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Synthesis and cytotoxicity of novel podophyllotoxin derivatives

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Abstract

In order to find novel synthetic antitumor agents with superior cytotoxicity and overcoming multidrug resistance, a novel series of 4β-N-substituted podophyllotoxin derivatives were synthesized and evaluated as potential antitumor agents. Seven novel podophyllotoxin derivatives were synthesized by linking 4β-amino-4-deoxypodophyllotoxin with N-substituted 5-methylindol-3-yl-glyoxyl chlorides and tested against K562 and K562/A02 using SRB methods *in vitro*, KB and KBV using MTT methods *in vitro*.

Keywords: Podophyllotoxin derivatives; Chemosynthesis; Antitumor; Multidrug resistance

During the course of cancer chemotherapy treatment, it has been found that prolonged treatment of carcinoma patients with certain anticancer medicines can result in an acquired resistance toward multiple drugs, which is known as multidrug resistance (MDR) [1]. The development of MDR is one of the major problems encountered in chemotherapy. The need to overcome MDR has fueled considerable interest in the development of novel synthetic antitumor agents with cytotoxicity against MDR cancer cell lines. Finding novel synthetic antitumor agents for overcoming MDR has currently become the focus of oncology.

Podophyllotoxin has important antineoplastic and antiviral properties. Because of its toxic side effects, extensive structure modifications had been performed since the 1950s. Podophyllotoxin derivatives possess antitumor activity, such as etoposide (VP-16) and teniposide (VM-26) have been widely used as anticancer drugs for clinical chemotherapy [2]. However, their low water solubility, acquired drug-resistance and severe gastrointestinal disturbances promoted people to search for new derivatives of podophyllotoxin [3]. The replacement of the C-4 sugar moiety of VP-16 with a non-sugar substitution has proven to be significant in overcoming the drug resistance of etoposide [4,5]. The C-4 non-sugar substitution can be linked through O-, S- or N-linkage. In general, the N-linked derivatives exhibits superior anticancer activity compared to VP-16 against some of the human cancer cell lines. Indoles are known for possessing various biological properties including antibacterial, cytotoxic, antioxidative and insecticidal activities [6]. Indibulin (D-24851, N-(pyridin-4-yl)-[1-(4-chlorobenzyl)-indol-3-yl]glyoxylamide) is a

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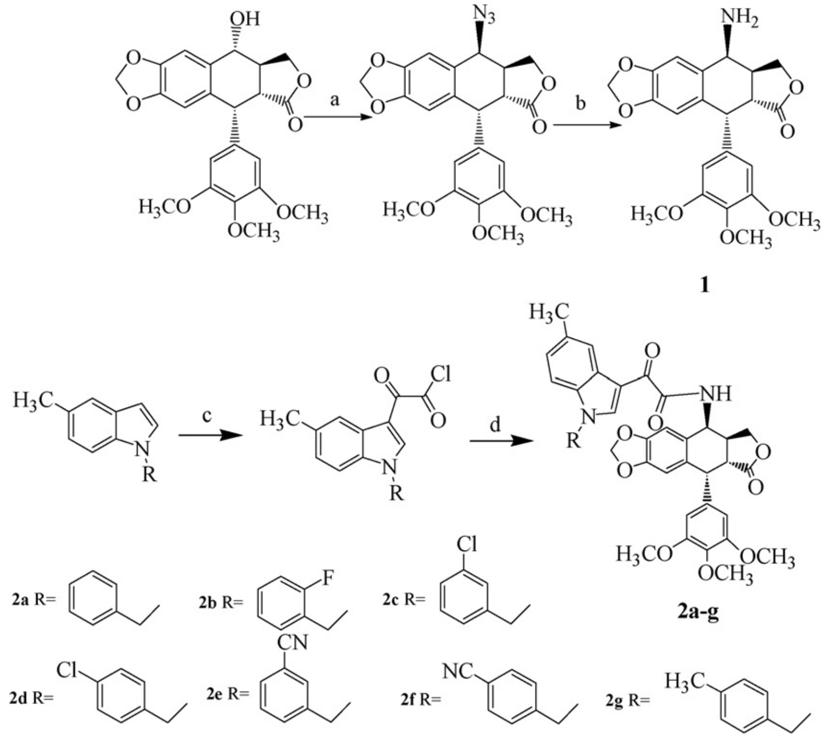

Scheme 1. Conditions and reagents: (a) NaN_3, CF_3COOH, CH_2Cl_2, r.t. 12 h; (b) $HCOONH_4$, 10% Pd/C, ethyl acetate, 50 °C, 5 h; (c) oxalyl chloride, $CH_3CH_2OCH_2CH_3$, r.t. 6 h; (d) 4β-amino-4-deoxypodophyllotoxin, triethylamine, CH_2Cl_2, r.t. 12 h.

synthetic small molecule tubulin inhibitor with significant antitumor activity against a variety of tumors *in vitro* and *in vivo*. And this clinical candidate is currently in clinical trials [7].

Based on the structure–activity relationships of podophyllotoxin and N-substituted indole is the activity group of indubulin, a series of derivatives of podophyllotoxin were prepared by linking 4β-amino-4-deoxypodophyllo-toxin with N-substituted 5-methylindol-3-yl-glyoxyl chlorides, structurally confirmed by TOF-MS, ^1H NMR and evaluated for their antitumor activities *in vitro*.

Compound **1** was obtained by two steps according to Refs. [8,9]. Oxalyl chloride (1.2 mmol) was added dropwise to a solution of N-substituted 5-methylindol (1 mmol) in dry diethyl ether (10 mL) at 0 °C and stirred for 6 h at room temperature. The reaction mixture was filtered and washed with petroleum. The crude product N-substituted 5-methylindol-3-yl-glyoxyl chloride, compound **1** (413 mg, 1 mmol), two drops of triethylamine in dry dichloromethane (10 mL) was stirred for 12 h. The reaction mixture was washed with water and dried over $MgSO_4$ and concentrated *in vacuo*. The residue was resolved in acetone and chromatographed on silica gel plate using petroleum-ethyl acetate to give the corresponding products compounds **2a–g**. All these compounds (**2a–g**) were characterized by TOF-MS and ^1H NMR (Scheme 1, Tables 1 and 2).

The antitumor activities of the derivatives were tested against K562 and K562/A02 by SRB methods *in vitro* and against KB and KBV by MTT methods *in vitro* (Table 3). All the seven derivatives were not reported by previous literature. Some of these derivatives have shown promising anticancer activity. The derivatives **2a**, **2b**, **2c**, **2d**, **2e**, **2f**

Table 1
Data of compounds 2**a**–**g**.

	Compound						
	2a	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**
Mp (°C)	172	164–165	162–163	181	171–172	186–187	179–180
Total yield (%)	57.2	49.8	55.1	62.8	54.3	53.3	51.6

Table 2
$[\alpha]_D^{19}$ (Rudolph Research Analytical Autopol II Automatic Polarimeter), HR-MS (Agilent 6210 LC-TOF) and ^{1}H NMR (Varian Mercury V × 300 MHz) data of **2a**–**g**.

Compound	$[\alpha]_D^{19}$ ($CHCl_3$)	TOF-MS (*m/z*)	^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm)
2a	−89.9 (*ca.* 0. 10)	689.2476 $(M+H)^+$	9.00 (s, 1H, –NH–), 8.19 (s, 1H, Ar–H), 7.94–7.96 (m, 1H, Ar–H), 7.31–7.39 (m, 3H, Ar–H), 7.19–7.23 (m, 3H, Ar–H), 7.12–7.15 (dd, 1H, *J* = 1.3, 8.4 Hz, Ar–H), 6.78 (s, 1H, 5–H), 6.33 (s, 1H, 8-H), 6.28 (s, 2H, 2′, 6′-H), 6.00 (d, 1H, *J* = 1.2 Hz, OCH_2O), 5.98–5.99 (d, 1H, *J* = 1.3 Hz, OCH_2O), 5.40 (s, 2H, Ar–CH_2–), 5.22–5.26 (d, 1H, *J* = 4.2 Hz, 4-H), 4.54 (br, 1H, 1-H), 4.43–4.48 (m, 1H, 11-H-a), 3.85–3.92 (m, 1H, 11-H-b), 3.81 (s, 3H, 4′–OCH_3), 3.75 (s, 6H, 3′, 5′–OCH_3), 2.97–3.10 (m, 2H, 2, 3-H), 2.52 (s, 3H, Ar–CH_3)
2b	−88.2 (*ca.* 0. 11)	707.2382 $(M+H)^+$	9.02 (s, 1H, –NH–), 8.18 (s, 1H, Ar–H), 7.98–8.01 (d, 1H, *J* = 8.0 Hz, Ar–H), 7.28–7.36 (m, 2H, Ar–H), 7.02–7.18 (m, 4H, Ar–H), 6.77 (s, 1H, 5-H), 6.25 (br, 3H, 8-H, 2′, 6′-H), 5.99 (d, 1H, *J* = 1.4 Hz, OCH_2O), 5.98 (d, 1H, *J* = 1.3 Hz, OCH_2O), 5.43 (s, 2H, Ar–CH_2–), 5.22–5.26 (d, 1H, *J* = 4.1 Hz, 4-H), 4.48–4.49 (d, 1H, *J* = 3.4 Hz, 1-H), 4.42–4.46 (m, 1H, 11-H-a), 3.85–3.91 (m, 1H, 11-H-b), 3.80 (s, 3H, 4′-OCH_3), 3.74 (s, 6H, 3′, 5′-OCH_3), 2.96–3.11 (m, 2H, 2, 3-H), 2.53 (s, 3H, Ar–CH_3)
2c	−89.0 (*ca.* 0. 10)	723.2087 $(M+H)^+$	8.98 (s, 1H, –NH–), 8.19 (s, 1H, Ar–H), 8.13–8.16 (d, 1H, *J* = 7.8 Hz, Ar–H), 7.25–7.32 (m, 2H, Ar–H), 7.14–7.22 (m, 3H, Ar–H), 7.05–7.09 (m, 1H, Ar–H), 6.78 (s, 1H, 5-H), 6.22 (s, 2H, 2′, 6′-H), 6.09 (s, 1H, 8-H), 6.00 (s, 2H, OCH_2O), 5.36 (s, 2H, Ar–CH_2–), 5.21–5.25 (d, 1H, *J* = 4.2 Hz, 4-H), 4.41–4.47 (m, 1H, 11-H-a), 4.39–4.40 (d, 1H, *J* = 4.8 Hz, 1-H), 3.85–3.91 (m, 1H, 11-H-b), 3.79 (s, 3H, 4′-OCH_3), 3.73 (s, 6H, 3′, 5′-OCH_3), 2.97–3.15 (m, 2H, 2, 3-H), 2.56 (s, 3H, Ar–CH_3)
2d	−134.2 (*ca.* 0. 11)	723.2090 $(M+H)^+$	8.98 (s, 1H, –NH–), 8.19 (s, 1H, Ar–H), 8.06–8.11 (m, 1H, Ar–H), 7.30–7.34 (m, 2H, Ar–H), 7.16–7.19 (m, 3H, Ar–H), 7.13 (m, 1H, Ar–H), 6.77 (s, 1H, 5-H), 6.24 (s, 2H, 2′, 6′-H), 6.19 (s, 1H, 8-H), 5.99 (s, 2H, OCH_2O), 5.36 (s, 2H, Ar–CH_2–), 5.21–5.25 (d, 1H, *J* = 4.1 Hz, 4-H), 4.42–4.47 (m, 2H, 1-H, 11-H-a), 3.84–3.91 (m, 1H, 11-H-b), 3.79 (s, 3H, 4′-OCH_3), 3.74 (s, 6H, 3′, 5′-OCH_3), 2.98–3.14 (m, 2H, 2, 3-H), 2.54 (s, 3H, Ar–CH_3)
2e	−80.1 (*ca.* 0. 09)	714.2427 $(M+H)^+$	9.01 (s, 1H, –NH–), 8.27–8.29 (d, 1H, *J* = 7.9 Hz, Ar–H), 8.29 (s, 1H, Ar–H), 7.60–7.63 (m, 1H, Ar–H), 7.41–7.53 (m, 3H, Ar–H), 7.13–7.20 (m, 2H, Ar–H), 6.79 (s, 1H, 5-H), 6.17 (s, 2H, 2′, 6′-H), 6.02 (d, 1H, *J* = 1.3 Hz, OCH_2O), 6.00–6.01 (d, 1H, *J* = 1.3 Hz, OCH_2O), 5.89 (s, 1H, 8-H), 5.42 (s, 2H, Ar–CH_2–), 5.21–5.25 (d, 1H, *J* = 4.3 Hz, 4-H), 4.40–4.45 (m, 1H, 11-H-a), 4.29–4.31 (d, 1H, *J* = 5.0 Hz, 1-H), 3.84–3.90 (m, 1H, 11-H-b), 3.77 (s, 3H, 4′-OCH_3), 3.71 (s, 6H, 3′, 5′-OCH_3), 2.98–3.20 (m, 2H, 2, 3-H), 2.59 (s, 3H, Ar–CH_3)
2f	−82.7 (*ca.* 0. 12)	714.2420 $(M+H)^+$	9.02 (s, 1H, –NH–), 8.22 (s, 1H, Ar–H), 8.20 (s, 1H, Ar–H), 7.62–7.66 (m, 2H, Ar–H), 7.27–7.30 (m, 2H, Ar–H), 7.10–7.18 (m, 2H, Ar–H), 6.78 (s, 1H, 5-H), 6.20 (s, 2H, 2′, 6′-H), 6.01 (s, 1H, 8-H), 6.01 (d, 1H, *J* = 1.5 Hz, OCH_2O), 6.00 (d, 1H, *J* = 1.4 Hz, OCH_2O), 5.47 (s, 2H, Ar–CH_2–), 5.22–5.26 (d, 1H, *J* = 4.3 Hz, 4-H), 4.40–4.46 (m, 1H, 11-H-a), 4.36–4.38 (d, 1H, *J* = 4.9 Hz, 1-H), 3.84–3.90 (m, 1H, 11-H-b), 3.79 (s, 3H, 4′-OCH_3), 3.73 (s, 6H, 3′, 5′-OCH_3), 2.97–3.18 (m, 2H, 2, 3-H), 2.57 (s, 3H, Ar–CH_3)
2g	−96.0 (*ca.* 0. 11)	703.2633 $(M+H)^+$	8.98 (s, 1H, –NH–), 8.18 (s, 1H, Ar–H), 8.09–8.12 (d, 1H, *J* = 7.9 Hz, Ar–H), 7.21–7.24 (m, 2H, Ar–H), 7.13–7.17 (m, 4H, Ar–H), 6.78 (s, 1H, 5-H), 6.23 (s, 2H, 2′, 6′-H), 6.16 (s, 1H, 8-H), 5.99 (s, 2H, OCH_2O), 5.34 (s, 2H, Ar–CH_2–), 5.21–5.25 (d, 1H, *J* = 4.1 Hz, 4-H), 4.42–4.47 (m, 2H, 1-H, 11-H-a), 3.85–3.91 (m, 1H, 11-H-b), 3.80 (s, 3H, 4′-OCH_3), 3.74 (s, 6H, 3′, 5′-OCH_3), 2.98–3.14 (m, 2H, 2, 3-H), 2.54 (s, 1H, Ar–CH_3)

and **2g** may overcome MDR compared to VP-16 against K562/A02 *in vitro*. The derivatives **2a** and **2d** may overcome MDR compared to VP-16 against KBV *in vitro*. We can assert that the promising results obtained for the new 4β-N-substituted podophyllotoxin derivatives described in this study make them potential candidates to take up further synthesis and evaluation of such new derivatives.

Table 3
The experimental data of compounds **2a–g** *in vitro*.

Compound	GI_{50} (10^{-6} mol/L)		Resistant factor (RF)	IC_{50} (10^{-6} mol/L)		Resistant factor (RF)
	K562	K562/A02		KB	KBV	
2a	7.15	8.24	1.15	2.90	3.07	1.06
2b	2.00	13.60	6.80	44.34	63.66	1.44
2c	9.35	13.00	1.39	>100	>100	–
2d	11.84	13.70	1.16	1.59	2.06	1.30
2e	2.42	10.10	4.17	24.41	34.48	1.41
2f	2.98	13.00	4.36	28.99	>100	–
2g	10.53	13.51	1.28	12.41	86.49	6.97
VP-16	0.67	22.00	32.84	0.32	7.07	22.09
VCR	–	–	–	4.99	>100	–
ADM	0.033 g/L	3.800 g/L	151.151	–	–	–

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