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DESIGN, SYNTHESIS, ACTIVITY, AND STRUCTURE OF A NOVEL CLASS OF MATRIX METALLOPROTEINASE INHIBITORS CONTAINING A HETEROCYCLIC P_2'-P_3' AMIDE BOND ISOSTERE[%]

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Abstract: A novel series of hydroxamate-based inhibitors of matrix metalloproteinases containing benzimidazole and imidazole heterocycles as amide bond isosteres have been prepared. Potent inhibition (in the low nanomolar range) and selectivity (> 100-fold) can be attained with inhibitors containing only **one** amide bond. X-ray crystal structures of matrilysin (MMP-7) with two different inhibitors bound confirm that imidazole is an excellent amide bond isostere.

The matrix metalloproteinases (MMPs) are a family of zinc-containing proteinases involved in extracellular matrix remodeling and degradation.[1] More than twelve different MMPs have been identified. They are classified into three groups - collagenases, stromelysins, and gelatinases - based primarily on protein substrate specificities. Collectively, these enzymes can degrade all the major components of the extracellular matrix. MMPs have been implicated in a wide variety of biological processes including diseases[2] such as rheumatoid arthritis and osteoarthritis, tumor metastases, periodontal diseases, and multiple sclerosis. A number of different classes of MMP inhibitors, such as hydroxamates, thiols, phosphorus-types, N-carboxyalkyls, and carboxylates have been reported.[2] These inhibitors consist of a moiety capable of chelating the essential zinc metal found at the catalytic site and a peptidic fragment that binds to a subset of the specificity pockets of the enzymes, usually at the S′ sites (nomenclature of Schechter and Berger[3]). Typically, hydroxamate based inhibitors that contain two amide bonds have nanomolar potencies. An example of such an inhibitor, BB-94, has been in clinical evaluation for tumor metastases.[4] Poor pharmacokinetics due to the hydrolyzable amide bonds may, however, be a problem. The P_2'-P_3' amide bond in N-carboxyalkyl peptide inhibitors, for example, has been reported to be sensitive to enzymatic hydrolysis.[5] Our strategy was to substitute the C-terminal amide bond with isosteres that would be hydrolytically stable and at the same time retain all the important protein binding interactions.

The schematic diagram of the active site of collagenase or matrilysin with a peptide inhibitor bound[2,6] is shown in Figure 1a. The binding determinants are zinc chelation, four hydrogen bonds to the protein, and hydrophobic interactions at the S_1', S_2', and S_3' sites. An essential requirement for an amide bond isostere is its ability to act as both hydrogen bond acceptor and donor while maintaining the proper orientation of the side chain. A nitrogen based heterocycle such as imidazole appears to be a promising candidate. Such a heterocycle has been incorporated into antagonists of substance P.[7] A recent report of an orally-bioavailable HIV protease inhibitor containing an imidazole-derived amide bond replacement[8] prompts us to disclose benzimidazole- and imidazole-

[%] The refined coordinates for the enzyme-inhibitor complexes will be deposited in the Protein Data Bank.
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derived inhibitors having the general structure **2** as effective MMP inhibitors (see Fig. 1b).

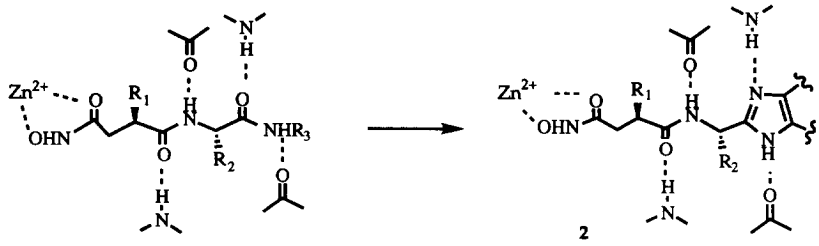
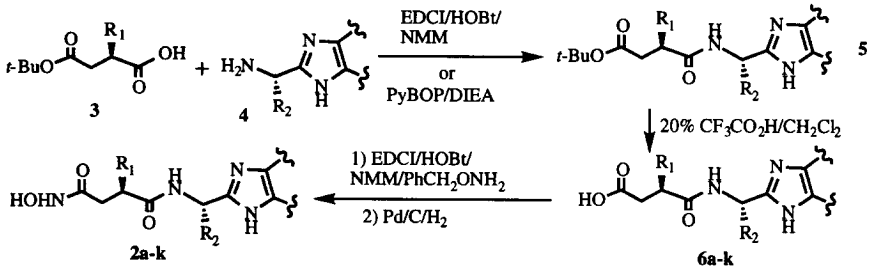


Figure 1. Schematic representation of the important interactions between the enzyme and (a) peptide inhibitor or (b) imidazole-based inhibitor.

The general strategy for the synthesis of **2** is illustrated in Scheme 1. The required chiral succinate derivative **3** containing the P_1' residue was prepared using Evan's asymmetric alkylation methodology described previously by us[9] and others.[2] The heterocyclic amine **4** was prepared from the CBZ-protected amino acid **7** according to Scheme 2. Coupling of **3** and **4** using HOBT/NMM/EDCI or PyBOP/DIEA, followed by treatment with TFA yielded the acid **6.** Coupling **6** with O-benzyl hydroxylamine followed by careful hydrogenolysis to avoid over-reduction of the hydroxamate to the parent amide, gave **2.**[10]

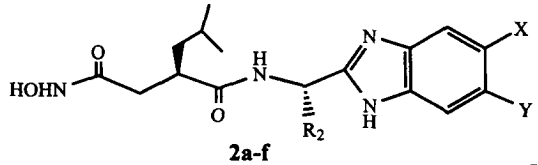


Scheme 1

The preparation of the heterocyclic amines (**10**, **13**, and **16**) involves the intramolecular cyclization of appropriate precursor amides (**8**, **11**, and **14**) followed by deprotection using Pd/C (Scheme 2). The cyclization of **8** to **9** was achieved by heating **8** in acetic acid at 65 °C for 15-20 min, in over 75% yield.[11] Refluxing **11** in acetic acid and concentrated ammonia[7] did not yield the desired product **12**. However, the use of solid ammonium acetate as the ammonia source[12] (refluxing of **11** with large excess (23-fold) of ammonium acetate in acetic acid for 5-8 h) did produce **12** in over 70% yield. Similarly, **15** was made through the ammonium acetate promoted cyclization of the acetal amide **14** (see Scheme 2).[13] Although the yield of **15** was much lower (20-30%) than that of **12**, it is comparable to that of the recently reported alternative procedure.[8]

The inhibitory potencies of the benzimidazole/imidazole-based compounds **2a-k** were assayed using the catalytic domains of human fibroblast stromelysin (HFS), human fibroblast collagenase (HFC) and matrilysin (MAT) by a fluorometric assay (Tables 1 and 2).[14] Replacement of the P_2'-P_3' amide bond in our dipeptide inhibitor RS 39066 (**1**) by the benzimidazole moiety resulted in only a 1 to 10-fold loss of potency (compare entry 1 and 2d), while substituents on benzimidazole at the 5 or 6 positions did not significantly affect the inhibitory

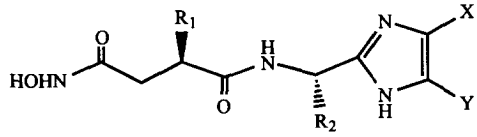
Table 1. Inhibition of MMPs by Benzimidazole-based Hydroxamates



2a-f

Cpd	R_2	X	Y	IC50 (nM) HFS	HFC	Mat
1	RS 39066 (see Fig. 1a)			0.8	6.9	0.23
2a	CH_2CHMe_2	H	H	8	29	2
2b	CH_2CHMe_2	H	CF_3	20	7	10
2c	CH_2CHMe_2	H	OCH_3	30	6	10
2d	CH_2CHMe_2	H	CO_2CH_3	5	3	1
2e	CH_2CHMe_2	Cl	Cl	68	40	33
2f	3-indolylmethyl	H	H	29	22	10

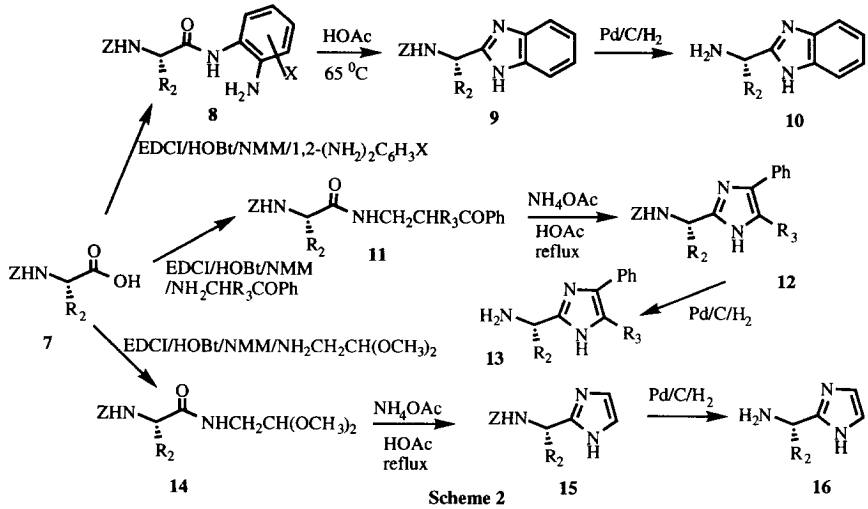
Table 2. Inhibition of MMPs by Imidazole-based Hydroxamates



2g-k

Cpd	R_1	R_2	X	Y	IC50 (nM) HFS	HFC	Mat
2g	CH_2CHMe_2	CH_2CHMe_2	H	H	320	9	50
2h	CH_2CHMe_2	CH_2CHMe_2	H	Ph	112	170	1
2i	CH_2CHMe_2	CH_2CHMe_2	CO_2Me	Ph	320	3500	70
2j	*c*-C_6H_{11}	CH_2CHMe_2	H	Ph	5900	480	3.1
2k	CH_2CHMe_2	3-indolylmethyl	H	H	280	3	18

potency. Selectivity and potency among different MMPs could be tuned by modification of the P_1' group, consistent with the trend observed for dipeptide based systems such as BB-94.[2,15]



Scheme 2

Both imidazole and substituted-imidazole derived compounds are also potent inhibitors of MMPs. The imidazole series is particularly sensitive to the ring substitution allowing development of very selective inhibitors (see Table 2). HFS accommodates large groups such as phenyl at the imidazole-4 position projecting towards S_3'. HFC is very sensitive to groups at this position on the imidazole ring (entries 2g-k, Table 2). The potency for MAT is attenuated by the substituents on the imidazole ring. Combining the optimum imidazole substitution, with the cyclohexyl at P_1' , which is preferred by MAT, leads to a remarkably selective inhibitor **2j**, which exhibits about 1000 fold selectivity for MAT versus HFS (see entry 2j, Table 2). These results suggest the possibility of achieving selectivity for a particular MMP by the variation of the substituents on the imidazole ring and P_1' group.

To confirm our design hypothesis and to assist in the structure-based design of more potent MMP inhibitors containing imidazole as the amide isostere, we have determined the crystal structures of matrilysin with two different inhibitors bound. Several inhibitors in Tables 1-2 were screened in our crystallization conditions.[16] Since carboxylates in the imidazole/benzimidazole series (**6a-k**) are in general weaker inhibitors of matrilysin (IC_{50} 1-100 μM), we also tried the co-crystallization of matrilysin with several carboxylates in order to permit the design of more potent carboxylate based inhibitors. Compounds **2k** and **6f** gave crystals large enough to collect X-ray diffraction data. The structures of matrilysin bound with compounds **2k** and **6f** (Fig. 2) were refined using data between 20.0-2.3 and 20.0-1.9 Å, respectively.[16] Both inhibitors bind as predicted, and as reported for peptide compounds.[6] In agreement with previous reports, the largest difference between carboxylate and hydroxamate-based inhibitors occurs at the methylene (0.8 Å difference) that presents the zinc ligand to the active site zinc.

When the C-terminal amide bond is replaced by a heterocycle isostere, the positions of the nitrogen atoms of the heterocycle correspond to those of the oxygen and nitrogen atoms of the amide bond (positional error of the structure is estimated by the Luzzatti plot[17] to be 0.25 Å). Although slight positional shifts were observed for residues neighboring the heterocycle, the potential hydrogen bond interactions are the same as reported previously

for dipeptide inhibitors (Fig. 2). From the structural point of view, imidazole and benzimidazole groups appear to be good amide replacements in these series of MMP inhibitors.

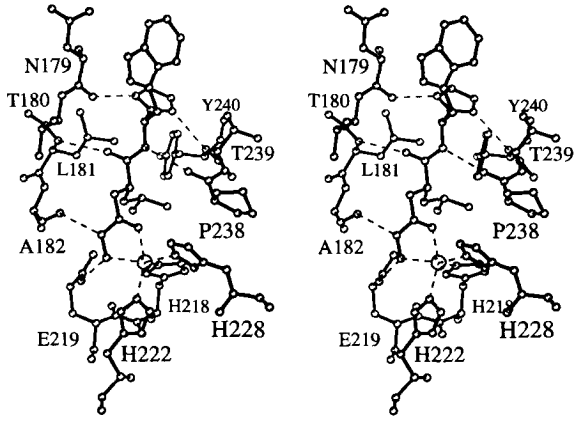


Figure 2. Stereo diagram of active site. Compound **2k** is at the center. Dashed lines represent polar interactions.

Results shown in Tables 1 and 2, coupled with the information obtained from the crystal structure studies clearly demonstrate that both imidazoles and benzimidazoles are suitable amide bond isosteres. Furthermore, the substitution pattern on the imidazole can be exploited to provide selectivity for a particular MMP. Both carboxylate and hydroxamate-based inhibitors containing imidazole and benzimidazole amide bond isosteres are in general about 1 to 100-fold less potent than the corresponding amide analogs. The difference in potency between imidazole and amide inhibitors may actually be less than observed; the imidazole group of the bound enzyme complex is unprotonated and this species is in equilibrium with the protonated form in the enzyme assay conditions (pH 7.5). This phenomenon was also reported in the studies of imidazole derived HIV proteinase inhibitors.[8] Factors contributing to the slight decrease in potency of **2a-k** may include a combination of the following: (1) decreased interaction resulting from replacing an O-H-N interaction with a N-H-N interaction, due to the lower electronegativity of nitrogen; (2) differences in electronic properties between an amide bond and an aromatic heterocycle which may effect the hydrogen bond forming ability of each group; (3) the pK_a of the heterocycle which will undoubtedly affect its ability to function as both a donor and an acceptor of a hydrogen bond at a given pH; and (4) the rigidity of the heterocyclic rings may prohibit the conformational adjustments necessary to optimize the interactions with the protein, as may be expected of the more flexible amide group.

In conclusion, we have successfully replaced the P_2'-P_3' amide bond in our dipeptide inhibitor RS 39066 by imidazoles and benzimidazoles while maintaining good potency against MMPs. These results and earlier reports[8] results clearly establish the viability of imidazole as a good amide isostere. Studies are in progress to determine the pharmacokinetic properties of our benzimidazole and imidazole based inhibitors and their *in vivo* activity in models of cartilage degradation and arthritis.

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10. All new compounds gave NMR and mass spectral data consistent with the assigned structures. Selected physical data for **2j**: ^{1}H NMR (DMSO-d_6) δ 8.7 (s, 1H), 8.0(d, 1H), 7.7(d, 2H), 7.65 (s, 1H), 7.3 (m, 2H), 7.15 (m, 1H), 4.95 (m, 1H), 2.5 (m, 1H), 2.2 (m, 2H), 1.6 (m, 8H), 1.4 (m, 1H), 1.1 (m, 5H), 0.85 (dd, 6H); FAB MS m/z 427.3 $(M+H)^+$.
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13. A typical procedure for the cyclization of **14** (R_2 = $CH_2CHC(CH_3)_2$) to **15** is as follows: **14** (50g, 142 mmol) and ammonium acetate (321g, 4 mol) were refluxed in acetic acid (321 mL) for 5 h. More acetic acid (50 mL) and ammonium acetate (50 g) were added and the mixture was refluxed for an additional 6 h. After removal of acetic acid, the residue was partitioned between ether (400 mL) and water (300 mL). The ethereal layer was separated and extracted with 2 *N* HCl (7 x 200 mL). The combined aqueous layers were carefully basified with potassium carbonate and extracted with ethyl actetate. The organic layer was washed with brine, dried with magnesium sulfate, and concentrated to give 12 grams of crude **15**. Pure **15** (7.3 g, 18%) was obtained by colum chromatography (35% ethyl acetate in dichloromethane) of the crude product: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 6.95 (s, 2H), 5.1 (m, 2H), 4.8 (m, 1H), 1.95 (m, 2H), 1.7 (m, 1H), 0.95 (2d, 6H); MS m/z 287 M^+.
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16. Crystals of the catalytic domain of matrilysin complexed with **2k** and **6f** were obtained by the hanging drop method with the following conditions: **2k** 0.1M MES pH 6.5, 1.6 M $MgSO_4$, 0.1% EtOH; **6f** 0.1 M Na acetate, 0.2 M $(NH_4)_2SO_4$, 20% PEG 4K. X-ray diffraction data were collected on a 30 cm MAR image plate using monochromatic Cu K_α radiation. The data were reduced using DENZO (ver 1.2.1) and scaled with SCALEPACK (ver 1.2.1). **2k** crystallized in space group $P3_221$; unit cell a = b = 61.7 Å, c = 174.8 Å, γ = 120°. Overall R_{merge} for 55848 observations was 6.7% to produce 17361 (97.0% complete) unique reflections to 2.3 Å. There are two molecules per asymmetric unit. **6f** crystallized in space group $C222_1$; unit cell a = 91.9 Å, b = 97.5 Å, c = 78.4 Å. Overall R_{merge} for 124560 measurements was 7.1% with 27615 (98.6 % complete) unique reflections to 1.9 Å. There are two molecules per asymmetric unit.

    Both structures were solved by molecular replacement using AMORE from CCP4 (ver 2.13)[18] using matrilysin as search model.[6] For **2k**, rotational search using all data between 10.0 and 2.3 Å resolution produced a peak with correlation coefficient of 20.9 (next highest peak had a correlation coefficient of only 7.7). Translational search using one molecule with this orientation confirmed the space group to be $P3_221$ in which the best search result had an R factor of 47.2%. The second molecule was located by a subsequent translational search with the first molecule fixed. After rigid body refinement the R factor dropped to 28.6%. Structure **6f** was similarly determined using all data between 10.0 - 2.5 Å resolution. The R factors after translation search for first and second molecule, and rigid body refinement were 47.3%, 36.3% and 35.3%. Inhibitors and metal ions were readily built into electron density maps (Fo-Fc) using the program O (ver 5.10).[19]
    Both structures were refined using X-PLOR (ver 3.1), including all data with $F>2\sigma$ between 20.0- 2.3 Å for **2k** and 20.0-1.9 Å for **6f**. Alternative cycles of model building and refinement proceeded smoothly. The final model of **2k** included 112 solvent molecules with R_{cryst} of 19.1% and free R_{free} of 26.7%; rms deviations from ideal geometry were 0.013 Å and 2.86° for bond length and bond angles. For **6f**, the final model included 195 solvent molecules with R_{cryst} of 19.5% and free R_{free} of 24.3%; rms deviations from ideal geometry were 0.014 Å and 2.86° for bond length and bond angles.
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