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LETTERS

PICKING THE S_1, S_1' AND S_2' POCKETS OF MATRIX METALLOPROTEINASES. A NICHE FOR POTENT ACYCLIC SULFONAMIDE INHIBITORS

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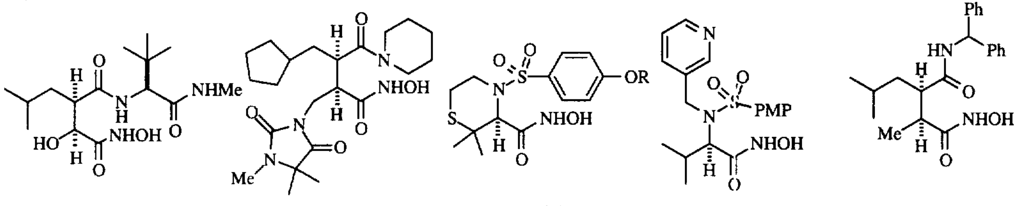
Abstract: A series of acyclic hydroxamic acids harboring strategically placed α-arylsulfonamido and thioether groups was synthesized and found to be potent inhibitors of various MMPs. An unprecedented cleavage of *t*-butyl hydroxamates to hydroxamic acids was found. © 1999 Elsevier Science Ltd. All rights reserved.

Matrix zinc proteinases (matrixins or MMPs) belong to the general family of metalloproteinases and are produced by various cell types in the body.[1] These fascinating enzymes have been implicated in a number of disruptive mechanisms leading to tumor metastasis,[2] rhumatoid arthritis,[3] osteoarthritis,[4] periodontal disease[5] and multiple sclerosis.[6] In particular, some MMPs have been implicated in the destruction of type IV collagen, which is believed to prevent tumor invasion. As a result, inhibitors of specific MMPs have been studied as a means to develop mechanism-based therapeutic agents to treat cancer, arthritis, and other life-threatening or debilitating diseases.[7]

Figure 1



R = 4-pyridyl

PMP = *p*-methoxyphenyl

1, Marimastat[1a] (British Biotech.)

2, Ro 32-3555[8] (Hoffmann LaRoche)

3, AG-3340[9] (Agouron)

4, CGS-27023A[10] (Ciba-Geigy/Novartis)

5, Dupont Merck[11]

One such enzyme, stromelysin-1 (MMP-3)[12] has been extensively studied, leading to the structural elucidation[13-18] of a truncated kDa catalytic domain by NMR spectroscopy and X-ray crystallography.[19,20]

Because of the importance of these enzymes and their implication in various disease processes, efforts toward the design and synthesis of inhibitor molecules have intensified in recent years.[7] A number of compounds containing hydroxamic acid moities such as **1**–**3**[1a,8-10] have clinical relevance (Figure 1). Among the various structural types reported as inhibitors of MMP-1 fibroblast collagenase are diphenylmethyl amides

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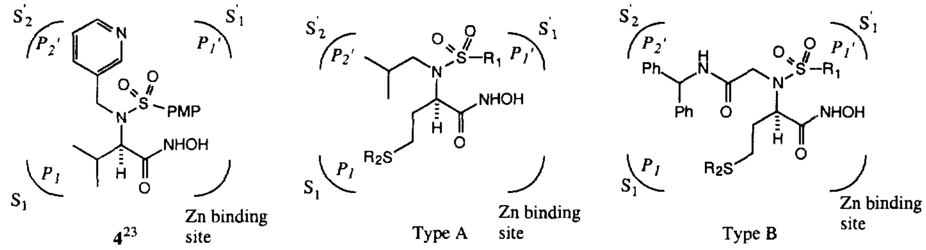
such as **5** (Figure 1),[11] acyclic α-*N*-sulfonylamino acids and their hydroxamic acid derivatives,[10,21] as well as cyclic analogs.[22] Gonella and coworkers,[23] reported on the biologically active conformation of CGS 27023, **4**, a potent non peptidic stromelysin inhibitor, in conjunction with multidimensional NOE measurements, and X-ray nucleus filtered NMR spectroscopy. After proper docking and orientation operations, it was shown that the long, narrow S_1' pocket of stromelysin is the site of interaction for the *p*-methoxyphenyl (PMP) ring of **4**. The pyridyl ring corresponding to the P_2' region occupies a shallow S_2' site, while the isopropyl group (P_1) resides in a solvent exposed wide hydrophobic surface corresponding to the S_1 pocket. Variations in the nature of substituents at these sites in the inhibitor validated the NMR predictions.[19]

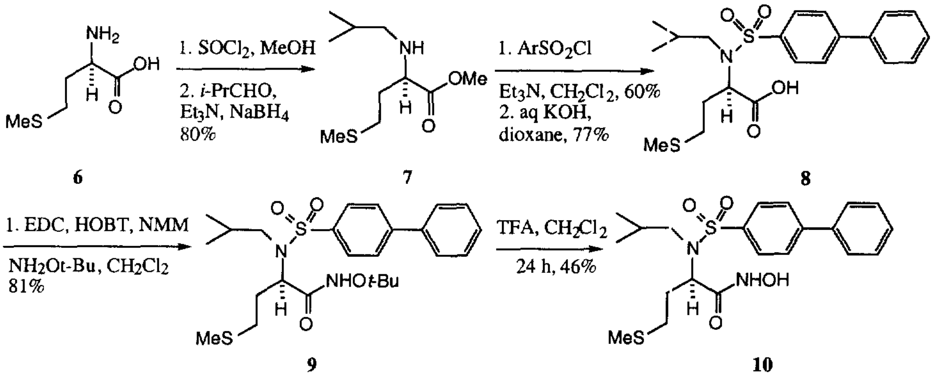
We wish to report a series of highly potent acyclic N-sulfonylamino hydroxamic acid derivatives related to **4** where the P_1 site is replaced by an ethylthioalkyl ether chain of a given length, and the pyridyl ring is replaced by an isobutyl group (P_2' site) (Figure 2, Type A). This relatively simple dual change in the nature of substituents has had a remarkably beneficial effect on the potency of these compounds as inhibitors of a variety of MMPs. A second prototype consists of the introduction of an arylalkylthioether and a bulky acetamide as can be found in **5**, (Figure 2, Type B).

Figure 2



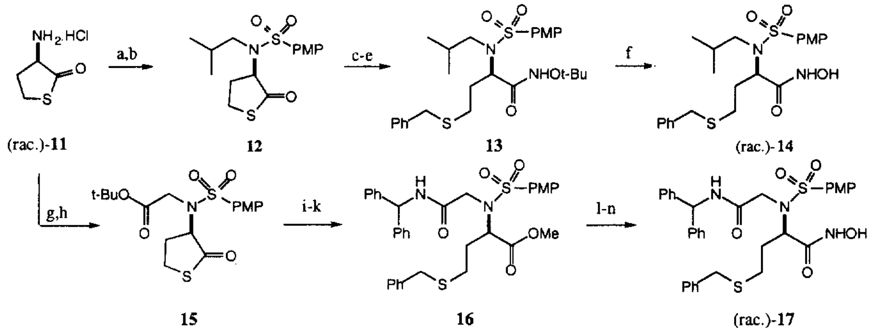
Synthesis: Schemes 1 and 2 illustrate the general approach to sulfonamides of Type A, which can be derived from D-methionine and DL-homocysteine. Thus, the readily available methyl ester **6** was reductively alkylated to afford **7** in excellent overall yield from the amino acid (Scheme 1).

Scheme 1



Treatment of **7** with diphenylsulfonyl chloride, followed by hydrolysis and acidification gave **8**. Conversion to the O-*t*-butyl hydroxamate ester **9** and treatment with TFA from a freshly opened bottle afforded the desired hydroxamic acid sulfonamide analog **10**. Other analogs varying in the sulfonamide and thioether moieties were similarly prepared (Table 1, entries **1-4**, **6**). Scheme 2 shows the synthesis of another analog starting with racemic homocysteine thiolactone methyl ester **11**. Reductive amination followed by sulfonamide formation led to the thiolactone **12**. Treatment with sodium methoxide and an alkyl halide followed by introduction of the hydroxamic acid unit allowed the preparation of a variety of thioethers. In the case of the S-benzyl analog **13**, hydrolysis with *fresh* TFA gave (rac.) **14**. The cleavage of O-*t*-butyl hydroxamates with TFA seems to be unprecedented, and a number of previous syntheses of hydroxamic acids have relied on other protective groups.[24]

Scheme 2



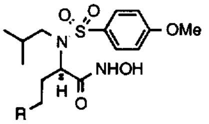
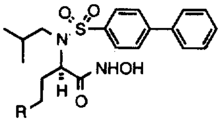
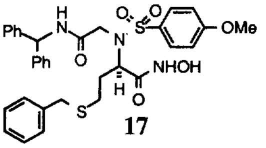
(a) *i*-Pr CH_2CHO,Et_3N, $NaBH_4$, 61%. (b) *p*-$MeOC_6H_4SO_2Cl$, NMM, CH_2Cl_2, 85%. (c) Na, MeOH, $PhCH_2Br$, 79%. (d) aq KOH, dioxane. (e) EDC, HOBT, NMM, NH_2Ot-Bu, CH_2Cl_2, 78%. (f) Fresh TFA, CH_2Cl_2, 50%. (g) $BrCH_2CO_2t$-Bu, *i*-Pr_2NEt_2, MeCN, 71%. (h) *p*-$MeOC_6H_4SO_2Cl$, pyr, 68%. (i) NaOMe, MeOH, $PhCH_2Br$, 95%. (j) TFA, CH_2Cl_2, 89%. (k) BOPCl, Ph_2CHNH_2, 81%. (l) aq KOH, dioxane, 76%. (m) EDC, HOBT, NMM, NH_2Ot-Bu, CH_2Cl_2, 82%. (n) Fresh TFA, CH_2Cl_2, 12-24h, 43%.

A prototype structure corresponding to the Type B motif was prepared by a variation of the methods described above and is shown in Scheme 2. Sequential treatment of DL-homocysteine thiolactone **11** with *t*-butyl bromoacetate and *p*-methoxyphenylsulfonyl chloride afforded the sulfonamide derivative **15**. Methanolysis of the thiolactone with concomitant thioetherification and conversion of the resulting ester **16** to the intended target **17** proceeded uneventfully.

Table 1 lists the IC_{50} values of the acyclic sulfonamides prepared according to the protocols shown in Schemes 1 and 2. Compounds listed in entries 1, 5, and 7 in particular exhibited excellent inhibition in the subnanomolar range for some of the MMPs.[25] The combination of the *p*-methoxyphenylsulfonamide and the benzhydrylamide groups in compound **17** proved to be particularly beneficial as evidenced in the excellent activity for the five MMPs tested. As a confirmation of the importance of the alkylthio ether group, the ethyl analog of **21**, (Table 1, entry 4), in which the methylthio group in **10** was replaced by hydrogen was only moderately active.

It was of interest to compare the proposed mode of binding of **4** to stromelysin[23] with that of a representative potent member of our series such as **18**, (Figure 3).[26] Assuming that the hydroxamic acid group in **18** binds to the same site as for **4**, and that the *p*-methoxyphenylsulfonamide moiety fits in the long narrow hydrophobic pocket defined by S_1', it can be seen that the P_1' and P_2' sites of **18** occupy the same positions as the isopropyl and 3-pyridylmethyl groups respectively in **4**. Thus, the wider hydrophobic S_1 pocket of the enzyme nicely accommodates the benzylthioethyl group in **18**. This pocket can accept thioethers of a given length although the *p*-phenyl-S-benzyl and *p*-benzyloxybenzyl analogs **19** and **20** (Table 1, entries 2,3) appear to be less tolerated. The shallow S_2' pocket seems to accept the isobutyl group in **18** in lieu of the 3-pyridyl of **4**. The benzhydrylamide analog **17**, which shows uniform potency for four MMPs, may also have the same bioactive conformation as **18**, with the bulky amide group occupying the P_2' site instead of the arylthioether in **18** or the isopropyl group in **4**.

Table 1

				IC_{50}[a]		
Entry		MMP-1	MMP-2	MMP-3	MMP-9	MMP-13
1.	R= SCH_2Ph, **18**	104 nM	0.7 nM	0.7 nM	2.5 nM	12 nM
2.	R = $SCH_2C_6H_4p\text{-}C_6H_5$, **19**	nt[b]	30 nM	20 nM	0.2 nM	nt
3.	R = $SCH_2C_6H_4p\text{-}OCH_2Ph$, **20**	nt	49 nM	29 nM	8 nM	20 nM
4.	R = H, **21**	384 nM	53 nM	45 nM	7 nM	96 nM
						
5.	R = SMe, **10**	156 nM	0.7 nM	37 nM	0.2 nM	3 nM
6.	R = SCH_2Ph, **22**	nt	4.1 nM[c]	90 nM	2.5 nM[c]	3.8 nM
7.	 **17**	nt	2.3 nM[d]	3.7 nM	0.5 nM[d]	4.5 nM
8.	Marimastat, **1**[1a]	1.5 nM	2 nM	25 nM	1.5 nM	3.5 nM
9.	CGS-27023 A, **4**[10]	96 nM	15 nM[e]	14 nM	10 nM[e]	12 nM

[a]See ref 25 for details; [b]not tested; [c]IC_{50} murine MMP zymography, 7.5 nM; [d]IC_{50} murine MMP zymography, 0.1 μM; [e]IC_{50} 1 μM murine MMP zymography.

In conclusion, we have shown that a series of acyclic α-*N*-alkyl, *N*-sulfonamido hydroxamic acids with α-ethylthioether substituents are excellent inhibitors of a variety of MMPs by measuring their IC_{50} values compared to Marimastat,[1a] (Table 1). It is possible that the substituents occupying the P_1' and P_2' positions are interchangeable, although more analogs are needed to unequivocally validate this suggestion.[27] Studies directed toward this goal and the synthesis of other designed prototypes in this series are ongoing.

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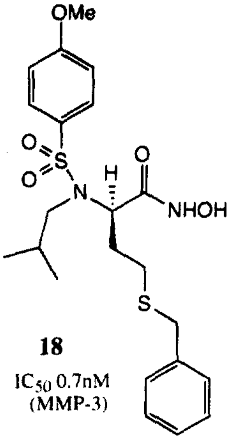

18

IC_{50} 0.7nM
(MMP-3)

Figure 3. Energy-minimized structure of **18** (orange) in the active site of stromelysin (MMP-3), showing key amino acids and the zinc atom (green); See refs 23 and 26.

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25. Human purified MMPs were purchased or acquired. MMP-2 gelatinase A and MMP-9 gelatinase B from Boehringer Mannheim (Meylan, France), MMP-1 interstitial collagenase and MMP-3 stromelysin 1 from Valbiotech (Paris, France), and MMP-13 collagenase 3 from Dr. Gillian Murphy (University of East Anglia, Norwich, U.K.). All enzymes were activated by APMA (4-aminophenylmercuric acetate). Inhibition of MMP-3 was quantified by using the peptidomimetic substrate (7-methoxycoumarine-4-yl)-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(Dnp)-NH_2 (Bachem, Bubbendorf, Switzerland) which is cleaved between Ala and Nva. For inhibition studies of the other enzymes, the substrate Dnp-Pro-Cha-Gly-Cys(Me)-His-Ala-Lys(Nma)-NH2 (Bachem), which is cleaved between amino acids Gly and Cys, was used. The fluorescent cleavage products were measured with a cytofluorometer (Cytofluor 2350, Millipore/PerSeptive Systems, Voisins le Bretonneux, France) equipped with a combination of 340 and 440 nm filters for excitation and emission, respectively.
26. The structure of **18** was optimized based on the parameters utilized for **4** and stromelysin MMP-3 (ref 23). Minimization using Sybyl 6.5 within the active site of the enzyme provided the orientation shown in Figure 3. For the X-ray structure of stromelysin, see Esser, C.K. and 27 coworkers, *J. Med. Chem.* **1997**, *40*, 1026.
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