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Regioselective Synthesis and Cytotoxicities of Camptothecin Derivatives Modified at the 7-, 10- and 20-Positions

Xian-dao Pan,[a,*] Rui Han[a] and Piao-yang Sun[b]

[a]*Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China*
[b]*Hengrui Pharmaceutical Co., Ltd., Lianyungang, Jiangsu 222002, China*

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Abstract—A series of 7-acyloxymethylcamptothecin and 20-*O*-acyl-7-acyloxymethylcamptothecin derivatives were regioselectively prepared on different solvents. 7-Acyloxymethylcamptothecins possess more efficacy than 20-*O*-acyl-7-acyloxymethylcamptothecins against six human cancer cell lines in vitro.

Camptothecin (CPT, **1**) and its semisynthetic analogues, irinotecan and topotecan, are among the most promising anticancer drugs used in clinics. Several other derivatives are at different stages of clinical trials currently and expected to launch to market within the next 2–4 years.[1] Mechanistic aspects of the action of these topoisomerase I inhibitors are also becoming clearer.[2] SAR studies have revealed that the C-7 substitutions can be used to increase CPT's lipophilicity, so lactone stabilization is further promoted by enhanced lipophilicity or lipid bilayer partitioning.[3] We have reported the esterification of (20*S*)-hydroxy of CPT can significantly reduce the toxicity of camptothecin derivatives.[4] A series of ester derivatives were designed on the basis of SAR results of CPT, and synthesized from the corresponding substituted CPT.

Camptothecin was firstly converted into 7-hydroxymethyl-CPT (**2**) according to Sawada et al.,[5] and then **2** was treated with acids to get corresponding 7-acyloxymethyl-CPT esters (**3a**–**3d**) and 7,20-diesters (**4a**–**4d**) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI)[6] and 4-dimethylamino pyridine (DMAP) (Scheme 1). The mono-esters (**5a**–**5b**) and di-esters (**6a**–**6b**) of 10-hydroxy-CPT (**7**) were also prepared in the same manner (Scheme 2). The ^{1}H NMR spectra of these CPT esters[7] showed the corresponding characteristic protons for their ester side chains. Unexpectedly, two kinds of esters were yielded in different solvents used. The di-esters were usually obtained in yields of 50–80% in a solvent of low polarity such as dichloromethane even if 1.2 equiv of acids were used. However, 7-acyloxymethyl-CPT or 10-acyloxy-CPT was obtained in yields of 40–60% in a aprotic polar solvent such as *N*,*N*-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO). 20-Hydroxy group is hard to esterify in this condition even if 3.0 equiv of acids were used.

This result may be explained to the structure of CPT. There is an intramolecular hydrogen bond in the E-ring of CPT molecule (Fig. 1). 20-Hydroxy group can be esterified besides 7-hydroxymethyl group owing to the weeker hydrogen bond in the low polar solvent. The stronger hydrogen bond is existed in the polar solvent such as DMF, the 20-hydroxy group is difficult to couple with the acid, so 7-acyloxymethyl ester of CPT was obtained.

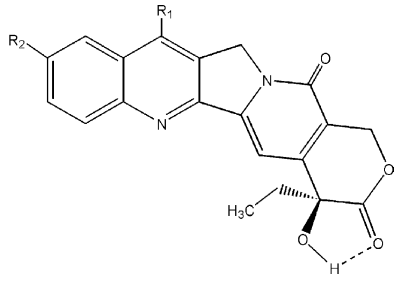

Figure 1.

*Corresponding author. Tel.: +86-10-6316-6764; fax: +86-10-6302-7389; e-mail: xdp@imm.ac.cn

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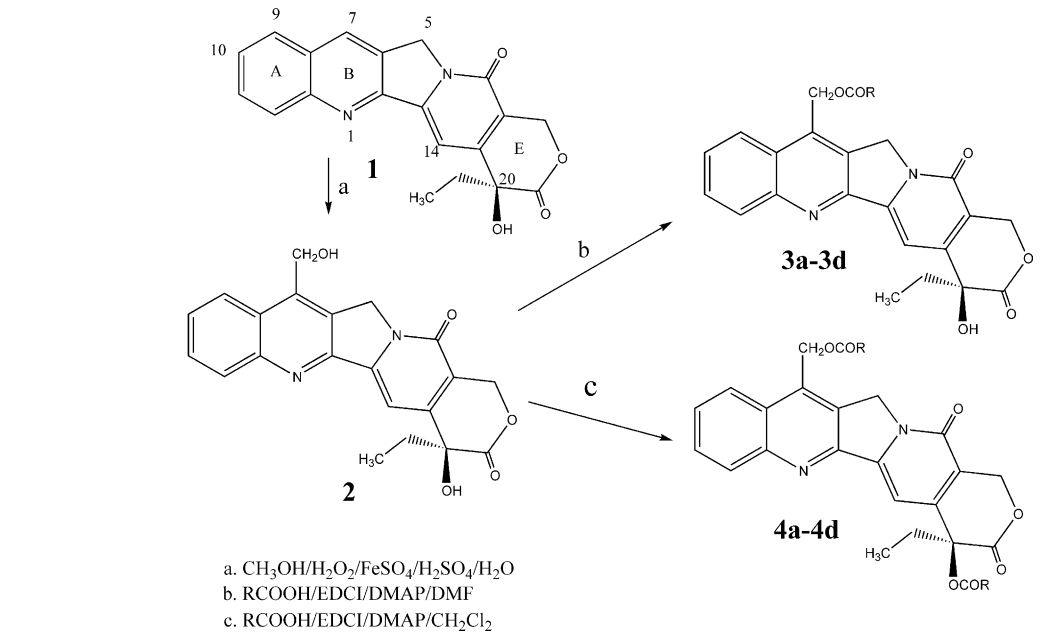
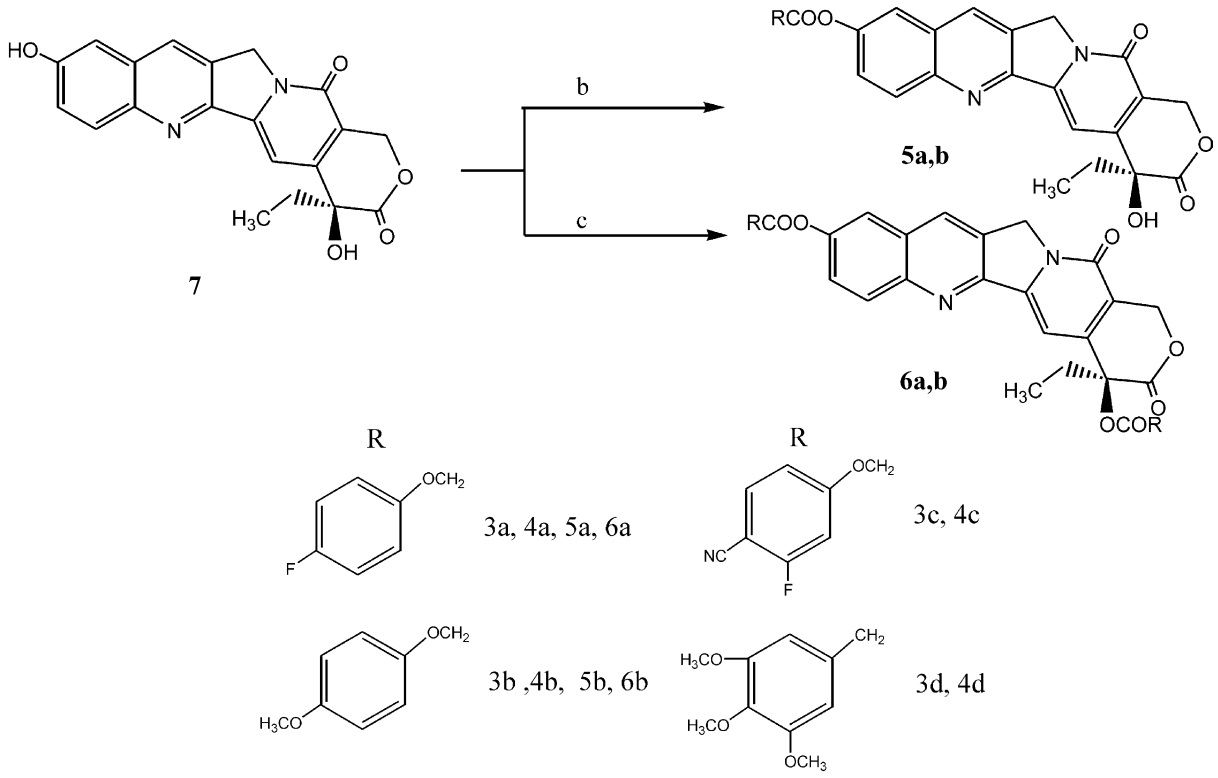


Scheme 1.



Scheme 2.

Cytotoxicities of these camptothecin ester derivatives were evaluated on six human cancer cell lines (KB, KB/VCR, A549, HCT-8, Bel7402, and A2780) using MTT assay. Topotecan and CPT were used as reference compounds. The phenoxyacetate (**3a–3c**, **4a–4c**) of CPT showed more active in vitro than the phenacetate of CPT (**3d**, **4d**). This result is consistent with our previous result.[4] 7-Acyloxymethylcamptothecins (**3a–3d**, Table 1) showed superior cytotoxic activity to topotecan. Other esters had comparable cytotoxic activity to topotecan. The data in Table 1 revealed that monoesters, especially for 7-acyloxymethylcamptothecins, possessed more efficacy in vitro than di-esters. This result is consistent with the SAR of CPT, that is large substituted groups at C-10 reduces the cytotoxicity of CPT, such as irinotecan. However, the substitutions at C-7 can improve the antitumor activity of CPT. All of these camptothecin esters possess poorer antitumor activity on KB/VCR (vincristine-resistant cancer cell line) than KB.

Table 1. Cytotoxicity of some di-esters and mono-esters of camptothecins

Compd		In vitro cytotoxicity (IC_{50}, μmol L^{-1})					
		KB	KB/VCR	A2780	A549	HCT-8	Bel7402
CPT		0.009	0.009	0.007	0.008	0.007	0.007
Topotecan		0.062	0.339	0.058	0.087	0.074	0.078
1	**3a**	0.007	0.049	0.008	0.057	0.008	0.009
	4a	0.072	>1.0	0.058	0.293	0.052	0.026
2	**3b**	0.008	0.051	0.009	0.069	0.009	0.008
	4b	0.310	0.267	0.071	0.348	0.076	0.084
3	**3c**	<0.001	0.437	<0.001	0.008	<0.001	<0.001
	4c	0.009	0.053	0.010	0.055	0.048	0.033
4	**3d**	0.036	0.340	0.061	0.160	0.055	0.072
	4d	0.078	0.947	0.068	0.098	0.076	0.082
5	**5a**	0.053	0.244	0.054	0.080	0.062	0.095
	6a	0.055	0.295	0.015	0.401	0.082	0.095
6	**5b**	0.047	0.275	0.065	0.190	0.061	0.074
	6b	0.069	0.460	0.088	0.718	0.097	0.200

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

In summary, we developed a simple and efficient method for preparing the mono- and di-acyl CPT derivatives. The mono-ester was synthesized in the polar solvent, and di-ester prepared in the lower polar solvent. Preliminary biological studies showed that 7-acyloxymethylcamptothecins possessed higher antitumor activity in vitro than 20-*O*-acyl-7- acyloxymethylcamptothecins and other CPT esters.

References and Notes

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7. **3a**. Yield 56.3%, mp 228–230 °C, ^{1}H NMR ($CDCl_3$) δ 8.25 (d, 1H, Ar-H), 8.05 (d, 1H, Ar-H), 7.84 (t, 1H, Ar-H), 7.66 (m, 2H, Ar-H), 6.91 (t, 2H, Ar-H), 6.80 (m, 2H, Ar-H), 5.82 (s, 2H, 7-CH_2), 5.73 (d, 1H, H17), 5.47 (s, 2H, H5), 5.34 (d, 1H, H17), 4.69 (s, 2H, OCH_2CO), 3.79 (s, 1H, 20-OH), 1.80 (m, 2H, 18-CH_2), 1.04 (t, 3H, 19-CH_3); **4a**. Yield 67.8%, mp 180–183 °C, ^{1}H NMR ($CDCl_3$) δ 8.33 (d, 1H, Ar-H), 8.12 (d, 1H, Ar-H), 7.92 (t, 1H, Ar-H), 7.74 (t, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 7.04–6.82 (m, 8H, Ar-H), 5.87 (s, 2H, 7-CH_2), 5.72 (d, 1H, H17), 5.48 (d, 1H, H17), 5.47 (s, 2H, H5), 4.85 (d, 2H, 20-OCH_2CO), 4.61 (s, 2H, 7-OCH_2CO), 2.26 (m, 2H, 18-CH_2), 1.02 (t, 3H, 19-CH_3); **3b**. Yield 62.5%, mp 168–170 °C, ^{1}H NMR ($CDCl_3$) δ 8.21 (d, 1H, Ar-H), 7.99 (d, 1H, Ar-H), 7.78 (t, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.61 (t, 1H, Ar-H), 6.75 (d, 2H, Ar-H), 6.69 (d, 2H, Ar-H), 5.77 (s, 2H, 7-CH_2), 5.69 (d, 1H, H17), 5.40 (s, 2H, H5), 5.30 (d, 1H, H17), 4.66 (d, 2H, OCH_2CO), 4.14 (bs, 1H, 20-OH), 3.68 (s, 3H, OCH_3), 1.89 (m, 2H, 18-CH_2), 1.01 (t, 3H, 19-CH_3); **4b**. Yield 83.3%, mp 202–204° (dec.), ^{1}H NMR ($CDCl_3$) δ 8.26 (d, 1H, Ar-H), 8.08 (d, 1H, Ar-H), 7.86 (t, 1H, Ar-H), 7.69 (t, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 6.89–6.69 (m, 8H, Ar-H), 5.81 (s, 2H, 7-CH_2), 5.67 (d, 1H, H17), 5.45 (s, 2H, H5), 5.43 (d, 1H, H17), 4.78 (d, 2H, 20-OCH_2CO), 4.67 (s, 2H, 7-OCH_2CO), 3.71 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 2.26 (m, 2H, 18-CH_2), 0.97 (t, 3H, 19-CH_3); **3c**. Yield 41.0%, mp 160–162 °C, ^{1}H NMR ($CDCl_3$) δ 8.26 (d, 1H, Ar-H), 8.01 (d, 1H, Ar-H), 7.86 (t, 1H, Ar-H), 7.67 (m, 2H, Ar-H), 6.78 (m, 3H, Ar-H), 5.85 (s, 2H, 7-CH_2), 5.72 (d, 1H, H17), 5.47 (s, 2H, H5), 5.34 (d, 1H, H17), 4.77 (s, 2H, OCH_2CO), 3.82 (s, 1H, 20-OH), 1.90 (m, 2H, 18-CH_2), 1.04 (t, 3H, 19-CH_3); **4c**. Yield 51.0%, mp 223–225 °C, ^{1}H NMR ($CDCl_3$) δ 8.29 (d, 1H, Ar-H), 8.04 (d, 1H, Ar-H), 7.91 (t, 1H, Ar-H), 7.70 (t, 1H, Ar-H), 7.51 (t, 1H, Ar-H), 7.39 (t, 1H, Ar-H), 7.20 (s, 1H, Ar-H), 6.78 (t, 2H, Ar-H), 6.66 (t, 2H, Ar-H), 5.85 (s, 2H, 7-CH_2), 5.67 (d, 1H, H17), 5.48 (s, 2H, H5), 5.44 (d, 1H, H17), 4.91 (q, 2H, OCH_2CO), 4.77 (s, 2H, $COCH_2O$), 2.26 (m, 2H, 18-CH_2), 0.99 (t, 3H, 19-CH_3); **3d**. Yield 54.6%, mp 178–181 °C, ^{1}H NMR ($CDCl_3$) δ 8.21 (d, 1H, Ar-H), 8.00 (d, 1H, Ar-H), 7.80 (t, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.62 (t, 1H, Ar-H), 6.37 (s, 2H, Ar-H), 5.70 (s, 2H, 7-CH_2), 5.69 (d, 1H, H17), 5.44 (s, 2H, H5), 5.31 (d, 1H, H17), 3.78 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3), 3.64 (s, 2H, Ar-CH_2CO), 1.89 (m, 2H, 18-CH_2), 1.02 (t, 3H, 19-CH_3), **4d**. Yield 68.4%, mp 98–100 °C, ^{1}H NMR ($CDCl_3$) δ 8.30 (d, 1H, Ar-H), 8.06 (d, 2H, Ar-H), 7.86 (t, 1H, Ar-H), 7.68 (t, 1H, Ar-H), 7.18 (s, 1H, Ar-H), 6.52 (s, 2H, Ar-H), 6.38 (s, 2H, Ar-H), 6.43 (d, 1H, Ar-H), 5.71 (s, 2H, 7-CH_2), 5.65 (d, 1H, H17), 5.44 (q, 2H, H5), 5.40 (d, 1H, H17), 3.85–3.64 (m, 22H, OCH_3, $ArCH_2CO$), 2.20 (m, 2H, 18-CH_2), 0.99 (t, 3H, 19-CH_3); **5a**. Yield 66.2%, mp 182–184 °C, IR (KBr) ν 3369, 2922, 1751, 1660, 1600, 1504, 1230, 1157, 829, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.33 (s, 1H, Ar-H), 8.22 (d, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.67 (s, 1H, Ar-H), 7.55 (d, 1H, Ar-H), 7.05 (t, 2H, Ar-H), 6.98 (m, 2H, Ar-H), 5.71 (d, 1H, H17), 5.33 (d, 1H, H17), 5.29 (s, 2H, H5), 4.94 (s, 2H, OCH_2CO), 3.91 (s, 1H, 20-OH), 1.90 (m, 2H, 19-CH_2), 0.98 (t, 3H, 18-CH_3); MS (EI): *m*/*z* 516 (M^+), 472, 364, 320, 305, 264, 235, 170, 125, 112, 95; HRMS (EI): *m*/*z* calcd for $C_{28}H_{21}FN_2O_7$ 516.1333, found 516.1331. **6a**. Yield 62.2%, mp 180–182 °C, IR (KBr) ν 3448, 1755, 1664, 1612, 1508, 1192, 1159, 1078, 829, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.37 (s, 1H, Ar-H), 8.28 (s, 1H, Ar-H), 7.77 (s, 1H, Ar-H), 7.61 (s, 1H, Ar-H), 7.15 (s, 1H, Ar-H), 7.05 (s, 2H, Ar-H), 6.99 (m, 4H, Ar-H), 6.60 (d, 2H, Ar-H), 5.64 (d, 1H, H17), 5.42 (d, 1H, H17), 5.29 (s, 2H, H5), 4.95 (s, 2H, $COCH_2O$), 4.81 (d, 2H, OCH_2CO), 2.21 (dm, 2H, CH_2), 0.97 (t, 3H, CH_3); MS (EI): *m*/*z* 668 (M^+), 170, 125, 95, 83; FAB-HRMS: *m*/*z* calcd for $C_{36}H_{26}F_2N_2O_9+H$ 669.1685, found 669.1695. **5b**. Yield 58.1%, mp 103–105 °C, ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 1H, Ar-H), 8.24 (d, 1H, Ar-H), 7.74 (d, 1H, Ar-H), 7.68 (s, 1H, Ar-H), 7.60 (d, 1H, Ar-H), 6.97 (d, 2H, Ar-H), 6.90 (d, 2H, Ar-H), 5.72 (d, 1H, H17), 5.33 (d, 1H, H17), 5.30 (s, 2H, H5), 4.92 (s, 2H, OCH_2CO), 3.80 (s, 3H, OCH_3), 3.64 (bs, 1H, 20-OH), 1.90 (m, 2H, 18-CH_2), 1.04 (t, 3H, 19-CH_3); **6b**. Yield 57.9%, mp 102–104 °C, ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1H, Ar-H), 8.26 (d, 1H, Ar-H), 7.75 (s, 1H, Ar-H), 7.62 (d, 1H, Ar-H), 7.21 (s, 1H, Ar-H), 6.84 (m, 8H, Ar-H), 5.65 (d, 1H, H17), 5.43 (d, 1H, H17), 5.25 (s, 2H, H5), 4.92 (s, 2H, OCH_2CO), 4.78 (d, 2H, OCH_2CO), 3.78 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 2.26 (m, 2H, 18-CH_2), 0.97 (t, 3H, 19-CH_3).