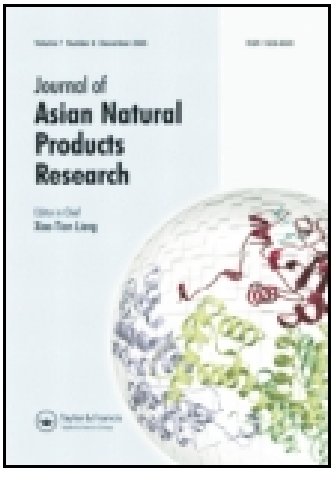

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Synthesis and cytotoxicity of novel 20-*O*-linked homocamptothecin ester derivatives as potent topoisomerase I inhibitors

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In an attempt to improve the antitumor activity of homocamptothecins (hCPTs), a series of novel 20-*O*-linked hCPT ester derivatives were first designed and synthesized based on a synthetic route, by which hCPTs are acylated with different substituted phenoxyacetic acid ester derivatives. Most of the derivatives were assayed for *in vitro* cytotoxicity against six human cancer cell lines KB, KB/VCR, A549, HCT-8, Bel7402, and A2780, and most of the assayed compounds exhibited good antiproliferative activity on these tumor cell lines especially on KB.

Keywords: 20-*O*-linked homocamptothecin ester; synthesis; cytotoxicity

1. Introduction

(20*S*)-Camptothecin (CPT), a cytotoxic quinoline alkaloid with topoisomerase I (Topo I) inhibitory, is a potent anticancer agent that was isolated from the Chinese tree *Camptotheca acumunata* and its structure was confirmed by Wani and Wall [1]. It has already been discovered that the cytotoxicity of camptothecin is due to a novel mechanism of action involving selective inhibition of DNA Topo I [2,3]. As a consequence of its broad-spectrum antitumour activity and particular cytotoxic mechanism, there has been a substantial effort toward the development of clinical CPT analogs. Two of its water-soluble analogs, topotecan and irinotecan (Figure 1), are used in clinics as anticancer agents and are now even being marketed in several countries, while several other analogs are the subject of ongoing clinical or preclinical evaluation (Figure 1) [4,5].

However, the severe toxicities, such as myelosuppression, nausea, vomiting, gastrointestinal disorders, and stomatitis [6,7] as well as the instability of lactone and other defects of CPTs [7], have promoted intensive efforts to reduce their toxicity in patients, increase the efficacy of these agents, and overcome the tumor drug resistance for this class of compounds. The most important discovery would be to modify the metabolically liable camptothecin lactone ring from a six-membered α-hydroxylactone ring to a seven-membered β-hydroxylactone ring, which leads to the development of the homocamptothecin (hCPT) family of compounds [8]. The hCPT is a semisynthetic analog of camptothecin with a seven-membered

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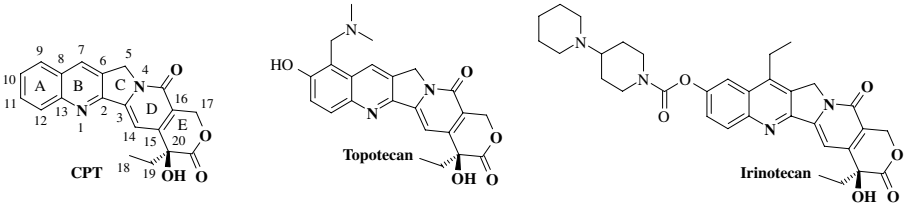


Figure 1. Structures of camptothecin and its representative analogs.

β-hydroxylactone ring resulting from the insertion of a methylene (—CH_2—) spacer between the alcohol moiety and the carboxyl group of the classical six-membered α-hydroxylactone ring of CPT. The hCPT represents a promising prototype for the next generation of Topo I-targeting agents.

Upgrading the highly reactive six-membered lactone to a more stable seven-membered ring is achieved without compromising the antitumor activity of hCPT, which not only retains superior antitumor activity, but also enhances the plasma stability of the drug and reinforces its capacity to inhibit Topo I compared with CPT [9]. Based on these encouraging results, many hCPT analogs have been synthesized and evaluated *in vitro* and *in vivo* as antitumor agents [10–13]. Among them, diflomotecan (BN80915) and elomotecan (BN80927) (Figure 2) are the representative ones and have been in clinical trials with encouraging results being obtained [14–16].

For CPT derivatives, a major issue is the opening of the lactone E-ring and the formation of an equilibrium between the closed lactone form and the open carboxylate form which is pH and also species dependent (Figure 3). Particularly, human serum albumin preferentially binds to the carboxylate form of camptothecin derivatives and shifts the equilibrium in favor of the carboxylate [17]. Some 20-*O*-alkyl esters [18] and amino acid esters of CPT [19] have been prepared to improve lactone ring stability and water solubility of CPT.

2. Results and discussion

In previous studies, we prepared many CPT oxyalkanoic acids [20] and substituted phenoxyacetic acid and phenoxybutyl acid ester derivatives [21]. These derivatives of hydroxyl group esterification of 20th positions were not only found to have better anticancer activities than that of irinotecan *in vitro* and *in vivo*, but also found to have much lower toxicities than that of CPT *in vivo*. Here, we report on the synthesis and the *in vitro* antitumor evaluation of CPTs and substituted phenoxyacetic acid ester derivatives of hCPTs, and several compounds that

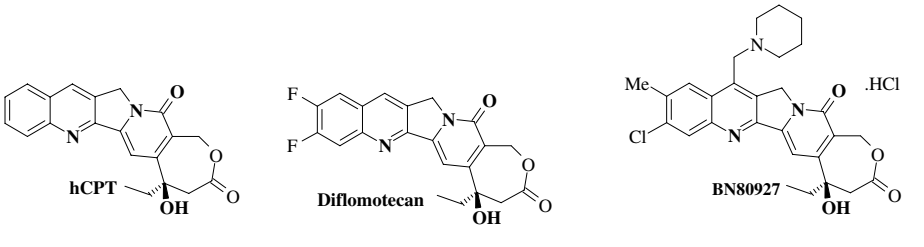


Figure 2. Structures of hCPT and its representative analogs.

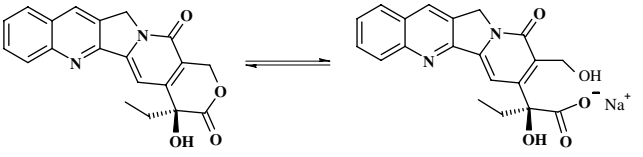
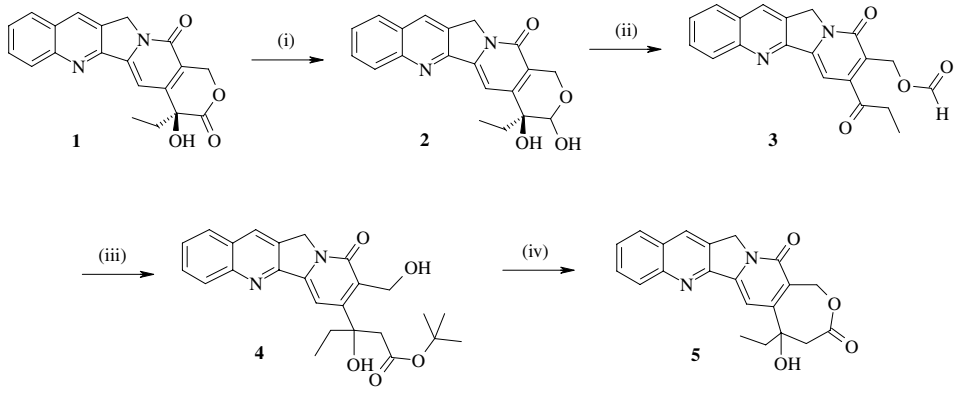


Figure 3. Structures of camptothecin (**1a**) and its water-soluble sodium salt.

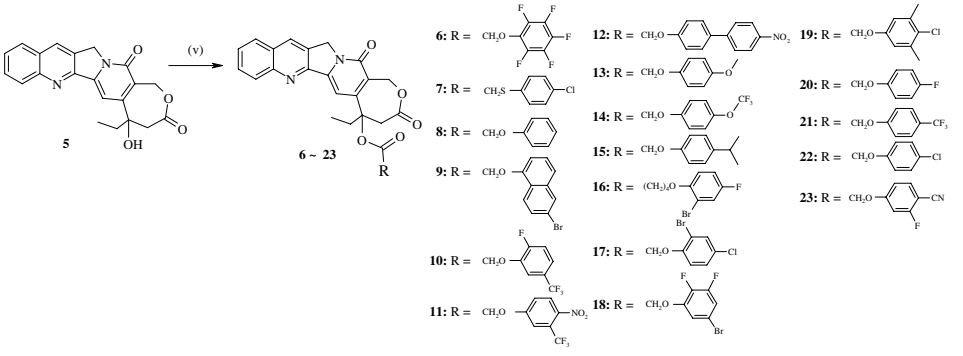
showed superior activity would be chosen to conduct further studies.

Preparation (Scheme 1) and purification of hCPTs **5** starting from CPT were done according to the previously published procedures [10,22]. hCPT ester derivatives **6**–**23** were prepared in proper yields by the straightforward acylation of hCPTs with the corresponding substituted phenoxyacetic acid and phenoxybutyl acid in the presence of a coupling agent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and a catalyst 4-(*N*,*N*-dimethylamino)pyridine (4-DMAP) at room temperature (Scheme 2).

Cytotoxicities of these hCPT ester derivatives were evaluated on six human cancer cell lines (KB, KB/VCR, A549, HCT-8, Bel7402, and A2780) by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to the previous method [23]. CPT, hCPT, and topotecan were used as reference compounds. Topotecan was used as a reference compound because it is a clinically effective CPT analog. CPT possesses the best antitumor activity *in vitro* in nanomolar concentration while topotecan and hCPT have poorer cytotoxic activity as compared with CPT. Most of these synthesized ester compounds (Table 1) showed good antiproliferative activity on these tumor cell lines, and several compounds showed comparable or superior cytotoxic activity to topotecan. The data in Table 1 exhibited the cytotoxicity of ester dependent on that of its parent compound. All of these assayed camptothecin esters possessed the lowest antitumor activity on vincristine-resistant KB (KB/VCR) and the highest antitumor activity on KB among these six human cancer cell lines. Esters **6**, **10**, **11**, **17**, **18**, and **22** had cytotoxities that were higher than toptecan (TPT) and hCPT and comparable with CPT on KB, HCT-8, Bel7402, and A2780. Ester **6** also possessed cytotoxity activity comparable with CPT on A549. In addition, compounds **12** and **13** showed comparable or a little



Scheme 1. Reagents and conditions: (i) $NaBH_4/CH_3OH$, r.t.; (ii) $NaIO_4$, r.t.; (iii) $Zn/Me_3ClSi/BrCH_2COOCMe_3/N_2$, reflux; (iv) CF_3COOH.



Scheme 2. Reagents and conditions: (v) R′COOH/EDCI/DMAP/CH_2Cl_2, r.t.

superior cytotoxities to TPT and hCPT on KB, A549, Bel7402, and A2780 with the same results for **19** and **20** on KB and Bel 7402 and for **14** and **15** on Bel 7402. Further investigations of the above esters with good cytotoxities would be carried out in the next step.

The insertion of a methylene spacer (—CH_2—) between the alcohol moiety and the carbonyl group of CPT lactone has two pronounced chemical consequences. One effect is due to the removal of the electronic induction of the hydroxyl oxygen atom on the carbonyl group, which results in a lower reactivity of the carboxyl function toward nucleophiles such as water, alcohols, or amines. The second effect stems from the thermodynamically disfavored closure of a seven-membered ring in comparison with a six-membered

Table 1. Cytotoxicities of 20(*S*)-*O*-linked hCPT ester derivatives against six human tumor cell lines.

	In vitro cytotoxicity (IC_{50}, $\mu mol\,l^{-1}$)					
Compound	KB	KB/VCR	A549	HCT-8	Bel7402	A2780
CPT	0.004	0.216	0.010	0.004	0.007	0.007
Topotecan	0.010	1.000	0.097	0.028	0.092	0.049
hCPT	0.76	0.36	1.25	4.37	0.37	0.28
6	0.004	>1	0.009	0.007	0.008	0.006
7	0. 051	>1	1.000	0.076	0.093	0.083
8	0.029	>1	0.093	0.076	0.080	0.063
9	0.059	0.900	0.776	0.083	0.212	0.197
10	0.005	0.846	0.082	0.008	0.009	0.008
11	0.005	0.476	0.100	0.007	0.009	0.007
12	0.010	0.697	0.086	0.051	0.073	0.058
13	0.008	0.548	0.097	0.033	0.074	0.050
14	0.027	0.846	0.100	0.068	0.074	0.069
15	0.051	>1	0.510	0.085	0.085	0.078
16	0.410	>1	1.000	0.758	0.654	0.694
17	0.005	0.641	0.170	0.008	0.008	0.007
18	0.006	0.401	0.279	0.010	0.032	0.035
19	0.008	>1	0.206	0.057	0.060	0.059
20	0.007	0.700	0.484	0.035	0.050	0.050
21	0.056	>1	>1	0.077	0.190	0.083
22	0.006	0.326	0.280	0.009	0.010	0.024

Note: KB, human epidermoid carcinoma of the nasopharynx; KB/VCR, vincristine-resistant KB; A549, human lung cancer; HCT-8, human colon cancer; Bel7402, human liver cancer; A2780, human ovarian cancer.

ring [24]. Here, the esterification of 20-*OH* of hCPT could strengthen these two notable chemical effects and further prohibit the seven-membered ring from opening under the physiological conditions. Taken together, these effects afford a mechanistic explanation for the slow and irreversible hydrolytic ring opening of hCPT, in contrast to the rapid hydrolysis of CPT to a pH-dependent equilibrium that hinders the antitumor activity of CPT.

In conclusion, we synthesized a series of 20-*O*-linked substituted phenoxyacetic acid ester derivatives of hCPT by acylating hCPT based on the expansion of the six-membered lactone ring of CPT to a seven-membered lactone ring and carried out the *in vitro* antitumor evaluation of the most esters by using MTT method. The above preliminary biological results indicated that the introduction of 20-*O*-phenoxyacetic acid esters of hCPT should lead CPT to increase the anticancer activity. The results provided further evidence for the previous induction [24] that the highly reactive α-hydroxylactone ring of CPT is not an absolute requirement for good Topo I-mediated activity, but homologation of the E-ring followed with esterification gives a more potent Topo I poison than TPT, an outstanding representative of CPTs, and exhibits high *in vitro* cytotoxicities. This novel E-ring modification also provides several more stable compounds that hydrolyze more slowly than the classical α-hydroxylactone of CPT. Although a potential ring-opening reaction with covalent binding to the enzyme is still possible, the lower reactivity of hCPT 20-*O*-ester derivatives may have some mechanistic implications. Furthermore, this kind of modification should influence the pharmacokinetics and pharmacodynamics of the drugs, and it would represent a promising template for the preparation of improved anticancer agents.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an XT4-100X micro-melting apparatus produced by Ningbo Biocotek Scientific Instrument Co., Ltd (Ningbo, China) and are uncorrected. The NMR spectra were recorded on a Varian Mercury-300 spectrometer (300 MHz for ^{1}H) produced by Varian Medical Systems (Salt Lake City, UT, USA). The mass spectra were obtained on a Finnigan LTQ FTMS (ESI) spectrometer produced by Thermo Electron Corporation (Waltham, MA, USA).

3.2 General procedures for compounds 2–5

hCPTs **2**–**5** were prepared (Scheme 1) according to the method reported by Lavergne *et al.* [10].

3.2.1 Compound 2

Pale yellow solid (89.5%); m.p. 281–283°C [lit. [24]: m.p. 280–283°C (dec.)], ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.64 (s, 1H, Ar-H), 8.13 (q, 2H, J = 7.5 Hz, Ar-H), 7.84 (t, 1H, J = 7.5 Hz, Ar-H), 7.68 (t, 1H, J = 7.5 Hz, Ar-H), 7.36 (s, 1H, Ar-H), 5.23 (s, 2H, 17-H), 4.99 (s, 1H, 21-H), 4.58 (q, 2H, J = 18.6 Hz, 5-H), 1.69 (q, 2H, J = 7.5 Hz, 19-CH_2), 0.90 (3H, t, J = 10.5 Hz, 18-CH_3).

3.2.2 Compound 3

Pale yellow solid (87.7%); m.p. 192–193°C [lit. [24]: m.p. 192–194°C (dec.)]; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.42 (s, 1H, Ar-H), 8.22 (d, 1H, J = 8.1 Hz, Ar-H), 8.09 (s, 1H, HCOO), 7.97 (d, 1H, J = 8.1 Hz, Ar-H), 7.85 (t, 1H, J = 8.1 Hz, Ar-H), 7.69 (t, 1H, J = 8.1 Hz, Ar-H), 7.33 (1H, s, Ar-H), 5.35 (s, 2H, 17-H), 5.32 (s, 2H, 5-H), 2.96 (q, 2H, J = 7.5 Hz, 19-CH_2), 1.27 (t, 3H, J = 7.5 Hz, 18-CH_3).

3.2.3 Compound **4**

Pale yellow solid (57.7%); m.p. 143–145°C [lit. [24]: m.p. 146–149°C]; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.38 (s, 1H, Ar-H), 8.22 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.92 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.82 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.65 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.43 (s, 1H, Ar-H), 5.28 (s, 2H, 17-H), 5.10 (m, 2H, 5-H), 3.08, 2.87 (d, 2H, $J = 16.2$ Hz, 20-CH_2COO), 1.99 (m, 2H, 19-CH_2), 1.38 (s, 9H, O—C(CH_3)$_3$), 0.93 (t, 3H, $J = 8.4$ Hz, 18-CH_3).

3.2.4 Compound **5**

Pale yellow solid (85.0%); m.p. 288–290°C (dec.) [lit. [24]: m.p. > 300°C]; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 8.68 (s, 1H, Ar-H), 8.13 (t, 2H, $J = 8.1$ Hz, Ar-H), 7.86 (t, 1H, $J = 8.1$ Hz, Ar-H), 7.71 (t, 1H, $J = 8.1$ Hz, Ar-H), 7.41 (s, 1H, Ar-H), 5.50, 5.41 (dd, 2H, $J = 15.0$ Hz, 17-H), 5.26 (s, 2H, 5-H), 3.44, 3.07 (dd, 2H, $J = 13.2$ Hz, 21-H), 1.84 (q, 2H, $J = 8.1$ Hz, 19-CH_2), 0.86 (t, 3H, $J = 7.2$ Hz, 18-CH_3).

3.3 General procedures for compounds 6–23

Taking compound **6** for example, a mixture of hCPT (15 mg, 0.041 mmol); 2,3,4,5,6-pentafluorophenoxyacetic acid (30 mg, 0.124 mmol); EDCI (30 mg, 0.157 mmol); 4-DMAP (12 mg, 0.098 mmol); and dichloromethane (5 ml) was stirred at room temperature for 8–12 h. The reaction was monitored by thin layer chromatography until the end. Then, chloroform (40 ml) was added, and organic phase was washed with water (35 ml), saturated sodium bicarbonate aqueous solution (35 ml) and brine (35 ml), and then dried over magnesium sulfate. After the solvent was removed under reduced pressure, the residue was taken up in chloroform and chromatographed (eluent: $CHCl_3$–CH_3OH 98:2) on silica gel to give 14 mg of compound **6** as a pale yellow solid.

3.3.1 Compound **6**

Pale yellow solid (57.7%); m.p. 165–167°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.46 (s, 1H, Ar-H), 8.30 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.96 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.88 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.71 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.38 (s, 1H, Ar-H), 5.83, 5.49 (dd, 2H, $J = 15.6$ Hz, 17-H), 5.29 (s, 2H, 5-H), 4.89 (m, 2H, 20-*O*—$COCH_2$—O—), 4.37, 3.15 (dd, 2H, $J = 13.2$ Hz, 21-H), 2.31, 1.98 (m, 2H, 19-CH_2), 1.16 (t, 3H, $J = 7.2$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 587.1227 $[M + H]^+$ (calcd for $C_{29}H_{20}F_5N_2O_6$, 587.1242).

3.3.2 Compound **7**

Pale yellow solid (72.9%); m.p. 258–260°C (dec.); ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.39 (s, 1H, Ar-H), 8.19 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.94 (d, 1H, $J = 7.8$ Hz, Ar-H), 7.85 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.68 (t, 1H, $J = 7.8$ Hz, Ar-H), 7.31 (d, 2H, $J = 7.5$ Hz, Ar-H), 7.24 (s, 1H, Ar-H), 7.07 (d, 2H, $J = 7.5$ Hz, Ar-H), 5.79, 5.46 (d, 2H, $J = 15.6$ Hz, 17-H), 5.25 (s, 2H, 5-H), 4.28,3.02 (dd, 2H, $J = 13.2$ Hz, 21-H), 3.66 (t, 2H, $J = 11.7$ Hz, 20-*O*—$COCH_2$—S—), 2.19, 1.88 (m, 2H, 19-CH_2), 1.09 (t, 3H, $J = 7.2$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 547.1090 $[M + H]^+$ (calcd for $C_{29}H_{24}ClN_2O_5S$, 547.1095).

3.3.3 Compound **8**

Pale yellow solid (41.8%); m.p. 112–114°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.44 (s, 1H, Ar-H), 8.30 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.96 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.88 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.71 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.41 (s, 1H, Ar-H), 7.22 (t, 2H, $J = 6.9$ Hz, Ar-H), 6.91 (t, 2H, $J = 7.5$ Hz, Ar-H), 6.78 (s, 1H, Ar-H), 5.84, 5.46 (dd, 2H, $J = 15.6$ Hz, 17-H), 5.28 (s, 2H, 5-H), 4.73 (d, 2H, $J = 7.8$ Hz, 20-*O*—$COCH_2$—O), 4.37, 3.09 (dd, 2H, $J = 13.2$ Hz, 21-H), 2.28, 1.93 (m, 2H, 19-CH_2), 1.12 (t, 3H, $J = 6.9$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 497.1703 $[M + H]^+$ (calcd for $C_{29}H_{25}N_2O_6$, 497.1713).

3.3.4 Compound **9**

Pale yellow solid (60.8%); m.p. 227–229°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.44 (s, 1H, Ar-H), 8.30 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.99 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.91 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.72 (t, 2H, $J = 8.4$ Hz, Ar-H), 7.57 (d, 1H, $J = 9.3$ Hz, Ar-H), 7.38 (t, 2H, $J = 8.4$ Hz, Ar-H), 7.22 (d, 1H, $J = 2.4$ Hz, Ar-H), 7.02 (m, 2H, Ar-H), 5.80–5.49 (m, 2H, 17-H), 5.24 (d, 2H, $J = 3.0$ Hz, 5-H), 4.91, 4.83 (d, 2H, $J = 16.2$ Hz, 20-*O*—$COCH_2$—O—), 4.36, 3.14 (dd, 2H, $J = 13.8$ Hz, 21-H), 2.32, 1.97 (m, 2H, 19-CH_2), 1.13 (t, 3H, $J = 7.8$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 625.0961 $[M + H]^+$ (calcd for $C_{33}H_{26}BrN_2O_6$, 625.0974).

3.3.5 Compound **10**

Pale yellow solid (48.0%); m.p. 162–164°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.43 (s, 1H, Ar-H), 8.24 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.95 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.86 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.69 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.33 (s, 1H, Ar-H), 7.17 (m, 3H, Ar-H), 5.84–5.48 (dd, 2H, $J = 15.3$ Hz, 17-H), 5.28 (s, 2H, 5-H), 4.83 (d, 2H, $J = 7.8$ Hz, 21-H), 4.40, 3.07 (dd, 1H, $J = 13.2$ Hz, 20-*O*—$COCH_2$—O—), 2.32, 1.96 (m, 2H, 19-CH_2), 1.13 (t, 3H, $J = 6.9$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 583.1481 $[M + H]^+$ (calcd for $C_{30}H_{23}F_4N_2O_6$, 583.1414).

3.3.6 Compound **11**

Pale yellow solid (60.5%); m.p. 190–192°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.46 (1H, s, Ar-H), 8.26 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.89 (d, 1H, $J = 7.8$ Hz, Ar-H), 7.81 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.72 (t, 1H, $J = 7.8$ Hz, Ar-H), 7.30 (s, 1H, Ar-H), 7.13 (m, 3H, Ar-H), 5.73, 5.54 (d, 2H, $J = 15.3$ Hz, 17-H), 5.29 (s, 2H, 5-H), 4.86 (m, 2H, 20-*O*—$COCH_2$—O), 4.22, 3.23 (dd, 2H, $J = 13.8$ Hz, 21-H), 2.34, 2.03 (m, 2H, 19-CH_2), 1.13 (t, 3H, $J = 7.8$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 610.1424 $[M + H]^+$ (calcd for $C_{30}H_{23}F_3N_3O_8$, 610.1437).

3.3.7 Compound **12**

Pale yellow solid (66.8%); m.p. 246–248°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.43 (s, 1H, Ar-H), 8.24 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.95 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.85 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.77 (d, 2H, $J = 8.4$ Hz, Ar-H), 7.69 (t, 1H, $J = 7.8$ Hz, Ar-H), 7.44 (t, 2H, $J = 6.3$ Hz, Ar-H), 7.36 (s, 1H, Ar-H), 7.20 (t, 1H, $J = 7.8$ Hz, Ar-H), 7.07 (t, 1H, $J = 8.4$ Hz, Ar-H), 6.97 (d, 2H, $J = 8.4$ Hz, Ar-H), 5.85, 5.49 (dd, 2H, $J = 15.6$ Hz, 17-H), 5.28 (d, 2H, $J = 6.9$ Hz, 5-H), 4.81 (dd, 2H, $J = 16.2$ Hz, 20-*O*—$COCH_2O$), 4.33, 3.14 (d, 2H, $J = 13.2$ Hz, 21-H), 2.27, 1.95 (m, 2H, 19-CH_2), 1.11 (t, 3H, $J = 6.9$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 619.1865 $[M + H]^+$ (calcd for $C_{35}H_{28}N_3O_8$, 619.1832).

3.3.8 Compound **13**

Pale yellow solid (57.4%); m.p. 139–141°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.38 (s, 1H, Ar-H), 8.23 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.93 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.84 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.67 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.27 (s, 1H, Ar-H), 6.79 (d, 2H, $J = 8.4$ Hz, Ar-H), 6.72 (d, 2H, $J = 6.3$ Hz, Ar-H), 5.82, 5.46 (dd, 2H, $J = 16.5$ Hz, 17-H), 5.24 (s, 2H, 5-H), 4.67 (d, 2H, $J = 2.4$ Hz, 20-*O*—$COCH_2$-O—), 4.38, 3.08 (dd, 2H, $J = 13.8$ Hz, 21-H), 3.52 (s, 3H, 4′-OCH_3), 2.28, 1.93 (m, 2H, 19-CH_2), 1.13 (t, 3H, $J = 7.8$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 527.1814 $[M + H]^+$ (calcd for $C_{30}H_{27}N_2O_7$, 527.1818).

3.3.9 Compound **14**

Pale yellow solid (70.5%); m.p. 163–165°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.41 (s, 1H, Ar-H), 8.23 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.95 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.86 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.69 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.03 (d, 2H, $J = 9.3$ Hz, Ar-H), 7.26 (s, 1H, Ar-H), 6.89 (d, 2H, $J = 9.3$ Hz, Ar-H), 5.81, 5.49 (dd, 2H, $J = 16.5$ Hz, 17-H), 5.27 (s, 2H, 5-H),

4.72 (d, 2H, $J = 3.9$ Hz, 20-*O*—$COCH_2$-O—), 4.34, 3.13 (dd, 2H, $J = 13.8$ Hz, 21-H), 2.29, 1.96 (m, 2H, 19-CH_2), 1.12 (t, 3H, $J = 7.8$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 581.1525 $[M + H]^+$ (calcd for $C_{30}H_{24}F_3N_2O_7$, 581.1536).

3.3.10 Compound **15**

Pale yellow solid (84.1%); m.p. 142–144°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.40 (s, 1H, Ar-H), 8.25 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.94 (d, 1H, $J = 8.7$ Hz, Ar-H), 7.85 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.68 (t, 1H, $J = 8.7$ Hz, Ar-H), 7.33 (s, 1H, Ar-H), 6.98 (d, 2H, $J = 8.7$ Hz, Ar-H), 6.80 (t, 2H, $J = 8.7$ Hz, Ar-H), 5.84, 5.45 (d, 2H, $J = 15.6$ Hz, 17-H), 5.24 (s, 2H, 5-H), 4.72 (m, 2H, 20-*O*—$COCH_2O$), 4.40, 3.08 (dd, 2H, $J = 13.2$ Hz, 21-H), 2.70 (m, 1H, 4′-$CHMe_2$), 2.34, 1.94 (m, 2H, 19-CH_2), 1.13 (t, 3H, $J = 6.9$ Hz, 18-CH_3), 1.01 (d, 6H, $J = 7.2$ Hz, 4′-$CH(\underline{CH_3)_2}$); HR-ESI-MS: *m/z* 539.2174 $[\overline{M + H}]^+$ (calcd for $C_{32}H_{31}N_2O_6$, 539.2182).

3.3.11 Compound **16**

Pale yellow solid (48.0%); m.p. 169–171°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.37 (s, 1H, Ar-H), 8.14 (d, 1H, $J = 7.8$ Hz, Ar-H), 7.91 (d, 1H, $J = 7.8$ Hz, Ar-H), 7.80 (t, 1H, $J = 7.8$ Hz, Ar-H), 7.65 (t, 1H, $J = 7.8$ Hz, Ar-H), 7.17 (m, 1H, Ar-H), 6.84 (m, 2H, Ar-H), 6.71 (m, 1H, Ar-H), 5.79, 5.45 (dd, 1H, $J = 15.6$ Hz, 17-H), 5.27 (s, 2H, 5-H), 4.33, 3.09 (dd, 2H, $J = 13.2$ Hz, 21-H), 3.93 (t, 2H, $J = 5.4$ Hz, $ArOCH_2C$), 2.54 (t, 2H, $J = 3.6$ Hz, 20-*O*—$COCH_2O$—), 2.26, 1.86 (m, 2H, 19-CH_2), 1.94 (m, 4H, —CH_2CH_2—),1.16 (t, 3H, $J = 7.2$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 635.1187 $[M + H]^+$ (calcd for $C_{32}H_{29}BrFN_2O_6$, 635.1115).

3.3.12 Compound **17**

Pale yellow solid (43.8%); m.p. 181–183°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.53 (s, 1H, Ar-H), 8.44 (d, 1H, $J = 6.3$ Hz, Ar-H), 8.01 (d, 1H, $J = 6.3$ Hz, Ar-H), 7.93 (t, 1H, $J = 6.0$ Hz, Ar-H), 7.76 (t, 1H, $J = 6.0$ Hz, Ar-H), 7.62 (s, 1H, Ar-H), 7.50 (d, 1H, $J = 1.5$ Hz, Ar-H), 7.14 (d, 1H, $J = 5.1$ Hz, Ar-H), 6.84 (d, 1H, $J = 6.6$ Hz, Ar-H), 5.81, 5.48 (dd, 2H, $J = 12.0$ Hz, 17-H), 5.31 (s, 2H, 5-H), 4.94, 4.78 (dd, 2H, $J = 12.0$ Hz, 20-*O*—$COCH_2O$—), 4.35, 3.13 (dd, 2H, $J = 10.2$ Hz, 21-H), 2.30, 1.95 (m, 2H, 19-CH_2), 1.15 (t, 3H, $J = 5.7$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 609.0425 $[M + H]^+$ (calcd for $C_{29}H_{24}BrClN_2O_6$, 609.0428).

3.3.13 Compound **18**

Pale yellow solid (43.6%); m.p. 206–208°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.44 (s, 1H, Ar-H), 8.26 (d, 1H, $J = 6.3$ Hz, Ar-H), 7.95 (d, 1H, $J = 6.3$ Hz, Ar-H), 7.87 (t, 1H, $J = 6.3$ Hz, Ar-H), 7.70 (t, 1H, $J = 6.3$ Hz, Ar-H), 7.34 (s, 1H, Ar-H), 6.87 (t, 1H, $J = 5.4$ Hz, Ar-H), 6.79 (d, 1H, $J = 4.5$ Hz, Ar-H), 5.83, 5.49 (dd, 2H, $J = 12.0$ Hz, 17-H), 5.29 (s, 2H, 5-H), 4.80 (q, 2H, $J = 12.6$ Hz, 20-*O*—$COCH_2$-O—), 4.38, 3.14 (d, 2H, $J = 10.2$ Hz, 21-H), 2.33, 1.98 (m, 2H, 19-CH_2), 1.15 (t, 3H, $J = 5.7$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 611.0625 $[M + H]^+$ (calcd for $C_{29}H_{22}BrF_2N_2O_6$, 611.0629).

3.3.14 Compound **19**

Pale yellow solid (43.8%); m.p. 152–154°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.40 (s, 1H, Ar-H), 8.20 (d, 1H, $J = 6.3$ Hz, Ar-H), 7.94 (d, 1H, $J = 6.3$ Hz, Ar-H), 7.84 (t, 1H, $J = 6.3$ Hz, Ar-H), 7.68 (t, 1H, $J = 6.3$ Hz, Ar-H), 7.26 (s, 1H, Ar-H), 6.59 (s, 2H, Ar-H), 5.82, 5.48 (dd, 2H, $J = 12.0$ Hz, 17-H), 5.26 (s, 2H, 5-H), 4.68 (d, 2H, $J = 3.0$ Hz, 20-*O*—$COCH_2$-O—), 4.37, 3.10 (dd, 2H, $J = 10.5$ Hz, 21-H), 2.33, 1.95 (m, 2H, 19-CH_2), 2.20 (6H, s, 3′-CH_3 and 4′-CH_3), 1.13 (t, 3H, $J = 5.4$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 559.1630 $[M + H]^+$ (calcd for $C_{31}H_{28}ClN_2O_6$, 559.1636).

3.3.15 Compound **20**

Pale yellow solid (70.4%); m.p. 132–134°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.40 (s, 1H, Ar-H), 8.24 (d, 1H, $J = 7.8$ Hz, Ar-H), 7.94 (d, 1H, $J = 7.5$ Hz, Ar-H), 7.86 (t, 1H, $J = 7.5$ Hz, Ar-H), 7.69 (t, 1H, $J = 7.5$ Hz, Ar-H), 7.24 (s, 1H, Ar-H), 6.84 (d, 2H, $J = 4.2$ Hz, Ar-H), 6.82 (d, 2H, $J = 4.2$ Hz, Ar-H), 5.86, 5.49 (dd, 2H, $J = 15.9$ Hz, 17-H), 5.26 (s, 2H, 5-H), 4.68 (d, 2H, $J = 6.0$ Hz, 20-*O*—$COCH_2$-O—), 4.35, 3.11 (dd, 2H, $J = 13.5$ Hz, 21-H), 2.24, 1.94 (m, 2H, 19-CH_2), 1.12 (t, 3H, $J = 7.5$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 515.1610 $[M + H]^+$ (calcd for $C_{29}H_{24}FN_2O_6$, 515.1618).

3.3.16 Compound **21**

Pale yellow solid (51.8%); m.p. 103–105°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.41 (s, 1H, Ar-H), 8.24 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.95 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.86 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.70 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.58 (m, 2H, Ar-H), 7.28 (s, 1H, Ar-H), 6.98 (m, 2H, Ar-H), 5.79, 5.49 (dd, 2H, $J = 15.3$ Hz, 17-H), 5.26 (s, 2H, 5-H), 4.79 (d, 2H, $J = 6.0$ Hz, 20-*O*—$COCH_2O$—), 4.77, 4.32 (dd, 2H, $J = 13.5$ Hz, 21-H), 2.32, 1.97 (m, 2H, 19-CH_2), 1.10 (t, 3H, $J = 4.5$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 565.1581 $[M + H]^+$ (calcd for $C_{30}H_{24}F_3N_2O_6$, 565.1587).

3.3.17 Compound **22**

Pale yellow solid (48.2%); m.p. 190–192°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 9.06 (d, 1H, $J = 7.5$ Hz, Ar-H), 8.92 (s, 1H, Ar-H), 8.15 (t, 2H, $J = 8.1$ Hz, Ar-H), 7.96 (m, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.08 (d, 1H, $J = 8.1$ Hz, Ar-H), 6.94 (d, 2H, $J = 8.1$ Hz, Ar-H), 5.80, 5.45 (dd, 2H, $J = 16.5$ Hz, 17-H), 5.43 (s, 2H, 5-H), 4.77 (m, 2H, 20-*O*—$COCH_2O$—), 4.33, 3.16 (dd, 2H, $J = 13.8$ Hz, 21-H), 2.26, 1.96 (m, 2H, 19-CH_2), 1.19 (t, 3H, $J = 7.5$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 531.1318 $[M + H]^+$ (calcd for $C_{29}H_{24}ClN_2O_6$, 531.1323).

3.3.18 Compound **23**

Pale yellow solid (65.0%); m.p. 200–202°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.42 (d, 1H, $J = 11.4$ Hz, Ar-H), 8.21 (d, 1H, $J = 8.4$ Hz, Ar-H), 7.95 (d, 1H, $J = 7.8$ Hz, Ar-H), 7.69 (t, 1H, $J = 8.4$ Hz, Ar-H), 7.43 (t, 1H, $J = 7.8$ Hz, Ar-H), 7.22 (s, 1H, Ar-H), 6.73 (m, 3H, Ar-H), 5.73, 5.53 (dd, 2H, $J = 15.9$ Hz, 17-H), 5.27 (s, 2H, 5-H), 4.77 (d, 2H, $J = 5.4$ Hz, 20-*O*—$COCH_2$-O—), 4.24, 3.20 (dd, 2H, $J = 14.1$ Hz, 21-H), 2.31, 2.00 (m, 2H, 19-CH_2), 1.11 (t, 3H, $J = 7.2$ Hz, 18-CH_3); HR-ESI-MS: *m/z* 540.1563 $[M + H]^+$ (calcd for $C_{30}H_{23}FN_3O_6$, 540.1571).

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References

[1] M.E. Wall, M.C. Wani, C.E. Cook, K.H. Palmer, A.T. McPhail, and G.A. Sim, *J. Am. Chem. Soc.* **88**, 3888 (1966).

[2] Y.H. Hsiang, R. Hertzberg, S. Hecht, and L.F. Liu, *J. Biol. Chem.* **260**, 14873 (1985).

[3] Y.H. Hsiang, M.G. Lihou, and L.F. Liu, *Cancer Res.* **49**, 5077 (1989).

[4] F. Zunino, S. Dallavalleb, D. Laccabuea, G. Berettaa, L. Merlinib, and G. Pratesi, *Curr. Pharm. Des.* **8**, 2505 (2002).

[5] Y. Pommier, *Chem. Rev.* **109**, 2894 (2009).

[6] C.J. Thomas, N.J. Rahier, and S.M. Hecht, *Bioorg. Med. Chem.* **12**, 1585 (2004).

[7] H. Ulukan and P.W. Swaan, *Drugs* **62**, 2039 (2002).

[8] O. Lavergne, L. Lesueur-Ginot, and F.P. Rodas, *Bioorg. Med. Chem. Lett.* **7**, 2235 (1997).

[9] C. Bailly, A. Lansiaux, L. Dassonneville, D. Demarquay, H. Coulomb, M. Huchet, O. Lavergne, and D.C. Bigg, *Biochemistry* **38**, 15556 (1999).

[10] O. Lavergne, D. Demarquay, C. Bailly, C. Lanco, A. Rolland, M. Huchet, H. Coulomb, N. Muller, N. Baroqqi, J. Camara, C.L. Breton, E. Manqinot, J.B.

Cazaux, and D.C. Bigg, *J. Med. Chem.* **43**, 2285 (2000).
[11] D. Bom, D.P. Curran, A.J. Chavan, S. Kruszewski, S.G. Zimmer, K.A. Fraley, and T.G. Burke, *J. Med. Chem.* **42**, 3018 (1999).
[12] R.S. Tangirala, S. Antony, K. Agama, Y. Pommier, B.D. Anderson, R. Bevins, and D.P. Curran, *Bioorg. Med. Chem.* **14**, 6202 (2006).
[13] L.J. Zhu, P.F. Cheng, N. Lei, J.Z. Yao, C. Q. Sheng, C.L. Zhuang, W. Guo, W.F. Liu, Y.Q. Zhang, G.Q. Dong, S.Z. Wang, Z.Y. Miao, and W.N. Zhang, *Arch. Pharm. Chem. Life Sci.* **344**, 726 (2011).
[14] H. Gelderblom, R. Salazar, J. Verweij, G. Pentheroudakis, M.J.D. Jonge, M. Devlin, C.V. Hooije, F. Seguy, R. Obach, J. Prunonosa, P. Principe, and C. Twelves, *Clin. Cancer Res.* **9**, 4101 (2003).
[15] D.M. Demarquay, H. Huchet, L. Coulomb, G. Lesueur, O. Lavergne, J. Camara, P.G. Kasprzyk, G. Prevost, and D.C. Bigg, *Cancer Res.* **64**, 4942 (2004).
[16] Z. Liao, R.W.J. Robey, K.K.T. Guirouilh-Barbat, O.S. Polgar, E. Bates, and Y. Pommier, *Mol. Pharmacol.* **73**, 490 (2008).
[17] T.G. Burke and Z. Mi, *Anal. Biochem.* **212**, 286 (1993).
[18] Z. Cao, N. Harris, and A. Kozielski, *J. Med. Chem.* **41**, 31 (1998).
[19] M.E. Wall and M.C. Wani, US Patent No. 5646159 (8 July 1997).
[20] L.X. Yang, X.D. Pan, and H.J. Wang, *Bioorg. Med. Chem. Lett.* **12**, 1241 (2002).
[21] X.D. Pan, H.Y. Liu, P.Y. Sun, C.G. Zhu, J. Yang, K.H. Yuan, and R. Han, *Acta Pharm. Sin.* **39**, 591 (2004).
[22] T.R. Govindachari and K.R. Ravindranath, *J. Chem. Soc., Perkin Trans. I.* **11**, 1215 (1974).
[23] X.D. Pan, R. Han, and P.Y. Sun, *Bioorg. Med. Chem. Lett.* **13**, 3739 (2003).
[24] L.G. Laurence, D. Daniele, K. Robert, G. K. Philip, D. Laurent, B. Christian, C. Jose, L. Olivier, and C.H.B. Dennis, *Cancer Res.* **59**, 2939 (1999).