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Discovery of Selective Phosphonamide-Based Inhibitors of Tumor Necrosis Factor-α Converting Enzyme (TACE)

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Abstract—A novel series of phosphonamide-based inhibitors of tumor necrosis factor-α converting enzyme (TACE) was discovered by structural modification of tetrahydroisoquinoline derivative **1b**, which was extremely weak inhibitor of TACE. (*S*)-Isomer at the phosphorus atom (**7b**) displayed potent inhibition for TACE, while selectivity sparing MMP-1, -3, and -9.

Introduction

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine, and elevated TNF-α levels are implicated in a number of pathologies such as rheumatoid arthritis, multiple sclerosis, non-insulin dependent diabetes mellitus (NIDDM), and inflammatory bowel disease.[1] TNF-α exists in two forms, a 26-kDa membrane-bound form (proTNF-α) and a 17-kDa soluble form processed by specific proteases. The enzyme responsible for the processing of the proTNF-α is TNF-α converting enzyme (TACE), which is a member of the ADAM familiy.[2,3] Therefore, small molecular weight inhibitors of TACE are considered to be attractive targets in drug discovery research.[1]

We recently reported the discovery of a series of phosphonamide-based metalloproteinases inhibitors represented by structure **1a** (Fig. 1).[4] Although compound **1a** shows potent inhibitory activity against TACE ($K_i = 7.15$ nM), it is also potent inhibitor of the matrix metalloproteinases (MMPs). It has been reported that side effects were observed in the clinical studies of broad-spectrum inhibitors of the MMPs,[5] selective TACE inhibitors are desirable in the long-term treatment of TNF-α mediated diseases. This paper will disclose the discovery of a new series of selective TACE inhibitors using a phosphonamide scaffold.

Inhibitor Design

In the previous paper, only the (*R*)-isomer at the phosphorus atom (**1a**) was found to be a potent inhibitor of TACE, collagenase-1 (MMP-1), stromelysin-1 (MMP-3) and gelatinase B (MMP-9), while the (*S*)-isomer **1b** was almost inactive for those enzymes (Table 1).[4a] After subsequent efforts, we have found that the introduction of fluorine atoms into the ester moiety of the (*S*)-isomers led to highly potent and selective inhibition against MMP-1 (**2b**, K_i: 6.23 nM for MMP-1, selectivities for MMP-3 and -9: >104- and 66-fold, respectively).[6] This unexpected appearance of the inhibitory activity of the (*S*)-isomer could be explained by the switching of the binding mode in the enzyme, as shown in Figure 2. We believe that the different binding mode of this type of compound would make the compound highly selective. Therefore, we have extended this observation to cover other types of metalloproteases, and chosen TACE as a prototypical enzyme since the active site of TACE was found to be very similar to those of MMPs.[1,7] Initially, we have tested a series of various ester derivatives of **1b**, however none of compounds showed inhibitory activity against TACE in the nanomolar range. Based on modeling studies using X-ray structure of TACE, it was anticipated that the ester groups could not be oriented

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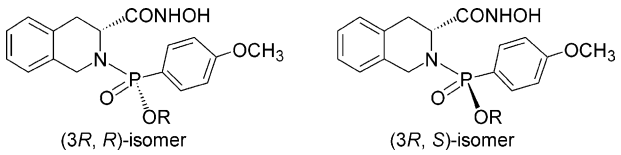
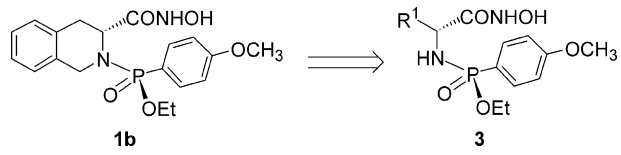


Figure 1. Structures of phosphonamide-based MMP inhibitors.



Scheme 1. Design of new phosphonamide derivatives.

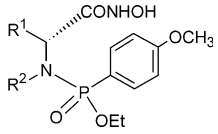
correctly for occupancy of the S1′ pokect due to the restriction of the bicyclic tetrahydroisoquinoline structure. We therefore considered that acyclic compounds such as **3** to allow greater flexibility of the ester groups in the compounds (Scheme 1). In this preliminary report, we wish to communicate the synthesis and biological data for a series of novel acyclic phosphonamide-based inhibitors which exhibit selective inhibition of TACE.

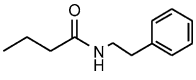
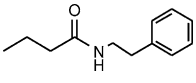
Chemistry

Compounds discussed below were prepared using synthetic routes shown in Schemes 2 and 3. Commercially available D-leucine benzyl ester **4** was coupled with 4-methoxyphenylchlorophosphonic acid ethyl ester[4] in the presence of *N*-methylmorpholine to give the phosphonamide **5** as a mixture of diastereomers (1:1) in 56% yield. Deprotection of the benzyl group in **5** with Pd/C (92% yield), followed by coupling with *O*-benzylhydroxylamine afforded **6** in 38% yield. Deprotection of the benzyl group in **6** with 10% Pd/C gave the diastereomerically pure two hydroxamic acids, which were successfully separated by HPLC purification (yield: 31% for **7a** and 24% for **7b**). The stereochemistries of the compounds were assigned by a characteristic signal pattern in ^{1}H NMR, especially in an aromatic region as reported previously.[4,6]

Alternatively, *N*-(*tert*-butoxycarbonyl)-D-glutamic acid 1-benzyl ester **8** was coupled with phenethylamine using

Table 1. In vitro profile of phosphonamide derivatives



Compd	R^1	R^2	K_i (nM)			
			TACE	MMP-1	MMP-3	MMP-9
1a (*R*) **1b** (*S*)	Tetrahydroisoquinoline ring		7.15 (41% @1000)[a]	4.59 (26% @1000)[a]	5.20 (9% @1000)[a]	5.05 (18% @1000)[a]
7a (*R*)		H	5.06	196	69.8	165
7b (*S*)		H	76.4	(10% @1000)[a]	(17% @1000)[a]	(17% @1000)[a]
12a (*R*)		H	27.6	40.4	7.29	23.7
12b (*S*)		H	100	(27% @1000)[a]	(47% @1000)[a]	(24% @1000)[a]

[a]% inhibition at the concentration (nM).

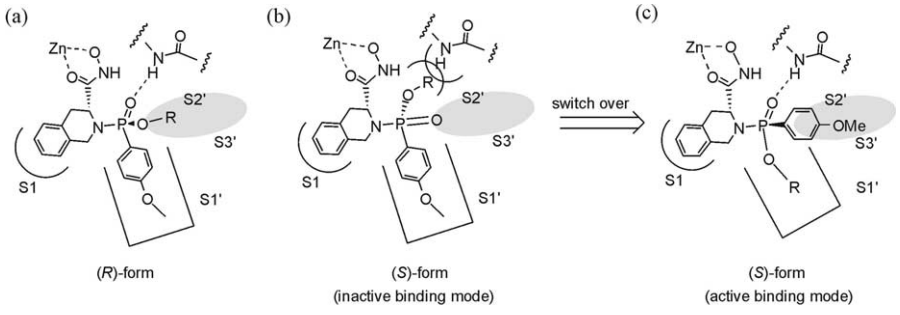
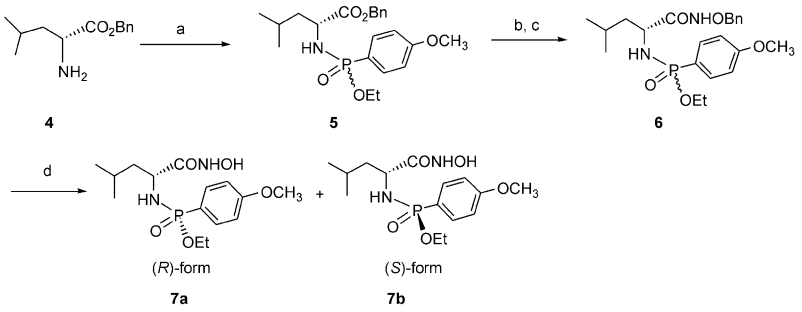
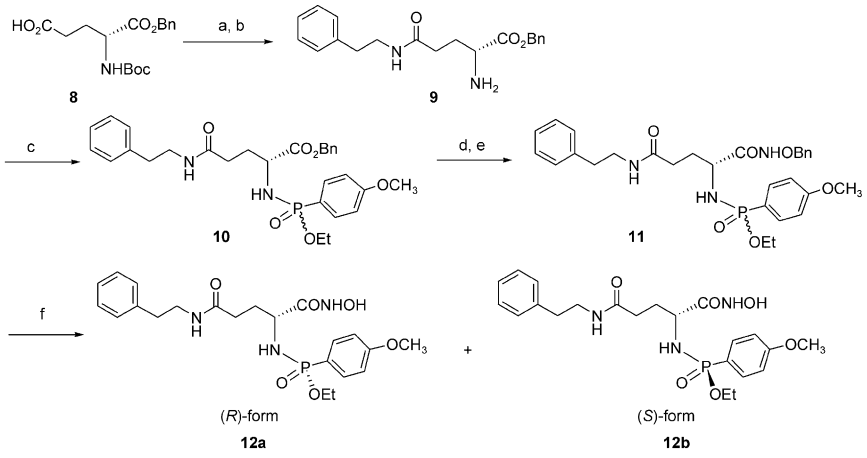


Figure 2. Expected binding mode of the phosphonamide inhibitors in MMPs.



Scheme 2. (a) 4-Methoxyphenylchlorophosphonic acid ethyl ester, *N*-methylmorpholine; (b) H_2, Pd/C; (c) *O*-benzylhydroxylamine hydrochloride, WSC, HOBt; (d) H_2, Pd/C.



Scheme 3. (a) Phenethylamine, WSC, HOBt; (b) 4 N HCl in AcOEt; (c) 4-methoxyphenylchlorophosphonic acid ethyl ester, *N*-methylmorpholine; (d) H_2, Pd/C; (e) *O*-benzylhydroxylamine hydrochloride, WSC, HOBt; (f) H_2, Pd/C.

WSC to yield **9**. Compound **9** was converted into the hydroxamates **12a** and **12b** using standard conditions as described above.

Results and Discussion

We firstly prepared D-leucine derivatives **7a** and **7b** in the anticipation of the hydrophobic interaction of the isobutyl group with the unprimed subsites (S1/S2) of the enzyme based on the modeling study.

The D-leucine derivatives **7a** and **7b** were tested in vitro for their ability to inhibit TACE, collagenase-1 (MMP-1), stromelysin-1 (MMP-3), and gelatinase B (MMP-9) (Table 1).[8] (*R*)-Isomer **7a** was potent inhibitor of TACE ($K_i = 5.06$ nM) and also moderate inhibitor of MMPs ($K_i = 196$, 69.8 and 165 nM for MMP-1, -3 and -9, respectively). It is noteworthy that whereas the (*S*)-isomer of the tetrahydroisoquinoline derivative **1b** showed no inhibition for TACE, the (*S*)-isomer of the D-leucine derivative **7b** exhibited inhibitory activity against TACE ($K_i = 76.4$ nM). Moreover this compound was extremely weak inhibitor against MMP-1, -3, and -9.

We believed that an additional substituent capable of interacting with S1/S2 site could provide an opportunity to modify not only potency but also the selectivity. Thus, D-gulutamate derivatives **12a** and **12b** were prepared and tested for their ability to inhibit these enzymes. (*R*)-Isomer **12a** was also potent inhibitor of TACE with K_i value of 27.6 nM. It is interesting to notice that while inhibition of TACE was decreased, the inhibition of MMPs was increased significantly compared to the leucine derivative **7a**. Contrary to our expectation, the inhibitory activity of compound **12b** was slightly decreased against TACE ($K_i = 100$ nM). Interestingly, compound **12b** was still selective against TACE, although the significant enhancement of potency for MMPs was observed in **12a**. Modeling study of **7b** with the active site of TACE suggested that the flexible

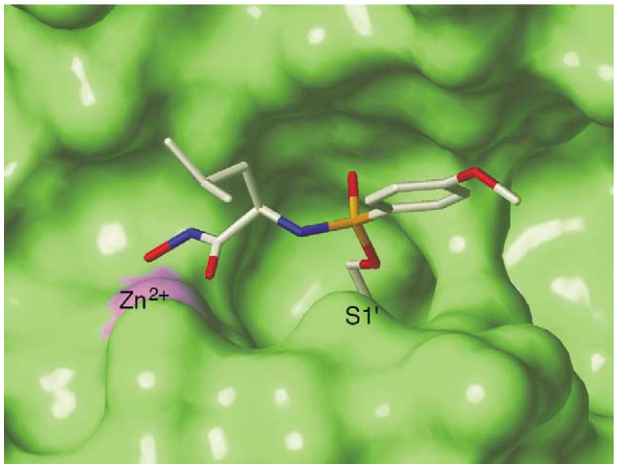


Figure 3. Computer model of **7b** in TACE.

structure of **7b** can adopt the switched binding mode as in Figure 2c (Fig. 3).[9]

In summary, we have discovered a novel series of phosphonamide-based TACE inhibitors. The (*S*)-form of the D-leucine derivative **7b** showed potent inhibitory activity against TACE with a highly selective profile. The different binding mode of this type of compound is likely to enhance selectivity for TACE. This study reveals the potential of the phosphonamide derivatives as a new type of MP inhibitor, and provides a novel concept for the design of selective inhibitors.

Acknowledgements

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8. Recombinant human TACE, collagenase-1 (MMP-1), stromelysin 1 (MMP-3), and gelatinase B (MMP-9) were used in our studies (see ref 4 for assay conditions).
9. Models of TACE complexed with the phosphonamide inhibitors were constructed based on the crystal structure of a TACE/peptide-based inhibitor complex (PDB code, 1BKC). Docking and energy minimizations were performed with the TRIPOS force field within the SYBYL program. All computations were performed on an Silicon Graphics workstation.