Discovery of Potent, Achiral Matrix Metalloproteinase Inhibitors

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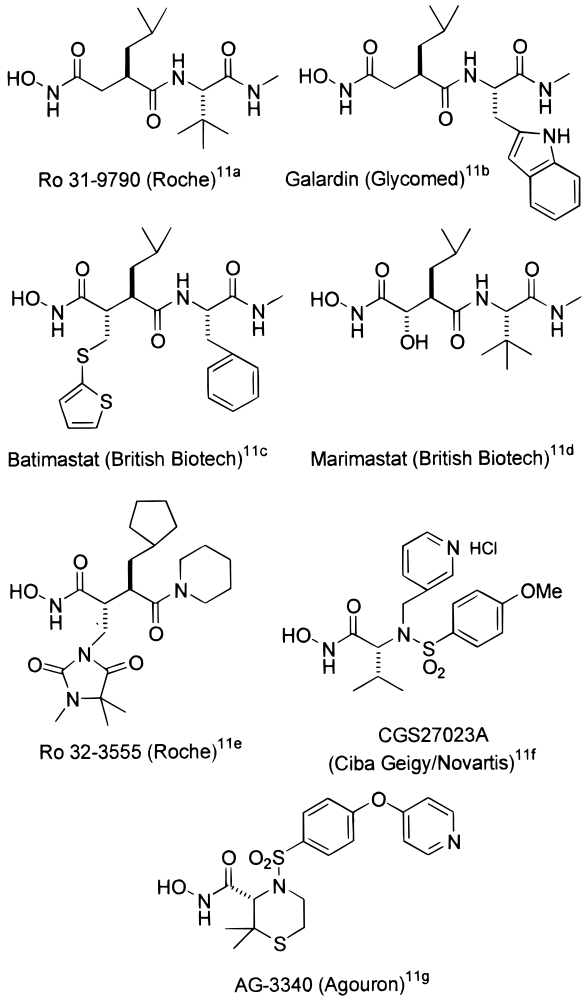
Received April 27, 1998

Introduction. The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that are capable of degrading many proteinaceous components of the extracellular matrix.[1] The enzymes have been implicated in several pathological processes including arthritis,[2,3] tumor growth and metastasis,[4] periodontal disease,[5] and multiple sclerosis.[6] Considerable research has been devoted to the discovery of potent MMP inhibitors which may act as potential disease-modifying agents in a number of important pathologies.[7] The phase II pancreatic cancer clinical trial data obtained with Marimastat, the most clinically advanced MMP inhibitor, has now been reported.[8,9]

The hydroxamic acid-based MMP inhibitors have been by far the most extensively studied class of inhibitors due to the ability of the hydroxamate group to efficiently complex the catalytic zinc and also to develop two hydrogen bonds to Glu-202 and Ala-165.[10] In addition to binding to the catalytic zinc, the hydroxamate-based MMP inhibitors require several more binding interactions for potent inhibition of the target enzymes. The S1′ and S2′ pockets have been extensively used for this purpose. While the design of MMP inhibitors is gradually becoming less complex, the chirality of such molecules demands extensive use of the chiral pool and/or stereoselective synthetic methods for their preparation. Structures of several MMP inhibitors that have progressed to early stages of clinical trial can serve as illustrative examples (see Chart 1).[11] We believed that the design of MMP inhibitors could be simplified by the introduction of a plane of symmetry. Such an approach also offered the potential for developing favorable binding at the S1 pocket. Indeed, this design, when applied to the sulfonamide-based MMP inhibitors,[11f] has led to the discovery of a novel series of simple, nonchiral, and potent in vitro inhibitors of the matrix metalloproteinases.[12,13]

Chart 1. Selected MMP Inhibitors



Chemistry. The synthesis of the MMP inhibitors was considerably simplified by the symmetry of the target molecules (see Scheme 1). Typically, a commercially available α,ω-diaminoalkane **1** was converted to the corresponding bis-sulfonamide **2** under the standard acylation conditions. In the next step the 1,3-diaza ring was formed by condensation of **2** with methyl glyoxalate polymer under the catalysis of sulfuric acid. Water formed during this reaction was removed via azeotropic distillation. The methyl ester **3** was then subjected to basic hydroxylamine solution[14] to provide the target hydroxamic acid **4** in 40−70% overall yield. X-ray crystallography data[15] obtained for compound **4e** (see Figure 1, top) confirmed the desired structure and symmetry of this series of MMP inhibitors. Compound **4e** was found to assume a chair conformation with the two methoxyphenylsulfonyl groups in equatorial positions and the hydroxamic acid group in an axial position. The arylsulfonyl groups were located in a trans relationship to each other.

In the case of compound **2d**, complete hydrolysis of the methyl ester occurred during the condensation step (see Scheme 2). The carboxylic acid **5** was cleanly re-esterified under standard conditions of thionyl chloride in methanol to afford methyl ester **6**, which upon

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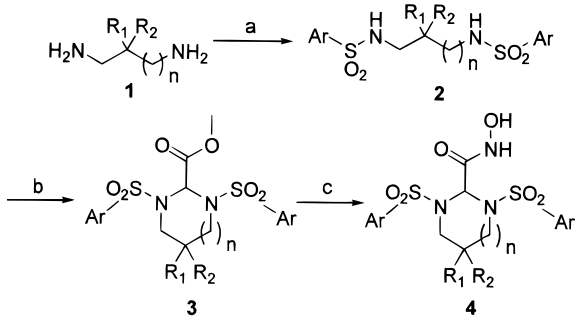
S0022-2623(98)00253-2 CCC: $15.00
Published on Web 08/22/1998

Scheme 1[a]



[a] Reagents: (a) $ArSO_2Cl$, Et_3N, CH_2Cl_2; (b) MeO_2CCHO, H_2SO_4, benzene, reflux; (c) NH_2OH, KOH, MeOH.

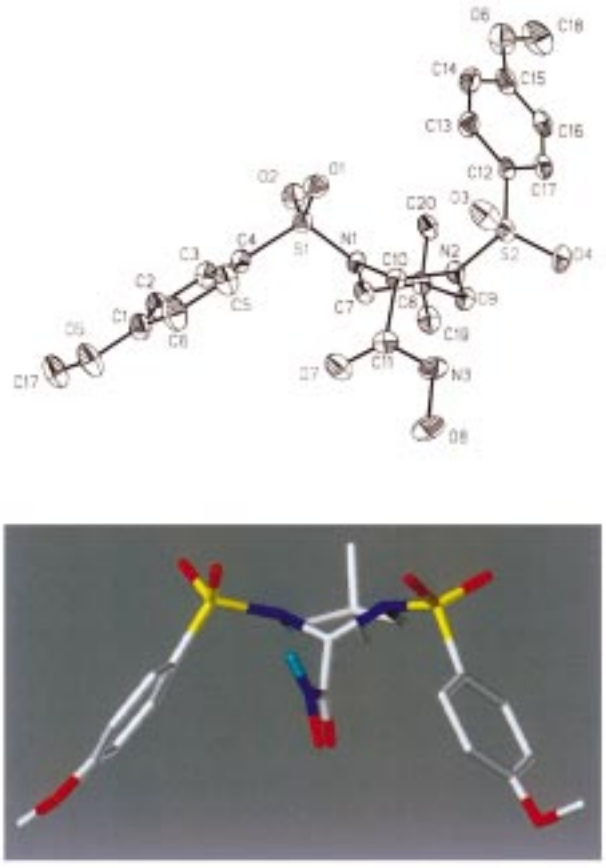


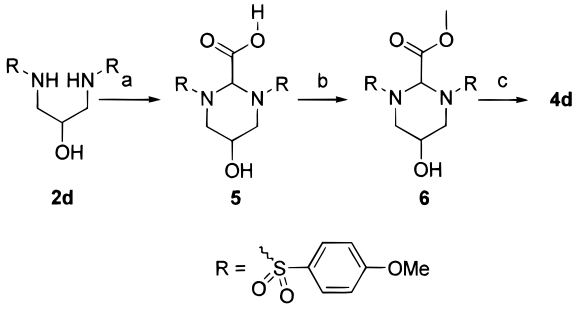
Figure 1. Conformation of **4e**: (top) free, an ORTEP view, and (bottom) stromelysin-bound.

treatment with hydroxylamine was directly converted to the target hydroxamic acid **4d**.

Compound **4f** was prepared from **2d** in four steps (Scheme 3). Jones oxidation of **2d** followed by 1,3-piperazine ring formation as described above (see Scheme 1) led to the keto ester **7**. The thioketal fragment was then introduced under standard conditions to give **8** which was directly converted to **4f** as described above.

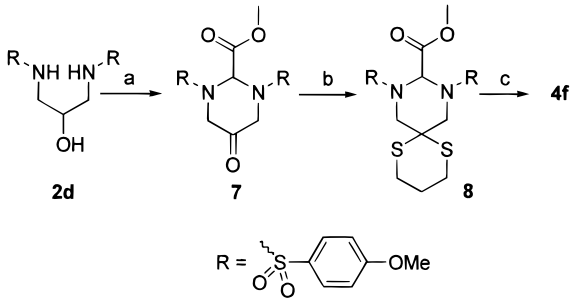
Biology. All compounds were tested for the inhibition of truncated collagenase-1 (MMP-1[16]), stromelysin (MMP-3[17]), matrilysin (MMP-7[16]), and gelatinase-B (MMP-9[16]), and the data are summarized in Table 1. At first we investigated simple bis(4-methoxyphenylsulfonamides) with different sizes of the central ring. It was gratifying to see a notable inhibition of collagenase and stromelysin by these simple, nonchiral molecules, especially the potent inhibition observed for **4b** containing a six-membered ring. Compounds with five-membered (**4a**) and seven-membered (**4c**) central rings were substantially less active with some preferential inhibition of MMP-1 by the seven-membered ring-based molecule over the five-membered ring compound. With the 1,3-piperazine emerging as the most promising ring size and a desire to maintain the plane of symmetry of the inhibitors, we decided to explore position C(5) as a site for further modification. Compounds **4d**−**f** were designed to test the MMP enzymes' tolerance for hydrophilic and lipophilic substituents. We found that addition of a hydroxyl group (compound **4d**) had no effect on potency compared to **4b**. However, the *gem*-dimethyl substituent present in **4e** noticeably improved potency for all enzymes, especially matrilysin. Interestingly, the larger size of substituent R_1 (compound **4f**) seemed to have a somewhat negative effect on potency especially for gelatinase-B. Another interesting effect was observed when the R_2 group was extended with the hope of expanding binding opportunities of the alkoxyarylsulfonamide in the S1′ pocket. Introduction of the *n*-butoxy group in place of the methoxy substituent (see compound **4g**) led to a dramatic loss of potency against collagenase and gelatinase-B and to a lesser extent against matrilysin while having no effect on the potency against stromelysin. One possible explanation for this effect is the inability of MMP-1 and MMP-7 to accommodate the *n*-butoxyphenyl substituent in their shallow S1′ pockets. However, a similar effect was observed for gelatinase-B, known to have a deep S1′ pocket.[18] This and the ability of both MMP-1 and MMP-7 to undergo a conformational shift to accommodate inhibitors with

Scheme 2[a]



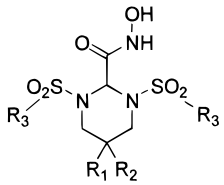
[a] Reagents: (a) MeO_2CCHO, H_2SO_4, benzene, reflux; (b) $SOCl_2$, MeOH; (c) NH_2OH, KOH, MeOH.

Scheme 3[a]



[a] Reagents: (a) 1. Jones reagent, Me_2CO, 2. MeO_2CCHO, H_2SO_4, benzene, reflux; (b) $HS(CH_2)_3SH$, $BF_3{-}OEt_2$; (c) NH_2OH, KOH, MeOH.

Table 1. Inhibition of MMP-1, -3, -7, and -9 by Compounds **4**



				IC$_{50}$ (nM)[a]			
compd	*n*	R_1, R_2	R_3	MMP-1	MMP-3	MMP-7	MMP-9
4a	0	H, H	C_6H_4−4-OMe	4650	1060	nd	nd
4b	1	H, H	C_6H_4−4-OMe	79	33	460	3.9
4c	2	H, H	C_6H_4−4-OMe	827	1290	nd	nd
4d	1	H, OH	C_6H_4−4-OMe	51	41	366	4.7
4e	1	Me, Me	C_6H_4−4-OMe	24	18.4	30	2.7
4f	1	$-S(CH_2)_3S-$	C_6H_4−4-OMe	79	19.5	123	15.6
4g	1	Me, Me	C_6H_4−4-OBu^n	2322	22	716	101
CGS27023A				49.5 (33)[b]	16.9 (43)[b]	106	4.3 (8)[b]

[a] Inhibitor concentrations were run in triplicate, and IC$_{50}$ determinations were calculated from a 4-parameter logistic fit of the data within a single experiment (see Supporting Information for details of the enzyme assays). nd, not determined. [b] K_i values reported[11] for CGS27023A are given in parentheses.

Figure 2. Stereoview of the active site of stromelysin with bound **4e** (all amino acid residues within 4-Å distance from the inhibitor are shown).

larger P1′ groups[10] might suggest that the P1 substituent is responsible for the selectivity observed with **4g**.

X-ray Crystallography. To better understand the interactions of this new series of MMP inhibitors with stromelysin we have obtained X-ray crystal data of compound **4e** with truncated stromelysin. The stereoview of the catalytic site of the enzyme with the bound inhibitor is shown in Figure 2.[19] The enzyme-bound **4e** has a plane of symmetry with a cis arrangement of the two arylsulfonyl groups as opposed to the trans arrangement observed in the crystal of **4e** itself (compare top and bottom panels in Figure 1). All interactions involving the hydroxamic acid group, the P1′ alkoxyaryl group, and the sulfonamide system linking them were found to be the same as those previously described.[20,10] However, several interactions unique for this series of MMP inhibitors can be observed with the second alkoxyarylsulfonyl group binding to the S1/S2 pocket (see Figure 3).[21] The two sulfonyl oxygen atoms and the imidazole ring of His-166 developed a network of hydrogen bonds involving a bridging molecule of water. This interaction seemed to compensate for the unfavorable interactions of the oxygens with the side chain of

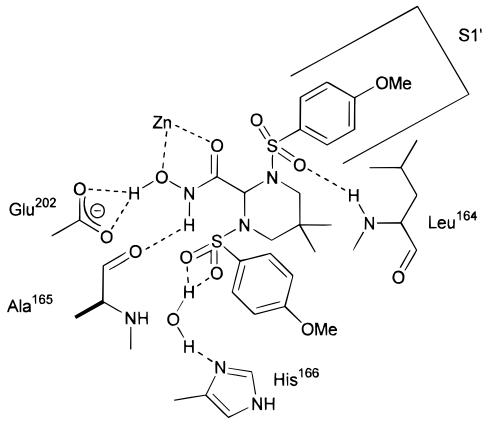


Figure 3. Binding interactions between **4e** and truncated stromelysin.

Val-163. The methoxyphenyl group developed several favorable van der Waals contacts including interactions of the phenyl ring with the His-166 side chain and the imidazole ring and between the methyl group and the aromatic ring of Phe-186. The C(5) carbon of the 1,3-piperazine ring was pointing out to the solvent; however,

the 5,5-*gem*-dimethyl substituents were still able to maintain favorable van der Waals distances to the side chains of both Val-163 and Pro-221. This interaction could explain a beneficial effect of the hydrophobic substituents at the C(5) position on potency against MMP-3 which was observed for **4e**−**g**. Overall, the X-ray structure of **4e** with stromelysin is consistent with the observed structure−activity relationsip (SAR), although it is not clear why inhibitors containing five- or seven-membered rings are much less potent compared to those with the six-membered ring.

Conclusion. In summary, we have introduced a symmetry element to the design of MMP inhibitors which led to the discovery of a novel series of MMP inhibitors that are both nonchiral and very easy to synthesize. The compounds based on the 1,3-piperazine heterocycle are potent inhibitors of collagenase, stromelysin, and gelatinase-B and moderate inhibitors of matrilysin. On the basis of X-ray crystallography of the inhibitor−enzyme complex, the binding of **4e** with stromelysin was found to involve several novel interactions at the S1/S2 pocket in addition to the interactions commonly observed for an arylsulfonamide−hydroxamic acid framework. Further exploration of this series is in progress and will be reported in due course.

Acknowledgment. The authors wish to thank the numerous P&GP scientists associated with the MMP project whose hard work and dedication over many years have contributed toward the completion of this work.

Supporting Information Available: Experimental procedures and NMR data for the synthesis of **4e**; details of enzyme expression, purification, and inhibition assays; X-ray crystallographic data for **4e** (8 pages). Ordering information is given on any current masthead page.

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JM980253R