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Synthesis and biological activity of selective azasugar-based TACE inhibitors

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Abstract—A series of azasugar-based hydroxamic acid derivatives bearing 2*R*,3*R*,4*R*,5*R*-configuration is described. Compound **4c** with 4,5-*O*-acetonide group showed excellent in vitro potency against TACE, with high selectivity over MMP-1 and moderate selectivity over MMP-3 and MMP-9.
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1. Introduction

Tumor necrosis factor α (TNF-α), a major immunomodulatory and proinflammatory cytokine, has been implicated in the pathogenesis of a number of inflammatory diseases including rheumatoid arthritis (RA),[1] multiple sclerosis,[2] type II diabetes,[3] and other human ailments. TNF-α is synthesized as a membrane-anchored 26 kD precursor and shed to a 17 kD soluble form by a specific proteolytic cleavage.[4] This processing step is performed by a TNF-α converting enzyme (TACE), a member of ADAM family of proteases classified as ADAM17.[5] Therefore, agents that block TACE, and thereby reduce levels of soluble TNF-α, may be effective in the treatment of these diseases.[6] Actually, data from human clinical trials have supported the therapeutic utility of anti-TNFα biologics, such as Enbrel® and Remicade®, in RA and Crohn's disease.[7]

A large number of non-specific inhibitors of matrix metalloproteases (MMPs) have been shown to be potent inhibitors of TACE.[8] Actually, several MMP/TACE inhibitors have already entered clinical trials for the treatment of RA and cancer.[9] However, in order to minimize detrimental side effects, such as muscle and joint pains, successful design of selective TACE inhibitors would be necessary. Recently, several TACE inhibitors including compound **1** with selectivity over MMP-1 bearing butyn-2-yloxy group have been reported (Fig. 1).[10]

We have already disclosed azasugar-based MMP/TACE inhibitors **2** with four asymmetric centers. The stereochemistry of C2-position is critical for showing potent inhibitory activities against MMPs and TACE, and needs to be fixed as *R*-configuration. Then there are eight stereoisomers based on three chiral centers at C3, C4 and C5-position (Fig. 2).[11,12] We have already prepared their several stereoisomers **3a**–**e** with different MMP/TACE inhibitory profiles, however, these compounds were not necessarily TACE selective inhibitors.

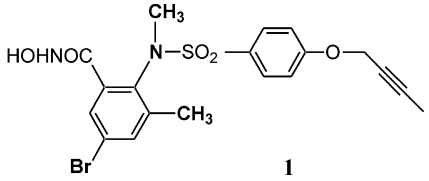

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Figure 1. Representative TACE inhibitor.

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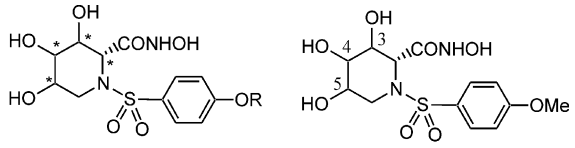


Figure 2. Azasugar-based metalloproteinase inhibitors designed.

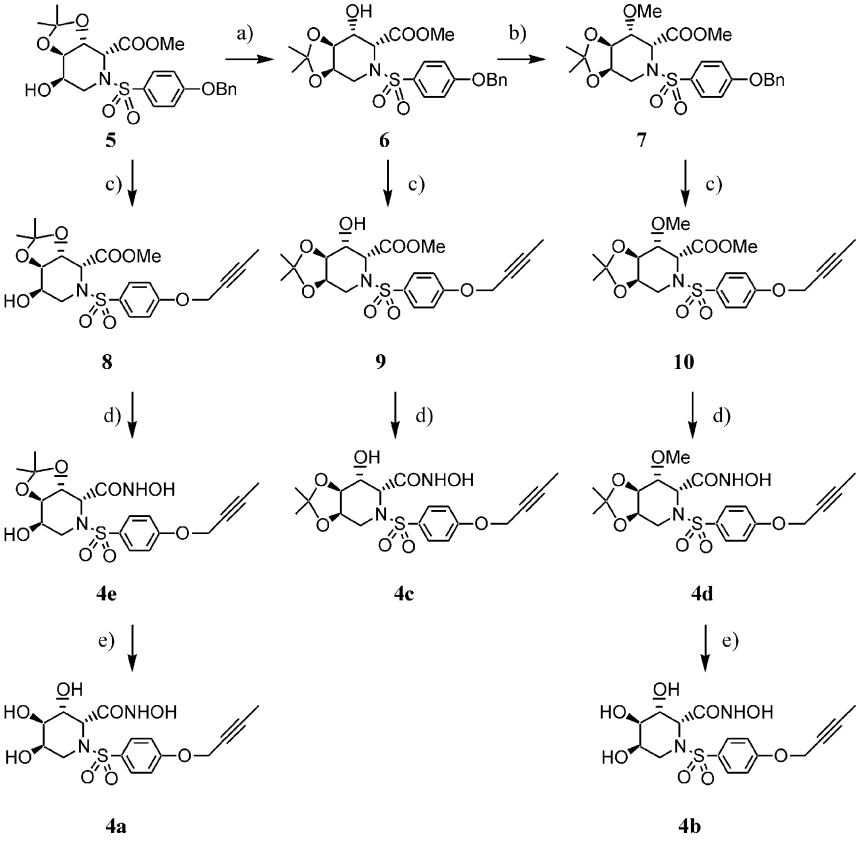
On the other hand, it was found that compounds bearing 3*R*,4*R*,5*R*-configuration such as **3e** were stable in aqueous solution, compared to those bearing 3*R*,4*R*,5*S*-configuration.[12] In addition, azasugar-based inhibitors including compound **3e** could be expected to show good water solubility, due to the hydroxyl group at C3, C4, C5 position. These properties are desirable for administration of drugs such as topical application and oral administration. Therefore, in order to discover TACE selective inhibitors successfully, we focused on the azasugar compounds having 3*R*,4*R*,5*R*-configuration. In this paper, we wish to report the systematic synthesis of azasugar-based TACE inhibitors (**4a–e**) bearing butyn-2-yloxy moiety and their TACE selectivity over MMPs.[12]

2. Chemistry

According to Scheme 1, the target compounds **4a–e** were prepared from key intermediate **5**.[12] The exchange of acetonide group of **5** was easily performed by the cleavage and successive reprotection of 4,5-*O*-acetonide in acidic condition to provide compound **6**. Compound **6** was subjected to the treatment of methyl iodide in the presence of silver oxide, to provide **7** in good yield. The hydrogenolysis of **5**, **6** and **7** with 10% Pd–C under hydrogen, and then the corresponding phenolic hydroxyl groups were alkylated with 1-bromo-2-butyne in the presence of potassium carbonate in CH_3CN to afford compounds **8**, **9** and **10**, respectively. The aminolysis of **8**, **9** and **10** with 50% hydroxylamine solution in the presence of sodium cyanide in MeOH, gave target compounds **4e**, **c** and **d** in moderate yields, respectively. Finally, the cleavage of acetonide groups of **4e** and **d** afforded the target compounds **4a** and **b** in good yield.[13]

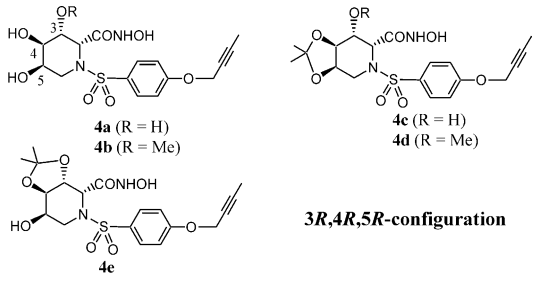
3. Discussion

Inhibitory activities toward MMP-1, MMP-3, MMP-9 and TACE of azasugar-based derivatives **4a–e** were summarized in Table 1.[14] Although, compound **4a** bearing butyn-2-yloxy moiety exhibited potent inhibitory activity toward TACE, it showed moderate-high selectivity over MMPs: 242-fold over MMP-1, 6-fold over MMP-3 and 27-fold over MMP-9, respectively. Compound **4b** having methoxy group at C3 position showed less selectivities over MMP-1, 3, 9, compared to **4a**.



Scheme 1. (a) Muromac®, MeOH, then 2,2-dimethoxypropane, *p*-TsOH, DMF; (b) MeI, Ag_2O, CH_2Cl_2; (c) 10% Pd–C/H_2, AcOEt, then 1-bromo-2-butyne, K_2CO_3, CH_3CN; (d) 50% NH_2OH, NaCN, MeOH; (e) Muromac®, MeOH.

Table 1. Inhibitory activities against MMP-1, 3, 9 and TACE



Compd	rMMP-1 Ki (nM)[a]	rMMP-3 Ki (nM)[a]	rMMP-9 Ki (nM)[a]	TACE Ki (nM)[a]
4a	128.22	3.30	14.24	0.53
4b	90	0.43	12	1.85
4c	>850	29.60	77.75	0.57
4d	552.4	11.5	98.2	0.84
4e	>850	58.05	118.37	1.85

[a] See ref 13 for assay conditions.

Interestingly, TACE inhibitory activity of 4,5-*O*-acetonide analogue **4c** was very potent and equal to that of **4c**, in addition, much improved selectivity (>1491-fold) over MMP-1 was found. Furthermore, its TACE inhibition was 52-fold selective over MMP-3, and 136-fold selective over MMP-9. Compound **4d** having methoxy group at C3 position also exhibited similar tendency as compound **4c**. This finding suggests that the difference of conformation between monocyclic analogues **4a** and **b**, and bicyclic rigid analogues **4c** and **d** would be essential for TACE selectivity over MMPs. On the other hand, 3,4-*O*-acetonide analogue **4e** displayed 3-fold less potent TACE inhibitory activity than **4c**, while inhibitory activities against MMPs of **4e** were similar to those of **4c**. This result indicates that the conformation fixed by 4,5-*O*-acetonide would be desirable for inhibitory activity against TACE, compared to that fixed by 3,4-*O*-acetonide.

4. Conclusion

In conclusion, we have succeeded in synthesizing a series of azasugar-based TACE inhibitors bearing 2*R*,3*R*,4*R*,5*R*-configuration, which exhibited excellent selectivity over MMP-1 and moderate selectivity over MMP-3 and MMP-9. Especially, compound **4d** with 4,5-*O*-acetonide group demonstrated very potent TACE inhibitory activity and good selectivity against MMPs compared to monocyclic compound **4a**. Now in order to obtain a better understanding about the difference of inhibitory activity profile of **4a**, **c** and **e**, docking study of these compounds with MMPs and TACE using computer technique is in progress.

Acknowledgements

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13. All new compounds obtained satisfactory characteristics data. Characteristics are given for a representative compound: **4c**: ^{1}H NMR (DMSO-d_6) δ: 1.17 (s, 3H), 1.23 (s, 3H), 1.84 (t, 3H, J=2.1 Hz), 3.44 (bs, 1H), 4.07 (d, 1H, J=6.0 Hz), 4.11 (dd, 1H, J=6.4, 7.1 Hz), 4.3–4.35 (m, 1H), 4.84 (q, 2H, J=2.1 Hz), 5.63 (bs, 1H), 7.15 (d, 2H, J=9.1 Hz), 7.71 (d, 2H, J=9.1 Hz), 8.92 (s, 1H), 10.78 (s, 1H). MALDI-TOF: 463 ($M+Na^+$), 479 ($M+K^+$).

14. (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.