J. Enzyme Inhibition, 1998, Vol. 13, pp. 79–101
Reprints available directly from the publisher
Photocopying permitted by license only

Review Article

MATRIX METALLOPROTEINASE INHIBITORS IN ARTHRITIS

K.M. BOTTOMLEY, W.H. JOHNSON and D.S. WALTER*

Roche Discovery Welwyn, Broadwater Road, Welwyn Garden City, Herts AL7 3AY, UK

(Received 22 September 1997)

Keywords: Collagenase; Stromelysin; Gelatinase; Inhibitor; Thiol; Hydroxamate; Phosphonamidates; Phosphonic acids; Carboxylic acids; Selectivity; Arthritis

INTRODUCTION

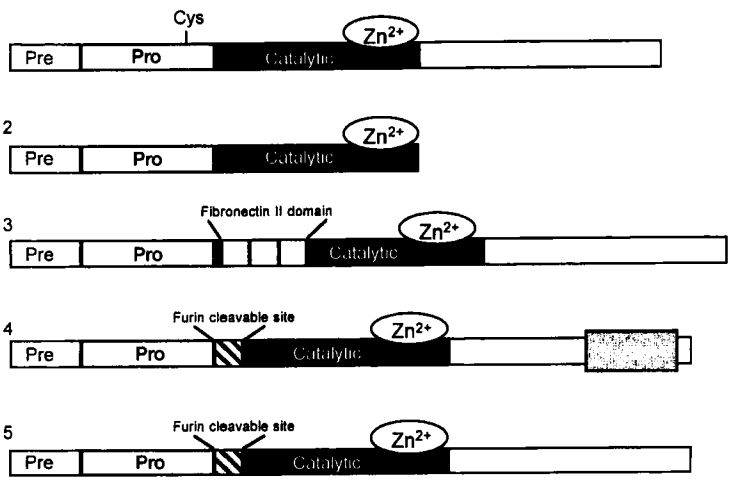
Matrix metalloproteinases (MMPs) (also known as matrixins), a subfamily of the metalloproteinase M10 family,[1] are closely related calcium requiring zinc endopeptidases of which 18 have been identified to date. The MMPs are generally subdivided (*i.e.* collagenases, gelatinases and stromelysins) according to the general macromolecular substrate requirements or the presence of structural features which anchor the enzyme to the cell membrane (membrane type matrix metalloproteinases, MT-MMPs). Collagenases 1, 2, 3 and 4 (MMPs 1, 8, 13 and 18) cleave triple helical collagen I and II at a single locus to produce a characteristic fragmentation pattern. Gelatinases A and B (MMPs 2 and 9) demonstrate a preference for cleavage of gelatin which is

* Corresponding author.

formed following proteolytic cleavage of collagens by collagenases. The stromelysins (1 and 2 (MMPs 3 and 10)) have a general and non-specific substrate requirement. The MT-MMPs (MMPs 14–17) are localised to the cell membrane by a transmembrane domain located towards the C-terminus of the enzyme molecule. However a recombinant, truncated and secreted form of MT1-MMP has been shown to degrade collagens I, II and III, to produce the products characteristic of the collagenases.[2] Consequently there is some overlap in the current criteria for subclassification of these enzymes. Of the remaining known human MPs, matrilysin, stromelysin 3, metalloelastase (MMPs 7, 11 and 12 respectively) and MMP-19[3] are generally unclassified.

The MMPs have a common basic modular structure consisting of an N-terminal signal peptide sequence followed by a pro-peptide sequence, a catalytic domain, and a C-terminal domain. The pro-domain confers latency on the enzyme as a result of an interaction between a conserved cysteine residue and the catalytic zinc ion. MMPs require the removal of this pro-domain for expression of catalytic activity. Stromelysin 3 and the MT-MMPs contain a furin recognition sequence [(R(R/Q)(K/R)R)] between the pro and catalytic domains. In the former case the intracellular action of furin (or a related enzyme) results in removal of the pro-domain and subsequent export from the cell of the corresponding active enzyme.[4] The MT-MMPs may be activated in a similar manner to that observed with stromelysin 3 thus being expressed at the cell surface as active enzymes. Remaining members of the MMP family are secreted from the cell as inactive zymogens and subsequent pericellular or extracellular proteolytic removal of the pro-domain leads to activation of the enzyme. The catalytic domain, contains a highly conserved Zn^{2+} binding motif consisting of three histidine residues and a conserved glutamate which in combination with a water molecule and the zinc ion, provide the enzyme's catalytic mechanism. The highly conserved Zn^{2+} binding motif (HExxHxxGxxH) is a feature of all the MMPs and characteristic of members of the M7, M11, M10 and M12 metalloproteinases.[1] C-terminal to the Zn^{2+} binding motif in MMPs is another conserved motif which includes a methionine that marks a characteristic turn (met turn) and is the feature which has contributed to the designation of these proteins as metzincins.[5] In both gelatinase A and B the catalytic domain also includes an additional fibronectin-like insert. The final C-terminal domain is variable both in sequence and, for those where a role has been established, function. For matrilysin the C-terminal domain is missing, whereas in the collagenases it is essential for matrix substrate recognition (the ability to cleave triple helical collagen I and II is dependent on the presence of this domain[6]).

Domain structure of the MMP family



1: Collagenases 1, 2, 3 & 4, Stromelysin 1 & 2 and metalloelastase. (MMPs-1, 8, 13, 18, 3, 10 and 12 respectively).
2: Matrilysin (MMP-7).
3: Gelatinase A & B (MMPs-2 and 9).
4: MT-MMP(1-4) (MMPs-14, 15, 16, and 17)
5: Stromelysin 3 (MMP-11).

In the case of gelatinase A the C-terminal domain is important for the normal activation of the enzyme[7] while for stromelysins, which have a broad range of matrix substrates, its contribution to enzyme function is unclear. Removal of this domain results in no significant changes of enzyme activity[8] although it does contribute to interactions with TIMPs[9] (Tissue Inhibitors of MMPs). The MT-MMPs contain a trans-membrane region within the C-terminal domain which serves to localise the enzyme in the cell membrane with the N-terminal portion of the molecule protruding into extracellular space[10]. The MT-MMPs appear important in the activation of other MMPs, specifically gelatinase A and collagenase 3.[11,12]

The MMPs acting collectively have the capacity to degrade all components of extracellular matrix. Their activity is tightly controlled at the transcriptional level through activation of the zymogen and by co-secretion of specific TIMPs. MMPs have important roles in the normal matrix turnover which occurs in tropoblast implantation, embryonic development, angiogenesis, growth, wound healing and the regulation of mammary gland function. Poorly regulated MMP activity has been implicated in a number of diseases including rheumatoid and osteoarthritis, cancer,[13] peridontal disease[14] and

inflammatory bowel disease.[15] Both rheumatoid and osteoarthritis are characterised by the loss of normal joint function, specifically due to destruction of the articulating cartilage. This destructive process is believed to be mediated largely by the actions of MMPs. Rheumatoid arthritis is a chronic inflammatory disease characterised, in part, by inflammation of the synovial lining with the resultant formation of pannus. Pannus is a rich source of both MMPs and pro-inflammatory cytokines, such as IL-1 and TNFα, which in turn induce production of MMPs by cartilage chondrocytes.[16–18] In the case of osteoarthritis, joint instability causes inappropriate cartilage remodelling and subsequent destruction of the tissue. In both of these diseases cartilage destruction involves loss of aggrecan and collagen II, the two major structural components of cartilage. Studies involving bovine nasal explants have demonstrated that potent, non-selective inhibitors of MMPs can prevent the breakdown of both of these cartilage components and many efforts have been made to elucidate the relative roles of the individual MMPs in these and other disease states.[19] Currently, attempts to use inhibitors of these enzymes to determine the contribution of sub-classes of the MMPs to normal and abnormal matrix remodelling have been hampered by the relative lack of selectivity of these compounds. A number of reviews covering general aspects of MMP inhibitors have appeared in the literature[20–25] and these issues will not be elaborated in this article. The purpose of this article is to summarise the specific application of inhibitors with varied selectivity profiles to the problem of treating arthritic disease as well as to summarise the design principals elucidated by this group and the rationale behind the development of collagenase inhibitors as novel therapies for rheumatoid and osteoarthritis.

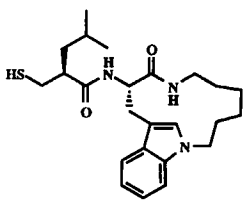
MMP INHIBITORS

The most effective low molecular weight MMP inhibitors reported in the chemical literature to date have been designed to mimic the conserved C-terminal triplet of the collagenase-mediated cleavage-site of collagen and without exception incorporate a zinc ligand in the place of the scissile amide bond. Of the ligands reported, hydroxamic acids generally provide the most potent inhibitors while carboxylic acids, thiols and various phosphorus containing ligands are effective in particular molecular environments but it would seem that additional lipophilic interactions are usually required to compensate for the intrinsically lower binding affinities of these ligands. This difference between zinc ligands can be used to introduce a bias towards

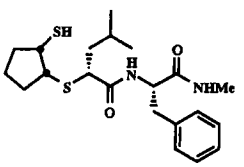
inhibition of enzymes with larger lipophilic enclosures and has proved especially important in the development of carboxylate based inhibitors.[26–33]

(a) Thiols

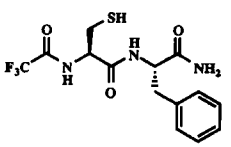
The well documented and successful development of thiol based inhibitors of angiotensin converting enzyme (ACE), a zinc endoprotease, led many groups to pursue the use of similar thiols as inhibitors of the matrix metalloproteases.[34–38] The relatively weak binding to the catalytic zinc atom of the MMPs has proven somewhat problematic in this series but advances in this area have been forthcoming. The conformationally restricted, indollactam-based inhibitor (**1**) is a very potent inhibitor of MMP-1 and showed considerable selectivity over MMP-9.[39] Similarly, dipeptidic inhibitors incorporating a thiol ligand have been prepared by Schwartz (**2**)[40,41] and workers at Glaxo have used solid phase chemistry to generate peptidic thiols (*e.g.* (**3**)) with useful activities and, once again, good selectivity for MMP-1 over MMP-9.[42]



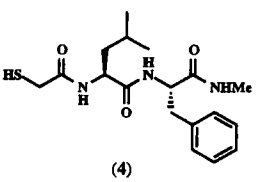
(1)



(2)



(3)



(4)

	IC_{50} (nM)				
	MMP-1	MMP-2	MMP-3	MMP-8	MMP-9
1	2.3				>>800
2	49	1.1	470	0.89	0.57*
3	40				>1000
4	420	1030	2610	50	90

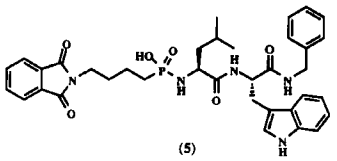
* Ki (nM)

Recently reported inhibitors developed by workers at Chiroscience[43–50] (*e.g.* (**4**)) are of some considerable interest as although they are essentially peptidic in nature, these compounds make use of a mercaptoamide moiety

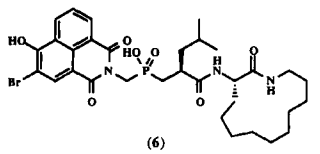
which not only binds to zinc to provide relatively potent inhibitors of a number of MMPs but is also claimed to confer good oral bioavailability and efficacy in animal models on this class of compounds. One of these inhibitors (D-5410) is currently in Phase I trials for rheumatoid arthritis and has been shown to be safe and well tolerated after oral doses of up to 2 g in human volunteers.[51]

(b) Phosphorus Ligands

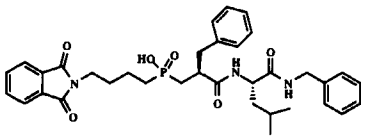
Inhibitors based on phosphorus ligands act as transition-state mimics and, in contrast to most of the other product-like inhibitors of the MMPs, incorporate some features of both the N- and C-terminal sides of the cleavage sites found in the natural substrates. Phosphonamidates and phosphinic acids such as (**5**), (**6**) (Ro 31-7467) and (**7**)[52–57] exhibit useful *in vitro* activity and also show some selectivity for collagenase in the case of the former two compounds and for stromelysin in the latter case. However, as a class, these compounds have far from ideal pharmacokinetic properties and none of these compounds have found any clinical application.



(5)



(6)

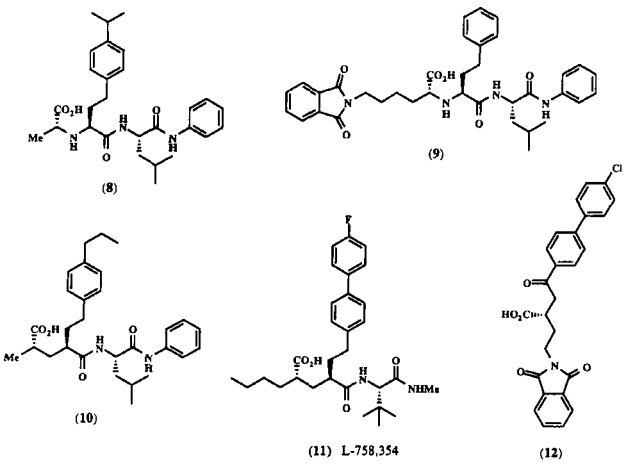


(7)

	IC_{50} (nM)		
	MMP-1	MMP-2	MMP-3
5	50		260
6	16	240	200
7	4100		430

(c) Carboxylic Acids

As was the case for the thiol based MMP inhibitors, the prior use of aminocarboxylates as effective orally available inhibitors of ACE led to the dedication of considerable effort, to the optimisation of this class of compounds as inhibitors of MMPs, specifically stromelysin.



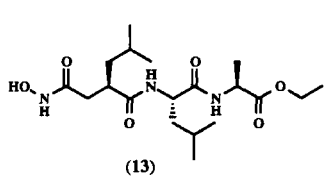
	Ki (nM)		
	MMP-1	MMP-2	MMP-3
8	>10000	130	180
9	720	86	8
10	>10000	310	68
11	>10000	22	7.6

Both stromelysin and gelatinase have a deep hydrophobic pocket at the S'_1 subsite[58–62] and it is this feature which provides the extra binding affinity required to produce potent inhibitors when using the relatively weaker binding carboxylate ligand. The size of this pocket also provides a handle for obtaining selectivity over collagenase which, in contrast, has a much smaller pocket in this position.[63–65] For example, compound (**8**) from the Merck laboratories,[28] has a *para*-substituted hydrophobic phenethyl moiety in the P'_1 position and showed selectivity for MMP-3 and MMP-2 over MMP-1. Substituents other than methyl in P_1 generally improved the potency of these compounds and in some cases a further bias toward selective inhibition of MMP-3 was observed (*e.g.* (**9**)).[30] Some of these compounds were assessed *in vivo* by monitoring the degradation of radiolabelled transferrin by activated human MMP-3 after injection into a murine pleural cavity but in most cases these compounds showed only small beneficial effects after oral dosing and subsequent efforts to find compounds with improved oral profiles were largely centred on the analogous C-carboxyalkyl series.[32] In general these compounds (*e.g.* (**10**)) were less potent inhibitors of MMP-3 but improved pharmacokinetics more than compensated for this loss of *in vitro* activity. Considerable optimisation of this series of compounds led workers at Merck to identify L-758,354 (**11**) as a potent stromelysin inhibitor both *in vitro* and

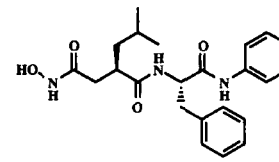
in the mouse pleural cavity assay (PLCAV). L-758,354 reportedly has an oral bioavailability of 78% in the mouse and an ED_{50} of 4.7 mg/kg i.v. and 11 mg/kg p.o. in the PLCAV model. However, on evaluation of this compound in various acute and chronic models of endogenous cartilage degradation, this compound was found to be ineffectual in preventing disease progression.[33,66] Some related compounds (*e.g.* (**12**)), developed at Bayer,[67] are also MMP-3 inhibitors (the selectivity profile of these compounds has not been reported) and one compound (BAY-129566) is apparently in phase II trials in the USA as a potential osteoarthritis therapy.

(d) Hydroxamic Acids

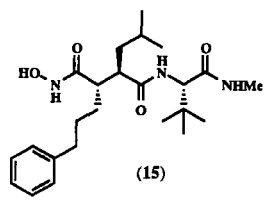
The majority of potent inhibitors with broad activity against matrix metalloproteinases that have been reported in the literature to-date are peptide mimics based on the natural matrix substrates and usually utilise a hydroxamic acid as the zinc-binding motif. Most incorporate a succinate moiety as a non-hydrolyzable amide isostere (*e.g.* (**13**)–(**16**)). Compounds (**13**) [Roche[20]] and (**14**) [British Biotech[69]] are close analogues of the endogenous peptidic cleavage products and are relatively potent inhibitors showing little preference for either collagenase, gelatinase or stromelysin (MMPs-1, 2, and 3 respectively). In cases where the carbon atom in the position α to the hydroxamic acid is substituted by a lipophilic group ((**15**) [Roche] and (**16**)



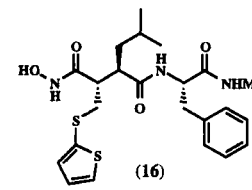
(13)



(14)



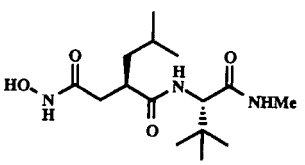
(15)



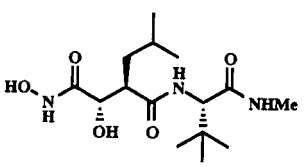
(16)

	IC_{50} (nM)				
	MMP-1	MMP-2	MMP-3	MMP-8	MMP-9
13	9		38		8
14	20	60	10		
15	1.5		8		2.6
16	3	4	20	10	1

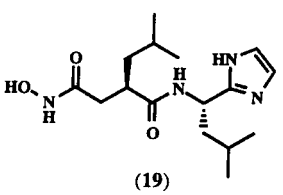
[British Biotech[24,70]]) the lack of selectivity persists but the compounds are somewhat more active under *in vitro* assay conditions. Despite the somewhat less than ideal pharmacokinetic profiles of many of the hydroxamic acids, it is this class of inhibitor which has made the most progress toward providing an effective clinical therapy for arthritic patients. Early tripeptidic, substrate mimics based on the hydroxamate moiety ((**17**)[71-73] and (**18**) [BB-2516 or Marimastat – currently in Phase III oncology trials[74,75]]) were shown to be highly potent against most of the MMPs with some considerable degree of selectivity for collagenases and gelatinases over stromelysin in particular. Due to their poor oral bioavailabilities, very few of these compounds were evaluated in animal models of arthritis although Ro 31-9790 (**17**) did progress as far as the clinic. The *t*-butyl glycine group in the P_2' position of this compound conferred improved proteolytic stability and hence sufficient oral bioavailability (3–7% in rats; 18% in marmosets) for this compound to show good *in vivo* activity in animal models of arthritis after oral dosing. The compound did not progress to phase II due to an undesirable drug-related, histopathological event. Substitution of the C-terminal amide bond by hydrolytically stable imidazole isosteres (*e.g.* (**19**)) has also been proposed[76] as a useful approach to the problem of compound stability in plasma but although such compounds retain a considerable degree of *in vitro* potency the pharmacokinetic benefits are unclear.



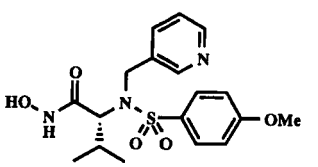
(**17**) Ro 31-9790



(**18**) BB-2516



(**19**)



(**20**) CGS 27023A

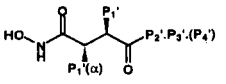
	IC_{50} (nM)			
	MMP-1	MMP-2	MMP-3	MMP-9
17	5		160	10
18	8	6	200	3
19	9		320	
20	33	11	13	7

In a novel departure from the peptidomimetic inhibitors discussed so far, workers at Ciba-Geigy reported a range of sulfonamide inhibitors based on (D)-amino acids.[77] The hydroxamic acid group was essential for good inhibitory activity and (**20**) [CGS 27023A], with Ki values in the 10–30 nM range against MMPs 1, 2 and 3, was shown to inhibit collagen loss from IL-1 treated cartilage explants and was also orally active in a stromelysin-induced rabbit model of proteoglycan degradation.[78,79] Some adverse side-effects (*e.g.* synovial fibrosis and osteophyte formation) have been associated with this compound[80] and its current development status as an arthritis therapy is unclear.The subsequent discussion summarises work carried out at Roche in which detailed structure-activity relationships for hydroxamic acids based on the succinyl framework were developed to provide a library of inhibitors with differing MMP selectivity profiles which, along with data gleaned from various animal models and from X-ray crystallography, were used to develop bioavailable non-peptidic analogues and ultimately to select a compound which is currently in development as a treatment for rheumatoid arthritis. The identification of the subsite requirements for hydroxamate containing inhibitors of collagenase 1, stromelysin 1 and gelatinases are detailed and key inhibitor-enzyme interactions are noted. Substituents in the P_1', P_2', P_1' (α) and P_3', (P_4') positions were systematically varied (see Table I) in order to identify selective inhibitors of each of the three classes of MMPs. The inhibitors thus obtained were subsequently used to determine the contribution of each of these classes of enzyme to the process of cartilage degradation observed in rheumatoid and osteoarthritis.

(i) Stromelysin Inhibitors

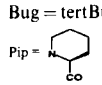
Modifying compound (**21**) by replacing the isobutyl group in the P_1' position with an *n*-pentyl group (**23**) resulted in a doubling of activity against stromelysin. As the length of this alkyl group is extended even further, activity increases proportionally and compounds containing seven (**24**) and nine (**25**) carbon chains in this position are significantly more potent than the parent. In the P_2' position, cyclohexylalanine (**27**) was less effective than *t*-butylglycine (**24**), but leucine (**29**), phenylalanine (**26**) and neopentylglycine (**28**) groups all gave some improvement in activity while compounds with smaller amino-acid residues in this position were only weakly active. Replacing the methylamide in (**29**) with a methyl ester (**30**) resulted in a 20-fold loss of activity but similar substitution with a leucine amide (**32**) or leucine ethylamide (**33**) was tolerated and gave a small increase in activity. Substitution in

TABLE I MMP inhibitors – selected SAR



No.	P_1'	$P_1'(\alpha)$	P_2'	$P_3'(P_4')$	MMP-1 collagen	IC50 (nM)* MMP-3 b-cas	MMP-3 fluor	MMP-9 hexa	MMP-9 fluor
21	$CH_2CH(CH_3)_2$	H	Bug	NHMe	5	470	160	12	10.4
22	"	CH_2N.Pth	"	"	< 0.5	37	7.5	4.3	—
23	$(CH_2)_4CH_3$	H	"	"	—	215	—	13	—
24	$(CH_2)_6CH_3$	"	"	"	—	54	31	1.2	1.3
25	$(CH_2)_8CH_3$	"	"	"	—	20	9	< 0.8	< 0.16
26	$(CH_2)_6CH_3$	"	Phe	"	—	39	16	1.95	—
27	"	"	Cha	"	—	71	—	—	—
28	"	"	Npg	"	—	29	5.6	< 1.0	—
29	"	"	Leu	"	—	50	23	1.7	—
30	"	"	"	OMe	—	2000	—	—	—
31	"	"	"	$NHCH_2{}^tBu$	—	—	—	3.4	—
32	"	"	"	$Leu.NH_2$	—	39	16	250	—
33	"	"	"	Leu.NHEt	—	33	8.2	320	310
34	$CH_2CH(CH_3)_2$	CH_3	Bug	NHMe	2.5	105	—	—	—
35	"	CH_2TMH	"	"	1	240	28	—	—
36	"	$(CH_2)_3Ph$	"	"	2.3	—	2.7	—	—
37	$(CH_2)_6CH_3$	"	"	"	—	—	< 0.4	—	< 0.1
38	$(CH_2)_8CH_3$	"	Npg	"	..	—	< 0.35	..	..
39	CH_2Ph	H	Bug	"	—			300	—
40	$(CH_2)_2Ph$	"	"	"	—			7.2	6.3
41	$(CH_3)_3Ph$	"	"	"	—			—	1.6
42	$(CH_3)_3Ph$	"	Phe	"	—			—	0.6
43	$(CH_2)_2C_6H_{11}$	"	Bug	"	—			255	355
44	$(CH_2)_3C_5H_9$	"	"	"	—			—	3.9
45	$(CH_2)_5CH(CH_3)_2$	"	Phe	"	—			—	0.85
46	$(CH_2)_5C(CH_3)_3$	"	"	"	—			—	5.5
47	$(CH_2)_6CH_3$	CH_2N.Pth	Bug	"	—			0.9	0.2
48	$(CH_2)_8CH_3$	$(CH_2)_3Ph$	"	"	—			—	< 0.1
49	$CH_2CH(CH_3)_2$	CH_2N.Succ	"	"	0.5				
50	"	CH_2N.Pth	Pip	"	2.5				
51	"	"	Piper		13.7				
52	"	CH_2TMH	"		10				
53	$CH_2C_3H_5$	"	"		33				
54	$CH_2C_4H_7$	"	"		7				
55	$CH_2C_5H_9$	"	"		7				
56	$CH_2C_6H_{11}$	"	"		23				

Abbreviations:
Bug = tertButylglycine Cha = cyclohexylalanine Npg = neopentylglycine TMH =  N.Succ = 

Pip =  Piper = 

*Assays used were cleavage of C14-labelled collagen, β-casein, and a hexapeptide for collagenase, stromelysin and gelatinase respectively. Fluorogenic assays later replaced the β-casein and hexapeptide assays to overcome mutual depletion problems observed in the case of potent inhibitors.

$P_1'(\alpha)$ generally led to increased activity but the extent of this effect was highly dependent on the nature of the group introduced at this position. A methyl group in $P_1'(\alpha)$ (**34**) results in a four-fold increase in activity and a phthaloylaminomethyl group (**22**) gives compounds that are up to 20 times more potent. Removing the imide portion of (**22**) as in the phenylpropyl substituted (**36**) is more effective yet (60-fold improvement over (**21**)) and illustrates some of the subtleties of the effects in this position. In most cases, the individual effects of groups in P_1', $P_1'(\alpha)$ and P_2' are independent and additive and compounds with P_1' = long alkyl chain; P_2' = hydrophobic residue; and $P_1'(\alpha)$ = phenylpropyl have IC_{50} values below the detection limit of the assay employed (*e.g.* (**37**) < 0.4 nM and (38) < 0.35 nM).

(ii) Gelatinase Inhibitors

As was observed with stromelysin, long alkyl groups in the P_1' position resulted in a substantial increase in activity against gelatinase (*e.g.* (**24**) and (**25**)). Although the benzylic group (**39**) was not tolerated, phenyl substituents were allowed on longer carbon chains ((**40**) and (**41**)). (This observation is in good agreement with work reported by the Celltech group[81]). Interestingly the phenyl group in (**40**) could not be replaced by cycloalkyl substituents (*e.g.* (**43**)), but in the case of the higher homologue (**41**), replacement of the aromatic moiety by cyclopentyl (**44**) led to only a negligible loss of activity. Branching at the more remote C_6 of the P_1' side-chain gave a small mark-up in activity in some cases (**45**) but very large groups reversed this effect (**46**). Altering the amino-acid residue in P_2' (*e.g.* (**24**), (**26**), (**28**) and (**29**)) had only minor consequences as did replacement of the methylamide in P_3' by a neopentylamide (**31**). Extension of the inhibitors into the P_3' and P_4' subsites ((**32**) and (**33**)) led to much less active inhibitors (250–320 nM) while incorporation of a $P_1'(\alpha)$ group into any of the compounds containing a long alkyl group in P_1' generally gave inhibitors with IC_{50} values below the limit of the assay (< 0.1 nM). The nature of the grouping in $P_1'(\alpha)$ was relatively flexible but simple alkyl or phenalkyl groups tended to be slightly more active than the analogous imides.

(iii) Collagenase Inhibitors

The sub-site requirements for collagenase inhibitors have been reviewed previously.[20] As with the aforementioned enzymes the addition of a $P_1'(\alpha)$ group results in a substantially more active series of inhibitors. The phthaloylaminomethyl group in (**22**) improved potency by an order of magnitude

and by comparison with the succinyl (**49**) (and the imide (**35**)) and phenylpropyl (**36**) substituted compounds it was obvious that the imide function was contributing to the improved binding observed. Incorporation of a proline methylamide in $P_2'P_3'$ gave weakly active inhibitors but the homologous pipecolic acid (**50**) was a potent inhibitor of collagenase (2.5 nM). Furthermore, removal of the terminal methylamide (*e.g.* (**51**)) led to inhibitors with useful activity and the trimethylhydantoin derivative (**52**) had an $IC_{50} = 10$ nM. Detailed structural studies of the collagenase catalytic domain suggested that the isobutyl group favoured in the P_1' position did not fill all of the available space in this region and the more bulky cycloalkyl groups ((**53**)–(**56**)) were introduced in its place. Some increase in potency was seen with the cyclobutyl and cyclopentyl analogues ((**54**) and (**55**)).

(iv) Selective Inhibitors

From the SARs outlined above it was apparent that the subsite requirements for the three enzymes of interest were sufficiently different (see summary in Figure 1) to allow the design of selective small molecule inhibitors (Table II). Of the three enzymes only stromelysin will tolerate large substituents in the $P_3'(P_4')$ position. This major difference, together with differing preferences in the other subsites allowed for the straightforward design of compounds

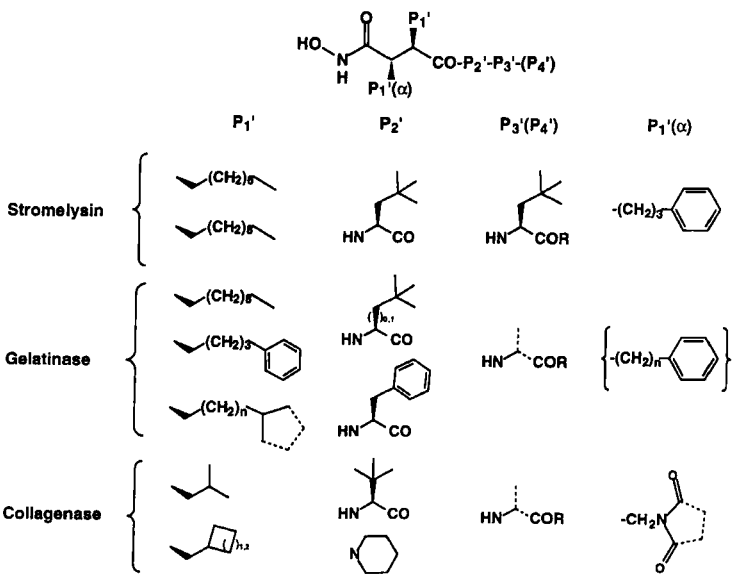
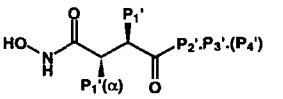


FIGURE 1 Subsite requirements for selective MMP inhibitors.

TABLE II Selective MMP Inhibitors



No.	P_1'	$P_1'(\alpha)$	P_2'	$P_3'(P_4')$	*Stromelysin fluor*	*Gelatinase fluor*	*Collagenase collagen*
21	$CH_2CH(CH_3)_2$	H	Bug	NHMe	160	10.4	5
57	$(CH_2)_6CH_3$	H	Npg	Npg.NHMe	4.7	252	8200
58	"	$(CH_2)_3Ph$	Leu	Leu.NHEt	< 0.19	19	>10000
25	$(CH_2)_8CH_3$	H	Bug	NHMe	9	< 0.16	320
52	$CH_2CH(CH_3)_2$	CH_2TMH	Piper		2175	240	10

with a high degree of selectivity (>50000) for stromelysin over collagenase ((**57**) and (**58**)). The closer resemblance of stromelysin to gelatinase (especially in the P_1' subsite) led to difficulties in obtaining compounds which were selective for gelatinase. The long alkyl group in P_1' easily gave selectivity for gelatinase over collagenase and some selectivity over stromelysin (>50) was noted when the chain length was increased from seven to nine carbon atoms (see (**24**) and (**25**)). Branching in this position led to more selective compounds but this effect was not optimised. Compounds with the relatively small isobutyl or cycloalkylmethyl groups in P_1', combined with an imide function in $P_1'(\alpha)$ and a tertiary amide replacing the $P_2'P_3'$ groups, were reasonably selective for collagenase. Compound (**52**) is selective for collagenase over stromelysin and gelatinase by factors of >200 and 24 respectively.

The three dimensional structure of the catalytic domain of fibroblast collagenase complexed with several inhibitors revealed some features which are crucial to an understanding of the enzyme–inhibitor interactions. No reliable model of gelatinase is available as this enzyme contains a large, structurally undefined amino-acid insertion but a model of stromelysin was successfully developed based on the collagenase structural data and this model was found to be in good agreement with more recently published X-ray data. The collagenase data and the stromelysin model successfully explain the preferences observed in the P_1' and $P_1'(\alpha)$ binding regions. When (**22**) was co-crystallised with collagenase a hydrogen bond was observed between one of the imide carbonyls and an asparagine residue (Figure 2(a)). In stromelysin this residue is replaced by valine (Figure 2(b)) and hence is consistent with the hydrophobic interaction observed in the SARs and selectivity data.

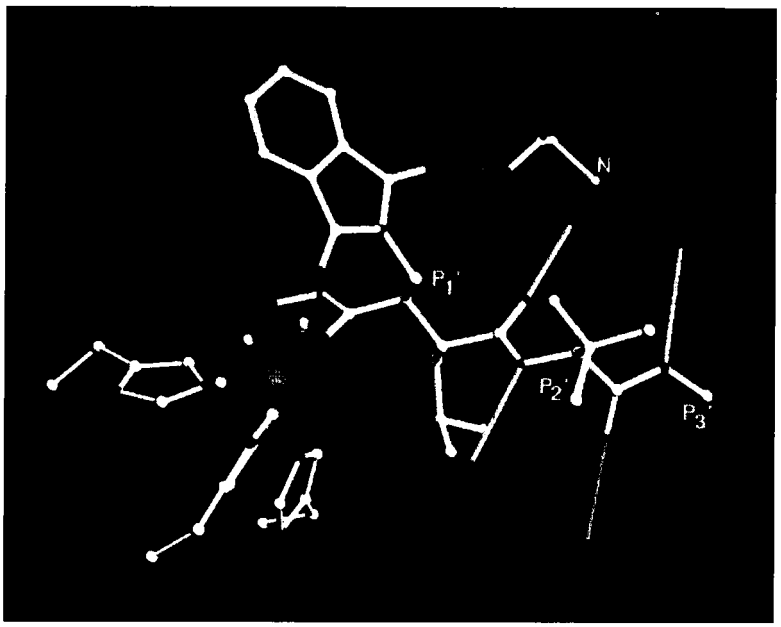


FIGURE 2(a) X-ray crystal structure of compound (**22**) co-crystallised with MMP-1. See Color Plate I.

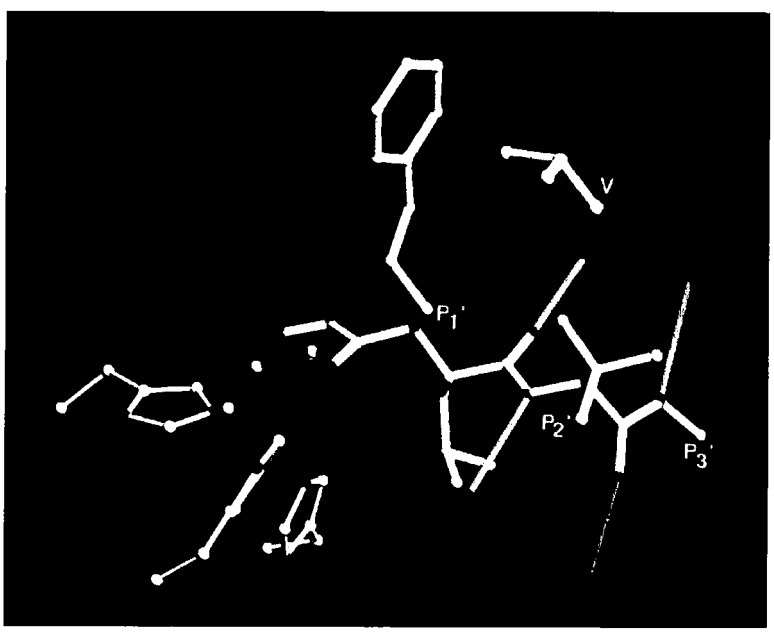


FIGURE 2(b) Computer-generated model of compound (**36**) bound to MMP-3. See Color Plate II.

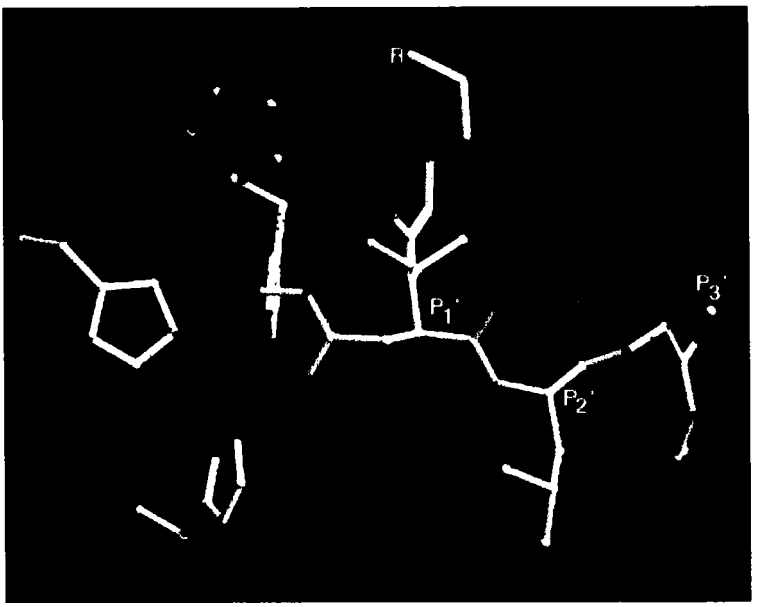


FIGURE 3(a) X-ray crystal structure of compound (**13**) co-crystallised with MMP-1. See Color Plate III.

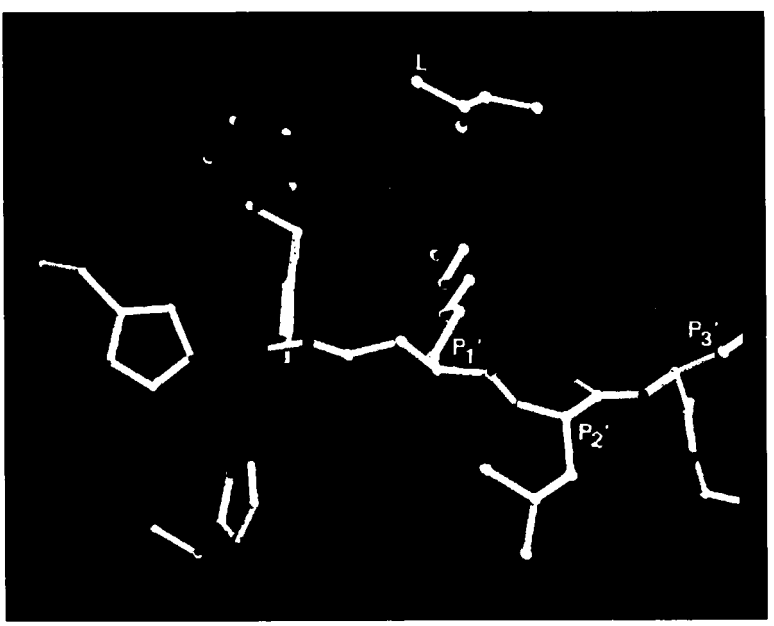


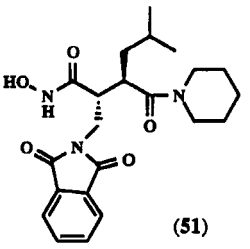
FIGURE 3(b) Computer-generated model of inhibitor with large alkyl chain in P_1' bound to MMP-3. See Color Plate IV.

In the P_1' hydrophobic binding site the collagenase data revealed a relatively shallow pocket bounded by a buried arginine residue (Figure 3(a)). Molecular modelling suggested that the larger cyclopentylmethyl group would be tolerated and this hypothesis was vindicated with compounds (**53**) and (**56**). In stromelysin this arginine residue is replaced by leucine and this smaller group does not appear to limit the depth of the pocket to the extent seen in collagenase. The pocket now goes through the enzyme (Figure 3(b)) and is consistent with the preference for long alkyl groups in the P_1' position of potent stromelysin inhibitors. It appears that the major differences in subsite selectivity between stromelysin and collagenase can thus be attributed to only two single-point mutations in the active site (*i.e.* Asn – Val and Arg – Leu).

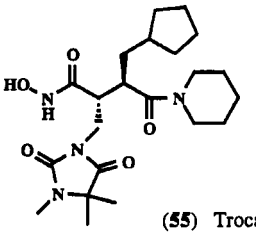
It is well documented that stromelysin mRNA and protein are found at elevated levels in the synovial fluid of patients suffering from rheumatoid and osteoarthritis[82,83] and it was thought that its associated proteolytic activity both in degradation of the matrix[84] and in the activation cascade of other MMPs[85] would make the inhibition of this enzyme a useful point of intervention for the regulation of the arthritic disease process. As a result a number of research groups invested considerable resource in the development of selective stromelysin inhibitors. The ability of such compounds (*e.g.* (**57**) and (**58**) to prevent aggrecan metabolism was studied using bovine nasal explants which had been stimulated with recombinant human IL-1α. A significant correlation ($r = 0.93$) between *in vitro* inhibition of MMP-3 and inhibition of aggrecan breakdown in the bovine cartilage explant model was observed (There was little or no correlation in the cases of MMP-9 and MMP-1 respectively) but a concomitant 1000-fold decrease in potency was noted in the latter assay.[19] As aggrecan metabolism is a rapidly reversible process, this data would suggest that for a stromelysin inhibitor to prove therapeutically useful, the attainment of unfeasibly high tissue concentrations would be required. Furthermore, no correlation between MMP-3 inhibition and collagen metabolism was observed. Considering this data and that reported by other groups using the carboxylate inhibitor class,[33] it seems highly unlikely that inhibition of stromelysin alone is sufficient to arrest the process of cartilage destruction which is typically associated with osteo- and rheumatoid arthritis. Similarly, selective inhibition of gelatinase also appears to be insufficient to prevent the degradation of the articular matrix.[19]

The cleavage of triple helical collagen II by collagenases is thought to be the key irreversible event in the degradation of cartilage and the process which leads to the loss of the functional and structural properties usually associated with normal articular cartilage.[86] The degradation of proteoglycan

is rapidly reversible in the presence of the supporting collagen network and consequently it is the initial destruction of the structural collagen II network that marks the irreversible step in the arthritic process. As a result, the selective inhibition of collagenases has been the focus of a number of research groups, our own included. The work outlined above has led to the discovery of a new series of potent non-peptidic inhibitors based on a disubstituted succinyl framework. A relatively small lipophilic residue in P_1' and an α side-chain bearing a hydrogen bonding imide moiety are the features which confer the selectivity of this series. Examples of this series (*e.g.* **(51)** and **(55)**) not only exhibit considerable selectivity for collagenase (over gelatinase as well as stromelysin) but also have greatly improved solubilities (>100 mg/ml in the case of **(55)**) and oral bioavailability (upto 41%). Unlike selective inhibitors of MMP-3 (and/or MMP-9), these selective collagenase inhibitors are potent inhibitors of cartilage collagen metabolism in both *in vitro* and *in vivo* models of arthritis. Trocade™ **(55)**[87–89] has been selected for further development and is currently in phase II trials as a cartilage protective agent. Trocade™ selectively inhibits all three members of the collagenase family (MMPs 1, 8 and 13) in preference to MMPs 2, 3 and 9; it inhibited interleukin-1α induced cartilage collagen degradation *in vitro* in bovine nasal cartilage explants ($IC_{50} = 60$ nM); it was well absorbed in rats when administered orally and systemic exposure was dose related with an oral bioavailability of 26% (25 mg/kg p.o.); it prevented granuloma-induced degradation of bovine nasal cartilage cylinders implanted subcutaneously into rats ($ED_{50} = 10$ mg/kg, b.i.d., p.o.); and it also successfully inhibited degradation of articular cartilage in a rat, *p.acnes* – induced monoarthritis model (dosed once daily for 14 days at 50 mg/kg p.o.). Trocade™ promises to break new ground in



(51)



(55) Trocade™

	IC_{50} (nM)					
	MMP-1	MMP-2	MMP-3	MMP-8	MMP-9	MMP-13
(51)	13.7		2900			
(55)	7.0	154	527	4.4	59	3.4

this field and has the potential to provide sufferers of osteo- and rheumatoid arthritis with the means to prevent the chronic joint debility associated with these diseases.

CONCLUDING REMARKS

Considerable advances in the understanding of the roles of individual MMPs in degradative arthritic processes have been made in recent years but many questions still remain unanswered. For example, although it appears that stromelysin and gelatinase do not play a significant role in the destruction of articular cartilage, the elusive aggrecanase is still being actively pursued[90,91] and some workers have recently published data which supports the view that a collagenase-like enzyme may have a role in the degradation of the proteoglycan core.[92] The role of the collagenases, on the other hand, is now fairly well established but there is evidence to suggest that inhibitors exhibiting selectivity within the collagenase family may also prove efficacious in treating arthritic disease. MMPs 1, 8 and 13 have all been found in articular cartilage and have all, in turn, been implicated in the degradation of the type II collagen, articular cartilage matrix. The relative contribution of each of the collagenases to the destruction of cartilage in rheumatoid and osteoarthritis has not yet been fully established but inhibitors with differing selectivities towards these enzymes will be crucial in determining such detailed information. Workers at Roche Bioscience have reported tht RS-113,456, a compound which inhibits MMPs 8 and 13 (Ki(nM) = 0.13 and 0.17) in preference to MMP-1 and -3 (Ki(nM) = 70 and 5), is effective in an *in vivo* model of articular degradation after oral dosing.[93] Finally, although not strictly an MMP, the recently characterised TNF-α converting enzyme (TACE)[94,95] is readily inhibited by hydroxamic acid inhibitors[95–98] of the MMP family and shows promise as an alternative therapeutic target in the area of rheumatoid and osteoarthritis.

Acknowledgements

The authors are most grateful to their colleagues Dr N. Borkakoti and Mr C. Nix for their contribution to the preparation of this manuscript and to the many members of the anti-inflammatory chemistry and biology departments who contributed to the synthesis and testing of many of the compounds mentioned in the text.

References

[1] Rawlings, N.D. and Barrett, A.J. (1995). *Meth. Enymol.*, **248**, 183.
[2] Ohuchi, E., Imai, K., Fujii, Y., Sato, H., Seiki, M. and Okada, Y. (1997). *J. Biol. Chem.*, **272**(4), 2446.
[3] Pendás, A.M., Knäuper, V., Puente, X.S., Llano, E., Mattei, M-G., Apte, S., Murphy, G. and López-Otin, C. (1997). *J. Biol. Chem.*, **272**, 4281.
[4] Pei, D. and Weiss, S.J., (1995). *Nature (Lond.)*, **375**, 244.
[5] Bode, W., Gomis-Ruth, F.X. and Stockler, W. (1993). *FEBBS Lett.*, **331**(1–2), 134.
[6] Clark, I.M. and Cawston, T.E. (1992). *Matrix Suppl.*, **1**, 73.
[7] Murphy, G., Nguyen, G.N., Cockett, M.I., Atkinson, S.J., Allan, J.A., Knight, C.G., Willenbrock, F. and Docherty, A.J. (1994). *J. Biol. Chem.*, **269**(9), 6632.
[8] Murphy, G., Allan, J.A., Willenbrock, F., Cockett, M.I., O'Connell, J.P. and Docherty, A.J. (1992). *J. Biol. Chem.*, **267**(14), 9612.
[9] Baragi, V.M., Fliszar, C.J., Conroy, M.C., Ye, Q.Z., Shipley, J.M. and Welgus, H.G. (1994). *J. Biol. Chem.*, **269**(17), 12692.
[10] Nguyen, Q., Willenbrock, F., Cockett, M.I., O'Shea, M., Docherty, A.J. and Murphy, G. (1994). *Biochemistry*, **33**(8), 2089.
[11] Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E. and Seiki, M. (1994). *Nature (Lond.)*, **370**, 61.
[12] Knäuper, V., Cowell, S., Smith, B., López-Otin, C., O'Shea, M., Morris, H., Zardi, L. and Murphy, G. (1997). *J. Biol. Chem.*, **272**, 7608.
[13] Coussens, L.M. and Werb, Z. (1996). *Chem. and Biol.*, **3**(11), 895.
[14] Haerian, A., Adonogianaki, E., Mooney, J., Docherty, J.P. and Kinane, D.F. (1995). *J. Clin. Periodontol.*, **22**, 505.
[15] Pender, S.L., Tickle, S.P., Docherty, A.J., Howie, D., Wathen, N.C. and MacDonald, T.T. (1997). *J. Immunol.*, **158**(4), 1582.
[16] MacNaul, K.L., Chartrain, N., Lark, M., Tocci, M.J. and Hutchinson, N.I. (1992). *Matrix Suppl.*, **1**, 198.
[17] Lohmander, L.S., Hoerrner, L.A. and Lark, M.W. (1993). *Arthritis Rheum.*, **36**(2), 181.
[18] Walakovits, L.A., Moore, V.L., Bhardwaj, N., Gallick, G.S., Lark, M.W. and Woessner, J.F. Jr. (1992). *Arthritis Rheum.*, **35**(1), 35.
[19] Bottomley, K.M., Borkakoti, N., Bradshaw, D., Brown, P.A., Broadhurst, M.J., Budd, J.M., Elliot, L., Eyers, P., Hallam, T.J., Handa, B.K., Hill, C.H., James, M., Lahm, H.W., Lawton, G., Merritt, J.E., Nixon, J.S., Röthlisberger, U., Whittle, A. and Johnson, W.H. (1997). *Biochem. J.*, **323**, 483.
[20] Johnson, W.H., Roberts, N.A. and Borkakoti, N. (1987). *J. Enzy. Inhib.*, **2**, 1.
[21] Schwartz, M.A. and Van Wart, H.E. (1992). *Prog. Med. Chem.*, **29**, 271.
[22] Morphy, J.R., Millican, T.A. and Porter, J.R. (1995). *Curr. Med. Chem.*, **2**, 743.
[23] Hagmann, W.K., Lark, M.W. and Becker, J.W. (1996). *Ann. Rep. Med. Chem.*, **31**, 231.
[24] Beckett, R.P., Davidson, A.H., Drummond, A.H., Huxley, P. and Whittaker, M. (1996). *Drug Discovery Today*, **1**, 16.
[25] Zask, A., Levin, J.I., Killar, L.M. and Skotnicki, J.S. (1996). *Curr. Pharm. Design.*, **2**, 624.
[26] Chapman, K.T., Kopka, I.E., Durette, P.L., Esser, C.K., Lanza, T.J., Izquierdo-Martin, M., Niedzwiecki, L., Chang, B., Harrison, R.K., Kuo, D.W., Lin, T-Y., Stein, R.L. and Hagmann, W.K. (1993). *J. Med. Chem.*, **36**, 4293.
[27] Brown, F.K., Brown, P.J., Bickett, D.M., Chambers, C.L., Davies, H.G., Deaton, D.N., Drewry, D., Foley, M., McElroy, A.B., Gregson, M., McGeehan, G.M., Myers, P.L., Norton, D., Salovich, J.M., Schoenen, F.J. and Ward, P. (1994). *J. Med. Chem.*, **37**, 674.
[28] Sahoo, S.P., Caldwell, C.G., Chapman, K.T., Durette, P.L., Esser, C.K., Kopka, I.E., Polo, S.A., Sperow, K.M., Niedzwiecki, L.M., Izquierdo-Martin, M., Chang, B.C., Harrison, R.K., Stein, R.L., MacCoss, M. and Hagmann, W.K. (1995). *Bioorg. Med. Chem. Lett.*, **5**(20), 2441.
[29] Caldwell, C.G., Sahoo, S.P., Polo, S.A., Eversole, R.R., Lanza, T.J., Mills, S.G., Niedzwiecki, L.M., Izquierdo-Martin, M., Chang, B.C., Harrison, R.K., Kuo, D.W., Lin, T-Y., Stein, R.L., Durette, P.L. and Hagmann, W.K. (1996). *Bioorg. Med. Chem. Lett.*, **6**(3), 323.

[30] Chapman, K.T., Wales, J., Sahoo, S.P., Niedzwiecki, L.M., Izquierdo-Martin, M., Chang, B.C., Harrison, R.K., Stein, R.L. and Hagmann, W.K. (1996). *Bioorg. Med. Chem. Lett.*, **6**(3), 329.
[31] Xue, C-B., He, X., Roderick, J., DeGrado, W.F., Decicco, C. and Copeland, R.A. (1996). *Bioorg. Med. Chem. Lett.*, **6**(4), 379.
[32] Chapman, K.T., Durette, P.L., Caldwell, C.G., Sperow, K.M., Niedzwiecki, L.M., Harrison, R.K., Saphos, C., Christen, A.J., Olszewski, J.M., Moore, V.L., MacCoss, M. and Hagmann, W.K. (1996). *Bioorg. Med. Chem. Lett.*, **6**(7), 803.
[33] Esser, C.K., Bugianesi, R.L., Caldwell, C.G., Chapman, K.T., Durette, P.L., Girotra, N.N., Kopka, I.E., Lanza, T.J., Levorse, D.A., MacCoss, M., Owens, K.A., Ponpipom, M.M., Simeone, J.P., Harrison, R.K., Niedzwiecki, L., Becker, J.W., Marcy, A.I., Axel, M.G., Christen, A.J., McDonnell, J., Moore, V.L., Olszewski, J.M., Saphos, C., Visco, D.M., Shen, F., Colletti, A., Krieter, P.A. and Hagmann, W.K. (1997). *J. Med. Chem.*, **40**, 1026.
[34] Beszant, B., Gaster, L.M., Harper, G.P., Hughes, I., Karran, E.H., Markwell, R.E., Miles-Williams, A.J. and Smith, S.A. (1993). *J. Med. Chem.*, **36**, 4030.
[35] Donald, D.K., Hann, M.M., Saunders, J. and Wadsworth, H.J. (1986). US Patent 4,595,700.
[36] Schwartz, M.A., Venkataraman, S., Ghaffari, M.A., Libby, A., Mookhtiar, K.A., Mallya, S.K., Birkedal-Hansen, H. and Van Wart, H.E. (1991). *Biochem. Biophys. Res. Commun.*, **176**, 173.
[37] Hughes, I., Harper, G.P., Karran, E.H., Markwell, R.E. and Miles-Williams, A.J. (1995). *Bioorg. Med. Chem. Lett.*, **5**(24), 3039.
[38] Hagmann, W.K. and Kopka, I.E. (1994). WO 9407481.
[39] Castelhano, A.L., Billedeau, R., Dewdney, N., Donnelly, S., Horne, S., Kurz, L.J., Liak, T.J., Martin, R., Uppington, R., Yuan, Z. and Krantz, A. (1995). *Bioorg. Med. Chem. Lett.*, **5**, 1415.
[40] Schwartz, M.A. (1996). *Protease Inhibitors: New Therapeutics and Approaches*, Baltimore, Maryland, November 6–7.
[41] Schwartz, M.A. and Van Wart, H.E. (1995). PCT WO 959620.
[42] Foley, M.A., Hassman, A.S., Drewry, D.H., Greer, D.G., Wagner, C.D., Feldman, P.L., Berman, J., Bickett, D.M., McGeehan, G.M., Lambert, M.H. and Green, M. (1996). *Bioorg. Med. Chem. Lett.*, **6**(16), 1905.
[43] Baxter, A.D., Montana, J., Watson, R.J. and Tiffin, P.D. (1997). PCT WO 9712861.
[44] Baxter, A.D., Bird, J., Bhogal, R., Massil, T., Minton, K.J., Montana, J. and Owen, D.A. (1997). *Bioorg. Med. Chem. Lett.*, **7**(7), 897.
[45] Baxter, A.D., Montana, J. and Owen, D.A. (1996). PCT WO 9635687.
[46] Baxter, A.D., Montana, J. and Owen, D.A. (1996). PCT WO 9635714.
[47] Baxter, A.D., Montana, J. and Owen, D.A. (1996). PCT WO 9635712.
[48] Baxter, A.D., Montana, J. and Owen, D.A. (1996). PCT WO 9635711.
[49] Montana, J., Baxter, A.D., Owen, D.A., Watson, R.J. and Phillipson, N. (1996). PCT WO 9611209.
[50] Montana, J., Dickens, J., Owen, D.A. and Baxter, A.D. (1995). PCT WO 9513289.
[51] R&D Focus, Drug Updates, (1996). IMS World Publications.
[52] Broadhurst, M.J., Handa, B.K., Johnson, W.H., Lawton, G. and Machin, P.J. (1988). Eur. Patent 276436.
[53] Kortylewicz, Z.P. and Galardy, R.E. (1990). *J. Med. Chem.*, **33**, 263.
[54] Morphy, J.R., Beeley, N.R.A., Boyce, B.A., Leonard, J., Mason, B., Millican, A., Millar, K., O'Connell, J.P. and Porter, J.R. (1994). *Bioorg. Med. Chem. Lett.*, **4**.
[55] Bird, J., De Mello, R.C., Harper, G.P., Hunter, D.J., Karran, E.H., Markwell, R.E., Miles-Williams, A.J., Rahman, S.S. and Ward, R.W. (1994). *J. Med. Chem.*, **37**, 158.
[56] Hunter, D.J., Bird, J., Cassidy, F., De Mello, R.C., Harper, G.P., Karran, E.H., Markwell, R.E., Miles-Williams, A.J. and Ward, R.W. (1994). *Bioorg. Med. Chem. Lett.*, **4**, 2833.
[57] Goulet, J.L., Kinneary, J.F., Durette, P.L., Stein, R.L., Harrison, R.K., Izquierdo-Martin, M., Kuo, D.W., Liu, T-Y. and Hagmann, W.K. (1994). *Bioorg. Med. Chem. Lett.*, **4**, 1221.
[58] Esser, C.K., Kopka, I.E., Durette, P.L., Harrison, R.K., Niedzwiecki, L.M., Izquierdo-Martin, M., Stein, R.L. and Hagmann, W.K. (1995). *Bioorg. Med. Chem. Lett.*, **5**, 539.

[59] Gowravaram, M.R., Tomczuk, B.E., Johnson, J.S., Delecki, D., Cook, E.R., Ghose, A.K., Mathiowetz, A.M., Spurlino, J.C., Rubin, B., Smith, D.L., Pulvino, T. and Wahl, R.C. (1995). *J. Med. Chem.*, **38**, 2570.
[60] Porter, J.R., Beeley, N.R.A., Boyce, B.A., Mason, B., Millican, A., Millar, K., Leonard, J., Morphy, J.R. and O'Connell, J.P. (1994). *Bioorg. Med. Chem. Lett.*, **4**, 2741.
[61] Gooley, P.R., O'Connell, J.F., Marcy, A.I., Cuca, G.C., Salowe, S.P., Bush, B.L., Hermes, J.D., Esser, C.K., Hagmann, W.K., Springer, J.P. and Johnson, B.A. (1994). *Nature: Struct. Biol.*, **1**, 111.
[62] Becker, J.W., Marcy, A.I., Rokosz, L.L., Axel, M.G., Burbaum, J.J., Fitzgerald, P.M.D., Cameron, P.M., Esser, C.K., Hagmann, W.K., Hermes, J.D. and Springer, J.P. (1995). *Protein Sci.*, **4**, 1966.
[63] Lovejoy, B., Cleasby, A., Hassell, A.M., Longley, K., Luther, M.A., Weigl, D., McGeehan, G., McElroy, A.B., Drewry, D., Lambert, M.H. and Jordan, S.R. (1994). *Science*, **263**, 375.
[64] Borkakoti, N., Winkler, F.K., Williams, D.H., D'Arcy, A., Broadhurst, M.J., Brown, P.A., Johnson, W.H. and Murray, E.J. (1994). *Nature: Struct. Biol.*, **1**, 106.
[65] Spurlino, J.C., Smallwood, A.M., Carlton, D.D., Banks, T.M., Vavra, K.J., Johnson, J.S., Cook, E.R., Falvo, J., Wahl, R.C., Pulvino, T.A., Wendoloski, J.J., Smith, D.L. (1994). *Proteins: Struct. Funct. Genet.*, **19**, 98.
[66] Durette, P.L. (1995). 2nd World Congress on Inflammation, Brighton, England, September.
[67] Kluender, H.C.E., Benz, G.H.H.H., Brittelli, D.R., Bullock, W.H., Combs, K.J., Dixon, B.R., Schneider, S., Wood, J.E., Vanzandt, M.C., *et. al.* (1996). PCT WO 9615096.
[68] R&D Focus, Drug Updates, (1996). IMS World Publications.
[69] Beckett, R.P., Whittaker, M., Miller, A. and Martin, F.M. (1995). PCT WO 9519956.
[70] Campion, C., Davidson, A.H., Dickens, J.P. and Crimmin, M.J. (1990). PCT WO 9005719.
[71] Broadhurst, M.J., Brown, P.A., Johnson, W.H. and Lawton, G. (1992). European Patent Application EP 497192-A.
[72] Soukup, M. and Wirz, B. (1995). European Patent Application EP 664284-A.
[73] Lewis, E.J., Bishop, J., Bottomley, K., Bradshaw, D., Broadhurst, M.J., Brown, P.A., Greenham, A.K., Hallam, T., Johnson, W.H., Lawton, G. and Nixon, J.S. (1993). *Rev. Esp. Rheumatol.*, **20** (suppl. 1), 494.
[74] Beckett, R.P., Whittaker, M., Miller, A. and Martin, F.M. (1995). PCT WO 9616931.
[75] Talbot, D.C. and Brown, P.D. (1996). *Eur. J. Cancer*, **32A**(14), 2528.
[76] Chen, J.J., Zhang, Y., Hammond, S., Dewdney, N., Ho, T., Lin, X., Browner, M.F. and Castelhano, A.L. (1996). *Bioorg. Med. Chem. Lett.*, **6**(13), 1601.
[77] MacPherson, L.J. and Parker, D.T. (1995). US Patent 5,455,258.
[78] O'Byrne, E.M., Parker, D.T., Roberts, E.D., Goldberg, R.L., MacPherson, L.J., Blancuzzi, V., Wilson, D., Singh, H.N., Ludewig, R. and Ganu, V.S. (1995). *Inflamm. Res.*, **4** (suppl. 2), S177.
[79] Spirito, S., Doughty, J., O'Byrne, E., Ganu, V. and Golberg, R.L. (1995). *Inflamm. Res.*, **44** (suppl. 2), S131.
[80] Ganu, V., Parker, D., MacPherson, L., Hu, S-I., Goldberg, R.A. and O'Byrne, E. (1994). *Osteoarthritis and Cartilage*, **2** (suppl. 1), 34.
[81] Miller, A., Askew, M., Beckett, R.P., Bellamy, C.L., Bone, E.A., Coates, R.E., Davidson, A.H., Drummond, A.H., Huxley, P., Martin, F.M., Saraglou, L., Thompson, A.J., van Dijk, S.E. and Whittaker, M. (1997). *Bioorg. Med. Chem. Lett.*, **7**(2), 193.
[82] Hasty, K.A., Reife, R.A., Kang, A.H. and Stuart, J.M. (1990). *Arthritis. Rheum.*, **33**. 388.
[83] Okada, Y., Shinmei, M., Tanaka, O., Naka, K., Kimura, A., Nakanishi, I., Bayliss, M.T., Iwata, K. and Nagase, H. (1992). *Lab. Invest.*, **66**, 680.
[84] Woessner, J.F. Jr. (1991). *FASEB J.*, **5**, 2145.
[85] Ito, A. and Nagase, H. (1988). *Arch. Biochem. Biophys.*, **267**, 211.
[86] Brinckerhoff, C.E. (1991). *Arthritis Rheum.*, **34**, 1073.
[87] Lewis, E.J., Bishop, J., Bottomley, K.M.K., Bradshaw, D., Brewster, M., Broadhurst, M.J., Brown, P.A., Budd, J.M., Elliott, L., Greenham, A.K., Johnson, W.H., Nixon, J.S., Rose, F., Sutton, B. and Wilson, K. (1997). *British. J. Pharm.*, **121**, 540.
[88] Broadhurst, M.J., Brown, P.A., Lawton, G., Ballantyne, N., Borkakoti, N., Bottomley, K.M., Cooper, M.I., Eatherton, A.J., Kilford, I.R., Malsher, P.J., Nixon, J.S., Lewis, E.J., Sutton, B.M. and Johnson, W.H. (1997). *Bioorg. Med. Chem. Lett.*, in press.

[89] Broadhurst, M.J., Brown, P.A. and Johnson, W.H., (1995). European Patent Application EP 0684240-A.
[90] Arner, E.C., Decicco, C.P., Cherney, R. and Tortorella, M.D. (1997). *J. Biol. Chem.*, **272**(14), 9294.
[91] Fosang, A.J., Last, K. and Maciewicz, R.A. (1996). *J. Clin. Invest.*, **98**(10), 2292.
[92] Brown, C.J., Rahman, S., Morton, A.C., Beauchamp, C.L., Bramwell, H. and Buttle, D.J. (1996). *J. Clin. Pathol: Mol. Pathol.*, **49**, M331.
[93] Eugui, E.M., Mirkovich, A., De Lustro, B., Haller, J., Rouhafza, S., Kim, Y., Broka, C.A., Walker, K.A.M., Hendricks, R.T., Lollini, L., Martin, R., Van Wart, H.E. and Caulfield, J.P. (1996). TIMP96 conference, Banff, Alberta, Canada, September 25–29.
[94] Black, R.A., Rauch, C.T., Kozlosky, C.J., Peschon, J.J., Slack, J.L., Wolfson, M.F., Castner, B.J., Stocking, K.L., Reddy, P., Srinivasan, S., Nelson, N., Boiani, N., Schooley, K.A., Gerhart, M., Davis, R., Fitzner, J.N., Johnson, R.S., Paxton, R.J., March, C.J. and Cerretti, D.P. (1997). *Nature* (*Lond.*), **385**, 729.
[95] Moss, M.L., Jin, S-L.C., Milla, M.E., Burkhart, W., Carter, H.L., Chen, W-J., Clay, W.C., Didsbury, J.R., Hassler, D., Hoffman, C.R., Kost, T.A., Lambert, M.H., Leesnitzer, M.A., McCauley, P., McGeehan, G., Mitchell, J., Moyer, M., Pahel, G., Rocque, W., Overton, L.K., Schoenen, F., Seaton, T., Su, J-L., Warner, J., Willard, D. and Becherer, J.D. (1997). *Nature* (*Lond.*), **385**, 733.
[96] Gearing, A.J.H., Beckett, P., Christodoulou, M., Churchill, M., Clements, J., Davidson, A.H., Galloway, W.A., Gilbert, R. *et al.* (1994). *Nature* (*Lond.*), **370**, 555.
[97] McGeehan, G.M., Becherer, J.D., Bast, R.C. Jr., Boyer, C.M., Champion, B., Connolly, K.M., Conway, J.G., Furdon, P., Karp, S. *et al.* (1994). *Nature* (*Lond.*), **370** 558.
[98] Mohler, K.M., Sleath, P.R., Fitzner, J.N., Cerretti, D.P., Alderson, M., Kerwar, S.S., Dauphine, S.T., Otten-Evans, C., Greenstreet, T. *et al.* (1994). *Nature* (*Lond.*), **370**, 218.