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Synthesis and Activity of Selective MMP Inhibitors with an Aryl Backbone

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Abstract—A series of novel, MMP-1 sparing arylhydroxamate sulfonamides with activity against MMP-2 and -13 is described.

Individual members of the matrix metalloproteinase (MMP) family of zinc-dependent enzymes catalyze a variety of important physiological processes, such as tissue regeneration, angiogenesis, and growth and development (Fig. 1).

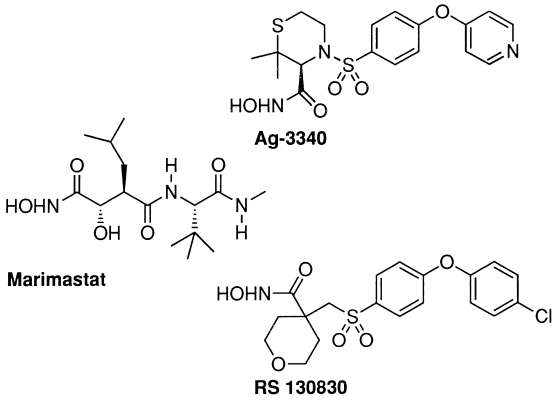

Figure 1. Structures of some reported MMP inhibitors.

Excessive MMP activity and the concomitant increased remodeling of the extracellular matrix is thought to exacerbate a number of pathologies, for example, the infiltration and metastasis of tumors, the erosion of the synovium in arthritis, and the loss of tone of cardiac tissue post myocardial infarction. Considerable research has been devoted towards identifying small-molecule MMP inhibitors with the hope that such compounds might prove clinically useful in attenuating excessive remodeling and improving the outcome of the above conditions and others.

Broad-spectrum inhibitors, such as British Biotech's marimastat, have been found to induce over the course of treatment a dose-limiting joint-stiffening, which must be reversed by putting the patient on a 'drug holiday'.[1] Since MMP-1 is ubiquitous[2] and is used in constitutive tissue housekeeping, there is keen interest in exploring inhibitors that spare MMP-1[3] while blocking those MMPs that have been associated with disease states, such as MMP-13 in arthritis[4] and MMP-2 in cancer metastasis.[4]

This paper describes a novel series of aryl hydroxamic acid derivatives that inhibit MMP-2 and -13 and spare MMP-1. The series was conceived *de novo*, upon consideration of the bond distances and geometry of the active site of these enzymes. Specifically, we felt that, with a hydroxamate bound to the zinc in the catalytic site, a two-carbon sp^2 linker joined to a sulfonamide should position an oxygen of that sulfonamide sufficiently close to the Leu 160 in the enzyme backbone to form a hydrogen bond. Such a bond is a key feature of essentially all published inhibitors.[5] The other planned design feature was to install a relatively long substituent

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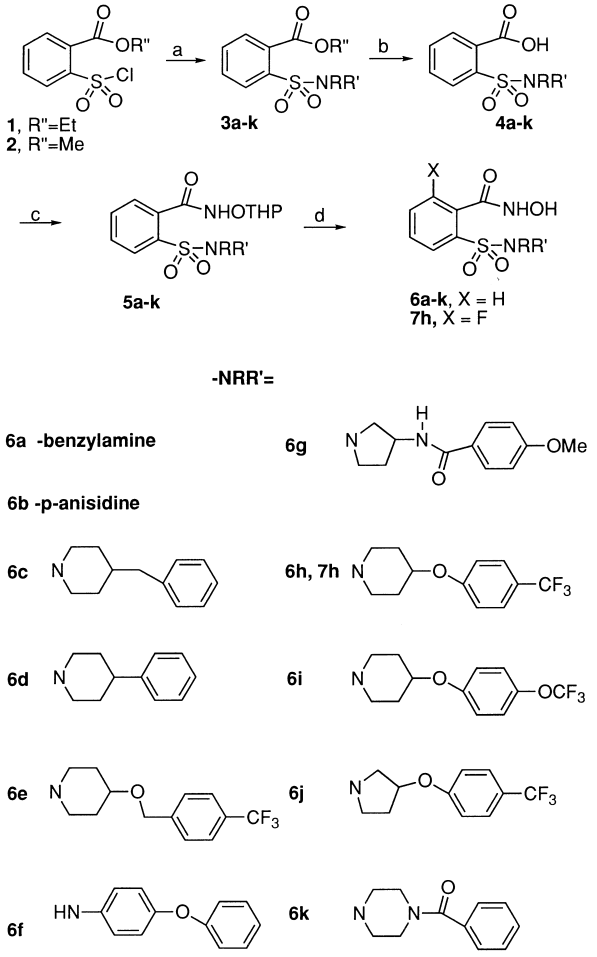
into P1′, to favor selectivity against MMP-1, as MMP-1 has a relatively more shallow pocket.

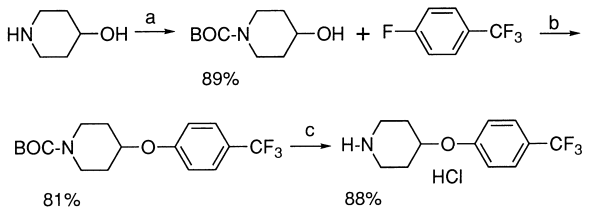
Chemistry

The requisite ester-sulfonyl chloride aryl scaffold (**1** or **2**) was prepared by the method of Nagasawa[6] or purchased[7] (Scheme 1) and was allowed to react with the requisite amines. The resulting sulfonamide esters (**3a**–**k**) were saponified to the corresponding acids (**4a**–**k**) and coupled with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine using EDC. Acid hydrolysis yielded the desired hydroxamates.



Scheme 1. (a) HNRR′, triethylamine, acetonitrile; (b) KOH, EtOH (aq); (c) EDC, HOBT, $THPONH_2$, DMF; (d) AcCl, MeOH.

The non-commercial amines leading to **6h**–**k** were prepared by routine protect/deprotect sequences or as shown in Scheme 2, by nucleophilic displacements of aryl fluorides.[8] The cesium carbonate conditions worked well in the example shown, but less reactive starting materials, such as 1-fluoro-4-trifluoromethoxybenzene required more forcing conditions (NaH/*N*-methylpyrrolidinone).



Scheme 2. (a) $(BOC)_2O$, THF, 0 °C; (b) Cs_2CO_3, 120 °C, DMF 2 days; (c) 4 N HCl, dioxane.

Crystallography

Figure 2 shows compound **7h** bound to the MMP-8 enzyme.[9] The complex was solved at 1.8 Å with an $R = 0.26$ and an $R_{free} = 0.33$. The sulfonyl group of the inhibitor makes a single hydrogen bond to the protein through the pro-*S* sulfonamide oxygen to the backbone amide hydrogen of Leu 160. In addition to the three zinc ligands derived from the histidine sidechains at positions 197, 201, and 207, the inhibitor ligates the zinc atoms through the oxygens of the hydroxamate moiety. The -OH group of the hydroxamate makes a hydrogen bond with the Glu 198 side chain. The piperidine-O-phenyl moiety extends into the S1′ subsite and makes van der Waals contact with the protein. Interestingly, *the sulfonyl group sits axial to the piperidine ring*; the *cis* conformation around the phenyl-SO_2 group, and the spatial constraints imposed by hydrogen bonding and the S1′ subsite do not allow steric-free positioning with an equatorial configuration. The selectivity against MMP-1 was achieved by making P1′ moieties of sufficient length to sterically interfere with the Arg residue in MMP-1 that is positionally equivalent to Leu 193 in MMP-8.

Results and Discussion

The aryl hydroxamates were assayed in vitro using previously described protocols.[10]

As shown in Table 1, it is clear that we were able to prepare compounds with potent inhibition of MMP-2 and -13 from a variety of different amines. The entries with very short P1′ subunits (**6a** and **6b**) showed poorer

Table 1. IC_{50}'s of the studied hydroxamates, in nM

Compound	MMP-2	MMP-13	MMP-1
6a	ND	>10,000	>10,000
6b	ND	>10,000	>10,000
6c	148	1000	>10,000
6d	70	520	>10,000
6e	35	900	>10,000
6f	3500	9000	>10,000
6g	2300	2200	>10,000
6h	1.3	28	>10,000
6i	2.0	33	>10,000
6j	1800	2000	>10,000
6k	1000	6700	>10,000
7h	3.3	12.2	>10,000
Marimastat	0.75	2.0	2.9
AG-3340	0.3	0.5	23.5
RS-130830	0.4	0.6	800

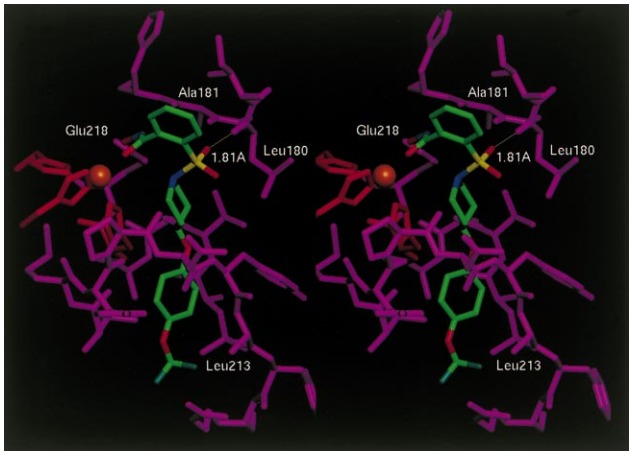


Figure 2. X-ray crystal structure of **7h** bound to hMMP-8.

potency across the board. Perhaps any van der Waals stabilization of P1′ from the pocket is minimal for these analogues. The most active analogues (**6h–i**, and **7h**) have P1′ subunits which reach down to the bottom of the pocket, having a length and volume similar to what is seen in, for example, Ag-3340 and RS-130630.

The potency of **6h** and **6i** towards the target enzymes and the extraordinary level of selectivity against MMP-1 has encouraged us to pursue this aryl series further.

Additional results will be reported in due course.[11]

References and Notes

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3. The hypothesis that sparing MMP-1 is necessary and sufficient to prevent the musculoskeletal stiffening has been called into question in view of recent clinical results where it is observed that certain inhibitors that inhibit MMP-1 nevertheless do not appear to show the side effect. See Shaw, T.; Nixon, J. S.; Bottomley, K. M. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1469.
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6. Nagasawa, H. T.; Kawle, S. P.; Elberling, J. A.; DeMaster, E. G.; Fukoto, J. M. *J. Med. Chem.* **1995**, *38*, 1865.
7. Sulfonyl chloride **2** is available from Lancaster, but in our hands reactions involving the ethyl ester seemed cleaner.
8. (a) Analogue **7h** was prepared by ortholithiation of the corresponding 3-fluorosulfonamide, followed by a carbon dioxide quench. The acid was transformed to the hydroxamate by making the acid chloride and reacting with tetrahydropyranhydroxylamine. Full details will be reported in a subsequent communication. (b) Sindelar, K.; Hrubantova, M.; Svatek, E.; Matousova, O.; Metysova, J. *Collect. Czech. Chem. Commun.* **1989**, *54*, 2240
9. Compound **7h** had an IC_{50} of 33 nM against this enzyme.
10. Assays were conducted at six dilutions with an N at least equal to 2. Inhibitors were tested against purified hMMP-13, hMMP-1 and hMMP-2 using an enzyme assay based on cleavage of the fluorogenic peptide MCA-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2. This is similar to conditions described by Knight, C. G.; Willenbrock, F.; Murphy, G. in *FEBS Lett.* **1992**, *296*, 263, except that 0.02% final concentration of 2-mercaptoethanol was used in the MMP-13 and MMP-1 assays.
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