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Inhibition of human leukocyte elastase by functionalized *N*-aryl azetidin-2-ones: substituent effects at C-3 and benzylic positions

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Summary — A series of functionalized *N*-aryl azetidin-2-ones with a latent alkylating group was prepared by a flexible four-step synthesis. They met criteria expected for a suicide-type inactivation of human leukocyte elastase (HLE) and porcine pancreatic elastase (PPE), with no inactivation of trypsin- and chymotrypsin-like proteases. The inhibition potency was dependent on the halogen substituents at C-3 (F, F; Cl, Cl; Br, Br) and the nature and the position relative to nitrogen of the latent benzylic leaving group (F, Cl, Br). Better inactivations of HLE compared with PPE were observed with azetidinones *gem*-disubstituted by Cl and Br rather than by F. Their protio analogs, which are devoid of the latent quinoniminium methide electrophile, behave as simple substrates of elastases.

elastase / inhibitor / suicide substrate / β-lactam / quinoniminium methide

Introduction

Human leukocyte elastase (HLE; EC 3.4.21.37) has been the focus of extensive studies aiming to develop various classes of low-molecular-weight inhibitors as potential drugs. This serine protease, released by azurophilic granules of polymorphonuclear neutrophils, has been implicated in the pathogenesis of a number of disease states [1], such as pulmonary emphysema [2], rheumatoid arthritis [3] and glomerulonephritis [4]. Synthetic inhibitors may be beneficial as a replacement or supplement to the major plasma inhibitor α_1-antiproteinase (α_1-PI) [5, 6]. These efforts have resulted in a wide variety of structural types of inhibitors, both reversible and irreversible [7]. Among irreversible inhibitors of HLE [8], the following mechanism-based inhibitors [9] are of particular interest: dihydrocoumarins [10], isocoumarins [11] and β-lactams [12], such as cephalosporin derivatives [13, 14], *N*-activated monocyclic β-lactams [15, 16] and *N*-aryl azetidinones [17, 18]. *N*-(2-Methylphenyl)-3,3-difluoroazetidin-2-one **1a** and *N*-(3-*tert*-butoxycarbonyl-6-methylphenyl)-3,3-difluoroazetidin-2-one **1b** (fig 1) have been shown to be substrates of HLE and porcine pancreatic elastase (PPE). The corresponding functionalized *N*-(2-chloromethylphenyl)-3,3-difluoroazetidin-2-one **2a**, which possesses a latent electrophilic quinoniminium methide ion [19, 20], was demonstrated to act as a suicide substrate of HLE [18] and was shown to prevent the degradation of lung elastic fibers and intradermal microvascular hemorrhage [17, 21, 22].

In this paper, we explore the effect of changing the nature of the C-3 substituents and the potential leaving group of functionalized 3,3-dihalogenoazetidin-2-ones on HLE and PPE inhibition. For this purpose, a new series of analogues of **1a**, **b** and **2a** have been prepared and studied. Compounds **3–5**, which form one of these series of molecules, have a methyl substituent at the *ortho* or *para* position to the nitrogen atom and are expected to behave as elastase substrates. The functionalized derivatives **2b–d**, **6a**, **b**, **7** and **8** (second series) possess a benzylic halogen

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Abbreviations: DMF, dimethylformamide; DMSO, dimethylsulfoxide; NBS, *N*-bromosuccinimide; TMS, tetramethylsilane; HLE (EC 3.4.21.37), human leukocyte elastase; PPE (EC 3.4.21.36), porcine pancreatic elastase; Suc-Ala_3-*p*-NA, succinylalanylalanylalanine-*p*-nitroanilide; Suc-Ala_2-Pro-Phe-*p*-NA, succinylalanylalanylprolylphenylalanine-*p*-nitroanilide MeO-Suc-Ala_2-Pro-Val-*p*-NA, methoxysuccinylalanylalanylprolylvaline-*p*-nitroanilide; Bz-Arg-*p*-NA, *N*-benzoylarginine-*p*-nitroanilide.

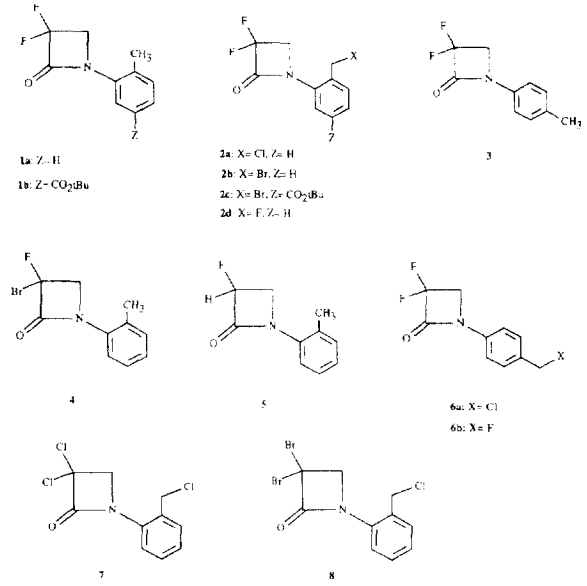


Fig 1. Structures of compounds **1–8**.

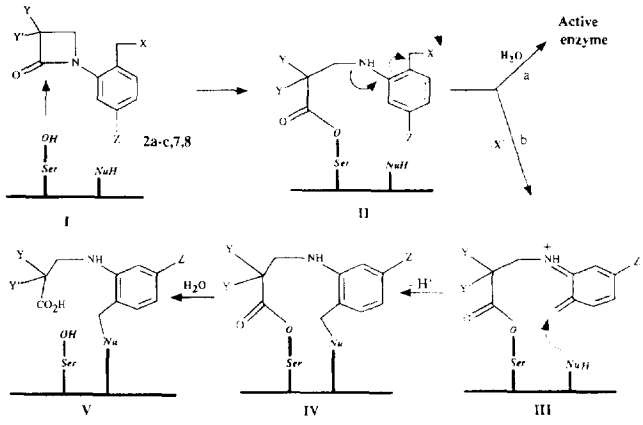


Fig 2. Postulated mechanism for the inactivation of elastase by functionalized *N*-aryl azetidinones. **I**: Michaelis complex; **II**: acyl-enzyme; **III**: quinoniminium methide intermediate; **IV** and **V**: inactivated enzymes.

atom which can be activated simultaneously with the formation of the acyl-enzyme according to the postulated mechanism shown in figure 2. Depending on the nature and the rate of elimination of this leaving group (F^-, Cl^- or Br^-), these compounds could behave as substrates (*Route a*) or mechanism-based inhibitors of elastases (*Route b*). In order to improve the presumed binding at the S_1 subsite, the steric bulk of the substituents (F, H; F, F; Cl, Cl; F, Br; and Br, Br) at the C-3 position of the β-lactam ring was varied. HLE has a larger S_1 primary binding site [23] than PPE and can better accommodate voluminous substituents [24].

Results

Chemistry

Using the Wasserman procedure [25], the ring-methylated *N*-aryl 3,3-dihaloazetidin-2-ones **1a, b** [26], **3** and **4** were prepared from the corresponding substituted 3-halopropionanilides [27] (fig 3). Debromination of 3-bromo-3-fluoroazetidinone **4** with tri-*n*-butyltin hydride [28] led to the 3-monofluoroazetidinone **5**.

The ortho- or *para*-aminobenzyl halides are highly reactive compounds [29] and cannot be directly used in the synthesis of the functionalized azetidinones. Trialkylsilyloxymethylanilines **11** and **11'** were prepared by selective silylation of the corresponding hydroxymethyl anilines by means of R_3SiCl-imidazole [30]. Acylation with 3-halogenopropionyl halides **9b–d** gave the substituted 3-halogenopropionanilides **12** and **12'** (fig 4). The crucial reaction in the flexible four-step synthesis of the functionalized *N*-aryl azetidinones is the cyclization of these anilides without removal of the potential leaving group [19]. Fortunately, no competitive elimination of the trialkylsilanolate group [31] occurred.

Treatment of the obtained silyl ether **13** with tetrabutylammonium fluoride led to β-lactam ring opening, presumably by an intramolecular attack of the *ortho*-alcoholate formed on the neighboring carbonyl. However, HF in aqueous acetonitrile [32] in which some β-lactams are stable [33], selectively cleaved the silyl protecting group. We observed that this reaction was catalyzed by glass powder. The resulting alcohols **14** and **14'** allowed us to prepare a series of halogen-

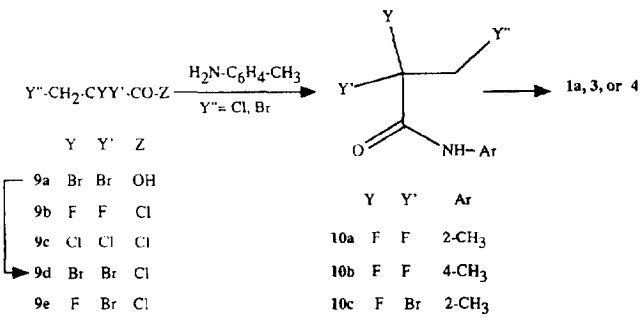


Fig 3. Synthetic scheme for the preparation of the *N*-aryl 2,2,3-trihalogenopropionanilides **10a–c** and unfunctionalized azetidinones **1a, 3** and **4**.

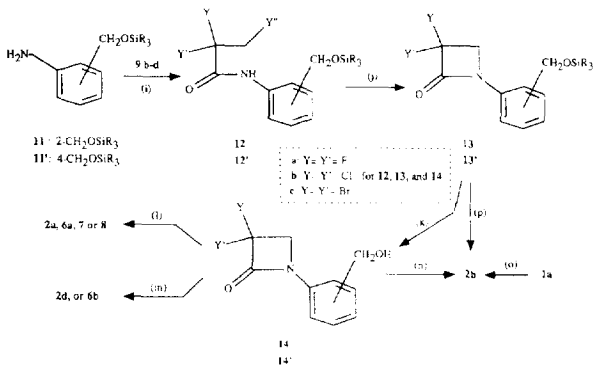


Fig 4. Synthetic scheme for the preparation of the functionalized azetidinones **2** and **6–8**: *(i)* 1 eq Et_3N/toluene/4°C; *(j)* NaH/DMF/CH_2Cl_2/–10°C; *(k)* HF/CH_3CN/H_2O/25°C; *(l)* $SOCl_2$/DMF; *(m)* Et_2NSF_3/CH_2Cl_2/–78°C; *(n)* 4 eq Me_3SiBr/25°C; *(o)* NBS/CCl_4; *(p)* $(C_6H_5)_3PBr_2$/CH_2Cl_2.

ated derivatives using different reagents: the chlorides **2a**, **6a**, **7** and **8** (treatment with $SOCl_2$/DMF [34]), the bromide **2b** (Me_3SiBr [35]), and the fluorides **2d** and **6b** (DAST [36]). In the reaction to **2d** and **6b** the temperature must be maintained at –78°C for 40 min so as to minimize the formation of an ether by-product (see *Experimental protocols*), probably resulting from the nucleophilic attack of the starting alcohol **14** or **14'** on an intermediate of type $ArCH_2OSF_2NEt_2$ which has a carbonium character [37]. Alternatively, the bromides **2b** and **2c** were prepared by selective benzylic bromination (*N*-bromosuccinimide) of the corresponding ring-methylated azetidinones **1a** and **1b**. In the present case, the presence of the *gem*-dihalogeno group probably prevents the radical substitution at C-4 of the β-lactam ring [38]. However, the yield of formation of the bromide **2b** was much higher when the silyl ether **12** was directly reacted with triphenylphosphine dibromide [39].

Enzymology

*Inhibition of elastases by **2b–d**, **6a**, **b**, **7** and **8***

When incubated in the presence of various excesses of the reagents, a time-dependent loss of the hydrolytic activity of HLE and PPE was observed. No significant reactivation (< 2%) was detected after elimination of the inhibitor by filtration-centrifugation (Centricon 10) and incubation at 4°C for 10 h. Addition of buffered hydroxylamine (0.75 M, pH 8.5) at room temperature did not reactivate the inhibited enzymes. The kinetic parameters of the inactivation were determined by progress curve analysis in the presence of the chromogenic substrate MeO-Suc-Ala_2-Pro-Val-*p*-NA for HLE and Suc-Ala_3-*p*-NA for PPE (fig 5A). The results are summarized in table I. The partition ratio ($r = k_{cat}/k_{inact}$), which represents the average number of enzyme turnovers per inactivation [9], was determined

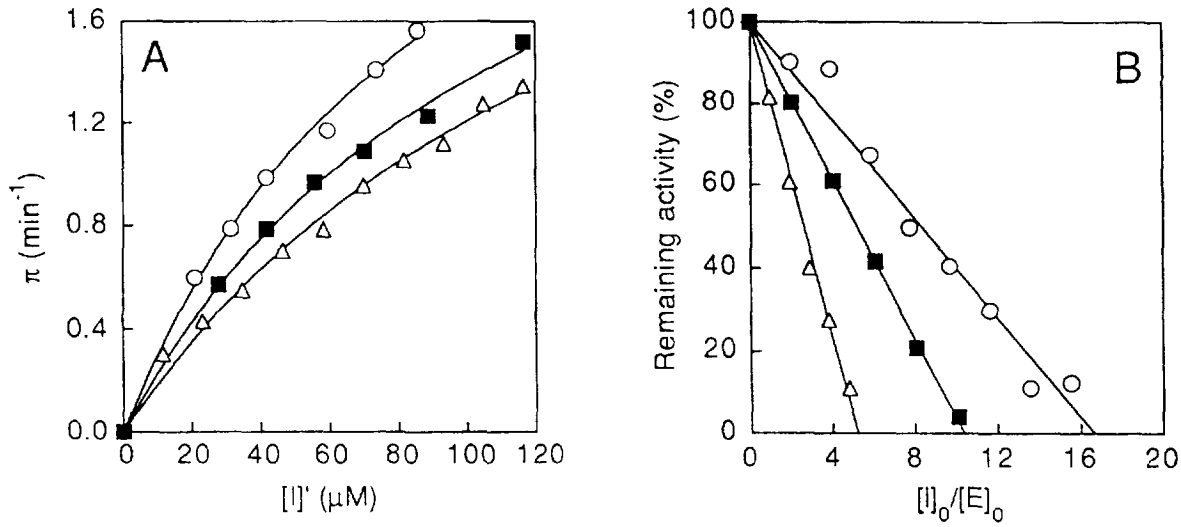
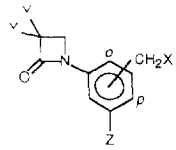


Fig 5. Inactivation of HLE by **2b** ( ), **7** (■) and **8** (Δ) pH 8.0 and 37°C. (**A**) Progress curve analysis: plots of π *versus* [I]' (see *Experimental protocols*). The kinetic parameters k_{inact} and K_I were calculated by iterative fits to equation [2]. (**B**) Determination of the partition ratio: plots of the remaining activity (%) at infinite time *versus* $[I]_0/[E]_0$.

Table I. Kinetic parameters for the inactivation of HLE and PPE by *N*-aryl azetidinones at pH 8.0 and 37°C.



Compound	*Y*	*Y'*	*CH_2X*	*Z*	*HLE*				*PPE*			
					$k_{inact} \times 10^3$ (s^{-1})	K_I (μM)	k_{inact}/K_I ($M^{-1}.s^{-1}$)	r[a]	$k_{inact} \times 10^3$ (s^{-1})	K_I (μM)	k_{inact}/K_I ($M^{-1}.s^{-1}$)	r[a]
2a[b]	F	F	*o*-CH_2Cl	H	35	120	292	18	80	270	296	11
2b	F	F	*o*-CH_2Br	H	57	107	533	16	69	94	740	10
2c	F	F	*o*-CH_2Br	CO_2tBu			11				58	
2d	F	F	*o*-CH_2F	H			29				< 1	
6a	F	F	*p*-CH_2Cl	H			26				117	
6b	F	F	*p*-CH_2F	H			31				53	
7	Cl	Cl	*o*-CH_2Cl	H	52	127	409	9			62	19
8	Br	Br	*o*-CH_2Cl	H	53	162	327	4			50	9

[a] $r = k_{cat}/k_{inact}$. [b] Reference [18]. Standard deviations were less than 15% (k_{inact} and K_I) and 7% for (k_{inact}/K_I).

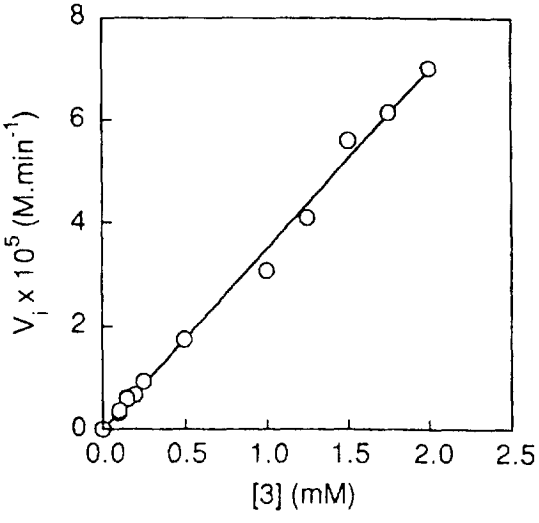


Fig 6. Hydrolysis catalyzed by PPE (312 nM) of **3** at pH 8.0 (4% v/v DMSO) and 37°C. v_i: initial rate.

from the intercept to the *x* axis minus 1 of the linear plot of the percentage of the remaining activity at infinite time *versus* the molar excess of the inhibitor over enzyme (fig 5B). It was verified that at a fixed inhibitor concentration, increasing amounts of the appropriate substrate protected the enzyme against inactivation.

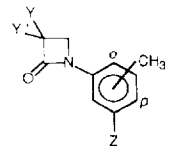
*Enzyme-catalyzed hydrolysis of **3–5***

A spectrophotometric evolution of **3** and **4** with time was observed in the presence of HLE and PPE allowing the determination of the kinetic parameters k_{cat} and K_M (fig 6; table II). Compound **5** was not hydrolyzed even at high enzyme concentrations (~1 µM). In all cases, no significant spontaneous hydrolysis in the buffer was detected within 1 h.

Discussion

We have previously designed functionalized carboxy-substituted *N*-aryl azetidinones possessing a latent quinoniminium methide function [20] as suicide substrates of serine β-lactamases. However, these β-

Table II. Kinetic parameters for the enzyme-catalyzed hydrolysis of *N*-aryl azetidinones (X = H) at pH 8.0 and 37°C.



Compound	*Y*	*Y'*	*Substituent position*	*Z*	*HLE*			*PPE*		
					$k_{cat} \times 10^3$ (s^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)	$k_{cat} \times 10^3$ (s^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
1a[a]	F	F	*o*	H			1100			500
1b[a]	F	F	*o*	CO_2tBu			1125			340
3	F	F	*p*	H	17 543	4747	3696			1874
4	F	Br	*o*	H	1510	882	1712	809	1127	718
5	F	H	*o*	H			NH			NH

[a]Reference [18]. NH: no hydrolysis. Maximum standard deviations for k_{cat}/K_M: ± 7%, for k_{cat} and K_M: ± 15%.

lactams behaved only as competitive inhibitors of the bacterial enzymes [19]. The presence of halogen substituents α to the carbonyl increased the $\nu_{C=O}$ IR frequencies of the 3-halogenated azetidinones obtained, but did not result in enzymic β-lactam ring opening [26].

On the other hand, we observed that *N*-(2-methylphenyl)-3,3-difluoroazetidin-2-ones **1a** and **1b** were substrates of HLE and PPE, and the *N*-(2-chloromethylphenyl)-3,3-difluoroazetidin-2-one **2a** was an efficient and selective inactivator of elastases [18].

We have now synthesized new analogues of this molecule using a flexible four-step synthesis. The crucial step, the cyclization of substituted 2,2,3-trihalogenopropionanilides, occurs without elimination of the potential trialkylsilanolate leaving group. A series of halogenated derivatives have been prepared from the deprotected benzylic alcohols obtained.

The functionalized *N*-aryl azetidinones **2** and **6–8** possess three essential structural features: 1) a *gem*-dihalogeno group which, through its inductive effect, lowers the pK_a of the conjugated acid of the substituted aniline leaving group [40] and thus favors the β-lactam ring opening; 2) an activated carbonyl group, which also results of the *gem*-dihalogeno substitution; and 3) a latent quinoniminium methide function, which can be unmasked simultaneously with the formation of the acyl-enzyme. Owing to the cyclic nature of the inhibitor, this electrophile would be tethered in the active site during the life-time of the acyl-enzyme.

The activation of the benzylic function results from the increased electron-releasing potency of the demasked amino substituent compared with that of the starting amido one ($\sigma^+_{p\text{-}NH_2} = -1.74$; $\sigma^+_{p\text{-}NHAc} = -0.58$ [41]). Substitution of *ortho*- and *para*-aminobenzyl systems with good leaving groups generally occurs by a dissociative mechanism (elimination addition; DN + AN [20]).

The inhibition of HLE and PPE by compounds **2b, c, 6a, 7** and **8** meets, as **2a** did [18], criteria expected for a mechanism-based inactivation (fig 2). This is supported by the kinetic data which are in agreement with a first-order process and saturation kinetics with inhibitor concentration. The inhibition is irreversible. The absence of reactivation in the presence of hydroxylamine excludes an inactivation due to the formation of a stable acyl-enzyme. The enzyme is protected against inactivation in the presence of increasing amounts of substrate at fixed inhibitor concentration. The inhibitors inactivate HLE without inhibiting chymotrypsin-like enzymes, such as bovine chymotrypsin, human cathepsin G and bovine trypsin. A better inactivation is observed with β-lactams displaying the functionalized methylene group in the *ortho* position (**2a**) than in the *para* one (**6a**). Consequently, compounds belonging to the *ortho* series were preferentially synthesized. For a given set of substituents at the C-3 position, the efficiency of the inhibition depends on the nature of the benzylic leaving group X. No significant inactivation is found with a fluorine atom at this position (**2d, 6b**). The brominated azetidinone **2b** is a better inactivator than

its chlorinated counterpart **2a** (increase of the k_{inact}/K_I inhibitor potency by a factor of 2 for HLE). The presence of a hydrophobic electron-attracting *tert*-butyl ester substituent (**2c**) leads to a 60-fold decrease of the inhibitory efficiency. This efficiency is also influenced by the nature of the substituents at C-3. Compared with the *gem*-difluoro compound **2a**, the *gem*-dichloro and *gem*-dibromo analogs **7** and **8** are more potent, but the increase in potency is moderate (factors of 1.3 and 1. 6 for HLE). The order in reactivity (Cl > Br > F) does not reflect the electron-withdrawing effects of these substituents. Obviously, the van-der-Waals radius of the substituents at C-3 plays a role in the inhibition of PPE, but not in that of HLE. Rather good values were obtained for partition ratios, especially for compound **8** (r = 4 for HLE and 9 for PPE). It should be noted that the structural factors do not affect the rate constant k_{inact} (35 to 57 x 10^{-3} s^{-1} for HLE). This suggests that the formation of the Michaelis complex is what differentiates the effectiveness of these inhibitors towards HLE.

For a suicide-type mechanism, the cleavage of the lactam amide bond is a prerequisite to demasking the latent alkylating group. The HPLC analysis of reaction mixtures of unfunctionalized *N*-(2-methylphenyl)-3,3-difluoroazetidin-2-ones **1a** and **1b** with PPE demonstrated that these compounds behave as substrates of this enzyme [18]. The influence of various substituents on β-lactam ring opening has been analyzed (table II). The *para*-methylated azetidinone **3** is more efficiently hydrolyzed than its *ortho*-analog **1a**. The inverse situation is observed for the inactivation potency of the corresponding functionalized molecules (**6a** compared with **2a**). No hydrolysis is detected when only one fluorine substituent is present at C-3. This demonstrates that the hydrolysis is facilitated by the strong electron-withdrawing effect of the *gem*-dihalogeno group at that position: either two fluorine atoms (**2a**) or a fluorine and a bromine atom (**4**). The hydrolysis of the β-lactam substrates is more efficiently catalyzed by HLE than by PPE. The *tert*-butyl ester **1b** is hydrolyzed by HLE as efficiently as the molecule devoid of that substituent (**1a**). Nevertheless, the corresponding inactivator is 57-fold less efficient (**2c** *versus* **2b**). Therefore, the efficiency of the enzyme-catalyzed hydrolysis of the unfunctionalized β-lactams do not quantitatively parallel the efficiency of the corresponding inactivators. However, the behavior of the unfunctionalized azetidinones as substrates constitutes a strong argument in favor of the postulated suicide mechanism of inactivation in the case of the functionalized *N*-aryl azetidinones. Interestingly, no inhibition of chymotrypsin- and trypsin-like proteinases was observed.

Experimental protocols

Chemistry

Melting points were obtained with a Metler FP 61 apparatus and infrared spectra were determined on a Perkin Elmer 1420 spectrophotometer. Proton NMR spectra were obtained on a Brucker AC 200-E apparatus at 200 MHz and are reported in ppm downfield from TMS. Carbon and fluorine NMR spectra were recorded at 50.3 and 188.3 MHz, respectively, and are reported downfield from TMS (^{13}C) and $CFCl_3$ (^{19}F). Analyses indicated by the symbols of the elements were within ±0.4% of theoretical values. Mass spectra were determined on a Kratos MS 50 instrument.

3-Bromo-2,2-difluoropropanoyl chloride **9b** and 2,3-dibromo-2-fluoropropanoyl bromide **9e** were prepared according to references [26] and [42] respectively.

2,2,3-Tribromopropanoic acid ***9a***

To 2-bromo-2-propenoic acid (3 g, 20 mmol) in CH_2Cl_2 was added bromine (4.79 g, 30 mmol). After stirring for 2 h at ambient temperature, the reaction mixture was washed with aqueous sodium sulfite, dried ($MgSO_4$) and evaporated to give a slightly yellow solid (5.7 g, 92%), mp = 91.3°C [43]. IR (CH_2Cl_2): 3340, 1720 cm^{-1}. ^1H NMR ($CDCl_3$): 4.25 (s, 2H, CBr_2CH_2Br), 10.6 (bs, 1H, CO_2H). Anal $C_3H_3Br_3O_2$ (C, H).

2,2,3-Trichloropropanoyl chloride ***9c***

A mixture of 2,2,3-trichloropropanoic acid [44], (5.2 g, 29 mmol) and thionyl chloride (6 ml) was refluxed for 1 h and cooled. After evaporation of the excess of $SOCl_2$, the title chloride was distilled as a colorless liquid (4.05 g, 66%), bp = 50°C/20 mmHg. IR (CH_2Cl_2): 1800, 1770 cm^{-1}. ^1H NMR ($CDCl_3$): 4.38 (s, 2H, CCl_2CH_2Cl).

2,2,3-Tribromopropanoyl chloride ***9d***

Starting from 2,2,3-tribromopropanoic acid, the title chloride was obtained according to the preceding procedure as a colorless liquid (4.35 g, 72%), bp = 66–70°C/20 mmHg. IR (CH_2Cl_2): 1800, 1770 cm^{-1}. ^1H NMR ($CDCl_3$): 4.35 (s, 2H, CBr_2CH_2Br). Anal $C_3H_2Br_3ClO$ (C, H, O).

4-tert-Butyldimethylsilyloxymethyl aniline ***11'***

To 4-aminobenzyl alcohol (123 mg, 1 mmol) in DMF (2 ml) was added *tert*-butyldimethylchlorosilane (180 mg, 1.2 mmol) and imidazole (170 mg, 2.5 mmol) at room temperature. The reaction mixture was stirred for 40 min and concentrated under vacuum (0.05 mmHg). Then the residue was taken up with water and the solution was extracted with ether. The organic phase was washed with water and brine, dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography (ether/pentane, 3:2) (173 mg, 73%). IR (CH_2Cl_2): 3430–3380, 2800, 1620, 1510, 1100 cm^{-1}. ^1H NMR ($CD_3)_2CO$: 0.05 ppm (s, 6H, $Si(CH_3)_2$), 0.85 (s, 9H, SiC_4H_9) 4.4 (bs, 2H, NH_2), 4.55 (s, 2H, CH_2O), 6.65 (d, $2H_{arom}$, J = 8.8 Hz), 6.95 (d, $2H_{arom}$, J = 8.7 Hz). Anal $C_{13}H_{23}NOSi$ (C: calcd 65.76 found 65.07, H, N).

N-Aryl 2,23-trihalopropanoic acid amides ***10a–c***, ***12a–c*** *and* ***12'a***. *General synthetic procedure*

An equimolar mixture of NEt_3 and aniline in toluene (2 ml/mmol) was added dropwise to 2,2,3-trihalopropanoyl halide **9b–e** (1.1 eq) in toluene (2 ml/mmol) at 4°C. The reaction mixture was stirred for 30 min at ambient temperature, then taken up with ether, washed with saturated $NaHCO_3$,and with brine. The organic phase was dried ($MgSO_4$) and evaporated

and the residue was purified by flash chromatography on silica gel.

N-(2-Methylphenyl)-3-bromo-2,2-difluoropropanoic acid amide ***10a***. Ether/pentane, 1:5 (174 mg, 63%); mp = 57.3°C. IR (CH_2Cl_2): 3395, 1705 cm^{-1}. ^1H NMR ($CDCl_3$): 2.3 ppm (s, 3H, CH_3Ar), 3.86 (t, 2H, J = 13.2 Hz, CF_2CH_2), 7.2 (m, $3H_{arom}$), 7.8 (d, $1H_{arom}$, J = 8.03 Hz), 7.95 (bs, 1H, N*H*). ^{19}F NMR (δ/$CFCl_3$): −105.3 ppm (t, 2F, J = 14.1 Hz, CF_2CH_2). ^{13}C NMR ($CDCl_3$): 17.4 (CH_3); 28 (t, J = 30 Hz, CF_2CH_2); 114 (t, J = 256 Hz, CF_2); 123.15; 126.15; 126.9; 129.62; 130.73; 133.3 (C_{arom}); 159.8 (t, J = 29 Hz, CF_2CO). MS m/z = 279, 277 ($M^{+\bullet}$ bromine isotopes), 142, 144, 134, 91, 77. Anal $C_{10}H_{10}BrF_2NO$ (C, H, N).

N-(4-Methylphenyl)-3-bromo-2,2-difluoropropanoic acid amide ***10b***. Ether/pentane, 1:5 (312 mg, 86%); mp = 98.8°C. IR (CH_2Cl_2): 3400, 1700 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 2.36 (s, 3H, $CH_3C_6H_4$), 4.14 (t, 2H, J = 14.34 Hz, CF_2CH_2), 7.23 (d, $2H_{arom}$, J = 8.2 Hz), 7.67 (d, $2H_{arom}$, J = 8.3 Hz), 9.89 (bs, N*H*). ^{19}F NMR: (δ/$CFCl_3$): −105 ppm (t, 2F, J = 14.1 Hz, CF_2CH_2). MS m/z = 279, 277 ($M^{+\bullet}$ bromine isotopes), 142–144, 134, 106, 91, 77, 65. Anal $C_{10}H_{10}BrF_2NO$ (C, H, N).

N-(2-Methylphenyl)-2,3-dibromo-2-fluoropropanoic acid amide ***10c***. Ether/pentane, 1:8 (355 mg, 98%); mp = 96.8°C. IR (CH_2Cl_2): 3400, 1700 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 2.26 (s, 3H, $ArCH_3$), 4.37 (dd, 1H, J_{AB} = 11.4 Hz, J_{AF} = 8.7 Hz), 4.67 (dd, 1H, J_{AB} = 11.4, J_{BF} = 30.8 Hz), 7.25 (m, $4H_{arom}$), 9.47 (bs, 1H, N*H*). ^{19}F NMR (δ/$CFCl_3$): 122 (ddd, J_{FB} = 30 Hz, J_{FA} = 8.9 Hz, $J_{F\text{-}NH}$ = 5.63 Hz). MS m/z = 337, 339, 341 ($M^{+\bullet}$ bromine isotopes), 197, 134, 107, 91, 77, 65. Anal $C_{10}H_{10}Br_2FNO$ (C, H, N).

N-(2-tert-Butyldimethylsilyloxymethylphenyl)-3-bromo-2,2-difluoropropanoic acid amide ***12a***. Ether/pentane, 1:11 (560, mg 68%); mp = 36.8°C. IR (CH_2Cl_2): 3400, 1700 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 0.17 ppm (s, 6H, $Si(CH_3)_2$), 0.96 (s, 9H, SiC_4H_9), 4.16 (t, 2H, J = 14 Hz, CF_2CH_2); 4.94 (s, 2H, CH_2O), 7.4 (m, $4H_{arom}$), 8.07 (bs, 1H, N*H*). ^{19}F NMR (δ/$CFCl_3$): −105.6 ppm (2F, t, J = 14.1 Hz, CF_2CH_2). MS m/z = 395, 393 (M-14), (350, 352), (230, 228), 130, 91, 77. Anal $C_{16}H_{24}BrF_2NO_2Si$ (C, H, N).

N-(2-tert-Butyldimethylsilyloxymethylphenyl)-2,2,3-trichloropropanoic acid amide ***12b***. Ether/pentane, 1:10, white solid (814 mg, 70%); mp = 41°C. IR (CH_2Cl_2): 3300, 1690, 1585 cm^{-1}. ^1H NMR ($CDCl_3$): 0.15 (s, 6H, $Si(CH_3)_2$), 0.95 (s, 9H, SiC_4H_9), 4.2 (s, 2H, CH_2Cl_2), 4.8 (s, 2H, CH_2O), 7.45 (m, $4H_{arom}$), 10.2 (bs, 1H, N*H*). MS m/z = 384, 382 (M-14), 340, 338, 268, 200, 192, 164, 132, 93, 75, 29. Anal $C_{16}H_{24}Cl_3NO_2Si$ (C, H, N).

N-(2-tert-Butyldimethylsilyloxymethylphenyl)-2,2,3-tribromopropanoic acid amide ***12c***. Ether/pentane, 1:8, white solid (120 mg, 76%); mp = 36.1°C. IR (CH_2Cl_2): 3290, 1680, 1520 cm^{-1}. ^1H NMR ($CDCl_3$): 0 (s, 6H), 0.8 (s, 9H), 4.35 (s, 2H, CH_2CBr_2), 4.69 (2H, s, CH_2O), 6.98 (s, $4H_{arom}$), 9.93 (bs, 1H, N*H*). Anal $C_{16}H_{24}Br_3NO_2Si$ (C, H, N).

N-(4-tert-Butyldimethylsilyloxymethylphenyl)-3-bromo-2,2-difluoropropanoic acid amide ***12'a***. Ether/pentane, 1:8 (230 mg, 70%); mp= 57.3°C. IR (CH_2Cl_2): 3400, 1700 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 0.17 (s, 6H, $Si(CH_3)_2$), 1 (s, 9H, SiC_4H_9), 4.16 (t, 2H, J = 14.3 Hz, CF_2CH_2), 4.82 (s, 2H, CH_2O), 7.43 (d, $2H_{arom}$, d, J = 8.5 Hz), 7.8 (d, $2H_{arom}$, J = 8.5 Hz), 8.78 (bs, 1H, N*H*). ^{19}F NMR (δ/$CFCl_3$): −120.8 (2F, t, J = 14 Hz, CF_2CH_2). Mass spectrum: m/z = 407 ($M^{+\bullet}$), 352, 350 (Br isotopes), 230, 228, 106, 90. HRMS calcd for $C_{16}H_{24}BrF_2NO_2Si$ 407.07283; found 407.0729.

N-Aryl 3,3-dihaloazetidin-2-ones ***1a***, ***3***, ***4***, ***13a–c*** *and* ***13'a***. *General synthetic procedure for the cyclization of N-aryl 2,2,3-trihalogenopropanoic acid amides*

A solution of an *N*-aryl 2,2,3-trihalogenopropanoic acid amide (1.5 mmol) in a 1:6 mixture of DMF and CH_2Cl_2 (9 ml) was slowly added (40 min) to sodium hydride (210 mg, 60% dispersion in oil, 5.25 mmol) suspended in the same solvent mixture (9 ml) at −10°C. After stirring for a further 40 min, the reaction mixture was quickly washed with aqueous saturated ammonium chloride until neutral. After drying over $MgSO_4$, the organic phase was concentrated under reduced pressure and the residue was purified by flash chromatography.

N-(2-Methylphenyl)-3,3-difluoroazetidin-2-one ***1a***. Ether/pentane, 1:6, colorless oil (230 mg, 78%) IR (CH_2Cl_2): 1780 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 2.31 (s, 3H, $CH_3C_6H_4$), 4.43 (t, 2H, J = 6.63 Hz, CH_2CF_2), 7.4 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −117 (t, 2F, J = 6.6 Hz). ^{13}C NMR ($CDCl_3$): 19 (CH_3, s), 57.192 (t, J = 26 Hz, CH_2CF_2),119 (t, CF_2, J_{CF} = 280 Hz) 122.932, 126.812, 127.964, 131.791, 132.38, 134 (C_{arom}), 158 (t, J = 30 Hz, CF_2CO). MS m/z = 197 ($M^{+\bullet}$), 133, 118, 103, 104, 91, 77. Anal $C_{10}H_9F_2NO$ (C, H, N).

N-(4-Methylphenyl)-3,3-difluoroazetidin-2-one ***3***. Ether/pentane, 1:4 (52 mg, 43%); mp = 88°C. IR (CH_2Cl_2): 1770 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 2.38 (s, 3H, $ArCH_3$), 4.42 (t, 2H, J = 6.4 Hz, CH_2CF_2), 7.34 ($2H_{arom}$, d, J = 8.4 Hz), 7.36 ($2H_{arom}$, d, J = 8.5 Hz). ^{19}F NMR (δ/$CFCl_3$): −115.5 (2F, t, J = 6.4 Hz). MS m/z = 197 ($M^{+\bullet}$), 133, 118, 91, 77. HRMS calcd for $C_{10}H_9F_2NO$ 197.06522; found, 197.0655.

N-(2-Methylphenyl)-3-bromo-3-fluoroazetidin-2-one ***4***. Ether/pentane, 1.5:5, colorless oil (119 mg, 76%), IR (CH_2Cl_2): 1770 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 2.48 (s, 3H, $CH_3C_6H_4$), 4.45 (dd, H_A, J_{AB} = 7.1, J_{AF} = 7 Hz, CH_AH_BCFBr), 4.7 (dd, H_b, J_{AB} = 7.3 Hz and J_{BF} = 9.2 Hz, CH_AH_BCFBr). ^{19}F NMR (δ/$CFCl_3$): −121.16 (dd, J = 9.3, 7.5 Hz). MS m/z = 259-257 ($M^{+\bullet}$ bromine isotopes), 133, 104, 105, 98, 77, 71. HRMS calcd for $C_{10}H_9BrFNO$, 256.98521; found, 256.9854.

N-(2-tert-Butyldimethylsilyloxymethylphenyl)-3,3-difluoroazetidin-2-one ***13a***. Ether/pentane, 1:10 white solid (303 mg, 53%); mp = 30.2°C. IR (CH_2Cl_2): 1775 cm^{-1}. ^1H NMR $(CD_3)_2CO$: 0.13 (s, 6H, $Si(CH_3)_2$), 0.95 (s, 9H, SiC_4H_9), 4.65 (t, 2H, J = 6.78 Hz, CH_2-CF_2), 4.91 (s, 2H, CH_2O), 7.41 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −117 (t, J = 6.5 Hz). MS m/z = 315 (M-15), 270, 162, 148, 117, 91, 77. Anal $C_{16}H_{23}F_2NO_2Si$ (C, H, N).

N-(2-tert-Butyldimethylsilyloxymethylphenyl)-3,3-dichloroazetidin-2-one ***13b***. Ether/pentane, 1:10, white solid (200 mg, 59%); mp = 39°C. IR (CH_2Cl_2): 1770 cm^{-1} (ν_{CO} β-lactam). ^1H NMR ($CDCl_3$): 0.15 (s, 6H, $Si(CH_3)_2$), 0.95 (s, 9H, SiC_4H_9), 4.5 (s, 2H, CH_2CCl_2), 4.8 (s, 2H, $ArCH_2O$), 7.5 (m, $4H_{arom}$). MS m/z = 360 ($M^{+\bullet}$), 346-344 (M-16), 304, 302 (M-58), 206, 192, 132, 93, 73, 29. Anal $C_{16}H_{23}Cl_2NO_2Si$ (C, H, N).

N-(2-tert-Butyldimethylsilyloxymethylphenyl)-3,3-dibromoazetidin-2-one ***13c***. Ether/pentane, 1:10 (400 mg, 66%); mp = 55.4°C. IR (CH_2Cl_2): 1765 (ν_{CO} β-lactam), 1760, 1593 cm^{-1}.

^{1}H NMR ($CDCl_3$): 0 (s, 6H, $Si(CH_3)_2$), 0.84 (s, 9H, SiC_4H_9), 4.57 (s, 2H, CH_2CBr_2), 4.66 (s, 2H, $ArCH_2O$), 7.3 (m, $4H_{arom}$). MS *m/z* = 395, 393, 391 ($M^{+\bullet}$ −56), 206, 193, 84, 49, 43.

N-(4-tert-Butyldimethylsilyloxymethylphenyl)-3,3-difluoroazetidin-2-one ***13'a***. Ether/pentane, 1:6 (72 mg, 51%), white solid mp = 48°C, IR (CH_2Cl_2): 1770 cm^{-1}. ^{1}H NMR ($CD_3)_2CO$: 0.2 (s, 6H, $Si(CH_3)_2$), 1 (s, 9H, SiC_4H_9), 4.49 (t, 2H, *J* = 6.42 Hz, CH_2CF_2), 4.87 (s, 2H, CH_2O), 7.55 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −111.6 (t, 2F, *J* = 6.4 Hz). MS *m/z* = 327 ($M^{+\bullet}$), 312, 270, 196, 168. HRMS calcd for $C_{16}H_{23}F_2NO_2Si$, 327.14661; found, 327.1471.

N-(2-Methylphenyl)-3-fluoroazetidin-2-one ***5***
Tri-*n*-butyltin hydride (161 µl, 0.6 mmol) and 2,2'-azobis(2-methylpropionitrile) (10^{-2} mmol) were added to azetidinone **4** (31 mg, 0.12 mmol) in toluene (2 ml) under argon, and then the mixture was stirred at 60°C for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel, ether/pentane, 3:8, (14.5 mg, 70%), solid mp = 61°C. IR (CH_2Cl_2): 1765 cm^{-1}. ^{1}H NMR ($CD_3)_2CO$: 2.35 (s, 3H, $ArCH_3$), 4 (ddd, 1H, *J* = 8.8, 6.6, 4.8 Hz, $CFHCH_2$, *trans*), 4.14 (ddd, 1H, *J* = 12.8, 6.6, 1.9 Hz, $CFHCH_2$, *cis*), 5.8 (ddd, 1H, J_{HF} = 55.3, 4.8, 1.8 Hz, $CHFCH_2$), 7.25 (m, $3H_{arom}$), 7.4 (dd, $1H_{arom}$, *J* = 8.2, 1.8 Hz). ^{19}F NMR (δ/$CFCl_3$): −198 (ddd, 1F, *J* = 54.5, 12.2, 9.0 Hz, $CFHCH_2$). MS *m/z* = 179 ($M^{+\bullet}$), 133, 118, 103, 104. HRMS calcd for $C_{10}H_{10}FNO$, 179.07464; found 179.0750.

N-(Hydroxymethylphenyl)-3,3-dihaloazetidinones ***14a-c*** *and* ***14'a***. *General synthetic procedure*
To a suspension of powdered glass (25 mg) in 40% aqueous HF solution (29 µl, 0.57 mmol) in a polyethylene flask, was added dropwise a solution of a silyl ether (**12a–c** or **16**) (0.19 mmol) in acetonitrile (1 ml). After stirring for 5 min at 20°C, the reaction mixture was neutralized with 5% $NaHCO_3$ and extracted with ether. The organic phase was quickly washed with brine. Drying ($MgSO_4$) and evaporation of the solvent gave the crude alcohol.

N-(2-Hydroxymethylphenyl)-3,3-difluoroazetidin-2-one ***14a***. This alcohol was not purified owing to its instability in the presence of silica. Oil (24 mg, 59%). IR (CH_2Cl_2): 3590, 3490, 1780 cm^{-1}. ^{1}H NMR ($CD_3)_2CO$: 4.58 (t, 2H, *J* = 6.8 Hz, CH_2CF_2), 4.76 (m, 3H, CH_2OH, *OH*), 7.4–7.62 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −112.66 (t, *J* = 6.7 Hz, CH_2CF_2). MS *m/z* = 213, 191, 106. HRMS calcd for $C_{10}H_9F_2NO_2$, 213.06013; found 213.0602.

N-(2-Hydroxymethylphenyl)-3,3-dichloroazetidin-2-one ***14b***. Ether/pentane, 1:2, colorless oil (132 mg; 70%). IR (CH_2Cl_2): 3580, 3480, 1765 cm^{-1} (ν_{CO} β-lactam). ^{1}H NMR ($CDCl_3$): 3 (s, 1H, $ArCH_2OH$), 4.5 (s, 2H, CH_2Cl_2), 4.75 (s, 2H, $ArCH_2OH$), 7.45 (m, 4H). MS *m/z* = 245, 191, 149, 106, 93. HRMS calcd for $C_{10}H_9Cl_2NO_2$, 245.00103; found 245.0011.

N-(2-Hydroxymethylphenyl)-3,3-dibromoazetidin-2-one ***14c***. Ether/pentane, 1:2, oil (250 mg, 82%). IR (CH_2Cl_2): 3580, 3480, 1760 (ν_{CO} β-lactam) cm^{-1}. ^{1}H NMR ($CDCl_3$): 4.55 (s, 2H, CH_2CBr_2), 4.6 (s, 2H, $ArCH_2OH$), 7.27 (m, 4H). MS *m/z* = 337, 335, 333 ($M^{+\bullet}$ bromine isotopes), 149, 105, 93, 77, 65, 51. HRMS calcd for $C_{10}H_9Br_2NO_2$, 332.90011; found, 332.9001.

N-(4-Hydroxymethylphenyl)-3,3-difluoroazetidin-2-one ***14'a***. Ether/pentane, 1:2, white solid (42 mg, 82%); mp = 124°C. IR (CH_2Cl_2): 3580, 3490, 1768 cm^{-1}. ^{1}H NMR ($CD_3)_2CO$: 4.35 (t, 1H, *J* = 5.7 Hz, CH_2OH), 4.45 (t, 2H, *J* = 6.3 Hz, CH_2CF_2), 4.68 (d, 2H, *J* = 5.7 Hz, CH_2OH), 7.5 (s, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −116.6 (t, 2F, *J* = 6.4 Hz, CH_2CF_2). MS *m/z* = 213 ($M^{+\bullet}$), 149, 106. HRMS calcd for $C_{10}H_9F_2NO_2$, 213.06014; found, 213.0604.

N-(Chloromethylphenyl)-3,3 dihaloazetidinones ***2a***, ***6a***, ***7*** *and* ***8***. *General synthetic procedure*
A mixture of thionyl chloride (14 mg, 0.12 mmol) and dry DMF (0.24 ml) was stirred for 5 min at 4°C, and then added to a solution of the alcohol **14a–c** or **14'a** (0.11 mmol) in DMF (0.3 ml) at 20°C. The stirring was continued for 20 min, the reaction mixture was evaporated under vaccum and the residue was purified by thin-layer chromatography on silica gel.

N-(2-Chloromethylphenyl)-3,3-difluoroazetidin-2-one ***2a***. Ether/pentane, 1:1.5, oil (14 mg, 51%). IR (CH_2Cl_2): 1784 cm^{-1} (ν_{CO} β-lactam). ^{1}H NMR ($CD_3)_2CO$: 4.6 (t, 2H, *J* = 6.8 Hz, CH_2CF_2), 4.97 (s, 2H, CH_2Ar), 7.63 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −112.8 (t, *J* = 6.7 Hz, CH_2CF_2). ^{13}C NMR ($CDCl_3$): 43.512 (CH_2Cl), 57.533 (t, $^2J_{CF}$ = 26.6 Hz, CF_2CH_2), 119.579 (t, CF_2, ^1J = 283 Hz, CF_2-CH_2), 123.50, 128.24, 129.78, 131.35, 132.08 (C_{arom}), 134.057 (t, $^4J_{CF}$ = 7 Hz, *C*N), 158.07 (t, $^2J_{CF}$ = 32 Hz, CF_2CO). MS *m/z* = 233, 231 ($M^{+\bullet}$), 167, 169, 132, 118, 92, 77. HRMS calcd for $C_{10}H_8ClF_2NO$, 231.02625; found, 231.0263.

N-(4-Chloromethylphenyl)-3,3-difluoroazetidin-2-one ***6a***. Ether/pentane, 1:2, white solid (16 mg, 55%); mp = 93°C. IR (CH_2Cl_2): 1775 cm^{-1} (ν_{CO} β-lactam). ^{1}H NMR ($CD_3)_2CO$: 4.48 (t, 2H, *J* = 6.5 Hz, CH_2CF_2), 4.79 (s, 2H, $ArCH_2Cl$), 7.57 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −115.5 (t, 2F, *J* = 6.6 Hz, CH_2CF_2). MS *m/z* = 233, 231 ($M^{+\bullet}$ isotopes Cl), 196, 167, 169, 168, 132, 118, 90, 77. HRMS calcd for $C_{10}H_8ClF_2NO$, 231.02614; found, 231.0260.

N-(2-Chloromethylphenyl)-3,3-dichloroazetidin-2-one ***7***. Ether/pentane, 1:5 white solid (124 mg, 67%); mp = 79°C. IR (CH_2Cl_2): 1780 cm^{-1} (ν_{CO} β-lactam). ^{1}H NMR ($CDCl_3$): 4.5 (s, 2H, CCl_2CH_2), 4.8 (s, 2H, $ArCH_2Cl$), 7.5 (m, $4H_{arom}$). ^{13}C NMR ($CDCl_3$): 43.28 (CH_2Cl), 55.220 (CCl_2), 64.604 (*C*N), 123.59, 126.06, 129.62, 131.15, 131.855, 132.01 (C_{arom}), 162.2 (CCl_2CO). MS *m/z* = 263, 261 ($M^{+\bullet}$ chlorine isotopes), 169, 167, 132, 91, 77, 40, 29. Anal $C_{10}H_8Cl_3NO$ (C, H, N).

N-(2-Chloromethylphenyl)-3,3-dibromoazetidin-2-one ***8***. Ether/pentane, 1:5, white solid (200 mg, 53%); mp = 112°C. IR (CH_2Cl_2): 1778 (ν_{CO} β-lactam), 1593 cm^{-1}. ^{1}H NMR ($CDCl_3$): 4.6 (s, 2H, CBr_2CH_2), 4.71 (s, 2H, $ArCH_2Cl$), 7.62 (s, $4H_{arom}$). ^{13}C NMR ($CDCl_3$): 43.46 (CH_2Cl), 46.48 (CBr_2), 65.60 (CH_2N), 123, 128, 129, 131, 132, 134 (C_{arom}), 160 (CBr_2CO). HRMS calcd for $C_{10}H_8Br_2ClNO$, 350.8660; found, 350.86622.

N-(Fluoromethylphenyl)-3,3 dihaloazetidinones ***2d*** *and* ***6b***. *General synthetic procedure*
Diethylaminosulfurtrifluoride (1 mmol) was slowly added to the alcohol **14a** or **14'a** (1 mmol) in dry CH_2Cl_2 (2 ml) at −78°C under argon. The reaction mixture was maintained for a further 45 min at −40°C, and was then allowed to warm at ambient temperature. The solvent was removed under reduced pressure and the residue was purified by thin-layer chromatography on silica gel.

N-(2-Fluoromethylphenyl)-3,3-difluoroazetidin-2-one ***2d***. Ether/pentane, 1:1.5, colorless oil (11 mg, 41%). IR (CH_2Cl_2): 1780 cm^{-1} (ν_{CO} β-lactam). ^{1}H NMR ($CD_3)_2CO$: 4.59 (t, 2H, *J* =

7 Hz, CH_2CF_2), 5.64 (d, 2H, J_{HF}^{gem} = 47.5 Hz, $ArCH_2F$), 7.59 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −112.6 (t, 2F, J = 6.8 Hz, CH_2CF_2), −205 (t, 1F, J = 47.5 Hz, $ArCH_2F$). MS m/z = 215 ($M^{+\bullet}$), 151, 109. HRMS calcd for $C_{10}H_8F_3NO$, 215.05580; found, 215.0562.

N-(4-Fluoromethylphenyl)-3,3-difluoroazetidin-2-one ***6b***. Ether/pentane, 1:1.5, solid (16 mg, 38%), mp = 104°C. IR (CH_2Cl_2): 1775 cm^{-1} (ν_{CO} β-lactam). ^1H NMR ($CD_3)_2CO$: 4.49 (t, 2H, J = 6.5 Hz, CH_2CF_2), 5.46 (d, 2H, J_{HF} = 48 Hz, $ArCH_2F$), 7.58 (s, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −115.5 (t, 2F, J = 6.6 Hz, CF_2CH_2), −200.39 (t, 1F, J_{HF} = 48 Hz, $ArCH_2F$). MS m/z = 215 ($M^{+\bullet}$), 151, 106, 109. HRMS calcd for $C_{10}H_8F_3NO$, 215.05580; found, 215.0559.

Bis 2-(3,3-difluoroazetidino)phenylmethyl ether ***15***. Ether/pentane, 1:2, colorless oil. IR (CH_2Cl_2): 1780 cm^{-1}. ^1H NMR ($CD_3)_2CO$: 4.6 (t, 4H, J = 7 Hz), 5.72 (t, 4H, CH_2OCH_2), 7.6 (m, $8H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −113 (t, J = 6.8 Hz, CF_2). MS m/z = 424 ($M^{+\bullet}$), 132, 109, 99.

N-(2-Bromomethylphenyl)-3,3-difluoroazetidin-2-one ***2b***
N-(2-*tert*-Butyldimethylsilyloxymethylphenyl)-3,3-difluoroazetidinone **12a** (75 mg, 0.22 mmol) in CH_2Cl_2 (0.7 ml) was added dropwise through a syringe to dibromotriphenylphosphorane (111 mg, 1.2 eq) suspensed in CH_2Cl_2 (0.5 ml) under argon. The reaction mixture was stirred for 3 h at room temperature and then directly purified by thin-layer chromatography on silica gel (AcOEt/cyclohexane, 1:4) to give the title bromide as a colorless oil (38 mg, 62%). IR (CH_2Cl_2): 1775 cm^{-1} (ν_{CO} β-lactam). ^1H NMR ($CD_3)_2CO$: 4.47 (t, 2H, J = 6.7 Hz, CH_2CF_2), 4.72 (s, 2H, $ArCH_2Br$), 7.37 (m, $4H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −115.6 (t, 2F, J = 6.6 Hz, CH_2CF_2). MS m/z = 277, 275 ($M^{+\bullet}$ bromine isotopes), 196 (M-Br), 132, 91, 77. HRMS calcd for $C_{10}H_8BrF_2NO$, 274.97579; found, 274.9760.

N-(2-Bromomethyl-5-tert-butyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one ***2c***
To a solution of *N*-(5-*tert*-butyloxycarbonyl-2-methylphenyl)-3,3-difluoroazetidin-2-one **1b** (22 mg, 0.075 mmol) [26] in CCl_4 (15 ml) was added NBS (17 mg, 1.3 eq) and benzoyl peroxide (1 mg). The mixture was refluxed and lighted with a 150 W lamp for 1 h. The succinimide was filtered off and the filtrate was cooled and evaporated *in vacuo* to give a residue which was purified on silica-gel thin-layer chromatography (ether/pentane, 1:6). The title bromide **2c** was obtained as a white solid (16 mg, 59%); mp = 110.9°C. IR (CH_2Cl_2): 1770, 1700 cm^{-1}. ^1H NMR ($CDCl_3$): 1.62 (s, 9H, C_4H_9), 4.5 (t, 2H, J = 6.7 Hz, CH_2CF_2), 4.7 (s, 2H, CH_2Br), 7.4 (m, $3H_{arom}$). ^{19}F NMR (δ/$CFCl_3$): −115 (t, 2F, J = 6 Hz). ^{13}C NMR ($CDCl_3$) 28.1 ($C(CH_3)_3$), 57.1 (t, J = 26.8 Hz, CF_2CH_2), 82.2 ($ArCH_2Br$), 119.3 (t, J = 28.3 Hz, CF_2CO), 124.3, 129.0, 131.4, 133.6 (C_{arom}), 134.2 (t, J = 2.9 Hz, C_{arom}-N) 136.8 (C_{arom}), 158.1 (t, J = 32.2 Hz, CF_2CO), 163.9 ($ArCO_2$). MS: m/z 377, 375 ($M^{+\bullet}$ bromine isotopes), 296, 240, 176. HRMS calcd for $C_{15}H_{16}BrF_2NO_3$ 375.02815; found 374.99366.

Enzymology

HLE, PPE and human cathepsin G were purchased from Elastin Products Co, Serva and Calbiochem, respectively; bovine α-chymotrypsin and trypsin were from Sigma. The molarity of the protease solutions was determined by active-site titrations as in the literature [18], except for cathepsin G (spectrophotometric determination using $A_{280}^{1\%}$ = 6.64 [45]). The enzymes were assayed spectrophotometrically with *p*-nitroanilide substrates: MeO-Suc-Ala-Ala-Pro-Val-*p*-NA (Sigma) for HLE; Suc-Ala-Ala-Ala-*p*-NA (Sigma) for PPE; Suc-Ala-Ala-Pro-Phe-*p*-NA (Sigma) for cathepsin G and chymotrypsin; and Bz-Arg-*p*-NA (Sigma) for trypsin. The amidolytic activities of the enzymes were followed at 405 nm with a Lambda 5 Perkin Elmer UV-vis spectrophotometer equipped with a thermostated holder in the following experimental conditions: 0.1 M Tris, 0.01 % $Brij_{35}$ (HLE, cathepsin G); 0.1 M Tris (PPE); 0.025 M sodium phosphate, 0.05 M KCl (chymotrypsin); 0.1 M Tris, 0.1 M $CaCl_2$ (trypsin). The kinetics were run at pH 8.0 and 37°C. Compounds **1–8** were dissolved in DMSO (Merck). Enzyme inhibition was analyzed by the progress curve method as described in [18] according to equation [1] where S is the chromogenic substrate, I the inhibitor, ES the Michaelis complex, E*I a steady-state inhibitor complex representing a kinetic composite of the non-covalent EI complex and the acyl-enzyme, and E-I is the inactivated enzyme.

$$\begin{array}{l} \overset{K_S \quad k_c}{S \rightleftarrows ES \rightarrow P} \\ + \\ E \\ + \quad \overset{K_I \quad k_{inact}}{I \rightleftarrows E^*I \rightarrow E\text{-}I} \end{array} \qquad [1]$$

The rate of change in absorbance at 405 nm, v, due to the hydrolysis of the chromogenic substrate was obtained using the computer-assisted spectrophotometer. A plot of ln(v) *versus* time gave a straight line with a slope of π. The kinetic parameters K_I and k_{inact} were calculated by iterative least-squares fits to equation [2] where K_M is the Michaelis constant for the chromogenic substrate and [I'] is equal to [I] x $K_M/[S]_O + K_M$.

$$\pi = k_{inact} \frac{[I]'}{[I]' + K_I} \qquad [2]$$

For HLE, the concentrations were $[E]_O$ = 30 nM, [MeO-Suc-Ala-Ala-Pro-Val-*p*-NA] = 100 μM; [**2b**] = 12–150 μM; [**2c**] = [**2d**] = [**6a**] = [**6b**] = 100–200 μM; [**7**] = 48–200 μM; [**8**] = 20–200 μM with 3.5% (v/v) DMSO. For PPE, the concentrations were $[E]_O$ = 200 nM, [Suc-Ala-Ala-Ala-*p*-NA] = 100 μM; [**2b**] = 8–150 μM; [**2c**] = [**2d**] = 100–200 μM; [**6a**] = 40–200 μM; [**6b**] = 100–200 μM; [**7**] = [**8**]= 32–400 μM with 3.5 % (V/V) DMSO.

To determine the partition ratio, the enzyme was incubated with various molar excesses $[I]_O/[E]_O$ for 4 half-lives of the inhibitor at 37°C and pH 8.0 (3.8%, v/v, DMSO) before measuring the remaining activity with an aliquot. The linear plot of this remaining activity *versus* $[I]_O/[E]_O$ gave the partition ratio r [9]. For HLE (1.3 μM), the concentrations of inhibitors were [**2b**] = 0–20.5 μM; [**7**] = 0–25.6 μM; [**8**] = 0–13 μM. For PPE (1 μM), the concentrations of inhibitors were [**2b**] = 0–14 μM; [**7**] = 0–20.5 μM; [**8**] = 0–6.4 μM. α-Chymotrypsin, cathepsin G and trypsin were incubated in the presence of the inhibitor (range 100–200 μM) during 1 or 15 min in the appropriate buffer before adding the corresponding chromogenic substrate. For α-CT: [E] = 40 nM, [Suc-Ala_2-Pro-Phe-*p*NA] = 40 μM; for cathepsin G: [E] = 50 nM, [Suc-Ala_2-Pro-Phe-*p*NA] = 500 μM; for trypsin: [E] = 620 nM, [Bz-Arg-*p*NA] = 250 M. In all cases, the final DMSO percentage in solution was 2% (v/v).

The enzyme hydrolysis of *N*-aryl azetidinones devoid of potential leaving group catalyzed by HLE and PPE was followed at 310 nm for **3** and 250 nm for **4**. The kinetics were run at pH 8.0 and 37°C. The kinetic parameters k_{cat} and K_M were calculated by iterative least-squares fits to the Michaelis equa-

tion. Experimental conditions were for [HLE] = 234 nM, [**3**] = 0.1–2 mM, 4% (v/v) DMSO. $\Delta\varepsilon = 418\ M^{-1}\cdot cm^{-1}$; HLE = 1 μM, [**4**] = 56–395 μM, 5% (v/v) DMSO, $\Delta\varepsilon = 4426\ M^{-1}\cdot cm^{-1}$; [PPE] = 312 nM, [**3**] = 0.1–2 mM, 4% (v/v) DMSO, $\Delta\varepsilon = 407\ M^{-1}\cdot cm^{-1}$; [PPE] = 976 nM, [**4**] = 56–395 μM, 5% (v/v) DMSO, $\Delta\varepsilon = 4234\ M^{-1}\cdot cm^{-1}$; for **5**: [HLE] = 1 μM, [PPE] = 300 μM, [**5**] = 0.1–2 mM.

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