

RESEARCH ARTICLE

Fluorobenzoyl dipeptidyl derivatives as inhibitors of the *Fasciola hepatica* cysteine protease cathepsin L1

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Abstract

Cathepsins are known to have many important physiological roles and provide a viable target for inhibition. Fluorobenzoyl dipeptide derivatives were synthesized and tested for biological activity in an effort to find an efficient inhibitor of the cysteine protease cathepsin L. Thirty-six novel inhibitors (**1–36**) were synthesized from protected amino acids via the standard DCC/HOBt coupling protocol, containing a benzyl ester or a nitrile as an electrophilic warhead. The activity of the inhibitors was evaluated against cathepsin L and IC₅₀ values calculated. Modification of both amino acids and terminal groups afforded compounds with single digit micromolar inhibition. Results utilizing the benzoyl-L-leucine-glycine nitrile backbone are comparable to that for the commercially available inhibitor **39**.

Keywords: Dipeptide; fluorine; benzyl ester; nitrile; protease inhibition; cathepsin L

Introduction

The group of cysteine proteases known as the cathepsins have been shown to play a crucial function in diseases such as osteoporosis, rheumatoid arthritis, cancer metastasis, and infectious diseases¹. Cathepsins are important targets for the development of inhibitors as therapeutic agents. Fasciolosis is caused by infection with *Fasciola hepatica* and *Fasciola gigantica* parasites. Not only is it an important human disease, but it also affects cattle and sheep worldwide, causing economic losses of approximately 2 billion dollars². It is caused by the ingestion of vegetation or water contaminated with the encysted infectious liver fluke larvae known as metacercariae³. It has become clear that the predominant protease activity in this parasite is associated with cells of the gut epithelium⁴. Like many other parasitic helminths, *Fasciola hepatica* liver flukes release many proteolytic enzymes that belong to the group of cysteine proteases. It has been suggested that these proteases may be involved in protecting the parasite against immune attack⁵.

Most of the current approaches to cathepsin inhibition involve molecules of a peptidic nature. The hydrolyzable amide is replaced by an electrophilic functionality and the catalytic thiol of the enzyme reacts with the inhibitor to form a covalent complex. Until recently, most potent cysteine protease inhibitors were irreversible inhibitors, with the electrophilic functionality alkylating the enzyme. Inhibitors containing electrophilic moieties such as an aldehyde, halomethyl ketone, or epoxide have been shown to be potent cysteine protease inhibitors⁶. Peptidic molecules containing the electrophilic benzyl ester and nitrile moieties have also been reported to be inhibitors of various cathepsins^{7–9}.

For this study, all of the compounds (**1–36**) prepared are based on a dipeptidyl scaffold. Previous work in these laboratories has identified *N*-benzoyl-dipeptidyl derivatives as potential inhibitors of the *Fasciola hepatica* protease, such as *N*-benzoyl-L-leucine-glycine **37** (IC₅₀ = 10.0 μM) and *N*-cinnamoyl-L-leucine-glycine nitrile **38** (IC₅₀ = 11.0 μM)¹⁰. The fluorobenzoyl moiety has been shown to be an effective *N*-terminal substituent in various potent inhibitors¹¹.

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Materials and methods

Chemistry

All chemicals were purchased from Sigma-Aldrich, Lennox Chemicals, or Fluorochem Limited, and used as received. When necessary, all solvents were purified and dried, and stored under argon. Tetrahydrofuran was distilled from Na/benzophenone and triethylamine distilled and stored over potassium hydroxide pellets. Commercial grade reagents were used without further purification procedures. Riedel-Haën silica gel was used for thin layer and column chromatography. Melting point determination was carried out using a Griffin melting point apparatus. Elemental analysis was carried out by the Microanalytical Laboratory at University College Dublin. Electrospray mass spectra were obtained on a Bruker Esquire 3000 series LC/MS (liquid chromatography/mass spectrometry) apparatus. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 400 NMR spectrometer operating at 400 MHz for ^1H -NMR, 376 MHz for ^{19}F -NMR, and 100 MHz for ^{13}C -NMR. The ^1H - and ^{13}C -NMR chemical shifts (δ) are comparative to tetramethylsilane, and the ^{19}F -NMR chemical shifts (δ) are relative to trifluoroacetic acid. All coupling constants (J) are in hertz (Hz).

N-2-fluorobenzoyl-L-leucine-glycine benzyl ester (1)

N-2-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) was dissolved in dichloromethane (50 mL) with glycine benzyl ester tosylate (1.12 g, 3.16 mmol), 1-hydroxybenzotriazole (0.43 g, 3.16 mmol), and triethylamine (0.44 mL). The mixture was cooled to 0°C, and 1,3-dicyclohexylcarbodiimide (0.65 g, 3.16 mmol) was added. After 30 min the solution was raised to room temperature and the reaction was allowed to proceed for 48 h. The precipitated *N,N*-dicyclohexylurea was removed by filtration and the filtrate was washed with 10% potassium hydrogen carbonate, 10% citric acid, and water and dried over magnesium sulfate. The solvent was evaporated *in vacuo*, and recrystallization from ethyl acetate/hexane furnished **1** as a white powder (0.45 g, 37%). M.p. = 101–103°C. $[\alpha]_{\text{D}}^{25} = -18.2^\circ$ (c, 3.0, EtOH). Anal. calcd. for $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_4\text{F}_1$: C, 65.98; H, 6.29; N, 6.99%. Found: C, 65.59; H, 6.04; N, 7.12%. Mass spectrum: $[\text{M} + \text{Na}]^+$ found 423.2. $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_4\text{F}_1\text{Na}_1$ requires 423.44. IR (KBr): ν 3290, 2957, 1720, 1638, 1542, 752 cm^{-1} . ^1H -NMR (400 MHz, DMSO): δ 8.49 (1H, t, $J = 6.0$ Hz, -NH-), 8.35 (1H, dd, $J = 2.8$ & 8.4 Hz, -NH-), 7.62–7.66 (1H, m, -ArH 6), 7.50–7.55 (1H, m, -ArH 4), 7.24–7.36 (7H, m, -ArH 3, 5 & -PhH), 5.12 (2H, s, -OCH₂-), 4.52–4.66 (1H, m, α -H), 3.99 (1H, dd, $J = 6.4$ & 17.2 Hz, -CH₂-), 3.88 (1H, dd, $J = 5.6$ & 17.6 Hz, -CH₂-), 1.50–1.73 (3H, m, -CH₂- & -CH-), 0.89 (3H, d, $J = 3.6$ Hz, -CH₃), 0.88 (3H, d, $J = 3.6$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO): δ 172.9 (-CONH-), 169.9 (-COOCH₂Ph), 164.0 (-ArCO-), 159.5 (d, -ArC 2), 136.1 (-PhC 1), 132.9 (d, -ArC 4), 130.5 (d, -ArC 6), 128.7 (-PhC 3 & 5), 128.4 (-PhC 4), 128.2 (-PhC 2 & 6), 124.7 (d, -ArC 5), 123.8 (d, -ArC 1), 116.4 (d, -ArC 3), 66.2 (-OCH₂-), 51.8 (α -C), 41.1 (-CH₂-), 41.0 (-CH₂-), 24.6 (-CH-), 23.3 (-CH₃), 21.7 (-CH₃). ^{19}F -NMR (376 MHz, DMSO): δ -38.7 to -38.8 (m).

N-3-fluorobenzoyl-L-leucine-glycine benzyl ester (2)

N-3-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and glycine benzyl ester tosylate (1.12 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **2** as a white powder (0.55 g, 45%). M.p. = 76–78°C. $[\alpha]_{\text{D}}^{25} = -21.3^\circ$ (c, 3.2, EtOH). Anal. calcd. for $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_4\text{F}_1$: C, 65.98; H, 6.29; N, 6.99%. Found: C, 65.72; H, 6.41; N, 6.88%. Mass spectrum: $[\text{M} + \text{Na}]^+$ found 423.1. $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_4\text{F}_1\text{Na}_1$ requires 423.44. IR (KBr): ν 3275, 2958, 1744, 1637, 1560, 754 cm^{-1} . ^1H -NMR (400 MHz, DMSO): δ 8.63 (1H, d, $J = 8.4$ Hz, -NH-), 8.49 (1H, t, $J = 6.0$ Hz, -NH-), 7.73–7.79 (2H, m, -ArH 2 & 6), 7.49–7.53 (1H, m, -ArH 5), 7.31–7.41 (6H, m, -ArH 4 & -PhH), 5.12 (2H, s, -OCH₂-), 4.54–4.58 (1H, m, α -H), 3.95 (1H, dd, $J = 6.0$ & 17.6 Hz, -CH₂-), 3.87 (1H, dd, $J = 5.6$ & 17.2 Hz, -CH₂-), 1.54–1.74 (3H, m, -CH₂- & -CH-), 0.89 (3H, d, $J = 6.4$ Hz, -CH₃), 0.85 (3H, d, $J = 6.0$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO): δ 173.1 (-CONH-), 170.0 (-COOCH₂Ph), 165.3 (d, -ArCO-), 162.2 (d, -ArC 3), 136.7 (d, -ArC 1), 136.2 (-PhC 1), 130.6 (d, -ArC 5), 128.7 (-PhC 3 & 5), 128.4 (-PhC 4), 128.2 (-PhC 2 & 6), 124.1 (d, -ArC 6), 118.5 (d, -ArC 4), 114.7 (d, -ArC 2), 66.2 (-OCH₂-), 52.0 (α -C), 41.1 (-CH₂-), 40.6 (-CH₂-), 24.6 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ^{19}F -NMR (376 MHz, DMSO): δ -37.8 to -37.9 (m).

N-4-fluorobenzoyl-L-leucine-glycine benzyl ester (3)

N-4-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and glycine benzyl ester tosylate (1.12 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **3** as a brown powder (0.80 g, 66%). M.p. = 108–110°C. $[\alpha]_{\text{D}}^{25} = -22.2^\circ$ (c, 3.2, EtOH). Anal. calcd. for $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_4\text{F}_1$: C, 65.98; H, 6.29; N, 6.99%. Found: C, 65.77; H, 6.31; N, 7.21%. Mass spectrum: $[\text{M} + \text{Na}]^+$ found 423.2. $\text{C}_{22}\text{H}_{25}\text{N}_2\text{O}_4\text{F}_1\text{Na}_1$ requires 423.44. IR (KBr): ν 3285, 2957, 1742, 1632, 1502, 695 cm^{-1} . ^1H -NMR (400 MHz, DMSO): δ 8.51 (1H, d, $J = 8.4$ Hz, -NH-), 8.43 (1H, t, $J = 6.0$ Hz, -NH-), 7.92–7.98 (2H, m, -ArH 2 & 6), 7.26–7.40 (7H, m, -ArH 3, 5 & -PhH), 5.10 (2H, s, -OCH₂-), 4.50–4.56 (1H, m, α -H), 3.93 (1H, dd, $J = 6.0$ & 17.6 Hz, -CH₂-), 3.84 (1H, dd, $J = 6.0$ & 17.2 Hz, -CH₂-), 1.52–1.71 (3H, m, -CH₂- & -CH-), 0.88 (3H, d, $J = 6.4$ Hz, -CH₃), 0.84 (3H, d, $J = 6.4$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO): δ 173.2 (-CONH-), 170.0 (-COOCH₂Ph), 165.6 (-ArCO-), 164.2 (d, -ArC 4), 136.1 (-PhC 1), 130.8 (-ArC 1), 130.6 (-ArC 2), 130.5 (-ArC 6), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.2 (-PhC 2 & 6), 115.4 (-ArC 3), 115.2 (-ArC 5), 66.1 (-OCH₂-), 51.9 (α -C), 41.1 (-CH₂-), 40.6 (-CH₂-), 24.7 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ^{19}F -NMR (376 MHz, DMSO): δ -34.1 to -34.2 (m).

N-pentafluorobenzoyl-L-leucine-glycine benzyl ester (4)

N-pentafluorobenzoyl-L-leucine (2.64 g, 8.1 mmol) and glycine benzyl ester tosylate (2.88 g, 8.1 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **4** as a white powder (1.56 g, 42%). M.p. = 165–167°C. $[\alpha]_{\text{D}}^{25} = -20.8^\circ$ (c, 1.5, EtOH). Anal. calcd. for $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_4\text{F}_5$: C, 55.93; H, 4.48; N, 5.93%. Found: C, 56.15; H, 4.62; N, 6.11%. Mass spectrum: $[\text{M} + \text{Na}]^+$ found 495.1. $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_4\text{F}_5\text{Na}_1$ requires 495.39. IR (KBr): ν 3287, 2966, 1745, 1662, 1546, 996 cm^{-1} . ^1H -NMR (400 MHz, DMSO): δ 9.13 (1H, d, $J = 8.4$ Hz, -NH-),

8.70 (1H, t, $J = 6.0$ Hz, -NH-), 7.31–7.38 (5H, m, -PhH), 5.13 (2H, s, -OCH₂-), 4.60–4.64 (1H, m, α -H), 4.03 (1H, dd, $J = 6.0$ & 17.2 Hz, -CH₂-), 3.84 (1H, dd, $J = 6.0$ & 17.6 Hz, -CH₂-), 1.49–1.70 (3H, m, -CH₂- & -CH-), 0.90 (3H, d, $J = 6.4$ Hz, -CH₃), 0.89 (3H, d, $J = 6.4$ Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO): δ 172.0 (-CONH-), 169.9 (-COOCH₂Ph), 156.6 (-ArCO-), 144.7–144.8 (m, -ArC 2), 140.3–142.8 (m, -ArC 4), 142.2 (m, -ArC 6), 138.4–138.6 (m, -ArC 3), 136.1 (-PhC 1), 135.9–136.1 (m, -ArC 5), 128.7 (-PhC 3 & 5), 128.4 (-PhC 4), 128.3 (-PhC 2 & 6), 112.7 (t, -ArC 1), 66.2 (-OCH₂-), 51.7 (α -C), 41.3 (-CH₂-), 40.1 (-CH₂-), 24.4 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO): δ -66.5 (dd, 2F, $J = 6.7$ & 22.9 Hz), -78.1 (t, $J = 16.1$ Hz), -86.4 to -86.5 (m, 2F).

N-2-fluorobenzoyl-L-leucine- β -alanine benzyl ester (5)

N-2-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and β -alanine benzyl ester tosylate (1.16 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **5** as a brown powder (0.73 g, 58%). M.p. = 69–71°C. $[\alpha]_D^{25} = -4.2^\circ$ (c, 1.4, EtOH). Anal. calcd. for C₂₃H₂₇N₂O₄F₁: C, 66.65; H, 6.56; N, 6.75%. Found: C, 66.60; H, 6.62; N, 6.77%. Mass spectrum: $[M + Na]^+$ found 437.2. C₂₃H₂₇N₂O₄F₁Na₁ requires 437.46. IR (KBr): ν 3268, 2913, 1727, 1642, 1468, 721 cm⁻¹. ¹H-NMR (400 MHz, DMSO): δ 8.31 (1H, dd, $J = 2.4$ & 8.0 Hz, -NH-), 8.16 (1H, t, $J = 5.6$ Hz, -NH-), 7.61–7.65 (1H, m, -ArH 6), 7.50–7.55 (1H, m, -ArH 4), 7.24–7.36 (7H, m, -ArH 3, 5 & -PhH), 5.09 (2H, s, -OCH₂-), 4.46–4.50 (1H, m, α -H), 3.29–3.44 (2H, m, β -CH₂), 2.54 (2H, t, $J = 6.8$ Hz, α -CH₂), 1.46–1.67 (3H, m, -CH₂- & -CH-), 0.89 (3H, d, $J = 2.8$ Hz, -CH₃), 0.87 (3H, d, $J = 2.8$ Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO): δ 172.2 (-CONH-), 171.5 (-COOCH₂Ph), 163.8 (-ArCO-), 159.5 (d, -ArC 2), 136.3 (-PhC 1), 132.8 (d, -ArC 4), 130.5 (d, -ArC 6), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.2 (-PhC 2 & 6), 124.7 (d, -ArC 5), 123.9 (d, -ArC 1), 116.4 (d, -ArC 3), 65.9 (-OCH₂-), 52.0 (α -C), 41.1 (-CH₂-), 35.1 (β -CH₂), 34.0 (α -CH₂), 24.6 (-CH-), 23.3 (-CH₃), 21.8 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO): δ -38.7 to -38.8 (m).

N-3-fluorobenzoyl-L-leucine- β -alanine benzyl ester (6)

N-3-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and β -alanine benzylester tosylate (1.16 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **6** as a brown powder (0.63 g, 50%). M.p. = 63–65°C. $[\alpha]_D^{25} = -9.1^\circ$ (c, 2.4, EtOH). Anal. calcd. for C₂₃H₂₇N₂O₄F₁: C, 66.65; H, 6.56; N, 6.75%. Found: C, 66.89; H, 6.90; N, 6.55%. Mass spectrum: $[M + Na]^+$ found 437.2. C₂₃H₂₇N₂O₄F₁Na₁ requires 437.46. IR (KBr): ν 3324, 2960, 1736, 1655, 1542, 752 cm⁻¹. ¹H-NMR (400 MHz, DMSO): δ 8.58 (1H, d, $J = 8.0$ Hz, -NH-), 8.17 (1H, t, $J = 5.6$ Hz, -NH-), 7.67–7.78 (2H, m, -ArH 2 & 6), 7.48–7.53 (1H, m, -ArH 5), 7.28–7.39 (6H, m, -ArH 4 & -PhH), 5.07 (2H, s, -OCH₂-), 4.46–4.52 (1H, m, α -H), 3.29–3.43 (2H, m, β -CH₂), 2.54 (2H, t, $J = 6.8$ Hz, α -CH₂), 1.47–1.72 (3H, m, -CH₂- & -CH-), 0.88 (3H, d, $J = 6.4$ Hz, -CH₃), 0.85 (3H, d, $J = 6.4$ Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO): δ 172.6 (-CONH-), 171.5 (-COOCH₂Ph), 165.3 (d, -ArCO-), 162.2 (d, -ArC 3), 136.7 (d, -ArC 1), 136.3 (-PhC 1), 130.6 (d, -ArC 5), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.2 (-PhC 2 & 6), 124.0

(d, -ArC 6), 118.4 (d, -ArC 4), 114.6 (d, -ArC 2), 65.9 (-OCH₂-), 52.0 (α -C), 40.7 (-CH₂-), 35.1 (β -CH₂), 34.0 (α -CH₂), 24.7 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO): δ -37.6 to -37.7 (m).

N-4-fluorobenzoyl-L-leucine- β -alanine benzyl ester (7)

N-4-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and β -alanine benzyl ester tosylate (1.16 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **7** as a brown powder (0.58 g, 46%). M.p. = 103–105°C. $[\alpha]_D^{25} = -12.3^\circ$ (c, 1.6, EtOH). Anal. calcd. for C₂₃H₂₇N₂O₄F₁: C, 66.65; H, 6.56; N, 6.75%. Found: C, 66.29; H, 6.35; N, 6.49%. Mass spectrum: $[M + Na]^+$ found 437.2. C₂₃H₂₇N₂O₄F₁Na₁ requires 437.46. IR (KBr): ν 3330, 2959, 1734, 1632, 1502, 857 cm⁻¹. ¹H-NMR (400 MHz, DMSO): δ 8.49 (1H, d, $J = 8.0$ Hz, -NH-), 8.14 (1H, t, $J = 5.6$ Hz, -NH-), 7.96–7.99 (2H, m, -ArH 2 & 6), 7.26–7.36 (7H, m, -ArH 3, 5 & -PhH), 5.07 (2H, s, -OCH₂-), 4.46–4.48 (1H, m, α -H), 3.29–3.39 (2H, m, β -CH₂), 2.54 (2H, t, $J = 6.8$ Hz, α -CH₂), 1.47–1.70 (3H, m, -CH₂- & -CH-), 0.88 (3H, d, $J = 6.4$ Hz, -CH₃), 0.85 (3H, d, $J = 6.4$ Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO): δ 172.6 (-CONH-), 171.5 (-COOCH₂Ph), 165.5 (-ArCO-), 164.3 (d, -ArC 4), 136.4 (-PhC 1), 130.9 (-ArC 1), 130.6 (-ArC 2), 130.5 (-ArC 6), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.2 (-PhC 2 & 6), 115.5 (-ArC 3), 115.3 (-ArC 5), 65.9 (-OCH₂-), 52.1 (α -C), 40.7 (-CH₂-), 35.1 (β -CH₂), 34.0 (α -CH₂), 24.7 (-CH-), 23.3 (-CH₃), 21.7 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO): δ -34.1 to -34.2 (m).

N-pentafluorobenzoyl-L-leucine- β -alanine benzyl ester (8)

N-pentafluorobenzoyl-L-leucine (2.93 g, 9.0 mmol) and β -alanine benzyl ester tosylate (3.33 g, 9.0 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **8** as a brown oil (1.99 g, 47%). $[\alpha]_D^{25} = -6.5^\circ$ (c, 2.1, EtOH). Anal. calcd. for C₂₃H₂₃N₂O₄F₅: C, 56.79; H, 4.76; N, 5.75%. Found: C, 56.61; H, 4.85; N, 6.01%. Mass spectrum: $[M + Na]^+$ found 509.2. C₂₃H₂₃N₂O₄F₅Na₁ requires 509.42. IR (KBr): ν 3267, 3083, 1745, 1638, 1560, 734 cm⁻¹. ¹H-NMR (400 MHz, DMSO): δ 9.16 (1H, d, $J = 8.4$ Hz, -NH-), 8.35 (1H, t, $J = 5.6$ Hz, -NH-), 7.31–7.38 (5H, m, -PhH), 5.10 (2H, s, -OCH₂-), 4.51–4.55 (1H, m, α -H), 3.30–3.44 (2H, m, β -CH₂), 2.56 (2H, t, $J = 6.4$ Hz, α -CH₂), 1.45–1.64 (3H, m, -CH₂- & -CH-), 0.90 (3H, d, $J = 6.4$ Hz, -CH₃), 0.89 (3H, d, $J = 6.4$ Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO): δ 171.4 (-COOCH₂Ph & -CONH-), 156.6 (-ArCO-), 144.5–144.7 (m, -ArC 2), 140.0–142.2 (m, -ArC 4), 142.2 (m, -ArC 6), 138.2–138.4 (m, -ArC 3), 136.4 (-PhC 1), 135.8–136.0 (m, -ArC 5), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.1 (-PhC 2 & 6), 112.7 (t, -ArC 1), 65.9 (-OCH₂-), 51.9 (α -C), 41.3 (-CH₂-), 35.1 (β -CH₂), 33.9 (α -CH₂), 24.4 (-CH-), 23.1 (-CH₃), 21.7 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO): δ -66.6 (dd, 2F, $J = 6.8$ & 23.6 Hz), -78.4 (t, $J = 21.6$ Hz), -86.6 to -86.7 (m, 2F).

N-2-fluorobenzoyl-L-leucine- γ -aminobutyric acid benzyl ester (9)

N-2-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and γ -aminobutyric acid benzyl ester tosylate (1.21 g, 3.16 mmol)

were used. Recrystallization from ethyl acetate/hexane furnished **9** as a brown oil (0.79 g, 63%). $[\alpha]_D^{25} = -19^\circ$ (c, 0.18, EtOH). Anal. calcd. for $C_{24}H_{29}N_2O_4F_1$: C, 67.27; H, 6.82; N, 6.53%. Found: C, 67.60; H, 6.58; N, 6.32%. Mass spectrum: $[M + Na]^+$ found 451.2. $C_{24}H_{29}N_2O_4F_1Na_1$ requires 451.49. IR (KBr): ν 3301, 2985, 1737, 1662, 1435, 728 cm^{-1} . 1H -NMR (400 MHz, DMSO): δ 8.30 (1H, dd, $J=2.4$ & 8.0 Hz, -NH-), 8.05 (1H, t, $J=5.6$ Hz, -NH-), 7.61–7.65 (1H, m, -ArH 6), 7.49–7.55 (1H, m, -ArH 4), 7.24–7.38 (7H, m, -ArH 3, 5 & -PhH), 5.08 (2H, s, -OCH₂-), 4.45–4.50 (1H, m, α -H), 3.13 (2H, q, $J=6.8$ Hz, γ -CH₂), 2.38 (2H, t, $J=7.6$ Hz, α -CH₂), 1.48–1.73 (5H, m, β -CH₂, -CH₂- & -CH-), 0.89 (6H, t, $J=6.4$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO): δ 172.9 (-CONH-), 172.1 (-COOCH₂Ph), 163.8 (-ArCO-), 159.5 (d, -ArC 2), 136.5 (-PhC 1), 132.8 (d, -ArC 4), 130.5 (d, -ArC 6), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.2 (-PhC 2 & 6), 124.7 (-ArC 5), 123.9 (d, -ArC 1), 116.3 (d, -ArC 3), 65.7 (-OCH₂-), 52.2 (α -C), 41.1 (-CH₂-), 38.1 (γ -CH₂), 31.1 (α -CH₂), 24.75 (-CH-), 24.72 (β -CH₂), 23.2 (-CH₃), 21.9 (-CH₃). ^{19}F -NMR (376 MHz, DMSO): δ -38.7 to -38.8 (m).

***N*-3-fluorobenzoyl-L-leucine- γ -aminobutyric acid benzyl ester (10)**

N-3-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and γ -aminobutyric acid benzyl ester tosylate (1.21 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **10** as a white powder (0.63 g, 50%). M.p. = 69–71 °C. $[\alpha]_D^{25} = -2.5^\circ$ (c, 1.2, EtOH). Anal. calcd. for $C_{24}H_{29}N_2O_4F_1$: C, 67.27; H, 6.82; N, 6.53%. Found: C, 67.65; H, 7.01; N, 6.38%. Mass spectrum: $[M + Na]^+$ found 451.2. $C_{24}H_{29}N_2O_4F_1Na_1$ requires 451.49. IR (KBr): ν 3330, 2954, 1725, 1634, 1534, 754 cm^{-1} . 1H -NMR (400 MHz, DMSO): δ 8.55 (1H, d, $J=8.0$ Hz, -NH-), 8.06 (1H, t, $J=5.6$ Hz, -NH-), 7.70–7.77 (2H, m, -ArH 2 & 6), 7.47–7.54 (1H, m, -ArH 5), 7.29–7.39 (6H, m, -ArH 4 & -PhH), 5.07 (2H, s, -OCH₂-), 4.44–4.50 (1H, m, α -H), 3.10 (2H, q, $J=6.4$ Hz, γ -CH₂), 2.37 (2H, t, $J=7.2$ Hz, α -CH₂), 1.50–1.73 (5H, m, β -CH₂, -CH₂- & -CH-), 0.89 (3H, d, $J=6.0$ Hz, -CH₃), 0.85 (3H, d, $J=6.0$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO): δ 172.9 (-CONH-), 172.4 (-COOCH₂Ph), 165.3 (d, -ArCO-), 162.2 (d, -ArC 3), 136.7 (t, -ArC 1), 136.5 (-PhC 1), 130.6 (d, -ArC 5), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.2 (-PhC 2 & 6), 124.0 (d, -ArC 6), 118.4 (d, -ArC 4), 114.6 (d, -ArC 2), 65.7 (-OCH₂-), 52.4 (α -C), 40.7 (-CH₂-), 38.1 (γ -CH₂), 31.1 (α -CH₂), 24.79 (-CH-), 24.77 (β -CH₂), 23.3 (-CH₃), 21.7 (-CH₃). ^{19}F -NMR (376 MHz, DMSO): δ -37.7 to -37.8 (m).

***N*-4-fluorobenzoyl-L-leucine- γ -aminobutyric acid benzyl ester (11)**

N-4-fluorobenzoyl-L-leucine (0.8 g, 3.16 mmol) and γ -aminobutyric acid benzyl ester tosylate (1.21 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **11** as a brown powder (0.73 g, 63%). M.p. = 83–85 °C. $[\alpha]_D^{25} = -2.9^\circ$ (c, 1.2, EtOH). Anal. calcd. for $C_{24}H_{29}N_2O_4F_1$: C, 67.27; H, 6.82; N, 6.53%. Found: C, 67.55; H, 7.13; N, 6.69%. Mass spectrum: $[M + Na]^+$ found 451.2. $C_{24}H_{29}N_2O_4F_1Na_1$ requires 451.49. IR (KBr): ν 3330, 2954, 1725, 1634, 1534, 754 cm^{-1} . 1H -NMR (400 MHz, DMSO): δ 8.49 (1H,

d, $J=8.0$ Hz, -NH-), 8.06 (1H, t, $J=5.6$ Hz, -NH-), 7.96–8.02 (2H, m, -ArH 2 & 6), 7.25–7.39 (7H, m, -ArH 3, 5 & -PhH), 5.08 (2H, s, -OCH₂-), 4.44–4.50 (1H, m, α -H), 3.11 (2H, q, $J=6.4$ Hz, γ -CH₂), 2.37 (2H, t, $J=7.2$ Hz, α -CH₂), 1.50–1.73 (5H, m, β -CH₂, -CH₂- & -CH-), 0.89 (3H, d, $J=6.4$ Hz, -CH₃), 0.86 (3H, d, $J=6.4$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO): δ 172.9 (-CONH-), 172.5 (-COOCH₂Ph), 165.6 (-ArCO-), 164.4 (d, -ArC 4), 136.5 (-PhC 1), 130.9 (-ArC 1), 130.6 (-ArC 2), 130.5 (-ArC 6), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.2 (-PhC 2 & 6), 115.4 (-ArC 3), 115.2 (-ArC 5), 65.7 (-OCH₂-), 52.3 (α -C), 40.8 (-CH₂-), 38.1 (γ -CH₂), 31.1 (α -CH₂), 24.8 (-CH-), 24.7 (β -CH₂), 23.3 (-CH₃), 21.7 (-CH₃). ^{19}F -NMR (376 MHz, DMSO): δ -34.1 to -34.2 (m).

***N*-pentafluorobenzoyl-L-leucine- γ -aminobutyric acid benzyl ester (12)**

N-pentafluorobenzoyl-L-leucine (2.69 g, 8.2 mmol) and γ -aminobutyric acid benzyl ester tosylate (3.15 g, 8.2 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **12** as a white powder (1.82 g, 46%). M.p. = 54–56 °C. $[\alpha]_D^{25} = -4.2^\circ$ (c, 1.2, EtOH). Anal. calcd. for $C_{24}H_{25}N_2O_4F_5$: C, 57.59; H, 5.04; N, 5.59%. Found: C, 57.32; H, 4.77; N, 5.91%. Mass spectrum: $[M + Na]^+$ found 523.1. $C_{24}H_{25}N_2O_4F_5Na_1$ requires 523.45. IR (KBr): ν 3329, 2962, 1736, 1655, 1508, 991 cm^{-1} . 1H -NMR (400 MHz, DMSO): δ 9.16 (1H, d, $J=8.4$ Hz, -NH-), 8.23 (1H, t, $J=5.6$ Hz, -NH-), 7.31–7.37 (5H, m, -PhH), 5.10 (2H, s, -OCH₂-), 4.48–4.51 (1H, m, α -H), 3.09–3.16 (2H, m, γ -CH₂), 2.40 (2H, t, $J=7.2$ Hz, α -CH₂), 1.47–1.75 (5H, m, β -CH₂, -CH₂- & -CH-), 0.90 (6H, t, $J=6.0$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO): δ 172.8 (-CONH-), 171.3 (-COOCH₂Ph), 156.6 (-ArCO-), 144.5–144.7 (m, -ArC 2), 140.0–142.6 (m, -ArC 4), 142.2 (m, -ArC 6), 138.2–138.5 (m, -ArC 3), 136.5 (-PhC 1), 135.8–136.0 (m, -ArC 5), 128.7 (-PhC 3 & 5), 128.3 (-PhC 4), 128.1 (-PhC 2 & 6), 112.7 (t, -ArC 1), 65.7 (-OCH₂-), 52.1 (α -C), 41.3 (-CH₂-), 38.1 (γ -CH₂), 31.0 (α -CH₂), 24.7 (β -CH₂), 24.5 (-CH-), 23.1 (-CH₃), 21.7 (-CH₃). ^{19}F -NMR (376 MHz, DMSO): δ -66.6 (dd, 2F, $J=6.8$ & 23.3 Hz), -78.4 (t, $J=22.1$ Hz), -86.6 to -86.7 (m, 2F).

***N*-2-fluorobenzoyl-L-leucine-glycine nitrile (13)**

N-2-fluorobenzoyl-L-leucine (1.01 g, 4 mmol) and glycine nitrile hydrochloride (0.37 g, 4 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **13** as a white powder (0.81 g, 69%). M.p. = 91–92 °C. $[\alpha]_D^{25} = -4.5^\circ$ (c, 2.1, EtOH). Anal. calcd. for $C_{15}H_{18}N_3O_2F_1$: C, 61.84; H, 6.23; N, 14.42%. Found: C, 61.98; H, 6.18; N, 14.17%. IR (KBr): ν 3277, 2961, 2372, 1637, 1534, 751 cm^{-1} . 1H -NMR (400 MHz, DMSO-*d*₆): δ 8.78 (1H, t, $J=5.6$ Hz, -NH-), 8.35 (1H, d, $J=7.6$ Hz, -NH-), 7.60–7.65 (1H, m, -ArH 6), 7.51–7.56 (1H, m, -ArH 4), 7.26–7.34 (2H, m, -ArH 3 & 5), 4.48–4.53 (1H, m, α -H), 4.17 (2H, d, $J=5.6$ Hz, -CH₂-), 1.47–1.72 (3H, m, -CH₂- & -CH-), 0.90 (6H, t, $J=3.6$ Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO-*d*₆): δ 172.9 (-CONH-), 164.2 (-ArCO-), 159.5 (d, -ArC 2), 132.8 (d, -ArC 4), 130.5 (d, -ArC 6), 124.6 (d, -ArC 5), 124.0 (d, -ArC 1), 117.9 (-CN), 116.4 (d, -ArC 3), 51.7 (α -C), 40.5 (-CH₂-, -ve DEPT), 27.4 (-CH₂-, -ve DEPT),

24.6 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -38.5 to -38.7 (m).

***N*-4-fluorobenzoyl-L-leucine-glycine nitrile (14)**

N-4-fluorobenzoyl-L-leucine (1.01 g, 4 mmol) and glycine nitrile hydrochloride (0.37 g, 4 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **14** as a white powder (0.75 g, 64%). M.p. = 138–139°C. [α]_D²⁵ = -8.6° (c, 2.2, EtOH). Anal. calcd. for C₁₅H₁₈N₃O₂F₁: C, 61.84; H, 6.23; N, 14.42%. Found: C, 61.72; H, 6.27; N, 14.29%. IR (KBr): ν 3286, 3070, 2346, 1674, 1634, 1505, 854 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.76 (1H, t, *J* = 5.6 Hz, -NH-), 8.43 (1H, d, *J* = 8.0 Hz, -NH-), 7.99–8.02 (2H, m, -ArH 2 & 6), 7.28–7.32 (2H, m, -ArH 3 & 5), 4.48–4.54 (1H, m, α-*H*), 4.14 (2H, d, *J* = 5.6 Hz, -CH₂-), 1.52–1.76 (3H, m, -CH₂- & -CH-), 0.90 (3H, d, *J* = 6.4 Hz, -CH₃), 0.86 (3H, d, *J* = 6.0 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 173.3 (-CONH-), 165.7 (-ArCO-), 164.3 (d, -ArC 4), 130.7 (-ArC 1), 130.66 (-ArC 2), 130.62 (-ArC 6), 117.9 (-CN), 115.5 (-ArC 3), 115.2 (-ArC 5), 51.9 (α-C), 40.2 (-CH₂-, -ve DEPT) 27.4 (-CH₂-, -ve DEPT), 24.7 (-CH-), 23.3 (-CH₃), 21.5 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -33.8 to -34.0 (m).

***N*-2-fluorobenzoyl-L-alanine-glycine nitrile (15)**

N-2-fluorobenzoyl-L-alanine (0.84 g, 4 mmol) and glycine nitrile hydrochloride (0.37 g, 4 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **15** as a white powder (0.55 g, 55%). M.p. = 109–111°C. [α]_D²⁵ = -12.2° (c, 1.6, EtOH). Anal. calcd. for C₁₂H₁₂N₃O₂F₁: C, 57.83; H, 4.85; N, 16.86%. Found: C, 57.91; H, 4.77; N, 16.56%. IR (KBr): ν 3336, 2944, 2364, 1677, 1638, 1534, 758 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.71 (1H, t, *J* = 5.6 Hz, -NH-), 8.51 (1H, d, *J* = 6.4 Hz, -NH-), 7.67–7.71 (1H, m, -ArH 6), 7.52–7.57 (1H, m, -ArH 4), 7.27–7.31 (2H, m, -ArH 3 & 5), 4.45–4.52 (1H, m, α-*H*), 4.18 (2H, d, *J* = 5.6 Hz, -CH₂-), 1.33 (3H, d, *J* = 7.2 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 173.0 (-CONH-), 163.8 (-ArCO-), 159.7 (d, -ArC 2), 133.0 (d, -ArC 4), 130.7 (d, -ArC 6), 124.7 (d, -ArC 5), 123.7 (d, -ArC 1), 117.9 (-CN), 116.4 (d, -ArC 3), 49.0 (α-C), 27.5 (-CH₂-, -ve DEPT), 18.0 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -38.4 to -38.6 (m).

***N*-pentafluorobenzoyl-L-alanine-glycine nitrile (16)**

N-pentafluorobenzoyl-L-alanine (1.13 g, 4 mmol) and glycine nitrile hydrochloride (0.37 g, 3.16 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **16** as a white powder (0.54 g, 42%). M.p. = 150–151°C. [α]_D²⁵ = -16.8° (c, 2.4, EtOH). Anal. calcd. for C₁₂H₈N₃O₂F₅: C, 44.87; H, 2.51; N, 13.08%. Found: C, 45.04; H, 2.44; N, 12.93%. IR (KBr): ν 3293, 3061, 2258, 1662, 1523, 999 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 9.30 (1H, d, *J* = 7.2 Hz, -NH-), 8.86 (1H, t, *J* = 5.6 Hz, -NH-), 4.47–4.54 (1H, m, α-*H*), 4.20 (2H, d, *J* = 5.6 Hz, -CH₂-), 1.30 (3H, d, *J* = 7.2 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.2 (-CONH-), 156.6 (-ArCO-), 144.7–144.8 (m, -ArC 2), 140.2–142.7 (m, -ArC 4), 142.2 (m, -ArC 6), 138.2–138.5 (m, -ArC 3), 135.8–136.0 (m, -ArC 5), 117.8 (-CN), 112.5 (t, -ArC 1), 48.9 (α-C), 27.4 (-CH₂-, -ve

DEPT), 18.1 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -66.3 (dd, 2F, *J* = 6.8 & 22.9 Hz), -77.9 (t, *J* = 15.4 Hz), -86.4 to -86.6 (m, 2F).

***N*-2-fluorobenzoyl-L-leucine-β-alanine nitrile (17)**

N-2-fluorobenzoyl-L-leucine (1.01 g, 4 mmol) and β-alanine nitrile (0.28 g, 4 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **17** as a white powder (0.83 g, 68%). M.p. = 84–85°C. [α]_D²⁵ = -8.3° (c, 1.2, EtOH). Anal. calcd. for C₁₆H₂₀N₃O₂F₁: C, 62.94; H, 6.60; N, 13.76%. Found: C, 63.31; H, 6.98; N, 13.40%. IR (KBr): ν 3293, 2960, 2247, 1655, 1542, 755 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.41 (1H, t, *J* = 5.6 Hz, -NH-), 8.37 (1H, d, *J* = 7.6 Hz, -NH-), 7.63–7.67 (1H, m, -ArH 6), 7.50–7.55 (1H, m, -ArH 4), 7.25–7.30 (2H, m, -ArH 3 & 5), 4.48–4.54 (1H, m, α-*H*), 3.27–3.42 (2H, m, β-CH₂), 2.66 (2H, t, *J* = 6.0 Hz, α-CH₂), 1.51–1.71 (3H, m, -CH₂- & -CH-), 0.92 (3H, d, *J* = 6.0 Hz, -CH₃), 0.90 (3H, d, *J* = 6.0 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.6 (-CONH-), 163.9 (-ArCO-), 159.5 (d, -ArC 2), 132.8 (d, -ArC 4), 130.6 (d, -ArC 6), 124.6 (d, -ArC 5), 124.0 (d, -ArC 1), 119.4 (-CN), 116.4 (d, -ArC 3), 52.0 (α-C), 41.1 (-CH₂-, -ve DEPT), 35.2 (-CH₂-, -ve DEPT), 24.6 (-CH-), 23.3 (-CH₃), 21.8 (-CH₃), 17.8 (α-CH₂, -ve DEPT). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -38.6 to -38.7 (m).

***N*-4-fluorobenzoyl-L-leucine-β-alanine nitrile (18)**

N-4-fluorobenzoyl-L-leucine (1.01 g, 4 mmol) and β-alanine nitrile (0.28 g, 4 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **18** as a white powder (0.64 g, 53%). M.p. = 119–120°C. [α]_D²⁵ = -17.5° (c, 1.5, EtOH). Anal. calcd. for C₁₆H₂₀N₃O₂F₁: C, 62.94; H, 6.60; N, 13.76%. Found: C, 63.14; H, 6.86; N, 13.62%. IR (KBr): ν 3326, 2957, 2249, 1649, 1604, 1502, 847 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.53 (1H, d, *J* = 8.4 Hz, -NH-), 8.36 (1H, t, *J* = 8.0 Hz, -NH-), 7.97–8.01 (2H, m, -ArH 2 & 6), 7.27–7.32 (2H, m, -ArH 3 & 5), 4.45–4.50 (1H, m, α-*H*), 3.25–3.36 (2H, m, β-CH₂), 2.64 (2H, t, *J* = 6.0 Hz, α-CH₂), 1.52–1.74 (3H, m, -CH₂- & -CH-), 0.90 (3H, d, *J* = 6.0 Hz, -CH₃), 0.87 (3H, d, *J* = 6.0 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 173.1 (-CONH-), 165.6 (-ArCO-), 164.3 (d, -ArC 4), 130.8 (d, -ArC 1), 130.6 (-ArC 2), 130.5 (-ArC 6), 119.5 (-CN), 115.5 (-ArC 3), 115.3 (-ArC 5), 52.1 (α-C), 40.7 (-CH₂-, -ve DEPT) 35.1 (β-CH₂, -ve DEPT), 24.7 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃), 17.8 (α-CH₂, -ve DEPT). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -34.0 to -34.1 (m).

***N*-2-fluorobenzoyl-L-alanine-β-alanine nitrile (19)**

N-2-fluorobenzoyl-L-alanine (0.84 g, 4 mmol) and β-alanine nitrile (0.28 g, 4 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **19** as a white powder (0.51 g, 49%). M.p. = 127–128°C. [α]_D²⁵ = -11.3° (c, 1.8, EtOH). Anal. calcd. for C₁₃H₁₄N₃O₂F₁: C, 59.31; H, 5.36; N, 15.96%. Found: C, 59.72; H, 5.67; N, 15.69%. IR (KBr): ν 3376, 3278, 2250, 1677, 1648, 1542, 901 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.36–8.39 (2H, m, -NH-), 7.68–7.72 (1H, m, -ArH 6), 7.51–7.56 (1H, m, -ArH 4), 7.27–7.31 (2H, m, -ArH 3 & 5), 4.44–4.48 (1H, m, α-*H*), 3.27–3.41 (2H, m, β-CH₂), 2.66 (2H, t, *J* = 6.4 Hz, α-CH₂), 1.33 (3H, d, *J* = 7.2 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.7 (-CONH-), 163.4 (-ArCO-), 159.7 (d, -ArC

2), 133.0 (d, -ArC 4), 130.7 (d, -ArC 6), 124.7 (d, -ArC 5), 123.6 (d, -ArC 1), 119.4 (-CN), 116.4 (d, -ArC 3), 49.1 (α -C), 35.1 (β -CH₂, -ve DEPT), 18.6 (-CH₃), 17.8 (α -CH₂, -ve DEPT). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -38.5 to -38.6 (m).

***N*-pentafluorobenzoyl-L-alanine- β -alanine nitrile (20)**

N-pentafluorobenzoyl-L-alanine (1.13 g, 4 mmol) and β -alanine nitrile (0.28 g, 4 mmol) were used. Recrystallization from ethyl acetate/hexane furnished **20** as a white powder (0.71 g, 53%). M.p. = 150–152°C. [α]_D²⁵ = -13.6° (c, 2.3, EtOH). Anal. calcd. for C₁₃H₁₀N₃O₂F₅: C, 46.57; H, 3.01; N, 12.54%. Found: C, 46.92; H, 3.23; N, 12.33%. IR (KBr): ν 3365, 3274, 2252, 1672, 1626, 1522, 992 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 9.19 (1H, d, *J* = 7.6 Hz, -NH-), 8.48 (1H, t, *J* = 5.6 Hz, -NH-), 4.45–4.53 (1H, m, α -H), 3.26–3.45 (2H, m, β -CH₂), 2.62–2.70 (2H, m, α -CH₂), 1.31 (3H, d, *J* = 7.2 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 171.9 (-CONH-), 156.5 (-ArCO-), 144.6–144.8 (m, -ArC 2), 140.1–142.6 (m, -ArC 4), 142.1–142.3 (m, -ArC 6), 138.3–138.5 (m, -ArC 3), 135.8–136.0 (m, -ArC 5), 119.4 (-CN), 112.7 (t, -ArC 1), 49.2 (α -C), 35.1 (β -CH₂, -ve DEPT), 18.7 (-CH₃), 17.8 (α -CH₂, -ve DEPT). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -66.4 (dd, 2F, *J* = 6.8 & 23.3 Hz), -78.2 (t, *J* = 15.8 Hz), -86.6 to -86.7 (m, 2F).

***N*-3-fluorobenzoyl-L-leucine-glycine nitrile (21)**

N-3-fluorobenzoyl-L-leucine (2.59 g, 10.24 mmol) and glycine nitrile hydrochloride (0.95 g, 10.24 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **21** as a white powder (1.72 g, 57%). M.p. 131–134°C. [α]_D²⁵ = -10.0° (c, 2.1, EtOH). Anal. calcd. for C₁₅H₁₈N₃O₂F: C, 61.84; H, 6.23; N, 14.42%. Found: C, 61.88; H, 6.16; N, 14.35%. IR (KBr): ν 3329, 2927, 2106, 1637, 1552, 1493, 1050, 842 cm⁻¹. Mass spectrum: [M + Na]⁺ found 314.1. C₁₅H₁₈N₃O₂FNa₁ requires 314.31. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.75 (1H, t, *J* = 5.6 Hz, -NH-), 8.69–8.71 (1H, d, *J* = 8 Hz, -NH-), 7.72–7.79 (2H, m, -ArH 2 & 6), 7.51–7.56 (1H, m, -ArH 5), 7.40–7.43 (1H, m, -ArH 4), 4.48–4.53 (1H, m, α -H), 4.14 (2H, d, *J* = 5.6 Hz, -CH₂-), 1.48–1.75 (3H, m, -CH₂- & -CH-), 0.86–0.92 (6H, dd, *J* = 6.4 & 18.8 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 173.2 (-CONH-), 165.5 (-ArCO-), 163.45 (d, -ArC 3), 136.5 (d, -ArC 1), 130.7 (d, -ArC 5), 124.2 (d, -ArC 6), 118.6 (d, -ArC 4), 118.0 (-CN), 114.8 (d, -ArC 2), 52.0 (α -C), 40.3 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.8 (-CH-), 23.4 (-CH₃), 21.5 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -36.94 to -37.01 (m).

***N*-2,3-difluorobenzoyl-L-leucine-glycine nitrile (22)**

N-2,3-difluorobenzoyl-L-leucine (1.33 g, 4.9 mmol) and glycine nitrile hydrochloride (0.45 g, 4.9 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **22** as a white powder (0.73 g, 48%). M.p. 103–105°C. [α]_D²⁵ = -13.0° (c, 2.1, EtOH). Anal. calcd. for C₁₅H₁₇N₃O₂F₂: C, 58.25; H, 5.54; N, 13.59%. Found: C, 57.95; H, 5.52; N, 13.36%. IR (KBr): ν 3280, 2928, 2198, 1637, 1548, 1483, 1272, 1050, 803 cm⁻¹. Mass spectrum: [M + Na]⁺ found 332.2. C₁₅H₁₇N₃O₂F₂Na₁ requires 332.3. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.82–8.85 (1H, t, *J* = 5.4 Hz,

-NH-), 8.74–8.76 (1H, d, *J* = 8 Hz, -NH-), 7.57–7.60 (1H, m, -ArH 6), 7.43–7.46 (1H, m, -ArH 5), 7.31–7.35 (1H, m, -ArH 4), 4.53–4.55 (1H, m, α -H), 4.21 (2H, d, *J* = 4 Hz, -CH₂-, -ve DEPT), 1.49–1.82 (3H, m, -CH₂- & -CH-), 0.94–0.96 (6H, t, *J* = 8.4 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.8 (-CONH-), 163.2 (-ArCO-), 151.2 (dd, -ArC 3), 146.3 (dd, -ArC 2), 126.5 (d, -ArC 1), 125.5 (d, -ArC 4), 125.1 (d, -ArC 5), 119.6 (d, -ArC 6), 117.9 (-CN), 51.8 (α -C), 40.5 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.6 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -62.40 to -62.51 (m), -64.0 to -64.10 (m).

***N*-2,4-difluorobenzoyl-L-leucine-glycine nitrile (23)**

N-2,4-difluorobenzoyl-L-leucine (1.47 g, 5.4 mmol) and glycine nitrile hydrochloride (0.5 g, 5.4 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **23** as a white powder (1.1 g, 66%). M.p. 108–111°C. [α]_D²⁵ = -10.9° (c, 2.1, EtOH). Anal. calcd. for C₁₅H₁₇N₃O₂F₂: C, 58.25; H, 5.54; N, 13.59%. Found: C, 57.96; H, 5.50; N, 13.37%. IR (KBr): ν 3201, 2987, 2110, 1640, 1507, 1301, 1089, 775 cm⁻¹. Mass spectrum: [M + Na]⁺ found 332.2. C₁₅H₁₇N₃O₂F₂Na₁ requires 332.3. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.77–8.79 (1H, t, *J* = 4 Hz, -NH-), 8.50–8.52 (1H, d, *J* = 9.2 Hz, -NH-), 7.71–7.73 (1H, m, -ArH 6), 7.31–7.40 (1H, m, -ArH 5), 7.17–7.25 (1H, m, -ArH 3), 4.45–4.55 (1H, m, α -H), 4.17 (2H, d, *J* = 5.2 Hz, -CH₂-), 1.46–1.78 (3H, m, -CH₂- & -CH-), 0.90–0.92 (6H, t, *J* = 6.8 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.9 (-CONH-), 163.4 (d, -ArC 4), 162.5 (-ArCO-), 158.9 (dd, -ArC 2), 132.4 (dd, -ArC 6), 120.8 (dd, -ArC 1), 117.9 (-CN), 111.8 (dd, -ArC 3), 104.7 (t, -ArC 5), 51.8 (α -C), 40.6 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.6 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -30.29 to -30.38 (m), -32.90 to -32.98 (m).

***N*-2,5-difluorobenzoyl-L-leucine-glycine nitrile (24)**

N-2,5-difluorobenzoyl-L-leucine (2.1 g, 7.75 mmol) and glycine nitrile hydrochloride (0.72 g, 7.75 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **24** as a white powder (1.43 g, 60%). M.p. 101–104°C. [α]_D²⁵ = -9.3° (c, 2.1, EtOH). Anal. calcd. for C₁₅H₁₇N₃O₂F₂: C, 58.25; H, 5.54; N, 13.59%. Found: C, 58.28; H, 5.56; N, 13.40%. IR (KBr): ν 3290, 2911, 2102, 1651, 1437, 1078, 985 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.75–8.78 (1H, t, *J* = 5.6 Hz, -NH-), 8.62–8.64 (1H, d, *J* = 7.6 Hz, -NH-), 7.36–7.46 (3H, m, -ArH 3, 4 & 6), 4.46–4.8 (1H, m, α -H), 4.17 (2H, d, *J* = 5.6 Hz, -CH₂-), 1.60–1.68 (3H, m, -CH₂- & -CH-), 0.89–0.92 (6H, t, *J* = 6.4 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.3 (-CONH-), 162.7 (-ArCO-), 157.5 (d, -ArC 2), 155.5 (d, -ArC 5), 125.1 (dd, -ArC 1), 118.9 (dd, -ArC 6), 117.9 (dd, -ArC 3), 117.5 (-CN), 116.3 (dd, -ArC 4), 51.5 (α -C), 39.9 (-CH₂-, -ve DEPT), 27.1 (-CH₂-, -ve DEPT), 24.2 (-CH-), 22.9 (-CH₃), 21.3 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -42.38 to -42.48 (m), -43.38 to -43.45 (m).

***N*-2,6-difluorobenzoyl-L-leucine-glycine nitrile (25)**

N-2,6-difluorobenzoyl-L-leucine (2.23 g, 8.23 mmol) and glycine nitrile hydrochloride (0.76 g, 8.23 mmol) were used.

Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **25** as a white powder (0.98 g, 39%). M.p. 159–163°C. $[\alpha]_D^{25} = -20.3^\circ$ (c, 2.1, EtOH). Anal. calcd. for $C_{15}H_{17}N_3O_2F_2$: C, 58.25; H, 5.54; N, 13.59%. Found: C, 58.18; H, 5.49; N, 13.51%. IR (KBr): ν 3190, 2875, 2055, 1675, 1688, 1402, 1075, 626 cm^{-1} . Mass spectrum: $[M + Na]^+$ found 332.1. $C_{15}H_{17}N_3O_2F_2Na_1$ requires 332.3. 1H -NMR (400 MHz, DMSO- d_6): δ 9.02 (1H, d, $J = 8.4$ Hz, -NH-), 8.85–8.88 (1H, t, $J = 5.6$ Hz, -NH-), 7.10–7.55 (1H, m, -ArH 4), 7.13–7.17 (2H, m, -ArH 3 & 5), 4.40–4.57 (1H, m, α -H), 4.18 (2H, d, $J = 2.4$ Hz, -CH₂-), 1.41–1.78 (3H, m, -CH₂- & -CH-), 0.89–0.92 (6H, dd, $J = 4.4$ & 2.5 Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 172.5 (-CONH-), 160.45 (d, -ArC 2), 159.96 (-ArCO-), 157.95 (d, -ArC 6), 131.91 (-ArC 4), 117.9 (-CN), 115.5 (t, -ArC 1), 112.1 (dd, -ArC 3 & 5), 51.5 (α -C), 40.8 (-CH₂-, -ve DEPT), 27.4 (-CH₂-, -ve DEPT), 24.5 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ^{19}F -NMR (376 MHz, DMSO- d_6): δ -37.54 to -37.58 (m).

N-3,4-difluorobenzoyl-L-leucine-glycine nitrile (**26**)

N-3,4-difluorobenzoyl-L-leucine (2.3 g, 8.5 mmol) and glycine nitrile hydrochloride (0.79 g, 8.5 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **26** as a white powder (0.52 g, 20%). M.p. 139–142°C. $[\alpha]_D^{25} = -19.6^\circ$ (c, 2.1, EtOH). Anal. calcd. for $C_{15}H_{17}N_3O_2F_2$: C, 58.25; H, 5.54; N, 13.59%. Found: C, 58.05; H, 5.51; N, 13.29%. IR (KBr): ν 3198, 2654, 2063, 1641, 1567, 1246, 1098, 803 cm^{-1} . 1H -NMR (400 MHz, DMSO- d_6): δ 8.76–8.82 (1H, t, $J = 11.4$ Hz, -NH-), 8.71–8.73 (1H, d, $J = 7.7$ Hz, -NH-), 7.97–8.00 (1H, m, -ArH 6), 7.81–7.84 (1H, m, -ArH 2), 7.54–7.61 (1H, m, -ArH 5), 4.47–4.52 (1H, m, α -H), 4.14 (2H, d, $J = 5.6$ Hz, -CH₂-), 1.59–1.75 (3H, m, -CH₂- & -CH-), 0.85–0.92 (6H, dd, $J = 6.4$ & 19.4 Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 173.1 (-CONH-), 164.64 (-ArCO-), 151.8 (dd, ArC 4), 149.3 (dd, -ArC 3), 131.55 (t, -ArC 1), 125.52 (dd, -ArC 2), 117.9 (-CN), 117.8 (d, -ArC 5), 117.3 (d, -ArC 6), 52.0 (α -C), 40.3 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.7 (-CH-), 23.3 (-CH₃), 21.5 (-CH₃). ^{19}F -NMR (376 MHz, DMSO- d_6): δ -58.33 to -58.41 (m), -62.09 to -62.20 (m).

N-3,5-difluorobenzoyl-L-leucine-glycine nitrile (**27**)

N-3,5-difluorobenzoyl-L-leucine (2.3 g, 8.5 mmol) and glycine nitrile hydrochloride (0.79 g, 8.5 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **27** as a white powder (0.62 g, 22%). M.p. 150–153°C. $[\alpha]_D^{25} = -16.0^\circ$ (c, 2.1, EtOH). Anal. calcd. for $C_{15}H_{17}N_3O_2F_2$: C, 58.25; H, 5.54; N, 13.59%. Found: C, 58.94; H, 5.37; N, 12.83%. IR (KBr): ν 3281, 2967, 2146, 1641, 1543, 1298, 1023, 754 cm^{-1} . 1H -NMR (400 MHz, DMSO- d_6): δ 8.78–8.80 (2H, m, -NH-), 7.64–7.67 (2H, m, -ArH 2 & 6), 7.53–7.45 (1H, m, -ArH 4), 4.47–4.53 (1H, m, α -H), 4.14 (2H, d, $J = 5.6$ Hz, -CH₂-), 1.55–1.74 (3H, m, -CH₂- & -CH-), 0.86–0.92 (6H, dd, $J = 6.4$ & 20 Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 172.99 (-CONH-), 164.27 (-ArCO-), 163.73 (d, -ArC 3), 161.27 (d, -ArC 5), 137.62 (t, -ArC 1), 117.94 (-CN), 111.35 (dd, -ArC 2 & 5), 107.22 (t, -ArC 4), 52.1 (α -C), 40.2

(-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.7 (-CH-), 23.3 (-CH₃), 21.5 (-CH₃). ^{19}F -NMR (376 MHz, DMSO- d_6): δ -32.61 to -32.68 (m).

N-2,3,6-trifluorobenzoyl-L-leucine-glycine nitrile (**28**)

N-2,3,6-trifluorobenzoyl-L-leucine (1.05 g, 3.63 mmol) and glycine nitrile hydrochloride (0.34 g, 3.63 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **28** as a white powder (0.28 g, 24%). M.p. 157–160°C. $[\alpha]_D^{25} = -27.2^\circ$ (c, 2.1, EtOH). Anal. calcd. for $C_{15}H_{16}N_3O_2F_3$: C, 55.05; H, 4.93; N, 12.84%. Found: C, 55.13; H, 4.98; N, 12.67%. IR (KBr): ν 3276, 2961, 2252, 1660, 1541, 1492, 1025, 814 cm^{-1} . 1H -NMR (400 MHz, DMSO- d_6): δ 9.13–9.15 (1H, d, $J = 8.2$ Hz, -NH-), 8.90–8.93 (1H, t, $J = 11.8$ Hz, -NH-), 7.55–7.64 (1H, m, -ArH 4), 7.18–7.24 (1H, m, -ArH 5), 4.51–4.57 (1H, m, α -H), 4.20 (2H, d, $J = 3.2$ Hz, -CH₂-), 1.41–1.78 (3H, m, -CH₂- & -CH-), 0.90–0.92 (6H, dd, $J = 5.4$ & 6.3 Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 172.3 (-CONH-), 158.77 (-ArCO-), 154.5 (d, -ArC 2), 147.9 (d, -ArC 6), 145.4 (t, -ArC 3), 118.70 (dd, -ArC 4), 117.87 (-CN), 117.0 (t, -ArC 1), 112.4 (d, -ArC 5), 51.5 (α -C), 40.8 (-CH₂-, -ve DEPT), 27.4 (-CH₂-, -ve DEPT), 24.5 (-CH-), 23.2 (-CH₃), 21.6 (-CH₃). ^{19}F -NMR (376 MHz, DMSO- d_6): δ -42.18 to -42.25 (m), -61.096 to -61.18 (m), -66.48 to -66.62 (m).

N-2,3,4-trifluorobenzoyl-L-leucine-glycine nitrile (**29**)

N-2,3,4-trifluorobenzoyl-L-leucine (1.17 g, 4 mmol) and glycine nitrile hydrochloride (0.37 g, 4 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **29** as a white powder (0.58 g, 44%). M.p. 116–119°C. $[\alpha]_D^{25} = -4.2^\circ$ (c, 2.1, EtOH). Anal. calcd. for $C_{15}H_{16}N_3O_2F_3$: C, 55.05; H, 4.93; N, 12.84%. Found: C, 54.43; H, 5.95; N, 12.58%. IR (KBr): ν 3287, 2964, 2249, 1657, 1547, 1496, 1045, 828 cm^{-1} . 1H -NMR (400 MHz, DMSO- d_6): δ 8.85–8.87 (1H, t, $J = 5.6$ Hz, -NH-), 8.75–8.77 (1H, d, $J = 8$ Hz, -NH-), 7.41–7.55 (2H, m, -ArH 5 & 6), 4.52–4.56 (1H, m, α -H), 4.21 (2H, d, $J = 5.6$ Hz, -CH₂-), 1.51–1.75 (3H, m, -CH₂- & -CH-), 0.93–0.97 (6H, dd, $J = 6.4$ & 10.4 Hz, -CH₃). ^{13}C -NMR (100 MHz, DMSO- d_6): δ 172.7 (-CONH-), 162.53 (-ArCO-), 151.7 (dd, ArC 4), 148.8 (dd, -ArC 2), 139.3 (dt, -ArC 3), 125.02 (dd, -ArC 5), 122.24 (t, -ArC 1), 117.89 (-CN), 113.02 (dd, -ArC 6), 51.8 (α -C), 40.5 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.6 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ^{19}F -NMR (376 MHz, DMSO- d_6): δ -55.67 to -55.79 (m), -58.92 to -59.02 (m), -84.95 to -85.08 (m).

N-2,4,5-trifluorobenzoyl-L-leucine-glycine nitrile (**30**)

N-2,4,5-trifluorobenzoyl-L-leucine (1.94 g, 6.7 mmol) and glycine nitrile hydrochloride (0.62 g, 6.7 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **30** as a white powder (1.20 g, 55%). M.p. 104–108°C. $[\alpha]_D^{25} = -5.1^\circ$ (c, 2.1, EtOH). Anal. calcd. for $C_{15}H_{16}N_3O_2F_3$: C, 55.05; H, 4.93; N, 12.84%. Found: C, 54.75; H, 4.90; N, 12.63%. IR (KBr): ν 3282, 2959, 2261, 1657, 1537, 1465, 1031, 824 cm^{-1} . 1H -NMR (400 MHz, DMSO- d_6): δ 8.81–8.84 (1H, t, $J = 5.4$ Hz, -NH-), 8.65–8.67

(1H, d, $J=8$ Hz, -NH-), 7.68–7.80 (2H, m, -ArH 3&6), 4.50–4.53 (1H, m, α -H), 4.20 (2H, d, $J=5.6$ Hz, -CH₂-), 1.55–1.73 (3H, m, -CH₂- & -CH-), 0.94–0.96 (6H, t, $J=6.4$ Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.7 (-CONH-), 162.3 (-ArCO-), 155.2 (dd, ArC 2), 150.8 (dd, -ArC 5), 146.0 (dd, -ArC 4), 120.85 (d, -ArC 1), 118.55 (d, -ArC 6), 117.90 (-CN), 107.20 (dd, -ArC 3), 51.9 (α -C), 40.6 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.6 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -37.97 to -38.07 (m), -54.57 to -54.70 (m), -66.76 to -66.90 (m).

***N*-3,4,5-trifluorobenzoyl-L-leucine-glycine nitrile (31)**

N-3,4,5-trifluorobenzoyl-L-leucine (2 g, 7 mmol) and glycine nitrile hydrochloride (0.65 g, 7 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **31** as a white powder (1.20 g, 52%). M.p. 146–149°C. $[\alpha]_D^{25} = -0.2^\circ$ (c, 2.1, EtOH). Anal. calcd. for C₁₅H₁₆N₃O₂F₃: C, 55.05; H, 4.93; N, 12.84%. Found: C, 54.83; H, 4.87; N, 12.73%. IR (KBr): ν 3247, 2943, 2253, 1653, 1529, 1476, 1040, 794 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.77–8.81 (2H, m, -NH-), 7.87–7.91 (2H, m, -ArH 2&6), 4.45–4.52 (1H, m, α -H), 4.14 (2H, d, $J=6$ Hz, -CH₂-), 1.53–1.73 (3H, m, -CH₂- & -CH-), 0.86–0.92 (6H, dd, $J=6$ & 20.8 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.9 (-CONH-), 163.6 (-ArCO-), 150.3 (dd, ArC 3&6), 141.2 (dt, -ArC 4), 130.43 (-ArC 1), 117.91 (-CN), 113.1 (dd, -ArC 2 & 6), 52.2 (α -C), 40.3 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.7 (-CH-), 23.3 (-CH₃), 21.5 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -58.22 to -58.30 (m), -80.76 to -80.91 (m).

***N*-2,3,4,5-tetrafluorobenzoyl-L-leucine-glycine nitrile (32)**

N-2,3,4,5-tetrafluorobenzoyl-L-leucine (1.54 g, 5 mmol) and glycine nitrile hydrochloride (0.46 g, 5 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **32** as a white powder (0.72 g, 42%). M.p. 97–101°C. $[\alpha]_D^{25} = -2.2^\circ$ (c, 2.1, EtOH). Anal. calcd. for C₁₅H₁₅N₃O₂F₄: C, 52.18; H, 4.38; N, 12.17%. Found: C, 52.02; H, 4.33; N, 12.06%. IR (KBr): ν 3294, 2962, 2252, 1648, 1521, 1482, 1361, 1238, 1031, 881 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.80–8.84 (2H, m, -NH-), 7.61–7.63 (1H, m, -ArH 6), 4.44–4.50 (1H, m, α -H), 4.17 (2H, d, $J=5.6$ Hz, -CH₂-), 1.48–1.73 (3H, m, -CH₂- & -CH-), 0.88–0.93 (6H, dd, $J=6.4$ & 17.6 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.5 (-CONH-), 161.5 (-ArCO-), 146.9 (d, -ArC 2), 144.5 (d, ArC 5), 142.0 (d, -ArC 4), 138.91–139.95 (d, -ArC 3), 120.64 (-ArC 1), 117.87 (-CN), 112.13 (d, -ArC 6), 51.9 (α -C), 40.6 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.6 (-CH-), 23.3 (-CH₃), 21.6 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -63.08 to -63.34 (m, 2F), -76.97 to -77.12 (m, 1F), -79.43 to -79.54 (t, 1F).

***N*-2,3,4,5,6-pentafluorobenzoyl-L-leucine-glycine nitrile (33)**

N-2,3,4,5,6-pentafluorobenzoyl-L-leucine (1.52 g, 6.6 mmol) and glycine nitrile hydrochloride (0.6 g, 6.6 mmol) were used.

Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **33** as a white powder (1.20 g, 51%). M.p. 131–133°C. $[\alpha]_D^{25} = -13.0^\circ$ (c, 2.1, EtOH). Anal. calcd. for C₁₅H₁₄N₃O₂F₅: C, 49.60; H, 3.88; N, 11.57%. Found: C, 49.70; H, 3.87; N, 11.40%. IR (KBr): ν 3265, 3077, 2962, 2252, 1654, 1554, 1521, 1343, 1115, 991 cm⁻¹. Mass spectrum: $[M + Na]^+$ found 386.1. C₁₅H₁₄N₃O₂F₅Na₁ requires 386.29. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 9.26–9.28 (1H, d, $J=7.2$ Hz, -NH-), 8.96–8.99 (1H, t, $J=5.6$ Hz, -NH-), 4.51–4.57 (1H, m, α -H), 4.20 (2H, d, $J=2.8$ Hz, -CH₂-), 1.60–1.68 (3H, m, -CH₂- & -CH-), 0.89–0.93 (6H, t, $J=6.8$ Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.1 (-CONH-), 156.9 (-ArCO-), 135.95–144.69 (m, -ArC 2, 3, 4, 5, & 6), 117.81 (-CN), 112.6 (t, -ArC 1), 51.7 (α -C), 40.8 (-CH₂-, -ve DEPT), 27.4 (-CH₂-, -ve DEPT), 24.5 (-CH-), 23.2 (-CH₃), 21.5 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ -65.65 to -66.11 (m, 2F), -76.99 to -77.11 (m, 1F), -85.50 to -86.71 (m, 2F).

***N*-2-trifluoromethylbenzoyl-L-leucine-glycine nitrile (34)**

N-2-trifluoromethylbenzoyl-L-leucine (2.27 g, 7.5 mmol) and glycine nitrile hydrochloride (0.70 g, 7.5 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **34** as a white powder (1.30 g, 51%). M.p. 163–166°C. $[\alpha]_D^{25} = -4.0^\circ$ (c, 2.1, EtOH). Anal. calcd. for C₁₆H₁₈N₃O₂F₃: C, 56.30; H, 5.32; N, 12.31%. Found: C, 56.07; H, 5.28; N, 12.17%. IR (KBr): ν 3292, 2958, 2247, 1642, 1547, 1341, 1283, 1173, 1121, 1068, 692 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.79–8.81 (1H, d, $J=8$ Hz, -NH-), 8.75–8.77 (1H, t, $J=5.6$ Hz, -NH-), 7.73–7.79 (2H, m, -ArH 4&5), 7.66–7.68 (1H, d, $J=7.6$ Hz, -ArH 3), 7.58–7.60 (1H, d, $J=7.2$ Hz, -ArH 6), 4.41–4.55 (1H, m, α -H), 4.19 (2H, d, $J=5.6$ Hz, -CH₂-), 1.44–1.79 (3H, m, -CH₂- & -CH-), 0.89–0.92 (6H, dd, $J=2.8$ & 6.4 Hz, -CH₃). ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 172.9 (-CONH-), 167.5 (-ArCO-), 136.2 (-ArC 1) 132.6 (-ArC 6), 130.2 (-ArC 4), 129.2 (-ArC 3), 126.5 (t, -ArC 5), 125.8 (d, -ArC 2), 122.7 (d, -CF₃), 117.9 (-CN), 51.6 (α -C), 40.4 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.5 (-CH-), 23.3 (-CH₃), 21.4 (-CH₃). ¹⁹F-NMR (376 MHz, DMSO-*d*₆): δ +18.08 (3F).

***N*-3-trifluoromethylbenzoyl-L-leucine-glycine nitrile (35)**

N-3-trifluoromethylbenzoyl-L-leucine (2.27 g, 7.5 mmol) and glycine nitrile hydrochloride (0.70 g, 7.5 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **35** as a white powder (0.9 g, 36%). M.p. 104–107°C. $[\alpha]_D^{25} = -33.5^\circ$ (c, 2.1, EtOH). Anal. calcd. for C₁₆H₁₈N₃O₂F₃: C, 56.30; H, 5.32; N, 12.31%. Found: C, 56.21; H, 5.34; N, 12.11%. IR (KBr): ν 3295, 2962, 2246, 1637, 1540, 1333, 1278, 1178, 1119, 1073, 696 cm⁻¹. ¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.94–8.96 (1H, d, $J=7.4$ Hz, -NH-), 8.82–8.85 (1H, t, $J=5.6$ Hz, -NH-), 8.33 (1H, s, -ArH 2); 8.26–8.27 (1H, d, $J=7.6$ Hz, -ArH 4), 7.94–7.96 (1H, d, $J=8$ Hz, -ArH 6), 7.75–7.79 (1H, t, $J=7.6$ Hz, -ArH 5), 4.55–4.62 (1H, m, α -H), 4.19 (2H, d, $J=5.6$ Hz, -CH₂-), 1.58–1.82 (3H, m, -CH₂- & -CH-), 0.90–0.96 (6H, dd, $J=6.4$ & 18 Hz, -CH₃).

^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$): δ 173.1 (-CONH-), 165.4 (-ArCO-), 135.05(-ArC1), 132.2 (-ArC4), 129.9 (-ArC5), 129.4 (d, -ArC3), 128.3 (d, -ArC6), 124.55 (d, -ArC2), 123.0 (-CF₃), 117.9 (-CN), 52.0 (α -C), 40.3 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.7 (-CH-), 23.3 (-CH₃), 21.5 (-CH₃). ^{19}F -NMR (376 MHz, $\text{DMSO}-d_6$): δ +14.94 (3F).

N-4-trifluoromethylbenzoyl-L-leucine-glycine nitrile (36)

N-4-trifluoromethylbenzoyl-L-leucine (1.52 g, 5 mmol) and glycine nitrile hydrochloride (0.46 g, 5 mmol) were used. Isolation by column chromatography and recrystallization from ethyl acetate/hexane furnished **36** as a white powder (0.5 g, 28%). M.p. 153–157°C. $[\alpha]_D^{25} = -26.0^\circ$ (c, 2.1, EtOH). Anal. calcd. C₁₆H₁₈N₃O₂F₃: C, 56.30; H, 5.32; N, 12.31%. Found: C, 56.19; H, 5.32; N, 12.17%. IR (KBr): ν 3291, 2968, 2249, 1635, 1544, 1335, 1281, 1176, 1112, 1074, 691 cm⁻¹. ^1H -NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.90–8.92 (1H, d, $J=8\text{ Hz}$, -NH-), 8.82–8.85 (1H, t, $J=5.6\text{ Hz}$, -NH-), 8.16 (2H, d, $J=8\text{ Hz}$, -ArH 2&6), 7.91 (2H, d, $J=8.4\text{ Hz}$, -ArH 3&5), 4.54–4.63 (1H, m, α -H), 4.19 (2H, d, $J=5.6\text{ Hz}$, -CH₂-), 1.57–1.83 (3H, m, -CH₂- & -CH-), 0.91–0.97 (6H, dd, $J=6.4$ & 17.2 Hz , -CH₃). ^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$): δ 173.1 (-CONH-), 165.7 (-ArCO-), 137.99 (-ArC1), 131.62 (d, -ArC4), 128.89 (-ArC2 & 6), 125.6 (q, -ArC3 & 5), 122.9 (-CF₃), 117.9 (-CN), 52.0 (α -C), 40.3 (-CH₂-, -ve DEPT), 27.5 (-CH₂-, -ve DEPT), 24.8 (-CH-), 23.3 (-CH₃), 21.5 (-CH₃). ^{19}F -NMR (376 MHz, $\text{DMSO}-d_6$): δ +14.71 (3F).

Biology

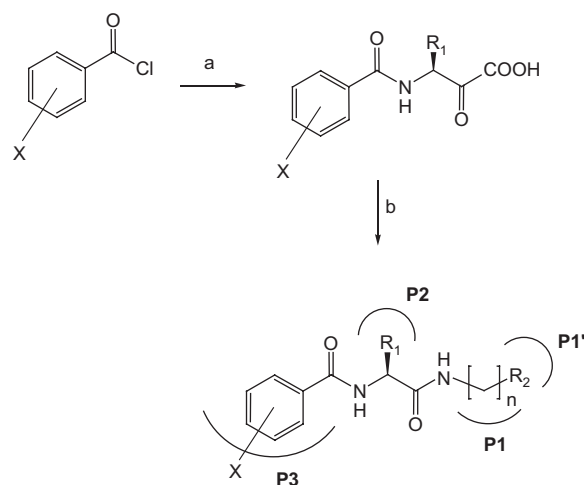
Enzyme assays with *Fasciola hepatica* cysteine cathepsin L endoprotease

Inactivated *Fasciola hepatica* cysteine cathepsin L endoprotease was isolated as previously reported¹². The protease was activated by the addition of dithiothreitol, ethylenediaminetetraacetic acid (EDTA), and sodium acetate followed by incubation at 37°C for 2 h. Proteinase activity was measured fluorometrically using *Z*-Phe-Arg-NHMec as substrate. The substrate was stored as a 1 mM stock solution in dimethyl sulfoxide (DMSO). Assays were carried out using a final concentration of 10 μM substrate in 0.1 M sodium acetate containing 0.1 M dithiothreitol and 0.5 M EDTA. The enzyme and inhibitor were incubated together for 15 min at 37°C, at which stage the substrate was added. The mixture was incubated at 37°C for 30 min. The reaction was stopped by the addition of 150 μL of a 10% acetic acid solution. The amount of 7-amino-4-methylcoumarin (-NHMec) released was measured using a PerkinElmer fluorescence spectrophotometer with excitation set at 370 nm and emission at 440 nm.

Results and discussion

Chemistry

The fluorobenzoyl amino acids were prepared using a Schotten–Baumann reaction furnishing the *N*-fluorobenzoyl amino acids in quantitative yields. The



Scheme 1. Reagents and conditions: (a) L-Leu or L-Ala, NaOH; (b) C-protected amino acid, DCC, HOBt, TEA, DCM.

dipeptidyl derivatives were synthesized using the standard DCC/HOBt protocol (Scheme 1)^{13–15}. Chiral integrity can be preserved by the use of 1-hydroxybenzotriazole (HOBt). HOBt acts as an auxiliary nucleophile and reduces the lifetime of the *O*-acylisourea intermediate, thus decreasing the occurrence of racemization. An acylating agent of lower potency is formed which is still reactive to aminolysis, but is less susceptible to racemization or other side reactions. 1-Hydroxybenzotriazole also offers an acidic hydrogen that can be more easily removed by bases than the proton at the chiral center.

The initial series of compounds synthesized concentrated on modification of the P1 position, with benzyl ester protection of the *C*-terminus (**1–12**). At this position, the methylene unit of the *C*-terminal amino acid glycine was increased in length. Using glycine benzyl ester, β -alanine benzyl ester, and γ -aminobutyric acid benzyl ester the chain length was extended. The optimal amino acid at the P2 position of the dipeptide was shown to be L-leucine^{9,10}. Compounds **1–12** were synthesized with the aim of optimizing the dipeptide backbone structure. The *ortho*-, *meta*-, and *para*-fluorinated derivatives were prepared along with the pentafluoro, for each of the three P1 amino acids.

The second series of inhibitors prepared involved modification of both the P1 and P2 positions of the dipeptide backbone. The fluorinated P3 positions were the same as the benzyl ester series. A nitrile moiety was introduced at the *C*-terminal. Compounds **13–20** were prepared by coupling either fluorinated benzoyl-L-leucine or benzoyl-L-alanine to glycine nitrile or β -alanine nitrile.

Biological results (see below) from this investigation showed compounds **13** and **14**, the leucine-glycine nitriles, to be the most active. A complete structure–activity relationship (SAR) study of these compounds was performed. By altering the number and position of fluorines at the P3 phenyl ring position, compounds **21–36** were synthesized by coupling the relevant fluorinated benzoyl-L-leucine with 2-aminoacetonitrile.

Biology

Protease inhibitory assays

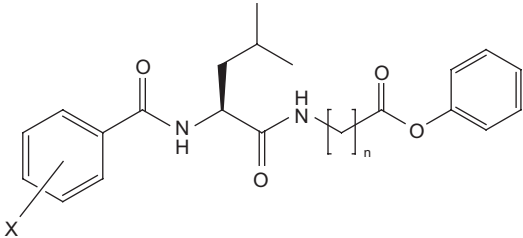
Proteolytic enzymes of the liver fluke *Fasciola hepatica* have been associated with cells of the gut epithelium. Accordingly these enzymes have been implicated in diverse functions such as feeding, tissue degradation, and protection of the parasite against attack. The first of the *Fasciola hepatica* secreted cysteine proteases (FhCL1) was isolated using gel filtration and ion exchange chromatography¹⁶. NH₂-terminal sequencing and substrate specificity studies revealed that the enzyme was a cathepsin L protease (FhCL1). Dowd *et al.* purified the second major secreted protease to homogeneity¹⁷. This enzyme is now termed cathepsin L2 (FhCL2). The involvement of these enzymes in key parasite functions renders them potential targets at which to direct novel antiparasite chemotherapy.

The exploitation of the leucine-glycine dipeptidyl scaffold has resulted in numerous cathepsin protease inhibitors¹⁸. The incorporation of an aromatic substituent at the *N*-terminus has been imperative to both the selectivity and potency of dipeptidyl inhibitors¹⁹. Previous work in these laboratories has identified dipeptidyl ester derivatives as inhibitors of *Fasciola hepatica* cathepsin L protease¹⁰. This prompted us to investigate the effect that fluorine substitution would have on the bioactivity of these compounds. The benefits of introducing fluorine into a biomolecule have been well documented²⁰. It was also anticipated that modification of the P1 side chain by one, two, and three methylene units would furnish more potent inhibitors of *Fasciola hepatica* cathepsin L endoproteases. Further studies involved the two other sites of possible modification of the dipeptide structure (P2 and P3). All the synthesized compounds underwent biological evaluation. The inhibitory activities of these compounds against the *Fasciola hepatica* cysteine cathepsin L endoproteases were determined using the fluorescent substrate *Z*-Phe-Arg-NHMec.

The inhibitory activities of compounds **1–12** are shown in Table 1. The γ -aminobutyric acid (ABA) derivatives were found to be the most effective inhibitors. The incorporation of three methylene units into the P1 position gave inhibitors with activity up to two-fold greater than their β -alanine analogs and as much as seven-fold greater than the glycine derivatives. The most active compound in this series was found to be *N*-2-fluorobenzoyl-*L*-leucine- γ -aminobutyric acid benzyl ester **9**, with an IC₅₀ value of 15.3 μ M, closely followed by its 4-fluorobenzoyl analog **11** that recorded an IC₅₀ value of 19.3 μ M. The P3 substitutions demonstrated a significant influence on the overall activity of these derivatives. In the case of the β -alanine and γ -ABA derivatives, mono-substitution of the benzoyl ring gave the most potent results, but in contrast, the incorporation of a pentafluorobenzoyl moiety furnished the best results with the glycine derivatives.

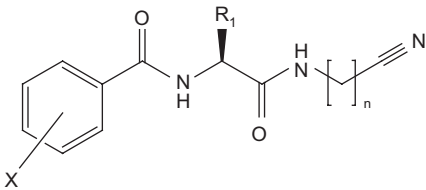
The inhibitory activities of the dipeptidyl nitriles **13–20** are shown in Table 2. The pentafluoro and 4-fluoro derivatives were shown to be more potent derivatives than the

Table 1. *In vitro* inhibitory activity of *N*-fluorobenzoyl dipeptidyl benzyl esters **1–12**, and compounds **37** and **38**, with the *Fasciola hepatica* cysteine cathepsin L endoprotease FhCL1.



Compound	X	n	IC ₅₀ (μ M)
1	2-F	1	>100
2	3-F	1	>100
3	4-F	1	43.4
4	Penta-F	1	39.5
5	2-F	2	29.4
6	3-F	2	24.8
7	4-F	2	62.3
8	Penta-F	2	57.7
9	2-F	3	15.3
10	3-F	3	40.1
11	4-F	3	19.3
12	Penta-F	3	21.4
37	—	—	10.0
38	—	—	11.0

Table 2. *In vitro* inhibitory activity of *N*-fluorobenzoyl dipeptidyl nitriles **13–20**, and compounds **37** and **38**, with the *Fasciola hepatica* cysteine cathepsin L endoprotease FhCL1.

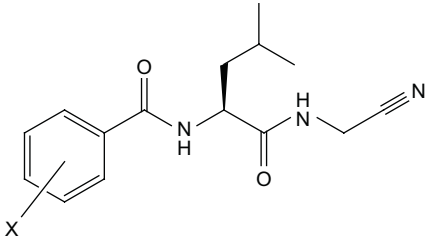


Compound	X	R1	n	IC ₅₀ (μ M)
13	2-F	<i>s</i> -Bu	1	3.0
14	4-F	<i>s</i> -Bu	1	2.8
15	2-F	-CH ₃	1	87.6
16	Penta-F	-CH ₃	1	68.3
17	2-F	<i>s</i> -Bu	2	24.3
18	4-F	<i>s</i> -Bu	2	21.6
19	2-F	-CH ₃	2	>100
20	Penta-F	-CH ₃	2	>100
37	—	—	—	10.0
38	—	—	—	11.0

2-fluoro analogs. A reduction of the P2 substituent size by using *L*-alanine resulted in a decrease in activity of up to 30-fold. By increasing the number of methylene units from one to two at position P1, the inhibitory activity was shown to decrease by up to eight-fold. This clearly shows that a glycine residue in the P1 position and a leucine residue in the P2 position are essential for effective inhibition.

The biological results of the completed SAR study (**21–36**) are outlined in Table 3. The IC₅₀ of the commercial

Table 3. *In vitro* inhibitory activity of *N*-fluorobenzoyl dipeptidyl nitriles **21–36**, and compounds **37** and **38**, with the *Fasciola hepatica* cysteine cathepsin L endoprotease FhCL1.



Compound	X	IC ₅₀ (μM)	Compound	X	IC ₅₀ (μM)
21	3-F	3.1	30	2,4,5-F	4.5
22	2,3-F	3.4	31	3,4,5-F	3.4
23	2,4-F	5.5	32	2,3,4,5-F	3.4
24	2,5-F	3.1	33	Penta-F	4.4
25	2,6-F	3.7	34	2-CF ₃	4.1
26	3,4-F	3.1	35	3-CF ₃	9.1
27	3,5-F	3.0	36	4-CF ₃	3.2
28	2,3,6-F	6.0	37	—	10.0
29	2,3,4-F	4.1	38	—	11.0

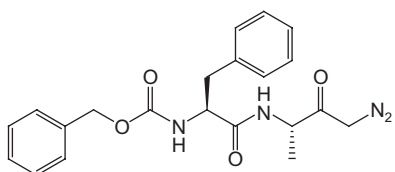


Figure 1. Commercially available inhibitor **39**.

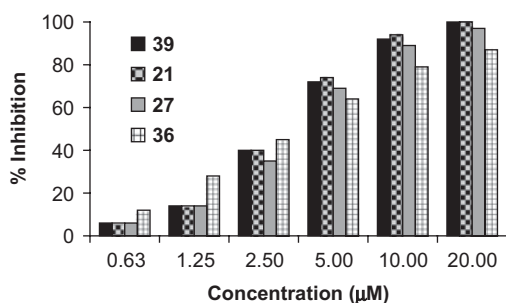


Figure 2. Bar chart illustration of percent inhibition of **39**, **21**, **27**, and **36** toward cathepsin L at varying concentrations (μM).

diazomethane inhibitor *Z*-Phe-Ala-CHN₂ **39** (Figure 1) is 3.0 μM¹⁰, which is comparable to the inhibitory concentrations found for the majority of the *N*-fluorobenzoyl-L-leucine-glycine nitrile derivatives. From the variable concentration assays performed, it can be seen that the commercially available inhibitor **39** is more potent at lower concentrations up to about 50% inhibition. Inhibitors **21**, **27**, and **36** showed comparable results which are displayed in a representative bar chart (Figure 2). It is clear that the introduction of fluorine atom(s) increases the biological activity of the compounds employed in this study. However, a direct correlation between number and position of the fluorine atoms on the aromatic ring with biological activity was not observed.

Conclusion

In summary, we have identified active inhibitors of the liver fluke cysteine protease cathepsin L. It has been shown that increasing the number of methylene units in the P1 position or reducing the size of the P2 substituent reduces the inhibitory activity of these compounds. It has also been shown that fluorobenzoyl dipeptidyl benzyl ester derivatives have the ability to be inhibitors of cathepsin L but are not as effective as the L-Leu-Gly nitrile motif. From the protease inhibition assays it has been found that at the *N*-terminal position P3, the location of the fluorine isostere may not be of major importance to the activity of the inhibitor. The number of fluorine substituents attached to the benzoyl group also gives little if any improvement to the biological activity. Despite this, it is evident that almost all of the fluorobenzoyl-L-leucine-glycine nitrile derivatives offer real inhibitory potency against the cathepsin L endoprotease. It is hoped that with further studies, greater improvements in bioactivity can be made. The inhibitory potency of the nitrile compounds such as *N*-3,5-difluorobenzoyl-L-leucine-glycine nitrile **27** relative to the commercial inhibitor *Z*-Phe-Ala-CHN₂ **39** shows that such benzoyl dipeptidyl derivatives may have a role to play as commercial inhibitors of *Fasciola hepatica* cathepsin L endoproteases. Subsequent reports will detail efforts to improve the activity of similar dipeptidyl derivatives with further modifications at the P3 position.

Acknowledgements

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Declaration of interest: The authors report no conflicts of interest.

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