J. Pharm. Pharmacol. 1998, 50: 593–600
Received October 20, 1997
Accepted February 18, 1998

Stereoselective Synthesis of Peptidyl Trifluoromethyl Alcohols and Ketones: Inhibitory Potency Against Human Leucocyte Elastase, Cathepsin G, Porcine Pancreatic Elastase and HIV-1 Protease

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Abstract

New fluorinated inhibitors have been designed to target two major proteases—human leucocyte elastase and HIV-1 protease.

Two series of β-peptidyl trifluoromethyl alcohols (TFMAs) Z-L-Val-NH-*CH(Y)-*CH(OH)-CF_3, where *C is the chiral centre, varied in the nature of the substituent Y, a phenylethyl [-$(CH_2)_2$-C_6H_5] or an isopropyl [-$CH(CH_3)_2$] group. These TFMAs were first synthesized as two pairs of the *syn* and *anti* diastereoisomers. The inhibitory effects of these mixtures were then assessed on three serine proteases chosen on the basis of the aromatic and aliphatic nature of the substituents—human leucocyte elastase (HLE), human cathepsin G (HCG) and porcine pancreatic elastase (PPE). In the presence of detectable inhibition, each epimer at C2 was separated to determine its inhibition constant (K_i) towards HLE, HCG and PPE. The stereoisomerically pure TFMAs were then oxidized into peptidyl trifluoromethyl ketones (TFMKs) for similar inhibition assays. The absolute configuration of the compounds remained unknown. One epimer at C2 of each *syn* and *anti* TFMA with the phenylethyl substituent behaved as a competitive inhibitor towards HLE and HCG with inhibition constants below the millimolar range, whereas their TFMK counterparts were non-inhibitors. In the second series, the two ketones inhibited both elastases with K_i values in the micromolar range, whereas only the *syn* TFMA was active towards HLE ($K_i = 5{\cdot}65 \times 10^{-4}$ M). The tested compounds also had structural properties compatible with recognition by HIV-1 protease. The inhibition of the enzyme was observed with TFMK only (IC50 = 15–200 μM). The phenylethyl substituent promoted inhibition by a factor of 10 (IC50 = 15 μM) compared with the isopropyl substituent (IC50 = 200 μM) leading to selective inhibition of HIV-1 protease.

Isomerically pure TFMKs were more potent towards HLE than the alcohols from which they were obtained. However, an enantiomerically pure TFMA selectively inhibited HLE unlike its TFMK analogue which also inhibited PPE. This last result together with the selective inhibition of HIV-1 protease by TFMK with a phenylethyl substituent might be relevant to the design of specific HLE and HIV-1 inhibitors as therapeutic agents.

In this study our three target serine proteases were human leucocyte elastase (HLE, EC 3.4.21.37), pancreatic porcine elastase (PPE, EC 3.4.21.36) and human leucocyte cathepsin G (HCG, EC 3.4.21.20). PPE and HLE share 43% sequence homology and are structurally very similar (Bode et al 1989). HLE and HCG originate from the azurophilic granules of polymorphonuclear neutrophils (Dewald et al 1975). HLE preferentially cleaves

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after aliphatic amino acid residues of medium size such as valine, PPE after aliphatic amino acids of smaller size such as alanine or norvaline; HCG has chymotrypsin-like specificity, favouring bulkier aliphatic or aromatic residues, such as phenylalanine, tryptophan or leucine, in this position (Powers et al 1977).

During inflammation, elastin and other essential components of the connective tissue are degraded by HLE released in abundance by polymorphonuclear neutrophils (Bieth 1989). HCG is thought to act in synergy with HLE in these degradative processes (Boudier et al 1981). The breakdown of the extracellular matrix by HLE is usually prevented by α_1-proteinase inhibitor, the primary physiological plasmatic inhibitor of this elastase (Travis & Salvesen 1983). However, genetic or functional deficiencies of α_1-proteinase inhibitor can occur and are considered to be responsible for inflammatory diseases such as pulmonary emphysema and chronic bronchial injury (Snider 1987).

In recent years, considerable efforts have been made to design synthetic inhibitors of HLE as drugs for elastase-induced diseases (Edwards & Bernstein 1994). The design of several peptidic inhibitors has been based on the structural requirements of the binding sites of HLE for its peptidic substrates (Bode et al 1989). HLE inhibitors include, among others, irreversible inhibitors such as peptidyl chloromethylketones (Powers et al 1977) and mechanism-based inhibitors such as monocyclic β-lactams (Maillard et al 1990; Doherty et al 1993). Efficient reversible inhibitors have also been reported, for example trifluoroacetylpeptide anilides (Mattos et al 1995; Amour et al 1996).

Transition-state analogues such as peptidyl trifluoromethyl ketones (TFMKs, Imperiali & Abeles 1986; Stein et al 1987) are a powerful class of inhibitors because they match the tetrahedral intermediate occurring during proteolytic hydrolysis of a peptide substrate and thus fit efficiently into the active site of the enzyme (Krantz 1992). Because it is the serine protease that transforms TFMKs into stable tetrahedral structures, they are considered to be irreversible inhibitors (Krantz 1992). They inhibit HLE by combining with the active-site serine (Ser-195) to form a hemiketal that resembles the transition state resulting from acylation of serine by peptide substrates. The mechanism proposed involves initial binding of the unhydrated TFMK to the enzyme followed by the reversible formation of the hemiketal by addition of the Oγ of Ser-195 (Brady & Abeles 1990). The X-ray crystal structures of the complexes of PPE with Ac-L-Ala-L-Pro-L-Val-CF_3 (Takahashi et al 1988), and of chymotrypsin with Ac-D,L-Phe-CF_3 or with Ac-L-Leu-D,L-Phe-CF_3 (Brady et al 1990) confirmed the formation of the tetrahedral ketal carbon of the inhibitors. A new method for the stereoselective synthesis of peptidyl trifluoromethyl ketones enabled the preparation of the two TFMKs of absolute stereochemistry Z-L-Val-L-Pro-L-Val-CF_3 and Z-L-Val-L-Pro-D-Val-CF_3 (Edwards 1992). Their respective inhibition activities were evaluated towards HLE. The isomer with the natural L configuration had a K_i of 0·8 nM, which was two hundred times more potent than the D isomer. This study thus revealed the importance of the chirality of the carbon Cβ adjacent to the ketone on the affinity with HLE. In contrast, only racemic mixtures of related β-peptidyl trifluoromethyl alcohols (TFMAs) have been evaluated towards chymotrypsin (Imperiali & Abeles 1986) and towards HLE (Stein et al 1987). Although they were found to be less active than their corresponding TFMKs, the influence of their stereochemistry on inhibitory potency remained unknown. Some difluoroketone peptides have been described as very potent inhibitors of HIV-1 protease (Sham et al 1993). This protease, activity of which is a prerequisite for viral replication, is a major target against AIDS. Because of their low molecular weights it is hoped that the biodisposition of TFMAs and TFMKs will be better than that of difluoroketone peptides.

In this study, we investigate the inhibition properties towards serine proteases and HIV-1 protease of enantiomerically pure TFMAs and compared their activity with those of the corresponding TFMKs. Two different series of TFMAs were first synthesized as mixtures of *syn* or *anti* enantiomers. The inhibition activities of these mixtures were then checked on HLE, HCG and PPE. When inhibition was detected, the enantiomers were separated, thus enabling the determination of the inhibition constants (K_i) of the stereoisomerically pure TFMAs. All four TFMKs were assayed as isomerically pure forms. The aim of this study was to compare the influence of the chirality of these compounds on their inhibition of the three serine proteases chosen. However, the absolute stereochemistry of all the compounds remained undefined.

In the first series, the four TFMA stereoisomers of Z-L-Val-NH-C*H(CH_2-CH_2-C_6H_5)-C*H(OH)-CF_3 were obtained—the *syn* pair **1a** and **1b** and the *anti* pair **2a** and **2b**. The corresponding ketones were **3a** and **3b**. In the second series, the ethylphenyl group was replaced by an isopropyl group. The TFMAs obtained were the *syn* pair **4a** and **4b** and the mixture of the *anti* stereoisomers **5**, epimers at C2. The two TFMK isomers **6a** and **6b** were also prepared.

Materials and Methods

Enzyme-inhibition procedures

The enzymes HLE, PPE and HCG were purchased from Elastin Products (Owensville, MO), Serva (Fontenay-sous-Bois, France) and Novabiochem (Meudon, France), respectively. Their active site concentrations were 20, 16 and 25 nM, respectively. Their chromogenic substrates (Sigma, Saint-Quentin-Fallavier, France) were methoxy- succinyl-alanyl-alanyl-prolyl-valyl-*p*-nitroanilide (CH_3O-Suc-Ala-Ala-Pro-Val-*p*-NA) used at concentrations ranging from 0·05 to 1 mM for HLE, *N*-succinyl-alanyl-alanyl-alanyl-*p*-nitroanilide (0·05 to 1 mM) for PPE and *N*-succinyl-alanyl-alanyl-prolyl-phenylalanyl-*p*-nitroanilide (5 to 50 μM) for HCG. The enzymes were assayed at pH 8·0 and 25 °C in 1 mL buffer—0·1 M HEPES, 0·5 M NaCl, 0·1% (v/v) Tween 80, 2% (v/v) DMSO for HLE and HCG and 0·1 M Tris, 4% (v/v) DMSO for PPE. The amidolytic activities of the three proteases towards their respective chromogenic substrates were followed by use of a Lambda 5 Perkin-Elmer UV-vis spectrophotometer equipped with a thermo- statted holder. The initial velocity, v, was determined by continuous monitoring of the release of *p*-nitroaniline at 405 nm in the presence of different concentrations of inhibitors: 0–120 μM for **1a**, 0–60 μM for **1b**, 0–300 μM for **2a**, 0–400 μM for **2b**, 0–300 μM for **3a**, 0–500 μM for **3b**, 0–300 μM for **4a**, 0–450 μM for **4b**, 0–200 μM for **5**, 0–100 μM for **6a** and 0–200 μM for **6b**. Control experiments without inhibitor were performed under the same conditions.

HIV-1 protease was kindly supplied by Rhône Poulenc Rorer (Vitry-sur-Seine, France). Kinetic assays were performed with the chromogenic substrate VSQNF(NO_2)PIV as described by Quasmi et al (1997). To determine IC50 (the concentration resulting in 50% inhibition), percentage inhibition was measured with different concentrations of inhibitors—1·25 to 12·5 μM **3a** and **3b** and 62·5–250 μM **6a** and **6b**.

Kinetic analysis of enzyme inhibition

The double-reciprocal plots of v against substrate concentration were straight lines with x intercept of $-1/K'_M$. The apparent Michaelis constants K'_M, equal to $K_M \times (1 + [I]_0/K_i)$, and the maximum velocity V_M were estimated by iterative least-squares fitting to the equation for competitive inhibition $v = V_M \times [S]_0/([S]_0 + K'_M)$ using the software package KaleidaGraph 2.1.3 (Abelbeck Software, Reading, PA). The constant K_i and its standard deviation were determined from the linear plot of K'_M/V_M against inhibitor concentration (least-squares analysis). Initial estimates of K_i were calculated from Dixon plots. Determination of IC50 values was performed using at least four concentrations of inhibitor, and by fitting the data to the equation:

$$\% \text{ inhibition} = (100 \times [I]_0)/(IC50 + [I]_0).$$

Chemical synthesis

Procedure. Melting points were recorded on a Köfler apparatus. ^{19}F NMR spectra were obtained at 84·6 MHz and ^{19}F chemical shifts are reported in ppm, negative up-field relative to internal $CFCl_3$. The ^1H NMR and ^{13}C NMR measurements were performed on Brüker AC200 and ARX400 instruments. The chemical shifts are reported in ppm, either downfield from tetramethylsilane (TMS) internal standard, or from the attributed chemical shifts of the deuterated solvents: $CDCl_3$ (7·26 ppm) and CD_3OD (3·50 ppm), as indicated. The coupling constants are given in Hz. Analytical thin-layer chromatography (TLC) was performed on aluminium plates coated with silica gel containing a fluorescent indicator (no. 5554, Merck; 254 nm). For all compounds visualization of the spots after TLC runs was performed by illumination with UV light (254 nm) or by spraying with ninhydrin and heating.

Because the Dess-Martin periodinane 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one is no longer commercially available it was prepared in our laboratory by the modified procedure reported elsewhere (Ireland & Liu 1993).

The *syn* and *anti* trifluoromethyl amino alcohols **7a** and **7b**, required for the peptidic coupling reaction, were prepared according to the general procedure reported by our laboratory (Bégué et al 1992).

syn *and* anti N-*Benzyloxycarbonyl*-N-*(3,3,3-trifluoro-2-hydroxy-1-(isopropyl)-propyl)* L-*valinamides* ***4*** *and* ***5***. A solution of trifluoromethyl amino alcohol **7a** or **7b** (0·5 g; 2·9 mmol), CBZ-L-valine (0·7 g; 2·8 mmol) and 1-hydroxybenzotriazole (HOBT; 0·4 g; 2·9 mmol) in CH_2Cl_2 (5 mL) was cooled to 0 °C and dicyclohexylcarbodiimide (DCC; 0·6 g; 2·9 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. After extraction with AcOEt, the organic layer was washed successively with 10% aqueous HCl, a saturated solution of $NaHCO_3$ and brine, and dried over $MgSO_4$. The residue was then chromatographed on silica gel (CH_2Cl_2-AcOEt, 85 : 15) to give alcohol **4** or **5**.

The mixture of the two diastereoisomers *syn* β-peptidyl alcohols **4a** and **4b** (0·9 g; 75%) were obtained as a white powder. The diastereoisomers were separated by re-crystallization from CH_2Cl_2-cyclohexane (1 : 5); mp **4a** 116–118 °C, **4b** 100–102 °C.

The mixture of the two diastereoisomers *anti* β-peptidyl alcohols **5** (0·6 g; 51%) were obtained as a white powder and were not separated; mp 150–152 °C.

syn *β-Peptidyl alcohols **4a** and **4b***

*Diastereomer **4a** (*RS *or* SR*).* ^{1}H NMR ($CDCl_3$; TMS): 0·95, d (6·8), 12H ($4CH_3$); 1·67, m, 2H (2NH); 2, m, 2H ($CH(CH_3)_2$); 3·8–4·1, m, 2H (CHNH); 5, AB system (12·1), 2H (OCH_2Ph); 5·45, m, 1H ($CHCF_3$); 7·2, m, 5H (H arom.). ^{13}C NMR ($CDCl_3$): 17·8; 17·9; 18·7; 18·9; 19·1; 29·9; 53·6; 61·1; 67·2; 68·5, q ($^2JCF = 29$ Hz), $CHCF_3$; 125, q ($^1JCF = 286$ Hz), CF_3; 127·8; 128·1; 128·4; 135·9; 156·8; 171·8. ^{19}F NMR ($CDCl_3$, $CFCl_3$): −77·1, d ($^3JHF = 6·9$ Hz). Analysis: calc. for $C_{19}H_{26}F_3N_2O_4$ C, 56·57%; H, 6·50%; N, 6·94%; found C, 56·60%; H, 6·54%; N, 7·08%.

*Diastereoisomer **4b** (*SR *or* RS*).* ^{1}H NMR ($CDCl_3$; TMS): 0·95, m, 12H ($4CH_3$); 2, m, 2H (2NH); 2·2, m, 2H ($CH(CH_3)_2$); 4–4·2, m, 2H (CHNH); 5·15, AB system (12·4), 2H (OCH_2Ph); 5·5, m, 1H ($CHCF_3$); 7·2, m, 5H (H arom.). ^{13}C NMR ($CDCl_3$): 18; 18·2; 18·7; 18·8; 20·2; 31; 54; 60; 68; 68·7, q ($^2JCF = 30$ Hz), $CHCF_3$; 124, q ($^1JCF = 286$ Hz, CF_3); 127·8; 128·2; 128·4; 136; 157; 172. ^{19}F NMR ($CDCl_3$, $CFCl_3$): −77·2, d ($^3JHF = 7$ Hz). Analysis: calc. for $C_{19}H_{26}F_3N_2O_4$, C, 56·57%; H, 6·50%; N, 6·94%, found C, 56·62%; H, 6·52%; N, 7·05%.

*Anti β-peptidyl alcohols **5** (*SS *and* RR*).* ^{1}H NMR ($CDCl_3$; TMS): 0·9, m, 12H ($4CH_3$); 1·65, m, 2H (2NH); 1·9–2·1, m, 2H ($CH(CH_3)_2$); 4, m, 2H (CHNH); 5, s, 2H (OCH_2Ph); 5·25, m, 1H ($CHCF_3$); 7·25, m, 5H (H arom.). ^{13}C NMR ($CDCl_3$): 19·6; 19·9; 20·2; 20·3; 29·4; 30·8; 57·7; 62·1; 68·3; 70, q ($^2JCF = 30$ Hz), $CHCF_3$; 124·6, q ($^1JCF = 290$ Hz), CF_3; 128·4; 129; 136; 136·8; 157·6; 173·9. ^{19}F NMR ($CDCl_3$, $CFCl_3$): −74·3, d ($^3JHF = 7$ Hz).

*Oxidation of β-peptidyl trifluoromethyl alcohols **1** and **4** to their corresponding ketones **3** and **6**.* A solution of alcohol **1** or **4** (0·4 mmol) in CH_2Cl_2 (5 mL) was added to a stirred suspension of Dess-Martin periodinane (4 equiv) in CH_2Cl_2 (5 mL) and stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and poured into saturated aqueous $NaHCO_3$ (20 mL) containing a sevenfold excess of $Na_2S_2O_3$ (15 mL). The organic layer was washed with brine and dried over $MgSO_4$. The residue was chromatographed on silica gel (CH_2Cl_2-*i*-PrOH, 95 : 5). The TFMA **1a** (0·2 g) was treated with Dess-Martin reagent (0·680 g) to give, after chromatography on SiO_2, the ketone **3a** (0·127 g; 93%) as a white powder, mp 146–148 °C. The TFMA **1b** (0·2 g) was treated with Dess-Martin reagent (0·680 g) to give, after chromatography on SiO_2, the ketone **3b** (0·120 g; 85%) as a white powder, mp 130–132 °C. The TFMA **4a** (0·160 g) was treated with Dess-Martin reagent (0·680 g) to give, after chromatography on SiO_2, the ketone **6a** (0·130 g; 80%) as a white powder, mp 100–102 °C. The TFMA **4b** (0·160 g) was treated with Dess-Martin reagent (0·680 g) to give, after chromatography on SiO_2, the ketone **6b** (0·140 g; 90%) as a white powder, mp 108–110 °C.

N-*Benzyloxycarbonyl*-N-*(3,3,3-trifluoro-2-hydroxy-2-oxo-1-(phenylethyl)-propyl)* L-*valinamides **3a** and **3b**. **3a** (*S *or* R*).* ^{1}H NMR (CD_3OD; TMS): 0·9, d (6·7), 6H ($2CH_3$); 1·7–1·95, m, 3H ($BnCH_2$ and $CH(CH_3)_2$); 2·5, m, 2H ($PhCH_2$); 3·9, m, 2H ($CHCOCF_3$ and CH-val); 5, AB system (12), 2H (OCH_2Ph); 7·2, m, 10H (H arom.). ^{19}F NMR (CD_3OD, $CFCl_3$): −76·2, s. Analysis: calc. for $C_{24}H_{27}F_3N_2O_4$ C, 62·06%; H, 5·86%; N, 6·03%; found C, 62·17%; H, 5·88%; N, 6·16%.

***3b** (*R *or* S*).* ^{1}H NMR (CD_3OD; TMS): 0·9, d (6·8), 6H ($2CH_3$); 1·8–2·1, m, 3H ($BnCH_2$ and $CH(CH_3)_2$); 2·6, m, 2H ($PhCH_2$); 3·8, m, 2H ($CHCOCF_3$ and CH-val); 5·2, AB system (12), 2H (OCH_2Ph); 7·4, m, 10H (H arom.). ^{19}F NMR (CD_3OD, $CFCl_3$): −76·1, s.

N-*Benzyloxycarbonyl*-N-*(3,3,3-trifluoro-2-hydroxy-2-oxo-1-(isopropyl)-propyl)* L-*valinamides **6a** and **6b**. **6a** (*S *or* R*).* ^{1}H NMR ($CDCl_3$; TMS): 0·9, m, 12H ($2(CH_3)_2$); 1·2–1·6, m, 2H (2NH); 2·1–2·3, m, 2H ($2CH(CH_3)_2$); 4·9, m, 1H ($CHCOCF_3$); 5·2, m, 1H (CH-*i*Pr); 5·1, s, 2H (OCH_2Ph); 7·3, m, 5H (H arom.). ^{13}C NMR ($CDCl_3$): 17·6; 17·9; 19·1; 19·6; 29·3; 30·7; 58·7; 60·4; 67·2; 120·0, q ($^1JCF = 285$ Hz), CF_3; 128; 128·2; 128·6; 136·1; 156·6; 171·88; 190·0, d ($^2JCF = 30$ Hz), $COCF_3$. ^{19}F NMR ($CDCl_3$, $CFCl_3$): −76·6, s. Analysis: calc. for $C_{19}H_{24}F_3N_2O_4$ C, 56·85%; H, 6·03%; N, 7·0%; found C, 56·89%; H, 6·07%; N, 7·06%.

***6b** (*R *or* S*).* ^{1}H NMR ($CDCl_3$; TMS): 0·9–1·2, m, 12H ($2(CH_3)_2$); 2·2–2·4, m, 2H ($2CH(CH_3)_2$); 4·1, m, 1H (CH-*i*Pr); 4·8, dd (6·2; 2), 1H ($CHCOCF_3$); 5·1, s, 2H (OCH_2Ph); 5·3, broad s, 2H (NH); 7·3, m,

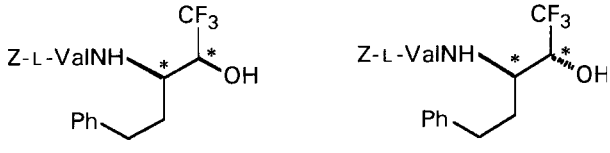
5H (H arom.). ^{13}C NMR ($CDCl_3$): 17·7; 17·8; 19·4; 19·9; 29·3; 30·5; 58·8; 60·0; 67·5; 118·8, q ($^1JCF = 285$ Hz), CF_3; 128; 128·2; 128·4; 128·7; 136·2; 156·7; 171·9; 192·0, d ($^2JCF = 28$ Hz), $COCF_3$. ^{19}F NMR ($CDCl_3$, $CFCl_3$): −76·5, s. Analysis: calc. for $C_{19}H_{24}F_3N_2O_4$ C, 56·85%; H, 6·03%; N, 7·0%; found C, 56·87%; H, 6·05%; N, 7·05%.

Results and Discussion

Chemistry

The synthesis of the homochiral *syn* and *anti* β-peptidyl TFMAs **1** and **2** (Figure 1) has previously been reported (Bégué et al 1994). The *N*-CBZ-protected dipeptide analogues **1a** and **1b** were oxidized to the corresponding α-peptidyl TFMKs **3a** and **3b** (Figure 2) by Dess-Martin periodinane (Ireland & Liu 1993) under Linderman conditions (Linderman & Graves 1989). It is important to underline that with the Dess-Martin reagent no epimerization at the chiral center α to the ketone occurred, according to ^{19}F NMR.

The *syn* and *anti* β-peptidyl TFMAs **4** and **5** were conveniently prepared as illustrated in Figure 3. Condensation of the *N*-CBZ-valine with the *syn*



1a: (*RS*) or (*SR*) **2a**: (*SS*) or (*RR*)
1b: (*SR*) or (*RS*) **2b**: (*RR*) or (*SS*)

Figure 1. Structures of the *syn* and *anti* β-peptidyl trifluoromethyl alcohols **1** and **2**.

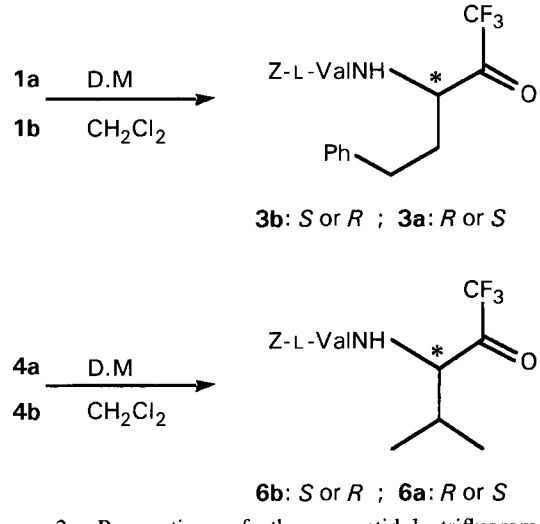


Figure 2. Preparation of the α-peptidyl trifluoromethyl ketones **3a**, **3b**, **6a** and **6b**. D.M = Dess-Martin reagent.

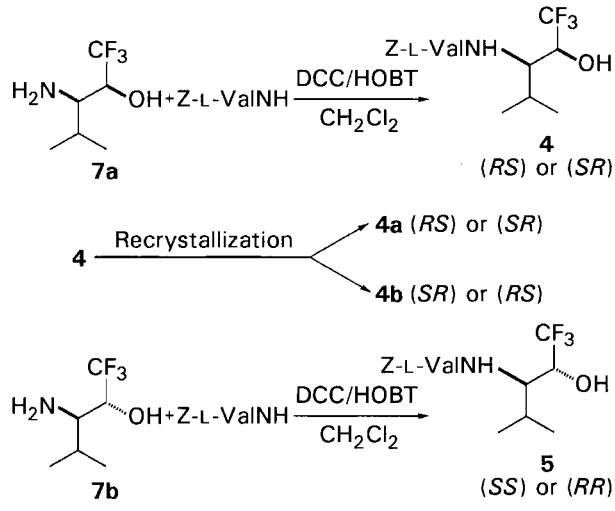


Figure 3. Preparation of the *syn* and *anti* β-peptidyl trifluoromethyl alcohols **4** and **5**.

trifluoromethyl amino alcohol **7a** in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazol (DCC-HOBT) in CH_2Cl_2 gave the *syn* peptidyl alcohols **4**, which were then separated by re-crystallization from a 1 : 5 mixture of CH_2Cl_2 and cyclohexane to give the homochiral alcohols **4a** and **4b**. In a similar manner the *anti* β-peptidyl TFMAs **5** were efficiently obtained as a mixture of stereoisomers from the *anti* trifluoromethyl amino alcohol **7b**. Oxidation of the homochiral alcohols **4a** and **4b** with Dess-Martin periodinane gave the corresponding α-peptidyl TFMKs **6a** and **6b** (Figure 2).

Biochemical studies

In the first series, TFMAs **1a** and **2a** competitively inhibited HLE and HCG (K_i between 0·13 and 0·94 mM) but not PPE whereas their respective enantiomers did not inhibit any of the enzymes. HLE and HCG have different structural requirements, yet the two enzymes had similar affinities towards **1a** and **2a** (Table 1). For both enzymes, **1a** had the highest potency. In comparison, the racemic mixture of TFMA Ac-Leu-*R,S*-CH($CH_2C_6H_5$)-*R,S*-CH(OH)-CF_3 also behaved as a reversible competitive inhibitor with a K_i of 0·2 mM towards chymotrypsin, whereas its ketone analogue Ac-Leu-D,L-Phe-CF_3 was a slow-binding inhibitor ($K_i = 1·2$ μM, Imperiali & Abeles 1986). However, neither of the ketone analogues of **1a** and **2a** inhibited chymotrypsin.

The second series of compounds containing an isopropyl group was designed to target HLE. Among the TFMAs, only **4a** competitively inhibited HLE (Figure 4); it had no inhibitory activity towards PPE or HCG. In contrast, TFMKs **6a** and

Table 1. K_i values (M) for inhibition of serine proteases and HIV-1 protease by trifluoromethyl alcohols and ketones at 25 °C and pH 8·0.

Configuration	Compound	Human leucocyte elastase	Porcine pancreatic elastase	Human cathepsin G	HIV-1 protease†
syn R,S**	**1a**	$1{\cdot}26 \times 10^{-4}$	–	$1{\cdot}98 \times 10^{-4}$	–
syn S,R**	**1b**	–	–	–	–
anti S,S**	**2a**	$9{\cdot}45 \times 10^{-4}$	–	$6{\cdot}08 \times 10^{-4}$	–
anti R,R**	**2b**	–	–	–	–
*S**	**3a**	–	–	–	$1{\cdot}5 \times 10^{-5}$
*R**	**3b**	–	–	–	$1{\cdot}5 \times 10^{-5}$
syn R,S**	**4a**	$5{\cdot}65 \times 10^{-4}$	–	–	–
syn S,R**	**4b**	–	–	–	–
anti S,S and *R,R*	**5**	–	–	–	–
*S**	**6a**	$2{\cdot}37 \times 10^{-6}$	$4{\cdot}39 \times 10^{-5}$	–	2×10^{-4}
*R**	**6b**	$8{\cdot}30 \times 10^{-6}$	$7{\cdot}69 \times 10^{-5}$	–	2×10^{-4}

The s.d. was < 25%. – = No inhibition. † IC50 (M) determined at 30 °C and pH 4·5.

6b inhibited HLE and PPE as reversible competitive inhibitors with affinities two orders of magnitude higher than that of **4a**. Inhibitor **6a** was more potent than **6b**, by factors of 3·5 and 1·8 for HLE and PPE, respectively (Figure 5). The chirality of the Cβ carbon of **6a** and **6b** thus had less effect on the inhibition of HLE than previously observed with the slightly longer TFMK Z-L-Val-L-Pro-L-Val-CF_3 and its epimer Z-L-Val-L-Pro-D-Val-CF_3 (Edwards 1992).

Both TFMAs and TFMKs can be considered as transition-state analogues that mimic the tetrahedral intermediate formed before the acylation and deacylation of the serine protease by a peptide substrate. However, TFMKs are covalently bound to the serine protease during the formation of the tetrahedral hemiketal with Ser-195 and are thus irreversible inhibitors (Krantz 1992), unlike TFMAs which are reversible inhibitors. Hence, TFMKs and TFMAs are members of two different classes of enzyme inhibitors. It was thus interesting to compare their respective inhibitory powers as isomerically pure forms. Our study was consistent with previous studies performed with racemic mixtures showing that TFMKs are overall much more potent as serine protease inhibitors than the TFMAs from which they are derived. To the best of our knowledge, the racemic mixture of a TFMK (ICI-200 880) is the only HLE-inhibitor to have progressed into clinical trials for the treatment and prevention of pulmonary emphysema (Edwards & Bernstein 1994). Our study also showed that in its effect on affinity and specificity towards HLE stereochemistry was more important for the TFMAs than for the related TFMKs.

Several studies of hydrolytic enzymes (acetylcholine esterase, carboxypeptidase, angiotensin convertase or pepsin) suggest that molecules containing a di- or trifluoromethyl ketone group are better inhibitors than similar compounds lacking fluorine (Gelb et al 1985). For aspartic proteases TFMA and TFMK might be considered as a structural mimics of the tetrahedral transition state of the hydrolytic process. The presence of a fluor-

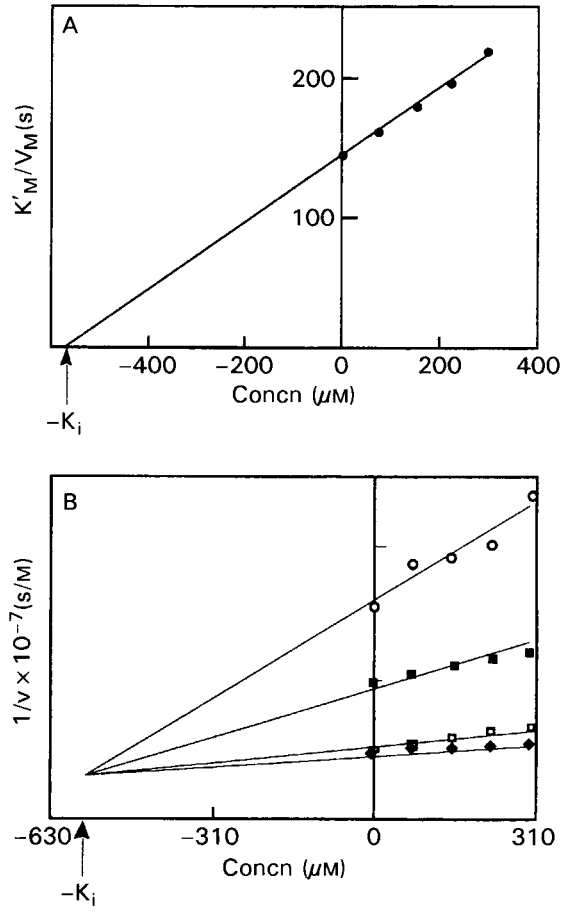


Figure 4. Inhibition of HLE by β-peptidyl trifluoromethyl alcohol **4a** at pH 8·0 and 25 °C. A. Plots of $K_{M\ app}/V_M$ against inhibitor concentration. The values of $K_{M\ app}/V_M$ were obtained from the slopes of the linear double-reciprocal plots of v against substrate concentration. B. Dixon plot of the inhibition of HLE by **4a** with CH_3O-Suc-Ala-Ala-Pro-Val-*p*-NA as substrate. Substrate concentrations (mM) were: ● 0·05; ○ 0·1; ■ 0·2; □ 0·5: ◆ 1·0.

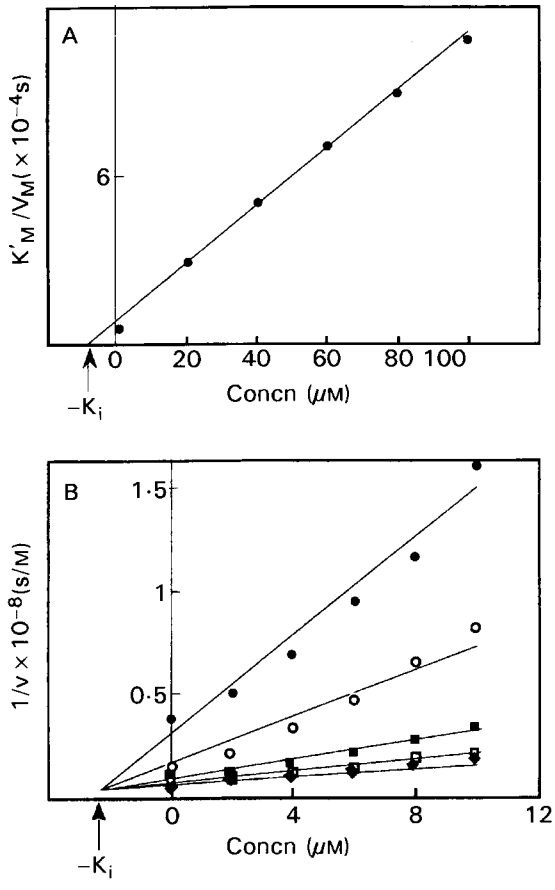


Figure 5. Inhibition of HLE by β-peptidyl trifluoromethyl ketones **6a** and **6b** at pH 8·0 and 25 °C. A. Plots of $K_{M\ app}/V_M$ against inhibitor concentration (**6b**). The values of $K_{M\ app}/V_M$ were obtained from the slopes of the linear double-reciprocal plots of v against substrate concentration. B. Dixon plot of the inhibition of HLE by **6a** with CH_3O-Suc-Ala-Ala-Pro-Val-*p*-NA as substrate. Substrate concentrations (mM) were: ● 0·1; ○ 0·2; ■ 0·3; □ 0·4; ◆ 0·5.

ine atom on the carbon adjacent to the carbonyl group induces an increase in the electrophilicity of the ketone group. Consequently, di- and trifluoromethyl ketones are mostly in a hydrated form in aqueous medium. TFMAs corresponding to TFMKs did not inhibit the protease within solubility limits. Inhibition of HIV-1 protease was observed only with TFMK compounds (IC50 = 15–200 μM). The phenylethyl substituent resulted in tenfold greater activity (IC50 15 μM) than did the isopropyl substituent (IC50 200 μM), whatever the chirality of the compound tested. Interestingly, TFMKs bearing a phenylethyl substituent selectively inhibited HIV-1 protease with no inhibition of HLE, PPE and HCG. This might be related to the structural requirements of HIV-1 protease to accommodate substrates and inhibitors in its active site (hydrophobic groups at the P1/P'1 position).

Acknowledgements

We are grateful to the Fondation pour la Recherche Scientifique for financial support of work on the inhibition of HIV-1 protease.

References

Amour, A., Smaoui, H., Heudes, D., Reboud-Ravaux, M. (1996) Protection of rat lung from elastase-induced elastic fiber degradation in vitro and from emphysema in vivo by a trifluoroacetylpeptide anilide inhibitor. Respiration 63: 277–282

Bégué, J.-P., Bonnet-Delpon, D., Sdassi, M. (1992) A versatile synthesis of amino trifluoromethyl ketones and alcohols. Tetrahedron Lett. 33: 1879–1882

Bégué, J.-P., Bonnet-Delpon, D., Fischer-Durand, N., Amour, A., Reboud-Ravaux, M. (1994) Stereoselective synthesis and inhibitor properties towards human leucocyte elastase of chiral β-peptidyl trifluoromethyl alcohols. Tetrahedron: Asymmetry 5: 1099–1110

Bieth, J. G. (1989) Human neutrophil elastase. In: Robert, L., Hornebeck, W. (eds) Elastin and Elastases. Vol. II, CRC Press, Boca Raton, Florida, pp 23–33

Bode, W., Meyer, E., Powers, J. C. (1989) Human leukocyte and porcine pancreatic elastase: X-ray crystal structures: mechanism, substrate specificity and mechanism-based inhibitors. Biochemistry 28: 1951–1953

Boudier, C., Holle, C., Bieth, J. G. (1981) Stimulation of the elastolytic activity of leukocyte elastase by leucocyte cathepsin G. J. Biol. Chem. 256: 10256–10258

Brady, K., Abeles, R. H. (1990) Inhibition of chymotrypsin by peptidyl trifluoromethyl ketones: determinants of slow-binding kinetics. Biochemistry 29: 7608–7617

Brady, K., Wei, A., Ringe, D., Abeles, R. H. (1990) Structure of chymotrypsin-trifluoromethyl ketone inhibitor complexes: comparison of slowly and rapidly equilibrating inhibitors. Biochemistry 29: 7600–7607

Dewald, B., Rindler-Ludwig, R., Bretz, U., Baggiolino, M. (1975) Subcellular localization and heterogeneity of neutral proteases in neutrophilic polymorphonuclear leukocytes. J. Exp. Med. 141: 709–723

Doherty, J. B., Shah, S. K., Finke, P. E., Dorn Jr, C. P., Hagman, W. K., Hale, J. J., Kissinger, A. L., Thompson, K. R., Brause, K., Chandler, G. O., Knight, W. B., Maycock, A. L., Ashe, B. M., Weston, H, Gale, P., Mumford, R., Andersen, F. O., Williams, R. H., Nolan, T. E., Frankenfield, D. L., Underwood, D., Vyas, K. P., Kari, P. H., Dahlgren, M. E., Mao, J., Fletcher, D. S., Dellea, P. S., Hand, K. M., Osinga, D. G., Peterson, L. B., Williams, D. T., Metzger, J. M., Bonney, R. J., Humes, J. L., Pacholok, S. P., Hanlon, W. A., Opas, E., Stolk, J., Davies, P. (1993) Chemical, biochemical, pharmacokinetic, and biological properties of L-680,833: a potent, orally active monocyclic β-lactam inhibitor of human polymorphonuclear leukocyte elastase. Proc. Natl Acad. Sci. USA 90: 8727–8731

Edwards, P. D. (1992) A method for the stereoselective synthesis of peptidyl trifluoromethyl ketones. Tetrahedron Lett. 33: 4279–4282

Edwards, P. D., Bernstein, P. R. (1994) Synthetic inhibitors of elastase. Med. Res. Rev. 14: 127–194

Gelb, M. M. H., Svaren, J. P., Abeles, R. H. (1985) Fluoroketone inhibitors of hydrolytic enzymes. Biochemistry 24: 1813–1817

Imperiali, B., Abeles, R. H. (1986) Inhibition of serine proteases by peptidyl fluoromethyl ketones. Biochemistry 25: 3760–3767

Ireland, R., Liu, L. (1993) Oxidation of fluoroalkyl-substituted carbinols by the Dess-Martin reagent. J. Org. Chem. 58: 2899

Krantz, A. (1992) A classification of enzyme inhibitors. Bioorg. Med. Chem. Lett. 2: 1323–1326

Linderman, R.-J., Graves, D.-M. (1989) An improved procedure for the preparation of the Dess-Martin periodinane. J. Org. Chem. 54: 661

Maillard, J. L., Favreau, C., Reboud-Ravaux, M., Kobaiter, R., Joyeau, R., Wakselman, M. (1990) Biological evaluation of the inhibition of granulocyte elastase by a synthetic β-lactam derivative. Eur. J. Cell Biol. 52: 213–218

Mattos, C., Giammona, D. A., Petsko, G. A., Ringe, D. (1995) Structural analysis of the active site of porcine pancreatic elastase based on the X-ray crystal structures of complexes with trifluoroacetyldipeptide-anilide inhibitors. Biochemistry 34:3193–3203

Powers, J. C., Gupton, B. F., Harkley, A. D., Nishino, N., Whitley, R. J. (1977) Specificity of porcine pancreatic elastase, human leukocyte elastase, and cathepsin G. Inhibition with peptide chloromethyl ketones. Biochim. Biophys. Acta 485: 158–166

Quasmi, D., de Rosny, E., René, L., Badet, B., Vergely, I., Boggetto, N., Reboud-Ravaux, M. (1997) Synthesis of *N*-glyoxyl peptides and their in vitro evaluation as HIV-1 protease inhibitors. Bio org. Med. Chem. Lett. 5: 707–714

Sham, H. L., Betebenner, D. A., Wideburg, N. E., Saldivar, A. C., Kohlbrenner, W. E., Kraig-Kennard, A., Vasavanonda, S., Kempf, D. J., Clement, J. J., Erickson, J. E., Plattner, J. J., Norbeck, D. W. (1993) Pseudosymmetrical difluoroketones. Highly potent and specific inhibitors of HIV-1 protease. FEBS Lett. 329: 144–146

Snider, G. L. (1987) Protease-antiprotease imbalance in the pathogenesis of emphysema and chronic bronchial injury: a potential target for drug development. Drug Dev. Res. 10: 235–253

Stein, R. L., Strimpler, A. M., Edwards, P. D., Lewis, J. J., Mauger, R. C., Schwartz, J. A., Stein, M. M., Trainor, D. A., Wildonger, R. A., Zottola, M. A. (1987) Mechanism of slow-binding inhibition of human leukocyte elastase by trifluoromethyl ketones. Biochemistry 26: 2682–2689

Takahashi, L. H., Radakrishnan, R., Rosenfield Jr, R. E., Meyer Jr, E. F., Trainor, D. M., Stein, M. (1988) X-ray diffraction analysis of the inhibition of porcine pancreatic elastase by a peptidyl trifluoromethyl ketone. J. Mol. Biol. 201: 423–428

Travis, J., Salvesen, G. S. (1983) Human plasma proteinase inhibitors. Annu. Rev. Biochem. 52: 655–709