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Design and synthesis of novel cytotoxic podophyllotoxin derivatives

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Abstract

In order to investigate the effect of different C4 linkage moieties on the cytotoxicity of podophyllotoxin derivatives, novel 4-*N*- and 4-*C*-substituted 4′-*O*-demethylepipodophyllotoxin derivatives were designed and synthesized. All the compounds were tested against A549 and MCF-7 tumor cells *in vitro*, and six compounds showed significant cytotoxicity. The most active compound **9f** was superior to GL-331, and exhibited potent cytotoxicity with IC_{50} value at 10^{-7} mol/L level.
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Keywords: Podophyllotoxin; C4-derivation; Cytotoxicity

Podophyllotoxin **1** is a bioactive lignan isolated from the roots of *Podophyllum peltatum* L (Fig. 1). Although the therapeutic application of podophyllotoxin is limited to topical use due to its high toxicity, its semisynthetic derivatives Etoposide **2** and Teniposide **3** have been widely used as important anticancer drugs in clinic, which has prompted extensive structural modification, particularly at the C4 position of podophyllotoxin. As highlights, C4 derivatives, GL-331 **4** and TOP-53 **5**, have displayed unique antitumor spectra and reached clinical trials [1].

Previous structure–activity relationship (SAR) indicated that bulky groups are well-tolerated at C4 and the moiety immediate to C4 would affect the antitumor spectra of podophyllotoxin derivatives significantly [1]. Although former modification has provided potent derivatives with both 4-*N*- (*e.g.* GL-331) and 4-*C*- (*e.g.* TOP-53) substitution, the effect of different C4 linkages on the pharmacological profiles of **1**-derivatives is still to be clarified. Accordingly, we designed two series of novel derivatives with C4 linkage units of either *p*-aminobenzamido or amido, which were extended with bulky tails to optimize the antitumor activity and incorporated with tertiary amino groups to improve the bioavailability profile of the target compounds.

4′-Demethylepipodophyllotoxin (DMEP, **6**) was synthesized from **1** stereoselectively through successive 4′-demethylation, 4-iodination, and nucleophilic substitution [2]. The intermediates **7** and **8** were prepared from DMEP as previously reported [3,4]. Briefly, DMEP was subjected to nucleophilic displacement by *p*-aminobenzoic acid and trimethylsilyl cyanide to provide **7** and **11** respectively, and **11** was then readily hydrolyzed to the carboxylic acid **8**. **7** and **8** were subsequently condensed with appropriate amines in the presence of 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIEA) to yield target compounds

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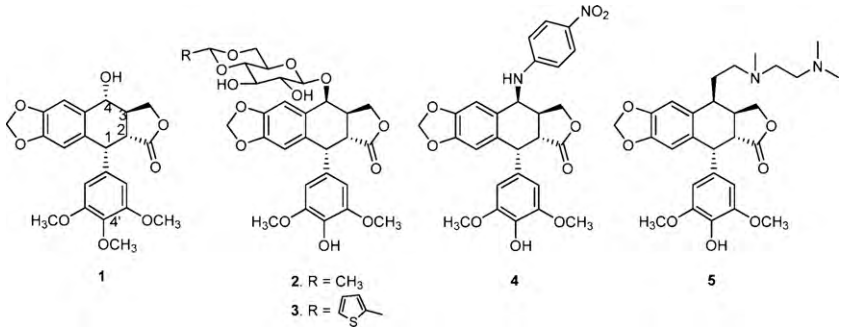
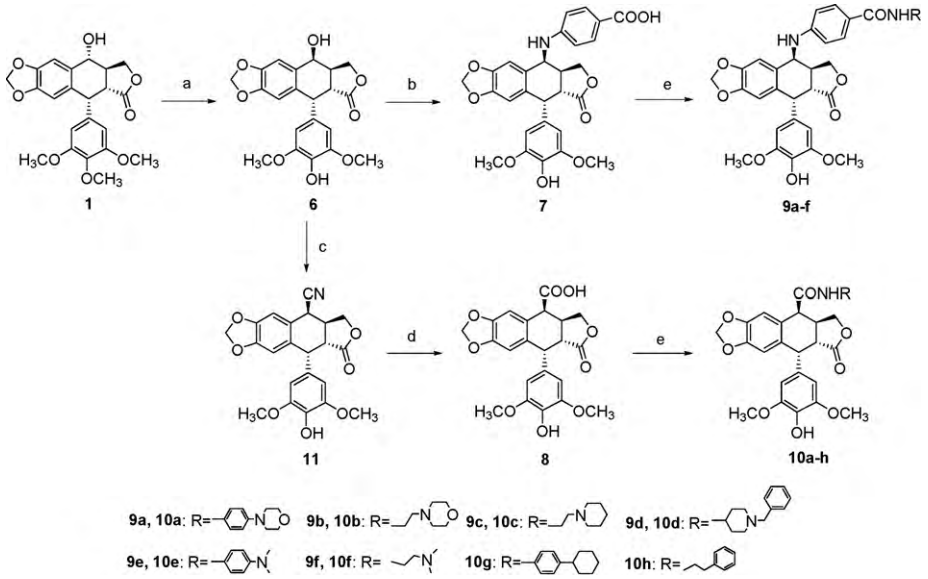


Fig. 1. Structures of Podophyllotoxin **1**, Etoposide **2**, Teniposide **3**, GL-331 **4**, and TOP-53 **5**.



Scheme 1. Conditions and reagents: (a) $MeSO_3H/NaI$; CH_2Cl_2, then $H_2O/BaCO_3$; acetone, 57%; (b) $MeSO_3H/NaI$; CH_2Cl_2, then *p*-aminobenzoic acid, $BaCO_3$, THF, 30%; (c) $BF_3{\cdot}Et_2O/Me_3SiCN/CHCl_3$, 57%; (d) $AcOH/HCl/H_2O$, 78%; and (e) HATU, DIEA, CH_2Cl_2, RNH_2.

Table 1
Data of compounds **9a–f** and **10a–h**.

No.	Yield%	Mp (°C)	$[\alpha]_D^{20}$ ($CHCl_3$)
9a	35.9	186–189	−119 (*c*, 0.05)
9b	87.3	168–169	−232 (*c*, 0.05)
9c	76.4	157–159	−127 (*c*, 0.05)
9d	72.7	165–168	−67 (*c*, 0.05)
9e	35.6	193–195	−70 (*c*, 0.05)
9f	74.8	241–243	−196 (*c*, 0.05)
10a	62.6	165–167	−17 (*c*, 0.05)
10b	79.3	124–127	−67 (*c*, 0.05)
10c	96.7	148–152	−119 (*c*, 0.1)
10d	59.3	182–185	−70 (*c*, 0.1)
10e	44.1	177–179	−132 (*c*, 0.1)
10f	64.5	190–191	−104 (*c*, 0.1)
10g	10.3	161–163	−96 (*c*, 0.1)
10h	53.8	228–231	−104 (*c*, 0.05)

9 and **10** (Scheme 1). All the 14 compounds are novel structures and their physical and spectral data are elaborated in Tables 1 and 2. In particular, the C4-configuration of **6**, **7** and **11** was deduced from the reaction mechanism as well as evidences from NMR data. It is generally accepted that the nucleophilic substitution occurred at C4 follows an S_N1 mechanism, and due to the steric effect posed by the bulky C1-substituent, C4β-configuration is usually preferred regardless of the reacting configuration in C4α or C4β [5]. It was also previously observed that C4α- and C4β-derivatives present different $J_{3,4}$ values. The C4β-substituted compounds show a $J_{3,4}$ around 4.0 Hz, whereas the

Table 2
Spectral date of compounds **9a–f** and **10a–h**.

No.	^{1}H NMR (300 MHz, δ) and HR-ESI-MS (m/z)
9a	$CDCl_3$: 7.76 (d, 2H, J = 8.7 Hz), 7.62 (s, 1H), 7.53 (d, 2H, J = 8.7 Hz), 6.94 (d, 2H, J = 7.8 Hz), 6.77 (s, 1H), 6.60 (d, 2H, J = 8.4 Hz), 6.55 (s, 1H), 6.33 (s, 2H), 5.98 (d, 2H, J = 6.0 Hz), 5.43 (s, 1H), 4.76–4.84 (m, 1H), 4.61 (d, 1H, J = 4.2 Hz), 4.40 (t, 1H, J = 7.8 Hz), 4.26 (d, 1H, J = 6.0 Hz), 3.88–3.96 (m, 5H), 3.82 (s, 6H), 3.09–3.15 (m, 6H); 680.2620 $[M+H]^+$ (calcd. 680.2608)
9b	$CDCl_3$: 7.67 (d, 2H, J = 8.4 Hz), 6.75 (s, 1H), 6.66 (s, 1H), 6.56 (d, 2H, J = 8.7 Hz), 6.53 (s, 1H), 6.32 (s, 2H), 5.97 (d, 2H, J = 4.2 Hz), 4.74–4.77 (m, 1H), 4.60 (d, 1H, J = 4.5 Hz), 4.39 (t, 1H, J = 7.2 Hz), 4.25 (d, 1H, J = 6.3 Hz), 3.94 (t, 1H, J = 10.2 Hz), 3.80 (s, 6H), 3.72 (m, 4H), 3.51–3.57 (m, 2H), 3.00–3.14 (m, 2H), 2.59–2.63 (m, 2H), 2.52 (m, 4H); 632.2612 $[M+H]^+$ (calcd. 632.2603)
9c	$CDCl_3$: 7.68 (d, 2H, J = 8.1 Hz), 6.87 (s, 1H), 6.76 (s, 1H), 6.52–6.57 (m, 3H), 6.32 (s, 2H), 5.96 (d, 2H, J = 3.0 Hz), 4.72–4.78 (m, 1H), 4.59 (d, 1H, J = 4.2 Hz), 4.38 (t, 1H, J = 8.1 Hz), 4.23 (d, 1H, J = 5.7 Hz), 3.96 (t, 1H, J = 9.6 Hz), 3.78 (s, 6H), 3.44–3.52 (m, 2H), 2.92–3.14 (m, 2H), 2.54–2.58 (t, 2H, J = 5.4 Hz), 2.45 (s, 4H), 1.38–1.66 (m, 6H); 630.2801 $[M+H]^+$ (calcd. 630.2810)
9d	$CDCl_3$: 7.64 (d, 2H, J = 8.1 Hz), 7.30–7.33 (m, 5H), 6.75 (s, 1H), 6.53–6.56 (m, 3H), 6.33 (s, 2H), 5.97 (d, 2H, J = 6.0 Hz), 5.87 (d, 1H, J = 7.5 Hz), 5.30 (s, 1H), 4.75 (m, 1H), 4.60 (d, 1H, J = 3.0), 4.36 (m, 1H), 4.22 (d, 1H, J = 5.7 Hz), 3.79 (s, 6H), 3.56 (s, 2H), 3.52 (m, 1H), 2.90–3.08 (m, 2H), 1.96–2.90 (m, 8H); 692.2978 $[M+H]^+$ (calcd. 692.2966)
9e	$CDCl_3$: 7.76 (d, 2H, J = 8.1 Hz), 7.64 (s, 1H), 7.47 (d, 2H, J = 8.4 Hz), 6.78 (s, 1H), 6.76 (d, 2H, J = 9.0), 6.60 (d, 2H, J = 8.1 Hz), 6.55 (s, 1H), 6.34 (s, 2H), 5.99 (d, 2H, J = 7.8 Hz), 5.31 (s, 1H), 4.79 (s, 1H), 4.60 (d, 1H, J = 3.6 Hz), 4.32–4.41 (m, 2H), 3.95 (t, 1H, J = 9.6 Hz), 3.82 (s, 6H), 3.10–3.16 (m, 2H), 2.95 (s, 6H); 638.2488 $[M+H]^+$ (calcd. 638.2497)
9f	DMSO-d_6: 8.31 (s, 1H), 7.99 (t, 1H, J = 5.4 Hz), 7.63 (d, 2H, J = 8.7 Hz), 6.77 (s, 1H), 6.70 (d, 2H, J = 8.7 Hz), 6.56 (s, 1H), 6.25 (s, 2H), 5.98 (d, 2H, J = 9.9 Hz), 4.92–4.96 (m, 1H), 4.51 (d, 1H, J = 4.8 Hz), 4.36 (t, 1H, J = 8.1 Hz), 3.63–3.67 (m, 7H), 3.23–3.28 (m, 3H), 3.00–3.10 (m, 1H), 2.37 (t, 2H, J = 7.2 Hz), 2.16 (s, 6H); 590.2490 $[M+H]^+$ (calcd. 590.2497)
10a	DMSO-d_6: 10.04 (s, 1H), 8.17 (s, 1H), 7.41 (d, 2H, J = 8.1 Hz), 7.01 (s, 1H), 6.57 (s, 1H), 6.89 (d, 2H, J = 8.1 Hz), 5.99 (s, 2H), 5.98 (s, 2H), 4.44–4.46 (m, 2H), 4.22 (d, 1H, J = 7.5 Hz), 3.94 (d, 1H, J = 9.9 Hz), 3.72 (m, 4H), 3.41 (s, 6H), 2.86 (m, 4H), 2.86–2.90 (m, 2H); 589.2191 $[M+H]^+$ (calcd. 589.2181)
10b	$CDCl_3$: 7.11 (s, 1H), 6.47 (s, 1H), 6.16 (s, 1H), 6.08 (s, 2H), 5.95 (d, 2H, J = 4.5 Hz), 4.39–4.45 (m, 1H), 4.33 (d, 1H, J = 5.1 Hz), 4.14 (d, 1H, J = 9.6 Hz), 3.87 (d, 1H, J = 7.8 Hz), 3.76 (s, 6H), 3.73 (s, 2H), 3.70 (m, 2H), 3.11–3.45 (m, 3H), 2.85 (dd, 1H, J = 4.8, 12.6 Hz), 2.45–2.51 (m, 6H); 541.2196 $[M+H]^+$ (calcd. 541.2181)
10c	$CDCl_3$: 7.08 (s, 1H), 6.60 (s, 1H), 6.48 (s, 1H), 6.11 (s, 2H), 5.93 (d, 2H, J = 2.1 Hz), 5.30 (s, 1H), 4.39–4.45 (m, 1H), 4.28 (d, 1H, J = 5.1 Hz), 4.13 (d, 1H, J = 9.9 Hz), 3.88 (d, 1H, J = 7.8 Hz), 3.76 (s, 6H), 3.15–3.36 (m, 3H), 2.88 (dd, 1H, J = 5.1, 12.6 Hz), 2.38–2.70 (m, 6H), 1.40–1.64 (m, 6H); 539.2390 $[M+H]^+$ (calcd. 539.2393)
10d	$CDCl_3$: 7.32 (m, 5H), 7.07 (s, 1H), 6.39 (s, 1H), 6.06 (s, 2H), 5.93 (s, 3H), 4.32–4.35 (m, 1H), 4.14 (d, 1H, J = 4.2 Hz), 4.04 (d, 1H, J = 10.2 Hz), 3.82 (d, 1H, J = 8.1 Hz), 3.73 (s, 6H), 3.52 (s, 2H), 3.17–3.23 (m, 1H), 2.79–2.85 (m, 2H), 2.70 (dd, 1H, J = 4.2, 12.0 Hz),1.42–2.12 (m, 7H); 601.2547 $[M+H]^+$ (calcd. 601.2544)
10e	$CDCl_3$: 7.71 (s, 1H), 7.35 (d, 2H, J = 8.4 Hz), 7.10 (s, 1H), 6.75 (d, 2H, J = 8.1 Hz), 6.43 (s, 1H), 6.08 (s, 2H), 5.96 (s, 2H), 5.38 (s, 1H), 4.42–4.47 (m, 1H), 4.35 (d, 1H, J = 4.5 Hz), 4.19 (d, 1H, J = 10.2 Hz), 3.88 (d, 1H, J = 7.8 Hz), 3.60 (s, 6H), 3.21–3.30 (m, 1H), 2.84–2.93 (m, 7H); 547.2077 $[M+H]^+$ (calcd. 547.2075)
10f	$CDCl_3$: 7.10 (s, 1H), 6.47 (s, 1H), 6.44 (s, 1H), 6.08 (s, 2H), 5.94 (d, 2H, J = 4.8 Hz), 4.40–4.45 (m, 1H), 4.27 (d, 1H, J = 4.5 Hz), 4.12 (d, 1H, J = 9.9 Hz), 3.85 (d, 1H, J = 7.8 Hz), 3.74 (s, 6H), 3.35–3.37 (m, 1H), 3.19–3.27 (m, 2H), 2.86 (dd, 1H, J = 5.1, 12.9 Hz), 2.38–2.46 (m, 2H), 2.28 (s, 6H); 499.2083 $[M+H]^+$ (calcd. 499.2075)
10g	$CDCl_3$: 8.45 (s, 1H), 7.41 (d, 2H, J = 7.8 Hz), 7.14 (d, 2H, J = 8.1 Hz), 7.06 (s, 1H), 6.33 (s, 1H), 6.02 (s, 2H), 5.96 (d, 2H, J = 10.2 Hz), 5.37 (s, 1H), 4.40–4.43 (m, 1H), 4.27 (d, 1H, J = 4.5 Hz), 4.14 (d, 1H, J = 9.9 Hz), 3.87 (d, 1H, J = 7.8 Hz), 3.53 (s, 6H), 3.20–3.30 (m, 1H), 2.76 (dd, 1H, J = 4.5, 12.0 Hz), 1.30–2.52 (m, 11H); 586.2449 $[M+H]^+$ (calcd. 586.2435)
10h	$CDCl_3$: 7.16–7.35 (m, 5H), 7.08 (s, 1H), 6.40 (s, 1H), 6.03 (s, 2H), 5.93 (d, 2H, J = 4.5 Hz), 5.70 (t, 1H, J = 4.5 Hz), 5.39 (s, 1H), 4.31–4.36 (m, 1H), 4.14 (d, 1H, J = 4.2 Hz), 3.98 (d, 1H, J = 9.9 Hz), 3.81 (d, 1H, J = 7.8 Hz), 3.74 (s, 6H), 3.42–3.66 (m, 2H), 3.08–3.12 (m, 1H), 2.83 (t, 2H, J = 5.4 Hz), 2.70 (dd, 1H, J = 5.1, 12.6 Hz); 532.1968 $[M+H]^+$ (calcd. 532.1966)

Table 3
Cytotoxicity of selected compounds.

IC_{50}[a] (10^{-6} mol/L)	**9b**	**9c**	**9d**	**9e**	**9f**	**10g**	**4**
A549	2.27	1.11	2.73	6.35	0.71	NA[b]	3.54
MCF-7	NA[b]	3.23	NA[b]	NA[b]	0.92	8.15	2.13

[a] IC_{50}: concentration that causes a 50% reduction of cell growth.
[b] NA: not active.

C4α-compounds display a $J_{3,4}$ above 10.0 Hz [6]. The $J_{3,4}$ values for **6**, **7** and **11** are 3.3, 4.2 and 5.7 Hz, respectively. Therefore, the C4-configuration of **6**, **7** and **11** is assigned as C4β.

All compounds were tested in parallel with GL-331 **4** against A549 and MCF-7 tumor cells with the MTT method *in vitro*. Preliminary results indicated that compound series **9** were generally more active than series **10**, and six compounds with considerable cytotoxicity are recorded in Table 3. The most active compounds **9c** and **9f** were comparable or superior to GL-331 in cytotoxicity.

In summary, based on previous SAR, two series of novel podophyllotoxin derivatives with different C4 linkage were designed and synthesized to enhance antitumor activity and improve bioavailability. It appears that the 4β-*p*-aminobenzamido series are more active than the 4β-amido series. Further analogue synthesis following this molecular design is ongoing and will be reported in due course.

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