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Discovery of Selective Hydroxamic Acid Inhibitors of Tumor Necrosis Factor-α Converting Enzyme

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Abstract—Modification of the P_1' substituent of macrocyclic matrix metalloproteinase (MMP) inhibitors provided compounds that are selective for inhibition of tumor necrosis factor-α converting enzyme (TACE) over MMP-1 and MMP-2. Several analogues potently inhibited the release of TNF-α in a THP-1 cellular assay. Compounds containing a trimethoxyphenyl group in the P_1' substituent demonstrated TACE selectivity across several series of hydroxamate-based inhibitors. © 2001 Elsevier Science Ltd. All rights reserved.

Tumor necrosis factor-α (TNF-α) is a cytokine produced mainly by activated monocyte/macrophages and released as a major mediator of inflammatory and immune responses.[1] The over-expression of TNF-α has been implicated in diseases such as rheumatoid arthritis, Crohn's disease, septic shock, AIDS, insulin resistance, cachexia, and cancer. Agents that block the action of TNF-α may therefore be effective in the treatment of these disease states. The approval of biologics such as TNF antibodies (Remicade®) and TNF soluble receptors (Enbrel®) for treatment of rheumatoid arthritis and Crohn's disease has validated the therapeutic effectiveness of TNF-α inhibition. Recently, a small molecule inhibitor of TNF-α processing through the selective inhibition of TNF-α converting enzyme (TACE) has entered clinical studies.[2]

TNF-α is produced by enzymatic cleavage of an Ala-Val bond of a 26 kDa membrane-bound pro-TNF releasing the 17 kDa mature protein. The major enzyme responsible for this proteolytic cleavage is the sheddase TACE, a zinc-containing metalloproteinase belonging to a disintegrin and metalloproteinase domain (ADAM) subfamily of the metzincin family of enzymes.[3] The active site of TACE was found to be very similar to the active site of another metzincin subfamily, the matrix metalloproteinases (MMPs), and several inhibitors of MMPs were found to inhibit TNF-α production through inhibition of TACE.[4] With the discovery of these dual MMP/TACE inhibitors, the objective of the current study was to identify structural substitutions of known MMP inhibitors which could differentiate these two activities. This effort ultimately led to the discovery of potent inhibitors that were selective for TACE relative to two MMPs, fibroblast collagenase (MMP-1) and gelatinase A (MMP-2).

The investigation of TACE inhibition began by exploring the SAR of a series of macrocyclic hydroxamic acid MMP inhibitors similar to those previously described in reports from our laboratories[5] and others.[6] The synthetic route to the macrocyclic hydroxamates is shown in Scheme 1. Succinate **1**[7] was alkylated with the silyl protected 4-bromobutanol to afford the diastereomer **2** after chromatography. Epimerization with LDA gave the desired diastereomer **3**. After coupling with L-tyrosine-*N*-methyl amide, deprotection of the silyl alcohol, and cyclization under Mitsunobu conditions, macrocycle **5** was obtained. Treatment with 9-BBN followed by coupling with various aryl bromides via palladium-catalyzed Suzuki reactions afforded analogues with modified P_1' substituents. Hydrolysis of the *tert*-butyl ester to the carboxylic acid followed by conversion to the hydroxamic acid gave analogues **7a**–**h**. Further investigation of the SAR of the macrocyclic hydroxamates was performed on phenyl ketone analogues. The synthetic strategy employed was similar to the method to prepare the above *N*-methyl amide analogues except

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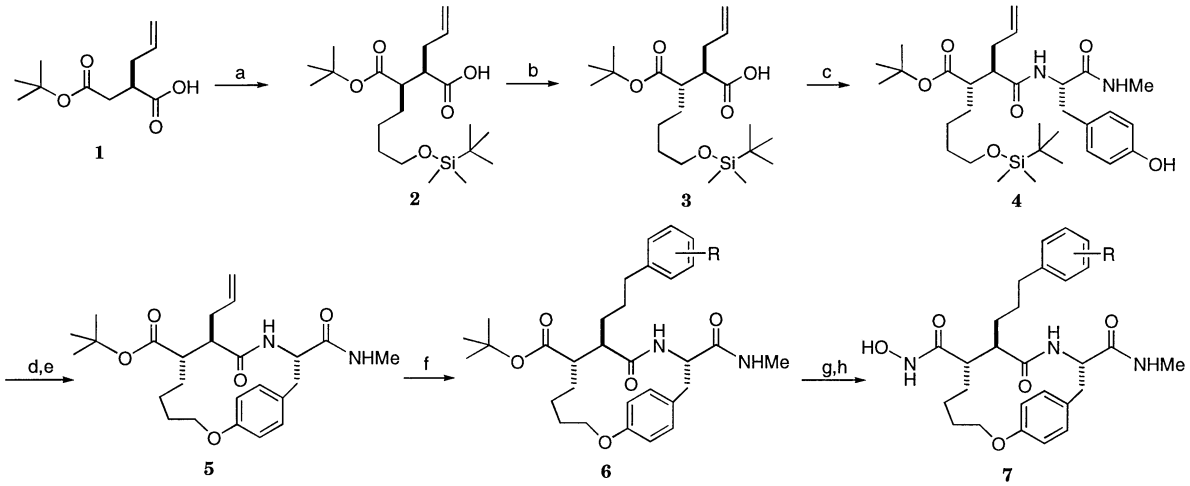
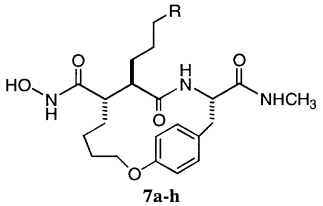
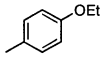
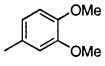
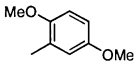
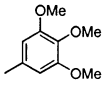
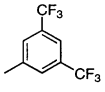
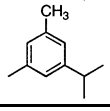


Scheme 1. Reagents: (a) LDA, THF, $I(CH_2)_4OSi(CH_3)_2tBu$, −78 °C to rt; (b) LDA, THF, −78 °C, MeOH quench; (c) 1-hydroxybenzotriazole (HOBT), DMF, L-Tyr-NHMe·HCl, 4-methylmorpholine (NMM), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), 0 °C; (d) tetrabutylammonium fluoride (TBAF), THF, 0 °C; (e) Bu_3P, 1,1′-(azodicarbonyl)dipiperidine, THF/benzene; (f) 9-BBN, THF/DMF, $PdCl_2$ (dppf), Cs_2CO_3, RPhBr; (g) TFA, CH_2Cl_2, 0 °C; (h) HOBT, DMF, 0 °C, NMM, EDC, *O*-(*t*-butyldimethylsilyl)hydroxylamine.

Table 1. Macrocyclic amide hydroxamic acids

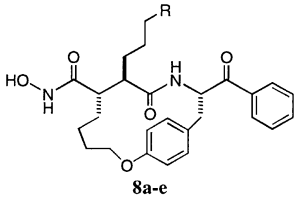


7a-h

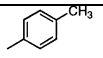
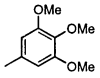
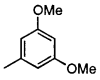
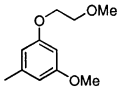
Compd	R	TACE IC_{50} (nM)	MMP-1 IC_{50} (nM)	MMP-2 IC_{50} (nM)	TNF-THP-1 IC_{50} (nM)
7a		3.4	2.3	0.26	440
7b		2.7	6.8	0.79	400
7c		5.2	230	10	180
7d		6.8	140	13	4100
7e		7.6	2300	110	140
7f		33	> 10,000	2200	300
7g		6.0	28	0.23	440
7h		12	> 1,000	96	220

succinate **3** was coupled to the amino ketone derivative of L-tyrosine.[8] Conversion to the hydroxamic acids afforded compounds **8a**–**e**.

Table 2. Macrocyclic ketone hydroxamic acids

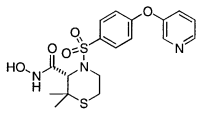
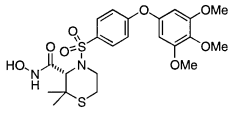
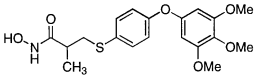
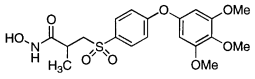


8a-e

Compd	R	TACE IC_{50} (nM)	MMP-1 IC_{50} (nM)	MMP-2 IC_{50} (nM)	TNF-THP-1 IC_{50} (nM)
8a		96	320	11	> 5000
8b		55	> 10,000	3400	3500
8c		43	> 10,000	1000	2900
8d		87	9200	230	> 5000
8e		74	> 10,000	4000	15,000

Selective inhibition of MMPs has been achieved for several series of inhibitors by varying the size of the P_1' substituent.[9] Fibroblast collagenase has a shallow S_1' pocket compared to gelatinase A and is generally intolerant of large P_1' substituents. The X-ray structure of the TACE catalytic domain with bound hydroxamate suggests the enzyme could accommodate inhibitors containing large P_1' substituents.[10] Therefore, compounds with substituted phenylpropyl groups were prepared to explore the different S_1' pockets of TACE, MMP-1, and MMP-2 and to identify P_1' modifications that provide TACE selectivity. The compounds were tested for TACE[11] and MMP[12] inhibition using fluorometric assay systems. Several of these macrocyclic

Table 3. Non-succinate hydroxamates containing the trimethoxyphenyl group

Compd		TACE IC_{50} (nM)	MMP-1 IC_{50} (nM)	MMP-2 IC_{50} (nM)	TNF-THP-1 IC_{50} (nM)
9 (Prinomastat)		7.9	5.7	0.048	8600
10		1.5	26	21	550
11		180	> 10,000	3400	Not tested
12		6.5	> 10,000	430	41,000

hydroxamates failed to demonstrate a trend toward TACE selectivity and, as shown in Table 1, potent inhibition for both TACE and MMP-2 was observed (**7a**–**d**). However, an approximate 10- to 20-fold selectivity for gelatinase A versus fibroblast collagenase was observed as expected for these compounds (**7a**–**d**). When the P_1' substituent was modified to contain the trimethoxyphenyl (TMP) group (**7e**) and the 3,5-bis(trifluoromethyl)phenyl group (**7f**), potent TACE activity was achieved and gelatinase A activity was substantially diminished providing compounds with overall TACE selectivity. The data suggest that substituents at the 3 and 5 phenyl positions are necessary and that bulky P_1' groups alone are not enough to decrease gelatinase A activity since the analogue with the hindered 1-naphthyl group (**7g**) does not provide TACE selectivity.

The macrocyclic hydroxamates **7a**–**h** were also evaluated for their ability to block TNF-α production in a THP-1 cellular assay.[13] The majority of these compounds were active in the whole cell assay with compound **7e**, containing the trimethoxyphenyl group, exhibiting an IC_{50} value of 140 nM.

Since it had been previously disclosed that ketones could replace the P_2'–P_3' amide bond of succinyl hydroxamate MMP inhibitors,[8] additional SAR of the macrocyclic hydroxamates was conducted on phenyl ketone analogues to determine whether the TACE selective aryl substitutions would have the same effect in another series. As shown in Table 2, potent inhibition and selectivity for TACE was observed with compounds containing various substituted phenylpropyl P_1' substituents (**8b**–**e**), compared to the reference compound **8a** containing a tolylpropyl P_1' group. The TMP ketone analogue **8b** demonstrated TACE selectivity comparable to the corresponding TMP-containing *N*-methyl amide **7e**. However, in contrast to the macrocyclic amide hydroxamates, the phenyl ketone analogues were found to be only weakly active in the TNF cellular assay.

In order to further investigate the capacity of substituted aryl P_1' groups to impart TACE selectivity, the trimethoxyphenyl group was incorporated onto several series of non-succinate hydroxamic acids and evaluated for TACE inhibition, TACE versus MMP selectivity, and cellular inhibition of TNF-α production.

As shown in Table 3, several TMP-containing non-succinate hydroxamates were synthesized and evaluated. The penicillamine derived sulfonamides[14] (**9** and **10**), the α-methyl sulfide[15] (**11**), and the α-methyl sulfone[15] (**12**) were prepared according to known procedures.

The reference compound prinomastat (**9**) demonstrated very potent inhibitory activity against MMP-2 as well as potent activity against MMP-1 and TACE. As observed in the macrocycle examples, the TMP-containing analogue (**10**) exhibited a decrease in the MMP-2 inhibitory potency. This 400-fold decrease, however, was not sufficient to provide significant TACE selectivity even with the slight increase in TACE potency because of the intrinsic selectivity for MMP-2 observed with this series. Other non-succinate TMP-containing compounds (**11**–**12**) were found to be potent inhibitors of TACE while differing in the degree of selectivity over the MMPs. Compound **11** was moderately potent against TACE with an IC_{50} value of 180 nM but was also less than 20-fold selective vs MMP-2. Compound **12** showed good selectivity over the MMPs (66-fold vs MMP-2 and > 1,500-fold versus MMP-1) and good potency against TACE with an IC_{50} value of 6.5 nM but failed to potently inhibit the release of TNF-α in the cellular assay.

In conclusion, several substituted propylphenyl P_1' groups were discovered that provide selectivity for inhibition of TACE over MMP-1 and MMP-2. Differences in the S_1' pockets may explain why these P_1' groups cause a decrease in the inhibitory activity of the MMPs while maintaining potent inhibitory activity of TACE.

TACE selectivity was demonstrated across several different series of hydroxamate-based inhibitors with compounds containing a TMP group in the P_1' substituent.

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