The synthesis and anticancer activities of peptide 5-fluorouracil derivatives

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A new series of peptide 5-fluorouracil derivatives was designed and synthesised in order to test in vitro anticancer activities. The results indicated that peptide 5-fluorouracil derivatives possessed anticancer activities against human HL-60 and Bel-7402 cell lines. The structures of the compounds were determined by means of ^{1}H NMR, ^{13}C NMR, IR, mass spectra and elemental analyses.

Keywords: peptide 5-fluorouracil, anticancer activities, crystal structure

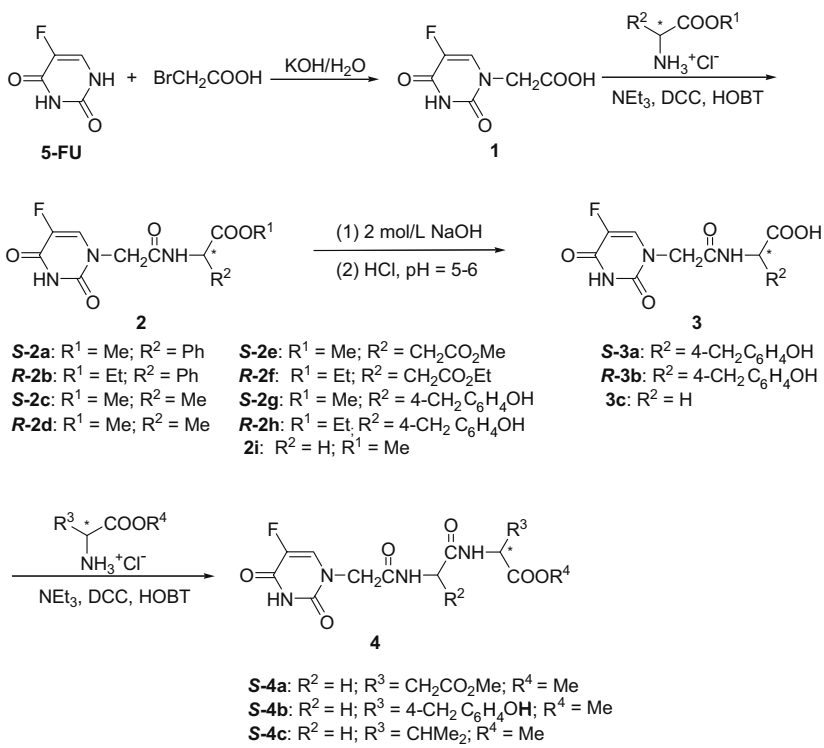
Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge at the start of the 21 st century. Therefore, identification of novel, potent selective, and less toxic anticancer agents remains one of the most pressing health problems.[1,2]

5-Fluorouracil (5-FU) has been used in treating cancer and squamous cell carcinoma of the head and neck.[3,4] However, the clinical dosage of (5-FU) is very close to its toxic one when given intravenously, resulting in strong toxicities to the gastric and intestinal mucosa and the bone marrow.[5] Novel prodrugs of 5-FU possessing a broader spectrum of antitumour activity and less toxicity have been sought diligently by many researchers.[6-8] The common feature of these prodrugs is that they are all N_1-modified derivatives through different biodegradable linkages.

Peptides play important roles in the metabolism of human beings and other organisms. They can function as hormones, enzyme inhibitors/substrates, growth promoters, inhibitors, neurotransmitters and immunomodulating agents as well as antibiotics, driving considerable pharmacological interests in the design and application of new drugs involving peptides.[9,10]

Based on the mutual prodrug concept, in continuation of our interest in the synthesis of 5-FU derivatives,[11-13] we have synthesised some peptide derivatives of 5-FU with the aim of finding appropriate biodegradable linkages and of improving tumour selectivity, efficiency and safety.

As shown in Scheme 1, compound **1** was prepared from 5-FU and 2-bromoacetic acid according to the literature.[14] Compound **2** was obtained by the reaction of amino acid hydrochlorides with compound **1** in the presence of dicyclohexyl carbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) at room temperature. Hydrolysis of **2** in aqueous sodium hydroxide and subsequent treatment with concentrated HCl afforded compound **3**. Compound **4** was prepared from **3** by a similar method to the transesterification of amino acid hydrochlorides used for the preparation of compound **2** (Scheme 1).



Scheme 1

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Compounds **2a**–**h** and **4a**–**c** were evaluated for percentage of inhibition of HL-60 and BEL-7402 cell growth. The antitumour activities in vitro for these compounds were evaluated by the SRB method for BEL-7402 cells and the MTT method for HL-60 cells. The activity data are presented in Tables 1 and 2. The results indicated that among the tested compounds, a majority of the newly prepared compounds showed a moderate inhibiting effect on the growth of the HL-60 and BEL-7402 cells at different concentrations of 10^{-4} mol L^{-1}, 10^{-5} mol L^{-1}, 10^{-6} mol L^{-1}, 10^{-7} mol L^{-1}, and 10^{-8} mol L^{-1}. The inhibiting effect of all the compounds against the HL-60 cells was different from that against the BEL-7402 cell lines, which suggested that these compounds possibly had different inhibition mechanisms against various tumor cells.[15] The anticancer activities of the *S*-configuration compounds were different from the *R*–analogues, which indicated that the anticancer activity was related to the configuration.[16] Also, inspection of the chemical structure and anticancer activity of **2e**, **2g**, **4a** and **4b** showed that the activities of longer peptide chain compounds (**4a** and **4b**) were significantly lower than those of the shorter ones (**2e** and **2g**).

All the products have been characterised by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and MS. Meanwhile, in order to confirm further the structure of compounds **3**, the crystal structure of **3b** as a representative example was determined by X-ray crystallography and an ORTEP view of the molecule together with the atomic numbering is shown in Fig. 1.

In conclusion, 15 5-FU derivatives (**2a**–**i**, **3a**–**c**, and **4a**–**c**) were synthesised and the crystal structure of **3b** was determined by X-ray diffraction analysis. The results of assay for the anticarcinogenic activity against the HL-60 and BEL-7402 cell lines indicated that some of the target compounds possessed significant anticancer activities. Further study in this area is underway in our laboratory.

Experimental

All chemicals and solvents were purchased from Aldrich, and Fluka. Melting points were recorded on a Digital Melting Point Apparatus WRS-1B and are uncorrected. TLC was performed using precoated silica gel 60 GF_{254} and column chromatography was performed using silica gel (300–400 mesh). IR spectra were taken on an EQUINOX-55 instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded on a AVANCE-300 instrument using tetramethylsilane (TMS) as an internal standard and DMSO-d_6 as the solvent at room temperature. Chemical shifts are given in δ relative to TMS, coupling constants (*J*) are expressed in Hz. Mass spectra were obtained on a DECAX-3000LCQ DecaXPPlus. The crystallographic data were collected on a Bruker Smart-Apex CCD diffractometer. Antimicrobial activities of the title compound were evaluated at The National Centre for Drug Screening in Shanghai, China.

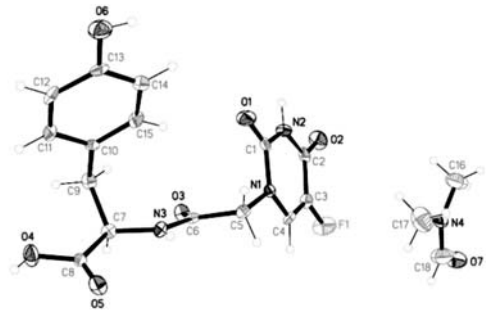

Fig. 1

General method for the synthesis of compound **2**

The starting material, 5-fluorouracil-1-acetic acid (5-fluoro-2,4-dioxo-1,2,3,4-tetrahydropyrimidine-1-acetic acid) was prepared from 5-FU and bromoacetic acid, in line with the literature method.[14] **2a**–**i** were synthesised from 5-fluorouracil-1-acetic acid, DCC, HOBt as follows: A solution of DCC (6.18 g, 30 mmol) in DMF (30 mL) was added dropwise to a DMF solution (50 mL) of 5-fluorouracil-1-acetic acid (3.76 g, 20 mmol) and HOBt (2.97 g, 22 mmol) at 0 °C over a period of 40 min. The resulting solution was stirred at room temperature for 5 h. The appropriate ester of the amino acid hydrochloride (20 mmol) and triethylamine (2.8 mL, 20 mmol) was added to the above mixture. After stirring 4 hours, a white solid was obtained. After filtration, the filtrate was concentrated under reduced

Table 1 Percentage of inhibition of HL-60 cell growth

Entry	Compound	Concentration/mol L^{-1}				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1	**2a**	63.1	0	5.4	9.7	2.3
2	**2b**	65.1	0	4.6	13.0	0.3
3	**2c**	59.1	0	0	0	1.2
4	**2e**	67.2	0	23.2	11.0	3.9
5	**2f**	55.8	2.7	12.8	5.4	5.9
6	**2g**	58.3	2.7	6.3	6.2	8.1
7	**2h**	68.3	25.5	9.8	12.7	8.9
8	**4a**	3.7	5.9	6.3	8.2	8.7
9	**4b**	11.0	6.5	9.5	4.0	7.9
10	**4c**	3.5	10.8	6.3	12.5	8.2

Table 2 Percentage of inhibition of BEL-7402 cell growth

Entry	Compound	Concentration/mol L^{-1}				
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1	**2a**	37.2	10.4	3.4	2.4	2.2
2	**2b**	46.4	7.7	6.9	9.8	9.6
3	**2c**	41.7	15.8	16.8	13.0	16.3
4	**2e**	47.5	16.1	0	11.8	5.6
5	**2f**	34.0	9.2	4.1	3.5	5.9
6	**2g**	34.2	9.7	2.3	7.3	7.3
7	**2h**	40.6	2.4	2.1	7.3	4.8
8	**4a**	19.9	0	5.5	7.4	0
9	**4b**	1.2	0	0	0	0
10	**4c**	11.4	6.5	0	0	0

pressure and the residue was separated by column chromatography to afford compound **2**.

(S)-Methyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)-2-phenyl acetate (**2a**): Yield: 56%, m.p. 184−186 °C. $[\alpha]_D^{20}$ = –105.4 (c 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3262 (N−H), 3054 (=C−H), 1671 (C=O), 1549 (C=N), 1378, 1243 (C−O−C), 975, 700. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.85 (d, J = 3.9 Hz, 1H), 9.16 (d, J = 7.2 Hz, 1H), 8.03 (d, J = 6.6 Hz, 1H), 7.36−7.39 (m, 5H), 5.46 (d, J = 7.2 Hz, 1H), 4.45 (d, J = 16.8 Hz, 1H), 4.38 (d, J = 16.8 Hz, 1H), 3.64 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.6, 166.6, 157.4 (d, $J_{C\text{-}F}$ = 25.7 Hz), 149.6,139.1 (d, $J_{C\text{-}F}$ = 226.7 Hz), 136.0, 131.0 (d, $J_{C\text{-}F}$ = 33.9 Hz,), 128.7, 128.3, 127.5, 56.2, 52.3, 49.2. ESI-MS (*m/z*): 334.1 ([M-1]$^+$, 100). Elemental anal. Calcd for $C_{15}H_{14}N_3FO_5$: C, 53.73; H, 4.18; N, 12.54. Found: C, 53.42; H, 4.14; N, 12.58%.

(R)-Ethyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)-2-phenylacetate (**2b**): Yield: 67%, m.p. 186−187 °C. $[\alpha]_D^{20}$ = + 72.4 (c 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3327 (N−H), 3075 (=C−H), 1708(C=O), 1545 (C=N), 1445 (−CH$_2$−), 1377 (C−H), 1233 (C−O−C), 977, 700. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.83 (s, 1H), 9.12 (d, J = 7.2 Hz, 1H), 8.03 (d, J = 6.9 Hz, 1H), 7.36−7.41 (m, 5H), 5.42 (d, J = 7.2 Hz, 1H), 4.44 (d, J = 16.5 Hz, 1H), 4.38 (d, J = 16.5 Hz, 1H), 4.02−4.19 (m, 2H,), 1.13 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.1, 166.6, 157.4 (d, $J_{C\text{-}F}$ = 25.4 Hz), 149.5, 139.1 (d, $J_{C\text{-}F}$ = 227.0 Hz), 136.0, 131.0 (d, $J_{C\text{-}F}$ = 31.8 Hz), 128.7, 128.3, 127.5, 61.0, 56.3, 49.2, 13.8. ESI-MS (*m/z*): 348.0 ([M-1]$^+$, 100). Elemental anal. Calcd for $C_{16}H_{16}N_3FO_5$: C, 55.01; H, 4.58; N, 12.03. Found: C, 54.96; H, 4.64; N, 12.12%.

(S)-Methyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido) propanoate (**2c**): Yield: 58%, m.p. 300−302 °C. $[\alpha]_D^{20}$ = -12.7 (*c* 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3437 (N−H), 3081 (=C−H), 2930 (C−H), 1709 (C=O), 1662 (C=C), 1379 (C−H), 1232 (C−O−C). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.85 (d, J = 5.1 Hz, 1H), 8.67 (d, J = 7.2 Hz, 1H), 8.02 (d, J = 6.6 Hz, 1H), 4.28−4.32 (m, 3H), 3.63 (s, 3H), 1.28 (d, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.7, 166.5, 157.5 (d, $J_{C\text{-}F}$ = 25.1 Hz), 149.6, 139.2 (d, $J_{C\text{-}F}$ = 226.7 Hz), 131.0 (d, $J_{C\text{-}F}$ = 34.1 Hz), 51.9, 49.2, 47.6, 17.1. ESI-MS (*m/z*): 272.1 (M$^+$, 100). ESI-MS (*m/z*): 272.1 (M$^+$, 100). Elemental anal. Calcd for $C_{10}H_{12}N_3FO_5$: C, 43.96; H, 4.40; N, 15.38. Found: C, 43.93; H, 4.42; N, 15.31%.

(R)-Methyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido) propanoate (**2d**). Yield: 49%, m.p. 301−303 °C. $[\alpha]_D^{20}$ = + 12.7 (*c* 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3437 (N−H), 3081 (=C−H), 2930 (C−H), 1709 (C=O), 1662 (C=C), 1379 (C−H), 1232 (C−O−C) ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.84 (d, J = 5.1 Hz, 1H), 8.67 (d, J = 7.2 Hz, 1H), 8.02 (d, J = 6.6 Hz, 1H), 4.29−4.32 (m, 3H), 3.63 (s, 3H), 1.28 (d, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.7, 166.5, 157.5 (d, $J_{C\text{-}F}$ = 25.1 Hz), 149.6, 139.2 (d, $J_{C\text{-}F}$ = 226.7 Hz), 131.0 (d, $J_{C\text{-}F}$ = 34.7 Hz), 51.9, 49.2, 47.6, 17.1. ESI-MS (*m/z*): 272.1 (M$^+$, 100). Elemental anal. Calcd for $C_{10}H_{12}N_3FO_5$: C, 43.96; H, 4.40; N, 15.38. Found: C, 43.87; H, 4.45; N, 15.20%.

(S)-Dimethyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido) succinate (**2e**): Yield: 69%, m.p. 109−110 °C. $[\alpha]_D^{20}$ = –14.4 (*c* 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3412 (N−H), 3046 (=C−H), 2849 (−CH$_2$−), 1753 (C=O), 1690 (C=O), 1522 (C=N), 1438 (−CH$_2$−), 1385 (C−H), 1249 (C−F), 1222 (C−O−C). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.85 (d, J = 4.8 Hz, 1H), 8.75 (d, J = 8.1 Hz, 1H), 8.01 (d, J = 6.9 Hz, 1H), 4.67 (dd, J = 6.3 Hz, J = 13.8 Hz, 1H), 4.33 (s, 2H), 3.61 (s, 3H), 3.64 (s, 3H), 2.69−2.85 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.7, 170.2, 166.7, 157.5 (d, $J_{C\text{-}F}$ = 25.0 Hz), 149.6, 139.2 (d, $J_{C\text{-}F}$ = 226.2 Hz), 130.9 (d, $J_{C\text{-}F}$ = 35.6 Hz), 52.2, 51.7, 49.3, 48.6, 35.7. ESI-MS (*m/z*): 330.2 (M$^+$, 100). Elemental anal. Calcd for $C_{12}H_{14}N_3FO_7$: C, 43.50; H, 4.32; N, 12.69. Found: C, 43.44; H, 4.64; N, 12.85%.

(R)-Diethyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido) succinate (**2f**): Yield: 70%, m.p.109−110 °C. $[\alpha]_D^{20}$ = + 16.0 (*c* 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3313.58 (N−H), 3075 (=C−H), 2989 (−CH$_2$−), 1720 (C=O), 1549 (C=N), 1467 (−CH$_2$−), 1377 (C−H), 1239 (C−F), 1167 (C−O−C). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.83 (d, J = 4.5 Hz, 1H), 8.72 (d, J = 7.8 Hz, 1H), 7.99 (d, J = 6.6 Hz, 1H), 4.64 (dd, J = 6.6 Hz, J = 14.1 Hz, 1H), 4.33 (s, 2H), 4.04−4.13 (m, 4H), 2.66−2.82 (m, 2H), 1.18 (t, J = 7.2 Hz, 3H), 1.17 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.2, 169.8, 166.7, 157.5 (d, $J_{C\text{-}F}$ = 24.5 Hz), 149.6, 139.2 (d, $J_{C\text{-}F}$ = 227.0 Hz), 130.9 (d, $J_{C\text{-}F}$ = 35.1 Hz), 61.0, 60.4, 49.3, 48.7, 35.9, 14.0, 13.9. ESI-MS (*m/z*): 358.2 (M$^+$, 100). Elemental anal. Calcd for $C_{14}H_{18}N_3FO_7$: C, 46.80; H, 5.01; N, 11.70. Found: C, 46.60; H, 4.94; N, 11.68%.

(S)-Methyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)-3-(4-hydroxyphenyl) propanoate (**2g**): Yield: 73%, m.p. 156−157 °C. $[\alpha]_D^{20}$ = + 38.4 (*c* 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3270 (N−H), 1738 (C=O), 1661 (C=C), 1552 (C=N), 1515, 1450 (−CH$_2$−), 1386 (C−H), 1230 (C−F), 1164 (C−O−C), 778. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.84 (s, 1H), 9.28 (s, 1H), 8.67 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 6.99 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H), 4.30−4.40 (m, 3H), 3.58 (s, 3H), 2.76−2.92 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.7, 166.7, 157.7, 156.1, 149.6, 139.2 (d, $J_{C\text{-}F}$ = 226.9 Hz), 131.0 (d, $J_{C\text{-}F}$ = 32.2 Hz), 130.1, 126.8, 115.1, 54.2, 51.9, 49.3, 36.1. ESI-MS (*m/z*): 364.6 (M$^+$, 100). Elemental anal. Calcd for $C_{16}H_{16}N_3FO_6$: C, 52.60; H, 4.38; N, 11.51. Found: C, 52.73; H, 4.26; N, 11.38%.

(R)-Ethyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)-3-(4-hydroxyphenyl) propanoate (**2h**): Yield: 66%, m.p. 159−160 °C. $[\alpha]_D^{20}$ = –29.7 (*c* 1.0, DMF).ν_{max} (KBr)/cm^{-1} 3336 (N−H), 3064 (=C−H), 2850 (−CH$_2$−), 1690 (C=O), 1532 (C=N), 1430 (−CH$_2$−), 1380 (C−H), 1220 (C−O−C), 972 (C−H). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.83 (s, 1H), 9.24 (s, 1H), 8.64 (d, J = 7.5 Hz, 1H), 7.95 (d, J = 6.9 Hz, 1H), 6.95 (d, J = 8.1 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H), 4.31−4.41 (m, 3H), 4.03 (q, J = 6.9 Hz, 2H), 2.82−2.87 (m, 2H), 1.10 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.2, 166.7, 157.5 (d, $J_{C\text{-}F}$ = 25.7 Hz), 156.1, 149.6, 139.2 (d, $J_{C\text{-}F}$ = 227.0 Hz), 131.0 (d, $J_{C\text{-}F}$ = 33.3 Hz), 130.1, 126.8, 115.1, 60.5, 54.2, 49.3, 36.2, 13.9. ESI-MS (*m/z*): 378.3 (M$^+$, 100). Elemental anal. Calcd for $C_{17}H_{18}N_3FO_6$: C, 53.83; H, 4.75; N, 11.08. Found: C, 53.91; H, 4.51; N, 10.86%.

Methyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetamido)acetate (**2i**): Yield: 83%, m.p. 192−195 °C. ν_{max} (KBr)/cm^{-1} 3362 (N−H), 3042 (=C−H), 2833 (C−H), 1735 (C=O), 1678 (C=C), 1383 (C−H), 1236 (C−O−C). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.87 (s, 1H), 8.67 (t, J = 5.9 Hz, 1H), 8.04 (d, J = 6.9 Hz, 1H), 4.34 (s, 2H), 3.90 (d, J = 6.0 Hz, 2H), 3.63 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.1, 167.4, 157.7 (d, $J_{C\text{-}F}$ = 25.8 Hz), 149.8, 139.5 (d, $J_{C\text{-}F}$ = 228.4 Hz), 131.0 (d, $J_{C\text{-}F}$ = 33.9 Hz), 51.9, 49.5, 40.7. ESI-MS (*m/z*): 258.0 (M$^+$, 100). Elemental anal. Calcd for $C_9H_{10}N_3FO_5$: C, 41.71; H, 3.86; N, 16.22. Found: C, 41.43; H, 3.84; N, 16.07%.

General method for the synthesis of compound **3**

2 (5 mmol) was dissolved in 2 mol L^{-1} NaOH (10 mL) (the solution maintains the pH at 10 to 11) and the solution was stirred at room temperature until **2** was fully hydrolysed (as monitored by TLC). The solution was then acidified with hydrochloric acid to pH = 2. The resulting precipitate was filtered, washed with ethyl acetate, dried, and recrystallised from ethanol.

(S)-2-(2-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetamido)-3-(4-hydroxyphenyl) propanoic acid (**3a**): Yield: 86%, m.p. 251−252 °C. $[\alpha]_D^{20}$ = + 97.7 (*c* 1.0, DMF).ν_{max} (KBr)/cm^{-1} 3372 (N−H), 3308 (N−H), 3213 (O−H), 1716 (C=O), 1663 (C=C), 1548 (C=N), 1515, 1482 (−CH$_2$−), 1381 (COOH), 1258 (C−F), 671. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.77 (s, 1H), 11.82 (d, J = 5.1 Hz, 1H), 9.23 (s, 1H), 8.51 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 6.9 Hz, 1H), 7.01 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.7 Hz, 2H), 4.25−4.39 (m, 3H), 2.92 (dd, J = 13.8 Hz, J = 4.8 Hz 1H), 2.78 (dd, J = 13.8 Hz, J = 8.7 Hz 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.5, 166.5, 157.5 (d, $J_{C\text{-}F}$ = 25.7 Hz), 156.0, 149.6, 139.1 (d, $J_{C\text{-}F}$ = 225.8 Hz), 131.0 (d, $J_{C\text{-}F}$ = 34.2 Hz), 130.1, 127.2, 115.0, 54.0, 49.3, 36.0. ESI-MS (*m/z*): 350.1 (M$^+$, 100). Elemental anal. Calcd for $C_{15}H_{14}N_3FO_6$: C, 51.28; H, 3.99; N, 11.97. Found: C, 51.53; H, 4.07; N, 11.808%.

(R)-2-(2-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetamido)-3-(4-hydroxy phenyl) propanoic acid (**3b**): Yield: 88%, m.p. 251−252 °C. $[\alpha]_D^{20}$ = –97.7 (*c* 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3372 (N−H), 3210 (O−H), 3092 (=C−H), 1724 (C=O), 1662 (C=C), 1550 (C=N), 1429 (−CH$_2$−), 1375 (COOH), 1256 (C−F), 825, 669. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.76 (s, 1H), 11.82 (d, J = 5.1 Hz, 1H), 9.22 (s, 1H), 8.51 (d, J = 7.5 Hz, 1H), 7.94 (d, J = 6.9 Hz, 1H), 7.00 (d, J = 8.1 Hz, 2H), 6.66 (d, J = 8.1 Hz, 2H), 4.30−4.39 (m, 3H), 2.92 (dd, J = 13.8 Hz, J = 8.4 Hz 1H), 2.78 (dd, J = 13.8 Hz, J = 8.1 Hz 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 172.6, 166.5, 157.5 (d, $J_{C\text{-}F}$ = 33.8 Hz), 156.0, 149.6, 139.2 (d, $J_{C\text{-}F}$ = 227.3 Hz), 131.0 (d, $J_{C\text{-}F}$ = 35.1 Hz), 130.1, 127.2, 115.0, 54.1, 49.3, 36.0. ESI-MS (*m/z*): 350.3 (M$^+$, 100). Elemental anal. Calcd for $C_{15}H_{14}N_3FO_6$: C, 51.28; H, 3.99; N, 11.97. Found: C, 51.30; H, 3.96; N, 12.07%.

2-(2-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetamido)acetic acid (**3c**): Yield: 86%, m.p. 257−259 °C.ν_{max} (KBr)/cm^{-1} 3490 (N−H), 3412 (O−H), 3070 (=C−H), 1701 (C=O), 1662 (C=O), 1541 (N−C=O), 1383 (COOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.64 (s, 1H), 11.83 (d, J = 4.5 Hz, 1H), 8.52 (t, J = 6.0 Hz, 1H), 8.02(d, J = 6.9 Hz, 1H), 4.34 (s, 2H), 3.80 (d, J = 6.0 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.8, 166.9, 157.5 (d, $J_{C\text{-}F}$ = 24.8 Hz), 149.6, 139.2 (d, $J_{C\text{-}F}$ = 226.8 Hz), 130.9 (d, $J_{C\text{-}F}$ =

34.0 Hz), 49.3, 40.6. ESI-MS (*m/z*): 244.0 (M^+, 100). Elemental anal. Calcd for $C_8H_8N_3FO_5$: C, 39.18; H, 3.27; N, 17.14. Found: C, 39.13; H, 3.14; N, 17.11%.

General method for the synthesis of compound **4**

A solution of DCC (3.09 g, 15 mmol) in DMF (15 mL) was added dropwise to a DMF solution (25 mL) of **3c** (2.45 g, 10 mmol) and HOBt (1.49 g, 11 mmol) at 0 °C over a period of 40 min. The resulting solution was stirred at room temperature for 5 h. The appropriate ester of the amino acid hydrochloride (10 mmol) and triethylamine (1.4 mL, 10 mmol) were added to the above mixture. After 4 h stirring, a white solid was obtained. After filtration, the filtrate was concentrated under reduced pressure and the residue was separated by column chromatography to afford compound **4**.

(S)-Dimethyl-2-(2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido)acetamido) succinate (**4a**): Yield: 48%, m.p. 195−196 °C. $[\alpha]_D^{20}$ = –14.0 (*c* 1.0, DMF).ν_{max} (KBr)/cm^{-1} 3365 (N−H), 1662 (C=O), 1543 (C=N), 1429 (−CH_2−), 1382 (C−H); 1237 (C−O−C). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.80 (s, 1H), 8.45 (t, *J* = 5.6 Hz, 1H), 8.39 (d, *J* = 7.8 Hz, 1H), 7.99 (d, *J* = 6.6 Hz, 1H), 4.67 (dd, *J* = 14.7 Hz, *J* = 6.9 Hz, 1H), 4.36 (s, 1H), 3.78 (d, *J* = 6.0 Hz, 2H), 3.63 (s, 3H), 3.61 (s, 3H), 2.67−2.85 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 170.9, 170.3, 168.4, 166.8, 157.5 (d, $J_{C\text{-}F}$ = 16.4 Hz), 149.6, 139.2 (d, $J_{C\text{-}F}$ = 227.6 Hz), 130.9 (d, $J_{C\text{-}F}$ = 33.1 Hz), 52.1, 51.6, 49.5, 48.4, 41.6, 35.6. ESI-MS (*m/z*): 387.1 (M^+, 100). Elemental anal. Calcd for $C_{14}H_{17}N_4FO_8$: C, 43.30; H, 4.38; N, 14.43. Found: C, 43.52; H, 4.14; N, 14.18%.

(S)-Methyl-2-(2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl) acetamido)acetamido)-3-(4-hydroxyphenyl)propanoate (**4b**): Yield: 55%, m.p. 168−169 °C. $[\alpha]_D^{20}$ = + 16.7 (*c* 1.0, DMF). ν_{max} (KBr)/cm^{-1} 3366 (N−H), 3025(=C−H), 1662 (C=O), 1543 (C=N), 1429 (−CH_2−), 1382 (C−H), 1238 (C−O−C), 672. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.84 (s, 1H), 9.23 (s, 1H), 8.32(t, *J* = 5.6 Hz, 1H), 8.31 (d, *J* = 6.9 Hz, 1H), 8.00 (d, *J* = 6.9 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 4.33−4.43 (m, 3H), 3.66−3.83 (m, 2H), 3.58 (s, 3H), 2.89 (dd, *J* = 13.5 Hz, *J* = 6.0 Hz, 1H), 2.78 (dd, *J* = 13.5 Hz, *J* = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.9, 168.4, 166.8, 157.5 (d, $J_{C\text{-}F}$ = 25.1 Hz), 156.0, 149.7, 139.3 (d, $J_{C\text{-}F}$ = 227.0 Hz), 130.9 (d, $J_{C\text{-}F}$ = 33.5 Hz), 129.9, 126.9, 115.1, 53.9, 51.8, 49.5, 41.5, 36.1. ESI-MS (*m/z*): 421.2 (M^+, 100). Elemental anal. Calcd for $C_{18}H_{19}N_4FO_7$: C, 51.18; H, 4.50; N, 13.27. Found: C, 51.26; H, 4.64; N, 12.92%.

(S)-Methyl-2-(2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetamido) acetamido)-3-methyl butanoate (**4c**): Yield: 45%, m.p. 200−201 °C. $[\alpha]_D^{20}$ = + 4.4 (*c* 1.0, DMF).ν_{max} (KBr)/cm^{-1} 3359 (N−H), 2967 (=C−H), 1743 (C=O), 1665 (C=C), 1430 (−CH_2−), 1383 (C−H), 1241 (C−O−C). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 11.86 (s, 1H), 8.46 (t, *J* = 5.7 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 6.9 Hz, 1H), 4.33 (s, 2H), 4.17 (dd, *J* = 7.5 Hz, *J* = 7.5 Hz, 1H), 3.83 (d, *J* = 5.7 Hz, 2H), 3.63 (s, 3H), 1.97−2.06 (m, 1H), 0.86 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ = 171.9, 168.6, 166.8, 157.5 (d, $J_{C\text{-}F}$ = 24.8 Hz), 149.7, 139.2 (d, $J_{C\text{-}F}$ = 227.0 Hz), 130.9 (d, $J_{C\text{-}F}$ = 35.2 Hz), 57.4, 51.6, 49.5, 41.6, 29.9, 18.9, 18.1. ESI-MS (*m/z*): 357.1 (M^+, 100). Elemental anal. Calcd for $C_{14}H_{19}N_4FO_6$: C, 46.92; H, 5.31; N, 15.64. Found: C, 47.12; H, 5.24; N, 15.58%.

Crystal data

Compound (**3b**). $C_{18}H_{21}FN_4O_7$, *M* 424.39, *T* 298(2) K, orthorhombic, Space group P 212121, *a* 4.8774(3), *b* 15.4880(10), *c* 25.4480(17) Å. *V* 1922.4(2) Å^3, *Dc* (Z 4) 1.466 g cm^{-3}, *F* (000) 888, *μ* 0.120 mm^{-1}, R^1 0.0991, wR^2 0.2955.

Crystal structure determination and refinement

Diffraction data were collected at 25(2) °C using a graphite monochromated Mo Kα (λ = 0.071073 nm) radiation with an ω-scan technique. Determination of the crystal class, orientation matrix, and cell dimensions was performed according to the established procedures. Lorentz polarisation and absorption corrections were applied. Empirical absorption corrections were performed with SADABS program. Most of the non-hydrogen atoms were located using direct methods, and those remaining were derived by subsequent Fourier syntheses. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were held stationary and included in the final stage of full-matrix least-squares refinement based on F^2 using SHELXS-97 and SHELXL-97 program packages.

CCDC contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request.cif.

Supplementary data (product NMR spectra) are available directly from the correspondent.

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