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INHIBITORS OF HUMAN COLLAGENASE: DIPEPTIDE MIMETICS WITH LACTAM AND AZALACTAM MOIETIES AT THE P_2'/P_3' POSITION

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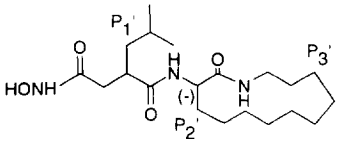
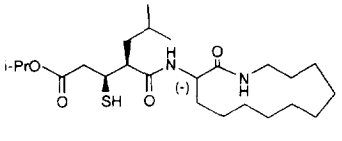
Abstract: A series of thiol-, aminophosphonic acid-, and hydroxamic acid-containing collagenase inhibitors, with lactam and azalactam P_2'/P_3' substituents has been prepared and evaluated *in vitro* as inhibitors of human fibroblast collagenase. The most potent inhibitor was the hydroxamic acid **17a** (IC_{50} 12 nM). Introduction of a basic amino function into the lactam ring had little effect on potency, but greatly enhanced aqueous solubility.

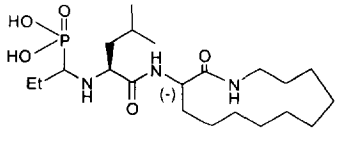
Collagenase (MMP-1) is a member of the family of zinc-containing matrix metalloproteinases (MMPs), and is thought to play a major role in the destruction of connective tissue components of articular cartilage in the arthritides.[1] The rational design of low molecular weight collagenase inhibitors has been reviewed,[2,3] and potent inhibitors have been described containing hydroxamic acid, thiol, and phosphorus oxyacid ligands to bind to the active site zinc ion in the enzyme. Previous studies have established that the preferred substituent at the P_1' position is an isobutyl group and that a variety of substituents are accommodated at the P_2' position including lysine,[2,4,5] arginine[4] and N^{ε}-protected lysine derivatives,[2,4] suggesting that this side chain points towards solvent rather than into the enzyme. This has been confirmed by recent MMP-inhibitor X-ray crystal structure determinations.[6]



1

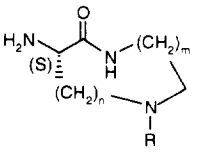


2



3

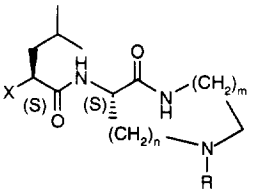
It has been reported that in a series of hydroxamic acids[2,7] and phosphinic acids[8] the P_2'/P_3' side chains may be joined to give lactams, such as **1**, with retention of inhibitory activity. Potency increased as the lactam ring size was increased from 7 to 9 and then to 13, and this increase in potency paralleled the amount of *trans*-amide conformation in the different lactams.[2] In this paper, we describe an extension of this work with the synthesis of the β-mercapto carboxylic acid derivative **2** and aminophosphonic acid **3** containing the 3-(-)-aminoazacyclotridecan-2-one moiety at the P_2'/P_3' position. We have found that such inhibitors are generally



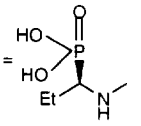
R = H, Me

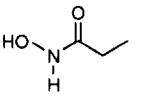
m = 2, 4, 5; n = 3, 4

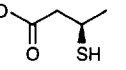
4



(R = H, Me)

5a, X = 

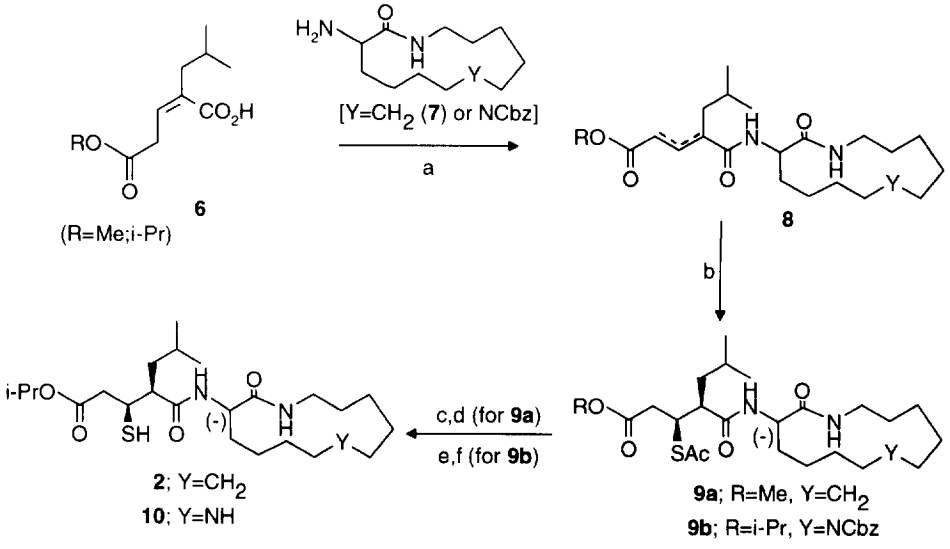
5b, X= 

5c, X = 

highly lipophilic and possess low aqueous solubility, features likely to hinder oral absorption in man.[9] Since basic groups are accommodated at the P_2' position in collagenase inhibitors, we synthesised[10] a novel series of conformationally constrained chiral cyclic mimics **4** of the amino acids lysine and ornithine, with ring sizes 11, 13 and 14, and we have now examined the effect on inhibitory potency of incorporating these aminoazalactams in collagenase inhibitors with aminophosphonic acid **5a**, hydroxamic acid **5b** and β-mercapto carboxylic ester zinc ligands **5c**.

The inhibitors were prepared as outlined in Schemes **1-3**. The carboxylic acids **6**,[11] **11**[4] and **14**[12]

Scheme 1

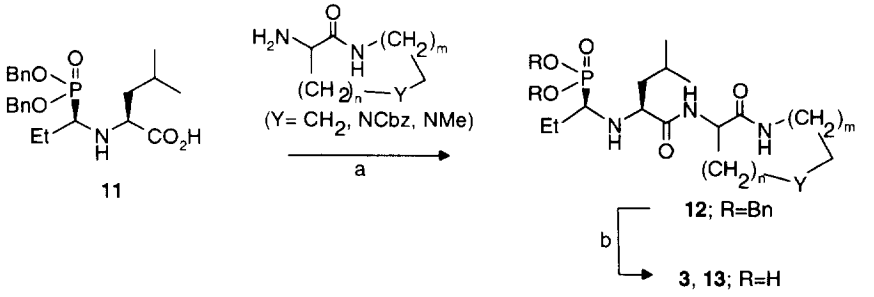


Reagents: (a) Im_2CO, CH_2Cl_2 (b) AcSH (c) NaOH-H_2O then HCl
(d) i-PrOH, $BF_3.Et_2O$ (e) Pd, HCO_2H, MeOH (f) NH_3, MeOH

were coupled with the appropriate 3-(*S*)-aminoazalactams **4** [prepared from the 3-*N*-*tert*-butoxycarbonyl derivatives[10]] and 3-(-)-aminoazacyclotridecan-2-one **7**.[7,8] The unsaturated amides **8** (mixture of four double bond isomers) were reacted with thiolacetic acid at room temperature and the least polar thioester diastereoisomers **9a,b** isolated by silica gel chromatography.[11] Basic hydrolysis of **9a**, followed by re-esterification (i-PrOH/BF_3.Et_2O)[13] of the resulting carboxylic acid gave the thiol **2**. Hydrogenolysis of **9b**, followed by treatment with ammonia in MeOH, gave thiol **10**. The stereochemistry of thiols **2** (*S,S,*-) and **10** (*S,S,S*) was confirmed[11] by their ^{1}H-NMR spectra (high field SH proton doublets).

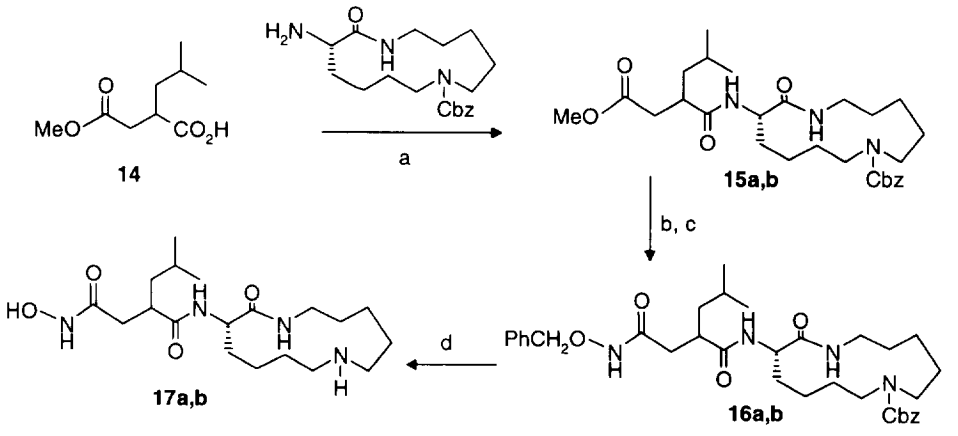
Hydrogenolysis of the dibenzyl phosphonate esters **12** furnished the diastereoisomerically pure aminophosphonic acids **3** and **13** (Scheme 2). The esters **15a** and **15b** (single diastereoisomers), were hydrolysed, coupled with *O*-benzylhydroxylamine giving **16a,b**, and hydrogenolysed to give hydroxamates **17a** and **17b** (Scheme 3).

Scheme 2



Reagents: (a) Im_2CO, MeCN (b) H_2, Pd-C

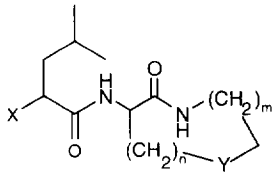
Scheme 3

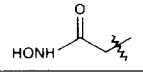
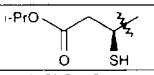
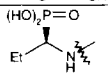
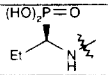
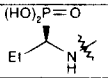
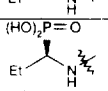
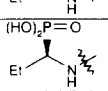
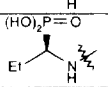
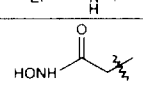
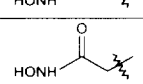
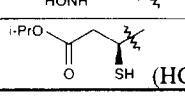


Reagents: (a) $(COCl_2)_2$, DMF, CH_2Cl_2, $(i\text{-}Pr)_2NEt$ (b) KOH, H_2O, 1,4-dioxan, then HCl, H_2O (c) EDC, $PhCH_2ONH_2$.HCl, $(i\text{-}Pr)_2NEt$ (d) H_2, Pd-C

The compounds were evaluated *in vitro* for their ability to inhibit the degradation of radiolabelled rat skin type-I collagen by semi-purified human lung fibroblast collagenase,[14,15] and the results are summarised in Table 1.[16]

Table 1. Inhibitory Potency of the Test Compounds



Cmpd.	X	n	m	ring size	Y	chirality	IC_{50} nM[b]
1		4	4	13	CH_2	R,(-)[a]	26[c]
2		4	4	13	CH_2	S,S,(-)[a]	51±18(3)
3		4	4	13	CH_2	R,S,(-)[a]	183±9(3)
13a		4	4	13	NH	R,S,S	460±145(5)
13b		3	5	13	NH	R,S,S	227±80(6)
13c		4	5	14	NH	R,S,S	307±127(5)
13d		4	4	13	NMe	R,S,S	510±133(2)
13e		4	2	11	NH	R,S,S	2230±1160(2)
17a		4	4	13	NH	(R or S),S	12±3(6)
17b		4	4	13	NH	(S or R),S	82±39(5)
10	 (HCl)	4	4	13	NH	S,S,S	44±19(4)

[a](-) corresponds to stereochemistry of 3-(-)-aminoazacyclotridecan-2-one. [b] Values are mean ± SEM of the number of experiments indicated in parenthesis. [c] From ref. 2.

The hydroxamate **1**, with an azacyclotridecan-2-one moiety at the P_2'/P_3' position, is reported[2] to have an IC_{50} value of 26 nM against human synovial collagenase. The isopropyloxycarbonyl thiol analogue **2**, and

the aminophosphonic acid **3** were less active with IC_{50} values of 51 and 183 nM, respectively. The thiol **2** possessed the preferred[11] (*S*)-configuration at both the thiol and P_1' centers, and the amino phosphonic acid **3** had a preferred[4] ethyl group at the P_1 position, and (*R,S,*-) stereochemistry. We have shown previously that both (*R*) and (*S*) P_1 alkyl groups are accommodated at the S_1 site of the enzyme.[4] Comparison of compounds **2** and **3** with their corresponding acyclic analogues containing P_2' aromatic L-amino acid substituents[4,11,17] showed that introduction of the P_2'/P_3' lactam ring resulted in a reduction in potency of approximately 5 fold in the thiol series, but there was no loss in potency with the aminophosphonic acid ligand.

These cyclic peptide mimetics may show improved *in vivo* stability[2] compared to acyclic dipeptide mimetics, by virtue of increased stability towards proteolytic cleavage, but incorporation of the highly lipophilic azacyclotridecan-2-one moiety at the P_2'/P_3' position greatly decreased the compounds' aqueous solubility. The calculated log P values[18] for the azacyclotridecan-2-ones were in the range 3.5 - 5.5. We reasoned that by incorporating a basic residue into the lactam ring, aqueous solubility would be greatly enhanced.

Compounds **13a-e** were prepared to investigate the optimum ring size and position of the basic N-atom in the azalactams. Comparison of the IC_{50} values for compounds **13a-c** indicated that incorporation of a 13- or 14-membered azalactam ring led to inhibitors with similar potency but the 11-membered ring analogue **13e** had reduced potency (IC_{50} 2230 nM). Comparison of compounds **13d** and **13a** (IC_{50} values of 510 and 460 nM respectively), indicated that methylation of the secondary amino function in the lactam ring had no effect on activity. Overall, inhibitor **13b** was the most potent (IC_{50} 227 nM) with similar potency to its non-basic parent **3** (IC_{50} 183 nM).

The two hydroxamic acid diastereoisomers **17a** and **17b**, bearing 13-membered azalactam ring P_2'/P_3' substituents, were both potent inhibitors (IC_{50} values 12 and 82 nM respectively) and it is likely that the more potent diastereoisomer **17a** possesses the natural (*R*) stereochemistry at the P_1' isobutyl center. The thiol **10** was also a potent inhibitor (IC_{50} 44 nM). Comparison of the IC_{50} values for hydroxamate **17a** and thiol **10** with those of their non-basic parents, **1** and **2**, confirmed that the introduction of a basic substituent into the lactam ring had little effect on inhibitory potency. However, the aminoazalactam derivatives **10**, **13a-e,** and **17a,b**, unlike their non-basic counterparts,[19] were found to possess high aqueous solubility (>10 mg/mL). These water-soluble, basic cyclic peptide mimetics may show improved bioavailability over their acyclic, more peptide-like, analogues. It is of interest that since the completion of this work, it has been reported that the addition of a tertiary amine at the C-terminus of acyclic dipeptide hydroxamate based metalloproteinase inhibitors results in significantly reduced biliary excretion and increased plasma half-life, compared to unfunctionalised inhibitors.[20] The chiral 3-aminoazalactams may have potential for incorporation into other biologically active peptides or pseudo-peptides.

Acknowledgement: We wish to thank the Analytical Sciences Department for spectroscopic determinations.

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16. All new compounds gave satisfactory ^{1}H NMR. FAB mass spectra data, and either high resolution FAB-MS or elemental analysis (± 0.4 %) was obtained for all compounds with the exception of **13e**.
17. For example, the analogue of thiol **2** with a TrpNHMe P_2' residue has IC_{50} of 9 nM and the analogue of the aminophosponic acid **3** with a PheNHMe P_2' residue has an IC_{50} of 230 nM.
18. MedChem 3.53, Medicinal Chemistry Project, Pomona College, Claremont, CA.
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