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In Situ "Click" Assembly of Small Molecule Matrix Metalloprotease Inhibitors Containing Zinc-Chelating Groups

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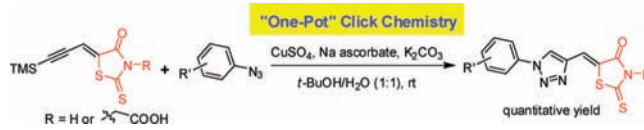
ABSTRACT



A panel of small molecule-based MMP inhibitors containing rhodanine warheads was assembled using "one-pot" click chemistry. Upon biological screening, moderate inhibitors were identified which specifically targets MMP-7 and MMP-13 over other MMPs.

Matrix metalloproteases (MMPs) consist of a family of zinc-dependent endoproteinases which are involved in tissue remodeling and degradation of the extracellular matrix (ECM), angiogenesis, and cell motility. Currently, at least 26 human MMPs have been discovered. Abnormal MMP activities are linked to many serious human diseases.[1] Therefore, the development of potent MMP inhibitors has received considerable interest in recent years.[2] In most cases, MMP inhibitors contain a well-known zinc-binding group (ZBG) that chelates to the catalytic Zn^{2+} ion located in the enzyme active site and a peptide or nonpeptide group that interacts with the surrounding sites. One of the most widely exploited ZBGs of MMP inhibitors is the hydroxamic acid group, which has produced numerous nanomolar inhibitors but has not been successful in clinical trials.[1,2] The reason is that inhibitors containing this ZBG normally exhibit very potent but broad-spectrum inhibition toward most metalloproteases rather than MMPs alone. Furthermore, they exhibit poor pharmacokinetic and bioavailability properties. As a result, there has been intensive research efforts in the discovery of new ZBGs which may be further developed into highly potent and selective MMP inhibitors.[3] Of particular interest is the work of Cohen et al., where they exploited the pyrone- and thiopyrone-containing moieties as potential ZBGs and systematically evaluated their inhibitory properties against various classes of metalloproteases including MMPs and Anthrax Lethal Factor (ALF).[3b−e] Our ongoing research interests in the use of high-throughput amenable chemistry (such as "click chemistry" and amide-forming reactions)[4] have recently led us to the development of "click" assembled, hydroxamate-containing peptide analogues which showed micromolar inhibitory activities against various MMPs.[5] Herein, we describe a "one-pot" click chemistry procedure for the rapid assembly of small molecule

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MMP inhibitors containing novel rhodanine ZBGs (Figure 1).

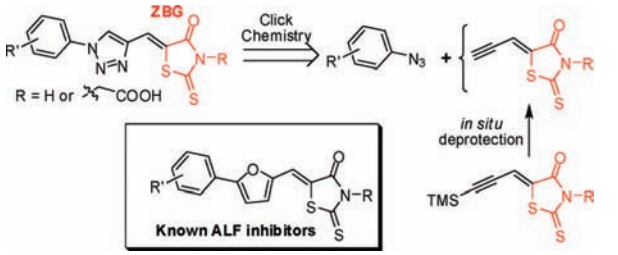
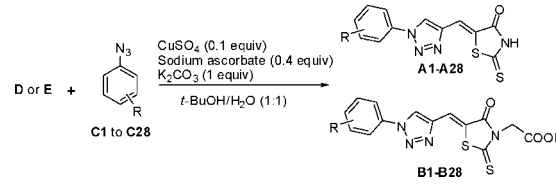


Figure 1. General structures of MMP inhibitors containing a rhodanine warhead and the "one-pot" click chemistry used in our approach. The rhodanine ZBG is highlighted (in red). Inset: known ALF inhibitors.

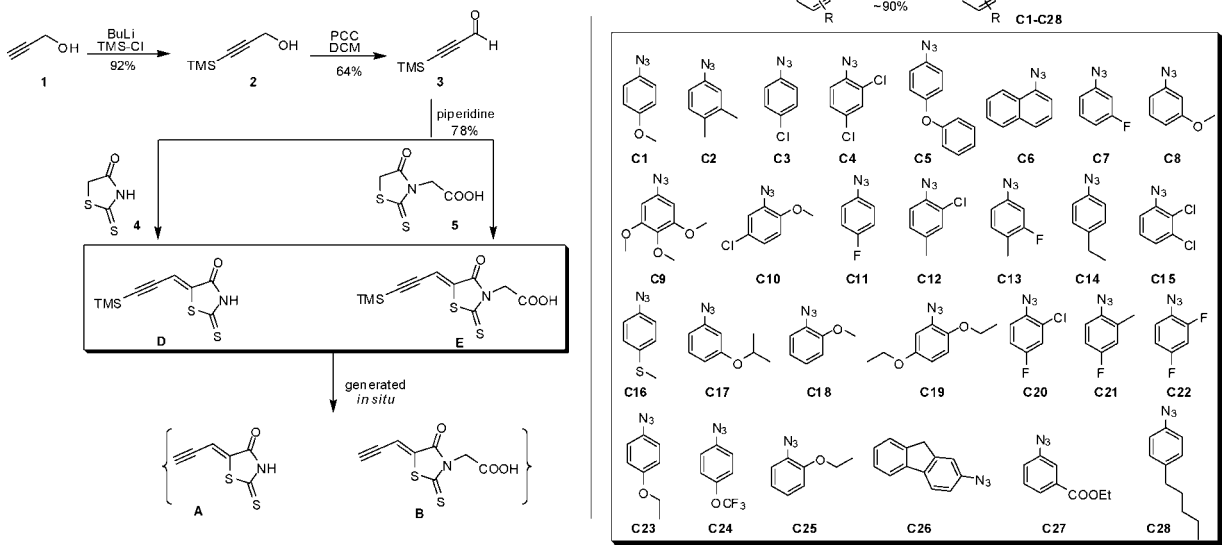
The design principle of our inhibitors was based on a recent report that rhodanine-based molecules (inset in Figure 1), identified from high-throughput screening (HTS), are efficient micromolar inhibitors of ALF.[6] Crystallographic analysis of the inhibitor−ALF complex further confirmed that the rhodanine ring is capable of interacting with Zn^{2+} ion via the thiazolidine sulfur atom. However, MMP inhibitors based on rhodanine ZBGs have yet to be reported in the literature. Our aims in this project are therefore to evaluate (1) whether rhodanine-containing small molecules constitute a novel class of potential MMP inhibitors and (2) whether our previously reported "click" approach could be used to rapidly assemble these inhibitors.[5] The Cu(I)-catalyzed 1,3-dipolar cycloaddition between an alkyne and azide is the prime example of "click chemistry" popularized by Sharpless.[7] In most cases, the 1,4-disubstituted-1,2,3-triazole ring is formed regioselectively by reacting a terminal alkyne with an azide in the presence of a Cu(I) catalyst. In recent years, interests have been drawn toward the "one-pot" click chemistry approaches using in situ generated alkynes or azides.[8] In our case, we found the two TMS-free rhodanine warheads **A** and **B** (Scheme 1) rapidly decomposed during column purification and could not be isolated in acceptable purity. We therefore developed a "one-pot" approach by using the TMS-protected rhodanine warheads instead (**D** and **E**; see also Figure 1) during click chemistry. Synthesis of the TMS-protected rhodanines involved three steps. Propargyl alcohol **1** was TMS-protected to give **2**, which was subsequently oxidized to give aldehyde **3**. The base-catalyzed Knoevenagel condensation between **3** and **4**/**5** then afforded the TMS-protected, alkyne-containing **D** and **E**. A total of 28 aromatic azides, **C1**−**C28**, were synthesized from the corresponding arylamines using standard azidation methods (Scheme 1).

For the "one-pot" click chemistry between the TMS-protected **D** and **E** and the azides **C** (Scheme 2), a variety of conditions were explored to ensure efficient TMS cleavage while maintaining minimum interference of the subsequent "click" assembly of the product followed by direct in situ

Scheme 2. "One-Pot" Click Chemistry of Rhodanine Inhibitors



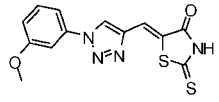
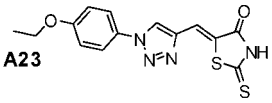
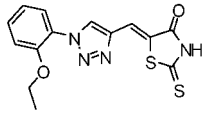
Scheme 1. (Left) Synthetic Routes of Alkyne Building Blocks **A** and **B**. (Right) Synthesis and List of Aromatic Azides Used



biological screening. We eventually found that **D** or **E** (1 equiv) and **C1**−**C28** (1.1 equiv) with K_2CO_3 (1 equiv; used for in situ deprotection of TMS) in the presence of $CuSO_4$ (0.1 equiv) and sodium ascorbate (0.4 equiv) in a solvent mixture of *t*-BuOH/H_2O (1:1) afforded the 56-membered, rhodanine-containing small molecule inhibitors in nearly quantitative yields with minimum side products, as judged by LCMS and NMR characterizations (see the Supporting Information). We further confirmed that these compounds were indeed suitable for direct in situ screening without further purifications.

We next obtained the inhibitor fingerprints of the 56-membered rhodanines against different MMPs (Supporting Information). A standard microplate-based enzymatic assay was adopted as previously described.[5] Representatives from different classes of MMPs, including collagenases (MMP-8 and MMP-13), matrilysins (MMP-7), gelatinases (MMP-9), and membrane-type MMPs (MMP-14), were tested. At an inhibitor concentration of 20 μM, most compounds showed good inhibition toward MMP-13 while displaying very weak/no inhibition toward MMP-7/-8 and MMP-9/-14, respectively (see the Supporting Information). The most potent inhibitors appeared to be those containing alkoxyl substituents at the aromatic ring (Table 1). These compounds were resynthesized, purified, characterized (LCMS and NMR), and further studied in detail (to obtain their IC_{50}/K_i); as shown in Table 1, **A25**, the most potent inhibitor, displayed K_i values of 14.1 and 20.9 μM against MMP-7 and MMP-13, respectively, and appeared to show some degrees of selectivity over other MMPs tested. These values are moderate at best but well expected if one considers that the parental inhibitor (inset in Figure 1) showed micromolar inhibition against ALF[6] and the previously reported "click-based" peptide hydroxamates were also micromolar MMP inhibitors.[5] Nevertheless, our results unambiguously establish the feasibility of using rhodanines as novel ZBGs of MMP inhibitors.

Table 1. Inhibition of the Three Selected Inhibitors

	IC_{50} (K_i) in μM		
	MMP-7	**MMP-8**	**MMP-13**
A8[a] 	ND	ND	ND
A23 	37.3 (17.6)	73.7 (ND)	42.5 (24.8)
A25 	24.0 (14.1)	97.9 (ND)	36.5 (20.9)

[a] Compound precipitated during the IC_{50}/K_i experiments; ND = not determined.

Molecular modeling was carried out for **A25** with MMP-7 and MMP-13. Compared to MMP-7 (which has a shallow S_1' pocket), MMP-13 has a relatively deep S_1' pocket. As shown in Figure 2, **A25** fit nicely into the active site of both enzymes, with the two sulfur atoms from the rhodanine warhead chelating to the zinc ion. In addition, the aromatic moiety in **A25** appeared to extend snugly into the S_1' binding site of both enzymes. The docking results thus appeared to indicate that rhodanines are likely a novel class of ZBGs of MMP inhibitors.

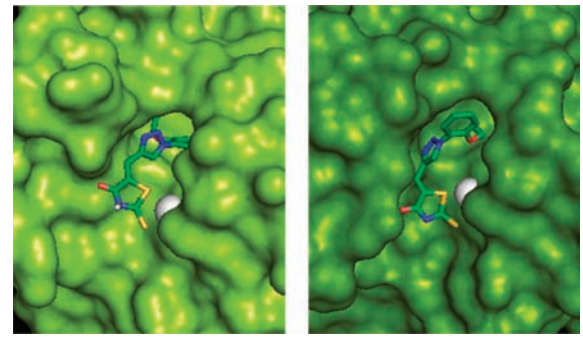


Figure 2. Molecular modeling of **A25** in the active site of MMP-7 (left) and MMP-13 (right). The zinc ion in the active site is shown as a white sphere.

Several key findings arose from our current work. First, we have been able to show rhodanines as a novel class of ZBGs of MMP inhibitors for the first time. Second, we have demonstrated the compatibility of these ZBGs with "click chemistry" for rapid assembly and direct in situ screening of potential MMP inhibitors. Finally, we have developed a "one-pot" click chemistry protocol for above applications. Our best inhibitors possess moderate inhibitory activities against MMPs currently, but upon further optimizations, they may emerge as a new class of potent small molecule-based MMP inhibitors.

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Supporting Information Available: Experimental details and characterization of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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