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MMP-13 Selective α-sulfone Hydroxamates: a Survey of P1' Heterocyclic Amide Isosteres

Thomas E. Barta
Pfizer Research & Development

Daniel P. Becker
Loyola University Chicago, dbecke3@luc.edu

Louis J. Bedell
Pfizer Research & Development

Alan M. Easton
Loyola University Chicago

A Survey of Isosteric Amide Replacements in a Series of Extended, Hydroxamate-containing, Selective MMP-13 Inhibitors

Thomas E. Barta, Daniel P. Becker, Louis J. Bedell, Alan M. Easton, Susan L. Hockerman, James Kiefer, Grace E. Munie, Karl J. Mathis, Madeleine H. Li, Joseph G. Rico, Clara I. Villamil, Jennifer M. Williams

Abstract: Seeking compounds preferentially potent and selective for MMP-13, we recently reported on a series of hydroxamic acids with a flexible benzamide tail groups.[1] In this Letter, we replace the amide moiety with non-hydrolyzable heterocycles in an effort to improve half-life. We identify a hydroxamate tetrazole **4e** that spares MMP-1 and -14, shows >400 fold selectivity *vs.* MMP-8 and >600 fold selectivity *vs.* MMP-2, and has a 4.8 h half-life in rats.

Matrix Metalloproteinases (MMPs) are a family of about 27 zinc-dependent enzymes responsible for the turnover of collagen in connective tissue. There are a variety of disease states where degradation of collagen contributes to the pathology, specifically, in osteo- and rheumatoid arthritis; in tumor angiogenesis and metastasis; in post-MI cardiovascular remodeling. In the previous Letter,[1a] we note that joint-stiffening, "musculoskeletal syndrome" (MSS), upon prolonged dosing with MMP inhibitors (MMPi's) has prevented approval of an MMPi. Efforts have been made to spare MMP isoforms that are thought to contribute to MSS, specifically, MMP-1 and -14 (MT1-MMP) (**SD-2590, fig. 1**),[1b] but a more focused approach toward realizing efficacious MMP inhibitors with reduced side effects should be to focus on optimizing a single MMP isoform that confers the most therapeutic benefit, reducing the probability of off-target protease binding. MMP-13, upregulated in osteoarthritic joints and in cancer, is an attractive isoform to target, and structural studies show that -13 differs from other MMP's deep in the S1' pocket, suggesting that lengthier inhibitors may confer selectivity.

With this in mind, we elaborated a series of rigid piperidino- ketones (compound **2**, **Figure 1**) with lengthier P1' subunits and, with optimization, we were able to achieve significant selectivity for MMP-13 vs. other MMP isoforms.[2] Conceptually dissecting the piperidine ring in compound **2**, we also wanted to explore acyclic-chain analogs such as amide **3** (**Figure 1**).[1]

While excellent MMP-13 selectivity (**Table 1**) was achieved with a number of amides related to compound **3**, the amides displayed short half-life in rats, presumably due to hydrolytic cleavage of the amide bond. In an effort to prepare compounds with suitable selectivity and PK, we incorporated the SAR findings from the acyclic amide series into a small series of compounds where the amide is replaced by isosteric heterocycles. This Letter summarizes that effort.

Among the isosteres we wanted to consider were tetrazoles. Entry into this series began by alkylating the requisite 5-phenyl-1H-tetrazoles (e.g. **5**, **scheme 1**) using O-tetrahydropyranyl-3-chloropropanol. Alkylation afforded predominantly the desired *2*-isomers for 5-[4-methoxyphenyl]- and 5-[4-trifluoromethoxyphenyl]-tetrazole, and the products were purified by chromatography. The THP protecting group could be removed with acid and the product alcohol

(e.g. **6**) reacted with the known aryl fluoride **7**.[1] The t-butyl ester **8** was cleaved and the resulting acid reacted with O-tetrahydropyranyl hydroxylamine in the presence of EDC. Removal of the THP gave the desired hydroxamic acids **4b**. In the case of 5-[4-methyl-2-pyridyl]-tetrazole, alkylation gave a 3:2 mixture, still favoring the *2*-position. The poor selectivity in the alkylation step afforded us the opportunity to isolate meaningful quantities of both *2*- and *1*- isomers and to carry the isomers individually on to final product (**4d** and **4e**, **table 1**).

Oxadiazoles (e.g **4f**, **Table 1**) were prepared by combining the known acid **9**[1] and 4-(trifluoromethoxy)benzamidoxime in the presence of EDC, then heating the coupled product to close the ring. After condensation of the oxadiazole, the *t*-butyl ester (**10, Scheme 2**) is carried on to the hydroxamic acid employing the same transformations used for the tetrazoles.

From **Table 1**, we see that replacing the amide in **3** (**Table 1**) with various aryl heterocycle combinations: tetrazoles (**4a-e**); oxadiazoles (**4f,g**); and imidazoles (**4h,i**) continues to demonstrate high levels of selectivity for MMP-13 *vs.* MMP-1, -2, -7, -8, -9, and -14. Selectivity *vs*. MMP-3 is lowered. Unexpectedly, we see that the *1,5*-isomer of the pyridyl analog (**4e**) has a selectivity profile very similar to its *2,5*-substituted isomer (**4d**). These results inspired us to make some similarly disposed imidazoles (**4j**). We also made an isomeric *1,5*-substituted tetrazole, where the tetrazole carbon connects to the acyclic chain, by a chemospecific method[5] (**scheme 2**) not involving N-alkylation (**4j**).

The crystal structure of tetrazole **4c** was determined at 1.9Å resolution (**Figure 2**).[6] The selectivity in this series derives from the depth and flexibility of the MMP-13 S1' pocket, which is distinct from other MMP family members such as MMP-1 and MMP-2, with shorter pockets. The tetrazole group forms a single direct hydrogen bond to the protein backbone at Thr245 (3.0Å); the remainder of the side chain interactions with the protein are van der Waals contacts. The S1' pocket of MMP-13 readily morphs to interact with inhibitors of various sizes and shapes, as we see in the case of tetrazoles **4d** and **4e**. We were able to achieve $>10^3$ selectivity *vs.* most other MMP family members with **4c**, but even more specific compounds may be possible with this or other scaffolds.

We were able to improve on the 0.59 h half-life we measured for the lead amide compound **3**; from **Table 1**, we see heterocyclic compounds with half-lifes ranging from 1.4 to 4.8 h, some compounds having good BA (e.g. tetrazole **4c**, 26%; and oxadiazole **4f**, 28%) and achieving encouraging C_{max}. Based on our data, we believe selected heterocyclic analogs in this Letter would be promising drug candidates for the treatment of arthritis, cancer, and post-MI left ventricular hypertrophy (CHF).

References and Notes

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Funckes-Shippy, C. L.; Heron, M.; Hockerman, S. L.; Howard, S. C.; Kiefer, J. R.; Li, M. H.; Mathis, K. J.; McDonald, J. J.; Mehta, P. P.; Munie, G. E.; Sunyer, T.; Swearingen, C. A.; Villamil, C. I.; Welsch, D.; Williams, J. M.; Yu, Y.; Yao, J. J. Med Chem. *in press*.

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6. Coordinates deposited in the protein data bank. Crystallographic conditions detailed in the Supplementary Material.

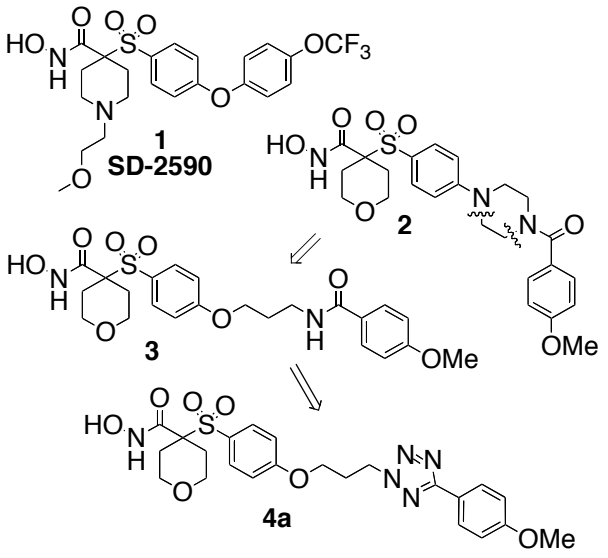
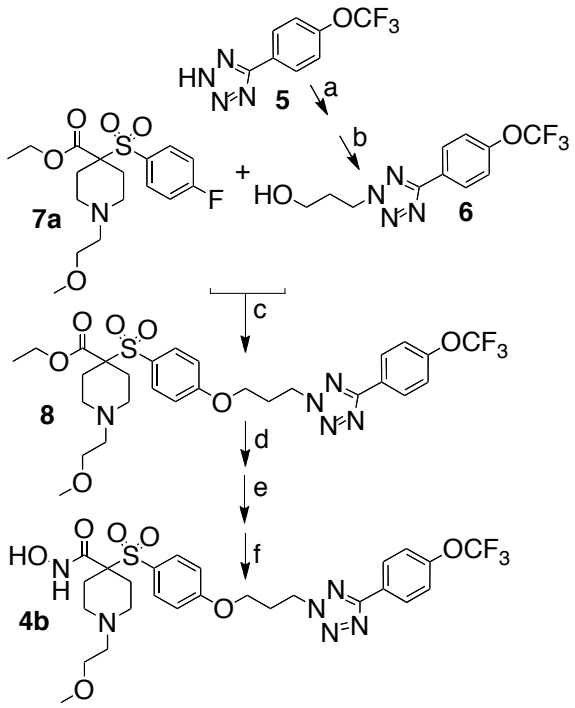
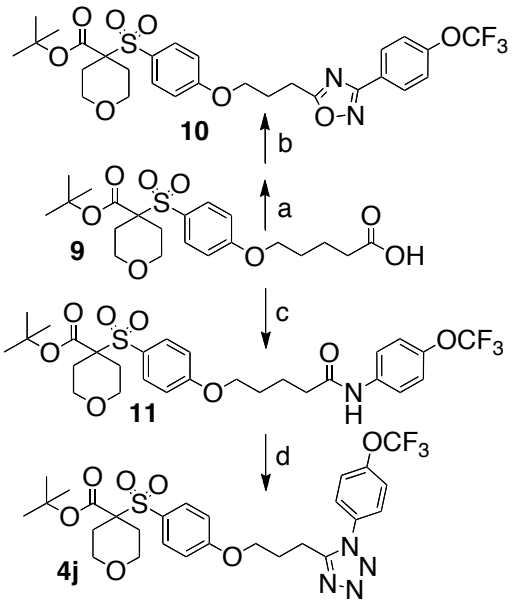


Figure 1. MMP inhibitors. Compound **1** (**SD-2590**) spares MMP-1; Compounds **2**, **3**, and **4a** are MMP-13 selective.

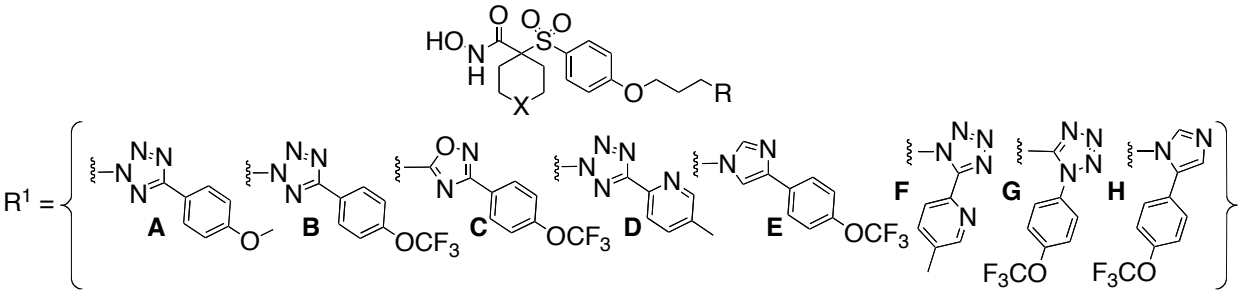


Scheme 1. Reagents and Conditions: (a) NaH (2.1 eq), NMP, rt; then 2-(3-chloropropoxy)tetrahydro-2H-pyran (1.1 eq) r.t. to 70°C (45%) (b) AcCl (3.8 eq), MeOH, rt (94%) (c) **6** (1.05 eq), NaH (1.1 eq), NMP, 55°C, 1h (70%) (d) NaOH (50% aq, 10 eq), THF, EtOH, rt to 60°C, then acidify w/ HCl, conc., azeotrope CH_3CN (e) triethylamine (XS), HOBT, $THPONH_2$ (1.5 eq based on **7a**), EDC (1.5), DMF, 40 h (65%) (f) AcCl (4 eq), MeOH, trit. ether (74%)



Scheme 2. Reagants and Conditions: (a) DMF, HOBT (1.4 eq), triethylamine (1.2 eq), 4-(trifluoromethoxy)benzamideoxime (1.2 eq), EDC (1.5 eq), rt, 2h (b) toluene, 90°C, 30 h (c) HOBT (1.33 eq), 4-(trifluromethoxy)aniline (1.33 eq), EDC (1.33 eq), DMF, rt, 4 h (80%); (d) PPh_3 (2.0 eq), dioxane; then DEAD (2.0 eq); then TMS-N_3 (2.0 eq), rt, 16 h (66%).

Table 1: Selectivity and PK of heterocyclic MMP-13 selective analogs.



		hMMP inhibition Ki (nM)[a]						Rat PK			
cmpd #[b,c]	R	-2	-3	-7	-8	-9	-13	BA%	$t_{1/2}$ (h)	C_{max} ng/mL (MPK)	Cl (mL/min/kg)
1	N/A	<0.1[c]	29[c]	7000	1.7[c]	0.2[c]	<0.1[c]	68	2.9	21900 (20)	
2	N/A	1300[c]	90[c]	>10k[c]	1200[c]	1200[c]	0.70[c]	14	1.2	4200 (20)	
3	N/A	550	900	-	900	>10k	1.6	1.5	0.59	0.02 (20)	
4a	A	48[c]	39[c]	-	190[c]	1100[c]	0.20[c]				
4b	B	410	28	>10k	170	1200	0.66	32	2.4	61 (1.5)	12
4c[b]	B	230	8.7	8600	8000	160	0.13	26	1.3	1400 (20)	
4d	D	110	44	3000	240	370	0.66	1.7	2.0	3500 (5)	46
4e	F	400	3.0	970	250	2400	0.60	5.0	4.8	3000 (5)	63
4f	C	410	31	>10k	180	1600	1.5	22	1.4	240 (20)	14
4g[b]	C	410	37	>10k	260	370	2.0	28	2.4	2200 (20)	19
4h	E	530	59	6000	74	1800	1.7				
4i	H	9200	130	>10k	140	>10k	3.7				
4j	G	430	9.9	7100	72	1700	1.2	7.3	4.0	3500 (5)	42

a.) MMP-1 and -14 >10k nM except for **1** (MMP-14 = 14 nM) and **4a** (MMP-1 = 5600 nM).
b.) For **4a-k,** X = O except: **4c**, where X = NCH_2CH_2OMe and **4g**, X = NCH_2CH_3
c.) IC_{50} instead of Ki.

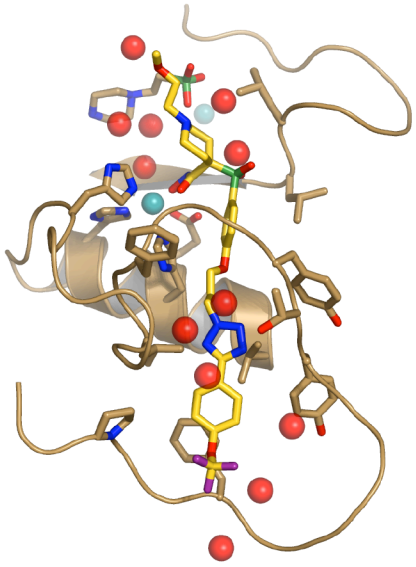


Figure 2. Structure of MMP-13 with compound **4c** bound at the catalytic site. The inhibitor binds deeply within the S1' pocket of the active site and causes a conformational change opening the loop to accommodate the compound. The hydroxamate moiety chelates the zinc ion at two positions, and Glu223 forms a hydrogen bond to the hydroxamate. Similarly, the hydroxamate nitrogen atom forms a hydrogen bond to the protein backbone, as does one of the sulfone oxygen atoms. A single hydrogen bond is formed between the tetrazole and the protein backbone. A HEPES buffer molecule bound adjacent to the inhibitor in two of the four protein molecules in the crystal, and an array of solvent atoms form an auxiliary van der Waals surface between the inhibitor and the protein.[6]

Supplementary Material:

Rod shaped crystals of the MMP-13 in complex with tetrahydro-N-hydroxy-4-[[4-(phenylmethyl)-1-piperazinyl]sulfonyl]-2H-pyran-4-carboxamide, hydrochloride (Getman, Daniel P.; Becker, Daniel P.; Barta, Thomas E.; Villamil, Clara I.; S. L. Hockerman; Bedell, Louis J.; Li, Madelleine H.; Freskos, John N.; Heintz, Robert M.; McDonald Joseph J. ; DeCrescenzo, Gary A. , "Thioaryl Sulfonamide Hydroxamic Acid Compounds" US 6,087,359, 2000.) complex were grown at 4°C by sitting drop vapor diffusion using 5 mg/mL protein and a reservoir solution of 1.4M lithium sulfate, 0.1M Hepes, pH 7.7. The ratio of protein to reservoir solution in the crystallization drop was 5:1. Crystals were pre-equilibrated in a soaking solution containing 1.5M lithium sulfate, 0.1M Hepes, pH 7.7 without inhibitor at room temperature for more than two hours. These crystals were then transferred to a second solution containing 1.5M lithium sulfate, 0.1M Hepes, pH 7.7 and 2.5mM of replacement inhibitor and were left to incubate at room temperature for several days. The cryo solution consisted of 20% sucrose, 1.5M lithium sulfate, and 0.1M Hepes, pH 7.7. Data were collected using a Rigaku Micromax 007 X-ray generator on a Mar Research MarCCD 165 detector. The diffraction data were integrated and scaled with HKL-2000 (Otwinowski, Z. and Minor, W, (**1997**). In Methods in Enzymology, 276, edited by C.W. Carter, Jr. & R.M. Sweet pp. 307-326, New York:Academic Press.), and the structures were determined by molecular replacement using a prior internal structure of MMP-13 as the initial model. The model adjustment occurred as in Kjeldgaard (Jones, T.A., Zou, J.Y., Cowan, S.W. and Kjeldgaard, M. Acta Cryst 1991 A47, 110-119.) and Coot (Emsley, P.; Cowtan, K. Acta Crystallographica Section D 2004, 60, 2126), and the structures were refined initially with X-PLOR (Brünger, A. T. 1992. X-plor version 3.1: a system for X ray crystallography and NMR. Yale University Press, New Haven, Conn.) and were further optimized in Refmac (Murshudov, G. N.; Vagin, A. A., and Dodson, E. I. Acta Cryst. **1997**, *D53*, 240).

Crystallographic Data and Refinement Statistics

PDB accession code	3O2X
Data collection statistics	
Radiation source	Rotating anode
Radiation detector	MAR CCD 165
Space group	$P2_12_12_1$
Resolution (Å)	20.0-1.9 (1.97-1.90)
Observed reflections	271,723
Unique reflections	56,980
Completeness (%)	84.3, 79.3
Mean I/σ_I	16.4, 3.8
R_{sym}%[b]	8.4, 27.1
Refinement statistics	
Resolution (Å)	20-1.9
No. protein + ligand atoms	5,486
No. solvent atoms	682
R (%), Rfree (%)	17.9, 21.1
Wilson B ($Å^2$), Refined <B> ($Å^2$)	12.9,
Rmsd ideal bond lengths (Å)	0.006
Bond angles (°)	1.095

[a] Highest resolution bin. [b] $R_{sym} = \Sigma(|I_i-<I>|)/\Sigma I_i$. All reflections with $I/\sigma_I < -1.0$ eliminated from scaling.