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POTENT MATRIX METALLOPROTEINASE INHIBITORS: AMINO-CARBOXYLATE COMPOUNDS CONTAINING MODIFICATIONS OF THE P1 RESIDUE

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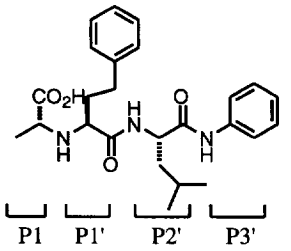
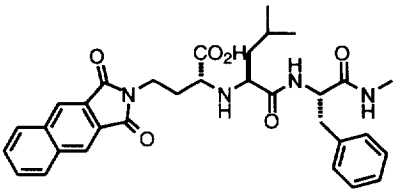
Abstract: A series of amino-carboxylate inhibitors of matrix metalloproteinases containing different P1 modifications have been synthesized. It was discovered that a toluenesulfonamide-type substituent at the P1 position considerably enhances the binding affinity of inhibitors for stromelysin. Replacements of the P2'-P3' residues with nonpeptide components result in loss of inhibitory activity.

Collagenases (MMP-1), stromelysins (MMP-3) and gelatinases (MMP-9) belong to a family of matrix metalloproteinases (MMPs) that are zinc dependent endoproteinases. The biological functions of these metalloproteinases involve the remodeling and degradation of the extracellular matrix in connective tissue.[1] Normally MMPs play an important role in matrix remodeling and maintenance. Unregulated expression of these enzymes, however, may result in connective tissue degradation and ultimate destruction. It has been found that the activities of collagenase and stromelysin were increased in the cartilage of patients with rheumatoid and osteoarthritis, and the activity correlates with the severity of the lesion.[2] In addition, elevated levels of stromelysin in human rheumatoid synovium have been observed.[3-4] Thus, stromelysin (MMP-3) appears to be a key enzyme in the destruction of mammalian cartilage and bone, and inhibition of this enzyme *in vivo* should have therapeutic potential for the treatment of arthritis.



I IC_{50}=0.32 μM (MMP-3)



II IC_{50}=0.09 μM (MMP-3)

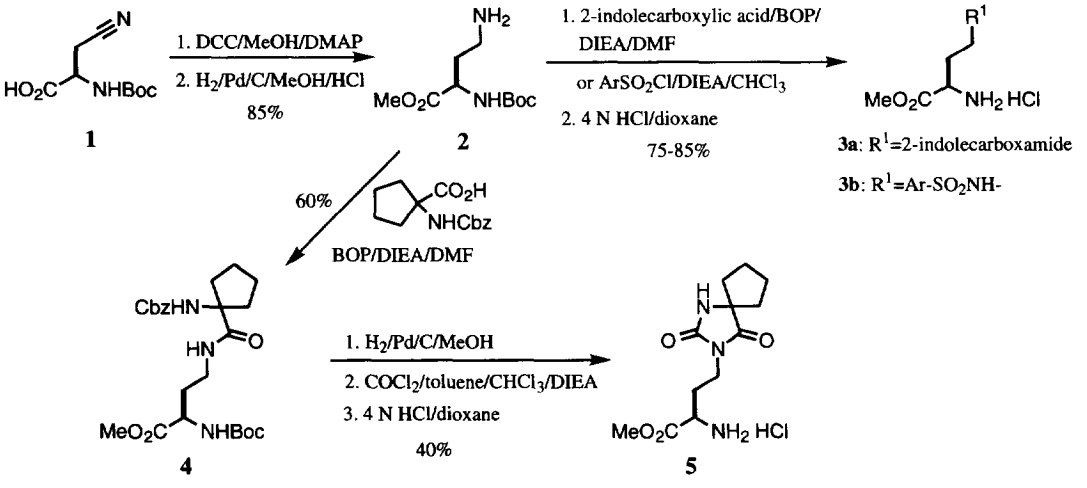
There has been considerable effort in the design of MMPs inhibitors, which have been extensively reviewed.[5-9] These inhibitors normally contain a hydroxamate, carboxylate, thiol, phosphinate, phosphonate or phosphoramidate as a zinc chelator. Among them, the amino-carboxylate series of MMP inhibitors have potential as oral therapeutic agents, as by analogy, amino-carboxylate inhibitors are effective oral agents for the inhibition of

angiotensin-converting enzyme, another zinc metalloproteinase.[10] Structure-activity relationships of amino-carboxylate inhibitors of the MMPs have been reported.[11-12] It was discovered that a phenethyl group at the P1' position (structure I)[11] gave compounds with high binding affinity. It has also been shown that a naphthalimide group at P1 (structure II)[12] imparts additional potency. However, the naphthalimide group was found to be unstable in aqueous solution, especially at high pH, due to base-catalyzed hydrolysis. As part of our efforts in the search for potent MMP-3 inhibitors, we were interested in the amino-carboxylate series of compounds with a novel and stable group at the P1 position. In this communication we report the synthesis and *in vitro* activity of an amino-carboxylate series of compounds based on structure I with a variety of different P1 modifications and P2'-P3' residue replacements.

Chemistry

The starting material D-cyanoalanine (**1**) was synthesized from N-*t*-butyloxycarbonyl-D-glutamine (Boc-D-Gln) using chemistry described previously.[13] The carboxylic acid of **1** was esterified using dicyclohexylcarbodiimide (DCC) in the presence of 4-dimethylaminopyridine in THF/MeOH and the nitrile group was reduced to an amine by catalytic hydrogenation to give **2**. Coupling of **2** with 2-indolecarboxylic acid using BOP[14] followed by acid treatment afforded intermediate **3a** while sulfonylation of **2** with arylsulfonyl chloride followed by acid treatment gave the intermediate **3b**. Coupling of **2** with N-benzyloxycarbonyl-1-amino-cyclopentanecarboxylic acid using BOP produced **4**. The Cbz group was removed by hydrogenation and the resulting amine was treated with phosgene to give a hydantoin ring. Deprotection of the Boc group using 4 N HCl afforded intermediate **5**.

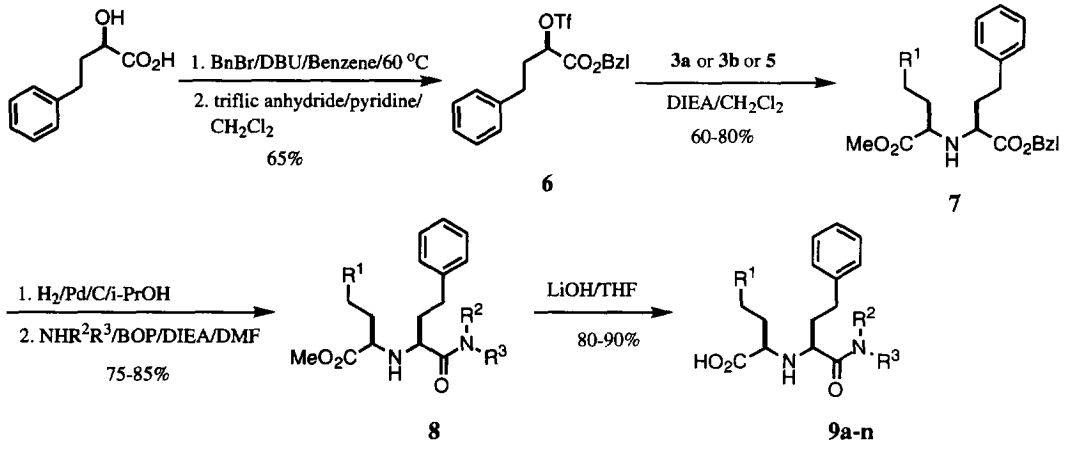
SCHEME 1



(R)-2-hydroxy-3-phenylpropionic acid was converted to its benzyl ester using benzyl bromide and DBU in benzene at 60 °C and the hydroxy group of the resulting ester was converted to a triflate **6**.[11] Displacement of the triflate **6** with **3a**, **3b** or **5** afforded intermediates **7**. The benzyl ester of **7** was removed by catalytic hydrogenation

and the resultant derivative was coupled with an amino acid amide or an amine derivative to give compounds **8**. Saponification of **8** using LiOH in THF afforded the target compounds **9a-n**.

SCHEME 2



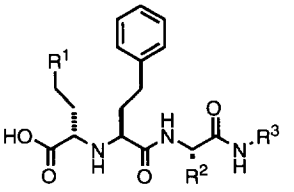
Structure Activity Relationships:

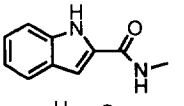
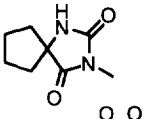
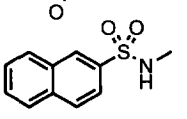
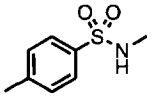
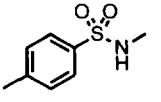
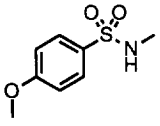
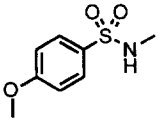
Molecular modeling studies using the X-ray crystal structure of prostromelysin[15] revealed the possibility of both hydrophobic and hydrogen-bonding interactions in the vicinity of the P1 side chain. We thus tried to pick up additional hydrogen bonding by incorporating three different types of substituents (2-indolecarboxamide, hydantoin, and sulfonamide) into the P1 residue of structure I. As can be seen from Table 1, the 2-indolecarboxamide analog (**9a**) and the hydantoin analog (**9b**), which have a phenethyl group as the P1' residue and a phenylalanine N-methylamide as the P2' and P3' residues showed similar activities, with an IC_{50} of 1.1 μM and 1.9 μM respectively for inhibition of MMP-3.[16] A 2-naphthalenesulfonamide in the P1 position (**9c**) gives an over 3 fold increase in potency relative to **9b**. Replacement of the 2-naphthalenesulfonamide with a *p*-toluenesulfonamide afforded compound **9d** which is 4 fold more potent than **9c**. Further refinement by replacement of the phenylalanine N-methylamide in **9d** with a leucine N-phenylamide results in a potent analog **9e** which has an IC_{50} of 50 nM. Replacement of the methyl group of the *p*-toluenesulfonamide in **9e** with a methoxy group produced an equipotent analog **9f**, indicating no extra hydrogen bond interaction of the methoxy group with the receptor. It has been reported[11] that the anilide group at the P3' position can be hydrolyzed by mouse blood to give an acid and an aniline. We thus utilized an unusual amide bond, an O-benzylhydroxamide group in **9g**, as a measure to avoid this metabolic liability, and compound **9g** is about 10 fold less potent than **9f**.

It has been discovered[17] that the amino acid amide residue at P2'-P3' position can be replaced with a morpholine to give potent inhibitors for collagenase in the hydroxamic acid series. In order to investigate the importance of this amino acid amide residue for the MMP-3 receptor in the amino-carboxylate series,[18] we synthesized a series of compounds which contain a nonpeptide component in the P2'-P3' region (Table 2). It was found that elimination of the amide bond between P2'- P3' residues results in significant loss in activity; compounds **9h** and **9i** have an IC_{50} of 14 μM and 18 μM respectively, which are over 100 fold less active relative

to **9d**. Replacement of the 4-benzylcarboxylic acid group in **9h** with a 4-(1-ethoxycarbonyl)piperidyl group leads to a less active analog **9j**. The importance of the N-H group of the amide bond between P1'-P2' residues was revealed by the syntheses of compounds **9k-9n**. These analogs contain a morpholine (**9k**), a 4-hydroxypiperidine (**9l**), a 4-ethoxycarbonylpiperazine (**9m**) and a 4-methanesulfonylpiperazine (**9n**) residue which replaces the phenylalanine N-methyl amide residue in **9d**, and they are inactive in the inhibition of MMP-3. These results indicated that the two primary amide bonds at P1'-P2' position and at P2'-P3' position are critical for high potency in the inhibition of MMP-3.

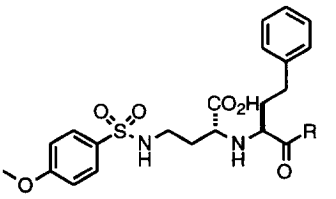
Table 1. *In Vitro* Activity of Amino-Carboxylate Analogs in the Inhibition of MMP-3

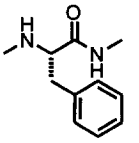
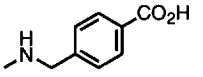
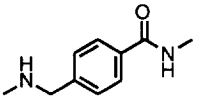
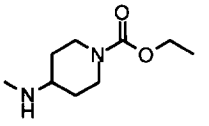
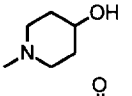
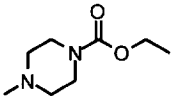
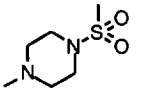


Compound*	R^1	R^2	R^3	IC_{50}, μM
9a		-CH_2Ph	-CH_3	1.10
9b		-CH_2Ph	-CH_3	1.90
9c		-CH_2Ph	-CH_3	0.51
9d		-CH_2Ph	-CH_3	0.12
9e		-$CH_2CH(CH)_3$	-Ph	0.05
9f		-$CH_2CH(CH)_3$	-Ph	0.05
9g		-$CH_2CH(CH)_3$	-OCH_2Ph	0.55

* Tested as TFA salts.

Table 2. *In Vitro* Activity of Amino-Carboxylate Analogs with Non-Peptide Component at P2'-P3' Residues in the Inhibition of MMP-3



Compound*	R	IC_{50}, μM
9d		0.12
9h		14.00
9i		18.00
9j		>20.00
9k		>20.00
9l		>20.00
9m		>20.00
9n		>20.00

* Tested as TFA salts

In summary, we have discovered an amino-carboxylate series of potent MMP-3 inhibitors which contain a novel modification in the P1 position. Among the P1 substituents investigated (2-indolecarboxamide, hydantoin, 2-naphthalenesulfonamide, *p*-toluenesulfonamide, and 4-methoxybenzenesulfonamide), the *p*-toluenesulfonamide-type subsituents (**9d-9f**) were found to afford the most potent analogs. The increase in potency in **9e** and **9f** compared with structure I might be due to hydrogen-bonding of the sulfonamide with the receptor. The studies explored in this work are very helpful for our understanding of the structure-activity relationships of MMP-3

inhibitors and the compounds described herein may prove very useful for the biological investigations on the role of stromelysin in cartilage degradation.

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