Bioorganic & Medicinal Chemistry 19 (2011) 2397–2406

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of novel irreversible serine protease inhibitors using amino acid based sulfonyl fluorides as an electrophilic trap

Arwin J. Brouwer, Tarik Ceylan, Anika M. Jonker, Tima van der Linden, Rob M. J. Liskamp *

Department of Medicinal Chemistry and Chemical Biology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 15 November 2010
Revised 2 February 2011
Accepted 9 February 2011
Available online 13 February 2011

Keywords:
Serine protease
Irreversible inhibitor
Peptido sulfonyl fluoride
Electrophilic trap
Chymotrypsin

ABSTRACT

We have designed and synthesized novel irreversible serine protease inhibitors containing aliphatic sulfonyl fluorides as an electrophilic trap. These substituted taurine sulfonyl fluorides derived from taurine or protected amino acids were conveniently synthesized from β-aminoethanesulfonyl chlorides using KF/18-crown-6 or from β-aminoethanesulfonates using DAST. Their potency of irreversible inhibition of serine proteases is described in different enzyme assays using chymotrypsin leading to binding affinities up to 22 μM.

1. Introduction

Irreversible enzyme inhibitors usually bind covalently to an enzyme using an electrophilic moiety ('warhead'). The Michael acceptor is perhaps the most widespread electrophilic moiety present in both many naturally occurring and man-made compounds.[1] The plethora of applications of the Michael acceptor is at least partly made possible by virtue of 'tuning' the reactivity of the Michael acceptors, which ranges from very reactive Michael acceptors such as acrylonitrile, via those present in natural products for example helenalin,[2] to the vinylsulfone[3] and vinylsulfonamide.[4] Despite the availability of several other electrophiles for incorporation into molecules towards biologically active and relevant compounds such as the chloromethylketone, aldehyde, beta-lactone, epoxide, epoxyketone, acyloxymethyl ketone, epoxysuccinate and borate moieties,[5] this number is limited. Furthermore, the reactivity of a potential electrophile is very important for selectivity and discriminativity. It is hypothesized that in general for selective biological applications one needs low-reactivity electrophiles, which do not react immediately with all kinds of abundantly present nucleophiles of for example proteins, as well as those present in the aqueous environment. Previously, we have developed an efficient synthesis of amino acid derived sulfonyl chlorides[6] which we have successfully employed for use in peptide-peptidomimetic hybrids,[7] synthetic receptors,[8] ligands for catalysis,[9] cyclic sulfonamides,[10] oligopeptidosulfonamides[11] and recently in the synthesis of sulfonyl azides.[12] These sulfonyl chlorides were relatively easy to prepare and turned out to be more stable than anticipated. The versatile synthesis and good stability of the sulfonyl chlorides, enticed us to attempt preparation of amino acid derived sulfonyl *fluorides* as possible *functionalized* analogs of the commonly used protease inhibitors PMSF and AEBSF (Fig. 1).

To our knowledge the synthesis of other functionalized sulfonyl fluorides has not been described in the literature together with applications as protease inhibitors or in other areas. This may be due to a relative lack of synthetic procedures for the mild preparation of aliphatic sulfonyl fluorides.

Recently, we reported the first amino acid derived sulfonyl fluorides and their potential as irreversible serine protease inhibitors was demonstrated by some preliminary investigations on the reactivity.[13] Herein, the ability of protease inhibition of the amino acid based sulfonyl fluorides will be evaluated using different enzyme assays. In addition, their synthesis and characterization is described, as well as further functionalization on the N-terminus.

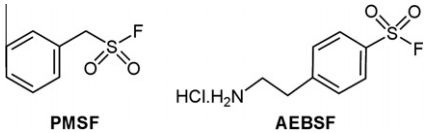


Figure 1. The structures of PMSF and AEBSF.

* Corresponding author. Tel.: +31 30 253 7396.
E-mail address: r.m.j.liskamp@uu.nl (R.M.J. Liskamp).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.02.014

2. Results and discussion

2.1. Chemistry

Natural Cbz- or Fmoc-protected amino acids with a variety of side chains were chosen as starting materials for the sulfonyl fluoride synthesis. First, Cbz- and Fmoc-protected amino acids **1b–f** (Ala, Val, Leu and Phe) were reduced to the corresponding alcohols **2b–f** (Scheme 1) using sodium borohydride treatment of the in situ prepared mixed anhydride.[14]

Mesylates **3b–f** were obtained in reasonable to high yields (Table 1) by reaction of alcohols **2b–f** with methanesulfonyl chloride in the presence of a base (Et_3N).

Next, thioacetates **4b–f** were prepared in good to high yields (65–91%) by reaction with in situ prepared cesium thioacetate. Oxidation of the thioacetates using aqueous acetic acid and hydrogen peroxide and subsequent addition of sodium acetate afforded sodium sulfonates **5b–f**. These sodium sulfonates were treated with a phosgene solution, to yield sulfonyl chlorides **6b–f**. For the synthesis of their corresponding sulfonyl fluorides we used three different synthesis routes.[13] The first method, a reaction with TBAF,[15] afforded sulfonyl fluoride **7f** only in low yield (30%). Furthermore, it was found that the yields were not reproducible due to varying quantities of residual water in the TBAF-solution. The second method, reaction with potassium fluoride using 18-crown-6,[16] was more successful, leading to reproducible and higher yields (45–67%, **7b**, **7d**, and **7e**). For the third method diethylamino sulfur trifluoride (DAST)[17] was used to convert the sodium sulfonates (**5b–e**) directly into sulfonyl fluorides **7b–e** in good yields (40–76%, 2 steps). Besides omitting one reaction step, another advantage of using DAST is that the acidic phosgene reaction is avoided, and therefore acid labile protecting groups for functional amino acids can be used.

All sulfonyl fluorides (**7a–f**) were characterized by ^1H, ^{13}C and 2D NMR spectroscopy, elemental analysis (CHNF) and mass spectroscopy. Besides elemental analysis, the presence of the fluorine was also evident from ^{19}F NMR and from a ^{13}C–F coupling in the ^{13}C NMR spectra.

Table 1
Yields (%) for the synthesis of sulfonyl fluorides **7b–f** from Cbz- or Fmoc-protected amino acids

Amino acid	3	4	6[a]	7[c]	7[a,d]
Cbz-Ala-OH (**b**)	82	91	61	65	65
Cbz-Val-OH (**c**)	50	65	51	Nd	40
Cbz-Leu-OH (**d**)		58[a]	44	45	76
Cbz-Phe-OH (**e**)	80[e]	86[e]	87[e]	67	62
Fmoc-Val-OH (**f**)	90	86	71	30[b]	Nd

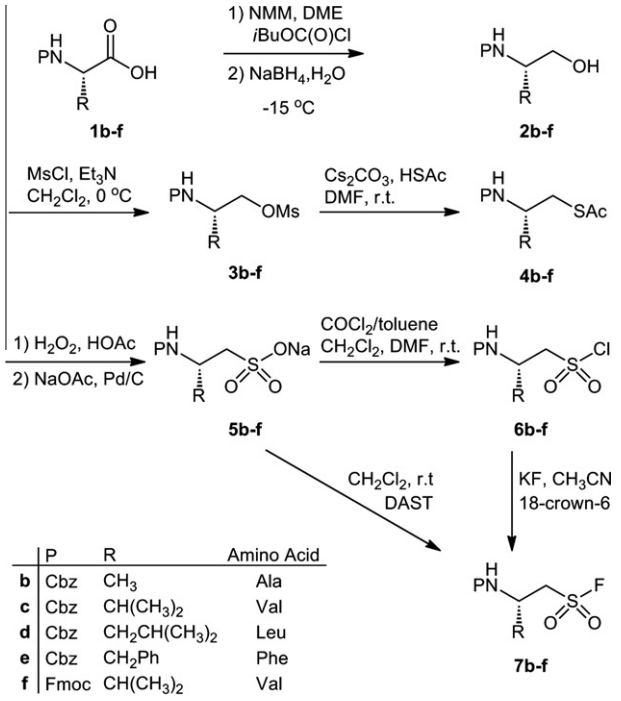
[a] Yield over two steps.
[b] Yield obtained using the TBAF procedure.
[c] Yield obtained using the KF/18-crown-6 ether procedure.
[d] Yield obtained using the DAST procedure.
[e] Yields from earlier work.[6]

It is expected that selective protease inhibitors can be prepared by further functionalization of the sulfonyl fluorides. To explore the possibility for coupling reactions at the N-terminus, the Cbz-group was cleaved from sulfonyl fluorides **7a** and **7d** using HBr in acetic acid (Scheme 2).

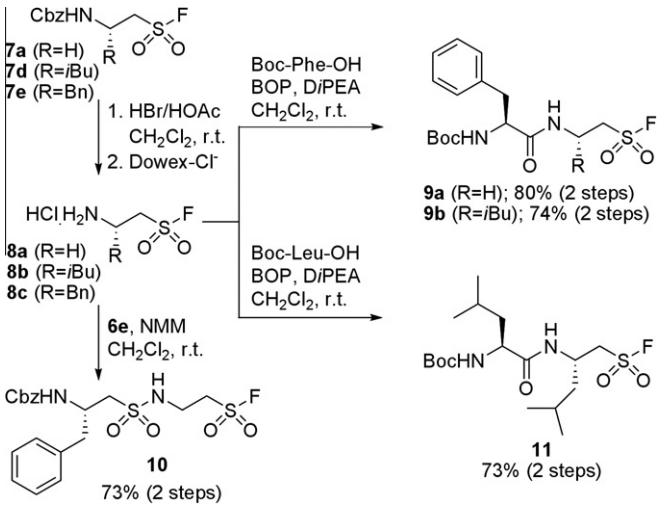
After treatment with HBr, an ion exchange resin was used to obtain the non-hygroscopic hydrochloride salts **8a–c**. Taurine derived hydrochloride salt **8a** was successfully coupled to an amino acid (Boc-Phe-OH) and a β-aminoethanesulfonyl chloride (**6e**) leading to sulfonyl fluorides **9a** and **10** in high yields (80% and 73%, respectively). As a proof of principle, two 'bulky' amino acids (Boc-Phe-OH and Boc-Leu-OH) were coupled to the more sterically demanding leucine derived sulfonyl fluoride **7d**, after Cbz-deprotection. Dipeptidosulfonyl fluorides **9b** and **11** were both isolated in high yields (74% and 73%, respectively), showing that in principle any sequence can be prepared. The ability of a sulfonyl fluoride to resist these harsh deprotection conditions and amine nucleophiles in basic conditions, points to its relative stability as a 'reactive' group.

2.2. Biological evaluation

Preliminary data on the reactivity clearly indicated the potential of the amino acid based sulfonyl fluorides as possible serine and cysteine protease inhibitors.[13] Thus, the next step was to examine the sulfonyl fluorides for inhibition of a serine protease. Chymotrypsin was chosen as a target enzyme for evaluation of the sulfonyl fluorides. Chymotrypsin is one of the most widely studied



Scheme 1. Synthesis of amino acid based sulfonyl fluorides **7b–f** starting from N-protected amino acids.



Scheme 2. Modification of the *N*-terminus of sulfonyl fluorides **7a** and **7d**.

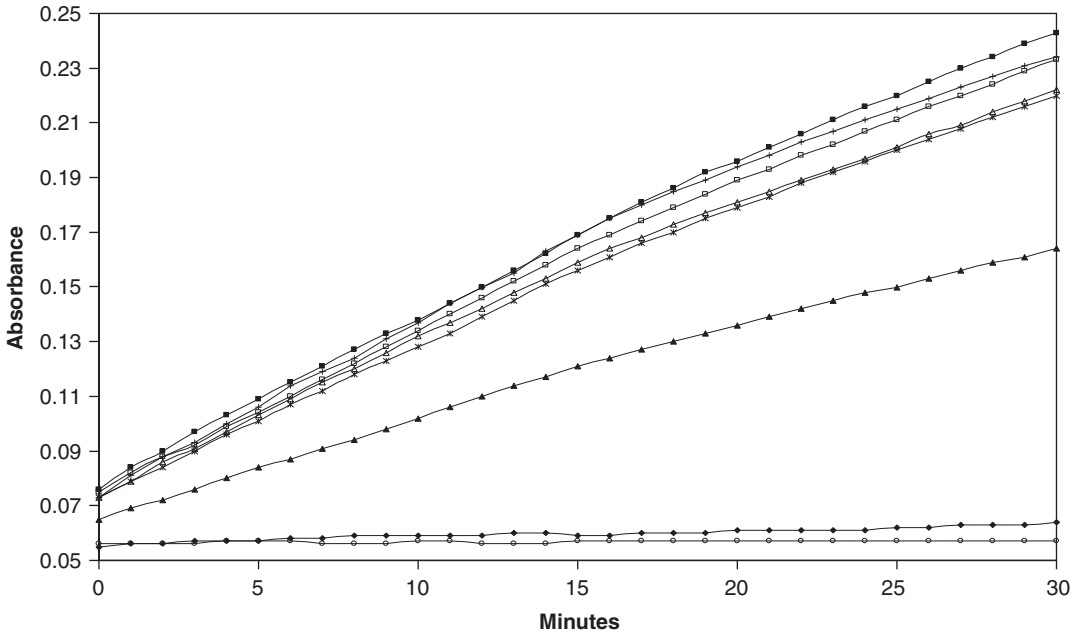
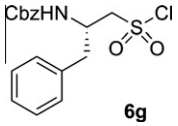


Figure 2. Progress curve of a chymotrypsin enzyme assay for comparison of the inhibitory activity of sulfonyl fluorides **7a**–**f** and PMSF (■, control; ▲, **7a**; □, **7b**; ∗, **7c**; △, **7d**; ♦, **7e**; +, **7f**; O, PMSF). [E] = 1.0 μM, [S] = 0.25 mM, [I] = 12.5 μM.



6g

Figure 3. Structure of Fmoc-protected phenylalanine derived sulfonyl chloride **6g**.[6]

members of the serine protease class of enzymes. First, sulfonyl fluorides **7a**–**f** (Fig. 8) were evaluated by an activity based enzyme assay. For this a colorimetric assay in 96-well plates was developed using a phosphate buffer (0.05 M, pH 7.0), and Bz-L-Tyr-pNA as a substrate. PMSF was used as a reference inhibitor. The optimal chymotrypsin and substrate concentrations were found to be 1.0 and 12.5 μM, respectively. Sulfonyl fluorides **7a**–**f** and PMSF were first pre-incubated for 1 h with chymotrypsin before addition of the substrate. Subsequently, the liberation of *p*-nitroaniline from Bz-L-Tyr-pNA was measured at 405 nm during 30 min to determine the residual enzyme activity. We were very pleased to see that our sulfonyl fluorides were indeed capable of decreasing the activity of chymotrypsin (Fig. 2).

Cbz-phenylalanine derived sulfonyl fluoride **7e** was clearly the best inhibitor, which might have been expected since the S1

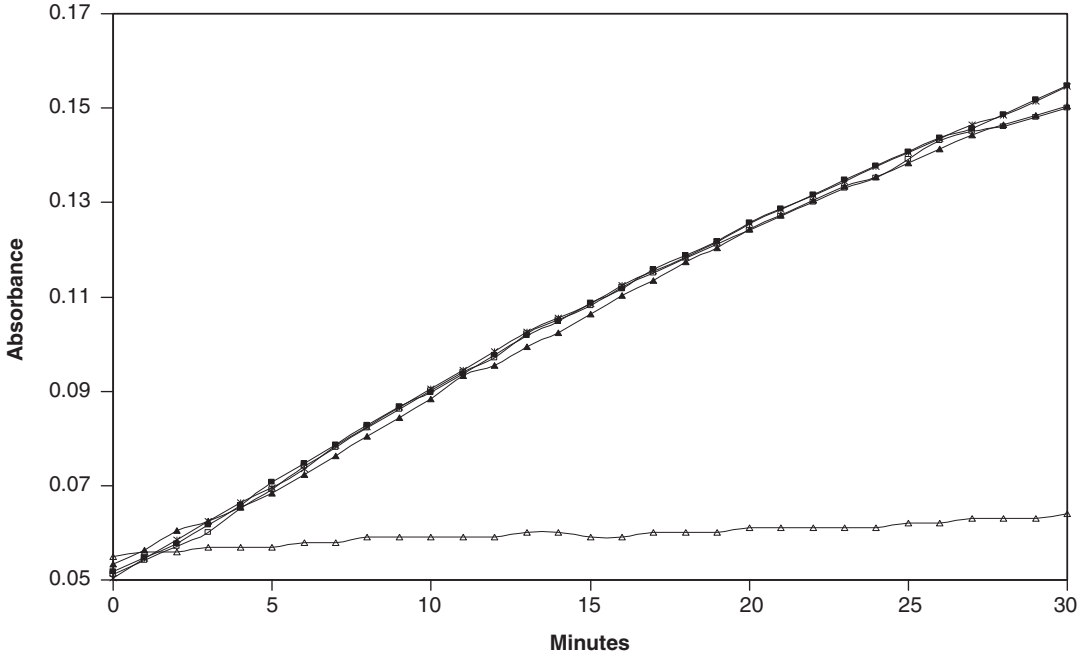


Figure 4. Progress curve of a chymotrypsin enzyme assay for comparison of the inhibitory activity of phenylalanine derived sulfonyl chlorides **6e** and **6g**, and sulfonyl fluorides **7e** and **8c** (■, control; ▲, **6e**; □, **6g**; ∗, **8c**; △, **7e**). [E] = 1.0 μM, [S] = 0.25 mM, [I] = 12.5 μM.

subsite of chymotrypsin has a preference for aromatic side chains. The inhibitory effect of **7e** was even comparable to PMSF, which is a known potent inhibitor. The inhibition results of the other sulfonyl fluorides also showed the influence of the sulfonyl fluoride side chain on the inhibition. Aliphatic side chain containing sulfonyl fluorides **7b**–**d** and **7f** (Fig. 8) showed the weakest inhibition, most likely due to low acceptance of the aliphatic group in the S1 subsite. Taurine derivative (**7a**), without side chain, was clearly inhibiting stronger. These results also showed that the aromatic ring from the Cbz-group from **7e** is not responsible for the high affinity since the weakly binding sulfonyl fluorides **7b**–**d** also contain a Cbz-group. The influence of the protecting group on the binding also seemed to be small since both Fmoc- and Cbz-valine derived sulfonyl fluorides gave similar inhibition. In order to further investigate the inhibition of phenylalanine derived sulfonyl fluoride **7e**, other derivatives were also evaluated. Thus, **7e** was compared with Cbz-deprotected sulfonyl fluoride **8c**, Cbz-protected sulfonyl chloride **6e** and Fmoc-protected sulfonyl chloride **6g**[6] (Figs. 3 and 4).

As expected both sulfonyl chlorides (**6e** and **6f**) showed only very weak inhibition, most likely due to fast hydrolysis of the much more reactive sulfonyl chloride moiety compared to the much more stable sulfonyl fluorides. The decrease in inhibitory activity of Cbz-deprotected sulfonyl fluoride **8c** compared to **7e** was remarkable. Apparently the Cbz-group in **7e** does participate in binding, or the positively charged nitrogen from **8c** reduces the binding affinity, possibly due to repulsion in the chymotrypsin binding pocket. Since taurine sulfonyl fluoride **7a** was the second best inhibitor, it was compared in the enzyme assay with Boc-Phe-Tau-F (**9a**), and Cbz-protected sulfonamide containing sulfonyl fluoride **10**, both with an aromatic side chain on the P2-position (Fig. 5).

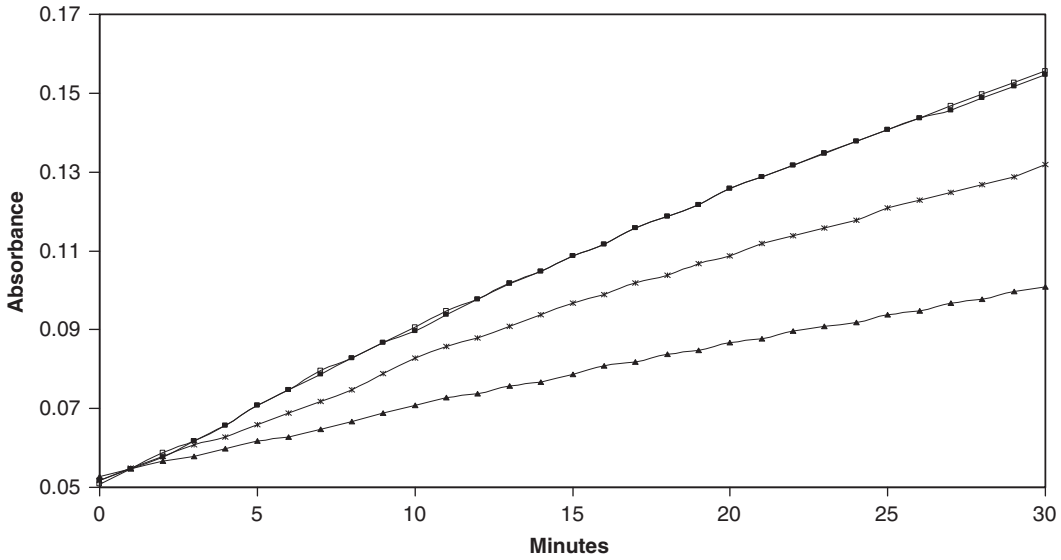


Figure 5. Progress curve of a chymotrypsin enzyme assay for comparison of the inhibitory activity of taurine derived sulfonyl fluorides **7a**, **9a** and **10**. (■, control; ▲, **7a**; □, **9a**; ∗, **10**). [E] = 1.0 μM, [S] = 0.25 mM, [I] = 12.5 μM.

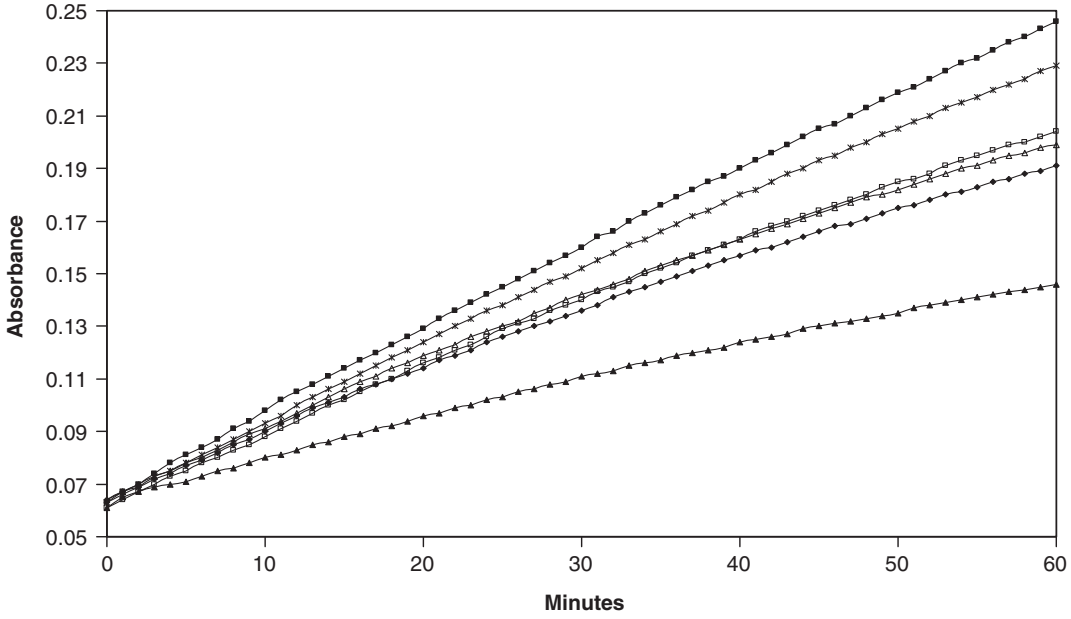


Figure 6. Progress curve of a papain enzyme assay for comparison of the inhibitory activity of sulfonyl fluorides **7a**–**e** (■, control; ▲, **7a**; □, **7b**; ∗, **7c**; △, **7d**; ♦, **7e**). [E] = 8.0 μM, [S] = 1.0 mM, [I] = 2.5 μM.

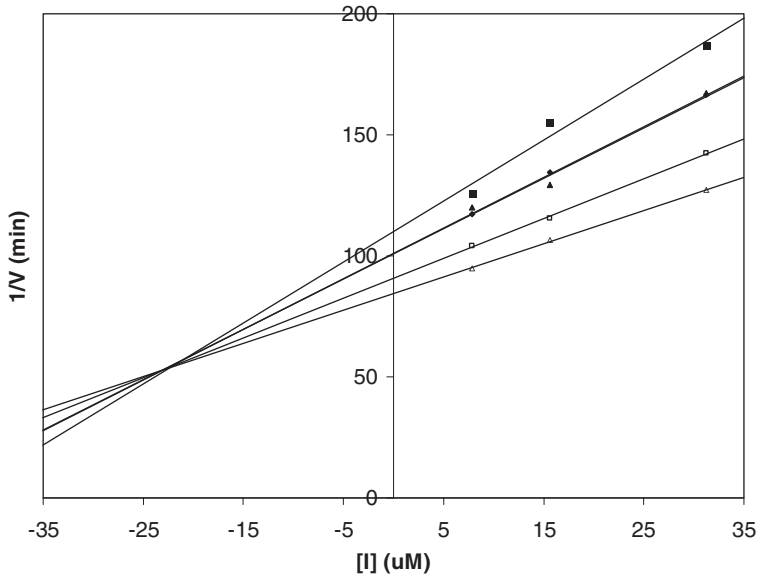


Figure 7. A Dixon plot of inhibition of chymotrypsin by sulfonyl fluoride **7e** ([S] (mM): ■, 1.5; ▲, 1.7; ♦, 1.9; □, 2.3; △, 2.5).

Table 2
Inhibitor constants and $k_{inactivation}$ values of sulfonyl fluorides **7a–e** (R-Ψ[CH_2SO_2]-F) and PMSF[a]

Sulfonyl fluoride	R	K_i (μM)	k_{inact} (min^{-1})
7b	Cbz-Ala	No inhibition	nd
7f	Fmoc-Val	No inhibition	nd
7d	Cbz-Leu	341 ± 35.6	0.053 ± 0.0017
7c	Cbz-Val	255 ± 6.8	0.043 ± 0.006
7a	Cbz-Gly	104 ± 10.7	0.13 ± 0.006
7e	Cbz-Phe	22 ± 1.6	0.33 ± 0.03
PMSF		13 ± 0.8[b]	0.32 ± 0.04[b]

[a] K_i values were determined using Dixon plots.[18]
[b] Literature K_i value: 28 μM; k_{inact} value: 0.32 min^{-1}.[19]

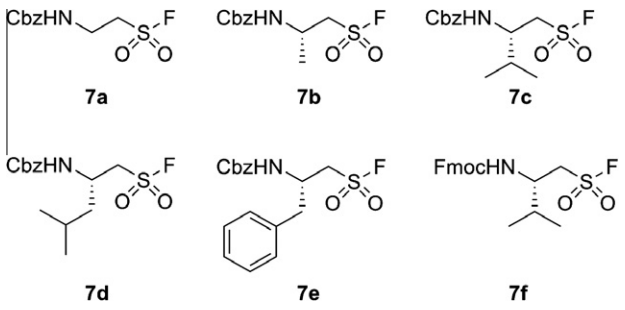


Figure 8. Structures of sulfonyl fluorides **7a–f**.

Both derivatives were inhibiting less good than **7a**, of which sulfonamide **10** was clearly the better one.

To determine whether the sulfonyl fluorides were also capable of inhibiting cysteine proteases, an enzyme assay was performed with papain, which is the most widely studied member of the cysteine protease class of enzymes. For this a colorimetric assay in 96-well plates was used. In this assay a phosphate buffer (0.10 M, pH 6.5) containing EDTA (1.5 mM) was used, and Bz-L-Arg-pNA (1.0 mM) as a substrate. DTT, normally used in papain enzyme assays to activate the enzyme, was not used because DTT can cleave the covalent papain-inhibitor linkage. A relatively high concentration of papain (8.0 μM) was used because only 10% of the papain was active without activation. Since papain has a preference for basic residues in the S1 subsite, low inhibition and low selectivity was expected for sulfonyl fluorides **7a–e**. We were very pleased to see clear inhibition for all sulfonyl fluorides, already at a relatively low concentration of inhibitor (2.5 μM) (Fig. 6).

As expected, aliphatic side chain containing sulfonyl fluorides **7b**, **7c** and **7d** were the weakest inhibitors. Phenylalanine derived sulfonyl fluoride **7e** was slightly better, but the best inhibitor was again the taurine derived one (**7a**). The lack of an aliphatic or aromatic side chain makes **7a** the best inhibitor. It is expected that lysine and arginine derived sulfonyl fluorides will inhibit much stronger.

Next, the chymotrypsin binding affinities were examined for sulfonyl fluorides **7a–f** (Fig. 8), and PMSF as a reference inhibitor. These inhibitors were subjected to an assay with different inhibitor and substrate concentrations. The remaining activities were measured and used in Dixon plots (Fig. 7), from which the K_i values were determined.[18] The K_i values found (Table 2) for the sulfonyl fluorides were in agreement with the results from the comparison assay (Fig. 2).

As expected, the best K_i values were found for phenylalanine derived sulfonyl fluoride **7e** (22 μM) and PMSF (13 μM, literature: 28 μM),[19] showing that the binding affinity of **7e** is similar to PMSF. We think that these low micromolar K_i values are very encouraging and that the affinity for the active site can be improved by modification of the *N*-terminus, for example, by introduction of (an) amino acid residue(s). Lineweaver–Burk plots from sulfonyl fluorides **7a** and **7e** showed that the mode of inhibition was competitive for the substrate.

$k_{inactivation}$ values were determined by plotting residual enzyme activity as a function of preincubation time. The lowest values were found for aliphatic sulfonyl fluorides **7c** and **7d** (0.043 and 0.053 min^{-1}, respectively). Glycine derived sulfonyl fluoride **7a** was clearly better (0.13 min^{-1}), but the highest $k_{inactivation}$ value was obtained for phenylalanine derived sulfonyl fluoride **7e** (0.33 min^{-1}). The obtained $k_{inactivation}$ value was equal to PMSF (0.32 min^{-1}, literature value[19]: 0.32 min^{-1}).

3. Conclusions

In conclusion, we have developed a successful synthesis of substituted β-aminoethanesulfonyl fluorides, which can be prepared starting from, in principle, any Cbz- or Fmoc-protected

amino acid. Different procedures were used for fluorination of which DAST and KF/18-crown-6 were the best. By using DAST for introduction of the fluorine atom, strong acidic conditions were avoided, which allows the use of acid labile protecting groups present in functional amino acid derivatives. The described sulfonyl fluorides were capable of irreversible competitive inhibition of the serine protease chymotrypsin. In addition, the affinities were found to be dependent on the side chain of the inhibitor, which points at the possibilities for tuning the selectivity and affinity. The binding affinities were good, up to 22 μM, of which the best one was comparable to PMSF (28 μM),[19] as well as the rate constants. Hardly any chymotrypsin inhibition was observed with substituted β-aminoethanesulfonyl chlorides, most likely due to fast hydrolysis. This shows the importance of the moderate reactivity needed for a suitable electrophilic trap for use in serine protease inhibitors. The sulfonyl fluorides were also capable of inhibition of the cysteine protease papain. We believe that these sulfonyl fluorides will become a new important class of irreversible serine and/or cysteine protease inhibitors of which the affinity can be further improved and/or tuned by using different amino acid side chains and/or by extending the *N*-terminus. Currently, we are preparing and evaluating small libraries of *N*-terminal functionalized amino acid based sulfonyl fluorides for finding inhibitors with higher affinities for biologically more relevant serine proteases.

4. Experimental

Peptide grade solvents for synthesis were purchased from Biosolve (The Netherlands) and were stored on molecular sieves (4 Å). Reactions were carried out at ambient temperature unless stated otherwise. TLC analysis was performed on Merck pre-coated Silica Gel 60 F-254 (0.25 mm) plates. Spots were visualized with UV light, ninhydrin or Cl_2-TDM.[20] Solvents were evaporated under reduced pressure at 40 °C. Column chromatography was performed on ICN Silica Gel 60 (32–63 μm). Melting points were measured on a Büchi Schmelzpunktbestimmungsapparat (according to dr. Tottoli) and are uncorrected. Electrospray mass spectra were recorded on a Shimadzu LC/MS-QP-8000 spectrometer, or a Finnigan LCQ Deca XP MAX spectrometer. Elemental analyses were carried out at Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany). ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) and COSY spectra were recorded on a Varian G-300 spectrometer. Chemical shifts are reported in ppm relative to TMS (0 ppm) or DMSO (2.50 ppm) for the ^{1}H NMR and to $CDCl_3$ (77 ppm) or DMSO (39.5 ppm) for the ^{13}C NMR spectra as internal standards. ^{13}C NMR spectra were recorded using the attached proton test (APT) pulse sequence.

4.1. General procedure for the synthesis of mesylates 3b–g

Mesylates **3b**–**g** were prepared following our earlier published protocol.[6] All products were recrystallized from CH_2Cl_2/hexanes, except **3d**, which was used directly in the next reaction without purification. Compounds **3e**–**g** were synthesized and characterized by as described.[6]

4.1.1. Cbz-Ala-ψ[CH_2O]-Ms (3b)

The reaction was performed on a 55.2 mmol scale to yield off-white crystals (11.9 g, 82%). R_f = 0.39 (EtOAc/CH_2Cl_2, 1:9); mp = 86 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.24 (d, 3H, $CHCH_3$), 2.96 (s, 3H, SO_2CH_3), 4.02 (m, 1H, NCH), 4.15 (dd, J_{gem} = 10.2 Hz, J_{vic} = 4.4 Hz, 1H, SOCH[a]), 4.24 (br dd, 1H, SOCH[b]), 4.92 (br s, 1H, NH), 5.10 (s, 2H, CH_2 (Cbz)), 7.35 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.1 ($CHCH_3$), 37.2 (SO_2CH_3), 46.1 (NCH), 66.8 (CH_2 (Cbz)), 71.6 ($SOCH_2$), 128.1, 128.2, 128.5, 136.2 (Ar-C (Cbz)), 155.5 (C=O (Cbz)). Anal. Calcd for $C_{12}H_{17}NO_5S$: C, 50.16; H, 5.96; N, 4.87. Found: C, 49.99; H, 6.05; N, 4.81; ESI-MS: *m*/*z* = 309.95 [M+Na]$^+$.

4.1.2. Cbz-Val-ψ[CH_2O]-Ms (3c)

The reaction was performed on a 25 mmol scale and purified using column chromatography (EtOAc/hexanes, 1:6) to yield a white solid (7.77 g, 50%). Depending on the purity of the crude mesylate, it can be purified either by crystallization or by column chromatography. R_f = 0.54 (EtOAc/hexanes, 5:95); mp = 64 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (2d, *J* = 7.7 Hz and *J* = 8.5 Hz, 6H, $CH(CH_3)_2$), 1.89 (m, 1H, $CH(CH_3)_2$), 2.93 (s, 3H, SO_2CH_3), 3.71 (m, 1H, NCH), 4.27 (d, *J* = 4.1 Hz, 2H, $SOCH_2$) 4.74 (br s, 1H, NH), 5.13 (s, 2H, CH_2 (Cbz)), 7.36 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.3, 19.2 ($CH(CH_3)_2$), 28.9 ($CH(CH_3)_2$), 37.2 (SO_2CH_3), 55.5 (NCH), 66.8 (CH_2 (Cbz)), 69.3 ($SOCH_2$), 128.0, 128.1, 128.5, 136.3 (Ar-C), 165.1 (C=O (Cbz)). Anal. Calcd for $C_{14}H_{21}NO_5S$: C, 53.32; H, 6.71; N, 4.44. Found: C, 53.19; H, 6.74; N, 4.28; ESI-MS: *m*/*z* = 353.95 [M+K]$^+$.

4.1.3. Cbz-Leu-ψ[CH_2O]-Ms (3d)

The reaction was performed on a 39.5 mmol scale to yield a yellow oil. The crude mesylate was directly used in the synthesis of thioacetate **4d**. A small sample was purified using column chromatography (EtOAc/hexanes, 1:6) for characterization. R_f = 0.78 (EtOAc/hexanes, 1:9); mp = 57 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.93 (d, *J* = 6.3 Hz, 6H, $CH_2CH(CH_3)_2$), 1.43 (m, 2H, $CH_2CH(CH_3)_2$), 1.70 (m, 1H, $CH_2CH(CH_3)_2$), 2.92 (s, 3H, SO_2CH_3), 3.94 (m, 1H, NCH), 4.14 (dd, J_{gem} = 9.9 Hz, J_{vic} = 4.0 Hz, 1H, SOCH[a]), 4.25 (dd, J_{gem} = 9.9 Hz, J_{vic} = 3.0 Hz, 1H, SOCH[b]), 4.95 (br s, 1H, CONH), 5.10 (s, 2H, CH_2 (Cbz)), 7.34 (m, 5H, C_6H_5 (Cbz)). Anal. Calcd for $C_{15}H_{23}NO_5S$: C, 54.69; H, 7.04; N, 4.25. Found: C, 54.66; H, 7.11; N, 4.21; ESI-MS: *m*/*z* = 367.95 [M+K]$^+$.

4.2. General procedure for the synthesis of thioacetates 4b–g

Thioacetates **4b**–**g** were prepared following our earlier published protocol.[6] All products were purified by recrystallization (from EtOAc/hexanes) and/or by column chromatography (10% hexanes/EtOAc). Compounds **4e**–**g** were synthesized and characterized as described before.[6]

4.2.1. Cbz-Ala-ψ[CH_2S]-Ac (4b)

The reaction was performed on a 31 mmol scale to yield off-white crystals (6.20 g, 91%). R_f = 0.30 (CH_2Cl_2); mp = 57 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.18 (d, *J* = 6.6 Hz, 3H, $CHCH_3$), 2.32 (s, 3H, $COCH_3$), 3.04 (d, *J* = 5.2 Hz, 2H, SCH_2), 3.93 (m, 1H, NCH), 4.90 (br s, 1H, NH), 5.08 (s, 2 H, CH_2 (Cbz)), 7.34 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 20.0 ($CHCH_3$), 30.4 ($COCH_3$), 34.8 (SCH_2), 47.0 (NCH), 66.5 (CH_2 (Cbz)), 128.0, 128.4, 136.4 (Ar-C), 155.5 (C=O (Cbz)), 195.6 ($COCH_3$). Anal. Calcd for $C_{13}H_{17}NO_3S$: C, 58.40; H, 6.41; N, 5.24. Found: C, 58.25; H, 6.29; N, 5.14; ESI-MS: *m*/*z* = 289.95 [M+Na]$^+$.

4.2.2. Cbz-Val-ψ[CH_2S]-Ac (4c)

The reaction was performed on a 36 mmol scale and purified by column chromatography followed by crystallization to yield white crystals (6.93 g, 65%). R_f = 0.35 (CH_2Cl_2); mp = 69 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.94 (2d, *J* = 6.9 Hz (both), 6H, $CH(CH_3)_2$), 1.82 (m, 1H, $CH(CH_3)_2$), 2.27 (s, 3H, $COCH_3$), 2.97 (dd, J_{gem} = 9.9 Hz, J_{vic} = 4.0 Hz, 1H, SOCH[a]), 3.06 (dd, J_{gem} = 9.9 Hz, J_{vic} = 3.0 Hz, 1H, SOCH[b]), 3.64 (m, 1H, NCH), 4.83 (d, *J* = 8.3 Hz, 1H, NH), 5.12 (2d, *J* = 12.1 Hz (both), 2H, CH_2 (Cbz)), 7.33 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.8, 19.1 ($CH(CH_3)_2$), 30.4 ($COCH_3$), 31.6 (SCH_2), 32.0 ($CH(CH_3)_2$), 56.5 (NCH), 66.5 (CH_2 (Cbz)), 127.8,

127.9, 128.3, 136.6 (Ar-C (Cbz)), 156.3 (C=O (Cbz)),195.9 ($COCH_3$). Anal. Calcd for $C_{15}H_{21}NO_3S$: C, 60.99; H, 7.17; N, 4.74. Found: C, 61.02; H, 7.20; N, 4.65; ESI-MS: m/z = 317.95 $[M+Na]^+$, 333.95 $[M+K]^+$.

4.2.3. Cbz-Leu-ψ[CH_2S]-Ac (4d)

The reaction was performed on a 38.4 mmol scale and purified by column chromatography to yield a white solid (6.80 g, 58%). R_f = 0.40 (CH_2Cl_2); mp = 52 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.90 (d, J = 6.6 Hz, 6H, $CH(CH_3)_2$), 1.28, 1.65 (2 m, 3H, $CHCH_2CH(CH_3)_2$), 2.29 (s, 3H, $COCH_3$), 2.97 (dd, J_{gem} = 14.0 Hz, J_{vic} = 7.2 Hz, 1H, SOCH[a]), 3.11 (dd, J_{gem} = 14.0 Hz, J_{vic} = 4.7 Hz, 1H, SOCH[b]), 3.90 (m, 1H, NCH), 4.75 (br d, 1H, NH), 5.14 (2d, J = 12.4 Hz (both), 2H, CH_2 (Cbz)), 7.33 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.1, 22.9 ($CH(CH_3)_2$), 24.8 ($CH(CH_3)_2$), 30.4 ($COCH_3$), 34.2 (SCH_2), 43.5 ($CH_2CH(CH_3)_2$), 49.2 (NCH), 66.5 (CH_2 (Cbz)), 127.9, 128.0, 128.4, 136.5 (Ar-C), 155.9 (C=O (Cbz)), 195.9 ($COCH_3$). Anal. Calcd for $C_{16}H_{23}NO_3S$: C, 62.11; H, 7.49; N, 4.53. Found: C, 62.08; H, 7.45; N, 4.42; ESI-MS: m/z = 332.00 $[M+Na]^+$, 347.95 $[M+K]^+$.

4.3. General procedure for the synthesis of sulfonyl chlorides 6b–g

Sulfonyl chlorides **6b**–**g** were prepared following our earlier published protocol.[6] After concentration of the reaction mixture, the residue was taken up in dichloromethane (50 mL) and washed once with water (30 mL) for removal of the salts. After separation, the organic layer was dried (Na_2SO_4) and concentrated in vacuo. All products were purified by a fast silica plug. Compounds **6a** and **6e**–**g** were synthesized and characterized by us before.[6]

4.3.1. Cbz-Ala-ψ[CH_2SO_2]-Cl (6b)

The reaction was performed on a 6.0 mmol scale to yield a white solid (1.07 g, 61%). Eluent silica plug: hexanes/EtOAc, 1:9, the purified product hydrolyzed clearly faster than sulfonyl chlorides with different side chains. R_f = 0.34 (hexanes/CH_2Cl_2, 1:9); mp = 85 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44 (br d, 3H, $CHCH_3$), 3.22 (br s, 1H, $CHCH_3$), 3.82, 4.08 (2 br d, 2H, CH_2SO_2Cl), 4.34 (m, 1H, NCH), 5.12 (s, 2H, CH_2 (Cbz)), 5.28 (m, 1H, NH), 7.35 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 19.6 ($CHCH_3$), 44.4 (NCH), 67.0 (CH_2 (Cbz)), 69.5 (CH_2SO_2Cl), 128.1, 128.2, 128.3, 128.5, 128.6, 128.8, 128.9, 129.3, 135.2, 135.9 (Ar-C (Cbz)), 155.3 (C=O (Cbz)); ESI-MS: m/z = 313.15 $[M+Na]^+$.

4.3.2. Cbz-Val-ψ[CH_2SO_2]-Cl (6c)

The reaction was performed on a 3.0 mmol scale to yield a white solid (490 mg, 51%). Eluent silica plug: hexanes/EtOAc, 3:10, the purified product contained some N-formylated side product which could be more easily separated after conversion to the sulfonyl fluoride. R_f = 0.45 (CH_2Cl_2); mp = 74 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.99 (2d, J = 6.7 Hz (both), 6H, $CH(CH_3)_2$), 2.10 (m, 1H, $CH(CH_3)_2$), 3.87–4.08 (m, 2H, CH_2SO_2Cl), 4.12 (m, 1H, NCH), 5.12 (s, 2H, CH_2 (Cbz)), 5.21 (br s, 1H, NH), 7.34 (m, 5 H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.9, 18.8 ($CH(CH_3)_2$), 31.4 ($CH(CH_3)_2$), 53.6 (NCH), 67.1 (CH_2SO_2Cl, CH_2 (Cbz)), 127.8, 128.0, 128.1, 128.2, 128.5, 136.0 (Ar-C (Cbz)), 155.7 (C=O (Cbz)).

4.3.3. Cbz-Leu-ψ[CH_2SO_2]-Cl (6d)

The reaction was performed on a 3.0 mmol scale to yield a white solid (440 mg, 44%). Eluent silica plug: hexanes/EtOAc, 3:7; R_f = 0.40 (hexanes/CH_2Cl_2, 2:8); mp = 45 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95 (d, J = 5.8 Hz, 6H, $CH_2CH(CH_3)_2$), 1.68, 1.71 (2 m, 3H, $CH_2CH(CH_3)_2$), 3.86 (dd, J_{gem} = 14.0 Hz, J_{vic} = 3.3 Hz, 1H, SOCH[a]), 4.08 (dd, J_{gem} = 14.0 Hz, J_{vic} = 6.1 Hz, 1H, SOCH[b]), 4.25 (m, 1H, NCH), 5.10 (s, 2H, (CH_2 (Cbz)), 5.21 (d, J = 8.0 Hz, 1H, NH), 7.34 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.5, 22.8 ($CH_2CH(CH_3)_2$), 24.7 ($CH_2CH(CH_3)_2$), 42.2 ($CH_2CH(CH_3)_2$), 46.9 (NCH), 67.0 (CH_2 (Cbz)), 69.0 (SCH_2), 128.0, 128.2, 128.5, 136.0 (Ar-C (Cbz)), 155.5 (C=O (Cbz)); ESI-MS: m/z = 396.75 $[M+CH_3CN+Na]^+$.

4.4. General procedures for the synthesis of sulfonyl fluorides 7a–f

Procedure 1 (using KF): To a solution of the sulfonyl chloride (**6a**–**b** and **6d**–**f**; 4,0 mmol) in acetonitrile (20 mL) was added potassium fluoride (465 mg, 8,0 mmol) and 18-Crown-6 (53 mg, 0,20 mmol). After stirring overnight at rt, the mixture was concentrated in vacuo and purified by column chromatography.

Procedure 2 (using DAST): Thioacetates **4b**–**f** were oxidized to sodium sulfonates **5b**–**f** following an earlier described protocol.[6] To a suspension of sulfonate salt (2.69 mmol) in DCM (14 mL) was added 640 μL DAST (4.84 mmol). After stirring overnight at rt, the mixture was concentrated in vacuo and purified by column chromatography.

Procedure 3 (using TBAF): To a solution of the sulfonyl chloride (**6a** or **6f**) (10.0 mmol, 3.91 g) in THF (200 mL) was added a tetrabutylammonium fluoride solution in THF (15 mL, 1.0 M). After stirring for 2 h at rt, HCl (200 mL, 1.0 M) was added and the THF was evaporated in vacuo. After addition of dichloromethane (400 mL), the layers were separated and the water layer was extracted with dichloromethane (400 mL). After drying (Na_2SO_4) and concentration in vacuo of the combined organic layer, the sulfonyl fluoride was purified by column chromatography.

4.4.1. Cbz-Tau-ψ[CH_2SO_2]-F (7a)

Eluent for column chromatography: CH_2Cl_2. Yield procedure 1: 70% (720 mg, white solid), yield procedure 2: 42% (110 mg, white solid), yield procedure 2 via the crude sulfonyl chloride: 60% (15.7 g, over 2 steps); yield procedure 3: 31% (328 mg, white solid). R_f = 0.30 (CH_2Cl_2); mp = 124 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.62 (m, 2H, SO_2CH_2), 3.72 (m, 2H, NCH_2), 5.12 (s, 2H, CH_2 (Cbz)), 5.38 (br s, 1H, NH), 7.35 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ = 36.6 (NCH_2), 50.6, 50.8 (SO_2CH_2), 67.3 (CH_2 (Cbz)), 128.1 128.4 128.6 135.8 (Ar-C), 156.1 (C=O (Cbz)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −120.9 (s). Anal. Calcd for $C_{10}H_{12}FNO_4S$: C, 45.97; H, 4.63; N, 5.36; F, 7.27. Found: C, 46.20; H, 4.57; N, 5.25; F, 7.21.

4.4.2. Cbz-Ala-ψ[CH_2SO_2]-F (7b)

Eluent for column chromatography: hexanes/CH_2Cl_2, 5:95. Yield procedure 1: 65% (900 mg, white solid), yield procedure 2: 65% (180 mg, white solid). R_f = 0.30 (CH_2Cl_2); mp = 85 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.42 (d, J = 6.9 Hz, 3H, $CHCH_3$), 3.51, 3.56 (2t, J_{gem} = 14.7 Hz, J_{vic} = 5.3 Hz, $^3J_{H^aF}$ = 5.3 Hz, 1H, SOCH[a]), 3.70 (dd, J_{gem} = 14.7 Hz, J_{vic} = 3.5 Hz, 1H, SOCH[b]), 4.25 (m, 1H, NCH), 5.10 (s, 2H, CH_2 (Cbz), 5.28 (s, 1H, NH), 7.34 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 19.2 (CH_3), 43.3 (NCH), 55.1, 55.3 (CH_2SO_2), 67.0 (CH_2 (Cbz)), 128.1, 128.3, 128.5, 135.9 (Ar-C (Cbz)), 155.2 (C=O (Cbz)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −115.6 (s). Anal. Calcd for $C_{11}H_{14}FNO_4S$: C, 47.99; H, 5.13; N, 5.09; F, 6.90. Found: C, 48.11; H, 5.08; N, 5.15; F, 6.82; ESI-MS: m/z = 313.95 $[M+K]^+$.

4.4.3. Cbz-Val-ψ[CH_2SO_2]-F (7c)

Eluent for column chromatography: hexanes/CH_2Cl_2, 3:7. Yield procedure 2: 40% (120 mg, white solid). R_f = 0.45 (CH_2Cl_2); mp = 123 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.98 (2d, J = 6.7 Hz (both), 6H, $CH(CH_3)_2$), 2.07 (m, 1H, $CH(CH_3)_2$), 3.57, 3.62 (2t, J_{gem} = 14.8 Hz, J_{vic} = 4.3 Hz, $^3J_{H^aF}$= 4.3 Hz, 1H, SOCH[a]), 3.71 (dd, J_{gem} = 14.8 Hz, J_{vic} = 7.4 Hz, 1H, SOCH[b]), 4.00 (m, 1H, NCH), 5.05, 5,08 (d, J = 8.8 Hz, 1H, NH), 5.12 (s, 2H, CH_2 (Cbz)), 7.35 (m, 5 H,

C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 18.0, 19.2 ($CH(CH_3)_2$), 31.1 ($CH(CH_3)_2$), 52.5, 52.6 (CH_2SO_2Cl), 52.7 (NCH), 67.1 (CH_2 (Cbz)), 128.0, 128.2, 128.5, 136.0 (Ar-C (Cbz)), 155.7 (C=O (Cbz)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −116.3 (s). Anal. Calcd for $C_{13}H_{18}FNO_4S$: C, 51.47; H, 5.98; N, 4.62; F, 6.26. Found: C, 52.01; H, 6.10; N, 5.26; F, 5.64; ESI-MS: m/z = 325.95 $[M+Na]^+$, 342.30 $[M+K]^+$.

4.4.4. Cbz-Leu-ψ[CH_2SO_2]-F (7d)

Eluent for column chromatography: hexanes/CH_2Cl_2, 3:7. Yield procedure 1: 45% (530 mg, white solid), yield procedure 2: 76% (334 mg, white solid). R_f = 0.40 (hexanes/CH_2Cl_2, 1:9); mp = 62 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 0.93 (3 lines, 6H, $CH_2CH(CH_3)_2$), 1.49, 1.70 (2 m, 3H, $CH_2CH(CH_3)_2$), 3.58, 3.64 (2t, J_{gem} = 14.8 Hz, J_{vic} = 5.1 Hz, $^3J_H{}^a{}_F$ = 5.1 Hz, 1H, SOCH[a]), 3.70, 3.74 (2t, J_{gem} = 14.8 Hz, J_{vic} = 5.6 Hz, $^3J_H{}^b{}_F$ = 1.4 Hz, 1H, SOCH[b]), 4.20 (m, 1H, NCH), 5.11 (s, 2H, (CH_2 (Cbz)), 5.15 (s, 1H, NH) 7.35 (m, 5H, C_6H_5 (Cbz)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.5, 22.8 ($CH_2CH(CH_3)_2$), 24.7 ($CH_2CH(CH_3)_2$), 41.9 ($CH_2CH(CH_3)_2$), 45.8 (NCH), 54.6, 54.7 (SCH_2), 67.1 (CH_2 (Cbz)), 128.0, 128.3, 128.6, 135.9 (Ar-C (Cbz)), 155.5 (C=O (Cbz)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −115.0 (s). Anal. Calcd for $C_{14}H_{20}FNO_4S$: C, 52.98; H, 6.35; F, 5.99; N, 4.41. Found: C, 53.06; H, 6.41; F, 6.03; N, 4.29; ESI-MS: m/z = 339.95 $[M+Na]^+$, 355.95 $[M+K]^+$.

4.4.5. Cbz-Phe-ψ[CH_2SO_2]-F (7e)

Eluent for column chromatography: hexanes/CH_2Cl_2, 2:8. Yield procedure 1: 67% (640 mg, white solid), yield procedure 2: 62% (520 mg, white solid). R_f = 0.39 (CH_2Cl_2); mp = 124 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 3.05 (m, 2H, $CHCH_2C_6H_5$), 3.55, 3.60 (2t, J_{gem} = 14.6 Hz, J_{vic} = 4.5 Hz, $^3J_H{}^a{}_F$ = 4.5 Hz, 1H, SOCH[a]), 3.75 (dd, J_{gem} = 14.6 Hz, J_{vic} = 5.9 Hz, 1H, SOCH[b]), 4.32 (m, 1H, NCH), 5.09 (s, 2H, CH_2 (Cbz)), 5.21 (d, J = 7.4 Hz, 1H, NH), 7.15–7.36 (m, 10H, 2xC_6H_5); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 38.9 ($CHCH_2CH_6H_5$), 48.9 (NCH), 52.8, 53.0 (CH_2SO_2F), 127.4, 128.0, 128.3, 128.5, 129.0, 129.2, 135.7, 135.9 (2 × C_6H_5), 155.4 (C=O (Cbz)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −115.7 (s). Anal. Calcd for $C_{17}H_{18}FNO_4S$: C, 58.11; H, 5.16; N, 3.99; F, 5.41. Found: C, 58.20; H, 5.22; N, 3.86; F, 5.26; ESI-MS: m/z = 389.90 $[M+K]^+$.

4.4.6. Fmoc-Val-ψ[CH_2SO_2]-F (7f)

Sulfonyl fluoride **7f** was purified by column chromatography (acetone/CH_2Cl_2, 1:9) followed by crystallization (CH_2Cl_2/hexanes). Yield procedure 3: 30% (211 mg, white solid). R_f = 0.46 (CH_2Cl_2); mp = 183 °C; ^1H NMR (300 MHz, $CDCl_3$/acetone-d_6): δ = 0.98 (d, J = 7.3 Hz, 6H, $CH(CH_3)_2$), 2.05 (m, 1H, $CH(CH_3)_2$), 3.68, 3.81 (2 m, 2H, SO_2CH_2), 4.10 (m, 1H, NCH), 4.22 (m, 1H, CH (Fmoc)), 4.41 (m, 2H, CH_2 (Fmoc)), 5.00 (d, J = 8.3 Hz, 1H, NH), 7.29–7.77 (m, 8H, Ar-CH (Fmoc)); ^{13}C NMR (75 MHz, $CDCl_3$/acetone-d_6): δ = 17.4, 18.6 ($CH(CH_3)_2$), 31.5 ($CH(CH_3)_2$), 46.9 (CH (Fmoc)), 52.1 (NCH), 52.2, 52.3 (SO_2CH_2), 66.2 (CH_2 (Fmoc)), 119.5, 124.6, 124.7, 126.5, 126.5, 127.3, 140.9, 143.4, 143.6 (Ar-C (Fmoc)), 155.6 (C=O (Fmoc)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −116.2 (s); In the ^1H NMR spectrum, also broad, low-intensity signals were observed, presumably due to the presence of the minor Fmoc-rotamer. Anal. Calcd for $C_{20}H_{22}FNO_4S$: C, 61.36; H, 5.66; N, 3.58; F, 4.85. Found: C, 61.27; H, 5.57; N, 3.49; F, 4.78; ESI-MS: m/z = 413.9 $[M+Na]^+$.

4.5. General procedure for the synthesis of Cbz-deprotected sulfonyl fluorides 8a–c

To a solution of the sulfonyl fluoride (1.0 mmol) in dichloromethane (10 mL) was added a solution of HBr in acetic acid (33%, 6.0 mL). After stirring at rt for 30 min, the solvents were removed in vacuo. The residue was dissolved in H_2O (10 mL), and Dowex 2 × 8 (600 mg, Cl-form) was added. Stirring for 5 min at rt, followed by filtration and concentration in vacuo, afforded the HCl-salt. The HCl-salts (**8a–c**) were obtained as white solids in very high yields (90–100%).

4.6. General procedure for coupling of an amino acid to Cbz-deprotected sulfonyl fluorides 8a and 8b

To a solution of HCl-salt (1.0 mmol, **8a**, **8b**) in dichloromethane (40 mL) was added a Boc-protected amino acid (1.0 mmol), BOP (464 mg, 1.05 mmol), and D*i*PEA (385 μL, 2.33 mmol). The mixture was stirred overnight at rt, and if necessary additional D*i*PEA was added to keep the mixture basic. After concentration, EtOAc (35 mL) was added the organic layer was washed with 1.0 M $KHSO_4$ (3 × 20 mL), 5% $NaHCO_3$ (3 × 20 mL, and brine. Drying (Na_2SO_4), followed by column chromatography afforded sulfonyl fluorides **9a**, **9b** and **11** as white solids.

4.6.1. Boc-Phe-Tau-F (9a)

Eluent for column chromatography: hexanes/ethyl acetate, 5:1. Yield: 80% (213 mg, white solid). R_f = 0.27 (acetone/CH_2Cl_2; 5:95); mp = 128 °C; ^1H NMR (300 MHz, $CDCl_3$/CD_3CN): δ = 1.41 (s, 9H, $C(CH_3)_3$), 2.88 (m, 1H, PhCH[a]), 3.00 (dd, J_{gem} = 13.8 Hz, J_{vic} = 6.3 Hz, 1H, PhCH[b]), 3.42–3.67 (m, 4H, $SO_2CH_2CH_2$), 4.28 (m, 1H, NCH), 5.21 (d, 1H, NHBoc), 7.00 (s, 1H, $NHCH_2CH_2SO_2$), 7.16 (m, 5H, Ar-CH); ^{13}C NMR (75.5 MHz, $CDCl_3$/CD_3CN): δ = 27.8, ($C(CH_3)_3$), 33.5 ($NHCH_2$), 38.0 (CH_2Ph), 49.5, 49.7 (CH_2SO_2), 55.3 (NCH) 79.8 ($C(CH_3)_3$) 126.6, 128.3, 128.9, 136.3 (Ar-C), 155.1 (C=O (Boc)), 172.1 (CHC(O)NH); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −121.6 (s). Anal. Calcd for $C_{16}H_{23}FN_2O_5S$: C, 51.32; H, 6.19; N, 7.48; F, 5.07. Found: C, 51.40; H, 6.21; N, 7.37; F, 5.01; ESI-MS: m/z = 275.0 $[M-Boc+Na]^+$.

4.6.2. Boc-Phe-Leu-ψ[CH_2SO_2]-F (9b)

The reaction was performed on 0.43 mmol scale to yield a white solid (138 mg, 74%). Eluent column chromatography: acetone/CH_2Cl_2, 1:99. R_f = 0.59 (acetone/CH_2Cl_2, 4:96); mp = 172 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 0.88–0.94 (m, 6H, $CH(CH_3)_2$), 1.20–1.69 (m, 3H, $CH_2CH(CH_3)_2$), 1.42 (s, 9H, $(CH_3)_3$), 3.06 (d, J = 7.2 Hz, 2H, $CH_2C_6H_5$), 3.34–3.39 (m, 1H, CH^aSO_2F), 3.58–3.65 (ddd, J_{gem} = 14.9 Hz, J_{vic} = 5.3 Hz, $^3J_H{}^b{}_F$ = 1.0 Hz, 1H, CH^bSO_2F), 4.25–4.42 (m, 2H, NCH (Phe), NCH (Leu)), 4.89 (br s, 1H, NHBoc), 6.30 (d, J = 8.5 Hz, 1H, NH (Leu)), 7.20–7.34 (m, 5H, Ar-CH); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.4, 22.9 ($CH(CH_3)_2$), 24.6 ($CH(CH_3)_2$), 28.2 ($(CH_3)_3$), 37.7 ($CH_2CH(CH_3)_2$), 41.4 ($CH_2C_6H_5$), 43.8 (NCH (Leu)), 54.4, 54.6 (CH_2SO_2F), 56.0 (NCH (Phe)), 80.6 ($C(CH_3)_3$), 127.1, 128.7, 129.2, 136.4 (Ar-C), 155.5 (C=O (Boc)), 171.4 (C=O (Phe)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −114.8 (s). Anal. Calcd for $C_{20}H_{31}N_2O_5SF$: C, 55.79; H, 7.26; N, 6.51. Found: C, 55.28; H, 7.23; N, 6.37; ESI-MS: m/z = 331.13 $[M-Boc+H]^+$.

4.6.3. Cbz-Phe-ψ[CH_2SO_2]-Tau-F (10)

Cbz-Tau-F (**7a**, 653 mg, 2.5 mmol) was Cbz-deprotected using the general procedure. To the HBr-salt, obtained after evaporation of HBr, was added Cbz-Pheψ[CH_2SO_2]-Cl (**6e**, 920 mg, 2.5 mmol), dichloromethane (25 mL) and *N*-methylmorpholine (825 μL, 7.5 mmol). The mixture was stirred for 40 min at rt, during which the mixture turned into a milky suspension. After evaporation of the dichloromethane, ethyl acetate (50 mL) and methanol (5 mL) were added. Washing with $KHSO_4$ (30 mL), water and brine was followed by drying (Na_2SO_4) and concentration in vacuo. To the crude product was added dichloromethane (40 mL), and the flask was rotated for 1 h at rt. Filtration followed by drying of the residue afforded sulfonyl fluoride **10** as a white solid (73%, 837 mg). R_f = 0.29 (acetone/CH_2Cl_2; 5:95); mp = 172 °C; ^1H NMR (300 MHz, CD_3CN): δ = 2.42 (dd, J_{gem} = 13.8 Hz, J_{vic} = 9.2 Hz, 1H, CH^aPh), 2.55

(dd, J_{gem} = 13.8 Hz, J_{vic} = 5.5 Hz, 2H, CH^bPh), 2.83 (dd, J_{gem} = 14.6 Hz, J_{vic} = 4.4 Hz, 1H, $NCHCH^aSO_2$), 2.90 (dd, J_{gem} = 14.6 Hz, J_{vic} = 8.3 Hz, 1H, $NCHCH^bSO_2$), 3.14 (m, 2H, NCH_2), 3.37 (m, 2H, SCH_2CH_2), 3.83 (m, 1H, NCH), 4.56 (s, 2H, CH_2 (Cbz)), 5.61 (m, 2H, 2 × NH), 6.83 (m, 10H, 2 × C_5H_5); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 38.4 (NCH_2), 41.1 ($NCHCH_2pH$), 50.4 (NCH), 52.3, 52.5 (CH_2SO_2F), 67.0 (CH_2 (Cbz)), 127.8, 128.7, 129.0, 129.6, 130.5, 138.4, 138.9 (2 × C_6H_5) 157.4 (C=O (Cbz)); ^{19}F NMR (282 MHz, CD_3CN): δ = −121.4 (s). Anal. Calcd for $C_{13}H_{18}FNO_4S$: C, 51.47; H, 5.98; F, 6.26; N, 4.62. Found: C, 52.01; H, 6.10; F, 5.64; N, 5.26; ESI-MS: m/z = 325.95 $[M+Na]^+$, 342.30 $[M+K]^+$.

4.6.4. Boc-Leu^1-Leu^2-ψ[CH_2SO_2]-F (11)

The reaction was performed on 0.71 mmol scale to yield a white solid (206 mg, 73%). Eluent column chromatography: gradient from acetone/CH_2Cl_2, 1:99, followed by acetone/CH_2Cl_2, 5:95. R_f = 0.49 (acetone/CH_2Cl_2, 4:96); mp = 161 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.86–0.95 (m, 12H, $(CH_3)_2$ (Leu^{1,2})), 1.37–1.82 (m, 6H, $CH(CH_3)_2$ (Leu^{1,2}), $NCHCH_2$ (Leu^{1,2})), 1.44 (s, 9H, $(CH_3)_3$), 3.56–3.64 (2t, J_{gem} = 14.9 Hz, J_{vic} = 5.2 Hz, $^3J_{H^aF}$ = 5.2 Hz, 1H, CH^aSO_2F), 3.71–3.78 (ddd, J_{gem} = 14.9 Hz, J_{vic} = 5.8 Hz, $^3J_{H^bF}$ = 0.9 Hz, 1H, CH^bSO_2F), 3.99–4.07 (m, 1H, NCH (Leu^1)), 4.41 (m, 1H, NCH (Leu^2)), 4.74 (br d, 1H, NHBoc), 6.57 (d, J = 8.3 Hz, 1H, NH (Leu^2)); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.5, 22.9 ($(CH_3)_2$ (Leu^{1,2})), 24.7 ($CH(CH_3)_2$ (Leu^{1,2})), 28.2 ($(CH_3)_3$), 40.2, 41.7 ($NCHCH_2$ (Leu^{1,2})), 44.0, 53.1 (NCH (Leu^{1,2}), 54.4, 54.6 (CH_2SO_2F), 80.4 ($C(CH_3)_3$), 155.8 (C=O (Boc)), 172.6 (C=O (Leu^1)); ^{19}F NMR (282 MHz, $CDCl_3$): δ = −115.0 (s); ESI-MS: m/z = 297.09 $[M-Boc+H]^+$.

4.7. Enzyme assays

4.7.1. General remarks for kinetic experiments

Buffer solutions were prepared using distilled water. Buffer solution for all chymotrypsin assays: sodium phosphate (0.05 M, pH 7.0); for the papain assay: sodium phosphate (100 mM, pH 6.5) containing EDTA (1.5 mM). Papain stock solutions were centrifuged before use. Papain and chymotrypsin were purchased from Sigma and were used without further purification. Bz-L-Arg-pNA and Bz-L-Tyr-pNA were purchased from Bachem (Switzerland). Kinetic enzyme assays were performed using 96-wells plates in a μQuant Biotek plate reader for 1 h at rt at 405 nm. All assays were carried out in triplicate or quadruplicate.

4.7.2. Progress curves

Chymotrypsin was dissolved in buffer (1.1 μM), the substrate (Bz-L-Tyr-pNA) was dissolved in DMSO (5.0 mM), and the inhibitors were dissolved in DMSO (250 μM). To each well was added inhibitor solution (10.0 μL) and enzyme solution (180.0 μL). For the controls DMSO was used instead of the inhibitor solution. After 1 h pre-inhibition a sample (95.0 μL) was taken from each well and added to wells containing substrate solution (5.0 μL), and subsequently the liberation *p*-nitroaniline was monitored during 30 min. Final concentrations in the wells were: enzyme: 1.0 μM; substrate: 0.25 mM; inhibitor: 12.5 mM.

4.7.3. K_i determination using Dixon plots[18]

An enzyme solution (5.0 μM) was made by dissolving chymotrypsin (1.25 mg, 0.05 μmol) in buffer (10 mL). A stock solution of the substrate (5.0 mM) was made by dissolving Bz-L-Tyr-pNA (2.03 mg, 5.0 μmol) in DMSO (1.0 mL). Dilutions of this stock solution were made using DMSO, resulting in substrate concentrations of 5.0, 4.6, 4.2, 3.8, 3.4 and 3.0 mM. For the inhibitor stock solution (12.5 mM), the inhibitor (5.0 μmol) was dissolved in DMSO (400 μL). DMSO was used for the dilutions, resulting in inhibitor concentrations of 625, 312.5 and 156.25 μM. In a typical assay to each well was added inhibitor solution (10 μL), substrate solution (10 μL), enzyme solution (20 μL) and buffer (160 μL). For the controls DMSO was added instead of inhibitor solution. The liberation of *p*-nitroaniline was measured directly at 405 nm for 15 min. Final concentrations in the wells were: enzyme: 0.5 μM; substrate: 0.25, 0.23, 0.21, 0.19, 0.17 or 0.15 mM; inhibitor: 31.25, 15.63 or 7.81 μM. The assays were performed in quadruplicate.

4.7.4. k_{inact} determination

An enzyme solution (5.55 μM) was made dissolving chymotrypsin (1.25 mg, 0.05 μmol) in buffer (9.0 mL). A stock solution of the substrate (2.78 mM) was made by dissolving Bz-L-Tyr-pNA (1.12 mg, 2.76 μmol) in DMSO (994 μL), which was diluted with DMSO to a concentration of 278 μM. For the inhibitor solution (12.5 mM), the inhibitor (5.0 μmol) was dissolved in DMSO (400 μL). In a typical assay, an enzyme–inhibitor mixture was first made taking inhibitor solution (16 μL), DMSO (84 μL) and enzyme solution (900 μL). From this mixture 30 μL was directly (t = 0 min) added to a well containing 270 μL substrate solution (278 μM). At t = 1 min, 30 μL of the enzyme–inhibitor mixture was added to a new well containing 270 μL substrate solution. This procedure was repeated until t = 5 min. At t = 6, the liberation of *p*-nitroaniline in all six wells was measured at 405 nm for 15 min. Final concentrations in the wells were: enzyme: 0.5 μM; substrate: 250 μM; inhibitor: 20 μM.

4.7.5. Papain assay

Papain was dissolved in buffer solution (80 μM) and after shaking the solution for 5 min, it was centrifuged. The substrate (Bz-L-Arg-pNA) was first dissolved in DMSO and then diluted twice (50.0 mM) with buffer. The inhibitors were dissolved in DMSO and diluted twice (125.0 μM) with buffer. To each well was added inhibitor solution (4.0 μL), buffer solution (172.0 μL) and enzyme solution (20.0 μL). For the controls a DMSO/buffer solution (1:1) was used instead of the inhibitor solution. After 1 h pre-inhibition a sample (98.0 μL) was taken from each well and added to wells containing substrate solution (2.0 μL), and subsequently the liberation *p*-nitroaniline was measured during 1 h. Final concentrations in the wells were: enzyme: 8.0 μM; substrate: 1.0 mM; inhibitor: 2.5 μM.

Acknowledgment

We thank Hans Hilbers for measuring some of the mass spectra.

References and notes

1. (a) Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305; (b) Rasnick, D. *Perspect. Drug Discov. Des.* **1996**, *6*, 47; (c)*Proteinase Inhibitors, Research Monographs in Cell and Tissue Physiology*; Barrett, A. J., Salvesen, G., Eds.; Elsevier: New York, 1986; Vol. 12,.
2. Lyss, G.; Knorre, A.; Schmidt, T. J.; Pahl, H. L.; Merfort, I. *J. Biol. Chem.* **1998**, *273*, 33508.
3. Bogyo, M.; McMaster, J. S.; Gaczynska, M.; Tortorella, D.; Goldberg, A. L.; Ploegh, H. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6629.
4. Roush, W. R.; Cheng, J.; Knapp-Reed, B.; Alvarez-Hernandez, A.; McKerrow, J. H.; Hansell, E.; Engel, J. C. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2759.
5. (a) Kettner, C. A.; Shenvi, A. B. *J. Biol. Chem.* **1984**, *259*, 15106; (b) Walker, B.; Lynas, J. F. *Cell. Mol. Life Sci.* **2001**, *58*, 596; (c) Powers, J. C.; Asgian, J. L.; Ekici, Ö. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639.
6. Brouwer, A. J.; Monnee, M. C. F.; Liskamp, R. M. J. *Synthesis* **2000**, 1579.
7. (a) De Bont, D. B. A.; Dijkstra, G. D. H.; den Hartog, J. A. J.; Liskamp, R. M. J. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 3035; (b) De Bont, D. B. A.; Moree, W. J.; Liskamp, R. M. J. *Bioorg. Med. Chem.* **1996**, *4*, 667; (c) De Bont, D. B. A.; Sliedregt-Bol, K. M.; Hofmeyer, L. J. F.; Liskamp, R. M. J. *Bioorg. Med. Chem.* **1999**, *7*, 1043; (d) Liskamp, R. M. J.; Kruijtzer, J. A. W. *Mol. Divers.* **2004**, *8*, 79.
8. (a) Löwik, D. W. P. M.; Mulders, S. J. E.; Cheng, Y.; Shao, Y.; Liskamp, R. M. J. *Tetrahedron Lett.* **1996**, *37*, 8253; (b) Löwik, D. W. P. M.; Weingarten, M. D.; Broekema, M.; Brouwer, A. J.; Still, W. C.; Liskamp, R. M. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1846.
9. Brouwer, A. J.; Van der Linden, H. J.; Liskamp, R. M. J. *J. Org. Chem.* **2000**, *65*, 1750.

10. Brouwer, A. J.; Liskamp, R. M. J. *J. Org. Chem.* **2004**, *69*, 3662.
11. (a) Monnee, M. C. F.; Marijne, M. F.; Brouwer, A. J.; Liskamp, R. M. J. *Tetrahedron Lett.* **2000**, *41*, 7991. ibid. *Tetrahedron Lett.* **2001**, *42*, 965; (b) De Jong, R.; Rijkers, D. T. S.; Liskamp, R. M. J. *Helv. Chim. Acta* **2002**, *85*, 4230.
12. (a) Merkx, R.; Brouwer, A. J.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2005**, *7*, 1125; (b) Brouwer, A. J.; Merkx, R.; Dabrowska, K.; Rijkers, D. T. S.; Liskamp, R. M. J. *Synthesis* **2006**, 455.
13. Brouwer, A. J.; Ceylan, T.; van der Linden, T.; Liskamp, R. M. J. *Tetrahedron Lett.* **2009**, *50*, 3391.
14. Rodríguez, M.; Llenares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *32*, 923.
15. Segall, Y.; Quistad, G. B.; Nomura, D. K.; Casida, J. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3301.
16. Dubbaka, S. R.; Vogel, P. *Tetrahedron* **2005**, *61*, 1523.
17. Segall, Y.; Quistad, G. B.; Casida, J. E. *Synth. Commun.* **2003**, *33*, 2151.
18. Dixon, M. *Biochem J.* **1972**, *129*, 197.
19. Tian, W-X.; Tsou, C.-L. *Biochemistry* **1982**, *21*, 1028.
20. von Arx, E.; Faupel, M.; Bruggen, M. *J. Chromatogr.* **1976**, *120*, 224.