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INHIBITION OF MATRIX METALLOPROTEINASES BY P_1 SUBSTITUTED *N*-CARBOXYALKYL DIPEPTIDES

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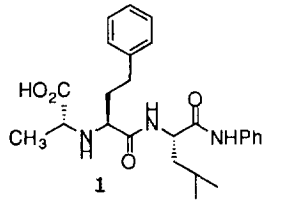
Abstract: Aroyl and arylacyl aminoalkyl substitutents at the P_1 position of *N*-carboxyalkyl dipeptides were found to enhance potency and selectivity for stromelysin-1 (MMP-3). In particular, the phthalimidobutyl and phenylpropanoylaminopropyl groups offered inhibitors of MMP-3 with K_i's of ~ 10 nM.

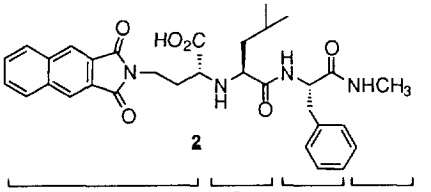
The matrix metalloproteinases (MMP's) are a family of zinc-containing, calcium-dependent mammalian proteinases that are capable of degrading the extracellular matrix of connective tissues and basement membranes.[1] These enzymes have been implicated in a variety of biological processes, including degradative diseases such as rheumatoid and osteoarthritis.[2] Many classes of MMP inhibitors have been described.[3] Two recent reports describe optimization of *N*-carboxyalkyl peptide inhibitors of stromelysin-1 (MMP-3) and collagenase-1 (MMP-1).[4,5] Potent inhibitors of gelatinase A (MMP-2) and MMP-3 have been achieved with arylalkyl substituents at P_1' (e.g., **1**)[4,6] and of MMP-1 and gelatinase B (MMP-9) with arylimidoethyl substitutents at P_1 (e.g., **2**).[5] Phthalimidobutyl substituents at P_1 were also found to be potency enhancing for MMP-3 in a series of phosphinic acid dipeptides.[7] Our initial studies of *N*-carboxyalkyl dipeptide inhibitors did not reveal any increased binding interactions with several MMP's with the typical amino acid side chains at P_1.[4] Herein we report the synthesis and enzyme inhibition of a series of potency enhancing substituents on the P_1 position of **1**.



1

P_1 P_1' P_2' P_3'

MMP-1 (HFC) Ki = 0.76 μM
MMP-2 (GEL-A) Ki = 0.20 μM
MMP-3 (HFS) Ki = 0.47 μM



2

P_1 P_1' P_2' P_3'

MMP-1 (HFC) Ki = 0.020 μM
MMP-9 (GEL-B) Ki = 0.005 μM
MMP-3 (HFS) Ki = 0.091 μM

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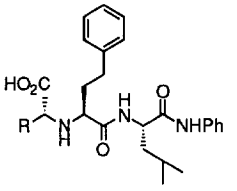
Experimental Procedures.

The inhibitors were prepared by previously described methodology.[4] Briefly, *N*-carboxyalkyl amino acid esters were prepared by reductive amination of an appropriately protected amino acid with a ketoester. The diastereomers were separated by reverse phase medium pressure column chromotography. Ester hydrolysis followed by condensation with L-leucine phenylamide and catalytic hydrogenolysis yielded *N*-carboxyalkyl dipeptides **3**. If the amino acid contained an aminoalkyl side chain, acylation was affected by treatment with an acid chloride in the presence of triethylamine to afford **4**. Compounds **1**, **3**, and **4** were evaluated as inhibitors of stromelysin-1 (MMP-3), collagenase-1 (MMP-1) and gelatinase A (MMP-2) according to published procedures[4] and the results with the active diastereomers are reported in Tables 1 and 2. All compounds displayed competitive, reversible inhibition.

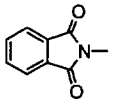
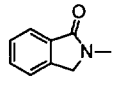
Results and Discussion

As seen in Table 1, increasing the length of the alkyl chain at P_1 (**3a-3c**) had little effect on the inhibition of MMP-3 compared to **1** but did result in a significant gain in selectivity over MMP-1 and, to a lesser extent,

Table 1. Inhibition of MMP's by P_1-Substituted *N*-Carboxyalkyl Dipeptides.



Compd. No.	R	MMP-3 Ki, μM (± SE)	MMP-1 Ki, μM (± SE)	MMP-2 Ki, μM (± SE)
1	CH_3	0.47 (0.08)	0.76 (0.22)	0.20 (0.04)
3a	C_2H_5	0.35 (.04)	1.8 (.2)	1.1 (.1)
3b	n-C_4H_9	0.32 (.03)	6.8 (.7)	1.1 (.2)
3c	n-C_6H_{13}	0.53 (.07)	43 @ 10	2.3 (0.3)
3d	$Ph(CH_2)_4$	0.12 (.02)	0.072 (.003) [c]	64% [d]
3e	Phth-$N(CH_2)_2$ [a]	0.033 (.004)	2.09 (.30)	0.020 (.003)
3f	Phth-$N(CH_2)_3$	0.14 (.02)	3.4 (0.4)	0.57 (.10)
3g	Phth-$N(CH_2)_4$	0.008 (.001)	0.72 (.12)	0.086 (.009)
3h	Phth[H_2]-$N(CH_2)_4$ [b]	0.012 (.001)	1.6 (.3)	0.11 (.02)
4a	Ac-Pro-$NH(CH_2)_4$	0.18 (.02)	1.1 (.1)	91% [d]

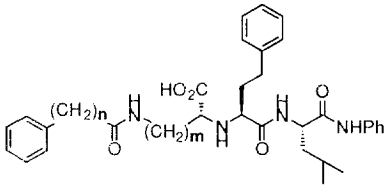
[a] Phth-N =  [b] Phth[H_2]-N = 

[c]Inhibition data reported as an IC_{50} as determined in reference 4. [d]Percent inhibition at 1 μM

MMP-2. The effects of chain length were more impressive in the compounds containing a P_1 phthalimidoalkyl group (Phth) **3e-3g**. The phthalimidobutyl substitutent in **3g** was the most potent and selective inhibitor of MMP-3 in the series. The benzolactam **3h**, lacking one of the carbonyls of the imide **3g**, inhibited all three MMP's with potencies comparable to **3g**. These results would suggest that only one of the carbonyl functionalities in the imide of **3g** and the lactam of **3h** is involved in binding to these enzymes. This is in contrast to the large loss of potency seen between a P_1 naphthylimide **2** and the corresponding naphthyl-lactam from a related series of *N*-carboxypropyl dipeptide inhibitors of MMP's wherein both carbonyls of the imide appear necessary for effective binding.[5]

It is intriguing to speculate that the P_1 substituents in compounds such as **2** and **3g** are "in register" with one another and are binding into the P_3 region that is often occupied by proline in many of the substrates of these enzymes. However, the P_1 (L)-proline analog **4a** is a much poorer inhibitor of MMP-3 than is the phthalimide **3g**. In addition, P_1 (D)- and (L)-(N-acetyl)prolylaminobutyl analogs in a related series of phosphinic acid inhibitors were found to be equipotent, suggesting that the binding interactions in this region are largely hydrophobic and relatively nonspecific.[7]

Table 2. Inhibition of MMP's by P_1 Arylacylaminoalkyl *N*-Carboxyalkyl Dipeptides.



Compd. No.	n	m	MMP-3 Ki, μM (± SE)	MMP-1 Ki, μM (± SE)	MMP-2 Ki, μM (± SE)
4b	0	2	0.12 (0.01)	1.09 (0.14)	72% [b]
4c	1	2	0.13 (0.02)	1.79 (0.21)	ND [c]
4d	2	2	0.11 (0.01)	3.33 (0.30)	41% [b]
4e	3	2	0.27 (0.6)	4.41 (0.61)	0.77 (0.10)
4f	0	3	0.27 (0.05)	50% [a]	50% [b]
4g	1	3	0.010 (0.002)	0.87 (0.07)	0.06 (0.005)
4h	2	3	0.0095 (0.0015)	2.2 (0.1)	87% [b]
4i	3	3	0.12 (0.02)	36% [a]	70% [b]
4j	0	4	0.032 (0.005)	5.8 (0.4)	0.120 (0.009)
4k	1	4	0.028 (0.009)	7.2 (0.7)	0.07 (0.01)
4l	2	4	0.19 (0.03)	13.0 (1.0)	49% [b]
4m	0	5	0.02 (0.002)	3.89 (0.55)	0.12 (0.01)
4n	1	5	0.047 (0.009)	5.81 (0.58)	0.35 (0.06)

[a]Percent inhibition at 10 μM. [b]Percent inhibition at 1 μM. [c]ND = Not determined.

In an effort to determine the optimum spacing for a P_1 substituent from the zinc ligand carboxylate to this putative hydrophobic domain, a series of P_1 acylated aminoalkyl substituents **4b-4n** were prepared (Table 2). This series varied both the length of the aminoalkyl chain as well as that of the phenylacyl group. Thus, this series of compounds would optimize placement of a phenyl group into the putative hydrophobic domain and would probe for potential polar interactions along the connecting spacer. As seen in Table 2, the P_1 phenylacylaminoethyl compounds (m = 2; **4b-4e**) were equipotent vs. MMP-3. The acylated aminopropyl (**4f-4i**) and aminobutyl analogs (**4j-4k**) were more potent vs. MMP-3 when m + n = 4 or 5. This P_1 substituent can be made too long as seen in the loss of potency when m + n = 6 in **4i** and **4l**. However, as seen in **4n** which has m + n = 6 and is still potent, exact conclusions of distance from one binding domain to another cannot be determined with such a nonrigid system.

A potency-enhancing hydrophobic domain in MMP-3 has been identified in the region of P_3 in *N*-carboxyalkyl derived inhibitors as well as phosphinic-acid containing inhibitors.[7] However, the intervening amide bond between what would be P_2 and P_1 in a peptidyl substrate was found to be unnecessary in these two inhibitor series; highlighting the dominant binding elements on the C-terminal side of a scissile bond. Selectivity for MMP-3 was achievable over MMP-1 but less so for MMP-2. Most of the potency and selectivity for MMP-3 (and MMP-2) over MMP-1 was more likely achieved by the P_1' phenethyl substituent than by interactions in the region of $P_{1(3)}$. Potent MMP-1 and MMP-9 *N*-carboxyalkyl peptide inhibitors containing large hydrophobic P_1 substituents with selectivity vs. MMP-3 were achieved with the smaller P_1' isobutyl group in **2**.[5]

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