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RAPID SYNTHESIS OF NOVEL DIPEPTIDE INHIBITORS OF HUMAN COLLAGENASE AND GELATINASE USING SOLID PHASE CHEMISTRY

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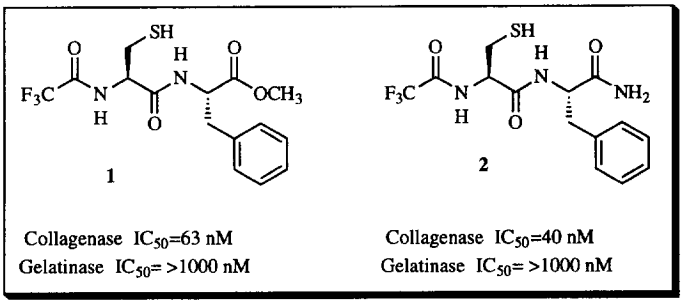
Abstract: Solid phase chemistry expedited the systematic modification of the C and N-terminal groups of cysteine derived lead compound **1** (collagenase IC_{50} 63nM), providing a series of matrix metalloproteinase inhibitors. Potent inhibitors of collagenase (**1-2**, **4-6**, and **10-13**) and gelatinase (**4-8**) were identified. Insights into the binding mode of selective inhibitors will be discussed. Copyright © 1996 Elsevier Science Ltd

Collagenase and gelatinase are members of the family of zinc containing matrix metalloproteinases (MMPs) that are capable of degrading extracellular matrix. The recent explosion of structural information about this family of enzymes reveals they are part of a superfamily of proteins structurally related to thermolysin and other bacterial zinc proteinases. Members of this superfamily have a common tertiary structure and the conserved sequence motif His-Glu-x-x-His (HExxH) in which the histidines serve as zinc binding ligands.[2] The name metzincins has been proposed for this family.[3] MMPs are involved in tissue remodeling during morphogenisis and wound healing. It has been shown that the overproduction of MMPs is associated with various pathologies,[4] including bone destruction, tumor metastasis, corneal ulceration, multiple sclerosis, and periodontal disease. Given the different pathologies linked to the overproduction of MMPs, selective inhibitors of family members would help to elucidate their physiological role. Synthetic small molecule inhibitors of MMPs, based upon the structure of the substrate cleavage site are of considerable interest.[5] A key feature of these inhibitors is the inclusion of a zinc binding ligand such as a thiol group. Herein, we report a versatile synthesis of compounds containing a zinc binding ligand using solid phase chemistry. This approach rapidly produced novel, potent, and selective inhibitors of collagenase and gelatinase.

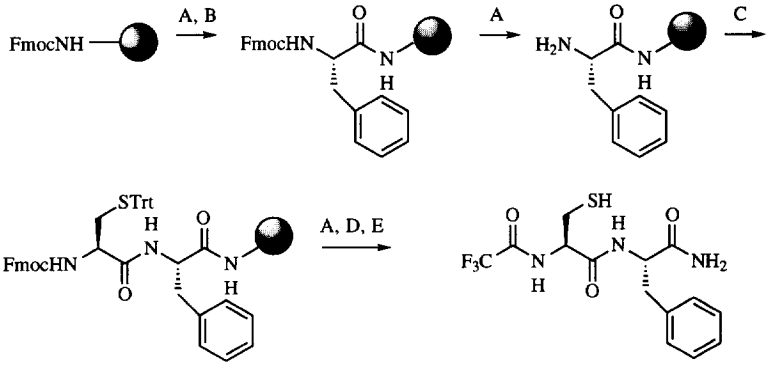
Lead compound **1**, obtained from a search of the Glaxo Wellcome compound portfolio for thiols, and amide analog **2**, synthesized for its adaptability to solid phase chemistry, proved to be potent inhibitors of collagenase (Figure 1).[6] Therefore, to synthesize a variety of MMP inhibitors our strategy was to generate diversity by sequentially varying three fragments of **2**, the C-terminal amino acid, the N-terminal substituent, and the zinc binding amino acid, while holding the other two fragments constant. From these studies, we hoped to discover the preferred substituents at each position and combine the best fragments from each iteration to obtain potent and selective inhibitors of collagenase and gelatinase. Modification of the C-terminal group of **2** was performed by replacing the phenylalanine residue with a variety of natural and unnatural amino acids. Synthesis of a second series of compounds capped the amino terminus of **2** with a variety of acids, isocyanates and sulfonyl chlorides. Lastly, the thiol fragment that presumably ligates zinc, was replaced by a carboxylic acid and an imidazole by replacing cysteine for aspartic acid, glutamic acid or histidine.

Figure 1



One advantage to using **2** as a lead is that it is amenable to solid phase synthesis. Several hundred compounds were synthesized on an Advanced Chem Tech Model 396 multiple peptide synthesizer. Syntheses were carried out using Rink resin as the solid support and Fmoc protected amino acids. The synthesis of **2** as a representative example is depicted in Scheme 1. Compounds were assayed against human fibroblast collagenase (MMP-1) and 92 kD gelatinase (MMP-9) according to a method described previously.[7]

Scheme 1

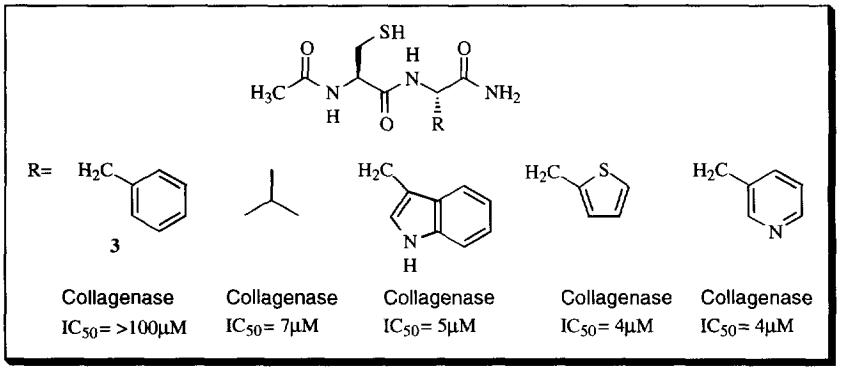


(A) The Fmoc group was removed with 20% piperdine in DMF; (B) N-Fmoc-L-Phenylalanine HOBt/ HBTu, all couplings were performed in NMP (0.25M), using 10 equivalents of amino acid and coupling reagent; (C) N-Fmoc-S-Trityl-L-cysteine, HOBt/HBTu, in NMP (0.25M), using 10 equivalents of amino acid and coupling reagent; (D) Trifluoroacetic acid anhydride, Et_3N; (E) King Reagent (TFA, phenol, thioanisole, ethandithiol, water).

In the first series of compounds the phenylalanine residue of **2** was replaced with a set of fifty natural and unnatural amino acids. We initially selected the acetyl group as the amino-terminal cap of cysteine for the first set of phenylanine replacements due to its ease of incorporation in the solid phase synthesis. Unfortunately, using the acetyl group as the N-terminal cap decreased the potency of the compounds significantly; for example acetyl analog **3** is 1000-fold less potent than **2** against collagenase. Despite the reduced binding affinity some general structure activity relationships (SAR) could be deduced from the

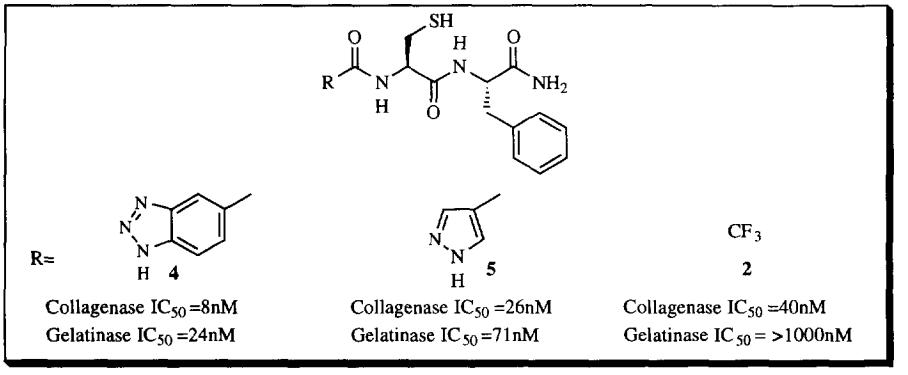
phenylalanine replacement series. Heteroaromatic and aliphatic side chains of L-amino acids demonstrated increased potency against collagenase compared with **3** [8] (Table 1)

Table 1



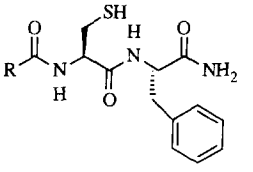
Intrigued by the difference in potency between **2** and **3** a second series of compounds was synthesized holding the phenylalanine moiety constant and capping the N-terminus of the dipeptide with approximately 150 different groups. Interestingly, in this series of compounds the collagenase and gelatinase SAR diverged widely with different N-terminal substituents. Collagenase is more effectively inhibited by dipeptides containing the trifluoroacetyl group (**2**) or groups derived from heteroaromatic acids (**4** and **5**) as the N-terminal substituent (Table 2).

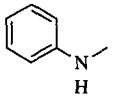
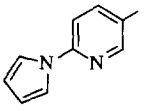
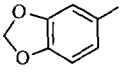
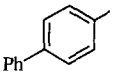
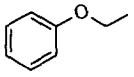
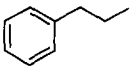
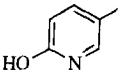
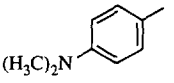
Table 2



In contrast, good inhibition of gelatinase was manifested with large lipophilic groups at the N-terminus. Aryl urea **6** (IC_{50} 11 nM) derived from phenyl isocyanate, biaryl compounds (**7** and **8**), and (**9**) derived from 3-phenylpropanoic acid are particularly noteworthy (Table 3). Compound **9** shows remarkable fidelity toward gelatinase.

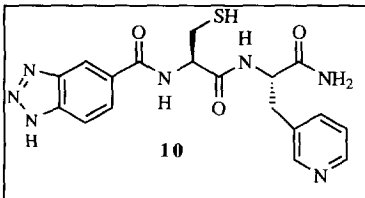
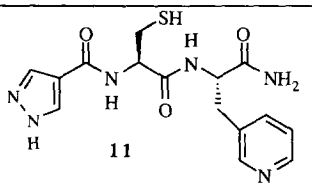
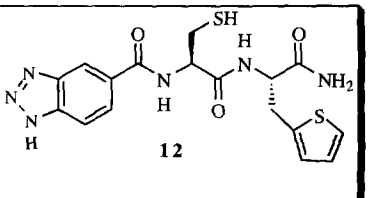
Table 3



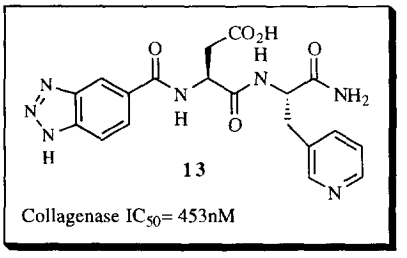
 **6**	 **7**		 **8**
Gelatinase IC_{50}= 11nM Collagenase IC_{50}= 40nM	Gelatinase IC_{50}= 15nM Collagenase IC_{50}= 250nM	Gelatinase IC_{50}= 19nM Collagenase IC_{50}= 215nM	Gelatinase IC_{50}= 21nM Collagenase IC_{50}= 425nM
	 **9**		
Gelatinase IC_{50}= 34nM Collagenase IC_{50}= 598nM	Gelatinase IC_{50}= 38nM Collagenase IC_{50}= 3498nM	Gelatinase IC_{50}= 41nM Collagenase IC_{50}= 83nM	Gelatinase IC_{50}= 41nM Collagenase IC_{50}= 93nM
			
Gelatinase IC_{50}= 49nM Collagenase IC_{50}= 105nM	Gelatinase IC_{50}= 82nM Collagenase IC_{50}= 130nM	Gelatinase IC_{50}= 84nM Collagenase IC_{50}= 171nM	Gelatinase IC_{50}= 80nM Collagenase IC_{50}= 147nM

A matrix of compounds that combined the best C- and N-terminal groups was prepared using the same parallel synthesis technique. The results demonstrated that phenylalanine at the C-terminus and phenyl urea at the N-terminus of the dipeptide (**6**) was the most potent inhibitor of gelatinase. Pyridylalanine or thienylalanine at the C-terminus along with amides derived from heteroaromatic acids at the N-terminus (**10-12**) proved to be potent inhibitors of collagenase (Table 4), however, none was better than compound **4.**

Table 4

 **10**	 **11**	 **12**
Gelatinase IC_{50}= 42nM Collagenase IC_{50}= 12nM	Gelatinase IC_{50}= 71nM Collagenase IC_{50}= 26nM	Gelatinase IC_{50}= 102nM Collagenase IC_{50}= 34nM

To obtain alternate zinc binding ligands, we synthesized another series of compounds in which the cysteine of residue of **10** was replaced with the D and L isomers of aspartic acid, glutamic acid, and histidine. The most potent compound of this series, **13**, is a moderate inhibitor of collagenase. This suggests that each series containing different zinc binding residues must be optimized independently.



13

Collagenase IC_{50}= 453nM

Several compounds were docked into a crystal structure of fibroblast collagenase [2a] in an effort to explain the structure activity relationship. The docking calculations were carried out with the MVP program, using a 2.4 Angstrom distance constraint between the cysteine sulfur and the active site zinc, and a build-up procedure to generate and energy minimize alternative orientations and conformations.[9] The predicted structure of **2** has the trifluoroacetyl group inserted into the P1' pocket, with the phenylalanine residue in a position and conformation similar to that of the P2' phenylalanine in the crystal structures.[2] This binding mode has six hydrogen bonds with the enzyme, a good zinc-sulfur geometry, and an additional interaction between a fluorine and the hydrogens of the P1' water molecule. Recent work indicates that fluorine can make significant interactions with hydrogen bonding groups.[10] The fluorine water interaction could be particularly strong here because the water molecule is oriented and polarized by two hydrogen bonds with arginine-214. The corresponding interaction with the methyl group of **3** would be much weaker, possibly explaining its poor activity against collagenase. A model for the 92 kD gelatinase was constructed from the crystal structure of collagenase with the MVP program. This model indicates that the active site residues are very similar to those of collagenase except that arginine-214 is mutated to leucine. This mutation leaves the P1' pocket much deeper and more hydrophobic. Any water molecule present in the P1' pocket of gelatinase would be disordered, greatly reducing any possible interaction with the trifluoroacetyl group. This could explain why **2** is not active against gelatinase. Bulky, lipophilic N-terminal substituents such as in **6-8** are more easily accommodated by the gelatinase P1' pocket than by the collagenase P1' pocket. Early docking calculations indicated that the bulky, polar N-terminal substituent of **4** could not be accommodated in the collagenase P1' pocket, even if the water molecule were displaced. However, recent crystal structures show that the P1' pocket of matrilysin (MMP-7) can adjust to accommodate large P1' substituents.[11] Conformational changes of this sort are difficult to model accurately, but it seems likely that the adjusted P1' pocket of collagenase would prefer polar substituents such as **4** and **5** due to the presence of arginine-214.

In summary, we have shown that potent and selective inhibitors of the matrix metalloproteinase enzymes collagenase (**2**) and gelatinase (**9**) are obtainable using solid phase synthesis. This methodology

coupled with the recently published structural information on MMPs may help to expedite the search for clinically useful MMP inhibitors.

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