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MACROCYCLIC HYDROXAMATE INHIBITORS OF MATRIX METALLOPROTEINASES AND TNF-α PRODUCTION

Robert J. Cherney,* Li Wang, Dayton T. Meyer, Chu-Biao Xue, Elizabeth C. Arner,‡ Robert A. Copeland, Maryanne B. Covington,‡ Karl D. Hardman, Zelda R. Wasserman, Bruce D. Jaffee,‡ and Carl P. Decicco

The DuPont Pharmaceutical Co.
Chemical and Physical Sciences and Inflammatory Diseases Research‡
Experimental Station, Wilmington, DE 19880-0500, U.S.A.

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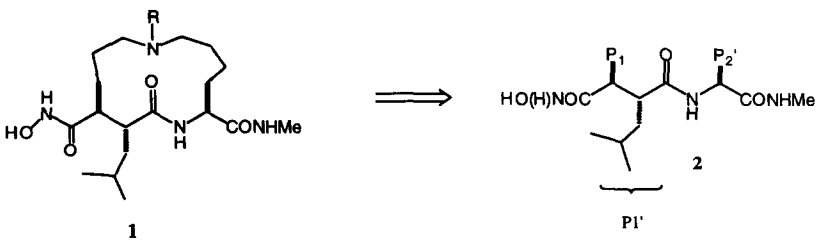
Abstract: Several macrocyclic, hydroxamate derivatives were synthesized and evaluated as inhibitors of matrix metalloproteinases (MMPs) and tumour necrosis factor-α (TNF-α) production. These macrocycles are anti-succinate based inhibitors linked from P1 to P2'. A variety of functionality was installed at the P1-P2' linkage, which gave inhibitors that displayed excellent MMP inhibition and good TNF-α suppression.

As part of our inflammatory disease program, we have investigated the inhibition of a large family of enzymes classified as the matrix metalloproteinases (MMPs).[1] These are endogenous, zinc-dependent endopeptidases that are responsible for the regulation of the extracelluar matrix. High levels of these enzymes have been found in a variety of diseases which are characterized by tissue and membrane destruction.[2] As a result, the MMPs have become the target of intense research within the pharmaceutical community.

It has also been shown that some MMP inhibitors can inhibit the shedding of tumour necrosis factor-α (TNF-α) from cells.[3] TNF-α is a pro-inflammatory cytokine that has been clinically linked to rheumatoid arthritis (RA)[4] and Crohn's disease.[5] Recently, a new metalloproteinase, TNF-α converting enzyme (TACE),[6] has been shown to cleave membrane bound pro-TNF, which results in the release of soluble TNF-α. Hence, we are also interested in evaluating our MMP inhibitors for their ability to suppress TNF-α processing.

We recently communicated our work on macrocyclic metalloproteinase inhibitors.[7] These are succinate based inhibitors that contain a hydroxamic acid used to ligate the active site zinc. In an effort to further optimize the potency and physical properties of these inhibitors,[8] we have explored nitrogen linked macrocycles of type **1**, which we report herein.



1 **2**

From in-house, enzyme-inhibitor crystallographic studies, we recognized that the macrocyclic linkage (N-R in **1**) resided in the solvent exposed area away from the active site. This provided an area of the inhibitor

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that was essentially free from contact with the enzyme, which we felt could be manipulated to affect the physical properties of the compounds. Changes in the macrocyle also have the ability to effect the conformation, thereby potentially optimizing the affinity of these inhibitors as well. From these considerations we chose a 13-membered sulfonamide (R = -SO_2Ph) linked macrocycle as our initial target. We felt the sulfonamide could aid synthetically in the macrocyclization, serve as a point of variation, and through its removal, provide access to other amine based analogues.

Synthesis

A general preparation of the macrocyclic sulfonamides is shown in Scheme 1. The previously reported[7,9] anti-succinate **3** served as the starting point of this work. With succinate **3** in hand, a hydroboration was performed with 9-BBN to provide the alcohol **4**. Hydrogenolysis of this material gave the carboxylate **5**, which was subsequently coupled to various lysine derivatives. For example, *L*-Lys-(SO_2Ph)-NHMe **6** was coupled to **5** under standard conditions to yield the amide **7**. After some experimentation, we found that Mitsunobu conditions could effect the direct macrocyclization of **7**. The best conditions were DIAD/PPh_3/THF, and when applied to **7** gave the macrocycle **8**. The efficiency of this cyclization varied with the R group of the sulfonamide, and Table 1 summarizes some of these unique Mitsunobu macrocyclizations. The synthesis was continued by deprotection of the *t*-butyl ester with TFA to afford the carboxylate **9**. This was converted to the benzyl protected hydroxamate **10**, which was hydrogenated[10] to the target hydroxamate **11**. Several macrocyclic sulfonamides were generated by this route and are shown in Table 2.

Scheme 1

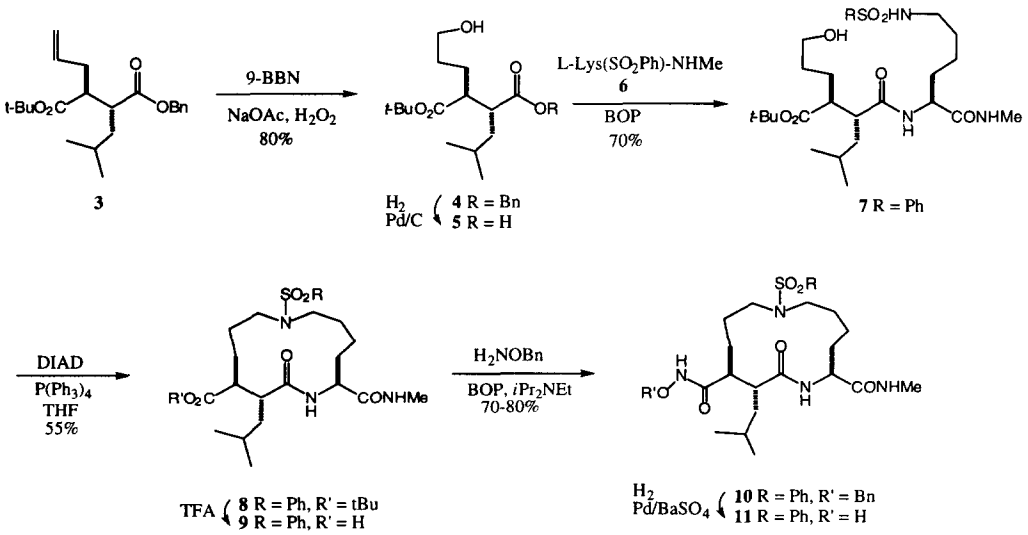
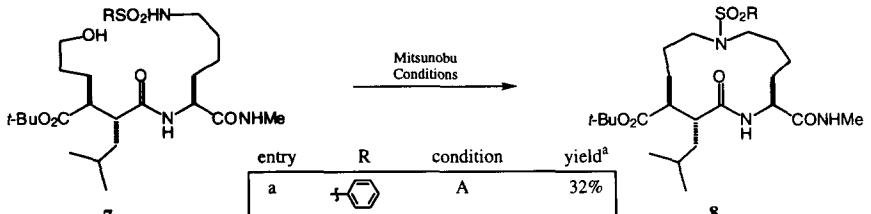
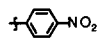


Table 1. *Mitsunobu Macrocyclizations*



7 8

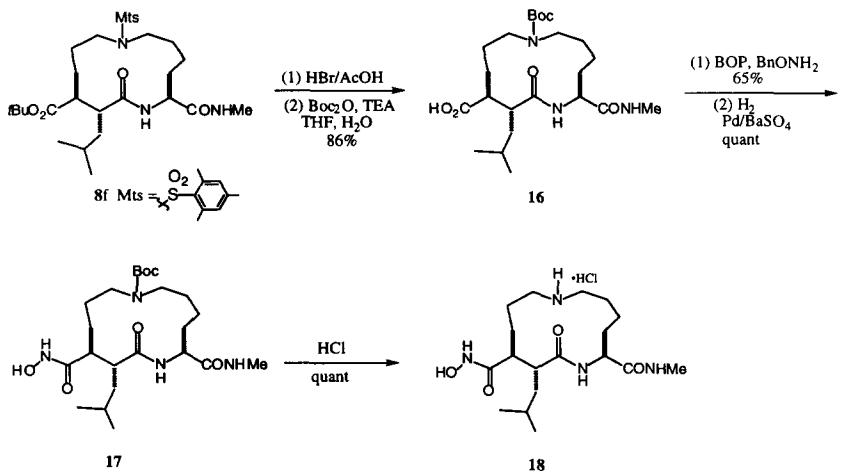
entry	R	condition	yield[a]
a		A	32%
b		B	25%
c		C	55%
d	CF_3	C	60%
e		C	30%
f		C	30%
g		C	52%

Mitsunobu Conditions
A. DIAD, PPh_3, toluene, -10°C
B. DEAD, PPh_3, THF, rt
C. DIAD, PPh_3, THF, rt

a. yields are unoptimized

We also discovered a method capable of converting a macrocyclic sulfonamide into either a carbamate or an amino derivative. As shown in Scheme 2, the N-mesitylenesulfonyl derivative **8f** was reacted with HBr/AcOH to give a nonisolated amino acid derivative, which in turn, was reacted with Boc_2O to yield the carbamate **16**. This material was converted to hydroxamate **17** via the benzyl protected hydroxamate under our standard protocol. Access to the amino derivative was accomplished by treatment of **17** with HCl to yield the macrocyclic amine **18**.

Scheme 2



Results and Discussion

The macrocyclic derivatives were tested in vitro as MMP inhibitors, and K_is were generated[7,11] for MMP 1, 3, and 9.[12] The parent benzenesulfonamide **11** was an excellent MMP inhibitor with a $Ki < 1$ for MMP-1 and MMP-9. This rivals linear inhibitors of this type and suggests the 13-membered sulfonamide provides an excellent platform for the extended conformation of the anti-succinate. As shown in Figure 1, the X-ray crystal structure of **11** bound in MMP-3 orients the phenyl of the sulfonamide back toward the protein. This area of MMP-3 is aromatic rich and may provide a site for additional interactions. To assess the benzenesulfonyl's interaction with the enzyme, we replaced the phenyl with a small trifluoromethyl group as in **12**. The trifluoromethyl derivative **12** was 30-fold less active in MMP-3 and at least fourfold less active in MMP-1, which suggests that the phenyl of **11** can contribute to the hydrophobic binding. In order to investigate this area further, a 4-amino group was attached to the phenylsulfonyl. This produced the aniline derivative **13**, which also proved to be 30-fold less active in MMP-3 than the parent **11**. Although the addition of the heteroatom was not tolerated in MMP-3, the aniline **13** did have improved physical properties. Therefore, we investigated small heterocycles like the imidazole of **15**. This proved to be a better compromise with respect to broad MMP activity, as the MMP-3 affinity of **15** was only threefold less than that for **11**. At this point, we decided to remove the sulfonamide and assess other groups in this area. The t-butyl carbamate **17** had a very similar MMP profile to the parent sulfonamide **11**. In addition, complete removal of the carbamate gave the secondary amine **18**, which displayed excellent MMP-1 affinity while having 12-fold less affinity for MMP-9 over the carbamate **17**.

Figure 1. *X-ray Crystal Structure of 11 bound in MMP-3*

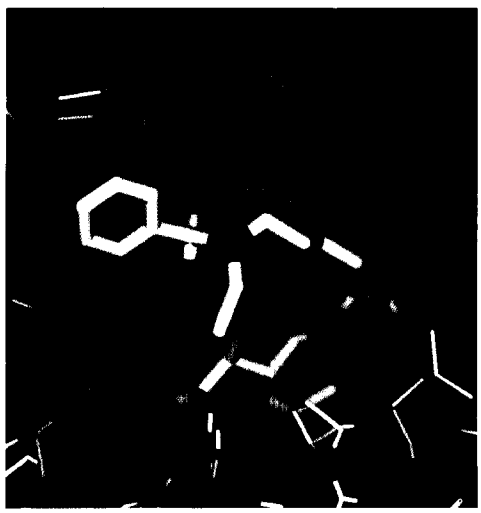
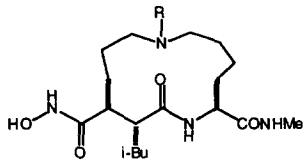


Figure 1 Description: Active site X-ray crystal structure of sulfonamide **11** (white carbons with blue nitrogens and red oxygens) bound in MMP-3 (white carbons with blue nitrogen and red oxygens - catalytic zinc is orange).

Table 2. *MMP Inhibition and TNF-α Suppression*



compd	R	Ki (nM) MMP-1	MMP-3	MMP-9	TNF-WBA IC_{50} μM
11	$-SO_2Ph$	<1	3.4	<1	1.3
12	$-SO_2CF_3$	4	118	<1	1.3
13	$-SO_2Ph-NH_2$	<1	99	1.6	0.95
14	-Mts	2.3	15	<1	1.6
15		<1	12	2.7	1.4
17	Boc	<1	3	<1	1.4
18	H•HCl	<1	NT	12.8	2.7

NT = not tested

These compounds were also assessed for their ability to suppress the release of TNF-α in human whole blood after stimulation with lipopolysaccharide (LPS). In this assay (TNF-WBA),[7,13] we are measuring the ability of the compounds to suppress the formation of TNF-α presumably by inhibiting TACE,[3,6] or some related metalloproteinase.[14] As shown in Table 2, all the sulfonamide macrocycles displayed good suppression of TNF-α in this cellular assay. The best sulfonamide was the aniline derivative **13**, which had an IC_{50} less than 1 μM. Again, the *t*-butyl carbamate **17** proved to be very similar to the sulfonamides with an IC_{50} just over 1 μM. However, the secondary amine **18** was about twofold less potent than the parent sulfonamide **11**.

Conclusions

We have introduced a new class of macrocyclic metalloproteinase inhibitors represented by the general structure **1**. Their synthesis features a unique macrocyclic Mitsunobu reaction involving sulfonamides as detailed in Table 1. These inhibitors displayed excellent inhibition of MMP-1, MMP-3, and MMP-9 as well as good potency in the TNF-WBA assay. Their novel binding and SAR should aid in the development of future inhibitors as potential therapeutics.

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