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Short communication

Alkyl and carboxylalkyl esters of 4′-demethyl-4-deoxypodophyllotoxin: synthesis, cytotoxic, and antitumor activity

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Abstract

Esters of 4′-demethyl-4-deoxypodophyllotoxin (DDPT) with alkanoic acids and alkanedioic acids were prepared and tested for cytotoxic and antitumor activity. Among 19 esters, esters of propanoic acid, tetradecanedioic acid, 13-carboxyundecanoic acid, and hexadecanedioic acid improved the antitumor activity compared with that of the starting compounds, DDPT.

Keywords: Deoxypodophyllotoxin; Esters; Cytotoxicity; Antitumor activity

1. Introduction

4-Deoxypodophyllotoxin (DPT) is a potent antimitotic agent first isolated from *Anthriscus sylvestris* [1]. It was shown to exhibit potent cytotoxicity against a wide variety of tumor cell lines [2,3]. Recently, we reported the presence of DPT in *Pulsatilla koreana*, one indigenous plant in Korea, and demonstrated that this antimitotic agent completely inhibited the tube-like formation of human umbilical venous endothelial cells (HUVEC) at a concentration considerably below the cytotoxic activity. Moreover, DPT also exhibited significant antitumor activity in BDF1 mice bearing murine Lewis lung carcinoma (3LL) cells with inhibition rate (IR) of 59% [4].

Previously, it was reported that 4′-demethyl-4-deoxypodophyllotoxin (DDPT) exerted a comparable in vitro potency as DPT [5]. In vivo experiments revealed a substantial loss of the antitumor activity of DDPT in the BDF_1/3LL model. We assumed that the free hydroxy group at the 4′ position in DDPT was not favorable for antitumor activity (Fig. 1). Indeed, in previous studies, we notified that the esterification of the phenolic hydroxy group with carbamic, carbonic and amino acids increased the activity [6]. Now we would like to report the synthesis of esters of alkanoic acids and alkanedioic acids as alternative protecting groups and tested their cytotoxic and antitumor activity.

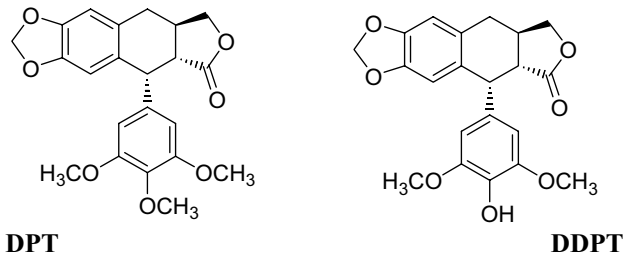

Fig. 1. Structures of DPT and of DDPT.

2. Chemistry

The starting material, DPT, was isolated from *A. sylvestris* (1.6 g DPT from 2 kg dried roots of *A. sylvestris*). 4′ Methoxy group of DPT was selectively demethylated with trimethylsilyl iodide (yield, 72%), which afforded DDPT (Fig. 2). Then, DDPT was esterified with various organic acids using DCC and DMAP (yield, 70–91%). The products were confirmed by the triplet peaks of α-methylene group at around 2.5 ppm (–$OCOCH_2C$–) on ^{1}H NMR. Esters with dicarboxylic acids were prepared by the similar methods (yield, 60–70%).

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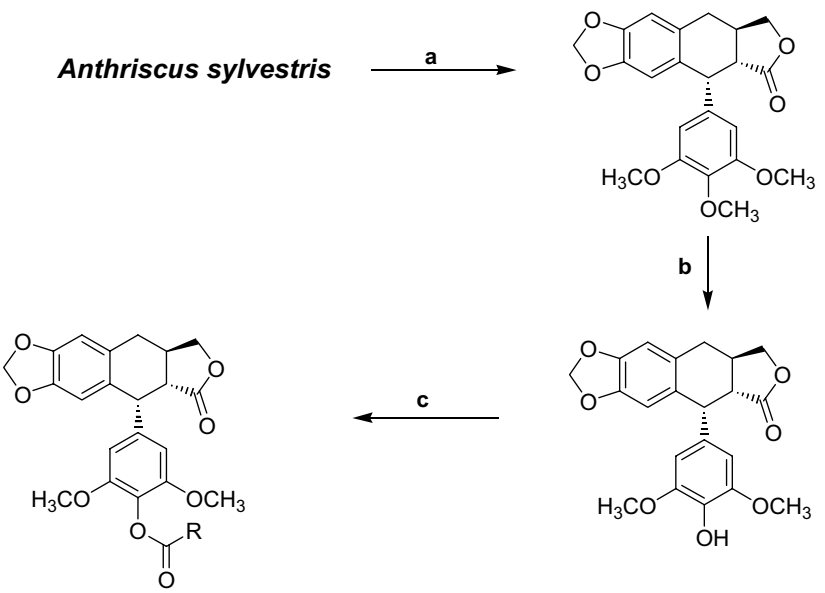

Fig. 2. (a) MeOH, 5 h, 3 times then silica gel column, cyclohexanes/EA (5:1); (b) TMSI, CH_2Cl_2, 0 °C, 5 h then $BaCO_3$, 30 min; (c) DCC, DMAP, CH_2Cl_2, 0 °C, 1 h.

3. Biological results and discussion

Biological activities are reported in Tables 1 and 2. For A549 cells, cytotoxic activity was dependent on chain length of the alkyl moiety; the shorter were the alkyl chains, the stronger were the cytotoxicities. Esters with acetic to octanoic acid (DOE1–DOE6; ED_{50}, 0.003–0.013 μg/ml) were more active than DDPT (ED_{50}, 0.023 μg/ml), while esters of longer alkanoic acids than hexadecanoic acid (DOE10–DOE13) showed weaker activity (ED_{50}, >2.670 μg/ml). A similar tendency was observed against SK-MEL-2 cells. Interestingly, among the 13 esters, nine showed higher cytotoxic activity on A549 than on SK-MEL-2 cells. Different from alkyl esters, esters with dicarboxylic acids seemed to have an optimal chain length at least on A549 cells: esters with sebacic acid to tetradecanedioic acid (DODE3–DODE5) having stronger activity (ED_{50}, 0.014–0.046 μg/ml) than shorter or longer dioic acids.

In animal tests, unlike cytotoxic activity, esters with shorter alkanoic acid showed weak antitumor activity except DOE2 (Table 1). On the other hand, medium chain length acids (DOE5–DOE10) (IR, 36–72%) were better for antitumor activity. In particular, antitumor activity of DOE5 (IR, 72%) was comparable with the positive control, etoposide (VP-16) (IR, 68%) at single injection (Fig. 3). This implies that DOE5 is pharmacokinetically beneficial because it re-

Table 1
Cytotoxicities and antitumor activities of 4′-*O*-alkanoyl-DDPTs (DOE1–DOE13)

Compounds	R	ED_{50} (μg/ml)		IR [a] (%)	Injection schedule
		A-549	SK-MEL-2		
DOE1	Acetyl	0.003	0.006	11	d1
DOE2	Propanoyl	0.004	0.010	70	d1, d8
DOE3	Butanoyl	0.005	0.017	21	d1, d9
DOE4	3-Methylbutanoyl	0.006	0.089	25	d1
DOE5	Heptanoyl	0.013	0.078	72	d1
DOE6	Octanoyl	0.009	0.030	45	d1
DOE7	Decanoyl	0.027	0.004	63	d1
DOE8	Dodecanoyl	0.030	0.025	57	d1
DOE9	Tetradecanoyl	0.041	0.050	36	d1, d8
DOE10	Hexadecanoyl	0.268	0.290	40	d1, d5
DOE11	Octadecanoyl	2.670	>5	–2	d1, d5, d9
DOE12	Eicosanoyl	>5	>5	11	d1, d5, d9
DOE13	Docosanoyl	>5	>5	–8	d1, d5, d9
	DPT	0.012	0.009	NT [b]	
	DDPT	0.023	0.015	<10	d1, d5, d9
	Etoposide (VP-16) [c]	1.102	NT [b]	68	d1, d5, d9

[a] Inhibition rate with a dose of 60 mg/kg except VP-16.
[b] Not tested.
[c] VP-16 was treated at 36 mg/kg/day.

Table 2
Cytotoxicities and antitumor activities of 4′-*O*-carboxyalkanoyl-DDPTs (DODE1–DODE6)

Compounds	R	ED_{50} (μg/ml)		IR [a] (%)	Injection schedule
		A-549	SK-MEL-2		
DODE1	5-Carboxypentanoyl	0.258	0.062	50	d1, d5, d9
DODE2	7-Carboxyheptanoyl	0.175	0.066	30	d1, d5, d9
DODE3	9-Carboxynonanoyl	0.014	0.015	36	d1, d5, d9
DODE4	11-Carboxydodecanoyl	0.046	0.068	25	d1, d5, d9
DODE5	13-Carboxyundecanoyl	0.019	0.070	86	d1, d5, d9
DODE6	15-Carboxypentadecanoyl (hexadecanedioic)	0.106	0.027	72	d1, d5, d9
	DPT	0.012	0.009	NT [b]	
	DDPT	0.023	0.015	<10	d1, d5, d9
	Etoposide (VP-16) [c]	1.102	NT [b]	84	d1, d5, d9

[a] Inhibition rate with a dose of 60 mg/kg except VP-16.
[b] Not tested.
[c] VP-16 was treated at 36 mg/kg/day.

mains in the body for a longer amount of time than VP-16. Among esters with alkanedioic acids, DODE5 (IR, 86%) showed the most potent antitumor activity which is comparable to VP-16 (IR, 84%) in a repeated animal test (Table 2). More interesting is the pattern of tumor growth treated with DODE5. As shown in Fig. 4, the antitumor activity of DODE5 showed a plateau from the day 7 at around 0.3 cm^{-3} of tumor volume crossing the tumor volume treated with VP-16 at the day 11. Considering the dosing frequency of DOE5 and DODE5, it was assumed that the activities of two compounds come from a different mechanism or pharmacokinetic profile.

In conclusion, introducing ester group, we successfully increased the in vivo antitumor activity of DDPT. Especially,

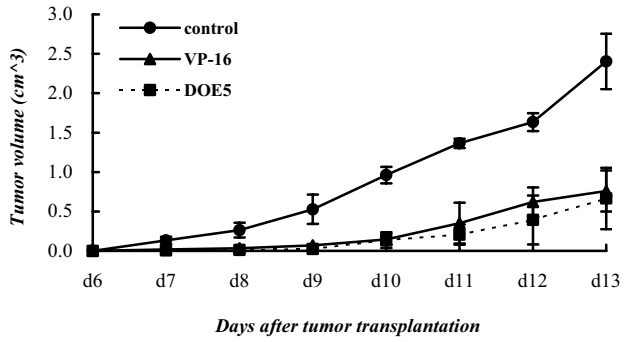

Fig. 3. Inhibition of tumor growth in BDF_1 mice by 4′-*O*-heptanoyl-DDPT (DOE5).

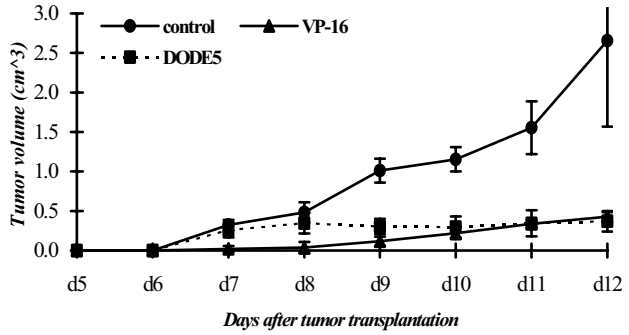

Fig. 4. Inhibition of tumor growth in BDF_1 mice by 4′-*O*-(13-carboxyundecanoyl)-DDPT (DODE5).

four esters of propanoic acid, tetradecanedioic acid, 13-carboxyundecanoic acid, and hexadecanedioic acid showed a promising antitumor activity.

4. Experimental protocol

The ^1H NMR spectra were recorded on a JEOL EX-90 (90 MHz) NMR spectrometer, and proton chemical shifts are relative to tetramethylsilane as an internal standard in $CDCl_3$. Mass spectra (MS) were collected on a Finnigan LCQ Advantage mass spectrometer using ion-trap MS with electrospray ionisation. All fractions from column chromatography (EM Kiesegel 60, 230–400 mesh) were monitored by thin layer chromatography (silica gel 60 GF-254, Merck, 230–400 mesh ASTM). All reagents, commercially available, were used without further purification unless otherwise stated.

4.1. Isolation of deoxypodophyllotoxin

Air-dried *A. sylvestris* (roots, 2 kg) was extracted with 5 l of ethyl acetate three times. The ethyl acetate extract was chromatographed over a silica gel column using cyclohexane/ethyl acetate (5:1) as an eluent to afford the crude DPT fraction. Recrystallization of the resulting crude fraction from MeOH afforded DPT (**1**, 4.2 g), which was confirmed by direct comparison with an authentic sample isolated from *P. koreana* and spectral data reported previously [4]. DPT obtained as such was used as a starting material for subsequent syntheses of the prodrugs.

4.2. Synthesis

4.2.1. Synthesis of 4′-demethyl-4-deoxypodophyllotoxin (DDPT)

The demethylation of DPT was performed under presence of trimethylsilyl iodide according to the method in a reference [7].

4.2.2. General procedures for the synthesis of esters of DDPT

The mixture of acid (1.2 mmole), 1,3-dicyclohexylcarbodiimide (1.2 mmole), 4-dimethylaminopyridine (0.4 mmole), and DDPT (1 mmole) in dried dichloromethane (20 ml) were stirred for 2 h at 0 °C. The resulting suspension was filtered, and distilled water (100 ml) was added. After extracting with dichloromethane (100 ml) three times, the organic layer was dried and concentrated to give the crude product, which then was purified by silica gel column with a mixture of cyclohexane and ethyl acetate (4:1).

*4.2.2.1. 4′-Demethyl-4′-*O*-acetyl-4-deoxypodophyllotoxin (DOE1).* Amorphous powder (85%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.49 (1H, s), 6.37 (2H, s), 5.91(2H, br s), 4.61(1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.29(3H, s); MS: $[M + H]^+$ 427.2 (calc. MS 426.1).

*4.2.2.2. 4′-Demethyl-4′-*O*-propanoyl-4-deoxypodophyllotoxin (DOE2).* Amorphous powder (83%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.49 (1H, s), 6.37 (2H, s), 5.91(2H, br s), 4.61(1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.27 (2H, q, J = 7.7 Hz), 1.24 (3H, t, J = 7.7 Hz); MS: $[M + H]^+$ 440.1 (calc. MS 441.2).

*4.2.2.3. 4′-Demethyl-4′-*O*-butanoyl-4-deoxypodophyllotoxin (DOE3).* Amorphous powder (90%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.49 (1H, s), 6.37 (2H, s), 5.91(2H, br s), 4.61(1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.55(2H, t, J = 7.4 Hz), 1.77 (2H, m), 1.02 (3H, t, J = 7.4 Hz); MS: $[M + H]^+$ 454.2 (calc. MS 455.3).

*4.2.2.4. 4′-Demethyl-4′-*O*-(3-methylbutanoyl)-4-deoxypodophyllotoxin (DOE4).* Amorphous powder (88%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.49 (1H, s), 6.37 (2H, s), 5.91(2H, br s), 4.61(1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.24 (2H, d, J = 5.7 Hz), 2.10–2.22 (1H, m), 1.06 (3H, s), 0.99 (3H, s); MS: $[M + H]^+$ 469.2 (calc. MS 468.2).

*4.2.2.5. 4′-Demethyl-4′-*O*-heptanoyl-4-deoxypodophyllotoxin (DOE5).* Amorphous powder (92%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.49 (1H, s), 6.37 (2H, s), 5.91 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.55 (2H, t, J = 7.1 Hz), 1.55–1.72 (2H, m), 1.30 (4H, m), 0.88 (3H, t, J = 6.0 Hz); MS: $[M + H]^+$ 497.3 (calc. MS 496.2).

*4.2.2.6. 4′-Demethyl-4′-*O*-octanoyl-4-deoxypodophyllotoxin (DOE6).* Amorphous powder (85%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.49 (1H, s), 6.37 (2H, s), 5.91 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.56 (2H, t, J = 7.4 Hz), 1.56–1.73 (2H, m), 1.30 (m, 8H), 0.87 (3H, t, J = 3.4 Hz); MS: $[M + H]^+$ 511.2 (calc. MS 510.2).

*4.2.2.7. 4′-Demethyl-4′-*O*-decanoyl-4-deoxypodophyllotoxin (DOE7).* Amorphous powder (87%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.49 (1H, s), 6.37 (2H, s), 5.91 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.56 (2H, t, J = 7.3 Hz), 1.55–1.72 (2H, m), 1.23 (12H, m), 0.92 (3H, t, J = 5.4 Hz); MS: $[M + H]^+$ 539.3 (calc. MS 538.3).

*4.2.2.8. 4′-Demethyl-4′-*O*-dodecanoyl-4-deoxypodophyllotoxin (DOE8).* Amorphous powder (91%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.51 (1H, s), 6.37 (2H, s), 5.91 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.56 (2H, t, J = 7.3 Hz), 1.55–1.72 (2H, m), 1.23 (16H, m), 0.88 (3H, t, J = 5.4 Hz); MS: $[M + H]^+$ 567.3 (calc. MS 566.3).

*4.2.2.9. 4′-Demethyl-4′-*O*-tetradecanoyl-4-deoxypodophyllotoxin (DOE9).* Amorphous powder (90%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.66 (1H, s), 6.53 (1H, s), 6.38 (2H, s), 5.91 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.22–2.54 (4H, m), 2.56 (2H, t, J = 7.3 Hz), 1.55–1.72 (2H, m), 1.25 (20H, m), 0.88 (3H, t, J = 5.4 Hz); MS: $[M + H]^+$ 595.3 (calc. MS 594.3).

*4.2.2.10. 4′-Demethyl-4′-*O*-hexadecanoyl-4-deoxypodophyllotoxin (DOE10).* Amorphous powder (85%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.63 (1H, s), 6.50 (1H, s), 6.37 (2H, s), 5.91 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.67 (6H, s), 3.22–2.54 (4H, m), 2.56 (2H, t, J = 7.3 Hz), 1.55–1.72 (2H, m), 1.25 (24H, m), 0.87 (3H, t, J = 5.4 Hz); MS: $[M + H]^+$ 623.3 (calc. MS 622.4).

*4.2.2.11. 4′-Demethyl-4′-*O*-octadecanoyl-4-deoxypodophyllotoxin (DOE11).* Amorphous powder (78%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.66 (1H, s), 6.52 (1H, s), 6.37 (2H, s), 5.91 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.68 (6H, s), 3.22–2.54 (4H, m), 2.57(2H, t, J = 7.3 Hz), 1.55–1.72 (2H, m), 1.25 (28H, m), 0.87 (3H, t, J = 5.4 Hz); MS: $[M + H]^+$ 651.3 (calc. MS 650.4).

*4.2.2.12. 4′-Demethyl-4′-*O*-eicosanoyl-4-deoxypodophyllotoxin (DOE12).* Amorphous powder (79%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.64 (1H, s), 6.50 (1H, s), 6.38 (2H, s), 5.90 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.67 (6H, s), 3.22–2.54 (4H, m), 2.56 (2H, t, J = 7.3 Hz), 1.55–1.72 (2H, m), 1.26 (32H, m), 0.87 (3H, t, J = 5.4 Hz); MS: $[M + H]^+$ 679.3 (calc. MS 678.4).

*4.2.2.13. 4′-Demethyl-4′-*O*-docosanoyl-4-deoxypodophyllotoxin (DOE13).* Amorphous powder (79%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.66 (1H, s), 6.52 (1H, s), 6.38 (2H, s), 5.93 (2H, br s), 4.61 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04

(1H, m), 3.68 (6H, s), 3.22–2.54 (4H, m), 2.57 (2H, t, J = 7.3 Hz), 1.55–1.72 (2H, m), 1.00–1.4 (36H, m), 0.88 (3H, t, J = 5.4 Hz); MS: $[M + H]^+$ 513.3 (calc. MS 512.2).

4.2.2.14. 4′-Demethyl-4′-O-(5-carboxypentanoyl)deoxypodophyllotoxin (DODE1). Amorphous powder (70%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.51 (1H, s), 6.36 (2H, s), 5.93 (2H, br s), 4.63 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.67 (6H, s), 3.02–2.5 (6H, m), 2.30–2.50 (2H, m), 1.70–1.90 (4H, m); MS: $[M + H]^+$ 541.2 (calc. MS 540.2).

4.2.2.15. 4′-Demethyl-4′-O-(7-carboxyheptanoyl)-4-deoxypodophyllotoxin (DODE2). Amorphous powder (65%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.50 (1H, s), 6.36 (2H, s), 5.92 (2H, br s), 4.59 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.67 (6H, s), 3.02–2.50 (4H, m), 2.56 (2H, t, J = 7.1 Hz), 2.33 (2H, t, J = 7.1 Hz), 1.55–1.80 (2H, m), 1.31 (8H, m); MS: $[M + H]^+$ 569.2 (calc. MS 568.2).

4.2.2.16. 4′-Demethyl-4′-O-(9-carboxynonanoyl)-4-deoxypodophyllotoxin (DODE3). Amorphous powder (63%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.65 (1H, s), 6.51 (1H, s), 6.36 (2H, s), 5.92 (2H, br s), 4.59 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.67 (6H, s), 3.02–2.50 (4H, m), 2.56 (2H, t, J = 7.1 Hz), 2.33 (2H, t, J = 7.1 Hz), 1.55–1.80 (2H, m), 1.31 (12H, m); MS: $[M + H]^+$ 597.3 (calc. MS 596.3).

4.2.2.17.4′-Demethyl-4′-O-(11-carboxyundecanoyl)-4-deoxypodophyllotoxin (DODE4). Amorphous powder (62%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.66 (1H, s), 6.52 (1H, s), 6.37 (2H, s), 5.92 (2H, br s), 4.60 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.66 (6H, s), 3.02–2.50 (4H, m), 2.57 (2H, t, J = 7.1 Hz), 2.34 (2H, t, J = 7.1 Hz), 1.55–1.80 (2H, m), 1.31 (16H, m); MS: $[M + H]^+$ 624.3 (calc. MS 625.3).

4.2.2.18. 4′-Demethyl-4′-O-(13-carboxytridecanoyl)-4-deoxypodophyllotoxin (DODE5). Amorphous powder (60%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.66 (1H, s), 6.52 (1H, s), 6.38 (2H, s), 5.93 (2H, br s), 4.60 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.69 (6H, s), 3.02–2.50 (4H, m), 2.57 (2H, t, J =7.1 Hz), 2.34 (2H, t, J = 7.1 Hz), 1.55–1.80 (2H, m), 1.31 (20H, m); MS: $[M + H]^+$ 653.4 (calc. MS 652.3).

4.2.2.19. 4′-Demethyl-4′-O-(15-carboxyheptadecanoyl)-4-deoxypodophyllotoxin (DODE6). Amorphous powder (63%); ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.66 (1H, s), 6.52 (1H, s), 6.37 (2H, s), 5.93 (2H, br s), 4.63 (1H, br s), 4.56–4.32 (1H, m), 3.72–4.04 (1H, m), 3.68 (6H, s), 3.02–2.50 (4H, m), 2.57 (2H, t, J =7.1 Hz), 2.33 (2H, t, J =7.1 Hz), 1.55–1.80 (2H, m), 1.31 (24H, m); MS: $[M + H]^+$ 681.3 (calc. MS 680.4).

4.2.3. Cytotoxicity

Tumor cells were maintained in plastic dishes in a RPMI-1640 medium supplemented with 10% fetal bovine serum. On day 0, 180 μl of a tumor cell suspension (3×10^4 cells/ml in culture medium) were seeded in each well of 96-well plates. The plates were incubated in a 5% CO_2 incubator at 37 °C for 24 h and then samples in a 20 μl culture medium were added at various concentrations. The plates were incubated for another 48 h. Cytotoxicity was measured by SRB method as described in literature [8] with slight modifications [4,9]. The values shown for these compounds are averages of three determinations.

4.2.4. Antitumor experiments

Antitumor experiments were carried out as described in our previous reports [4,9]. Briefly, 3LL cells were inoculated subcutaneously into BDF_1 mice on the day 0 (1×10^7 cells/mouse/0.2 ml PBS). Esters were dissolved in a medium comprising of 5% DMSO and 20% Cremophor® to make a concentration of 6 mg/ml. 0.2 ml of the solution was intraperitoneally injected per mouse (20 g) corresponding to a dose of 60 mg/kg/day, and the injection schedules were adjusted by the change of body weight as in Tables 1 and 2. Body weights of mice were tracked every day and tumor sizes were measured with calipers from day 9. Tumor volumes were calculated by the following equation: tumor volume (mm^3) = (length (mm) × width $(mm)^2$)/2. The inhibition rate was evaluated as $(1–T/C) \times 100\%$ (where T is the mean tumor volume of the treated group and C is the mean tumor volume of the control group). Each group consisted of six mice. Etoposide was administered at day 1, 5, and 9 at 36 mg/kg/day.

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