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Design, Synthesis and Evaluation of Novel Azasugar-Based MMP/ADAM Inhibitors

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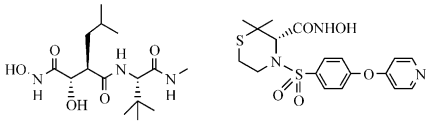
Abstract—In order to verify whether azasugar would be a useful scaffold for inhibitory activity against metalloproteinases, we synthesized some azasugar-based compounds. As a result, it is clarified that azasugar moiety could function as successful inhibitor of matrix metalloproteinase-1, -3 and -9 and TACE.

Metalloproteinases, a family of zinc-containing enzymes, are classified into two types. One is matrix metalloproteinases (MMPs), comprised of collagenases, stromelysins, gelatinases and membrane-type MMPs (MT-MMPs), which mediate the breakdown of connective tissue and are therefore targets for therapeutic inhibitors in many inflammatory, malignant and degenerative diseases.[1–4] The other is a disintegrin and metalloproteinases (ADAMs),[5] which mediate the processing of membrane-bound cytokine such as tumor necrosis factor α (TNF-α) into soluble form. TNF-α is a major mediator of inflammatory and immune responses[6] and a strong inducer of other cytokines such as IL-lβ, IL-6 and IL-8. Elevated TNF-α levels are implicated in pathologies of rheumatoid arthritis,[7] multiple sclerosis,[8] type II diabetes,[9] and other human ailments. Therefore, TNF-α converting enzyme (TACE or ADAM17) inhibitor is an attractive target for medicinal chemists.[10]

Actually, there have been considerable efforts in the design and synthesis of MMP/ADAM inhibitors and a lot of small molecule inhibitors have been reported and showed blocking activities against both MMPs and TACE.[10] The most widely pursued approach toward MMP inhibition has been the design of substrates that bind to the catalytic site of the enzymes. Namely, peptidemimetics that incorporate a zinc ligand and P1′ side chain are the most common class of MMP inhibitors such as Marimastat[11] (Chart 1). Then, as a second generation, sulfonamide-based inhibitors, including AG3340[12] (Chart 1) have been studied. On the other hand, based on their structural relationships to sugars, azasugars, namely polyhydroxypiperidine derivatives, are interesting candidates for the inhibition of various glycosidase. For example, 1-deoxynojirimycin is a well-known specific inhibitor for glucosidase[13] (Chart 1). However, azasugar scaffold has never been adapted in the field of metalloproteinase inhibitors yet.



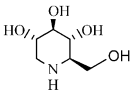


Chart 1.

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In this letter, we describe the design and synthesis of azasugar-based MMP/ADAM inhibitors bearing a novel scaffold and their biological profiles.

Chemistry

Our interest is to verify whether azasugar scaffold would be useful for inhibitory activities against metalloproteinases, including TACE and MMP-1, -3, -9. We focused on an azasugar scaffold bearing a six-membered ring as shown in Figure 1. For many of sulfonamide-based MMP inhibitors, the zinc binding group and the P1′-substituent appear to be primarily responsible for the interactions with MMPs and ADAMs. Moreover, in the case of sulfonamide derivatives including AG3340, it was well-known that *R*-configuration at hydroxamic acid moiety would be important for desirable inhibitory activity against metalloproteinases.[14] In addition to the importance of *R*-configuration of spatially oriented hydroxamic acid group at the C-2 position, the introduction of arylsulfonyl group at the N-1 position would become crucial for practical design of the potential metalloproteinase inhibitors. We designed a new series of MMP/ADAM inhibitors **1-4** based on azasugar scaffold readily obtainable from the L-Threitol, illustrated in Figure 2. In this study, at first, the stereochemistry of trihydroxy-piperidine unit was fixed as 2*R*,3*S*,4*R*,5*S*-configuration, according to the literature.[15a]

After deprotonation of glycine ester **5** with an excess of lithium diisopropylamide (LDA) and subsequent transmetalation with tin chloride, addition of chiral aldehyde **6**, prepared easily from L-Threitol, gave rise to the aldol product **7** as an epimeric mixture at the C-2 position. As anticipated, it was demonstrated that highly stereoselective reaction at the C-3 position was performed by using of the condition reported previously.[15a] Compound **7** was subjected to acetylation followed by deprotection of silyl group using tetrabutylammonium fluoride (TBAF), to afford diasteromerically pure **8** after purification with column chromatography in 37% yield. Cyclization of **8** under Mitsunobu condition [diethyl azodicarboxylate (DEAD)-triphenylphospine (PPh_3)][16] provided azasugar derivatives **9** in good yield without epimerization at C-2 position. After hydrogenolysis of compound **9** in the presence of a catalyst, 10% palladium-carbon, under hydrogen atmosphere, corresponding carboxylic acid was subject to the condensation with benzyloxyamine hydrochloride (NH_2OBn) in the presence of 1-(3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride (WSC) and 1-hydroxy- triazole (HOBt), followed by the treatment of sodium methoxide to give compound **10** in 59% yield. Compound **10** was subjected to deprotection of isopropylidene group using DOWEX-H^+, followed by the treatment of 2,2-dimethoxypropane in the presence of *p*-TsOH to afford compound **12** in good yield. Finally, deprotection of benzyl group for compound **12** in the presence of a catalyst, 10% palladium-carbon, under hydrogen atmosphere provided the target compound **1** bearing 3,4-*O*-isopropylidene group.[17] On the other hand, compound **10** was hydrogenated to afford target compound **2** in excellent yield.[18] Next, triol derivative **11** was transformed, by removal of protective group, to hydroxamic acid **3** in 96% yield.[17] Finally, compound **9** was subjected to deprotection of benzyl ester, condensation of NH_2OBn in the presence of WSC and HOBt followed by hydrogenolysis to provide target compound **4** in 63% yield.[17] The configuration of compound **4** was comfirmed by small coupling constants, $J_{2,3} = 1.8$ Hz between 2-H and 3-H, and $J_{3,4} = 2.2$ Hz between 3-H and 4-H (Scheme 1).

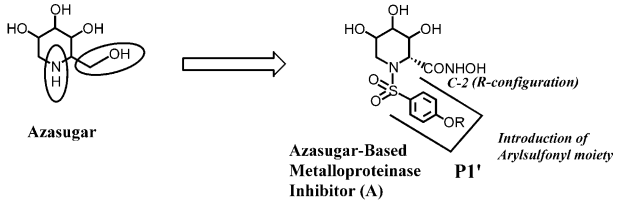


Figure 1. Design of novel azasugar-based metalloproteinase inhibitor.

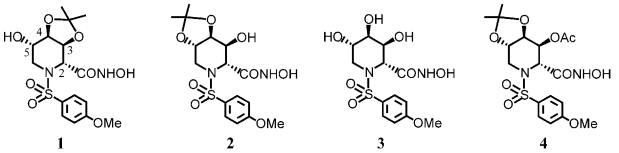
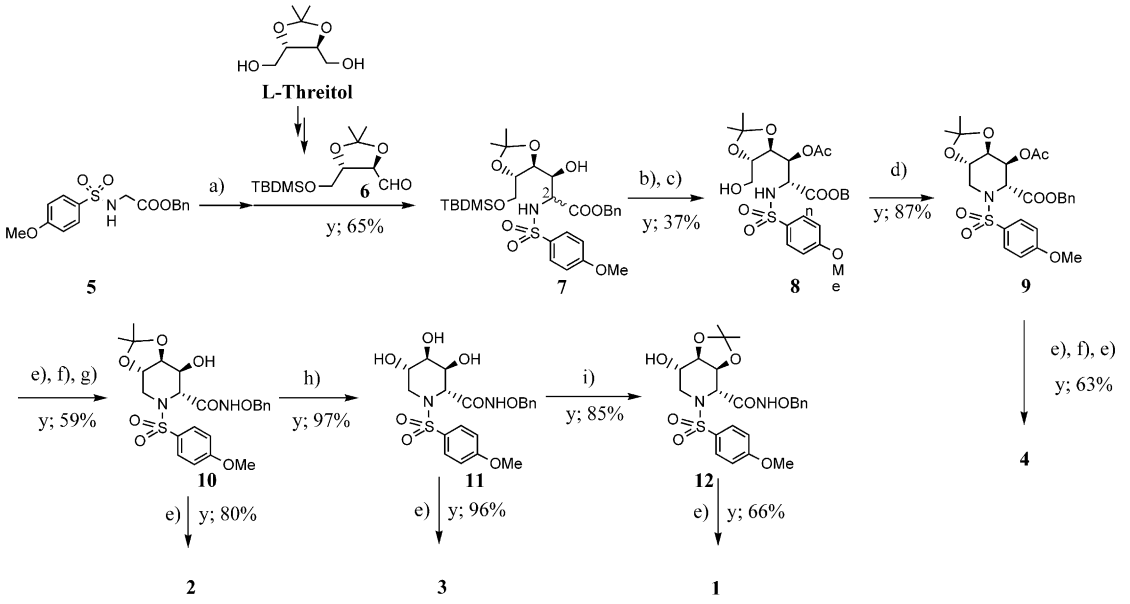


Figure 2. Structure of azasugar-based metalloproteinase inhibitors with 2*R*,3*S*,4*R*,5*S*-configuration.

Biological Evaluation

Inhibitory activities of compound **1–4** against TACE and MMPs (MMP-1, MMP-3, MMP-9) were summarized in Table 1.[18] Compound **3** having three hydroxyl groups exhibited moderate inhibitory activities against all metallo-proteinase (MMP-1, -3, -9 and TACE), K_i values were 84 nM against MMP-1, 17 nM against MMP-3, 157 nM against MMP-9 and 71 nM against TACE, respectively. On the other hand, compound **1** and **2**, having 3,4-*O*-isopropylidene group or 4,5-*O*-isopropylidene group, also showed potent inhibitory activity against target enzymes. Interestingly, compound **2** with 4,5-*O*-isopropylidene group exhibited 12–21 times more potent inhibitory activity against MMP-1, MMP-3 and MMP-9 than those of compound **1**. This result indicated that structural difference based on isopropylidene group at the 3,4-position or 4,5-position would play important role for interaction with MMP-1, -3, -9. Compound **4** bearing acetyl group showed 6–13 times weak activity against MMP-1 and -3 than compound **2**. In addition, regarding the inhibitory activity toward MMP-3, it was found that azasugar compounds synthesized exhibited more potent than representative MMP inhibitor, Marimastat. In this investigation, it was clarified that azasugars could function as a useful scaffold for inhibition toward metalloproteinases.

In conclusion, we synthesized novel azasugar-based metalloproteinase inhibitors **1-4** which exhibited desirable inhibitory activities against TACE and MMP-1, -3, -9. This result suggests that azasugar skeleton could be useful as



Scheme 1. (a) LDA, $SnCl_2$; (b) Ac_2O, pyridine; (c) TBAF, AcOH, THF; (d) DEAD, Ph_3P, THF; (e) Pd/C, H_2; (f) NH_2OBn, WSC, HOBt; (g) NaOMe, MeOH; (h) DOWEX-H+; (i) 2,2-dimethoxypropane, *p*-TsOH, DMF.

Table 1. Inhibitory activities of azasugar derivatives **1–4** against MMPs and TACE

Compd	rMMP-1 K_i (nM)[a]	rMMP-3 K_i (nM)[a]	rMMP-9 K_i (nM)[a]	TACE K_i (nM)[a]
1	554	44	310	22
2	26	3.7	21	40
3	84	17	157	71
4	162	50	47	21
Marimastat	1.1	84	11	0.40

[a]See ref 18 for assay conditions.

scaffold for the synthesis of successful metalloproteinase inhibitors. Moreover, sulfonaminyl azasugars could attain much improvement of water solubility, compared to other class of metalloproteinase inhibitor. Therefore, these findings would be useful for design of novel metalloproteinase inhibitors. We are now investigating optimization of azasugar compounds.

Acknowledgements

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17. All new compounds gave satisfactory characteristics data. Characteristics are given for a representative compound: **3**; ^1H NMR (DMSO-d_6, 250 MHz) δ 3.25–3.7 (m, 5H), 3.83 (s, 3H), 4.34 (d, 1H, $J=2.0$ Hz), 4.7–5.2 (m, 3H), 7.05 (d, 2H, $J=8.9$ Hz), 7.72 (d, 2H, $J=8.9$ Hz), 8.88 (s, 1H), 10.86 (s, 1H). MALDI-TOF: 385 ($M+Na^+$).
18. (a) Recombinant human collagenase-1 (MMP-1), stromelysin-1 (MMP-3), gelatinase B (MMP-9) and TNF-α converting enzyme (TACE) were used in our studies. Assay

conditions were referred as below: Sawa, M.; Kiyoi, T.; Kurokawa, K.; Kumihara, H.; Yamamoto, M.; Miyasaka, T.; Ito, Y.; Hirayama, R.; Inoue, T.; Kirii, Y.; Nishiwaki, E.; Ohmoto, H.; Maeda, Y.; Ishibushi, E.; Inoue, Y.; Yoshino, K.; Kondo, H. *J. Med. Chem.* **2002**, *45*, 919. (b) Yoshiizumi, K.; Yamamoto, M.; Miyasaka, T.; Ito, Y.; Kumihara, H.; Sawa, M.; Kiyoi, T.; Yamamoto, T.; Nakajima, F.; Hirayama, R.; Kondo, H.; Ishibushi, E.; Ohmoto, H.; Inoue, Y.; Yoshino, K. *Bioorg. Med. Chem.* **2003**, *11*, 433.