

# Glucoside Derivatives Of Podophyllotoxin: Synthesis, Physicochemical Properties, And Cytotoxicity

This article was published in the following Dove Press journal:  
*Drug Design, Development and Therapy*

Cheng-Ting Zi<sup>1,2,\*</sup>  
Liu Yang<sup>2,\*</sup>  
Qing-Hua Kong<sup>2</sup>  
Hong-Mei Li<sup>2</sup>  
Xing-Zhi Yang<sup>2</sup>  
Zhong-Tao Ding<sup>3</sup>  
Zi-Hua Jiang<sup>4</sup>  
Jiang-Miao Hu<sup>2</sup>  
Jun Zhou<sup>2</sup>

<sup>1</sup>Key Laboratory of Pu-Er Tea Science, Ministry of Education, College of Science, Yunnan Agricultural University, Kunming, 650201, People's Republic of China;

<sup>2</sup>State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China; <sup>3</sup>Key Laboratory of Medicinal Chemistry for Nature Resource, Ministry of Education, School of Chemical Science and Technology, Yunnan University, Kunming 650091, People's Republic of China;

<sup>4</sup>Department of Chemistry, Lakehead University, Thunder Bay ON P7B 5E1, Canada

\*These authors contributed equally to this work

Correspondence: Zi-Hua Jiang  
Department of Chemistry, Lakehead University, 955 Oliver Road, Thunder Bay ON P7B 5E1, Canada  
Tel +1 807 766 7171  
Fax +1 807 346 7775  
Email [zjiang@lakeheadu.ca](mailto:zjiang@lakeheadu.ca)

Jiang-Miao Hu  
State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, No. 132, Lanhei Road, Kunming 650201, People's Republic of China  
Tel +86 871 6522 3264  
Fax +86 871 6522 3261  
Email [hujiangmiao@mail.kib.ac.cn](mailto:hujiangmiao@mail.kib.ac.cn)

**Background:** Widespread concern of the side effects and the broad-spectrum anticancer property of podophyllotoxin as an antitumor agent highlight the need for the development of new podophyllotoxin derivatives. Although some per-butyrylated glucosides of podophyllotoxin and 4 $\beta$ -triazolyl-podophyllotoxin glycosides show good anticancer activity, the per-acetylated/free of podophyllotoxin glucosides and their per-acetylated are not well studied.

**Methods:** A few glucoside derivatives of PPT were synthesized and evaluated for their in vitro cytotoxic activities against five human cancer cell lines, HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer), as well as the normal human pulmonary epithelial cell line (BEAS-2B). In addition, we investigated the structure–activity relationship and the physicochemical property–anticancer activity relationship of these compounds.

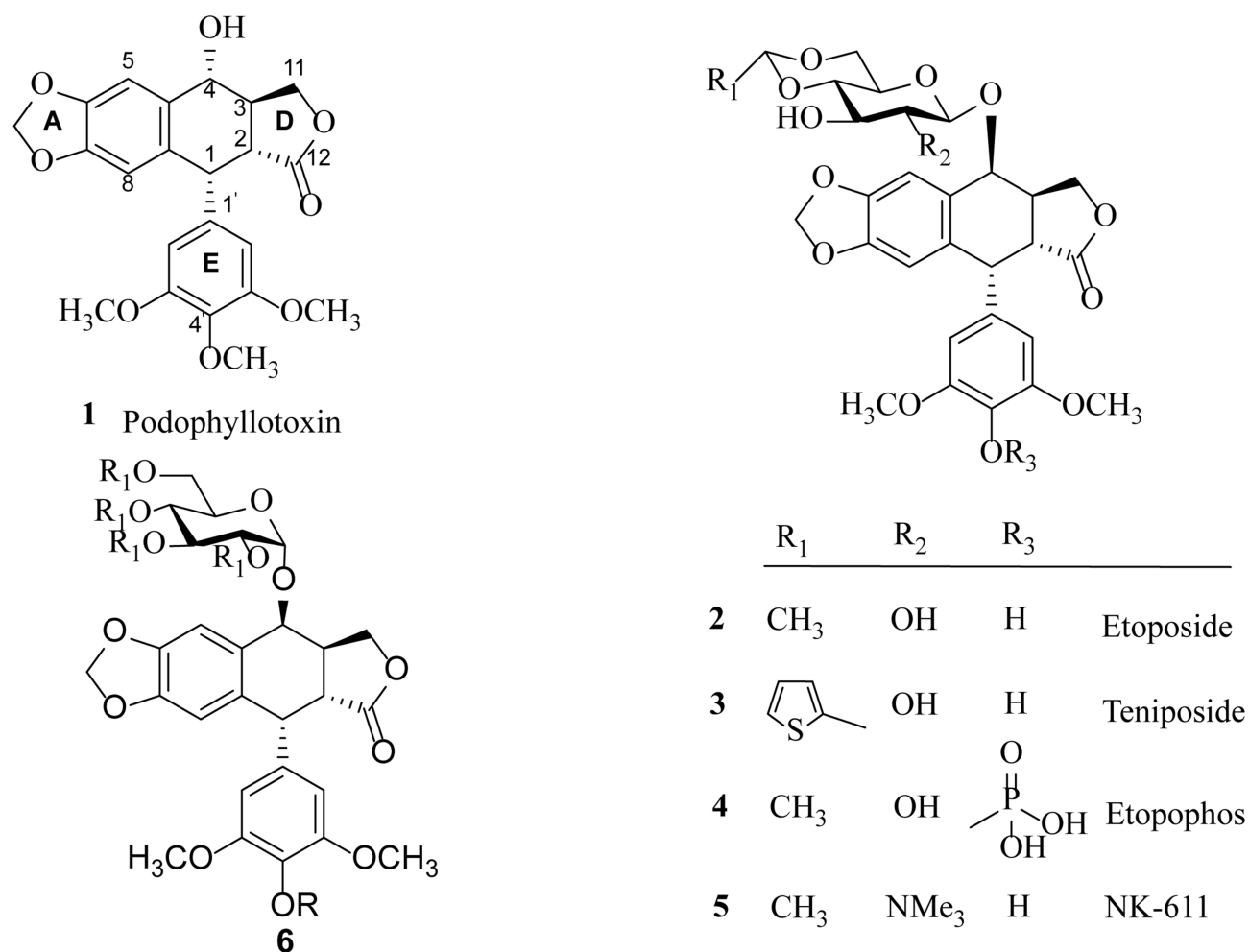
**Results:** Compound **6b** shows the highest cytotoxic potency against all five cancer cell lines tested, with IC<sub>50</sub> values ranging from 3.27 $\pm$ 0.21 to 11.37 $\pm$ 0.52  $\mu$ M. We have also found that **6b** displays higher selectivity than the etoposide except in the case of HL-60 cell line. The active compounds possess similar physicochemical properties: MSA > 900, %PSA < 20, ClogP > 2, MW > 700 Da, and RB > 10.

**Conclusion:** We synthesized several glucoside derivatives of PPT and tested their cytotoxicity. Among them, compound **6b** showed the highest cytotoxicity. Further studies including selectivity of active compounds have shown that the selectivity indexes of **6b** are much greater than the etoposide except in the case of HL-60 cell line. The active compounds possessed similar physicochemical properties. This study indicates that active glucoside analogs of podophyllotoxin have potential as lead compounds for developing novel anticancer agents.

**Keywords:** podophyllotoxin, glucoside, synthesis, cytotoxicity, physicochemical properties

## Introduction

Cancer is the second leading cause of death in the worldwide and remains one of the most difficult diseases to combat.<sup>1</sup> Developing new anticancer drugs and more effective treatment strategies for cancer is of great importance in medicinal chemistry. Natural products with diverse structures and unique biological activities are valuable sources for drug discovery. Close to 60% clinical drugs are either natural products or structural analogs of natural products with improved pharmacological activity.<sup>2–4</sup> Podophyllotoxin (PPT, **1**, Scheme 1), a well-known naturally occurring aryltetralin lignan, is mainly isolated from the roots of the North American *Podophyllum peltatum* Linnaeus, the Tibetan *P. emodi* Wall, or the Taiwanese species *Podophyllum peltatum*.<sup>5</sup> It shows strong cytotoxic activity against various cancer cell lines and acts at the colchicine-binding site on tubulin.<sup>6</sup>



**Scheme 1** Structure of compounds **1–6**: podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**), etopophos (**4**), NK-611 (**5**), and podophyllotoxin glucosides (**6**).

Due to its high toxicity and poor water solubility, podophyllotoxin has limited application as an anticancer drug. Based on its potent anticancer activity, **PPT** has served as a lead compound for the discovery and development of new anticancer agents. For example, the two semisynthetic glucosidic cyclic acetals of **PPT**, etoposide (**2**) and teniposide (**3**) (Scheme 1), are in clinical use for the treatment of a variety of cancers, including small-cell lung cancer, non-Hodgkin's lymphoma, leukemia, Kaposi's sarcoma, neuroblastoma, and soft-tissue sarcoma.<sup>7–10</sup> The mechanism of action for etoposide and teniposide is different from that of **PPT** in that both etoposide and teniposide block the DNA topoisomerase-II by stabilizing the enzyme–DNA complex.<sup>11–14</sup> However, the therapeutic use of etoposide and teniposide is often hindered by problems such as acquired drug resistance, myelosuppression, and their poor water solubility. To overcome the problems of etoposide and teniposide, further structure modifications of **PPT** have been carried out, which led to the synthesis of other **PPT**

derivatives, such as etopophos (**4**) and NK-611 (**5**) (Scheme 1), which reached clinical studies.<sup>15</sup> The clinically useful podophyllotoxin-derived glucosides **2–5** possess a 4,6-cyclic acetal moiety and various other substitutions on the sugar residue, suggesting the important role of substituents in modifying the biological activities of these podophyllotoxin derivatives.

In recent years, we have been working on the structural modification of podophyllotoxin and focused on glycosides of podophyllotoxin (such as **6**, Scheme 1) and 4 $\beta$ -triazolyl-podophyllotoxin.<sup>16–19</sup> Per-butyrylated glucosides of podophyllotoxin<sup>16</sup> as well as the glucosides of 4 $\beta$ -triazolyl-podophyllotoxin and their acylated analogues show good cytotoxicity.<sup>19,20</sup> The glucosides of podophyllotoxin and their per-acetylated analogues are less well studied.<sup>21</sup> In this article, a few glucoside derivatives of **PPT** were synthesized (Table S1) and evaluated for their in vitro cytotoxic activity against five human cancer cell lines,

HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer). To evaluate the selectivity of these compounds between tumor cells and normal cells, their growth inhibitory effect was tested on normal human pulmonary epithelial cell lines (BEAS-2B). In addition, the physicochemical properties of these compounds were calculated and correlated with their anticancer activity.

## Results And Discussion

### Chemical Synthesis

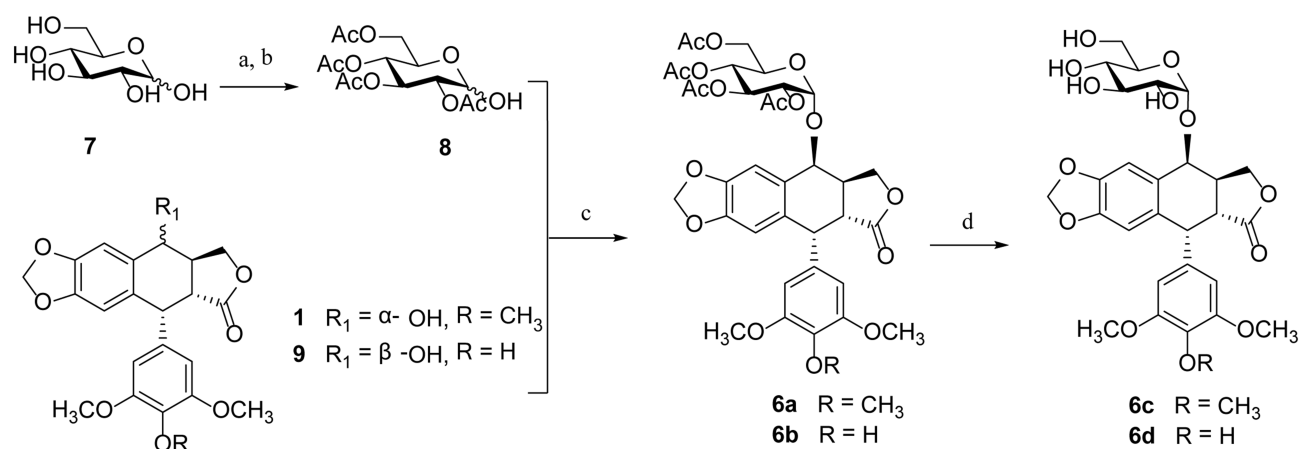
There have been several reports on constructing the glucosidic linkages of podophyllotoxin according to known literatures.<sup>22–25</sup> The synthesis of glucoside derivatives of podophyllotoxin **6a** – **6d** following a similar method is reported in the literature and is shown in Scheme 2. 1,2,3,4,6-Penta-*O*-acetyl- $\alpha/\beta$ -D-glucopyranose (mainly  $\alpha$ -form)<sup>26</sup> was treated with ammonia solution (25%) in acetonitrile to give 2,3,4,6-tetra-*O*-acetyl- $\alpha/\beta$ -D-glucopyranose (**8**) as an anomeric mixture ( $\alpha/\beta$  ratio = 6:1) in 46% yield.<sup>27,28</sup> Then, compound **8** was allowed to react with podophyllotoxin (**1**) and 4'-demethylepipodophyllotoxin (**9**)<sup>29</sup> in the presence of trifluoroborane etherate ( $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ) at  $-78^\circ\text{C}$  to give the per-acetylated glucoside derivatives of podophyllotoxin **6a** and **6b** in 58–62% yield.<sup>16</sup> Compounds **6a** and **6b** were treated with sodium methoxide in methanol at room temperature for 2 hrs to yield podophyllotoxin glucosides **6c** and **6d** in 78–80% yields.<sup>30</sup>

All the glucoside derivatives of PPT were characterized by  $^1\text{H}$  and  $^{13}\text{C}$ -NMR, electrospray ionization mass spectrometry (ESI-MS), and high-resolution mass spectrometry (HRESI-MS). The characteristic  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -

NMR data of compounds **6a** – **6d** are shown in Table 1. In the  $^1\text{H}$ -NMR spectra, the proton at C-4 of  $4\beta$ -substituted compounds appears as a doublet at 4.72–4.96 ppm, usually with a coupling constant  $J_{3,4} < 4.0$  Hz, indicating a *cis*-relationship between  $\text{C}^3\text{-H}$  and  $\text{C}^4\text{-H}$ .<sup>31</sup> The coupling constant of the anomeric proton of the glucose residue ( $J_{1'',2''}$ ) is typically  $< 4.0$  Hz, which confirms that the glycosidic linkage is  $\alpha$ -linkage.

### Evaluation Of Biological Activity

The per-butyrylated glucoside derivatives of podophyllotoxin **6e** and **6f** have been previously documented.<sup>16</sup> Per-acetylated glucoside derivatives of podophyllotoxin (**6a** and **6b**) and podophyllotoxin glucosides (**6c** and **6d**) were tested for their cytotoxicity against five human cancer cell lines, including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer). Etoposide (**2**) and cisplatin were taken as control drugs, and their  $\text{IC}_{50}$  data are presented in Figure 1 and Table 2. Compounds **6c** and **6d** having a free glucose residue show weak activity (all having  $\text{IC}_{50} > 40 \mu\text{M}$ ), while peracetylated glucoside derivatives **6a** and **6b** show improved activity. Among these derivatives, compound **6b** shows the highest cytotoxicity against five cancer cells, with their  $\text{IC}_{50}$  values ranging from  $3.27 \pm 0.21$  to  $11.37 \pm 0.52 \mu\text{M}$ , which is more potent than the control drug etoposide against the MCF-7 and SW480 cell lines. In our previous study, we reported that the cytotoxic activity of  $4\beta$ -triazolyl-podophyllotoxin derivatives with a peracetylated glucose residue mostly shows weak activity.<sup>19</sup> Furthermore, compound **6b** with a hydroxy group at the C-4' position in the E ring is more



**Scheme 2** Synthesis of glucoside derivatives of PPT **6a** – **6d**. Reagents and conditions: (A)  $\text{Ac}_2\text{O}$ , sodium acetate,  $100^\circ\text{C}$ , 20 mins, ~99%; (B)  $\text{NH}_3 \cdot \text{H}_2\text{O}$ ,  $\text{CH}_3\text{CN}$ , rt, overnight, 46%; (C)  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ ,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$  to rt, 58–62%; (D)  $\text{CH}_3\text{ONa}$ ,  $\text{CH}_3\text{OH}$ , 2 hrs, rt, 78–80%.

**Table 1** The Characteristic  $^1\text{H}$ -NMR And  $^{13}\text{C}$ -NMR Data Of Compounds **6a** – **6d**

Compound	$^1\text{H}$ -NMR				$^{13}\text{C}$ -NMR		4-Configuration
	C <sup>4</sup> -H (ppm)	J <sub>3-4</sub> (Hz)	C <sup>1'</sup> -H (ppm)	J <sub>1'-2''</sub> (Hz)	C-4 (ppm)	C-1' (ppm)	
6a	4.76	2.7	5.22	3.2	75.7	95.7	$\beta$
6b	4.72	3.8	5.35	3.2	75.7	95.7	$\beta$
6c	4.78	3.4	5.01	3.6	75.3	99.4	$\beta$
6d	4.77	3.4	4.99	3.6	75.3	99.4	$\beta$
PPT <sup>a</sup>	4.96	7.4	–	–	72.6	–	$\alpha$

Note: <sup>a</sup>Data from Hartwell et al.<sup>38</sup>

active than compound **6a** which has a methoxyl group at the C-4' position.

Cancer chemotherapy is often associated with low/non-selectivity of cancer drugs which attack cancer cells as well as normal cells, leading to serious side effects. In order to test their selectivity, compounds **6a** and **6b** were tested for their growth inhibitory effects on a normal human bronchial epithelial cell line (BEAS-2B) (Table 2). The selectivity index (SI) was expressed as the ratio of the IC<sub>50</sub> value of the compound in normal cell line over that in cancer cell line. A larger SI value indicates that the drug displays higher selectivity toward cancer cells over normal cells.<sup>32,33</sup>

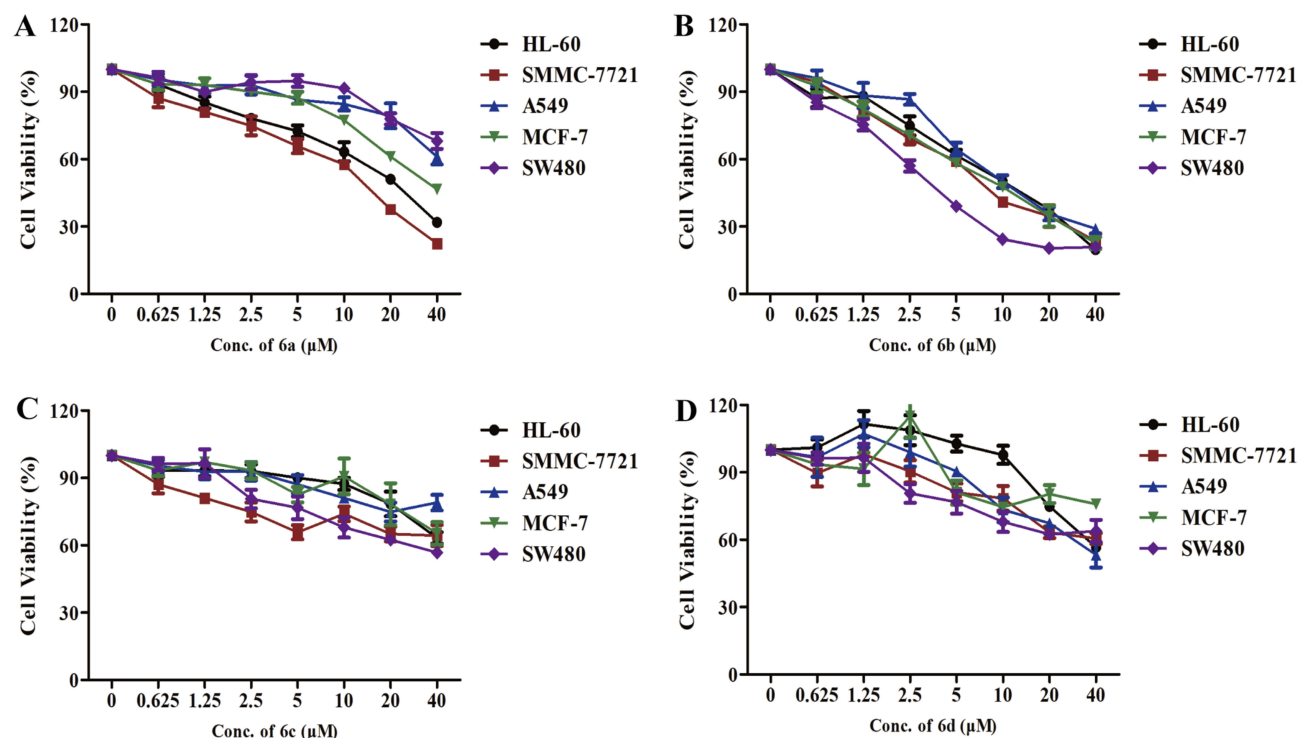
The SI values of compound **6a**, **6b** and etoposide are presented in Table 3. Compound **6b** shows moderate selectivity

toward cancer cells with SI values in the range of 1.9–6.7 in all cells tested. Compound **6b** displays higher selectivity than etoposide in four of the five cancer cell lines tested except an HL-60 cell line. Among these derivatives, **6b** shows the highest potency (IC<sub>50</sub> 3.27±0.21  $\mu\text{M}$ ) and highest selectivity (SI 6.7) in SW480 cell line, suggesting that **6b** may be a promising therapeutic agent for colon cancer.

## Physicochemical Property–Cytotoxicity Relationship

### Values Of Partition Coefficient Of The Compounds

The logarithm of the octanol–water partition coefficient investigation (log*P*) is an important pharmaceutical parameter in evaluating solvency, absorption, and transport of drugs; the



**Figure 1** Inhibitory effects of podophyllotoxin derivatives on cancer cells. (A–D) The inhibitory effects of compounds **6a** – **6d** on HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer) cells, as evaluated by the MTT assay.

**Table 2** Cytotoxicity Of Podophyllotoxin Derivatives **6a** – **6f** In Vitro<sup>a</sup>

Compound	IC <sub>50</sub> (μM)					
	HL-60	SMMC-7721	A-549	MCF-7	SW480	BEAS-2B
6a	21.36±0.38	14.50±0.56	>40	36.55±0.78	>40	30.60±0.54
6b	11.37±0.52	8.41±0.48	10.74±0.37	9.18±0.49	3.27±0.21	21.78±0.36
6c	>40	>40	>40	>40	>40	NT
6d	>40	>40	>40	>40	>40	NT
6e <sup>b</sup>	>40	>40	>40	>40	>40	NT
6f <sup>b</sup>	16.87±0.32	16.82±0.12	16.04±0.73	39.13±0.52	38.72±0.92	NT
2	0.31±0.24	8.12±0.72	11.92±0.12	32.82±0.44	17.11±0.67	11.17±0.56
Cisplatin	1.17±0.34	6.43±0.57	9.24±0.36	15.56±0.52	13.42±0.44	NT

**Note:** <sup>a</sup>Values are means of three independent experiments; <sup>b</sup>Experimental data of compounds **6e** and **6f** from ref.<sup>16</sup>

**Abbreviation:** NT, not tested.

**Table 3** Selectivity Of The Cytotoxicity Of Compounds **6a**, **6b**, And Etoposide To Cancer Cells As Compared With BEAS-2B Normal Cells

Compound	Selectivity Index (SI) <sup>a</sup>				
	HL-60	SMMC-7721	A-549	MCF-7	SW480
6a	1.4	2.1	–	0.8	–
6b	1.9	2.6	2.0	2.4	6.7
2	36.0	1.4	0.9	0.3	0.7

**Note:** <sup>a</sup>Selectivity index (SI) = IC<sub>50</sub> of the compound in BEAS-2B cell line/IC<sub>50</sub> of the compound in cancer cell line.

preferred log $P$  value is less than 5.<sup>11</sup> Compounds etoposide (**2**) and the most potent compound **6b** were measured for values of log $P$ . Solutes were equilibrated between octanol and water. The concentration of compounds in octanol was determined by the HPLC method.<sup>12,13</sup> The log $P$  values of compounds **2** and **6b** were determined to be 1.44 and 1.78 at 30°C. As shown in Table 4 (see [supporting information](#) for the details), compound **6b** expressed the log $P$  value and was close to the calculated value of 2.24.

### Solubility

Poor water solubility is a common problem in developing podophyllotoxin derivatives for therapeutic use. Compounds with glucose residue are slightly soluble in water. The solubility of podophyllotoxin (**1**) and compounds **6b** in aqueous at temperature 25°C are reported **1** has a solubility of 2.2 mg/mL in water, while **6b** with a peracetylated glucoside residue has a solubility of 1.7 mg/mL in water (see [supporting information](#) for the details). The solubility values obtained for **6b** become unfairly soluble in water.

### Physicochemical Property

The physicochemical properties of a drug can largely affect the pharmacokinetics and efficacy of a drug. The physico-

chemical properties of glucoside derivatives of podophyllotoxin **6a** – **6d** and **6e** – **6f**<sup>19</sup> were calculated and compared with etoposide **2**, which include molecular weight (MW), molecular surface area (MSA), polar surface area (PSA), relative polar surface area (%PSA), calculated partition coefficient (ClogP), calculated distribution coefficient at pH 7.4 (ClogD<sub>7.4</sub>), hydrogen bond donor (HD), hydrogen bond acceptor (HA), and rotatable bond (RB) (Table 4). Noteworthy is that almost all active compounds (having IC<sub>50</sub> < 40 μM) are relatively lipophilic (MSA > 900, % PSA < 20, ClogP > 2), and since they have a higher molecular weight (MW > 700 Da) and a larger number of rotatable bonds (RB > 10), with the exception of compound **6e**, they are placed at an advantage for further optimization. By contrast, inactive compounds **6c** and **6d** (having a free glucose residue) have %PSA values >22, ClogD<sub>7.4</sub> <0, and a smaller number of rotatable bonds (RB < 10). It is obvious that derivatives with free glucose residues (**6c** and **6d**) are relatively more polar, and this might account for the general lack of activity for these compounds. This result suggests that the peracetylated/perbutyrylated derivatives of podophyllotoxin glucosides may, therefore, be more suitable for further optimization.

**Table 4** Physicochemical Properties Of Glucoside Derivatives Of Podophyllotoxin

Compound	Physicochemical Properties								
	MW	MSA	PSA	%PSA <sup>a</sup>	ClogP <sup>b</sup>	ClogD7.4 <sup>c</sup>	H-D <sup>d</sup> /H-A <sup>e</sup>	RB <sup>f</sup>	logP <sup>g</sup>
6a	745	1006	196.1	19.5	2.57	1.62	0/12	15	logP <sup>g</sup>
6b	731	968	207.0	21.4	2.24	1.47	1/12	14	NT
6c	576	755	171.8	22.8	0.43	-0.15	4/12	7	2.14
6d	562	716	182.8	25.5	0.29	-0.30	5/12	6	NT
6e	857	1257	196.1	15.6	6.80	6.20	0/12	23	NT
6f	843	1218	207.1	17.0	6.47	6.05	1/12	22	NT
2	588	758	160.8	21.2	0.03	1.16	3/12	5	NT

**Note:** <sup>a</sup>%PSA: relative polar surface area = (PSA/MSA) × 100; <sup>b</sup>ClogP: calculated partition coefficient; <sup>c</sup>ClogD<sub>7.4</sub>: calculated distribution coefficient at pH 7.4; <sup>d</sup>HD: hydrogen bond donor count; <sup>e</sup>HA: hydrogen bond acceptor count; <sup>f</sup>RB: rotatable bond count; <sup>g</sup>logP: value for log octanol-water partition coefficients.

**Abbreviations:** MW, molecular weight; MSA, molecular surface area; PSA, polar surface area; NT, Not tested.

## Chemical Stability Investigation

The most potent compound **6b** was selected for investigations of chemical stability in aqueous phase with comparison of podophyllotoxin (**1**). The results indicate that compound **6b** exhibits better chemical stability under the specific conditions (37°C, pH = 7.0, Figure 2) (see [supporting information](#) for the details). Obviously, **6b** showed considerable stability with podophyllotoxin.

## Experimental

### General

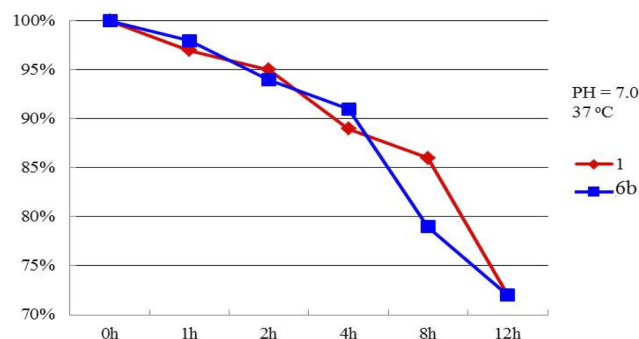
All cancer cells (HL-60, SMMC-7721, A-549, MCF-7, and SW480) were obtained from a Shanghai cell bank in China. D-glucose was purchased from Aladdin Chemical Co., Ltd (Guangzhou, China); podophyllotoxin was obtained from Chengdu Proifa Technology Development Co., Ltd (Chengdu, China); boron trifluoride etherate was obtained from J&K Chemical Technology Co., Ltd (Beijing China); 3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane and acetonitrile were distilled

over calcium hydride. All reagents were commercially available and used without further purification unless indicated otherwise. The melting points were measured by an X-4 melting point apparatus and were uncorrected. Optical rotations were obtained with a Jasco P-1020 Automatic Digital Polariscopes. MS data were obtained in the ESI mode on API Qstar Pulsar instrument; HRMS data were obtained in the ESI mode on LCMS-IT-TOF (Shimadzu, Kyoto, Japan); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded on Bruker AVANCE III 400 MHz, or 600 MHz (Bruker BioSpin GmbH, Rheinstetten, Germany) instruments, using tetramethylsilane (TMS) as an internal standard: chemical shifts (δ) are given in ppm, coupling constants (J) in Hz, and the solvent signals were used as references (CDCl<sub>3</sub>: δ<sub>C</sub> = 77.2 ppm; residual CHCl<sub>3</sub> in CDCl<sub>3</sub>: δ<sub>H</sub> = 7.26 ppm; CD<sub>3</sub>OD: δ<sub>C</sub> = 49.0 ppm; residual CH<sub>3</sub>OH in CD<sub>3</sub>OD: δ<sub>H</sub> = 4.78 ppm). Column chromatography (CC): silica gel (200–300 mesh; Qingdao Makall Group CO., LTD; Qingdao, China). All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates.

## Chemistry

### Synthesis Of 2,3,4,6-Tetra-O-Acetyl-α/β-D-Glucopyranose (**8**)

D-glucose (1.8 g, 10 mmol) was suspended in acetic anhydride (9.5 mL, 100 mmol) and anhydrous sodium acetate (0.9 g, 11 mmol) was added, and the resulting mixture was heated at 100°C for 20 mins. The reaction was quenched (saturated aqueous sodium bicarbonate, 20 mL) and diluted with dichloromethane (30 mL); the organic layer was washed with brine (3 × 30 mL) and dried with sodium sulfate. The solvent was evaporated, and the residue dried in vacuo to give the crude 1,2,3,4,6-penta-O-acetyl-D-glucopyranose.

**Figure 2** Chemical stability investigation of compounds **1** and **6b**.

The crude 1,2,3,4,6-penta-*O*-acetyl- $\alpha$ -D-glucopyranose was dissolved in acetonitrile (20 mL), and 25% ammonia solution (0.4 mL, 20 mmol) was added dropwise slowly. The mixture was stirred at room temperature for 6 hrs. The solvent was evaporated, and the brown oily residue was passed a short pad of silica column (petroleum ether/ethyl acetate 4:1, *v/v*) to afford the product **8** (1.6 g, 46% yield for two steps).  $\alpha/\beta$  ratio = 6:1.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.20 (d, 1/7H,  $J = 8.0$  Hz,  $\text{C}^1\text{-H}_\beta$ ), 5.54 (t, 1H,  $J = 9.6$  Hz,  $\text{C}^3\text{-H}$ ), 5.47 (d, 6/7H,  $J = 3.2$  Hz,  $\text{C}^1\text{-H}_\alpha$ ), 5.08 (t, 1H,  $J = 9.6$  Hz,  $\text{C}^4\text{-H}$ ), 4.91 (dd, 1H,  $J = 3.2$  Hz, 10.0 Hz,  $\text{C}^2\text{-H}$ ), 4.27–4.23 (m, 2H,  $\text{C}^6\text{-CH}_2$ ), 4.14–4.12 (m, 1H,  $\text{C}^5\text{-H}$ ), 2.10–2.00 (m, 12H, 4  $\times$   $\text{OCH}_3$ );  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{Cl}$ , 400 MHz)  $\delta$  170.8 (C=O), 170.2 (C=O), 170.1 (C=O), 169.7 (C=O), 95.5 (C-1 $_\beta$ ), 90.1 (C-1 $_\alpha$ ), 73.2 (C-5 $_\beta$ ), 72.1 (C-4 $_\beta$ ), 72.0 (C-2 $_\beta$ ), 71.9 (C-5 $_\alpha$ ), 69.8 (C-4 $_\alpha$ ), 68.4 (C-2 $_\alpha$ ), 68.3 (C-3 $_\beta$ ), 67.2 (C-3 $_\alpha$ ), 61.9 (C-6), 20.7 ( $\text{OCH}_3$ ), 20.7 ( $\text{OCH}_3$ ), 20.6 ( $\text{OCH}_3$ ), 20.5 ( $\text{OCH}_3$ ); ESIMS:  $m/z$  371  $[\text{M} + \text{Na}]^+$ .

#### Synthesis Of 4'-Demethylepipodophyllotoxin (9)

4'-Demethylepipodophyllotoxin (**9**) was prepared according to the literature.<sup>29</sup>

### General Procedure For The Synthesis Of Compounds 6a – 6b

To a mixture of 2,3,4,6-tetra-*O*-acetyl- $\alpha/\beta$ -D-glucopyranose (0.2 mmol) and podophyllotoxin/4'-demethylepipodophyllotoxin (0.2 mmol) in dry  $\text{CH}_2\text{Cl}_2$  (3 mL) was added of  $\text{BF}_3 \cdot \text{H}_2\text{O}$  (25  $\mu\text{L}$ , 0.02 mmol) at  $-78^\circ\text{C}$ , and the resulting mixture was stirred for 1 hr. Then, triethylamine (0.1 mL) was added to the mixture, and acetic acid (0.1 mL) was added. The solvent was evaporated, and the residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 2:1, *v/v*) to afford the major product **6a** or **6b** as white powder.

#### 4-O-(2',3',4',6'-Tetra-O-Acetyl- $\alpha$ -D-Glucopyranosyl)-Epipodophyllotoxin (6a)

White powder; yield 58%; mp 167–168  $^\circ\text{C}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.99 (s, 1H,  $\text{C}^6\text{-H}$ ), 6.55 (s, 1H,  $\text{C}^8\text{-H}$ ), 6.23 (s, 2H,  $\text{C}^{2'}$ ,  $\text{C}^{6'}$ -H), 6.00–5.98 (m, 2H,  $\text{OCH}_2\text{O}$ ), 5.33 (t, 1H,  $J = 8.0$  Hz), 5.22 (d, 1H,  $J = 3.2$  Hz,  $\text{C}^{1''}$ -H), 5.08–5.02 (m, 2H), 4.76 (d, 1H,  $J = 2.7$  Hz,  $\text{C}^4\text{-H}$ ), 4.66–4.65 (m, 1H), 4.30 (d, 1H,  $J = 2.1$  Hz,  $\text{C}^1\text{-H}$ ), 4.28–4.18 (m, 2H), 4.14–4.03 (m, 1H), 3.79 (s, 3H,  $\text{C}^{4'}\text{-OCH}_3$ ), 3.76 (s, 6H,  $\text{C}^{3'}$ ,  $\text{C}^{5'}\text{-OCH}_3$ ), 3.45–3.42 (m, 1H,  $\text{C}^3\text{-H}$ ), 3.01–2.98 (m, 1H,  $\text{C}^2\text{-H}$ ), 2.14–2.04 (m, 12H, 4  $\times$   $\text{OCH}_3$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  177.8 (C-12), 170.6 (C=O), 170.0

(C=O), 169.3 (C=O), 169.2 (C=O), 153.4 (C-3', C-5'), 147.9 (C-6), 146.7 (C-7), 137.5 (C-4'), 136.9 (C-1'), 131.5 (C-9), 128.4 (C-10), 109.7 (C-8), 106.8 (C-5), 105.4 (C-2', C-6'), 101.3 ( $\text{OCH}_2\text{O}$ ), 95.7 (C-1'), 75.7 (C-4), 72.3, 71.0, 69.4, 68.2, 67.7 (C-11), 60.8 (4'- $\text{OCH}_3$ ), 60.7, 56.2 (3', 5'- $\text{OCH}_3$ ), 44.9 (C-2), 44.2 (C-1), 38.1 (C-3), 20.7 ( $\text{OCH}_3$ ), 20.6 ( $\text{OCH}_3$ ), 20.6 ( $\text{OCH}_3$ ), 20.5 ( $\text{OCH}_3$ ); ESIMS:  $m/z$  767  $[\text{M} + \text{Na}]^+$ , HRESIMS: calcd for  $\text{C}_{36}\text{H}_{40}\text{O}_{17}\text{Na}$   $[\text{M} + \text{Na}]^+$  767.2285, found 767.2286.

#### 4-O-(2',3',4',6'-Tetra-O-Acetyl- $\alpha$ -D-Glucopyranosyl)-4'-Demethylepipodophyllotoxin (6b)

White powder; yield 62%; mp 172–174  $^\circ\text{C}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  6.94 (s, 1H,  $\text{C}^6\text{-H}$ ), 6.55 (s, 1H,  $\text{C}^8\text{-H}$ ), 6.45 (s, 2H,  $\text{C}^{2'}$ ,  $\text{C}^{6'}$ -H), 5.97–5.95 (m, 2H,  $\text{OCH}_2\text{O}$ ), 5.40 (t, 1H,  $J = 8.0$  Hz), 5.35 (d, 1H,  $J = 3.2$  Hz,  $\text{C}^{1''}$ -H), 5.10–5.07 (m, 1H), 4.83–4.80 (m, 1H), 4.72 (d, 1H,  $J = 3.8$  Hz,  $\text{C}^4\text{-H}$ ), 4.41 (d, 1H,  $J = 2.1$  Hz,  $\text{C}^1\text{-H}$ ), 4.32–4.31 (m, 1H), 4.22–4.21 (m, 1H), 4.09–4.07 (m, 1H), 3.75 (s, 6H,  $\text{C}^{3'}$ ,  $\text{C}^{5'}\text{-OCH}_3$ ), 3.67–3.64 (m, 1H), 3.45–3.42 (m, 1H,  $\text{C}^3\text{-H}$ ), 3.00–2.97 (m, 1H,  $\text{C}^2\text{-H}$ ), 2.15–2.09 (m, 12H, 4  $\times$   $\text{OCH}_3$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$  177.7 (C-12), 170.5 (C=O), 170.0 (C=O), 169.3 (C=O), 169.2 (C=O), 152.3 (C-3', C-5'), 148.0 (C-6), 146.8 (C-7), 140.4 (C-4'), 140.4 (C-1'), 131.1 (C-9), 128.4 (C-10), 109.8 (C-8), 106.8 (C-5), 104.9 (C-2', C-6'), 101.3 ( $\text{OCH}_2\text{O}$ ), 95.7 (C-1'), 75.7 (C-4), 71.0, 69.5, 68.2, 67.8, 67.5 (C-11), 60.7, 56.2 (3', 5'- $\text{OCH}_3$ ), 45.0 (C-2), 44.3 (C-1), 38.0 (C-3), 20.7 ( $\text{OCH}_3$ ), 20.6 ( $\text{OCH}_3$ ), 20.6 ( $\text{OCH}_3$ ), 20.5 ( $\text{OCH}_3$ ); ESIMS:  $m/z$  756  $[\text{M} + \text{Na}]^+$ , HRESIMS: calcd for  $\text{C}_{35}\text{H}_{38}\text{O}_{17}\text{Na}$   $[\text{M} + \text{Na}]^+$  756.2123, found 756.2126.

### General Procedure For The Synthesis Of Compounds 6c – 6d

To a solution of **6a/6b** (0.1 mmol) in methanol (1.5 mL) was added sodium methoxide (0.03 mmol) at  $0^\circ\text{C}$ , and the resulting mixture was stirred for 2 hrs. The reaction was slowly quenched (anhydrous Amberlite ion-exchange resin IRA-400), and the resin was removed by filtration. The filtrate was concentrated under vacuum, and the residue was purified by flash chromatography on silica gel (chloroform/methanol 9:1, *v/v*) to afford compound **6c** or **6d** as white powder.

#### 4-O-( $\alpha$ -D-Glucopyranosyl)-Epipodophyllotoxin (6c)

White powder; yield 80%; mp 190–191  $^\circ\text{C}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 600 MHz)  $\delta$  7.07 (s, 1H,  $\text{C}^6\text{-H}$ ), 6.51 (s, 2H,

C<sup>2'</sup>, C<sup>6'</sup>-H), 6.48 (s, 1H, C<sup>8</sup>-H), 5.94–5.93 (m, 2H, OCH<sub>2</sub>O), 5.01 (d, 1H, *J* = 3.6 Hz, C<sup>1''</sup>-H), 4.78 (d, 1H, *J* = 3.4 Hz, C<sup>4</sup>-H), 4.47–4.45 (m, 1H), 4.42 (t, 1H, *J* = 9.6 Hz), 4.36 (d, 1H, *J* = 2.2 Hz, C<sup>1</sup>-H), 3.77 (s, 6H, C<sup>3'</sup>, C<sup>5'</sup>-OCH<sub>3</sub>), 3.76 (s, 3H, C<sup>4'</sup>-OCH<sub>3</sub>), 3.69–3.63 (m, 2H), 3.55–3.53 (m, 1H), 3.41–3.38 (m, 2H), 3.36–3.33 (m, 1H), 3.24–3.21 (m, 1H, C<sup>3</sup>-H), 3.16–3.13 (m, 1H, C<sup>2</sup>-H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  178.7 (C-12), 154.7 (C-3', C-5'), 149.0 (C-6), 148.2 (C-7), 139.5 (C-4'), 137.9 (C-1'), 132.6 (C-9), 131.2 (C-10), 110.1 (C-8), 108.2 (C-5), 106.9 (C-2', C-6'), 102.5 (OCH<sub>2</sub>O), 99.4 (C-1'), 75.3 (C-4), 75.0, 74.3, 73.8, 71.1, 70.0 (C-11), 61.7, 61.1 (4'-OCH<sub>3</sub>), 56.6 (3', 5'-OCH<sub>3</sub>), 46.6 (C-2), 45.4 (C-1), 38.7 (C-3); ESIMS: *m/z* 575 [M - H]<sup>-</sup>, HRESIMS: calcd for C<sub>28</sub>H<sub>32</sub>O<sub>13</sub> [M - H]<sup>-</sup> 576.1843, found 576.1846.

#### 4-O-( $\alpha$ -D-Glucopyranosyl)-4'-Demethylepipodophyllotoxin (6d)

White powder; yield 78%; mp 201–203 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  7.07 (s, 1H, C<sup>6</sup>-H), 6.49 (s, 1H, C<sup>8</sup>-H), 6.47 (s, 2H, C<sup>2'</sup>, C<sup>6'</sup>-H), 5.93–5.92 (m, 2H, OCH<sub>2</sub>O), 4.99 (d, 1H, *J* = 3.6 Hz, C<sup>1''</sup>-H), 4.82–4.81 (m, 1H), 4.77 (d, 1H, *J* = 4.4 Hz, C<sup>4</sup>-H), 4.46–4.39 (m, 2H), 4.33 (d, 1H, *J* = 2.2 Hz, C<sup>1</sup>-H), 3.78 (s, 6H, C<sup>3'</sup>, C<sup>5'</sup>-OCH<sub>3</sub>), 3.70–3.62 (m, 2H), 3.56–3.53 (m, 1H), 3.41–3.34 (m, 3H), 3.36–3.33 (m, 1H), 3.26–3.23 (m, 1H, C<sup>3</sup>-H), 3.20–3.18 (m, 1H, C<sup>2</sup>-H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  178.8 (C-12), 149.5 (C-3', C-5'), 148.9 (C-6), 148.2 (C-7), 135.5 (C-4'), 133.9 (C-1'), 132.9 (C-9), 131.2 (C-10), 110.2 (C-8), 108.1 (C-5), 106.4 (C-2', C-6'), 102.5 (OCH<sub>2</sub>O), 99.4 (C-1'), 75.3 (C-4), 75.0, 74.3, 73.8, 71.1, 69.9 (C-11), 61.7, 56.8 (3', 5'-OCH<sub>3</sub>), 46.5 (C-2), 45.6 (C-1), 38.7 (C-3); ESIMS: *m/z* 561 [M - H]<sup>-</sup>, HRESIMS: calcd for C<sub>27</sub>H<sub>30</sub>O<sub>13</sub> [M - H]<sup>-</sup> 561.1686, found 561.1684.

#### Cytotoxicity Assay

The following five human cancer lines were used in the cytotoxicity assay: human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480). All the cells were cultured in RMPI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% FBS (Hyclone, USA) in 5% CO<sub>2</sub> at 37°C. The cytotoxicity assay was performed according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] method in 96-well microplates.<sup>34</sup> Briefly, adherent cells (100  $\mu$ L) were seeded into each well of a 96-well

cell culture plate and allowed to adhere for 12 hrs before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of  $1 \times 10^5$  cells/mL in 100  $\mu$ L of the medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 hrs. After the incubation, MTT (100  $\mu$ g) was added to each well, and the incubation continued for 4 hrs at 37°C. The cells were lysed with SDS (200  $\mu$ L) after removal of 100  $\mu$ L of the medium. The optical density of lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). IC<sub>50</sub> values were calculated by Reed and Muench's method.<sup>35,36</sup>

#### Calculated Molecular Physicochemical Properties

All structures of podophyllotoxin derivatives were built and energy minimized by the Tripos force field with 0.05 kcal/(mol Å). The Gasteiger–Huchel method was used to calculate charges. Energy minimization was performed by the Powell method with 2000 iterations. Molecular surface area (MSA), polar surface area (PSA), calculated partition coefficient (ClogP), calculated solubility (ClogS), hydrogen bond donor (HD), hydrogen bond acceptor (HA) and rotatable bond (RB) were obtained from MarvinSketch version 5.3.8. ([www.chemaxon.org](http://www.chemaxon.org)).<sup>37</sup>

#### Conclusion

In conclusion, we synthesized a few glucoside derivatives of podophyllotoxin and screened for cytotoxicity against a panel of five human cancer cell lines including HL-60 (leukemia), SMMC-7721 (hepatoma), A-549 (lung cancer), MCF-7 (breast cancer), and SW480 (colon cancer). Derivatives having a free glucose residue show weak activity (IC<sub>50</sub> > 40  $\mu$ M), while the peracetylated derivative **6b** shows the highest cytotoxic potency against all five cancer cell lines tested, with IC<sub>50</sub> values ranging from 3.27 $\pm$ 0.21 to 11.37 $\pm$ 0.52  $\mu$ M. Compound **6b** also displays moderate selectivity toward cancer cells over normal human pulmonary epithelial cells (BEAS-2B). The calculated physicochemical properties of these PPT derivatives indicated that more lipophilic compounds are generally more cytotoxic to cancer cells. Our results suggest that some of these compounds have potential as lead compounds for developing novel anticancer agents.

#### Acknowledgment

We are grateful to the National Nature Science Foundation of China for financial support (No. 21602196); the Yunnan

Provincial Science and Technology Department (Nos. 2017ZF003, 2017FD084, and 2017FG001-046); and Yunnan Agricultural University Natural Science Foundation for Young Scientists (No. 2015ZR08).

## Disclosure

The authors declare no conflicts of interest in this work.

## References

- Gomes TRH, Fernando MH, Regnia CM. Global trends in nanomedicine research on triple negative breast cancer: a bibliometric analysis. *Int J Nanomedicine*. 2018;13:2321–2336. doi:10.2147/ijn.s164355
- Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. *J Nat Prod*. 2003;66:1022–1037. doi:10.1021/np030096l
- Newman DJ. Natural products as leads to potential drugs: an old process or the new hope for drug discovery? *J Med Chem*. 2008;51:2589–2599. doi:10.1021/jm0704090
- Ojima I. Modern natural products chemistry and drug discovery. *J Med Chem*. 2008;51:2587–2588. doi:10.1021/jm701291u
- MacRae WD, Hudson JB, Towers BH. The antiviral action of lignans. *Planta Med*. 1989;55:531–535. doi:10.1055/s-2006-962087
- Umesha B, Basavaraju YB, Mahendra C. Synthesis and biological screening of pyrazole moiety containing analogs of podophyllotoxin. *Med Chem Res*. 2015;24:142–151. doi:10.1007/s00044-014-1100-3
- You YJ. Podophyllotoxin derivatives: current synthetic approaches for new anticancer agents. *Curr Pharm Des*. 2005;11:1695–1717. doi:10.2174/1381612053764724
- Gordaliza M, Garcia PA, Miguel Del Corral JM, Castro MA, Gomez-Zurita MA. Podophyllotoxin: distribution, sources, applications and new cytotoxic derivatives. *Toxicol*. 2004;44:441–459. doi:10.1016/j.toxicol.2004.05.008
- Lv M, Xu H. Recent advances in semisynthesis, biosynthesis, biological activities, mode of action, and structure-activity relationship of podophyllotoxins: an update (2008–2010). *Mini-Rev Med Chem*. 2011;11:901–909. doi:10.2174/138955711796575461
- Zhang X, Rakesh KP, Shantharam CS, et al. Podophyllotoxin derivatives as an excellent anticancer aspirant for future chemotherapy: a key current imminent needs. *Bioorg Med Chem*. 2018;26:340–355. doi:10.1016/j.bmc.2017.11.026
- Lee KH, Imakura Y, Haruna M, et al. Antitumor agents. 107. New cytotoxic 4-alkylamino analogues of 4'-demethyl-epipodophyllotoxin as inhibitors of human DNA topoisomerase II. *J Nat Prod*. 1989;52:606–613. doi:10.1021/np50063a021
- Le KH, Beers SA, Mori M, et al. Antitumor agents. 111. New 4-hydroxylated and 4-halogenated anilino derivatives of 4'-demethylepipodophyllotoxin as potent inhibitors of human DNA topoisomerase II. *J Med Chem*. 1990;33:1364–1368. doi:10.1021/jm00167a013
- Wang ZQ, Kuo YH, Schnur D, et al. Antitumor agents. 113. New 4 beta-arylamino derivatives of 4'-O-demethylepipodophyllotoxin and related compounds as potent inhibitors of human DNA topoisomerase II. *J Med Chem*. 1990;33:2660–2666. doi:10.1021/jm00171a050
- Jardine I. Podophyllotoxins. In: Cassidy JM, Douros JD, editors. *Anticancer Agents Based on Natural Product Models*. New York, NY, USA: Academic Press; 1980:319–351.
- Reddy DM, Srinivas J, Chashoo G, Saxena AK, Sampath Kumar HM. 4β-[(4-Alkyl)-1,2,3-triazol-1-yl] podophyllotoxins as anticancer compounds: design, synthesis and biological evaluation. *Eur J Med Chem*. 2011;46:1983–1991. doi:10.1016/j.ejmech.2011.02.016
- Zi CT, Yang D, Dong FW, et al. Synthesis and antitumor activity of novel per-butyrylated glycosides of podophyllotoxin and its derivatives. *Bioorg Med Chem*. 2015;23:1437–1446. doi:10.1016/j.bmc.2015.02.021
- Zi CT, Liu ZH, Li GT, et al. Synthesis, and cytotoxicity of perbutyrylated glycosides of 4β-triazolopodophyllotoxin derivatives. *Molecules*. 2015;20:3255–3280. doi:10.3390/molecules20023255
- Zi CT, Li GT, Li Y, et al. Synthesis and anticancer activity of 4β-triazole-podophyllotoxin glycosides. *Nat Prod Bioprospect*. 2015;5:83–90. doi:10.1007/s13659-015-0057-3
- Zi CT, Xu FQ, Li GT, et al. Synthesis and anticancer activity of glucosylated podophyllotoxin derivatives linked via 4β-triazole rings. *Molecules*. 2013;18:13992–14012. doi:10.3390/molecules181113992
- Reddy PB, Paul DV, Agrawal SK, Saxena AK, Kumar HM, Qazi GN. Design, synthesis, and biological testing of 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxin analogues as antitumor agents. *Arch Pharm*. 2008;341:126–131. doi:10.1002/ardp.200700116
- Stähelin HF, von Wartburg A. The chemical and biological route from podophyllotoxin glucoside to etoposide: ninth Cain memorial Award lecture. *Cancer Res*. 1991;51:5–15.
- Gu XY, Chen L, Wang X, et al. Direct glycosylation of bioactive small molecules with glycosyl iodide and strained olefin as acid scavenger. *J Org Chem*. 2014;3:1100–1110. doi:10.1021/jo402551x
- Daley L, Guminski Y, Demerseman P, et al. Synthesis and antitumor activity of new glycosides of epipodophyllotoxin, analogues of etoposide, and NK 611. *J Med Chem*. 1998;41:4475–4485. doi:10.1021/jm9800752
- Allevi P, Anastasia M, Ciuffred P, Sanvito AM, Macdonald P. A short and simple synthesis of the antitumor agent etoposide. *Tetrahedron Lett*. 1992;33:4831–4834. doi:10.1016/S0040-4039(00)61297-2
- Kuhn M, von Wartburg A. Über ein neues glykosidierungsverfahren synthese von epipodophyllotoxin-β-D-glucopyranosid. 21. Mitt. Über mitosehemmende Naturstoffe. *Helv Chim Acta*. 1968;51:1631–1641. doi:10.1002/hlca.19680510719
- Cohen RB, Tsou KC, Rutenburg SH, Seligman AM. The colorimetric estimation and histochemical demonstration of beta-d-galactosidase. *J Biol Chem*. 1952;195:239–249.
- Kartha KP, Field RA. Iodine: a versatile reagent in carbohydrate chemistry IV. Per-O-acetylation, regioselective acylation and acetylation. *Tetrahedron*. 1997;53:11753–11766. doi:10.1016/S0040-4020(97)00742-4
- Fiandor J, García-López MT, De Las Heras FG, Méndez-Castrillón PP. A facile regioselective 1-O-deacylation of peracylated glycopyranoses. *Synthesis*. 1985;12:1121–1123. doi:10.1055/s-1985-31446
- Hansen HF, Jesen RB, Willumsen AM, et al. New compounds related to podophyllotoxin and congeners: synthesis, structure elucidation and biological testing. *Acta Chem Scand*. 1993;47:1190–1200. doi:10.3891/acta.chem.scand.47-1190
- Zi CT, Yang L, Gao W, et al. Click glycosylation for the synthesis of 1,2,3-triazole-linked picropodophyllotoxin glycoconjugates and their anticancer activity. *ChemistrySelect*. 2017;2:5038–5044. doi:10.1002/slct.201700347
- Brewer CF, Loike JD, Horwitz SB, Sternlicht H, Gensler WJ. Conformational analysis of podophyllotoxin and its congeners. Structure-activity relationship in microtubule assembly. *J Med Chem*. 1979;22:215–221. doi:10.1021/jm00190a007
- de Oliveira PF, Alves JM, Damasceno JL, Oliveira RAM, Dias HJ, Crotti AEM. Cytotoxicity screening of essential oils in cancer cell lines. *Rev Bras Farmacogn*. 2012;22:88–93.
- Shi JF, Wu P, Jiang ZH, Wei XY. Synthesis and tumor cell growth inhibitory activity of biotinylated annonaceous acetogenins. *Eur J Med Chem*. 2014;71:219–228. doi:10.1016/j.ejmech.2013.11.012
- Malich G, Markovic B, Winder C. The sensitivity and specificity of the MTS tetrazolium assay for detecting the in vitro cytotoxicity of 20 chemicals using human cell lines. *Toxicology*. 1997;124:179–192. doi:10.1016/S0300-483X(97)00144-3
- Zamoiskii EA. Evaluation of Reed-Muench method in determination of activity of biological preparations. *Zh Mikrobiol Epidemiol Immunobiol*. 1956;27:77–83.

36. Stanic MA. simplification of the estimation of the 50 percent end-points according to the Reed and Muench method. *Pathologia Et Microbiologia (basel)*. 1963;26:298–302.
37. Jeong YC, Anwar M, Moloney MG. Synthesis, antibiotic activity and structure–activity relationship study of some 3-enaminetetramic acids. *Bioorg Med Chem Lett*. 2014;24:1190–1200. doi:10.1016/j.bmcl.2014.03.013
38. Hartwell JL, Schrecker AW. Components of Podophyllin V. The Constitution of Podophyllotoxin. *J Am Chem Soc*. 1951;73:2909–2916. doi:10.1021/ja01150a143

## Drug Design, Development and Therapy

Dovepress

### Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which has also

been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/drug-design-development-and-therapy-journal>