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Letters

Potent and Selective Carboxylic Acid-Based Inhibitors of Matrix Metalloproteinases

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Abstract: A novel series of carboxylic acids containing a substituted piperidine were synthesized and tested for inhibition of selected matrix metalloproteinases. Multiple analogues prepared based on this novel design were found to inhibit the target MMPs (MMP-2, -3, -8, -9, and -13) with unprecedented, low nanomolar potency while, at the same time, sparing MMP-1 and MMP-7. Solubility and plasma protein binding of several members of this new series of carboxylic acids were also investigated.

Introduction. The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes that are capable of degrading many proteinaceous components of the extracellular matrix.[1] The enzymes have been implicated in several pathological processes including arthritis,[2,3] tumor growth and metastasis,[4] periodontal disease,[5] multiple sclerosis,[6] and congestive heart failure.[7,8] Considerable research has been devoted to the discovery of potent MMP inhibitors, which may act as potential disease modifying agents in a number of important pathologies.[9,10]

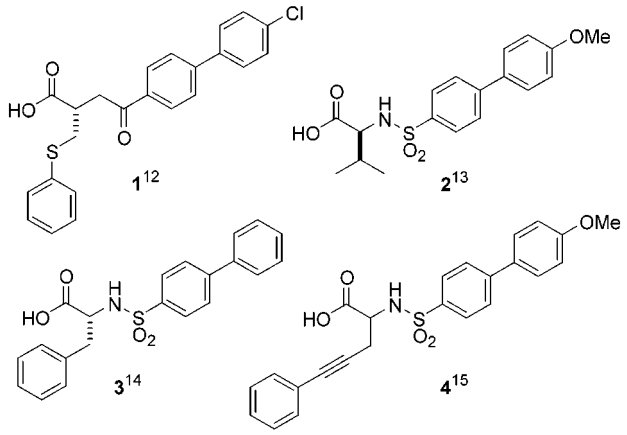

Figure 1. Representative carboxylate-based MMP inhibitors.

The high potency of the majority of the published MMP inhibitors has traditionally been associated with the presence of a hydroxamic acid functionality in the molecule.[9] The ability of the hydroxamate group to efficiently complex the catalytic zinc and also to develop two hydrogen bonds to Glu-202 and Ala-165[11] provides a major potency advantage over other zinc binding groups. However, pharmacokinetic challenges associated with the hydroxamic acid group prompted many research teams to look for alternative zinc-binding groups.[9] Out of several groups explored, the carboxylic acid-based inhibitors have attracted considerable interest, leading to the discovery of several scaffolds[12−15] (see Figure 1). While these compounds represent considerable progress in the design of carboxylate-based MMP inhibitors, their relatively low potency has left plenty of room for improvement.

After spending considerable time searching for potent and selective hydroxamate-based MMP inhibitors,[16] we chose to examine carboxylic acid-based inhibitors with the primary goal of improving their inhibitory potency.[15,17] Very early in our design process we realized that additional binding interactions between the inhibitor and the target enzyme would have to be identified to compensate for the loss in potency observed with the

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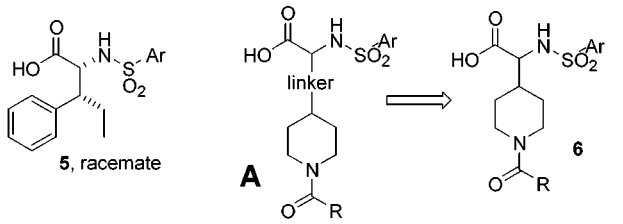


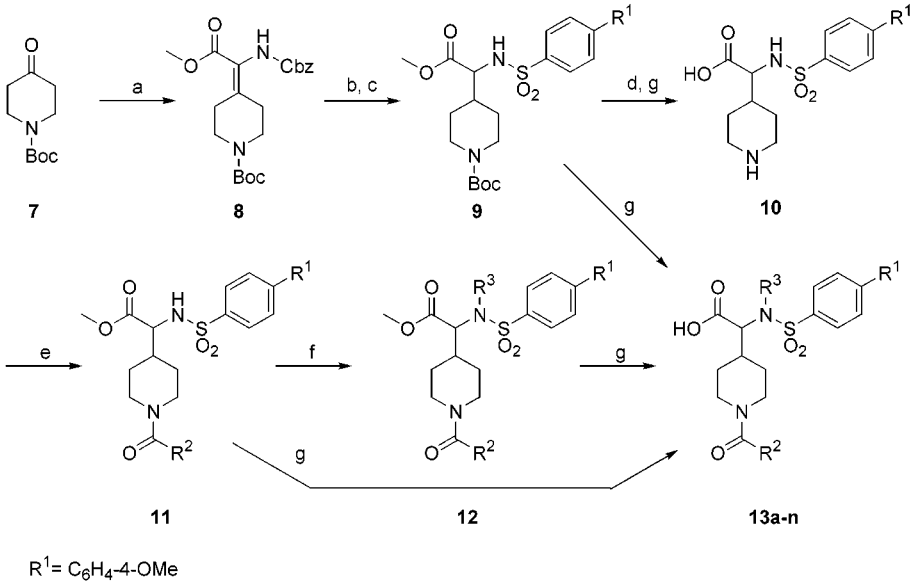
Figure 2. Design of piperidine-based carboxylic acids.

hydroxamate to carboxylate switch. Using both molecular modeling and low resolution X-ray crystallographic data obtained for the complex of our model carboxylic acid **5** with stromelysin, we decided to explore piperidine-based structures as represented by **A** (Figure 2). After examination of a series of homologues, compound **6** was selected for experimental verification of our design. The backbone was flexible enough for the carboxylic group to effectively complex with the catalytic zinc, yet the compound possessed the desired conformational stability due to the presence of a ring at the α position to the carboxylic acid. This compound was also projected to have favorable binding interactions in the S2 pocket of stromelysin. Herein we report that, indeed, this design led to the discovery of a novel series of carboxylate-based inhibitors of matrix metalloproteinases with unprecedented potency and selectivity.

Chemistry. The target MMP inhibitors were synthesized as shown in Scheme 1. The commercially available N-Boc-piperidone (**7**) was allowed to react with the glycine-derived phosphonate in the presence of DBU to give the α,*β*-unsaturated ester **8** in >60% yield. Compound **8** was next subjected to palladium-catalyzed hydrogenolysis to reduce the double bond and remove the Cbz protecting group. The biphenylsulfonyl group was then introduced using the corresponding sulfonyl chloride in the presence of triethylamine to provide methyl ester **9** in >70% overall yield. Quantitative removal of the Boc protecting group was accomplished with trifluoroacetic acid, and the resulting piperidine **10** could then be derivatized using standard acylation conditions. Finally, the ester group of **11** was hydrolyzed with lithium hydroxide in methanol to provide the target carboxylic acid **13**. In the case of substituted sulfonamides, N-alkylation was accomplished using the appropriate alkyl halide in the presence of cesium or potassium carbonate in dimethylformamide, and the resulting intermediate **12** was hydrolyzed as described above for the synthesis of ester **11**. All target compounds prepared using this methodology were racemic mixtures.

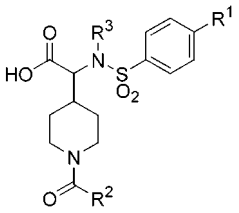
In Vitro SAR. All compounds were tested for the inhibition of collagenases-1, -2, and -3 (MMPs -1, -8, and -13, respectively), gelatinases A and B (MMPs -2 and -9, respectively), stromelysin (MMP-3), and matrilysin (MMP-7) as described previously.[15,16] All binding data were obtained at pH 7.4 and are summarized in Table 1. Compound **13d** containing both a Boc-protected piperidine ring and the methoxybiphenysulfonyl as a P1′ group was prepared as the first analogue. It was very gratifying to see the inhibitory potency of **13d** fall below 10 nM against MMPs -2, -8, -9, and -13, and below 100 nM against stromelysin. This unprecedented level of potency was accompanied by rather limited binding to MMP-1 and MMP-7, the two enzymes we wanted to spare due to concerns about potential side effects. A series of analogues (**13a**−**c**) with progressively smaller alkyl carbamate groups further confirmed the high potency and selectivity associated with the piperidine-based design. Although the methyl carbamate **13a** was less potent than the *tert*-butyl carbamate **13d**, it was still considerably more potent than the unsubstituted piperidine **10a**. This clearly indicated that the carbamate group played an important role in binding to the MMPs. The importance of the carbamate group was further confirmed by the lower potency of amide **13e** as compared to the carbamate analogue **13c**. Interestingly, the urea **13f** and the methoxyethoxy carbamate **13g**, with the P2 substituents modified to increase water solubility, showed only limited reduction in potency against MMP-3 and MMP-9 but maintained low nano-

Scheme 1[a]



[a] (a) $MeO_2CCH(NHCbz)PO(OMe)_2$, DBU, CH_2Cl_2, RT; (b) H_2, Pd/C, MeOH; (c) $R^1C_6H_4SO_2Cl$, Et_3N, CH_2Cl_2; (d) TFA, CH_2Cl_2; (e) R^2COCl, Et_3N, CH_2Cl_2; (f) $R^3Br(I)$, Cs_2CO_3 (K_2CO_3), DMF; (g) LiOH, MeOH.

Table 1. In Vitro Inhibitory Activity of Carboxylic Acids



				MMP (IC_{50}, nM)[a]						
compd	R^1	R^2	R^3	1	2	3	7	8	9	13
10				3890	53	4840	4240	30.2	1760	264
13a	C_6H_4-4-OMe	OMe	H	5210	2.8	657	5650	6.7	176.5	29.5
13b	C_6H_4-4-OMe	OEt	H	2760	1.0	162	9870	2.7	94.9	5.4
13c	C_6H_4-4-OMe	O*i*-Pr	H	1400	<0.4	41.8	1740	1.5	11.7	1.0
13d	C_6H_4-4-OMe	O*t*-Bu	H	1420	0.5	56.4	2590	1.2	5.4	0.7
(*R*)-13d	C_6H_4-4-OMe	O*t*-Bu	H	1370	<0.4	33.7	3660	1.5	6.6	0.4
(*S*)-13d	C_6H_4-4-OMe	O*t*-Bu	H	3490	7.2	345	8510	3.0	71.5	6.9
13e	C_6H_4-4-OMe	CH_2*i*-Pr	H	4900	3.2	739	4370	5.3	137	13.9
13f	C_6H_4-4-OMe	morpholine	H	1760	1.1	221	8570	3.0	50.8	1.8
13g	C_6H_4-4-OMe	OCH_2CH_2OMe	H	2590	0.8	133	4110	3.4	55	3.5
13h	C_6H_4-4-SMe	OCH_2CH_2OMe	H	1080	0.4	40.6	1020	1.8	7.3	1.0
13i	Ph	OCH_2CH_2OMe	H	6050	24.2	418	>10000	44.8	415	55.3
13j	OPh	OCH_2CH_2OMe	H	>10000	58.1	399	>10000	25.8	597	140
13k	O-*n*-Bu	OCH_2CH_2OMe	H	>10000	549	678	>10000	294	2990	791
13l	C_6H_4-4-OMe	OCH_2CH_2OMe	Me	1850	0.9	69.6	3950	3.6	13.9	2.4
13m	C_6H_4-4-OMe	OCH_2CH_2OMe	3-picolyl	3310	1.0	86.1	3640	3.2	18.7	3.0
13n	C_6H_4-4-OMe	OCH_2CH_2OMe	CH_2CH_2OMe	1220	0.6	45.5	2650	2.4	9.1	1.0
14i[b]	C_6H_4-4-OMe	OCH_2CH_2OMe	H	8.1	<0.4	13.8	20	0.5	<0.4	0.4
1				>10000	91.3	7950	>10000	303	3110	1570
2				8710	6.6	1890	>10000	10.6	747	24.2
4				1960	12.5	2280	>10000	9.4	553	21.0

[a] All assays were performed at pH 7.4; see ref 15 for assay protocols. Standard deviations are less than 30% of the mean values. [b] This is a corresponding hydroxamic acid.

Table 2. Solubility and Plasma Protein Binding of Selected Inhibitors

compd	solubility (mg/mL)	protein binding (%)[a]
10a	0.59	62.0
13b	6.79	98.8
13c	0.13	99.0
13d	1.35	99.6
13e	nd	98.4
13f	18.65	97.0
13g	8.07	96.9
13h	3.50	98.2
13l	16.38	96.8
13m	0.36	93.2
13n	11.09	90.7

[a] Error for this measurement is 0.2%; nd − not determined.

molar potency against MMP-13. With both **13f** and **13g** showing very good aqueous solubility (see Table 2), we selected carbamate **13g** for further exploration of the SAR in this series.

Next, the role of the P1′ group was briefly investigated and, as expected, the inhibitory potency was observed to be highly dependent on the type of substituents in this position. Replacement of the methoxy group on the biphenyl ring with a thiomethyl group produced a slight improvement in potency (compare **13h** versus **13g**). However, removal of the methoxy group and replacement of the biphenyl group by aryloxy or alkoxy phenyl groups resulted in a significant decrease of activity (compounds **13i**, **13j**, and **13k**, respectively).

Modifications to the sulfonamide NH position were also performed in order to determine the role this group has on enzyme inhibition. It was reported earlier that introduction of alkyl groups in this position had a detrimental effect on the inhibitory potency of carboxylic acids.[18] Therefore, we were surprised to observe that not only small but also larger N-alkyl groups appear to be well tolerated in the piperidine-based series (compare compound **13l** and **13m** with *N*-methyl and *N*-picolyl groups,[19] respectively). In fact even more unexpected was the increase in potency observed for the methoxyethyl derivative **13n**.

All of the analogues described so far were prepared and tested as racemic mixtures. To know if the activity of these compounds is associated with one particular enantiomer, both antipodes of **13d** were prepared[20] (see **(*R*)-13d** and **(*S*)-13d**). Interestingly, the two isomers demonstrated a rather small 1 order of magnitude difference in potency of inhibition of our primary targets MMP-3 and MMP-13. The potency of compound **(*R*)-13d**, the more active enantiomer and the most active analogue in the series, was similar to the level observed for hydroxamic acids with analogous structure (see **14i**). In addition, the low nanomolar values observed for the inhibition of target enzymes were nicely complemented by high selectivity against MMP-1 and MMP-7.

Selected compounds were also tested for solubility in pH 7 buffer and for binding to plasma proteins, and the data are summarized in Table 2. Most of the analogues possessed good solubility and easily met or exceeded our minimum goal of 1 mg/mL. However, plasma protein binding was found to be rather high (>90%) for all compounds with the substituted piperidine ring. This was in a stark contrast to relatively low protein binding of 62% observed for the unsubstituted analogue **10a**. Interestingly, the N-substituted sulfonamides showed relatively lower protein binding compared to the unsubstituted NH sulfonamides, yielding two inhibitors

13m and **13n** with acceptable levels of protein binding (93.2% and 90.7%, respectively).

Conclusion. In summary, a new series of potent and selective carboxylic acid-based matrix metalloproteinase inhibitors were discovered. The new design is based on a substituted piperidine ring attached to the sulfonylated glycine at the α position to the carboxylic group. Many inhibitors based on this design featured unprecedented, sub- or low-nanomolar binding to MMP-2, -3, -8, -9, and -13 when measured at physiological pH of 7.4. These potent interactions were contrasted by relatively weak, micromolar binding to MMP-1 and -7. SAR studies revealed the importance of the carbamate group associated with the piperidine ring as well as the substituents in the P1′ position. The SAR studies also demonstrated the potential beneficial effects of N-alkyl substituents on the sulfonamide group. Very good aqueous solubility of several inhibitors was achieved. However, this higher solubility did not always lead to lower binding to plasma protein. Further exploration of this series is in progress and will be reported in due course.

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Supporting Information Available: Experimental procedures for the synthesis of **13n** and **(*R*)-13d** and analytical data of the target inhibitors described in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) Both enantiomers were prepared from the corresponding, commercially available N-α-Fmoc-α-[1-(*tert*-butoxycarbonyl)-4-piperidinyl]glycines as follows: (1) $TMSN_2$, MeOH; (2) H_2, Pd/C, EtOH, 45 psi; (3) $MeOC_6H_4C_6H_4SO_2Cl$, Et_3N, THF; (4) LiOH, THF−H_2O. Optical purity was determined to be at least 98:2 based on ^1H NMR of Mosher esters derived from alcohols obtained from the corresponding acids **(*R*)-13d** and **(*S*)-13d** through reduction with lithium aluminum hydride.

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