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MERCAPTOACYL MATRIX METALLOPROTEINASE INHIBITORS: THE EFFECT OF SUBSTITUTION AT THE MERCAPTOACYL MOIETY

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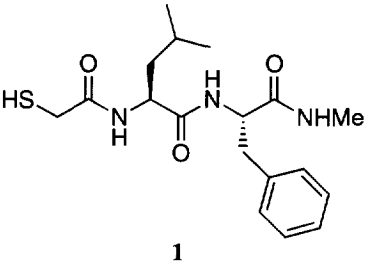
Abstract: The *in vitro* potency of orally-active mercaptoacyl matrix metalloproteinase inhibitors is increased by the introduction of appropriate substituents on the mercaptoacyl moiety. © 1997 Elsevier Science Ltd.

In our previous paper[1] we outlined the discovery of a novel series of mercaptoacyl matrix metalloproteinase (MMP) inhibitors for the treatment of inflammatory disorders and cancer.[2,3] Our research identified inhibitors such as compound **1** that had modest *in vitro* potency against a number of MMP enzymes, but which showed a truly disease-modifying effect in an *in vivo* model of arthritis. A limited amount of structural modification established that the mercaptoacyl unit was critical for the activity of these inhibitors: modification or deletion of this unit led to a large decrease in activity. However, we also identified that appropriate modification of the peptide residue leads to compounds of improved activity.



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We considered that the P_1' leucine and P_2' phenylalanine residues of compound **1** would be already favourable for interaction with the MMP enzymes as they closely mimic the backbone of the natural substrate for these enzymes. Therefore we postulated that a greater opportunity to enhance potency may arise outside these regions. In particular we wanted to examine the area close to the mercaptoacyl zinc-binding moiety.

Taking compound **1** as our lead, in this paper we describe the work towards a systematic examination of the substituent effects at positions close to the mercaptoacyl unit, and the corresponding effect on the *in vitro* activity. While the substrate specificity in the area close to the mercaptoacyl unit (equivalent to the S_1 binding

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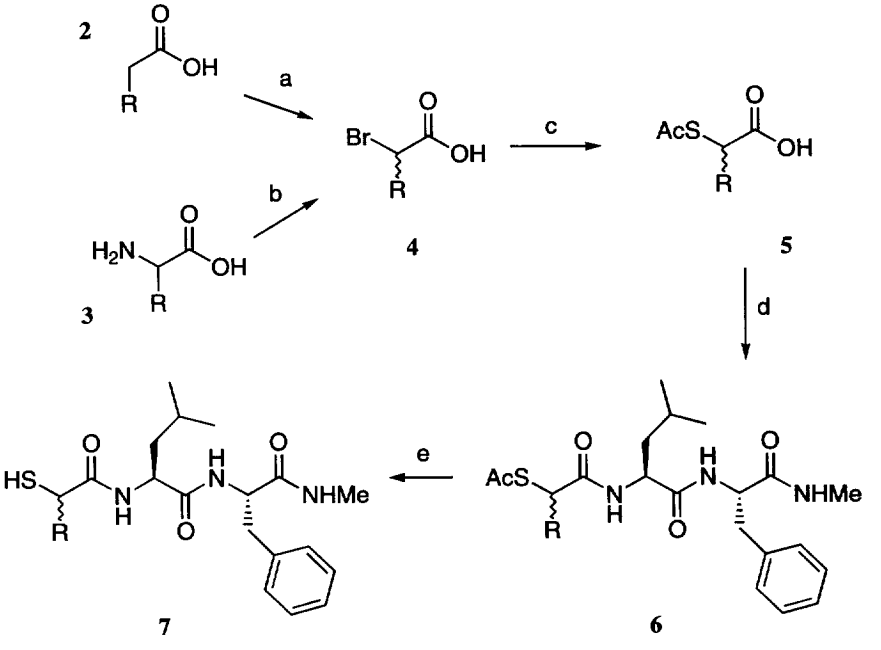
site) is not well defined, computer-aided drug design (CADD) suggested that there was an opportunity to form hydrogen bonds with residues on the enzyme surface close to the mercaptoacyl group. For example, there was the possibility of interacting with Ser 172 of MMP-8 (neutrophil collagenase).

The main focus of the work described in this communication is the preparation of substituted compounds **7**, and determination of their activities against MMP-8, MMP-3 (stromelysin) and MMP-9 (gelatinase B). The target compounds **7** bear a substituent between the sulfur and carbonyl of the mercaptoacyl unit, and were prepared as shown in **Scheme 1**. Direct bromination of an appropriate carboxylic acid **2**, or diazotisation of an amino acid **3** in the presence of potassium bromide gave an α-bromo acid **4**, if the desired α-bromo acid was not available commercially. Compound **4** was treated with potassium thiolacetate to give the thiolacetate acid **5**, which was then coupled with an appropriate dipeptide under standard peptide coupling conditions to give thiolacetate **6**, followed by deprotection to provide the target compound **7**.

Scheme 1 Preparation of α-substituted compounds **7**

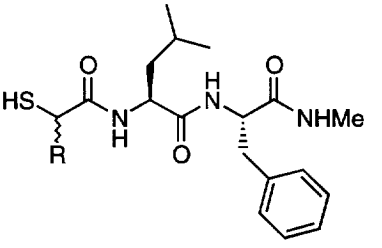


Reagents: (a) i. $SOCl_2$ ii. NBS, $SOCl_2$ iii. $NaHCO_3$ (b) $NaNO_2$, H_2SO_4, KBr, 0 °C (c) KSAc, DMF (d) Leu-Phe-NHMe, EDAC, HOBT, THF (e) NH_4OH, H_2O, MeOH

Initially we examined the introduction of lipophilic substituents into the mercaptoacyl unit. The data we obtained for the compounds prepared (**7a-c**) are shown in **Table 1**.[4] It was found that, in general, these substituents had little effect, showing only a modest increase in activity for the compounds when compared to the unsubstituted compound **1**. Having established that the presence of such a substituent was of little benefit to activity, we next explored the introduction of hydrogen bonding substituents on to the side chain. The phthalimide (NPhth) was chosen as a representative hydrogen-bonding group, and the effect of carbon chain

length was examined. The data for these compounds (**7d-f**) are shown in **Table 2**, and show that this substituent significantly improves the *in vitro* potency of the compounds. The chain length also affects the activity, being optimal at three carbons for those chain lengths examined.

Table 1 Activities of the Compounds with Lipophilic Substituents against MMP Enzymes[5]



		IC_{50} (μM)		
	R	**MMP-3**	**MMP-8**	**MMP-9**
1	H	2.60	0.05	0.09
7a	CH_2Ph	0.97	0.02	0.04
7b	CH_2CH_2Ph	2.60	0.08	0.05
7c	Pr^i	1.40	0.07	0.03

Table 2 Activities of the Compounds with Hydrophilic Substituents against MMP Enzymes[5]

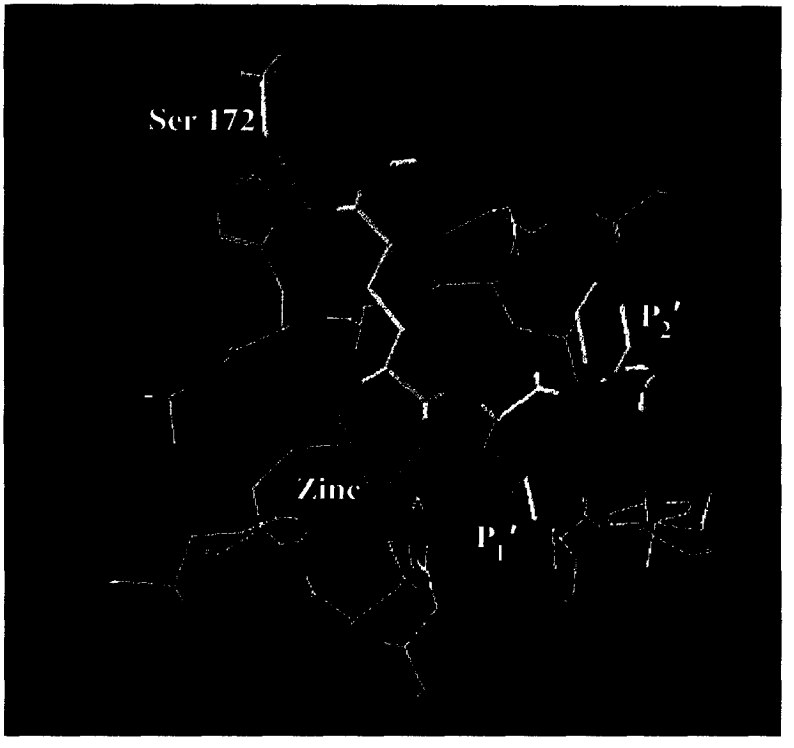
		IC_{50} (μM)		
	R	**MMP-3**	**MMP-8**	**MMP-9**
1	H	2.60	0.050	0.090
7d	$[CH_2]_2NPhth$	0.86	0.015	0.031
7e	$[CH_2]_3NPhth$	0.55	0.025	0.003
7f	$[CH_2]_4NPhth$	0.50	0.125	0.030
7g	$[CH_2]_3CO_2Me$	0.49	0.014	0.001
7h	$[CH_2]_3NHSO_2Me$	0.55	0.019	0.006
7i	$[CH_2]_3CO_2H$	4.28	0.015	0.032

For this optimal chain length, the variation of hydrogen bonding group was then found to lead to changes in activity profile: esters and sulfonamides could be tolerated (**7g-h**), but the introduction of an acid (**7i**) led to a significant loss of MMP-3 activity.

The crystal structure of MMP-8[6] was employed for CADD studies to investigate the nature of the protein-inhibitor interactions. Compound **1** was placed in the binding site using the bound inhibitor as a guide. The

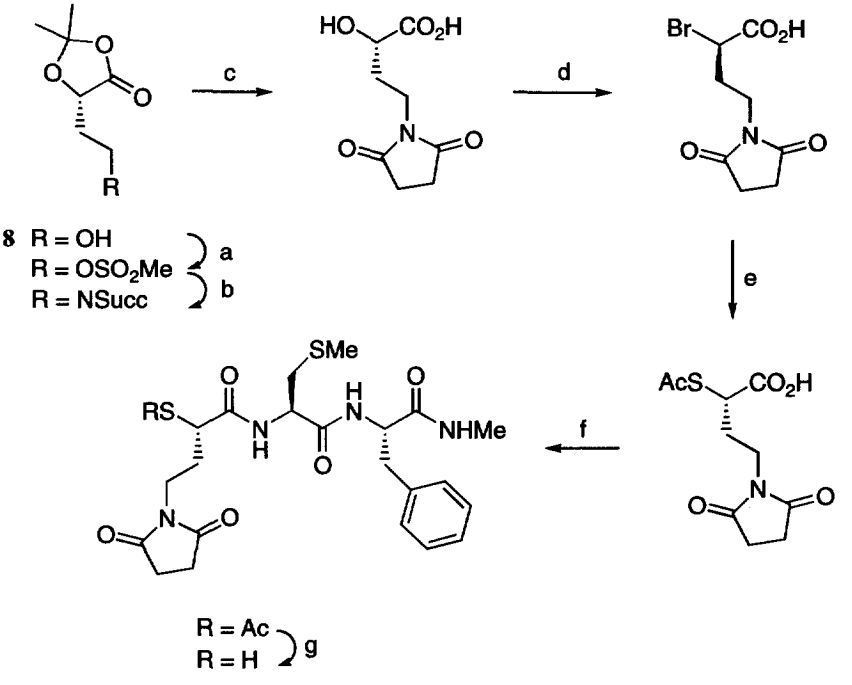
propyl-ester substituent was constructed using the MacroModel package[7] on to the docked structure of **1** employing the *S* configuration, as the *R* isomer formed a steric clash with the binding site. Following this, a Monte Carlo conformational search[8] was conducted on the sidechain using the AMBER forcefield,[9] with the addition of appropriate forcefield parameters. This involved 10 steps of conjugate gradient minimization per conformer, generating a total of 100 conformers. Examination of the lowest energy conformers indicated a possible hydrogen bond between the ester carbonyl and Ser 172 (**Figure 1**), suggesting that the improved affinity of this compound compared to **1** may be the result of an increase in binding energy gained from an additional hydrogen bond between the substituent and the protein.

Figure 1 Proposed Mode of Binding of Compound **7g** in MMP-8



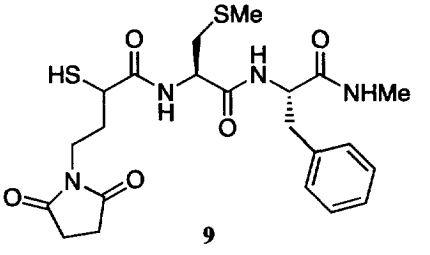
All of these compounds had been prepared initially with a racemic mercaptoacyl side-chain and were thus 1 : 1 mixtures of diastereoisomers. The stereochemical preference at this centre was examined for the succinimidoethyl substituted analogue **9**. Both isomers of the appropriate optically active side-chain **2** (R = $[CH_2]_2$NSucc) could be obtained from the corresponding isomers of known malic acid derivative **8**[10] and the target was then completed as described above (**Scheme 2**). In this case we used an alternative dipeptide unit which had, in our previous work,[1] been shown to give equivalent or improved activity against the MMP enzymes. The data we obtained for these compounds are shown in **Table 3**. As expected from our CADD investigation, there is a strong stereochemical preference for one isomer: the *S*-isomer is 6-10 times more potent than the corresponding *R*-isomer.

Scheme 2 Preparation of Single Isomer Target from Compound **8**



Reagents: (a) $MeSO_2Cl$, Et_3N, –78 °C → 25 °C (b) KNSucc, DMF, 80 °C (c) Dowex® H^+, MeOH, H_2O (d) HBr, HOAc (e) KSAc, DMF (f) EDAC, HOBT, Cys(SMe)-Phe-NHMe (g) NH_4OH, MeOH

Table 3 The Effect of Chirality on the Activity of Compound **9**[5]



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	IC_{50} (μM)		
Stereochemistry	**MMP-3**	**MMP-8**	**MMP-9**
R	0.270	0.030	0.030
RS	0.082	0.010	0.009
S	0.045	0.003	0.005

In summary, the work described this paper identifies the important structural constraints of the mercaptoacyl moiety, and demonstrates that the *in vitro* activity of the series may be improved significantly by the introduction of hydrogen-bonding substituents on a stereochemically defined chain. Work towards modifying the MMP inhibition selectivity profile and oral activity of these compounds will be the subject of future publications.

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