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Prodrugs of 4′-Demethyl-4-deoxypodophyllotoxin: Synthesis and Evaluation of the Antitumor Activity

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Abstract—A series of prodrugs of 4′-demethyl-4-deoxypodophyllotoxin (DDPT) including carbamates (**3**–**8**), a carbonate (**9**) and water-soluble amino acid derivatives (**10**–**17**) were prepared and tested for their antitumor activity. The carbamate **6** (2-hydroxyethylcarbamoyl-DDPT), carbonate **9** (2-chloroethyloxycarbonyl-DDPT), and most of amino acid prodrugs (**12**–**17**) showed enhanced antitumor activity.

Introduction

4-Deoxypodophyllotoxin (DPT, **1**, Fig. 1) 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 **1** 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 ones. Moreover, **1** 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, **2**) exerted a comparable in vitro potency with **1**.[5] However, in vivo experiments revealed a substantial loss of the antitumor activity of **2** in the same BDF1/3LL model. These results indicate that the free hydroxy group at the 4′ position in DDPT was not favorable for its antitumor activity. We therefore envisioned that transformation of the hydroxy group into bioreversible functionalities might improve the in vivo activity of DDPT. In this paper, the synthesis and antitumor activity of a series of carbamate, carbonate, and amino acid prodrugs of DDPT are presented and discussed.

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Results and Discussion

Chemistry

Previously, it had been reported that DPT was abundant in *A. sylvestris*.[6] Thus, we took this advantage and used this plant as a source of DPT. Briefly, the air-dried materials (the whole plant, 3 kg) were extracted with ethyl acetate. 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,** 6.8 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.

The general methods employed for the preparation of DDPT and its derivatives **3**–**17** are outlined in Scheme 1. Selective demethylation[7] of DPT **1** with trimethylsilyl iodide (TMS) gave DDPT (**2**) in 72% yield. Compound

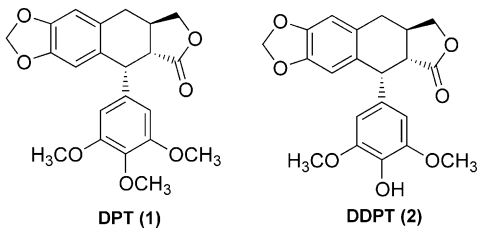

Figure 1. Structures of DPT (**1**) and 4′-DDPT (**2**).

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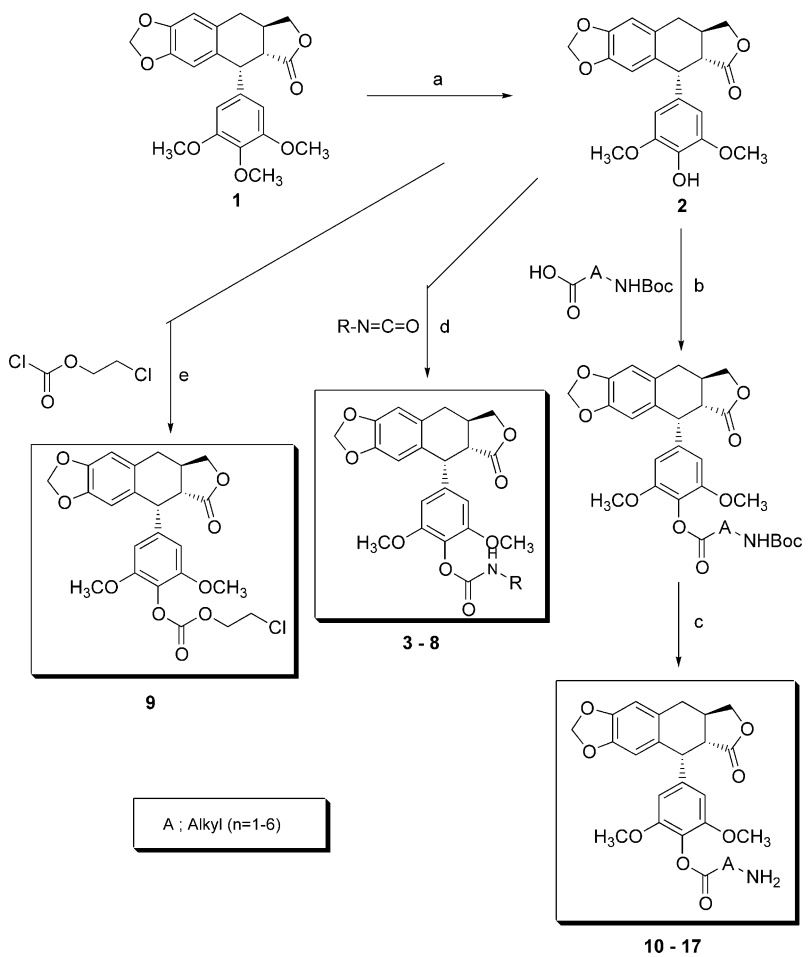


Scheme 1. (a) TMSI, CH_2Cl_2, 0 °C, 5 h, then $BaCO_3$, 30 min; (b) DCC (5 equiv), DMAP (2.5 equiv), CH_2Cl_2, 0 °C, 1 h; (c) 50% TFA/CH_2Cl_2, rt, 1 h; (d) TEA, 0 °C, 40 min; (e) DMAP, CH_2Cl_2, 0 °C, 5 h.

2 was reacted with various isocyanates in the presence of triethylamine (TEA) to give 4′-demethyl-4′-*O*-(carbamoyl)-DPT derivatives (**3**–**8**) in 66–80% yields. In the presence of 4-dimethylaminopyridine (DMAP) as a base, DDPT **2** was esterified with 2-chloroethyl chloroformate to give 4′-demethyl-4′-*O*-(2-chloroethylcarbonyloxy)-DPT (**9**) in 78% yield. 4′-Demethyl-4′-*O*-(aminoacyl)-DPT derivatives **10**–**17** were prepared in 73–83% yields by reacting DDPT **2** with various Boc-protected amino acids in the presence of dicyclohexylcarbodiimide (DCC) and DMAP, and successive removal of the Boc protecting group by trifluoroacetic acid (TFA). All compounds were unambiguously confirmed by spectroscopic methods.[8]

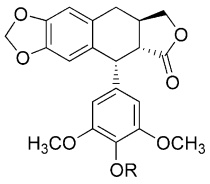
Bioactivity

Cytotoxicity of the synthesized compounds was measured against three cancer cell lines (A549, human lung carcinoma; SK-MEL-2, human melanoma; and MCF-7, human breast carcinoma). The results, expressed as ED_{50} values (the concentration that produces a 50% reduction in cell growth), are summarized in Table 1. For the comparison purpose, the average ED_{50} values (AC) of each compound in three cancer cell lines were calculated and the results are included in Table 1. Generally, the prodrugs showed decreased cytotoxicity (AC values up to 171 nM) compared to the parent compound **2** (AC, 15 nM). The carbamates **3**–**5** were the least cytotoxic with approximately 10-fold drops in the ED_{50} values relative to that of **2**. The results indicate that under cell culture conditions these carbamates were not conversed to the parent compound **2** or the rate of conversion was too slow. Of exception were the compounds **10** and **11**, which showed the same potency (AC values of 14 and 29 nM, respectively) with **2**. The potent cytotoxicity of **10** and **11** suggests that these derivatives were sufficiently conversed to the parent compound **2** under cell culture conditions. Similar phenomena have been observed previously.[10]

The prodrugs were evaluated for the antitumor activity in BDF1/3LL model. Due to the limited solubility in solvent system (5% DMSO, 15% Cremophore® in saline), the carbamates **3**–**8** and were evaluated at doses of 0.06 mmol/kg/day and the carbonate **9** was administered at 0.03 mmol/kg/day. The amino acid prodrugs (**10**–**17**) were injected at the maximum tolerated doses (Table 1). Etoposide, a clinical anticancer drug, was administered at 0.06 mmol/kg/day and used as a positive control. The results are summarized in Table 1.

Our rationale for the preparation of carbamates **3**–**8** was based on the previous reports that carbamates could be used as effective prodrugs to obtain prolonged

Table 1. Cytotoxicity and antitumor activity of the synthesized prodrugs



Compd	R	Cytotoxicity[a] (ED_{50}, nM)[b]				Antitumor activity
		A549	SK-MEL-2	MCF7	AC[c]	IR[d]
3	Ethylcarbamoyl	132	143	142	139	NA[e] (0.06)[f]
4	Isopropylcarbamoyl	153	165	166	161	NA (0.06)
5	Cyclohexylcarbamoyl	163	152	197	171	NA (0.06)
6	2-Hydroxyethylcarbamoyl	62	59	46	56	**95** (0.06)
7	4-Fluorophenylcarbamoyl	86	78	76	80	69 (0.06)
8	4-Methoxyphenylcarbamoyl	96	89	93	93	NA (0.06)
9	2-Chloroethyloxycarbonyl	92	72	88	84	**88** (0.03)
10	Alanyl	22	9	11	14	41 (0.3)
11	Glycinyl	12	34	31	29	33 (0.3)
12	Phenylalanyl	87	59	72	73	**80** (0.36)
13	3-Aminopropanoyl	97	79	83	86	**85** (0.3)
14	4-Aminobutanoyl	92	91	89	91	**85** (0.36)
15	6-Aminohexanoyl	89	77	72	79	**89** (0.06)
16	8-Aminooctanoyl	74	61	69	68	**80** (0.42)
17	4-Aminophenylmethyl	114	152	99	122	**80** (0.3)
DPT (**1**)		23	14	11	16	59 (0.06)
DDPT (**2**)		15	12	18	15	13 (0.06)
Etoposide		1900				**78** (0.06)

[a]A549, human lung carcinoma; SK-MEL-2, human melanoma; MCF-7, human breast carcinoma.
[b]Drug concentration required to inhibit the growth of cancer cells by 50%, see ref 14.
[c]AC; average of ED_{50} value.
[d]IR, inhibition rate (%), determined as described in ref 15.
[e]NA; not active.
[f]Numbers in parenthesis represents the highest formulable doses (**3**–**9**) or maximum tolerated doses (**10**–**17**) in mmol/kg/day.

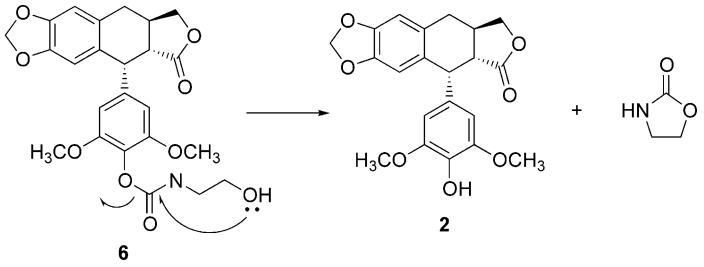


Figure 2. Possible intramolecularly cyclative hydrolysis of **6** leading to the release of the parent compound **2**.

duration of action of the parent drug,[11] high stability against unspecific enzymes, or to protect the parent drugs against hepatic first pass metabolism.[12] However, as shown in Table 1, four carbamates (**3**–**5** and **8**) did not show any antitumor activity. These results again indicate that the carbamates concerned were not sufficiently conversed to the parent compound **2**. Of exception was the analogue **6** which exhibited very potent antitumor activity with IR of 95%, much higher than that of etoposide (IR, 78%). Previously, it was reported that 3-azido-3-deoxythymidin-5-yl-*O*-(2-hydroxyethyl) carbonate showed increased anti-HIV potency compared to its parent drug, zidovuldin (AZT). This was attributed to the facile release of the parent compound, AZT through intramolecular cyclic rearrangement of the prodrug.[13] Likewise, it could be postulated that in the analogue **6**, intramolecular cyclic rearrangement of the hydroxy side chain (Fig. 2) might be an important factor facilitating the hydrolysis of its 4′-carbamate linkage, resulting in the more effective release of the cytotoxic compound **2** into foci and thus leading to more potent antitumor activity of this compound compared to other carbamates. Noteworthy, a carbonate analogue **9** also exerted potent antitumor activity with IR of 89%, suggesting that the carbonates might be more effectively hydrolyzed than the carbamates under physiological conditions to release the active parent inside the cancer cell.

Though the two compounds **6** and **9** of the synthesized carbamates and carbonates showed potent antitumor activity, their water solubility proved to be relatively poor for drug formulation for further in vivo evaluation. We envisioned that esterification of the 4′-hydroxy

group with amino acids would substantially improve the solubility of the compounds in aqueous system. In addition, the amino acid derivatives were expected to be sufficiently reversible, partly due to the folding effects of the side chain amino group, similar to the case of **6**. This prompted us to synthesize 8 additional amino acid prodrugs (**10**–**17**) of **2**.

The synthesized amino acid prodrugs showed excellent water solubilities. For example, the water solubility of 4′-demethyl-4′-*O*-(8-aminohexanoyl)-DPT (**15**), a representative selected from the 4′-demethyl-4′-*O*-(aminoacyl)-DPT compounds (**10**–**17**), was 50 times higher than that of the parent compound DDPT (data not shown).[9] The high water solubility allowed these compounds to be administered at the maximum tolerated doses. In the BDF1/3LL model, most of the amino acid prodrugs (**12**–**17**) showed potent antitumor activity with IR of 80–89%, higher than the IR value (78%) of etoposide (Table 1). However, compounds **10** and **11** showed relatively weak activity with IR values of 41 and 33%, respectively, much lower than that of other amino acid derivatives (**12**–**17**). The weak in vivo activity of **10** and **11** could be due to their premature hydrolysis caused by the electron-withdrawing effects of the α-protonated amino group. In contrast, the presence of the phenyl moiety in **12** might somewhat lower the rate of hydrolysis of this compound compared to **10** and **11**, resulting in its potent antitumor activity. In summary, we have reported the synthesis of a series of prodrugs of compound **2** (4′-demethyl-4-deoxypodophylotoxin). Eleven compounds among 15 prodrugs prepared showed enhanced in vivo antitumor activity compared to the parent compound **2**. The carbamate **6** offered the best antitumor activity with IR of 95%, followed by the carbonate **9** (IR value of 89%) and amino acid ester **15** (IR values of 87%). The results demonstrate that the design and synthesis of the presented prodrugs are beneficial for therapeutic values of the compound **2**. The approaches may be applicable for other antitumor agents, which possess similar functional features with **2**.

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8. All newly synthesized compounds gave satisfactory analytical and spectroscopic data. **6**; ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.68 (s, 1H), 6.53 (s, 1H), 6.39 (s, 2H), 5.93 (s, 2H), 4.90 (br, 1H), 4.60 (d, J = 3.80 Hz, 1H), 4.52–4.40 (m, 1H), 3.99–3.62 (m, 9H), 3.25–2.69 (m, 6H). Anal. calcd for $C_{24}H_{25}NO_9$: C, 61.14; H, 5.34; N, 2.97; found: C, 61.03; H, 6.5.38; N, 2.95. **9**; ^{1}H NMR (90 MHz, $CDCl_3$): δ 6.67 (s, 1H), 6.53 (s, 1H), 6.37 (s, 2H), 5.91 (s, 2H), 4.66 (d, J = 3.81 Hz, 1H), 4.52–4.41 (m, 1H), 3.98–3.62 (m, 9H), 3.24–2.72 (m, 6H). Anal. calcd for $C_{24}H_{23}ClO_9$: C, 58.72; H, 4.72; found: C, 58.62; H, 4.68. **15**; ^{1}H NMR (90 MHz, $CDCl_3$): δ 7.68 (br, 2H), 6.65 (s, 1H), 6.49 (s, 1H), 6.37 (s, 2H), 5.92 (s, 2H), 4.58 (d, J = 3.84 Hz, 1H), 4.44–4.34 (m, 1H), 3.99–3.66 (m, 7H), 3.02–2.54 (m, 8H), 1.90–1.26 (m, 6H). Anal. calcd for $C_{27}H_{31}NO_8$: C, 65.18; H, 6.28; N, 2.82; found: C, 65.00; H, 6.22; N, 2.79.
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