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SYNTHESIS OF A NEW CLASS OF CAMPTOTHECIN DERIVATIVES, THE LONG-CHAIN FATTY ACID ESTERS OF 10-HYDROXYCAMPTOTHECIN, AS A POTENT PRODRUG CANDIDATE, AND THEIR *IN VITRO* METABOLIC CONVERSION BY CARBOXYLESTERASES

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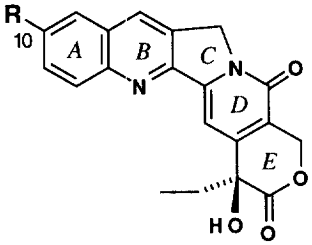
Abstract Five (20*S*)-10-hydroxycamptothecin derivatives carrying the long-chain fatty acid esters were prepared for the development of a new class of prodrug-type agents. *In vitro* experiments using three kinds of purified carboxylesterase isozymes from the liver microsomes of rat, pig, and human demonstrated that these derivatives were efficiently metabolized by enzymes compared with CPT-11.

Camptothecin derivatives such as topotecan[1] and irinotecan (CPT-11)[2] began to be used as clinical antitumor agents, which possessed a novel mechanism of action involving the inhibition of DNA topoisomerase I. CPT-11, a carbamate derivative of 10-hydroxy-7-ethylcamptothecin, can be classified into the category of prodrug, because we and other investigators have found the conversion of CPT-11 to its active metabolite, the 10-hydroxy derivatives, by liver carboxylesterase in various mammals and humans.[3] Other prodrug approaches, which utilize the 20-hydroxy group in camptothecin, have recently been examined.[4] During our chemical investigation of camptothecin (**1**) and its related molecules,[5] we planned the synthesis of a new class of prodrug-type 10-hydroxycamptothecin derivatives, which serve as test samples for the development of a potent anticancer chemotherapeutic agent. The biological evaluation of 10-hydroxycamptothecin, the active principle of the prodrug, has been studied.[1a, 6] In this communication, we describe the synthesis of (20*S*)-10-hydroxycamptothecin derivatives (**3**-**7**) carrying the long-chain fatty acid esters in the molecules as well as the preliminary *in vitro* evaluation of the carboxylesterase activities for a series of these novel compounds.



R=H; camptothecin **1**
R=OH; **2**
R=OCO$(CH_2)_8CH_3$; **3**
R=OCO$(CH_2)_{10}CH_3$; **4**
R=OCO$(CH_2)_{14}CH_3$; **5**
R=OCO$(CH_2)_{16}CH_3$; **6**
R=OCO$(CH_2)_{18}CH_3$; **7**

The Friedlaender condensation strategy was adopted for the construction of the target molecules (**3**-**7**) in the final stage of the synthesis. Initially, the 5-acyloxy-anthranilaldehyde derivatives (**15**-**19**), corresponding to the A-ring

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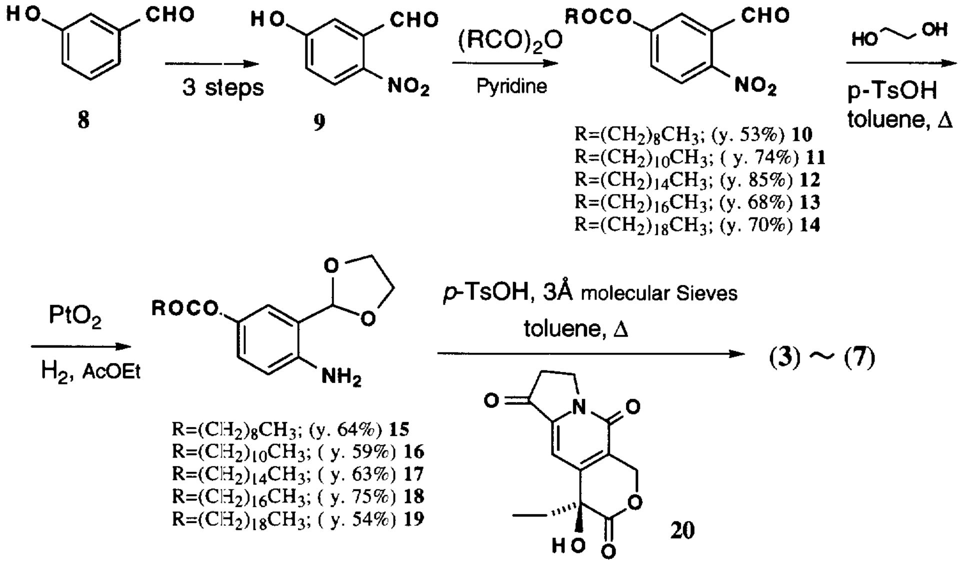
moiety in the compounds (**3**-**7**), were prepared from the commercially available *m*-hydroxybenzaldehyde (**8**) as follows. Nitration of the ethoxycarbonyl derivative of **8** with conc. HNO_3/H_2SO_4 followed by alkaline hydrolysis gave 5-hydroxy-2-nitrobenzaldehyde (**9**) in 43% overall yield from **8**,[7] which was then acylated with five kinds of fatty acid anhydride, *i.e.*, capric-, lauric-, palmitic-, stearic-, and arachidic-anhydride, to give the corresponding esters (**10**-**14**) in good to moderate yields. After the aldehyde function in **10**-**14** was respectively protected as ethyleneacetal, the nitro group was reduced by catalytic hydrogenation (H_2, PtO_2) to afford the primary amines (**15**-**19**), which were one of two fragments for the Friedlaender condensation, while a chiral tricyclic ketone (**20**), the CDE-ring system in (**3**-**7**), was prepared according to the method developed by Ejima *et al.*[8] Finally, heating of **20** with each amines (**15**-**19**) in the presence of a catalytic amount of *p*-TsOH in refluxing toluene furnished the desired 10-acyloxycamptothecin derivatives (**3**-**7**) in good yield. The structures of these new compounds were fully characterized by physico- and spectroscopic analyses.[9]



The purification and characterization of the carboxylesterase isozymes were done as previously described.[3] Three carboxylesterase isozymes were examined for the ability of hydrolyzing the prodrug of the 10-hydroxycamptothecin derivatives (**3**-**7**). The purified carboxylesterase isozymes used in this study were rat RH1, pig P1 and human HU1. Incubation of the carboxylesterase isozymes with the 10-hydroxycamptothecin derivatives (**3**-**7**) was carried out in 30mM HEPES buffer (pH7.4) at 37°C. A stock solution of each 5mM 10-hydroxycamptothecin derivative (**3**-**7**) was dissolved in dimethyl sulfoxide. The reaction mixture consisting of 100µl of 60mM HEPES buffer (pH7.4), 2µl of stock solution of each substrate, 10µl of the enzyme solution at a suitable concentration, and 88µl of distilled water was incubated for 60min at 37°C. After incubation, the reaction was stopped by the addition of 200µl of MeOH with 7-ethyl-10-hydroxycamptothecin (SN-38) as the internal standard. Following incubation, 30 µl of the mixture was injected into a high-performance liquid chromatography

(HPLC) system (Hitachi L-6000 system). The enzymatic product, (20*S*)-10-hydroxycamptothecin and internal standard was separated using a reverse-phase column (YMC A-402, 4.6ϕ x 15cm) and detected by a fluorospectrometer (Jasco 821-FP intelligent spectrofluorometer) set at an excitation wavelength of 380 nm and an emission wavelength of 556 nm. The mobile phase consisted of acetonitrile-1%acetic acid (25:75, v/v).

Table 1 Hydrolysis of (20*S*)-10-hydroxycamptothecin derivatives by purified carboxylesterase isozymes from liver microsomes of rat, pig and human

		Specific activity (nmol/mg/min)					
Species	Isozymes	caploxy- (**3**)	lauroxy- (**4**)	palmitoyloxy- (**5**)	stearoxy- (**6**)	arachidiroxy- (**7**)	CPT-11[3]
Rat	RH1	17.4	19.1	2.33	0.698	0.183	0.259
Pig	P1	37.4	42.1	9.13	3.02	0.854	2.147
Human	HU1	27.9	15.3	1.58	1.27	1.07	0.129

Since carboxylesterases are important for the metabolic activation of prodrugs and detoxification of xenobiotics,[3] differences in substrate specificity of these enzymes are important for the design of prodrugs. Table 1 shows the hydrolytic activity towards five kinds of 10-hydroxycamptothecin derivatives and CPT-11. Pig carboxylesterase (P1) was found to have the highest specific activity towards these camptothecin derivatives, except arachidiroxy-20(S)-camptothecin (**7**). Of all the 10-hydroxycamptothecin derivatives, 10-caproxy-, 10-lauroxy-, and 10-palmitoyloxy-20(S)-camptothecin proved to be more effective prodrugs than CPT-11. Human carboxylesterase (HU1) possesses higher hydrolytic activities towards all the 10-hydroxycamptothecin derivatives than CPT-11. These results suggest that the long-chain fatty acid esters of 10-hydroxycamptothecin can be useful prodrugs for anticancer chemotherapeutic agents. Furthermore, the structure activity relationship in the metabolic activation of prodrugs by carboxylesterases of mammals and human could be clarified in this experiment.

In conclusion, the findings in the present study suggest that a new class of prodrug type 10-hydroxycamptothecin derivatives are efficiently converted *in vitro* by the liver carboxylesterase of mammals and humans. These observations should be generally useful in the design of new ester-type prodrugs. Further studies are in progress in our laboratories to clarify the *in vivo* metabolism of these 10-hydroxycamptothecin derivatives.

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9. **3:** 46% yield, yellow prisms, mp 212-215°C (5%MeOH/$CHCl_3$-AcOEt). EI-MS m/z : 518 (M^+). ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.32 (1H, s), 8.24 (1H, d, *J*=9.2 Hz), 7.68 (1H, d, *J*=2.4 Hz), 7.66 (1H, s), 7.57 (1H, dd, *J*=9.2 and 2.6 Hz), 5.75, 5.31 (2H, ABq, *J*=16.2 Hz), 5.29 (2H, s), 3.78 (1H, br), 2.65 (2H, t, *J*=7.5 Hz), 1.96-1.84 (2H, m), 1.81 (2H, quint, *J*=7.5 Hz), 1.46-1.29 (12H, m), 1.05 (3H, t, *J*=7.5 Hz), 0.89 (3H, t, *J*=7.0 Hz). **4:** 53% yield, yellow prisms, mp 214-217°C (5%MeOH/$CHCl_3$-AcOEt). EI-MS m/z : 546 (M^+). ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.33 (1H, s), 8.24 (1H, d, *J*=9.0 Hz), 7.68 (1H, d, *J*=2.4 Hz), 7.66 (1H, s), 7.57 (1H, dd, *J*=9.2 and 2.5 Hz), 5.75, 5.31 (2H, ABq *J*=16.2 Hz), 5.30 (2H, s), 3.76 (1H, br), 2.65 (2H, t, *J*=7.5 Hz), 1.96-1.84 (2H, m), 1.81 (2H, quint, *J*=7.5 Hz), 1.46-1.28 (16H, m), 1.05 (3H, t, *J*=7.5 Hz), 0.89 (3H, t, *J*=6.8 Hz). **5:** 59% yield, yellow prisms, mp 214-216°C (5%MeOH/$CHCl_3$-AcOEt). EI-MS m/z : 602 (M^+). ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.34 (1H, s), 8.24 (1H, d, *J*=9.0 Hz), 7.69 (1H, d, *J*=2.5 Hz), 7.67 (1H, s), 7.57 (1H, dd, *J*=9.3 and 2.5 Hz), 5.76, 5.31 (2H, ABq, *J*=16.3 Hz), 5.30 (2H, s), 3.72 (1H, br), 2.65 (2H, t, *J*=7.5 Hz), 1.96-1.86 (2H, m), 1.81 (2H, quint, *J*=7.5 Hz), 1.46-1.26 (24H, m), 1.10 (3H, t, *J*=7.1 Hz), 0.88 (3H, t, *J*=6.8 Hz). **6:** 78% yield, yellow prisms, mp 212-215°C (5%MeOH/$CHCl_3$-AcOEt). EI-MS m/z : 630 (M^+). ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.34 (1H, s), 8.24 (1H, d, *J*=9.0 Hz), 7.69 (1H, d, *J*=2.5 Hz), 7.67 (1H, s), 7.57 (1H, dd, *J*=9.3 and 2.5 Hz), 5.76, 5.31 (2H, ABq, *J*=16.3 Hz), 5.30 (2H, s), 3.72 (1H, br), 2.65 (2H, t, *J*=7.5 Hz), 1.96-1.86 (2H, m), 1.81 (2H, quint, *J*=7.5 Hz), 1.46-1.26 (28H, m), 1.10 (3H, t, *J*=7.1 Hz), 0.88 (3H, t, *J*=6.8 Hz). **7:** 60% yield, yellow prisms, mp 214-217°C (5%MeOH/$CHCl_3$-AcOEt). EI-MS m/z : 658 (M^+). ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.33 (1H, s), 8.24 (1H, d, *J*=9.2 Hz), 7.68 (1H, d, *J*=2.6 Hz), 7.66 (1H, s), 7.57 (1H, dd, *J*=9.1 and 2.6 Hz), 5.75, 5.31 (2H, ABq, *J*=16.2 Hz), 5.30 (2H, s), 3.74 (1H, br), 2.65 (2H, t, *J*=7.5 Hz), 1.96-1.84 (2H, m), 1.81 (2H, quint, *J*=7.4 Hz), 1.46-1.26 (32H, m), 1.05 (3H, t, *J*=7.5 Hz), 0.88 (3H, t, *J*=7.0 Hz).