Recent advances in matrix metalloproteinase inhibitor research

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The matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that degrade all of the major components of the extracellular matrix. Over-expression and activation of MMPs have been linked with a range of diseases for which good therapeutic approaches are currently sought, such as arthritis, cancer and multiple sclerosis. Inhibition of MMPs has therefore become the focus of considerable interest, and potential therapeutic applications continue to grow. Orally active, broad-spectrum inhibitors have been identified, and some of these are undergoing clinical evaluation. Structural information on MMP–inhibitor complexes is now available, enabling the structure-based design of selective MMP inhibitors.

The matrix metalloproteinases (MMPs) are a family of potentially very damaging enzymes, and their activity is closely regulated in nature. Expression is under tight control by pro- and antiinflammatory cytokines and/or growth factors and, once produced, the enzymes are usually secreted as inactive zymogens. Upon activation (removal of the inhibitory propeptide region of the molecules) they are subject to control by locally produced inhibitors (the tissue inhibitors of metalloproteinases or TIMPs, of which there are at least three types)[1,2]. The hallmark of diseases involving the enzymes appears to be a stoichiometric imbalance between active enzymes and TIMPs, leading to excessive tissue disruption, and often degradation[3]. It is this imbalance that a pharmacological approach, based on specific enzyme inhibitors, promises to redress.

The MMP family has grown substantially since commercial projects based on inhibition of this type of enzyme were first initiated, probably at G.D. Searle in the early 1980s. The target enzyme then was 'collagenase', a partially characterized enzyme with the ability to hydrolyse triple-helical collagen at neutral pH, which was thought to be involved in the loss of bone and cartilage in rheumatoid arthritis. We now know that there are at least 11 cloned family members, and others are emerging regularly. The allocation of MMPs into subfamilies is somewhat arbitrary because the true physiological substrates for the individual enzymes remain a matter of debate. Three 'collagenases' have, however, now been identified on the basis of sequence similarities and their ability to hydrolyse the fi-brillar collagens (types I, II and III); these are fibroblast and neutrophil collagenase (MMP 1 and MMP 8, respectively)[1,2] and collagenase 3 (MMP 13), recently cloned from a human breast cancer cDNA library[4]. A second subfamily of MMPs comprising stromelysins 1 and 2 (MMP 3 and MMP 10) and matrilysin (MMP 7) can be identified on the basis of their predilection for nonfibrillar collagens, laminins and fibronectins as substrates[1,2]. A third subfamily, containing 72 kDa gelatinase (gelatinase A; MMP 2) and 92 kDa gelatinase (gelatinase B; MMP 9), also exists; members of this group are

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characterized by their preference for the basement membrane collagens (types IV and V)[1,2]. The three other cloned members, metalloelastase (MMP 12), membrane-type MMP (MT-MMP; MMP 14) and stromelysin 3 (MMP 11), do not fit neatly into any of these subfamilies, although the elastin-hydrolysing properties of metalloelastase[5] are shared by matrilysin and 92 kDa gelatinase[6]. The recent finding that 72 kDa gelatinase may act as a 'collagenase' (hydrolysing triple-helical collagen) when rendered free of TIMP (Ref. 7) emphasizes the considerable substrate overlap between MMP enzymes, at least *in vitro*.

Two of the most recently identified enzymes, MT-MMP and stromelysin 3, are interesting in that they illustrate how disparate the family members can be and how much remains to be learned about MMP biology. Only MT-MMP (Ref. 8) and stromelysin 3 (Ref. 9) have been shown to contain an insert bearing a furin-processing motif (Arg–Xaa–Lys–Arg). In principle, this should enable both enzymes to mature in the Golgi and to appear on or at the cell surface as active proteinases, rather than as inactive zymogens [as demonstrated for stromelysin 3 (Ref. 10)]. Moreover, MT-MMP, which can activate pro-72 kDa gelatinase[8], is the only member of the family so far identified that is not a secreted product. It contains a membrane-spanning domain and, on its cytoplasmic side, a protein kinase C phosphorylation motif (Arg–Xaa–Xaa–Thr*–Xaa–Arg)[11], perhaps indicating that its activity is subject to intracellular regulation. Even more fundamentally opposed to MMP dogma, stromelysin 3 has no activity towards any extracellular matrix substrate yet examined but hydrolyses (and hence inactivates) some serpins (serine proteinase inhibitors), such as α_1-antiproteinase and α_2-antiplasmin[12]. In principle, this should lead to activation of some serine proteinases and predispose towards haemostatic disorders and to the secondary sequelae of inflammatory conditions, such as emphysema. The serpinase activity of stromelysin 3, observed *in vitro*, is shared by most, if not all, MMP family members but, as with stromelysin 3, the physiological and pathological roles of these hydrolytic actions *in vivo* remain unclear[13–15].

The ability of the MMP family to hydrolyse non-matrix substrates is further emphasized by the fact that a range of MMPs can hydrolyse the membrane-bound precursor of the pro-inflammatory cytokine tumour necrosis factor α (TNF-α)[16]. This extracellular cleavage yields mature soluble TNF-α and the inhibitors of MMPs can block production of TNF-α both *in vitro* and *in vivo*[16–18]. This important pharmacological action is a probable contributor to the antiarthritic action of this class of compounds seen in animal models[19] and to their ability to protect mice from the lethal effects of bacterial endotoxin[17]. It considerably broadens the potential uses of MMP inhibitors, embracing diseases not generally considered to be associated with matrix degradation. It is unclear whether the so-called 'TNF convertase(s)' is a known MMP or a novel specific enzyme. Even if a single TNF convertase operating under normal physiological conditions can be identified, it is possible that, under pathological conditions in which many MMPs are overexpressed, any of a number of MMPs might drive the production of soluble TNF-α.

A central issue in this field is whether broad-spectrum MMP inhibitors or selective inhibitors, targeted against individual MMP enzymes, represent the most appropriate pharmacological strategy. Selective inhibitors against some MMP family members have been identified and, in principle, should provide greater specificity, and hence safety, than broad-spectrum MMP inhibitors. This assumes, however, that a particular enzyme can be unequivocally identified as the cause of a particular disease. Such data are very difficult to obtain and, in general, human and animal studies using a range of approaches (histological, immunological, molecular biological or pharmacological) have revealed that in disease states, some MMP family members (with overlapping substrate selectivities) are often co-expressed[3,20,21].

Pseudopeptide inhibitor design and structure–activity relationships

Two approaches to the identification of MMP inhibitors have been followed: substrate-based design of pseudopeptide derivatives and random screening of compound libraries and natural products. The initial guide for substrate-based design was the sequence around the glycine–isoleucine and glycine–leucine cleavage sites in the collagen molecules that are hydrolysed by collagenase (Figure 1). The key to obtaining potent enzyme inhibition has been the incorporation of a zinc-binding group (ZBG), to chelate the active-site zinc(II) ion, into peptide analogues of the sequence on either the left-hand side (LHS) or the right-hand side (RHS), or both sides, of the cleavage site (Figure 1). At an early stage, it was found that RHS inhibitors featuring a hydroxamic acid ZBG, such as **1** (Searle; the structures of the compounds discussed are shown in Figure 2), are particularly potent in terms of their *in vitro* activity[22]. Considerable insight into MMP–ligand interactions has been obtained from the study of inhibitor structure–activity relationships (SAR)[23,24], elegant studies of enzyme–substrate specificity[25–28] and peptidyl inhibitors based on the MMP prodomain region[29]. The use of high-resolution X-ray

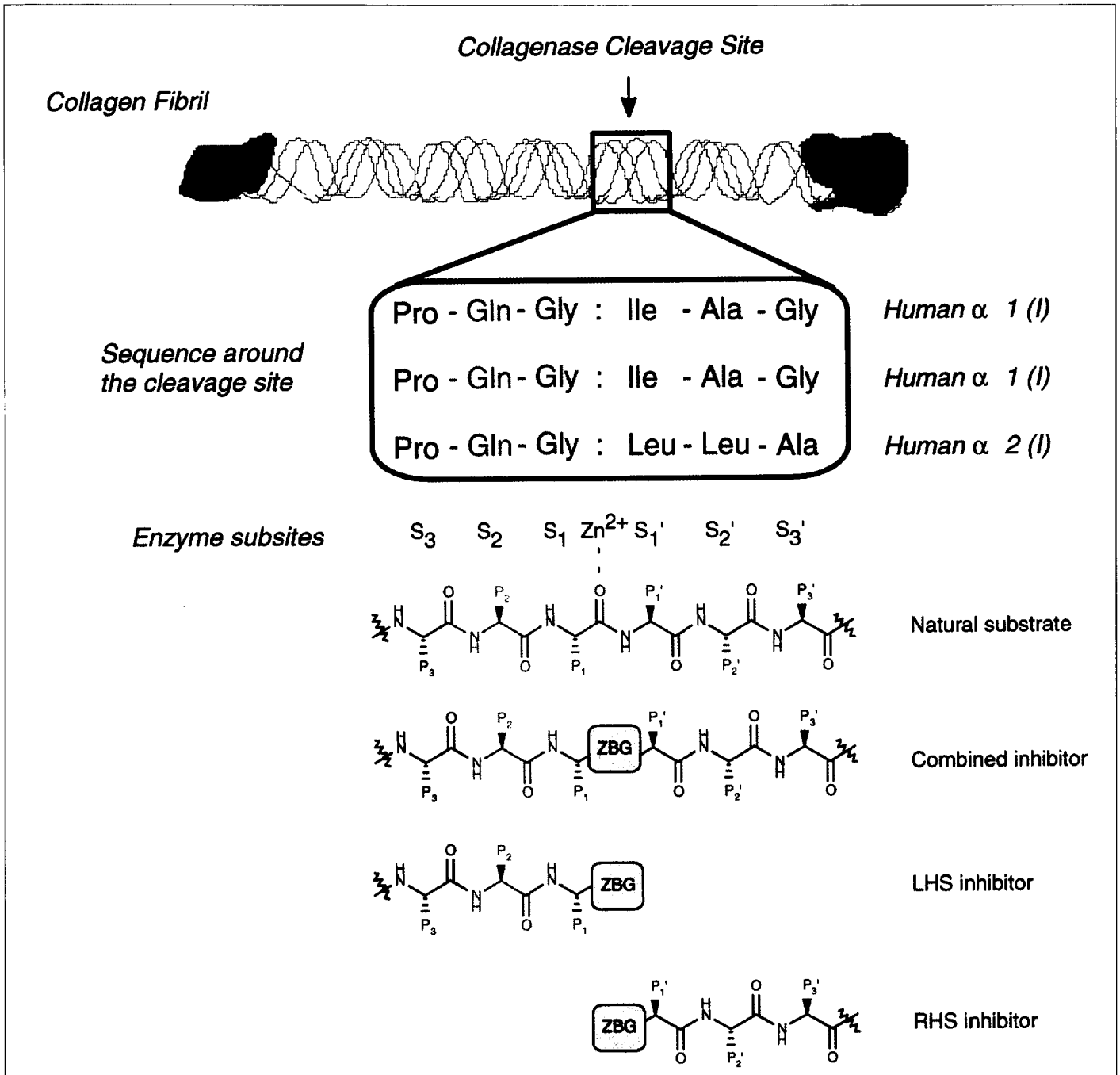

Figure 1. *Design of matrix metalloproteinase inhibitors on the basis of the cleavage site of collagen.*

crystallography[30–37] and NMR spectroscopy[38] in the elucidation of structures (Box 1) is, however, now providing new paradigms for the design of inhibitors in general and selective inhibitors in particular. The SAR for RHS inhibitors such as **1** and analogues (Figures 2 and 3; Table 1) is discussed below in the light of recent structural information (Box 1).

Zinc-binding and P_1 groups

The selection of suitable ZBGs has been the subject of intense research, and several different ZBGs (hydroxamate, carboxylate, aminocarboxylate, sulphydryl and derivatives of phosphorus acids) have been identified[23,24]. A comparison of different ZBGs suggests the following preference in terms of

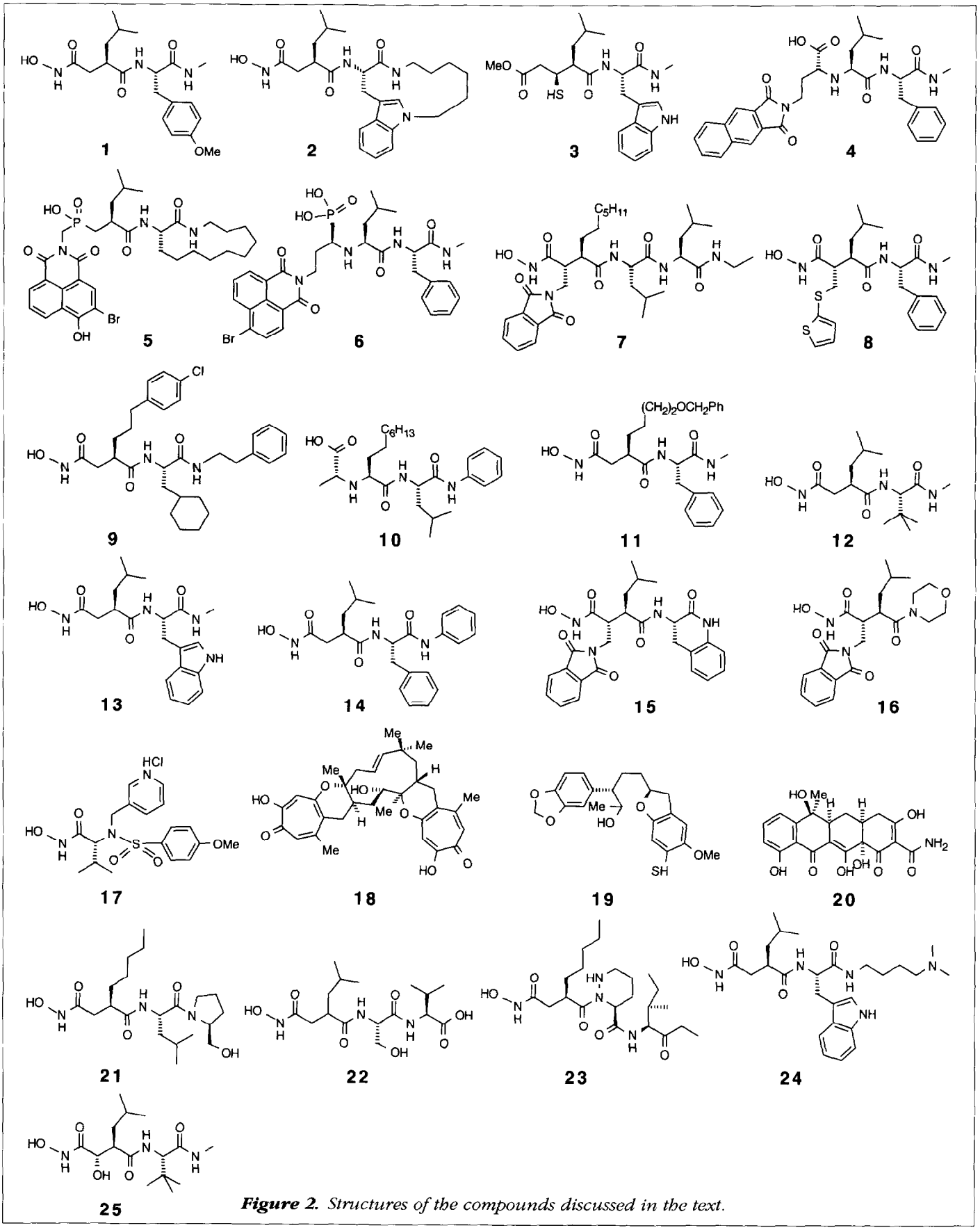

Figure 2. *Structures of the compounds discussed in the text.*

Box 1. Structure of matrix metalloproteinases (MMPs)

X-ray structures of neutrophil collagenase and fibroblast collagenase have been published by several groups[30–35], and an NMR structure of stromelysin 1 is also in the public domain[38]. Superposition studies with the collagenase and stromelysin 1 structures show that they have an almost identical tertiary fold; the only significant differences in the region of the active site arise from a small number of amino acid substitutions and insertions.

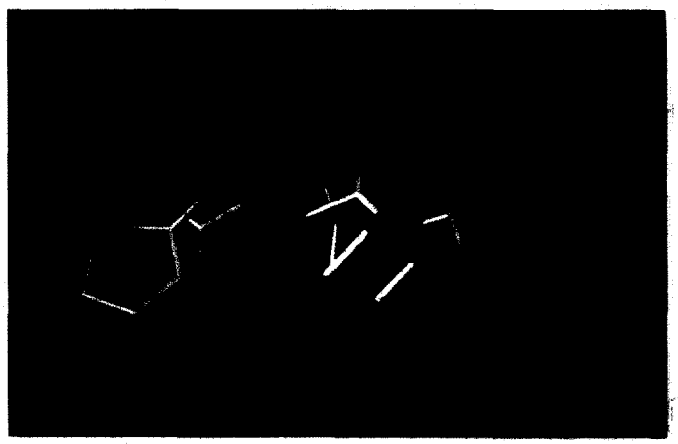

Figure I. *X-ray crystal structure of neutrophil-collagenase–batimastat complex[37] viewed with a Connoly surface (green); the active-site zinc(II) (magenta) lies behind the thienyl P_1 group. Note the deep S_1' pocket going into the plane of the page and the P_2' phenylalanine side chain sitting in the S_2' cleft and exposed to solvent.*

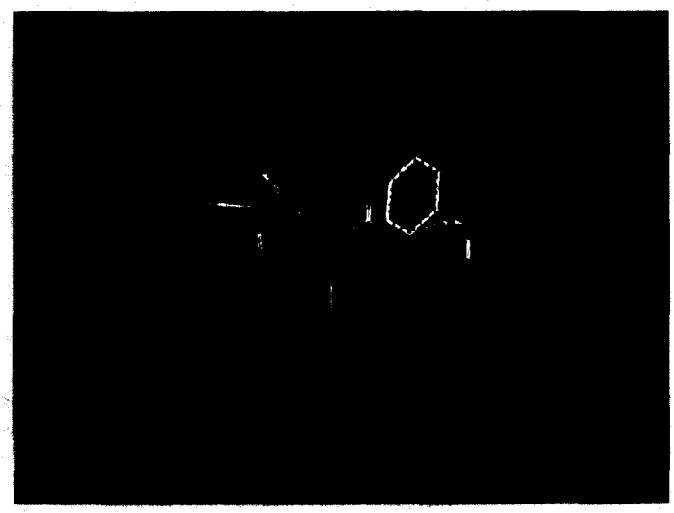

Figure II. *Hydrogen bonding interactions between batimastat (**8**) and the β4 sheet (green) and the proline turn (blue) and hydroxamate chelation of zinc(II) (magenta) for the neutrophil collagenase inhibitor complex[37].*

Figures I and II illustrate the binding of the hydroxamate inhibitor batimastat (**8**) to the active site of neutrophil collagenase as determined by X-ray crystallography[37]. The active site comprises a cleft between a large upper N-terminal domain and a smaller lower C-terminal domain. The upper surface of the cleft is formed by the backbone and side chain groups of the β4 sheet and the lower surface by turn residues (the 'proline-turn' residues) separating helix II (the zinc-binding helix) and helix III. The floor of the cleft is formed by helix II, which includes the zinc-binding HEXXH residues characteristic of the MMP family.

The S_1' subsite is a deep hydrophobic pocket penetrating into the surface of the enzyme to the right of the catalytic zinc. There is significant variation in the amino acids that form this pocket between the individual MMPs; in particular, the pocket is shorter for fibroblast collagenase. The S_2' subsite is a shallow cleft exposed to solvent, formed on the upper side by the β4 residues Gly158–Ile159 and on the lower side by the 'proline-turn' residues Pro217 and Asn218. The active-site cleft opens out in the S_3' region to provide a shallow 'pocket'. To the left of the active-site zinc(II) ion is an open region with no real S_1 or S_2 pockets, although the S_3 pocket is well defined and ideally shaped to accommodate the P_3 proline residue found in substrates.

inhibition of fibroblast collagenase: hydroxamate (e.g. **2**; Syntex) >> formylhydroxylamine > sulphydryl > phosphinate > aminocarboxylate > carboxylate[39]. Comparison of X-ray crystal structures of **2** and its corresponding carboxylate and sulphodiimine analogues bound to matrilysin emphasizes the dominant role the ZBG plays in determining the inhibitory potency[36]. The hydroxamate acts as a bidentate ligand with each oxygen an optimum distance (1.9–2.3 Å) from the active-site zinc(II) ion. The position of the hydroxamate nitrogen suggests that it is protonated and forms a hydrogen bond with a carbonyl oxygen of the enzyme backbone of neutrophil collagenase[32,37], fibroblast collagenase[34] and matrilysin[36].

Potency may be enhanced by a P_1 substituent: a β-carbonyl group increases the activity of sulphydryl compounds (e.g. **3**; SmithKline Beecham)[40], and a cyclic imido substituent increases the activity of amino carboxylates (e.g. **4**; Glaxo)[41], phosphinates (e.g. **5**; Ro 31-7467; Roche)[42], phosphonic acids (e.g. **6**; SmithKline Beecham)[43] and hydroxamates (e.g. **7**; Roche)[44,45]. The role of the cyclic imido group is not entirely clear. In fibroblast collagenase S_1 asparagine

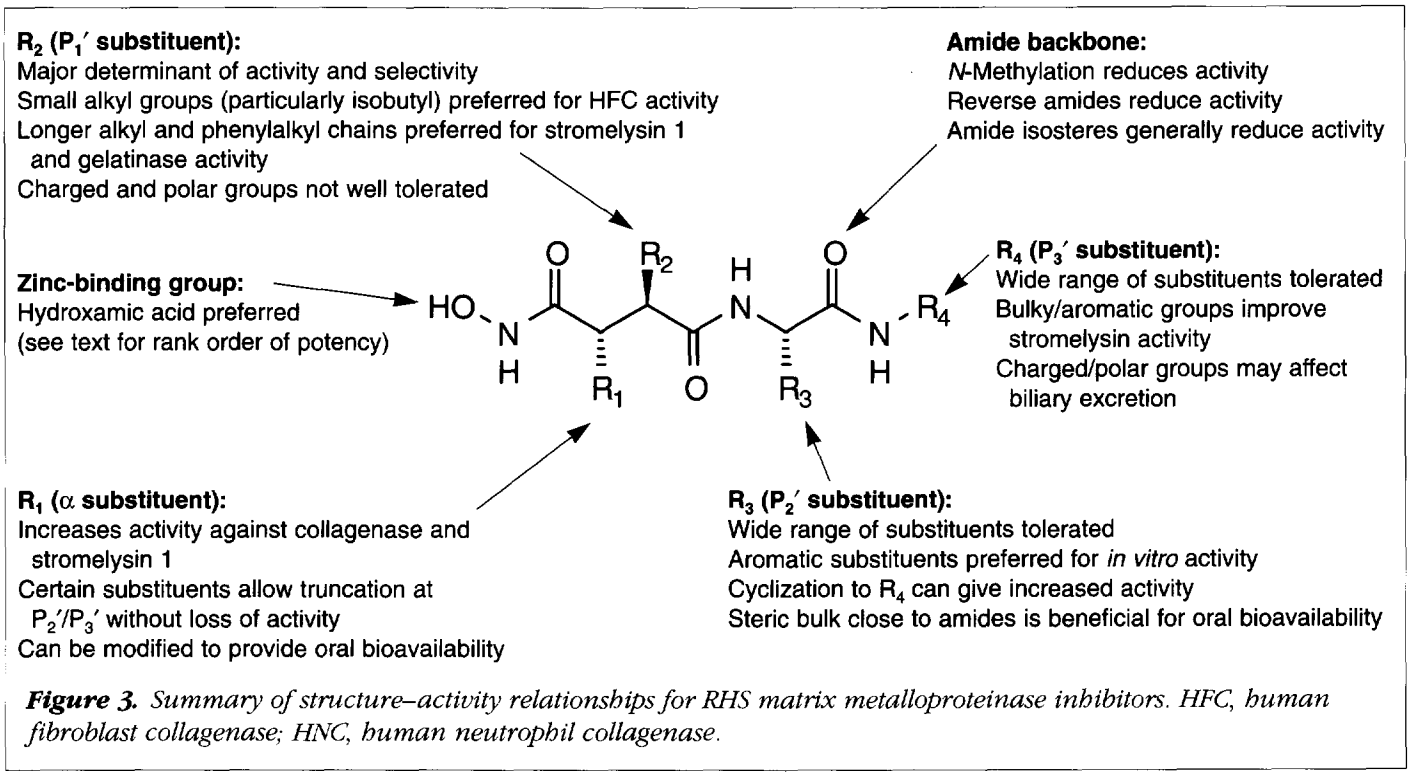

Figure 3. *Summary of structure–activity relationships for RHS matrix metalloproteinase inhibitors. HFC, human fibroblast collagenase; HNC, human neutrophil collagenase.*

(Asn180; residue 159 in Table 2) and histidine (His183) residues have been shown, by analysis of an X-ray crystal structure, to hydrogen bond to the carbamate carbonyl group of an amino carboxylate inhibitor possessing a P_1 benzyloxycarbonylaminoalkyl substituent[30]. It has been speculated that one of the carbonyl groups of a P_1 cyclic imido group could also hydrogen bond to the S_1 asparagine in fibroblast collagenase[43]. However, cyclic imido P_1 substituents also increase activity against stromelysin 1 (Refs 41,45), where the corresponding residue is valine. Furthermore, lipophilic P_1 groups[45,46] that cannot take part in hydrogen bonding improve activity against both fibroblast collagenase and stromelysin 1 (e.g. **8**; batimastat; British Biotech)[46].

P_1' group

The S_1' pocket, of all the subsites, appears to offer the greatest opportunity for selective inhibitor design because there is considerable variation between the MMPs in the residues that line the pocket. For example, the location of a single arginine residue is critical in determining the depth of the pocket in neutrophil and fibroblast collagenase[32]. In both enzymes, an arginine side chain lines the bottom of the pocket, and modelling studies suggest that the guanidine groups lie in the same region of space. In neutrophil collagenase, the arginine residue (Arg222) is exposed to solvent on the proline turn, whereas in fibroblast collagenase, the arginine residue (Arg214; residue 193 in Table 2) is located on the zinc-binding helix in the interior of the protein[32,35]. The exposed arginine side chain of neutrophil collagenase can thus rotate out of the pocket to avoid long P_1' groups, whereas the core arginine residue of fibroblast collagenase cannot and forms a rigid cap to the bottom of the pocket[32,35]. This difference is clearly seen in published SAR data which shows that neutrophil collagenase tolerates larger P_1' groups than fibroblast collagenase. Structural studies and homology modelling indicate that the matrilysin S_1' pocket is possibly occluded by a tyrosine side chain (Tyr214; residue 194 in Table 2). There is a valine residue at this position in stromelysin 1, 72 kDa and 92 kDa gelatinase (Table 2). The small size of the valine side chain results in a deep pocket, the end of which is exposed to solvent[47]. This also is reflected in SAR data showing that these enzymes tolerate large hydrophobic P_1' side chains: a P_1' 3-phenylpropyl group provides selective inhibition of 72 kDa gelatinase over fibroblast collagenase and stromelysin 1 for carboxylates, phosphonates and hydroxamates (e.g. **9**; Celltech)[48,49]; for amino carboxyls (e.g. **10**; Merck) a P_1' *n*-octyl group provides selective inhibition of 72 kDa gelatinase and stromelysin 1 over fibroblast collagenase[50]; for hydroxamates a similar length alkyl chain at P_1' (e.g. **7**; Roche) enhances inhibition of stromelysin 1 and 92 kDa gelatinase[45];

Table 1. *In vitro* activities of matrix metalloproteinase inhibitors[d,e]

Compound[a]	HFC MMP 1	HNC MMP 8	72 kDa Gel MMP 2	92 kDa Gel MMP 9	Stromelysin 1 MMP 3	Refs
1	20	–	–	–	–	22
2	0.1[b]	0.4[b]	–	0.2[b]	9[b]	39
3	2.5	–	–	–	–	40
4	20	–	–	5	91	41
5	17.6	–	239	–	–	42
6	20	–	–	–	–	43
7	–	–	–	–	0.59	45
8	3	10	4	1	20	46
9	2440[b]	–	0.03[b]	–	7.31[b]	48
10	>10 000[b]	–	340[b]	–	570[b]	50
11	1450[b]	2[b]	–	3.3[b]	15[b]	47,51
12	10	–	8	–	700	58
13	2	–	–	0.2	28	54
14	20	–	60	–	10	58
15	0.0025	–	18	–	23	60
16	10	–	400	–	4500	61
18	–	–	–	–	31000	64
19	75[c]	–	–	44[c]	600	65
20	510 000	31 000	–	–	–	67
21	190	300	–	330	1700	68
22	–	–	590	850	650	69
23	–	–	1 700	570	350	70
24	3[b]	–	–	–	–	71
25	5	–	6	3	200	74

[a]No data are available for compound **17**

[b]K_i values (nM)

[c]Inhibition (%) at 10 μM

[d]Expressed as IC_{50} nM values except where indicated

[e]HFC, human fibroblast collagenase; HNC, human neutrophil collagenase; Gel, gelatinase

and hydroxamates featuring benzyloxyalkyl P_1' substituents (e.g. **11**; Sterling Winthrop) are 100–1000-fold more potent inhibitors of neutrophil collagenase, stromelysin 1 and 92 kDa gelatinase than of fibroblast collagenase[47,51].

Attempts to obtain fibroblast collagenase-selective inhibitors by the introduction of a group at P_1' that can interact with the arginine S_1' pocket 'cap' have been unsuccessful[51]. As might be expected, there is a preferred orientation for the P_1' group relative to the ZBG, which can now be rationalized by structural information. For example, incorrect conformational restraint of the P_1' group of **1** results in a loss of inhibitory activity[52,53].

P_2' and P_3' groups

Various α-amino acid residues can be tolerated at P_2', which suggests that the P_2' side chain does not play a dominant role in enzyme binding. For fibroblast collagenase, however, a bulky *tert*-butyl group at P_2', as in Ro 31-9790 (**12**; Roche), enhances inhibition in comparison with other P_2' alkyl groups[44], and a P_2' tryptophan, as in Galardin™ (**13**; Glycomed)[54] and in the thiol-based inhibitor **3** (Ref. 40), is also advantageous. For neutrophil collagenase, the S_2' subsite is a narrow cleft formed on the upper side by two residues of the β4 sheet (Gly158–Ile159) and on the lower side by two residues on the 'proline turn' (Pro217–Asn218)[32,33,35,37]. Although the proline residue is conserved, the remaining residues are not conserved either in sequence identity or in similarity between the various MMPs. It is unclear how significant these amino acid substitutions are for either substrate or inhibitor specificity. Because all four side chains are exposed to solvent, and the P_2' side chain is also exposed to solvent, the selectivity which can be obtained by exploiting these

Table 2. Alignment of residues in the P_1', P_2' and P_3' pockets of human neutrophil collagenase (HNC), human fibroblast collagenase (HFC), stromelysin 1, 72 kDa and 92 kDa gelatinases and matrilysin[a,b]

Pocket	Matrix metalloproteinase	Residue					
		157	**158**	**160**	**188**	**189**	**219**
P_3'	HNC	Asn	Gly	Leu	Asn	Tyr	Tyr
P_3'	HFC	Gly	Gly	Leu	Glu	Tyr	Tyr
P_3'	Stromelysin 1	Gly	Asn	Leu	Gly	Thr	Tyr
P_3'	72 kDa Gelatinase	Asp	Gly	Leu	Gly	Tyr	Tyr
P_3'	92 kDa Gelatinase	Asp	Gly	Leu	Gly	Tyr	Tyr
P_3'	Matrilysin	Gly	Asn	Leu	Gly	Gly	Tyr
		158	**159**	**217**	**218**		
P_2'	HNC	Gly	Ile	Pro	Asn		
P_2'	HFC	Gly	Asn	Pro	Ser		
P_2'	Stromelysin 1	Asn	Val	Pro	Leu		
P_2'	72 kDa Gelatinase	Gly	Leu	Pro	Ile		
P_2'	92 kDa Gelatinase	Gly	Leu	Pro	Met		
P_2'	Matrilysin	Asn	Thr	Pro	Thr		
		193	**194**	**197**	**219**	**220**	**222**
		H2	H2	H2	T	T	T
P_1'	HNC	Leu	Val	His	Tyr	Ala	Arg
P_1'	HFC	Arg	Val	His	Tyr	Thr	Ser
P_1'	Stromelysin 1	Leu	Val	His	Tyr	His	Leu
P_1'	72 kDa Gelatinase	Leu	Val	His	Tyr	Thr	Thr
P_1'	92 kDa Gelatinase	Leu	Val	His	Tyr	Arg	Thr
P_1'	Matrilysin	Leu	Tyr	His	Tyr	Gly	Gly

[a]Residue numbering follows the human neutrophil collagenase numbering convention used in Ref. 32
[b]Residues in the P_1' pocket are assigned to either helix II (H2) or the proline turn (T)

differences is likely to be modest. Although the S_2' subsite is a shallow cleft, the inhibition of stromelysin 1 by simple tryptophan derivatives lacking an obvious ZBG (e.g. Cbz–L–Trp–OH; Parke-Davis) has been explained in terms of binding to this subsite[55].

At P_3', a methyl group is usually preferred, although a P_3' phenyl group can enhance inhibition of stromelysin 1 for aminocarboxyls[56], phosphinates[57] and hydroxamates (e.g. **14**; British Biotech)[58] and of matrilysin[59]. This could reflect an increase in the size of the stromelysin 1 S_3' pocket caused by the substitution of the neutrophil collagenase Asn188–Tyr189 residues with the smaller Gly192–Thr193 amino acids (residues 188 and 189 respectively in Table 2) in the stromelysin 1 sequence.

Early SAR data derived for RHS MMP inhibitors suggested that the backbone amide bonds are important for inhibition[23], and it has subsequently been discovered from structural studies that the P_1'–P_2' C=O and N–H and the P_2'–P_3' C=O and N–H are all involved in hydrogen bonding interactions with the enzyme (Box 1). When the P_2' and P_3' groups form a cyclic lactam, a correlation is found between the increase in inhibition of fibroblast collagenase observed with the larger rings (as in Ro 31-7467) and the amount of *trans* amide[23], which is the required geometry for effective hydrogen-bonding interactions between enzyme and inhibitor. Indolactam cyclization between P_2' and P_3' (e.g. **2**; Syntex) also confers *trans* amide geometry and increases activity by at least ten-fold relative to that of the acyclic analogues[39]. High potencies are, however, reported for carbostyril derivatives (e.g. **15**; Otsuka) in which the amide geometry is *cis*[60], and nonpeptide compounds that lack not only the P_2'–P_3' C=O and N–H but also the P_1'–P_2' N–H (Refs 41,61–63). The presence of a cyclic imide group at P_1 enables inhibition of fibroblast collagenase to be maintained for aminocarboxyls[41] and hydroxamates (e.g. **16**; Roche)[61] in which the P_2' amino acid is deleted or replaced by a nitrogen heterocycle.

These recent results suggest that, at least for fibroblast collagenase, the hydrogen-bonding interactions provided by a P_2'–P_3' amide are not an absolute requirement for activity if other interactions are introduced. Interestingly, sulphonamide derivatives that also lack a P_2' amino acid (e.g. **17**; CGS 27023A; Ciba) are claimed to be potent, and orally active, inhibitors of stromelysin 1 (Refs 62,63).

Natural products

Natural product MMP inhibitors discovered by screening include pycnidione (**18**; Merck)[64], futoenone derivatives (e.g. **19**; OsteoArthritis Sciences)[65] and tetracyclines, such as aranciamycin and minocycline, for which chemical modification (e.g. **20**; CMT 1) has enabled the separation of MMP activity from antibiotic activity[66,67]. It is possible that, for such compounds, a ring hydroxyl and/or carbonyl chelates the active-site zinc(II) ion because for the futoenone derivatives, replacement of a ring carbonyl by a sulphydryl, as in **19**, enhances inhibition of stromelysin 1, presumably as a result of stronger zinc(II) ion chelation[65].

The more potent natural products, actinonin (**21**; Rhone-Poulenc)[68], BE16627B (**22**; Banyu)[69] and matlystatin B (**23**; Sankyo)[70], are structurally similar to the RHS hydroxamates obtained by substrate-based design. Except for these pseudopeptides, however, the screening of natural products has provided only moderately active leads, and in the absence of structural information revealing their interaction with the enzyme active site, it is unclear how best to optimize such compounds.

Orally active inhibitors

The discovery of MMP inhibitors with good oral bioavailability has been a high priority because most of the potential clinical indications would require chronic therapy. Excellent *in vitro* potencies have been reported for pseudopeptide MMP inhibitors but the early lead compounds, without exception, had poor oral bioavailability. The factors that could limit the oral bioavailability of pseudopeptide MMP inhibitors include degradation by digestive enzymes within the gut, poor absorption across the gastrointestinal mucosa and extraction by the liver (first-pass metabolism), all of which could be affected by the physicochemical properties of the molecule (aqueous solubility, log*P* and molecular weight). It has, for example, been hypothesized that the high biliary excretion rate of the hydroxamate inhibitor **1** could be reduced by the introduction of a basic group at P_3' (Ref. 71). From the synthesis of a series of analogues of **1**, it was found that for the tertiary amino analogue (**24**; Sterling Winthrop) the plasma half-life in rats was increased threefold and the biliary excretion reduced by more than fourfold, resulting in an oral bioavailability of 8.5%, while maintaining *in vitro* inhibitory activity[71]. In a separate study, it was reported that a P_3' morpholino alkyl group enhanced oral activity[72].

Conradi and coworkers have shown in studies of the passage of small peptides across confluent monolayers of Caco-2 cells (as a model of the gastrointestinal mucosa) that it is the amide backbone, and particularly the number of hydrogen bond donors, that limits absorption[73]. It was suggested that the energy barrier caused by desolvation of the peptide backbone on passage from the aqueous environment to the lipid environment of the membrane is a determining factor for absorption[73]. This energy barrier should be lowered by introducing groups that can participate in internal hydrogen bonding and/or shield the amide backbone such that hydration is disrupted but enzyme interactions are unaltered. Indeed, the replacement of the α-thienylthiomethylene substituent of batimastat (**8**) with an α-hydroxy group, which may participate in internal hydrogen bonding, improves oral bioavailability[74]. Furthermore, the combination of a P_2' *tert*-butyl (an amide shielding device) with the favourable α-hydroxy substituent has, a synergistic effect on oral absorption of the compound BB-2516 (**25**; British Biotech)[74]. A P_2' *tert*-butyl group alone can also impart a degree of oral absorption. This is a feature of Ro 31-9790 (**12**; Roche)[44] and has been incorporated into other inhibitors[45,51,59,75], presumably to achieve oral activity.

Clinical trials

Four pseudopeptide MMP inhibitors are known to have been evaluated in the clinic: Galardin™ (**13**; Glycomed), Ro 31-9790 (**12**; Roche), batimastat (**8**; British Biotech) and BB-2516 (**25**; British Biotech). Tetracycline and its derivatives minocycline and doxycycline, which are only weakly active MMP inhibitors, have also been evaluated in periodontal disease and rheumatoid arthritis. In periodontal disease, the presence of bacteria results in the release of MMPs, and the dual antibiotic and MMP inhibitory activity of the tetracyclines appears to be of benefit[76]. Mixed results, however, have been obtained from trials of tetracyclines in arthritis; mino-cycline was found to be of benefit in an open study in rheumatoid arthritis, whereas no benefit was observed following tetracycline treatment in an earlier double-blind placebo-controlled trial[77].

The potent pseudopeptide MMP inhibitors may hold more promise for the treatment of arthritis because they are

effective in animal models of arthritis[19,77]. It has recently been observed that antibodies to TNF-α have a significant clinical benefit in rheumatoid arthritis[78] and that several MMP inhibitors will block TNF-α production[16–18]. Roche selected Ro 31-9790 (**12**) for development for arthritis and have recently completed a Phase I trial but no data have been published[79].

Galardin™ (**13**; Glycomed) is currently in clinical development as a topical treatment for corneal ulceration[54]. Studies in rabbits had shown that Galardin™ reduced the degree of corneal damage following either chemical or bacterial challenge[54]. A Phase I trial was performed in normal volunteers where it was shown that concentrations of up to 800 μg ml^{-1} given for 14 days (24 drops in 12 h) produced no significant adverse reactions[54]. A Phase II/III clinical trial in patients with corneal ulcers caused by bacterial infection has been completed but a US NDA has yet to be filed[79].

Celltech may have suspended development of the gelatinase-selective compound CDP-845 as a potential cancer therapy[79]. The structure of this compound and any preclinical results have not been disclosed. The broad-spectrum MMP inhibitor batimastat (**8**; British Biotech) was selected for development as an anticancer agent on the basis of promising results in models of metastasis[80], angiogenesis[81] and tumour progression[82–84]. In particular, intraperitoneal administration of batimastat was very effective at resolving the malignant ascites that formed in the peritoneal cavity in a murine xenograft model of human ovarian carcinoma; an increase in survival was recorded[82]. These results led to a study (started 1993) of batimastat in patients with symptomatic malignant ascites; the compound was administered as a suspension (150–1350 mg m^{-2}) in dextrose into the peritoneal cavity following drainage. Initial results indicated that the compound was reasonably well tolerated both locally and systemically. This form of administration gave rise to high and sustained plasma concentrations. A single injection of 1000 mg m^{-2} produced peak batimastat levels of 1000 ng ml^{-1} falling to 100–200 ng ml^{-1} after 28 days[85]. This exceeds the levels of batimastat associated with efficacy in animal models of cancer[84]. A second study with batimastat (intrapleural administration) has also been performed in patients with malignant effusion in the pleural cavity[86]. Although these were both Phase I studies, signs of efficacy were observed in that the frequency of drainage was lower than that seen before patients entered the study. Phase II studies are now in progress.

An earlier Phase I study of batimastat in breast cancer patients using an oral formulation resulted in relatively low blood levels, but significant oral absorption has since been

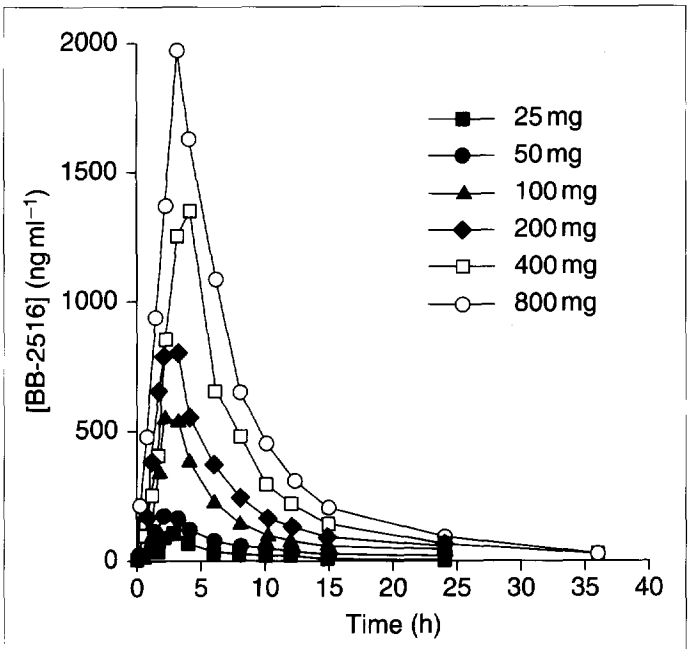

Figure 4. *Plasma concentrations of BB-2516 in human volunteers after a single oral dose.*

obtained in healthy volunteers with the broad-spectrum follow-up compound BB-2516 (**25**; British Biotech)[87]. This Phase I single-dose rising study was initiated in 1994 and indicated a terminal elimination half-life for BB-2516 of approximately 9.5 h (Figure 4). In a second study in healthy volunteers, the compound was given twice daily for 6 days at doses of 50, 100 and 200 mg. The drug was well tolerated, and the 50 mg dose resulted in levels which were maintained above the IC_{90} for fibroblast collagenase throughout the 6-day period[88]. Pilot Phase II studies in a variety of tumour types were initiated in March 1995.

Conclusion

MMP inhibitors are at an interesting stage of development. It is becoming clear that MMP or MMP-like enzymes are involved in processes other than connective tissue breakdown, such as TNF-α production. Inhibitors could, therefore, have a role in the treatment of disease states such as multiple sclerosis, cachexia and type II diabetes, as well as cancer, arthritis and cardiovascular disorders where aberrant matrix degradation is already known to occur. The advances in the understanding of inhibitor SAR coupled with structural data from NMR spectroscopy and X-ray crystallography may assist in the design of inhibitors selective for individual members of this family of enzymes. Thus it may be possible to determine whether particular enzymes are important in different disease states.

In animal models of cancer and arthritis, data are available showing the effectiveness of broad-spectrum MMP inhibitors. The early clinical trials of these compounds indicate an absence of significant toxicity, and with the advent of orally active inhibitors it will not be long before the critical question 'Are MMP inhibitors efficacious in the treatment of human diseases?' can be answered.

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