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Solid-Phase Synthesis of Di- and Tripeptidic Hydroxamic Acids as Inhibitors of Procollagen C-proteinase

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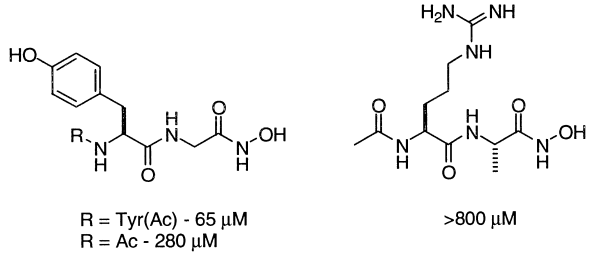
Abstract—A solid-phase approach to the rapid synthesis of di- and tripeptide-like hydroxamic acids is presented. These compounds are shown to be potent inhibitors of procollagen C-proteinase (PCP).

Overproduction of collagen can lead to many fibrotic diseases, including arthritis, adult respiratory distress syndrome, and surgical adhesions. Procollagen C-proteinase has been shown to cleave the C-propeptide from procollagen to ultimately produce collagen fibrils. Prevention of excessive formation of collagen through inhibition of PCP could, therefore aid in treatment of these inflammatory conditions.[1]

Our investigation for the development of inhibitors of PCP began with small peptidic analogues that were designed based on the natural cleavage site of procollagen as shown in Figure 1.[2] A hydroxamic acid functionality was incorporated as the zinc ligand at the cleavage site and the peptide was built towards the N-terminal end and succinates were constructed towards the C-terminal end.

Schemes 1 and 2 illustrate that close analogues based on the cleavage site were not potent inhibitors of PCP. A number of potential inhibitors were prepared as shown in Scheme 3 by varying AA3-AA2 and leaving AA1 as Gly, without any improvement in the potency. However, high throughput screening (HTS) revealed the Cbz protected dipeptide hydroxamic acid, Cbz-Phe-Trp-NHOH, as a low micromolar PCP inhibitor (IC_{50} = 17 μM).

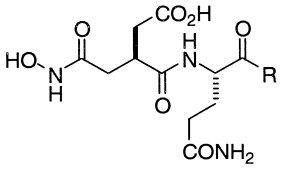


Scheme 1. IC_{50}'s of N-terminal mimics of the natural substrates.

Follow up on HTS lead, Cbz-Phe-Trp-NHOH, included Cbz-Trp-NHOH, which was considerably less potent (67 μM), as well as the enantiomer, Cbz-D-Phe-D-Trp-NHOH, which was also less active (18 μM). At this juncture a wide array of nearly 1000 di- and tripeptidic substrates were prepared on solid support.[3] ArgoGel® was the resin of choice since it led to, in the majority of

	AA4-AA3-AA2-AA1---AA1'-AA2'
Pro-α2(I)	Phe-Tyr-Arg-Ala --- Asp-Gln
Pro-α1(I)	Tyr-Tyr-Arg-Ala --- Asp-Asp
Pro-α1(II)	Tyr-Met-Arg-Ala --- Asp-Gln
Pro-α1(III)	Tyr-Tyr-Gly --- Asp-Glu

Figure 1. Human C-proteinase cleavage sites of Pro-α chains.



Scheme 2. Initially synthesized C-terminal mimics of the natural substrates.

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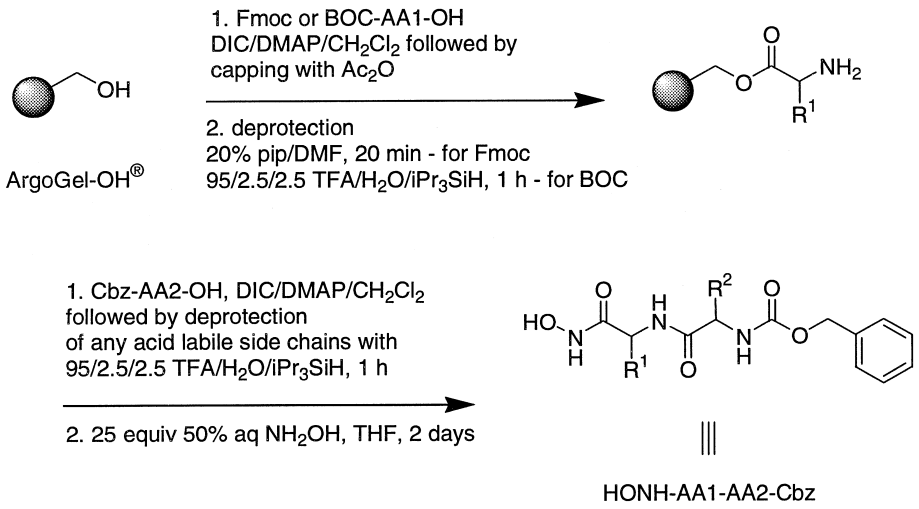


Scheme 3. For HONH-AA1-AA2-AA3-Cbz: repeat first two steps with either Fmoc or BOC-AA2-OH then use Cbz-AA3-OH utilizing the last two steps.

cases, hydroxamic acids that were >80% pure by HPLC.[4] The amino acids were coupled to the resin using standard coupling conditions and the hydroxamic acids were formed as previously described.[5]

In order to follow up on the HTS hit, the first library, consisting of >100 individual compounds, was prepared with the first amino acid (AA1) constant as Trp with variation at AA2 (Scheme 3). This investigation was carried out with both D- and L-Trp. From this library it can be seen that the natural amino acids are more active at both positions AA1 and AA2. Branched or straight chain alkyl groups of at least three carbons are the most preferred substituent at AA2. A sample of this library showing the five most active with either L- or D-Trp as AA1 is illustrated in Table 1.

Using the optimized substituent at AA2 (Ile) the Cbz group was substituted in a library of 40 different amides or ureas synthesized as shown in Scheme 4. In general the amides were more potent than the ureas, however there was only slight improvement over the original Cbz substituent (0.73 μM) (Table 2).

The next variation was on AA1 using the optimized AA2 group as Ile or Val with the Cbz end cap. A library of ~150 compounds was prepared using the chemistry as outlined previously in Scheme 3, where R^2 is derived from L-Ile or L-Val.

The Ile analogues were consistently more potent than the Val analogues, some of the most active of which are depicted in Table 3. The potency of these dipeptidic

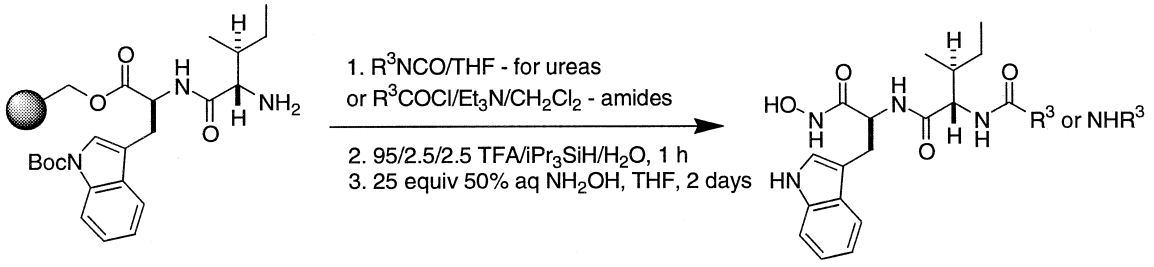
Table 1. Most potent analogues from variation of AA2 on Cbz-AA2-(L or D)-Trp-NHOH

Cbz-AA2-Trp-NHOH		Cbz-AA2-D-Trp-NHOH	
AA2	PCP IC_{50} (μM)	AA2	PCP IC_{50} (μM)
Ile	0.73	Thr	15
Nva[b]	0.87	D-Phe[a]	18
Phg	1.2	Trp	19
Val	1.9	Abu[b]	19
Glu	3	D-Tyr	20

[a]Prepared in solution phase.
[b]Nva = norvaline, Abu = aminobutyric acid.

Table 2. Most potent analogues from variation of end cap (R^3) on R^3-Ile-Trp-NHOH

Amides		Ureas	
R^3	PCP IC_{50} (μM)	R^3	PCP IC_{50} (μM)
3-BrPh	0.5	2-NO_2Ph	2.1
3,4-Cl_2Ph	0.75	$PhCH_2CH_2$	3.6
3-CF_3Ph	2.8	2-ClPh	5
3-ClPh	3.4	3,5-Cl_2Ph	5.1
3-NO_2Ph	2.5	3-BrPh	15



Scheme 4.

Table 3. Most potent analogues from variation of AA1 on Cbz-Ile-AA1-NHOH[a]

AA1	PCP IC_{50} (μM)	AA1	PCP IC_{50} (μM)
Dpr(CBz)[a]	0.061	4-BrPhe	0.2
Dpr(Bz)[a]	0.094	4-NO_2hPhe	0.2
4-Taz[a]	0.11	5-Br-2-Thi[a]	0.21
Tyr(*t*-Bu)	0.13	2-Pal[a]	0.22
3-ClPhe	0.19	4-NO_2Phe	0.22
4-PhPhe	0.2	His	0.25

[a]Dpr = 2,3-diaminopropionic acid, Taz = thiazolylalanine, Thi = thiophenylalanine, Pal = pyridylalanine.

Table 4. Most potent analogues from variation of end caps on R^3-Ile-4-Taz-NHOH

Sulfonamides		Carbamates	
R^3	PCP IC_{50} (μM)	R^3	PCP IC_{50} (μM)
2,5-Cl_2Ph	0.029	3,4-Cl_2Bn	0.073
3,4-Br_2Ph	0.030	2-BrBn	0.078
4,5-Br_2-2-thiophene	0.032	3-CF_3Bn	0.090
2,4-Me_2,4-ClPh	0.043	Bn	0.11
2,4,5-Cl_3Ph	0.049	3,5-Cl_2Bn	0.15

analogues was dramatically enhanced by optimization of AA1. Thus, combinatorial methods allowed rapid determination of the optimal AA1 as Dpr (Table 3, entries 1 and 2), which was found to be 10× more active than the Trp analogue (Table 1, entry 1). Overwhelmingly preferred at this position is an aromatic or heteroaromatic substituent. Interestingly, only one D-amino acid (D-His(Bn), 0.43 μM) has shown up with submicromolar activity of which the corresponding L-isomer was 0.37 μM.

Additional libraries were prepared by variation of the second amino acid (AA2) or the end capping group using the optimal AA1 groups in Table 3. Nearly 500 compounds were prepared using 4-Taz as AA1. These included tripeptides, peptoids,[6] and dipeptides with end capping groups including sulfonamides and carbamates.

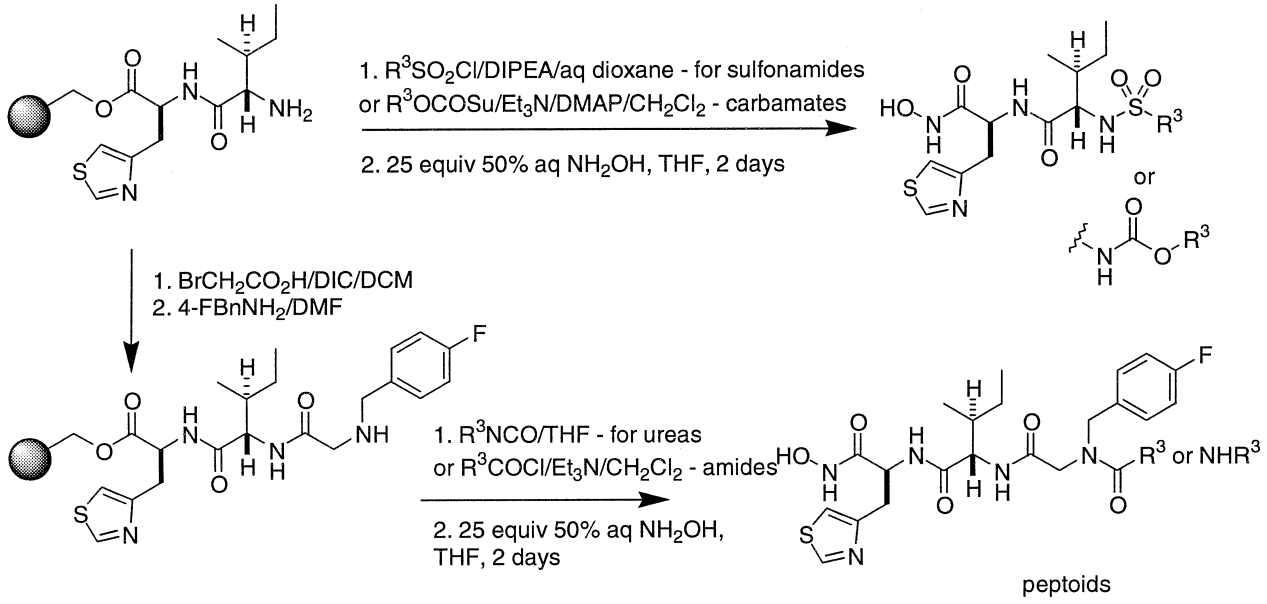
As seen in Table 4, halogenated aromatics provide the most active end cap functionality for the dipeptide, with a potency advantage seen for the sulfonamides. These compounds were prepared as illustrated in Scheme 5. Table 5 illustrates a series of tripeptides and peptoids that were prepared.

Table 5. Most potent analogues from variation of AA3 on Cbz-AA3-Ile-4-Taz-NHOH and peptoids: RCON(4-FBn)CH_2-Ile-4-Taz-NHOH

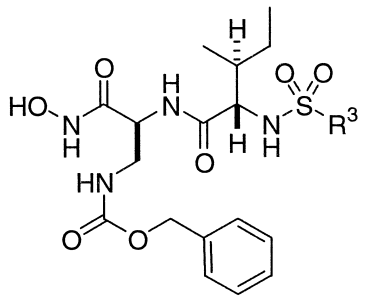
Tripeptides		Peptoids	
AA3	PCP IC_{50} (μM)	R	PCP IC_{50} (μM)
Met	0.043	4-ClPh	0.05
hPhe	0.044	3-ClPh	0.053
Trp	0.067	4-BrPh	0.089
4-FPhe	0.078	4-FPh	0.090
Ser(Bn)	0.078	3-MePh	0.110
Leu	0.110	4-*t*-BuPh	0.14

The tripeptides and peptoids have some active compounds but are less potent than the lower molecular weight sulfonamides in Table 4.

The last series of compounds prepared was based on the finding which determined Dpr as the optimal AA1 (Table 3). The four best sulfonamides (Table 4) were prepared and showed a modest increase in PCP inhibition as compared to the thiazole series. This library gave rise to the most active compound to date as illustrated in Scheme 6 and Table 6.



Scheme 5.



Scheme 6.

Table 6. Variation of R^3

R^3	PCP IC_{50} (μM)
3,4-Br_2Ph	0.026
4,5-Br_2-2-thiophene	0.052
2,5-Cl_2Ph	0.065
2,5-Me_2-4-ClPh	0.067

Overall a greater than 650-fold increase in activity was obtained through the utilization of solid-phase parallel synthesis for the preparation of potent PCP inhibitors. These peptide-like inhibitors can be used as tools to derive smaller nonpeptidic inhibitors of PCP and may aid in the SAR development of such compounds.

Experimental

Coupling of AA to resin. Suspend ArgoGel-OH in CH_2Cl_2 in an empty solid-phase extraction vial, fitted with a stopcock. Add 3 equiv protected amino acid, 3 equiv of DIC and 0.05 equiv DMAP. Place reaction on a spinner and rotate overnight. The unreacted resin sites are then capped off with an Ac_2O capping solution (19 mL Ac_2O, 9 mL DIPEA, 0.8 g HOBt, 400 mL NMP) for 1 h. Additional amino acids are coupled on similarly.

Removal of BOC. Suspend resin in a solution of 95/2.5/2.5 TFA/H_2O/triisopropyl silane for 1–2 h.

Removal of FMOC. Suspend resin in 20% piperidine in DMF for 20 min.

Preparation of sulfonamides. Suspend resin in 90% aq dioxane then add 10 equiv of RSO_2Cl and 20 equiv of DIPEA. The reaction is then placed on a spinner and rotated overnight.

Preparation of ureas. Suspend resin in THF then add 3 equiv of RNCO. The reaction is then placed on a spinner and rotated overnight.

Preparation of amides. Use carboxylic acid conditions outlined above for coupling to resin, or suspend resin in CH_2Cl_2 then add 3 equiv of RCOCl and 3 equiv of Et_3N. The reaction is then placed on a spinner and rotated overnight.

Preparation of carbamates. Suspend resin in CH_2Cl_2 then add 3 equiv of the desired succinimidylcarbonate, 3 equiv Et_3N and 0.05 equiv 4-DMAP. [Alternatively, the resin is treated with 90% aq dioxane, then 10 equiv of the desired chloroformate and 20 equiv DIPEA were added.] The reaction is then placed on a spinner and rotated overnight.

Preparation of peptoids. Suspend resin in CH_2Cl_2 then add 12 equiv of $BrCH_2CO_2H$ and 13 equiv of DIC. The reaction is then placed on a spinner and rotated for 2 h. The reaction is then filtered by suction filtration and washed with CH_2Cl_2, DMSO, and CH_2Cl_2. Suspend resin in DMSO then add 40 equiv or the desired primary amine. The reaction is then placed on a spinner and rotated overnight.

NH_2OH cleavage. Suspend resin in THF then add 25 equiv of 50% aq NH_2OH and rotate for 2 days. The resin is removed by filtration and washed with CH_2Cl_2, MeOH, and then CH_2Cl_2. The filtrate is then concentrated.

The solvent volume in all cases is a sufficient amount to swell the resin (~12.5 mL/g of resin). Also, all reactions were worked up by suction filtration followed by washing with 3×CH_2Cl_2, 3×MeOH, 1×50% HOAc/CH_2Cl_2, 3×MeOH and then 3×CH_2Cl_2 and dried, unless otherwise noted.

Acknowledgements

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References and Notes

1. Kessler, E. *Proc. Indian Acad. Sci. (Chem. Sci.)* **1999**, *111*, 197 and references therein.
2. Lee, S.-T.; Kessler, E.; Greenspan, D. S. *J. Biol. Chem.* **1990**, *265*, 21992.
3. These compounds were all characterized by reversed-phase HPLC/MS and a select few were independently synthesized using solution phase. They were characterized by NMR, MS, HPLC and found to be identical to the dipeptide prepared on polymer support.
4. For compounds less than 80% pure the following conditions were used for purification: Zorbax SB-Phenyl (Prep.Column), 21.2 mm×7.5 cm, 20 mL/min, 30–75% CH_3CN/0.1% aq TFA gradient over 12 min.
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