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Novel Inhibitors of Procollagen *C*-Proteinase. Part 2: Glutamic Acid Hydroxamates☆

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Abstract—Glutamic acid derived hydroxamates were identified as potent and selective inhibitors of procollagen C-proteinase, an essential enzyme for the processing of procollagens to fibrillar collagens. Such compounds have potential therapeutic application in the treatment of fibrosis.

The production of extracellular matrix (ECM) is a tightly regulated process required for normal wound healing. Excessive accumulation of ECM, particularly fibrillar collagens, results in a variety of chronic fibrotic conditions, including pulmonary, renal and liver fibrosis, and scleroderma.[1–7]

Procollagen C-Proteinase (PCP), a member of the astacin family of metalloproteases,[8] catalyzes the cleavage of the solubilizing C-terminal globular domain from procollagens, resulting in the formation of insoluble, fibrillar collagens.[9,10] Inhibition of PCP may present an attractive approach for interfering in the progression of fibrosis, especially when matrix metalloproteinases responsible for collagen degradation are uninhibited. While peptidic inhibitors of PCP are known,[11] few examples of small molecule inhibitors of PCP have been reported. We therefore sought to develop potent and selective inhibitors of PCP as potential therapeutic agents for the treatment of fibrosis.

In Part 1, hydroxamate derivatives of several diamino acids[12] were described as potent inhibitors of PCP. Herein we disclose the synthesis and structure–activity relationships (SAR) around PCP inhibitors prepared by derivatization of glutamic acid. Structural features that led to selectivity over matrix metalloproteinases will also be described.

To facilitate structure–activity studies, a solid-phase approach that allowed for the exploration of multiple sites of diversity around glutamic acid was utilized. As several solid-phase approaches to the synthesis of hydroxamic acids had been reported in the literature,[13] we opted to apply an analogous route employing hydroxylamine chlorotrityl resin.

Hydroxamic acid derivatives of D-glutamic acid were synthesized by coupling the *N*-sulfonylated D-glutamic acid γ-methyl esters to hydroxylamine chlorotrityl resin under standard conditions. The starting sulfonamides were prepared in two steps from D-glutamic acid. Following loading, the sulfonamides were further derivatized by *N*-alkylation under Mitsunobu conditions. Optimized conditions involved the use of ADDP/PPh_3 in THF.[14] Deprotection of the γ-carboxylate methyl

☆For Part 1, see ref. 12.
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esters was accomplished by treatment with potassium trimethylsilanoate[15,16] in THF for 2 h under nitrogen atmosphere. This reaction was quite sensitive, in that significant reductions in yield were observed at longer reaction times. The starting resin was divided over 48 filter wells and subjected to library synthesis, using the Robbins FlexChem® system. The free carboxylic acids were coupled to a variety of primary and secondary amines under standard conditions.[17] After shaking overnight, the library was cleaved from the resin with dilute TFA to afford the crude target products (Scheme 1).

Attempts to extend the approach in Scheme 1 to the preparation of D-aspartic acid derivatives was unsuccessful, presumably due to the facile formation of *N*-hydroxysuccinimide derivatives and autolysis from the resin on activation of the β-carboxylic acid. However, a single aspartic acid analogue **6** was synthesized in solution-phase for comparative purposes.

Taking advantage of the polyfunctional nature of glutamic acid, an alternative route installing the hydroxamate functionality at the γ-carboxylic acid was developed. In this case, the α-allyl ester of D- or L-Fmoc-glutamic acid was coupled to the resin through the γ-carboxylic acid under standard conditions (HOAt/HATU/DIEA). Deprotection of the Fmoc protecting group was followed by sulfonylation of the resin bound substrate. Mitsunobu alkylation as before afforded the N-alkylated sulfonamide derivatives. Deprotection of the allyl ester was accomplished using trimethylsilylazide, tetrabutylammonium fluoride and tetrakis(triphenylphosphine)palladium (0).[18] The free acid was then subjected to coupling and TFA cleavage in library format as before (Scheme 2).

All library compounds were purified by reverse-phase HPLC using mass-triggered fraction collection (LC/MS).[19] Compounds obtained in >85% purity, as determined by analytical LC/MS using 2 UV channels, were tested for their ability to inhibit PCP in an in vitro assay utilizing a synthetic peptide as substrate.[20] Selectivity of the compounds for PCP versus matrix metalloproteases was determined in vitro against MMP-1 and MMP-8.[20]

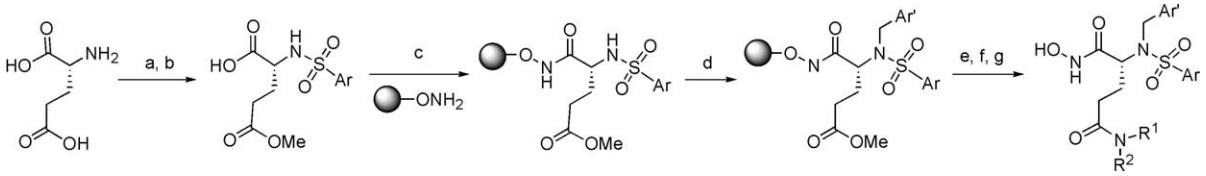
Initially, the effect of the *N*-aralkyl substituent on the activity of *N*-4-methoxybenzenesulfonyl (MBS) glutamic and aspartic acid methyl ester derivatives was explored. For the glutamic acid analogues, a clear preference for the piperonyl moiety was found. The corresponding aspartic acid analogue **6** was 3-fold less active than the glutamic acid derivative **5** (Table 1).

Next, amide derivatives of the γ-carboxylic acid of glutamic acid were prepared while holding the MBS and piperonyl substituents constant. A variety of primary and secondary amines were incorporated, providing secondary and tertiary amides, respectively.

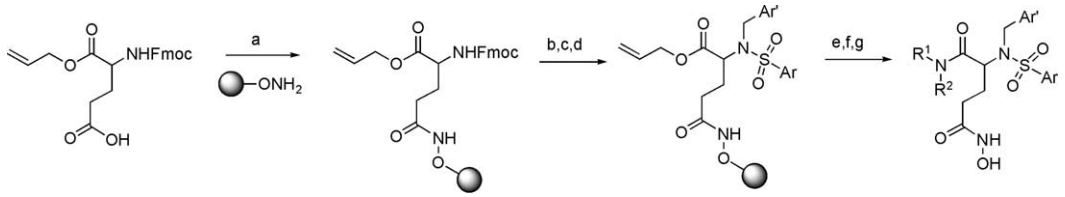
For the secondary amides, larger lipophilic substituents were preferred (Table 2). For example, the isoamyl amide **10** was 14-fold more active than the corresponding isobutyl amide **9**, which was in turn 4-fold more active than the ethyl amide **7**. The diphenylmethyl amide **16**, with a K_i of 11 nM, was 10-fold more potent than benzyl derivative **12**. The picolyl amides **13**–**15** were somewhat weaker than **12**, indicating the presence of basic functionality at this site was detrimental to activity.

In the case of the tertiary amides, similar activity trends were observed (Table 2). The *N*-cyanoethyl benzylamide **21** was approximately 3-fold more potent than the secondary benzylamide **12**, though this trend was not seen for 3-picolyl derivatives **14** and **22**. Incorporation of the 3-picolyl sidechain in compound **22** resulted in a 15-fold loss of activity versus benzyl derivative **21**, again indicating that basic substituents were disfavored. Cyclic secondary amides, such as morpholine analogue **23** and piperidine analogue **24**, had comparable activity to **12**.

A series of piperazine analogues was prepared to further probe the amide SAR (Table 3). As seen in the previous series, compounds containing basic functionality were also less active in the piperazine series. As illustrated in

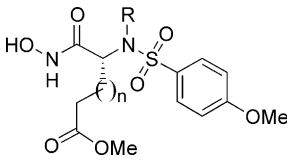


Scheme 1. (a) $SOCl_2$, MeOH; (b) $ArSO_2Cl$, aq NaOH, dioxane/H_2O; (c) 100–200 mesh hydroxylamine chlorotrityl resin, HATU, HOAt, DIEA, DMF; (d) Ar′CH_2OH, Ph_3P, ADDP, THF; (e) TMSOK, THF; (f) R^1R^2NH, HATU, HOAt, DIEA, DMF; (g) 10% TFA/DCM.



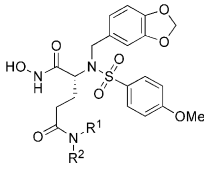
Scheme 2. (a) 100–200 mesh hydroxylamine chlorotrityl resin, HATU, HOAt, DIEA, DMF; (b) 20% piperidine/DMF; (c) $ArSO_2Cl$, 2/1 DCM/pyridine; (d) Ar′CH_2OH, Ph_3P, ADDP, THF; (e) $TMSN_3$,TBAF, $(Ph_3P)_4Pd$, DCE; (f) R^1R^2NH, HATU, HOAt, DIEA, DMF; (g) 5% TFA/DCM.

Table 1. Effect of *N*-aralkyl substituents for N-MBS Glu(OMe) and N-MBS Asp(OMe) analogues **1**–**6**



Compd	*n*	R	PCP K_i (nM)
1	1	4-Bromobenzyl	>40,000
2	1	4-Methyl-3-nitrobenzyl	>40,000
3	1	4-Trifluoromethylbenzyl	>40,000
4	1	4-Methoxybenzyl	15,000
5	1	Piperonyl	300
6	0	Piperonyl	950

Table 2. Effect of amide side chain on activity of *N*-(piperonyl)MBS Glu(NRR') analogues **7**–**24**



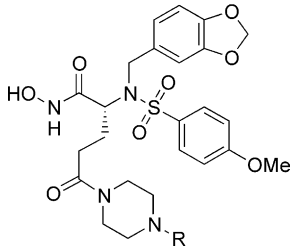
Compd	R^1	R^2	PCP K_i (nM)
7	H	Ethyl	1200
8	H	c-Propylmethyl	660
9	H	*i*-Butyl	280
10	H	*i*-Amyl	20
11	H	c-Hexylmethyl	410
12	H	Benzyl	98
13	H	2-Picolyl	530
14	H	3-Picolyl	160
15	H	4-Picolyl	1170
16	H	Diphenylmethyl	11
17	Methyl	Methyl	330
18	Ethyl	Ethyl	1300
19	Benzyl	Benzyl	400
20	Phenethyl	Benzyl	100
21	2-Cyanoethyl	Benzyl	33
22	2-Cyanoethyl	3-Picolyl	480
23	4-Morpholino		190
24	4-Benzylpiperidinyl		120

Table 3, activity increased as the basicity was attenuated. Thus, the *N*-phenyl piperazine **27** was 3-fold more active than *N*-methyl piperazine **26**. Removal of the basic center by formation of amide, carbamate, or urea derivatives resulted in compounds with good potency.

In contrast to the α-hydroxamates, derivatives of both D- and L-γ-glutamic acid had weak activity versus PCP. The most potent γ-glutamic acid analogues were only micromolar inhibitors of PCP. The series was therefore not pursued.

Having identified compounds with promising inhibitory activity against PCP, we next turned our attention to assessing selectivity for PCP versus other metalloproteinases. Initial MBS analogues were also found to be potent inhibitors of MMP-8. Since the sulfonamide moiety of related MMP inhibitors is known to occupy the narrow S1′ sub-site,[21] modification of the sulfonamide substituent was explored as a means to enhance selectivity for PCP.

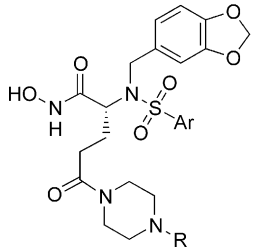
Table 3. Effect of N4-substituents on piperazine amides **25**–**40**



Compd	R	PCP K_i (nM)
25	H	1200
26	Methyl	1000
27	Phenyl	340
28	2-pyridyl	210
29	Benzyl	740
30	2-Picolyl	>1000
31	3-Picolyl	620
32	4-Picolyl	>1000
33	Acetyl	200
34	Benzoyl	35
35	2-Furanoyl	40
36	Methanesulfonyl	340
37	Ethoxycarbonyl	64
38	Benzyloxycarbonyl	260
39	(4-Methoxyphenyl)aminocarbonyl	90
40	(3-Methoxyphenyl)aminocarbonyl	105

Replacement of the MBS moiety with the sterically hindered 4-methoxy-2,3,6-trimethylbenzenesulfonyl (MTMBS) group was found to enhance PCP selectivity by up to three orders of magnitude (Table 4) versus MMP-1 and MMP-8. For example, acetyl piperazine **33** was 20-fold selective for MMP-8 versus PCP. Incorporation of the MTMBS group produced compound **41**, which was greater than 100-fold selective for PCP versus MMP-1 and MMP-8. Selectivity was similarly enhanced for the MTMBS ethoxycarbonyl derivative **42** versus MBS analogue **37.** This modification also increased binding potency towards PCP by 2–5-fold in most cases.

Table 4. Effect of sulfonamide moiety on PCP versus MMP selectivity



Compd	R	Ar	PCP K_i (nM)	MMP1 K_i (nM)	MMP8 K_i (nM)
33	Acetyl	MBS	200	120	9
41	Acetyl	MTMBS	63	37,000	7000
37	COOEt	MBS	64	90	6
42	COOEt	MTMBS	43	32,000	30,000
43	COOBn	MTMBS	100	>40,000	35,000
44	Phenyl	MTMBS	110	>40,000	13,000

The solid-phase synthetic approaches described above allowed for the facile synthesis of several hundred hydroxamate analogues of glutamic acid, leading to the identification of a novel series of PCP inhibitors with selectivity over MMP-1 and MMP-8. Together with the diamino acid analogues described previously by our co-workers,[12,22] these compounds are the first non-peptidic inhibitors reported with low nanomolar inhibitory potency against PCP. The therapeutic utility of these and other inhibitors will be reported in due course.

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