Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors

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Contents

I. Matrix Metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs), also called matrixins, are a family of structurally related zinc-containing enzymes that mediate the breakdown of connective tissue and are therefore targets for therapeutic inhibitors in many inflammatory, malignant, and degenerative diseases.[1−3] Long before the individual enzymes were isolated and characterized, researchers had been interested in the activity of tissue remodeling, both in physiological and disease processes. One of the earliest descriptions of MMPs in 1949 was as depolymerizing enzymes which, it was proposed, could facilitate tumor growth by making connective tissue stroma, including that of small blood vessels, more fluid.[4] Some 13 years later, the first vertebrate MMP, collagenase, was isolated and characterized as the enzyme responsible for the resorption of tadpole tails.[5] During the next 20 years, several mammalian enzymes were partially purified, but it was not until 1985 that the field really developed when structural homologies became apparent, allowing many new members to be identified through the techniques of molecular biology.

The mammalian MMP family is now known to include at least 20 enzymes (Table 1). Three collagenases have been identified, interstitial collagenase (MMP-1), neutrophil collagenase (MMP-8), and collagenase-3 (MMP-13). These enzymes can degrade the fibrillar collagens, which are generally resistant to proteolysis, making a characteristic 3/4 length break in the α-chain.[6,7] There are two type IV collagenases,[8,9] now termed gelatinase A (MMP-2) and gelatinase B (MMP-9), which, as described by Liotta and colleagues,[10] can degrade type IV collagen of basal laminae as well as other nonhelical collagen domains and proteins such as fibronectin and laminin. The gelatinases have also been shown to degrade native insoluble elastin.[11] Three enzymes have been classified as stromelysins, although only stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) are closely related functionally, degrading various proteoglycan components of the extracellular matrix as well as fibronectin and laminin.[12,13] Stromelysin-3 (MMP-11) was identified in the tissue surrounding invasive breast carcinoma.[14] Its preferred substrate remains a matter of debate, but it has continued to be of interest due to its apparent induction in malignant tissue.[15,16] It does not appear to be particularly active in degrading known extracellular matrix proteins, but it is effective in degrading the serine proteinase inhibitor (serpin) α-1 antitrypsin and in doing so may potentiate the action of serine proteinases such as urokinase-type plasminogen activator (uPA).[17] This serpinase activity is also displayed by other MMPs and supports the hypothesis that the metallo- and serine proteinase families can function in overlapping cascades of inhibition and activation.

Two enzymes have been identified which, on the basis of sequence homology, do not belong in the

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three subgroups described above. These are matrilysin (MMP-7, formerly known as PUMP)[18] and macrophage metalloelastase (MMP-12).[19] Matrilysin is a truncated proteinase which can degrade nonfibrillar collagen, fibronectin, and laminin. Metalloelastase is a relatively nonspecific enzyme capable of degrading many substrates including elastin.[20] Recently, the MMP family has grown by the addition of a new subgroup, the membrane-type or MT-MMPs. Currently four members have been identified (MMP-14 to MMP-17).[21−24] These proteinases have a C-terminal transmembrane domain that allows them to be anchored in the cell membrane. The substrates for most of these enzymes have yet to be established; however, MT1-MMP and MT3-MMP (MMP-14, MMP-16) appear to be specific activators of latent gelatinase A[23,25] and MT1-MMP has been shown to degrade

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Andrew Gearing was born in Derbyshire (U.K.) in 1957. He received his PhD in Immunobiology from the University of Aston in 1983. He spent 6 years at the National Institute of Biological Standards and Control studying inflammatory and hematopoietic cytokines, joining British Biotech in 1989 to run the Immunology group. He has been particularly involved in investigating the role of MMPs in inflammatory disease and their interactions with the cytokine network. He is currently Head of Research Strategy.

a variety of matrix molecules including collagen.[26] More recently, MMP-19 (also known as RASI-1), an enzyme with some homology to the stromelysins,[27,28] enamelysin (MMP-20),[29] two genomic sequences on chromosome 1 (MMP-21 and MMP-22),[30] and a short MMP cloned from an ovary cDNA library (MMP-23)[31] have been added to the family, and it is likely that other members will be discovered in the next few years.

Another recent development is the finding that some MMP inhibitors can inhibit the cleavage of the membrane-bound precursor form of tumor necrosis factor-α (TNF-α) as well as the shedding of various other cytokines, growth factors, and receptors.[32−34] Several members of the MMP family show some activity in this form of cytokine processing, and a novel metalloproteinase disintegrin (TNF convertase or TACE; ADAM-17), distinct from the MMP family, that processes precursor TNF-α has recently been cloned.[35,36] The physiological and pathological rel-

Table 1. Matrix Metalloproteinase Enzymes and Their Substrates

MMP No.	enzyme	principle substrate(s)[a]
MMP-1	Fibroblast collagenase	fibrillar and nonfibrillar collagens (types I, II, III, VI, X), gelatins
MMP-2	72 kDa gelatinase (gelatinase A)	basement membrane and nonfibrillar collagens (types IV, V, VII, X), fibronectin, elastin
MMP-3	Stromelysin-1	proteoglycan, laminin, fibronectin, collagen (types III, IV, V, IX), gelatins, pro-MMP-1
MMP-7	Matrilysin (PUMP)	fibronectin, gelatins, proteoglycan, pro-MMP-1
MMP-8	Neutrophil collagenase	fibrillar collagens (types I, II, III)
MMP-9	92 kDa gelatinase (gelatinase B)	basement membrane collagens (types IV, V), gelatins
MMP-10	Stromelysin-2	fibronectin, collagen (types III, IV), gelatins, pro-MMP-1
MMP-11	Stromelysin-3	serpin
MMP-12	Metalloelastase	elastin
MMP-13	Collagenase-3	fibrillar collagens (types I, II, III), gelatins
MMP-14	Membrane-type 1 (MT1-MMP)	pro-72 kDa gelatinase
MMP-15	Membrane-type 2 (MT2-MMP)	to be determined
MMP-16	Membrane-type 3 (MT3-MMP)	pro-72 kDa gelatinase
MMP-17	Membrane-type 4 (MT4-MMP)	to be determined
MMP-18	Collagenase-4 (xenopus MMP)	to be determined
MMP-19	Novel MMP (RASI 1)	gelatin
MMP-20	Enamelysin	amelogenin (dentine), gelatin
MMP-21	MMP identified on chromosome 1	to be determined
MMP-22	MMP identified on chromosome 1	to be determined
MMP-23	From human ovary cDNA	to be determined

[a] The principal substrates listed are only helpful as a guide; in practice the substrate specificity shown in vitro is broad with considerable overlap between MMPs.

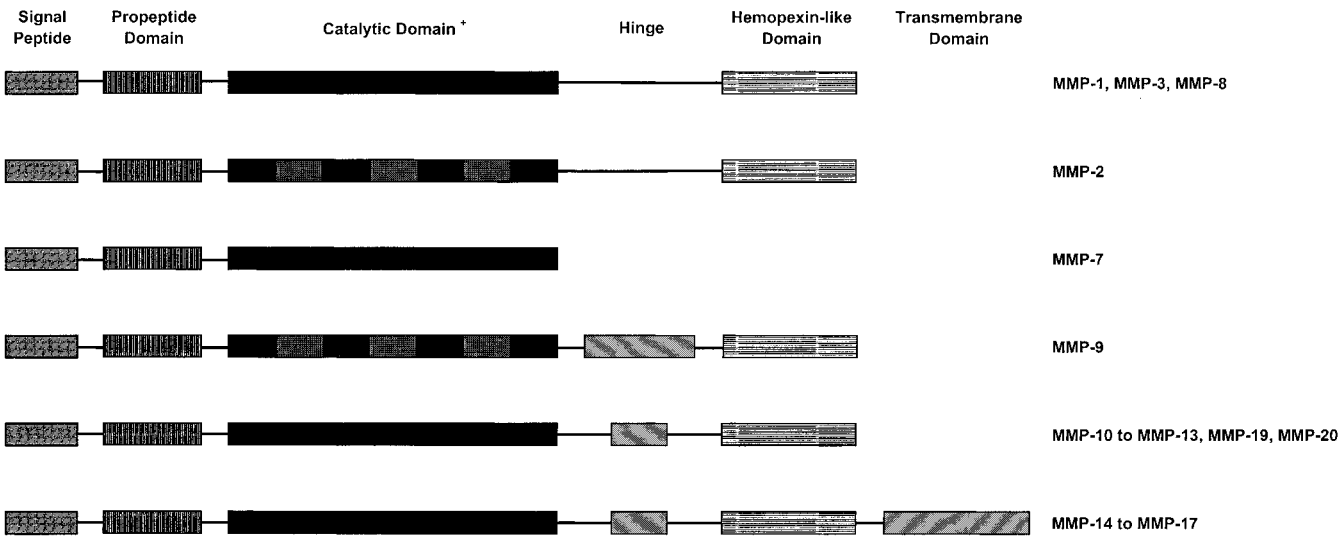


Figure 1. Schematic representation of the general domain topology of selected MMPs.

evance of this activity remains to be determined. MMP inhibitors have also been shown to inhibit soluble CD23 production[37−40] and transforming growth factor α (TGF-α) production.[41] CD23 is a low-affinity IgE receptor that is expressed on the surface of mature cells. Production of soluble CD23 is associated with autoimmune disease and allergy. Both the membrane-bound protein and the soluble fragments have been implicated in the regulation of IgE production; the former through negative feedback inhibition of B-cells and the latter through cytokine-like activities. TGF-α has been implicated as a causative factor in angiogenic and fibrotic disease states. The relative contributions of MMPs and disintegrin metalloproteinases to the shedding of cell surface molecules is not clear and is the subject of intense study.

The domain structures of the MMP family members are illustrated schematically in Figure 1. The catalytic domain of the MMPs contains two zinc atoms. One of these atoms plays a catalytic role and the other a structural role. The catalytic zinc atom coordinates with three histidine residues contained within the conserved −VAAHEXGHXXGXXH− sequence of the catalytic domain. A second conserved sequence −PRCGXPD− is found in the propeptide amino-terminal domain. The thiol group of the cysteine residue within the propeptide coordinates to the catalytic zinc atom and thereby confers latency. This interaction must be broken by proteolytic cleavage of the amino-terminal domain, or conformational modification, before the metalloproteinase can degrade matrix proteins. The activation step can be initiated by several possible mechanisms involving either an enzymatic activation cascade (e.g., by the action of plasmin) and/or cell surface regulation (e.g., by the action of MMP-14 (MT1-MMP)). In the case

of MMP-11 (stromelysin-3) and MMP-14, the presence of a furin-processing motif (Arg−Xaa−Lys−Arg) suggests that these enzymes can be activated intracellularly in the Golgi vesicles.[15,21] With the exception of MMP-7 and MMP-23, all of the human MMPs contain a conserved carboxy-terminal domain which shows homology to hemopexin (Figure 1).[3] This domain appears to be important in substrate binding and in interactions with the natural tissue inhibitors of metalloproteinases (TIMPs).[42] In addition, the two gelatinases contain a gelatin-binding domain, with three fibronectin type II-like repeats, which also appears to play a role in substrate binding.[43]

Once activated, MMPs are subject to inhibition by endogenous proteinase inhibitors such as α2-macroglobulin and more importantly the family of tissue inhibitors of metalloproteinases, TIMPs 1−4.[44−47] These negative regulatory controls are clearly important for a family of enzymes with such destructive potential. Finely regulated MMP activity is associated with processes of ovulation,[48] trophoblast invasion,[49] skeletal[50] and appendageal development,[51] and mammary gland involution.[52] However, it appears that these controls do not always operate as they should, and there is now a substantial body of observational and experimental data which indicates that inappropriate expression of MMP activity constitutes part of the pathogenic mechanism in several diseases. These include the destruction of cartilage and bone in rheumatoid and osteoarthritis,[1,53] tissue breakdown and remodeling during invasive tumor growth and tumor angiogenesis,[54] degradation of myelin-basic protein in neuroinflammatory diseases,[55,56] opening of the blood-brain barrier following brain injury,[57] increased matrix turnover in restenotic lesions,[58] loss of aortic wall strength in aneuryms,[59] tissue degradation in gastric ulceration,[60] and breakdown of connective tissue in peridontal disease.[61] As the role of MMPs in disease has become better understood, interest in the control of their activity has increased. This has led to considerable effort, largely on the part of the pharmaceutical industry, in the development of MMP inhibitors. This review describes progress in the design of MMP inhibitors and outlines the results of early clinical trials.

II. MMP Substrate Selectivity

The amino acid sequence around the collagenase cleavage site (glycine∼isoleucine or glycine∼leucine) in collagen has been taken as the initial guide for the substrate-based design approach for synthetic MMP inhibitors. As discussed above, the various MMPs exhibit different selectivities for the various matrix proteins. There has been considerable interest in understanding such substrate selectivity not only to identify optimized peptide substrates for assay development but also as an aid to the design of selective MMP inhibitors. Initial studies focused on determining the sequence about the cleavage site in protein substrates for individual enzymes.[62−64] More detailed information on the relative substrate selectivity has come from cleavage studies of short peptides.[65−74] In studies by Van Wart and co-workers, the comparative cleavage rates for a series of fluorescent octapeptide substrates were determined for a variety of MMPs (MMP-1, MMP-2, MMP-7, MMP-8, and MMP-9).[65,66] The data from this work is summarized in Figure 2 for changes in the amino acids at the P3−P3′ positions. Stein and co-workers conducted a separate, less extensive, study examining substrate cleavage rates for MMP-3.[68] This group provided a comparison of their results with those of Van Wart, and these have also been included in Figure 2. A possible limitation is that the effect of changing an amino acid at a particular position was studied while the remainder were kept constant, since it does not allow for synergistic effects between substitutions at different positions. Navre and coworkers used a different technique of bacteriophage peptide display libraries in which the amino acids at all positions were varied simultaneously.[69] It is also worth noting that the selection of peptides in the earlier independent sites studies were based on knowledge of the known MMP substrates whereas there was no such bias in this combinatorial study. However, the Navre group studied the selectivity for cleavage by only two MMP enzymes (MMP-3 and MMP-7) and then prepared a series of optimized synthetic peptide substrates based on the phage clones.[69] A few general conclusions may be made on the basis of these three studies concerning substrate selectivity. In most cases, variation of substitution provides a gradation of selectivity and there are very few substitutions that provide significant differential selectivity between the enzymes. In all studies the preferred amino acid at P3 is proline for all of the enzymes examined. From the substrate specificity studies, Arg is preferred at P2 for MMP-2 selectivity and Met appears to be good for MMP-7. The cleavage of peptide substrates prepared on the basis of phage display results indicate that Phe is preferred over Leu and Met at P2 for MMP-3 whereas Leu and Met are preferred for MMP-7.[69] For all enzymes, P1 Val results in negligible cleavage. At the P1 position, Glu provides significant cleavage by MMP-7 and MMP-8 and negligible by MMP-1, MMP-2, and MMP-9. At P1′, the presence of a Tyr residue results in highly selective cleavage by MMP-8. From the same studies Leu and Met appear to be preferred for broad-spectrum cleavage; however, in contrast, the phage display results suggest that Met at P1′ gives minimal cleavage with MMP-7. Furthermore, the substrate specificity studies suggest that P1′ Phe is preferred for cleavage by MMP-3 over the other enzymes whereas the phage display results indicate that P1′ Phe provides negligible cleavage. At P2′, all studies suggest that there is little to be gained in selectivity terms by the various substitutions at this position and that generally Trp is preferred for efficient cleavage. The same is true for amino acid changes at P3′ although there appears to be conflicting data from the substrate and phage studies; the former suggests a strong preference for P3′ Met for selective cleavage by MMP-7,[66] whereas the latter indicates that Met at P3′ results in poor cleavage by MMP-7.[69]

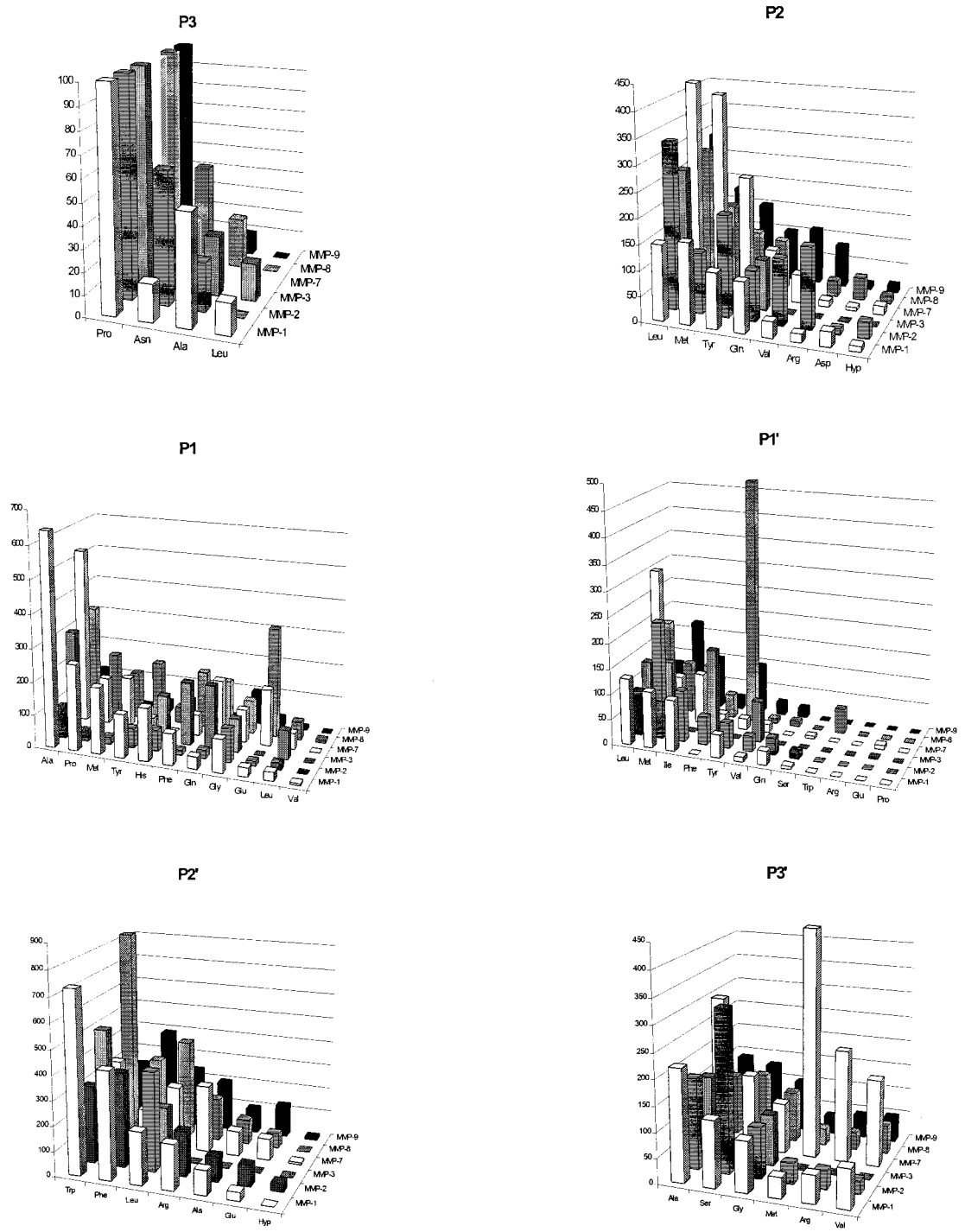


Figure 2. Comparative substrate specificities for amino acid substitutions at P3−P3′ Note: *Z*-axes are % activity (k_c/K_m relative to k_c/K_m for the reference peptide), *X*-axes are amino acid identities, and *Y*-axes are MMP identities. Data for MMP-1, MMP-2, MMP-7, MMP-8, and MMP-9 is from refs 65 and 66, and data for MMP-3 is from ref 68. The data set for MMP-3 is less extensive. No data is available at P3 for MMP-2 (Leu), MMP-7 (Ala and Leu), MMP-8 (Leu), MMP-9 (Leu), at P2 for MMP-3 (Arg, Asp, Hyp, Met, Tyr, Val), at P1 for MMP-3 (Glu, Pro, Met, Tyr, Val), MMP-7 (His), at P1′ for MMP-3 (Arg, Gln, Ile, Ser, Trp, Tyr), at P2′ for MMP-3 (Ala, Arg, Glu, Hyp, Phe), and at P3′ for MMP-3 (Arg, Met, Ser, Val).

Combinatorial peptide synthesis has been employed in studies of synthetic MMP substrates that feature unnatural amino acids.[70−74] McGeehan and co-workers identified Dnp−Pro−Cha−Abu∼Smc−His−Ala−D-Arg−NH_2 (where Dnp is 2,4-dinitrophenyl, Cha is cyclohexylalanine, Abu is α-aminobutyric acid, and Smc is *S*-methylcysteine) as an optimal substrate for MMP-1.[70,71] Singh and co-workers studied the cleavage by MMP-1 and MMP-3 of controlled-pore glass-bound combinatorial peptide libraries.[72,73] Using a genetic algorithm-directed optimization procedure, COP−Ala−Gly−Pro−Ser−Thr∼Ser(Bn)−Thr−$(Acp)_5$−βAla−AMP−CPG (where COP is 7-hydroxycoumarin-4-propanoyl, Ser(Bn) is *O*-benzylserine, Acp is 6-aminocaproyl, AMP is 3-aminopropyl, and CPG is controlled-pore glass) was identified as a particularly good substrate for MMP-3.[73] In addition to Ser(Bn), a range of other extended P1′ substituents

resulted in substrates that were effectively processed by MMP-3.[73] In a separate study, Dive and co-workers have shown that MMP-11 and MMP-14 cleave substrates containing in their P1′ position unusual amino acids with extremely long side chains more efficiently than the corresponding substrates with natural phenylalanine or leucine amino acids.[74]

Selective replacement of the catalytic zinc of MMP-3 catalytic domain with other transition metals (e.g., Co^{2+}, Mn^{2+}, Cd^{2+}, and Ni^{2+}) results in retention of protease activity.[75] However, it was found that substitution of the catalytic metal influences the substrate specificity of the enzyme.[75] Presumably, the differences in coordination properties of the different metal ions alter active-site geometry and hence affect substrate binding.

III. MMP Structural Studies

Considerable insight into MMP ligand interactions has been obtained from studies of enzyme substrate specificity. However, the advent of high-resolution X-ray and NMR structures has provided new paradigms for the design of MMP inhibitors in general and selective MMP inhibitors in particular.[76] The first report of the structure of a matrix metalloproteinase (the catalytic domain of MMP-1) inhibitor complex by Lovejoy and co-workers[77] has been followed by further structures for truncated MMP-1[78−80] and structures for inhibitor complexes of the catalytic domains of MMP-8,[81−84] MMP-3,[85−89] MMP-7,[90] and MMP-13.[91] A recent report describes the structure of the full-length proform of MMP-2 in which the catalytic glutamic acid (Glu-404; MMP-2 numbering) was mutated to alanine to ensure stability against autoproteolysis during crystallization.[92] The catalytic domain in these structures all possess a similar overall spherical topology characterized by a twisted five-stranded β-sheet, containing four parallel strands and one antiparallel strand and three long α-helices. Notably, the catalytic domain of proMMP-2 is unaffected by the insertion of the fibronectin domains.[92] The conserved active-site sequence motif HEXXHXXGXXH coordinates the catalytic zinc(II) ion and contains the glutamic acid residue which facilitates catalysis. The substrate binding groove which is relatively open at S3−S1 and S3′ narrows at S1′ and S2′ with the S1′ site being a well-defined pocket that penetrates the surface of the enzyme. The studies also indicate the presence of a second "structural" zinc(II) ion and two or three calcium(II) ions. Clearly difficulties have been experienced in obtaining recombinant full-length enzymes suitable for structural determination. An exception is MMP-7, one of the smallest members of the MMP family which does not possess a C-terminal domain, whose inhibitor-complexes possess broadly similar structures to that of the catalytic domains of MMP-1, MMP-3, and MMP-8.[90] There may be subtle structural differences between the truncated forms of the MMPs and the mature full-length enzymes in relation to the role of the second "structural" zinc(II) ion. To overcome problems of autoproteolysis when attempting to crystallize full-length active HFC (domains II and III), mutation of two amino acids which chelate the "structural" zinc(II) ion was found to result in a loss of catalytic activity.[93] However, while analysis of the zinc contents of truncated forms of prostromelysin-1 and progelatinase-A indicates the presence of two zinc(II) ions, only one zinc(II) ion is detected in the corresponding full-length enzymes, and it has been suggested that the C-terminal domain performs the role proposed for the "structural" zinc(II) ion in maintaining the structure of the enzyme catalytic domain.[94] However, a recent structure of full-length porcine synovial collagenase (MMP-1), which includes the haemopexin-like domain III, reveals the presence of both "catalytic" and "structural" zinc(II) ions in the crystalline form.[95] Both zinc(II) ions are observed in the structure of proMMP-2.[92] A recent analysis of the binding motifs within MMPs for the structural zinc and calcium ions reveals that there are at least four different motifs for structural zinc binding but that the calcium binding motif is more strictly conserved.[96] These motifs are absent in other members of the metzincin family of enzymes and may be involved in macromolecular substrate recognition.[97] The haemopexin C-terminal domain which is present in all the MMPs except for MMP-7 and MMP-23 appears to regulate enzyme activity. For MMP-1, it is suggested that the haemopexin domain plays a role in unravelling the scissile strand from the triple helix of collagen since truncated forms of MMP-1 lacking the haemopexin domain, although still capable of degrading gelatin and casein, are unable to cleave triple-helical collagen.[98] The porcine synovial MMP-1 haemopexin domain possesses a calcium-linked four-bladed β-propellor structure[95] in common with the haemopexin-like C-terminal domain of MMP-2[92,99,100] and MMP-13.[101] The activation of the MMPs involves the removal of a propeptide portion which features an unpaired cysteine residue that chelates the catalytic zinc(II) ion.[87,92] X-ray crystal structures of the complex between MMP-3 catalytic domain and TIMP-1[42] and MMP-14 catalytic domain and TIMP-2[102] also reveals ligation of the catalytic zinc(II) ion by the thiol of a cysteine residue. The proMMP-2 structure reveals interactions between the propeptide and the third fibronectin domain.[92] These interactions may mimic the binding of gelatin to the fibronectin domains and could possibly be exploited in the design of specific inhibitors.[92]

The reaction mechanism for proteolysis by MMPs[103] has been rationalized on the basis of structural information (Figure 3).[79] It is proposed that the scissile amide carbonyl coordinates to the active-site zinc(II) ion. This carbonyl is attacked by a water molecule that is both hydrogen bonded to a conserved glutamic acid (Glu-198 in MMP-8) and coordinated to the zinc(II) ion. The water donates a proton to the Glu residue which transfers it to the nitrogen of the scissile amide. This is followed by the Glu residue shuttling the remaining proton from the water molecule to the nitrogen of the scissile amide with resultant peptide bond cleavage. During this process the positively charged zinc(II) ion helps to stabilize negative charge at the carbon of the scissile amide and a conserved alanine (Ala-161 in MMP-8) residue

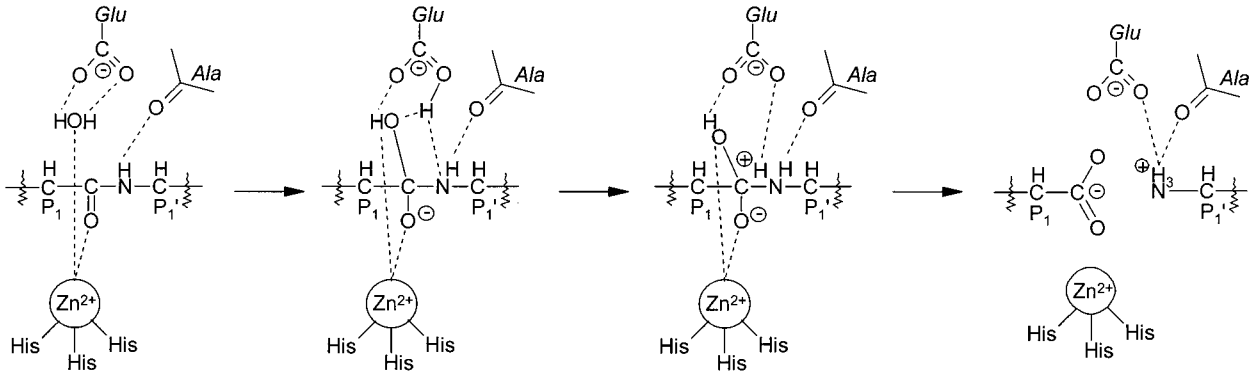


Figure 3. Reaction mechanism for proteolysis by MMPs after Lovejoy et al.[79]

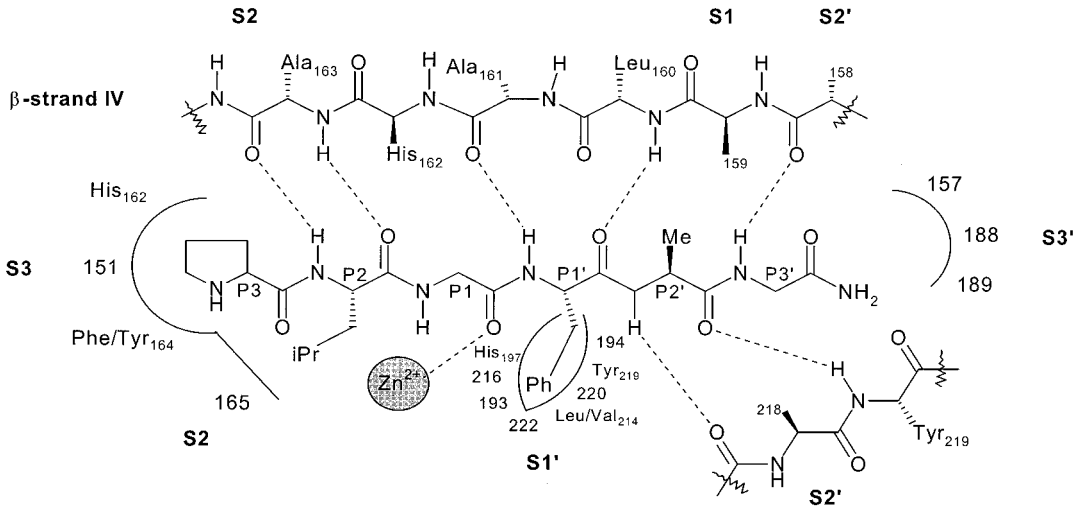


Figure 4. Schematic representation of MMP active-site after Grams et al.[83] Numbering follows that for MMP-8.

Table 2. Selected Variable Residues in the Active Site of MMPs

residue no.[a]	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-11	MMP-12	MMP-13	MMP-14
151	Ser	Tyr	Tyr	Tyr	Ser	Tyr	Tyr	Leu	His	Tyr	Thr
157	Gly	Asp	Gly	Gly	Asn	Asp	Gly	Gly	Gly	Ser	Gly
158	Gly	Gly	Asn	Asn	Gly	Gly	His	Gly	Gly	Gly	Gly
159	Asn	Leu	Val	Thr	lle	Leu	Ser	lle	lle	Leu	Phe
165	Gln	Ala	Ala	Ala	Gln	Pro	Pro	Phe	Gly	Pro	Phe
188	Glu	Gly	Gly	Gly	Asn	Gly	Gly	Gly	Gly	Gly	Gly
189	Tyr	Tyr	Thr	Ile	Tyr	Tyr	Thr	Thr	Thr	Tyr	Asn
193	Arg	Leu	Leu	Tyr	Leu	Leu	Leu	Gln	Leu	Leu	Leu
194	Val	Val	Val	Ala	Val	Val	Val	Val	Thr	Val	Val
218	Ser	lle	Leu	Thr	Asn	Met	Leu	Phe	Thr	lle	Phe
220	Thr	Thr	His	Gly	Ala	Arg	Asn	Thr	Lys	Thr	Gln
222	Ser	The	Leu	Gly	Arg	Thr	Phe	Arg	Val	Thr	Met

[a] Numbering follows that for MMP-8.

helps to stabilize positive charge at the nitrogen of the scissile amide.[79]

Comparison of the structures of the various MMP inhibitor complexes reveals valuable information on the differences in the active sites of the MMPs studied and on the binding modes of inhibitors.[76] A schematic representation of a hexapeptide substrate bound into an MMP active site is given in Figure 4, and the variable residues in the active-site region are given in Table 2 for selected MMPs. The S3−S1 subsites form a shallow region bordered on one side by the β-strand IV and which features a hydrophobic proline binding cleft at S3. Proline is a preferred P3 group in MMP substrates, and the structure of the left-hand side inhibitor Pro−Leu−Gly−NHOH ligated to the catalytic domain of MMP-8[81,83] illustrates the tight complementarity in proline binding at S3. This inhibitor binds antiparallel to the β-strand IV by two backbone hydrogen bond interactions (via NH of P3−P2 amide and carbonyl of P2−P1 amide) with the P2 leucine residing in a shallow cleft and the carbonyl of the P3−P2 amide and NH of the P2−P1 amide exposed to solvent.[81,83] Differences between the various MMPs in the S3−S1 region are relatively subtle. However, substantial differences in enzyme selectivity have been reported for thiadiazolyl MMP inhibitors, which have been observed from structural studies to bind to the unprimed enzyme subsites.[104,105] A significant interaction between the MMPs and their substrates or inhibitors occurs between the S1′

subsite and the P1′ residue. There is variation between the MMPs in the amino acid residues that form the S1′ pocket (Table 2). It is apparent that inhibitors can be obtained which exhibit degrees of enzyme selectivity for the known MMPs, and there has been considerable debate concerning the possible therapeutic advantages of selective inhibition (vide infra). From X-ray crystallographic analysis and homology modeling the MMPs may be classified as falling into two broad structural classes dependent on the depth of the S1′ pocket. This "selectivity pocket" is relatively deep for the majority of the enzymes (e.g., MMP-2, MMP-9, MMP-3, MMP-8, MMP-13, etc.), but for certain enzymes (e.g., MMP-1, MMP-7, and MMP-11) it is partially or completely occluded due to an increase in the size of the side chain of the amino acid at position 193 (MMP-8 numbering) from leucine to arginine (MMP-1), tyrosine (MMP-7), glutamine (MMP-11), or one of the amino acid residues that forms the pocket. The Van Wart group has shown that mutation of the S1′ subsite tyrosine of MMP-7 to leucine changes the substrate specificity to be more like that of the deep pocket enzyme MMP-3.[106] The main type of selectivity that has been obtained is for inhibition of the deep pocket enzymes over the short pocket enzymes, and this is achieved by the incorporation of an extended P1′ group (e.g., biphenyl), whereas, generally speaking, the presence of smaller P1′ groups lead to broad-spectrum inhibition. However, there are exceptions to this rule. While finer degrees of enzyme selectivity have been reported (e.g., inhibition of MMP-2 over MMP-3[107]), the origin of such selectivity is not so readily explained. A recent report of homology models for MMP-2 and MMP-9 based on the structure of MMP-3 suggests that there may be differences in the shape of the bottom of the S1′ subsite for the deep pocket enzymes.[108] In the case of MMP-2, the S1′ pocket may be a channel with no bottom, whereas that for MMP-9 is said to be a pocket-like subsite. Unfortunately, no comment is made on the S1′ pocket shape in the report on the structure of proMMP-2.[92] The S2′ subsite is a solvent-exposed cleft with a general preference for hydrophobic P2′ residues in both substrates and inhibitors. The S3′ subsite is a relatively ill-defined solvent exposed region. While there are some variations in residues for this subsite for the various MMPs, the introduction of different P3′ substituents in general tends to have only a modest effect on inhibitor selectivity. This is in contrast to the effects of P3′ substituents on the inhibition of the TNF-α converting enzyme (TACE). Recently, an X-ray crystal structure has been reported for TACE.[109] This reveals that the active site of TACE shares properties with the MMPs but exhibits differences, particularly in the S3′ subsite which is a deep pocket that merges with the S1′ specificity pocket within the core for the protein.

Pseudopeptide inhibitors that bind to the primed subsites (e.g., batimastat BB-94 (**1**)[84]) tend to do so in an extended manner forming hydrogen bonds with the protein backbone. The hydrogen bond between the carbonyl oxygen of Pro-217 (MMP-8 numbering) and the P2′ N−H is seen to lengthen to >3.3 Å in certain structures, suggesting that it may be a weaker interaction.[76] An NMR structural study of MMP-1 catalytic domain suggests that substantial structural changes occur in the active-site cleft on the binding of an inhibitor.[110] Conformational changes have also been observed in the active site between X-ray structures of MMP-3 catalytic domain with different inhibitors bound.[111] Thus, caution needs to be employed when using structural information in a predictive manner in the design of MMP inhibitors. Detailed aspects of MMP enzyme−inhibitor interactions have been previously reviewed in this journal,[76] and in the present article only selected aspects of MMP structural information are discussed that illustrate aspects of inhibitor design and SAR.

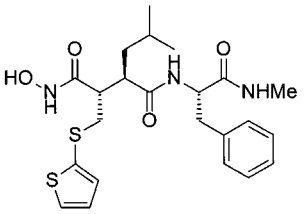
IV. Matrix Metalloproteinase Inhibitors

Both small molecules (synthetic and natural products) and macromolecular endogenous inhibitors such as TIMP-1[44] and TIMP-2[45] have been considered as potential therapies for diseases in which excess MMP activity has been implicated. Various research groups including those at Celltech, Rhone-Poulenc, and Molecular Oncology have attempted large-scale production of TIMPs with a view to therapeutic trials. However, technical difficulties with the production and use of these proteins have so far prevented their development, and the work described in this review focuses solely on the design and testing of small molecule inhibitors.

The principal approach taken for the identification of synthetic MMP inhibitors is the substrate-based design of peptide derivatives derived from information of the sequence about the cleavage site. The screening of natural products has generally not resulted in the discovery of potent compounds, and the most active natural products are structurally similar to certain of the peptide derivatives obtained by the substrate-based design. Recently, non-peptide-based inhibitors have been identified. This has, in part, been aided by the advent of detailed structural information of MMP-inhibitor complexes which is enabling rational inhibitor design. Medicinal chemists working in the MMP field have had to face two difficult questions: (i) should they make selective or broad-spectrum inhibitors? and (ii) how can they make these compounds orally active? This section reviews recent advances in the medicinal chemistry of MMP inhibitors dealing in turn with the various approaches to inhibitor design and the issues that chemists working in this field are facing.

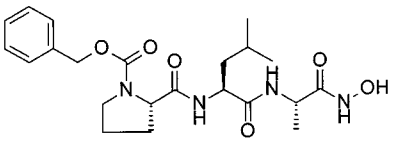
The distinction between compounds designed by the substrate-based approach and those designed by the structure-based approach is often rather artificial. This is because for the most part the few examples of MMP inhibitors that have been reported to date as being discovered by the process of structure-based design are analogues of compounds obtained by the substrate-based approach. However, there are now a number of non-peptidyl compounds which, although not necessarily designed de novo, have been optimized on the basis of direct or indirect MMP structural information.

Scheme 1



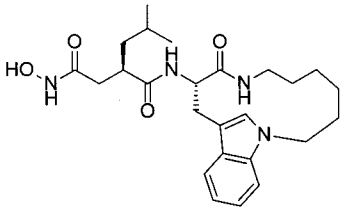
1

IC_{50} MMP-1 10 nM
MMP-2 4 nM
MMP-3 20 nM
MMP-8 10 nM
MMP-9 1 nM
MMP-14 3 nM



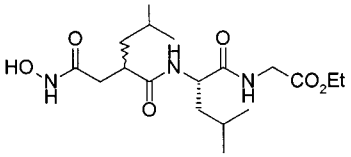
2

IC_{50} MMP-1 8000 nM
MMP-2 8000 nM
MMP-3 3500 nM



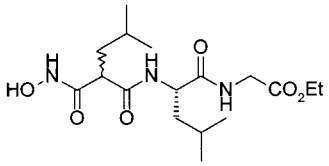
3

IC_{50} MMP-1 0.1 nM
MMP-3 9 nM
MMP-8 0.4 nM
MMP-9 0.2 nM



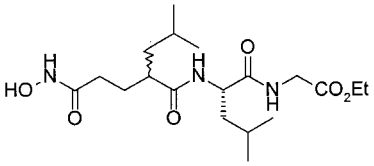
4

IC_{50} MMP-1 40 nM



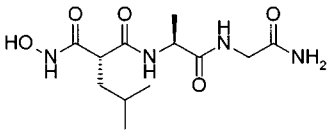
5

IC_{50} MMP-1 29 000 nM



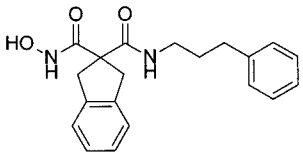
6

IC_{50} MMP-1 > 10 000 nM



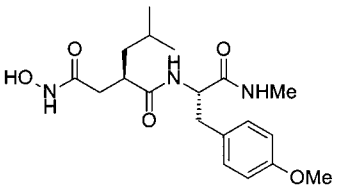
7

IC_{50} MMP-8 121 000 nM



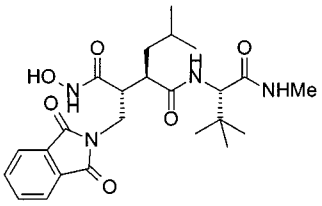
8

IC_{50} MMP-8 300 nM



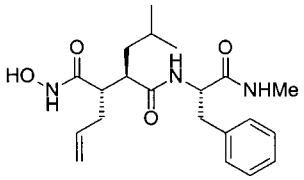
SC-44463 (9)

IC_{50} MMP-1 20 nM
MMP-2 6 nM
MMP-3 30 nM
MMP-7 30 nM



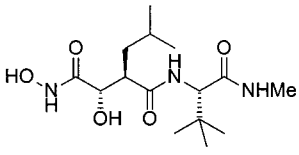
Ro 32-0554 (10)

IC_{50} MMP-1 0.5 nM
MMP-3 9.1 nM
MMP-9 4.3 nM



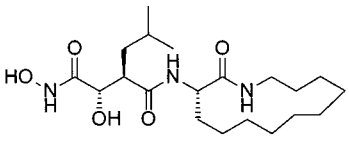
BB-1101 (11)

IC_{50} MMP-1 10 nM
MMP-2 5 nM
MMP-3 30 nM
MMP-7 30 nM
MMP-8 3 nM
MMP-9 3 nM
Cellular TACE 550 nM



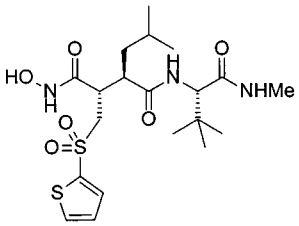
BB-2516 (12)

IC_{50} MMP-1 5 nM
MMP-2 6 nM
MMP-3 200 nM
MMP-7 20 nM
MMP-8 2 nM
MMP-9 3 nM
MMP-14 1.8 nM
Cellular TACE 3800 nM



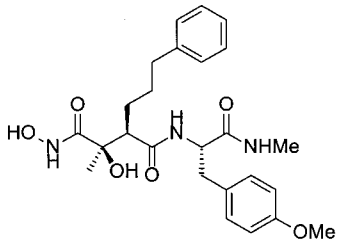
13

IC_{50} MMP-1 30 nM
MMP-2 20 nM
MMP-3 500 nM
MMP-7 200 nM
MMP-8 20 nM
Cellular TACE 2250 nM



14

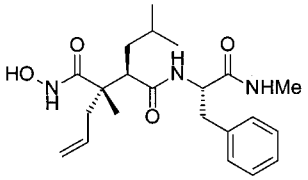
IC_{50} MMP-1 2 nM
MMP-2 20 nM
MMP-3 30 nM
MMP-7 20 nM
MMP-9 7 nM
Cellular TACE 800 nM



15

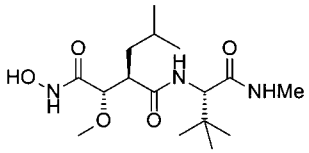
K_i MMP-1 2 nM
MMP-3 3 nM
MMP-9 < 1 nM

Scheme 1 (Continued)



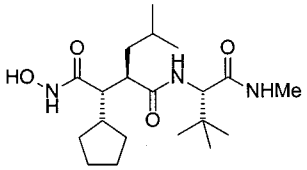
16

IC_{50} MMP-1 3.1 nM
MMP-2 4.2 nM
MMP-3 25 nM



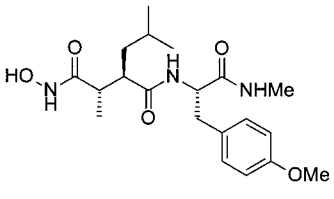
17

IC_{50} MMP-1 6 nM
MMP-2 30 nM
MMP-3 40 nM



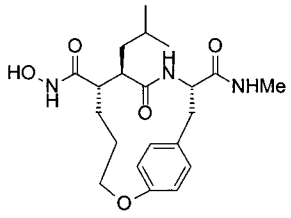
18

IC_{50} MMP-1 4 nM
MMP-2 3 nM
MMP-3 30 nM
MMP-7 20 nM
MMP-8 20 nM
MMP-9 9 nM
Cellular TACE 900 nM



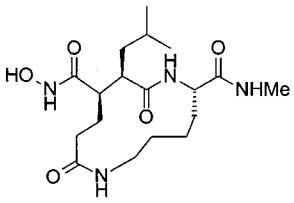
BB-16 (19)

IC_{50} MMP-1 5 nM
MMP-2 10 nM
MMP-3 40 nM
MMP-7 60 nM
MMP-8 7 nM
Cellular TACE 3000 nM



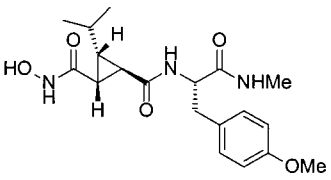
20

K_i MMP-1 1.2 nM
MMP-3 32.7 nM
MMP-9 1.8 nM
Cellular TACE (IC_{50}) 1200 nM



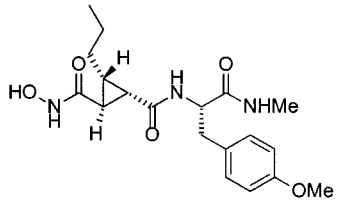
SC903 (21)

K_i MMP-1 2.8 nM
MMP-3 24.1 nM
MMP-9 2.6 nM
Cellular TACE (IC_{50}) 6500 nM



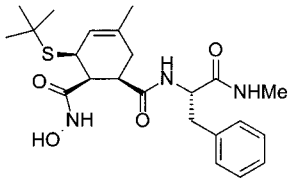
22

IC_{50} MMP-9 50 000 nM

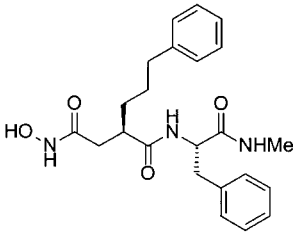


23

K_i MMP-1 200 nM

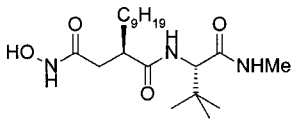


24



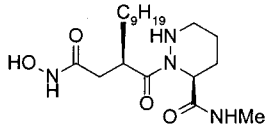
25

IC_{50} MMP-1 1000 nM
MMP-2 15 nM
MMP-3 500 nM
MMP-7 10 000 nM
MMP-8 30 nM
MMP-9 15 nM



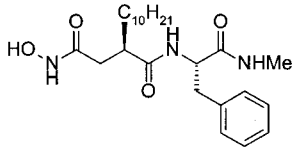
26

IC_{50} MMP-1 375 nM
MMP-2 <0.15 nM
MMP-3 18 nM
MMP-9 1.5 nM



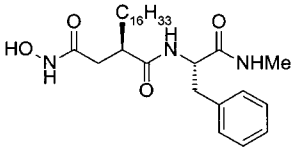
R-94138 (27)

IC_{50} MMP-2 38 nM
MMP-9 1.2 nM



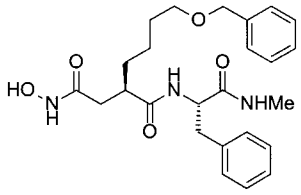
28

IC_{50} MMP-1 20 nM
MMP-2 2 nM
MMP-3 100 nM
MMP-9 2000 nM



29

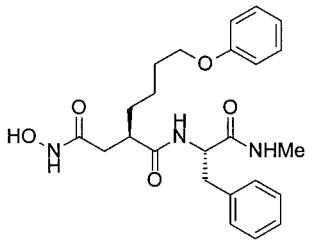
IC_{50} MMP-1 20% @ 100 000 nM
MMP-2 20 nM
MMP-3 300 nM
MMP-7 20% @ 100 000 nM
MMP-9 1 nM



30

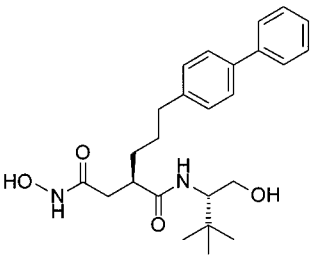
K_i MMP-1 1450 nM
MMP-3 15 nM
MMP-8 2 nM
MMP-9 3 nM

Scheme 1 (Continued)



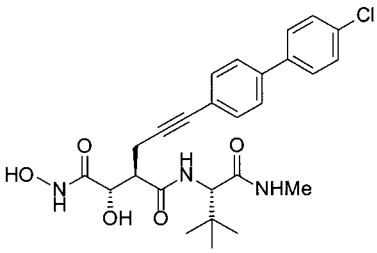
31

K_i	MMP-1	8 nM
	MMP-3	28 nM
	MMP-8	<2 nM
	MMP-9	1 nM



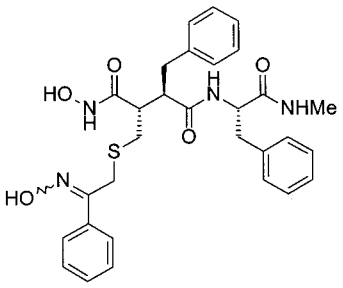
32

K_i stromelysin-1 1.5 nM



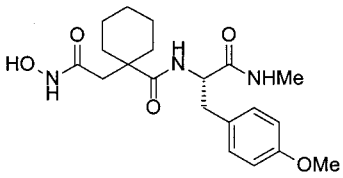
33

IC_{50}	MMP-1	100 nM
	MMP-2	0.07 nM
	MMP-3	3 nM
	MMP-7	700 nM
	MMP-8	4 nM
	MMP-9	1 nM



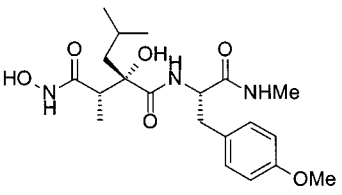
34

IC_{50}	MMP-1	> 10 000 nM
	sCD23 production	130 nM



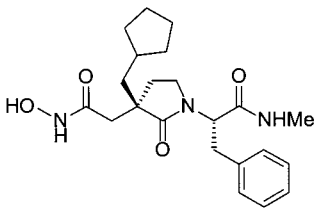
35

IC_{50} MMP-1 150 nM



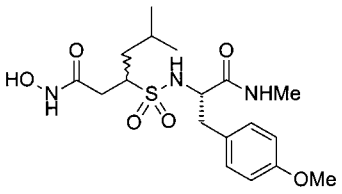
36

IC_{50}	MMP-1	5 nM
	MMP-2	1 nM
	MMP-3	15 nM
	MMP-9	1 nM
	Cellular TACE	1000 nM



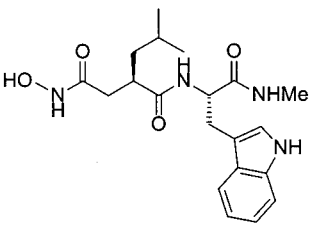
37

Ki	MMP-1	1.3 nM
	MMP-2	1.1 nM
	MMP-3	187 nM



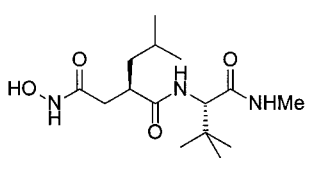
38

K_i	MMP-1	770 nM
	MMP-2	620 nM
	MMP-3	4100 nM
	MMP-9	620 nM



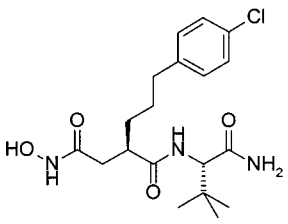
GM6001 (39)

K_i	MMP-1	0.4 nM
	MMP-2	0.39 nM
	MMP-3	26 nM
	MMP-8	0.18 nM
	MMP-9	0.57 nM



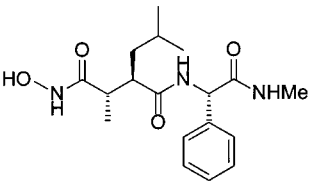
Ro 31-9790 (40)

IC_{50}	MMP-1	10 nM
	MMP-2	8 nM
	MMP-3	700 nM
	MMP-14	1.9 nM



CT1746 (41)

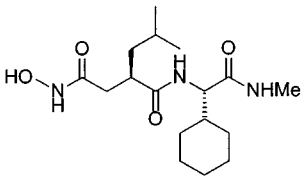
K_i	MMP-1	122 nM
	MMP-2	0.04nM
	MMP-3	10.9 nM
	MMP-7	136 nM
	MMP-9	0.17 nM



KB-R7785 (42)

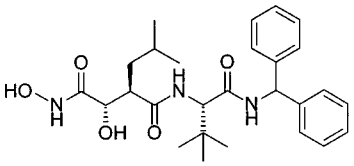
IC_{50}	MMP-1	3 nM
	MMP-2	7.5 nM
	MMP-3	1.9 nM
	MMP-9	3.9 nM
	MMP-14	56 nM

Scheme 1 (Continued)



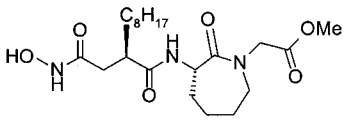
43

IC_{50}	MMP-1	5.4 nM
	MMP-2	8.4 nM
	MMP-3	2.3 nM
	MMP-9	5 nM
	MMP-14	2.3 nM



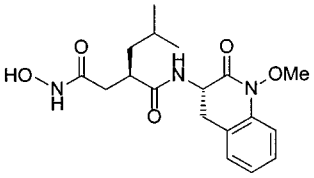
44

IC_{50}	MMP-1	600 nM
	MMP-2	3000 nM
	MMP-3	50 nM
	MMP-7	4 nM



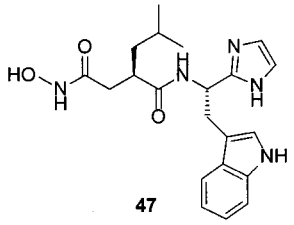
45

IC_{50}	MMP-1	11 nM
	MMP-3	1040 nM



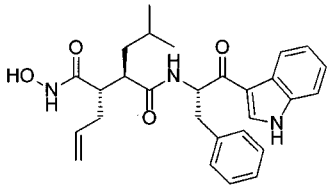
OPB-3206 (**46**)

IC_{50}	MMP-1	700 nM
	MMP-2	5000 nM
	MMP-3	2000 nM
	MMP-9	500 nM



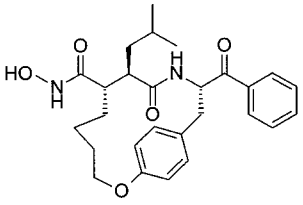
47

IC_{50}	MMP-1	3 nM
	MMP-3	280 nM
	MMP-7	18 nM



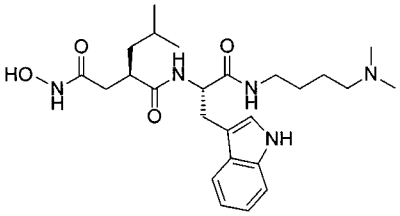
48

IC_{50}	MMP-1	1.1 nM
	MMP-2	1.1 nM
	MMP-3	2.3 nM
	MMP-7	2.2 nM



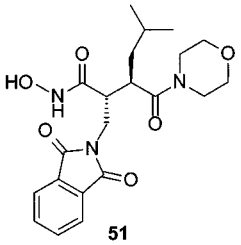
49

IC_{50}	MMP-1	13 nM
	MMP-2	9.5 nM
	MMP-3	8.9 nM
	MMP-7	3.3 nM



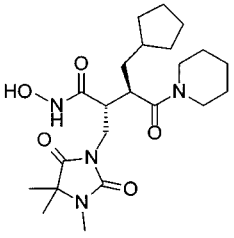
50

K_i MMP-1 3nM



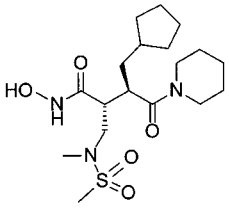
51

IC_{50}	MMP-1	10 nM
	MMP-2	400 nM
	MMP-3	4500 nM



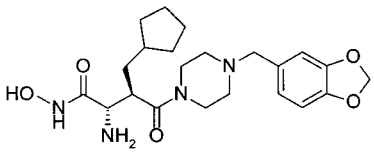
Ro 32-3555 (52)

K_i	MMP-1	3 nM
	MMP-2	154 nM
	MMP-3	527 nM
	MMP-8	4 nM
	MMP-9	59 nM
	MMP-13	3 nM



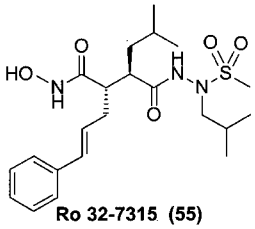
53

IC_{50}	MMP-1	6 nM
	MMP-2	900 nM
	MMP-3	200 nM
	MMP-7	200 nM
	MMP-8	200 nM
	MMP-9	2000 nM
	MMP-13	400 nM



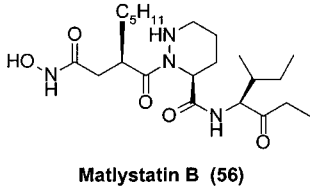
54

K_i	MMP-1	6.5 nM
	MMP-2	20 nM
	MMP-3	240 nM



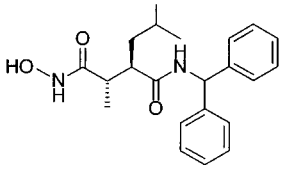
Ro 32-7315 (55)

IC_{50}	MMP-1	500 nM
	MMP-2	250 nM
	MMP-3	210 nM
	MMP-7	310 nM
	MMP-9	100 nM
	MMP-12	11 nM
	MMP-13	110 nM
	TACE	5 nM



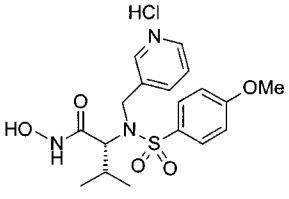
Matlystatin B (56)

IC_{50}	MMP-2	1700 nM
	MMP-9	570 nM



57

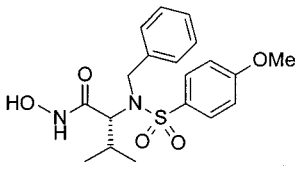
K_i	MMP-3	148 nM
	MMP-8	1.9 nM



CGS 27023A (58)

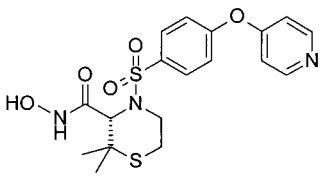
K_i	MMP-1	33 nM
	MMP-2	20 nM
	MMP-3	43 nM
	MMP-9	8 nM

Scheme 1 (Continued)



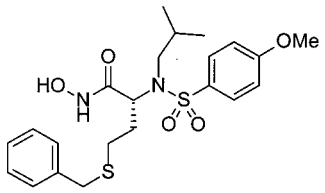
CGS 25966 (59)

K_i MMP-3 92 nM



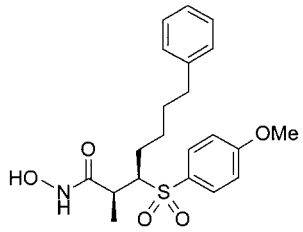
AG3340 (60)

K_i	MMP-1	8.2 nM
	MMP-2	0.083 nM
	MMP-3	0.27 nM
	MMP-7	54 nM
	MMP-13	0.038 nM



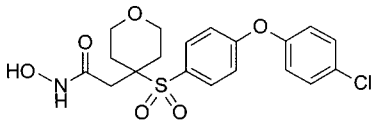
61

IC_{50}	MMP-1	104 nM
	MMP-2	0.7 nM
	MMP-3	0.7 nM
	MMP-9	2.5 nM
	MMP-13	12 nM



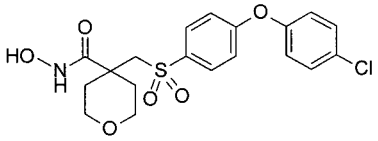
62

K_i	MMP-1	2000 nM
	MMP-2	10 nM
	MMP-3	500 nM
IC_{50}	PDE4	30 nM



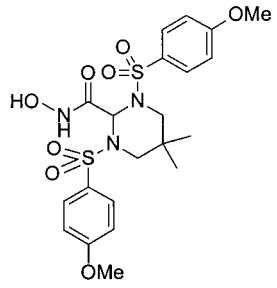
RS-113,456 (63)

K_i	MMP-1	70 nM
	MMP-2	0.054 nM
	MMP-3	5.2 nM
	MMP-7	240 nM
	MMP-8	0.13 nM
	MMP-9	0.065 nM
	MMP-12	0.15 nM
	MMP-13	0.17 nM



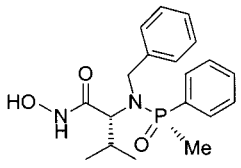
RS-130,830 (64)

K_i	MMP-1	590 nM
	MMP-2	0.22 nM
	MMP-3	9.3 nM
	MMP-7	1200 nM
	MMP-9	0.58 nM
	MMP-13	0.52 nM



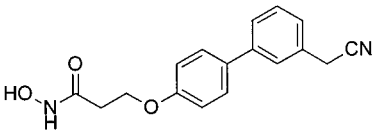
PGE-4410186 (65)

IC_{50}	MMP-1	24 nM
	MMP-3	18.4 nM
	MMP-7	30 nM
	MMP-9	2.7 nM



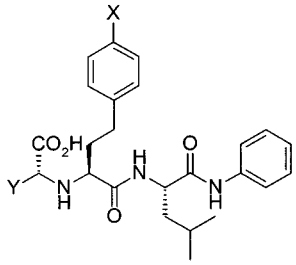
66

IC_{50}	MMP-1	20.5 nM
	MMP-2	13.3 nM
	MMP-3	24.4 nM
	MMP-7	886 nM
	MMP-8	5.3 nM
	MMP-9	20.6 nM
	MMP-13	7.4 nM



67

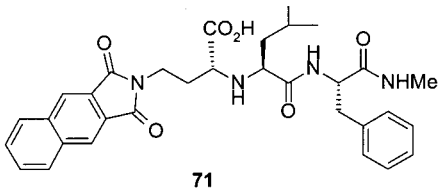
IC_{50} MMP-3 15 nM



68 X = H: Y = Me K_i MMP-1 760 nM; MMP-2 200 nM; MMP-3 470 nM

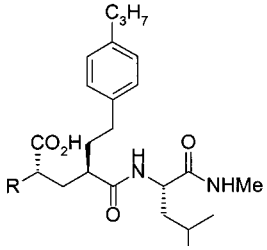
69 X = C_4H_9; Y = Me MMP-1 5900 nM; MMP-2 3.5 nM; MMP-3 18 nM

70 X = H: Y = Phthbutyl MMP-1 720 nM; MMP-2 86 nM; MMP-3 8 nM



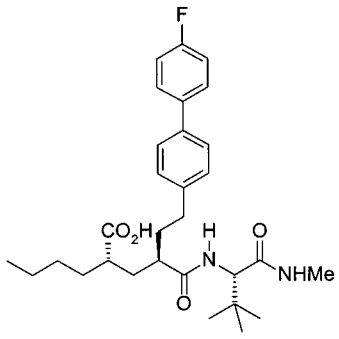
71

K_i MMP-1 20 nM; MMP-3 91 nM; MMP-9 91 nM



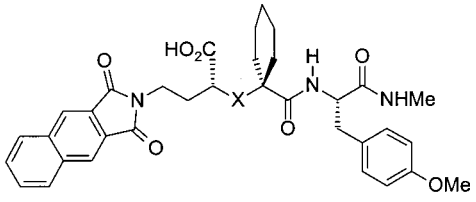
72 R = [H2]-Phthbutyl K_i MMP-1 >10 000 nM; MMP-2 6 nM; MMP-3 0.36 nM

73 R = Me MMP-1 >10 000 nM; MMP-2 310 nM; MMP-3 68 nM



L-758,354 (74)

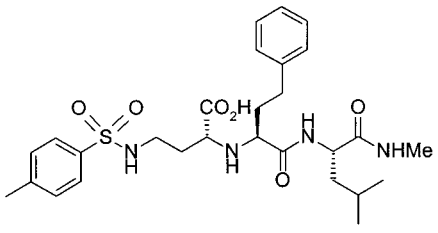
K_i MMP-2 17 nM; MMP-3 10 nM



75 X = NH IC_{50} MMP-1 90 nM

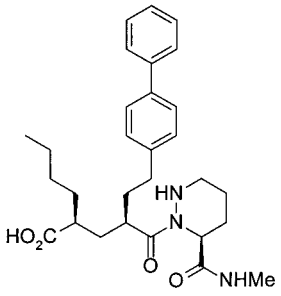
76 X = CH_2 IC_{50} MMP-1 380 nM

Scheme 1 (Continued)



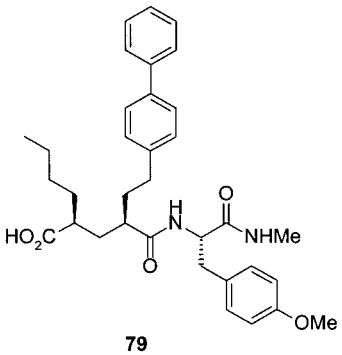
77

IC_{50} MMP-3 50 nM



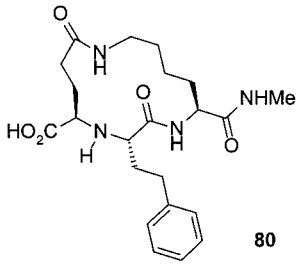
78

K_i MMP-3 42 nM



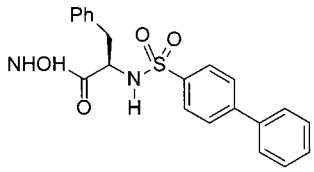
79

K_i MMP-3 21 nM



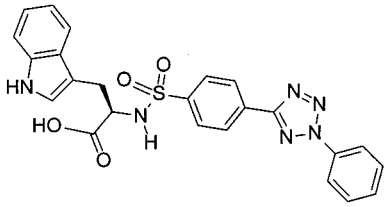
80

K_i	MMP-1	2500 nM
	MMP-2	8100 nM
	MMP-3	12 500 nM
	MMP-8	17 nM
	MMP-9	6600 nM



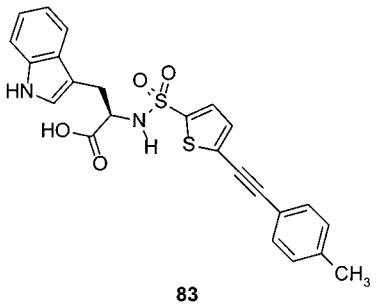
81

IC_{50}	MMP-1	970 nM
	MMP-2	12 nM
	MMP-3	>1000 nM
	MMP-7	800 nM
	MMP-9	16 nM
	MMP-14	17 nM



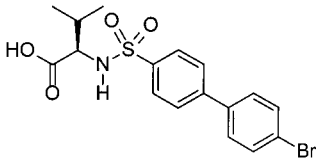
82

IC_{50}	MMP-1	>1000 nM
	MMP-2	19 nM
	MMP-3	>1000 nM
	MMP-7	>1000 nM
	MMP-9	32 nM



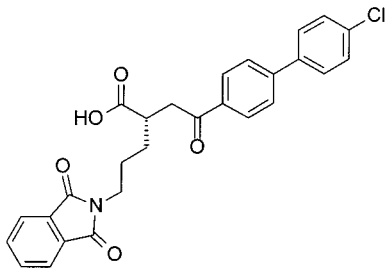
83

IC_{50} MMP-2 2 nM
MMP-9 10 nM



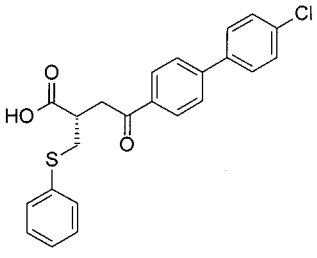
84

IC_{50}	MMP-1	3200 nM
	MMP-2	5 nM
	MMP-3	12 nM
	MMP-9	8300 nM



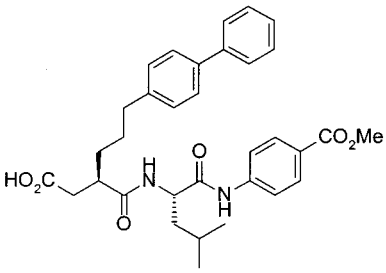
85

K_i	MMP-2	0.9 nM
	MMP-3	15 nM
	MMP-9	3 nM



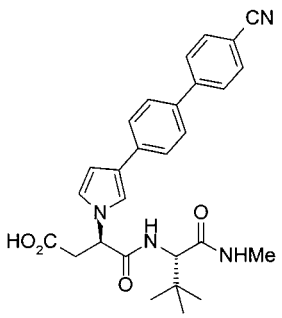
BAY 12-9566 (86)

K_i	MMP-1	>5000 nM
	MMP-2	11 nM
	MMP-3	143 nM
	MMP-9	301 nM
	MMP-13	1470 nM



AG3067 (87)

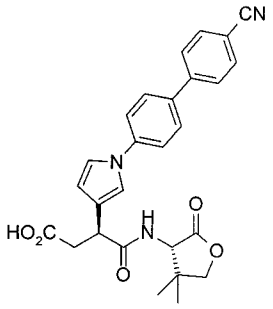
K_i	MMP-1	>1000 nM
	MMP-2	16 nM
	MMP-3	2 nM
	MMP-7	614 nM



AG3365 (88)

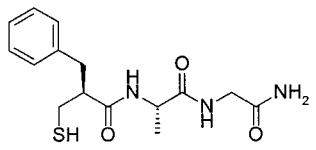
K_i	MMP-2	0.04 nM
	MMP-3	1.5 nM
	MMP-7	305 nM
	MMP-13	0.05 nM

Scheme 1 (Continued)



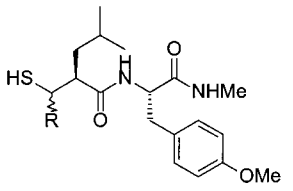
AG3433 (89)

K_i MMP-2 0.9 nM
MMP-3 19 nM
MMP-7 4545 nM
MMP-13 3.3 nM



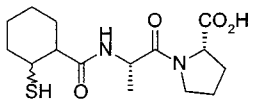
90

K_i MMP-8 1200 nM

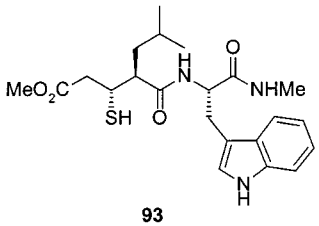


91

R = H IC_{50} MMP-1 360 nM
R = Me 220 nM

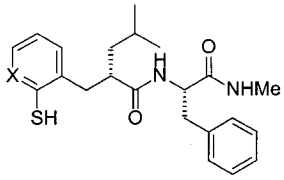


92



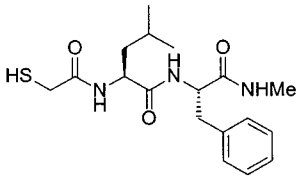
93

IC_{50} MMP-1 2.5 nM



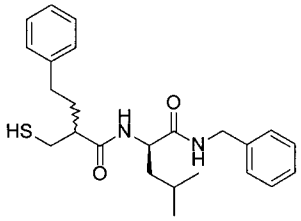
94

X = CH IC_{50} MMP-1 30 nM
X = N > 100 μM



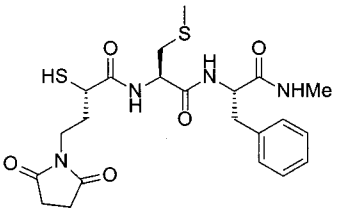
95

IC_{50} MMP-3 260 nM
MMP-8 50 nM
MMP-9 90 nM



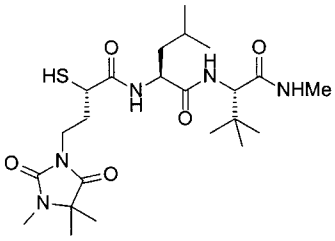
96

IC_{50} MMP-3 2500 nM



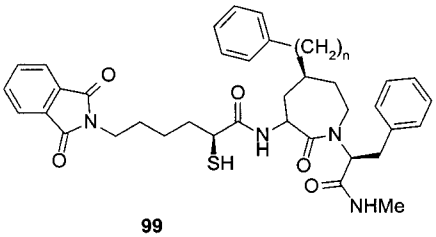
97

IC_{50} MMP-3 45 nM
MMP-8 3 nM
MMP-9 5 nM



D2163 (**98**)

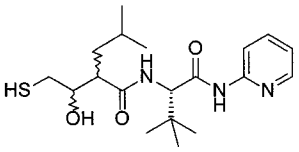
IC_{50} MMP-1 25 nM
MMP-2 41 nM
MMP-3 157 nM
MMP-8 10 nM
MMP-9 25 nM
MMP-13 4 nM



99

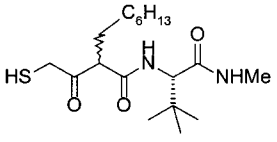
K_i n = 0 MMP-2 1.2 nM
MMP-3 39 nM
MMP-12 18 nM

n = 1 MMP-3 210 nM



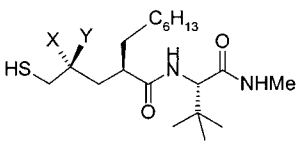
100

IC_{50} MMP-1 890 nM
MMP-3 4600 nM
MMP-9 4500 nM



101

IC_{50} MMP-1 15 nM
MMP-3 16 nM
MMP-9 0.3 nM

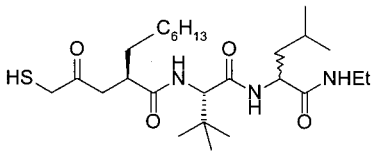


102 X,Y = O IC_{50} MMP-1 10 nM
MMP-2 8 nM
MMP-9 0.1 nM

103 X = OH Y = H IC_{50} MMP-1 140 nM
MMP-3 430 nM
MMP-9 12 nM

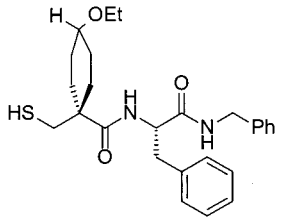
105 X = H Y =O H IC_{50} MMP-1 5 nM
MMP-3 9 nM
MMP-9 0.14 nM

Scheme 1 (Continued)



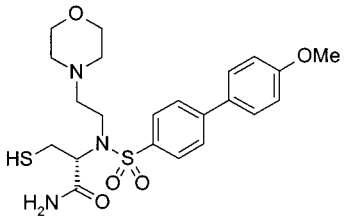
104

IC_{50} MMP-1 >10 000 nM
MMP-3 36 nM
MMP-9 20 nM



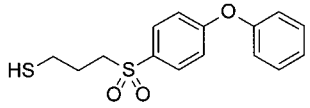
106

IC_{50} MMP-1 823 nM
MMP-3 207 nM
MMP-9 26 nM



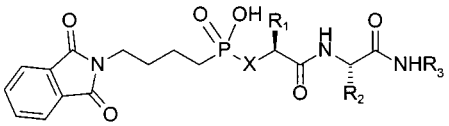
107

IC_{50} MMP-1 70 nM
MMP-13 0.1 nM



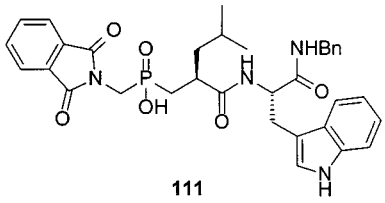
108

IC_{50} MMP-1 1500 nM
MMP-3 500 nM
MMP-8 4 nM
MMP-13 0.5 nM

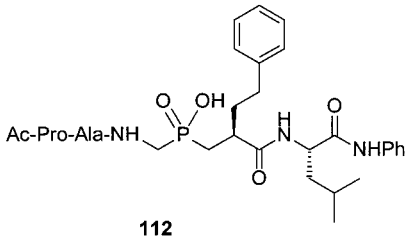


109 X = NH; $R_1 = iC_4H_9$ K_i MMP-3 7 nM
R_2 = 2-naphthyl R_3 = Me

110 X = CH_2 $R_1 = Ph(CH_2)_2$ IC_{50} MMP-1 >10 000 nM
$R_2 = iC_4H_9$; R_3 = Ph MMP-2 20 nM
MMP-3 1.4 nM

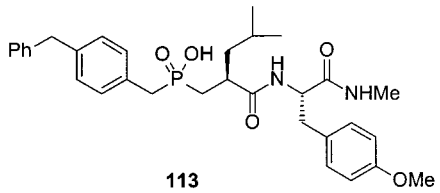


111



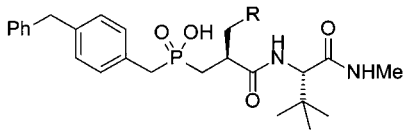
112

K_i MMP-1 3000 nM
MMP-3 6 nM



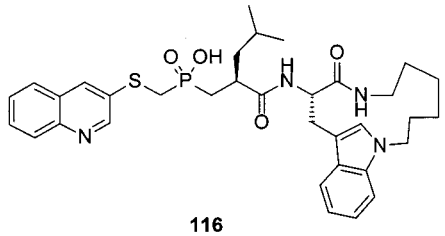
113

IC_{50} MMP-1 270 nM



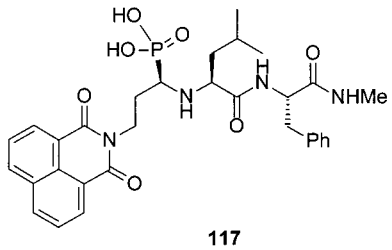
114 R = $PhO(CH_2)_3$ IC_{50} MMP-1 >30 000 nM
MMP-13 30 nM

115 R = $PhCH_2$ IC_{50} MMP-1 690 nM
MMP-3 1200 nM
MMP-13 14 nM



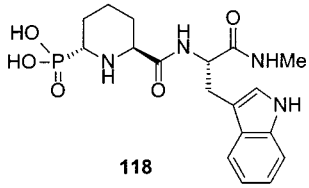
116

K_i MMP-1 5 nM
MMP-2 14 nM
MMP-3 806 nM
MMP-7 78 nM
MMP-8 4 nM



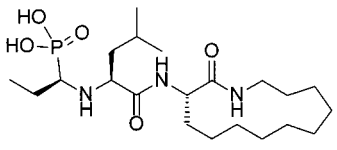
117

IC_{50} MMP-1 20 nM



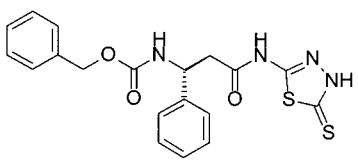
118

IC_{50} MMP-1 >100 000 nM
MMP-2 >100 000 nM
MMP-9 >100 000 nM



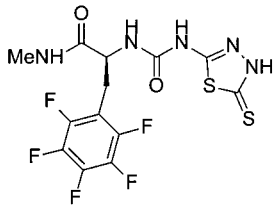
119

IC_{50} MMP-1 180 nM



PNU-141803 (120)

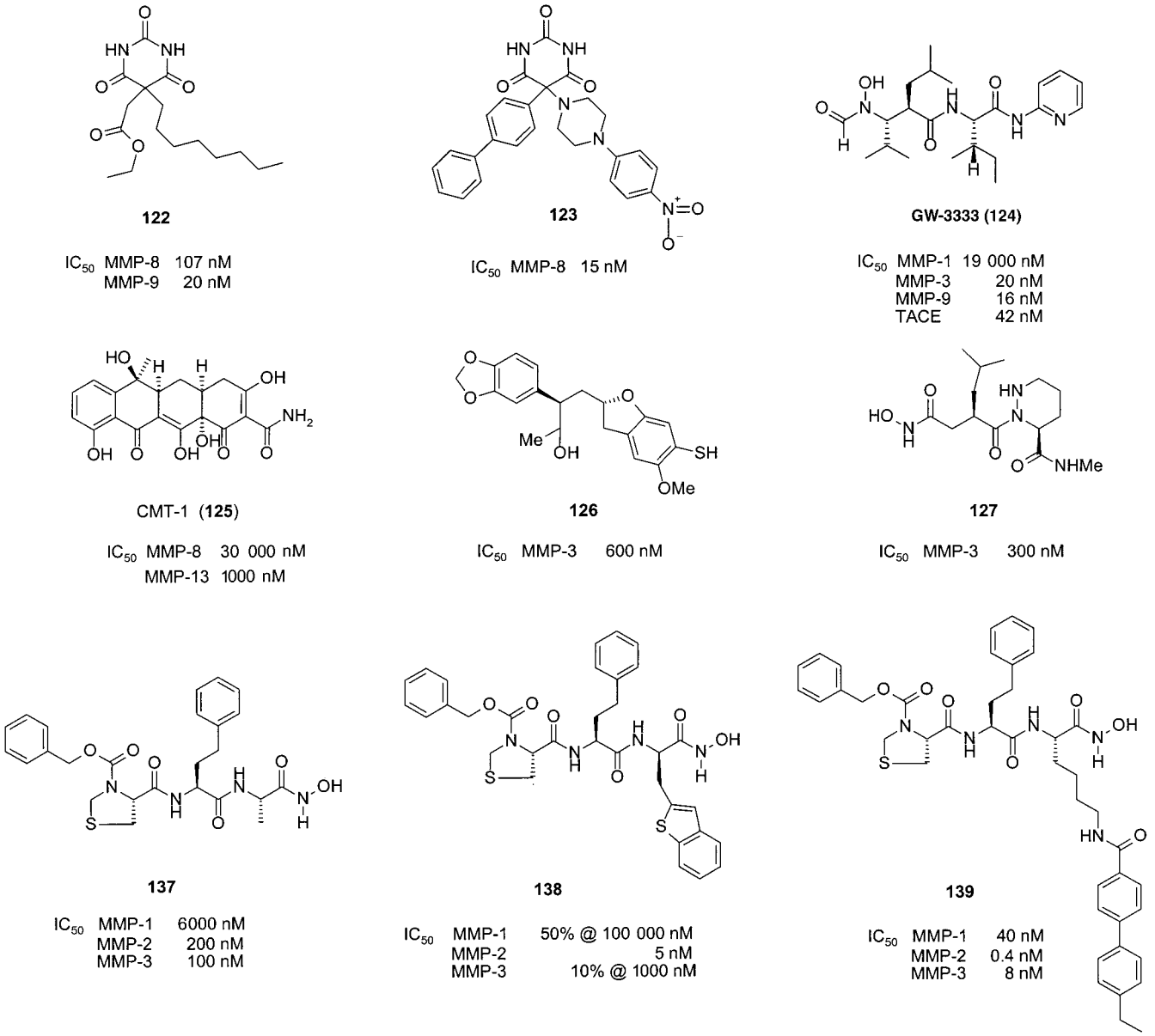
K_i MMP-1 inactive
MMP-2 49 500 nM
MMP-3 310 nM



PNU-142372 (121)

K_i MMP-1 inactive
MMP-2 3000 nM
MMP-3 18 nM

Scheme 1 (Continued)



The requirement for a molecule to be an effective inhibitor of the MMP class of enzymes is a functional group (e.g., carboxylic acid, hydroxamic acid, and sulfhydryl, etc.) capable of chelating the active-site zinc(II) ion (this will be referred to as zinc binding group or ZBG), at least one functional group which provides a hydrogen bond interaction with the enzyme backbone, and one or more side chains which undergo effective van der Waals interactions with the enzyme subsites. It is now apparent that this requirement can be satisfied by a variety of different structural classes of MMP inhibitors which have been discovered by a number of methods including structure-based design[76] and combinatorial chemistry.[112,113] The discovery of MMP inhibitors such as batimastat (**1**; Scheme 1) predated these methods and followed a substrate-based approach to inhibitor design which had been pioneered by early workers in the field.[114] The earliest MMP inhibitors were designed from a knowledge of the amino acid sequence of human triple helical collagen at the site of cleavage by MMP-1 (Figure 5) and subsequently using information derived from substrate specificity studies (vide supra). In the substrate-based design, a ZBG is attached to peptide derivatives which mimic part of the cleaved sequence. Three classes of compounds have been developed, those in which the ZBG is flanked on both sides by amino acid residues and those in which the amino acid residues are present on only the left-hand side or only the right-hand side of the ZBG. The variety of different MMP inhibitors that have been identified following this approach incorporate a range of different ZBG functionality and have been summarized in a number of early reviews.[114−118] It was generally found that compounds which mimic the sequence to the right-hand side of the active-site (P1′ and P2′) and incorporate a hydroxamic acid ZBG exhibited particularly potent inhibition. In contrast, the corresponding left-hand side inhibitors were reported to possess only modest inhibitory potency. Compounds of this class were first described in 1986[119,120] but have not received as much

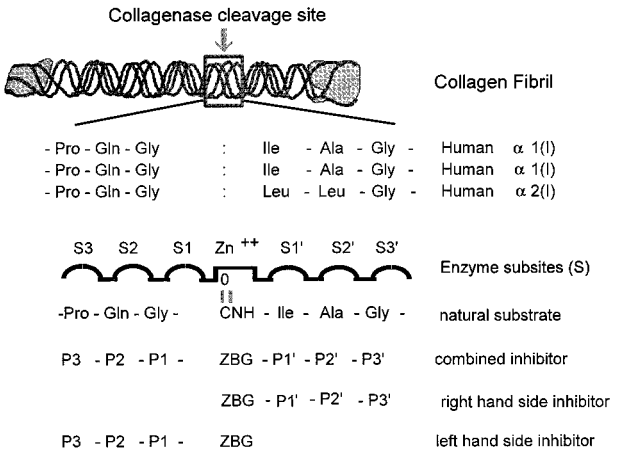


Figure 5. Design of matrix metalloproteinase inhibitors based on the sequence of the collagen substrate cleavage site.

attention[121−123] as right-hand side MMP inhibitors. The early investigations resulted in the identification of left-hand side hydroxamates with only micromolar inhibitory activity such as Z−Pro−Leu−Ala−NHOH (**2**).[119,120]

A key question that medicinal chemists working in the area have tried to answer is "which ZBG is best"? This issue has been addressed by comparing different ZBGs while keeping the rest of the inhibitor structure constant. Using this approach, Castelhano and co-workers arrived at the following preference in terms of inhibition of MMP-1: hydroxamate (e.g., **3**) ≫ formylhydroxylamine > sulfhydryl > phosphinate > aminocarboxylate > carboxylate.[124] Comparison of X-ray crystal structures of **3** and its corresponding carboxylate and sulfodiimine analogues bound to MMP-7 emphasizes the dominant role the ZBG plays in determining the inhibitory potency. The geometries of various ZBGs have been reviewed previously.[76] The hydroxamate acts as a bidentate ligand with each oxygen an optimal distance (1.9−2.3 Å) from the active-site zinc(II) ion, and the position of the hydroxamate nitrogen suggests that it is protonated and forms a hydrogen bond with a carbonyl oxygen of the enzyme backbone. As will become apparent from the discussion below, optimization of the inhibitor structure for many of the above ZBGs can lead to nanomolar inhibition of selected MMPs.

We consider that there are four broad classes of MMP inhibitors as follows: (a) Succinyl hydroxamates, (b) Sulfonamide hydroxamates and related structures, (c) Non-hydroxamates, and (d) Natural products and their derivatives.

While this classification is somewhat arbitrary, it does, we feel, reflect the structural classes of MMP inhibitors that have been investigated. Each of these classes are discussed in turn below with particular emphasis on more recent results.

A. Succinyl Hydroxamates

Early studies conducted by Johnson and co-workers demonstrated that succinyl hydroxamic acid derivatives (e.g., **4**) are more potent inhibitors of MMP-1 than either the corresponding malonyl (e.g., **5**) or glutaryl derivatives (e.g., **6**).[114] The insertion of a single methylene spacer between the ZBG and the carbon bearing the P1′ substituent also showed an improvement in activity for other ZBGs (thiol, formylhydroxylamine, and phosphonate) investigated.[114]

Interestingly, while X-ray analyses of enzyme/succinyl hydroxamate inhibitor complexes have revealed substrate-like binding modes, X-ray analysis of a malonyl hydroxamate bound to MMP-8 reveals a nonsubstrate-like binding mode.[83] It was discovered that the peptidic tail of the weak inhibitor (2S)-HONH−Mal(i-Bu)−Ala−Gly−NH_2 (**7**) bound into the deep S1′ pocket of MMP-8.[83] This insight has led to the discovery of more potent nonpeptidic malonyl hydroxamates (e.g., **8**),[125−127] but the inhibitory activity of these compounds is not as great as that generally obtained with succinyl hydroxamates. The succinyl hydroxamates may be subdivided into peptidic derivatives that possess a P2′ amino acid residue and non-peptidyl compounds in which this group is not an amino acid.

1. Succinyl Hydroxamates with a P2′ Amino Acid Residue

Independently of Johnson and co-workers,[114] the group of Dickens also disclosed that succinyl hydroxamates, such as SC-44463 (**9**), possess potent MMP inhibitory activity.[128] The structure−activity relationships (SAR) for succinyl hydroxamates possessing a P2′ amino acid residue have been extensively explored. An overview of the SAR is given in Figure 6 and discussed in more detail below.

a. P1 Modifications. We and others have found that generally the introduction of a P1 substituent (substituent α to hydroxamic acid) confers the property of broad-spectrum activity against a variety of MMP enzymes.[129] A beneficial effect is conferred by both lipophilic substituents and those capable of undergoing hydrogen bonding. A P1 alkylcyclic imido substituent increases the potency of not only succinyl hydroxamate MMP inhibitors but also MMP inhibitors with amino carboxylate, phosphinate, and phosphonic acid ZBGs. The role of the cyclic imido group is not entirely clear. In MMP-1, analysis of an X-ray crystal structure has shown that the S1 asparagine (Asn-180; MMP-1 numbering) residue hydrogen bonds to the carbamate carbonyl group of an amino carboxylate inhibitor that possesses a P1 benzyloxycarbonylaminoalkyl substituent.[77] A similar interaction is observed between the carbonyl of a P1 phthalimido methyl substituent and Asn-180 in the X-ray structure of Ro 32−0554 (**10**) complexed to MMP-1 catalytic domain.[130] However, a beneficial effect of a P1′ substituent is often observed for the inhibition of MMP-3 even though the corresponding residue to the asparagine is valine which cannot partake in such a hydrogen bonding interaction.

In our own research program, investigation of P1 substituents led to the discovery of batimastat (BB-94) (**1**),[131,132] BB-1101 (**11**),[133] and later marimastat (BB-2516) (**12**).[129,134] Batimastat possesses a thienylthiomethylene α-substituent and BB-1101 features a smaller allyl α-substituent, while the α-substituent for marimastat is a hydroxyl group. All three compounds are broad-spectrum inhibitors which have

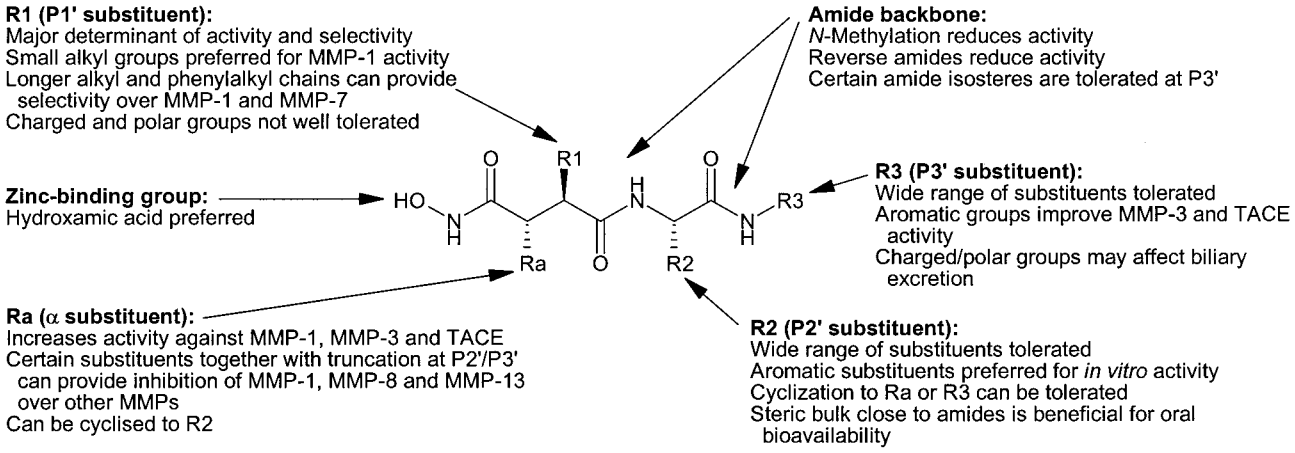


Figure 6. Summary of structure−activity relationships for right-hand side MMP inhibitors.

displayed efficacy in animal models of human disease (vide infra). Unlike marimastat, batimastat and BB-1101 are not orally available. We have attributed the oral availability of marimastat in part to the increase in aqueous solubility achieved by the introduction of the α-hydroxyl group. An X-ray crystal structure of BB-1909 (**13**), an analogue of marimastat, complexed to the catalytic domain of human neutrophil collagenase reveals that the hydroxyl is directed away from the protein surface and is hydrogen-bonded to a solvent molecule.[135] We later found that the presence of certain α-substituents such as allyl (as in BB-1101) and thienylsulfonylmethylene (as in BB-3103 (**14**)) had a beneficial effect on the inhibition of TACE. This dual activity may be of benefit in diseases which involve both inflammation and matrix remodeling and has been implicated in the pharmacological activity of succinyl hydroxamate compounds, such as BB-1101 (**11**) in animal models of arthritis[136] and multiple sclerosis.[137]

Recently, analogues of marimastat have been reported in which the α-position is disubstituted, e.g., **15**.[138] These compounds feature an α-hydroxy group and an α-methyl group and there is a strong stereochemical preference at the α-position, which is opposite to that of marimastat with respect to the orientation of the hydroxyl.[138] Furthermore, phenylpropyl is reported to be the optimal substituent at P1′ and, rather surprisingly, provides potent inhibition of both the short pocket enzyme MMP-1 and the deep pocket enzymes MMP-3 and MMP-9. An X-ray crystal structure of **15** complexed to the catalytic domain of MMP-3 reveals a hydrogen bond between the α-hydroxy group and the backbone of Ala-165 (MMP-3 numbering), as predicted by modeling, and also a van der Waals (VDW) interaction between the P1′ aryl group and His-201. The preparation of the succinate portion of these compounds has been published by the Evans group and involves the catalytic asymmetric aldol reaction between methyl pyruvate and the appropriate enolsilane.[139] Gem-disubstitution at the α-position has also been reported for analogues of BB-1101 (**11**).[140] The combination of α-methyl and α-allyl with *S* stereochemistry (e.g., **16**) is well tolerated, whereas activity is reduced when the stereochemistry at the α-position is *R* and by *gem*-diallyl substition.[140] The pharmacokinetics were investigated for compound **16**, but it was found that the quarternary α-carbon did not confer any benefit compared to BB-1101. We have replaced the α-hydroxy group of marimastat, respectively, with an α-alkoxy, e.g., **17**,[141] and an α-cycloalkyl group, e.g., **18**.[142] Compound **18** is orally available in the rat and the marmoset and inhibits TNF-α production following oral administration in a rat lipopolysaccharide (LPS) challenge model.[142] The 2,3-disubstituted succinate of compound **18** was prepared by a stereoselective Ireland−Claisen rearrangement.[142,143] The synthesis by solid-phase methods of marimastat analogues in which the α-hydroxy group is replaced by a substituted α-amino group has been recently reported.[144]

From analysis of the X-ray crystal structure of batimastat complexed to the active site of recombinant MMP-8 catalytic domain, it is apparent that the α-thienylthiomethylene substituent points away from the enzyme as does the P2′ phenylalanine side chain.[84] Similarly, it has been observed in the X-ray structure of BB-16 (**19**) complexed to MMP-3 catalytic domain that the P1 and P2′ substituents are directed away from the active site into solvent.[145] These observations suggest the possibility of joining the P1 and P2′ side chains together to form a cyclic inhibitor. Similar cyclization strategies have been followed by Xue and co-workers[145] and by Steinman and co-workers.[146] Both groups identified the same compound, SE205 (**20**), as possessing similar potency to uncyclized analogues. Interestingly, this cyclization strategy resulted in a substantial increase in aqueous solubility (SE205 13 mg/mL vs BB-16 0.3 mg.ml).[145] Increasing the ring size by insertion of one or two methylenes in the alkyl chain from the α-position was well tolerated.[146] Similar inhibitory activity was obtained for the 13-member amide-linked derivative SC903 (**21**).[145] Alternative P1 to P2′ macrocyclization strategies have been reported that involve amine formation.[147,148] Depending on the nature of the macrocyclic amine, a degree of selectivity can be obtained for MMP-9 and MMP-8 over MMP-1 and MMP-3[147] or activity enhanced against TACE.[148] The introduction of conformational restraint by the construction of a three-membered ring between the α and P1′ positions (e.g., **22**) has been reported by Martin and co-workers to result in a reduction in the

inhibition of MMP-9.[149] Ghose and co-workers investigated a variety of approaches for the introduction of conformational restraint into the succinyl group in order to determine the pharmacophoric geometry for MMP-1 inhibition.[150] A cyclopropyl derivative (**23**) that possesses improved in vitro potency in comparison to **22** was identified in this study.[150] The introduction of a six-membered ring between the α and P1′ positions (e.g., **24**) resulted in ineffective compounds.[151]

b. P1′ Modifications. As discussed earlier, the S1′ pocket is considered to be the selectivity pocket for the MMP inhibitors. This is confirmed by SAR data which shows that certain MMPs tolerate large hydrophobic P1′ side chains: a P1′ 3-phenylpropyl group provides selective inhibition of MMP-2 over MMP-1 and MMP-3 for succinyl hydroxamates (e.g., **25**)[152] and carboxylates and for phosphonate ZBG MMP inhibitors.[153] This seminal discovery by Porter, Morphy, and co-workers was made before structural data on the MMPs revealed that the S1′ subsite is a deep pocket for the majority of the enzymes (e.g., MMP-2, MMP-3, MMP-8, etc.) but is occluded for a few of the MMPs (e.g., MMP-1 and MMP-7) (vide supra). It is intriguing that compound **25**, one of the first deep pocket selective MMP inhibitors, should show greater potency for the inhibition of MMP-2 over MMP-3. This tendency for lower IC_{50} values against MMP-2 (and the other gelatinase MMP-9) than MMP-3 is exhibited by the majority of MMP inhibitors with extended P1′ groups. The discovery that the incorporation of extended P1′ groups can provide potent deep pocket selective MMP inhibitors has been embraced and enhanced by medicinal chemists working in this field. An extended alkyl group at P1′ provides deep pocket selectivity. Broadhurst and co-workers showed that a C_9 alkyl chain at P1′, as in compound **26**, gives reduced in vitro inhibition of MMP-1 while maintaining potent inhibitory activity against MMP-2, MMP-3, and MMP-9.[154] For a series of matlystatin derivatives, a C_9 alkyl chain at P1′, as in R-94138 (**27**), provides at least 10-fold greater potency than C_8 or C_{10} for the inhibition of MMP-9.[155] In analogous succinyl hydroxamates featuring a *n*-nonyl P1′ substituent, the C_9 chain length provides at least a 500-fold selectivity for MMP-2 inhibition over inhibition of MMP-1[107,154] yet extending the P1′ substituent to C_{10}, as in compound **28**, results in potent inhibition of MMP-1.[107] Increasing the length of the P1′ side chain further to C_{16}, as in compound **29**, results in a loss of activity against MMP-1.[107] A similar switch in the inhibition of MMP-1 has been observed within a series of succinyl hydroxamate MMP inhibitors with extended P1′ substituents containing heteroatom-based modifications.[156−158] The benzyl ether **30** is a weak MMP-1 inhibitor, whereas the corresponding phenyl ether **31** is a potent MMP-1 inhibitor.[156−158] These results indicate that selected extended P1′ substituents can be accommodated in the S1′ pocket of MMP-1. In the case of compound **31** it has been proposed that the S1′ blocking residue of MMP-1, Arg-214 (MMP-1 numbering), might be displaced by a $\pi-\pi$ interaction between the electron-rich phenolic group and the electron-poor guanidinium group.[158] Thus, the occlusion of the S1′ pocket for MMP-1 (and presumably that for MMP-7 and MMP-11) is not absolute since the pocket can undergo conformational changes to accommodate certain extended P1′ substituents.[76,91] Interestingly, disubstitution at the α-position has been observed to increase MMP-1 potency for a P1′ 3-phenylpropyl compound (**15**)[138] in comparison to a des-α analogue (e.g., **25**).[152] Biphenylalkyl P1′ substituents have been incorporated into MMP inhibitors with amino carboxyl and carboxylic acid ZBGs.[76,159,160] This modification has also been successfully applied to succinyl hydroxamate compounds (e.g., **32**). Similar deep pocket selectivity is observed for MMP inhibitors that feature the related rigid arylalkynylmethylene P1′ substituents as in compound **33**.[161]

In a series of α-unsubstituted succinyl hydroxamic acid derivatives, phenyl, benzyl, or 2-naphthylmethyl are P1′ substituents of choice for the inhibition of soluble CD23 formation.[162] Selectivity for the inhibition of soluble CD23 formation over inhibition of MMP-1 has been achieved by the combination of P1′ benzyl with an oxime group at P1 as in compound **34**.[163] P1′ phenyl substitution has also been reported for succinyl hydroxamic acid MMP inhibitors by Robinson and co-workers.[164] They also investigated P1′ C-α gem-disubstitution and found that this modification led to a loss of potency relative to the corresponding P1′ isobutyl compounds with the least detrimental effect being observed for a P1′ *gem*-cyclohexyl compound **35**.[164] However, a P1′ quaternary carbon is tolerated when one of the substituents is hydroxyl as in compound **36**[165] or when one of the substituents is cyclized onto the nitrogen of the P1′−P2′ amide as in compound **37**.[166]

Replacement of the P1′−P2′ amide bond of succinyl hydroxamic acid MMP inhibitors by a sulfonamide bond, as in compound **38**, results in a substantial loss of MMP inhibitory activity.[167] This has been explained in terms of the hydrogen bond from the N−H of the conserved leucine (Leu-160 for MMP-8) to the sulfonyl oxygen being less energetically accessible due to the pyramidal nature of the sulfonamide.[167] The P1′−P2′ amide has also been replaced by a urea functionality, but these analogues were found to be unstable and prone to acid-catalyzed hydantoin formation.[167]

c. P2′ Modifications. X-ray crystallographic analysis of MMP-inhibitor complexes reveals that the P2′ group of peptidyl succinyl hydroxamic acid based MMP inhibitors points out of the enzyme, making few contacts with the S2′ cleft.[76] Indeed, analysis of SAR indicates that modification of the P2′ group has, in general, a modest effect on in vitro activity. Tryptophan at P2′, as in GM6001 (**39**),[168,169] yields more potent inhibitors than other amino acid side chains.[170] The group at P2′ can, however, have an effect on the pharmacokinetic properties of the inhibitors. We have previously suggested that the oral activity of marimastat results from the beneficial combination of a sterically bulky *tert*-butyl group and an α-hydroxy group which increases aqueous solubility.[129] We argued that the bulky P2′ group shields the adjacent amide bonds reducing hydration[171] and, hence, the

desolvation energy barrier of the peptide backbone associated with absorption from an aqueous environment to the lipid environment of cell membranes.[172] A P2′ *tert*-butyl group is also a feature of the orally available compounds Ro 31−9790 (**40**)[173] and CT1746 (**41**).[174,175] The phamacokinetics of Ro 31−9790 has been studied in man, and it has been found that the main metabolite is the amide which arises from dehydroxylation of the hydroxamic acid moiety.[176] The deep pocket selective compound CT1746 has been shown to be effective in animal models of cancer following oral administration.[174,175] Babine and Bender suggest that the P2′ *tert*-butyl group is probably inferior with respect to VDW interactions compared to other, more extended side chains but that this is offset by more facile desolvation of the adjacent peptide linkages and a conformational effect that preorganizes the compound for binding.[76] Ikeda and co-workers describe compounds that feature a P2′ phenyl substituent, e.g., KB−R7785 (**42**).[177] KB−R7785 is orally active as determined both by an ex vivo MMP-1 inhibition assay in mice and demonstration of efficacy in a rat adjuvant arthritis assay. The beneficial effect of the P2′ phenyl group on absorption is attributed to it being an amide shielding moiety.[177] The same researchers have also investigated the SAR for the inhibition of an MMP-14 mutant lacking the transmembrane domain.[178] It was found that the phenylglycine derivative **42** was a weaker inhibitor of MMP-14 than the corresponding cyclohexylglycine derivative **43**, BB-94 (**1**), BB-2516 (**12**), or Ro 31−9790 (**40**). A homology model of MMP-14 suggests that the S1 and S2′ subsites are narrower than those of other MMPs.[178] This results in the phenylglycine compound **42** binding in a conformer which is not at an energy minimum whereas the cyclohexylglycine compound **43** and the *tert*-butylglycine compounds **12** and **14** can bind to MMP-14 in a low-energy comformation.[178]

The P2′ and P3′ substituents may be cyclized to form a lactam, and it is found that there is a correlation between the inhibitory potency and ring size for compounds with both hydroxamic acid and phosphonic acid ZBGs.[114,179] This has been attributed to trans amide geometry for the P2′−P3′ being the required geometry for effective hydrogen bonding interactions between enzyme and inhibitor. The inhibitory potency was found to increase as the lactam ring size was increased from 7 to 9 and then to 13 atoms.[114] Trans geometry is observed in the complex between MMP-8 catalytic domain and BB-1909 (**13**), which features a 13-membered lactam ring between P2′ and P3′.[135] Indolactam cyclization between P2′ and P3′ (e.g., **3**) also confers trans amide geometry and increases activity by at least 10-fold relative to the acyclic analogues.[124]

d. P3′ Modifications. The S3′ region of the MMP enzymes is a relatively open area and a wide range of groups may be introduced at P3′. Heteroaryl and aryl groups appear to enhance MMP-3 and TACE inhibitory activity. We found that the introduction of a benzhydryl group at P3′, e.g., **44**, leads to compounds that are selective for MMP-7 and MMP-3 relative to MMP-1 and MMP-2.[180,181] Disubstitution of the P2′−P3′ amide tends to reduce inhibitory activity,[114,179] except for P2′−P3′ caprolactam derivatives.[182] It has been found that the weak inhibitory activity of P2′−P3′ caprolactam derivatives unsubstituted at P3′[114] may be increased substantially by the introduction of a P3′ methyl acetate substituent as in compound **45**.[182] Interestingly, this compound is a selective inhibitor of MMP-1 over MMP-3 despite possessing an extended C_8 alkyl substituent at P1′. Removal of the P3′ ester carbonyl to provide the corresponding methyl ethyl ether results in a reversal of selectivity for inhibition of MMP-3 over that of MMP-1.[182] A P2′ dihydrocarbostyril derivative, OPB-3206 (**46**), exhibits weak MMP inhibitory activity but is orally available in the rat.[183]

The P2′−P3′ amide is not strictly required for inhibitory activity since it may be replaced by a variety of alternative functionalities. For example, the P2′ amino acid may by replaced by a β-amino alcohol, as in compound **32**, but this generally results in a 10−50-fold loss of activity.[76] From the X-ray crystallographic analysis of **32** complexed to the catalytic domain of MMP-3, it is apparent that the C-terminal hydroxy group accepts a hydrogen bond from the N−H of Tyr-240 (MMP-3 numbering).[76]

The replacement of the C-terminal amide group with a nitrogen heterocycle has been a successful modification. An X-ray crystal structure of a C-terminal imidazole **47** complexed to MMP-7 has been reported.[76,184] The use of imidazole to replace the C-terminal *N*-methyl amide is said to result in a 5-fold reduction in potency against all MMPs tested for this analogue of GM6001.[76] Examination of the crystal structure shows that the imidazole makes hydrogen bonds to the carbonyl of Asn-179 and to the N−H of Tyr-240 (MMP-7 numbering) without any apparent perturbation of the inhibitor backbone in comparison to a related structure in which the C-terminal *N*-methyl amide is retained.[76,184] The introduction of a phenyl substituent at the 5-position of the imidazole ring provided selective inhibition of MMP-7 over MMP-1 and MMP-3, whereas a P3′ benzimidazole group provided broad-spectrum inhibition of the three MMPs tested.[184]

The replacement of the P2′−P3′ amide by an aryl ketone or heteroaryl ketone group, as in the P3′ indole ketone **48** and the P1−P2′ cyclized P3′ phenyl ketone **49**, is tolerated.[185] Compound **48** is an analogue of BB-1101 (**11**) which possesses negligible oral availability. In contrast, the indole ketone **48** is 12% bioavailable in the monkey following dosing at 10 mg/kg p.o. and has a half-life of 20 h.[185] Two earlier studies of P2′−P3′ amide derivatives showed that the nature of the P3′ substituent can have an effect on the oral availability of succinyl hydroxamates.[186,187] In one study, the effect of P3′ substituents on biliary excretion in the rat was examined for a series of GM6001 analogues.[186] It was found that the presence of a tertiary amine at P3′ reduced biliary excretion and increased plasma half-life.[186] The tertiary amine **50** was found to be 8.5% orally bioavailable in the rat.[186] In the other study, it was found that oral availability as measured in a mouse pleural cavity assay was significantly enhanced by the introduction

of an alkyl morpholino P3′ substituent.[187] The beneficial effect of the morpholino group was attributed to its basicity.[187] Epimerisation-free amide coupling conditions have been developed by Fray and Ellis to facilitate the introduction of a wide range of P3′ substituents into a *N*-succinyl-*tert*-leucine intermediate for the preparation of succinyl hydroxamates.[188]

B. Non-Peptidic Succinyl Hydroxamates

Truncation of the P2′−P3′ group of pseudo-peptide succinyl hydroxamic acid derivatives leads to MMP inhibitors which tend to be selective for the collagenases. Broadhurst and co-workers discovered that potent inhibition of the collagenases could be achieved when a cyclic imide group is introduced at P1, as in the phthalimido derivative **51**.[189] Subsequent optimization of this series led to the discovery of Ro 32−3555 (**52**), which was selected for development for the treatment of arthritis.[190,191] Presumably, for compound **51** and Ro 32−3555 (**52**), a favorable balance between active-site interactions and solvation is maintained despite the removal of three hydrogen bonding groups in comparison to succinyl hydroxamates with P2′ groups. The presence of the cyclic imide group at P1, a feature of earlier MMP inhibitors identified by the same workers and by other groups (vide supra), appears to also be important for activity and may compensate for the loss of the hydrogen bonds as observed in the X-ray structure of Ro 32−0554 (**10**) complexed to the active site of MMP-1.[130] The cyclopentylmethyl P1′ group of Ro 32−3555 was chosen on the basis of X-ray crystal structure data for MMP-1.[190] The introduction of cyclopentylmethyl provides a modest increase in potency over an analogue of Ro 32−3555 with an isobutyl group at P1′ suggesting improved complementarity with the S1′ pocket.[190] Ro 32−3555 exhibits an oral bioavailability of 26% in the rat and inhibits articular cartilage degradation in a rat monoarthritis model.[192] Ro 32−3555 (Trocade) has been referred to as a cartilage protective agent (CPA).[193] An improved synthesis of the chiral 2,3-disubstituted succinate of Ro 32−3555 has been reported[194] based on the earlier succinate alkylation protocol of Crimmin and co-workers.[195] We found that incorporation of a sulfonamide moiety at P1 in combination with P2′−P3′ truncation, as in compound **53**, can provide selective inhibition of MMP-1 over other the MMPs that we evaluated.[196] Analogues of **53** possess different selectivity profiles depending on the nature of the sulfonyl substituent.

Alpegiani and co-workers found that an α amino group in conjunction with a P2′ piperazinyl moiety, as in compound **54**, provided compounds with good oral availability.[197] Compound **54** exhibits 58% oral bioavailability in the rat and 34% in the cynomolgus monkey.[197] Broadhurst and co-workers have found that the P2′−P3′ amino acid residue may be replaced by a hydrazide moiety as in Ro 32−7315 (**55**).[198,199] This potent TACE selective inhibitor has been selected for clinical development.[199] Cyclic hydrazide compounds such as the piperazic acid derivative matlystatin B (**56**) and its analogues have been previously identified as natural product MMP inhibitors.[155,200−202] Matlystatin analogues have been prepared in which the ZBG has been altered,[203] the P3′ group modified,[155,204] and the P1′ substituent changed.[155] As described above, the P1′ nonyl derivative **27** is a potent inhibitor of the gelatinases, in contrast to the modest activity exhibited by the parent molecule matlystatin B (**56**).[155]

The P2′ amino acid residue of succinyl hydroxamic acid MMP inhibitors may be replaced with a benzhydryl group, as in compound **57**.[111] A combinatorial chemistry approach was employed in this study that first involved the exploration of P2′ modifications for a series of *N*-carboxyalkyl amino acid based inhibitors (vide infra). Modeling of the optimal P2′ group that had been identified by this approach led to the identification of benzhydryl as a preferred substituent, which was then introduced into the corresponding succinyl hydroxamic acid derivative **57**. An X-ray crystal structure of this compound bound to the catalytic domain of MMP-3 revealed an unexpected conformational shift in the 222−231 loop region (MMP-3 numbering).[111] This illustrates that subtle changes in binding can occur with variation of inhibitor structure and that these are very difficult to predict on the basis of modeling alone. Inhibition of MMP-3 was further increased by replacing one of the phenyl groups of compound **57** with 3-pyridyl.[111]

C. Sulfonamide Hydroxamates and Related Structures

N-Sulfonyl amino acid hydroxamates were independently identified as inhibitors of MMPs by two research groups.[205,206] The first such compound to enter development is the orally available broad-spectrum inhibitor CGS 27023A (**58**).[205] Key structural features of CGS 27023A are said to be the isopropyl substituent which slows down metabolism of the adjacent hydroxamic acid group and the basic 3-pyridyl substituent which may aid partitioning into the hydrated negatively charged environment of cartilage.[205] SAR for the inhibition of macrophage metalloelastase (MMP-12) by CGS 27023A and analogues has been reported.[207] This reveals that CGS 27023A is a potent inhibitor of MMP-12, an enzyme that has been implicated in the development of emphysema that results from chronic inhalation of cigarette smoke.[208] The binding mode of CGS 27023A and analogues to MMP-3 has been investigated by NMR spectroscopy.[209−211] The *p*-methoxyphenyl substituent of CGS 27023A occupies, but does not fill, the S1′ specificity pocket, while the pyridylmethyl and isobutyl substituents occupy the S2′ and S1′ subsites, respectively.[211] An X-ray crystallographic analysis of a related compound CGS 25966 (**59**), a close analogue of CGS 27032A, complexed to the catalytic domain of MMP-1 has been reported by Babine and Bender.[76] The observed binding mode is broadly in agreement with the NMR studies in that the 4-methoxyphenyl group resides in the S1′ pocket and the isopropyl group is located in the S1 subsite. The X-ray structure indicates that the isopropyl group is relatively close to the *N*-benzyl substituent.[76] By introducing a six-membered ring to provide beneficial ligand preorganization[76] and extending the P1′ sub-

stituent, the potent MMP inhibitor AG3340 (**60**) was derived.[212,213] This compound was selected for development based on its superior efficacy in a murine model of cancer growth and metastasis in comparison to a number of analogues and because it showed a favorable pharmacokinetic profile with 18% oral bioavailability in rats.[213] There has been considerable interest from other researchers in analogues of CGS 27023A and AG3340.[141] For example, it has been recently reported by Hanessian and co-workers that modification of the substituent α to the hydroxamic acid in CGS 27023A leads to increases in the inhibition of the deep pocket MMPs, e.g., thioether derivative **61**.[214] Other analogues of particular interest are sulfone derivatives,[215−218] bis-sulfonamides,[219,220] and phosphinamides.[221] Groneberg, Burns, and co-workers have identified sulfone hydroxamic acids (e.g., **62**) which are inhibitors of both MMPs and the enzyme phosphodiesterase type 4 (PDE4).[216] Inhibition of PDE4 results in increased intracellular concentration of cyclic AMP and consequently in antiinflammatory activity.[222] Analogues of compound **62** have been identified, by both combinatorial methods (vide infra)[215] and traditional analogue synthesis,[216] that provide selective inhibition of PDE4 over MMP inhibition by the introduction of a 3,4-dimethoxyphenylsulfonyl group. The reverse selectivity for MMP inhibition over that of PDE4 is achieved by the incorporation of a cyclic quaternary center α to the sulfonyl moiety.[215] This is a structural feature of the compound RS-113,456 (**63**) identified by Campbell and co-workers.[217,218] Oral availability and half-life were improved in this series by shifting the cyclic group to be α to the hydroxamic acid as in the development compound RS-130,830 (**64**).[217] Separate X-ray crystallographic analyses of both RS-113,456 and RS-130,830 bound to the catalytic domain of MMP-13 reveal that the two compounds adopt virtually identical conformations.[91] An X-ray crystal structure has also been determined for RS-104,966, an analogue of **63** lacking the chloro substituent, bound to the catalytic domain of MMP-1. This shows that induced fit of MMP inhibitors with large P1′ substituents can occur by Arg-214 (MMP-1 numbering) adopting a new position, creating a larger open S1′ pocket.[91] RS-113,-456 (**63**) dosed orally diminishes flow-mediated arterial enlargement in a rat arteriovenous fistula model,[217] and RS-130,830 (**64**) is being investigated in the clinic as a therapeutic agent for the treatment of osteoarthritis.[218] In contrast to the majority of MMP inhibitors, both RS-113,456 and RS-130,830 lack any stereocenters yet retain potent inhibitory activity for the deep pocket MMPs. In an alternative approach based on symmetrical bis-sulfonamides, Pikul and co-workers identified another series of nonchiral MMP inhibitors (e.g., **65**).[219] The 1,3-piperazinyl derivative PGE−4410186 (**65**) exhibits broad-spectrum inhibitory activity against the enzymes tested.[219] An X-ray crystal structure of PGE−4410186 complexed to the catalytic domain of MMP-3 reveals similar interactions previously observed in the crystal structure of CGS 25966 complexed to the catalytic domain of MMP-1[76] in terms of one of the 4-methoxyphenylsulfonyl groups residing in the S1′ pocket.[219] The second 4-methoxyphenylsufonyl binds to the S1/S2 pocket with the two sulfonyl oxygen atoms of this group interacting with the imidazole ring of His-166 (MMP-3 numbering) via a hydrogen-bonded bridging water molecule.[219] PGE−4410186 and analogues have been evaluated in an in vitro cartilage permeation model.[220] It was found that permeability across articular cartilage was increased for analogues of PGE−4410186 with increasing hydrophilicity.[220] Pikul and co-workers have also investigated analogues of CGS 27023A in which the sulfonamide moiety is replaced by a phosphinamide group as in compound **66**.[221] This compound is a potent inhibitor of MMP-3, the collagenases (MMP-1, MMP-8, MMP-13), and the gelatinases (MMP-2, MMP-9) but is less effective at inhibiting MMP-7. An X-ray crystal structure of phosphinamide **66** bound to MMP-3 catalytic domain reveals that the phosphinamide phenyl group is accommodated into the S1′ pocket and that the phosphinamide oxygen is within hydrogen bonding distance to the N−H of Leu-164 and Ala-165 (MMP-3 numbering).[221] This provides an explanation for the observation that optimum enzyme inhibitory activity is achieved when the configuration at the phosporus chiral center is *R*.[221] However, hydrolysis of the phosphinamide bond which occurs at low pH may limit the potential of these compounds to be developed into orally available drugs.

A new drug discovery technique involving multidimensional NMR spectroscopy, known as "SAR by NMR", was first used to identify potent analogues of the immunosuppressant FK506.[223] Application of this technique has been extended to the field of MMP inhibition, where it has been used to identify a series of MMP-3 inhibitors.[224,225] In this study, two ligands that bind weakly to proximal sites on MMP-3 were identified. Acetohydroxamic acid binds to the active-site zinc(II) ion and 3-cyanomethyl-4′-hydroxybiphenyl binds to the S1′ pocket.[224] On the basis of the NMR-derived structural information, the two molecules were linked together to give the potent MMP-3 inhibitor **67**.[224]

D. Non-Hydroxamates

Due to the intense competition in the area of hydroxamic acid MMP inhibitors there has been considerable interest in compounds with alternative zinc binding groups. For the purpose of this review these are subdivided as follows: (1) Carboxylic acid and N-carboxyalkyl ZBGs, (2) Thiol ZBGs, (3) Phosphorus-based ZBGs, (4) Novel zinc binding groups.

1. Carboxylic Acids and N-Carboxyalkyl ZBGs

With the exception of a few families of hydroxamic acids derived directly by solid-phase procedures, all the other examples of MMP inhibitors described in the preceding sections will have been prepared by converting a carboxylic precursor into the corresponding hydroxamic acid. Consequently, a vast volume of test data has been built up on the value of carboxylic acid containing structures as potential MMP inhibitors. A typical example is the carboxylic acid precursor of compound **29**; this is selective for MMP-2 over MMP-1, -3, and -7 (IC_{50} of 30 nM vs

>100 mM).[107] Although the carboxylate group is a less effective binding group toward zinc—Babine and Bender suggest a 3.5 kcal/mol advantage in hydroxamate binding[76]—many carboxylates have been shown to be effective MMP inhibitors and some appear to have promise as clinical candidates.

Hagmann and colleagues at Merck were among the earliest to disclose MMP inhibitors with an *N*-carboxyalkyl zinc binding group,[226] but to date no compounds of this class have reached the clinic. The most likely reasons are weak in vitro potency, relative to hydroxamic acids, and poor oral bioavailability. This series of compounds, exemplified by **68**, was targeted against MMP-3 but inhibit both MMP-1 and MMP-3 (IC_{50}s of 60 and 300 nM, respectively, for **68**). Replacement of the phenethyl group at P1′ with linear alkyl chains removed the MMP-1 inhibitory activity without affecting the activity against MMP-2 and MMP-3.[227] A similar effect was achieved by para-substitution of the phenyl ring of the phenethyl group with small linear alkyl changes as in **69**.[228] A similar series of P3′ esters has been identified by a combinatorial approach.[159] Extending the P1 methyl to groups such as a phthalamidobutyl, as in **70**, increased the activity against MMP-3 and further increased the selectivity compared to MMP-1 (K_i of 8 vs 720 nM).[229] A comparable observation had been made previously with the carboxylic acid **71**.[230] Replacing the nitrogen atom of the amino—carboxylate with a carbon provided a series of glutaric acid derivatives with similar in vitro potency but much improved oral bioavailability and in vivo pharmacological properties.[231] In this series, compound **72** which incorporates the two features that promoted activity against MMP-3—a phthalimidobutyl group (or the monodeoxy equivalent) at P1 and a 4-propylphenethyl group at P1′—was among the most potent compounds as measured by in vitro activity (K_i 0.36 nM vs MMP-3). Compounds containing this P1 group showed poor in vivo activity in a mouse pleural cavity assay. However, compound **73**, containing only a methyl group as the P1 substituent, was active when administered orally. Further refinement of this series of compound has given the lead compound L-758,-354, **74**.[160] L-758,354 incorporates a *tert*-butyl group at P2′ and a methyl group at P3′ and has an ED_{50} of 11 mg/kg p.o. for the inhibition of MMP-3 in the mouse pleural cavity assay. L-758,354 was active in an acute model of arthritis (MMP-3 injection into rabbit joints; ED_{50} = 6 mg/kg i.v.) but not in chronic models (rat adjuvant induced arthritis and mouse collagen induced arthritis).

While the above carboxylate inhibitors were directed particularly against MMP-3, Robinson has elaborated a similar backbone to produce inhibitors of MMP-1. The P1′ *gem*-cyclohexyl compound **75**, developed from compound **71**, exhibited an IC_{50} of 90 nM against MMP-1, a 4-fold loss of activity compared to the equivalent P1′ isobutyl compound. This is a relatively small reduction compared to the 80-fold reduction noted in the succinate hydroxamate **35**.[164] Because the naphthalimide is susceptible to hydrolysis by a mechanism involving the P1′ NH, the glutaric acid derivative **76** was also prepared and was only slightly less potent than **75**.[232] More radical changes to the P1 substituent have been examined. A tosylaminoethyl group at P1 and a phenethyl at P1′ gave the potent MMP-3 inhibitor **77**.[233]

Cherney and Decicco have combined the *N*-carboxyalkyl and carboxyalkyl modifications, described above, with the P2′ piperazic acid group in the matlystatins to provide another series of MMP-3 inhibitors, e.g., **78**.[204] As with some of the non-peptide hydroxamates described earlier and the carboxylates described above, the interaction of the P1′ biphenyl substituent with the S1′ pocket is the dominant factor contributing to the inhibitor binding. As part of this study, an X-ray structure of the acyclic analogue **79** with MMP-3 revealed an important interaction between the terminal phenyl of the biphenyl group and the side chain of His-224 (MMP-3 numbering). Related structures in which the P1 substituent is linked directly to P3′ to form macrocycles have been prepared. The ring size of the resultant macrocycle can, not surprisingly, have an effect on the activity and selectivity against a range of MMPs.[234] From a series of differing sized macrocycles containing a biphenylethyl group as a constant at P1′, **80** was identified as a selective inhibitor of MMP-8.

As indicated above, the *N*-sulfonyl amino acid hydroxamates have been a fruitful area of investigation in the search for new MMP inhibitors. The equivalent carboxylic acids are also proving to show activity in their own right. In almost all cases, when the two classes are compared, the hydroxamic acids prove to be more potent in vitro. However, there is a possibility that any advantages that the hydroxamic acid may possess in terms of intrinsic activity may be counterbalanced by a poorer pharmacokinetic profile. Whether this is true is still a matter for debate, and it may be that some of the current interest in carboxylic acids, especially in recent patent applications, simply represents the intensity of interest in hydroxamic acids.

Tamura and colleagues at Shionogi have prepared a series of biphenylsulfonyl derivatives of D-amino acids. The hydroxamic acid **81** was a potent inhibitor of MMP-2 and MMP-9, whereas the equivalent carboxylic showed a 40-fold loss of potency. However, they found compound **81** to be unstable in vivo undergoing metabolism to produce hydroxylamine. The hydroxamic acids were also poorly absorbed in a mouse model, in contrast to the carboxylic acids. In light of these observations, further investigations concentrated on the carboxylic acids. The tetrazole derivative **82** suppressed colonization of Lewis lung carcinoma cells in mice. Compound **82** also prolonged survival of Ma44-bearing mice (26% increased life span) following daily oral dosing.[235] In contrast to this declared preference toward the carboxylic acid series, a recent report from the same group has described the effect of the hydroxamic acid **81** in mouse cancer models. Following daily oral dosing, compound **81**, which has in vitro activity against MMP-2, -9, and -14 but not MMP-1, -3, or-7, inhibits tumor-induced angiogenesis, primary tumor growth (48% inhibition in B16−BL6 melanoma model), and liver metastasis. Compound **81** also inhibited liver metastasis of C-1H

human colon carcinoma cells.[236] This family of carboxylic acids has been used as probes for evaluating a recent homology model of the S1′ subsites of the gelatinases developed by the Shionogi group.[108] They concluded that whereas MMP-9 has a pocket-like S1′ subsite, in MMP-2 it is more channel-like and consequently more tolerant of increased length of the sulfonamide substituent. Thus, although extending the terminal methyl substituent of compound **83** to a *n*-butyl group has little effect on the activity against MMP-2, there is a 300-fold loss of activity against MMP-9 (IC_{50} 9.6 vs 2700 nM).[108]

The carboxylic acid **84** derived from D-valine exhibits selective inhibition of MMP-2 and −3.[237] 4-Substitution of the biphenyl ring helped to increase potency over the unsubstituted compound and also helped to improve the pharmacokinetic profile. Carboxylate **84** could achieve high plasma concentrations with a long half-life (38−48 h) and was able to inhibit left ventricular dilation in a rat model of congestive heart failure.[237]

The antiinflammatory drug Fenbufen has provided the starting point for a series of carboxylic acid MMP inhibitors under investigation by Hibner and coworkers at Bayer in a program originally aimed at investigating new approaches to the treatment of osteoarthritis. Although the initial target enzyme was MMP-3, compounds such as **85** were potent inhibitors of MMP-2 and -9. Replacement of biphenyl by phenyl reduced activity. 4′-Substitution of the biphenyl with chloro and small linear alkoxy substituents improves activity.[238] This strongly suggests that the biphenyl group occupies the S1′ pocket. Support for this hypothesis is provided by the lack of activity against MMP-1. BAY 12−9566 (**86**) is currently in phase II clinical trials for osteoarthritis and cancer. Like many of the compounds prepared within this series, the compound exhibits modest in vitro activity against most MMPs, notably having no activity against MMP-1 and good activity against MMP-2. Bay 12−9566 (**86**) inhibited the invasion of human HT1080 fibrosarcoma cells in vitro (40−65% at 10^{-6} M concentration) and is not cytotoxic. Oral treatment of mice (50 mg/kg for 7 days) inhibited angiogenesis induced by Matrigel and bFGF in a subcutaneous pellet assay (40% decrease in haemorrhage, 66% reduction of haemoglobin in the pellet).[239]

Possible concerns about the metabolic stability and bioavailability of peptide hydroxamates have led to Bender and colleagues at Agouron to focus on a series of succinyl carboxylates.[240] An early lead AG3067 (**87**) provided a starting point for further investigation. The P1′ biphenylpropyl group naturally provided selectivity for the deep pocket enzymes. Adding a 4′-cyano group to the biphenyl group slightly improved MMP-2 activity and prevents potential enzymatic oxidation. Incorporation of a *tert*-leucine *N*-methylamide group at P2′/P3′ improved activity and solubility. By replacing the propyl group at P1′ with five-membered heterocycles dramatic improvements in activity were obtained. AG3365 (**88**) is a potent selective inhibitor. Replacing the P2′/P3′ fragment with aminopantolactone to reduce the peptidic character together with reorientation of the P1′ pyrrole ring led to the orally bioavailable compound AG3433 (**89**).[241] This compound is currently under preclinical investigation.

2. Thiol ZBGs

Thiols are an attractive ZBG for incorporation in MMP inhibitors. Babine and Bender have suggested that although the intrinsic affinity of a monodentate thiol ZBG is less than that of the bidentate groups such as carboxylate or hydroxamate, lower dissolution costs and easier ionization tend to make such inhibitors only slightly less potent than the hydroxamate class of MMP inhibitors.[76] The structure determination of the inhibitor **90** with MMP-1 has indicated that it is a RHS inhibitor whose side-chain binding geometry is similar to that obtained with substrate-like hydroxamates.[83] Consequently much of the SAR determined for the hydroxamate structures has been applied in the design of thiolate inhibitors.

Among the earliest examples of modern substrate-based thiol MMP-1 inhibitors are the dipeptide analogues, such as **91** introduced by Searle.[242] Incorporation of a substituent α to the thiol (**91**, R = Me) led to an improvement in activity. Explorations about thiol dipeptide framework fragment has shown a preference for the *S*-stereochemistry at the P1′ position, which relates to the L-stereochemistry of the natural substrate when this substituent is present α to the thiol.[243] In its absence, compounds possessing the *R*-stereochemistry were more active than the *S*-analogues, though these compounds tended to show lower activities than similar compounds containing an α-substituent.[244] Derivatives incorporating a linked P1−P1′ substituent, as in compound **92**, show a total loss of activity,[245] although a similar strategy had been successful in the design of ACE inhibitors. However, the incorporation of a carboxyalkyl methyl group in the P1 position led to a significant increase in activity. The *S*,*S*-stereochemistry at P1,P1′, as in compound **93**, was optimal, and some members of the series were reported to show inhibition of collagenolysis in vivo. Although the increased activity of this series of compounds could be a consequence of a highly beneficial S1 interaction, it is also possible that the ester carbonyl and the thiol group may participate in bidentate coordination to the active-site zinc.[245]

Hughes and colleagues have also prepared thiophenol derivatives such at **94**.[246] Having noted that the phenyl α-substituted thiol (**91**, R = Ph) showed comparable activity to the corresponding methyl derivative (**91**, R = Me), it was concluded that a phenyl group could be included at the P1 position. They also showed it was possible to transpose the thiol about the aryl group. Modeling studies with low-energy conformations of thiophenol **94** indicated that the thiol group can indeed approach the active-site zinc. In contrast, the 2-pyridyl analogue (**94**, X = N) is inactive, possibly as a consequence of it existing predominantly as the thione tautomer.[246]

Several groups have followed up on these concepts of involving the thiol in a bidendate binding group and moving it from the position it occupies in

compound **93**. Montana and co-workers have identified a series of inhibitors incorporating a mercaptoacyl ZBG.[247] The initial lead **95** resembles **93** with the carbonyl oxygen and the thiol groups transposed. Compound **95** is a moderate inhibitor against a range of deep pocket enzymes and is orally active in a rat adjuvant arthritis model.[247] Alkylation of the sulfur significantly reduced activity as did increasing the spacing between the mercapto and acyl group by incorporating an extra methylene ($IC_{50} > 100\ \mu M$ vs MMP-3). Interestingly, Hagmann and Kopka had shown previously that incorporating a phenethyl group α to the carbonyl in the truncated β-mercaptoethylacyl compound **96** allowed some of the inhibitory activity to be recovered.[248]

A modeling investigation on compound **95** docked into MMP-8, based on the known conformation of an enzyme-bound hydroxamate inhibitor,[82] confirmed that the thiol and acyl carbonyl could cooperate in binding to the active zinc site.[249] The amino acid side chains could be accommodated into the S1′ and S2′ pockets in a similar manner as those of succinyl hydroxamic acids. The model indicated that *S*-substitution α to the thiol and acyl group would lead to preferential occupation of the S1 pocket. In compound **97**, the *S*-stereochemistry at the α-carbon provides 6−10 times the potency of the *R*-isomer. The model also highlights the possibility of an interaction between the imide carbonyl and the Ser-172 (MMP-8 numbering) of the enzyme. Montana et al. at Darwin-Chiroscience have followed up on this work by filing new patent applications on several series of compounds. In these applications the mercaptoacyl group is retained as the ZBG, though it is often present in its acylated form which presumably can act as a prodrug for the parent thiol, and the phthalamidoalkyl group is a common P1 substituent. Among other areas the applications cover modifications to the P2′ amino acid group including deletion of the C-terminal amide, replacement by imidazole, and replacement of the P2′ amino acid by a heterocycle.[141] Most of these strategies are similar to those previously utilized by others in modifications of the succinyl hydroxamates. Unfortunately, no biological data was included with these applications so it is not yet possible to comment on the relative merits of these MMP inhibitors. Two compounds from the Darwin-Chiroscience series, D2163 (**98**) and D1927, have entered clinical development. D2163 contains a trimethylhydantoinylethyl group at the P1 position and a leucinyl-*tert*-butylglycinyl backbone. The structural relationship to BB-2516 (**12**) and Ro 32−3555 (**52**) is noteworthy. D2163 is reported to have an oral activity of 30 mg/kg in a rat model of cancer and in human phase I trials showed dose-related plasma levels in excess of the IC_{50} 12 h after administration.[250,251] The structure of D1927 has not been released by the company. Warshawsky and co-workers at Hoechst have produced a variant of the Montana series in which the P2′ amide nitrogen is linked to the P1′ group.[252] Compound (**99**, $n = 0$) shows good activity against MMP-2, -3, and -12. Extending the P1′ side chain (**99**, $n = 1$) tends to reduce the activity against MMP-3.

Campbell and Levin have also prepared a novel series of inhibitors developed from initial observations on the amino acid α-mercaptoamides similar to **95**.[253] As they found these mercaptoamides to be unstable in solution, probably owing to proteolysis of the acetamide bond, a series of mercapto alcohols and mercaptoketones were prepared. The mercapto alcohols **100** exhibited modest activity against MMP-1, MMP-3, and MMP-9, while the equivalent mercaptoketones could be optimized to active broad-spectrum inhibitors such as **101**. A related series of succinyl mercaptoalcohols and mercaptoketones were also prepared by the same group.[254] Compounds such as **102** and **103** were broad-spectrum inhibitors, while dipeptide derivatives such as **104** were totally devoid of MMP-1 inhibitory activity. Although there is no direct evidence for the binding mode of these compounds, the results seem consistent with the mercaptoalcohols and mercaptoketones acting as bidendate zinc ligands. This partly explains the different activities exhibited by the syn alcohol **102** and the anti alcohol **103**. No evidence was presented that any of these compounds showed in vivo activity.

The β-mercaptoacylamide **106** was prepared as one of a series of α-cyclohexyl derivatives.[255] This compound was most active against MMP-9 in vitro and exhibited some oral activity in rats, the plasma levels of **106** exceeding the IC_{50} values for MMP-3 at 4 h after administration. The 4-alkoxysubstituent on the cyclohexane group improved activity against all the enzymes investigated over the corresponding compound containing an unsubstituted cyclohexane group. Replacing the 4-ethoxy substituent with 4-propyloxy led to a significant reduction in MMP-1 activity and improvement in selectivity for MMP-3 over MMP-9. The equivalent cyclopentyl compounds were inactive, suggesting little penetration of the S1′ enzyme subsite. The mercaptoamides, exemplified by **107**, described in a Monsanto patent application, appear to be an interesting attempt to incorporate a bidendate thiolate ZBG into the sulfonamidocarboxylate framework,[256] though the simple γ-sulfone thiols, e.g., **108** described by Freskos, which lack this bidendate zinc-binding element also exhibit potent activity against MMP-13 and-8.[257] Constrained analogues of **108** with comparable activity and selectivity for MMP-13 over MMP-1 have recently been described.[258]

N-Acetylcysteine has been reported to affect the process of tumor cell invasion and metastasis by the inhibition of MMP-2 and MMP-9.[259] L-Cysteine-2-phenylethylamide is a more effective inhibitor, with analysis by modeling indicating that the phenethyl group fills the S1′ pocket of MMP-8.[260] Other cysteine derivatives have been explored by several groups. Foley and colleagues have used solid-phase chemistry to prepare several libraries of cysteine-containing dipeptide derivatives of the form RCO−Cys−AA−NH_2. Variation of the acyl and second amino acid led to activities against differing MMPs. Modeling studies indicate that the group R interacts at the S1′ subsite (vide infra).[261] Navre and co-workers have used solid-phase synthesis to prepare diketopiperazines bearing a pendant methylenethiol. These com-

pounds are described as having activity against MMP-1 and −8 (vide infra).[262,263]

3. Phosphorus-Based ZBG

Inhibitors based on phosphorus ZBGs have long been a favored area for investigation.[264] A variety of functionalities have been exploited to produce potent inhibitors against a range of MMPs, though so far none seem effective enough to have reached the clinic. Phosphonamidates as a ZBG can bind to the enzyme to form complexes in which either one of both of the oxygen atoms can coordinate to active site zinc. Z-Phe^{p}−Leu−Ala inhibits thermolysin effectively (K_i 68 pm) with one of the phosphonamidate oxygens binding strongly to the zinc ion and the other being more weakly coordinated with a longer Zn−O bonding distance (2.2−2.6 Å).[265] In the MMP inhibitor area, a typical example of an early phosphonamidate inhibitor is phthaloyl−CH_2−PO_2−Ile−Trp−NHBzl which inhibits a crude MMP-1 extract (K_i = 34 nM).[266] The related compound **109** has increased bulk in both the P1 and P2′ positions and is a slow binding inhibitor of MMP-3.[267] The Hagmann group also prepared phosphonate and phosphinic acid variants of compound **109**.[268] Whereas the phosphonates were inactive, the phosphinic acids were more potent than the corresponding phosphonamidates, a ranking of activity which differs from that found for thermolysin. Compounds such as **110** in which the group at P1′ was phenethyl were inhibitors of MMP-2 and MMP-3 but inactive against MMP-1.[268] The contribution that the phthalamidobutyl groups makes to binding to the S1−S3 sites is highlighted by the 1000-fold loss in MMP-3 activity when it is replaced by a methyl group. The crystal structure of the phosphinate **111** with MMP-7 has been determined. One of the phosphorus atoms is coordinated to the zinc atom (Zn−O bonding distance 1.96 Å), whereas the other oxygen points away from the zinc (Zn−O distance of 3.32 Å) and H-bonds to Glu-219 carboxyl (MMP-7 numbering).[76]

Effective phosphinic acid inhibitors have been developed by exploiting extra binding to the LHS binding sites S1−S3. Amino acid substituents have been used to improve MMP-3 selectivity in **112**.[269] This study indicated the need to involve all three unprimed sites in inhibitor binding to obtain optimal activities; shorter amino acid sequences led to >10-fold loss of activity. The S1−S3 subsites can also be explored by the use of substituted aromatic groups.[270] Reiter and co-workers have prepared compounds such as **113** where the 4-benzyl substituent was included so as to fill the S2 pocket.[270] Loss of activity was noted when the benzyl group was omitted or replaced by small aliphatic or cyclohexyl methyl groups, the latter being presumably too large for the S2 pocket. The binding mode of compound **113** was confirmed by its crystal structure with MMP-1, which showed the benzyl group at S2 and the isobutyl filling the S1′ pocket in a manner similar to other substrate-like inhibitors. The binding of the phosphinate group to the zinc was similar to that found with **111** and MMP-7. Selectivity for deep pocket enzymes was achieved by varying the P_1' position in a manner analogous to that described for hydroxamic acid derivatives. Thus, compound **114** which has a 4-phenoxybutyl group as the P_1' substituent is a selective deep pocket inhibitor. Of interest is compound **115** which is selective for MMP-13 over both MMP-1 and another deep pocket enzyme MMP-3. The authors suggest that this selectivity is determined by the interaction with the S2 binding sites.[270] Cyclic P_2'−P_3' phosphonate variants have been constructed analogous to similar hydroxamate structures.[179,271]

Castlehano has introduced the novel arylthiomethylene phosphinic acid ZBG.[124] Compounds such as the P2′−P3′ linked species **116** showed useful activities. However, they were less active against all MMPs than the corresponding hydroxamic acids (e.g., **3**).

N-Phosphonoalkyl dipeptides related to these phosphinates have been prepared by Hunter et al.,[272] who identified such compounds as potent inhibitors of MMP-1 (e.g., **117**). Conformationally restricted versions of compound **117** in which the P_1 and P_1' substituents are incorporated within a piperidine-2-carboxylic template (e.g., **118**) have been prepared.[273] As in the hydroxamic acid series, this cyclization strategy abolishes activity as an MMP inhibitor.

Morphy and co-workers have examined the effect of a number of ZBGs in a pseudodipeptide template. While the hydroxamic acid ZBG produced the most potent MMP-2 inhibitor, both the carboxylic acid and phosphonic acid derivatives were potent and selective inhibitors (MMP-2 over MMP-1).[153] Clearly any slight differences in the geometries which the various ZBGs assume on coordination to the zinc atom must be transmitted to the side-chain orientations in any general template. Consequently, while some idea of the relative binding efficiency of different ZBGs may be inferred from such studies, a true measure of their effect can only be really deduced by some side chain optimization in each particular case.

A similar study on an azalactam P2′/P3′ scaffold identified the aminophosphonic acid **119**, which was ∼10-fold less active against MMP-1 than the corresponding hydroxamic acid. Variation of the lactam ring size and the incorporation of a basic residue with the intent of increasing aqueous solubility reduced the activity of the aminophosphonates.[274]

4. Novel Zinc Binding Groups

One of the recurring themes of all investigations into new MMP inhibitors is the evaluation of potential new ZBGs: although most of this interest may be driven by the search for a proprietary intellectual position, new ZBGs may also offer a scaffold on which to better design true enzyme selective inhibitors and to overcome real or perceived liabilities with the currently used functionalities.

As described earlier, 5-substituted-1,3,4-thiadiazole-2-thiones have been identified from screening compound collections for inhibition of MMP-3.[104,105] Previously the known metal binding properties of this group have led to it being incorporated into antioxidants useful for paints. Stockman and Finzel have optimized early screening leads into two distinct series of MMP-3 inhibitors, the amides as in PNU-141803 (**120**) and the ureas as with PNU-142372

(**121**). An NMR[105] and crystal[104] determination of the urea **121** bound to MMP-3 has shown it to act as a LHS inhibitor with the aromatic ring extending into the hydrophobic S3 pocket, the thiadiazole sulfur forming a binding interaction with the catalytic Zn. The Zn−S−C angle is approximately 90° and the Zn−S bond length 2.3 Å. Both the nitrogen atoms of the heterocycle form H-bonding interactions with the enzyme; the N^4 accepts a hydrogen bond from the Ala-167 (MMP-3 numbering) backbone amide, while the N^3−H forms a bifurcated H bond to the carboxylate of Glu-202. Because of the coplanarity of the ring and the functional groups, the amide NH of **120** and the urea NHs of **121** are both able to form H bonds to the carbonyl oxygen of Ala-167. Alkylation of any of these inhibitor nitrogens or substitution by a carbon is reported to remove the inhibitory activity; this lends support to the conclusions of the physical studies. An SAR study on the amino acid side chain of derivatives of the urea **121** has demonstrated the requirement for a phenethyl group over smaller substituents.[275] The phenyl group can participate in a $\pi-\pi$ stacking interaction with the Tyr-155 (MMP-3 numbering). Enhanced stacking interaction along with hydrogen bonding via a water molecule to the His-166 both contribute to the unique potency of the pentafluoro derivative **121**. The replacement of the S3 tyrosine by a serine in MMP-1 (Table 2; residue 151) may explain the lack of activity against collagenase. While differential inhibition between MMP-1 and the S1′ deep pocket enzymes can be achieved by selective binding to that pocket as described earlier, this approach on its own may not be able to provide effective selectivity between the deep pocket enzymes, whatever the ZBG. The observations with these LHS inhibitors show that the unprimed sites may offer better selectivity between these enzymes, although the binding pockets do intrinsically provide less effective binding. This suggests that the unprimed subsites would be better used to modulate the binding primarily occasioned by the complexation of the ZBG and interaction with the S1′ pocket.

Pyrimidin-2,3,4-trione is a novel ZBG recently described in patent applications from Grams et al. at Boehringer Mannheim.[276,277] The binding mode is unclear, but both **122** and **123** show good activity against MMP-8 and MMP-9. A tetrapeptide sequence designed to mimic the HEXXH sequence that binds to the zinc active site has been investigated by Ferry.[278] The pseudo peptide H−His−ϵAhx−β-Ala−His−OH was identified from a combinatorial library (vide infra) as a weak MMP-2 inhibitor.[279] It is probable that these compounds do not act as true substrate inhibitors but instead chelate the active-site zinc, so converting the enzyme to an inactive form lacking the zinc ion. Potential ZBGs such as hydroxyamidines, amidopyridines, and amidomidazoles were examined as part of a comparison of the effect of different groups on a standard dipeptide framework that had given good MMP-2 inhibitory activity when attached to a hydroxamic acid group.[153] Activity was lost with the above groups which even with the caveats noted early probably is a true reflection of their potential.

Although the *N*-formylhydroxylamine ZBG was utilized in certain early MMP inhibitors,[114] it has not received a great deal of attention. This may have been due to apparent weaker activity than the corresponding hydroxamic acid compounds.[114,124] There may have also been concern about the possible metabolic liability of the *N*-formylhydroxylamine ZBG since 5-lipoxygenase inhibitors that feature the related *N*-acetylhydroxylamine group exhibit short half-lives in vivo.[280] Recently, Moss and co-workers have identified a dual MMP and TACE inhibitor, GW-3333 (**124**), which features the *N*-formylhydroxylamine ZBG.[281] This compound is orally available in the rats (bioavailability 25−50%; $t_{1/2}$ 2−4 h) and was orally active in a TNF-dependent peptidoglycan−polysaccharide (PGPS) reactivation arthritis model in the rat.[281]

E. Miscellaneous Natural Products

Screening has led to the discovery of both synthetic and natural product MMP inhibitors. The latter include tetracyclines such as aranciamycin and minocycline, for which it has been found that chemical modification can separate MMP activity from antibiotic activity,[282,283] i.e., CMT-1 (**125**) (IC_{50}s 30 μM vs MMP-8; 1 μM vs MMP-13). Tetracyclines which inhibit MMP-13 in vitro are also highly inhibitory of glycosaminoglycan release from IL-1-stimulated cartilage explants in culture.[284] This may reflect the ability of some tetracyclines to inhibit bond destruction in rat models. Other natural products include catechin derivatives,[285] pycnidione,[286] and a series of futoenone derivatives (e.g., **126**).[287] It is not immediately apparent as to how these diverse compounds interact with MMP enzymes. It is possible that a ring hydroxyl and/or carbonyl chelates the active-site zinc(II) ion. Indeed, it has been found for the futoenone derivatives that replacement of an oxygen substituent by a sulfhydryl, as in **126**, enhances inhibition of stromelysin presumably as a result of stronger zinc(II) ion chelation.[287]

Three groups of pseudopeptides Actinonin,[288] BE-166278 [289] (Banyu), and matlystatin B (**56**)[155] have been identified as potent MMP inhibitors. All of these compounds are hydroxamic acids that bear close structural similarity to similar structures obtained by substrate-based design. Although modifications to the antibiotic actinonin have not led to significant improvement in the MMP inhibitory activity, the more potent matlystatins have been a more fruitful starting point for the development of new inhibitor series.[201−203] The piperazic acid derivative **127** is a modest inhibitor of MMP-3; the terminal amide establishes H-bonds between the carbonyl and amide NH of Tyr-223 (MMP-3 numbering) and the amide NH and the enzyme's Asp-162 carbonyl oxygen. Presumably the NH of the piperazic acid also provides a H-bonding interaction to the enzyme which compensates for the loss of the interaction provided by the amide NH group present between the P1′ and P2′ groups in classical succinate structure. As described above, the related compound R-94138 (**27**) is a potent inhibitor of MMP-2 and MMP-9.[155] Few of

the other leads identified from natural products have been as amenable to optimization. Among the more interesting are nicotianamine,[290] betulinic acid,[291] glycyrrhetinic acid,[292] and rifampicin.[293] All of these compounds exhibit weak inhibitory activity, and in the absence of any information about their interaction with the enzymes active site, it is unclear how to best optimize their activities.

V. Combinatorial Synthesis of MMP Inhibitors

The application of combinatorial methods in the identification and optimization of bioactive compounds is now an integral part of the drug discovery process. The methodology and philosophy of combinatorial approaches have developed considerably over the past decade and have been the subject of intense interest.[294] At one level, combinatorial libraries can be large, random, diverse prospecting libraries which are used mainly to seek out new lead structures. Such libraries may be arrays of single compounds which may be assayed directly or defined mixtures of compounds from which active leads may be identified by an iterative deconvolution process. Another class of library is a targeted or focused library in which some concept of functionality, derived from substrate specificity and/or structural information, is incorporated into the individual library members.[216]

Recently, there have been a number of reports on the application of combinatorial methods to the inhibition of therapeutically relevant enzymes including MMPs. Several reviews of the topic have appeared.[112,113,295] For the most part, the approach taken has been to prepare targeted libraries, though some examples of the prospecting library approach have appeared. The majority of libraries feature a particular ZBG, and the effect of the attached substituents on potency and selectivity has then been investigated. The traditional substrate-based approach to inhibitor design is to attach a ZBG to a fragment of a preferred peptide substrate. This results in peptidic-based inhibitors which are highly amenable to preparation by combinatorial methods. As described above, this is a highly effective approach for the investigation of enzyme selectivity. However, a disadvantage of such an approach is that it is very difficult to obtain oral activity since the N−H group of amides is a known factor in limiting oral absorption. Furthermore, compounds based on natural amino acids may be prone to rapid proteolytic metabolism in vivo and, hence, have an unacceptably short half-life.[172] Thus, there is increasing interest in the preparation of nonpeptidic MMP inhibitor libraries.

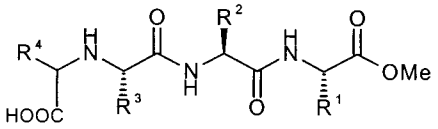
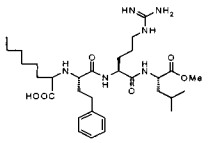
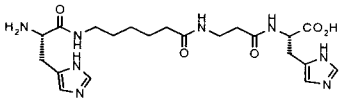
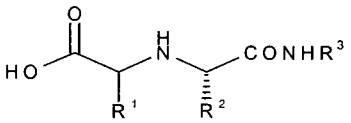
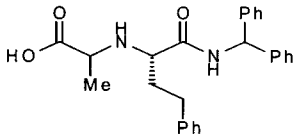
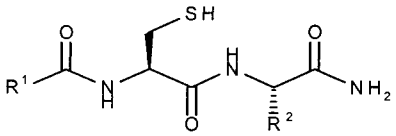
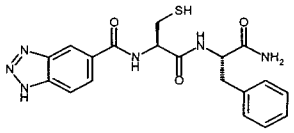
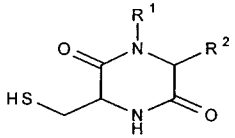
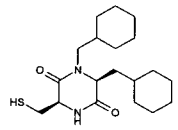
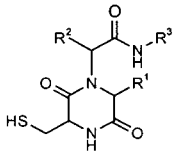
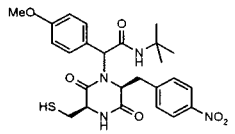
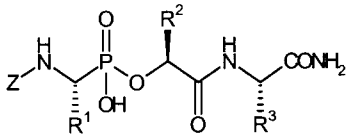
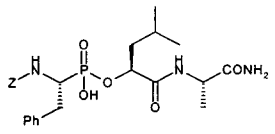
A 20 000 member library of *N*-carboxylalkyl tripeptides has been prepared by solid-phase synthesis following a combination of split/pool and indexed techniques (SpAM).[159,296] The library was screened against MMPs-1, -2, and -3 as 100 mixtures each of 200 compounds. Due to the indexed library approach for R_4 and R_3 introduction, SAR information was obtained directly for the P1−P1′ modifications and this was consistent with literature data. Deconvolution resulted in the identification of active inhibitors (e.g., **128**) (Table 3). In a similar manner, a group at Servier identified the compound H−His−ϵAhx−βAla−His−OH (**129**) as a weak inhibitor of MMP-2 and MMP-9 from an initial 13 824 member library (Table 3).[278,279] In contrast to the two previous libraries, Decicco and DeGrado used solid-phase chemistry to prepare a focused 100 member library incorporating the *N*-carboxyalkyl ZBG. This library was targeted to identify variations of the C-terminal amide substituent of the *N*-carboxyalkyl library. Weak inhibition of stromelysin-1 (MMP-3) was observed for a benzhydryl P2′ derivative (e.g., **130**; Table 3). In subsequent noncombinatorial studies, introduction of this modification into succinyl hydroxamic acid derivatives gave more potent compounds.[111] The synergy of the application of structure-based ligand design to the combinatorial approach was highlighted by the authors.

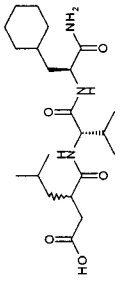
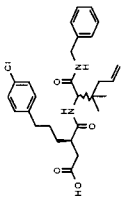
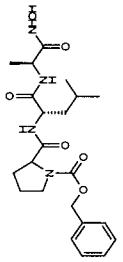
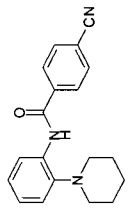
Foley and colleagues at Glaxo-Wellcome have identified cysteine-containing dipeptide amides (RCO−Cys−AA−NH_2) as MMP inhibitors.[261] They prepared several hundred compounds using parallel solid-phase synthesis on a Rink resin support. Broad-spectrum activity (e.g., **131**; Table 3) and selectivity against different enzymes was achieved by systematic variation of the general structure. A trifluoromethyl analogue ($R_1 = CF_3$; $R_2 = CH_2Ph$) is selective for MMP-1 over MMP-9 (IC_{50} 40 nM; >1000 nM) possibly because the CF_3 group can hydrogen bond to the Arg-214 of the collagenase P_1' pocket via a water molecule. In contrast, a phenylpropyl analogue ($R_1 = Ph(CH_2)_3$; $R_2 = CH_2Ph$) is selective for MMP-9 over MMP-1 (38 nM vs 3.5 μM), the phenylpropyl being much more suitable for filling the deep P_1' pocket of the gelatinases.

A different series of thiol ZBG libraries derived from cysteine has been described by Campbell and colleagues at Affymax. A pharmacophore model constructed from the crystal structures of succinyl hydroxamates bound to MMPs led to the identification of 2,5-diketopeperazines DKP as a scaffold with the potential of delivering the appropriate substituents to the binding sites of the enzymes. Fortunately, DKPs have been investigated extensively in earlier combinatorial studies and the synthesis on solid-phase is well developed.[297] From 684 compounds in pools of 19, compounds such as **132** with useful activity and moderate selectivity have been identified.[262] The incorporation of an Ugi-reaction step in the synthesis allowed extra functionality to be introduced onto the DKP scaffold.[263] Nine microtiter plates of discreet compounds were prepared, and the IC_{50}s against MMP-1 were determined by a protocol that normalized measured values against product concentration. Although the majority of the compounds were inactive, several compounds derived from 4-nitrophenylalanine showed good activity. Compound **133** was a potent inhibitor of MMP-1 and 60-fold less active against MMP-8 (IC_{50}s 21 nM vs MMP-1; 1300 nM vs MMP-9). The authors suggest that the selectivity for MMP-1 may be a consequence of an interaction between the aromatic nitro group and the Arg-214 (MMP-1 numbering) of the MMP-1 S1′ pocket.[263]

The Affymax group explored libraries of MMP inhibitors focused around ZBGs other than thiols,

Table 3. Matrix Metalloproteinase Inhibitor Libraries

ref	general structure	Library Size	Active Compound Identified		Inhibitory Activity
159, 296		100 x 200		128	MMP-3 IC_{50} 0.4 μM
278, 279	Tetrapeptide	24 x 13,824		129	MMP-2 IC_{50} 400 μM MMP-9 IC_{50} 300 μM
111		100		130	MMP-3 33% inhibition at 100 μM 72% inhibition at 200 μM
261		"Several hundred"		131	MMP-1 IC_{50} 8 nM MMP-2 IC_{50} 24 nM
262		2 x 684		132	MMP-1 IC_{50} 300 nM MMP-3 IC_{50} 2300 nM MMP-7 IC_{50} 590 nM MMP-9 IC_{50} 113 nM
263		920		133	MMP-1 IC_{50} 21 nM MMP-2 IC_{50} 1300 nM
299		540		134	Thermolysin K_i 49 nM

301	324	135	MMP-7 IC_{50} 165 nM	
302	> 90	136	MMP-2 IC_{50} 60 nM	
207	500	2	MMP-1 IC_{50} 8 μM MMP-2 IC_{50} 8.0 μM MMP-3 IC_{50} 3.5 μM	
316	1,600	140	MMP-1 IC_{50} 55,000 nM	

including phosphonates[298,299] and more recently carboxylates.[300] In the former study, compounds were screened against the bacterial metalloenzyme thermolysin while attached to beads using an enzyme depletion assay procedure. Iterative deconvolution was then employed for identification of active compounds (e.g., **134**). In the latter study, a library of 324 dipeptide succinates was prepared on Tentagel beads following a "split-mix" protocol using secondary amine encoding tags.[301] Following photolytic cleavage, the library was screened against matrilysin in a novel high-density nanowell array format. This study provided direct SAR for the P2′ and P3′ amino acids, and the re-synthesis of two inhibitors led to the identification of carboxylate **135** as an inhibitor of MMP-7 (Table 3).[301]

Related succinate inhibitors have been prepared by parallel solution-phase synthesis using the Ugi multicomponent reaction.[302,303] The carboxylic acid products, while active as MMP inhibitors (e.g., **136**; Table 3), can be converted to the more potent hydroxamic acids. The transformation to the hydroxamic acids is not a very efficient process and requires product purification by preparative HPLC, which, however, does separate the diastereoisomers. In the special case where a hydroxy group is present α to the hydroxamic acid as in analogues of marimastat (**12**), the desired hydroxamates were obtained directly in a one-pot five-component condensation.[303]

The importance of the hydroxamic acid group as a potent ZBG and the frequency of its incorporation in diverse series of MMP inhibitors led us to develop a versatile solid-phase route to compounds bearing this group.[207] Since our first reports on the use of hydroxylamine presenting resins, they have been the subject of intense interest.[304−313] In particular, we have used such a modified Wang resin in the preparation of a 500 member library of left-hand side tripeptide hydroxamates $Z-AA_3-AA_2-AA_1-NHOH$.[207,314,315]

To identify optimal amino acids for each subsite and so improve the inhibitory potency of the literature lead compound Z−Pro−Leu−Ala−NHOH (**2**), a 500 member library was generated as 10 mixtures each of 50 compounds in which the identity of the amino acid at P1 was defined but varied at the P2 and P3 positions (Figure 7). The tripeptide library mixtures, prepared using standard Fmoc protocols, were cleaved from the resin to give the product hydroxamic acids. Although assay of the mixtures against MMP-1, MMP-2, and MMP-3 suggested that D-leucine at P1 would give selectivity for MMP-2 while L-isoleucine at this position would provide potent broad-spectrum activity (Figure 8), iterative deconvolution of the mixtures with P1 D-leucine and P1 L-isoleucine in each case did not lead to the identification of compounds with improved activity over the literature lead compound **2**. This result probably reflects insufficient diversity within the library or cooperative effects between the mixture components on assay. With this experience, we decided to concentrate on producing single compounds by parallel synthesis methods. From the synthesis of over 200 individual tripeptides, it was found that substitution of L-proline by L-thioproline

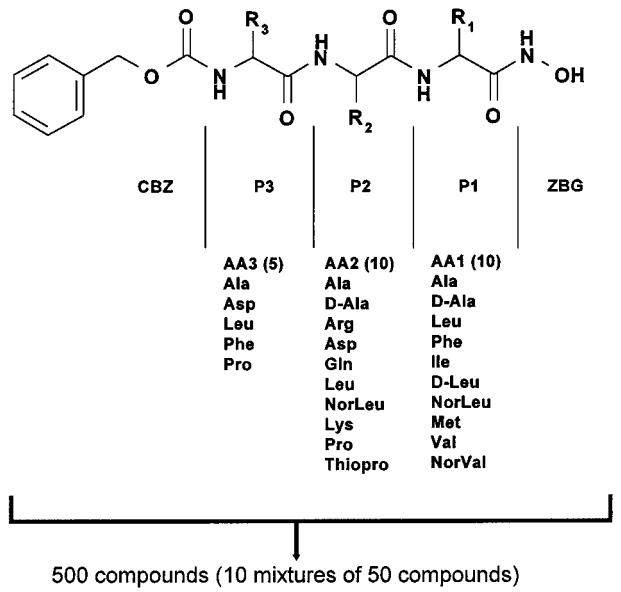


Figure 7. Left-hand side matrix metalloproteinase inhibitor library.

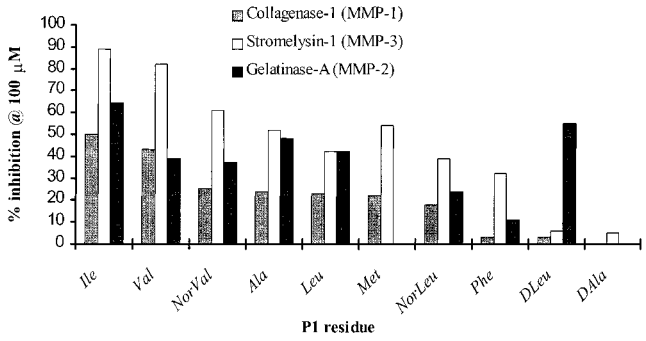


Figure 8. First-level assay of left-hand side matrix metalloproteinase inhibitor library.

at P3 and L-leucine by L-homophenylalanine at P2 gave a significant improvement in the inhibition of MMP-2 and MMP-3 (compound **137**). We found that L-stereochemistry and an extended side chain at P1 gave nanomolar inhibition of the three enzymes evaluated (compounds **138** and **139**). While there is a X-ray crystal structure of a tripeptide (Pro−Leu−Ala−NHOH) bound into the left-hand side (unprimed region) of the active site of MMP-8,[83] it does not necessarily follow that the more potent analogues adopt a similar binding mode. Although this study provided useful SAR and demonstrated the ability to optimize inhibitory activity by combinatorial methods, the peptidic nature of the compounds limits their utility as pharmaceutical agents.

The introduction of the hydroxamate ZBG into succinyl-based MMP inhibitors has been achieved, albeit as a mixture of regioisomers, by the reaction of succinic anhydrides with an *O*-hydroxylaminetrityl resin.[305] A novel series of hydroxamic acid sulfones (e.g., **62**) have been prepared using a solid-phase method by Burns and co-workers.[215] The precursor carboxylic acids were prepared by the addition of a thiophenol to a Wang resin bound acrylic acid and subsequent oxidation and, following cleavage from the resin, converted them to hydroxamic acids by capture and release from hydroxylamine resin. The compounds obtained included both broad-spectrum and selective MMP inhibitors.

A solution-phase prospecting library of 1600 compounds in pools of 40s was prepared using a technique that provided an internal method of deconvolution. The amide **140** was identified as a weak inhibitor of MMP-1.[316] The absence of subsequent patent applications on this series would suggest it has not proven to be a suitable lead into a new MMP inhibitor series.

VI. Pharmacological Effects in Disease Models

MMP inhibitors have been evaluated in a wide range of animal models of human disease. As with all in vivo pharmacological experiments, difficulties have often been encountered in ensuring sufficient exposure to the MMP inhibitors. This is particularly a problem in rodent models where rapid compound metabolism can often occur. Nevertheless, promising pharmacological effects have been observed with MMP inhibitors in animal models. In this section, an overview is given on the effects of MMP inhibitors in animal models of cancer and inflammation. There have been a number of reviews which specifically discuss the role of MMPs in cancer[317−324] and inflammatory diseases.[1,325−328]

A. Cancer

The ability of MMP inhibitors to restrict invasive tumor growth and metastasis has been demonstrated in a wide variety of animal cancer models. In early studies, the broad-spectrum MMP inhibitor SC-44463 (**9**) was shown to block organ colonization, or experimental metastasis, in mice innoculated with B16 murine melanoma cells.[329] More recently, MMP inhibitors have been tested in more complex and clinically relevant cancer models. In a study of a rat mammary carcinoma, Eccles and co-workers demonstrated effective suppression of micrometastatic disease with the broad-spectrum MMP inhibitor batimastat (**1**).[330] Animals were treated with short (7 days) and long (58 days) courses of batimastat starting just prior to the removal of the primary tumor grown adjacent to the mammary fat pad. Animals treated with the short course developed significantly fewer lung metastases than animals receiving a vehicle control, but approximately half of these animals developed local and distant lymph node metastases which led to significant morbidity. These lymphatic metastases, however, remained as silent micrometastases in animals receiving the long course of batimastat. This is a finding with obvious relevance for the adjuvant treatment of breast cancer.

Other studies have shown inhibition of the growth of human carcinomas established as xenografts either subcutaneously or by orthotopic implantation in the tissue of the human primary site. Both batimastat[331] and the selective inhibitor CT1746 (**41**)[175] have been shown to inhibit the local invasive growth and spread of orthotopically implanted human colorectal carcinoma. Batimastat was shown to inhibit the local regrowth of MBA-MD-435 human breast carcinoma following resection in nude mice[332] and to reduce

tumor growth and prolong survival in a xenograft model of human pancreatic cancer.[333] Batimastat also has a cytostatic effect in an orthotopic metastatic human hepatocellular carcinoma model in nude mice.[334]

The deep pocket MMP selective inhibitor AG3340 (**60**) has shown a range of effects in animals including inhibition of tumor growth in models of human glioma,[335] human colon carcinoma,[336] Lewis lung carcinoma,[213] and human nonsmall cell lung cancer.[337] When dosed intraperitoneally in the Lewis lung carcinoma model, AG3340 abolished primary tumor growth in four out of six mice. In addition, the formation of secondary tumors greater than 5 mm in diameter was reduced by 90%.[213] The deep pocket selective inhibitor BAY 12−9566 (**86**) has shown similar activity in models of Lewis lung carcinoma and B16 melanoma.[338] Both broad-spectrum (batimastat)[339] and selective MMP inhibitors (BAY 12−9566)[340] have shown antiangiogenic activity when tested in the Matrigel implant model.

MMP inhibitors have also been studied in combination with cytotoxic chemotherapies in animal cancer models. In these studies, the antitumor effects appear to be additive without additional marked toxicity. This has been observed for CT1746 (**41**) in combination with cisplatin or cyclophosphamide in the Lewis lung carcinoma model,[174] for AG3340 (**60**) in combination with carboplatin in a human lung cancer model,[341] for batimastat in combination with cisplatin in a human ovarian cancer model,[342] and for R-94138 (**27**) in combination with mitomycin C in a human gastric cancer model.[343] In the latter study, a combination of R-94138 and cisplatin was also investigated, but this was no more effective than cisplatin alone.[343]

On the basis of these experimental results and from theoretical considerations it would seem that the use of MMP inhibitors in combination with chemotherapies in patients with minimal or microscopic disease may be of benefit. Similar arguments have been made for other nonchemotherapy based cancer treatments such as the monoclonal antibody 17-A which has been tested in colorectal patients with minimal residual disease.[344] Finally, there are also indications that MMP inhibitors may have a chemopreventative potential. In a *Min* model of intestinal tumorigenesis, batimastat was able to suppress the development of tumors by nearly 50%.[345]

B. Inflammation

The migration of leucocytes through connective tissues, tissue destruction, remodeling, and angiogenesis observed in inflammatory diseases mirror similar MMP-driven processes in cancer.[346] There is now a considerable body of evidence that inflammatory leucocytes in culture and inflamed tissues in vivo express multiple MMPs.[326] The inhibition of the TNF-α converting enzyme (TACE, ADAM-17) also confers an added antiinflammatory potential to some MMP inhibitors,[33] such as BB-1101 (**11**). It is therefore not surprising that MMP inhibitors have been shown to be effective in a number of animal models of inflammatory disease, described below. The relative contributions of MMP inhibition and TACE inhibition will remain unclear until compounds which are specific for TACE and MMPs are tested. We have briefly reviewed below those diseases for which there is animal model data indicative of MMP inhibitor efficacy.

1. Arthritis

Degradation of the extracellular matrix is a characteristic of both rheumatoid arthritis and osteoarthritis.[1] Adjuvant arthritis is an arthritis-like syndrome that can be induced in rodents following injection with complete Freunds adjuvant. Batimastat (**1**) given intraperitoneally from onset of symptoms significantly reduced paw oedema bone degradation and cartilage breakdown.[347] BB-1101 (**11**) given orally also reduced paw oedema and bone degradation.[136] The reduction in paw swelling was attributed in part to the antiinflammatory effect of inhibiting TACE.[136] A succinyl hydroxamate, GI168, administered prior to symptom onset significantly reduced ankle swelling, bone and cartilage destruction, and the deposition of new bone and pannus.[348] The thiol MMP inhibitor **95** was also reported to reduce paw oedema in the adjuvant arthritis model.[249] The sulfonamide hydroxamate CGS 27023A (**58**) inhibited cartilage proteoglycan loss in the rabbit following direct injection of stromelysin into the knee joint[205,349] and also in a partial menisectomy model of osteoarthritis.[53,350] The collagenase-selective compound Ro 32−3555 (**52**) inhibited the degradation of articular cartilage in a rat monoarthritis model induced by an intra-articular injection of *Propionibacterium* acnes.[192] Interestingly, Ro 32−3555 did not show an effect in an adjuvant arthritis model.[192] However, the compound inhibited both cartilage and bone changes in a mouse model of osteoarthritis.[351] The potent TACE inhibitor GW-3333 (**124**) inhibited joint swelling, limp response, and body weight loss in a peptidoglycan−polysaccharide reactivation arthritis model in the rat following oral dosing.[281]

2. Restenosis

Restenosis is a complication of balloon catheter angioplasty, which is used to treat atherosclerosis. Restenosis also occurs in rats following balloon angioplasty. Batimastat (**1**) given for 7−14 days post angioplasty was shown to significantly reduce thickening of the carotid artery.[352] In similar studies, treatment with GM6001 (**39**) caused a transient effect on vessel thickening,[353] and reduced collagen deposition in the vessel wall.[354]

3. Aortic Aneurysm

Experimental aortic aneurysm can be induced in rats following a brief perfusion of elastase. Batimastat (**1**) reduced the extent of vascular inflammation and aortic enlargement associated with this model.[355] In a rodent arteriovenous fistula model, administration of the deep pocket selective MMP inhibitor RS-113,456 (**63**) limited flow-mediated arterial enlargement.[217]

4. Glomerulonephritis

Glomerulonephritis, a nephritic syndrome which results in destruction and fibrosis of the kidney, can

Table 4. Matrix Metalloproteinase Inhibitors in Development[a]

company	compound	indication(s)	status	structure
Agouron	AG3340	Cancer	Phase III	**60**
		Macular degeneration	Phase II	
Bayer	BAY 12-9566	Cancer	Phase III	**86**
British Biotech	Marimastat (BB-2516)	Cancer	Phase III	**12**
British Biotech	BB-3644	Cancer	Phase I	NR
Chiroscience	D1927	Cancer	Preclinical	NR
Chiroscience	D2163 (BMS 275291)	Cancer	Phase I	**98**
CollaGenex	Metastat	Cancer	Preclinical	NR
Novartis	CGS 27023A	Arthritis/cancer	Phase I	**58**
Roche	Ro 32-3555	Arthritis	Phase II	**52**
Roche Bioscience	RS-130,830	Osteoarthritis	Phase I	**64**

[a] NR: Structure has not been released.

be generated in rats by injection of an antibody against Thy-1.1. BB-1101 (**11**) given prior to disease induction significantly reduced the inflammatory response and kidney damage but was less effective given postinduction.[356]

5. Multiple Sclerosis

In experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS), rodents are immunized with myelin or one of its protein components in adjuvant, resulting in an autoimmune inflammation of the brain and spinal cord. Adoptive transfer of activated myelin-specific T cells can also result in a similar disease with recurrent episodes of paralysis. An MS-like lesion can also be induced in the brain by the generation of a delayed-type hypersensitivity response (DTH).

Gijbels and co-workers have shown that GM-6001 (**39**) given continuously or from disease onset can reduce symptoms in EAE.[357] Hewson and co-workers using Ro 31−9790 (**40**) showed a reduction in disease severity in both EAE and in an adoptive transfer EAE model when given from the start of the model.[358] BB-1101 (**11**) reduced the severity of disease in EAE[137] and both the severity and incidence of relapse in chronic relapsing EAE.[359] BB-1101 was also shown to inhibit the inflammation and demyelination associated with the DTH model.[360]

6. Guillain Barré Syndrome

Guillain Barré syndrome (GBS) is an acute inflammatory paralytic disease of the peripheral nervous system in which leucocytes infiltrate nerves causing demyelination and oedema. An animal model of GBS, experimental autoimmune neuritis (EAN), is induced in rodents by immunization with peripheral nerve myelin. This results in a T cell-dependent inflammation in peripheral nerves leading to paralysis. BB-1101 (**11**) given from initiation in EAN prevented the development of symptoms and reduced the inflammation, demyelination, and weight loss.[361] When given from onset of symptoms, the compound significantly reduced disease severity.

7. Stroke

Animal models of stroke usually involve clipping or blocking the mid-cerebral artery to give either permanent or temporary occlusion and reperfusion. Alternatively, injection of blood or bacterial collagenase into the brain can cause a local haemorrhage. An inflammatory infiltrate is associated with the damaged region in these models. BB-1101 (**11**) reduces the early phases of blood-brain barrier leakage in an ischemia reperfusion model in the rat and the secondary brain oedema which occurs following haemorrhage.[362,363]

8. Bacterial Meningitis

Rodents can develop bacterial meningitis following infection with bacteria. Batimastat (**1**) was effective in reducing intracranial pressure and blood-brain barrier breakdown in a model of meningococcal meningitis.[364]

9. Uveoretinitis

Uveoretinitis is an autoimmune inflammatory disease of the eye. Rodent models of uveitis involve immunization with retinal antigens in adjuvant. Treatment with BB-1101 (**11**) was shown to reduce retinal damage in experimental autoimmune uveitis.[365]

10. Graft-Versus-Host Disease

Graft-versus-host disease (GVHD) can be a major complication following allogeneic bone marrow transplantation. In a mouse model of lethal acute GVHD administration of KB−R7785 (**42**) reduced mortality.[366] This effect was attributed to the inhibition of both TNF-α and Fas ligand release by KB−R7785.

11. Noninsulin-Dependent Diabetes Mellitus

TNF-α is a key mediator of insulin resistance in noninsulin-dependent diabetes mellitus. In a mouse model of insulin resistance, administration of KB−R7785 (**42**) resulted in a significant decrease in both plasma glucose and insulin levels.[367] It is suggested that KB−R7785 exerts its antidiabetic effect by ameliorating insulin sensitivity through the inhibition of TNF-α production.

VII. Clinical Development of MMP Inhibitors

The clinical development of MMP inhibitors has to date been focused largely on cancer, although more recently these compounds are being tested in a range of other indications (Table 4). The earliest MMP inhibitors to be tested in patients were characterized

Table 5. Single-Dose Pharmacokinetic Profiles for Matrix Metalloproteinase Inhibitors in Healthy Volunteers

compound	dose (mg)	C_{max} (μg/L)	T_{max} (h)	$AUC_{0-\infty}$	$t_{1/2}$ (h)
AG-3340	10	127	1.5	274 μg·h/L	3−6
	50	620		1440 μg·h/L	
BAY 12-9566	400	29 mg/L		527 mg·h/L[a]	90−100
Marimastat	50	192	1.9	992 μg·h/L[a]	7−10
	400	1413	2.8	9904 μg·h/L[a]	
Ro 32-3555	50	1272	0.6	6140 μg·h/L	24

[a] 0−24 h AUC.

by their poor oral bioavailability. Consequently, alternative routes of administration were explored. Ilomastat (GM6001) (**39**) was administered in eye drops to normal volunteers and subsequently to patients with corneal ulcers.[368] Batimastat (BB-94) (**1**) was administered directly into the peritoneum or pleural space of patients with malignant effusions. A phase I study of intraperitoneal batimastat was conducted in patients with symptomatic malignant ascites. Patients with any form of malignancy who required paracentesis for symptomatic relief were eligible for the study. Patients received a single intraperitoneal dose of batimastat in 500 mL 5% dextrose (150−1350 mg/m^2) after paracentesis. In this study, batimastat was generally well tolerated and there were early signs of efficacy with several patients requiring no further paracentesis for over 3 months. The intraperitoneal administration of batimastat also gave rise to an unexpectedly high and sustained plasma concentrations of the drug with 100−200 ng/mL batimastat still detectable 28 days after a single administration.[369] Further development of batimastat for this indication was hindered by peritoneal irritation and poor tolerability.

A second phase I study of batimastat of similar design was conducted in patients with malignant pleural effusion. Patients with symptomatic malignant pleural effusion received lower doses of batimastat (15−135 mg/m^2), given intrapleurally in 50 mL 5% dextrose after aspiration of the effusion. Batimastat was well tolerated, and again there were early signs that the drug might be effective in palliation of this condition. In patients receiving batimastat at 60−135 mg/m^2 there was a significant reduction in the number of aspirations.[370]

Another series of early clinical trials involved the use of tetracycline derivatives with anticollagenase activity. In a placebo-controlled study, lymecycline in combination with conventional nonsteroidal anti-inflammatory treatment resulted in a decrease in the severity and duration of reactive arthritis triggered by *Clamydia trachomatis*.[371] Low-dose doxycycline (20 mg twice daily) was also reported to decrease urinary pyridinoline collagen cross-link excretion in patients with rheumatoid arthritis.[372] In a randomized study of patients with periodontal disease, tetracycline and minocycline gels were shown to significantly reduce the concentration of stromelysin in gingival crevicular fluid. This effect was not observed with metronidazole antimicrobial treatment.[373] Recently, Periostat (doxycycline hydrate) has been licensed for periodontal disease in the United States.[374] The relative importance of the antimicrobial and antimetalloproteinase properties in mediating these different effects will become clearer with the development and testing of nonantimicrobial tetracycline derivatives such as Metastat (Table 4).

In the past 2−3 years a number of second-generation orally bioavailable MMP inhibitors have started clinical trials including marimastat (BB-2516) (**12**), BAY 12−9566 (**86**), AG3340 (**60**), CGS-27023A (**58**), D2163 (**98**), and Ro 32−3555 (**52**). Pharmacokinetic parameters from phase I studies in healthy volunteers are presented for some of these compounds in Table 5. Marimastat and AG3340 appear to give broadly similar plasma exposure as measured by total drug AUC (area under the curve).[375−377] In contrast, BAY 12−9566 gives much higher plasma exposure with a prolonged elimination half-life.[378,379] This prolonged half-life may be attributable to high plasma protein binding. The pharmacokinetics of BAY 12−9566 are similar in both healthy volunteers and cancer patients, and there are indications of saturation of absorption.[380] The plasma exposure of marimastat is higher in patients with advanced cancer than in healthy volunteers.[381]

Results from later stage trials have only been presented for the treatment of patients with cancer. Since this class of agent is noncytotoxic, conventional oncology measures, such as cytoreductive tumor responses, could not be used to establish activity. In early trials with marimastat, cancer antigens were used as surrogate markers of disease stabilization. Using this approach, a combined analysis of a series of six similarly designed phase II trials has revealed a dose-dependent reduction in the rate of rise of these serum markers.[381−383] This has helped to define a biologically active dose range of 10−50 mg administered twice daily. The upper limit of this dose range was further refined by the finding that a dose of 50 mg twice daily was not well tolerated for longer than 1 month. In these and other studies, the principal side effect of marimastat has been identified as musculoskeletal pain and inflammation. This is manifested most commonly as tenderness, pain, and restriction of movement in the upper shoulder girdle, although other joints in the arms and legs have been affected.[381,384] The condition was shown to be largely reversible, and patients have continued treatment after a 2−4 week drug holiday.

Recently, we used a rodent model to investigate the effect of MMP inhibitor selectivity on the development of tendinitis.[385] In this model, broad-spectrum MMP inhibitors cause tendinitis, whereas this is not observed for collagenase selective inhibitors or deep pocket selective inhibitors. Interestingly, broad-spectrum MMP inhibitors which are also powerful inhibitors of TACE did not cause tendinitis. In the

tendinitis model, inhibitor blood levels were maintained at 150−300 ng/mL. In parallel studies of anticancer efficacy in the B16 melanoma model, only the broad-spectrum and broad-spectrum plus sheddase inhibitors inhibited tumor growth (inhibitor blood levels were 50−190 ng/mL). This suggests that some product of the sheddase reaction may contribute to the tendinitic syndrome. In these comparative studies in the rat tendinitis model and the B16 melanoma model, care was taken to ensure similar exposure levels to the MMP inhibitors under investigation. This was achieved by continuous administration of compounds using osmotic minipumps to ensure that similar blood levels were maintained with each compound throughout each study. We consider that it is necessary to carefully control exposure in this manner in order to make a valid comparison between the pharmacology of broad-spectrum and selective MMP inhibitors. It is possible that at high exposure levels such compounds are no longer selective and may in fact exhibit broad-spectrum activity. This may explain studies in which MMP inhibitors with deep pocket selectivity have been shown to be effective at inhibiting tumor growth in the B16 model.[213,236] Contrary to our results, Bird and co-workers proposed that it is the inhibition of non-MMP metalloproteinase enzymes such as TACE which gives rise to the musculoskeletal side effects.[250]

In a separate study, patients with advanced gastric cancer were examined endoscopically before and after 4 weeks treatment with marimastat given at 25 mg once daily or 50 mg twice daily. Treatment at both doses was shown to be associated with changes in the macroscopic and histological appearance of the tumors, consistent with an increase in the quantity of fibrotic stromal tissue. The changes were very similar to those seen in various cancer models, and several of the patients appeared to benefit from these alterations in tumor/stroma ratio.[386]

The safety and tolerability of marimastat has also been studied in combination with cytotoxic chemotherapies. Results from a trial of marimastat and carboplatin in patients with advanced ovarian cancer showed that the two agents could be administered in combination without apparent potentiation of the side effects of either drug.[387] Similarly, marimastat and gemcitabine appear to be well tolerated in combination in patients with pancreatic cancer.[388] These results are important since animal cancer model data indicate that it is likely that MMP inhibitors will be most effective if used early in combination with chemotherapy.

An alternative approach to the use of cancer antigens as pharmacodynamic markers has been reported for BAY 12-9566. In a phase I study in patients with advanced malignancy plasma levels of the angiogenesis-related growth factors, VEGF and bFGF were measured along with urinary levels of pyridinoline and deoxypyridinoline collagen cross links. Unfortunately, no effect on these markers was detected in the first 11 patients with doses of up to 400 mg 3 times daily.[389]

Marimastat, AG3340, and BAY 12−9566 are currently being tested in a range of randomized placebo-controlled studies in patients with advanced pancreatic, prostatic, gastric, lung, breast, and ovarian cancers, as well as in patients with glioblastoma. Although advanced cancer may not be the ideal setting for a tumorostatic agent, these trials may show a progression-free or overall survival advantage for patients receiving MMP inhibitor treatment and should also provide the sort of controlled safety data that will be required to conduct studies in the adjuvant setting, where from a theoretical basis one might expect the effects of these inhibitors to be greatest. Preliminary results from the first of these studies have been released. The trial compared the effect on overall survival of three different doses of marimastat to a standard regimen of gemcitabine in patients with advanced pancreatic cancer. None of the marimastat doses showed superiority to gemcitabine, and the median survival of patients at all three marimastat doses was inferior to that of gemcitabine patients. However, the 12 month survival of patients receiving the high dose of marimastat was the same as the gemcitabine patients, and a Cox proportional hazards analysis suggested that this dose was superior to the two lower doses.

VIII. Conclusion

The worldwide effort on MMP research has led to the discovery of a remarkably diverse group of compounds which are effective inhibitors of these enzymes. Despite this structural diversity, it has not yet been possible to identify specific inhibitors for each of the MMP enzymes. However, a number of compounds have been identified which exhibit a preference for the inhibition of MMPs with a deep S1′ pocket over those with a short S1′ pocket. The merits of such selectivity will ultimately only be discovered in the clinic, but there are indications that selective inhibitors are less effective than broad-spectrum MMP inhibitors in animal models of cancer. This is not surprising given the multiple expression of MMPs in cancer and other diseases.

Thus, MMP inhibitors may be an important new class of therapeutic agents for the treatment of diseases characterized by excessive extracellular matrix degradation and/or remodeling, such as cancer and chronic inflammatory diseases such as rheumatoid arthritis, atherosclerosis, and MS. However, rather than treating the primary cause of the disease, they will serve as disease-modifying agents which should stabilize the condition. In addition, they may also be useful in more acute inflammatory settings such as stroke and meningitis. Results from randomized clinical trials which will become available in the next few years will define the true utility of MMP inhibitors.

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