheme 4. Synthesis of Coumarin Thioacid



The coumarin thioacid shows a reactivity profile similar to that for the thiobenzoic acid on which the structure was based (product **3a**, Table 1); it reacts with the sulfenamide quantitatively within 1 h but shows absolutely no reactivity with the disulfide even after 48 h at room temperature (Scheme 5).

Scheme 5. Synthesis of the Coumarin−Peptide Conjugate



Fluorescence spectroscopy of the coumarin−cysteine conjugate (**6**) and coumarin thioacid (**5**) shows a marked increase in fluorescence only when the coumarin−cysteine conjugate is formed, with an excitation maximum at 392 nm and an emission maximum at 422 nm (Figure 1).

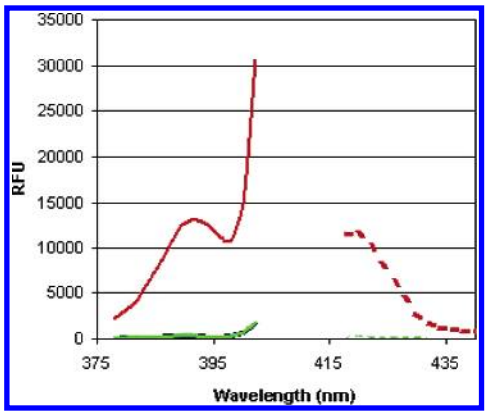


Figure 1. Excitation (solid) and emission (dashed) spectra of **6** (red) and **5** (green).

In conclusion, we have applied a screening strategy to identify several classes of nucleophiles which chemoselectively react with isothiazolidinones but not disulfides. One nucleophile, a thioacid, was modified to act as a fluorescent detector of isothiazolidinones. This strategy could be used to develop PTP-1B inhibitors as well as to elucidate the biological role of isothiazolidinones with fluorescence spectroscopy.

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Supporting Information Available: Experimental procedures and characterizations of compounds **3a**−**l**, **5**, and **6** (^{1}H NMR, ^{13}C NMR, MS). This material is available free of charge via the Internet at http://pubs.acs.org.

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