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Novel Inhibitors of Procollagen C-Terminal Proteinase. Part 1: Diamino Acid Hydroxamates

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Abstract—The parallel synthesis of novel inhibitors of procollagen C-terminal proteinase is described. The synthetic strategy allowed for the facile synthesis of a large number of side-chain diversified diamino acid hydroxamates, of which the D-diaminopropionic acid derivatives were shown to be single digit nanomolar PCP inhibitors.
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The different types of collagens are synthesized as procollagen precursor molecules having amino- and carboxy-terminal peptide extensions, which are specifically removed by two different proteolytic enzymes, procollagen N- and C-proteinases. Removal of the solubilizing globular domain from the C-terminus of procollagen by procollagen C-terminal proteinase (PCP) results in the production of insoluble fibrillar collagen. The excessive deposition of collagen is associated with a variety of fibrotic diseases, such as interstitial pulmonary fibrosis, kidney fibrosis, hepatitis and ARDS and hence inhibition of PCP is an attractive approach for interfering in the progression of fibrosis.[1]

Pzrocollagen C-terminal protease is a member of the metzincin enzyme family, as are the matrix metalloproteases (MMPs).[2] Potent inhibition of MMPs by hydroxamic acids is extensively documented and several hydroxamates have entered clinical development for cancer and arthritis.[3] In our hands, targeted screening of zinc-ligand containing compounds resulted in the identification of sulfonylated, alkylated D-amino acid hydroxamates as PCP inhibitors.[4] In particular, *N*-piperonyl 4-methoxybenzene sulfonamide derivatives were shown to confer PCP inhibitory potency. Since these derivatives of β-*N*-benzoyl Dpr had shown PCP inhibition, we decided to introduce an additional site of diversity, by varying the side-chain length and substitution pattern. The compound libraries reported here and in a subsequent paper were synthesized with the goal of optimizing inhibitory potency vs. the PCP enzyme as well as addressing the physicochemical properties of the initial leads.

The synthetic strategy we employed allowed for the rapid, parallel synthesis of hydroxamic acid derivatives of polyfunctionalized amino acids, leading to the facile incorporation of multiple sites of diversity. Because of the successful application of hydroxamic acids as MMP inhibitors, several synthetic approaches, both in solution and on solid support have been reported.[5] In our case, compounds were synthesized using the commercially available hydroxylamine chlorotrityl resin, affording the well known advantages of solid-phase synthesis: ease of manipulation of compound libraries, use of the resin as an orthogonal protecting group, removal of non-resin bound impurities or side-products by filtration and the opportunity to drive reactions to completion by use of excess reagents.[6]

Hydroxamic acid derivatives of diamino acids (Lys, Orn and Dab) (Scheme 1) were synthesized by coupling of the α-Fmoc, side-chain Dde protected amino acid[7] to deprotected hydroxylamine chlorotrityl resin. Adequate

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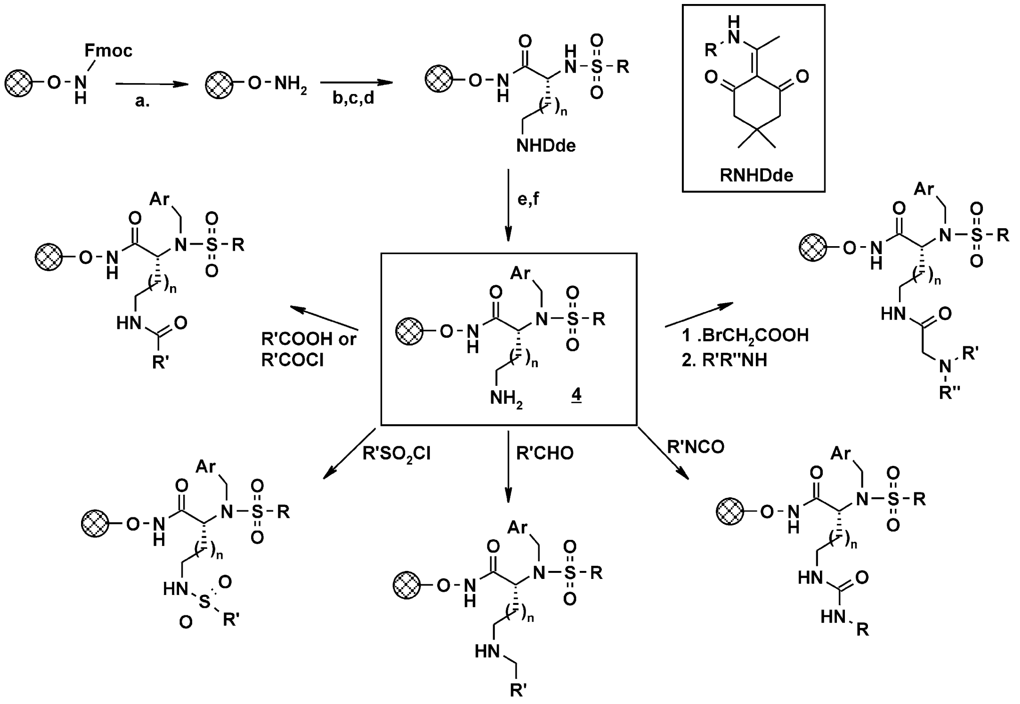
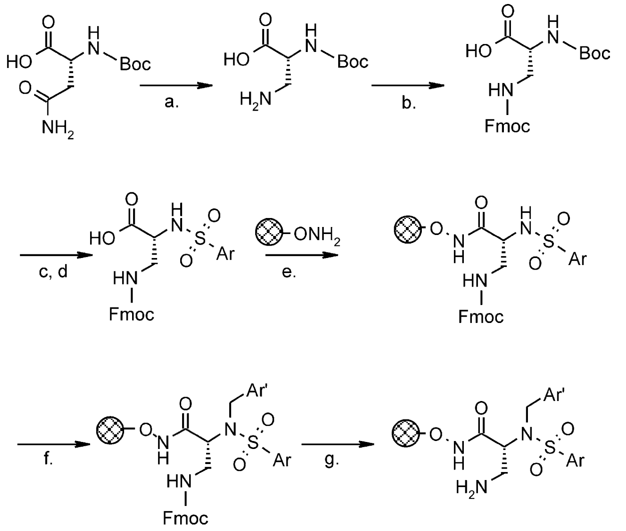


Scheme 1. Synthesis of sulfonylated diamino acid hydroxamate derivatives. Conditions: (a) 20% Piperidine/DMF, 2×20 min; (b) Fmoc-D-AA(Dde)-OH, HATU, HOAt, DIEA, DMF; (c) 20% Piperidine/DMF, 20 min; (d) RSO_2Cl, Pyridine/DCM 1/2; (e) $ArCH_2OH$, Ph_3P, ADDP, THF; (f) 2% NH_2NH_2/DMF. Abbreviations: Dde: 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl; ADDP: 1,1′- (azodicarbonyl)diperidine.

swelling of the resin (several hours in DCM) prior to Fmoc deprotection of the starting resin was shown to be critical for efficient loading of the resin. In addition, removal of the Fmoc protecting group from the commercial hydroxylamine resin (see Scheme 1, step a) required optimization, since incomplete deprotection was observed under standard conditions. Increased piperidine treatment times were necessary to consistently obtain fully deprotected resin. It is thought that a hydrophobic collapse is responsible for the reduced reactivity. Loading of the protected amino acid was followed by Fmoc removal under standard conditions. The α-amino group was sulfonylated, followed by alkylation of the resulting sulfonamide under Mitsunobu conditions. Evaluation of several activating agents resulted in the selection of 1,1′- (azodicarbonyl)diperidine)/PPh_3 as the optimal reagents for the Mitsunobu alkylation. Attempts to obtain the *N*-substituted sulfonamides by reductive alkylation, followed by reaction with sulfonyl chlorides proved sluggish, under several conditions evaluated. Removal of the side-chain Dde group by hydrazine treatment, followed by modification of the amino group led to the target compounds. Modifications of the side-chain amino group included acylation, sulfonylation, reductive alkylation and urea formation. Alternatively, the amino group was acylated with bromoacetic acid, followed by bromide displacement with amines. While the sulfonylation and Mitsunobu reactions were performed in batch mode, the side-chain derivatization reactions were performed in library format, using the Robbins FlexChem® system. The starting amino-deprotected resin was divided dry over 48 filter wells, reswollen in DCM and reacted with the appropriate reagents for derivatization of the side-chain amine. Cleavage of the library from the resin by treatment with dilute TFA and collection of the cleavage cocktails in deep well plates afforded the crude target products.

In the case of diaminopropionic acid, modifications to this synthetic route were required to avoid migration of the Dde group from the β to α amino position (Scheme 2). For this class of compounds, the sulfonylated Fmoc-side chain protected derivatives were generated prior to attachment to the resin. Sulfonylated D-Dpr(Fmoc) was prepared in three steps from Boc-D-Asn via Curtius rearrangement, followed by Fmoc protection of the



Scheme 2. Synthesis of D-Dpr derivatives. Conditions: (a) iodosobenzene diacetate, EtOAc/AcN/H_2O 2/2/1; (b) Fmoc-Cl, Na_2CO_3, H_2O/dioxane; (c) TFA; (d) $ArSO_2Cl$, DIEA, dioxane/H_2O; (e) HATU, HOAt, DIEA, DMF; (f) $Ar'CH_2OH$, Ph_3P, ADDP, THF; (g) 20% Piperidine/DMF.

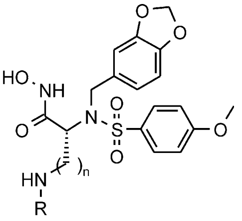
side-chain amine, α-amine Boc deprotection and sulfonylation. As before, alkylation of the resin-bound sulfonamide under Mitsunobu conditions was followed by side-chain deprotection and derivatization. Treatment with dilute TFA again released the target compounds from the solid support. In addition to the α-amine sulfonylated D-Dpr derivatives, the regioisomeric β-sulfonylated L- and D-Dpr derivatives were also prepared by essentially the same approach. All compounds were purified (>85% purity) by automated LC-MS using acetonitrile gradients in dilute aqueous TFA solutions. Instability of the Lys hydroxamic acid derivatives in TFA solutions required the use of less acidic elution buffers, such as aqueous NH_4OAc.

This straightforward, solid phase synthetic approach allowed for the facile synthesis of several hundred analogues around different diamino acids, resulting in the evaluation of a wide range of side-chain functionalities. The compounds were tested for their ability to inhibit PCP by an in vitro assay utilizing a synthetic peptide as substrate.[8]

For the diamino acid derivatives, the optimal length of the side-chain functionality was first investigated. Side-chain benzoylated compounds derived from D-Lys, D-Orn, D-Dab and D-Dpr were synthesized and tested for PCP inhibition. As can be seen in Table 1, significant differences in activity were observed, with the shortest chain length ($n=1$, Dpr) resulting in the most potent compounds. Interestingly, chain extension with one methylene group ($n=2$, Dab) resulted in considerable loss in activity (~40-fold), which was partially regained by further elongation of the chain ($n=3$, Orn).[9]

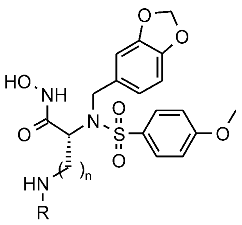
Additional analogues in both the Dpr and Dab series were prepared, and the data is exemplified in Tables 2–3. As can be seen in Table 2 for D-Dab, submicromolar inhibitors were obtained with a wide variety of functionalities, such as *para* and *ortho*-substituted benzoyl (Table 2, entries 1 and 3), heterocyclic derivatives (entries 2 and 5) and more extended functionalizations (entries 4 and 6). In addition, non-polar (entry 1), as well as basic side-chain functionalities were tolerated (entry 6), although incorporation of the most basic, 2-aminopyrazine-acetyl (entry 7), resulted in a substantial loss in potency. Surprisingly, the most potent compound obtained incorporated the relatively small 5-methyl-4-isoxazoyl (entry 5). Due to the wide range of tolerated functionality, we hypothesized that the side-chain of D-Dab was likely directed into solvent accessible space and was not directly contributing to binding.

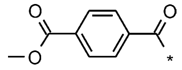
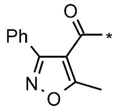
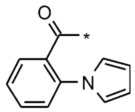
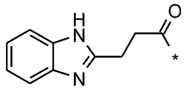
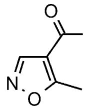
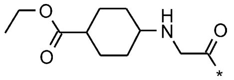
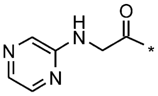
Table 1. Effect of side-chain length on PCP inhibitory potency



Entry	*n*	R	K_i (nM)
Lys	4	Benzoyl	350
Orn	3	Benzoyl	100
Dab	2	Benzoyl	470
Dpr	1	Benzoyl	14

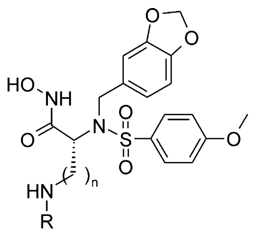
Table 2. PCP inhibitory activity of D-Dab derivatives

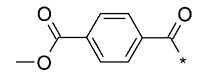
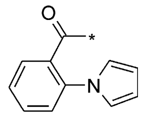
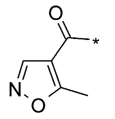
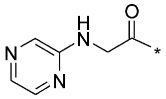
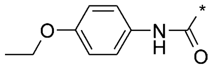
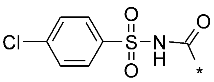
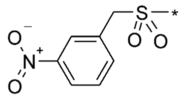
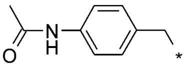


Entry	*n*	R	K_i (nM)
1	2		840
2	2		960
3	2		530
4	2		410
5	2		86
6	2		470
7	2		2500

For the D-Dpr derivatives, the activity level for similar substitutions to the D-Dab compounds was dramatically different (Table 3). While the 4-carbomethoxy benzoyl derivative led to a two-fold decrease in potency versus the benzoyl derivative in the Dab series (Table 2, entry 1), this substitution for Dpr gave rise to a low nanomolar inhibitor (Table 3, entry 1), as did incorporation of 2-(1-pyrrole)benzoyl (entry 2). Incorporation of the most active moiety from the Dab series led to a 140-fold reduction in potency (entry 3), while incorporation of the basic 2-aminopyrazine moiety resulted in severely diminished activity (entry 4). To evaluate the role of the side-chain amide linker, libraries of alternatively derivatized compounds were also synthesized. Low nanomolar inhibitors incorporating urea (entry 5), sulfonylurea (entry 6) and a sulfonamide linkers (entry 7) were

Table 3. PCP inhibitory activity of D-Dpr derivatives



Entry	*n*	R	K_i (nM)
1	1		7
2	1		8
3	1		1960
4	1		250
5	1		22
6	1		18
7	1		28
8	1		1010

identified. Amine-linked compounds generally were significantly less potent (entry 8).

Further investigation of the Dpr derivatives included the regioisomeric compounds or β-Dpr analogues. Generally, the compounds were substantially less potent than the α-Dpr derivatives and no clear preference for the D- or L-Dpr epimers could be seen. The most potent analogue in this series incorporated one of the preferred side-chains identified in the α-Dpr series, namely 2-(1-pyrrolo)-benzoyl ($K_i = 67$ nM).

Based on these results, we postulated that the contribution of the different D-Dpr side-chain modifications was likely due to a combination of binding interactions, conformational effects and exposure to solvent accessible space.

In conclusion, potent PCP inhibitors derived from diaminopropionic acid were obtained. Together with the Orn derivatives we described previously, these compounds are the first non-peptidic molecules to be reported with low nanomolar inhibitory potency for PCP.

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