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Novel highly potent, structurally simple γ-trifluoromethyl γ-sulfone hydroxamate inhibitor of stromelysin-1 (MMP-3)

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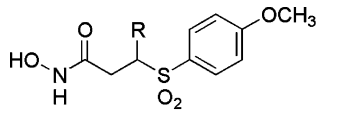
Abstract—The γ-trifluoromethyl γ-sulfone hydroxamate **1** was synthesized both in racemic and enantiomerically pure forms by means of a thia-Michael reaction of *p*-methoxythiophenol on achiral and chiral 3,3,3-trifluorocrotonoyl Michael acceptors. The (*R*)-**1** enantiomer was the most potent inhibitor of MMP-3 (stromelysin-1), showing an IC_{50} = 3.2 nM, as well as the most selective with respect to MMP-9 (65-fold).

Matrix metalloproteinases (MMPs) are a family of zinc metallo-endopeptidases secreted by cells, which are responsible for much of the turnover of matrix components.[1]

A common feature of many serious diseases, such as heart failure and cancer, is that MMPs have been reported to play a key-role, in combination with their natural tissue inhibitors (TIMPs). Progression and growth of both pathologies, mainly in the early stages, is favoured by the proteolytic activity of several MMPs, such as stromelysin-1 (MMP-3) and gelatinase-B (MMP-9). For these reasons inhibition of MMPs is actively studied as a promising therapeutic target for heart failure and cancer therapy.[2]

Some years ago Groneberg, Salvino, et al.[3] and Freskos et al.[4] described as structurally very simple class of hydroxamate inhibitors bearing an arylsulfone moiety in γ-position, such as **A** (Fig. 1), which showed nanomolar inhibitory potency against MMP-2, 3 and 13.



R = H, alkyl, cycloalkyl, etc. **(A)**
R = CF_3 **(1)**

Figure 1.

The R side chain was found to be critical not only for potency, but it could also dramatically influence the enzyme selectivity profile of the inhibition.

Within the frame of a research project aimed at a better understanding and rationalization of the 'fluorine-effect' in peptidomimetic structures, particularly those displaying activity as protease inhibitors,[5] we decided to investigate the effect of a trifluoromethyl (Tfm) group, positioned as R substituent of structures **A**, on the inhibitory potency of MMPs. It is in fact widely accepted that the Tfm group is at the same time highly hydrophobic and sterically demanding.[6] Moreover its high electron-density could provide additional interactions within the MMPs active sites, possibly including hydrogen bonding.[7] This choice was encouraged by the observation that compounds **A** bearing large hydrophobic groups R (such as alkyl, cycloalkyl and arylalkyl groups) showed low nanomolar, and even subnanomolar affinity for

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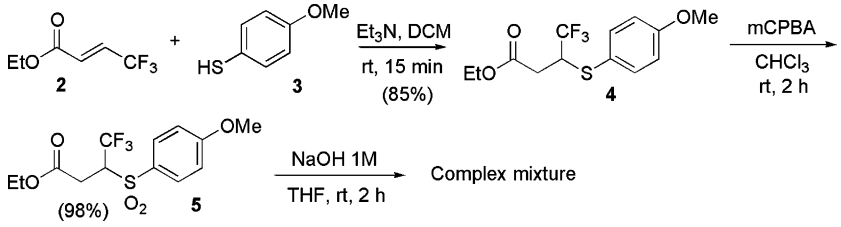


Scheme 1.

MMP-2, 3 and 13, and excellent selectivity with respect to MMP-1.[3,4]

In this communication we describe the synthesis of the racemic target structure **1**, as well as a route to both its pure enantiomers, and the determination of their inhibitory activity towards MMP-3 and MMP-9.

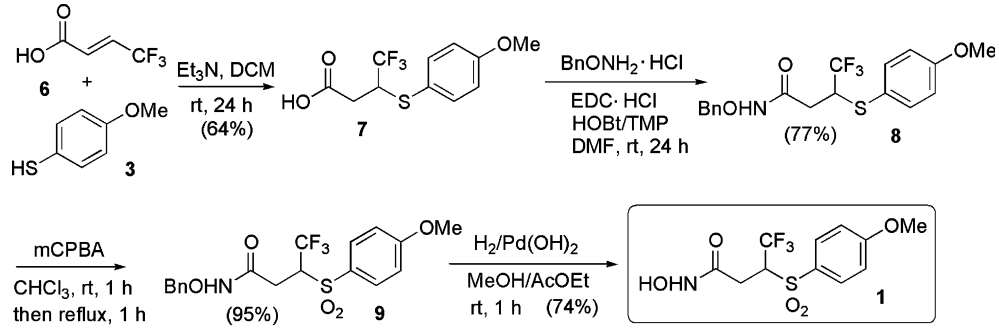
First attempts to synthesize racemic **1** were disappointing. Thia-Michael addition of thiol **3** (Scheme 1) to ethyl 3,3,3-trifluorocrotonate **2** occurred efficiently providing **4** in good yields.[8] Then, oxidation to the sulfone **5** occurred nearly quantitatively. However, the α-Tfm centre of **5** proved to be rather labile at basic pH, owing to the strong acidity of the proton in α-position to the strongly electronwithdrawing Tfm and sulfone moieties. Therefore, attempts to perform an alkaline hydrolysis of the ester function of **5** invariably produced intractable mixtures of by-products.

Further attempts to hydrolyze the sulfide **4** under the same conditions gave rise to a retro-Michael reaction.

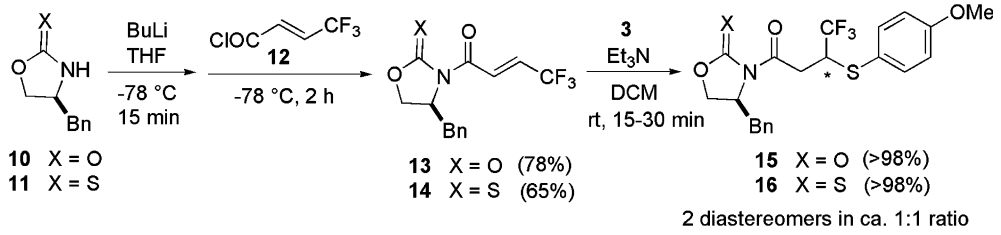
We therefore decided to use 3,3,3-trifluorocrotonic acid **6** (Scheme 2) as starting material, that would allow to skip the 'difficult' hydrolysis step. The thia-Michael addition of **3** to **6** occurred in reasonable yields, delivering the carboxylic acid **7**.[9] Coupling with *O*-Bn hydroxylamine delivered the *O*-Bn hydroxamate **8** in satisfactory yield, and the subsequent oxidation to the sulfone **9** took place in nearly quantitative yield. Hydrogenolysis with the Pearlman's catalyst afforded the target racemic hydroxamic acid **1** in good overall yields.[10]

Next, we turned our attention to the synthesis of (*R*) and (*S*)-**1**. It was decided to explore the use of both an oxazolidin-2-one[11] **10** (Scheme 3) and an oxazolidine-2-thione[12] **11** as chiral auxiliaries, since they are known to feature a remarkably different chemistry at the cleavage stage (the oxazolidin-2-one is more chemically robust but more resistant to exocyclic cleavage).[13]

Acylation of lithiated **10** and **11** with 3,3,3-trifluorocrotonoyl chloride **12** occurred smoothly affording the Michael acceptors **13** and **14**, respectively.[14] Thia-Michael addition of thiophenol **3** delivered in quantitative yields the oxazolidine-2-one **15** and the oxazolidine-2-thione **16**, in both cases as nearly equimolar mixtures of diastereomers, which could be obtained in stereo- and chemically pure form by flash chromatography. No attempts were made to improve the stereocontrol, as both the epimeric forms were needed for biological evaluation. The stereochemistry of (*S*,*S*)-**15** was assessed by X-ray diffraction of suitable single crystals (Fig. 2).[15]



Scheme 2.



Scheme 3.

The diastereomer (*S*,*S*)-**15** was subjected to exocyclic cleavage of the oxazolidin-2-one auxiliary, affording the carboxylic acid (*S*)-**7** (Scheme 4) in fair yields.[16] Coupling with *O*-Bn hydroxylamine occurred efficiently, and the resulting hydroxamate (*S*)-**8** was quantitatively oxidized to the sulfone (*S*)-**9**. The enantiomerically pure hydroxamic acid (+)-(*S*)-**1** was obtained in good yields by hydrogenolysis. An identical synthetic sequence was performed from (*S*,*R*)-**15** to provide (−)-(*R*)-**1**.[17]

In order to improve the synthetic process, and particularly the auxiliary cleavage step, we next focused our attention on the oxazolidine-2-thione intermediate (*S*,*S*)-**16** (Scheme 5). Satisfactorily, we found that the hydroxamate (*S*)-**8** could be obtained directly, in reasonable yields, by reaction with *O*-Bn-hydroxylamine, that could be then converted into the final hydroxamate (*S*)-**1** through the identical sequence described above. Analogously, the epimer (*S*,*R*)-**16** was converted in comparable yields into (*R*)-**8**, and then into the enantiomer (−)-(*R*)-**1**.

With racemic **1**, as well as both the single enantiomers in hand, we next addressed the inhibition tests on the catalytic domains of MMP-3 and MMP-9.[18]

The (*R*) enantiomer of **1** is the most potent compound (Table 1), but it is worth noting that all of them are single digit nanomolar inhibitors of MMP-3. Moderate selectivity was observed with respect to MMP-9, in particular (*R*)-**1** that was ca. 65-fold selective. Interestingly, both the pure enantiomers of **1** showed better inhibitory potency than the racemic compound. The reasons for this surprising finding are presently under investigation.[19] It is apparent however that the inhibitory potency of **1** is independent of its stereochemistry. This could be the result of an easy interchange of position of the Tfm and sulfone moieties in two different enzyme pockets, most likely S'_1 and S'_2.[20] These findings also suggest that judicious introduction of a Tfm group, and possibly of other fluoroalkyl groups (such as CF_2Cl, C_2F_5, etc.), onto the backbone of protease inhibitors could represent a successful strategy in order to improve and modulate the inhibitory activity.

We are currently investigating the synthesis and the inhibitory activity of other fluoroalkyl analogues of **1**

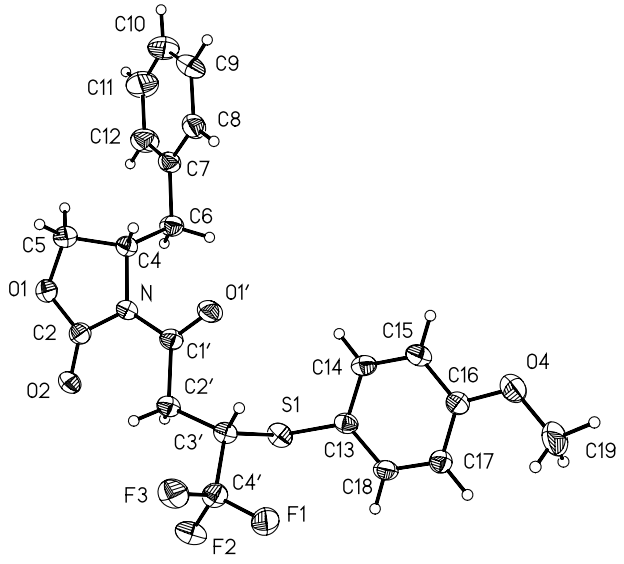
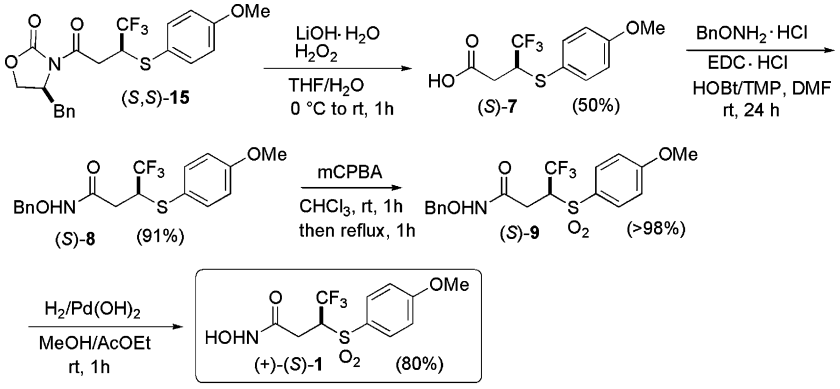


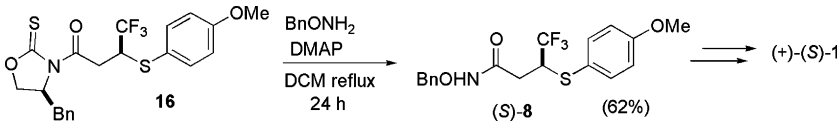
Figure 2. ORTEP view of 4-benzyl-3-[4,4,4-trifluoro-3-(4-methoxy-phenylsulfanyl)-butyryl]-oxazolidin-2-one (*S*,*S*)-**15**.

Table 1. Inhibition tests

Compound	IC_{50}/MMP-3 (nM)	IC_{50}/MMP-9 (nM)
(*R*)-**1**	3.2	209.3
(*S*)-**1**	4.3	51.9
Racemic **1**	8.4	515.9



Scheme 4.



Scheme 5.

on a wider range of MMPs both in vitro and in vivo, in order to have a more complete picture of the effect of fluorine in this particular class of protease inhibitors.

Acknowledgements

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9. To a stirred solution of **6** (5.25 mmol) in DCM (10 mL) thiol **3** (3.5 mmol) and Et_3N (8.75 mmol) were added. After stirring for 24 h the reaction mixture was washed with a 1 M solution of HCl and then with a 5% aqueous solution of $NaHCO_3$. The aqueous solution was acidified with a 1 M solution of HCl until pH 1–2 was reached and then extracted with AcOEt. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo, affording pure **7** (64% yield): R_f: 0.40 ($CHCl_3$/MeOH, 9:1); $[\alpha]_D^{23}$ (*R* enantiomer) −5.6 (*c* 0.78, $CHCl_3$); $[\alpha]_D^{23}$ (*S* enantiomer) +5.1 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.7–9.20 (very br s, 1H), 7.53 (d, 2H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.5 Hz), 3.81 (s, 3H), 3.77 (m, 1H), 2.94 (dd, 1H, J = 17.0 and 3.8 Hz), 2.69 (dd, 1H, J = 17.0 and 10.6 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) δ: 175.4, 160.8, 137.1, 126.8 (q, J = 276.3 Hz), 121.6, 114.8, 55.3, 48.5 (q, J = 29.7 Hz), 34.1; ^{19}F NMR (235.3 MHz, $CDCl_3$) δ: −71.6 (d, 3F, J = 8.9 Hz); MS (DIS EI 70 eV) *m/z* (%): 280 [M^+] (15), 260 (20), 139 (100).
10. To a stirred solution of **9** (0.41 mmol) in a MeOH/AcOEt 4:1 mixture (5 mL), a catalytic amount of $Pd(OH)_2$/C was added and the reaction mixture was kept vigorously stirred under hydrogen atmosphere at rt for 1 h. The palladium powder was filtered over Celite pad, washing the dark solid with MeOH. The solvent was removed in vacuo and the residue was purified by flash chromatography ($CHCl_3$/MeOH, 95:5), affording **1** (74% yield): R_f: 0.36 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (400 MHz, CD_3OD) δ: 7.86 (d, 2H, J = 8.9 Hz), 7.14 (d, 2H, J = 8.9 Hz), 4.62 (m, 1H), 3.89 (s, 3H), 2.92 (dd, 1H, J = 16.2 and 5.9 Hz), 2.63 (dd, 1H, J = 16.2 and 6.1 Hz); ^{13}C NMR (400 MHz, $CDCl_3$, 305 K) δ: 169.3, 168.9, 135.1, 132.9, 127.1 (q, J = 279.1 Hz), 118.4, 66.7 (q, J = 28.1 Hz), 59.0, 31.1; ^{19}F NMR (235.3 MHz, $CDCl_3$) δ: −64.1 (d, 3F, J = 8.2 Hz); MS (DIS EI 70 eV) *m/z* (%): 327 [M^+] (3), 295 (12), 155 (100).
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15. The absolute configuration of (*S*,*S*)-**15** was confirmed on the basis of the Flack parameter (x = 0.0329 (0.0284) for the selected configuration and x = 1.0281 (0.0380) for the opposite one): Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876–881, Full data (excluding structure factors) of the crystal structure have been deposited with Cambridge Crystallographic Data Centre as supplementary publication no. 263271. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
16. Although the cleavage reaction of **15** was conducted under strictly controlled conditions, it is likely that competitive oxidation of the sulfur atom by action of hydrogen peroxide is responsible for the rather modest yields.
17. The following values were recorded by polarimetric analysis: (*R*)-**1**: $[\alpha]_D^{23}$ −16.9 (*c* 0.88, EtOH); (*S*)-**1**: $[\alpha]_D^{23}$ +16.1 (*c* 1.1, EtOH).
18. The catalytic domains of MMP-3 and MMP-9 enzymes were produced in *E. coli*, transfected with cDNAs corresponding to the respective human sequences. Proteins were purified by affinity chromatography and the inhibitory potencies of racemic **1** and single enantiomers were assayed with synthetic, general MMP fluorescent substrate (Mca-PLGLDpaAR, Tebu-bio) using a FL600 Avantec fluorimeter. For MMP-3 see: (a) Ye, Q. Z.; Johnson, L. L.; Hupe, D. J.; Baragi, V. *Biochemistry* **1992**, *31*, 11231–11235; For MMP-9 see: (b) Ye, Q. Z.; Johnson, L. L.; Yu, A. E.; Hupe, D. *Biochemistry* **1995**, *34*, 4702–4708.
19. The tests were reproducibly repeated several times on chemically pure compounds, therefore this result is unlikely to be an artifact.
20. We thank one of the referees for this suggestion.