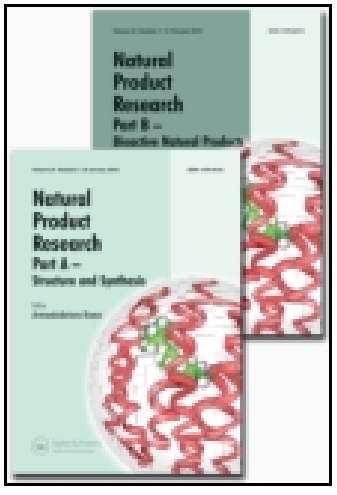

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Design and synthesis of novel camptothecin/5-fluorouracil conjugates as cytotoxic agents

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Design and synthesis of novel camptothecin/5-fluorouracil conjugates as cytotoxic agents

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In an effort to overcome several limitations associated with the synthesis of camptothecin (CPT), seven conjugates (**10a**–**10g**) composed of CPT and a 5-fluorouracil derivative joined by suitable dipeptide linkages were synthesised, and their cytotoxic activity against four human tumour cell lines as well as an *in vitro* pharmacokinetic determination of their lactone stability were studied. Among these compounds, most tested conjugates showed cytotoxic activities comparable or superior to CPT-11 (**2**), but they were less potent when compared with CPT (**1**). Interestingly, all of the compounds showed selective inhibitory activities against BGC-823, with IC_{50} values lower than 0.1 μmol, which is more potent than CPT-11 (**2**). Also, the *in vitro* pharmacokinetic determination of the lactone levels of the representative compound **10b** showed that the biological life span of their lactone forms in human and mouse plasma were significantly increased when compared with their mother compound CPT (**1**).

Keywords: camptothecin; 5-fluorouracil; antineoplastic properties; conjugates

1. Introduction

A challenging research focus at present in cancer therapy is the discovery of molecules that could be selective for tumour cells and that could be characterised by reduced undesirable effects. Among the various strategies to improve the therapeutic index of different drugs, conjugation of cytotoxic drug components has proven to be a promising approach to enhance the activity and selectivity of some monomeric leads by forming bivalent heterodimers (Liu, Yang, & Tian, 2007; Tietze, Bell, & Chandrasekhar, 2003). This concept is now accepted as an effective strategy for designing ligands, inhibitors and other drugs that influence biological systems. On the basis of this theory, some interesting results have been reported by our group as well as others in recent years (Chen, Xiang, Liu, & Tian 2009; Liu, Liu, Tian, & Yang, 2008; Ohtsu, Nakanishi, Bastow, F. Lee, & K. Lee, 2003; Shi et al., 2001; Tatsuzaki et al., 2007). In our previous study, we explored the

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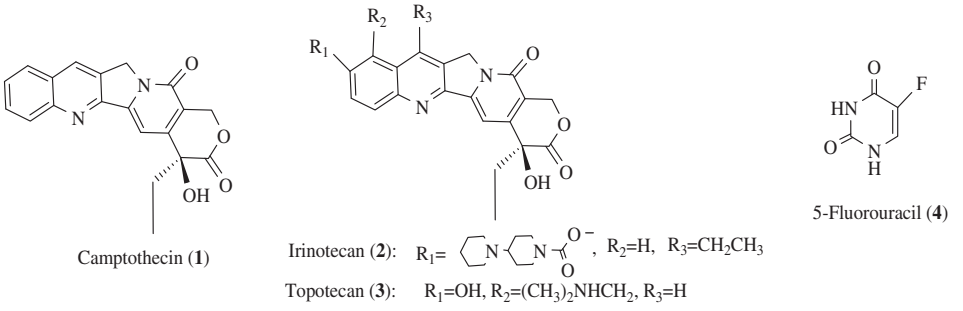


Figure 1. Structures of CPT (**1**), irinotecan (**2**), topotecan (**3**) and 5-fluorouracil (**4**).

syntheses and evaluation of heterodimer conjugates by the combination of two kinds of antitumour drugs through various linkages, which led to a large increase in potency over their monomeric counterparts (Chen et al., 2009; Liu et al., 2007, 2008).

Camptothecin (CPT) (**1**) is a topoisomerase I-targeting cytotoxic alkaloid with significant antineoplastic activity (Liew & Yang, 2008; Slichenmyer, Rowinsky, Donehower, & Kaufmann, 1993; Takimoto, Wright, & Arbuck, 1998). Hydrophilisation of camptothecin molecules results in the identification and development of topotecan (**2**) and irinotecan (**3**) (Figure 1), which have been clinically proven for use in the treatment of ovarian and colon cancers, respectively (Li, Zu, Shi, & Yao, 2006; Lorence & Nessler, 2004). However, as an important class of anticancer compounds, both poor water-solubility and lactone instability appear to be the predominant reason for the difficulty in clinically developing CPTs and therefore, these compounds are suitable for incorporation as parent drugs in a prodrug approach (Driver & Yang, 2005; Du, 2003; Verma & Hansch, 2009). Using the prodrug concept, the water solubility of the most active CPTs can be adequately enhanced so as to allow parenteral administration in an aqueous medium. The acylation of the 20-hydroxyl group may also render the lactone more stable towards ring opening. This may increase the availability of an active drug that contains the intact lactone.

Antimetabolite 5-fluorouracil (5-FU) (**4**) is one of the major anticancer agents used clinically for the treatment of stomach, colorectal, head and neck cancers (Campos, Domínguez, Gallo, & Espinosa, 2000). But 5-FU is poorly tumour selective, so its therapy causes high incidences of toxicity in the bone marrow, gastrointestinal tract, central nervous system and skin, which has prompted efforts in the development of derivatives aiming at reducing the adverse effects of 5-FU. Among the various attempts to optimise the efficacy of 5-FU, one of the main strategies that potentiates its antitumour activity is its chemical and biochemical modulation, by combination with other cytotoxic agents or with biochemical modulators (Longley, Harkin, & Johnston, 2003; Peters et al., 2000). Also, considerable data from clinical studies have shown that the combined use of CPT-related analogues and 5-FU resulted in higher response rates than for either agent alone, and simultaneously circumvented some faults, such as decreasing the amount of repair needed for CPT-induced DNA damage and increasing the induction of

apoptosis, or increasing DNA adduct formation in cancer cells (Guichard, Hennebelk, Bugat, & Cana, 1998; Peters et al., 2000).

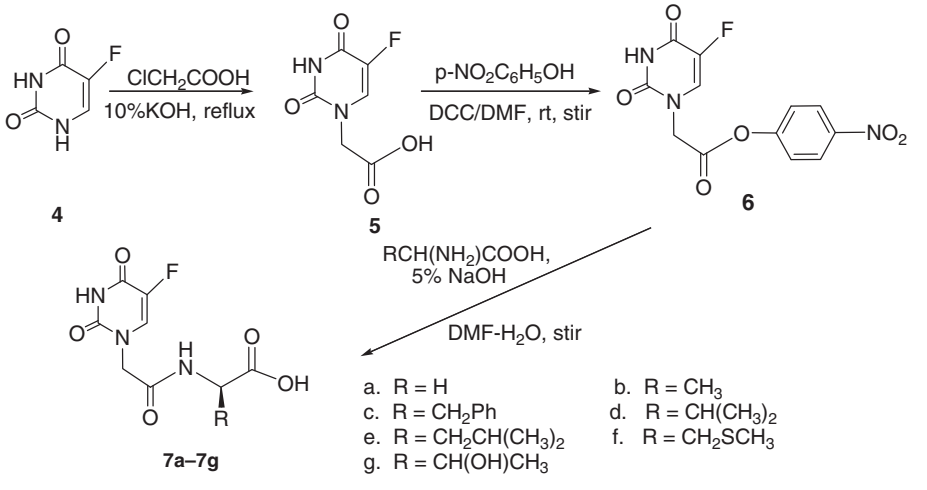
Based on the abovementioned information, the conjugation of 5-FU and CPT with dipeptide spacers might provide new classes of antitumour drug candidates with improved tumour selectivity, efficiency and safety.

2. Results and discussion

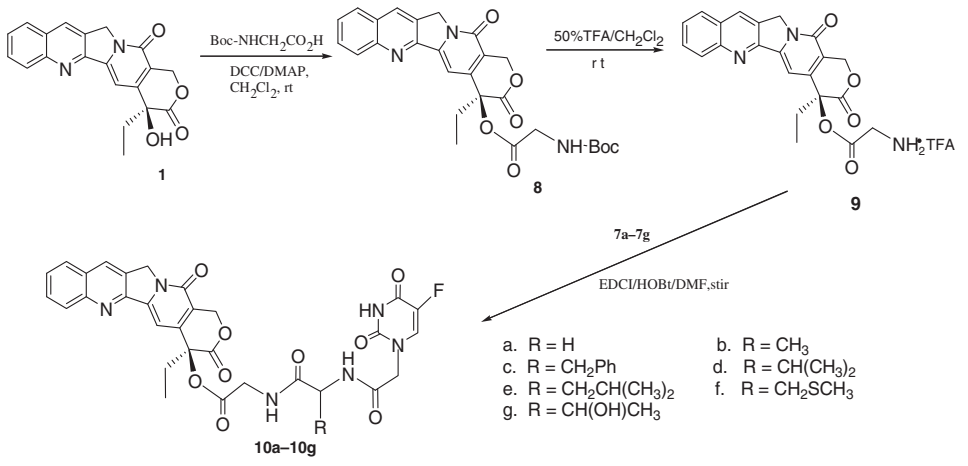
2.1. *Chemistry*

The reaction of 5-FU (**4**) with chloroacetic acid in the presence of potassium hydroxide gave the corresponding 5-fluorouracil-1-acetic acid (**5**) in an almost excellent yield (Liu, Yang, & Tian, 2006), which upon treatment with *p*-nitrophenol using *N*,*N*-dicyclohexylcarbodiimide (DCC) afforded activated ester **6**. Sequential treatment of **6** with a series of L-amino acids in alkaline DMF–H_2O solvent produced the corresponding [(5-fluorouracil-1-yl)acetyl]-L-amino acids **7a**–**7g** (Scheme 1) (Chen, Tian, & Tu, 2004).

CPT (**1**) was isolated from a Chinese medicinal plant, *C. acuminata*, and it served as the starting material for the preparation of all the derivatives. The conversion of the isolated available CPT to the CPT-20-ester of *N*-Boc-glycine derivative **8** in the presence of DCC and 4-dimethylaminopyridine (DMAP) as catalysts was accomplished by a modified version of Greenwald's method (Greenwald et al., 1998), followed by the removal of the *N*-Boc group of **8** with TFA in CH_2Cl_2 (1 : 1), forming the TFA salt **9**. The desired compounds **10a**–**10g** were achieved by treating the TFA salt **9** with the corresponding [(5-fluorouracil-1-yl)acetyl]-L-amino acids **7a**–**7g** using 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (EDCI) as the coupling agent (Scheme 2). The desired compounds **10a**–**10g** were purified by standard flash chromatography on silica gel and characterised by m.p., IR, ^{1}H-NMR and HRMS analyses. The analytical and spectral data are illustrated in Tables 1 and 2, respectively.



Scheme 1. Synthesis of [(5-fluorouracil-1-yl)acetyl]-L-amino acids **7a**–**7g**.



Scheme 2. Synthesis of the target compounds **10a**–**10g**.

Table 1. Analytical data of compounds **10a**–**10g**.

Compound	Yield (%)	m.p. (°C)	$[\alpha]_D^{20}$ (°) ($c = 0.5$, DMF)	HRMS (m/z)
10a	73	186–188	−79	Calcd for $C_{30}H_{25}N_6O_9F$: 633.1740 $[M+H]^+$; found: 633.1740 $[M+H]^+$
10b	69	189–191	−80	Calcd for $C_{31}H_{27}N_6O_9F$: 647.1896 $[M+H]^+$; found: 647.1896 $[M+H]^+$
10c	56	190–192	−71	Calcd for $C_{37}H_{31}N_6O_9F$: 745.209 $[M+Na]^+$; found: 745.2014 $[M+Na]^+$
10d	52	186–188	−90	Calcd for $C_{33}H_{31}N_6O_9F$: 675.2209 $[M+H]^+$; found: 675.2219 $[M+H]^+$
10e	45	176–178	−77	Calcd for $C_{34}H_{33}N_6O_9F$: 689.2366 $[M+H]^+$; found: 689.2366 $[M+H]^+$
10f	68	192–194	−67	Calcd for $C_{33}H_{31}N_6O_9SF$: 729.1749 $[M+Na]^+$; found: 729.1738 $[M+Na]^+$
10g	62	192–194	−75	Calcd for $C_{32}H_{29}N_6O_{10}F$: 699.1821 $[M+Na]^+$; found: 699.1825 $[M+Na]^+$

2.2. *Cytotoxic activities*

The cytotoxicities of compounds **10a**–**10g** were measured on four different human cancer cell lines (SGC-7901, BGC-823, A-549 and HePG-2) using an *in vitro* MTT assay. Irinotecan (**2**) and CPT (**1**) were used as reference compounds. The results are listed in Table 3.

As shown in Table 3, all the conjugates were less potent than **1** in the cytotoxic assay, which is almost a common feature of prodrugs, whereas most of the compounds exhibited cytotoxic activities comparable or superior to irinotecan (**2**). Significantly, the compounds were more sensitive against the BGC-823 cell line than against the other cell lines, which is consistent with the clinical behaviour of CPT derivatives. For the BGC-823 cell line, all the compounds showed high inhibitory activities with IC_{50} values lower than 0.1 μM, which was more potent than

Table 2. Spectral data of compounds **10a**–**10g**.

Compound	IR KBr, ν_{max} (cm^{-1})	^{1}H-NMR: (DMSO-d_6) δ (ppm)
10a	3463 (NH), 3073, 1619, 1595, 1559 (ArH), 1762, 1702, 1664 (C = O), 123 6(C–F), 1184, 115 5(ester linkage –O–C)	0.89 (t, 3H, H-18), 2.13 (q, 2H, H-19), 4.04 and 4.23 (2d, 4H, 2 × CH_2–Gly-Gly), 4.29 (s, 2H, 5-FU–N–CH_2), 5.47 (s, 2H, H-17), 5.26 (s, 2H, H-5), 7.14 (s, 1H, H-14), 7.70 (t, 1H, H-11), 7.84 (s, 5-FU-ring–H-6), 7.935 (t, 1H, H-10), 8.123(d, 1H, H-12), 8.460 (d, 1H, H-9), 8.661 (s, 1H, 1H, H-7), 11.80 (s, 1H, 5-FU-ring–NH)
10b	3359 (NH), 3067, 1600, 1558 (ArH), 1749, 1698, 1662 (C = O), 1237 (C–F), 1187, 1158 (ester linkage –O–C)	0.92 (t, 3H, H-18), 1.24 (d, 3H, L-alanine–CH_3), 2.15 (q, 2H, H-19), 4.05 (d, 2H, CH_2–Gly), 4.25(m, 1H, L-alanine–CH), 4.35 (s, 2H, 5-FU–N–CH_2), 5.31 (s, 2H, H-5), 5.50 (s, 2H, H-17), 7.17 (s, 1H, H-14), 7.73 (t, 1H, H-11), 7.85 (s, 1H, 5-FU-ring–H-6), 7.89 (t, 1H, H-10), 8.15 (d, 1H, H-12), 8.48 (d, 1H, H-9), 8.71 (s, 1H, H-7), 11.80 (s, 1H, 5-FU-ring–NH)
10c	3293 (NH), 3065, 1598, 1559 (ArH), 1750, 1699, 1662 (C = O), 1237(C–F), 1188, 1161 (ester linkage –O–C)	0.92 (t, 3H, H-18), 2.15 (q, 2H, H-19), 3.03 (m, 2H, L-phenylalanine–CH_2), 4.08 (d, 2H, CH_2–Gly), 4.35 (s, 2H, 5-FU–N–CH_2), 4.56 (m, 1H, L-phenylalanine–CH), 5.30 (s, 2H, H-5), 5.51 (s, 2H, H-17), 7.19 (s, 1H, H-14), 7.23 (m, 5H, L-phenylalanine–ArH), 7.73 (t, 1H, H-11), 7.85 (s, 1H, 5-FU-ring–H-6), 7.88 (t, 1H, H-10), 8.15 (d, 1H, H-12), 8.54 (d, 1H, H-9), 8.70 (s, 1H, H-7), 11.77 (s, 1H, 5-FU-ring–NH)
10d	3311 (NH), 3067, 1599, 1557 (ArH), 1751, 1699, 1661 (C = O), 1236 (C–F), 1186, 1156 (ester linkage –O–C)	0.83 (dd, 6H, L-valine–CH(CH_3)$_2$), 0.92 (t, 3H, H-18), 1.97 (m, H, L-valine–CH(CH_3)$_2$), 2.15 (q, 2H, H-19), 4.14 (d, 2H, CH_2–Gly), 4.36 (s, 2H, 5-FU–N–CH_2), 4.42 (m, 1H, L-valine–CH), 5.31 (s, 2H, H-5), 5.50 (s, 2H, H-17), 7.17 (s, 1H, H-14), 7.73 (t, 1H, H-11), 7.87(s, 1H, 5-FU-ring–H-6), 7.89 (t, 1H, H-10), 8.16 (d, 1H, H-12), 8.60 (d, 1H, H-9), 8.71 (s, 1H, H-7), 11.78 (s, 1H, 5-FU-ring–NH)
10e	3291 (NH), 3069, 1598, 1557 (ArH), 1751, 1700, 1661 (C = O), 1237 (C–F), 1188, 1160 (ester linkage –O–C)	0.81 (m, 6H, 2 × L-leucine–CH_3), 0.91 (t, 3H, H-18), 1.48 (m, 2H, L-leucine–CH_2), 1.59 (m, 1H, L-leucine–CH(CH_3)$_2$), 2.13 (q, 2H, H-19), 4.06 (d, 2H, CH_2–Gly), 4.19 (m, 1H, L-leucine–α-CH), 4.37 (s, 2H, 5-FU–N–CH_2), 5.31 (s, 2H, H-17), 5.50 (s, 2H, H-5), 7.17 (s, 1H, H-14), 7.73 (t, 1H, H-11), 7.97 (s, 1H, 5-FU-ring–H-6), 8.17 (t, 1H, H-10), 8.40 (d, 1H, H-9), 8.59 (d, 1H, H-12), 8.71 (s, 1H, H-7), 11.79 (s, 1H, 5-FU-ring–NH)
10f	3307 (NH), 3066, 1599, 1541 (ArH),1752, 1702, 1660 (C = O), 1236 (C–F), 1180, 1154 (ester linkage –O–C)	0.91 (t, 3H, H-18), 1.92 (s, 3H, L-methionine–CH_3), 2.13 (m, 3H, L-methionine–CH_2SCH_3 and H-19), 2.41 (m, 2H, L-methionine–$CH_2CH_2SCH_3$), 4.09 (d, 2H, CH_2–Gly), 4.30 (m, 1H, L-methionine–α-CH), 4.38 (s, 2H, 5-FU–N–CH_2), 5.29 (s, 2H, H-17), 5.48 (s, 2H, H-5), 7.15 (s, 1H, H-14), 7.72 (t, 1H, H-11), 7.94 (s, 1H, 5-FU-ring–H-6), 8.15 (t, 1H, H-10),

(*Continued*)

Table 2. Continued.

Compound	IR KBr, ν_{max} (cm^{-1})	^{1}H-NMR: (DMSO-d_6) δ (ppm)
		8.44 (d, 1H, H-9), 8.54 (d, 1H, H-12), 8.68 (s, 1H, H-7), 11.78 (s, 1H, 5-FU-ring–NH)
10g	3421, 3308 (NH, OH), 3072, 1589, 1562 (ArH), 1755, 1699, 1659 (C = O), 1238 (C–F), 1182, 1162 (esterlinkage –O–C)	0.91 (t, 3H, H-18), 1.05 (m, 3H, L-threonine–$CH(OH)CH_3$), 2.17 (q, 2H, H-19), 4.07 (d, 2H, CH_2–Gly), 4.12 (m, 1H, L-threonine–$CH(OH)CH_3$), 4.19 (m, 1H, L-threonine–α-CH), 4.28 (s, 2H, 5-FU–N–CH_2), 5.50 (s, 2H, H-17), 5.31 (s, 2H, H-5), 7.19 (s, 1H, H-14), 7.75 (t, 1H, H-11), 7.87 (s, 1H, 5-FU-ring–H-6), 7.95 (t, 1H, H-10), 8.18 (d, 1H, H-12), 8.38 (d, 1H, H-9), 8.71 (s, 1H, H-7), 11.79 (s, 1H, 5-FU-ring–NH)

Table 3. *In vitro* cytotoxicity of compounds **10a**–**10g** against human tumour cell lines (IC_{50} values).

	Cytotoxic activities (IC_{50}, μM)			
Compound	SGC-7901	BGC-823	A-549	HePG-2
10a	3.10	0.089	2.45	4.41
10b	2.35	0.045	0.45	3.29
10c	4.62	0.033	0.57	2.35
10d	2.92	0.067	0.38	1.21
10e	2.38	0.041	1.43	8.48
10f	5.99	0.074	5.21	5.44
10g	8.01	0.058	5.37	4.63
1	0.625	0.030	0.091	8.75
2	7.60	1.720	1.17	19.96

irinotecan. The different cytotoxic activity range of compounds **10a**–**10g** indicated that the substituents of dipeptide linkages obviously affected the selectivity as well as the activity against the four tested cancer cell lines. This investigation further highlighted that the constitution of the dipeptide spacers has a major impact on the cytotoxic activity of such analogues. Hence, a systemic, predictable correlation could be made between the nature of dipeptides and anticancer activities.

2.3. In vitro *determination of lactone levels in human and mouse plasma for 1 and 10b*

The result of the *in vitro* determination of lactone levels in human and mouse plasma buffers for **10b** and CPT (**1**) based on the method described in the literature (He et al., 2004) are shown in Table 4.

As shown in Table 4, the percentage of lactone of CPT (**1**) in human blood is 28.4 after 4 h, 10.9 after 8 h and 3.8 after 24 h. In other words, the active form of **1** is significantly decreased in a relatively shorter time period after oral administration.

Table 4. Comparison between the values of the percentage of lactone of the representive prodrug **10b** and **1** in human and mouse plasma.

	Time (h)					
	0	2	4	6	8	24
Human plasma						
Percentage of lactone for **10b**	100.0	81.9	70.3	54.3	42.5	10.5
Percentage of lactone for **1**	100.0	61.4	28.4	19.4	10.9	3.8
Mouse plasma						
Percentage of lactone for **10b**	100.0	83.8	80.7	73.1	52.6	17.9
Percentage of lactone for **1**	100.0	55.1	40.5	24.5	14.6	5.3

This is in contrast to what is observed in mice, for which the active drug form (i.e. the closed lactone form) lasts for a relatively longer time period. For example, the closed lactone form of **1** in mouse plasma is still 40.5% even after 4 h. The closed lactone form of **10b** in human plasma is much more stable than its mother compound **1**. For example, 70.3% of **10b** is still detected as the closed lactone form even after 4 h. CPT (**1**), in terms of lactone level, shows a difference between mouse and human plasma. Similarly, this kind of difference is also observed for prodrug **10b**. From the results, it is further evident that the biological life span of the lactone forms of these compounds in human and mouse plasma significantly increased when compared with their mother compound CPT (**1**). Meanwhile, the cleavage of **10b** in both mouse and human plasma was monitored by HPLC, which clearly showed that compound **10b** was cleaved to afford mainly its parental compound of CPT in both mouse and human plasma, and the cleavage of **10b** is the result of the combination of chemical and enzymatic effects in both mouse and human plasma. This was also the case in phosphate buffer at pH 7.4.

Another important aspect which has to be addressed is that these compounds would be cleaved to release active CPT and 5-FU in both mouse and human plasma. Such an assumption was supported by employing the HPLC experimental method, which simultaneously implied that these conjugates might act as mutual prodrugs and exert their cytotoxicities by hydrolysing to their parent compounds CPT and 5-FU, and they could have synergetic effects in biological systems.

3. Experimental

The values of melting points were taken on a Kofler melting point apparatus and were uncorrected; IR spectra were obtained on a NIC-5DX spectrophotometer; mass spectral analysis was performed on a ZAB-HS and Bruker Daltonics APEXII49e instrument. Optical rotations were determined on a Perkin Elmer 341 spectropolarimeter. NMR spectra were recorded on a Bruker AM-400 spectrometer at 400 MHz using TMS as the reference (Bruker Company, USA). The starting CPT was isolated from the Chinese medicinal plant *Camptotheca acuminata* and was purified before being used. The [(5-fluorouracil-1-yl)acetyl]-L-amino acids **7a**–**7g**

used for the experiments were prepared by following a modified previous procedure (Chen et al., 2004; Liu et al., 2006).

3.1. *Synthesis of CPT-20-glycinate TFA salt 9*

t-Boc-glycine (377 mg, 2.1 mmol) was dissolved in 200 mL of anhydrous dichloromethane at room temperature, and to this solution CPT (250 mg, 0.72 mmol), DCC (444 mg, 2.1 mmol) and DMAP (87 mg, 0.72 mmol) at 0°C were added. The reaction mixture was stirred for 1 h and subsequently left overnight at room temperature. The solution was washed with 0.1N HCl, dried, and evaporated under reduced pressure to yield a white solid, which was recrystallised from methanol to give CPT-20-ester of *t*-Boc-glycine (**8**), The *t*-Boc protection group was removed by dissolving it in a mixture of methylene chloride (15 mL) and TFA (15 mL) and stirring at room temperature for 1 h. The solvent was removed and the solid was recrystallised from methylene chloride and ether to give CPT-20-glycinate TFA salt **9**. The spectral data for **9** are identical to those reported by Greenwald (1998).

3.2. *General procedure for synthesis of target compounds (10a–10g)*

[(5-fluorouracil-1-yl)acetyl]-L-amino acids **7a**–**7g** (0.047 mol) were dissolved in 50 mL of anhydrous DMF and cooled to 0°C. 1-Hydroxybenzotriazole (HOBt) (0.07 mol) and 0.056 mol of *N*-ethyl-*N*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) were added, and the mixture was stirred for 30 min at 0°C. Subsequently, 0.039 mol of CPT-20-glycinate TFA salt **9** and finally 24.3 mL *N*-ethyl diisopropylamine were added. The mixture was stirred for 16 h at ambient temperature. The solvent was removed under reduced pressure and the residue was separated by flask-column chromatography (gradient elution with mixtures of chloroform–methanol) on silica gel and was monitored by TLC. The synthesised target compounds **10a**–**10g** were characterised by m.p., IR, ^{1}H-NMR and high-resolution mass spectrometry (HRMS) analyses. Their analytical and spectral data are given in Tables 1 and 2, respectively.

3.3. *Cytotoxicity assays*

Cytotoxicity assays were performed on four different human cancer cell lines (SGC-7901, BGC-823, A-549 and HePG-2). Cells (6000–10,000) in 100 μL culture medium per well were seeded into 96-well microtest plates (Falcon, CA). Cells were treated in triplicate with a gradient concentration of test compounds and incubated at 37°C for 48 h (or 72 h). A microculture tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT; Sigma, St. Louis, MO) assay was performed to measure the cytotoxic effects. The drug concentration required for 50% growth inhibition (IC_{50}) of tumour cells was determined from the dose–response curve.

3.4. In vitro *determination of lactone levels in human and mouse plasma for 1 and 10b*

The test compounds (0.2 mL, 100 μg mL^{-1}) in acetonitrile were added to 0.8 mL pre-incubated human and mouse plasma, respectively. The mixture was incubated

at 37°C, and 100 μL aliquots were taken at time points 2, 4, 6, 8 and 24 h. To precipitate plasma protein, 400 μL of acetonitrile (−20°C) was added, vortexed for 20 s and centrifuged at 10,000 rpm for 5 min. The supernatant was transferred to a glass vial and stored at −20°C immediately until HPLC analysis was complete. HPLC (HP 1100) analysis: 20 μL of solution obtained as abovementioned was injected onto a C_{18} column (Zobax SB, 4.6 × 150 mm) and chromatographed with methanol/water/0.1% acetic acid as mobile phase. CPT (**1**) and **10b** were detected (detector: DAB) at 254 nm. The percentage of lactone was determined by the ratio of the lactone levels measured at different time points to the lactone levels measured at the starting point ($t = 0$ h).

4. Conclusions

In summary, we synthesised seven CPT/5-FU conjugates joined by dipeptide linkages, based on the effective combination principle. The compounds selected for the *in vitro* determination of lactone levels showed that their biological life span in human and mouse plasma was much longer than that of their parent compound CPT. Meanwhile, most compounds exhibited potent cytotoxic activity against tumour cell replication. Interestingly, all the compounds were selectively active against BGC-823, with IC_{50} values lower than 0.1 μmol. These results are encouraging and suggest that the design and synthesis of these compounds will be beneficial for the therapeutic values of CPT analogues; the approach should be applicable for other antitumour agents, and it is worthwhile to explore the antitumour potential of these and similar types of compounds. In-depth mechanistic studies and the development of new CPT/5-FU conjugates are actively underway in our laboratory.

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