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DESIGN AND SYNTHESIS OF CONFORMATIONALLY-CONSTRAINED MMP INHIBITORS

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Abstract: A novel series of conformationally constrained matrix metalloprotease inhibitors was identified. The potencies observed for these inhibitors were highly dependent upon the substitution pattern on the caprolactam ring as well as the succinate moiety. © 1998 Elsevier Science Ltd. All rights reserved.

Introduction

Matrix metalloproteases (MMPs) containing a divalent Zn^{2+} at the active site are an important class of endoproteinases involved in extracellular matrix remodeling. Under normal physiological conditions, the proteolytic activities of the enzymes are controlled by tissue inhibitors of matrix metalloproteases (TIMPs).[1-6] In pathological conditions this fine balance is tipped more towards catabolism leading to degradation of the matrix components. MMPs have been implicated in numerous disease states involving matrix degradation which include arthritis,[7,8,9] cancer,[10,11] and periodontal diseases.[12] Consequently, there has been significant interest in developing MMP inhibitors which may control the aberrant regulation of MMP production.[13]

The Design Concept of Constrained Inhibitors

Our initial strategy was to design MMP inhibitors containing a heterocyclic moiety. A literature search revealed that Roche had investigated peptidomimetic structures containing a caprolactam ring system, but with no substitution at the ring nitrogen.[14] The binding potency reported for this caprolactam-based inhibitor (see compound **1**, Table) was low, indicating that some additional enzyme-substrate interactions would be required to improve the potency. We therefore focused our attention on substitution of the caprolactam ring nitrogen and the succinate moiety with various groups. In this preliminary report we wish to share our early SAR work which produced a series of potent inhibitors with a unique range of selectivities for the various MMPs.

Chemistry

The majority of the metalloprotease inhibitors disclosed in this report were prepared by a convergent route by coupling a succinate moiety with an amino containing caprolactam. A representative example of the preparation of these inhibitors is shown below. The synthesis begins with L-α-amino-ε-caprolactam which was first protected as the *t*-Boc derivative. The amide proton was selectively removed and the resulting anion was quenched with methyl bromoacetate to provide caprolactam **2**. Subsequent deprotection with trifluoroacetic acid provided the desired amino-caprolactam **3**. The *t*-butyl succinate **4**, which was prepared following Evan's oxazolidinone chemistry,[15,16] was directly coupled to the amino-caprolactam **3** providing amide **5**. The *t*-butyl ester in **5** was then transformed in 3 steps to the desired hydroxamic acid **11**. The alkyl ethers **16 - 21** were prepared via an analogous route using the corresponding alkyl bromide.

We next turned our attention to the preparation of disubstituted succinate derivatives.[17] The acid **7** was alkylated at the α-position with an electrophile to provide the corresponding disubstituted product as a mixture of diastereomers **8** which were not readily separable at this stage. In order to enrich the mixture with the

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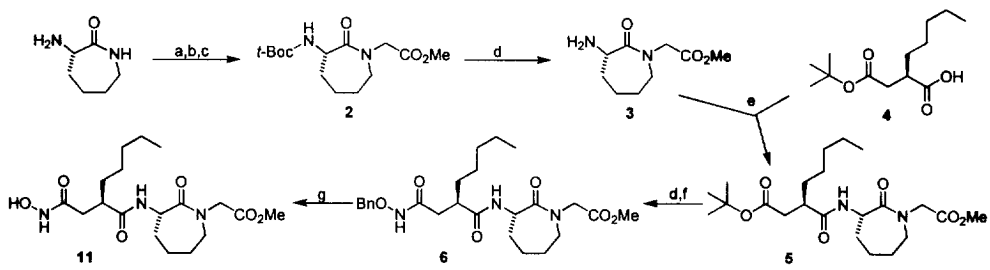
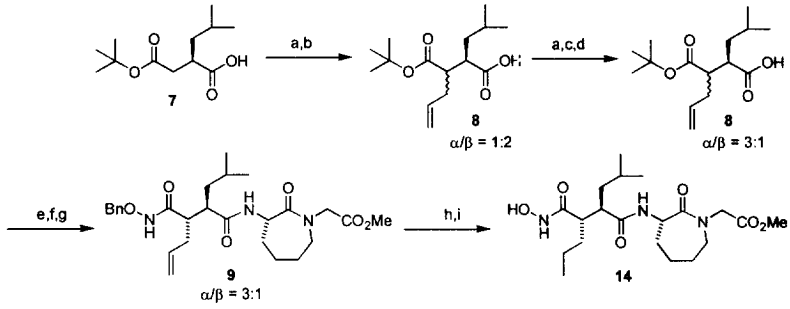
Scheme 1



Reagents/Conditions: (a) $(t\text{-Boc})_2O$, DMSO; (b) $LiN(TMS)_2$, THF, -78 °C; (c) $BrCH_2CO_2Me$; (d) CF_3CO_2H; (e) EDAC, HOBT; (f) EDAC, HOBT, $BnONH_2{\cdot}HCl$; (g) H_2, Pd-C

desired *S*-isomer, the crude product was subjected to a repeated deprotonation-protonation sequence. The enriched acid mixture **8** (α:ß = 3:1) was then directly coupled to the substituted caprolactam followed by deprotection of the *t*-butyl ester under acidic conditions. The acid, thus obtained, was converted to the corresponding benzyl hydroxamate **9**. The diastereomeric benzyl hydroxamates were readily separable by crystallization at this stage. Debenzylation of the α-isomer under controlled hydrogenolysis conditions provided the desired hydroxamic acid **14** as the major product. For SAR purposes, both diasteromers could be obtained using this methodology.

Scheme 2



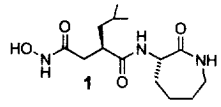
Reagents/Conditions: (a) LDA, THF; (b) allyl bromide; (c) MeOH; (d) Repeat steps a and c; (e) **3**, EDAC, HOBT; (f) CF_3CO_2H; (g) EDAC, HOBT, $BnONH_2{\cdot}HCl$; (h) separate diastereomers; (i) H_2, Pd-C

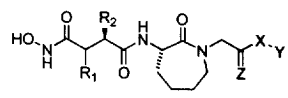
Results and Discussion

A summary of the in vitro binding potencies for selected examples against MMP's 1 and 3 is presented in the Table. The low binding potency exhibited by **1** was attributed to a lack of enzyme-substrate interactions in the S3' pocket. As depicted in the Table, the addition of various substituents on the caprolactam ring nitrogen did improve the binding potencies for both enzymes. The first analogs were prepared with a methyl acetate substituent at the P3' site (see compounds **10** - **15**). Although no significant change in potency for MMP-3 was observed, the affinity of compound **10** for MMP-1 was 17 times higher than that of the original analog **1**. In general, this series of caprolactams was found to be selective for MMP-1. We expected that as the chain length of R_2 increased, we would see a fall in potency for MMP-1 and a concomitant rise in affinity for MMP-3. The S1' pocket in MMP-1 is believed to be shallow due to the presence of a rigid Arg residue.[14] Interestingly, as the size of the R_2 group increased, so too did the corresponding affinity for MMP-1. The data obtained with **12** obviously contradicts this notion.

The introduction of an additional substituent at R_1 had a profound effect on the affinity of the inhibitor for MMP-3 (see **10** vs. **13**). Even a small methyl group helped enhance the potency by a factor of 10. The addition of substituents at both R_1 and R_2 had a synergistic effect producing a series of non-selective MMP inhibitors like **14** and **15**. The introduction of a longer chain did not provide any significant boost in potency (see **15**).

Table: In vitro potencies of caprolactam-based MMP inhibitors





Compound	R_1	R_2	X	Y	Z	IC_{50} (nM) MMP-1[a]	MMP-3[a]
1	-	-	-	-	-	3900	11700
10	H	*i*-butyl	O	Methyl	O	225	2900
11	H	*n*-pentyl	O	Methyl	O	129	824
12	H	*n*-octyl	O	Methyl	O	11	1040
13	(S)-Me	*i*-butyl	O	Methyl	O	49	215
14	(S)-*n*-Pr	*i*-butyl	O	Methyl	O	96	58
15	(S)-Me	*n*-octyl	O	Methyl	O	57	21
16	H	*i*-butyl	O	Phenyl	H_2	117	518
17	H	*i*-butyl	CH_2	Phenoxy	H_2	140	1420
18	H	*n*-octyl	O	Methyl	H_2	1890	250
19	(R)-Me	*n*-octyl	O	Methyl	H_2	4360	108
20	(S)-Me	*n*-octyl	O	Methyl	H_2	112	30
21	(S)-*n*-Pr	*i*-butyl	O	Methyl	H_2	83	94
22	(S)-$(CH_2)_3OH$	*i*-butyl	O	Methyl	H_2	51	104

[a]All in vitro binding data were obtained as single determinations.[18,19]

The strategy at the second phase of the project was primarily focused on modifying those substituents which may be prone to metabolic degradation. We chose to convert the methyl acetate unit to a non-hydrolyzable ether unit. Compounds **16** and **17** served as probes for checking the depth of the S3' pocket. It appears that no dramatic change in binding potency can be attained by elongating the chain. The removal of the ester carbonyl (see compounds **12** and **18**), had a significant effect on the enzyme selectivity for these molecules. For the first time we were able to reverse the enzyme selectivity, producing a potent and selective MMP-3 inhibitor. The addition of an (*R*)-methyl at R_1 (compound **19**) did not dramatically affect the potency or the selectivity profile however, the addition of an (S)-methyl substituent at the same position greatly improved the potency while maintaining a slight bias towards MMP-3 (see **20**). The addition of a larger *n*-propyl group at R_1 in combination with an *i*-butyl unit at R_2 did not have a significant effect either on potency or selectivity (compound **21**).

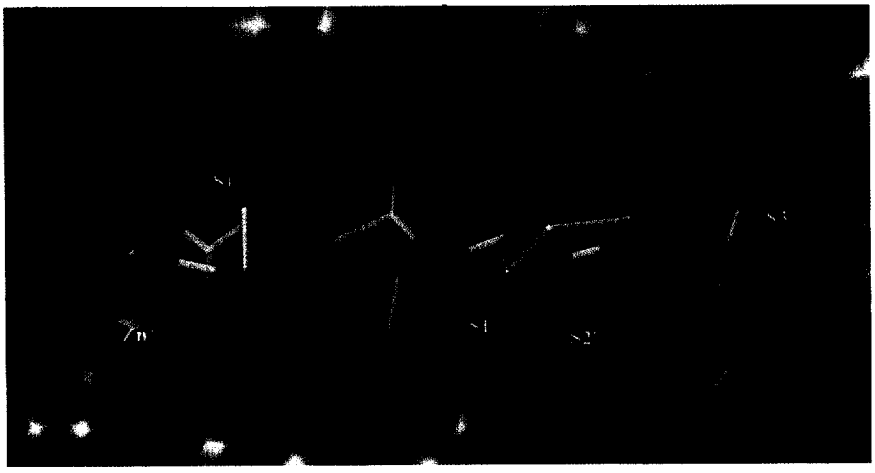
The dramatic reversal of selectivity seen between **12** and **18** can not be easily explained at this stage. It is clear that the ester carbonyl is playing a role in preserving the MMP-1 selectivity observed with **12**. There is also an equally perplexing issue as to why an MMP inhibitor like **12** with a large *n*-octyl substituent at R_2 is demonstrating the high selectivity observed for MMP-1.[20]

The crystal structure of compound **22** bound to truncated stromelysin (MMP-3) has been obtained (Figure 1) and helps to explain our SAR observations.[21] The hydroxamate unit binds to the active site zinc atom and the *i*-butyl group extends into the deep S1' pocket as expected. The caprolactam ring portion of the molecule is oriented with the amide carbonyl available for a hydrogen bond to Tyr-223 and the ring carbons extending into S2'. This arrangement allows the methoxyethyl group to occupy the S3' pocket. Finally, the hydroxypropyl substituent on the succinate extends into the solvent exposed S1 pocket.

Conclusion

Conformationally restricted metalloprotease inhibitors based on an amino-caprolactam scaffold have been synthesized and tested for biological activity. Substitution of the amino-caprolactam with an ester group produced a series of MMP-1 selective inhibitors with enhanced potency over the parent caprolactam **1**. The addition of a long aliphatic chain at the P1' site lead to a significant increase in potency for MMP-1 for this series. When a disubstituted succinate is employed in the inhibitor backbone, the compounds were more potent, but less selective for MMP-1. A series of ether substituted caprolactams was also prepared. Several of these compounds exhibit a remarkable reversal in enzyme selectivity when compared to the corresponding ester substituted caprolactams.

Figure 1 X-Ray structure of truncated MMP-3 / **22** complex.



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