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Synthesis and Inhibition of Human Leucocyte Elastase by Functionalized *N*-Aryl Azetidin-2-ones: Effect of Different Substituents on the Aromatic Ring

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Abstract

N-aryl-3,3-difluoroazetidin-2-ones featured by a latent electrophilic methylene quinoniminium function have been synthesized and evaluated as inhibitors of human leucocyte elastase.

To promote hydrophobic interactions with the enzyme, to increase the rates of β-lactam ring opening and of benzylic group departure, or to induce hydrosolubility, these compounds incorporate on their aromatic ring either an alkyl moiety, a methoxy substituent or a carboxylic group.

Some of these β-lactams proved to be good inactivators of human leucocyte elastase.

Human leucocyte elastase (HLE, EC 3.4.21.37) is a serine proteinase which is released from polymorphonuclear neutrophils by inflammatory stimuli (Jennings & Crystal 1992). A poor regulation of the enzyme by endogenous inhibitors can lead to the degradation of the extracellular matrix components. An increasing body of evidence suggests that HLE is implicated in the pathogenesis of pulmonary emphysema (Weinbaum et al 1991), rheumatoid arthritis (Ekerot & Ohlsson 1984), glomerulonephritis (Sanders et al 1980), cystic fibrosis (Meyer et al 1991), adult respiratory distress syndrome (ARDS) (Merrit et al 1983), and other degradative pathologies (Edwards 1994). Due to their considerable potential for use in therapy, the design and development of synthetic inhibitors of this enzyme is a major field of research.

Several mechanism-based inhibitors, such as cephalosporin derivatives, fluorinated β-lactams, *N*-activated monocyclic β-lactams and *N*-aryl-3,3-dihaloazetidinones display a typical β-lactam ring. In particular, *N*-(2-chloromethylphenyl)-3,3-difluoroazetidin-2-one (**1**) (Table 1, Z = H) possessing a latent electrophilic methylene quinoniminium function, was shown to be a selective inactivator of HLE (Fig. 1) and an effective modulator of elastic fibres degradation and microvascular haemorrhage (Maillard et al 1992; Reboud-Ravaux et al 1992). We recently reported the effects of varying the nature of the halogen substituents at C-3 and that of the potential leaving group at the benzylic position on the inhibition of HLE by these azetidinones (Vergely et al 1995).

We present herein the results concerning the effects of a Z substituent *meta* to the nitrogen and *para* to the benzylic position, together with the introduction of other potential leaving groups X, on the inhibiting efficiency of the *N*-aryl-3,3-difluoro azetidinones (Table 1).

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Materials and Methods

Chemical synthesis

Melting points were obtained with a Mettler 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 tetramethylsilane (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 (MS) and high resolution mass spectra (HRMS) were determined on a Kratos MS 50 instrument. The reactions were monitored by TLC on silica gel 60 F_{254} (Merck). Silica gel (Kieselgel 60, 70–230 mesh, Merck) was used for flash chromatography.

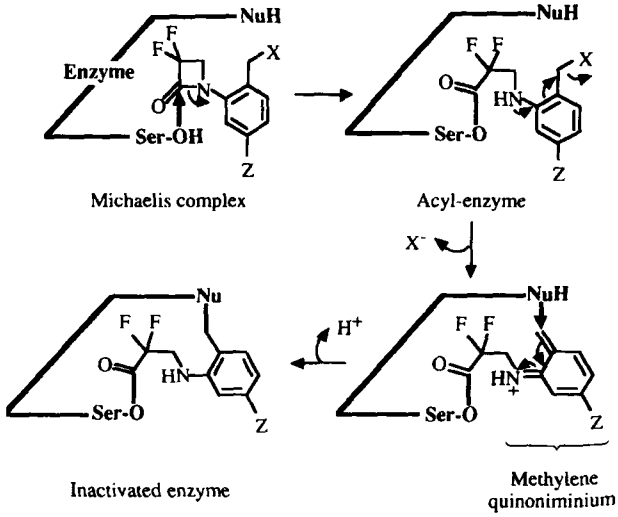
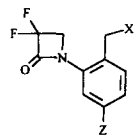


FIG. 1. Postulated mechanism for the inactivation of HLE by *N*-aryl azetidin-2-ones.

Table 1. Structure of compounds **1**, **2**, **2a–d**, **3a–d**, **4–7**, **4a–6a** and **7b**.



Compound	X	Z
1	Cl	H
2	H	H
2a	H	OCH_3
2b	H	$CO_2C_6H_{13}$
2c	H	$CO_2(CH_2)_3CO_2$-*t*-Bu
2d	H	$CO_2(CH_2)_3CO_2H$
3a	Cl	OCH_3
3b	Cl	$CO_2C_6H_{13}$
3c	Cl	$CO_2(CH_2)_3CO_2$-*t*-Bu
3d	Cl	$CO_2(CH_2)_3CO_2H$
4	F	H
4a	F	OCH_3
5	OAc	H
5a	OAc	OCH_3
6	$OC(O)C_6H_3$–2,6–$(CF_3)_2$	H
6a	$OC(O)C_6H_3$–2,6–$(CF_3)_2$	OCH_3
7	Br	H
7b	Br	$CO_2C_6H_{13}$

Synthesis of precursors

tert-*Butyl 4-bromobutyrate*. In a thick glass tube equipped with a screwing cap, were introduced successively 4-bromobutyric acid (6·7 g; 40 mmol), *tert*-butanol (80 μL), concentrated sulphuric acid (100 μL) and 2-methylpropene (6 mL) by condensation. After being stirred for 72 h at room temperature in the firmly closed flask, the reaction mixture was taken up with CH_2Cl_2 and neutralized with 5% aqueous $NaHCO_3$ (10 mL). The organic phase was washed with brine (10 mL), dried ($MgSO_4$), evaporated under reduced pressure and the residue was distilled. Colourless oil (6·12 g; 69%) bp_{11mm} 58°C. IR (CH_2Cl_2) 1720 cm^{-1}. ^1H NMR ($CDCl_3$) 1·38 (9H, s, $OC(CH_3)_3$); 2·06 (2H, m, $CH_2-CH_2-CH_2$); 2·33 (2H, t, J = 7·6 Hz, CH_2-CO_2); 3·38 (2H, t, J = 6·4 Hz, $Br-CH_2$). Anal calc for $C_8H_{15}BrO_2$: C, 43·09; H, 6·78. Found: C, 42·85; H, 6·69.

tert-*Butyl 4-acetoxybutyrate*. A mixture of powdered potassium acetate (2·94 g; 30 mmol), *tert*-butyl 4-bromobutyrate (1·34 g; 6 mmol) and Aliquat 336 (5 mg) was stirred at 40°C for 24 h. The whole mixture was directly flash chromatographed (ether). Colourless oil (1·07 g; 88%). IR (CH_2Cl_2) 1730, 1725, 1665 cm^{-1}. ^1H NMR ($CDCl_3$) 1·37 (9H, s, $OC(CH_3)_3$); 1·88 (2H, m, $CH_2-CH_2-CH_2$); 1·98 (3H, s, $OCOCH_3$); 2·23 (2H, t, J = 7·4 Hz, CH_2-CO_2); 4·01 (2H, t, J = 6·4 Hz, CH_2OCO).

tert-*Butyl 4-hydroxybutyrate*. A mixture of *tert*-butyl 4-acetoxybutyrate (134 mg; 0·66 mmol) and 2-aminoethanol (40 mg; 0·66 mmol) was stirred vigorously at 100°C for 8 h. The crude oil was purified on preparative thin layer chromatography (ether : pentane: 1 : 1). Colourless oil (67 mg; 64%). IR (CH_2Cl_2) 3610, 2930, 1715 cm^{-1}. ^1H NMR ($CDCl_3$) 1·38 (9H, s, $OC(CH_3)_3$); 1·81 (2H, m, $CH_2-CH_2-CH_2$); 2·78 (2H, t, J = 7·1 Hz, CH_2-CO_2); 3·61 (2H, t, J = 6 Hz, CH_2-OH); 5·23 (1H, s, O–H).

Hexyl 4-methyl-3-nitrobenzoate. To 4-methyl-3-nitrobenzoic acid (5·43 g; 30 mmol) and concentrated H_2SO_4 (0·05 mL) in toluene (200 mL) was added hexanol (6·4 g, 6 mL) in toluene (50 mL). The mixture was stirred overnight at room temperature. After evaporation of the solvent, the residue was flash chromatographed (ether : pentane: 1 : 7). Yellowish oil (7·4 g; 91%). IR (CH_2Cl_2) 1700, 1605, 1520, 1350 cm^{-1}. ^1H NMR ($CDCl_3$) 0·86 (3H, t, J = 6·6 Hz, CH_2-CH_3); 1·35 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·71 (2H, m, OCH_2-CH_2); 2·59 (3H, s, $Ar-CH_3$); 4·27 (2H, t, J = 6·7 Hz, OCH_2-CH_2); 7·36 ($1H_{arom}$, d, J = 8 Hz); 8·1 ($1H_{arom}$, dd, J = 1·6, 8 Hz); 8·53 ($1H_{arom}$, d, J = 1·6 Hz). Anal calc for $C_{14}H_{19}NO_4$: C, 63·45; H, 7·23; N, 5·29. Found: C, 63·24; H, 7·05; N, 4·99.

3-tert-*Butoxycarbonylpropyl 4-methyl-3-nitrobenzoate*. To a suspension of 4-methyl-3-nitrobenzoic acid (1·45 g; 8 mmol) in toluene (5 mL) under stirring at 60°C were added successively dropwise 1,8-diazabicyclo[5.4.0] andec-7-ene (1·22 g; 8 mmol) in toluene (2 mL) and *tert*-butyl 4-bromobutyrate (1·80 g; 8 mmol) in the same solvent. After being stirred for 24 h at the same temperature, the mixture was filtered, the solution was concentrated and the residue was purified by flash chromatography (ether : pentane: 2 : 5). White crystals (2 g; 77%), mp 60°C. IR (CH_2Cl_2) 3670, 3370, 2920, 1780, 1740, 1715 cm^{-1}. ^1H NMR ($CDCl_3$) 1·38 (9H, s, $OC(CH_3)_3$); 2·04 (2H, m, $CH_2-CH_2-CH_2$); 2·32 (2H, t, J = 7·4 Hz, CH_2-CO_2); 2·6 (3H, s, $Ar-CH_3$); 4·31 (2H, t, J = 6·4 Hz, CH_2-OCO); 7·37 ($1H_{arom}$, d, J = 8 Hz); 8·08 ($1H_{arom}$, dd, J = 1·7, 8 Hz); 8·51 ($1H_{arom}$, d, J = 1·6 Hz). ^{13}C NMR ($CDCl_3$) 20·5 ($Ar-CH_3$); 24·04 ($-CH_2-$); 27·98 ($C(CH_3)_3$); 31·85 (CH_2-CO_2); 64·5 (CO_2-CH_2); 80·5 ($(CH_3)_3C-O$); 125·5, 130·0, 132·95, 133·2 (C_{arom}); 139·5 ($C_{arom}-N$); 165·1 (CO_2); 171·84 ($Ar-CO_2$). Anal calc for $C_{16}H_{21}NO_6$: C, 59·43; H, 6·55; N, 4·33; O, 29·69. Found: C, 59·60; H, 6·47; N, 4·13; O, 29·65.

Preparation of the substituted anilines

Preparation of the benzylic bromide—general synthetic procedure. To a methylated aromatic compound in CCl_4 (0·8 M), was added *N*-bromosuccin-imide (NBS) (1·5 eq), and a catalytic amount of benzoyl peroxide. The mixture was refluxed in the presence of a 150-W lamp for 7 h under argon. The succinimide was filtered off, the filtrate was concentrated and the crude oil was flash chromatographed.

4-Bromomethyl-3-nitroanisole (12). Ether : pentane: 1 : 3. White solid (632 mg; 64%), mp 64·2°C. IR (CH_2Cl_2) 1615, 1505 cm^{-1}. ^1H NMR ($CDCl_3$) 3·81 (3H, s, $O-CH_3$); 4·73 (2H, s, $Br-CH_2$); 7·06 ($1H_{arom}$, dd, J = 2·8, 8·6 Hz); 7·39 ($1H_{arom}$, d, J = 8·6 Hz); 7·49 ($1H_{arom}$, d, J = 2·8 Hz). Anal calc for $C_8H_8BrNO_3$, C: 39·06; H: 3·28; N: 5·69; found C: 39·24; H: 3·47; N: 5·66.

Hexyl 4-bromomethyl-3-nitrobenzoate (16). Ether : pentane: 1 : 9. Colourless oil (4·9 g; 28·5%). IR (CH_2Cl_2) 1690, 1500 cm^{-1}. ^1H NMR ($CDCl_3$) 0·86 (3H, t, J = 6·5 Hz, CH_2-CH_3); 1·35 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·71 (2H, m, OCH_2-CH_2); 4·29 (2H, t, J = 4·3 Hz, $O-CH_2$); 4·78 (2H, s, $Br-CH_2$); 7·6 ($1H_{arom}$, d, J = 8 Hz); 8·2 ($1H_{arom}$, dd, J = 1·6, 8 Hz); 8·58 ($1H_{arom}$, d, J = 1·6 Hz). Anal calc for $C_{14}H_{18}BrNO_3$, C: 48·86; H: 5·1; N: 3·76; O: 18·30; found C: 48·84; H: 5·27; N: 4·06; O: 18·59.

Substitution of the benzyl bromides by the acetate anion—general synthetic procedure. To a suspension of potassium acetate in dimethylformamide (DMF) was added dropwise the brominated derivative in DMF (0·25 M, 0·1 eq). After being stirred for 1 h at room temperature, the mixture was concentrated and the residue was purified by flash chromatography.

4-Acetoxymethyl-3-nitroanisole (13). Ether : pentane: 1 : 2. White crystals (34 mg; 66%), mp 50·1°C. IR (CH_2Cl_2) 1735, 1705, 1525 cm^{-1}. ^1H NMR ($CDCl_3$) 2·06 (3H, s, $OCOCH_3$); 3·81 (3H, s, OCH_3); 5·35 (2H, s, $Ar-CH_2O$); 7·09 ($1H_{arom}$, dd, J = 2·7, 8·7 Hz); 7·4 ($1H_{arom}$, d, J = 8·6 Hz); 7·54 ($1H_{arom}$, d, J = 2·7 Hz). Anal calc for $C_{10}H_{11}NO_5$, C: 53·38; H: 4·93; N: 6·23; found C: 53·57; H: 5·02; N: 6·32.

Methyl 4-acetoxymethyl-3-nitrobenzoate (21). Obtained from the methyl 4-bromomethyl-3-nitrobenzoate 20 (Zrihen et al 1983) AcOEt-pentane: 1-3. white solid (757 mg, 69%), mp 78°C; IR (CH_2Cl_2) 1740, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) 2·2 (3H, s, $OCOCH_3$); 4 (3H, s, OCH_3); 5·6 (2H, s, $ArCH_2O$); ⟨8.3⟩ ($3H_{arom}$,m). Anal calc for $C_{11}H_{11}NO_6$, C: 52·22; H: 4·38; N: 5·54; found C: 52·14; H: 4·31; N: 5·54.

Methanolysis of the benzyl esters—general synthetic procedure. To the benzyl acetate in methanol (0·13 M) was added a catalytic amount of sodium methoxide. The mixture was stirred for 2 h at room temperature, concentrated and the residue was purified by flash chromatography.

4-Hydroxymethyl-3-nitroanisole (14). Ether : pentane: 1 : 1. White solid (350 mg; 86%), mp 74·4°C. IR (CH_2Cl_2) 3670, 3590, 1525 cm^{-1}. ^1H NMR ($CDCl_3$) 2·65 (1H, bs, O–*H*); 3·81 (3H, s, OCH_3); 4·78 (2H, s, $Ar-CH_2O$); 7·11 ($1H_{arom}$, dd, J = 2·6, 8·5 Hz); 7·49 ($1H_{arom}$, d, J = 8 Hz); 7·52 ($1H_{arom}$, d, J = 2·9 Hz). Anal calc for $C_8H_9NO_4$, C: 52·50; H: 4·96; N: 7·65; found C: 52·36; H: 4·9; N: 7·63.

Hexyl 4-hydroxymethyl-3-nitrobenzoate (18). A mixture of bromide **16** (1·7 g; 5 mmol) and sodium formate (442 mg; 6·5 mmol) was heated at 150°C for 3 h in the presence of Aliquat 336 (31 mg). The suspension, containing the formate **17**, was dropped on a column of neutral activated alumina (50 g) and percolated with a mixture CH_2Cl_2–MeOH (97 : 3) which allowed the selective methanolysis of the formyl group to take place. Concentration of the collected effluent yielded a crude oil **18** which was purified by flash chromatography, (ether : pentane: 1 : 1). Colourless oil (1·06 g; 75%). IR (CH_2Cl_2) 1690, 1500 cm^{-1}. ^1H NMR ($CDCl_3$) 0·85 (3H, t, J = 6·5 Hz, CH_2-CH_3); 1·3 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·7 (2H, m, OCH_2-CH_2); 1·94 (1H, s, CH_2-OH); 4·29 (2H, t, J = 7·3 Hz, OCH_2-CH_2); 5·0 (2H, s, $Ar-CH_2O$); 7·84 ($1H_{arom}$, d, J = 1·6 Hz); 8·24 ($1H_{arom}$, dd, J = 1·6, 8 Hz); 8·65 ($1H_{arom}$, d, J = 1·6 Hz). Anal calc for $C_{14}H_{19}NO_5$, C: 60·24; H: 6·83; N: 4·77; found C: 59·77; H: 6·80; N: 4·97.

Methyl 4-hydroxymethyl-3-nitrobenzoate (22). AcOEt-pentane: 2·5-7, colourless oil (1 g, 76%); IR (CH_2Cl_2) 3585–3320, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) 2·6 (1H, s, CH_2OH); 4 (3H, s, OCH_3); 5,2 (2H, s, $ArCH_2OH$); ⟨8.3⟩ ($3H_{arom}$, m).

Reduction of the nitro group. General synthetic procedure. A suspension of the nitro compound and platinum oxide hydrate (0·1 eq) in 95% ethanol (0·75 M) were stirred under hydrogen pressure (3 atm) for 30 min at room temperature. The solid was filtered off. Concentration of the solution yielded the expected aniline which was used in the following condensation step without further purification.

5-methoxy-2-methylaniline (8′a). Colourless oil (217 mg; 48%). IR (CH_2Cl_2) 3670, 3380, 1615, 1505 cm^{-1}. ^1H NMR ($CDCl_3$) 2·04 (3H, s, $Ar-CH_3$); 3·66 (2H, s, large, NH_2); 3·68 (3H, s, OCH_3); 6·2 ($1H_{arom}$, s); 6·23 ($1H_{arom}$, d, J = 2·5 Hz); 6·88 ($1H_{arom}$, d, J = 8·3 Hz). Anal calc for $C_8H_{11}NO$, C: 70·13; H: 8·09; N: 10·22; found C: 70·13; H: 8·14; N: 10·33.

Hexyl 3-amino-4-methylbenzoate (8′b). Ether : pentane: 1 : 7. White solid (2·2 g; 94%), mp 63·1°C. IR (CH_2Cl_2) 1695, 1615, 1500, 1450 cm^{-1}. ^1H NMR ($CDCl_3$) 0·86 (3H, t, J = 6·6 Hz, CH_2-CH_3); 1·35 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·71 (2H, m, OCH_2-CH_2); 2·59 (3H, s, $Ar-CH_3$); 3·45 (2H, s, large, NH_2); 4·27 (2H, t, J = 6·7 Hz, OCH_2-CH_2); 7·36 ($1H_{arom}$, d, J = 8 Hz); 8·1 ($1H_{arom}$, dd, J = 1·6, 8 Hz); 8·53 ($1H_{arom}$, d, J = 1·6 Hz).

tert-*Butoxycarbonylpropyl 3-amino-4-methylbenzoate (8′c).* White solid (800 mg; 90%), mp 108·9°C. IR (CH_2Cl_2) 3670, 3380, 1715, 1705 cm^{-1}. ^1H NMR ($(CD_3)_2CO$) 1·28 (9H, s, $C(CH_3)_3$); 1·87 (2H, m, $CH_2-CH_2-CH_2$); 2·03 (3H, s, $Ar-CH_3$); 2·23 (2H, t, J = 7·3 Hz, CH_2-CO_2); 4·12 (2H, t, J = 6·4 Hz, CO_2-CH_2); 4·52 (2H, s large, NH_2); 6·91 ($1H_{arom}$, d, J = 7·7 Hz); 7·1 ($1H_{arom}$, d, J = 1·7 Hz); 7·22 ($1H_{arom}$, s). ^{13}C NMR [$(CD_3)_2CO$]: 17·98 ($Ar-CH_3$); 25·98 ($-CH_2-$); 28·44 ($C(CH_3)_3$); 32·56 (CH_2-CO_2); 64·30 ($O-CH_2$); 80·61 ($(CH_3)_3C-O$); 115·79, 119·08, 127·98, 129·85, 131·0 (C_{arom}); 174·31 ($C_{arom}-N$); 167·35 (CO_2); 172·17 ($Ar-CO_2$). Anal calc for $C_{16}H_{23}NO_4$, C: 65·58; H: 7·91; N: 4·78; found, C: 65·34; H: 7·81; N: 4·96.

tert-*Butoxycarbonylpropyl 3-amino-4-thexyldimethylsilyloxymethylbenzoate (8c).* Colourless oil (250 mg; 98%). IR (CH_2Cl_2) 3660, 3440, 3360, 2940, 1725, 1710 cm^{-1}. ^1H NMR ($CDCl_3$) 0·01 (6H, s, $Si(CH_3)_2$); 0·83 (12H, m, $Si-C_6H_{12}$); 1·33 (9H, s, $OC(CH_3)_3$); 1·54 (1H, m, $-CH-$); 1·92 (2H, m, $CH_2-CH_2-CH_2$); 2·3 (2H, t, J = 7·3 Hz, CH_2-CO_2); 4·2 (2H, t, J = 6·4 Hz, CO_2-CH_2); 4·6 (2H, s, $Ar-CH_2O$); 6·92 ($1H_{arom}$, d, J = 8·1 Hz); 7·3 ($2H_{arom}$, dd, J = 1·5, 8·2 Hz). Anal calc for $C_{24}H_{41}NO_5Si$, C: 63·91; H: 9·16; N: 3·11; found, C: 63·91; H: 8·88; N: 3·38.

5-Methoxy-2-hydroxymethylaniline (15). White crystals (260 mg; 88%), mp 78·4°C. IR (CH_2Cl_2) 3570, 3440, 3370, 1630, 1505 cm^{-1}. ^1H NMR ($CDCl_3$) 1·68 (1H, bs, O–*H*); 3·68 (3H, s, $O-CH_3$); 4·1 (2H, bs, NH_2); 4·50 (2H, s, $Ar-CH_2O$); 6·21 ($2H_{arom}$, m); 6·90 (H_{arom}, d, J = 2·4 Hz). Anal calc for $C_8H_{11}NO_2$, C: 62·80; H: 7·25; N: 9·16; found, C: 63·09; H: 6·98; N: 9·39.

Hexyl 3-amino-4-hydroxymethylbenzoate (19). White solid (1·65 g; 60%), mp 61·8°C. IR (CH_2Cl_2) 3380, 3300, 2900, 1710, 1610 cm^{-1}. ^1H NMR ($(CD_3)_2CO$) 0·80 (3H, t, J = 5·6 Hz, CH_2-CH_3); 1·25 (6H, m, $CH_2CH_2CH_2-CH_3$);

1·7 (2H, m, OCH_2-CH_2); 4·15 (2H, t, J = 6·5 Hz, OCH_2-CH_2); 4·6 (2H, s large, NH_2); 4·65 (2H, s, $Ar-CH_2O$); 7·2 ($3H_{arom}$, m). Anal calc for $C_{14}H_{21}NO_3$, C: 66·54; H: 8·4; N: 5·41; found C: 66·90; H: 8·4; N: 5·57.

Preparation of the mixed benzyltrialkylsilyl ethers—general synthetic procedure. To a hydroxymethylated compound in dry DMF (0·12 M), were added *tert*-butyldimethyl or dimethylthexylsilylchlorosilane (1·3 eq) and imidazole (2·5 eq). After being stirred at room temperature for 90 min, the mixture was concentrated under reduced pressure (0·1 mm Hg) and purified by flash chromatography.

5-Methoxy-2-thexyldimethylsilyloxymethylaniline (8a). Ether: pentane: 1 : 4. Yellowish oil (383 mg; 90%). IR (CH_2Cl_2) 3660, 3440, 3360, 2950, 1610, 1505 cm^{-1}. ^1H NMR ($CDCl_3$) 0·01 (6H, s, $Si(CH_3)_2$); 0·67 (12H, m, $Si-C_6H_{12}$); 0·82 (1H, m, $-CH-$); 3·67 (3H, s, OCH_3); 4·5 (2H, s, $Ar-CH_2O$); 6·13 ($2H_{arom}$, m); 6·78 ($1H_{arom}$, d, J = 8·6 Hz). ^{13}C NMR ($CDCl_3$) − 3·2 ($Si(CH_3)$; −1·47 ($Si(CH_3)$; 18·49, 20·11, 20·25, 24·85 (CH_3); 25·07 (C_q); 34·12 (−CH−); 54·99 (OCH_3); 64·16 (OCH_2); 101·56, 102·77, 118·42, 129·48 (C_{arom}); 147·39 ($C_{arom}-N$); 160·26 ($C_{arom}-O$).

*Hexyl 3-amino-4-*tert*-butyldimethylsilyloxymethylbenzoate (8b).* Ether : pentane: 1 : 4. Colourless oil, (246 mg; 70%). IR (CH_2Cl_2) 3690, 3458, 3370, 1708, 1620 cm^{-1}. ^1H NMR ($(CD_3)_2CO$) 0·0 (6H, s, $Si(CH_3)_2$); 0·81 (3H, t, J = 5.6 Hz, CH_2-CH_3); 0·81 (9H, s, $Si-C_4H_9$); 1·25 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·7 (2H, m, OCH_2-CH_2); 4·15 (2H, t, J = 6·5 Hz, OCH_2-CH_2); 4·64 (2H, bs $Ar-CH_2O$); 7·16 ($2H_{arom}$, d, J = 1·1 Hz); 7·26 ($1H_{arom}$, s). Anal calc for $C_{20}H_{35}NO_6Si$, C: 65·74; H: 9·59; N: 3·71; found C: 65·70; H: 9·64; N: 3·83.

Methyl 3-nitro-4-thexyldimethylsilyloxymethylbenzoate (23). Colourless oil (1·92 g; 82%). IR (CH_2Cl_2) 3585, 3320, 1725 cm^{-1}. ^1H NMR ($CDCl_3$) 0·01 (6H, s, $Si(CH_3)_2$); 0·82 (12H, m, $Si-C_6H_{12}$); 1·52 (1H, m, $-CH-$); 3·82 (3H, s, CO_2-CH_3); 4·99 (2H, s, $Ar-CH_2O$); 7·88 ($1H_{arom}$, d, J = 8·2 Hz); 8·16 ($1H_{arom}$, dd, J = 1·7, 7·6 Hz); 8·59 ($1H_{arom}$, d, J = 1·6 Hz).

3-Nitro-4-thexyldimethylsilyloxymethylbenzoic acid (24). To methyl 3-nitro-4-thexyldimethylsilyloxymethylbenzoic acid (1·47 g; 4·16 mmol) dissolved in the minimal amount of methanol was added aqueous 1 M NaOH (1·2 eq). After being stirred for 1 h at room temperature, the mixture was acidified (0·2 M HCl) and extracted with ether. The combined organic phases were dried ($MgSO_4$) and evaporated: white solid (1·23 g; 87%), mp 85·4°C. IR (CH_2Cl_2) 3380, 1700 cm^{-1}. ^1H NMR ($CDCl_3$) 0·01 (6H, s, $Si(CH_3)_2$); 0·81 (12H, m, $Si-C_6H_{12}$); 1·51 (1H, m, $-CH-$); 5·07 (2H, s, $Ar-CH_2O$); 8·2 ($3H_{arom}$, m).

tert-*Butyloxycarbonylpropyl 3-nitro-4-thexyldimethylsilyloxymethylbenzoate (25).* To a solution of 3-nitro-4-thexyldimethylsilyloxymethylbenzoic acid (341 mg; 1 mmol) in CH_2Cl_2 (8 mL) was added successively *tert*-butyl 4-hydroxybutyrate (160 mg; 1 mmol) in CH_2Cl_2, *N,N'*-dicyclohexylcarbodiimide (DCC) (227 mg; 1·1 mmol) and 4-dimethylaminopyridine (DMAP) (7 mg). The mixture was stirred for 24 h at room temperature, then the solid was filtered off. The crude oil obtained after concentration of the filtrate was purified by flash chromatography (ether : pentane: 1 : 5). Colourless oil (280 mg; 58%). IR (CH_2Cl_2) 3370, 2940, 1725, 1710 cm^{-1}. ^1H NMR ($CDCl_3$) 0·01 (6H, s, $Si(CH_3)_2$); 0·83 (12H, m, $Si-C_6H_{12}$); 1·32 (9H, s, $OC(CH_3)_3$); 1·54 (1H, m, $-CH-$); 1·93 (2H, m, $CH_2-CH_2-CH_2-$); 2·25 (2H, t, J = 7·4 Hz, CH_2-CO_2); 4·25 (2H, t, J = 6·4 Hz, CO_2-CH_2); 5·03 (2H, s, $Ar-CH_2O$); 7·87 ($1H_{arom}$, d, J = 8·1 Hz); 8·16 ($1H_{arom}$, dd, J = 1·5, 8·2 Hz); 8·56 ($1H_{arom}$, d, J = 1·6 Hz). Anal calc for $C_{24}H_{39}NO_7Si$, C: 59·93; H: 8·17; N: 2·91; found, C: 59·77; H: 7·94; N: 3·23.

Preparation of the 3-bromo-2,2-difluoropropionanilides—general synthetic procedure. To the 3-bromo-2,2-difluoropropanoyl chloride in toluene (2 mL $mmol^{-1}$) was added an equimolecular mixture of NEt_3 and a substituted aniline dissolved in toluene (1 mL $mmol^{-1}$). The mixture was stirred for 50 min at room temperature, taken up with ether, washed sequentially with 0·2 M aq $NaHCO_3$ until neutral and with brine, and dried ($MgSO_4$). The residue obtained after evaporation of the solvents was purified by flash chromatography.

N-*(2-Thexyldimethylsilyloxymethyl-5-methoxyphenyl)-3-bromo-2,2-difluoropropionamide (9a).* Ether : pentane: 1 : 4. Colourless oil (408 mg; 81%). IR (CH_2Cl_2) 3670, 3320, 1695, 1610, 1585 cm^{-1}. ^1H NMR ($CDCl_3$) 0·01 ppm (6H, s, $Si(CH_3)_2$); 0·68 (12H, m, $Si-C_6H_{12}$); 1·54 (1H, m, $-CH-$); 3·67 (3H, s, OCH_3); 3·72 (2H, t, J = 13·3 Hz, CF_2-CH_2); 4·58 (2H, s, $Ar-CH_2O$); 6·51 ($1H_{arom}$, dd, J = 2·6, 8·3 Hz); 6·89 ($1H_{arom}$, d, J = 8·3 Hz); 7·74 ($1H_{arom}$, d, J = 2·6 Hz); 9·87 (1H, bs, NH). ^{19}F NMR ($CDCl_3$) − 115,8 (t, J = 13·2 Hz, CF_2-CH_2). ^{13}C NMR ($CDCl_3$) − 3·36 ($Si(CH_3)_2$); 18·33, 20·02 (CH_3); 25·17 (−CH−); 28·3 (CH_2Br, t, J = 30·7 Hz); 33·89 (C_q); 55·31 (OCH_3); 64·54 (CH_2O); 107·26, 110·26 (C_{arom}); 114·23 (CF_2, t, J = 272·6 Hz); 121·98, 128·75 (C_{arom}); 137·03 ($C_{arom}-N$); 159·76 ($C_{arom}-O$); 159·85 (CF_2-CO, t, J = 27·5 Hz).

N-*(2-Methyl-5-methoxyphenyl)-3-bromo-2,2-difluoropropionamide (9'a).* Ether-pentane: 1-3. White crystals (108 mg; 88%), mp 78·9°C. IR (CH_2Cl_2) 3670, 3380, 1710, 1615, 1530 cm^{-1}. ^1H NMR ($CDCl_3$) 2.16 (3H, s, $Ar-CH_3$); 3.73 (3H, s, $O-CH_3$); 3.80 (2H, t, J = 13.3 Hz, CF_2-CH_2); 6.64 ($1H_{arom}$, dd, J = 2·7, 8·4 Hz); 7·05 ($1H_{arom}$, d, J = 8·4 Hz); 7·46 ($1H_{arom}$, d, J = 2·7 Hz); 7·83 (1H, bs, NH). ^{19}F NMR ($CDCl_3$) − 116·23 (t, J = 13·3 Hz, CF_2-CH_2). ^{13}C NMR ($CDCl_3$) 17·5 ($Ar-CH_3$); 28·3 (CH_2Br, t, J = 30·6 Hz); 56·2 (CH_3O); 107·2, 112·3 (C_{arom}); 114·23 (CF_2, t, J = 272·6 Hz); 122·0, 128·5 (C_{arom}); 131·1 ($C_{arom}-N$); 159·7 ($C_{arom}-O$); 159·85 (CF_2-CO, t, J = 27·50 Hz). MS m/z = 307($M^{+\cdot}$), 164, 136, 121. HRMS calc for $C_{11}H_{12}BrF_2NO_2$: 307·0019; found: 307·0020.

N-*(2-*tert*-Butyldimethylsilyloxymethyl-5-hexyloxycarbonylphenyl)-3-bromo-2,2-difluoropropionamide (9b).* Ether : pentane: 1 : 4. Colourless oil (881 mg; 82%). IR (CH_2Cl_2) 3680, 3315, 1710, 1580 cm^{-1}. ^1H NMR ($CDCl_3$) 0·01 (6H, s, $Si(CH_3)_2$); 0·77 (3H, t, J = 8 Hz, CH_2-CH_3); 0·80 (9H, s, $Si-C_4H_9$); 1·3

(6H, m, $CH_2CH_2CH_2$–CH_3); 1·69 (2H, m, OCH_2–CH_2); 3·76 (2H, t, J = 13·2 Hz, CH_2Br); 4·20 (2H, t, J = 6·8 Hz, OCH_2–CH_2); 4·71 (2H, s, CH_2–O); 7·12 ($1H_{arom}$, d, J = 7·8 Hz); 7·71 ($1H_{arom}$, dd, J = 1·5, 7·8 Hz); 8·74 ($1H_{arom}$, d, J = 1·5 Hz); 10·0 (1H, bs, N*H*). ^{19}F NMR ($CDCl_3$) – 103·8 (t, dd, J = 1·9, 13·2 Hz, CF_2–CH_2). ^{13}C NMR ($CDCl_3$) 13·81; 18·02; 22·34; 25·46; 27·81 (CH_2Br, t, $^2J_{CF}$ = 30·4 Hz); 28·46; 31·26; 64·88 (CH_2O); 65·21 (OCH_2); 76·29; 77·56; 114·14 (CF_2, t, $^1J_{CF}$ = 283 Hz); 122·62, 126·27, 127·74, 131·03, 134·11, 135·95 (C_{arom}); 160·1 (CF_2CO, t, $^2J_{CF}$ = 27·6 Hz); 165·66 (CO_2).

N-*(2-Methyl-5-hexyloxycarbonylphenyl)-3-bromo-2,2-difluoropropionamide (9′b)*. Ether : pentane: 1 : 3. White crystals (2·4 g; 98%), mp 51·6°C. IR (CH_2Cl_2) 3425, 3400, 1711 cm^{-1}. ^1H NMR ($(CD_3)_2CO$) 0·76 (3H, t, J = 6·8 Hz, CH_2–CH_3); 1·22 (6H, m, $CH_2CH_2CH_2$–CH_3); 1·62 (2H, m, OCH_2–CH_2); 2·23 (3H, s, Ar–CH_3); 3·98 (2H, t, J = 14 Hz, CH_2Br); 4·15 (2H, t, J = 6·7 Hz, OCH_2–CH_2); 7·3 ($1H_{arom}$, d, J = 8 Hz); 7·72 ($1H_{arom}$, dd, J = 1·7, 8 Hz); 7·91 ($1H_{arom}$, d, J = 1·7 Hz); 9·6 (1H, bs, N*H*). ^{19}F NMR ($(CD_3)_2CO$) – 103·8 (t, J = 13·8 Hz, CF_2–CH_2). Anal calc for $C_{17}H_{22}BrF_2NO_3$, C: 50·25; H: 5·46; N: 3·45; found, C: 50·45; H: 5·46; N: 3·11.

N-*[2-Thexyldimethylsilyloxymethyl-5-(3*-tert-*butyloxycarbonyl)-propyloxycarbonylphenyl]-3-bromo-2,2-difluoropropionamide (9c)*. Ether-pentane: 1-3. Colourless oil (275 mg; 87%). IR (CH_2Cl_2) 3670, 2950, 1715, 1595, 1585 cm^{-1}. ^1H NMR ($CDCl_3$) 0·0 (6H, s, $Si(CH_3)_2$); 0·71 (12H, m, Si–C_6H_{12}); 1·30 (9H, s, $C(CH_3)_3$); 1·54 (1H, m, –CH–); 1·88 (2H, m, CH_2–CH_2–CH_2); 2·24 (2H, t, J = 7·4 Hz, CH_2–CO_2); 3·73 (2H, t, J = 13·21 Hz, CF_2–CH_2); 4·20 (2H, t, J = 6·41 Hz, CO_2–CH_2); 4·66 (2H, s, Ar–CH_2O); 7·09 ($1H_{arom}$, d, J = 7·9 Hz); 7·66 ($1H_{arom}$, dd, J = 1·5, 7·9 Hz); 8·66 ($1H_{arom}$, d, J = 1·4 Hz); 9·86 (1H, bs, N*H*). ^{19}F NMR ($CDCl_3$) – 115·7 (t, J = 13·20 Hz, CF_2–CH_2). ^{13}C NMR ($CDCl_3$) – 3·34 ($Si(CH_3)_2$); 18·36, 20,04 (CH_3); 24·23 (–CH–); 25·25 ($-CH_2-$); 28·04 ($C(CH_3)_3$); 28·06 (CH_2Br, t, J = 30·6 Hz); 31·98 (CH_2–CO_2); 33·92 (C_q); 64·29 (CH_2O); 64·75 (CO_2–CH_2); 80·45 (C–O); 115·1 (CF_2, t, J = 251·6 Hz); 122·93, 126·56, 128·00, 130·35, 134·68 (C_{arom}); 136·01 (C_{arom}–N); 160·05 (CF_2–CO, t, J = 27·3 Hz); 165·70 (CH_2–CO_2); 172·09 (Ar–CO_2).

N-*[2-Methyl-5-(3*-tert-*butyloxycarbonyl)propyloxycarbonylphenyl]-3-bromo-2,2-difluoropropionamide (9′c)*. Ether : pentane: 1 : 1. Yellowish oil (550 mg; 94%). IR (CH_2Cl_2) 3670, 3400, 1765, 1715, 1705 cm^{-1}. ^1H NMR ($(CD_3)_2CO$) 1·29 (9H, s, $C(CH_3)_3$); 1·88 (2H, m, CH_2–CH_2–CH_2); 2·23 (3H, s, Ar–CH_3); 2·26 (2H, t, J = 7·3 Hz, CH_2–CO_2); 3·38 (2H, t, J = 14·1 Hz, CH_2Br); 4·19 (2H, t, J = 6·5 Hz, CO_2–CH_2); 7·3 ($1H_{arom}$, d, J = 8 Hz); 7·72 ($1H_{arom}$, dd, J = 1·75, 8 Hz); 7·91 ($1H_{arom}$, d, J = 1·6 Hz); 9·71 (1H, bs, N*H*). ^{19}F NMR ($(CD_3)_2CO$) – 115·8 (t, J = 14·1 Hz, CF_2–CH_2). ^{13}C NMR ($(CD_3)_2CO$) 18·31 (Ar–CH_3); 24·97 ($-CH_2-$); 28·22 (CH_3); 28·71 (CH_2Br, t, J = 30·3 Hz); 32·32 (CH_2–CO_2); 64·75 (OCH_2); 80·48 (C–O); 115·5 (CF_2, t, J = 251·6 Hz); 127·90, 128·76, 129·64, 131·78, 135·31 (C_{arom}); 140·34 (C_{arom}–N); 161·1 (CF_2–CO, t, J = 27·5 Hz); 166·08 (CO_2); 172·53 (Ar–CO_2).

Preparation of the N-*aryl azetidinones*

General method A. The anilide (1·5 mmol) dissolved in a mixture of DMF-CH_2Cl_2 (1–6; 9 mL) was added dropwise (40 min) to a suspension of NaH (60% dispersion in oil; 3·5 eq) in the same mixture of solvent at –10°C. After stirring for a further 40 min, the mixture was quickly washed with aqueous saturated ammonium chloride until neutral. After drying ($MgSO_4$) and concentration under reduced pressure (0·1 mmHg), the crude azetidinone was purified by flash chromatography.

General method B. To an anilide (0·5 mmol) dissolved in dry acetone (3 mL) was added K_2CO_3 (207 mg, 1·5 mmol). The mixture was stirred under reflux and under argon for 3 h. The insoluble material was filtered off, the solution was concentrated and the residue was purified by flash chromatography.

N-*(2-Thexyldimethylsilyloxymethyl-5-methoxyphenyl)-3,3-difluoroazetidin-2-one (10a) (method B)*. Ether : pentane: 1 : 4. Yellowish oil (89 mg; 90%). IR (CH_2Cl_2) 3380, 2950, 1775, 1605, 1505 cm^{-1}. ^1H NMR ($CDCl_3$) 0·0 (6H, s, $Si(CH_3)_2$); 0·77 (12H, m, Si–C_6H_{12}); 1·54 (1H, m, –CH–); 3·73 (3H, s, O–CH_3); 4·25 (2H, t, J = 6·4 Hz, CF_2–CH_2); 4·56 (2H, s, Ar–CH_2O); 6·70 ($1H_{arom}$, dd, J = 2·3, 8·5 Hz); 7·09 ($1H_{arom}$, d, J = 2·3 Hz); 7·18 ($1H_{arom}$, d, J = 8·5 Hz). ^{19}F NMR ($CDCl_3$) – 116·30 (t, J = 6·4 Hz, CF_2–CH_2). ^{13}C NMR ($CDCl_3$) – 3·4 – ppm ($Si(CH_3)_2$); 18·33; 19·98 (CH_3); 24·86 (–CH–); 33·85 (C_q); 55·28 (CH_3–O); 57·5 (CF_2–CH_2, t, J = 37·7 Hz); 62·03 (CH_2–O); 108·1, 112·54 (C_{arom}); 119·9 (CF_2, t, J = 272·6 Hz); 125·09, 130·8 (C_{arom}); 134·2 (C_{arm}–N); 159·26 (C_{arom}–O); 159·85 (CF_2–CO, t, J = 27·5 Hz).

N-*(2-Methyl-5-methoxyphenyl)-3,3-difluoroazetidin-2-one (2a) (method A)*. Ether : pentane: 1 : 3. White crystals (160 mg; 55%), mp 55·4°C. IR (CH_2Cl_2) 3670, 3380, 1775, 1615, 1505 cm^{-1}. ^1H NMR ($CDCl_3$) 2·2 (3H, s, Ar–CH_3); 3·7 (3H, s, O–CH_3); 4·1 (2H, t, J = 6·3 Hz, CF_2–CH_2); 6·7 ($1H_{arom}$, dd, J = 2·6, 8·4 Hz); 6·8 ($1H_{arom}$, d, J = 2·6 Hz); 7·0 ($1H_{arom}$, d, J = 8·4 Hz). ^{19}F NMR ($CDCl_3$) – 116·23 (t, J = 6·3 Hz, CF_2–CH_2). ^{13}C NMR ($CDCl_3$) 18·05 (Ar–CH_3); 55·4 (O–CH_3); 57·4 (CF_2–CH_2, t, J = 37·4 Hz); 108·2, 113·57 (C_{arom}); 119·2 (CF_2, t, J = 272·5 Hz); 123·4, 132·25 (C_{arom}); 134·5 (C_{arom}–N); 158·05 (C_{arom}–O); 159·2 (CF_2–CO, t, J = 29·4 Hz).

N-*(2*-tert-*Butyldimethylsilyloxymethyl-5-hexyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one (10b) (method A)*. Ether : pentane: 1 : 4. Colourless oil (176 mg; 45%). IR (CH_2Cl_2) 3545, 3400, 1785, 1705 cm^{-1}. ^1H NMR ($(CD_3)_2CO$) 0·0 (6H, s, $Si(CH_3)_2$); 0·81 (3H, t, J = 7·6 Hz, CH_2–CH_3); 0·82 (9H, s, Si–C_3H_9); 1·20 (6H, m, $CH_2CH_2CH_2$–CH_3); 1·72 (2H, m, OCH_2–CH_2); 4·20 (2H, t, J = 6·6 Hz, CF_2–CH_2); 4·50 (2H, t, J = 6·8 Hz, OCH_2–CH_2); 4·84 (2H, s, CH_2O); 7·64 ($1H_{arom}$, d, J = 7·8 Hz); 7·84 ($1H_{arom}$, dd, J = 1·5, 7·8 Hz); 8·1 ($1H_{arom}$, d, J = 1·5 Hz). ^{19}F NMR ($(CD_3)_2CO$)-115 (t, J = 6.6 Hz, CF_2–CH_2). ^{13}C NMR ($CDCl_3$) 13·80; 18·05; 22·41; 25·32; 28·41; 57·27 (CF_2–CH_2, t, $^2J_{CF}$ = 26·6 Hz); 64·88 (CH_2–OSi); 65·34 (O–CH_2); 76·29; 77·29; 119·6 (CF_2, t, $^1J_{CF}$ = 283 Hz); 122·52, 126·37, 127·84, 131·13, 134·15, 135·85 (C_{arom}); 159·4 (CF_2–CO, t, J = 31·6 Hz); 165·6 (Ar–CO_2).

N-(2-Methyl-5-hexyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one (2b) (method A). Ether : pentane: 1 : 4. White crystals (1·17 g; 72%), mp 54·7°C. IR (CH_2Cl_2) 3680, 3400, 1785 1710 cm^{-1}. ^1H NMR ($CDCl_3$) 0·86 (3H, t, J = 6·7 Hz, CH_2-CH_3); 1·39 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·73 (2H, m, OCH_2-CH_2); 2·36 (3H, s, Ar−CH_3); 4·16 (2H, t, J = 6·5 Hz, CF_2-CH_2); 4·22 (2H, t, J = 6·6 Hz, OCH_2-CH_2); 7·26 ($1H_{arom}$, d, J = 8 Hz); 7·83 ($1H_{arom}$, dd, J = 1·7, 8 Hz); 7·84 ($1H_{arom}$, d, J = 1·7 Hz). ^{19}F NMR ($CDCl_3$) − 113·55 (t, J = 6·5 Hz, CF_2-CH_2). ^{13}C NMR ($CDCl_3$) 13·73; 19·10 (Ar−CH_3); 22·27; 25·38; 28·36; 29·44; 31·17; 56·62 (CF_2-CH_2, t, J = 26·8 Hz); 65·25 (O−CH_2); 119·4 (CF_2, t, $^1J_{CF}$ = 280 Hz); 123·42, 128·49, 129·16, 131·60 (C_{arom}); 137·59 (C_{arom}−N, t, J = 6·9 Hz); 158·01 (CF_2-CO, t, J = 30 Hz); 165·27 (Ar−CO_2). HRMS calc for $C_{17}H_{21}F_2NO_3$, 325·1489; found, 325.1486.

N-[2-Thexyldimethylsilyloxymethyl-5-(3-tert-butyloxycarbonyl)-propyloxycarbonyl-phenyl]-3,3-difluoroazetidin-2-one (10c) (method B). Ether : pentane: 1 : 3. Yellowish oil (30 mg; 86%). IR (CH_2Cl_2) 2950, 1785, 1715, 1705 cm^{-1}. ^1H NMR ($CDCl_3$) 0·00 (6H, s, $Si(CH_3)_2$); 0·74 (12H, m, Si−C_6H_{12}); 1·32 (9H, s, $C(CH_3)_3$); 1·54 (1H, m, −CH−); 1·90 (2H, m, $CH_2-CH_2-CH_2$); 2·27 (2H, t, J = 7·35 Hz, CH_2-CO_2); 4·16 (2H, t, J = 6·34 Hz, CF_2-CH_2); 4·23 (2H, t, J = 6·40 Hz, CO_2-CH_2); 4·66 (2H, s, Ar−CH_2O); 7·47 ($1H_{arom}$, d, J = 7·7 Hz); 7·85 ($2H_{arom}$, m). ^{19}F NMR ($CDCl_3$)-115·90 (t, J = 6·4 Hz, CF_2-CH_2).

N-[2-Methyl-5-(3-tert-butyloxycarbonyl)-propyloxycarbonyl-phenyl]-3,3-difluoroazetidin-2-one (2c) (method B). Ether : pentane: 1 : 3. Yellowish crystals (133 mg; 87%), mp 133·9°C. IR CH_2Cl_2) 3680, 3660, 3600, 2950, 1785, 1715, 1705 cm^{-1}. ^1H NMR ($CDCl_3$) 1·36 (9H, s, $C(CH_3)_3$); 1·98 (2H, m, $CH_2-CH_2-CH_2$); 2·31 (2H, t, J = 7·4 Hz, CH_2-CO_2); 2·34 (3H, s, Ar−CH_3); 4·17 (2H, t, J = 6·36 Hz, CF_2-CH_2); 4·26 (2H, t, J = 6·41 Hz, CO_2-CH_2); 7·27 ($1H_{arom}$, s); 7·79 ($2H_{arom}$, d, J = 7·5 Hz). ^{19}F NMR ($CDCl_3$) − 115·78 (t, J = 6·3 Hz, CF_2-CH_2). ^{13}C NMR ($CDCl_3$) 19·11 (Ar−CH_3); 23·94 (−CH_2−); 27·82 ($C(CH_3)_3$); 31·79 (CH_2-CO); 56·65 (CH_2-CF_2, t, J = 29·3 Hz); 64·19 (O−CH_2); 80·32 (C−O); 119·5 (CF_2, t, J = 272·6 Hz); 123·52, 128·52, 128·86, 131·63, 133·5 (C_{arom}); 137·78 (C_{arom}−N); 158·5 (CF_2-CO, t, J = 27·7 Hz); 165·13 (CH_2-CO_2); 171·84 (Ar−CO_2).

Functional modifications of the N-aryl azetidinones

Cleavage of the hydroxyl protective group – 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 the silyl ether (0·19 mmol) dissolved in CH_3CN (1 mL). After stirring for 10 min at 20°C, the mixture was neutralized with 5% $NaHCO_3$ and extracted with ether. The combined organic layers were quickly washed with brine, dried ($MgSO_4$) and evaporated to dryness to yield the expected alcohol which was used in subsequent steps without further purification.

N-(2-Hydroxymethyl-5-methoxyphenyl)-3,3-difluoroazetidin-2-one (11a). Whitish solid (16 mg; 77%), mp 56°C. IR (CH_2Cl_2) 3590, 1775, 1605, 1505 cm^{-1}. ^1H NMR ($CDCl_3$) 2·59 (1H,b s, O−H); 3·75 (3H, s, O−CH_3); 4·22 (2H, t, J = 6·2 Hz, CF_2-CH_2); 4·50 (2H, s, Ar−CH_2O); 6·75 ($1H_{arom}$, d, J = 2·5 Hz); 7·28 ($2H_{arom}$, d, J = 8·8 Hz). ^{19}F NMR ($CDCl_3$) − 116·17 (t, J = 6·4 Hz, CF_2-CH_2). ^{13}C NMR ($CDCl_3$) 55·5 (ArOCH_3), 57·27 (t, J = 26·6 Hz, CF_2CH_2); 61·46 (ArCH_2OH); 107·71–134·37 (C_{arom}); 119·19 (t, J = 280·8 Hz, CF_2), 158·66 (t, J = 30·8 Hz, CF_2CO); 159·88 (C_{arom}−O). MS: m/z = 243 ($M^{+\cdot}$), 164, 123. HRMS for $C_{11}H_{11}FNO_3$ 243·0707; found 243·0697.

N-(2-Hydroxymethyl-5-hexyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one (11b). Colourless oil (70 mg; 72%). IR (CH_2Cl_2) 3590, 3410, 1785, 1715 cm^{-1}. ^1H NMR ($(CD_3)_2CO$) 0·89 (3H, t, J = 6·2 Hz, CH_2-CH_3); 1·36 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·75 (2H, m, OCH_2-CH_2); 3·34 (2H, t, J = 6·6 Hz, CF_2-CH_2); 4·60 (2H, t, J = 6·8 Hz, OCH_2-CH_2); 4·79 (2H, s, CH_2−OH); 7·72 ($1H_{arom}$, d, J = 8 Hz); 7·95 ($1H_{arom}$, dd, J = 1·6, 8 Hz); 8·20 ($1H_{arom}$, d, J = 1·5 Hz).

N-[(5-tert-Butyloxycarbonyl)propyloxycarbonyl-2-hydroxymethyl-phenyl]-3,3-difluoroazetidin-2-one (11c). Colourless oil (34 mg, 73%). IR (CH_2Cl_2) 3670, 3584, 1776, 1710 cm^{-1}. ^1H NMR ($CDCl_3$) 1·38 (9H, s, $C(CH_3)_3$); 2·01 (2H, m, $CH_2CH_2CH_2$); 2·32 (2H, t, J = 7·1 Hz, CH_2CO_2); 4·25 (2H, t J = 6·4 Hz, CF_2CH_2); 4·30 (2H, t, J = 6·5 Hz, OCH_2); 4·65 (2H, s, ArCH_2OH); 7·53–7·94 ($3H_{arom}$, m). ^{19}F NMR ($CDCl_3$) − 115,77 (t, J = 6·3 Hz, CF_2-CH_2). MS m/z = 399($M^{+\cdot}$), 362, 343, 279, 240, 220, 205, 167, 147. HRMS for $C_{19}H_{23}F_2NO_6$: 399·1493; found 399·1493.

Conversion of benzyl alcohol to chloride – general synthetic procedure. To the alcohol dissolved in DMF (0·5 M), was added dropwise the Vilsmeier reagent (1 M, 1·3 eq) prepared by treatment of dry DMF with $SOCl_2$ for 5 min at 4°C. The mixture was stirred for 40 min at room temperature and evaporated to dryness under vacuum (0·1 mm). The crude chloride was purified by thin layer chromatography on silica gel.

N-(2-Chloromethyl-5-methoxyphenyl)-3,3-difluoroazetidin-2-one-(3a). Ether : pentane: 1 : 1. Colourless oil (93 mg; 65%). IR (CH_2Cl_2) 1773, 1600 cm^{-1}. ^1H NMR ($CDCl_3$) 3·74 (3H, s, ArOCH_3); 4·22 (2H, t, J = 6·4 Hz, CF_2CH_2); 4·60 (2H, s, ArCH_2Cl); 6·73–7·26 (m, $3H_{arom}$). ^{19}F NMR ($CDCl_3$) − 116·13 (t, J = 6·3 Hz, CF_2-CH_2). ^{13}C NMR ($CDCl_3$) 43·61 (ArCH_2Cl), 55·64 (ArOCH_3); 57·66 (t, J = 26·6 Hz, CF_2CH_2); 109·27–135·18 ($5C_{arom}$); 119·58 (t, J = 281·9 Hz, CF_2); 159·88 (t, J = 31·2 Hz, CF_2CO); 160·61 (C_{arom}−O). MS m/z = 261($M^{+\cdot}$), 226, 198, 162, 121. HRMS for $C_{11}H_{10}F_2ClNO_2$ 261·0392; found, 261·0368.

N-(2-Chloromethyl-5-hexyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one (3b). Ether : pentane: 1 : 1. Colourless oil (18 mg; 42%). IR (CH_2Cl_2) 1780, 1710 cm^{-1}. ^1H NMR ($CDCl_3$) 0.79 3H, t, J = 6·2 Hz, CH_2-CH_3); 1·35 (6H, m, $CH_2CH_2CH_2-CH_3$); 1·77 (2H, m, OCH_2-CH_2); 4·25 (2H, t, J = 6·4 Hz, CF_2-CH_2); 4·26 (2H, t, J = 6·4 Hz, OCH_2-CH_2); 4·71 (2H, s, Ar−CH_2Cl); 7·46 ($1H_{arom}$, d, J = 8 Hz); 7·86 ($1H_{arom}$, dd, J = 1·6, 8 Hz); 7·93 ($1H_{arom}$, d, J = 1·6 Hz). ^{19}F NMR ($CDCl_3$)-115·0 (t, J = 6·5 Hz, CF_2-CH_2). ^{13}C NMR ($CDCl_3$) 13·78; 22·31; 25·40; 28·37; 31·19; 42·91 (CH_2Cl);

57·53 (CF_2–CH_2, t, J = 26·6 Hz); 65·64 (OCH_2); 119·6 (CF_2, t, $^1J_{CF}$ = 283 Hz); 124·04, 128·70, 131·19, 134·23 (C_{arom}); 136·39 (C_{arom}–N, t, J = 7 Hz); 158·02 (CF_2–CO, t, J = 32 Hz); 165·04 (Ar–CO_2). HRMS for $C_{17}H_{20}ClF_2NO_3$ 359·1099; found 359·1098.

N-*(5-tert-Butyloxycarbonylpropyloxycarbonyl-2-chloromethylphenyl)-3,3-difluoroazetidin-2-one (3c)*. Ether : pentane: 1 : 2. Colourless oil (35 mg; 75%). IR (CH_2Cl_2) 1779, 1709 cm^{-1}. ^1H NMR ($CDCl_3$) 1·37 [9H, s, $C(CH_3)_3$]; 2·00 (2H, m, $CH_2CH_2CH_2$); 2·32 (2H, t, J = 7.1 Hz, CH_2CO_2); 4·26 (2H, t, J = 6·4 Hz, CH_2CF_2); 4·31 (2H, t, J = 6·4 Hz, OCH_2); 4·71 (2H, s, $ArCH_2Cl$); 7·45–7·93 ($3H_{arom}$). ^{19}F NMR ($CDCl_3$)-116·03 (t, J = 6·4 Hz). ^{13}C NMR ($CDCl_3$): 24·07 ($CH_2CH_2CH_2$), 28·00 [$C(CH_3)_3$], 31·95 (CH_2CO_2), 42·99 ($ArCH_2Cl$), 57·17 (t, J = 26·9 Hz, CF_2CH_2), 64·72 ($ArCO_2CH_2$), 80·58 [$C(CH_3)_3$], 119·30 (t, J = 283·6 Hz, CF_2), 124·23–136·69 ($6C_{arom}$), 157·77 [t, J = 33·9 Hz, (CF_2CO)], 164·73 ($ArCO_2$), 171·90 (CO_2tBu). MS m/z = 417 ($M^{+\cdot}$), 361, 258, 194, 149. HRMS for $C_{19}H_{22}ClF_2NO_5$ 417·1153; found 417·1154.

Preparation of functionalized azetidinones with miscellaneous leaving groups at the benzylic position

N-*(2-Fluoromethyl-5-methoxyphenyl)-3,3-difluoroazetidin-2-one (4)*. To diethylaminosulphur trifluoride (DAST; 31 μL, 0·24 mmol) in CH_2Cl_2 at − 65°C was added dropwise (10 min) the alcohol (**11a**) (40 mg, 0·16 mmol) in CH_2Cl_2. The reaction temperature was allowed to rise to 0°C (90 min). The crude oil obtained after concentration under reduced pressure was chromatographed by thin layer chromatography on silica gel to yield the expected fluoride (**4a**). Ether : pentane: 1 : 2 (Rf = 0.31). Colourless oil (11·3 mg; 29%). IR (CH_2Cl_2) 1773, 1600 cm^{-1}. ^1H NMR ($CDCl_3$) 3·77 (3H, s, $ArOCH_3$); 4·24 (2H, dt, J = 0·7, 6·4 Hz, CF_2CH_2); 5·31 (2H, d, J = 48·5 Hz, $ArCH_2F$); 6·76–7·29 ($3H_{arom}$, 0.31 m). ^{19}F NMR ($CDCl_3$) − 116·05 (2F, t, J = 6·3 Hz, CF_2), − 212·49 (1F, t, J = 48·6 Hz, $ArCH_2F$). ^{13}C NMR ($CDCl_3$) 55·67 ($ArOCH_3$), 58·16 (dt, J = 3·4, 28·6Hz, CF_2CH_2); 81·60 (d, J = 165·4 Hz, $ArCH_2F$); 108·53, 113·01 (d, J = 1·7 Hz); 119·81 (t, J = 282·9 Hz,CF_2CO); 120·31 (d, J = 17·5 Hz); 132·81 (d, J = 6·1 Hz), 135·83 (d, J = 3·4 Hz); 158·53 (t, J = 31·6 Hz, CF_2CO); 161·13 (d, J = 3·0 Hz, C_{arom}–O). MS m/z = 245 ($M^{+\cdot}$), 181, 166, 153, 139. HRMS for $C_{11}H_{10}F_3NO_2$ 245·0568; found 245·0663. The symetrical ether by-product (**26**) was similarly isolated, ether : pentane: 1 : 2 (R_f = 0·08) colourless oil (18·7 mg, 50%). IR (CH_2Cl_2) 1775, 1602 cm^{-1}; ^1H NMR ($CDCl_3$) 3·73 (s, 3H, $ArOCH_3$), 4·11 (t, J = 6·4 Hz, 2H, CF_2CH_2), 4·39 (s, 2H, $ArCH_2O$), 6·7–7·18 (m, $3H_{arom}$); ^{19}F NMR ($CDCl_3$)-118·05 (t, J = 6·4 Hz, CF_2); ^{13}C NMR ($CDCl_3$) 55·56 ($ArOCH_3$), 57·76 (t, J = 26·6 Hz; CF_2CH_2), 68·75 [$(ArCH_2)_2O$], 108·76, 112·99 ($2C_{arom}$), 119·80 (t, J = 282·4 Hz, CF_2), 122·45, 132·32 ($2C_{arom}$), 135·19 (t, J = 3·8 Hz, C_{arom}–N), 158·1 (t, J = 31·1 Hz, CF_2CO), 160·10 (C_{arom}–O); MS m/z = 452, 450, 309, 285, 264, 242, 227.

N-*(2-Acetoxymethyl-5-methoxyphenyl)-3,3-difluoroazetidin-2-one* (**5a**). A mixture of *N*-(2-hydroxymethyl-5-methoxyphenyl)-3,3-difluoroazetidin-2-one (**11a**) (50 mg, 0·2 mmol), DMAP (13·7 mg; 0·12 mmol) and acetic anhydride (26 μL, 0·26 mmol) in CH_2Cl_2 was stirred at room temperature for 1 h. After concentration under reduced pressure, the residual oil was purified by thin layer chromatography on silica gel (ether : pentane: 1 : 1) (Rf = 0·40). Yellowish oil (53 mg; 90%). IR (CH_2Cl_2) 3665, 2920, 1775 1730, 1605 cm^{-1}. ^1H NMR ($CDCl_3$) 1·99 (3H, s, CH_3–CO), 3·75 (3H, s, OCH_3); 4·17 (2H, t, J = 6·38 Hz, CF_2–CH_2); 5·04 (2H, s, Ar–CH_2O); 6·78 ($1H_{arom}$, dd, J = 2·5, 8·5 Hz); 6·92 ($1H_{arom}$, d, J = 2·6 Hz); 7·28 ($1H_{arom}$, d, J = 8·5 Hz). ^{19}F NMR ($CDCl_3$) − 116·17 (t, J = 6·3 Hz). ^{13}C NMR ($CDCl_3$) 20·59 (CH_3CO), 55·33 ($ArOCH_3$), 57·43 (t, J = 26·6 Hz, CF_2CH_2), 62·76 ($ArCH_2O$), 108·62–132·61 (C_{arom}), 119·57 (t, J = 283·2 Hz, CF_2), 134 (t, J = 3·8 Hz, C–N), 158·05 (t, J = 31·7 Hz, CF_2CO), 160·18 (C_{arom}–O), 170·21 (CH_3CO_2). MS m/z = 285 ($M^{+\cdot}$), 242, 162, 121, 77, 43. HRMS for $C_{13}H_{13}F_2NO_4$ 285·0811; found 285·0813.

N-*[2-(2,6-Bis(trifluoromethyl)benzoyloxymethyl)-5-methoxyphenyl]-3,3-difluoroazetidin-2-one (6a)*. To chloride (**3a**) (49 mg, 0·19 mmol) dissolved in DMF (0·6 mL), was added dry KF (23 mg, 0·38 mmol). After stirring for 3 min at room temperature, 2,6-bis-trifluoromethylbenzoic acid was added and the stirring was maintained for 15 h. The crude oil obtained after evaporation under reduced pressure was purified by thin layer chromatography on silica gel (ether : pentane: 1 : 2). White solid (67 mg, 73%), mp 85·5°C. IR (CH_2Cl_2) 1774, 1732, 1601 cm^{-1}. ^1H NMR ($CDCl_3$): 3·69 (3H, s, $ArOCH_3$), 4·11 (2H, t, J = 6·4 Hz, CF_2CH_2), 5·34 (2H, s, $ArCH_2O$), 6·71–7·79 (m, $6H_{arom}$). ^{19}F NMR ($CDCl_3$) − 59·72 [s, $(CF_3)_2Ar$], − 116·45 (t, J = 6.3 Hz, $COCF_2$). ^{13}C NMR ($CDCl_3$) 55·32 ($ArOCH_3$); 57·28 (t, J = 26·6 Hz, CF_2CH_2); 65·07 ($ArCH_2O$), 108·21–119·22 (C_{arom}); 119·53 (t, J = 282·4 Hz, CF_2CH_2); 122·66 [q, J = 274·3 Hz, $Ar(CF_3)_2$]; 128·61 (q, J = 32·6 Hz, C_{arom}–CF_3); 129·80–135·67 (C_{arom}); 158·23 (t, J = 32·1 Hz, CF_2CO); 160·70 (C_{arom}–O); 164·42 ($ArCO_2$). MS m/z = 483 ($M^{+\cdot}$), 242, 226, 175, 162, 91. HRMS for $C_{20}H_{13}F_8NO_4$ 483·07193; found 483·07170.

N-*(2-Bromomethyl-5-hexyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one (7b)*. To *N*-(2-methyl-5-hexyloxycarbonylphenyl)-3,3-difluoroazetin-2-one (**2b**) (48 mg; 0·14 mmol) dissolved in the minimum amount of dry CCl_4 was added NBS (33 mg; 0·28 mmol) and benzoyl peroxide (3 mg, 0·1 eq). The mixture was illuminated with a lamp (150 W) and stirred under reflux for 10 h. The succinimide was filtered off and the filtrate was evaporated under reduced pressure. The residue was purified by thin layer chromatography on silica gel (ether : pentane: 1 : 12). Colourless oil (25 mg; 42%). IR (CH_2Cl_2) 3680, 3600, 3080, 1780, 1710 cm^{-1}. ^1H NMR ($CDCl_3$) 0·87 (3H, t, J = 6·7 Hz, CH_2–CH_3); 1·35 (6H, m, $CH_2CH_2CH_2$–CH_3); 1·73 (2H, m, OCH_2–CH_2); 4·14 (2H, t, J = 6·4 Hz, CF_2–CH_2); 4·26 (2H, t, J = 6·8 Hz, OCH_2–CH_2); 4·62 (2H, s, Ar–CH_2Br); 7·43 ($1H_{arom}$, d, J = 8 Hz); 7·83 ($1H_{arom}$, dd, J = 1·6, 8 Hz); 7·91 ($1H_{arom}$, d, J = 1·6 Hz). ^{19}F NMR ($CDCl_3$) − 116·33 (t, J = 6·6 Hz, CF_2–CH_2). ^{13}C NMR ($CDCl_3$) 13·71; 22·24; 25·33; 28·30; 29·53; 31·12; 41·34 (CH_2Br); 56·87 (CF_2–CH_2, t, J = 26·8 Hz); 65·57 (OCH_2); 119·4 (CF_2, t, $^1J_{CF}$ = 280 Hz); 124·14, 128·75, 131·26, 131·74 (C_{arom}); 136·9 (C_{arom}–N, t, J = 7 Hz); 158·01 (CF_2–CO, t, J = 30 Hz); 164·64 (Ar–CO_2). MS m/z = 403 ($M^{+\cdot}$), 384, 375, 324. HRMS calc for $C_{17}H_{20}BrF_2NO_3$ 403·0590; found 403·0595.

Preparation of the free acids (2d) and (3d).
N-(2-methyl-5-carboxypropyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one (**2d**). To a solution of *N*-(2-methyl-5-*tert*-butoxycarbonylpropyloxycarbonylphenyl)-3,3-difluoroazetidin-2-one (**2c**) (15 mg; 0·04 mmol) in CH_2Cl_2 (0·5 mL) at $-15°C$, was added TFA (50 μL; 20 eq). The reaction temperature was allowed to rise to room temperature (30 min). The residue obtained after evaporation under reduced pressure was triturated with ether and the acid was isolated by filtration as a white solid (9·4 mg, 73%). Mp 126·7°C. IR (CH_2Cl_2) 1785, 1715, 1705 cm^{-1}. ^1H NMR ($CDCl_3$) 2·18 (2H, m, $CH_2-CH_2-CH_2$); 2·45 (3H, s, $Ar-CH_3$); 2·54 (2H, t, J = 6·9 Hz, CH_2-CO_2); 4·27 (2H, t, J = 6·34 Hz, CF_2-CH_2); 4·40 (2H, t, J = 6·2 Hz, CO_2-CH_2); 7·34 ($1H_{aromo}$, d, J = 7·9 Hz); 7·90 ($2H_{arom}$, dd, J = 1·6, 9·7 Hz); 10·3 (1H, s, CO_2H). ^{19}F NMR ($CDCl_3$) − 115·78 (t, J = 6·3 Hz, CF_2-CH_2). MS m/z = 327(M^+.) 223, 177, 160, 104, 77, 69. HRMS calc for $C_{15}H_{15}F_2NO_5$ 327·0915; found 327·0918.

N-(5-Carboxypropyloxycarbonyl)-2-chloromethylphenyl)-3,3-difluoroazetidin-2-one (3d). This acid was obtained by acidolysis of *N*-(5-*tert*-butyloxycarbonylpropyloxycarbonyl-2-chloromethylphenyl)-3,3-difluoroazetidin-2-one (**4c**) according to the procedure described for the preparation of compound **2d**. Colourless oil (26 mg, 93%). IR (CH_2Cl_2) 1774, 1705 cm,$^{-1}$. ^1H NMR ($CDCl_3$) 2·06 (2H, m, $CH_2CH_2CH_2$), 2·46 (2H, t, J = 7·1 Hz, CH_2CO_2H); 4·27 (2H, t, J = 6·4 Hz, CF_2CH_2); 4·34 (2H, t, J = 6·2 Hz, CO_2CH_2); 7·44–7·91 (m, $3H_{arom}$); 8·09 (1H, bs, CO_2H). ^{19}F NMR ($CDCl_3$) − 115·92 (t J = 6·3 Hz, CF_2). ^{13}C NMR ($CDCl_3$) 23·75 ($CH_2CH_2CH_2$), 29·53 (CH_2CO_2H), 42·86 (CH_2Cl), 57·18 (t, J = 26·8 Hz, CH_2CF_2), 119·21 (t, J = 282·9 Hz, CF_2), 124·15–136·50 (6 C_{arom}), 157·84 (t, J = 32·8 Hz, CF_2CO), 164·65 (CO_2 and CO_2H). MS m/z = 361(M^+.), 297, 257, 229, 211, 194, 176, 159, 149, 103. HRMS calc for $C_{15}H_{14}ClF_2NO_5$ 361·05612; found 361·05607.

Enzymatic studies

Materials. HLE, PPE and human cathepsin G were purchased from Elastin Products Co, Serva and Calbiochem. Bovine α-chymotrypsin and trypsin were from Sigma. Active-site titrations were performed as described in Wakselman et al (1991) except for cathepsin G (spectrophotometric determination using $A_{280}^{1\%} = 6·64$; Travis et al 1978). The enzymes were assayed spectrophotometrically with *p*-nitroanilide substrates: methoxysuccinyl-alanyl-alanyl-prolyl-valyl-*p*-nitroanilide (MeOSuc-Ala_2-Pro-Val-*p*NA, Sigma) for HLE, succinyl-alanyl-alanyl-alanyl-*p*-nitroanilide (Suc-Ala_3-*p*NA, Sigma) for PPE, succinyl-alanyl-alanyl-prolyl-phenylalanyl-*p*-nitroanilide (Suc-Ala_2-Pro-Phe-*p*NA, Sigma) for human cathepsin G and α-chymotrypsin, benzoyl-arginyl-*p*-nitroanilide (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% $Brij_{35}$, or 0·1 M Hepes, 0·5 M NaCl, 0·1% Tween 80 for HLE and cathepsin G (for each enzyme, identical activities were obtained in both buffers); 0·1 M Tris for PPE; 0·025 M sodium phosphate, 0·05 M KCl for α-chymotrypsin; 0·1 M Tris, 0·1 M $CaCl_2$ for trypsin. The kinetics were run at pH 8·0 and 37°C. The compounds were previously dissolved in organic cosolvent (DMSO).

Spontaneous hydrolysis of N-*aryl-azetidin-2-ones in buffer at pH 8·0 and 37°C.* The decomposition of the synthetic compounds in the buffer solution was measured by following the change of absorbance with time at the appropriate wavelength. Except for **3a** and **4a**, monophasic reaction progress curves were observed corresponding to first-order kinetics described by the kinetic constant k which was calculated by iterative least-squares fits to equation (1):

$$\text{Abs.} = (\epsilon_A - \epsilon_B)[A]_0 e^{-kt} + \epsilon_B[A]_0 \qquad (1)$$

where Abs. is the absorbance of the solution, and ε_A and ε_B the molar absorption coefficients of A and B species (reactant and hydrolysis product, respectively). For these experiments, $[A]_0$ was in the range 30–250 μM. The wavelengths (nm) used were: 290 for **1** and **7**; 240 for **2a**; 322 for **2d**; 310 for **3a** and **4a**; 340 for **3d** and **7b** and 300 for **4** and **5**.

Determination of kinetic parameters for inactivation of HLE and PPE by N-*aryl-azetidin-2-ones at pH 8·0 and 37°C.* For the most active compounds, enzyme inhibition was analysed by the progress curve method as described in Wakselman et al (1991) according to the scheme of equation (2):

$$\begin{array}{l} S \underset{}{\overset{K_S}{\rightleftarrows}} ES \xrightarrow{k_c} P \\ + \\ E \\ + \\ I \underset{}{\overset{K_I}{\rightleftarrows}} E^*I \xrightarrow{k_{inact}} E - I' \end{array} \qquad (2)$$

where S is the chromogenic substrate, I is the inhibitor, ES is the Michaelis complex, E*I is a steady-state inhibitor complex representing a kinetic composite of the non-covalent EI complex and of the acyl-enzyme, and E-I′ is the inactivated enzyme. In our experimental conditions, the Michaelis constant K_M for hydrolysis of MeOSuc-Ala_2-Pro-Val-*p*NA by HLE and Suc-Ala_3-*p*NA by PPE was respectively $1·4 \times 10^{-4}$ M and 2·6 mM at pH 8·0 and 37°C. For experiments with HLE, the concentrations were [E] = 30 nM, [MeO Suc-Ala_2-Pro-Val-*p*NA] = 100 μM, [**3d**] = 320–1600 μM; [**4a**] = 87–583 μM. For experiments with PPE, the concentrations were [E] = 200 nM, [Suc-Ala_3-*p*NA] = 100 μM, [**3a**] = [**3d**] = [**7b**] = 100–200 μM. In all cases, the final DMSO concentration in the reaction mixture was 4–5% (v/v), a concentration which did not significantly affect HLE and PPE activities.

For weaker inhibitors, the classical method of preincubating enzyme and inhibitor was used. The loss of the enzymatic activity with time is a pseudo-first order process characterized by the rate constant k_{obs} which varies with the inhibitor concentration [I] according to equation (3):

$$k_{obs} = k_{inact}[I]/([I] + K_I) \qquad (3)$$

This method was used for the analysis of the inactivation of HLE (1 μM) by **3a**, **3b**, **3c** and **7b** (concentrations in the range 200–800 μM). In all cases, final DMSO percentage in solution was 4-5% (v/v).

Selectivity of inactivation. α-Chymotrypsin, cathepsin G and trypsin were incubated in presence of the inhibitor (range 100–200 μM) during 1 or 15 min in the appropriate buffer before adding the corresponding chromogenic substrate (final DMSO percentage in solution was 2% v/v). For α-chymotrypsin:

[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 μM.

Enzymatic hydrolysis of substrates by elastases at pH 8·0 and 37°C. The enzyme hydrolysis of *N*-aryl-azetidinones catalysed by HLE and PPE was monitored at the following wavelengths: 270 (**2a**), 325 (**2b**), 320 (**2c**, **2d**), 300 (**5**, **6**) and 304 (**5a**, **6a**) nm. The kinetic parameters k_{cat} and K_M were calculated by iterative least-square fits to the Michaelis equation expressed using the active site concentration of enzyme. The initial rate values V, were obtained from equation (4):

$$V(M\ s^{-1}) = \Delta Abs./\Delta t(s)/\Delta\epsilon(M^{-1}\ cm^{-1}) \quad (4)$$

where $\Delta\varepsilon$ is the difference between the molar absorption coefficients of substrate and the product of hydrolysis. Experimental conditions for the kinetic analyses performed with HLE (0·1 M Tris, 0·01% $Brij_{35}$, or 0·1 M Hepes, 0·5 M NaCl, 0·1% Tween 80) and PPE (0·1 M Tris) are listed below (4–5% (v/v) except for **6** –8% v/v): HLE-**2a**: [E] = 3·3 μM, [**2a**] = 40–600 μM, $\Delta\varepsilon = 1542\ M^{-1}\ cm^{-1}$; HLE-**2b**: [E] = 2·7 μM, [**2b**] = 20–65 μM, $\Delta\varepsilon = 2900\ M^{-1}\ cm^{-1}$; HLE-**2c**: [E] = 1·8 μM, [**2c**] = 15–125 μM, $\Delta\varepsilon = 2270\ M^{-1}\ cm^{-1}$; HLE-**2d**: [E] = 2·2 μM, [**2d**] = 70–1400 μM, $\Delta\varepsilon = 2500\ M^{-1}\ cm^{-1}$; HLE-**5**: [E] = 294 nM, [**5**] = 40–1000 μM, $\Delta\varepsilon = 1504\ M^{-1}\ cm^{-1}$; HLE-**5a**: [E] = 32 nM, [**5a**] = 120–800 μM, $\Delta\varepsilon = 2410\ M^{-1}\ cm^{-1}$; HLE-**6**: [E] = 294 nM, [**6**] = 10–50 μM, $\Delta\varepsilon = 2433\ M^{-1}\ cm^{-1}$; HLE-**6a**: [E] = 1·5 μM, [**6a**] = 15–60 μM, $\Delta\varepsilon = 1029\ M^{-1}\ cm^{-1}$; PPE-**2a**: [E] = 1·8 μM, [**2a**] = 40–880 μM, $\Delta\varepsilon = 1463\ M^{-1}\ cm^{-1}$; PPE-**2c**: [E] = 9·7 μM, [**2c**] = 15–125 μM, $\Delta\varepsilon = 2158\ M^{-1}\ cm^{-1}$; PPE-**2d**: [E] = 1·5 μM, [**2d**] = 70–1400 μM, $\Delta\varepsilon = 2393\ M^{-1}\ cm^{-1}$; PPE-**5**: [E] = 252 nM, [**5**] = 40–1000 μM, $\Delta\varepsilon = 1518\ M^{-1}\ cm^{-1}$; PPE-**5a**: [E] = 236 nM, [**5a**] = 100–800 μM, $\Delta\varepsilon = 2126\ M^{-1}\ cm^{-1}$; PPE-**6**: [E] = 252 nM, [**6**] = 10–50 μM, $\Delta\varepsilon = 2347\ M^{-1}\ cm^{-1}$; PPE-**6a**: [E] = 2·0 μM; [**6a**] = 10–80 μM; $\Delta\varepsilon = 1603\ M^{-1}\ cm^{-1}$.

Results

Synthesis

The *N*-aryl azetidinones have been prepared by an efficient four-step synthesis starting from an appropriately substituted aniline: acylation by the 3-bromo-2,2-difluoropropanoyl chloride (Fig. 2, step a), cyclisation of the resulting propionanilide to give the β-lactam ring (step b), deprotection of the hydroxyl group (step c) and functional modification at the benzylic carbon (step d).

The radical bromination of a methylated *N*-aryl azetidinone is an alternative access to functionalized compounds (Zrihen et al 1983). The presence of the *gem*-difluorosubstituted C_3 was expected to discriminate against a possible radical formation at C_4, position which is known to be sensitive to NBS treatment (Easton & Pitt 1990). Indeed we observed that the bromination of compound **2b** occurred only at the benzylic carbon and not at C_4. However, the obtained bromide **7b** has to be separated from the starting unreacted compound and the benzylic *gem*-dibromo derivative.

Preparation of anilines 8a–c

The different routes used to obtain the substituted anilines **8a–c** are shown in Table 2. The 5-methoxy-2-thexyldimethylsilyloxymethylaniline **8a** was prepared starting from 4-methyl-3-nitroanisole. The benzylic bromide **12** resulting from bromination using NBS was substituted with potassium acetate suspended in DMF (Bocchi et al 1979). Methanolysis

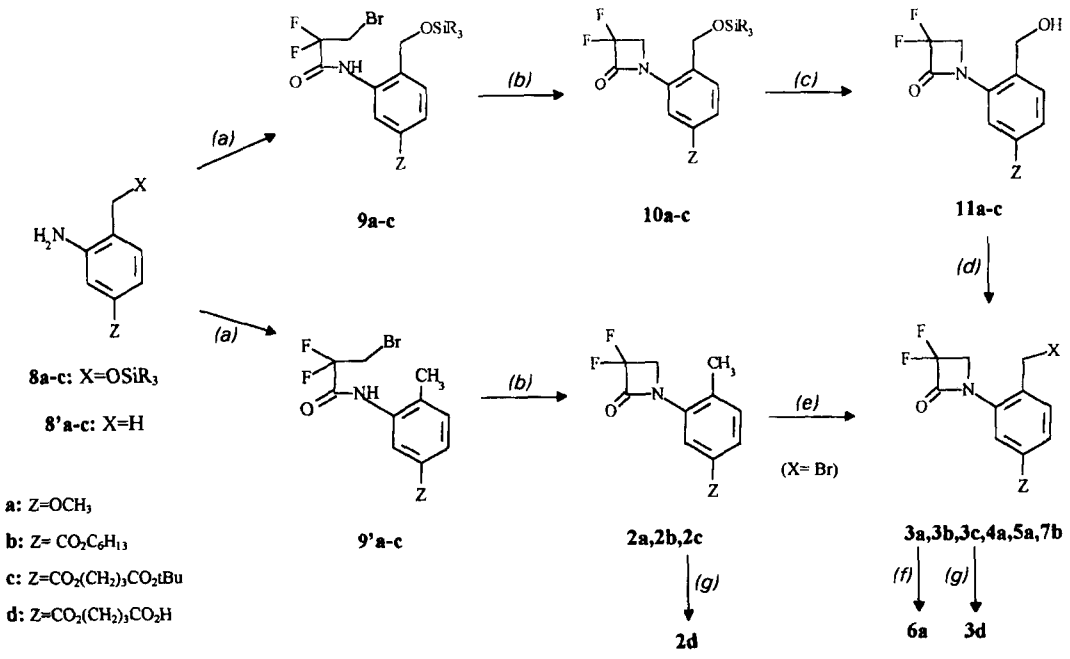
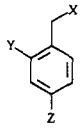


FIG. 2. Synthesis of compounds **2a–d**, **3a–d**, **4a**, **5a**, **6a** and **7b**: (*a*) 1 eq Et_3N/toluene/4°C; (*b*) NaH/DMF/CH_2Cl_2/ − 10°C (method A) or K_2CO_3/acetone/reflux (method B); (*c*) HF/CH_3CN/H_2O/25°C; (*d*) $SOCl_2$/DMF for **3a–c**; Et_2NSF_3/CH_2Cl_2/ − 78°C for **4a**; Ac_2O/DMAP for **5a**; (*e*) NBS/CCl_4/bz_2O for **7b**; (*f*) 2,6-$(CF_3)_2C_6H_4CO_2H$/KF/DMF/25°C; (*g*) TFA/CH_2Cl_2/ − 15°C.

Table 2. Preparation of substituted anilines **8a–c**: R_3: dimethylthexyl; R'_3: dimethyl-*t*-butyl.



Z = OMe	Compound	**12**	→	**13**	→	**14**	→	**15**	→	**8a**		
	Y	NO_2		NO_2		NO_2		NH_2		NH_2		
	X	Br		OAc		OH		OH		$OSiR_3$		
$Z = CO_2C_6H_{13}$	Compound	**16**	→	**17**	→	**18**	→	**19**	→	**8b**		
	Y	NO_2		NO_2		NO_2		NH_2		NH_2		
	X	Br		OCHO		OH		OH		$OSiR'_3$		
$Z = CO_2R$	Compound	**20**	→	**21**	→	**22**	→	**23**	→	**24**	→	**25**	→	**8c**
	Y	NO_2		NO_2		NO_2		NO_2		NO_2		NO_2		NH_2
	X	Br		OAc		OH		$OSiR_3$		$OSiR_3$		$OSiR_3$		$OSiR_3$
	R	Me		Me		Me		Me		H		$(CH_2)_3CO_2tBu$		$(CH_2)_3CO_2tBu$

of the nitroacetate **13** yielded the corresponding nitroalcohol **14** which was catalytically hydrogenated. Then, the aniline **15** was selectively *O*-silylated by the thexyldimethylchlorosilane in presence of imidazole in DMF (Wetter & Oertle 1985).

The hexyl 3-amino-4-*tert*-butyldimethylsilyloxymethylbenzoate **8b** was obtained starting from the corresponding hexyl 4-bromomethyl-3-nitrobenzoate **16** (Table 2). Treatment of **8b** with sodium formate then by methanol/activated alumina (Harris & Bull 1985) allowed a smooth selective cleavage of the benzyl formate function in the presence of the benzoic ester group. The obtained nitro alcohol **18** was then hydrogenated and the *ortho*-hydroxymethylaniline **19** silylated.

By using a bulky protective group for the benzylic hydroxy function, we succeeded to preserve it during the saponification of the ester function of methyl 3-nitro-4-thexyldimethylsilyloxymethylbenzoate **23** (Table 2). After esterification of the acid **24** using DCC with a catalytic amount of DMAP, the nitro substituent was reduced to yield the aniline **8c**. In the esterification step, the alcoholic component, the *tert*-butyl 4-hydroxybutyrate, was prepared by substitution of the *tert*-butyl 4-bromobutyrate with potassium acetate in the presence of Aliquat, then treatment by ethanolamine.

Preparation of the anilides **9** *and* **9′**

The propionanilides **9a–c** and **9′a–c** have been obtained through acylation of the functionalized anilines **8a–c** and of the corresponding methylated anilines **8′a–c** by the 3-bromo-2,2-difluoropropanoyl chloride (Joyeau et al 1988) in presence of triethylamine in toluene.

Preparation of the N*-aryl azetidinones* **2a–c** and **10a–c**

The formation of the β-lactam ring by cyclization of the β-halopropionamides according to Wasserman's procedure features the slow addition of the amide dissolved in a mixture of DMF and CH_2Cl_2 to NaH suspended in the same solvent mixture (mode A) (Wasserman et al 1979). This method proved to be unsuitable for the cyclization of *N*-(5-methoxy-2-thexyldimethylsilyloxymethylphenyl)-3-bromo-2,2-difluopropionamide **9a**. Under these conditions, very polar species were formed, probably resulting from β-lactam ring opening. The NH of a 2,2-difluoropropionanilide being more acidic than that of an unhalogenated propionamide, we anticipated that the substitution could take place using milder basic conditions. Indeed, treatment by K_2CO_3 in refluxing acetone, recommended for β-lactam ring closing through N-C_4 bond formation from propionamide derivatives possessing an acidic NH (Evans & Sjogren 1986) led to the expected azetidinone **10a** with a good yield (90%; mode B). The method has been also successfully applied to the preparation of azetidinones **2c**, **10c** and **10′c**.

Selective cleavage of the trialkylsilyl groups

In the β-lactam field, hydrofluoric acid is known to cleave alkoxytrialkylsilane functions without ring opening (Ongania 1985). In aqueous acetonitrile, the thexyldimethylsilyl- and *tert*-butyldimethylsilyl ethers **10a–c** yielded the alcohols **11a–c** while safeguarding the β-lactam nucleus, and also the *tert*-butyl ester group in the case of compound **11c**.

Functional modifications at the benzylic position

Alcohols **11a–c** were easily converted to the chlorides **3a–c**, using the Vilsmeier reagent ($DMF/SOCl_2$) (Hudson & de Spinoza 1976). The treatment of the alcohol **11a** with DAST yielded the fluoride **4a** along with the symmetrical ether **26**, from which it was easily separated (Hudlicky 1988). The acetate **5a** was prepared from **11a** using acetic anhydride in presence of DMAP. The 2,6-*bis*-(trifluoromethyl)-benzoate group was introduced through substitution of the benzylic chloride **3a** by the acid in presence of potassium fluoride, to give azetidinone **6a** (Fig. 2, step f). The synthesis of unsubstituted parent azetidinones **2**, **4** and **7** (Z = H) has been previously reported (Vergely et al 1995), and that of azetidinones **5** and **6** will be described in due course.

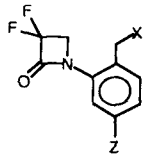
Other transformations

For the azetidinones **2c** and **3c**, the deprotection of the *tert*-butyl ester group to give the corresponding acids **2d** and **3d** (Fig. 2, step g) was achieved by treatment with trifluoroacetic acid.

Enzymatic studies

Solubility and stability of studied compounds. Although compounds **2d** and **3d** were hydrosoluble, they were studied in the the presence of DMSO (4–5% v/v) in order to run kinetics in

Table 3. Kinetic parameters for the inactivation of HLE and PPE by N-arylazetidin-2-ones at pH 8·0 and 37°C. Half-life times $t_{\frac{1}{2}}$ of the spontaneous hydrolysis of the synthetic compounds in the buffer (pH 8·0 and 37°C).



				HLE			PPE		
n°	X	Z	$t_{\frac{1}{2}}$ (min)	$k_{inact} \times 10^3$ (s^{-1})	K_I (μM)	k_{inact}/K_I ($M^{-1} s^{-1}$)	$k_{inact} \times 10^3$ (s^{-1})	K_I (μM)	k_{inact}/K_I ($M^{-1} s^{-1}$)
1*		H	151	35	120	292	80	270	296
3a		OCH_3	***			43			25
3b	Cl	$CO_2C_6H_{13}$	n.d.			1			n.d.
3c		$CO_2(CH_2)_3CO_2tBu$	n.d.			< 1			n.d.
3d		$CO_2(CH_2)_3CO_2H$	210	16	402	40			24
4**	F	H	345			29			< 1
4a		OCH_3	***	26	387	67			n.d.
5	OAc	H	196			0			0
5a		OCH_3	> 500			0			0
6	$OCOC_6H_3(CF_3)_2$	H	> 500			0			0
6a		OCH_3	> 500			0			0
7**	Br	H	19	57	107	533	69	94	740
7b		$CO_2C_6H_{13}$	2			3			25

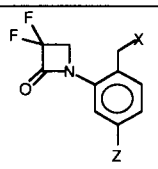
* Wakselman et al (1991); ** Vergely et al (1995); *** more than two consecutive reactions during hydrolysis; n.d.: not determined; standard deviations were less than 7% for $t_{\frac{1}{2}}$, 20% for k_{inact}, 25% for K_I, and 18% for k_{inact}/K_I (five to ten inhibitor concentrations were tested for each experiment).

similar conditions for all compounds. Molecules **2b–c** and **3d** displaying an ester substituent Z, and **6** and **6a** with a benzoate derivative as leaving group were the less soluble molecules (solubility limit were 65, 125, 50, 60 and 100 μM, respectively). The hydrolysis of the amide bond of the *N*-arylazetidin-2-ones with subsituent Z ≠ OMe resulted in a characteristic increased absorption within the range 280–300 nm. Unhydrolyzed compounds with Z = OMe (**3a**, **4a**, **5a**, **6a**) displayed a peak of absorbance at ~290 nm due to the contribution of the +M effect. At 37°C and pH 8·0, most of the studied *N*-arylazetidin-2-ones (**1–6a**) are chemically stable with a half-life time in the range 114 (**2a**) to > 500 min (**2**, **2b**, **2c**, **5a**, **6**, **6a**) (Tables 3 and 4). When X = Br (**7**, **7b**), the chemical stability was decreased ($t_{\frac{1}{2}}$ = 19 and 2 min, respectively). Instability was related to the nature of the leaving group X and of the aromatic substituent Z (see Tables 3 and 4). In conclusion, no noticeable spontaneous hydrolysis occurred during the course of enzyme inhibition experiments.

Inactivation of elastases by 1, 3a–d, 4–4a, 5–5a, 6–6a, 7–7b. All compounds with a halogen atom as leaving group (**1**, **3a–d**, **4–4a**, **7–7b**) behaved as irreversible inhibitors of HLE and PPE. When the leaving group was acetate (**5**, **5a**) or a benzoate derivative (**6**, **6a**), no inhibition of either elastase was observed. Nevertheless, when they were incubated with these enzymes, enzymatic hydrolysis was detected with an increased absorption at characteristic wavelengths allowing an easy determination of the kinetic constants for this enzymatic hydrolysis. The inactivators fulfilled criteria expected for suicide inactivation. Inhibitions of elastases were a first-order process with saturation kinetics. They were irreversible since elimination of the inhibitor by filtration-centrifugation (Centricon 10) did not lead to recovery of enzyme activity. Substrates protected the enzymes against inactivation by *N*-arylazetidin-2-ones. No reactivation after treatment of the inactivated enzymes by buffered hydroxylamine was observed, that was in agreement with a chemical modification of a residue at the active site. The kinetic parameters k_{inact} and K_I were determined using the progress curve or the preincubation methods (Table 3). Electronegativity and steric factors of the leaving group influenced the inhibitory efficiency of these compounds: comparison of results for **1**, **4**, **5**, **6** and **7**. With Z ≠ H, the inhibitory efficacy was decreased by a factor ~4 to ~300 towards HLE and ~12 to ~300 towards PPE when the leaving group was Cl or Br. Conversely, with X = F, an increase of HLE inactivation was observed (**4a** compared to **4**). The most active compound was **7**. When observed, the inactivation of elastases was specific since chymotrypsin- and trypsin-like enzymes were not inactivated. In conclusion, the inhibitory efficiency of these compounds were influenced by the properties of the leaving group (electronegativity and steric factors).

Enzyme-catalysed hydrolysis of 2–2d, 5–5a and 6–6a. The kinetic parameters for the enzymatic hydrolysis are summarized in Table 4. A similar efficiency of hydrolysis in the absence (X = H, **2** and **2a**) and the presence of the leaving group $OCOC_6H_3(CF_3)_2$ (**6–6a**) was noticed. A complex hydro-

Table 4. Kinetic parameters for the enzyme-catalyzed hydrolysis of *N*-arylazetidin-2-ones (X ≠ halogen) at pH 8·0 and 37°C. Half-life times $t_{\frac{1}{2}}$ of the spontaneous hydrolysis of the synthetic compounds in the buffer (pH 8·0 and 37°C).



				HLE			PPE		
n°	X	Z	$t_{\frac{1}{2}}$ (min)	$k_{cat} \times 10^3$ (s^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1} s^{-1}$)	$k_{cat} \times 10^3$ (s^{-1})	K_M (μM)	k_{cat}/K_M ($M^{-1} s^{-1}$)
2*	H	H	> 500			1100			500
2a		OCH_3	114			619	1598	4033	396
2b		$CO_2C_6H_{13}$	114			130			N.H.
32c		$CO_2(CH_2)_3CO_2tBu$	> 500	206	304	678	18	135	133
2d		$CO_2(CH_2)_3CO_2H$	295	5551	7061	786			92
5**	OAc	H	196	15 739	3911	4024			2160
5a**		OCH_3	> 500			5393			1704
6	$OCOC_6H_3(CF_3)_2$	H	> 500			2232			544
6a		OCH_3	> 500			710	69	91	760

* Wakselman et al (1991); N. H.: no hydrolysis; standard deviations were less than 7% for $t_{\frac{1}{2}}$, 14% for k_{cat}, 23% for K_M, and 9% for k_{cat}/K_M (five to ten substrate concentrations were tested for each experiment); ** approximate molar absorption coefficient (see text).

lytic process was observed for *N*-aryl-azetidin-2-ones displaying an acetate as leaving group (**5**, **5a**): two successive reactions were detected, the first one being the hydrolysis of the amide bond of the lactam ring. In these cases, approximated values of the molar absorption coefficient were used in calculations. As a specific cleavage at the amide bond of the β-lactam ring was demonstrated, we could assume that the same initial cleavage occurred in the course of inactivation by the structurally related functionalized *N*-arylazetidin-2-ones. If Z group is a methoxy, a neutral ester or an ester bearing a carboxylate anion, the efficiency of hydrolysis by both elastases was significantly decreased (compared to Z = H). A better affinity for elastases was observed for the neutral ester (**2c**) than for the charged analog (**2d**) but this advantage was counterbalanced by a lower k_{cat} value. Lower ratios k_{cat}/K_M for PPE compared to that for HLE were frequently observed, particularly with bulky ester substituents (**2b–d**). The best example was the absence of hydrolysis of the compound **2b** in the presence of PPE.

Discussion

Variation of the nature of the Z substituent

The methoxy group as Z substituent was introduced to enhance the departure of the leaving group X, thus to increase the efficiency of the inhibition. However, the expected effect was not observed. Substituents Z as ester (neutral or charged) were tested as we assumed that this group could fit the hydrophobic cavity of the active site of HLE (subsites S_3–S_5). We postulated that the anionic charge of the ester of **3d** could interact with Arg217 located in S_4–S_5 subsites. These inactivators (**3b–d**) have a lower inhibitory efficiency than compound **1** (Z = H). Enzymatic hydrolysis of the corresponding susbrates (**2a–d**) was observed with a ratio k_{cat}/K_M close to that of compound **2** (Z = H). This demonstrates that the weak inhibitory potency of compounds **3b–d** is not due to the absence of enzyme recognition or a lack of formation of the acyl-enzyme. During the lifetime of the acyl-enzyme, the alkylation is probably prevented by an incorrect position of the benzylic group towards His57 due to the steric hindrance of the Z group. We previously demonstrated that this active site amino acid group was alkylated by the *N*-arylazetidin-2-one **1** with X = Cl and Z = H (Vergely 1994).

Variation of the nature of the leaving group X

Inactivation is found to be dependent on the nature and nucleofugicity of the leaving group, especially when X is not an halogen atom. Compounds **5–5a** and **6–6a** synthesized as potential inactivators behave only as substrates of elastases. The ester leaving groups OAc (**5–5a**) and $OCOC_6H_3(CF_3)_2$ (**6–6a**) display some differences: acetate is a small and poor leaving group (pKa = 4·76; Albert & Serjeant 1971) whereas the disubstituted benzoate is a bulky and apparently better leaving group (pKa = 0·58; Krantz et al 1991). With X = OAc, we hypothesize that either OAc is not eliminated, or a nucleophilic attack mediated by the enzyme occurs on the carbonyl group of acetate. The second hypothesis seems to be probable since two successive reactions are observed when **5** and **5a** are incubated in presence of enzyme. Interestingly, in a series of cyclopeptidic suicide substrates of trypsin-like enzymes (Reboud-Ravaux et al 1991; Wakselman et al 1993), compounds with an acetate leaving group (c[-Gly_2-Arg(or-Lys)-3-Abz(6-CH_2OCOCH_3)-Gly_2-]) do not behave as inactivators. In the case of the bis(trifluoromethyl)benzoates, steric hindrance probably prevents nucleophilic attack on the carbonyl function. For peptidyl affinity label inactivators (Smith et al 1988; Krantz et al 1991) and benzisothiazolones (Subramanyam et al 1994) bearing this benzoate derivative as leaving group, a lack of inhibition of HLE was also noticed.

For compounds **6–6a**, either the leaving group is not sufficiently labile, or the departure of the benzoyl group is impeded by stabilization in the active site by interaction between enzyme and the *ortho* CF_3 groups.

Acknowledgements

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