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Design and synthesis of procollagen C-proteinase inhibitors

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ABSTRACT

Non-peptidic inhibitors of procollagen C-proteinase (PCP) were designed from substrate leads. Compounds were optimized for potency and selectivity, with N-substituted aryl sulfonamide hydroxamates having the best combination of these properties. Compounds **89** and **60** have IC_{50} values of 10 and 80 nM, respectively, against PCP; excellent selectivity over MMP's 1, 2, and 9; and activity in cell-based collagen deposition assays.

Fibrosis is a pathological condition in which the normal wound healing process goes awry, culminating in the excessive production and deposition of collagen, the major component of scar tissue, in the extracellular matrix.[1,2] The disregulated formation of scar tissue may occur in any organ, hindering function and potentially leading to organ failure. Post operative and dermal scarring may result in a variety of complications including physical restriction or disfigurement. Currently, there are no adequate therapies for fibrotic conditions; new anti-fibrotics are needed for both systemic and topical applications.

Procollagen C-proteinase (PCP, also known as bone morphogenetic protein-1) is a zinc metallo endopeptidase of the astacin family that cleaves both pro-collagen and non-collagen substrates involved in forming functional collagen fibrils. As such it is an attractive anti-fibrotic target.[3–5] Types I–III collagens are initially excreted as pro-collagen peptides which contain solubilizing N and C terminal sequences. Cleavage of the globular C-terminal sequence is necessary to convert soluble pro-collagens into fully formed, insoluble, collagen fibrils. In addition, PCP processes prolysyl oxidase to its active form, which catalyzes the crosslinking of collagens, contributing to the structural stability of collagen fibrils. Inhibition of PCP is expected to disrupt fibril formation and stability, preventing the excess collagen deposition associated with fibrosis.[6,7]

Herein we describe the evolution and SAR of a series of potent, selective, non-peptidic PCP inhibitors as part of our anti-fibrosis programs. Starting from peptide-based PCP inhibitors and substrates, we devised alternative scaffolds which were elaborated through iterative modifications into promising leads.

Design of initial peptidic inhibitors was guided by studies of truncated PCP substrates. PCP cleavage sequences in known enzyme substrates types I, II, and III collagen indicate a substrate preference for P3: Met or Tyr; P2: Arg or Tyr; P1: Ala or Gly; P1′: Asp, P2′: Asp, Glu, or Gln; P3′: Ala or Pro. A series of short peptides derived from procollagen I–III sequences were examined as possible PCP substrates, measuring relative rates of cleavage to identify preferred peptide structures (Table 1). Of those tested, an octapeptide derived from the α1 strand of type III collagen, **1**, demonstrated the highest rate of cleavage. Limited truncation of **1** at either the C or N termini retained activity, though further deletions lead to substantially reduced cleavage by PCP. In addition, an alanine scan of peptide **1** confirmed that the P1′ aspartic acid was critical for recognition in this sequence, as illustrated by the complete lack of activity against peptide **9**.

Replacing the scissile bond in analogs of peptide **1** with zinc binding groups such as thiols, hydroxamic acids, and carboxylic acids provided initial inhibitors (Fig. 1). The hydroxamate capped aspartate–glutamate mimetic **10**, a mixture of 2 diastereomers with an IC_{50} of 40 μM, was the most effective peptide-based inhibitor.[8] Other zinc chelating functionalities were less potent: a carboxylic acid analog **11**, IC_{50}: 1500 μM, was substantially lower in activity while thiol **12** showed only moderate activity with an IC_{50} of 85 μM. Incorporation of a hydroxamate at the carboxy terminus of the scissile bond of peptide **1** was also effective: the tetra-peptide hydroxamate **13** inhibited PCP with an IC_{50} of 42 μM. Concurrent with this work others reported hydroxamate dipeptides mimicking P1′ and P2′ side chains, which were more effective then those derived from a N-terminal sequence.[9]

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Table 1
Relative order of PCP activity on pro-collagen peptides

Substrate	Peptide sequence	Collagen chain	PCP activity[a]
1	Ac-PYYG-DEPM-NH_2	α1(III)	++++
2	Ac-PYYG-DEP-NH_2	α1(III)	+++
3	Ac-FYRA-DQPR-NH_2	α2(I)	++
4	Ac-YYG-DEPM-NH_2	α1(III)	++
5	Ac-PYYG-DE-NH_2	α1(III)	++
6	Ac-YYRA-DDAN-NH_2	α1(I)	+
7	Ac-FAPYYG-DEPMDF-NH_2	α1(III)	+
8	Ac-PYYG-D-NH_2	α1(III)	–
9	Ac-PYYG-AEPM-NH_2	–	–

[a] Relative rates of peptide cleavage by PCP.

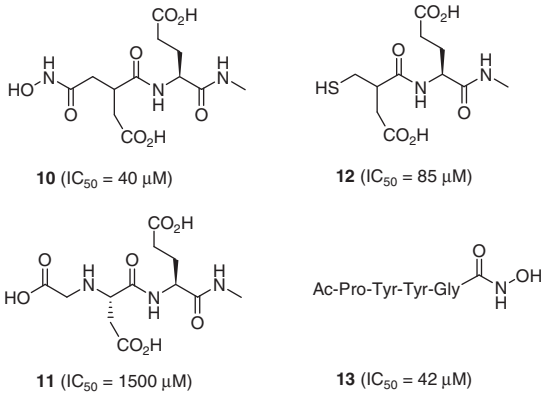


Figure 1. Peptidic PCP inhibitors derived from peptide 1.

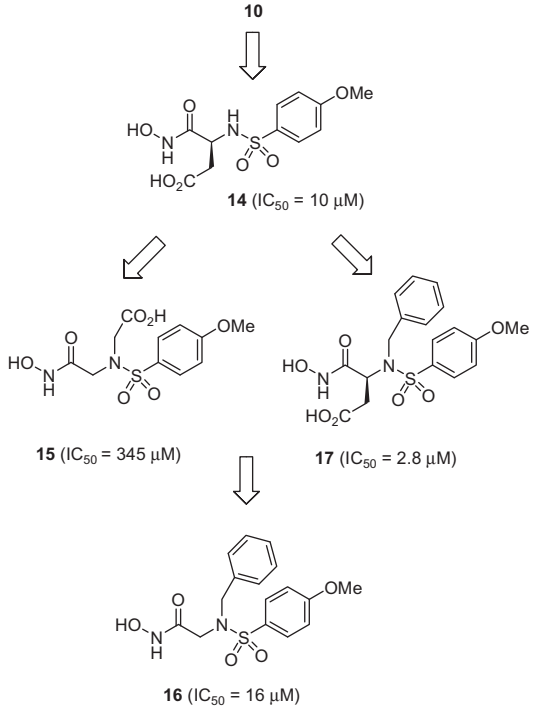


Figure 2. Preliminary sulfonamide based leads.

Other core scaffolds were explored using hydroxamates as the key zinc chelating group. Modifying dipeptides such as **10** by replacing the central amide with a sulfonamide was envisioned to display potential P_1', P_2' mimicking side chains and zinc binding functionality from an alternative scaffold. Surprisingly, our initial survey of N-substituted sulfonamide hydroxamates revealed that the diacid functionalities preferred in substrates were not necessary for potent inhibitory activity. Similar findings have been reported by others where hydroxamate bearing scaffolds with hydrophobic substituents have demonstrated potent binding.[10–14]

A hydroxamate inhibitor utilizing a *N*-arylsulfonamide aspartic acid core, **14**, IC_{50}: 10 μM, showed greater potency then our initial peptidyl Asp-Glu mimetic **10** (Fig. 2). Examples with acidic functionality displayed from the sulfonamide nitrogen resulted lower affinity, **15**: IC_{50} of 345 μM, but replacing this acidic group with a benzyl group retained comparable binding, **16**: IC_{50} of 16 μM. Compounds containing both a carboxylic acid at the C-α position and a *N*-benzyl functionality further increased inhibitory potency, **17**: IC_{50} of 2.8 μM, but had liabilities of decreased activity in cell-based assays and potential stability concerns due to observed intramolecular cyclization to a less active species. Therefore, **16** was chosen as an starting point for optimization.

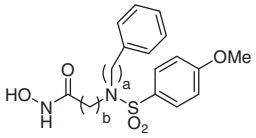
To determine optimal placement of aromatic and zinc chelating groups, a homologous series of sulfonamides was synthesized around compound **16**. Hydrocarbon spacers of varying lengths were incorporated between the sulfonamide nitrogen and both the hydroxamate functionality and the phenyl substituent. Of those tested, ethyl spacers were identified as preferable for both substituents. Compound **22**, $a = 2$, $b = 2$, with an IC_{50} of 900 nM, displayed a substantial improvement over the initial peptidyl hydroxamate inhibitors (Table 2).

The compounds in Tables 2–4 were prepared via three general synthetic methods. (Fig. 3, schemes I–III) In the first route, an amino ester **26** and an aldehyde **27** were condensed via a reductive amination using sodium triacetoxyborohydride or sodium cyanoborohydride as the hydride source. In the second route, Michael addition of a primary amine **30** into an acrylate ester provided a N-propanoate ester functionalized amine **28**. In both routes, the resulting secondary amine, **28**, was next coupled with the appropriate sulfonyl chloride in the presence of a base, typically triethylamine, to form the elaborated tertiary sulfonamide. Alternatively, in the third method, the secondary sulfonamide **32** is formed first by reacting amine **30** and sulfonyl chloride **31** in the presence of base, followed by alkylation of the sulfonamide nitrogen with an alkylbromide ester. In all three routes, the esters of the penultimate compounds were converted to the desired hydroxamic acids **29** by displacement of the alkoxy substituent using excess hydroxyl amine in methanol at room temperature.

Optimization of the sulfonamide substituent R^1 was undertaken to identify substituent preference at this position (Table 3). Varying the R^1 group in 2 series, which contained either methylene or ethylene linked hydroxamic acids ($b = 1,2$), showed that the ethylene spacer was preferred. Mid micromolar potency inhibitors could be obtained with methylene linked hydroxamates, but examples such as **41**, showed a much higher potency against MMP's thought to be critical to wound healing such as MMP 2 and 9 where it had IC_{50} values of 0.14 and 0.05 μM respectively, with moderate PCP activity, IC_{50} = 19 μM. In the ethylene linked hydroxamic acids series, a variety of alkyl and aryl functionality retained micromolar potency for this class, with the most potent of these compounds bearing a phenethyl, **24**, or *para*-methoxy phenethyl, **48**, at the R^1 position. Retaining these preferred substrates at R^1, optimization of the aryl sulfonamide portion of the molecule, R, was examined next.

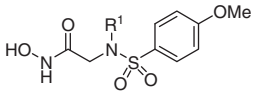
While a variety of R groups retain micromolar activity, appropriate substitution at the *para* position of phenylsulfonyl groups provided several sub-micromolar inhibitors (Table 4). The *para*-phenylsulfonyl hydroxyl imidamide analog, **60,** IC_{50} = 80 nM, showed excellent potency and selectivity versus MMP 1, 2, and 9. Similarly, a *para*-methyl sulfonyl group, **65:** IC_{50} = 0.29 μM, was moderately potent and displayed excellent selectivity when

Table 2
Optimization of spacers for sulfonamide series



Compound number	a: $(CH2)_x$ spacer length	b: $(CH_2)_x$ spacer length	IC_{50} (μM)
18	0	1	69
16	1	1	16
19	2	1	18
20	0	2	3
21	1	2	4.5
22	2	2	0.9
23	0	3	28
24	1	3	10
25	2	3	4.9

Table 3
Optimization of spacers for sulfonamide series



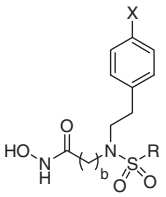
Compound	b: $(CH_2)_x$ spacer	R^1	IC_{50} (μM)
15	1	CH_2CO_2H	345
33	1	Adamantyl	[a]
34	1	*sec*-Butyl	112
35	1	CH_2(4-F-Ph)	34
36	1	CH_2CO_2n-Bu	167
37	1	4-MeO-Ph	73
38	1	CH_2CH_2(4-MeO)Ph	18
39	1	CH_2(4-MeO-Ph)	29
40	1	CH_2(4-CF_3-Ph)	67
41	1	CH_2(4-Cl-Ph)	19
42	1	$CH(Ph)_2$	102
24	2	CH_2CH_2Ph	0.9
43	2	CH_2CH_2-N-morpholinyl	81
44	2	*sec*-Butyl	1.7
45	2	CH_2-cyclhexane	1.0
46	2	CH_2CH_2(2-pyridyl)	8.1
47	2	4-MeO-Ph	1.7
48	2	CH_2CH_2(4-MeO-Ph)	1.0
49	2	CH_2CH_2(3-MeO-Ph)	2.3
50	2	CH_2CH_2(2-MeO-Ph)	[b]
51	2	CH_2CH_2(4-NO_2-Ph)	11
52	2	CH_2CH_2(4- NH_2SO_2-Ph)	23
53	2	CH_2CH_2(3,4-diMeO-Ph)	42
54	2	H	166

[a] 9–21% Inhibition at 10 μM.
[b] 45% Inhibition at 10 μM.

counter screened against MMP 2 & 9 (IC_{50} >6 μM for both MMPs). The scaffold of **65** was utilized to screen alternative zinc chelating functionalities.

Hydroxamate containing compounds have potential liabilities as systemic drugs: rapid metabolism or excretion, hydrolysis to release hydroxyl amine, and promiscuity for other zinc utilizing enzymes.[15–18] Therefore, alternative zinc binding functionalities were incorporated into this scaffold to determine effects on potency (Table 5). The syntheses of several non-hydroxamic acids analogs of **65** are summarized in Figure 4. The synthesis of the α-keto thiols **68** and **69** are shown in the first scheme, 4-I. The secondary sulfonamide **74**, synthesized by condensing 4-methoxyphenethylamine with 4-(methylsulphonyl)benzene-sulfonyl chloride, was alkylated

Table 4
Optimization of sulfonamide substituent R



Compound	X	b: $(CH_2)_x$	R	IC_{50} (μM)
55	OMe	2	*n*-Bu	6.7
56	OMe	1	*n*-Bu	55
57	H	2	Ph	2.2
58	H	2	4-CO_2H-Ph	1.0
59	OMe	2	2-CO_2H-Ph	[a]
60	OMe	2	4-(C(=NOH)NH_2)-Ph	0.08
61	OMe	2	3-(C(=NOH)NH_2)-Ph	0.40
62	OMe	2	2-(C(=NOH)NH_2)-Ph	7.1
63	OMe	2	4-(Ph-C(=O)NH)-Ph	0.41
64	OMe	2	4-(Ph-SO_2-NH)-Ph	0.47
65	OMe	2	4-SO_2Me-Ph	0.29
66	OMe	2	2-SO_2Me-Ph	9.1
67	H	2	4-*t*Bu-Ph	17

[a] 32% inhibition at 10 μM.

with either allyl bromide or 4-bromo-1-butene. The resulting alkenes were then epoxidized with *m*CPBA to give **75**. The epoxide rings were opened with thioacetic acid, and oxidized with PCC to produce the acetate protected α-keto thiols. The acetate was removed with hydrazine, which minimized competing β-elimination of the sulfonamide in the case of **69**, to produce compounds **68** and **69**.

The synthesis of *N*-hydroxy urea **70** was accomplished as shown in Figure 4-II. The sulfonamide **73** was alkylated with bromoacetaldehyde diethyl acetal. The protected aldehyde was unmasked under acidic conditions and condensed with hydroxylamine to give the oxime **75**. The oxime was reduced to the hydroxylamine and N-acylated with TMS-isocyanate, which upon TMS exchange furnished the desired N-hydroxy urea **70**. Thiols **71** and **72** were prepared as depicted in Figure 4-III. In the case of **71,** N-alkylation of **73** with 1-chloro-3-iodopropane and subsequent conversion of the chloropropane substituent into a mercaptopropane via displacement with thioacetic acid and hydrolysis to afford the desired propanethiol **71**. In the case of **72**, a hydroxyethyl substituted sulfonamide was synthesized by mesylation of 2-(4-methoxyphenyl)ethanol, N-alkylation of ethanol amine, and reaction of the secondary amine with 4-(methylsulfonyl)benzene sulfonyl chloride to give the ethanol substituted sulfonamide **76**. The alcohol was then mesylated, displaced with thiolacetic acid, and hydrolyzed to give the desired ethanethiol **72.**

As shown in Table 5, replacement of the hydroxamate functionality of **65** with other zinc chelating functionalities, including thiols, α-keto thiols, hydroxy-ureas, and carboxylic acids substantially decreased inhibitory potency. Variation in linker length produced only minor changes in potency. While thiol containing compounds **68** and **72** retained micromolar activity, potential limitations of thiols as drug-like functionality prioritized continued optimization of our hydroxamates leads.

In the next iterative optimization, we fixed 4-methoxyphenethyl as the R^1 group and ethyl-hydroxamic acid as the zinc binding functionality while the *para* position of the aryl sulfonamide was diversified further, (Table 4) since compounds with hydroxyl imidamide, amide, and sulfonamide moieties provided sub-micromolar inhibition and improved selectivity. For example, compound **60** showed excellent potency; selectivity versus select MMPs, where

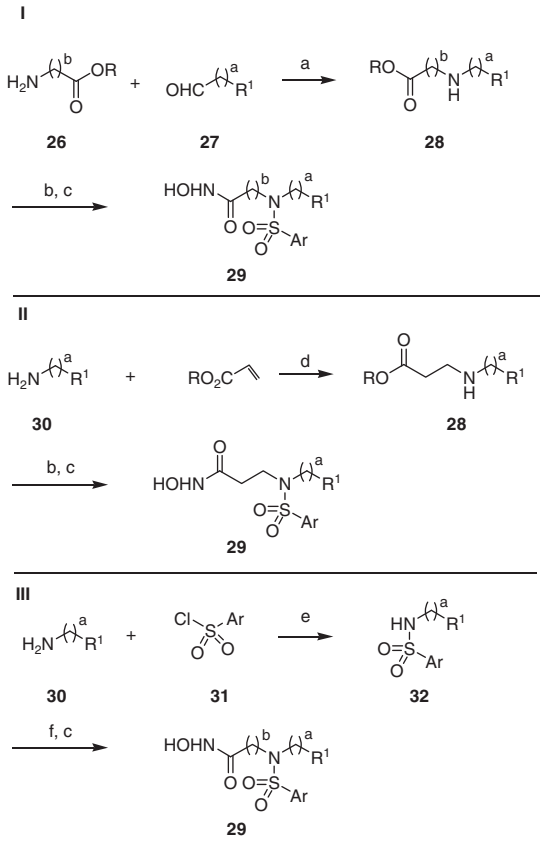
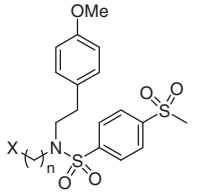


Figure 3. General synthesis of hydroxamates **15–67** and conditions: R = Et or Me; (a) $NaHB(OAc)_3$, TEA, DCM, rt, or $NaBH_3CN$, AcOH, MeOH, rt; (b) $ClSO_2Ar$, TEA, DCM, rt; (c) NH_2OH, MeOH, rt; (d) EtOH, reflux; (e) TEA, DCM, rt; (f) $Br(CH_2)_bCO_2R$, NaH, NaI, DMF, 90 °C.

Table 5
Comparison of zinc chelating groups incorporated into compound **65** scaffold



Compound Number	*n*	X	IC_{50}[a] (μM)
65	2	–CONHOH	0.29
68	1	$-COCH_2SH$	4.7
69	2	$-COCH_2SH$	13
70	2	$-N(OH)CONH_2$	>100
71	3	–SH	14
72	2	–SH	9.4

IC_{50}'s for MMP 1, 2, and 9 were all greater than 25 μM; and low cytotoxicity, as measured against proliferation of 3T3-L1 cells, with an IC_{50} of 150 μM. Therefore we examined polar linking groups for the attachment of diversifying R^2 and R^3 groups at this position.

Utilizing a urea to display hydrophobic substituents from the 4-postion of the phenylsufonamide, **80,** resulted in several promising compounds. Syntheses of sulfonamide–ureas were accomplished by two general methods (Fig. 5). First, the isocyante of 4-isocyanate-phenylsulfonylchloride, **77**, was chemoselectively coupled to an amine to produce the appropriate di or tri substituted urea–sulfonyl chloride **78**, which was then elaborated via our standard

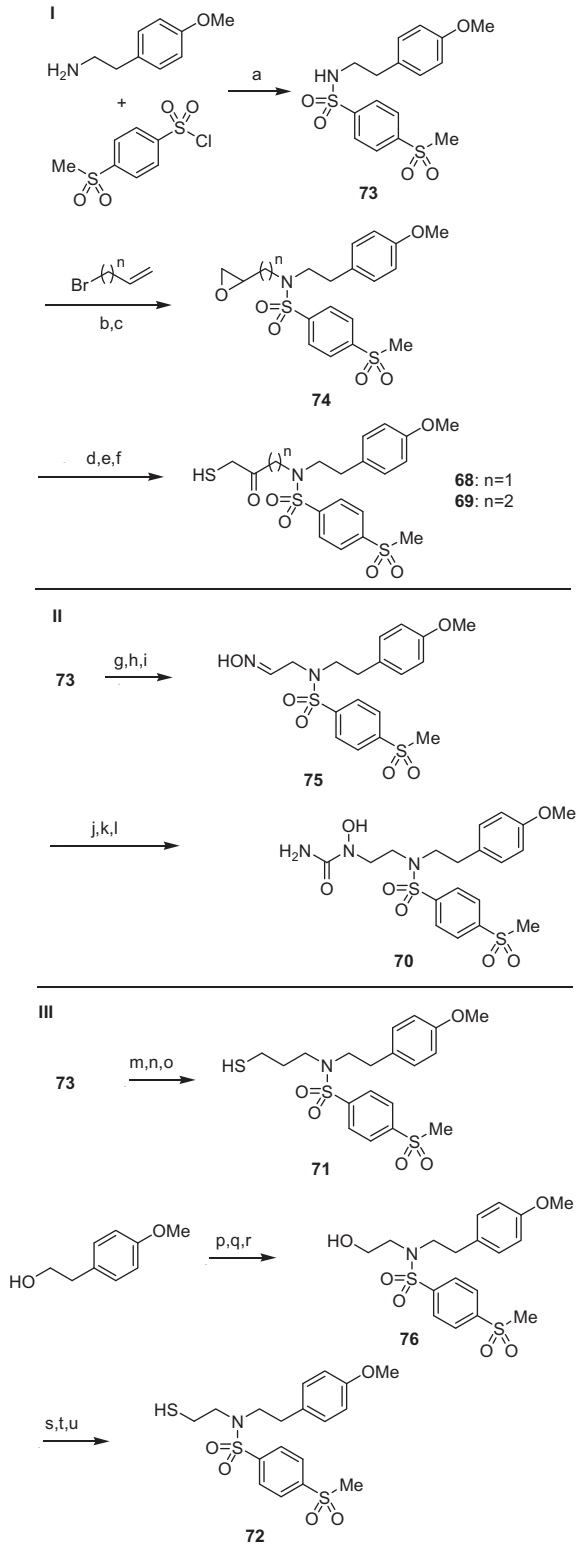


Figure 4. Synthesis of non-hydroxamate inhibitors. *n* = 1 or 2; R = CH_2CH_2(4-MeOPh); Conditions (yields, n = 1 and n = 2): (a) TEA, DCM, rt (93%); (b) NaH, DMF, rt (*n* = 1); NaH, DMF, TBAI, 60 °C (*n* = 2), (50%); (c) *m*CPBA, DCM, rt (26% two steps, 75%); (d) AcSH, rt to 40 °C (69%, 65%); (e) PCC, celite, DCM, rt, (88%, 96%); (f) Hydrazine, THF/DMF, −10 to −18 °C (91%, 90%); (g) NaH, $BrCH_2(OEt)_2$, TBAI, DMF, 80 °C (50%); (h) TsOH, Acetone, reflux (76%); (i) NH_2OH, $NaHCO_3$, EtOH/THF, rt, (97%); (j) $PyrBH_3$, THF 0 °C (90%); (k) TMS-NCO, dioxane, rt (quant); (l) H_2O, (40%); (m) NaH, ICH_2CH_2Cl, DMF, rt; (n) AcSH, K_2CO_3, DMF, 60 °C (40% for 2 steps); (o) LiOH, THF/H_2O, rt (42%); (p) MsCl, TEA, DCM, 0 °C to rt (70%); (q) ethanolamine, TEA, THF, rt (92%); (r) $ClSO_2Ph(4\text{-}SO_2Me)$, TEA, DMF, rt (85%); (s) MsCl, TEA, DCM, 0 °C to rt (94%); (t) AcSH, DMF, Cs_2CO_3, rt (92%); (u) LiOH, THF/H_2O/EtOH, rt, (72%).

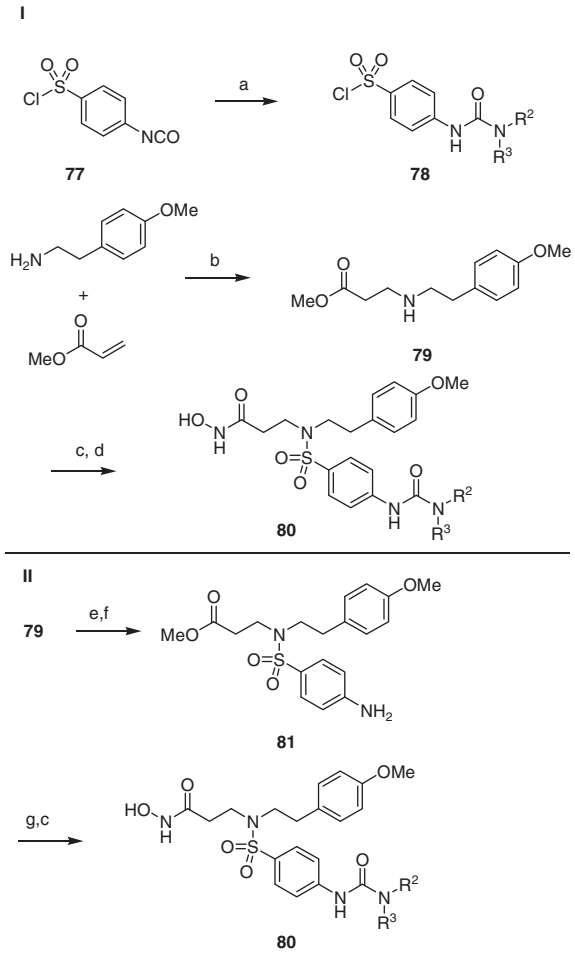
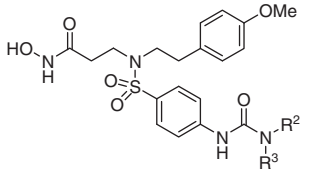


Figure 5. Synthesis of ureas. Conditions: (a) NHR^2R^3; THF, 0 °C; (b) EtOH, reflux; (c) **78**, TEA, rt; (d) NH_2OH, MeOH, rt; (e) $ClSO_2Ph\text{-}NO_2$, TEA, DCM, rt; (f) HCO_2NH_4, Pd/C, MeOH/EtOAc, reflux; (g) R-NCO, DCM, reflux.

Table 6
Optimization of spacers for sulfonamide series **81**



Compound	R^2	R^3	IC_{50} (μM)
82	Ph	H	0.093
83	4-MeO-Ph	H	0.35
84	4-CF_3-Ph	H	0.042
85	Bn	Bn	0.38
86	Ph	Ph	0.78
87	4-Ph-Ph	H	0.073
88	4-Cl-Ph	H	0.30
89	Bn	H	0.010
90	CH_2CH_2Ph	H	0.030
91	Me	H	0.060

reaction sequences to hydroxamates **80**. In the second method, an 4-aminophenylsulphone intermediate **81** was prepared from reduction of a 4-nitro precursor. Reaction of the amine with various isocyanates gave disubstituted ureas. The final compounds were, again, produced by conversion of the esters to hydroxamic acids **80** with hydroxyl amine.

The ureas gave substantial improvement over the previous *para*-substituted analogs, with IC_{50} values in the low to mid nanomolar range (Table 6). Trisubstituted ureas bearing a benzyl or phenyl substituent at R^3 were less potent then the corresponding disubstituted analogs, as seen in compounds **85** versus **89** and **82** versus **86**.

The urea **89** was identified as a potent inhibitor of PCP with an IC_{50} of 10 nM. When tested in a cellular assay, measuring inhibition of collagen deposition in HFF cell cultures,[19] **89** displayed an IC_{50} of 1 μM. The compound showed excellent selectivity versus several matrix metalloproteinases (MMP-1 IC_{50} >25 μM; MMP-2 IC_{50} >25 μM; and MMP-9 IC_{50} >25 μM). Compounds **89** and **60** possess promising properties for the development of topically applied anti-scarring therapeutics. The additional optimization of this class of inhibitors to improve the DMPK properties may also provide new systemic anti-fibrotic agents.

References and notes

1. Hinz, B.; Gabbiani, G. *F1000 Biol. Report* **2010**, *2*, 78.
2. Zavoico, G. B. *Drug Market Dev.* **1999**, *10*, 2.
3. Lapiere, C. M.; Lenarers, A.; Kohn, I. D. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 3054.
4. Li, S.; Sieron, A. L.; Fertala, A.; Hojima, Y.; Arnold, W. V. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5127.
5. Uzel, M. I.; Scott, I. C.; Babakhanlou-Chase, H.; Palamakumbura, A. H.; Pappano, W. N.; Hong, H.-H.; Greenspan, D. S.; Trackman, P. C. *J. Biol. Chem.* **2001**, *276*, 22537.
6. Prockop, D. J.; Sieron, A. L.; Li, S. *Matrix Biol.* **1998**, *16*, 399.
7. Turtle, E.; Ho, W.-B. *Expert Opin. Ther. Pat.* **2004**, *14*, 1185.
8. IC_{50} determination for PCP and MMP's: A fluorogenic peptide containing a consensus PCP cleavage site was used to assess the inhibitory activity of compounds on PCP. In a similar assay, a fluorogenic peptide with the sequence Mca-P-L-G-L-Dap(Dnp)-A-R-NH_2 (λ_{ex} = 360 nm, λ_{em} = 465 nm; Bachem catalog no. M-1895) was used to measure the inhibitory activity of compounds on MMP-1, MMP-2 and MMP-9. The latter enzymes were activated according to the manufacturer's instructions using *p*-amino phenylmercuric acetate. BMP-1, and activated MMP-1, -2, and -9 were incubated for 1 h at 37 °C in the presence of inhibitors and 50 mM of the appropriate fluorogenic peptide substrate, and the increase in fluorescence was used to determine initial rates.
9. Ovens, A.; Joule, J. A.; Kadler, K. E. J. *Peptide Sci.* **2000**, *6*, 489.
10. Dankwardt, S. M.; Martin, R. L.; Chan, C. S.; VanWart, H. E.; Walker, K. A. M.; Delae, N. G.; Robinson, L. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2085.
11. Robinson, L. A.; Wilson, D. M.; Delaet, N. G. J.; Bradley, E. K.; Dankwardt, S. M.; Campbell, J. A.; Martin, R. L.; Van Wart, H. E.; Walker, K. A. M.; Sullivan, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2381.
12. Delaet, N. G. J.; Robinson, L. A.; Wilson, D. M.; Sullivan, R. W.; Bradley, E. K.; Dankwardt, S. M.; Martin, R. L.; Van Wart, H. E.; Walker, K. A. M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2101.
13. Fish, P. V.; Allan, G. A.; Bailey, S.; Blagg, J.; Butt, R.; Collis, M. G.; Greiling, D.; James, K.; Kendall, J.; McElroy, A.; McCleverty, D.; Reed, C.; Webster, R.; Whitlock, G. A. *J. Med. Chem.* **2007**, *50*, 3442.
14. Bailey, S.; Fish, P. V.; Billotte, S.; Bordner, J.; Greiling, D.; James, K.; McElroy, A.; Mills, J. E.; Reed, C.; Webster, R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6562.
15. Hajduk, P. J.; Shuker, S. B.; Nettesheim, D. G.; Craig, R.; Augeri, D. J.; Albert, D. H.; Guo, Y.; Meadows, R. P.; Xu, L.; Michaelides, M.; Davisen, K.; Fesik, S. W. *J. Med. Chem.* **2002**, *45*, 5628.
16. Singh, J.; Conzentino, P.; Cundy, K.; Gainor, J. A.; Gilliam, C. L.; Gordon, T. D.; Johnson, J. A.; Morgan, B. A.; Schneider, E. D.; Wahi, R. C.; Whipple, D. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 337.
17. Flipo, M.; Charton, J.; Hocine, A.; Dassonneville, S.; Deprez, B.; Deprez-Poulain, R. *J. Med. Chem.* **2009**, *52*, 6790.
18. Becker, D. P.; Barta, T. E.; Bedell, L. J.; Boehm, T. L.; Bond, B. R.; Carroll, J.; Carron, C. P.; Decrescenzo, G. A.; Easton, A. M.; Freskos, J. N.; Funckes-Shippy, C. L.; Heron, M.; Hockerman, S.; Howard, C. P.; Kiefer, J. R.; Li, M. H.; Mathis, K. J.; McDonald, J. J.; Mehta, P. P.; Munie, G. E.; Sunyer, T.; Swearingen, C. A.; Villamil, C. I.; Welsch, D.; William, J. M.; Yu, Y.; Yao, J. *J. Med. Chem.* **2010**, *53*, 6653.
19. Franklin, T. J.; Morris, W. P.; Edwards, P. N.; Large, M. S.; Stephenson, R. *Biochem. J.* **2001**, *353*, 333.