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Amino Acid Derived Sulfonamide Hydroxamates as Inhibitors of Procollagen C-Proteinase. Part 2: Solid-Phase Optimization of Side Chains

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Abstract—Optimization of the amino acid side chain and the *N*-alkyl group of the sulfonamide of amino acid derived sulfonamide hydroxamates is discussed. The solid-phase synthesis of these potent inhibitors of procollagen C-proteinase (PCP) is presented. In addition, novel carboxylic acid sulfonamides were discovered to be PCP inhibitors.

Procollagen C-proteinase (PCP) causes proteolytic cleavage of the C-propeptide from procollagen en route to the formation of collagen fibrils.[1] This makes inhibition of PCP an interesting target for prevention of abnormal fibrotic or inflammatory conditions, such as adult respiratory distress syndrome and surgical adhesions.

Previously we have disclosed peptidic[2] and small molecule[3] approaches toward this target, and herein present further optimization of our small molecule, amino acid derived, sulfonamide hydroxamates as potent inhibitors of PCP.

Other related work in the field include two Fibrogen patents that discloses less potent sulfonamides[4] and *N*,*N*′-disubstituted hydantoin hydroxamates (IC_{50}'s as low as 59 nM)[5] as inhibitors of PCP. Kadler et al. have also recently revealed dipeptide (succinate) type hydroxamic acids as inhibitors of PCP with potencies in the 0.1–10 μM range.[6] A recent Pfizer patent also discloses some small molecule ox(adi)azolyl-hydroxamic acids as inhibitors of PCP, however, no biological data is presented.[7]

Our lead compound has the basic structure as illustrated in Figure 1. The goal of our research program was to optimize the substituent at the alpha position of the amino acid (R) as well as the alkyl group on the sulfonamide nitrogen (R^1).

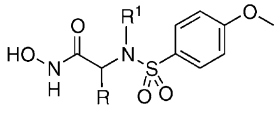

Figure 1.

It was decided the best way to optimize this lead structure would be through the utilization of solid-phase techniques. A few of the analogues were prepared initially in solution phase, however, the majority were prepared as shown in Scheme 1.[8]

Compounds with variation at R (i.e., different amino acids) were prepared where R^1 was constant as piperonyl and in the optimization of R^1, the amino acid used was D-alanine (R = Me). D-Alanine, though not the most potent amino acid we have as yet found, was chosen due to the relative synthetic ease of preparing the substrates and the consistent high purity of products obtained. Only D-amino acids were prepared, as it had been shown in other analogues that the L-series lacked potency. Piperonyl was chosen as the constant for R^1 when varying the amino acid, due to its high potency in other analogues previously described.

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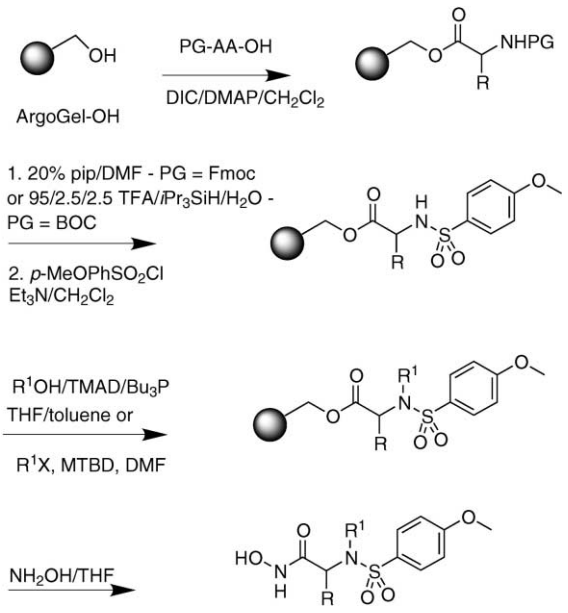

Scheme 1. Synthetic scheme for high throughput synthesis of analogues. (X = Br or Cl-added cat. $Bu_4N^+I^-$ with X = Cl). Abbreviations: TMAD = azodicarboxylic acid bis(dimethylamide), MTBD = 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene.

For the initial two libraries prepared, 69 different R groups and 161 different R^1 groups were utilized. Lastly, a few libraries having the 'optimal' R and R^1 groups were investigated. Figure 2 and Table 1 illustrate the results from the variation of the amino acid (R).[9]

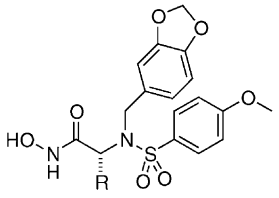

Figure 2.

Table 1. Twenty most potent compounds prepared by variation of amino acid (R group), sorted descending potency

Compd	D-Amino acid	PCP, IC_{50} (nM)
1	Dpr(Fmoc)	0.024
2	2-Thi	0.2
3	Orn(2-ClCbz)	3.9
4	Dpr(2-ClCbz)	4.6
5	Orn(Cbz)	9
6	Dpr(Cbz)	14
7	Lys(Cbz)	16
8	Dpr(2-BrCbz)	18
9	Ser(*t*-Bu)	23
10	Dpr(Alloc)	23
11	Orn(2-BrCbz)	24
12	His(Bn)	27
13	4-NO_2Phe	32
14	Cys(pMB)	32
15	His(Trt)	36
16	Tyr(Me)	40
17	Cys(pTol)	45
18	His(Me)	50
19	Orn(BOC)	52
20	Val	53

Abbreviations: Dpr, 2,3-diaminopropionic acid; Thi, thienylalanine; pMB, 4-methoxybenzyl.

Table 1 clearly indicates that carbamate substituted hetero alkyl (especially Dpr and Orn derived amino acids) and heteroaromatic substituents are highly preferred R groups in these inhibitors.

Figure 3 and Table 2 illustrate the effect of varying the alkyl group, R^1 on the sulfonamide, using D-Ala as the amino acid. For a reference point the D-Ala compound with R^1 = piperonyl has an IC_{50} of 0.3 μM.

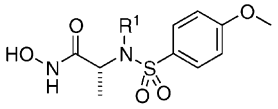

Figure 3.

Table 2. Twenty most potent compounds prepared by variation of alkyl group on nitrogen of sulfonamide (R^1 group), sorted by descending potency

Compd	R^1	PCP, IC_{50} (nM)
21	2-CF_3Bn	6
22	3-NO_2-4-ClBn	30
23	4-BrBn	37
24	3-PhO(thiophene)-2-CH_2	40
25	3,4-Me_2(thieno[2,3-*b*]thiophene-2-CH_2	60
26	3,4-F_2Bn	76
27	4-MeSBn	80
28	1-NaphthylCH$_2$	90
29	3-BrBn	93
30	3,4-Cl_2Bn	120
31	2-NO_2-3-MeBn	120
32	7-AllylO-2-naphthylCH$_2$	130
33	3,5-F_2Bn	140
34	1-Pyrrole$(CH_2)_2$	170
35	2-Cl-thiazole-5-CH_2	180
36	2,3-Me_2-4-MeOBn	220
37	3,5-$(CF_3)_2$Bn	220
38	6-Me-2-naphthylCH$_2$	220
39	5-Me-isoxazole-3-CH_2	230
40	4-*i*PrBn	230

Substituted benzylic or heteraryl CH_2 compounds proved to provide the most active alanine analogues (Table 2). The SAR surrounding the substitution pattern on the aromatic portion of R^1 was unpredictable. It appeared that no position (*o*, *m*, or *p*) was preferred. Variation of the electronic nature of these groups also did not predictably improve or decrease potency of these compounds. In general, alkylation with non-aromatic heterocyclic or aliphatic electrophiles led to considerably less potent compounds.

Based on the data presented in Table 1, further studies were explored using 2-thienylalanine derived sulfonamides. Lead optimization was already in progress with Orn[3] and Dpr[10] as they had previously been determined to be active compounds. Unfortunately, out of a library of 29 compounds, none of the R^1 groups which were superior to piperonyl in the D-Ala series gave any increase in activity in the 2-Thi series as shown in Figure 4 and Table 3. However, some of the more potent analogues in the alanine series also showed up as some of the more potent compounds in the thienylalanine series, but not in the same rank order.

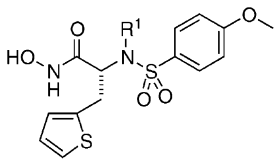

Figure 4.

Table 3. Twenty most potent compounds prepared by variation of alkyl group on nitrogen of sulfonamide (R^1 group), sorted by descending potency

Compd	R^1	PCP, IC_{50} (nM)
41	Piperonyl	0.2
42	3-NO_2,4-MeBn	60
43	4-BrBn	70
44	3,4-F_2Bn	80
45	2,3-F_2Bn	100
46	4-MeBn	110
47	4-MeO_2CBn	110
48	2-F,3-MeBn	150
49	2,4,5-F_3Bn	180
50	4-VinylBn	190
51	3-BrBn	260
52	7-AllylO-2-naphthylCH_2	290
53	4-IBn	360
54	3-ClBn	390
55	3,4-Cl_2Bn	440
56	4-(1,2,3-Thiadiazolyl)Bn	440
57	3-Me,4-MeOBn	490
58	3,5-$(CF_3)_2$Bn	590
59	1-Pyrrole$(CH_2)_2$	610
60	2,4-Cl_2Bn	860

During the course of this study many carboxylic acids were separated from the final hydroxamic acids as side products. These were also submitted for biological study and some interesting carboxylic acid lead compounds were also discovered. Though in general the carboxylic acids were less potent, they were the first carboxylic acids to show significant potency against this enzyme (Fig. 5 and Table 4).

In conclusion, solid-phase parallel synthesis allowed rapid incorporation of a variety of side chains into the sulfonamide lead compound, providing previously unknown leads to be further explored. Based on the original D-alanine compound with the piperonyl side chain, the potency was increased 50-fold by variation of the alkyl chain (R^1) and by 12,500-fold by variation of the D-amino acid (R). Also the generation of unexpectedly potent carboxylic acid compounds was accomplished.

Acknowledgements

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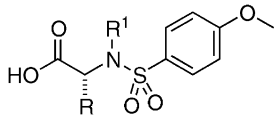

Figure 5.

Table 4. Twenty most potent carboxylates derived from above libraries by variation of alkyl group on nitrogen of sulfonamide (R^1 group) and by variation of amino acid (R group), sorted by descending potency

Compd	D-Amino acid	R^1	PCP, IC_{50} (nM)
61	PropargylGly	Piperonyl	10
62	2-Thi	Piperonyl	24
63	4-Pip(BOC)Gly	Piperonyl	26
64	Arg$(Et)_2$	Piperonyl	28
65	Ala	3,4-Me_2(thieno[2,3-*b*]thiophene-2-CH_2	50
66	2-Thi	5-Me-isoxazole-3-CH_2	120
67	hArg(Bu,Me)	Piperonyl	200
68	Ala	2,3-Me_2-4-MeOBn	200
69	2-Thi	4-MeO_2CBn	210
70	2-Thi	3-BrBn	230
71	Cys(pMB)	Piperonyl	250
72	Ala	3-IBn	280
73	Ala	4-*i*PrBn	350
74	Ser(Bn)	Piperonyl	380
75	Ala	2-NO_2,3-MeBn	380
76	Glu(cHex)	Piperonyl	470
77	2-FurylAla	Piperonyl	470
78	Ala	2-F,4-BrBn	490
79	4-PyridylAla	Piperonyl	592
80	5-Br-2-Thi	Piperonyl	670

Abbreviations: 4-Pip(BOC)Gly, 4-(BOC)piperidinylglycine.

References and Notes

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9. All compounds were analyzed by HPLC/MS and if <80% pure were further purified by RPHPLC.
10. Manuscript in preparation.