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Rapid Assembly of Matrix Metalloprotease Inhibitors Using Click Chemistry

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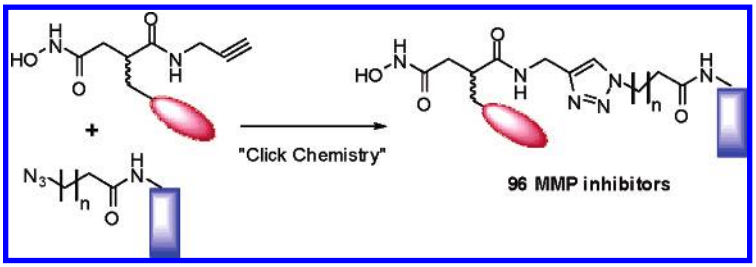
ABSTRACT



A panel of 96 metalloprotease inhibitors was assembled using "click chemistry" by reacting eight zinc-binding hydroxamate warheads with 12 azide building blocks. Screens of the bidentate compounds against representative metalloproteases provided discerning inhibition fingerprints, revealing compounds with low micromolar potency against MMP-7. The relative ease and convenience of the strategy in constructing focused chemical libraries for rapid in situ screening of MMPs is thereby demonstrated.

Matrix metalloproteases (MMPs) are a family of zinc-containing metalloproteases which play critical roles in a variety of physiological processes. Tight regulation of these enzymes is essential in maintaining normal cellular function and development. As one of the most important classes of therapeutic targets, MMPs are responsible for a variety of human diseases, including arthritis, Alzheimer's disease, cancer, and heart diseases.[1] One of the most widely exploited scaffolds of MMP inhibitors is the peptide-based succinyl hydroxamate.[2] Inhibitors containing this kind of zinc-binding groups normally exhibit broad-spectrum inhibition toward most metalloproteases, rather than MMPs alone. This is largely due to the structural similarity in the active sites of metalloproteases which possess highly conserved zinc-binding residues essential for enzyme catalysis. Therefore, it has been an ongoing challenge to develop highly efficient synthetic strategies that allow rapid generation and screening of small molecule inhibitors possessing not only high potency but more importantly good selectivity toward MMPs.[1]

Fragment-based assembly, which allows medicinal chemists to explore N^2 possibilities with N+N combinations, is widely employed for high-throughput drug discovery.[3] It typically involves a two-step process including fragment identification and fragment linkage. A number of methods have been developed to assist in this process,[4] including NMR/X-ray approaches,[4a] the MS-based tethering strategy,[4b] and "click chemistry" coupled with in situ screening.[4c] Among them, approaches based on "click chemistry" [5] were

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shown to be a highly versatile and effective choice for rapid synthesis and identification of inhibitors against a number of biological targets, including HIV protease,[6a,b] sulfotransferases,[6c] fucosyltransferases,[6d] protein tyrosine phosphatases,[6e] acetylcholinesterase,[6f] and others.[6g] However, it remains to be seen whether this approach is applicable against other important therapeutic targets, e.g., matrix metalloproteases. Herein, we describe, for the first time, a "click chemistry" approach for the rapid synthesis/assembly of a small molecule library based on different succinyl hydroxamates, and subsequent in situ screening for identification of candidate hits which possess moderate inhibitory activity against MMPs over other metalloproteases. Our approach thus lays the foundation for future exploration of more potent and selective MMP inhibitors, in high throughput, using "click chemistry".

Our library design was based on the general structure of hydroxamate-based MMP inhibitors (Figure 1). With this

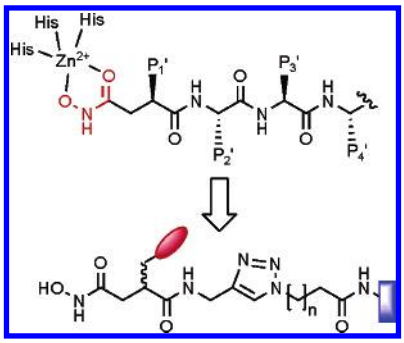

Figure 1. Structure of (top) general hydroxamate inhibitors and (bottom) "click chemistry" inhibitors reported herein against MMPs.

class of inhibitors, it was previously shown that (1) hydrophobic P_1' residues are in general preferred, (2) a variety of substitutions are tolerated at P_2' and P_3' positions, and (3) hydrophobic P_4' residues are preferred and sometimes could confer a good degree of specificity among different MMPs.[2] As such, a total of eight succinyl hydroxamates bearing a variety of alkyl, cycloalkyl, and aromatic side chains were synthesized as shown in Scheme 1. A common alkyne handle was tethered to each warhead, facilitating the subsequent assembly with twelve different azides (Scheme 2) using "click chemistry". The azides bear a hydrophobic moiety connected via a linker with a varying alkyl length ($n = 2-4$). By changing the linker length of the azides, our design facili-

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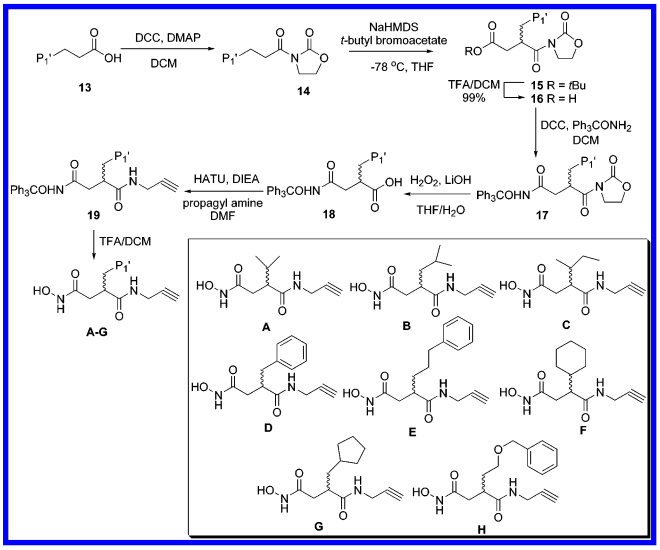
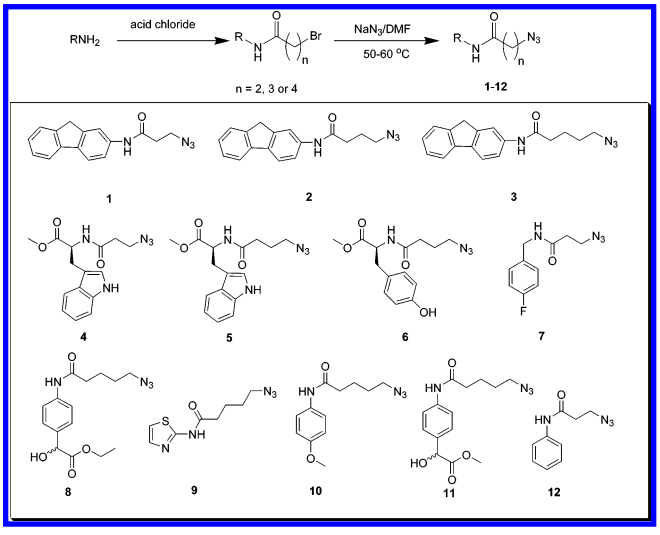
Scheme 1. Synthesis of Eight Alkyne-Containing Warheads



tates the projection of the hydrophobic moiety into the P_4' binding pocket of a targeted MMP, thereby improving both potency and specificity of the resulting inhibitors.

When devising a suitable route for the warhead synthesis, two key criteria were considered: (1) the strategy has to be general, enabling the facile incorporation of a wide variety of P_1' side chains and, when necessary, with precise stereospecific control of the chiral center located in the warhead; (2) the resulting warhead must be compatible with standard solid-phase chemistry, allowing future large-scale library synthesis. Both criteria were fulfilled by the method shown in Scheme 1, recently developed by us for the synthesis of **18B** (a hydroxamate warhead bearing an isobutyl side chain).[7] The strategy is based on the well-established enolate chemistry with Evan's oxazolidinone auxiliary.[8] The hydroxamate was protected with a trityl group, ensuring its compatibility with standard Fmoc peptide chemistry/TFA cleavage procedures.

Scheme 2. Synthesis of 12 Azides



Overkleeft and co-workers have independently reported a similar method based on the synthesis of *N*-Boc-*O*-TBS-hydroxamates.[9] Two additional steps were performed to introduce an alkyne handle in the warhead for use in the subsequent "click chemistry". As shown in Scheme 1, a total of eight different hydrophobic warheads were synthesized (**A**-**H**).

Briefly, a suitable carboxylic acid, **13**, was converted to **14** with oxazolidinone, followed by enolate chemistry to introduce the succinyl template **15**. In our case, a racemic mixture of **15** was synthesized in order to save cost. If desired, the same chemistry could be directly applied for the synthesis of chiral warheads using the corresponding chiral oxazolidinone. Next, selective removal of the *tert*-butyl group under acidic conditions (to give **16**), followed by coupling of trityl-protected hydroxylamine (to give **17**) and base hydrolysis with H_2O_2/LiOH, gave **18A**−**H**. Subsequently, **18A**−**H** were coupled to propagylamine using the HATU/DIEA coupling method, giving **19A**−**H** in excellent yields. Final deprotection of the trityl group with TFA furnished the final warheads, **A**−**G**, which were unambiguously characterized by LC−MS and ^{1}H and ^{13}C NMR (Supporting Information). All steps shown in Scheme 1 gave good to excellent yields, except the last step, where TFA was used to remove the trityl group. Upon closer examination, a prominent side product was consistently observed. Three of these side products were isolated, further characterized by NMR and ESI-MS, and confirmed to be the cyclic adducts **20**−**22** (Figure 2). They are presumably generated

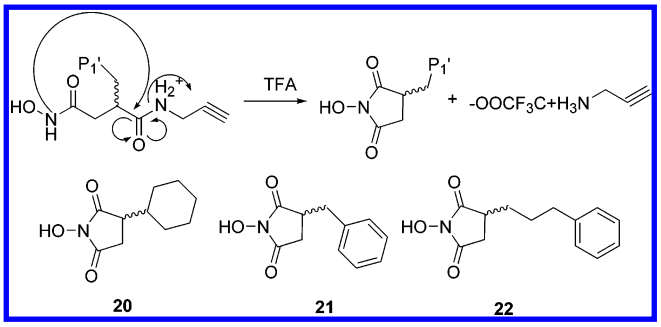


Figure 2. Proposed mechanism of the side reaction during TFA deprotection of warheads.

from the acid-catalyzed cyclization reaction of the warheads as proposed in Figure 2. Nevertheless, moderate yields (40−55%) were routinely obtained in this step.

The 12-member azide library was synthesized via a highly efficient two-step procedures (Scheme 2). Twelve different amines, each bearing a different hydrophobic moiety with varied functional groups, were first acylated with either 2-bromoacetyl chloride, 3-bromopropionyl chloride, or 5-bromopentanoyl chloride, followed by an S_{N2} substitution reaction with sodium azide in DMF to generate the corresponding azides in excellent yields.

Next, a 96-member inhibitor library was assembled using "click chemistry" in a 96-deep well block. Each of the eight alkyne warheads was mixed with each of the twelve azides (in slight excess; see the Supporting Information for details) in a *t*-BuOH/H_2O solution, followed by addition of catalytic amounts of sodium ascorbate and $CuSO_4$. The "click chemistry" proceeded with high efficiency at room temperature for >12 h. LC−MS confirmed, in almost all cases, the complete consumption of the alkynes and quantitative formation of the desired triazole products, thus ensuring that they may be used directly for subsequent in situ enzymatic screening without any further purification.

MMP-7 is one of the few MMPs that is secreted by cancer cells and contributes to proliferation of intestinal adenomas as well as pancreatic cancer.[1] It was chosen for this study, together with collagenase and thermolysin that have roles in the progression bacterial corneal keratitis and the metabolism of *Bacillus* sp., respectively.[10] All three enzymes were screened in a high-throughput, automated fashion against the 96-member library panel using standard fluorescence assays in microplates.[6] The inhibitor potency was evaluated from the reduction in fluorescence output when introduced in standard enzymatic assays with quenched substrates. The resulting inhibition fingerprints obtained are displayed in Figure 3a, demonstrating how such enzymes

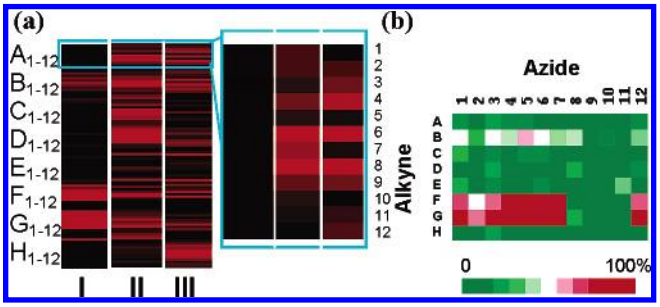


Figure 3. (a) Inhibitor fingerprints of (I) MMP-7, (II) thermolysin, and (III) collagenase, represented as "barcodes". Black: minimum inhibition; Red: maximum inhibition. (b) Screening of "clicked" inhibitors against MMP-7. Heat map obtained using TreeView displays the inhibition fingerprint, with most potent inhibitors indicated in bright red.

may be easily discerned through their unique inhibition "barcodes", which differ from previous fingerprint profiles generated by other methods[11] in that our current method is able to directly reflect an enzyme's inhibition profiles (potency and selectivity). By taking advantage of such inhibition fingerprints, it would become possible to characterize and group

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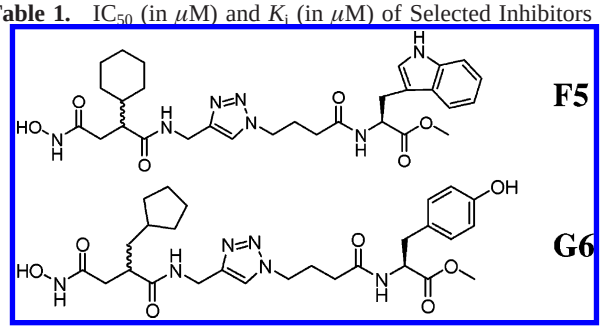
proteins in an activity-dependent manner. Furthermore, such analysis may prove useful in distinguishing not only inhibitors that are potent against specific enzyme classes, but also those that provide good discriminatory potential, thereby minimizing off-target effects of potential drug candidates.

A broader evaluation of the inhibition profile obtained against MMP-7 (as shown in Figure 3b) revealed a unique and consistent trend. Hydroxamate warheads containing alkyl and cycloalkyl side chains at the P_1' position (i.e., isobutyl, cyclohexyl and cyclopentyl in **B**, **F**, and **G**, respectively) contributed highly to potency of inhibitors against MMP-7. The strongest inhibition was observed for scaffolds containing the latter two unnatural cyclic analogues. This correlates well with generic inhibitors against metalloproteases such as GM6001 and batimastat that are designed with a small hydrophobic residue (Leu), i.e., isobutyl in the P_1' pocket.[12] Furthermore, both thermolysin and collagenase (profiles provided in the Supporting Information) do not display such an exclusive and distinctive preference for these small cyclic residues. Our results thus highlight the potential of such pharmacophores in the design of novel inhibitors against MMP-7 and possibly other MMPs. Another observation is that, azides having a 4-carbon linker ($n = 4$; **F8**−**11**, **G8**−**11** in Figure 3b) appeared to contribute negatively to the inhibitor potency, which indicates the linker might be too long to properly project the hydrophobic moiety of the azides into P_4' pocket of MMP-7.

To unambiguosly confirm the potency of these scaffolds from the preliminary screen, two compounds,**F5** and **G6**, representing each of the cyclohexyl and cyclopentyl warheads were selected for detailed evaluations. These molecules were purified and fully characterized by NMR and LC−MS, before being evaluated against the panel of metalloproteases to elucidate the relevant inhibition constants. As shown in Table 1, **F5** and **G6** indeed inhibited MMP-7 strongly with

Table 1. IC_{50} (in μM) and K_i (in μM) of Selected Inhibitors



	MMP-7		thermolysin	collagenase
	K_i	IC_{50}	IC_{50}	IC_{50}
F5	3.8	12.5	>50	>50
G6	1.4	6.5	>50	>50

a K_i of 1.4 and 3.8 μM, respectively. More importantly, these inhibitors were 10−35 times more potent toward MMP-7 than the bacterial metalloproteases tested, demonstrating the potential of the strategy in elucidating inhibitors with both good potency and high selectivity against MMPs.

We docked **G6**, the most potent inhibitor identified from our screening, against the MMP-7 active site using the Sybyl software, on the FlexX suite.[13] As would be expected, the optimized docking configuration of the inhibitor/enzyme complex shows the inhibitor adopts an extended conformation, fitting comfortably in the enzyme active site (Figure 4). The hydroxamate group from the inhibitor was shown to

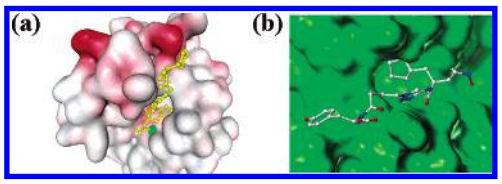


Figure 4. In silico docking displays the possible binding mode of **G6**/MMP-7 complex. The hydroxamic group in the inhibitor chelates with the zinc atom (green sphere) in the enzyme active site, with the inhibitor projecting into the S′ pockets of the enzyme. Docking was performed using Sybyl v7.2 (Tripos, MO) with electrostatic surface images generated using WebLab ViewerLite (Accelrys, San Diego, CA).

chelate to the target zinc atom. The cyclopentyl P_1' side chain also fits nicely into the S_1' pocket. As we were hoping in the original design, the phenolic ring from the azide component appeared to sit in the S_4' pocket as well. In addition, a number of favorable hydrogen bonds were also evident in the complex (Supporting Information).

In conclusion, we have developed a high-throughput strategy for assembling and screening MMP inhibitors. The panel of compounds we have synthesized show promise as a valuable resource for screening various MMPs and obtaining unique inhibition fingerprints. Moreover, we have identified specific scaffolds that show good potency and moderate selectivity for MMP-7 over other metalloproteases. We anticipate that the pharmacophore we have uncovered in this study may lead to future development of more potent and selective inhibitors against other MMPs. We are thus extending our experiments against a wider panel of MMPs to confirm the selectivity patterns obtained against MMP-7 and these results will be reported in due course.

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

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