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Carbamates of 4′-demethyl-4-deoxypodophyllotoxin: Synthesis, cytotoxicity and cell cycle effects

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ABSTRACT

In an attempt to generate compounds with superior bioactivity and reduced toxicity, 12 carbamates of 4′-demethyl-4-deoxypodophyllotoxin, *N*-(1-oxyl-4′-demethyl- 4-deoxypodophyllic)-α-amino acids amides, were synthesized and evaluated for antiproliferative activity and cell cycle effects. These synthesized compounds proved to be more hydrophilic, as well as improved or comparable in vitro cytotoxicities against four cell lines (A-549, HeLa, SiHa, and HL-60) compared with either parent DPT or anti-cancer drug VP-16. Furthermore, flow cytometric analysis exhibited that *N*-(1-oxyl-4′-demethyl-4-deoxypodophyllic)-D-α-methine amide (**15f**) induced cell cycle arrest in the G2/M phase in A-549 cells.

Cancer is a leading cause of human death and can be triggered by environmental pollutants and genetic mutations. A number of natural products, with diverse chemical structures, have been isolated as anticancer agents until now.[1] Several potential lead molecules, such as podophyllotoxin (PPT, **1**), camptothecin (**2**), taxol (**3**), combretastatin A-4 (**4**), vincristine, vinblastine, etc., have been isolated from plants and many of them have been modified to yield analogues endowed with better activity, lower toxicity or higher water-solubility. For example, etoposide (**5**, VP-16), teniposide (**6**) and the water-soluble prodrug, etoposide phosphate (**7**) derived from PPT are presently in clinical use for the treatment of small cell lung cancer, testicular carcinoma, non-Hodgkin's lymphoma, and Kaposi's sarcoma.[2]

4-Deoxypodophyllotoxin (DPT, **8**), an analogue of PPT, is a potent antimitotic agent first isolated from *Anthriscus sylvestris*.[3] It has been reported that DPT exhibits antiproliferative and antitumor activities,[4–8] anti-inflammatory,[9,10] anti-viral activities[11,12] and broad insecticidal activity.[13] The mechanism studies revealed that DPT inhibits tubulin polymerization and induces cell cycle arrest at G2/M, followed by apoptosis through multiple cellular processes, involving the activation of ATM, upregulation of p53 and Bax, activation of caspase-3 and -7, and accumulation of PTEN resulting in the inhibition of the Akt pathway.[14,15] Besides, DPT inhibits migration and MMP-9 via MAPK pathways in TNF-α-induced HASMC.[16]

Previously, it was reported that 4′-demethyl-4-deoxypodophyllotoxin (DDPT, **9**, Fig. 1) exerted a comparable in vitro potency with DPT. However, in vivo experiments revealed a substantial loss of the antitumor activity of DDPT in the BDF1/3LL model.[17] The results indicated that the free hydroxy group at the C-4′ position in DDPT was not favorable for antitumor activity. Lately, Ahn and co-workers envisioned that transformation of the hydroxyl group into bioreversible functionalities might improve the in vivo activity of DDPT. Thus, they synthesized and evaluated in vivo antitumor activity of many prodrugs of DDPT, including carbamates, carbonate, esters (such as alkyl, carboxyl alkyl, unsaturated fatty acid esters, and water-soluble amino acid esters). It was reported that the esters showed increased in vivo antitumor activity despite the lower in vitro activity than DDPT.[18–20]

Inspired by previous work[21–27] as well as the fact that amino acids possess good water solubility, we here present the synthesis of twelve new α-amino acids carbamates derivatives of DDPT (**15a–l**) and our measurements of their cytotoxic activity against A-549, HeLa, SiHa, and HL-60 cell lines. Furthermore, the effect of **15f** on A-549 cells was also investigated to reveal its actions on the cell cycle.

Amino acids amides **12a–l** were prepared as depicted in Scheme 1. Compounds **11a–h** were prepared by Boc-protected amino acid **10** reacted with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-

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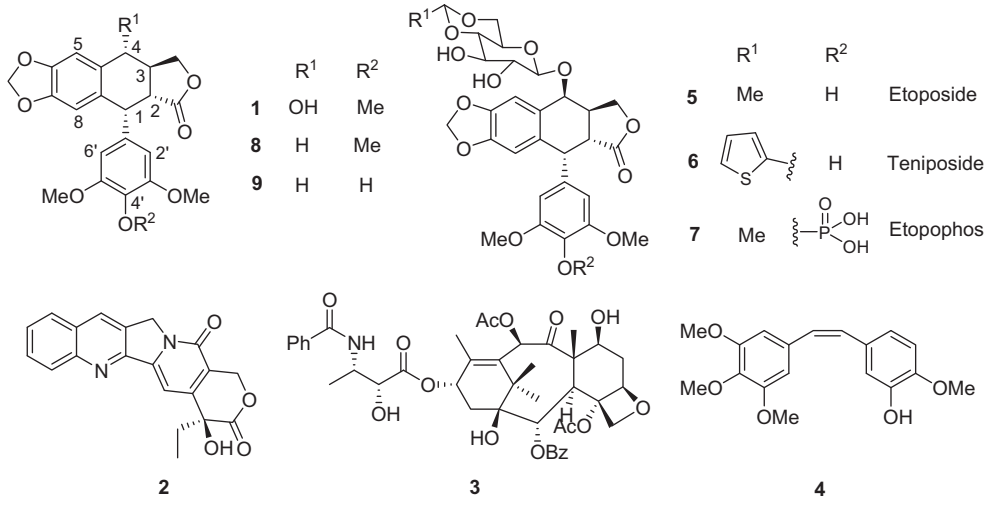
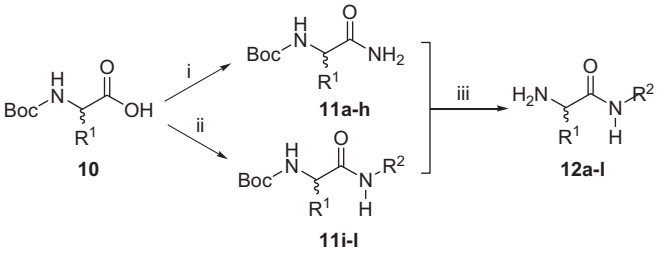


Figure 1. Structures of compounds **1**–**9**.



Scheme 1. Synthesis of **12a**–**l**. Reagents and conditions: (i) EEDQ, NH_4HCO_3, $CHCl_3$, rt; (ii) EDCI, DIEA, amine; (iii) TFA, CH_2Cl_2.

Table 1
Biological evaluation of compounds **15a**–**l**

Compounds	Cytotoxicity (IC_{50}, μM) [a]				log*P*
	A-549[b]	SiHa[b]	HeLa[c]	HL-60[c]	
15a	4.23	0.696	0.743	0.397	−0.09
15b	1.49	2.89	3.83	1.03	0.84
15c	9.93	0.877	0.703	0.67	1.26
15d	1.77	3.70	5.28	1.02	1.36
15e	0.86	4.32	2.03	0.711	0.28
15f	0.887	0.614	0.528	0.0893	0.26
15g	3.41	1.07	1.72	0.94	1.35
15h	1.61	0.416	0.575	0.231	1.33
15i	2.31	0.432	0.433	0.213	1.45
15j	1.91	14.6	20.3	1.03	2.57
15k	3.37	1.12	0.693	0.416	1.95
15l	2.69	6.87	3.07	0.988	2.06
VP-16	24.9	40.4	2.76	2.85	0.69
DPT	1.38	6.01	1.98	0.47	3.16
DDPT	1.8	53.3	43.0	2.96	2.92

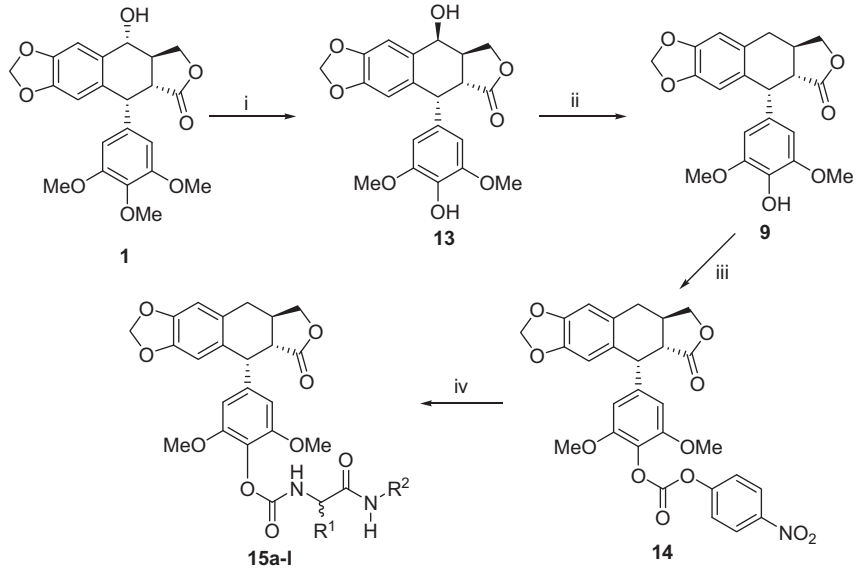
[a] Data are the mean of three independent experiments.
[b] MTT method with 48 h drug exposure.
[c] CCK-8 method with 48 h drug exposure.

quinoline (EEDQ) under ammonium hydrogencarbonate in chloroform at room temperature.[28] Boc-amino acid amides **11i–l** were easily obtained through Boc protected L-valenine (or L-phenylalanine) was reacted respectively with cyclopropylamine (or cyclopentylamine) under 1-[3-(dimethylamino) propyl]-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of diisopropylethylamine (DIEA) in dichloromethane.[29] Then, the Boc protection group of compounds **11a–l** were easily removed using trifluoroacetic acid (TFA) in dichloromethane and afford compounds **12a–l**, the later were used directly for next reaction without further purified.

The synthetic route to the target compounds first involved generation of the intermediate DDPT, which was prepared from **1** as described in previous publication (Scheme 2).[27] Briefly, the intermediate **9** is prepared through 4′-demethylation of **1** with HBr gas in CH_2Cl_2,[30] and subsequent 4-deoxylation of **13** by hydrogenolysis with 10% Pd/C in HAc.[31] Then treatment of **9** with 4-nitrophenyl chloroformate in the presence of pyridine provides the intermediate **14** in high yield.[32] Subsequently, introduction of the carbamate chains was performed by reacting **14** with α-NH_2 of the appropriate α-amino acid amide **12a–l** in the presence of 4-*N*,*N*-dimethylaminopyridine (DMAP) and triethylamine (Et_3N), affording *N*-(1-oxyl-4′-demethyl-4-deoxypodophyllic)-α-amino acids amides **15a–l** with high or moderate yields. The structures of the final products **15a–l** were identified by IR, ^1H NMR, ^{13}C NMR and HR-MS.

The biological activities of the DPT derivatives **15a–l** were evaluated by an in vitro cytotoxicity test carried out with a panel of four human tumor cell lines (A-549, lung carcinoma; HeLa, cervical carcinoma; SiHa, cervical squamous cell carcinoma; and HL-60, human premyelocytic leukemia).[33] The results expressed as IC_{50} values are summarized in Table 1.

As illustrated in Table 1, the DPT derivatives **15a–l** were generally showed more potent or comparable activity in comparison with DPT, DDPT and VP-16 in their in vitro cytotoxicity to these four cell lines. These compounds were more effective in HL-60 cells, and had lower potency in A-549 cells, although the order of potency varied in each cell line. In Table 1, we also show that different substituent at the α-carbon of the amino acids in compounds **15a–h** have different effects on the activity of the resultant compound. Notably, the compounds with D-amino acids incorporated appear to be more potent than those with L-amino acid (**15e** vs **15f**, **15g** vs **15h**) on their anti-proliferative activity. In addition, carbamates of deoxypodophyllotoxin with cyclopropyl amine incorporated showed more potent anti-proliferative activity in the in vitro assay than those of cyclopentyl amine except A-549 cell line (**15i** vs **15j**, **15k** vs **15l**). In the previous reports, the aliphatic or aromatic carbamates of DDPT showed the lower cytotoxic with approximately 10-fold drops in the in vitro activity relative to that of DDPT. The authors considered the prodrugs of DDPT exhibited less in vivo antitumor activity because they were stable in under cell culture conditions.[18] These α-amino acids carbamates of DDPT showed more potent or comparable in vitro activity in



Compounds	R^1	R^2	Compounds	R^1	R^2
15a	*L*-Me	H	**15g**	*L*-$CH_2C_6H_5$	H
15b	*L*-$CHMe_2$	H	**15h**	*D*-$CH_2C_6H_5$	H
15c	*L*-CH_2CHMe_2	H	**15i**	*L*-$CHMe_2$	*cyc*-Pr
15d	*L*-CH(Me)CH_2Me	H	**15j**	*L*-$CHMe_2$	*cyc*-Pen
15e	*L*-CH_2CH_2SMe	H	**15k**	*L*-$CH_2C_6H_5$	*cyc*-Pr
15f	*D*-CH_2CH_2SMe	H	**15l**	*L*-$CH_2C_6H_5$	*cyc*-Pen

Scheme 2. Synthesis of **15a–l**. Reagents and conditions: (i) HBr, acetone/H_2O–$BaCO_3$; (ii) H_2, 10% Pd/C, HAc; (iii) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2; (iv) **12a–l**, DMAP/Et_3N, CH_2Cl_2.

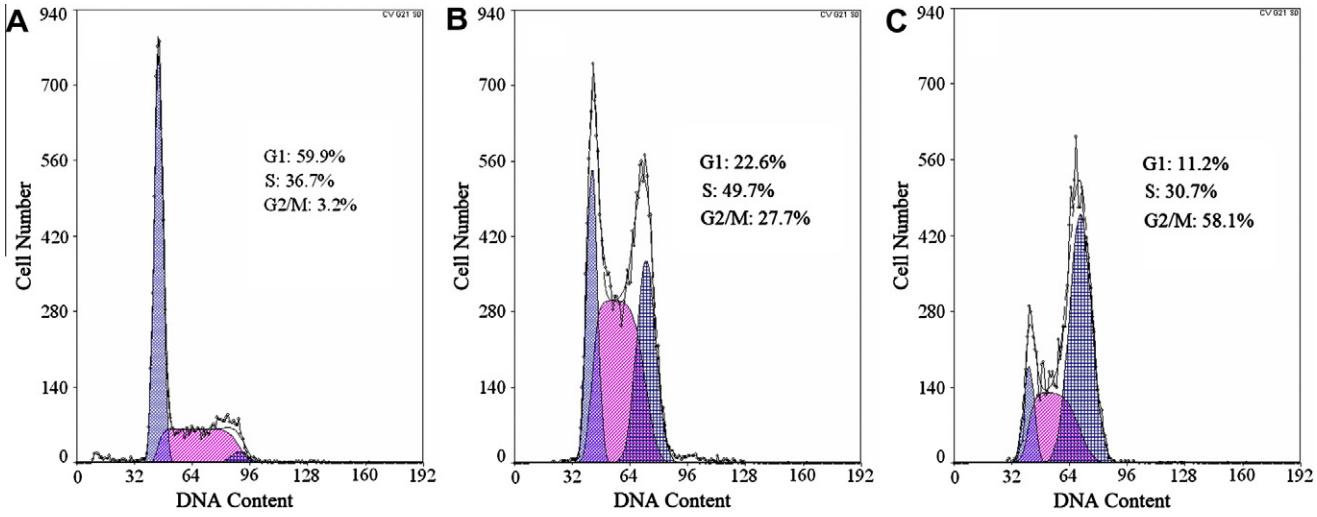


Figure 2. Effects of **15f** on cell cycle progression. Values indicate the percentage of the cell population at the phase of the cell cycle. (A) Control A-549 cells; (B) A-549 cells treated with 0.4 μM **15f** at 12 h; (C) A-549 cells treated with 0.4 μM **15f** at 24 h.

comparison with DPT, DDPT and VP-16, so they are a series of α-amino acids carbamates derivatives, not prodrugs of DDPT.

The octanol–water partition coefficients of **15a–l** were also determined[34] and results were shown in Table 1. In line with

expectations, the experimental log*P* values of the newly synthesized carbamoyl derivatives **15a–l** are significantly lower than those of DPT and DDPT, which means that they are all more hydrophilic than the parent compound. The result was in according with the previously reported results,[18] derivatives of DDPT formed by amino acids conjugated to DDPT, either carbamates or esters, may improve the hydrophilic and solubility.

The effects of **15f** on cell cycle progression were also determined by FACS analysis in propidium iodide-stained A-549 cells. As shown in Figure 2, treatment with **15f** (0.4 μM) led to a time-dependent accumulation of cells in the G2/M phase with a concomitant decrease in the population of G1 phase cells. 27.7% (Fig. 2B) and 58.1% (Fig. 2C) of the cells were in G2/M phase after 12 h and 24 h treatment with **15f**, respectively, compared with 3.2% (Fig. 2A) in untreated cultures. These results demonstrate that these carbamates of DPT interfere with cell proliferation by arresting the cell cycle, and that the cell cycle is arrested in same phases than those identified in previous studies.[14,15]

In summary, the podophyllotoxin class of compounds is a promising group of putative antitumor chemotherapeutics. In this present work, most of water-soluble carbamates of deoxypodophyllotoxin exhibited improved or comparable antiproliferation against A-549, HeLa, SiHa and HL-60 cells as compared with etoposide and DPT. In addition, compounds **15f** could induce cell-cycle arrest in G2/M phase in A-549 cells. Further biological evaluation is in progress to clarify the stability in physiological conditions, the toxicity and the mechanism of **15f** action and to define its effects on tubulin polymerization.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.024.

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