Communications to the Editor

Structure-Based Design and Synthesis of a Potent Matrix Metalloproteinase-13 Inhibitor Based on a Pyrrolidinone Scaffold
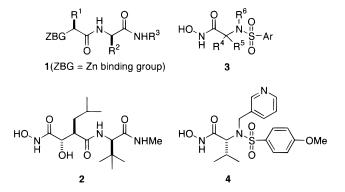
Ralph P. Robinson,* Ellen R. Laird, James F. Blake,
Jon Bordner, Kathleen M. Donahue,
Lori L. Lopresti-Morrow, Peter G. Mitchell,
Matthew R. Reese, Lisa M. Reeves,
Ethan J. Stam, and Sue A. Yocum

*Central Research Division, Pfizer Inc.,
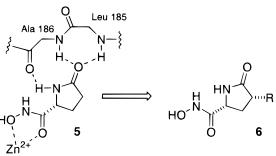Eastern Point Road, Groton, Connecticut 06340*

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Introduction. The inhibition of matrix metalloproteinases (MMPs) is an attractive approach toward the treatment of a variety of diseases.[1–3] For example, inhibitors of MMP-2 and MMP-9 are sought for prevention of cancer tumor growth,[4] while inhibitors of MMP-13 show potential for reducing cartilage loss in osteoarthritis.[5] Several MMP inhibitors are now in advanced clinical trials. However, there remains considerable room for optimization of oral absorption and duration of action while maintaining potency and selectivity. The vast majority of reported MMP inhibitors can be viewed as being derivatives of either the substrate-like peptidic motif **1** (e.g., marimistat, **2**) or the aryl sulfonamide-based hydroxamic acids **3** (e.g., CGS 27023A, **4**).[6] Although substantial modification of **1** and **3** is often tolerated, the discovery of new structural classes should prove valuable in the search for clinically useful MMP inhibitors. We now describe the structure-based design and synthesis of a novel, potent, and selective inhibitor of MMP-13 that utilizes a pyrrolidinone ring as a scaffold.[7]

Results and Discussion. X-ray crystal structures of MMPs (including MMP-13) with bound inhibitors reveal that inhibitor binding interactions typically include coordination of the active site Zn atom by a ligand (e.g., hydroxamic acid) and occupancy of the S1′ pocket with a hydrophobic group.[8–14] Another interaction often observed involves hydrogen bonding between an acceptor on the inhibitor and main chain NHs of amino acid residues flanking the catalytic site, specifically Leu-185 and Ala-186 (MMP-13 numbering). A few inhibitors also possess a proton donor that interacts with Ala-186.[8,10] The close proximity of the latter two interaction sites led us to consider lactams that could make both hydrogen bond-donating and -accepting contacts and, being cyclic, would possess the entropic advantage of ring constraint. Using a homology model of the catalytic domain of MMP-13 based on the X-ray structure of MMP-8,[15,16] five- and six-membered ring lactams were examined to identify the position most favorable for attaching a ligand for the active site Zn atom. Among the various possibilities, the pyrrolidin-5-one **5** bearing hydroxamate at the 2-position was especially attractive (Chart 1). As determined using a Monte Carlo fragment positioning algorithm,[17] this structure is able to adopt an energetically favorable orientation capable of direct-
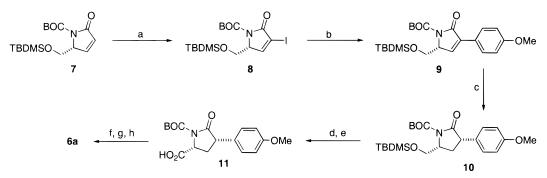
Chart 1

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* To whom correspondence should be addressed. Tel: 860-441-4923. Fax: 860-715-2469. E-mail: ralph_p_robinson@groton.pfizer.com.

Scheme 1[a]

[a] Reagents: (a) I_2, pyridine, CCl_4 (31%); (b) 4-MeO(C_6H_4)B(OH)$_2$, cat. Pd(PPh$_3$)$_4$, aq Na$_2$CO$_3$, toluene, $\triangle$ (77%); (c) H_2 (3 atm), 20% Pd(OH)$_2$/C, EtOH (100%); (d) aq 0.5 M HCl (1 equiv), THF (48%); (e) H_5IO_6, cat. CrO$_3$, wet CH$_3$CN/0 °C (98%); (f) PhCH$_2$ONH$_2$·HCl, BOP, *i*-Pr$_2$NEt, CH$_2$Cl$_2$ (73%); (g) HCl(g)/CH$_2$Cl$_2$ (80%); (h) H_2 (3 atm), 5% Pd/BaSO$_4$, MeOH (96%).

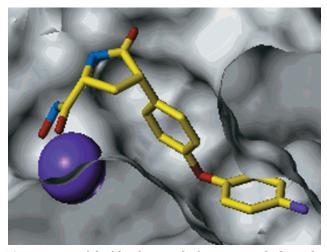ing a group (R) from the cis 4-position into the S1′ pocket as in **6** (Chart 1; see also Figure 1).

Figure 1. Model of binding mode for compound **6b** in the active site of MMP-13. The protein is rendered as a solvent-accessible surface and is clipped to reveal the S1′ pocket. The catalytic zinc is portrayed as a space-filled violet sphere.
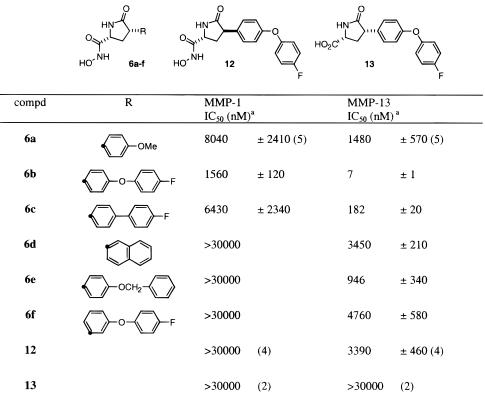
On the basis of the reported MMP binding mode of **4** and related aryl sulfone analogues (in which an aryl group fills the S1′ pocket),[14,18] as well as the availability of starting materials, compound **6a** (R = 4-methoxyphe-nyl) was first prepared. The synthesis began with the α,β-unsaturated γ-lactam **7**[19] which was α-iodinated[20] to obtain **8** (Scheme 1). Suzuki coupling[21] introduced the 4-methoxyphenyl group, providing **9**,[22] and hydrogena-tion, giving **10**, established the cis relationship required between the substituents on the pyrrolidinone ring.[23] Selective removal of the *tert*-butyldimethylsilyl protect-ing group[24] and oxidation of the intermediate alcohol[25] gave the carboxylic acid **11**. Final conversion to **6a** was carried out in three steps involving coupling to *O*-benzyl-hydroxylamine and removal of the BOC and benzyl[26] protecting groups.

Testing of **6a** against MMP-13 showed the compound to have modest activity (IC$_{50}$ = 1480 nM, Table 1). This result suggested that the pyrrolidinone scaffold could be functioning as proposed and that appropriate sub-stitution of the phenyl ring with larger hydrophobic groups might improve potency by filling the S1′ pocket more completely. Accordingly, compounds **6b–f** were prepared by routes paralleling that in Scheme 1.[27] On the basis of the reported SAR among analogues of **3**, as

well as docking studies using the MMP-13 homology model, the 4-(4-fluorophenoxy)phenyl compound **6b** was of particular interest (Figure 1). Indeed, this compound exhibited high potency against MMP-13 (IC$_{50}$ = 7 nM). As for **6a**, inhibition of MMP-1 by **6b** was weak, a potentially desirable result in consideration of side effects attributed to MMP-1 inhibition by "broad-spectrum" MMP inhibitors.[4] Additionally, **6b** showed modest to high selectivity for MMP-13 versus MMP-2, MMP-3, and MMP-9 (Table 2).

Because the pyrrolidinones **6** are conformationally restricted, it was expected that the potency of a given analogue against various MMPs would be particularly dependent on the degree to which the enzyme must reorganize (or its capacity to do so) upon ligand binding. Insofar as the S1′ site involves a large area of interac-tion, the involvement of amino acid residues defining this pocket is particularly relevant. The observation that **6b** is a very potent inhibitor of MMP-13 suggests that the residues comprising the MMP-13 S1′ pocket are pre-organized to accept this inhibitor without requiring energetically demanding movement. For the other MMPs, residues that may affect access to (or the flexibility of) the S1′ pocket can be identified to explain the decreases in the activity of **6b**. In MMP-1, an arginine side chain (Arg-214) residing within S1′ normally blocks large groups from entry resulting in a preference by MMP-1 for small P1′ substituents. Although in some cases large hydrophobic groups such as a diphenyl ether can be accommodated by displacement of Arg-214, binding potency is often reduced.[14] Similarly, in MMP-3, a tyrosine side chain (Tyr-223) has been observed to restrict access to S1′.[28] In MMP-2 and MMP-9 there are fewer residues in the loop linking helices B and C that forms part of S1′.[29] Therefore, the loop may be less flexible and so may be more restricted in accommodating rigid ligands.

Compounds **6c–f** were significantly less potent (at least ∼25-fold) than **6b** against MMP-13 (Table 1). The tight SAR is consistent with occupation of a well-defined pocket such as the S1′ site. An examination of **6c** and **6d** within the homology model shows that although the aryl substituents can be accommodated, they are less complementary than the naturally bent 4-(4-fluorophe-noxy)phenyl group of **6b** (Figure 1). The 4-benzyloxy-phenyl group of **6e** exhibits a similar geometry to **6b**, and therefore, the modest potency of **6e** against MMP-13 is surprising. The low potency of **6f** is consistent with

Table 1. Inhibition of MMP-1 and MMP-13 by **6a–f**, **12**, and **13**

compd	R	MMP-1 IC$_{50}$ (nM)[a]		MMP-13 IC$_{50}$ (nM)[a]	
6a	phenyl-OMe	8040	± 2410 (5)	1480	± 570 (5)
6b	phenyl-O-phenyl-F	1560	± 120	7	± 1
6c	phenyl-phenyl-F	6430	± 2340	182	± 20
6d	naphthyl	>30000		3450	± 210
6e	phenyl-OCH$_2$-phenyl	>30000		946	± 340
6f	phenyl-O-phenyl-F	>30000		4760	± 580
12		>30000	(4)	3390	± 460 (4)
13		>30000	(2)	>30000	(2)

[a] Values are ±SD of 3 determinations unless otherwise noted (*n*).

Table 2. Inhibition of Various MMPs by **6b**

enzyme	IC$_{50}$ (nM)[a]
MMP-1	1560 ± 120
MMP-2	39 ± 17
MMP-3	703 ± 170
MMP-9	82 ± 16
MMP-13	7 ± 1

[a] Values are ±SD of 3 determinations.

the model since a poor fit of the 3-(4-fluorophenoxy)-phenyl group within S1′ is predicted.

As expected, the trans (2*R*,4*R*) compound **12**[30] was considerably less potent versus MMP-13 (IC$_{50}$ = 3390 nM) than its cis stereoisomer **6b**. The carboxylate analogue of **6b** (**13**)[31] was devoid of MMP-13 activity in keeping with the SAR in other series where the carboxylates possess markedly less MMP inhibitory activity than the corresponding hydroxamates.

Conclusion. In summary, we have discovered a potent inhibitor of MMP-13 by a structure-based approach using a homology model of the enzyme derived from MMP-8. A pyrrolidinone template was selected based on an analysis of hydrogen-bonding interactions made by known MMP inhibitors and the vectors required for correct projection of established MMP binding groups (hydroxamate for Zn chelation, substituted aryl for occupation of the S1′ site). Despite the availability of several crystal structures of MMPs, few reports of their explicit use in the design of scaffolds exist.[32] The novel structure of **6b**, a 4-arylpyrrolidin-5-one-2-carboxylic acid hydroxyamide, represents a significant departure from those of the majority of reported MMP inhibitors. Although our work focused on inhibition of MMP-13, it seems likely that improved inhibition of other MMPs by analogues in this structural class may

be possible by optimization of the P1′ (R) group. Furthermore, the conformational rigidity of the series may be advantageous as a means to obtain selectivity. Thus **6b** may be regarded as a novel, nonpeptidic, and low-molecular-weight lead for continued pursuit of MMP inhibitors as drugs.

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(23) Proof of the assigned stereochemistry was obtained from single-crystal X-ray structure analysis of racemic **6f** (prepared via Suzuki coupling of **8** with 3-(4-fluorophenoxy)phenylboronic acid in refluxing toluene for 16 h). Comparison of the ^{1}H NMR spectra of **6a−e** to the spectrum of **6f** showed a common pattern of chemical shifts and coupling constants for the pyrrolidinone ring protons. The pattern was clearly distinguishable from that exhibited by **12**.

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