# Potent and Selective Nonpeptidic Inhibitors of Procollagen C-Proteinase

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6-Cyclohexyl-*N*-hydroxy-3-(1,2,4-oxadiazol-5-yl)hexanamides were previously disclosed as inhibitors of procollagen C-proteinase (PCP) culminating in the identification of amide **1**. Our objective was to discover a second inhibitor that would have improved affinity for PCP and to optimize properties for transepidermal delivery (TED) to intact skin. Further investigation of this template identified a number of potent PCP inhibitors ($IC_{50}$ values of 2−6 nM) with improved TED flux. Sulfonamide **56** had excellent PCP enzyme activity when measured with a peptide substrate ($K_i$ 8.7 nM) or with the endogenous substrate procollagen ($IC_{50}$ 3.4 nM) and demonstrates excellent selectivity over MMPs involved in wound healing (>10 000-fold). In the fibroplasia model, **56** inhibited deposition of insoluble collagen by 76 ± 2% at 10 $\mu$M and was very effective at penetrating human skin in vitro with a TED flux of 1.5 $\mu$g/cm$^2$/h, which compares favorably with values for agents that are known to penetrate skin well in vivo. Based on this profile, **56** (UK-421,045) was selected as a candidate for further preclinical evaluation as a topically applied, dermal anti-scarring agent.

## Introduction

Fibrosis is a complex process that is characterized by excessive accumulation of extracellular matrix (ECM[a]), principally type I collagen. Recent advances in the understanding of the molecular mechanisms of fibrosis have revealed that, rather than being a distinct pathological event, fibrotic ECM deposition results from disregulation of normal tissue repair processes.[1,2] In the case of wounds to the skin, dermal fibroblasts respond to activation by transforming growth factor-$\beta$ (TGF-$\beta$1) by releasing soluble procollagens and procollagen C-proteinase (PCP;[3,4] EC 3.4.24.19, also known as Bone Morphogenetic Protein-1, BMP-1).[5,6] Proteolytic cleavage of the C-terminal propeptide of types I, II, and III procollagens by PCP at Gly−Asp and Arg−Asp sites affords collagen, which undergoes self-assembly into fibrils, followed by cross-linking to give an insoluble collagen matrix. The cross-linking process is primarily driven by prolyl hydroxylase-mediated hydroxylation of the proline-rich collagen molecules. In addition to processing procollagens, PCP is also able to cleave latent prolyl oxidase to its active form and, therefore, may contribute further to ECM formation.[7]

The excessive accumulation of ECM by these processes leads to the formation of scar tissue, which can range in severity from being grossly disfiguring to cosmetically unpleasant. Clinically significant scars include hypertrophic/keloid scars and those that occur following burns. These latter categories can often lead to functional impairment due to skin contraction, especially if they occur across joints. Unsightly scars, particularly on the face or hands, can cause significant psychological distress in many cases.

Hence, we identified PCP as an attractive point at which to intervene in the collagen deposition pathway and initiated a research program aimed at identifying small, reversible inhibitors of this enzyme suitable for *topical application* as antiscarring agents.

In the context of dermal scars, selectivity over inhibition of several other proteolytic enzymes that are found in the wound environment was felt to be important. For instance, matrix metalloproteinase-1 (MMP-1) is expressed in keratinocytes at the migrating front of acute wounds and is required, along with MMP-2 and -9, for keratinocyte migration (i.e., wound re-epithelialisation in vivo). MMP-2 is expressed in fibroblasts and endothelial cells in acute wounds and is required for fibroblast and endothelial migration (i.e., granulation and angiogenesis). MMP-9 has also been suggested to be involved in cell migration, while MMP-14 is involved in the activation of MMP-2. Therefore, an agent designed to prevent dermal scarring without effects on the rate or quality of wound healing would need to be selective over MMPs.[8]

While this work was in progress, two types of low molecular weight inhibitors of PCP have been reported with good inhibition of enzyme activity (Chart 1). These inhibitors incorporate either a hydroxamic acid (e.g., **3**−**5**) or phosphinate (e.g., **6**) as the zinc binding ligand in the catalytic site of the enzyme.[9] Our own efforts identified succinyl hydroxamates **1** (UK-383,367) and **2** as potent and selective inhibitors of PCP.[10]

The continuing results of these efforts are presented in this article. The compounds described herein are potent, competitive, and reversible inhibitors of PCP enzymatic activity, with high selectivity for PCP relative to MMPs.

## Chemistry

The general method for the synthesis of the oxadiazole hydroxamates disclosed in Tables 1−6 (compounds **7**−**71**) is outlined in Scheme 1. Nitriles **72** were reacted with $HONH_2$ to

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[a] Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase; PCP, procollagen C-proteinase; TED, transepidermal delivery; CDI, 1,1-carbonyldiimidazole; DCM, dichloromethane; DIPE, diisopropyl ether; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; THF, tetrahydrofuran; TFA, trifluoroacetic acid.

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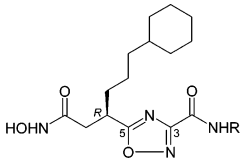
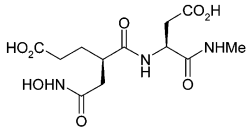
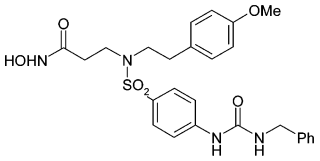
**Chart 1.** PCP Inhibitors



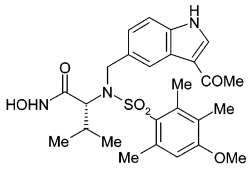
**1**: R = H, $IC_{50}$ = 44 nM
**2**: R = $CH_2CO_2H$, $IC_{50}$ = 21 nM



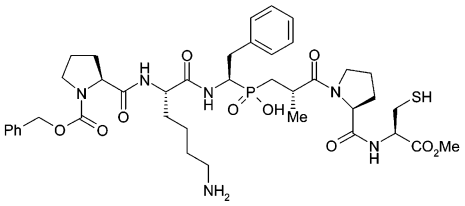
**3**: $IC_{50}$ = 100 nM



**4**: $IC_{50}$ = 10 nM

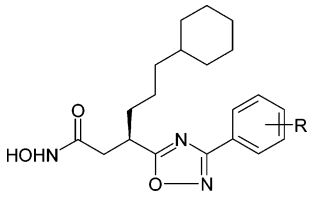


**5**: $IC_{50}$ = 26 nM



**6**: $IC_{50}$ = 4 nM

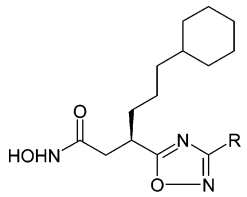
**Table 1.** Substituted Aryl Oxadiazoles



| cmpd | R | PCP $IC_{50}$[a] (nM) |
|---|---|---|
| **7** | H | 116 |
| **8** | 4-$CO_2$Et | 154 |
| **9** | 4-$CO_2$H | 32 |
| **10** | 4-$SO_2NMe_2$ | 75 |
| **11** | 4-$NHSO_2$Me | 3 |
| **12** | 3-$CO_2$Et | 184 |
| **13** | 3-$CO_2$H | 6 |

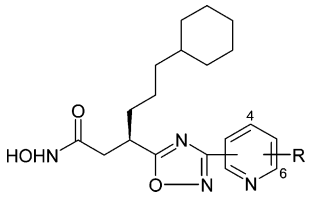
[a] Mean of at least two experiments.

**Table 2.** Heteroaromatic Substituted Oxadiazoles



| cmpd | R | PCP $IC_{50}$ (nM) |
|---|---|---|
| **7** | Ph | 116 |
| **14** | 2-Py | 25 |
| **15** | 3-Py | 23 |
| **16** | 4-Py | 2 |
| **17** | 6-methyl-3-pyridazinyl | 30 |
| **18** | 2-pyrimidinyl | 23 |
| **19** | 5-pyrimidinyl | 14 |
| **20** | 2-pyrazinyl | 14 |
| **21** | 3-pyrazolyl | 74 |
| **22** | 4-pyrazolyl | 39 |
| **23** | 1-methyl-2-imidazolyl | 92 |
| **24** | 2-furanyl | 46 |
| **25** | 5-uracilyl | 10 |

**Table 3.** Substituted Pyridyl Oxadiazoles



| cmpd | R | PCP $IC_{50}$ (nM) |
|---|---|---|
| | 2-Pyridyl | |
| **26** | 4-$CO_2$H | 6 |
| **27** | 4-$CONH_2$ | 17 |
| **28** | 4-CONHMe | 30 |
| **29** | 4-$CONMe_2$ | 17 |
| | 3-Pyridyl | |
| **30** | 5-$CO_2$H | 17 |
| **31** | 6- $CO_2$H | 59 |
| **32** | 6-$NH_2$ | 18 |
| **33** | 6-NHMe | 13 |
| **34** | 6-$NMe_2$ | 28 |
| **35** | 6-(4-methyl-1-piperazinyl) | 8 |
| **36** | 6-$NHSO_2$Me | 27 |

give the corresponding *N*-hydroxy amidines **73**, which were then coupled with (*R*)-succinic acid mono-ester **74**[11] to give (presumably) the *O*-acyl derivatives **75**. Thermal cyclodehydration of **75** in xylene created the 1,2,4-oxadiazoles **76** and deprotection of the *t*-butyl esters gave carboxylic acids **77**. Finally, activation of the carboxylic acids **77** with either 1,1-carbonyldiimidazole (CDI) or *i*-BuOCOCl, followed by reaction with $TMSONH_2$ and methanolic workup, gave compounds **7**, **8**, **12**, **14**−**25**, **37**−**39**, **50**, **56**, **65**, and **66**. Oxadiazole esters **8** and **12** were converted to the corresponding carboxylic acids **9** and **13**, respectively, by mild base hydrolysis.

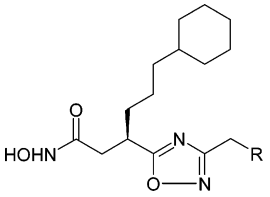
The remaining inhibitors were also prepared by the general synthetic sequence outlined in Scheme 1, except that additional functional group transformations were undertaken of appropriate intermediates (Schemes 2−7).

Arylsulfonamides **10** and **11** were prepared from aniline **80** (Scheme 2). Reduction of **79** with $SnCl_2$ gave aniline **80** and then deprotection of the *t*-butyl ester with TFA gave **81**. The aniline **81** was then converted to reversed sulfonamide **82** via a diazonium salt. Thus, diazotization of **81** with $NaNO_2$/cHCl, followed by treatment with $SO_2$ in the presence of $CuCl_2$ gave the corresponding sulfonyl chloride, which then reacted with $HNMe_2$ to give **82**. Acid **82** was then converted to hydroxamate **10** by standard methods. Alternatively, reaction of aniline **80** with $MeSO_2Cl$ gave sulfonamide **83**, and then reaction with TFA and introduction of the hydroxamate gave **11**.

2-Pyridyl acid **26** and amides **27**−**29** were prepared from ester **85** (Scheme 3). Acid **26** was prepared in a similar manner to benzoic acid **9** by introduction of the hydroxamate and then mild base hydrolysis of the ethyl ester. Ester **85** was treated with $NH_3$, $NH_2Me$ or $NHMe_2$ to yield the corresponding carboxamides **86**−**88**, which were then converted to **27**−**29** by standard methods.

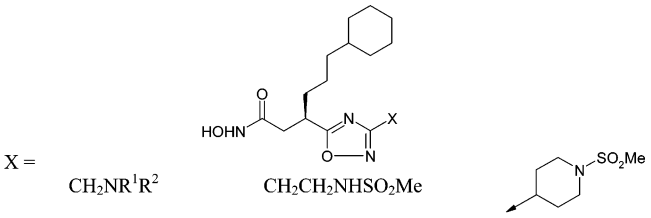
The synthesis of the 3-pyridyl acid **30** was prepared by the exact same procedure as 2-pyridyl acid **26** except that ethyl 5-cyanonicotinate was used as the starting material. The synthesis of the 3-pyridyl compounds **31**−**36** is described in Scheme 4. Pd-mediated ethoxycarbonylation of 6-chloropyridine

**Table 4.** Pyridylmethyl and Aminomethyl Oxadiazoles



| cmpd | R | PCP $IC_{50}$ (nM) |
|---|---|---|
| **37** | 2-Py | 6 |
| **38** | 3-Py | 6 |
| **39** | 4-Py | 28 |
| **40** | $NH_2$ | 122 |
| **41** | NHMe | 23 |
| **42** | NHEt | 21 |
| **43** | NH-*i*-Pr | 29 |
| **44** | NH-*t*-Bu | 10 |
| **45** | NH-*c*-Pr | 6 |
| **46** | NH-*c*-Bu | 6 |
| **47** | $NHC(Me)_2CH_2OH$ | 8 |
| **48** | $NMe_2$ | 31 |
| **49** | 1-pyrrolidinyl | 24 |
| **50** | 4-morpholinyl | 5 |
| **51** | 4-hydroxy-1-piperidinyl | 13 |

**Table 5.** Substituted Aminomethyl Oxadiazoles




**52-64** **65** **66**

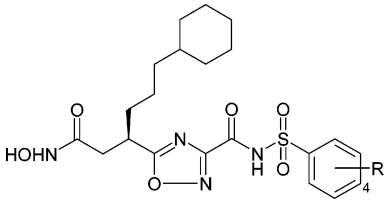
| cmpd | $R^1$ | $R^2$ | PCP $IC_{50}$ (nM) |
|---|---|---|---|
| **52** | H | COMe | 19 |
| **53** | Me | COMe | 3 |
| **54** | H | $CONH_2$ | 324 |
| **55** | H | $SO_2NH_2$ | 98 |
| **56** | H | $SO_2Me$ | 11 |
| **57** | Me | $SO_2Me$ | 38 |
| **58** | H | $SO_2Et$ | 26 |
| **59** | H | $SO_2$-*i*-Pr | 21 |
| **60** | H | $SO_2Ph$ | 26 |
| **61** | H | $SO_2$-2-Py | 107 |
| **62** | H | $SO_2$-3-Py | 26 |
| **63** | H | $SO_2$-4-pyrazole | 88 |
| **64** | | $-SO_2CH_2CH_2CH_2-$ | 15 |
| **65** | | | 20 |
| **66** | | | 3 |

**89** gave the corresponding 6-ester **90**, and then conversion to acid **31** followed standard methods. The 6-chloropyridine derivative **89** (or **92**) was reacted with a range of amines to give the corresponding 6-aminopyridines **93**−**95**. However, the attempted aminolysis of **89** with $NH_3$ was unsuccessful, and so, amine **91** was prepared according to Scheme 1, where the 6-aminopyridine group was installed early in the sequence (**73**: R = 6-amino-3-pyridyl). Aminopyridines **91** and **93**−**95** were converted to **32**−**35** by standard methods. Amine **91** was converted to sulfonamide **36** by reaction with $MeSO_2Cl$ to give the bissulfonamide **96**, and NaOH hydrolysis then, simultaneously, removed one of the $MeSO_2-$ groups and cleaved the *t*-Bu ester to give acid **97**.

3-(Aminomethyl)oxadiazoles **40**−**49** and **51** were all prepared from tosylate **99** (Scheme 5). Hydride reduction of ester **98**,[10] followed by tosylation of the resulting alcohol, gave **99**. Reaction of tosylate **99** with ammonia in a sealed vessel at 50 °C cleanly gave amine **100** in high yield. To convert **100** into **40**, it was necessary to protect the primary amino group (as **101**) to prevent self-condensation with the activated acid during the reaction with $HONH_2$ used to introduce the hydroxamate group. It proved unnecessary to protect *sec*- and *tert*-amines in this reaction. Thus, tosylate **99** underwent reaction with a variety of alkyl amines and amino alcohols to give amines **102**, which were then converted to **41**−**49** and **51** by standard methods.

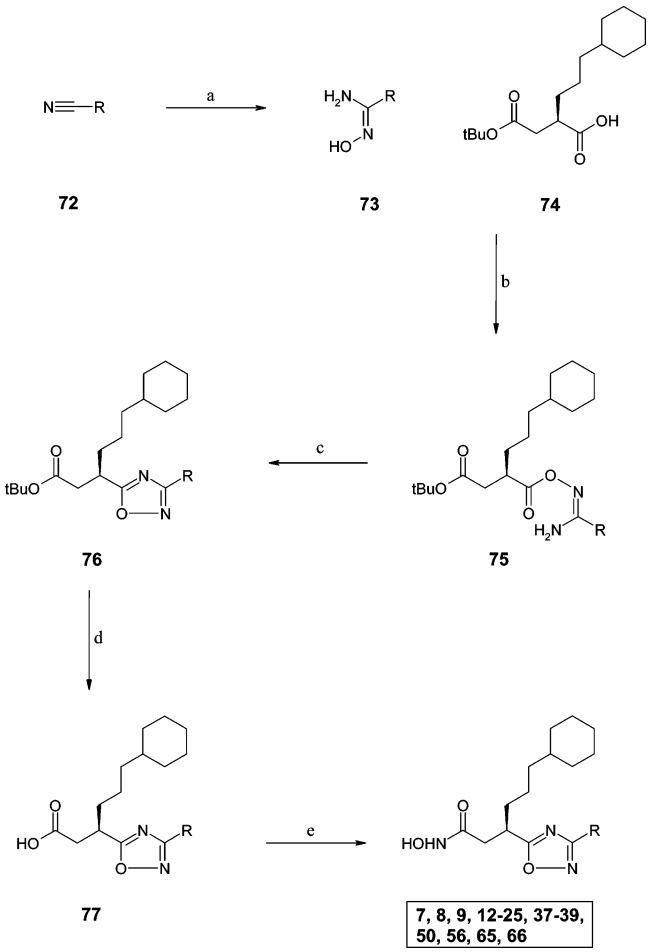
Oxadiazole amines **100** and **103** proved to be useful intermediates and were converted to carboxamides (**52**, **53**), urea

**Table 6.** Oxadiazole Acylsulphonamides



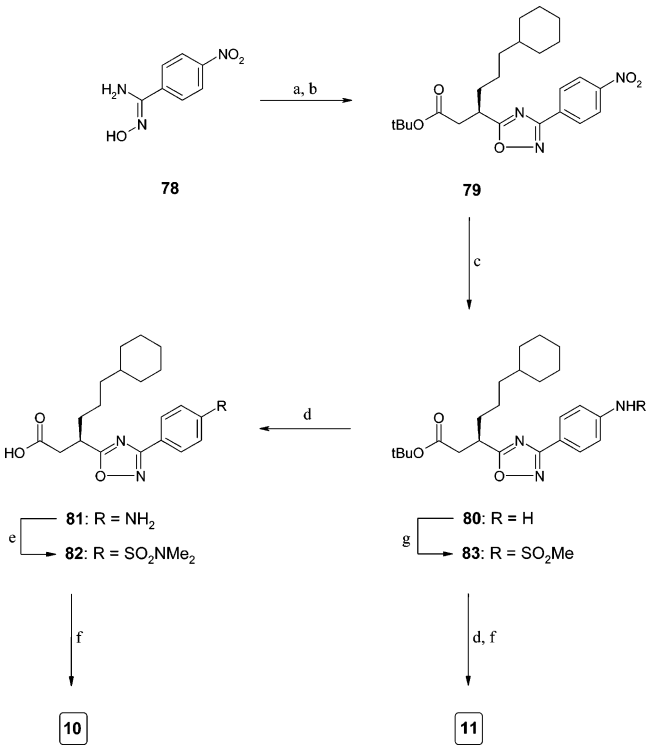
| cmpd | R | PCP $IC_{50}$ (nM) |
|---|---|---|
| **67** | H | 7 |
| **68** | 4-Me | 14 |
| **69** | 4-OMe | 17 |
| **70** | 3,4-$(OMe)_2$ | 1.8 |
| **71** | 4-F | 33 |

**Scheme 1.** General Method for the Synthesis of Inhibitors[a]



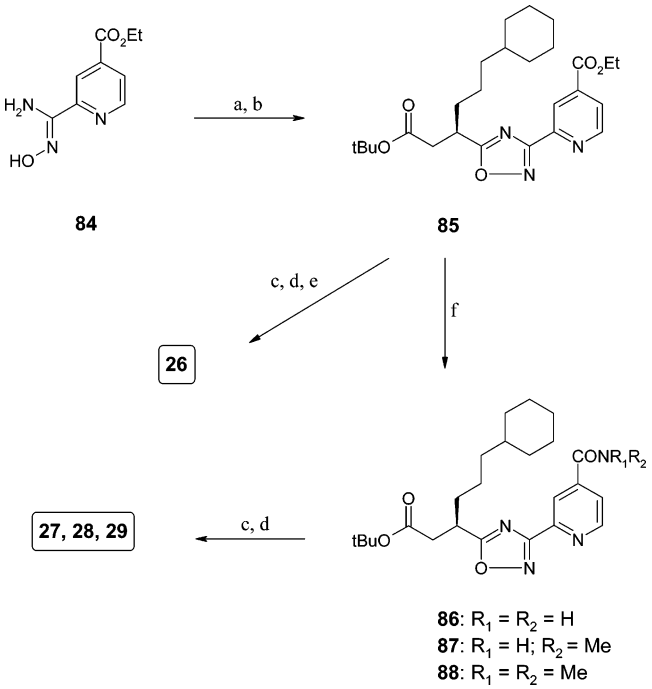

[a] Reagents and conditions: (a) $HONH_2$; (b) CDI; (c) xylene, 130 °C; (d) TFA; (e) *i*-BuOCOCl or CDI, then $TMSONH_2$, then MeOH.

**Scheme 2.** Synthesis of Compounds **10** and **11**[a]




[a] Reagents and conditions: (a) CDI, **74**; (b) xylene, 130 °C; (c) $SnCl_2$; (d) TFA; (e) (i) cHCl, $NaNO_2$, −10 °C; (ii) $SO_2$, $CuCl_2$, −10 °C; (iii) $NHMe_2$; (f) *i*-BuOCOCl or CDI, then $TMSONH_2$, then MeOH; (g) $MeSO_2Cl$.

**Scheme 3.** Synthesis of Compounds **26**−**29**[a]




[a] Reagents and conditions: (a) CDI, **74**; (b) xylene, 130 °C; (c) TFA; (d) *i*-BuOCOCl or CDI, then $TMSONH_2$, then MeOH; (e) LiOH; (f) $NHR_1R_2$, 23−60 °C.

(**54**), sulfonyl urea (**55**), sulfonamides (**57**−**63**), and sultam (**64**; Scheme 6). The amines **100** and **103** underwent reaction with a suitable reagent (see the scheme) to yield amino derivatives **104**, which were then converted to **52**−**55** and **57**−**63** by standard methods. Amine **100** was coupled with $Cl(CH_2)_3SO_2Cl$ to give the corresponding sulfonamide **104** (R = H; X = $SO_2(CH_2)_3Cl$), treatment with NaOMe simultaneously created the sultam and cleaved the *t*-butyl ester to yield **105** (R, X = $SO_2(CH_2)_3$), which was converted to **64** by standard methods.

The synthesis of acyl sulfonamide derivatives **67**−**71** proved to be problematic, and our preferred method is shown in Scheme 7. Treatment of amide **106** (which is readily available from **98**)[10] with 2 equiv of NaH at 0 °C gave the carboxamide anion, and reaction in situ with an arylsulphonyl chloride gave the corresponding acyl sulfonamide **107**, which were converted to **67**−**71** by standard methods.

## Compound Design

At the outset of the work described in this article, no small molecule inhibitors of PCP have been described in the literature. to identify new leads, compounds from the Pfizer file, prepared in previous metalloproteinase projects, were screened for their ability to inhibit PCP (Figure 1). From this targeted screening, a number of inhibitors with $IC_{50}$ values < 10 $\mu$M were identified and, of particular interest, were compounds **I** and **II**, which are derived from the classical succinic acid group of metalloprotease inhibitors.[11]

Compound **I** displayed only modest activity against PCP and was inversely selective against MMP-2. However, it was noted that replacement of the phenyl ring of **I** with a cyclohexane, to give **II**, gave a 10-fold increase in potency against PCP and also led to a significant drop off in activity against MMP-2. A second hit from the screening was **III**, which demonstrated moderate and balanced potency against both PCP and MMP-2. Oxadiazole **III** can also be considered as a succinic acid derivative in which the $C_1$-carboxy terminus has been masked as a heterocycle. Hence, combining these two pieces of SAR, as an initial venture, we prepared **IV** as the direct analogue of **III**, where the phenyl ring was replaced by cyclohexyl. Remarkably, this simple modification gave a 180-fold increase in inhibitory activity against PCP and further attenuated MMP-2 activity. Hence, it was clear that the (cyclohexyl)propyl side chain of **IV** played an important role in regulating affinity for PCP and confirming selectivity over MMP-2. Finally, replacement of the chemically and metabolically vulnerable ester group of **IV** with 3-carboxamides on the oxadiazole ring identified primary amide **1** as a potent and selective inhibitor of PCP.[10]

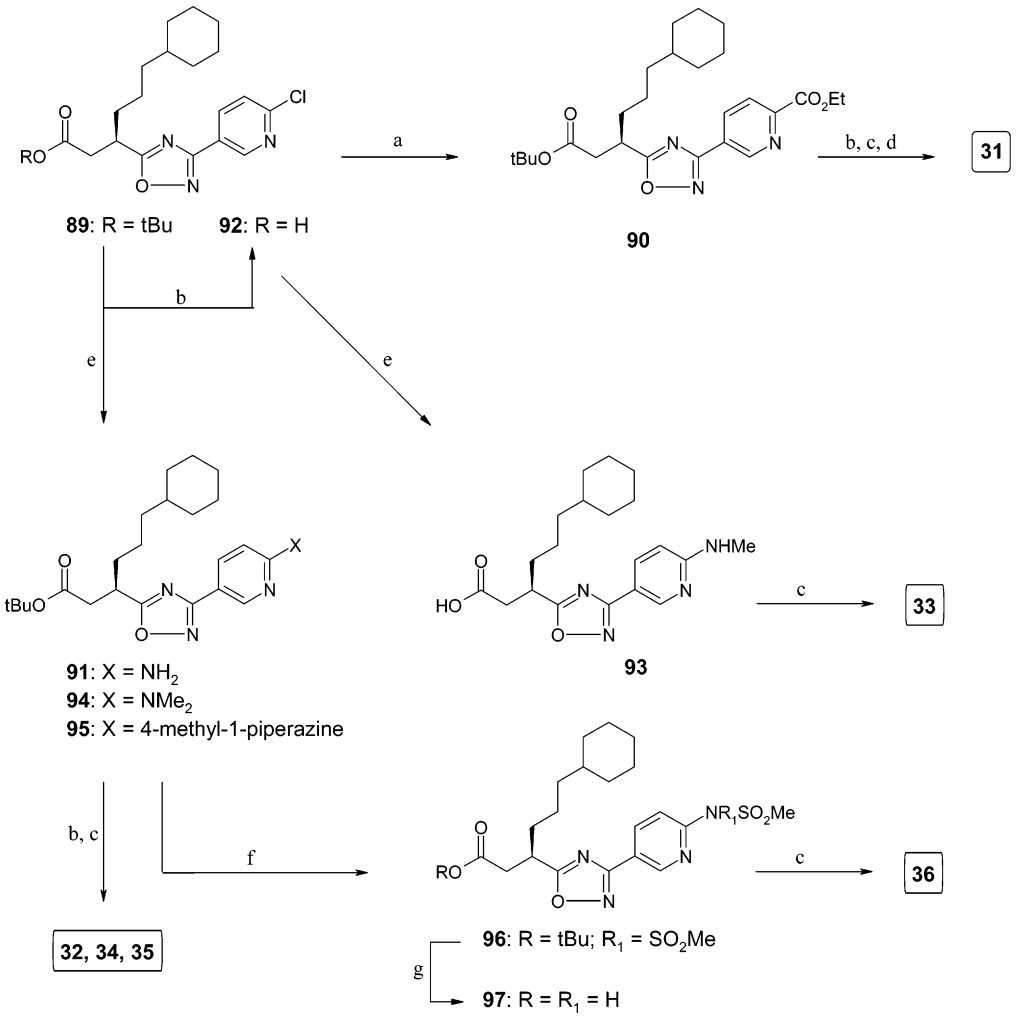
With compound **1** selected for early clinical development as a *topically* applied dermal anti-scarring agent, our objective was to discover a second agent that would have improved affinity for PCP and would optimize physicochemical properties suitable for the transepidermal delivery (TED) to intact skin.[12] It was also important that we retain desirable properties, such as high selectivity over the MMPs, crystallinity, hydrolytic stability, and to enhance solubility in formulations suitable for TED.

In the first instance, target compounds were tested for their ability to inhibit the PCP-mediated cleavage of a fluorogenic substrate and key physicochemical properties were measured. Selected compounds were then screened for selectivity against MMP-2 and, if required, additional MMPs. Finally, preferred compounds were assessed in an in vitro cell-based model of collagen deposition (fibroplasia model; vide infra) and for their ability to penetrate human skin in vitro.

The ability of a compound to penetrate the stratum corneum and epidermis of human skin (flux, $J$; $\mu g/cm^2/h$) is a function of the intrinsic permeability of the compound (IP) and the concentration of the compound in the vehicle ($C_o$; eq 1).[13]

$$J = \mathrm{IP} \times C_o \qquad (1)$$

**Scheme 4.** Synthesis of Compounds **31**−**36**[a]



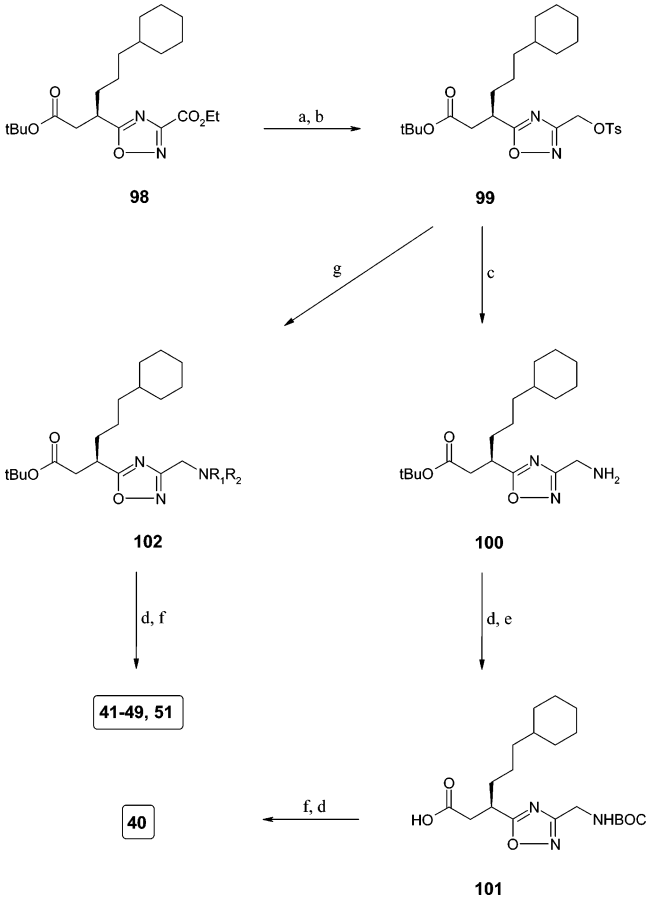

[a] Reagents and conditions: (a) Pd cat., CO, EtOH; (b) TFA; (c) *i*-BuOCOCl or CDI, then $TMSONH_2$, then MeOH; (d) LiOH; (e) $NHR_1R_2$, 90 °C; (f) $MeSO_2Cl$; (g) NaOH.

Intrinsic permeability is related to the physicochemical properties of the molecule, such as molecular size (as measured by molecular weight, (mw)), lipophilicity (Log $D_{7.4}$), H-bond donor/acceptor ability, charge, and degree of ionization (p$K_a$).[14−16] In general, intrinsic permeability will increase with increasing lipophilicity, but will decrease as molecular weight, H-bonding ability, or degree of ionization are increased.[14−16] A second important parameter in defining TED flux is the concentration/solubility of the compound in the vehicle, which is again a function of physicochemical properties of the compound. We opted to try to maximize solubility in both aqueous and organic media so as to ensure flexibility in developing a formulation suitable for topical application and clinical development. Our objective was to seek an agent with TED flux >0.1 $\mu g/cm^2/h$ in vitro, which would compare favorably with values for agents that are known to penetrate skin well in vivo.[14,17]

Hence, the design of inhibitors with suitable physicochemical properties was a key aspect of the program. We were seeking to identify potent PCP-i ($IC_{50}$ < 10 nM) that were crystalline solids (mp > 110 °C) with good aqueous solubility (>50 $\mu g$/mL) and satisfactory aqueous stability under autoclave conditions (121 °C). An inhibitor that met these criteria would compare favorably with amide **1** ($IC_{50}$ 44 nM; mw 324; p$K_a$ 9.0 −CONHO*H*; Log $D_{7.4}$ 2.6; aq. sol. 33 $\mu g$/mL; mp 134−140 °C). Any inhibitor with greatly improved solubility (>1000 $\mu g$/mL) was likely to offer a significant advantage in improving TED flux. The preferred values of other physicochemical parameters, such as molecular weight, lipophilicity, H-bonding ability, charge, or degree of ionization were less clearly defined and, wherever possible, it was our intention to assess their influence experimentally.

The 6-cyclohexyl-*N*-hydroxyhexanamide backbone of this class of PCP inhibitors confers good affinity for PCP and selectivity over MMPs. It also confers quite a burden in terms of lipophilicity and tends to dominate the physicochemical properties of the inhibitors. Our attempts to modify this backbone by introduction of polar groups, rigidifying the many rotatable bonds or downsizing the lipophilic chain, resulted with a significant reduction in affinity for PCP.[10] Hence, as there was scope to further explore the 3-position of the oxadiazole ring, we decided to investigate a wider range of substituents beyond carboxamides. In terms of affinity for PCP, our experience from the carboxamide series had shown that a variety of amide substitutions were tolerated, however, it proved difficult to improve activity below a plateau of $IC_{50}$ <20−40 nM. In terms of physicochemical properties, there proved to be a fine balance between compounds that were crystalline solids and those that were amorphous solids, low melting waxes, or gums. Hence, it was evident that these substituents would need to be quite polar in nature to furnish inhibitors with appropriate physicochemical properties for TED and clinical development.

**Scheme 5.** Synthesis of Compounds **40**−**49** and **51**[a]



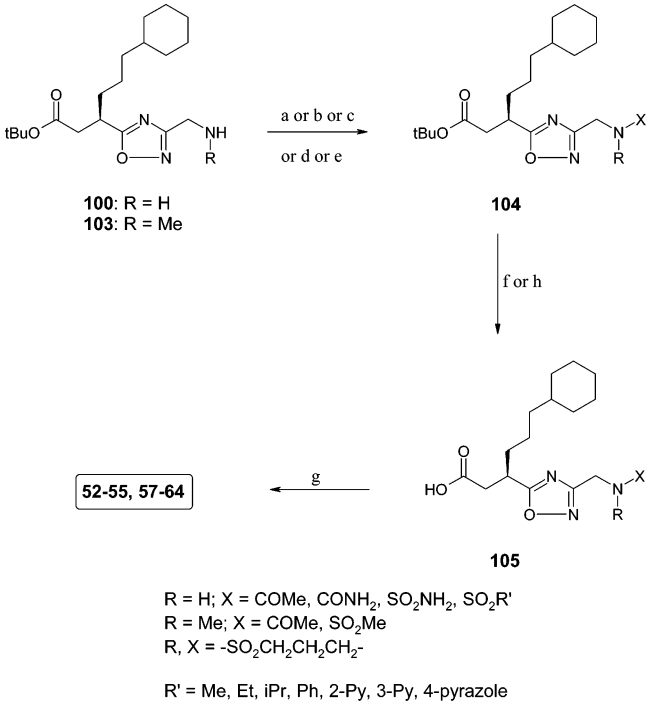

[a] Reagents and conditions: (a) $NaBH_4$; (b) TsCl; (c) $NH_3$, 50 °C; (d) TFA; (e) BOC-on; (f) *i*-BuOCOCl or CDI, then $TMSONH_2$, then MeOH; (g), $NHR_1R_2$, 40 °C.

Our earlier studies with the carboxamides highlighted opportunities for suitable starting points. The incorporation of heterocycles, acidic groups, and basic groups linked through the amide were tolerated, and these classes of compounds tended to have more acceptable physicochemical properties. Glycine derivative **2** ($IC_{50}$ 21 nM; mp 141−142 °C) was one of the more potent compounds from this series, and so, we decided to prepare a series of carboxylic acids and acid surrogates whereby the amide linker was to be replaced by a phenyl ring (Table 1).

The first analogue, compound **7**, demonstrated modest potency for PCP ($IC_{50}$ 116 nM) and potency was further enhanced by the incorporation of a carboxylic acid at either the 4- (**9**) or more preferably the 3-position (**13**, $IC_{50}$ 6 nM). The replacement of the carboxylic acid with a weakly acidic isostere such as sulfonamide **11** ($IC_{50}$ 3 nM; calculated p$K_a$ 9.5 -N*H*$SO_2$Me) was also tolerated. These preliminary results gave encouragement that our objective of significantly increasing activity for PCP inhibition was achievable. However, none of these phenyl derivatives combined sufficient potency against PCP with a suitable solid form. Hence, the next step was to replace the phenyl ring by heterocycles, with the aim of improving solid form by the introduction of more polar groups capable of promoting crystallinity through favorable H-bonding interactions.

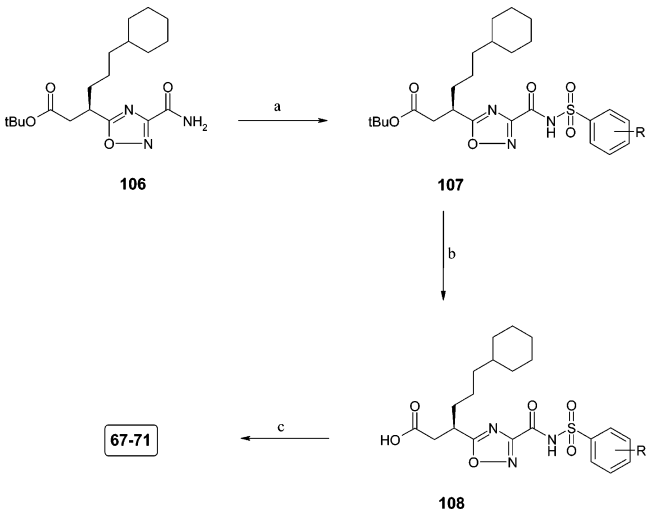
The phenyl ring was successfully replaced with a variety of 6- and 5-ring heterocycles directly attached to the oxadiazole (**14**−**25**, Table 2), with the six-membered rings demonstrating a clear advantage in affinity (e.g., **16**, $IC_{50}$ 2 nM). However,

**Scheme 6.** Synthesis of Compounds **52**−**55** and **57**−**64**[a]




[a] Reagents and conditions: (a) AcCl; (b) O═C(OSuc)$_2$, $NH_3$; (c) $ClSO_2NHtBu$; (d) R′$SO_2Cl$; (e) $Cl(CH_2)_3SO_2Cl$; (f) TFA; (g) *i*-BuOCOCl or CDI, then $TMSONH_2$, then MeOH; (h) NaOMe.

**Scheme 7.** Synthesis of Compounds **67**−**71**[a]




[a] Reagents and conditions: (a) NaH, $RC_6H_4SO_2Cl$; (b) TFA; (c) *i*-BuOCOCl or CDI, then $TMSONH_2$, then MeOH.

most of these heterocyclic derivatives were either noncrystalline or the few examples that were solids had very low aqueous solubility. Uracil **25** had the best balance of improved affinity for PCP and solubility ($IC_{50}$ 10 nM; mw 391; p$K_a$ 7.6 −CON*H*CO−, 9.4 -CONHO*H*; Log $D_{7.4}$ 2.1; aq. sol. 72 $\mu$g/mL; mp 210 °C) when compared to amide **1** and so was selected for further evaluation. The pyridyl analogues **14** and **15** also represented suitable structures for further modification (Table 3).

It had been shown that substitution of a phenyl ring with an appropriately positioned carboxylic acid had resulted in an increase in affinity (cf. **9**, **13** vs **7**) and a similar tactic was applied in the 2- and 3-pyridyl series. The effects with the pyridyl acids (**26** vs **14**; **30** vs **15**) were not so pronounced as with the phenyl acids, although, once again, a *meta*-arrangement

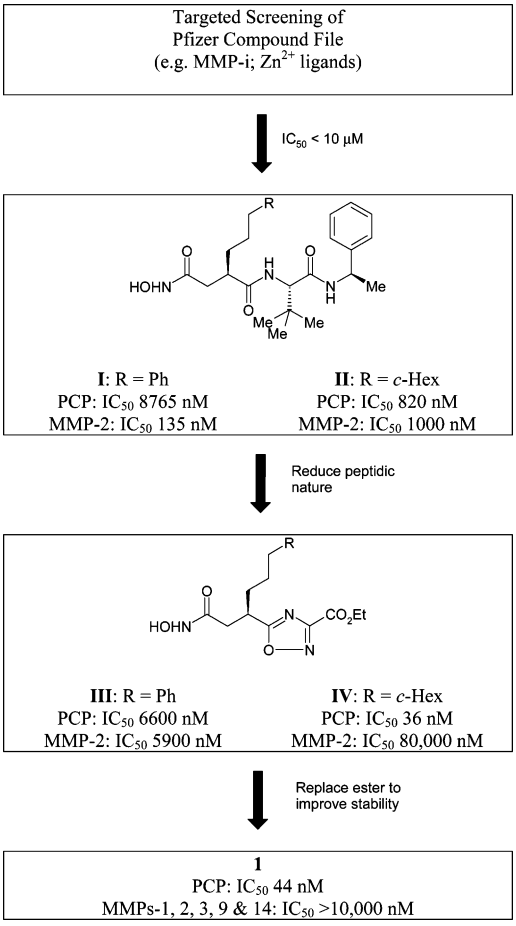



**Figure 1.** Identification of lead series and compound **1**.

between the oxadiazole and acid substituent was preferred (**26**, **30** vs **31**). Pyridyl acid **26** had good affinity for PCP ($IC_{50}$ 6 nM) combined with excellent aqueous solubility (mw 402; p$K_a$ 2.8 −COO*H*, 9.05 −CONHO*H*; Log $D_{7.4}$ 0.6; aq. sol. > 60 000 μg/mL at pH 7.0; mp 117 °C) and so was selected for further evaluation. The conversion of 2-pyridyl acid **26** to a series of amides (**27**−**29**) resulted in a small drop in affinity for PCP. A more beneficial modification was through the introduction of an amino group as was observed with aminopyridines (**32**−**35**). A 6-(1-piperazinyl) group was the preferred substituent in the 3-pyridine series **35** ($IC_{50}$ 8 nM), and this compound demonstrated that a basic center was well tolerated (calculated p$K_a$ 8.4 $R_2N^+$*H*Me). The introduction of a sulfonamide **36** as an acidic isostere was also tolerated in the pyridine series ($IC_{50}$ 27 nM; p$K_a$ 6.5 −N*H*$SO_2$Me, 9.3 −CONHO*H*), but was inferior to the phenyl analogue **11**.

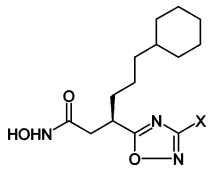
The result that inhibitors containing a basic group were well tolerated prompted further exploration of compounds of this type, because the introduction of a basic amino group at an appropriate position should furnish compounds with improved aqueous solubility. The amine group was attached to the oxadiazole, employing a $-CH_2-$ linker so as to maintain the basic nature of the N by reducing the influence of the electron-withdrawing oxadiazole group and to mimic a similar spatial arrangement of the N atom of the carboxamides (e.g., **1**). The corresponding homologues 3-(2-aminoethyl)oxadiazoles were avoided, as these have been shown to be unstable to rearrangement.[18] The first examples to be prepared were the pyridylmethyl analogues **37**−**39** (Table 4), as directly linked pyridines had been quite potent (e.g., **16**). All three isomers showed good affinity, with **38** having the best balance of potency ($IC_{50}$ 6 nM) with crystallinity (mw 372; p$K_a$ 4.4 −Py*H*$^+$, 9.4 −CONHO*H*; Log $D_{7.4}$ 3.3; aq. sol. 27 μg/mL; mp 112 °C), although no improvement in aqueous solubility was achieved with these weakly basic compounds. The preparation of a series of alkyl amines **40**−**51** demonstrated good affinity, with toleration of a wide variety of groups. The amino group could be substituted with small linear (**41**, **42**), branched (**43**, **44**, **47**), or cyclic (**45**, **46**) alkyl groups. The alkyl groups could be further substituted with an O atom as either alcohols (**47**, **51**) or ethers (**50**). The amines could be secondary (**41**−**47**), tertiary (**48**), or incorporated into a ring (**49**−**51**). Only the primary amine **40** had relatively poor affinity ($IC_{50}$ 122 nM). Amino alcohol **47** had the best balance of affinity for PCP ($IC_{50}$ 8 nM) and physicochemical properties (mw 382; p$K_a$ 6.4 $-CH_2N^+$*H*HR, 9.0 −CONHO*H*; Log $D_{7.4}$ 2.6; aq. sol. 1080 μg/mL at pH 7.1; mp 49 °C as free base) and so was selected for further evaluation. Unfortunately, it was subsequently discovered that all the amines **37**−**51** failed to demonstrate satisfactory aqueous stability under autoclave conditions (121 °C, 15 min, pH 3−6), with the major route of decomposition being hydrolysis of the hydroxamate to the corresponding carboxylic acid. All the other inhibitors disclosed in Tables 1−3, 5, and 6 were quite stable under these conditions.

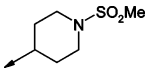
A series of derivatives of the methylamino group were then prepared with the aim of improving hydrolytic stability of this class (Table 5). It has been shown that carboxamides (e.g., **1**) had excellent aqueous stability under these conditions, and so, we thought that increasing the $sp^2$ character of the N atom would confer additional aqueous stability. Initially, a few representative examples were prepared; carboxamides **52** and **53** had good affinity but were not of a suitable solid form, and urea **54** and sulfonyl urea **55** were not of sufficient potency. Only sulfonamide **56** had good activity for PCP inhibition ($IC_{50}$ 11 nM) combined with improved aqueous solubility and a crystalline solid form (mw 388; p$K_a$ 8.9 −CONHO*H*, 10.2 −N*H*$SO_2$Me; Log $D_{7.4}$ 2.3; aq. sol. 130 μg/mL; mp 115 °C). The preparation of a series of sulfonamide analogues by alkylation of the N atom (**57**), variation of the sulfonyl group (**58**−**63**), or tying the sulfonamide into a ring (sultam **64**) failed to identify a compound with a superior profile to **56**. However, modification of the linker between the oxadiazole and the sulfonamide was successful; piperidine sulfonamide **66** was the most potent compound in the sulfonamide series ($IC_{50}$ 3 nM; mw 442; Log $D_{7.4}$ > 2; aq. sol. 21 μg/mL; mp 148 °C) although no improvement in aqueous solubility was achieved. Compounds **56** and **66** demonstrated excellent aqueous stability and were selected for further evaluation.

To further explore the scope around the sulfonamide series, a small set of acyl sulfonamides **67**−**71** were prepared (Table 6). These compounds were designed as hybrids of carboxamide **1** and sulfonamide **60**, with the result that the acyl sulfonamide **67** would incorporate an acidic, ionisable group (−CON*H*$SO_2$-Ph) that should enhance aqueous solubility compared to **1** or **60**. The acyl sulfonamides **67**−**71** had good affinity for PCP and compound **67** was selected for further evaluation ($IC_{50}$ 7 nM; mw 464; p$K_a$ 3.2 −CON*H*$SO_2$Ph, 9.9 −CONHO*H*; Log $D_{7.4}$ 1.0; aq. sol. 3980 μg/mL; mp 149 °C) as a representative lead from this class as it had much improved aqueous solubility.

The program had identified a number of preferred compounds with improved affinity for PCP combined with a satifactory crystalline solid form and increased aqueous solubility; these included neutral compounds (**56**, **66**), acids (**26**, **67**), weakly acidic heterocyle (**25**), weakly basic heterocycle (**38**), and amine

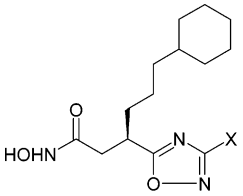
**Table 7.** MMP Selectivity and Efficacy in the Fibroplasia Model



| cmpd | X | PCP $IC_{50}$ (nM) | MMP inhibition[a,b,c] $IC_{50}$ (nM) | fibroplasia model[d] |
|---|---|---|---|---|
| **1** | $CONH_2$ | 44 | MMP-1: >10 000<br>MMP-2: >10 000<br>MMP-3: >10 000<br>MMP-9: >10 000<br>MMP-14: >10 000 | 76 ± 5% I at 10 $\mu$M |
| **25** | 5-uracil | 10 | MMP-2: 79 600<br>MMP-3: 78 000<br>MMP-9: 50 000<br>MMP-13: 49 600<br>MMP-14: >100 000 | 56 ± 12% I at 30 $\mu$M |
| **26** | 4-carboxy-2-pyridine | 6 | MMP-1: >100 000<br>MMP-2: >30 000 | [e] |
| **38** | $CH_2$-3-Py | 6 | MMP-2: >100 000 | 87 ± 11% I at 10 $\mu$M |
| **47** | $CH_2NHC(Me)_2CH_2OH$ | 8 | MMP-1: >100 000<br>MMP-2: >30 000 | 36 ± 8% I at 10 $\mu$M |
| **56** | $CH_2NHSO_2Me$ | 11 | MMP-1: >100 000<br>MMP-2: >100 000<br>MMP-3: 91 000<br>MMP-9: >100 000<br>MMP-13: >100 000<br>MMP-14: >100 000 | 76 ± 2% I at 10 $\mu$M |
| **66** |  | 3 | MMP-2: >100 000 | 88 ± 3% I at 10 $\mu$M |
| **67** | $CONHSO_2Ph$ | 7 | MMP-1: >100 000<br>MMP-2: 92 000<br>MMP-3: 76 000<br>MMP-9: 61 000<br>MMP-13: >100 000<br>MMP-14: >100 000 | 56 ± 11% I at 30 $\mu$M |

[a] <50% I at 10 $\mu$M. [b] <50% I at 100 $\mu$M. [c] <50% I at 30 $\mu$M. [d] Mean ± sem ($n = 3$). [e] Compound **26** failed to consistently demonstrate reduction in collagen deposition.

**Table 8.** In Vitro Penetration through Human Skin in 50% Aqueous Propylene Glycol



| cpmd | X | mw | LogD | p$K_a$[a] | pH | satd solubility, $C_o$ ($\mu$g/mL) | TED flux ($\mu g/cm^2/h$)[b] | TED flux ratio[c] |
|---|---|---|---|---|---|---|---|---|
| **1** | $CONH_2$ | 324 | 2.6 | | 6.5 | 670 | 0.304 | 0.09 |
| **9** | 4-carboxy-phenyl | 401 | | 3.7 | 4.8 | 260 | NFD[d] | |
| **25** | 5-uracil | 391 | 2.1 | 7.6 | 5.0 | 4100 | 0.209 | 0.70 |
| **26** | 4-carboxy-2-pyridine | 402 | 0.6 | 2.8 | 3.8 | 290 | 0.107 | 0.025 |
| | | | | 2.7[e] | 4.8 | 570 | NFD[d] | |
| | | | | | 7.5 | 2970 | NFD[d] | |
| **47** | $CH_2NHC(Me)_2CH_2OH$ | 382 | 2.6 | 6.4 | 6.0 | 33 000 | 1.33 | 1.7 |
| **56** | $CH_2NHSO_2Me$ | 388 | 2.3 | 10.2 | 4.3 | 4560 | 0.028 | 0.14 |
| **66** | $CH(CH_2CH_2)_2NSO_2Me$ | 442 | >2 | | 4.6 | 610 | 0.110 | 0.06 |
| **67** | $CONHSO_2Ph$ | 464 | 1.0 | 3.2 | 5.0 | 3900 | 0.128 | 0.46 |
| **109** | $CONMe_2$ | 352 | 3.6 | | 6.5 | 8260 | 1.40 | 1.0 |

[a] Aqueous p$K_a$ of ionized group X. The p$K_a$ of the hydroxamic acid was 8.9−9.9 (see text for values). [b] Mean of 6−10 experiments. [c] Compared to an internal standard (**109**). The disconnection between TED flux and TED flux ratio is a function of the variability of skin samples (see text). [d] No flux detected. [e] p$K_a$ measured in 50% aqueous propylene glycol.

(**47**). These preferred compounds were then evaluated in MMP selectivity assays (Table 7) in an in vitro cell-based model of collagen deposition (fibroplasia model)[19] (Table 7) and for their ability to penetrate human skin in vitro (Table 8).

Compounds **25**, **26**, **38**, **47**, **56**, **66**, and **67** all demonstrate excellent selectivity over the MMP enzymes with at least 5000-fold selectivity (Table 7). Initial screening was performed with MMP-2 as a representative member of the MMP family because

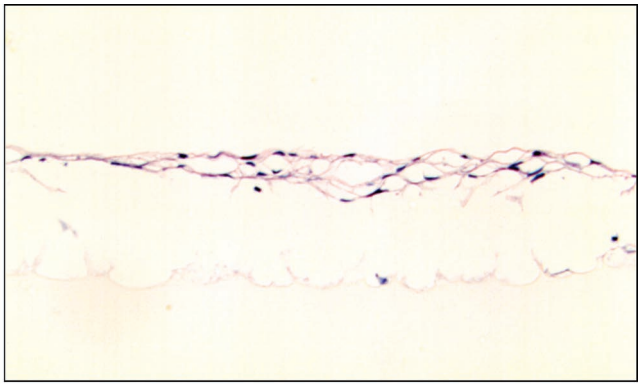
of the key role it plays in the tissue healing process, and then additional profiling of other MMPs was performed as necessary. We believe the principle origin of this selectivity is due to the lipophilic propyl(cyclohexane) side chain that is likely to bind in a deep lipophilic pocket adjacent to the catalytic $Zn^{2+}$ in the active site of PCP. This binding model is supported by hydroxamate inhibititors such as **4** and **5**, which also incorporate a very lipophilic side chain. Compounds related to **5** are selective for PCP over the MMPs[9] but not to the same degree as the compounds listed in Table 7. Compound **56** had a slight advantage in its selectivity profile with >10 000-fold verses MMP-1, -2, -9, -13, and -14 and 9000-fold verses MMP-3.

The fibroplasia model was used to approximate the processes leading to excessive collagen deposition and scar tissue formation in man. This in vitro cell-based model of collagen deposition[19] uses adult dermal fibroblasts cultured in the presence of proline, ascorbate, and TGF-$\beta$1. Under these conditions, the fibroblasts proliferate, stratify, and deposit ECM, including type 1 collagen (Figure 2a,b). The collagen deposition was quantified by HPLC determination of 4-hydroxyproline content of the insoluble culture fraction. These biochemical effects were confirmed by histological analysis and quantification of cell counts and cell viability to confirm that test compounds had no direct effects on the cells other than to inhibit collagen deposition. It should be noted that a correlation between the deposition of collagen in the in vitro fibroplasia model and the process in vivo has not yet been established.

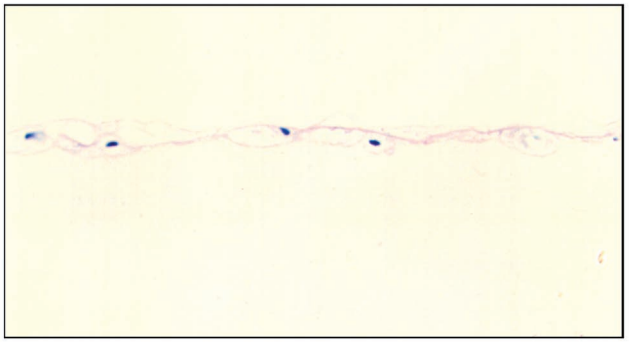
Evaluation of preferred compounds in the fibroplasia model furnished some unexpected results (Table 7). Interestingly, inhibitors that incorporate a partially ionized group (at pH 7.4), whether acidic (e.g., **25**, **26**, **67**) or basic (e.g., **47**), were inferior at inhibiting the deposition of insoluble collagen deposition when compared to neutral inhibitors (e.g., **1**, **38**, **56**, **66**), despite all compounds having excellent affinity for the PCP enzyme. The neutral inhibitors were able to reduce collagen deposition by >75% inhibition at 10 $\mu$M, whereas the ionized inhibitors could only reach a significant level of inhibition (>50%) at a higher concentration of 30 $\mu$M; performance in this model proved to be a deciding factor in compound selection. Compound **56** inhibited deposition of insoluble collagen in a dose-dependent manner and by 76 ± 2% at 10 $\mu$M (mean ± sem; $n = 3$; Figure 2c). In addition, **56** had no effect on cell number in the model, confirming the effect was not due to inhibition of cell proliferation or compound cytotoxicity.

The disconnection between enzyme affinity and efficacy in the fibroplasia model is not fully understood, however, we would suggest that the following two factors are making an important contribution. First, the fibroplasia model is a cell-based system, and so, a significant proportion of the inhibitor concentration will be lost to binding to the cell culture media. Second, as PCP is an extracellular but cell surface bound enzyme, it may reside in a localized lipophilic environment.[20] If the inhibitor binds to the active site in the neutral form to penetrate this environment, ionized compounds will be at a disadvantage. Hence, these two factors could combine to significantly reduce the free concentration of the neutral form of ionized compounds with the results that they are less effective in this model.

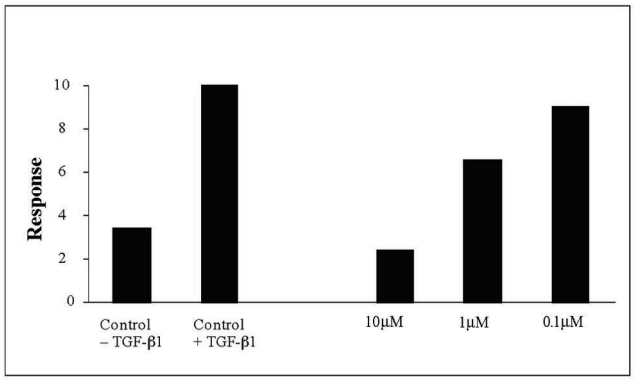
The ability of preferred compounds to penetrate human skin in vitro was measured (Table 8). Human cadaver skin was mounted in a Franz diffusion cell, and a saturated solution of the test compound in 50% aqueous propylene glycol was added to the donor compartment. Samples of receptor fluid were taken at intervals between 0 and 24 h to determine flux. The vehicle also contained [$^{14}$C]-mannitol to test for membrane integrity and



(a) Control with TGF-β1 (day 8).



(b) Control with TGF-β1 plus 10 μM compound **56** (day 8).




(c) Effect of **56** at 0.1, 1.0 and 10 μM compared to control ± TGF-β1.

**Figure 2.** Effect of **56** in human dermal fibroplasia model. (a, b) Histological analysis: images are taken from representative experiments (×40-fold magnification). (c) Compound **56** inhibited deposition of insoluble collagen in a dose-dependent manner and by 76 ± 2% at 10 $\mu$M (mean ± sem; $n = 3$).

an internal standard (**109**) to allow comparison of results. The direct comparison of TED flux results was not appropriate due to the high variability in the nature of human skin (donor, site, and condition), and so, the TED flux ratio standardized to an internal reference was used to assess ability to penetrate skin.

In general, the solubility of the compound in the vehicle was the most important parameter in determining flux (Figure 3). This trend was independent of charge as neutral (**1**, **25**, **56**, **66**, **109**), acidic (**67**), and weakly basic (**47**) compounds demonstrated good TED flux ratios. The ability of **56** to demonstrate improved skin penetration compared to **1** could be attributed to the higher saturated solubility of **56** in the vehicle as they were similar in all other respects. The poor flux of acids **9** and **26** was partly attributed to low solubility in the vehicle although additional factors were involved. The TED flux ratio of **26** was further investigated by performing experiments at higher pHs,

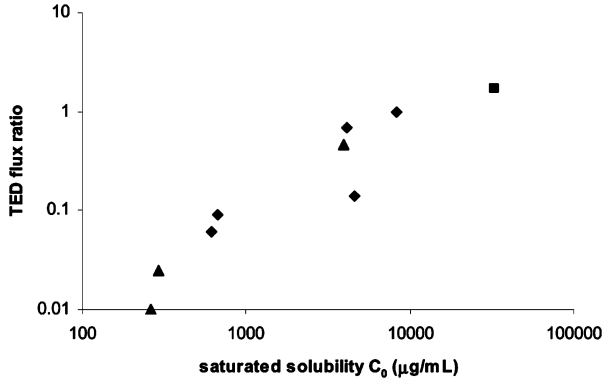



**Figure 3.** Plot of TED flux ratio vs saturated solubility in 50% aqueous propylene glycol. Compound classes were assigned as follows: acids (▲) and bases (■) have >50% ionization at the pH of the experiment, whereas neutrals (♦) are either nonionized or have <50% ionization.

which increased solubility up to 2970 $\mu$g/mL; however, these changes had a detrimental effect on skin penetration. Acid **26** had better flux at lower pH, suggesting increased ionization had an adverse effect. Comparing **26** with **67**, both acids would be 99% ionized at pH 5, and so, the degree of ionization was not the only factor. The failure of **26** to demonstrate reasonable skin penetration was probably related to a combination of poor solubility, degree of ionization, and the high molecular weight of the template because, in general, small acids have satisfactory TED flux (cf nicotinic acid, $J = 2.2$ $\mu$g/cm$^2$/h).[14]

A more detailed analysis of the ability of **56** to penetrate human skin was then performed. Compound **56** had excellent solubility in alternative formulations suitable for TED delivery, which translated to a significant increase in TED flux. When propylene glycol−citrate buffer−ethanol (80:10:10) was employed as the TED vehicle, the saturated solubility increased to 72 000 $\mu$g/mL and the TED flux increased to 1.50 $\mu$g/cm$^2$/h (TED flux ratio 9.19; all measured at pH 4.8).

## Conclusion

From these experiments, **56** emerged as having the superior combination of required properties when compared to the alternative lead compounds. Sulfonamide **56** had excellent PCP enzyme activity when measured with a peptide substrate ($IC_{50}$ 11.3 ± 3.7 nM; calcd $K_i$ 8.7 ± 2.9 nM; mean ± sem; $n = 4$) or with the endogenous substrate procollagen ($IC_{50}$ 3.4 ± 1.0 nM; mean ± sem; $n = 3$) and demonstrates excellent selectivity over the MMPs involved in wound healing (>10 000-fold). We believe the (cyclohexyl)propyl side chain played an important role in confirming activity for PCP and regulating selectivity over the MMPs by binding in a deep lipophilic pocket adjacent to the catalytic $Zn^{2+}$ in the active site of PCP. In the fibroplasia model, **56** inhibited deposition of insoluble collagen by 76 ± 2% at 10 $\mu$M (mean ± sem; $n = 3$) and was superior to the ionized PCP inhibitors **25**, **26**, **47**, and **67**, which were less effective even at higher concentrations; performance in this model proved to be the deciding factor in compound selection. Compound **56** was a crystalline solid with good solubility in both aqueous and organic solvents suitable for TED formulation and had satisfactory aqueous stability under autoclave conditions. Furthermore, **56** was very effective at penetrating human skin in vitro with a TED flux of 1.5 $\mu$g/cm$^2$/h, which compares favorably with values for agents that are known to penetrate skin well in vivo. Based on this profile, **56** (UK-421,045) was selected as a candidate for further preclinical evaluation as a topically applied, dermal antiscarring agent.

## Experimental Section

**Biology. PCP Inhibition.** To determine potency of PCP inhibitors, a fluorogenic PCP cleavage assay was used. This assay is based on the method of Beekman[21] using a fluorogenic substrate. The substrate (Dabcyl-Arg-Tyr−Tyr-Arg-Ala-Asp−Asp-Ala-Asn-Val-Glu(EDANS)-$NH_2$) contains the cleavage site of human PCP.[22] Human PCP was purified from supernatant of stable transfected CHO cells using hydrophobic interaction column followed by Superdex 200 gel filtration.[23] Total protein (4 $\mu$g) of this enzyme preparation was incubated with various concentrations of the substance to be tested and $3 \times 10^{-6}$ M substrate in assay buffer (50 mM Tris-Base, pH 7.6 containing 150 mM NaCl, 5 mM $CaCl_2$, 1 $\mu$M $ZnCl_2$, and 0.01% Brij 35). The assay was performed in 96-well black fluorimeter plates and fluorescence was read continuously in a fluorimeter over 2.5 h ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 485$ nm) at a constant 37 °C with shaking. Release of the fluorogenic signal was in linear correlation to PCP activity. Reading of the mean velocity from 30 min after start of experiment until 2.5 h was calculated by the Biolise software.[24] $IC_{50}$ values were calculated by plotting % inhibition values against compound concentration using Tessela add-in for Excel spreadsheet. The $IC_{50}$ values reported in Tables 1−7 are a mean of at least two experiments, and a difference of <2-fold should not be considered significant. The inhibition constant $K_i$ was calculated from the $IC_{50}$ using the Cheng−Prusoff equation $K_i = IC_{50}/(1 + ([S]/K_m))$, where the $K_m$ of the substrate was 10.29 $\mu$M.

**MMP Inhibition.** The assays for MMPs 1, 2, 3, 9, 13, and 14 have been described previously.[11] In brief, the assays for MMPs 2, 3, 9, and 14 were based upon the original protocol described by Knight et al. (*Fed. Euro. Biochem. Soc.* **1992**, *296* (3), 263−266). Assays for MMPs 2, 3, and 9 used the Nagase substrate (*J. Biol. Chem.* **1994**, *269*, 20952−20957), whereas the assay for MMP-14 (purchased from Prof. Tschesche, Department of Biochemistry, Faculty of Chemistry, University of Bielefeld, Germany) used the substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-$NH_2$ (Bachem Ltd, Essex, U.K.), as described by Will et al. (*J. Biol. Chem.* **1996**, *271* (29), 17119−17123). The assay for MMP-1 inhibition used the substrate Dnp-Pro-$\beta$-cyclohexyl-Ala-Gly-Cys(Me)-His-Ala-Lys(N-Me-Ala)-$NH_2$, as originally described by Bickett et al. (*Anal. Biochem.* **1993**, *212*, 58−64). The assay for MMP-13 inhibition used the substrate Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(NMA)-$NH_2$. Enzyme and substrate concentrations were typically 1 nM and 5−10 $\mu$M, respectively.

**Procollagen Cleavage Assay.** This assay is based on the method of Greenspan and co-workers.[25] PCP activity was assessed by measuring C-terminal peptide cleavage of the natural substrate, procollagen-1. The C-terminal peptide was radioactively labeled on Tryptophan residues, which are only present in this part of procollagen. Human PCP (16 $\mu$g) was incubated with 0.01% bovine serum albumin and $^3$H-procollagen (27 $\mu$g, specific activity 16.9 $\times 10^6$ cpm/mg) in 50 mM Tris, pH 7.4, 150 mM NaCl, 15 mM $CaCl_2$, and 0.02% Brij 35 for 18 h at 37 °C. Procollagen was then precipitated by incubation with 0.01% sirius red at room temperature for 30 min. After centrifugation, the radioactivity in the supernatant was measured by scintillation counting. $IC_{50}$ values were calculated by plotting % inhibition values against compound concentrations using Tessela add-in for Excel spreadsheet.

**Fibroplasia Model.** This assay is based on that of Clark[19a] and utilizes adult human dermal fibroblasts (Clonetics). Cells were seeded at confluency in 24-well plates ($1 \times 10^5$ cells/well/mL) and cultured in Dulbecco's modified eagle's medium (Gibco BRL), supplemented with 10% fetal calf serum (PAA laboratories), 50 IU/mL penicillin, 50 $\mu$g/mL streptomycin (Gibco BRL), 300 $\mu$M proline (Sigma), 50 $\mu$g/mL ascorbate (Sigma), and 3 ng/mL TGF-$\beta_1$(Sigma), at 37 °C and 5% $CO_2$. The inhibitor was added at varying concentrations and medium was changed twice a week. Samples for collagen analysis and histology were taken after 8 days of culture. For collagen analysis, cell pellets were washed twice in water and then processed for hydroxy-4-proline analysis using HPLC.

**In Vitro Transepidermal Penetration Experiments.** The ability of test compounds to penetrate human cadaver skin was assessed using a Microette Transdermal Diffusion Cell autosampling system with static Franz diffusion cells. Experiments were routinely performed at pH 4.3−6.5 to mimic the natural pH of healthy adult skin. The receptor compartments and reservoirs were filled with receptor fluid (degassed phosphate buffered saline, pH 7.4) at 37 ± 1 °C, resulting in a skin surface temperature of 32 ± 1°C. Split thickness human cadaver skin (300−500 μm) was sandwiched between the donor and the receptor compartments, with the stratum corneum side facing the donor compartment. The apparatus was left to equilibrate for 1 h prior to application of a saturated solution of test compound in 50% v/v aqueous propylene glycol (PG; 200 μL). [$^{14}$C]Mannitol (4 μCi/mL) was added to the formulation to test for membrane integrity. 5-{(1*R*)-4-Cyclohexyl-1-[2-(hydroxylamino)-2-oxoethyl]butyl}-1,2,4-oxadiazole-3-carboxamide (**109**), which had been shown to flux well across the skin, was added at a fixed concentration (90% saturated solubility) to the test vehicles to act as internal reference compound to adjust for variability in skin donor or experimental conditions. Samples of receptor fluid from each cell were collected automatically at preprogrammed intervals between 0 and 24 h. The receptor fluid samples removed were replaced with fresh phosphate buffered saline from reservoirs. Concentrations of test compound were measured by LC-MS−MS analysis and membrane integrity was determined by the concentration of $^{14}$C-mannitol in the receptor fluid. Membranes were classified as intact when the percentage mannitol penetrating did not exceed 3% of the total applied dose after 24 h. The flux across skin at steady state (μg/cm$^2$/h) was calculated by taking the gradient of the linear portion of a plot of the cumulative amount of compound against time (15−24 h) and dividing by the exposed surface area of the skin (0.63 cm$^2$). The TED flux values are the mean of 6−10 experiments. The flux ratio was then calculated by dividing the flux of the test compound by the flux of the reference (**109**) over the same time period. Experiments with propylene glycol/citrate buffer/ethanol (80:10:10 gelled with 2.5% Carbopol) as the TED formulation were performed in a similar matter.

**Solubility in Phosphate Buffered Saline (PBS).** The test compound (ca. 1−5 mg) was weighed into a 2 mL eppendorf tube in triplicate and then phosphate buffered saline (400 μL, 0.01M; pH adjusted to pH 6.5) was added. These samples were shaken at room temperature overnight and were then centrifuged at 15 000 rpm for 1 h. The supernatant was transferred to a fresh eppendorf tube and centrifuged again at 15 000 rpm for 1 h. The pH of the final supernatant was recorded and then diluted appropriately in 20/80 acetonitrile/water. Samples were analyzed by HPLC (Luna (Phenomenex) C18, 150 × 4.6 mm, 5 μ pore size; gradient elution 20−90% 0.1% TFA MeCN/0.1% TFA water; flow rate = 1 mL/min; detection $\lambda$ = 220 nm).

**Solubility in TED Formulation.** Sufficient test compound was weighed into 20 mL glass centrifuge tubes to ensure saturated solubility. Aqueous propylene glycol (2 mL, 50% v/v) was added and the tubes rotated on a blood tube rotator (model SB1, Stuart Scientific, U.K.) for 72 h to ensure equilibration. The tubes were inspected after 24 h to ensure an excess of test compound was present. Tubes were centrifuged twice at 4000 rpm for 60 min, and the supernatant was retained for use in penetration experiments. A sample of the supernatant was taken, diluted using acetonitrile−Milli-Q water (20:80) at ratios of 1 in 10, 1 in 100, and 1 in 1000 and then analyzed by HPLC. Experiments with propylene glycol/citrate buffer/ethanol (80:10:10 gelled with 2.5% Carbopol) as the TED formulation were performed in a similar matter.

**Hydrolytic Stability Measurement.** The autoclave solution stability of test compounds was determined between the pH range of 1 to 12.6 in 0.2 M aqueous buffers. The test compound was dissolved at 50 μg/mL in a number of aqueous buffers and autoclaved at 121 °C for 15 min. HPLC analysis was used to examine compound solution stability by comparing the percent main band of test compound remaining with a control sample ($T_0$, stored at 4 °C).

**Chemistry. General Details.** Melting points (mp) were determined using open glass capillary tubes and either Gallenkamp or Electrothermal melting point apparatus and are uncorrected. Proton nuclear magnetic resonance ($^1$H NMR) data were obtained using a Varian Unity 300 or a Varian Inova 400. Low-resolution mass spectral (LRMS) data were recorded on a Fisons Instruments Trio 1000 (thermaspray) or a Finnigan Mat. TSQ 7000 (APCI). The calculated and observed ions quoted refer to the isotopic composition of lowest mass. Elemental combustion analyses (Anal.) were determined by Exeter Analytical U.K. Ltd. Column chromatography was performed using Merck silica gel 60 (0.040−0.063 mm). Log $D_{7.4}$ determinations were similar to those described by Stopher and McClean.[26] p$K_a$s were determined by Sirius Analytical Instruments Ltd (U.K.). When it was not possible to measure ionization constants, values were calculated using Advanced Chemical Development, Inc. (Toronto) software (ACD/p$K_a$ DB).

**General Methods for the Synthesis of PCP Inhibitors 7−71. Preparation of *N*-Hydroxy Amidines 73. (Method A):** To a solution of nitrile **72** (1.0 equiv) in methanol cooled to 0 °C were added hydroxylamine hydrochloride (1.0 equiv) and triethylamine (1.0 equiv), and the mixture was stirred at room temperature (or heated to reflux) overnight. The mixture was cooled in an ice-bath and the solid was collected by filtration, washed with methanol, and dried to give the *N*-hydroxy amidine product **73**.

**Preparation of *N*-Acyloxy Amidines 75. (Method B):** To a solution of (2*R*)-2-(2-*tert*-butoxy-2-oxoethyl)-5-cyclohexylpentanoic acid **74** (1.0 equiv) in dichloromethane (DCM) was added CDI (1.0 equiv), and the reaction was stirred at room temperature overnight. The corresponding *N*-hydroxy amidine **73** (1.0 equiv) was added, and the mixture was stirred at room temperature for 72 h (or heated at reflux for 2 to 8 h). The solvent was removed in vacuo and the residue was dissolved in ethyl acetate and washed with water. The organic layer was dried ($Na_2SO_4$) and the solvent was evaporated. The residue was taken crude onto the next stage.

**Preparation of Oxadiazoles 76. (Method C):** A solution of of *N*-acyloxy amidine **75** (1.0 equiv) in xylene was heated to reflux overnight. The reaction mixture was cooled to room temperature, the solvent was removed in vacuo, and the residue was purified by flash chromatography on silica gel to afford the oxadiazole product **76**.

**Preparation of Succinic Acid 77. (Method D):** To a solution of succinate *t*-butyl ester **76** (1 equiv) in DCM was added TFA in a 1:1 ratio, and the resulting solution was stirred at room temperature for 2 h. The solvent and excess TFA were removed in vacuo, and the residue was azeotroped with toluene to give a solid that was triturated with diisopropyl ether (DIPE). The solid was collected by filtration and dried under high vacuum to give the succinic acid product **77**.

**Preparation of Hydroxamic Acid with *iso*-Butylchloroformate. (Method E):** To a solution of succinic acid **77** (1 equiv) in THF was added triethylamine (2 equiv). The reaction was cooled to 0 °C in an ice bath, then *iso*-butylchloroformate (1 equiv) was added and a precipitate began to form immediately. The mixture was stirred for 1 h, then *O*-trimethylsilyl hydroxylamine (3.2 equiv) was added, and the reaction was warmed to room temperature and stirred overnight. Methanol was added, stirring was continued for 1.5 h, and then the solvent was removed in vacuo. The residue was dissolved in ethyl acetate and washed with water. The organic layer was dried ($MgSO_4$) and the solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel to give the hydroxamic acid product.

**Preparation of Hydroxamic Acid with 1,1-Carbonyldiimidazole. (Method F):** To a solution of succinic acid **77** (1 equiv) in THF was added CDI (1.2 equiv). The mixture was stirred for 1 h, then *O*-trimethylsilyl hydroxylamine (3.2 equiv) was added and the reaction was stirred at room temperature overnight. Methanol was added, stirring was continued for 1 h, and then the solvent was removed in vacuo. The residue was purified by chromatography on silica gel to give the hydroxamic acid product.

Complete experimental procedures, along with spectroscopic and analytical data, for the preparation of all intermediates and target

compounds for the PCP inhibitors **7**−**71** can be found in the Supporting Information.

**Preparation of PCP Inhibitors 1, 25, 26, 47, 56, 66, and 67. 5-{(1*R*)-4-Cyclohexyl-1-[2-(hydroxyamino)-2-oxoethyl]butyl}-1,2,4-oxadiazole-3-carboxamide (1).** The preparation of **1** has been reported previously:[10] mp 136−138 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.80 (2H, m), 1.27−0.99 (8H, m), 1.73−1.46 (7H, m), 2.56−2.39 (2H, m), 3.47 (1H, m), 8.05 (1H, br s), 8.26 (1H, br s), 8.81 (1H, br s), 10.50 (1H, br s); LRMS *m*/*z* 323 ($MH^+$); Anal. ($C_{15}H_{24}N_4O_4\cdot H_2O$) C, H, N.

**(3*R*)-6-Cyclohexyl-3-[3-(2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-1,2,4-oxadiazol-5-yl]-*N*-hydroxyhexanamide (25). *N*′-Hydroxy-2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinecarboximidamide (73: R = 5-Uracil).** A mixture of 5-cyanouracil (2.0 g, 14.6 mmol), hydroxylamine hydrochloride (1.4 g, 20.0 mmol), and triethylamine (2.8 mL, 20.0 mmol) in MeOH (50 mL) was heated at reflux for 18 h. The solid was collected by filtration, washed with MeOH, and dried to give the *N*-hydroxy amidine as a pale yellow solid (1.8 g, 72%). $^1$H NMR (DMSO-$d_6$) $\delta$ 5.71 (2H, br s), 7.62 (1H, s), 11.0−11.4 (1H, br s); LRMS *m*/*z* 193 ($MNa^+$), 171 ($MH^+$).

***tert*-Butyl (3*R*)-6-Cyclohexyl-3-[3-(2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-1,2,4-oxadiazol-5-yl]hexanoate (76: R = 5-Uracil).** To a solution of **74** (0.45 g, 1.5 mmol) in DCM (20 mL) was added 1-hydroxybenzotriazole (0.245 g, 1.8 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.34 g, 1.8 mmol), followed by *N*-hydroxy amidine **73** (R = 5-uracil; 0.305 g, 1.8 mmol). Dimethylformamide (10 mL) was added to aid solubility, and the mixture was stirred at room temperature for 18 h. The solvents were removed by evaporation in vacuo, and the residue was dissolved in water (100 mL) and extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed by evaporation in vacuo to afford **75** (R = 5-uracil). Compound **75** (R = 5-uracil) was suspended in xylene (20 mL) and heated to reflux for 4 h. The reaction mixture was cooled to room temperature, the solvent was removed by evaporation in vacuo, and the residue was purified by chromatography on silica gel using DCM−methanol (95:5) as eluent to afford **76** (R = 5-uracil) as a pale yellow solid (0.60 g, 92%): $^1$H NMR (DMSO-$d_6$) $\delta$ 0.73−0.95 (2H, m), 1.03−1.20 (17H, m), 1.52−1.73 (7H, m), 2.64−2.74 (2H, m), 8.00 (1H, s), 11.0−11.7 (2H, br s); LRMS *m*/*z* 455 ($MNa^+$).

**(3*R*)-6-Cyclohexyl-3-[3-(2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-1,2,4-oxadiazol-5-yl]hexanoic Acid (77: R = 5-Uracil).** To a solution of **76** (R = 5-uracil; 0.58 g, 1.34 mmol) in DCM (20 mL) was added TFA (10 mL), and the solution was stirred at room temperature for 2 h. The solvents were removed by evaporation in vacuo, and the residue was azeotroped with toluene (×3) to afford a brown solid that was triturated with DIPE. The solid was collected by filtration and dried to give **77** (R = 5-uracil) as a white solid (0.40 g, 79%): mp 240−244 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.72−0.89 (2H, m), 1.03−1.17 (8H, m), 1.53−1.72 (7H, m), 2.66−2.81 (2H, m), 3.36−3.47 (1H, m), 8.01 (1H, s), 11.32 (1H, br s), 11.38 (1H, br s), 12.05−12.30 (1H, br s); LRMS *m*/*z* 375 (M − H).

**(3*R*)-6-Cyclohexyl-3-[3-(2,4-dioxo-1,2,3,4-tetrahydro-5-pyrimidinyl)-1,2,4-oxadiazol-5-yl]-*N*-hydroxyhexanamide (25).** *iso*-Butylchloroformate (0.11 mL, 0.9 mmol) was added to a stirred solution of **77** (R = 5-uracil; 0.30 g, 0.8 mmol) and triethylamine (0.225 mL, 1.6 mmol) in THF (10 mL) at 0 °C, and the mixture was stirred for 1 h. *O*-Trimethylsilyl hydroxylamine (0.32 mL, 2.6 mmol) was added, and the mixture was warmed to room temperature and stirred for 2 h. MeOH (10 mL) was then added and the reaction was stirred for 1 h. The solvents were evaporated in vacuo, and the residue was partitioned between aqueous HCl (20 mL, 2 M) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo. The residue was purified by chromatography on silica gel with DCM−methanol−acetic acid (90:10:0.5) as eluent to give **25** as a white solid (0.19 g, 61%): mp 210 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.74−0.89 (2H, m), 1.06−1.24 (8H, m), 1.53−1.69 (7H, m), 2.66−2.81 (2H, m), 3.39−3.47 (1H, m), 8.05 (1H, s), 10.9−11.4 (2H, br s); LRMS *m*/*z* 414 ($MNa^+$); Anal. ($C_{18}H_{24}N_5O_5\cdot H_2O\cdot 0.1CH_2Cl_2\cdot 0.1$AcOH) C, H, N.

**2-(5-{(1*R*)-4-Cyclohexyl-1-[2-(hydroxyamino)-2-oxoethyl]butyl}-1,2,4-oxadiazol-3-yl)isonicotinic Acid (26). Ethyl 2-[Amino(hydroxyimino)methyl]isonicotinate (84).** Compound **84** was obtained as a pale yellow solid (2.8 g) from ethyl 2-cyanoisonicotinate by general method A: $^1$H NMR (DMSO-$d_6$) $\delta$ 1.28 (3H, t), 4.34 (2H, q), 5.87 (2H, s), 7.78 (1H, d), 8.25 (1H, s), 8.70 (1H, d), 10.03 (1H, s); LRMS *m*/*z* 210 ($MH^+$).

**Ethyl 2-{5-[(1*R*)-1-(2-*tert*-Butoxy-2-oxoethyl)-4-cyclohexylbutyl]-1,2,4-oxadiazol-3-yl}isonicotinate (85).** Compound **85** was obtained as a colorless oil (18.9 g) from compounds **74** and **84** by general methods B then C: $^1$H NMR ($CDCl_3$) $\delta$ 0.80−0.92 (2H, m), 1.08−1.48 (20H, m), 1.57−1.71 (5H, m), 1.71−1.94 (2H, m), 2.69 (1H, dd), 2.92 (1H, dd), 3.53−3.61 (1H, m), 4.46 (2H, q) 7.95 (1H, d), 8.64 (1H, s), 9.92 (1H, d); LRMS *m*/*z* 494 ($MNa^+$), 472 ($MH^+$); Anal. ($C_{26}H_{37}N_3O_5\cdot$EtOAc) C, H, N.

**(3*R*)-6-Cyclohexyl-3-{3-[4-(ethoxycarbonyl)-2-pyridinyl]-1,2,4-oxadiazol-5-yl}hexanoic Acid (77: R = 4-Ethoxycarbonyl-2-pyridine).** (3*R*)-6-Cyclohexyl-3-{3-[4-(ethoxycarbonyl)-2-pyridinyl]-1,2,4-oxadiazol-5-yl}hexanoic acid was obtained as a pale yellow oil (4.6 g) by general method D: $^1$H NMR ($CDCl_3$) $\delta$ 0.74−0.87 (2H, m), 1.04−1.38 (8H, m), 1.41 (3H, t), 1.55−1.70 (5H, m), 1.70−1.91 (2H, m), 2.83 (1H, dd), 3.06 (1H, dd), 3.53−3.62 (1H, m), 4.43 (2H, q), 8.04 (1H, d), 8.67 (1H, s), 8.90 (1H, d); LRMS *m*/*z* 416 ($MH^+$).

**Ethyl 2-(5-{(1*R*)-4-Cyclohexyl-1-[2-(hydroxyamino)-2-oxoethyl]butyl}-1,2,4-oxadiazol-3-yl)isonicotinate.** Ethyl 2-(5-{(1*R*)-4-cyclohexyl-1-[2-(hydroxyamino)-2-oxoethyl]butyl}-1,2,4-oxadiazol-3-yl)isonicotinate was obtained as a colorless oil (0.44 g) by general method E: $^1$H NMR ($CDCl_3$) $\delta$ 0.75−0.89 (2H, m), 1.11−1.40 (8H, m), 1.45 (3H, t), 1.53−1.97 (7H, m), 2.68 (1H, dd), 2.84 (1H, dd), 3.67−3.79 (1H, m), 4.48 (2H, q), 7.98 (1H, d), 8.61 (1H, s), 8.90 (1H, d); LRMS *m*/*z* 453 ($MNa^+$), 431 ($MH^+$).

**2-(5-{(1*R*)-4-Cyclohexyl-1-[2-(hydroxyamino)-2-oxoethyl]butyl}-1,2,4-oxadiazol-3-yl)isonicotinic acid (26).** To a solution of ethyl 2-(5-{(1*R*)-4-cyclohexyl-1-[2-(hydroxyamino)-2-oxoethyl]butyl}-1,2,4-oxadiazol-3-yl)isonicotinate (0.44 g, 1.0 mmol) in MeOH (20 mL) was added a solution of LiOH (0.09 g, 2.0 mmol) in water (7 mL), and the reaction was stirred at room temperature for 2 h. The solvent was removed by evaporation in vacuo, and the residue was partioned between water (20 mL) and DCM (20 mL). The aqueous layer was separated, acidified to pH 4 with aqueous HCl (2 M), and then extracted with DCM (2 × 20 mL). The combined organic extracts were dried ($MgSO_4$), and the solvent was removed in vacuo to give **26** as a white solid (0.04 g): mp 117−122 °C; $^1$H NMR ($CD_3OD$) $\delta$ 0.80−0.93 (2H, m), 1.06−1.47 (8H, m), 1.53−1.75 (5H, m), 1.75−1.93 (2H, m), 2.62 (1H, dd), 2.74 (1H, dd), 3.62−3.73 (1H, m), 8.07 (1H, d), 8.62 (1H, s), 8.89 (1H, d); LRMS *m*/*z* 403 ($MH^+$).

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-(3-{[(2-hydroxy-1,1-dimethylethyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanamide (47). *tert*-Butyl (3*R*)-6-Cyclohexyl-3-(3-{[(2-hydroxy-1,1-dimethylethyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanoate (76: R = $CH_2NHC(Me)_2CH_2OH$).** A solution of tosylate **99** (3.71 g, 7.32 mmol) and 2-amino-2-methyl-1-propanol (2.10 mL, 22.0 mmol) in THF (8 mL) was heated at 40 °C in a sealed vessel for 2 h. The reaction mixture was cooled to room temperature and partioned between EtOAc (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The organic layer was washed with saturated brine (50 mL) and dried ($MgSO_4$), and the solvents were evaporated in vacuo to afford **76** (R = $CH_2NHC(Me)_2CH_2OH$) as a colorless oil (2.52 g, 81%): $^1$H NMR ($CDCl_3$) $\delta$ 0.84 (2H, m), 1.10 (6H, s), 1.15−1.35 (9H, m), 1.40 (9H, s), 1.60−1.80 (6H, m), 2.60 (1H, dd), 2.70 (1H, br s), 2.79 (1H, dd), 3.29 (2H, s), 3.42 (1H, m), 3.83 (2H, s); LRMS *m*/*z* 424 ($MH^+$).

**(3*R*)-6-Cyclohexyl-3-(3-{[(2-hydroxy-1,1-dimethylethyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanoic Acid (77: R = $CH_2NHC(Me)_2CH_2OH$).** (3*R*)-6-Cyclohexyl-3-(3-{[(2-hydroxy-1,1-dimethylethyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanoic acid was obtained as a colorless oil (2.95 g) by general method D: $^1$H NMR ($CDCl_3$) $\delta$ 0.82 (2H, m), 1.10−1.30 (8H, m), 1.39 (6H, d), 1.60−

1.80 (7H, m), 2.78 (1H, dd), 2.92 (1H, dd), 3.42 (1H, m), 3.63 (2H, s), 4.24 (2H, m); LRMS *m*/*z* 368 ($MH^+$).

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-(3-{[(2-hydroxy-1,1-dimethylethyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanamide (47).** Compound **47** was obtained as a white foam (980 mg, 52%) by general method E: mp 49−51 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.80 (2H, m), 0.98 (6H, s), 1.05−1.25 (8H, m), 1.50−1.70 (7H, m), 2.40 (2H, m), 3.20 (2H, m), 3.40 (1H, m), 3.73 (2H, s), 4.43 (1H, br s), 8.62 (1H, br s), 10.38 (1H, br s); LRMS *m*/*z* 383 ($MH^+$); Anal. ($C_{19}H_{34}N_4O_4 \cdot 0.05H_2O$) C, H, N.

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-(3-{[(methylsulfonyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanamide (56). (1*Z*)-*N*′-Hydroxy-2-[(methylsulfonyl)amino]ethanimidamide (73: R = $CH_2NHSO_2Me$).** A solution of *N*-(cyanomethyl)methanesulfonamide (10.85 g, 81.0 mmol) in EtOH (370 mL) was treated with hydroxylamine hydrochloride (5.63 g, 81.0 mmol) followed by a solution of NaOH (3.24 g, 81.0 mmol) in water (125 mL), and the mixture was stirred at room temperature for 20 h. All volatile solvents were removed by evaporation under reduced pressure. The residue was extracted with hot EtOH (×3) and decanted from the solid residues (NaCl). The combined organic extracts were cooled, which gave a precipitate. The solid was collected by filtration, washed with $Et_2O$, and dried to afford the *N*-hydroxy amidine as white crystals (9.77 g, 72%): $^1$H NMR (DMSO-$d_6$) $\delta$ 2.87 (3H, s), 3.49 (2H, s), 5.22 (2H, br s), 7.09 (1H, br s), 9.00 (1H, s); LRMS *m*/*z* 190 ($MNa^+$); Anal. ($C_3H_9N_3O_3S \cdot 0.1H_2O$) C, H, N.

***tert*-Butyl (3*R*)-6-Cyclohexyl-3-(3-{[(methylsulfonyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanoate (76: R = $CH_2NHSO_2Me$).** Sodium (2*R*)-2-(2-*tert*-butoxy-2-oxoethyl)-5-cyclohexylpentanoate (i.e., **74** sodium salt; 18.68 g, 58.4 mmol) was partitioned between 10% aqueous citric acid solution (190 mL) and EtOAc (190 mL). The organic layer was washed with brine, dried ($MgSO_4$), and filtered, and the solvents were removed under reduced pressure to afford the acid **74** (quant) as a colorless oil. A solution of **74** in DCM (185 mL) was treated CDI (9.46 g, 58.4 mmol), and the reaction mixture was stirred at room temperature for 1.5 h. *N*-Hydroxy amidine **73** (R = $CH_2NHSO_2Me$; 9.75 g, 58.4 mmol) was added portionwise, but required the addition of DMF (50 mL) to dissolve the solid completely. The reaction mixture was stirred at room temperature for 18 h. The solvents were removed by evaporation under reduced pressure. The residue was dissolved on EtOAc (500 mL), washed with water (2 × 500 mL) and brine (200 mL), and dried ($MgSO_4$), and the solvent was removed by evaporation under reduced pressure to afford compound **75** (R = $CH_2NHSO_2Me$) as a white solid (26.3 g, ∼100%): $^1$H NMR (DMSO-$d_6$) $\delta$ 0.81 (2H, m), 1.00−1.30 (9H, m), 1.37 (9H, s), 1.40−1.65 (6H, m), 2.39 (1H, dd), 2.55 (1H, dd), 2.79 (1H, m), 2.94 (3H, s), 3.62 (2H, s), 6.19 (2H, br s), 7.30 (1H, br s); LRMS: *m*/*z* 470 ($MNa^+$). Compound **75** (R = $CH_2NHSO_2Me$; 26.0 g, 58.2 mmol) was dissolved in xylene (500 mL) and heated at 130 °C under a nitrogen atmosphere for 20 h. The solvents were evaporated in vacuo, and the crude reaction mixture was purified by column chromatography on silica gel using pentane then a gradient of DCM−MeOH (100:0 to 90:10) as eluent. Appropriate fractions were combined, and the solvent was removed under reduced pressure to give a brown residue that was then triturated with pentane to afford the oxadiazole **76** (R = $CH_2NHSO_2Me$) as a white solid (9.43 g, 38%): $^1$H NMR ($CD_3OD$) $\delta$ 0.83 (2H, m), 1.10−1.30 (9H, m), 1.38 (9H, s), 1.60−1.75 (7H, m), 2.67 (1H, dd), 2.79 (1H, dd), 2.98 (3H, s), 3.42 (1H, m), 4.39 (2H, s); LRMS *m*/*z* 452 ($MNa^+$).

**(3*R*)-6-Cyclohexyl-3-(3-{[(methylsulfonyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanoic Acid (77: R = $CH_2NHSO_2Me$).** A solution of **76** (R = $CH_2NHSO_2Me$; 9.36 g, 21.8 mmol) in toluene (100 mL) was treated with TFA (50 mL) and stirred under a nitrogen atmosphere at room temperature for 20 h. The solvent was removed under reduced pressure and azeotroped with EtOAc. The residue was dissolved in EtOAc (250 mL) and washed with $H_2O$ followed by brine. The organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was triturated with $Et_2O$ to yield a solid that was collected by filtration and dried to afford acid **77** (R = $CH_2NHSO_2Me$) as an off-white solid (7.06 g, 87%): $^1$H NMR ($CD_3OD$) $\delta$ 0.83 (2H, m), 1.10−1.35 (8H, m), 1.60−1.80 (7H, m), 2.75 (1H, dd), 2.85 (1H, dd), 2.97 (3H, s), 3.48 (1H, m), 4.39 (2H, s); LRMS *m*/*z* 396 ($MNa^+$); Anal. ($C_{16}H_{27}N_3O_5S$) C, H, N.

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-(3-{[(methylsulfonyl)amino]methyl}-1,2,4-oxadiazol-5-yl)hexanamide (56).** A solution of **77** (R = $CH_2NHSO_2Me$; 6.80 g, 18.2 mmol) and 2,6-lutidine (2.3 mL, 20 mmol) in THF (100 mL) at 0 °C under a nitrogen atmosphere was treated with *iso*-butyl chloroformate (2.6 mL, 20 mmol) and stirred at 0 °C for 1.5 h. *O*-(Trimethylsilyl)hydroxylamine (4.9 mL, 40.0 mmol) was added, and the reaction mixture was stirred for 18 h, warming to room temperature over this period of time. MeOH was then added and the reaction mixture was stirred for an additional 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in EtOAc (400 mL). The organic solution was washed with dilute HCl (350 mL, 1 M) and brine (200 mL), dried ($MgSO_4$), and then evaporated in vacuo. The solid residue was recrystallized from hot EtOAc to afford hydroxamic acid **56** as a white solid (6.19 g, 88%): $[\alpha]^{25}_D$ +18.5 (*c* 2.0, MeOH); mp 115−116 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.80 (2H, m), 1.00−1.15 (8H, m), 1.50−1.70 (8H, m), 2.50−2.55 (2H, obs), 2.89 (3H, s), 3.41 (1H, m), 4.24 (2H, d), 7.63 (1H, br s), 8.62 (1H, br s), 10.38 (1H, br s); LRMS *m*/*z* 411 ($MNa^+$); Anal. ($C_{16}H_{28}N_4O_5S$) C, H, N.

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-{3-[1-(methylsulfonyl)-4-piperidinyl]-1,2,4-oxadiazol-5-yl}hexanamide (66). 1-(Methylsulfonyl)-4-piperidinecarbonitrile (72: R = 1-(Methylsulfonyl)-4-piperidinyl).** 1-(Methylsulfonyl)-4-piperidinecarbonitrile was obtained as a white solid (2.48 g, 42%; $Et_2O$): $^1$H NMR ($CD_3OD$) $\delta$ 1.85 (2H, m), 2.01 (2H, m), 2.81 (3H, s), 2.95 (1H, m), 3.18 (2H, m), 3.41 (2H, m); LRMS *m*/*z* 211 ($MNa^+$); Anal. ($C_7H_{12}N_2O_2S$) C, H, N.

***N*′-Hydroxy-1-(methylsulfonyl)-4-piperidinecarboximidamide (73: R = 1-(Methylsulfonyl)-4-piperidinyl).** *N*′-Hydroxy-1-(methylsulfonyl)-4-piperidinecarboximidamide was obtained as a white solid by general method A and was used in the next step without further purification.

***tert*-Butyl(3*R*)-3-{[({(*Z*)-amino[1-(methylsulfonyl)-4-piperidinyl]methylidene}amino)oxy]carbonyl}-6-cyclohexylhexanoate (75: R = 1-(Methylsulfonyl)-4-piperidinyl).** *tert*-Butyl (3*R*)-3-{[({(*Z*)-amino[1-(methylsulfonyl)-4-piperidinyl]methylidene}amino)oxy]-carbonyl}-6-cyclohexylhexanoate (1.50 g, 1.83 mmol) was prepared from **74** (0.55 g, 1.83 mmol) and **73** (R = 1-(methylsulfonyl)-4-piperidinyl); 1.20 g, 2.0 mmol) by general method B.

***tert*-Butyl (3*R*)-6-Cyclohexyl-3-{3-[1-(methylsulfonyl)-4-piperidinyl]-1,2,4-oxadiazol-5-yl}hexanoate (76: R = 1-(Methylsulfonyl)-4-piperidinyl).** A solution of **75** (R = 1-(methylsulfonyl)-4-piperidinyl; 1.50 g, 1.83 mmol) in toluene (100 mL) was treated with pyridine (90 $\mu$L, 1.83 mmol) and anhydrous $ZnCl_2$ (150 mg, 1.83 mmol), and the mixture was heated at reflux for 20 h. The cooled reaction mixture was diluted with EtOAc (100 mL) and washed with water (100 mL) and saturated brine (100 mL). The organic layer was dried ($Na_2SO_4$) and the solvent was evaporated in vacuo. The residue was purified by chromatography on silica gel using cyclohexane−EtOAc (90:10 to 50:50) as eluent to give **76** (R = 1-(methylsulfonyl)-4-piperidinyl) as an oil, which was recrystallization from pentane as a white solid (320 mg, 36%): $^1$H NMR ($CDCl_3$) $\delta$ 0.83 (2H, m), 1.10−1.35 (8H, m), 1.40 (9H, s), 1.60−1.80 (7H, m), 1.99 (2H, m), 2.14 (2H, br d), 2.60 (1H, dd), 2.71−2.80 (4H, dd and s), 2.95 (3H, m), 3.41 (1H, m), 3.74 (2H, m); LRMS *m*/*z* 506 ($MNa^+$); Anal. ($C_{24}H_{41}N_3O_5S$) C, H, N.

**(3*R*)-6-Cyclohexyl-3-{3-[1-(methylsulfonyl)-4-piperidinyl]-1,2,4-oxadiazol-5-yl}hexanoic Acid (77: R = 1-(Methylsulfonyl)-4-piperidinyl).** (3*R*)-6-Cyclohexyl-3-{3-[1-(methylsulfonyl)-4-piperidinyl]-1,2,4-oxadiazol-5-yl}hexanoic acid was obtained as a white solid (245 mg, 89%; triturated with pentane) by general method D: $^1$H NMR ($CDCl_3$) $\delta$ 0.81 (2H, m), 1.05−1.35 (8H, m), 1.55−1.85 (7H, m), 1.97 (2H, m), 2.10 (2H, m), 2.70−2.80 (4H, dd and s), 2.93 (4H, m), 3.45 (1H, m), 3.72 (2H, m); LRMS *m*/*z* 426 (M − H); Anal. ($C_{20}H_{33}N_3O_5S$) C, H, N.

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-{3-[1-(methylsulfonyl)-4-piperidinyl]-1,2,4-oxadiazol-5-yl}hexanamide (66).** (3*R*)-6-Cyclohexyl-*N*-hydroxy-3-{3-[1-(methylsulfonyl)-4-piperidinyl]-1,2,4-oxadiazol-5-yl}hexanamide was obtained as fluffy white solid (204 mg, 85%; triturated with DIPE) by general method E: $^1$H NMR ($CD_3OD$) $\delta$ 0.83 (2H, m), 1.05−1.35 (8H, m), 1.55−1.75 (7H, m), 1.88 (2H, m), 2.09 (2H, m), 2.48 (1H, dd), 2.60 (1H, dd), 2.82 (3H, s), 2.98 (3H, m), 3.52 (1H, m), 3.70 (2H, m); LRMS *m*/*z* 465 ($MNa^+$); Anal. ($C_{20}H_{34}N_4O_5S$) C, H, N.

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-(3-{[(phenylsulfonyl)amino]carbonyl}-1,2,4-oxadiazol-5-yl)hexanamide (67). *tert*-Butyl (3*R*)-6-Cyclohexyl-3-(3-{[(phenylsulfonyl)amino]carbonyl}-1,2,4-oxadiazol-5-yl)hexanoate (107: R = H).** To a suspension of sodium hydride (0.65 g of 60% dispersion in mineral oil, 16.4 mmol) in THF (80 mL) at 0 °C was added dropwise a solution of **106** (3.0 g, 8.2 mmol) and freshly distilled benzenesulfonyl chloride (1.15 mL, 9.0 mmol) in THF (20 mL). The reaction was stirred at 0 °C for 3 h then quenched with aqueous HCl (2 M) and warmed to room temperature. The mixture was diluted with water (50 mL) and then extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried ($Na_2SO_4$), and the solvent was removed in vacuo. The residue was purified by chromatography on a Biotage 405 cartridge with DCM−methanol (100:0 then 95:5) as eluent to give **107** (R = H) as a clear oil (2.8 g, 67%): $^1$H NMR ($CDCl_3$) $\delta$ 0.78−0.91 (2H, m), 1.11−1.55 (18H, m), 1.55−1.79 (6H, m), 2.65 (1H, dd), 2.80 (1H, dd), 3.44−3.52 (1H, m), 7.56 (2H, dd), 7.66 (1H, dd), 8.17 (2H, d); LRMS *m*/*z* 504 (M − H); Anal. ($C_{25}H_{35}N_3O_6S\cdot 0.5H_2O$) C, H, N.

**(3*R*)-6-Cyclohexyl-3-(3-{[(phenylsulfonyl)amino]carbonyl}-1,2,4-oxadiazol-5-yl)hexanoic Acid (108: R = H).** A solution of **107** (R = H; 2.65 g, 1.0 mmol) and TFA (6 mL) in DCM (30 mL) was stirred at room temperature for 2 h. A further portion of TFA (1 mL) was added and stirring was continued for an additional 1 h. The solvents were evaporated in vacuo, and the residue was azeotroped with toluene (×3) to give **108** (R = H) as an oil (2.28 g): $^1$H NMR (DMSO-$d_6$) $\delta$ 0.72−0.85 (2H, m), 1.01−1.26 (8H, m), 1.52−1.69 (7H, m), 2.67−2.81 (2H, m), 3.39−3.50 (1H, m), 7.56−7.68 (2H, m), 7.68−7.76 (1H, m), 7.97 (2H, d); LRMS *m*/*z* 448 (M − H).

**(3*R*)-6-Cyclohexyl-*N*-hydroxy-3-(3-{[(phenylsulfonyl)amino]carbonyl}-1,2,4-oxadiazol-5-yl)hexanamide (67).** To a solution of **108** (R = H; 2.24 g, 5.0 mmol) in THF (40 mL) was added 2,6-lutidine (1.07 g, 10.0 mmol), the solution was cooled to 0 °C, and then *iso*-butylchloroformate (0.66 mL, 5.05 mmol) was added. The reaction was stirred at 0 °C for 1 h, then *O*-trimethylsilyl hydroxylamine (1.83 mL, 15.0 mmol) was added, and the reaction was warmed to room temperature and stirred overnight. Methanol (25 mL) was added, and stirring was continued for 30 min. The solvent was evaporated in vacuo, and the residue was partitioned between ethyl acetate (75 mL) and aqueous HCl (35 mL, 2 M). The aqueous layer was extracted with ethyl acetate (2 × 60 mL), and the combined organic extracts were then dried ($Na_2SO_4$) and the solvent was removed in vacuo. The residue was purified by chromatography on a Biotage CP-18 reverse phase 40S cartridge with acetonitrile−water (30:70 to 50:50) as eluent to afford **67** as a foam. Recrystallization from toluene gave **67** as a white crystalline solid (1.0 g, 51%): mp 149 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.74−0.89 (2H, m), 1.06−1.24 (8H, m), 1.53−1.69 (7H, m), 2.66−2.81 (2H, m), 3.39−3.47 (1H, m), 8.05 (1H, s), 10.9−11.4 (2H, br s); LRMS *m*/*z* 414 ($MNa^+$); Anal. Calcd. for $C_{18}H_{24}N_5O_5\cdot H_2O\cdot 0.1CH_2Cl_2\cdot 0.1AcOH$) C, H, N.

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**Supporting Information Available:** Complete experimental details, along with spectroscopic and analytical data, for the preparation of all intermediates and target compounds for the PCP inhibitors **7**−**71**. This material is available free of charge via the Internet at http://pubs.acs.org.

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