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Antitumor Activity of Unsaturated Fatty Acid Esters of 4′-Demethyldeoxypodophyllotoxin

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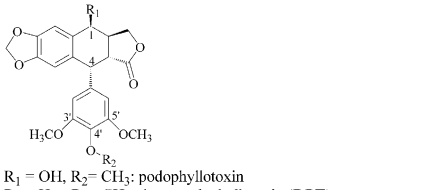
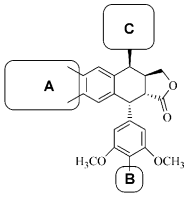
Abstract—Unsaturated fatty acid esters of 4′-demethyldeoxypodophyllotoxin (DDPT) were prepared and tested for antitumor activity. The esters showed increased in vivo antitumor activity despite the lower in vitro activity than DDPT. Especially, the ester (DFE12) of all-*cis*-11,14-eicosadienoic acid was much better (IR, 83%) than VP-16 (IR, 60%) without loss of body weight. Unsaturated fatty acids could be evaluated to be good carrier vehicles of DDPT.

Podophyllotoxin, natural cyclolignan, shows strong cytotoxic activity on cancer cell lines,[1] and its derivatives, etoposide (VP-16) and teniposide (VM-26), are currently applied for small-cell lung cancer,[2] testicular carcinoma,[3] non-Hodgkin's lymphoma,[4] and Kaposi's sarcoma.[5] The cytotoxic mechanims of podophyllotoxin derivatives are known as inhibitors of both tubulin polymerization and DNA topoisomerase-II.[6,7] Of the two, DNA topoisomerase II inhibitory effect contributes to an antitumor activity in vivo.[6] Structure activity relationship for DNA topoisomerase-II inhibition has been disclosed: portion A intercalates between DNA double helix; portions B and C bind miner groove; portion C is a variable site.[8] Phenolic hydroxy group at 4′ is essential for topoisomerase-II inhibition.[6] Thus, the studies have been mainly focused on the variation of portion C.



R_1 = OH, R_2= CH_3: podophyllotoxin
R_1 = H, R_2= CH_3: deoxypodophyllotoxin (**DPT**)
R_1= H, R_2= H : 4′-demethyldeoxypodophyllotoxin (**DDPT**)



Among deoxypodophyllotoxin (DPT) derivatives, 4′-demethyldeoxypodophyllotoxin (DDPT) attracted our attention due to its bioactivity profiles: it does not show in vivo activity in spite of strong cytotoxicity on S-180 cells (IC_{50}, 0.03 μM) based on the inhibitory activities on tubulin polimerization (IC_{50}, 2.0 μM) and DNA topoisomerase II (IC_{50}, 24.5 μM).[6] In our previous studies, some of carbamates, carbonates, esters on portion B of DDPT expressed better in vivo activity, which partially owed to the delay of metabolic excretion.[9,10]

Unsaturated fatty acids are biologically active and used as carrier vehicles for some drugs. Certain unsaturated fatty acids showed antitumor activities both in vitro and in vivo: linoleic and linolenic acids inhibited proliferation of cancer cells and oleic, linoleic and palmitoleic acids prolonged the life spans of Ehrlich ascites carcinoma-bearing mice.[11–13] More interestingly, some of natural fatty acids are taken up avidly by tumors for use as biochemical precursors and energy sources. These properties were successfully applied for selective delivery of parclitaxel to tumor tissue by conjugation of paclitaxel with DHA.[14]

On the basis of these facts, esters of DDPT with various unsaturated fatty acids were prepared and tested for antitumor activity with the hope that the esterification with the fatty acids would delay the metabolic excretion and increase the tumor selectivity (Fig. 1).

Starting material, DPT, was isolated from *Anthricus sylvestris* (1.6 g from 2 kg of dry *A. sylvestris*) as

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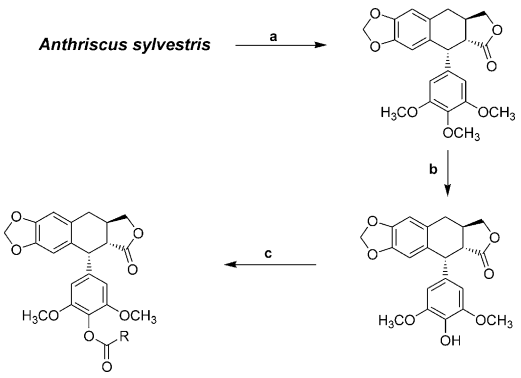
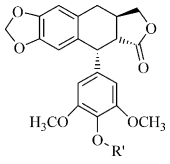


Figure 1. (a) MeOH, 5 h, three times then silica gel column, cyclohexanes/EA (5:1); (b) TMSI, CH_2Cl_2, 0 °C, 5 h then $BaCO_3$, 30 min; (c) DCC, DMAP, CH_2Cl_2, 0 °C, 1 h.

described in our previous paper.[9] Briefly, 4′ methoxy group of DPT was selectively demethylated with trimethylsilyl iodide yielding DDPT (yield, 72%). Then, DDPT was esterified with various unsaturated fatty acids using DCC and DMAP (yield, 70–92%). The products were confirmed by the triplet peaks around 2.5 ppm from α-proton (–$OCOCH_2C$–) of acid moieties on ^{1}H NMR. Double bonds of unsaturated fatty acids were safe during the esterification.

Cytotoxicities on A549 and SK-MEL-2 cell lines are summarized in Table 1[15]. There are some interesting results. First of all, the esters with long-chain acids (C16–C22) expressed weaker cytotoxicity (ED_{50}, 0.084–> 5 μg/mL) than short alkanoic acid (C2–C6) esters (ED_{50}, 0.003–0.013 μg/mL).[10] The bulky long chain acid moiety of the esters might interfere the binding of DDPT to the active site of a target molecule, possibly topoisomerase-II or tubulin. Secondly, the alkenoic acid esters showed a more potent cytotoxic activity than the alkanoic acid esters at the same carbone numbers [DOE11 (ED_{50}, > 5 μg/mL) vs DFE2 (ED_{50}, 0.159 μg/mL) on SK-MEL-2]. As the number of double bond increases, the cytotoxicity also tends to increase [DFE14 (ED_{50}, 2.7 μg/mL) vs DFE15 (ED_{50}, 0.119 μg/mL) on A549]. This could be attributed to the easier dispersive property of alkenoic acid moiety. Thirdly, among the octadecenoic acid esters the *cis*-forms are stronger than the trans-froms in cytotoxic activity. (DFE2 > DFE3, DFE4 > DFE5, DFE6 > DFE7). Since most of the unsaturated fatty acids occuring in cell surface are of *cis*-configuration rather than trans,[16] the *cis*-acid esters are assumed to be more favorable to be taken up into the cell[3].

Table 1. Cytotoxicity and antitumor activity of 4′-*O*-alkenoyl-4′-demethyldeoxypodophyllotoxin derivatives (DFE1–DFE16)



Compd	R′	Carbon	ED_{50} (μg/mL)		IR[a]
			A-549	SK-MEL-2	
DOE10	Hexadecanoyl	16	0.268	0.290	40[d]
DFE1	*cis*-9-Hexadecenoyl	16	0.733	0.065	83[c]
DOE11	Octadecanoyl	18	2.670	> 5	−2[d]
DFE2	*cis*-9-Octadecenoyl	18	0.179	0.159	84[b]
DFE3	*trans*-9-Octadecenoyl	18	0.367	0.550	89[b]
DFE4	*cis*-11-Octadecenoyl	18	0.335	0.057	90[b]
DFE5	*trans*-11-Octadecenoyl	18	1.010	0.253	81[b]
DFE6	All-*cis*-9,12-octadecanedienoyl	18	0.090	0.030	54[b]
DFE7	All-*trans*-9,12-Octadecanedienoyl (linoleaidic)	18	0.259	0.110	82[b]
DFE8	All-*cis*-9,12,15-Octadecantrienoyl (linolenic)	18	0.084	0.048	62[b]
DFE9	All-*cis*-6,9,12-Octadecantrienoyl	18	0.085	0.041	69[b]
DOE12	Eicosanoyl	20	> 5	> 5	11[d]
DFE10	*cis*-11-Eicosenoyl	20	> 5	> 5	62[c]
DFE11	*trans*-11-Eicosenoyl	20	1.660	1.130	28[c]
DFE12	All-*cis*-11,14-Eicosadienoyl (eicosadienoic)	20	0.870	0.347	77[c]
DFE13	All-*cis*-5,8,11,14-Eicosatetraenoyl (arachidonic)	20	0.910	0.184	76[c]
DOE13	Docosanoyl	22	> 5	> 5	−8[d]
DFE14	*cis*-13-Docosenoyl	22	2.710	0.561	15[c]
DFE15	All-*cis*-4,7,10,13,16,19-docosahexaenoyl	22	0.119	0.035	58[c]
DPT			0.012	0.009	
DDPT			0.023	0.015	< 10[d]
VP-16			1.102		

[a]Dose: 60 mg/kg except DFE7 (45 mg/kg); the standard injection schedule was d1, d5, d9 but changed according to the loss of body weight (DOE10, DFE1,4: d1, d5; DFE6, 8, 9: d1, d7; DFE7: d1, d9).
[b]IR of positive control (VP-16) is 75%.
[c]IR of VP-16 is 59%.
[d]IR of VP-16 is 68% and data adopted from ref 10. The experimental details on the inhibition of tumor growth were described on ref 9.

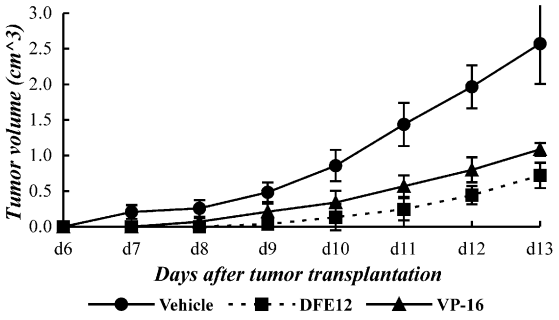


Figure 2. Inhibition of LL/2 tumor growth in BDF_1 mice by DFE12.

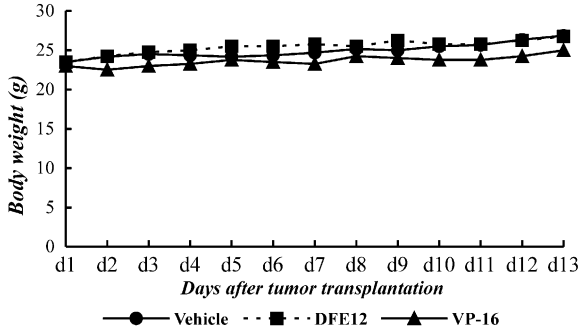


Figure 3. Body weight changes of BDF_1 mice treated with DFE12, administered on d1, d5, d9.

As shown in the last column of Table 1, most of the esters were found to be comparable or better in vivo antitumor activity than positive control, etoposide (VP-16), except DFE6, DFE8, and DFE9.

In Figures 2 and 3, the antitumor activity and body weight changes of DFE12 were presented representatively, where IR of DFE12 was 77%, while it was 59% for VP-16 at day 13. Injection of DFE12 seemed not to influence the body weight of the animals.

As it was so in cytotoxicity, the esters of unsaturated fatty acids (DFE1–15) were better also in terms of in vivo antitumor activity than the esters of alkanoic acids (DOE10–13) at the same carbon number. This might be due to easier uptake of the alkenoic acid eaters as mentioned above for their higher cytotoxicity.

On the other hand, the esters with long chain unsaturated fatty acids (C16–C22), despite lower cytotoxicity [DOE1], were better in vivo antitumor activity than short alkanoic acid (C2–C6) esters[10] (ED_{50}, 0.003 μg/mL on A549; IR, 11%) vs DFE2 (ED_{50}, 0.159 μg/mL on A549; IR, 84%)]. According to the previous report,[17] theophylline esters of short alkanoic acids were degraded much faster than unsaturated fatty acid esters in human plasma. Eventually the DDPT esters with short alkanoic acids might be easily hydrolyzed to lose the protecting role of phenolic hydroxy group before anchoring in active site. On the contrary, the long chain unsaturated fatty acid esters seem to be more resistant to esterase than short chain alkanoic acid esters and thus they are degraded relatively slowly. This might give a favorable pharmacokinetic property, resulting in long duration in animal body.

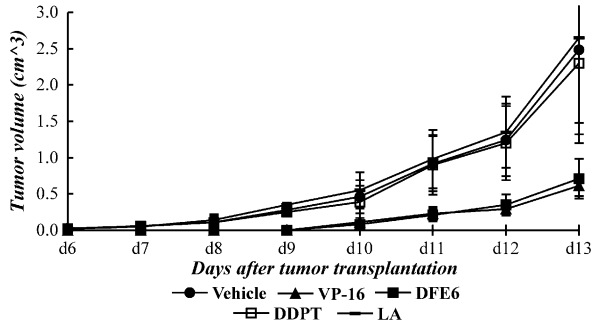


Figure 4. Inhibition of LL/2 tumor growth in BDF_1 mice by DFE6, DDPT, linoleic acid (LA).

Another interesting thing is that, contrary to the cytotoxic activity, the esters with unsaturated fatty acids bearing one double bond (DFE2–5) showed higer IR values than esters with two or more double bonds (DFE6–9).

It was discovered that, among the derivatives synthesized, DFE12 showed a promising antitumor activity, being statistically better in vivo antitumor activity than VP-16 and causing no change of body weight (Figs. 2 and 3). Possibly, all-*cis*,*cis*-11,14-eicosadienoic acid moiety in DFE12 was assumed to carry DDPT molecule selectively to the tumor tissue as DHA (all-*cis*-4,7,13,16,19-docosahexaenoic acid) did to paclitaxel.

To clarify the in vivo effect of unsaturated fatty acid itself, antitumor activity of linoleic acid (LA, all-*cis*-9,12-octadecanedienoic acid) was compared with DFE6 and DDPT. The same molar doses of DDPT and LA as DFE6 were administered respectively. As shown in Figure 4, each of LA and DDPT was ineffective when applied alone. As a result, the in vivo activity of DFE6 or other active esters might not be ensued from an enhancing effect of LA.

In conclusion, the esters with alkenoic acids of DDPT showed more potent cytotoxic activity and in vivo antitumor activity than esters with long alkanoic acids. Among them, DEF12 showed a remarkable antitumor activity without toxicity. It was assumed that these interesting results might owe to the delay of metabolic excretion of phenolic hydroxy group, increased solubility, and possibly selective targeting to tumor tissue. For clarifying this assumption, more detailed studies are needed.

Acknowledgements

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