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# A NOVEL SERIES OF MATRIX METALLOPROTEINASE INHIBITORS FOR THE TREATMENT OF INFLAMMATORY DISORDERS

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**Abstract:** The preparation of a novel series of matrix metalloproteinase inhibitors is described, based on the use of a mercaptoacyl zinc binding moiety. The compounds have been tested in a model of rheumatoid arthritis and show good oral activity.


The matrix metalloproteinases (MMPs) are a large and expanding family of zinc dependant endopeptidases that have attracted much attention as targets for drug discovery over the last decade.[1] In part, this interest is spurred by the central role of these enzymes in the degradation and remodelling of the extracellular matrix.[1,2] The enzymes are divided into four main groups:[3]

| | | |
|---|---|---|
| *collagenases* | interstitial collagenase | MMP-1 |
| | polymorphonuclear neutrophil (PMN) collagenase | MMP-8 |
| | collagenase-3 | MMP-13 |
| *gelatinases* | gelatinase A, 72 kDa-gelatinase, type IV collagenase | MMP-2 |
| | gelatinase B, 92 kDa-gelatinase | MMP-9 |
| *stromelysins* | stromelysin-1, transin, proteoglycanase | MMP-3 |
| | stromelysin-2 | MMP-10 |
| | stromelysin-3 | MMP-11 |
| *membrane-type matrix metalloproteinases* | | |
| | MT-1 | MMP-14 |
| | MT-2 | MMP-15 |
| | MT-3 | MMP-16 |
| | MT-4 | MMP-17 |

The discovery that the MMP enzymes are over-expressed in many pathological conditions has led to the belief that inhibitors of the MMPs could be useful for the treatment of a range of inflammatory disorders for which there is an unmet therapeutic need.[4,5] Thus, inhibitors of MMPs could be useful in the treatment of rheumatoid arthritis, osteoarthritis, Crohn's disease, ulcerative colitis, peridonitis, gingivitis, psoriasis, dermatitis, Alzheimer's disease and multiple sclerosis as well as in the treatment of cardiovascular disorders and cancer.

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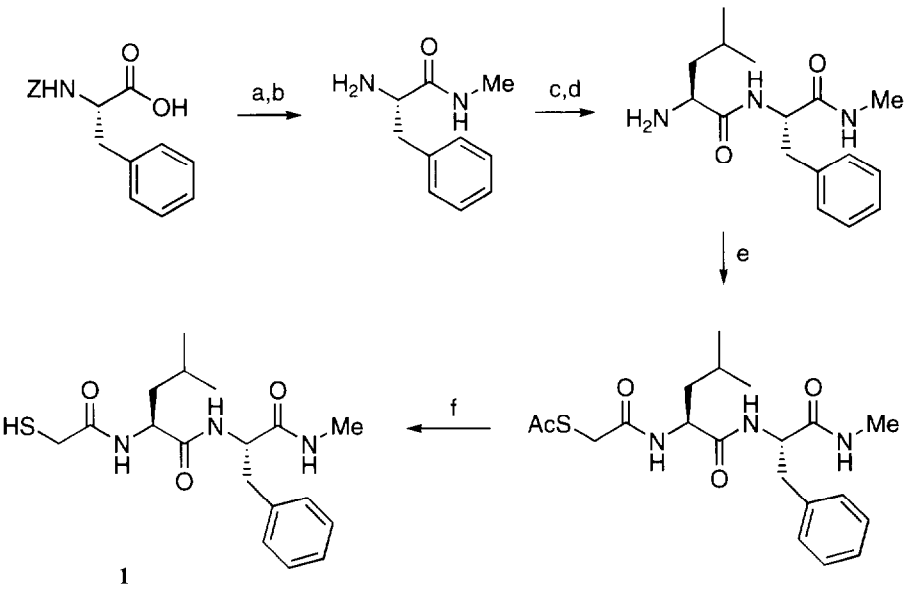
Many of the synthetic inhibitors of MMPs explored in detail to date have been substrate-based inhibitors containing a hydroxamic acid as the zinc binding group, with enzyme recognition being provided by $P_1'$, $P_2'$, and in some cases $P_3'$ interactions.[5] Until recently, compounds of this class have shown poor oral activity in animal models, with low bioavailability and poor physico-chemical properties. Alternative zinc binding groups such as phosphinates, aminocarboxylates, and thiol based ligands have, in general, demonstrated reduced potency against the MMP enzymes while still failing to address these underlying problems.

In this paper we describe our work on a novel series of MMP inhibitors based on the mercaptoacyl zinc binding group.[6] The synthesis of the lead we identified, compound **1**, is shown in **Scheme 1**.[7]

**Scheme 1** The Synthesis of Compound **1**

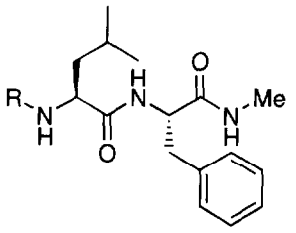



**1**

**Reagents**: (Z = $PhCH_2OCO$-) (a) i. DCC, *N*-hydroxysuccinimide, THF ii. $MeNH_2$, $H_2O$, THF (b) $H_2$, Pd–C (c) Z-Leu-OH, DCC, 1-hydroxybenzotriazole, THF (d) $H_2$, Pd–C (e) $AcSCH_2CO_2H$, EDAC, 1-hydroxybenzotriazole, THF (f) $NH_4OH$, $H_2O$, MeOH

Compound **1** is a modestly active, broad spectrum inhibitor of the MMP enzymes, as shown by its *in vitro* potency (**Table 1**).[8] We explored the requirements of the zinc binding group, and it was discovered that the introduction of a metabolically stable sulfur substituent such as in the *S*-methyl derivative **2** was detrimental to activity. The position of the thiol relative to the amide group was also explored, and the chain extended derivative **3** demonstrated that further separation of the thiol and carbonyl group also reduced potency. We also evaluated the amide **4**, which was inactive, demonstrating that the thiol group is essential for activity (**Table 1**).

**Table 1** Activities of Compounds Exploring Zinc Interaction[8]



| Compound | R | $IC_{50}$ (μM) MMP-3 | MMP-8 | MMP-9 |
|---|---|---|---|---|
| **1** | $HSCH_2CO$ | 2.6 | 0.05 | 0.09 |
| **2** | $MeSCH_2CO$ | >100 | 2.6 | 4.6 |
| **3** | $HSCH_2CH_2CO$ | >100 | 5.1 | 9.6 |
| **4** | $MeCH_2CO$ | >100 | >100 | 30 |

It is postulated that zinc binding is achieved in this series *via* a bidentate interaction involving both the amide carbonyl group and the thiol. Compound **1** was docked manually into the catalytic site of PMN collagenase using the X-ray bound conformation of a known hydroxamic acid MMP inhibitor as a guide (**Figure 1**).[9] The conformation of the mercaptoacyl moiety of **1** placed the carbonyl and sulfur atoms at suitable binding distances from the zinc atom suggesting a bidentate interaction. The sulfur–zinc bond length corresponded to that observed in other zinc–sulfur complexes.

While suffering from a reduction in potency against the MMP enzymes compared to the corresponding hydroxamic acid, this compound is active orally in animal models of arthritis, and the effects of the compound have been shown to be disease modifying. Compound **1** displays good oral activity in an adjuvant arthritic rat model of rheumatoid arthritis.[10] When dosed at 40 mg/kg p.o. daily, compound **1** produces a 40% inhibition of hind paw swelling (**Figure 2a**) with a corresponding improvement in the observed X-rays as measured by a visual analogue scoring (VAS) method, (**Figure 2b**) illustrating truly disease-modifying activity.

A limited amount of work was carried out in this series of compounds exploring the requirements of the $P_1'$ and $P_2'$ substituents (**Table 2**). While the threonine **5** was detrimental to activity, an increase in potency was realised by replacing the $P_1'$ leucine in **1** with *S*-methyl cysteine in **6**. However, the oxidation of **6** to the sulfoxide **7** led to a significant reduction in activity. These data are consistent with the known hydrophobic nature of the $S_1'$ pocket. Replacing the $P_2'$ phenylalanine with tryptophan (in **8** and **9**) or the 4-thiazolylalanine moiety in **10** was also well tolerated by the MMP enzymes, as expected for the open $S_2'$ position.

**Figure 1** Proposed Mode of Binding of **1** in MMP-8[9]

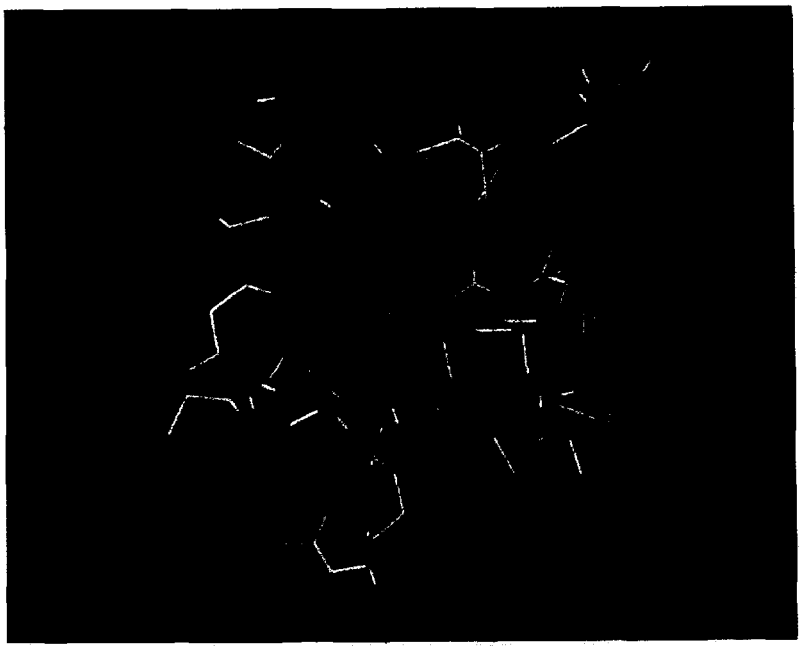
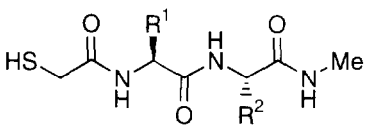


**Table 2** Activities of Compounds with Varied $P_1'$ and $P_2'$ Substituents[8]




| Compound | $R^1$ | $R^2$ | $IC_{50}$ (μM) MMP-3 | MMP-8 | MMP-9 |
|---|---|---|---|---|---|
| **1** | *i*-Bu | $CH_2Ph$ | 2.6 | 0.05 | 0.09 |
| **5** | CHMe(OH) | $CH_2Ph$ | >100 | 4.1 | 1.7 |
| **6** | $CH_2SMe$ | $CH_2Ph$ | 0.65 | 0.006 | 0.013 |
| **7** | $CH_2SOMe$ | $CH_2Ph$ | 8.5 | 0.48 | 0.35 |
| **8** | *i*-Bu | $CH_2$-(3-indolyl) | 0.43 | 0.08 | 0.03 |
| **9** | $CH_2SMe$ | $CH_2$-(3-indolyl) | 0.81 | 0.02 | 0.12 |
| **10** | *i*-Bu | $CH_2$-(4-thiazolyl) | 0.43 | 0.012 | 0.007 |

**Figure 2a** Effect of **1** on Paw Volume Increase

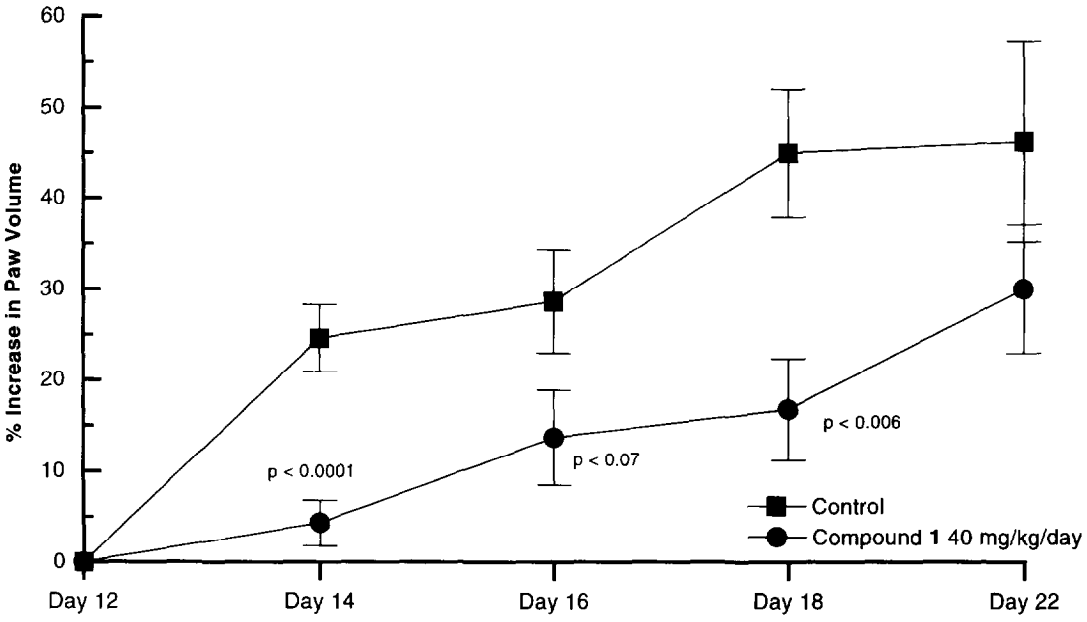
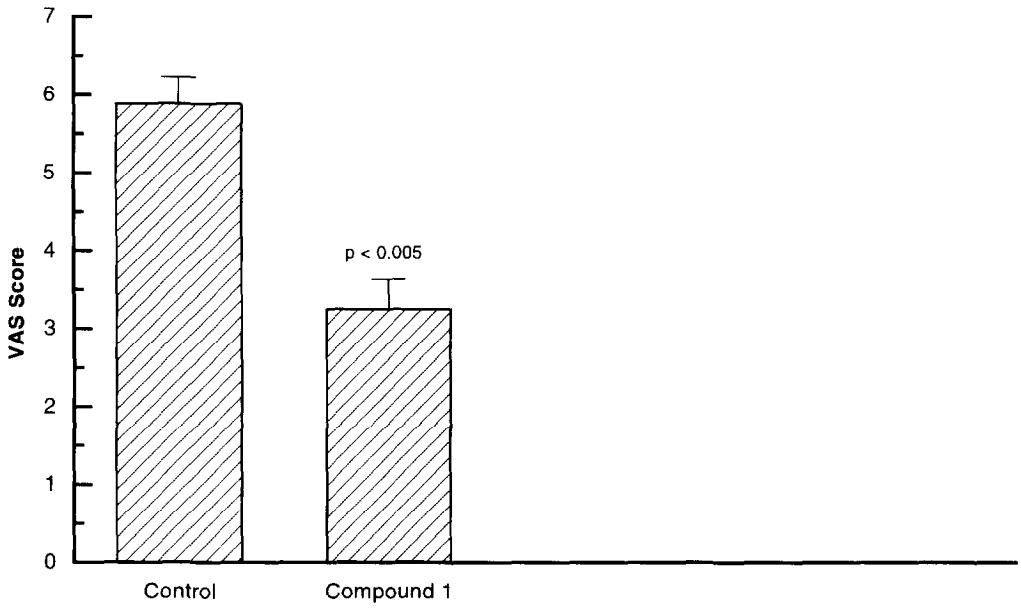



**Figure 2b** Effect of **1** on X-ray VAS




Control animals were dosed daily with vehicle, 1% methyl cellulose, and treated animals dosed with compound **1** orally daily at 40 mg/kg/day

In summary, at Chiroscience we have identified a novel series of MMP inhibitors capable of displaying good oral activity in an animal model of rheumatoid arthritis. Our strategy to improve the enzyme potency further and enhance the oral activity of this series of compounds, along with an overview of how the physico-chemical properties can be manipulated and selectivity achieved between the different MMP enzymes will be the subject of future publications.

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