Complementarity of Combinatorial Chemistry and Structure-Based Ligand Design: Application to the Discovery of Novel Inhibitors of Matrix Metalloproteinases

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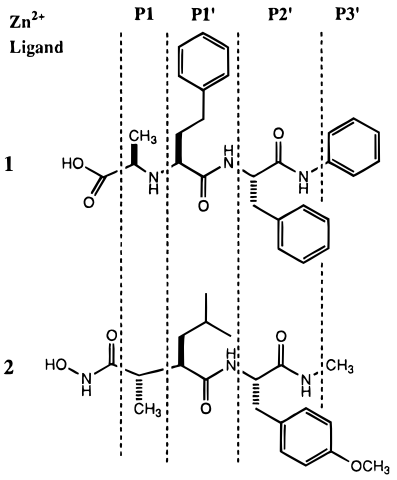
Received March 19, 1996

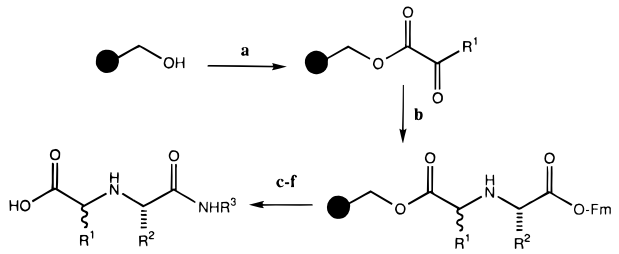
Combinatorial chemistry[1] and structure-based ligand design[2] have recently emerged as powerful methods for the discovery and optimization of ligands for a variety of enzymes and receptors. In this paper, we describe the complementarity of these two techniques in the design of a novel class of inhibitors of the matrix metalloproteinases human stromelysin (MMP-3: EC 3.4.24.17) and human neutrophil collagenase (MMP-8: EC 3.4.24.34). The cleavage specificity of these two enzymes has recently been shown to occur in the peptide sequence linking the G1 and G2 domains[3] of proteoglycan, a major macromolecular constituent of human cartilage. It has been postulated that inhibition of this enzymatic activity will lead to molecules that can function as cartilage protectants giving rise to a new class of drugs for the treatment of arthritis.[4]

Previous workers have described two classes of MMP inhibitors comprising the *N*-carboxyalkyl peptides[5] and the hydroxamic acids,[6] typified by **1** and **2**, respectively. Both classes of inhibitors are derivatives of peptides and, hence, have limited bioavailability and plasma half-lives. We therefore used combinatorial methods to discover a substitute for the right-hand, peptidic half of the molecule, which binds into the P2′ and P3′ regions of the enzyme active site.[7] To facilitate the preparation of large numbers of potential inhibitors, we devised a solid phase three-step synthesis of *N*-carboxyalkyl amino acid derivatives related to Enalapril, involving condensation with the support, reductive amination, and amide bond formation (Scheme 1). The synthesis allows independent variation of three functional groups. This procedure allows the synthesis of the targets as 1:1 mixtures of epimers with a purity of greater than 50% as assessed by HPLC and mass spectrometry (see supporting information).



	MMP-3 (Ki, nM)	MMP-8 (Ki, nM)
1	350	35
2	3	0.5

Scheme 1[a]



[a] (a) HO_2CCOR^1, HBTU, DIEA/DMF; (b) HCl $NH_2CH(R^2)COOFm$, $NaCNBH_3$, DIEA/0.5 M HOAc/DMF; (c) 20% piperidine/DMF; (d) HSpfp, DIC/DMF:CH_2Cl_2 (1:1); (e) NH_2R^3, DIEA, HOBT/DMF; (f) 4 N HCl/dioxane.

Previous studies have shown that compounds with $R^1 = CH_3$ and R^2 = 2-phenylethyl have good potency against MMP-3, and preliminary libraries supported these findings. Therefore, these functional groups were held constant, and a library of diverse amines was coupled onto this common framework. The compounds were screened for inhibition of MMP-3[8] without purification at a nominal concentration of 200 μM, and inhibition constants (K_I) were determined for purified compounds active in the initial screen. After screening over 100 R^3 substituents, the (*R*,*S*)-1-phenylethyl group was found to give a weakly active compound (10% inhibition of MMP-3 at 100 μM). The inhibition was stereospecific; while the *R*-isomer **3** inhibited MMP-3 6% at 100 μM, the corresponding value for the *S*-isomer **4** showed 33% inhibition at the same concentration.

We next attempted to model the mode of interaction of **4** with MMP-3 on the basis of the known structure of a complex of **2** with this enzyme. The fit was not satisfactory, and without knowledge of this inhibition, compound **4** would not have been investigated as an inhibitor candidate. Nevertheless, the best

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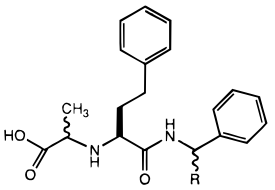
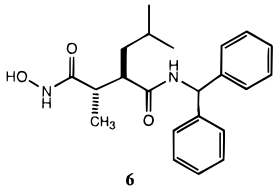
S0002-7863(96)00897-9 CCC: $12.00



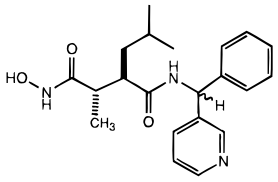
3, R = ***R***-CH_3
4, R = ***S***-CH_3
5, R = phenyl

docking suggested that the terminal phenyl group of **4** might be binding in the S2′ site of the enzyme. If this binding orientation was correct, then the affinity might be enhanced by substituting the terminal methyl group of **4** with a side chain known to be a good P2′ substituent.[5,6] A phenyl group was therefore introduced into this site, providing the benzhydrylamine derivative **5**, which gave 72% inhibition of MMP-3 at 200 μM. Finally, the benzhydrylamine group was introduced into the more potent hydroxamic acid template, leading to compound **6**, which inhibits MMP-3 and MMP-8 with K_I = 148 nM and 1.9 nM, respectively.



6

The high affinity of **6** for MMP-3 and -8 was puzzling in light of our computer modeling, which failed to show a well-defined hydrophobic pocket capable of binding the phenyl rings. We therefore determined the 1.8 Å resolution crystal structures of **6** bound to MMP-3 and compared it to the corresponding complex with the peptide-based hydroxamic acid **2**. Both compounds bind to MMP-3 in reasonably similar conformations. As in the complex with **2**, the pro-*S*-phenyl group of **6** projects into the P2′ side chain site, and the pro-*R*-phenyl group lies in a groove that binds the P2′ amide. However, a loop region (residues 222−231) in the protein undergoes an unexpected conformational shift, leading to major differences in the interactions that stabilize the two complexes (Figure 1).[9] While the *N*-methylamide of **2** hydrogen bonds to the backbone amides of MMP-3, the corresponding pro-*R*-phenyl of **4** is bound via favorable van der Waals and hydrophobic interactions. These differences arise from the very different conformations of the 222−231 loop in the two structures. Further careful examination of the enzyme complex with **6** suggested that a hydrogen bond acceptor would better facilitate inhibitor binding. Stereoisomers **7** and **8** were thus prepared, and a stereospecific binding preference for one diastereomer was observed (K_I = 9 nM) representing a 16-fold improvement in affinity for this enzyme. The antipode with the opposite stereochemistry alpha to the pyridyl ring bound more weakly (K_I = 150 nM).



7 and 8

Structure-based ligand design is a promising technique in which the three-dimensional structure of an active site is used to guide the design of novel ligands. While this technique has had many notable successes, it is often difficult to envision alternative modes of binding that involve deep-seated conformational changes. For instance, it would have been difficult to have predicted that a phenyl group would be an effective surrogate for the P3′ amide in **2**. *However, once combinatorial methods established this finding, modeling and crystallographic studies helped define the binding mode.* The crystallographic complex may now be used for additional rounds of design, synthesis, and evaluation. The discovery of the benzhydryl surrogate represents a much desired deviation from the more general structural elements common to most MMP inhibitors. Of major note, the removal of the P3′ amide bond without a significant decrease in enzyme affinity is an attractive feature. This compound represents an important starting point for the development of more potent compounds with high therapeutic potential.

(9) In this region, eight of the residues adopt $\phi\psi$ angles from different minima in the Ramachandran plot.

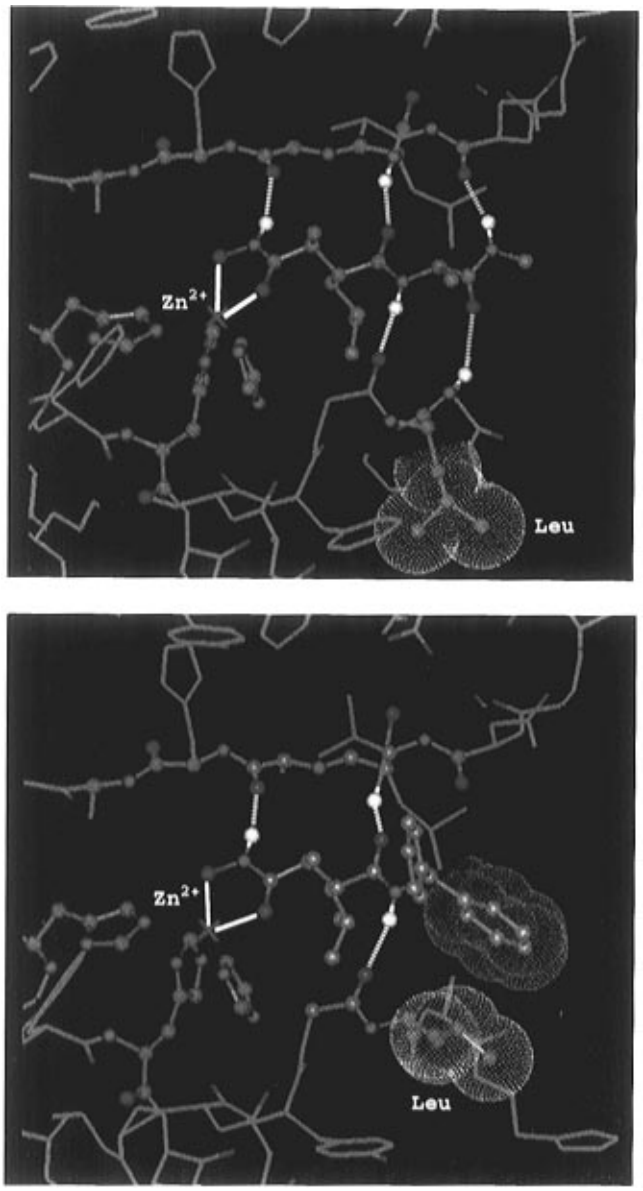


Figure 1. Crystal structure of the complex of compound **2** with MMP-3 (A). For clarity, the P2′ substituent is truncated at the β-carbon. Dashed lines indicate the positions of hydrogen bonds; yellow lines highlight the hydrogen bonds not present in the complex with the benzhydrylamine-based compound **6**. In the complex with **6** (B), a Leu residue (highlighted with white dots) swings up to interact with the phenyl rings of the benzhydrylamine.

Supporting Information Available: Experimental and spectroscopic details (4 pages). See any current masthead page for ordering and Internet access instructions.

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