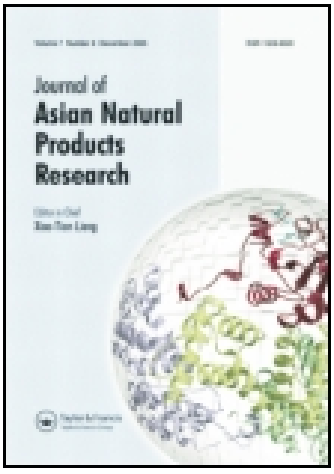

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Synthesis and antitumor activity of novel podophyllotoxin derivatives against multidrug-resistant cancer cells

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Published online: 29 Apr 2011.

To cite this article: Yong-En Guo , Hong Chen , Song Zuo , Dai-Lin Liu , Yan-Ling Lu , Jing-Jing Lv , Shao-Peng Wen & Tong-Cun Zhang (2011) Synthesis and antitumor activity of novel podophyllotoxin derivatives against multidrug-resistant cancer cells, Journal of Asian Natural Products Research, 13:05, 417-424, DOI: 10.1080/10286020.2011.568941

To link to this article: http://dx.doi.org/10.1080/10286020.2011.568941

Journal of Asian Natural Products Research
Vol. 13, No. 5, May 2011, 417–424

Synthesis and antitumor activity of novel podophyllotoxin derivatives against multidrug-resistant cancer cells

Yong-En Guo[a]*, Hong Chen[bc1], Song Zuo[bc], Dai-Lin Liu[b], Yan-Ling Lu[bc], Jing-Jing Lv[b], Shao-Peng Wen[d] and Tong-Cun Zhang[d2]

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(*Received 19 December 2010; final version received 2 March 2011*)

Seven novel 4β-N-substituted podophyllotoxin derivatives with indole rings were prepared and evaluated for cytotoxicity against human cancer cell lines HeLa, KB, KBV, K562, and K562/AO2. Most of them demonstrated improved antitumor activity and weak multidrug resistance compared to the drugs currently available.

Keywords: antitumor; podophyllotoxin; indole; prepare; cytotoxicity

1. Introduction

Prolonged treatment of carcinoma patients with certain anticancer medicines can result in the acquired multiple drug resistance (MDR) [1], a case associated with the overproduction of specific tubulin isotypes and the decreased expression of topoisomerases, etc. [2,3]. Increasingly, severe MDR of tumor is one of the main factors to cause abortive chemotherapy. Seeking novel synthetic antitumor agents to overcome MDR has currently been the focus of oncology.

Podophyllotoxin **1** has important antineoplastic and antiviral properties [4,5]. Since the 1950s, extensive structure modifications have been performed to reduce its toxic side effects. Its derivatives such as etoposide (VP-16, **2**) and teniposide (VM-26) have been widely used as anticancer drugs clinically to treat small cell lung cancer, testicular carcinoma, and lymphoma [6–8]. However, their low water solubility, acquired drug resistance, and severe gastrointestinal disturbances promoted researchers to search after new podophyllotoxin derivatives [9]. NK_{611}, GL_{331}, and TOP_{53} are under the clinical trials [10].

Previous studies showed that replacement of the C-4 sugar moiety in VP-16 with a non-sugar substituent through O-, S-, or N-linkage was significant in overcoming MDR. Generally, 4β-N-substituted podophyllotoxin derivatives exhibit superior anticancer activity compared to VP-16 against some of human cancer cell lines, but the O- and S-linked derivatives are not so active as their N-linked congeners [11–14].

Indibulin **3** also shows significant antitumor activity against a variety of tumors *in vitro* and *in vivo*. It destabilizes microtubules in the tumor cells and in a cell-free system [15]. Further research revealed that it was neither a substrate of *p*-gp170 nor of multidrug resistance-associated protein

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.568941
http://www.informaworld.com

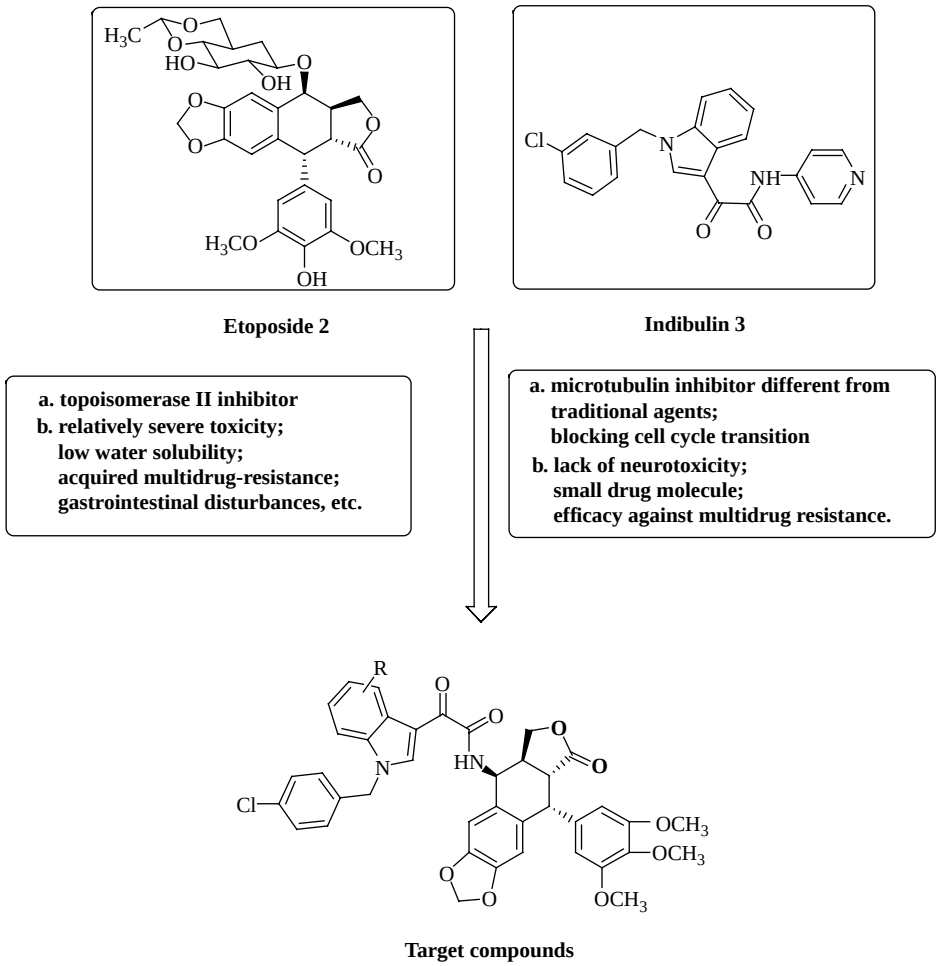
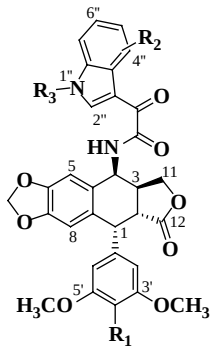


Chart 1. Design of the novel podophyllotoxin derivatives.

(MRP), and retained its antitumor efficacy in cell lines with MDR or MRP phenotypes. Additionally, the administration of indibulin to rats revealed no deficiency in motor function and no change in nerve conduction velocity (NCV). It suggested that indibulin is free of neurotoxicity [16].

The microtubulin-binding sites of podophyllotoxin and indibulin are different. To overcome MDR by inhibiting human DNA topoisomerase II and lower podophyllotoxin's toxicity, some podophyllotoxin derivatives based on indol-3-yl-glyoxyl substituents were designed by replacing the pyridin-4-ylamino moiety of indibulin with the structure of 4β-amino podophyllotoxin, as illustrated in Chart 1.

Recently, in our group many podophyllotoxin derivatives were synthesized and evaluated, including 4-O-substituents and 4-N-substituents of podophyllotoxin parent nucleus with electron-rich or electron-poor substituents in indole rings [17]. It comes out that combination of indoles (with N-electron-rich or aromatic ring electron-rich substituents) and 4-N-substituted podophyllotoxin derivatives can get potential candidates. In this paper, seven unreported 4β-N-indol-3-ylglyoxyl-substituted podophyllotoxin



Compound	R_1	R_2	R_3
9a	OCH_3	4"-carbomethoxy	H
9b	OCH_3	4"-carbomethoxy	2'''-fluorobenzyl
9c	OH	4"-carbomethoxy	4'''-methylbenzyl
9d	OH	5"-fluoro	2'''-chlorobenzyl
9e	OCH_3	5"-fluoro	2'''-chlorobenzyl
9f	OCH_3	4"-carbomethoxy	benzoyl
9g	OCH_3	4"-carbomethoxy	acetyl

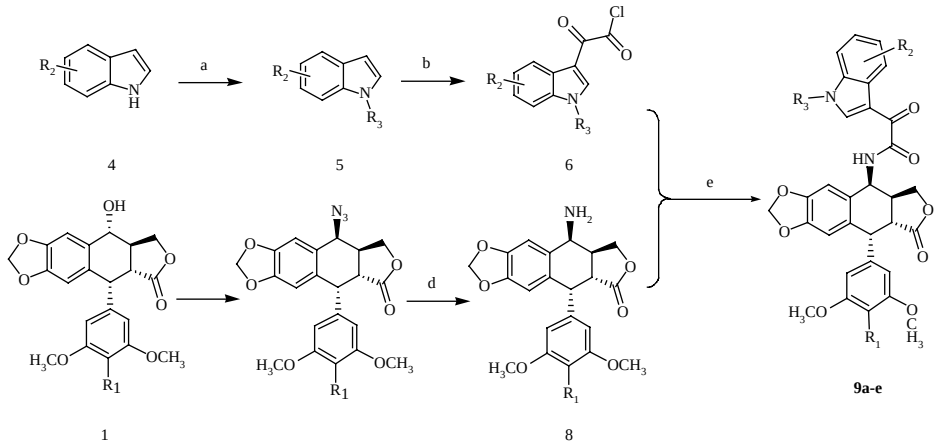
Figure 1. Structures of synthesized podophyllotoxin derivatives.

derivatives as shown in Figure 1 were synthesized and evaluated against human cancer cell lines.

2. Results and discussion

The podophyllotoxin compounds **9a–e** as shown in Figure 1 were prepared according to Scheme 1. N-substituted indoles **5**, readily available from indole **4** and aralkyl chlorides with NaH as catalyst, reacted with oxalyl chloride to afford 2-(*N*-aralkylindol-3-yl)glyoxyloyl chlorides **6**, as important intermediates to get compounds **9a–e**. In our research, Friedel–Crafts acylation of indoles with oxalyl chloride gave 3-substituted products (via the more stable carbocation intermediates with two complete phenyl rings as the resonants) in higher yields. Ether was the most effective solvent, allowing precipitation of the glyoxyloyl chlorides [18]. N-acylation of compound **9a**, a very weak secondary amine, afforded corresponding compounds **9f–g**, as shown in Scheme 2. Most probably more side reactions, i.e. acetylation at the methoxyphenyl groups, occurred when stronger acylation agent acetyl chloride was used in comparison with benzoyl chloride. However, when milder acetic anhydride was applied under the same reaction condition with 5-methylindole as the substrate instead, 80% yield of product was obtained [19]. Acetic anhydride will be tried in our future experiment.

Using compound **9a** as the example of target compounds, its ^{1}H NMR spectrum revealed proton signals of three methoxy groups, respectively, at δ 3.76 (s, 6H, 3′, 5′-OCH_3) and 3.82 (s, 3H, 4′-OCH_3), eight aromatic protons at δ 6.31 (s, 2H, H-2′, 6′),

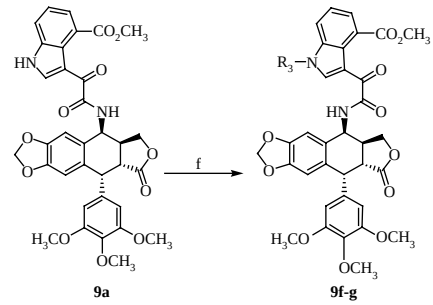


Scheme 1. Synthetic route to compounds **9a–e**: (a) aralkyl chloride, NaH, DMF, 0°C; (b) oxalyl chloride, Et_2O, 0°C; (c) CF_3CO_2H, NaN_3, CH_2Cl_2, rt; (d) 10% Pd/C, HCO_2NH_4, CH_2Cl_2, rt; and (e) DMAP, TEA, CH_2Cl_2, rt.

6.48 (s, 1H, H-8), 6.79 (s, 1H, H-5), and 7.35–7.77 (m, 4H, H-2″, 5″, 6″, 7″), successively. The signals of methylene group at δ 5.98 (d, 1H, $J = 1.0$ Hz, OCH_2O) and 5.99 (d, 1H, $J = 1.0$ Hz, OCH_2O), and the other aliphatic proton signals at δ 3.97 (s, 3H, CO_2CH_3), 4.10 (m, 1H, H-11β), 4.42 (m, 1H, H-11α), 2.99–3.02 (m, 2H, H-2, 3), 4.52 (d, 1H, $J = 3.4$ Hz, H-1), and 5.21 (dd, 1H, $J = 3.8$, 8.1 Hz, H-4) were also detected in the ^{1}H NMR spectrum. The reported signals of H-4, H-3, H-2, and H-1 of podophyllotoxin are located at δ 4.59 (m, 1H, H-4), 2.85 (m, 2H, H-2, 3), and 4.75 (d, 1H, $J = 8.8$ Hz, H-1) [20]. Stronger van der Waals effects, due to H-11α's more closeness to H-2, H-4 and other groups in space than H-11β, explain why its ^{1}H NMR signal appears downfield and it is coupled with H-11β. Two protons at dioxymethylene group come into resonance at different frequencies, and are coupled to each other. Each of their signals is split into doublet. The assignment of the configurations at C-4 for our target compounds **9a–g** was based on the coupling constants of H-3/H-4. The 4α-substituted podophyllotoxins have a $J_{3,4} \geq 10.0$ Hz as H-3 is *trans* to H-4. However, $7.8\,\text{Hz} \leq J_{3,4} \leq 8.1\,\text{Hz}$ in ^{1}H NMR of the target compounds **9a–g** suggests that they have 4β-configurations, because of a *cis* relationship between H-3 and H-4 [21].

As shown in Table 1, compounds **9a**, **9d,** and **9e** exhibit strong biological activity against HeLa cells, obviously better than that of reference compounds, while derivatives **9b**, **9c**, **9d**, **9f**, and **9 g** are more effective against K562 and K562/AO2 cells *in vitro* than VP-16.

In conclusion, compounds **9b–g** showed promising anticancer activity, especially an important advantage in anti-multidrug resistance activity. In the preliminary structure–activity relationship (SAR) study, we predicted that the N-substituted



Scheme 2. Synthetic route to compounds **9f–g**: (f) acyl chlorides, TEA, CH_2Cl_2, rt.

Table 1. IC_{50} values of the novel podophyllotoxin derivatives.

Compound	IC_{50} (μmol/L)				
	HeLa	K562	K562/AO2	KB	KBV
9a	1.05	0.99 ± 0.60	4.04 ± 0.97	3.99 ± 1.28	6.28 ± 2.05
9b	5.237	0.29 ± 0.19	0.12 ± 0.06	2.49 ± 0.94	3.49 ± 1.05
9c	3.805	0.23 ± 0.16	0.70 ± 0.02	0.88 ± 0.23	3.62 ± 2.17
9d	0.15	0.40 ± 0.14	0.29 ± 0.26	1.00 ± 0.78	3.16 ± 1.46
9e	0.73	1.22 ± 0.35	0.62 ± 0.28	4.99 ± 1.36	5.46 ± 3.65
9f	10.67	0.31 ± 0.18	0.22 ± 0.20	1.03 ± 0.38	2.59 ± 0.28
9 g	11.11	0.46 ± 0.37	0.10 ± 0.01	1.62 ± 1.25	2.30 ± 2.17
VP-16	2.11 ± 1.17	0.9 ± 0.08	7.05 ± 5.08	3.36 ± 1.05	55.47 ± 4.36
VCR	–	–	–	0.93 ± 0.26	50.30 ± 3.84
ADM	–	1.77 ± 0.1	>167.6	–	–

Notes: (a) Cytotoxicity against HeLa, KB, and KBV cell lines and against K562 and K562/AO2 cell lines was measured, respectively, by the standard MTT and SRB assay method. (b) Each value represents the mean ± SD of three independent experiments.

electron-rich substituents in indole were the key groups contributing much to antitumor activity, while R_1 substituents in podophyllotoxin parent nucleus showed no significant influence on their bioactivity.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-4 digital melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker ARX-300 spectrophotometer, with tetramethylsilane (TMS) as the internal standard. Mass spectral data were obtained on Agilent 6210 TOF MS. All materials and reagents were used without further purification unless stated. Ninety-eight percent of podophyllotoxin was purchased from the Nanjing Qingze Corporation, Nanjing, China. CH_2Cl_2 was refluxed over P_2O_5 and distilled. Et_2O was distilled over sodium with benzophenone as monitor.

3.2 General synthetic methods

3.2.1 4β-2-[N-(2‴-Fluorobenzyl)-4″-carbomethoxyindol-3″-yl]glyoxylamido 4-deoxypodophyllotoxin (9b)

4-Carbomethoxy indole (0.19 g, 1.0 mmol) in anhydrous DMF (*N,N*-dimethylformamide; 2 ml) was added dropwise to a mixture of 70% NaH (51 mg, 1.5 mmol) in anhydrous DMF (1 ml) at 0°C. After the mixture was stirred at room temperature (rt) for 1 h and cooled to 0°C, 2-fluorobenzyl chloride (0.15 g, 1.05 mmol) was added, and the solution was stirred at rt for an additional 3.5 h. The reaction solution was poured into icy water (50 ml), and stirring continued until precipitation appeared. The solid was filtered and washed with water. N-substituted indole was obtained, dried, and used for the next step without further purification.

Oxalyl chloride (0.14 g, 1.1 mmol) was added dropwise to a solution of *N*-(2-fluorobenzyl)-4-carbomethoxyindole (0.27 g, 0.90 mmol) in anhydrous ether (10 ml) at 0°C, and stirring continued at rt for 6 h. The solid was filtered and washed with dry petroleum ether. The mixture of above crude 2-[*N*-(2-fluorobenzyl)-4-carbo-methoxyindol-3-yl]glyoxyloyl chloride (0.35 g, 0.90 mmol), compound **8** (0.41 g, 1.0 mmol), two drops of triethyl amine, and DMAP (4-dimethylaminopyridine, 3.0 mg) in dry dichloromethane (10 ml) was stirred for 12 h. The reaction mixture was washed with water, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in acetone and chromatographed on silica-gel plate using petroleum ether and ethyl

acetate (1:1) as eluent to give compound **9b** (0.51 g, overall yield 67% in three steps of reactions) as light yellow solid. For ^{1}H NMR spectral data ($CDCl_3$) (see Table 2). HR-ESI-MS *m/z*: 751.2305 $[M + H]^+$ (calculated for $C_{41}H_{36}FN_2O_{11}$, 751.2303).

Compounds **9a** and **9c–e** were prepared as described above.

Compound **9a**: White solid, overall yield 75% in two steps of reactions, mp 273.9–274.8°C. ^{1}H NMR spectral data ($CDCl_3$) (see Table 2). HR-ESI-MS *m/z*: 643.1906 $[M + H]^+$ (calculated for $C_{34}H_{31}N_2O_{11}$, 643.1928).

Compound **9c**: White solid, overall yield 70% in three steps of reactions, mp

Table 2. ^{1}H NMR spectral data for the target compounds **9a–g**.

Compound	^{1}H NMR (δ/ppm)
9a	2.99–3.02 (m, 2H, H-2, 3), 3.76 (s, 6H, 3′, 5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.97 (s, 3H, CO_2CH_3), 4.10 (m, 1H, H-11β), 4.42 (m, 1H, H-11α), 4.52 (d, 1H, J = 3.4 Hz, H-1), 5.21 (dd, 1H, J = 3.8, 8.1 Hz, H-4), 5.98 (d, 1H, J = 1.0 Hz, OCH_2O), 5.99 (d, 1H, J = 1.0 Hz, OCH_2O), 6.31 (s, 2H, H-2′, 6′), 6.48 (s, 1H, H-8), 6.79 (s, 1H, H-5), 7.35–7.77 (m, 4H, H-2″, 5″, 6″, 7″), 8.87 (d, 1H, J = 3.8 Hz, CONH), 10.21 (s, 1H, NH in indolyl ring).
9b	2.99–3.02 (m, 2H, H-2, 3), 3.76 (s, 6H, 3′, 5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 3.97 (s, 3H, CO_2CH_3), 4.11 (m, 1H, H-11β), 4.44 (m, 1H, H-11α), 4.63 (d, 1H, J = 4.7 Hz, H-1), 5.21 (dd, 1H, J = 3.8, 8.1 Hz, H-4), 5.48 (s, 2H, CH_2 in benzyl group), 5.98 (d, 1H, J = 1.0 Hz, OCH_2O), 5.99 (d, 1H, J = 1.0 Hz, OCH_2O), 6.31 (s, 2H, H-2′, 6′), 6.53 (s, 1H, H-8), 6.79 (s, 1H, H-5), 7.04–7.33 (m, 4H, H-3‴, 4‴, 5‴, 6‴ in benzyl group), 7.35–7.62 (m, 4H, H-2″, 5″, 6″, 7″), 8.94 (d, 1H, J = 3.8 Hz, CONH).
9c	2.33 (s, 3H, CH_3), 2.99–3.02 (m, 2H, H-2, 3), 3.76 (s, 6H, 3′, 5′-OCH_3), 3.97 (s, 3H, CO_2CH_3), 4.12 (m, 1H, H-11β), 4.42 (m, 1H, H-11α), 4.49 (**s**, 1H, H-1), 5.21 (dd, 1H, J = 4.0, 7.9 Hz, H-4), 5.39 (s, 2H, CH_2 in benzyl group), 5.98 (d, 1H, J = 1.3 Hz, OCH_2O), 5.99 (d, 1H, J = 1.3 Hz, OCH_2O), 6.28 (s, 2H, H-2′, 6′), 6.43 (s, 1H, H-8), 6.78 (s, 1H, H-5), 7.07–7.17 (m, 4H, H-2‴, 3‴, 5‴, 6‴ in benzyl group), 7.34–7.70 (m, 4H, H-2″, 5″, 6″, 7″), 8.91 (d, 1H, J = 4.0 Hz, CONH).
9d	3.00–3.10 (m, 2H, H-2, 3), 3.79 (s, 6H, 3′, 5′-OCH_3), 4.08 (m, 1H, H-11β), 4.44 (m, 1H, H-11α), 4.65 (d, 1H, J = 4.0 Hz, H-1), 5.25 (dd, 1H, J = 4.3, 8.1 Hz, H-4), 5.56 (s, 2H, CH_2 in benzyl group), 5.98 (d, 1H, J = 1.2 Hz, OCH_2O), 6.01 (d, 1H, J = 1.2 Hz, OCH_2O), 6.32 (s, 2H, H-2′, 6′), 6.55 (s, 1H, H-8), 6.75 (s, 1H, H-5), 7.02–7.33 (m, 4H, H-3‴, 4‴, 5‴, 6‴ in benzyl group), 7.34–8.07 (m, 4H, H-2″, 4″, 6″, 7″), 9.06 (d, 1H, J = 4.3 Hz, CONH).
9e	3.00–3.05 (m, 2H, H-2, 3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3), 4.12 (m, 1H, H-11β), 4.42 (m, 1H, H-11α), 4.60 (d, 1H, J = 3.2 Hz, H-1), 5.25 (dd, 1H, J = 3.6, 7.8 Hz, H-4), 5.51 (s, 2H, CH_2 in benzyl group), 5.98 (d, 1H, J = 1.0 Hz, OCH_2O), 5.99 (d, 1H, J = 1.0 Hz, OCH_2O), 6.30 (s, 2H, H-2′, 6′), 6.46 (s, 1H, H-8), 6.77 (s, 1H, H-5), 7.02–7.33 (m, 4H, H-3‴, 4‴, 5‴, 6‴ in benzyl group), 7.34–8.08 (m, 4H, H-2″, 4″, 6″, 7″), 9.05 (d, 1H, J = 3.6 Hz, CONH).
9f	2.99–3.05 (m, 2H, H-2, 3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3), 3.97 (s, 3H, CO_2CH_3), 4.12 (m, 1H, H-11β), 4.42 (m, 1H, H-11α), 4.49 (d, 1H, J = 4.5 Hz, H-1), 5.22 (dd, 1H, J = 4.4, 7.9 Hz, H-4), 5.98 (d, 1H, J = 1.8 Hz, OCH_2O), 5.99 (d, 1H, J = 1.8 Hz, OCH_2O), 6.32 (s, 2H, H-2′, 6′), 6.56 (s, 1H, H-8), 6.85(s, 1H, H-5), 7.31–7.62 (m, 5H, H-2‴, 3‴, 4‴, 5‴, 6‴ in benzoyl group), 7.67–8.08 (m, 4H, H-2″, 5″, 6″, 7″), 8.91 (d, 1H, J = 4.4 Hz, CONH).
9 g	2.20 (s, 3H, $COCH_3$), 2.99–3.05 (m, 2H, H-2, 3), 3.76 (s, 6H, 3′, 5′-OCH_3), 3.81 (s, 3H, 4′-OCH_3), 3.96 (s, 3H, CO_2CH_3), 4.10 (m, 1H, H-11β), 4.36–4.54 (**m**, 1H, H-1), 4.42 (m, 1H, H-11α), 5.20 (m, 1H, H-4), 5.98 (s, 1H, OCH_2O), 5.99 (s, 1H, OCH_2O), 6.29 (s, 2H, H-2′, 6′), 6.47 (s, 1H, H-8), 6.87(s, 1H, H-5), 7.44–7.90 (m, 4H, H-2″, 5″, 6″, 7″), 8.96 (d, 1H, J = 3.8 Hz, CONH).

146.0–147.0°C. ^{1}H NMR spectral data ($CDCl_3$) (see Table 2). HR-ESI-MS *m/z*: 733.2448 $[M + H]^+$ (calculated for $C_{41}H_{37}N_2O_{11}$, 733.2397).

Compound **9d**: Light yellow solid, overall yield 48% in three steps of reactions, mp 154.0–154.2°C, ^{1}H NMR spectral data ($CDCl_3$) (see Table 2). HR-ESI-MS *m/z*: 713.1717 $[M + H]^+$ (calculated for $C_{38}H_{31}ClFN_2O_9$, 713.1702).

Compound **9e**: White solid, overall yield 52% in three steps of reactions, mp 167.0–168.0°C, ^{1}H NMR spectral data ($CDCl_3$) (see Table 2). HR-ESI-MS *m/z*: 727.1835 $[M + H]^+$ (calculated for $C_{39}H_{33}ClFN_2O_9$, 727.1859).

3.2.2 Preparation of 4β-2-(N-benzoyl-4″-carbomethoxyindol-3″-yl)glyoxylamido 4-deoxypodophyllotoxin (9f)

After the solution of compound **9a** (0.64 g, 0.97 mmol), benzoyl chloride (0.14 g, 1 mmol), two drops of triethyl amine, and DMAP (3.0 mg) in anhydrous CH_2Cl_2 (10 ml) are stirred at rt for 6 h, the reaction mixture was washed with water, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was dissolved in acetone and chromatographed on silica-gel plate using petroleum ether and ethyl acetate (1:1) as eluent to afford 0.67 g of compound **9f** as white powder, yield 90%. ^{1}H NMR spectral data ($CDCl_3$) (see Table 2). HR-ESI-MS *m/z*: 747.2212 $[M + H]^+$ (calculated for $C_{41}H_{35}N_2O_{12}$, 747.2190).

Compound **9 g** was prepared in the same manner. Yield 46%, mp 159.0–159.6°C, ^{1}H NMR spectral data ($CDCl_3$) (see Table 2). HR-ESI-MS *m/z*: 685.2055 $[M + H]^+$ (calculated for $C_{36}H_{33}N_2O_{12}$, 685.2033).

3.3 Biological evaluation

The biological activity of these podophyllotoxin derivatives was evaluated by *in vitro* cytotoxicity test, carried out with a panel of five human cancer cell lines including HeLa (cervical cancer cells), K562 (human erythroleukemia cells), K562/AO2 (multidrug-resistant human erythroleukemia cells induced by doxorubicin), KB (human oral squamous carcinoma cells), and KBV (MDR human oral squamous carcinoma cells) using vincristine sulfate, VP-16 **2**, and adriamycin as reference compounds. The screening procedure was based on the standard 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) or sulforhodamine B (SRB) method [22]. IC_{50} values of compounds **9a–g** (see Table 1).

4. Conclusion

The promising results obtained from these new derivatives make them become potential candidates. It is valuable for us to further prepare and evaluate their new derivatives.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 30873363), the Key Project of Science Foundation of Tianjin (09ZCKFNC01200), and the Program of Science Foundation of Tianjin (08JCYBJC070000).

Notes

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References

[1] S.B. Kaye, *Curr. Opin. Oncol* **10**, S15 (1998).
[2] C. Ferlini, G. Raspaglio, S. Mozzeti, L. Cicchillitti, F. Fillippetti, D. Gallo, C. Fattorusso, G. Campiani, and G. Scambia, *Cancer Res* **65**, 2397 (2005).
[3] I. Wessel, P.B. Jensen, J. Falck, S.E.L. Mirski, S.P.C. Cole, and M. Sehested, *Cancer Res* **57**, 4451 (1997).
[4] K.H. Lee, Y. Iwakura, M. Haruna, S.A. Beers, and L.S. Thurston, *J. Nat. Prod* **52**, 606 (1989).
[5] X.M. Zhou, Z.W. Wang, J.Y. Zhang, H.X. Chen, and K.H. Lee, *J. Med. Chem* **34**, 3346 (1991).
[6] M. Gordaliza, M.A. Castro, and A.S. Feliciano, *Curr. Pharmaceut. Res* **6**, 1811 (2000).

[7] D.S.V. Vilet, Y. Tachibana, K.F. Bestow, E.S. Huang, and K.H. Lee, *J. Med. Chem* **44**, 1422 (2001).
[8] L. Schacter, *Semin. Oncol* **23**, 1 (1996).
[9] O. Pagani, M. Zucchetti, C. Sessa, J.D. Jong, M. D'Incalci, M.D. Fusco, A. Kaeser-Fröhlich, A. Hanauske, and F. Cavalli, *Cancer Chemother. Pharmacol* **38**, 541 (1996).
[10] K.R. Handel, *Eur. J. Cancer* **34**, 1514 (1998).
[11] A.K. Mukherjee, S. Basu, N. Sarkar, and A.C. Ghosh, *Curr. Med. Chem* **8**, 1467 (2001).
[12] K.H. Lee, S.A. Beers, M. Mori, Z.Q. Wang, Y.H. Kuo, L. Li, S.Y. Liu, J.Y. Chang, F.S. Han, and Y.C. Cheng, *J. Med. Chem* **33**, 1364 (1990).
[13] Z.Q. Wang, Y.H. Kuo, D. Schnur, J.P. Bowen, S.Y. Liu, F.S. Han, and K.H. Lee, *J. Med. Chem* **33**, 2660 (1990).
[14] Z. Xiao, Y. Li, and J. Feng, *J. Med. Chem* **45**, 2294 (2002).
[15] A. Kiselyov, K.V. Balakin, S.E. Tkachenko, N. Savchuk, and A.V. Ivachtchenko, *Anti-Cancer Agents Med. Chem* **7**, 189 (2007).
[16] G. Becher, B. Bickel, P. Emig, and S. Seeber, *Cancer Res* **61**, 392 (2001).
[17] P.F. Yu, H. Chen, J. Wang, C.X. He, B. Cao, M. Li, N. Yang, Z.Y. Lei, and M.S. Cheng, *Chem. Pharm. Bull* **56**, 831 (2008).
[18] G.C. Condie, M.F. Channon, A.J. Ivory, N. Kumar, and D.S. Black, *Tetrahedron* **61**, 4989 (2005).
[19] P.Z. Yu, L.P. Wang, and Z.N. Chen, *J. Nat. Prod* **54**, 1422 (1991).
[20] S. Kirchberg, R. Fröhlich, and A. Studer, *Angew. Chem. Int. Ed* **48**, 4235 (2009).
[21] C.J. Cao, Y.Y. Chen, Y.G. Wang, and Y.Z. Chen, *Chin. Chem. Lett* **10**, 1003 (1999).
[22] H. Chen, W.C. Li, and B. Cao, *Eur. J. Pharmacol* **627**, 69 (2010).