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BROAD SPECTRUM MATRIX METALLOPROTEINASE INHIBITORS: AN EXAMINATION OF SUCCINAMIDE HYDROXAMATE INHIBITORS WITH P_1 C_α GEM-DISUBSTITUTION

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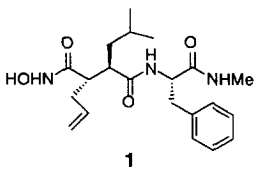
Abstract: A series of P_1 C_α gem-disubstituted succinamide hydroxamate matrix metalloproteinase inhibitors were prepared stereoselectively and evaluated in vitro for their ability to inhibit MMP-1, MMP-2, and MMP-3. It was found that while methyl/allyl substitution as in **2** and **18** provided compounds that were broad spectrum inhibitors and nearly equipotent with parent inhibitor **1**, a larger group such as bis-allyl as in **13** or gem-cyclopentyl as in **14** significantly reduced enzyme inhibition.

Introduction

The matrix metalloproteinases (MMP's) are a family of zinc-containing endopeptidases that are capable of hydrolyzing the extracellular matrix components of connective tissues and basement membranes.[1] While their roles in the normal and pathological turnover of these tissues are not completely understood, elevated levels of these enzymes have been implicated in several disease states including arthritis, multiple sclerosis, cancer angiogenesis, and metastasis.[2] Accordingly, it has been suggested that potent, long-lived and bioavailable MMP inhibitors may constitute an important therapeutic approach to control these pathological processes.

Of the various classes of MMP inhibitors reported to date, the succinamide hydroxamates (e.g., **1** or BB-1101) have been the most extensively investigated and include several clinical candidates.[3] However, many succinamides are poorly bioavailable and short-lived, in some cases due to the rapid in vivo hydrolysis or reduction of the hydroxamic acid functionality.[4] As a result, we investigated the effect of a newly introduced quaternary center adjacent to this group, as in analog **2**, as a means of preventing this metabolism and, perhaps, increasing the compounds' bioavailability and plasma half-life. During the course of this work, a group at Pfizer reported using a quaternary center adjacent to the central carbonyl of a family of succinamide hydroxamates (e.g., **3**) as an "amide-shielding device" to increase oral activity;[5] this modification resulted in a minimum 80-fold loss in potency and it was not disclosed if an improvement in bioavailability resulted.



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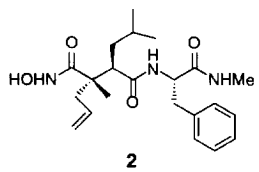

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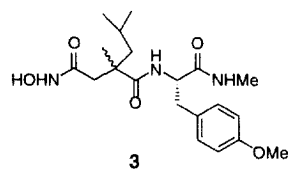

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Chemistry

The synthesis of the target MMP inhibitors began with the preparation of the appropriately trisubstituted succinates as shown in Scheme 1. As previously reported,[6] the dianion of (2*R*)-succinate **4** was allylated at low

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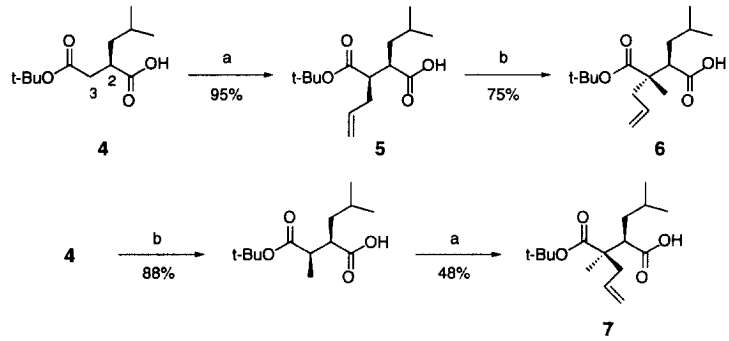
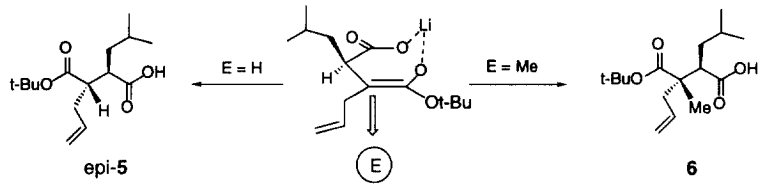
Scheme 1



Reagents and conditions: (a) 2.2 equiv LDA, THF, -78 °C; allyl bromide, -78 °C to rt; (b) 2.2 equiv LDA, THF, -78 °C; methyl iodide, -78 °C to rt.

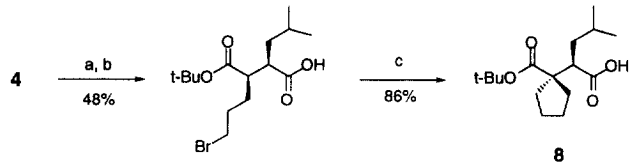
temperature to give (2*R*,3*R*)-disubstituted succinate **5** in high yield and diastereoselectivity of >20:1 at the newly created stereocenter. In our work it was found that deprotonation of **5** at –78 °C followed by treatment with methyl iodide gave (2*R*,3*S*)-succinate **6** with stereoselectivity of >20:1 as determined by NMR.[7] This is in contrast to the deprotonation and methanol protonation of **5**, which in our hands gave a ca. 2.5:1 *S*:*R* epimeric mixture at the allyl center. These results can be explained by invoking the proposed chelated cyclic intermediate of Crimmin[6] (Figure 1) in which the second electrophile (H or Me) adds to the enolate face opposite to the

Figure 1



bulky isobutyl group arriving with the same 'stereosense' as the first electrophile (allyl in this example). Consistent with this hypothesis is the fact that the larger methyl electrophile adds with much higher selectivity than the proton. In addition, quaternary succinate **6** was formed with high stereoselectivity regardless of whether succinate **5**, 3-epi-**5** or an epimeric mixture of the two was used. It was also found that reversing the order of addition of the two electrophiles, methylation followed by allylation, gave (2*R*,3*R*)-succinate **7**, although the stereoselectivity of the second alkylation was not as high (ca. 6:1).

Scheme 2

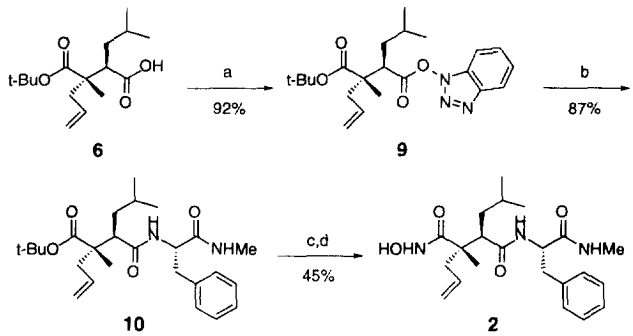


Reagents and conditions: (a) 2.2 equiv LDA, THF, -78 °C; 1-bromo-4-chlorobutane, -78 °C to rt; (b) NaBr, CH_2Br_2, DMF, 100 °C; (c) 2.2 equiv LDA, THF, -78 °C; methanol, -78 °C to rt.

Using the same procedure, the gem-dimethyl and gem-diallyl succinates were prepared along with gem-cyclopentyl succinate **8**, the synthesis of which is shown in Scheme 2.[8]

Unlike disubstituted succinates, the trisubstituted succinates could not be efficiently coupled with amino acids using standard coupling techniques; at room temperature the conversion could not be driven greater than approximately 20% and elevated temperatures gave mixtures of compounds. It was found, however, that treatment of succinates such as **6** with NMM, EDC and HOBt gave, after work up and chromatographic purification, the stable active ester **9** in >90% yield. Treatment of this ester with a variety of amino acid amides in DMF at 50 °C for 2 days gave the desired amides such as **10** in yields of 80–90%. This procedure was also compatible with bulky amino acid residues including *t*-butylleucine and cyclohexylalanine. Removal of the *t*-butyl ester and installation of the hydroxamate functionality with *O*-*t*-butyldimethylsilylhydroxylamine under standard conditions gave the desired hydroxamic acids such as **2** in modest but reliable yields after aqueous workup.[9]

Scheme 3

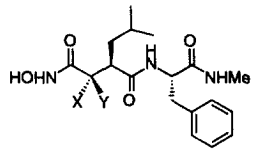


Reagents and conditions: (a) EDC, HOBt, NMM, CH_2Cl_2, rt; (b) L-phenylalanine N-methylamide, DMF, 50 °C, 2 d; (c) TFA, CH_2Cl_2, rt; (d) EDC, HOBT, NMM, $TBDMSONH_2$, DMF, 0 °C to rt.

Results and Discussion

The in vitro inhibition of enzymes MMP-1 (collagenase-1), MMP-2 (gelatinase A), and MMP-3 (stromelysin-1)[10] by the target compounds bearing an L-phenylalanine N-methylamide are presented in Table 1. For comparison, the enzyme data for reference compounds **1**, **1′** and **11** are included. Similar to the reference compounds, the P_1 C_α disubstituted analogs are broad spectrum inhibitors being equally potent for MMP-1 and MMP-2.[11] It was found that the addition of a P_1 C_α methyl to **1** giving inhibitor **2** was well tolerated leading to only a 2-fold loss of potency versus all three enzymes. The same addition to **11** to afford gem-dimethyl analog **12** led to a slightly larger 7-fold loss in activity. Inversion of the P_1 C_α center of **2** to give analog **2′** gave an expected 16-fold attenuation of potency, consistent with the activity difference between **1** and its P_1 C_α epimer **1′** (5- to 19-fold) and the rule of thumb that (*S*) is the desired configuration at this stereocenter. It was also found that while the gem-diallyl inhibitor **13** was 50- to 250-fold less active against the three enzymes than reference compound **1**, the gem-cyclopentyl inhibitor **14** caused a much smaller 15-fold loss in activity.

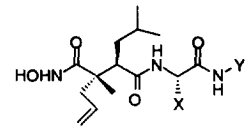
Table 1: Variation of C_α Disubstitution



			Enzyme Inhibition IC_{50} (nM)		
Compound	X	Y	MMP-1	MMP-2	MMP-3
1	allyl	H	2.2	1.8	12
1'	H	allyl	35	35	59
2	allyl	Me	3.1	4.2	25
2'	Me	allyl	52	66	420
11	Me	H	1.3	1.3	50
12	Me	Me	9.4	2.3	170
13	allyl	allyl	550	110	2600
14	$-CH_2CH_2CH_2CH_2-$		37	50	170

In an attempt to further improve the potency of these compounds and further characterize the structure activity relationships involved, the amino acid residue of **2** was varied and the inhibition profiles are shown in Table 2. It can be seen that while the L-phenylalanine N-phenylamide analog **15** and the L-cyclohexylalanine N-(phenethyl)amide **16** gave unacceptable losses in potency, *L*- cyclohexylalanine analog **17** and *t*-butylleucine N-methylamide **18** had excellent potency for MMP-1 inhibition.

Table 2: Variation of the Amino Acid Residue.

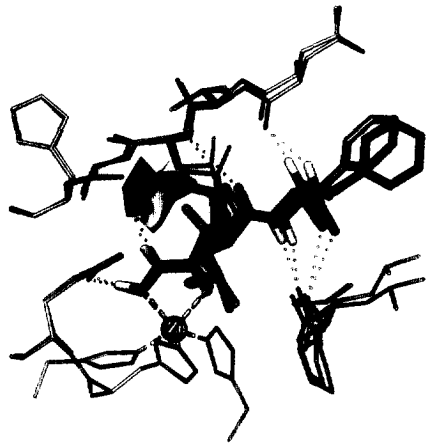


			Enzyme Inhibition IC_{50} (nM)		
Compound	X	Y	MMP-1	MMP-2	MMP-3
15	$PhCH_2$	Ph	120	52	91
16	c-$C_6H_{11}CH_2$	$PhCH_2CH_2$	110	64	160
17	c-$C_6H_{11}CH_2$	$H_2NSO_2PhCH_2CH_2$	6.9	23	15
18	*t*-Bu	Me	2.7	2.5	480
19	*t*-Bu	Ph	50	6.2	95

The interactions of several of the quaternary succinamide hydroxamates with MMP-3 were modeled using the previously published crystal structure of the stromelysin catalytic domain with bound carboxylic acid inhibitor L-702,842.[12] Molecular dynamics calculations indicated that compound **2** should bind to the enzyme in a similar fashion as reference inhibitor **1** with the quaternary methyl group being accommodated by a small shift in both the enzyme and ligand thus keeping the key binding interactions intact (Figure 2; **1** in green; **2** in blue).

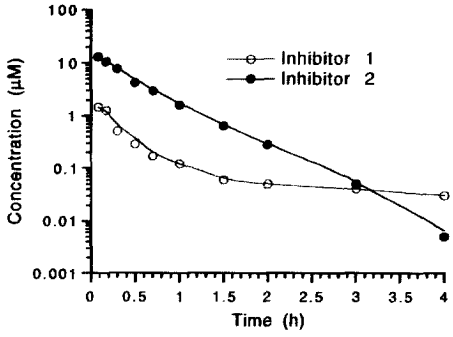
In contrast, bis-allyl analog **13** appeared to suffer from negative van der Waals interactions between the additional allyl moiety and several residues of the enzyme. The result of these interactions appear to be the shifting of the ligand somewhat out of the active site causing sub-optimal P1′ interaction and the loss of several inhibitor/enzyme hydrogen bonds. Modeling of gem-cyclopentyl analog **14** also suggested negative interactions between the additional α-substituent and the enzyme, although the interactions in this case appear to be less severe than that for **13** and result in a noticeable but less significant shift of the ligand in the active site pocket. Thus, the calculations qualitatively agree with the relative binding potencies of **1**, **2**, **13**, and **14**, and indicate that quaternary substituents larger than methyl are not tolerated well, even if confined in a ring.

Figure 2. Molecular modeling of inhibitors **1** and **2** with MMP-3.



In order to determine if P_1 C_α disubstitution would improve the pharmokinetic/pharmacodynamic profile of this family of hydroxamate inhibitors, compound **2** was dosed intravenously (10 mg/kg) and orally (30 mg/kg) in rats and the compound half-life and bioavailability were assessed. It was found, however, that similar to inhibitor **1**, compound **2** did not give measurable blood levels after oral dosing and, in fact, possessed a shorter half-life in this species (0.8 h vs. 2.7 h for **1**) (Figure 3).

Figure 3. Pharmacokinetic comparison of **1** and **2** in rats (iv, 10 mg/kg, n = 2).



In summary, a series of succinamide hydroxamates bearing a quaternary center adjacent to the zinc chelating group was prepared. Quantification of these compounds' ability to inhibit several MMP's indicated that while methyl substitution such as in **2** or **18** provided compounds that are broad spectrum and nearly equipotent with the known inhibitor **1**, P_1 C_α substitution with larger groups such as allyl or a conformationally restricted moiety such as a gem-cyclopentyl ring significantly reduces enzyme inhibition. Despite the nanomolar potencies of these compounds, it was found that this modification did not improve the pharmacokinetic profile or bioavailabiltiy of these compounds in rats.

Acknowledgement

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References and Notes

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7. The stereochemical assignments of succinates **6** and **7** were based on the work in ref 6.
8. Attempts at making the three- and four-membered gem-cycloalkyl succinates were unsuccessful using several different synthetic approaches.
9. All compounds reported in Tables 1 and 2 were characterized by ^1H and ^{13}C NMR, mass spectroscopy, elemental analysis and optical rotation.
10. The IC_{50} values were determined using fluorimetric assays for MMP enzyme inhibition run such that the cleavage of the peptide substrate Gly-Glu(EDANS)-Gly-Pro-Leu-Gly-Leu-Tyr-Ala-Lys(DABCYL)-Gly (10 μM) was monitored using a concentration of enzyme which results in 5–10% cleavage in control wells in 40 min. Cleavage of the Gly-Leu bond results in a 40-fold increase in fluorescence which was monitored in a microtiter plate fluorescence reader (ex WL, 335 nm; em WL, 485 nm). The concentration of compound which results in 50% inhibition was determined by plotting the log[Inh] vs. the logit function of the % inhibition. IC_{50} values were determined using a regression analysis of the concentration/inhibition data. The IC_{50} values for all compounds except **2** (n = 53) are from a single determination.
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