

Article

Synthesis and Antitumor Activity of Amino Acid Ester Derivatives Containing 5-Fluorouracil

Jing Xiong ^{1,2,*}, Hai-Feng Zhu ¹, Ya-Juan Zhao ¹, Yun-Jun Lan ¹, Ji-Wang Jiang ¹,
Jing-Jing Yang ² and Shu-Feng Zhang ^{2,*}

¹ College of Chemistry and Materials Engineering, Wenzhou University, Wenzhou 325000, China

² State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012, China

* Author to whom correspondence should be addressed; E-mails: xiongjing@wzu.edu.cn (J.X.), Zhangshf@chem.dlut.edu.cn (S.-F.Z.); Tel.: +86-13738390567.

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Abstract: A series of amino acid ester derivatives containing 5-fluorouracil were synthesized using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and *N*-hydroxybenzotriazole (HOBt) as a coupling agent. The structures of the products were assigned by NMR, MS, IR etc. The *in vitro* antitumor activity tests against leukaemia HL-60 and liver cancer BEL-7402 indicated that (*R*)-ethyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-(4-hydroxyphenyl) propanoate showed more inhibitory effect against BEL-7402 than 5-FU.

Keywords: 5-fluorouracil; amino acid ester; antitumor activity

1. Introduction

5-Fluorouracil (5-FU) is an antimetabolite of the pyrimidine analogue type, which is frequently used for treating solid tumors, such as colorectal, gastric tract, and liver carcinomas [1–3]. However, the clinical applications of 5-FU are greatly limited by its short plasma half-life, poor tumor affinity, myelosuppression, and strong intestinal toxicity. Consequently, numerous research efforts have focused on the discovery of suitable carrier-linked prodrugs, in which 5-FU is conjugated with a wide spectrum of low- or high- molecular-weight carriers including glucose, peptides, and biodegradable polymers such as polysaccharides, liposomes, etc [4–10]. In general prodrug systems the drug is bound

to the carrier through a spacer that incorporates a predetermined breaking point that allows the bound drug to be released at the cellular target site. Therefore, the optimization physicochemical properties of a carrier, the modification of the carrier with 5-FU to preserve the targeting properties of the carrier and ensure a controlled release of 5-FU inside or outside the tumor cells are the critical aspects of 5-FU prodrug design [3,11].

Peptides play an important role in human metabolism. Some peptide derivatives of 5-FU have been reported as an approach to develop chemotherapeutic agents with improved physicochemical and biological characteristics [4,12,13], and we also have previously reported some peptide derivatives of 5-FU [14–16]. In continuation of the research, we now describe our studies on the synthesis and assessment of some amino acid ester derivatives containing 5-FU with the aim of finding appropriate biodegradable linkages.

2. Results and Discussion

2.1. Chemistry

The synthetic route to the target compounds **2a–o** is shown in Scheme 1 and Figure 1. The starting material 2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl) acetic acid (or 5-fluorouracil-1-yl acetic acid) (**1**) could be easily prepared by carboxymethylation of 5-fluorouracil according to the literature [17]. Treatment of compound **1** with a series of amino acid esters using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDC·HCl) and *N*-hydroxybenzotriazole (HOBt) as a coupling agent yielded a series of 2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-aceto amino acid ester derivatives **2**. HOBt was reported as a racemisation suppressant in peptide coupling reactions with carbodiimide coupling reagents [18–20].

Scheme 1. Synthesis of 2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-aceto amino acid ester derivatives **2**.

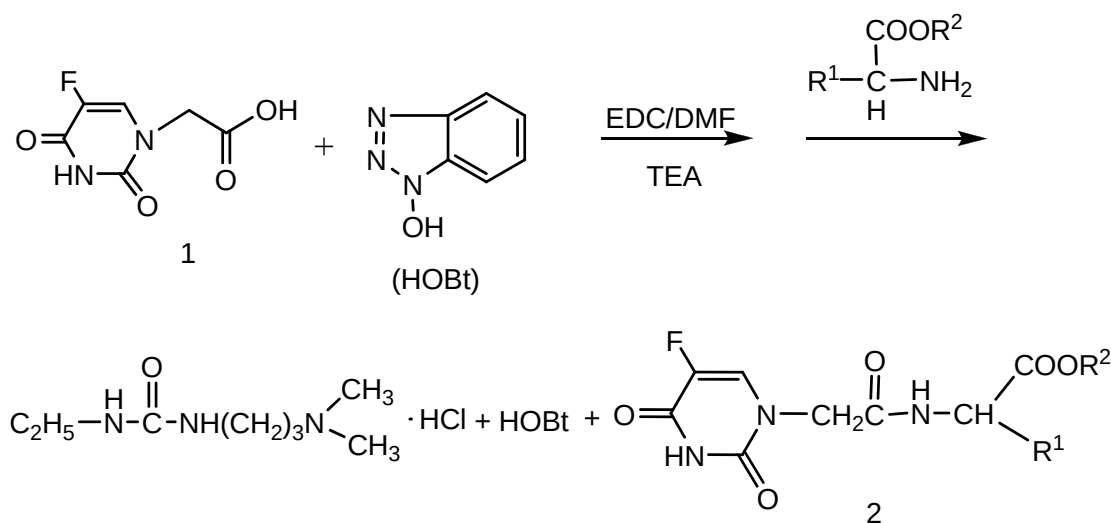
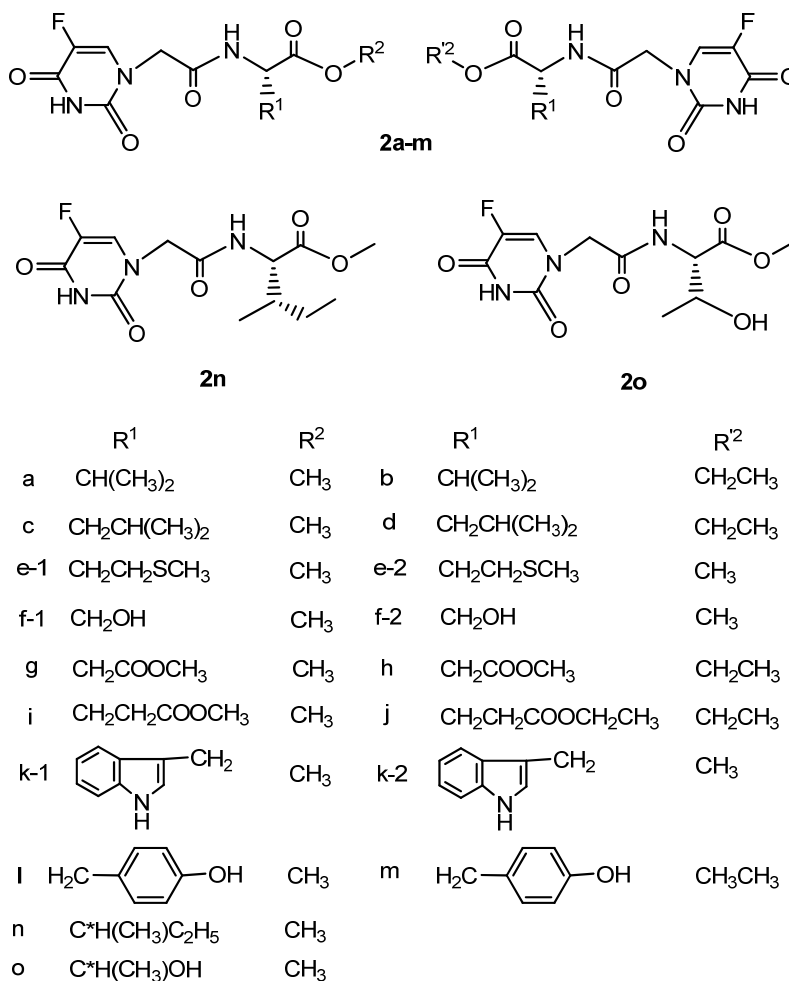


Figure 1. Structural formulae of compounds **2**.

The purity and structures of compounds **2a-o** were established on the basis of their melting points, specific rotations and spectral data, which were in full agreement with the proposed molecular structures. The ¹H-NMR spectra of all compounds showed doublets at 7.93–8.02 ppm, which corresponded to the coupling of fluorine and hydrogen signals in the FC=CH moieties. Compounds **2e-1** and **2e-2**, for example, almost have the same melting point (139–140 °C), the same spectral data, but opposite specific rotations of $[\alpha]_D^{18.1}$ -10.4 and $[\alpha]_D^{18.1}$ +10.4, respectively. In the ¹H-NMR their CH₂SCH₃ fragment methylene protons were observed as multiplets at δ 2.50–2.41 ppm, which overlapped with the signal of the solvent DMSO-*d*₆. The ¹³C-NMR of **2c** and **2d** displayed signals at δ 39.7 ppm from the methylene carbon from the CH₂CH(CH₃)₂ moiety which overlapped as well with that of the solvent DMSO-*d*₆. The assignment of the above four compounds were further proven by ¹³C-¹H COSY spectra.

2.2. *In vitro* antitumor activity

All target compounds **2a-o** were evaluated for their *in vitro* antitumor activity against the HL-60 leukaemia and BEL-7402 liver cancer cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazoliumbromide (MTT) [21] and Sulforhodamine B (SRB) assay methods [22], respectively, with 5-FU and the prodrug FT-207 being used for comparisons (Tables 1 and 2).

As shown in Table 1, all compounds' *in vitro* inhibition rates against HL-60 were significantly lower than that of 5-FU, except for the R-type compounds **2h**, **2j**, **2k-2** and **2m**, which exhibited equivalent inhibitory effect as 5-FU at 10^{-4} mol/L concentration, but the activity decreased rapidly when the concentration declined. The results indicate that these compounds were less sensitive to HL-60 at lower concentrations when the *N*-1 position of 5-FU was occupied.

Table 1. Inhibitory rates (%) against HL-60.

Compounds	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
2a	2.0	8.0	9.6	6.9	8.3
2b	3.7	5.2	6.2	9.7	2.8
2c	33.1	0.1	9.9	9.8	0
2d	27.3	0	1.7	0	10.0
2e-1	22.4	11.2	7.1	4.2	1.2
2e-2	18.5	15.3	3.4	10.5	0
2f-1	3.3	10.8	6.6	12.3	0
2f-2	31.8	9.2	3.7	6.2	0
2g	36.1	10.0	5.7	5.0	2.5
2h	55.7	19.6	23.1	2.3	8.6
2i	2.8	6.7	1.5	8.3	0.6
2j	55.8	12.8	2.7	5.4	5.9
2k-1	29.2	0	3.8	8.2	2.4
2k-2	51.2	15.5	9.8	12.7	8.9
2l	42.4	7.9	5.2	9.9	5.7
2m	65.1	0	12.6	13.0	0.3
2n	11.4	0	0	0	0
2o	22.4	11.2	7.1	4.2	1.2
5-FU	57.4	33.5	0	7.0	10.4
FT-207	0	0	0	0	0

Table 2. Inhibitory rates (%) against BEL-7402.

Compounds	Concentration (mol/L)				
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
2a	0	0	0	0	0
2b	9.0	8.1	0	0	1.8
2c	50.0	13.2	5.7	5.2	0
2d	13.2	0	0	0	0
2e-1	41.2	9.7	8.8	8.2	9.1
2e-2	38.4	9.1	8.8	0	5.3
2f-1	17.4	10.5	16.6	14.0	4.6
2f-2	41.2	9.8	0	0	0
2g	36.1	11.1	7.0	7.4	2.4
2h	52.6	15.9	2.4	0.7	0
2i	14.5	8.7	8.0	4.6	11.7
2j	34.0	9.2	4.1	3.5	5.9
2k-1	35.9	0	6.9	3.0	0.7
2k-2	22.4	11.9	7.1	4.2	1.2
2l	36.2	10.2	4.9	0.5	0
2m	71.7	68.3	60.4	43.1	24.3
2n	8.2	4.5	5.2	0	0
2o	9.7	0	8.8	8.2	9.1
5-FU	72.6	53.8	35.0	23.8	16.6
FT-207	58.0	8.1	0	0	0

In Table 2, almost all the compounds showed less sensitivity to BEL-7402, except **2m**, which showed more potent inhibitory effect than 5-FU. The reason maybe was the *R*-conformation of **2m** with a moderately rigid stereo structure, being composed of the pyrimidine ring and the phenyl ring, so it could release 5-FU sufficiently, while other compounds showed either more flexible configurations (such as **2a-e**), or a more rigid structure such as the case of **2k** [23]. The different inhibition against HL-60 and BEL-7402 between *R*-type and *S*-type compounds suggested the complexity of the antitumor mechanism.

3. Experimental

3.1. General

Melting points of synthesized compounds were determined on a Digital Melting Point Apparatus X-4 and were uncorrected. Mass spectra were obtained on a DECAX-30000 LCQ DecaXP Plus instrument. IR spectra were recorded (in KBr) on a Bruker EQUINOX 55. ¹H-NMR and ¹³C-NMR were recorded on Bruker AVANCE-300 at 300 and 75 MHz, respectively in DMSO-*d*₆ solutions with TMS as internal standard.

3.2. General procedure for the synthesis of compounds **2a-o**

Synthesis of compounds **2a-o** was accomplished as shown in Scheme 1. 2-(5-Fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl) acetic acid (10 mmol), HOBT (10 mmol) and DMF (50 mL) were added to a round-bottom flask, then EDC·HCl (13 mmol), L- or D-amino acid ester hydrochloride (10 mmol), and triethylamine (20 mmol) were added to the above mixture. After 10 h reaction at room temperature with thin layer chromatography (TLC) monitoring, the white solid 5-fluorouracil-1-yl-aceto amino acid esters **2a-o** were obtained after filtration, reduced pressure distillation of DMF, and silica gel column chromatography separation.

(S)-Methyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-methylbutanoate (**2a**). Yield: 68%; m.p. 109-110°C; ¹H-NMR δ: 11.80 (s, 1H, NH of 5-FU), 8.54 (d, 1H, NH, *J* = 8.1 Hz), 8.01 (d, 1H, FC=CH, ³*J*_{FH} = 6.9 Hz), 4.39 (d, 2H, NCH₂, *J* = 16.8 Hz), 4.22 (t, 1H, NCH, *J* = 7.2 Hz), 3.65 (s, 3H, OCH₃), 2.09-1.98 (m, 1H, CCH, *J* = 6.6 Hz), 0.89 (d, 3H, CH₃, *J* = 6.6 Hz), 0.87 (d, 3H, CH₃, *J* = 6.6 Hz); ¹³C-NMR δ: 171.9, 167.1, 157.7 (d, ²*J*_{FC} = 25.6 Hz), 149.8, 139.3 (d, ¹*J*_{FC} = 226.7 Hz), 131.3 (d, ²*J*_{FC} = 33.6 Hz), 57.7, 51.9, 49.5, 30.4, 19.0, 18.3; IR (cm⁻¹) ν: 3456, 3280, 2969, 1722, 1666, 1560, 1467, 1379, 1227, 1146, 783; MS (ESI) *m/z*: 300 (M⁺); [α]_D²⁰ -22.0 (c 1.0, DMF).

(R)-Ethyl 2-(2-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-methylbutanoate (**2b**). Yield: 65%; m.p. 136-137 °C; ¹H-NMR δ: 11.83 (s, 1H, NH of 5-FU), 8.53 (d, 1H, NH, *J* = 8.1 Hz), 8.02 (d, 1H, FC=CH, ³*J*_{FH} = 6.6 Hz), 4.38 (d, 2H, NCH₂, *J* = 16.8 Hz), 4.19 (dd, 1H, NCH, *J* = 8.1, 6.3 Hz), 4.16-4.05 (m, 2H, COOCH₂, *J* = 7.2 Hz), 2.11-1.98 (m, 1H, CCH), 1.19 (t, 3H, OCH₂CH₃, *J* = 7.2 Hz), 0.89 (d, 3H, CH₃, *J* = 6.9 Hz), 0.88 (d, 3H, CH₃, *J* = 6.6 Hz); ¹³C-NMR δ: 174.0, 169.6, 160.5 (d, ²*J*_{FC} = 25.3 Hz), 151.6, 141.2 (d, ¹*J*_{FC} = 228.1 Hz), 132.8 (d, ²*J*_{FC} = 33.5 Hz), 63.3, 59.9, 51.6;

31.6, 20.0, 19.2, 15.2; IR (cm⁻¹) ν : 3295, 3253, 2977, 1707, 1552, 1467, 1377, 1225, 1148; MS (ESI) m/z : 314(M⁺); [α]_D^{18.0} +14.0 (*c* 0.1, DMF).

(*S*)-Methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-4-methylpentanoate (**2c**). Yield: 75%; m.p. 144–145°C; ¹H-NMR δ : 11.83 (d, 1H, NH of 5-FU, ⁴*J*_{FH} = 5.4 Hz), 8.60 (d, 1H, NH, *J* = 7.8 Hz), 8.02 (d, 1H, FC=CH, ³*J*_{FH} = 6.9 Hz), 4.32 (s, 2H, NCH₂), 4.35–4.27 (m, 1H, NCH), 3.62 (s, 3H, OCH₃), 1.66–1.59 (m, 1H, CCH), 1.54–1.46 (m, 2H, CCH₂), 0.88 (d, 3H, CH₃, *J* = 6.3 Hz), 0.83 (d, 3H, CH₃, *J* = 6.3 Hz); ¹³C-NMR δ 172.9, 167.0, 157.8 (d, ²*J*_{FC} = 25.5 Hz), 149.8, 139.4 (d, ¹*J*_{FC} = 226.6 Hz), 131.3 (d, ²*J*_{FC} = 33.8 Hz), 52.2, 50.5, 49.6, 39.7, 24.3, 22.9, 21.6; IR (cm⁻¹) ν : 3323, 3046, 2959, 1666, 1543, 1472, 1384, 1244, 1155; MS (ESI) m/z : 314(M⁺); [α]_D^{16.1} -19.2 (*c* 1.0, DMF).

(*R*)-Ethyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-4-methylpentanoate (**2d**). Yield: 72%; m.p. 146–147°C; ¹H-NMR δ : 11.81(d, 1H, NH of 5-FU, ⁴*J*_{FH} = 5.1 Hz), 8.57(d, 1H, NH, *J* = 7.8 Hz), 8.01(d, 1H, FC=CH, ³*J*_{FH} = 6.9 Hz), 4.32(s, 2H, NCH₂), 4.30–4.24(m, 1H, NCH), 4.08(q, 2H, OCH₂, *J* = 7.2 Hz), 1.67–1.58(m, 1H, CCH), 1.56–1.48(m, 2H, CCH₂), 1.17(t, 3H, OCH₂CH₃, *J* = 7.2 Hz), 0.89(d, 3H, CH₃, *J* = 6.3 Hz), 0.84(d, 3H, CH₃, *J* = 6.3 Hz); ¹³C-NMR(75 MHz) δ : 172.3, 166.8, 157.7(d, ²*J*_{FC} = 25.2 Hz), 149.7, 139.3(d, ¹*J*_{FC} = 226.4 Hz), 131.2 (d, ²*J*_{FC} = 33.9 Hz), 60.7, 50.6, 49.5, 39.7, 24.3, 22.8, 21.5, 14.1; IR(KBr, cm⁻¹) ν : 3328, 2963, 2818, 1690, 1637, 1555, 1473, 1377, 1238, 1196, 1150; MS(ESI) m/z : 328(M⁺); [α]_D^{17.7} +11.6(*c* 1.0, DMF).

(*S*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-4-(methylthio)butanoate (**2e-1**). Yield: 62%; m.p. 139–140°C; ¹H-NMR(300 MHz) δ : 11.84(s, 1H, NH of 5-FU), 8.64(d, 1H, NH, *J* = 7.5 Hz), 8.02(d, 1H, FC=CH, ³*J* = 6.9 Hz), 4.46–4.39(m, 1H, NCH), 4.33(s, 2H, NCH₂), 3.63(s, 3H, OCH₃), 2.50–2.48(m, 2H, CH₂S), 2.02(s, 3H, SCH₃), 1.97–1.79(m, 2H, CCH₂); ¹³C-NMR(75 MHz) δ : 172.2, 167.1, 157.8(d, ²*J*_{FC} = 25.7 Hz), 149.9, 139.5(d, ¹*J*_{FC} = 226.5 Hz), 131.2(d, ²*J*_{FC} = 33.8 Hz), 52.3, 51.1, 49.7, 30.9, 29.5, 14.8; IR(KBr, cm⁻¹) ν : 3345, 3042, 2983, 1687, 1662, 1542, 1474, 1428, 1386, 1233; MS(ESI) m/z : 332(M⁺); [α]_D^{18.1} -10.4(*c* 1.0, DMF).

(*R*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-4-(methylthio)butanoate (**2e-2**). Yield: 59%; m.p. 138–140 °C; ¹H-NMR(300 MHz) δ : 11.83(s, 1H, NH of 5-FU), 8.63(d, NH, *J* = 7.6 Hz), 8.02(d, 1H, FC=CH, ³*J*_{FH} = 6.0 Hz), 4.43–4.38(m, 1H, NCH), 4.33(s, 2H, NCH₂), 3.64(s, 3H, OCH₃), 2.47–2.43(m, 2H, CH₂S), 2.03(s, 3H, SCH₃), 1.95–1.87(m, 2H, CCH₂); ¹³C-NMR(75 MHz) δ : 172.1, 167.1, 157.8(d, ²*J*_{FC} = 25.7 Hz), 149.8, 139.5(d, ¹*J*_{FC} = 226.7 Hz), 131.2 (d, ²*J*_{FC} = 33.7 Hz), 52.3, 51.1, 49.6, 30.9, 29.5, 14.7; IR(KBr, cm⁻¹) ν : 3348, 3237, 2962, 1725, 1660, 1569, 1478, 1425, 1381, 1345, 1305, 1246, 1162, 794; MS(ESI) m/z : 332(M⁺); [α]_D^{18.1} +10.4(*c* 1.0, DMF).

(*S*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-hydroxypropanoate (**2f-1**). Yield: 54%; m.p. 190–191°C; ¹H-NMR(300 MHz) δ : 11.83(d, 1H, NH of 5-FU, ⁴*J*_{HH} = 4.5 Hz), 8.63(d, 1H, NH, *J* = 8.1 Hz), 8.01(d, 1H, FC=CH, ³*J*_{FH} = 6.9 Hz), 5.11(t, 1H, OH, *J* = 5.4 Hz), 4.38(s, 2H, NCH₂), 4.42–4.36(m, 1H, NCH), 3.65(s, 3H, OCH₃), 3.75–3.58(m, 2H, CH₂OH, *J* = 5.4, 10.8 Hz); ¹³C-NMR(75 MHz) δ : 170.9, 167.0, 157.6(d, ²*J*_{FC} = 25.7 Hz), 149.8, 139.3(d, ¹*J*_{FC} = 226.7 Hz),

131.2(d, $^2J_{\text{FC}} = 33.7$ Hz), 61.4, 54.8, 52.0, 49.4; IR(KBr, cm^{-1}) ν : 3459, 3295, 2850, 1713, 1692, 1563, 1467, 1416, 1382, 1277, 1251, 1185, 1071; MS(ESI) m/z : 288(M $^+$). $[\alpha]_D^{10.7} -10.0$ (c 1.0, DMF).

(*R*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-hydroxypropanoate (**2f-2**). Yield: 55%; m.p. 190-192°C; $^1\text{H-NMR}$ (300 MHz) δ : 11.81(s, 1H, NH of 5-FU), 8.62(d, 1H, NH, $J = 7.5$ Hz), 8.00(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.9$ Hz), 5.12(t, 1H, OH, $J = 5.4$ Hz), 4.37(s, 2H, NCH₂), 4.41-4.35(m, 1H, NCH), 3.63(s, 3H, OCH₃), 3.74-3.56(m, 2H, CH₂OH, $J = 5.4$, 10.8 Hz); $^{13}\text{C-NMR}$ (75 MHz) δ : 171.0, 167.0, 157.7(d, $^2J_{\text{FC}} = 25.6$ Hz), 149.8, 139.3(d, $^1J_{\text{FC}} = 226.6$ Hz), 131.4(d, $^2J_{\text{FC}} = 33.5$ Hz), 61.5, 54.9, 52.1, 49.5; IR(KBr, cm^{-1}) ν : 3459, 3295, 2850, 1713, 1692, 1563, 1467, 1416, 1382, 1277, 1251, 1185, 1071; MS(ESI) m/z : 288(M $^+$). $[\alpha]_D^{11.0} +10.0$ (c 1.0, DMF).

(*S*)-dimethyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)succinate (**2g**). Yield: 65%; m.p. 145-147 °C; $^1\text{H-NMR}$ (300 MHz) δ : 11.85(d, 1H, NH of 5-FU, $^4J_{\text{FH}} = 5.1$ Hz), 8.76(d, 1H, NH, $J = 7.8$ Hz), 8.01(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.6$ Hz), 4.68(dd, 1H, NCH, $J = 7.5$, 6.6 Hz), 4.33(s, 2H, NCH₂), 3.64(s, 3H, OCH₃), 3.62(s, 3H, OCH₃), 2.85-2.70(m, 2H, CCH₂, $J = 6.6$, 7.5, 16.8 Hz); $^{13}\text{C-NMR}$ (75 MHz) δ : 170.9, 170.5, 166.9, 157.7(d, $^2J_{\text{FC}} = 25.7$ Hz), 149.8, 139.4(d, $^1J_{\text{FC}} = 228.0$ Hz), 131.1 (d, $^2J_{\text{FC}} = 33.9$ Hz), 52.4, 51.9, 49.5, 48.7, 35.9; IR(KBr, cm^{-1}) ν : 3348, 3191, 2850, 1755, 1697, 1526, 1430, 1380, 1337, 1216, 1047, 980; MS(ESI) m/z : 330(M $^+$); $[\alpha]_D^{11.0} -14.4$ (c 1.0, DMF).

(*R*)-diethyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)succinate (**2h**). Yield: 68%; m.p. 116-118 °C; $^1\text{H-NMR}$ (300 MHz) δ : 11.82(d, 1H, NH of 5-FU, $^4J_{\text{FH}} = 4.8$ Hz), 8.71(d, 1H, NH, $J = 7.8$ Hz), 7.99(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.8$ Hz), 4.63(dd, 1H, NCH, $J = 6.6$, 7.5 Hz), 4.33(s, 2H, NCH₂), 4.12-4.03(m, 4H, OCH₂, $J = 6.9$ Hz), 2.81-2.65(m, 2H, CCH₂, $J = 6.6$, 16.5 Hz), 1.17(t, 3H, CH₃, $J = 6.9$ Hz), 1.16(t, 3H, CH₃, $J = 6.9$ Hz); $^{13}\text{C-NMR}$ (75 MHz) δ : 170.4, 170.0, 166.9, 157.7(d, $^2J_{\text{FC}} = 25.7$ Hz), 149.8, 139.4(d, $^1J_{\text{FC}} = 228.3$ Hz), 131.2 (d, $^2J_{\text{FC}} = 33.8$ Hz), 61.2, 60.6, 49.5, 48.9, 36.1, 14.2, 14.1; IR(KBr, cm^{-1}) ν : 3314, 3217, 2990, 1721, 1691, 1549, 1467, 1378, 1339, 1240, 1167, 1021, 792; MS(ESI) m/z : 358(M $^+$); $[\alpha]_D^{9.7} +16.0$ (c 1.0, DMF).

(*S*)-dimethyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)pentanedioate (**2i**). Yield: 42%; m.p. 147-148 °C; $^1\text{H-NMR}$ (300 MHz) δ : 11.85(s, 1H, NH of 5-FU), 8.63(d, 1H, NH, $J = 7.8$ Hz), 8.02(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.9$ Hz), 4.32(s, 2H, NCH₂), 4.37-4.34(m, 1H, NCH), 3.63(s, 3H, OCH₃), 3.58(s, 3H, OCH₃), 2.37(t, 2H, CH₂CH₂CO, $J = 7.5$ Hz), 2.06-1.76(m, 2H, CH₂CH₂CO); $^{13}\text{C-NMR}$ (75 MHz) δ : 172.7, 171.9, 168.0, 157.7(d, $^2J_{\text{FC}} = 25.7$ Hz), 149.8, 139.4(d, $^1J_{\text{FC}} = 226.7$ Hz), 131.1(d, $^2J_{\text{FC}} = 33.7$ Hz), 52.2, 51.5, 51.3, 49.6, 29.6, 26.4; IR(KBr, cm^{-1}) ν : 3328, 2964, 1716, 1660, 1541, 1449, 1348, 1261, 800; MS(ESI) m/z : 344(M $^+$); $[\alpha]_D^{17.8} -8.39$ (c 0.5, DMF).

(*R*)-diethyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)pentanedioate (**2j**). Yield: 45%; m.p. 108-109 °C; $^1\text{H-NMR}$ (300 MHz) δ : 11.84(s, 1H, NH of 5-FU), 8.61(d, 1H, NH, $J = 7.8$ Hz), 8.01(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.9$ Hz), 4.33(s, 2H, NCH₂), 4.30-4.26(m, 1H, NCH), 4.08(q, 2H, OCH₂CH₃, $J = 6.9$ Hz), 4.04(q, 2H, OCH₂CH₃, $J = 7.2$ Hz), 2.36(t, 2H, CH₂CH₂CO, $J = 7.5$ Hz), 2.03-1.78(m, 2H, CH₂CH₂CO), 1.17(t, 3H, OCH₂CH₃, $J = 6.9$ Hz), 1.16(t, 3H, OCH₂CH₃, $J = 7.2$ Hz);

^{13}C -NMR(75 MHz) δ : 172.2, 171.4, 167.0, 157.7(d, $^2J_{\text{FC}} = 25.7$ Hz), 149.8, 139.4(d, $^1J_{\text{FC}} = 226.9$ Hz), 131.2(d, $^2J_{\text{FC}} = 33.7$ Hz), 60.9, 60.1, 51.5, 49.6, 29.9, 26.5, 14.2, 14.1; IR(KBr, cm^{-1}) ν : 3304, 3213, 2924, 1725, 1675, 1546, 1468, 1416, 1379, 1250, 1176, 1023; MS(ESI) m/z : 372 (M^-); $[\alpha]_D^{8.7} +12.40$ (c 1.0, DMF).

(*S*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-(1*H*-indol-3-yl)propanoate (**2k-1**). Yield: 50%; m.p. 205-206 °C; ^1H -NMR(300 MHz) δ : 11.84(s, 1H, NH of 5-FU), 10.90(s, 1H, NH of indole), 8.72(d, 1H, NH, $J = 7.2$ Hz), 7.93(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.8$ Hz), 7.48(d, 1H, Ar-H, $J = 7.5$ Hz), 7.33(d, 1H, Ar-H, $J = 8.1$ Hz), 7.16(d, 1H, =CHN, $J = 2.2$ Hz), 7.07(t, 1H, Ar-H, $J = 8.1, 6.9$ Hz), 6.99(t, 1H, Ar-H, $J = 7.5, 6.9$ Hz), 4.54(dd, 1H, NCH, $J = 7.2, 6.6$ Hz), 4.33(d, 2H, NCH₂, $J = 16.5$ Hz), 3.56(s, 3H, OCH₃), 3.19-3.03(m, 2H, CCH₂, $J = 7.5, 6.0, 14.4$ Hz); ^{13}C -NMR(75 MHz) δ : 172.2, 166.9, 157.8(d, $^2J_{\text{FC}} = 25.7$ Hz), 149.9, 139.4(d, $^1J_{\text{FC}} = 228.1$ Hz), 136.3, 131.3(d, $^2J_{\text{FC}} = 33.8$ Hz), 127.3, 124.1, 121.2, 118.7, 118.2, 111.7, 109.2, 53.6, 52.1, 49.5, 27.4; IR(KBr, cm^{-1}) ν : 3386, 3312, 3069, 2977, 1702, 1545, 1381, 1233, 1062; MS(ESI) m/z : 387 (M^-); $[\alpha]_D^{17.9} +36.4$ (c 1.0, DMF).

(*R*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-(1*H*-indol-3-yl)propanoate (**2k-2**). Yield: 53%; m.p. 205-206 °C; ^1H -NMR(300 MHz) δ : 11.84(d, 1H, NH of 5-FU, $^4J_{\text{FH}} = 5.1$ Hz), 10.90(s, 1H, NH of indole), 8.72(d, 1H, NH, $J = 7.5$ Hz), 7.93(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.9$ Hz), 7.48(d, 1H, Ar-H, $J = 7.8$ Hz), 7.34(d, 1H, Ar-H, $J = 8.1$ Hz), 7.16(s, 1H, =CHN), 7.07(t, 1H, Ar-H, $J = 7.2, 7.8$ Hz), 6.99(t, 1H, Ar-H, $J = 7.2$ Hz), 4.55(dd, 1H, NCH, $J = 6.9, 6.6$ Hz), 4.33(d, 2H, NCH₂, $J = 16.5$ Hz), 3.57(s, 3H, OCH₃), 3.20-3.03(m, 2H, CCH₂, $J = 7.5, 6.0, 14.8$ Hz); ^{13}C -NMR(75 MHz) δ : 172.2, 166.9, 157.8 (d, $^2J_{\text{FC}} = 25.7$ Hz), 149.9, 139.4(d, $^1J_{\text{FC}} = 228.2$ Hz), 136.3, 131.3(d, $^2J_{\text{FC}} = 33.8$ Hz), 127.3, 124.1, 121.2, 118.7, 118.2, 111.7, 109.2, 53.5, 52.1, 49.5, 27.4; IR(KBr, cm^{-1}) ν : 3392, 3320, 3075, 1732, 1648, 1542, 1446, 1385, 1355, 1248, 1219, 1099, 744; MS(ESI) m/z : 387 (M^-); $[\alpha]_D^{17.8} -36.4$ (c 1.0, DMF).

(*S*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-(4-hydroxyphenyl)propanoate (**2l**). Yield: 72%; m.p. 192-193 °C; ^1H -NMR(300 MHz) δ : 11.85(s, 1H, NH of 5-FU), 9.28(s, 1H, OH), 8.68(d, 1H, NH, $J = 7.5$ Hz), 7.94(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.9$ Hz), 6.99 (d, 2H, Ar-H, $J = 8.4$ Hz), 6.66 (d, 2H, Ar-H, $J = 8.4$ Hz), 4.39(dd, 1H, NCH, $J = 6.3, 7.5$ Hz), 4.31(d, 2H, NCH₂, $J = 16.8$ Hz), 3.59(s, 3H, OCH₃), 2.93-2.77(m, 2H, CH₂Ar, $J = 6.3, 8.1, 13.8$ Hz); ^{13}C -NMR(75 MHz) δ : 171.9, 166.9, 157.7(d, $^2J_{\text{FC}} = 25.7$ Hz), 156.3, 149.8, 139.4(d, $^1J_{\text{FC}} = 226.9$ Hz), 131.2(d, $^2J_{\text{FC}} = 33.8$ Hz), 130.3, 127.0, 115.3, 54.4, 52.0, 49.5, 36.3; IR(KBr, cm^{-1}) ν : 3271, 1739, 1712, 1661, 1552, 1516, 1451, 1386, 1231, 1164, 778; MS(ESI) m/z : 364 (M^+); $[\alpha]_D^{17.8} +16.6$ (c 1.0, DMF).

(*R*)-ethyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-(4-hydroxyphenyl)propanoate (**2m**). Yield: 70%; m.p. 156-158 °C; ^1H -NMR(300 MHz) δ : 11.82(s, 1H, NH of 5-FU), 9.23(s, 1H, OH), 8.63(d, 1H, NH, $J = 7.5$ Hz), 7.94(d, 1H, FC=CH, $^3J_{\text{FH}} = 6.6$ Hz), 6.99(d, 2H, Ar-H, $J = 8.4$ Hz), 6.65(d, 2H, Ar-H, $J = 8.4$ Hz), 4.36(dd, 1H, NCH, $J = 7.5, 6.9$ Hz), 4.31(d, 2H, NCH₂, $J = 16.8$ Hz), 4.02(q, 2H, OCH₂, $J = 7.2$ Hz), 2.89-2.77(m, 2H, CH₂Ar, $J = 7.5, 6.0, 13.8$ Hz), 1.10(t,

3H, CH₃, $J = 7.2$ Hz); ¹³C-NMR(75 MHz) δ : 171.4, 166.9, 157.7(d, $^2J_{FC} = 25.7$ Hz), 156.3, 149.8, 139.4(d, $^1J_{FC} = 228.1$ Hz), 131.2(d, $^2J_{FC} = 33.9$ Hz), 130.3, 127.0, 115.3, 60.7, 54.4, 49.5, 36.4, 14.1; IR(KBr, cm⁻¹) ν : 3336, 3065, 2851, 1691, 1532, 1380, 1340, 1220, 801; MS(ESI) m/z : 378(M⁺); $[\alpha]_D^{9.7} - 24.4$ (c 1.0, DMF).

(2*S*,3*S*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-methylpentanoate (**2n**). Yield: 70%; m.p. 152-154 °C; ¹H-NMR(300 MHz) δ : 11.82(d, 1H, NH of 5-FU, $^4J_{FH} = 5.1$ Hz), 8.57(d, 1H, NH, $J = 8.1$ Hz), 8.01(d, 1H, FC=CH, $^3J_{FH} = 6.9$ Hz), 4.36(d, 2H, NCH₂, $J = 16.8$ Hz), 4.25(dd, 1H, NCH, $J = 8.1, 6.6$ Hz), 3.63(s, 3H, OCH₃), 1.77-1.72(m, 1H, CCH), 1.43-1.09(m, 2H, CCH₂), 0.84(t, 3H, CH₂CH₃, $J = 7.2$ Hz), 0.83(d, 3H, CHCH₃, $J = 6.9$ Hz); ¹³C-NMR(75 MHz) δ : 172.0, 167.1, 157.7(d, $^2J_{FC} = 25.7$ Hz), 149.8, 139.3(d, $^1J_{FC} = 227.9$ Hz), 131.4(d, $^2J_{FC} = 33.8$ Hz), 56.6, 52.0, 49.5, 36.9, 24.9, 15.6, 11.3; IR(KBr, cm⁻¹) ν : 3275, 3083, 2968, 1712, 1666, 1571, 1460, 1378, 1340, 1243, 1149, 976, 700; MS(ESI) m/z : 314(M⁺); $[\alpha]_D^{17.5} - 3.0$ (c 0.5, DMF).

(2*S*,3*S*)-methyl 2-(2-5-(fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)acetamido)-3-hydroxybutanoate (**2o**). Yield: 47%; m.p. 207-208 °C; ¹H-NMR(300 MHz) δ : 11.79(d, 1H, NH of 5-FU, $^4J_{FH} = 5.1$ Hz), 8.44(d, 1H, NH, $J = 8.4$ Hz), 8.01(d, 1H, FC=CH, $^3J_{FH} = 6.6$ Hz), 5.03(d, 1H, OH, $J = 5.1$ Hz), 4.42(dd, 2H, NCH₂, $J = 6.6, 16.5$ Hz), 4.32(dd, 1H, NCH, $J = 8.4, 3.3$ Hz), 4.15-4.08(m, 1H, CHOH, $J = 3.3, 5.1, 6.3$ Hz), 1.05(d, 3H, CH₃, $J = 6.3$ Hz); ¹³C NMR(75 MHz) δ 171.0, 167.4, 157.7(d, $^2J_{FC} = 25.6$ Hz), 149.8, 139.3(d, $^1J_{FC} = 226.6$ Hz), 131.4(d, $^2J_{FC} = 33.5$ Hz), 66.5, 58.1, 52.0, 49.6, 20.2; IR(KBr, cm⁻¹) ν : 3484, 3312, 2977, 1718, 1663, 1558, 1376, 1283, 1238, 1140; MS(ESI) m/z : 302(M⁺); $[\alpha]_D^{17.6} - 2.0$ (c 0.1, DMF).

4. Conclusions

A series of amino acid ester derivatives containing 5-fluorouracil were synthesized by EDC/HOBt method and characterized. The *in vitro* antitumor activity tests indicated that the synthesized compounds had less inhibition rates against HL-60 and BEL-7402 than 5-FU except compound **2m**, which showed more potent inhibitory effect against BEL-7402 than 5-FU. This might be explained by the R configuration of compound **2m** with the moderate rigid framework composed of pyrimidine ring and hydroxyphenyl ring, which may be easily to give 5-fluorouracil.

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Sample Availability: Samples of the compounds are available from the authors.

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