European Journal of Medicinal Chemistry 85 (2014) 498–507

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and evaluation of novel podophyllotoxin derivatives as potential antitumor agents

Wei-Hua Cheng [a], Bo Cao [b], Hai Shang [a], Cong Niu [b], Li-Ming Zhang [c], Zhong-Heng Zhang [c], Dan-Li Tian [d], Shi Zhang [b], Hong Chen [b, c, **], Zhong-Mei Zou [a, *]

[a] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, PR China
[b] Pharmacognosy Division, Medical College of Chinese People's Armed Police Force, Tianjin 300162, PR China
[c] Tianjin Key Laboratory on Technologies Enabling Development of Clinical Therapeutics and Diagnostics, School of Pharmacy, Tianjin Medical University, Tianjin 300070, PR China
[d] Tianjin First Central Hospital, Tianjin 300192, PR China

ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form
24 July 2014
Accepted 4 August 2014
Available online 4 August 2014

Keywords:
Podophyllotoxin
Cytotoxicity
Apoptosis
Multidrug resistance
MDR-1

ABSTRACT

Cancer multidrug resistance (MDR) is a common cause of treatment failure in cancer patients. Increased expression of permeability glycoprotein (P-gp), which is also known as *MDR-1*, is the main cause of multidrug resistance. Podophyllotoxin derivatives hold great promise in the battle to overcome multidrug resistance, as they can induce cytotoxicity through multiple mechanisms. Here, we synthesized sixteen novel podophyllotoxin derivatives and evaluated their cytotoxicities in human cancer cell lines, HeLa, K562 and K562/A02. Some of these compounds were more potent than etoposide, a clinically relevant inhibitor of DNA repair enzymes. In particular, compound **5p** exhibited the most potent activity toward drug-resistant K562/A02 cells, as it robustly inhibited tumor cell proliferation and induced apoptosis. Furthermore, preliminary investigation suggested that **5p** inhibited the expression of *MDR-1* in K562/A02 cells more effectively than etoposide.

© 2014 Elsevier Masson SAS. All rights reserved.

1. Introduction

Prolonged cancer chemotherapy treatment can result in an acquired resistance toward multiple drugs, which is known as multidrug resistance (MDR) [1]. The development of MDR is a major factor of mortality following failed chemotherapy; as such, intense efforts are underway to develop novel synthetic antitumor agents with cytotoxicity against MDR cancer cell lines.

Podophyllotoxin (PPT, **1**), an abundant naturally occurring cyclolignan isolated mainly from *Podophyllum peltatum* and *Podophyllum hexandrum*, has important antineoplastic and antiviral properties [2]. However, its antimitotic activity proved to be of the greatest interest to researchers [3]. Because of its toxic side effects, extensive modifications to the original structure have been carried out over several decades. Podophyllotoxin derivatives with antitumor activity, such as etoposide (VP-16, **2**) and teniposide (VM-26, **3**) have been widely used as anticancer drugs for clinical chemotherapy [4]. However, their low water solubility, induction of acquired drug-resistance and severe gastrointestinal disturbances prompted a search for new podophyllotoxin derivatives [5]. In order to obtain better therapeutic agents, a great number of podophyllotoxin analogs have been synthesized. This has led to the development of NK-611 (**4**) andGL-331 (**5**) (Fig. 1); the latter compound entered phase II clinical trials for the treatment of various cancers [6]. Recently, several novel podophyllotoxin derivatives showed a potent anti-tumor activity [7–9].

Two alternative molecular mechanisms are generally involved in the antineoplastic activity of podophyllotoxin analogs: prevention of the assembly of tubulin into microtubules and inhibition of the catalytic activity of DNA topoisomerase II (Topo II) [10,11].

The investigation of the structure–activity relationships of PPT indicates that the *trans*-lactone, the 4β-substituted moiety, and the 4′-demethyl moieties are essential for TOP-II inhibitory activity [2,11,12]. 4β-N-substituted derivatives of PPT have attracted much interest owing to their improved cytotoxicity relative to PPT [13]. In this regard, we have discovered 4β-N-substituted derivatives of PPT that show both higher antitumor activity and greater solubility

* Corresponding author. Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100193, PR China.
** Corresponding author. Pharmacognosy Division, Medical College of Chinese People's Armed Police Force, Tianjin 300162, PR China.
E-mail addresses: chenhongtian06@163.com (H. Chen), zmzou@implad.ac.cn (Z.-M. Zou).

http://dx.doi.org/10.1016/j.ejmech.2014.08.006
0223-5234/© 2014 Elsevier Masson SAS. All rights reserved.

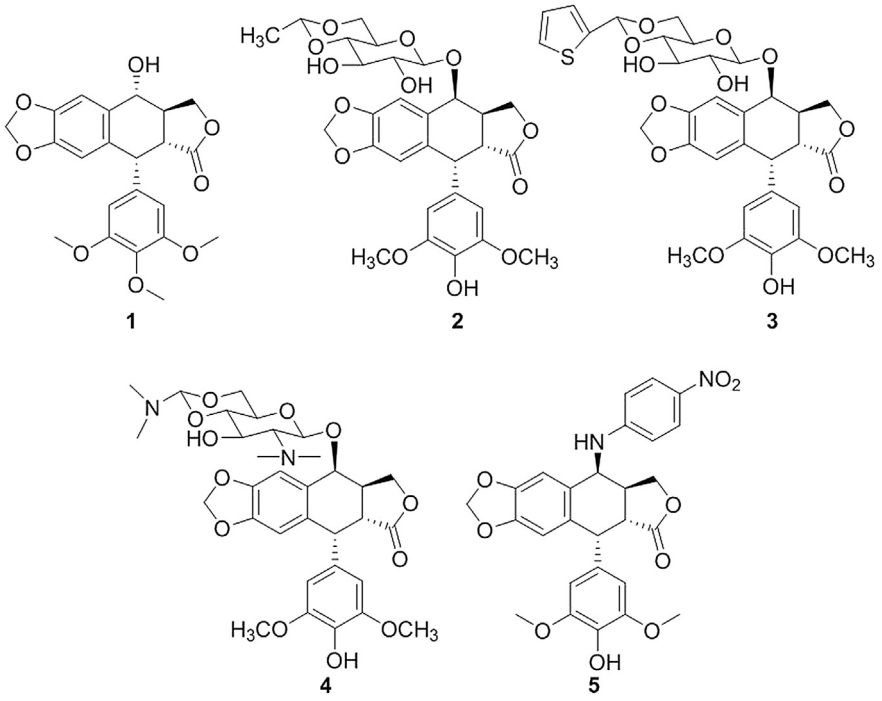


Fig. 1. Structures of podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**), NK611(**4**) and GL331(**5**).

than VP-16 [14–16]. However, these novel podophyllotoxin analogs are subject to rapid metabolic turnover *in vivo* [16]. In order to address this limitation, we synthesized additional 4β-N-substituted derivatives of PPT by means of a Schiff base reaction, and evaluated their cytotoxicities against a panel of three human cancer cell lines. The toxicity of compound **5p** was 10 times higher than that of VP-16 in K562/A02 tumor cells. This was reflected by the resistance index of 0.905 for 5p, but 5.667 for podophyllotoxin and 69.29 for VP-16. Our preliminary results indicate that compound **5p** may overcome MDR by reducing the expression of *MDR-1*.

2. Results and discussion

2.1. Chemistry

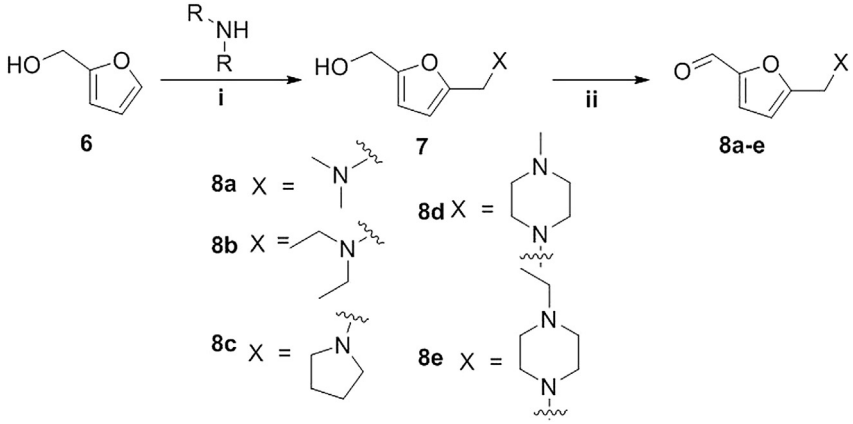
The synthesis of compounds **8a**–**e** is outlined in Scheme 1. Compound **7** was prepared by means of a Mannich reaction with secondary amine in the presence of glacial acetic acid and formaldehyde aqueous solution. Then, compound **7** was reacted with active dioxide manganese to give the intermediate **8**.

The synthetic route (Scheme 2) to the target compounds **5a**–**p** involved the intermediate **3**, which was prepared from **1**. In the presence of sodium azide, compound **2** was derived from compound **1** [17]. Then, compound **3** was derived from **2** through a reduction azide reaction. Next, **3** was combined with one of compounds **8a**–**e** in the presence of absolute methanol and a catalytic amount of glacial acetic acid to provide the intermediate **4**. Then, compounds **5a**–**p** was derived from **4** through a reduction reaction. The structures of the target compounds were identified by HRMS, ^{1}H NMR, and ^{13}C NMR spectral analysis.

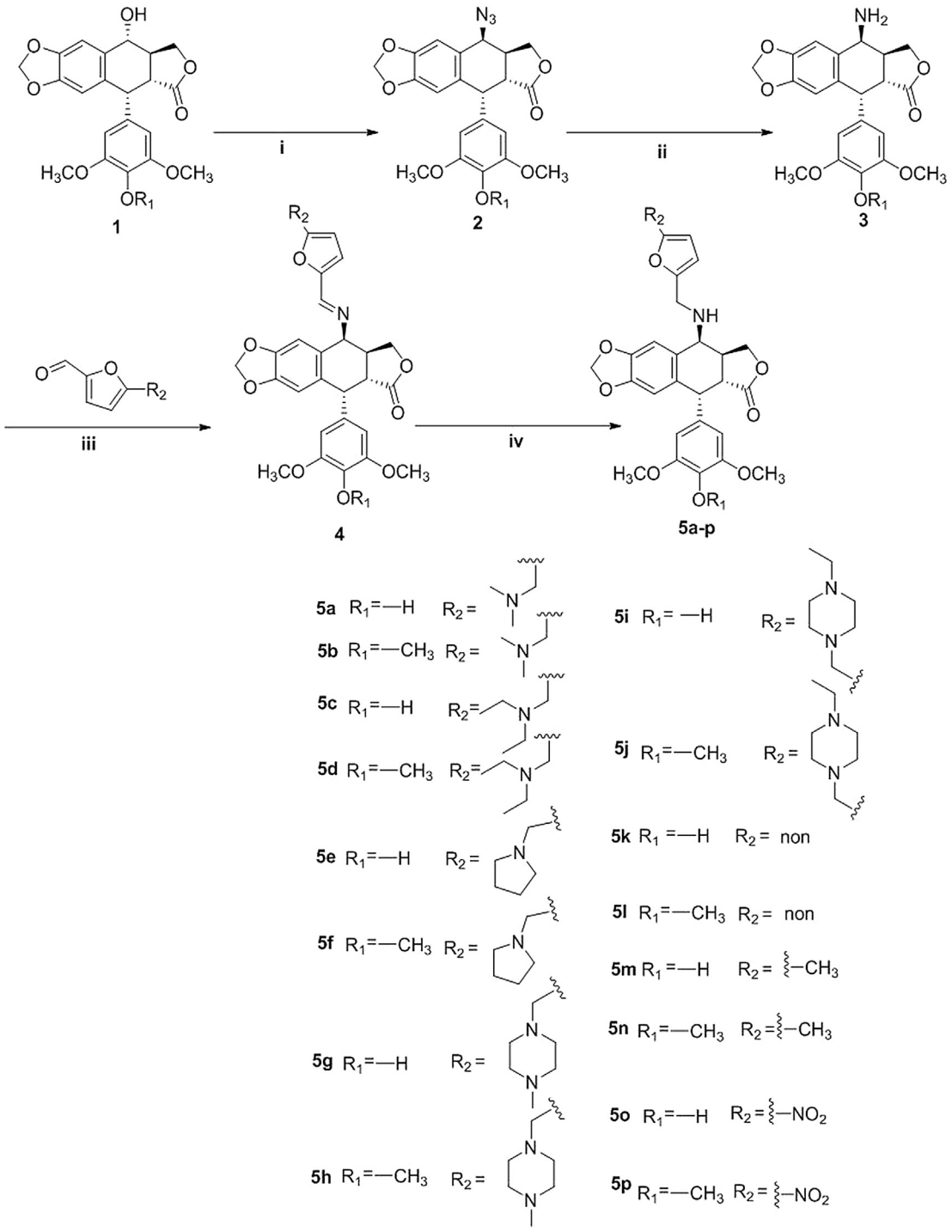
2.2. Biological activity

2.2.1. In vitro cytotoxicity assay

The evaluation of the cytotoxicities of derivatives **5a**–**p** was carried out with a panel of three tumor cell lines (HeLa, K562, and



Scheme 1. Reagents and conditions: (i) CH_3COOH/HCHO/amines; (ii) CH_2Cl_2/MnO_2.



Scheme 2. Reagents and conditions: (i) NaN_3, CF_3COOH, CH_2Cl_2; (ii) Pd/C, $HCOONH_4$, CH_2Cl_2; (iii) CH_3COOH, MeOH; (iv) $NaBH_4$, MeOH.

K562/A02) using the MTT assay. As a comparison, we used the cytotoxic podophyllotoxin and its derivative, VP-16. The results are summarized in Table 1, where it can be seen that compounds **5a** and **5i** were more cytotoxic towards HeLa cells than the positive control, VP-16. The IC_{50} value of compound **5a** was 0.51 μM, whereas compounds **5e**, **5f**, **5l** and **5n** displayed more moderate cytotoxicities (i.e., higher IC_{50}) in both HeLa and K562 cell lines. The derivatives **5e**, **5i**, **5n** and **5p** appear to be more cytotoxic to the MDR cell line, K562/A02, when compared to VP-16. In particular, compound **5p** significantly inhibited proliferation of K562/A02, which likely explains its ability to abrogate drug resistance.

We were also able to deduce some preliminary structure–activity relationships. First, the 4'-OH compounds were more cytotoxic than the corresponding 4'-OMe analogues (compare **5a** and **5b**, **5c** and **5d**, **5e** and **5f**, and **5g** and **5h**). This observation is in accord with previously reported activities of closely-related structures [13]. Second, the introduction of an amine group, such as dimethylamine, diethylamine, pyrrolidine, 1-methylpiperazine, or 1-ethylpiperazine, resulted in a considerable increase in cytotoxicity in HeLa and K562. However, **5l**, **5n** and **5p**, which lack the amide group, exhibited stronger activity against K562/A02 tumor cells than did the positive control, VP-16. We thus infer that

Table 1
Cytotoxicities of podophyllotoxin derivatives in three cancer cell lines.

Comp.	IC_{50} (μmol/L)[a,b]			RF
	HeLa	K562	K562/A02	
5a	0.51	7.89	70.12	8.89
5b	14.2	6.91	35.33	5.11
5c	6.38	8.25	>1000	>100
5d	>100	>100	>1000	>100
5e	1.21	5.28	24.66	4.67
5f	1.40	6.25	40.23	6.43
5g	2.42	8.13	200.12	24.61
5h	15.3	>100	>1000	>100
5i	0.67	7.45	123.21	16.54
5j	>100	>100	>1000	>100
5k	70.46	>100	>1000	>100
5l	1.53	5.78	10.94	1.89
5m	60.45	65.91	>1000	34.23
5n	1.94	6.79	10.08	1.48
5o	6.64	9.24	59.24	6.41
5p	7.55	5.99	5.42	0.905
Podophyllotoxin	0.10	0.03	0.17	5.667
VP-16	6.27	3.39	234.7	69.29

RF: resistance factor was calculated from the ratio of the growth inhibition constant (IC_{50}) of the resistant cell subline to that of the parental cell line.
[a] The value is the average of three replicates.
[b] IC_{50}: Concentration that causes a 50% reduction of cell growth.

removal of the amine group might increase the cytotoxicity of podophyllotoxins in K562/A02 tumor cells. Conversely, an excessive number of amino groups may engender multidrug resistance, although this hypothesis requires further testing.

Previous studies indicate that VP-16 and VM-26, which have a free hydroxyl group at the E-ring, are inhibitors of topoisomerase II [18–21]. The derivatives we prepared also have free hydroxyl groups at the E-ring (**5a**, **5c**, **5e**, **5g**, **5i**, **5k**, **5m** and **5o**). It was suggested that these derivatives were inhibitors of topoisomerase II [22], whereas podophyllotoxin targets microtubules [23]. In contrast, and analogous to podophyllotoxin, the derivatives (**5b**, **5d**, **5f**, **5h**, **5j**, **5l**, **5n** and **5p**) do not have free hydroxyl groups at the E-ring, which is consistent with our previous finding that they also target microtubules [22].

2.2.2. Study of the pharmacological mechanism-of-action

Our *in vitro* cytotoxicity studies indicated that compound **5p** markedly inhibited the growth of K562/A02 tumor cells. We therefore studied this compound further in order to elucidate the mechanism underlying its anti-MDR activity.

2.2.2.1. Cell growth. Cell growth assays indicated that **5p** inhibited the growth of cells in a concentration dependent manner. Indeed, **5p** was able to block the growth of both parental K562 cells and their multidrug-resistant derivatives (K562/A02) in a dose dependent fashion (Fig. 2).

2.2.2.2. Morphological evaluation of apoptosis. As shown in Fig. 3, untreated K562/A02 cells exhibited normal growth characteristics (Fig. 3A). Interestingly, we found alterations in the structure, size, and shape of the nuclei in K562/A02 cells treated with 6.0 μM etoposide or **5p** for 48 h (Fig. 3B), with large cytoplasmic vacuoles, abnormal mitotic figures, multinucleation and formation of large cells being typical (Fig.3C and D). However, HeLa cells treated with 12.0 μM of **5p** for 48 h developed typical apoptotic features, such as membrane blebbing, cell shrinkage and detachment, and nuclear condensation and fragmentation (Fig. 3E). These hallmarks of cell death, which increased concomitantly with increasing drug concentration, are indicated by red arrows.

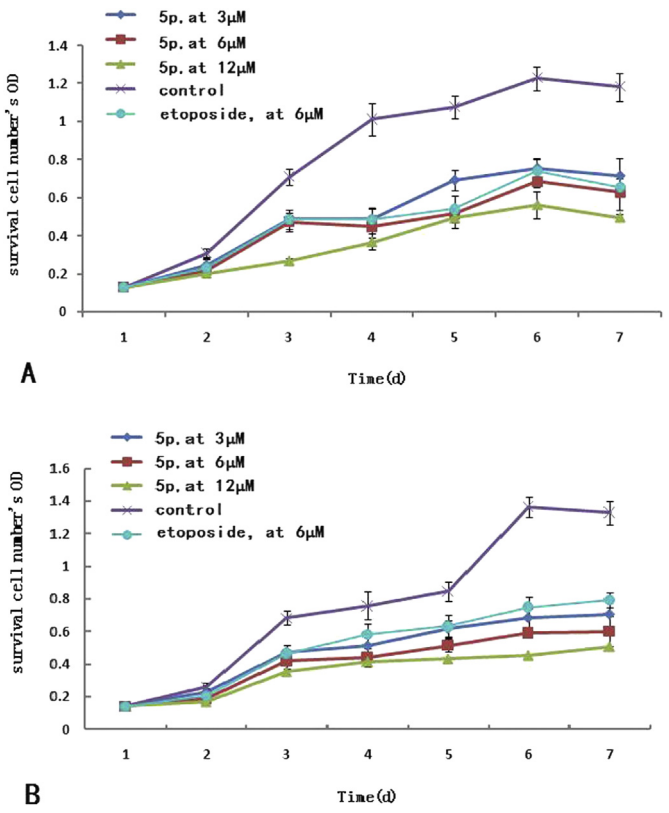


Fig. 2. Time-dependent effects of **5p** on cell growth and viability. (A) Treatment of K562 cells; (B) Treatment of K562/A02 cells.

2.2.2.3. Apoptosis-specific marker induction in response to compounds. The nuclear morphology of treated cells suggested that **5p** triggered apoptosis. To confirm that **5p** induced apoptosis in K562/A02 cells, we performed double staining with Annexin V-FITC and propidium iodide (PI). This revealed an increase in the percentage of apoptotic cells when cells were treated with either **5p** or etoposide for 48 h. We found that a 48 h treatment with 12.0 μM **5p** greatly enhanced the apoptosis of K562/A02 cells (65.35 ± 1.35% compared with 7.9 ± 1.34% in the control group; $P < 0.01$) (Fig. 4).

*2.2.2.4. Effect of **5p** on MDR-1 mRNA expression in K562/A02 and K562 cells.* The permeability glycoprotein (P-gp), encoded by *MDR-1* is a170 kDa, 1280-residue polypeptide chain organized into two homologous halves. The putative ATP-dependent export pump function of P-gp, which results in the translocation of small molecules from the cell interior, was deduced based on sequence homology to bacterial ABC transporters [24]. The highest *MDR-1* mRNA level was found in K562/A02, while K562 expressed only low levels of *MDR-1*($P < 0.001$). Strikingly, *MDR-1* mRNA was down regulated in a dose dependent manner upon treatment of K562/A02 with **5p**. Specifically, high-dose (12 μM) **5p** treatment significantly reduced *MDR-1* mRNA to 31.17% of the levels found in control cells ($P < 0.01$). As shown in Fig. 5, compound **5p** was much more effective in down regulating *MDR-1* when compared with etoposide at an equimolar concentration.

*2.2.2.5. Effect of compounds **5c**, **5e** and **5p** on MDR-1 mRNA expression in K562/A02 and K562 cells.* We also evaluated the effect of other synthetic compounds on the expression of *MDR-1*. K562/A02 cells were treated with 6 μM **5c**, **5e**, **5p**, or VP-16. All compounds elicited a reduction in *MDR-1* levels compared to the

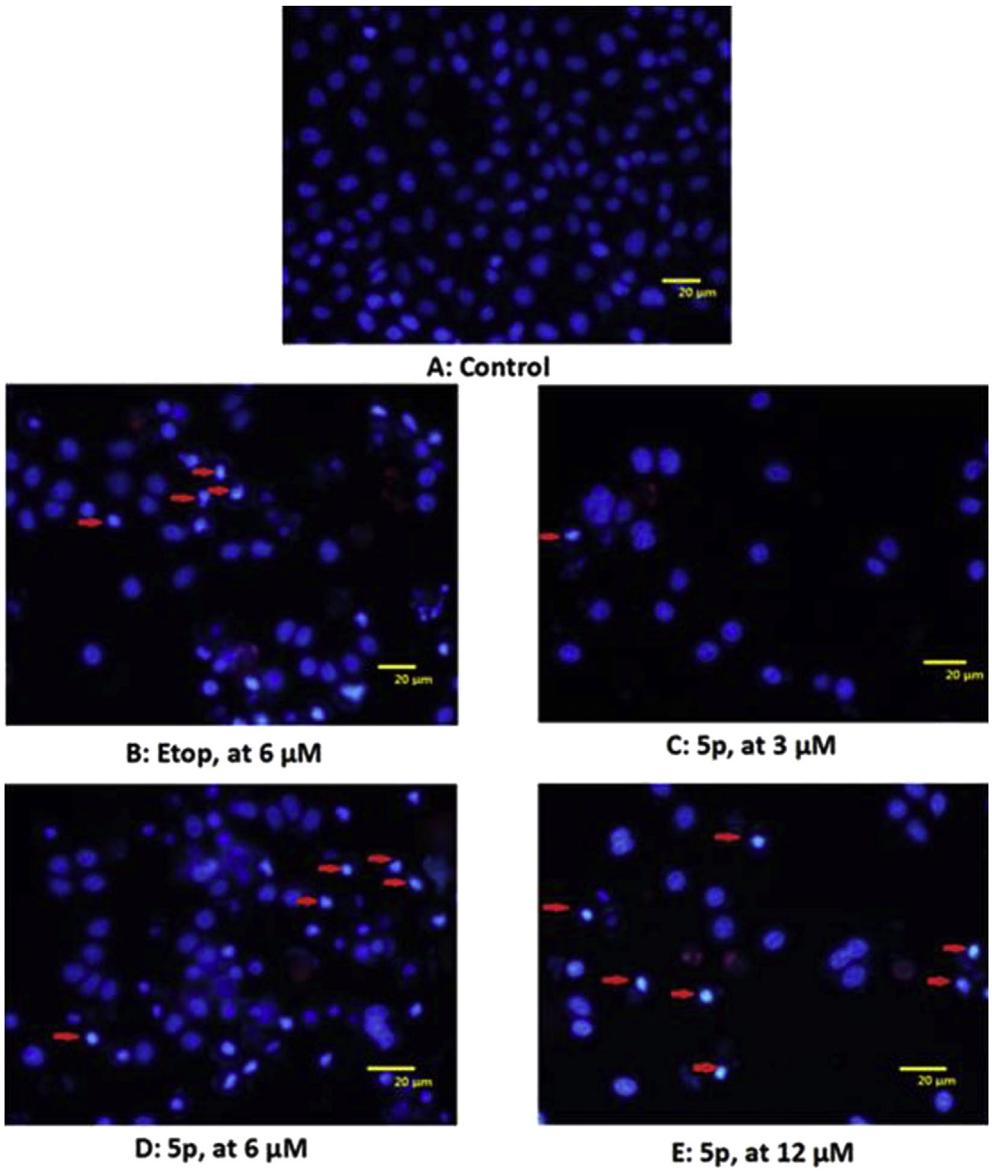


Fig. 3. Chromatin condensation and nuclear fragmentation typical for apoptosis induction were visualized by fluorescence microscopy following Hoechst 33342 staining. (A) Control K562/A02 cells; (B) K562/A02 cells treated with 6 μM etoposide; (C) K562/A02 cells treated with 3 μM **5p**; (D) K562/A02 cells treated with 6 μM **5p**; (E) K562/A02 cells treated with 12 μM **5p**. Magnification 400×.

control group ($P < 0.05$). Of note, **5p** lead to a greater reduction (~70%) than did **5c** (~35%), which indicates that the structure of **5p** may be more suitable for further anti-MDR drug development (Fig. 6).

3. Conclusion

Here, we synthesized sixteen novel podophyllotoxin derivatives **5a**–**p**, most of which showed promising cytotoxicity against a panel of cancer cell lines *in vitro*. Our studies indicate that **5p** markedly inhibited the growth of the MDR human cancer cell line K562/A02 *in vitro*. This was associated with both reduced proliferation and induction of apoptosis, as revealed by colorimetric tests, morphological analysis, and flow cytometry. Our data suggest that the mechanism-of-action of compounds **5p**, **5c** and **5e** may involve the inhibition of *MDR-1* expression, although further mechanistic research must be performed to confirm this hypothesis. Together, our results suggest that these novel compounds have the potential to be developed into anticancer agents.

4. Experimental

4.1. Chemistry

All materials and reagents were obtained from commercial sources and used without further purification unless stated. Podophyllotoxin (98% purity) was purchased from Qingze Corporation of Nanjing in China. CH_2Cl_2 was redistilled over P_2O_5. Melting points were determined on an electric X-4 digital visual melting point apparatus. The ^1H NMR and ^{13}C NMR spectra were obtained using a Bruker ARX-300 instrument (300 MHz, 400 MHz). Chemical shifts (d) are reported in ppm downfield from internal TMS standard, and the multiplicities were marked as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra data were obtained on Agilent 6210 TOP-MS and are reported as *m*/*z*.

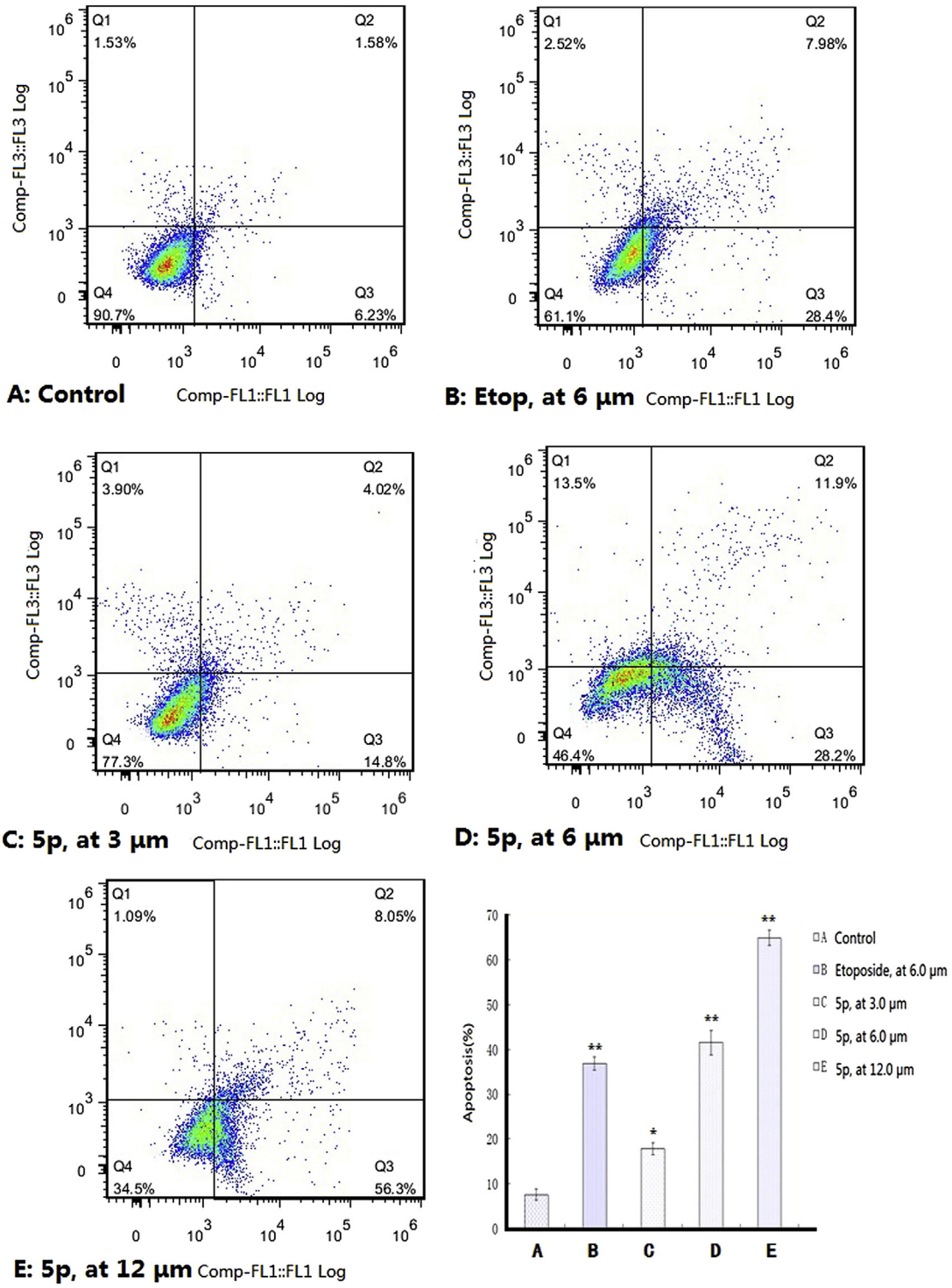


Fig. 4. **5p** induces apoptosis in K562/A02 cells. (A) Control K562/A02 cells; (B) K562/A02 cells treated with 6 μM etoposide; (C) K562/A02 cells treated with 3 μM **5p**; (D) K562/A02 cells treated with 6 μM **5p**; (E) K562/A02 cells treated with 12 μM **5p**. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the control.

*4.1.1. General procedure for the synthesis of compounds **8a–e***

Compounds (**8a–e**) were synthesized by means of a Mannich reaction. Formaldehyde (1.2 mmol) was added to a mixture of furfuryl alcohol (1 mmol) and corresponding secondary amine (1.2 mmol) in glacial acetic acid (15 mL) and stirred for 4 h at 40 °C. After completion of the reaction was confirmed by thin layer chromatography (TLC), the solvent was evaporated and glacial acetic acid was recovered before neutralization with saturated aqueous NaOH and extraction with ethyl acetate (3 × 30 mL). The combined organic fractions were washed with water followed by brine, dried over Na_2SO_4. Then, to a stirred solution of compound **7** (1 mmol) and active dioxide manganese (10 mmol) in dry CH_2Cl_2 (20 mL) at room temperature for 2h, purified by column chromatography on silica gel using petroleum ether and ethyl acetate to afford the yellow solids **8a–e**.

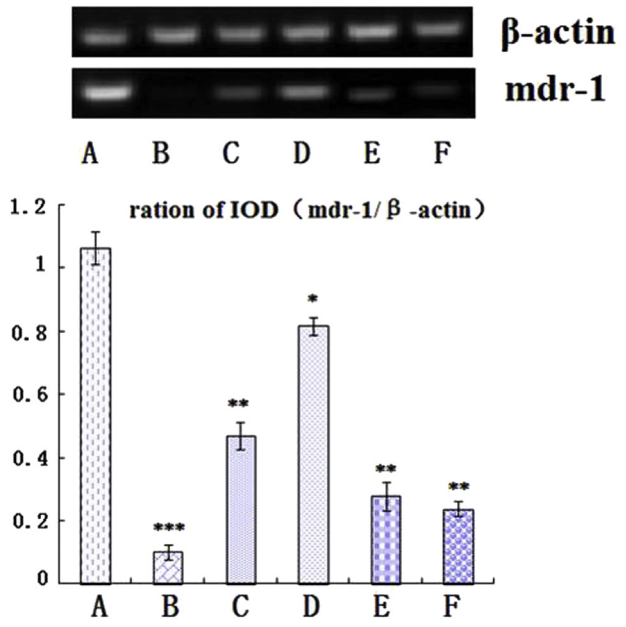


Fig. 5. Effect of **5p** on MDR-1 mRNA expression in K562A and K562 cells. (A) Control K562/A02 cells; (B) Control K562 cells; (C) K562/A02 cells treated with 6 μM etoposide; (D) K562/A02 cells treated with 3 μM **5p**; (E) K562/A02 cells treated with 6 μM **5p**; (F) K562/A02 cells treated with 12 μM **5p**. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.01$ compared with the control.

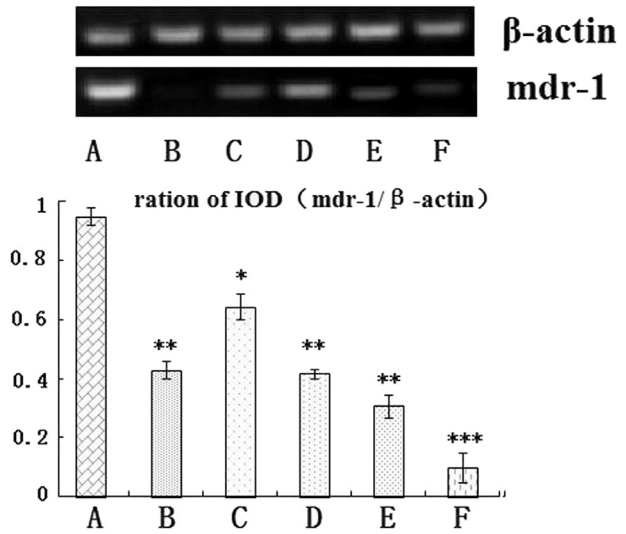


Fig. 6. Effect of compounds **5c**, **5e** and **5p** on *MDR-1* mRNA expression in K562A and K562 cells. (A) Control K562/A02 cells; (B) K562/A02 cells treated with 6 μM etoposide; (C) K562/A02 cells treated with 6 μM **5c**; (D) K562/A02 cells treated with 6 μM **5e**; (E) K562/A02 cells treated with 6 μM **5p**; (F) Control K562 cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.01$ compared with the control.

*4.1.2. General procedure for the synthesis of compounds **5a–j***

A mixture of podophyllotoxin **1** (10 mmol), NaN_3 (40 mmol), and CF_3COOH (10 mL) was stirred in dry CH_2Cl_2 (10 mL) for in ice bath for 15 min under nitrogen. Then, the mixture was stirred for 4 h at room temperature. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$. The organic extracts were dried over anhydrous Na_2SO_4, concentrated, and crystallized from CH_2Cl_2/acetic ether (1:1) to give a crystalline material **2**.

To a solution of compound **2** (10 mmol) in ethyl acetate (20 mL), 10% Pd/C (1.00 g) and $HCOONH_4$ (40 mmol) were added, and the mixture was stirred at 60 °C for 8 h. After completion of the reaction was confirmed by TLC, the solvent was washed with saturated brine, dried over anhydrous Na_2SO_4, and then removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and purified on silica gel to afford the product **3**.

A mixture of one of the compounds **8a–e** (1.5 mmol), compound **3** (1.0 mmol), and glacial acetic acid (60 μL) was stirred in dry MeOH (15 mL) for 12 h at room temperature. Then, $NaBH_4$ (4 mmol) was added and the mixture was stirred for 4 h at 0 °C.The reaction mixture was neutralized with 1 M HCl, and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic fraction was evaporated. The residue was separated by column chromatography on silica gel with CH_2Cl_2–MeOH to afford compounds **5a–j**.

*4.1.2.1. 4β-N-[5-{(Dimethylamino)methyl)furan-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5a**).* Yield: 65%; white powder solid; mp: 223–224 °C; $[\alpha]_D^{25}$ − 52° (c 0.1 CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6.33 (s, 1H), 6.25 (s, 2H), 6.19 (d, *J* = 3.1 Hz, 2H), 5.91 (dd, *J* = 6.1, 1.3 Hz, 2H), 4.50 (d, *J* = 5.1 Hz, 1H), 4.35–4.17 (m, 2H), 3.95–3.83 (m, 2H), 3.75 (s, 6H), 3.60 (d, *J* = 14.0 Hz, 1H), 3.53–3.44 (m, 2H), 3.31 (dd, *J* = 13.0, 5.1 Hz, 1H), 2.84–2.67 (m, 1H), 2.30 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 153.0, 151.7, 147.5, 147.1, 146.2, 134.0, 132.4, 131.8, 131.2, 110.2, 109.7, 108.6, 108.5, 108.3, 101.5, 69.0, 57.1, 56.6, 55.4, 47.4, 45.7, 44.3, 42.2, 39.5, 31.8. HRMS (ESI) 537.2249 for $[M+H]^+$ (calcd. 537.2237 for $C_{29}H_{32}N_2O_8$).

*4.1.2.2. 4β-N-[5-{(Dimethylamino)methyl)furan-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5b**).* Yield: 62%; white powder solid; mp: 226–227 °C; $[\alpha]_D^{25}$ − 48° (c 0.1 CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6.34 (s, 1H), 6.24 (s, 1H), 6.19 (d, *J* = 3.2 Hz, 1H), 5.98–5.81 (m, 2H), 4.51 (d, *J* = 5.3 Hz, 1H), 4.36–4.18 (m, 2H), 3.95–3.82 (m, 2H), 3.78 (s, 3H), 3.72 (s, 6H), 3.61 (d, *J* = 14.9 Hz, 1H), 3.53–3.45 (m, 2H), 3.32 (m, 1H), 2.85–2.67 (m, 1H), 2.30 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 153.5, 152.3, 147.5, 147.2, 145.4, 135.6, 134.3, 132.3, 131.7, 110.7, 110.3, 108.7, 101.6, 69.0, 61.4, 57.0, 56.2, 55.6, 47.5, 45.3, 44.5, 42.1, 39.6, 31.8. HRMS (ESI) (551.2413 for $[M+H]^+$ (calcd. 551.2393for $C_{30}H_{34}N_2O_8$).

*4.1.2.3. 4β-N-[5-{(Diethylamino)methyl)furan-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5c**).* Yield: 72%; white powder solid; mp: 230–231 °C; $[\alpha]_D^{25}$ − 69° (c 0.1 CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6.34 (s, 1H), 6.25 (s, 2H), 6.18 (s, 2H), 5.91 (dd, *J* = 6.5, 1.3 Hz, 2H), 4.50 (d, *J* = 5.2 Hz, 1H), 4.26 (dd, *J* = 9.1, 4.0 Hz, 2H), 3.93–3.84 (m, 2H), 3.74 (s, 6H), 3.69 (d, *J* = 5.2 Hz, 2H), 3.60 (d, *J* = 14.0 Hz, 1H), 3.30 (dd, *J* = 14.0, 5.2 Hz, 1H), 2.80–2.69 (m, 1H), 2.64–2.50 (m, 4H), 1.09 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 152.0, 146.6, 146.3, 145.3, 133.0, 131.4, 130.7, 130.1, 109.0, 107.4, 107.0, 100.2, 67.4, 55.4, 53.7, 48.0, 45.8, 45.6, 42.5, 40.3, 37.6, 10.5. HRMS (ESI) 565.2637 for $[M+H]^+$ (calcd. 565.2550 for $C_{31}H_{36}N_2O_8$).

*4.1.2.4. 4β-N-[5-{(Diethylamino)methyl)furan-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5d**).* Yield: 70%; white powder solid; mp: 233–235 °C; $[\alpha]_D^{25}$ − 58° (c 0.1 CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6.34 (s, 1H), 6.24 (s, 2H), 6.19 (s, 2H), 5.91 (dd, *J* = 6.0, 1.4 Hz, 2H), 4.52 (d, *J* = 5.2 Hz, 1H), 4.36–4.19 (m, 2H), 3.94–3.88 (m, 1H), 3.86 (s, 1H), 3.78 (s, 3H), 3.72 (s, 6H), 3.70 (d, *J* = 5.2 Hz, 2H), 3.64–3.55 (m, 1H), 3.41–3.26 (m, 1H), 2.81–2.69 (m, 1H), 2.65–2.53 (m, 4H), 1.10 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 152.0, 151.4, 146.6, 146.3, 136.1, 134.7, 131.4, 130.5, 109.0, 108.6, 107.4, 107.4, 107.3, 100.3, 67.4, 59.7, 55.2, 53.7, 48.0, 45.8, 45.6, 42.7, 40.2, 37.7, 10.5. HRMS (ESI) 579.2696 for $[M+H]^+$ (calcd. 579.2706 for $C_{32}H_{38}N_2O_8$).

*4.1.2.5. 4β-N-[5-{(Pyrrolidin-1-yl)methyl)furan-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5e**).* Yield: 60%; white powder solid; mp: 222–223 °C; $[\alpha]_D^{25}$ − 68° (c 0.1 CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6.30 (s, 1H), 6.25 (s, 2H), 6.19 (d, *J* = 5.4 Hz, 2H), 5.91 (dd, *J* = 6.4, 1.3 Hz, 2H), 5.41 (s, 1H), 4.50 (d, *J* = 5.2 Hz, 1H), 4.32–4.18 (m, 2H), 3.94–3.84 (m, 2H), 3.75 (s, 6H), 3.69 (s, 2H), 3.59 (d, *J* = 14.9 Hz, 1H), 3.31 (dd, *J* = 13.7, 5.2 Hz, 1H), 2.82–2.67 (m, 1H), 2.61 (s, 4H), 1.82 (dd, *J* = 6.6, 3.4 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 153.3, 147.6, 147.2, 146.3, 133.9, 132.3, 131.8, 131.2, 110.1, 108.6, 108.4, 107.9, 101.3, 68.4, 56.4, 54.7, 53.7, 46.6, 43.5, 41.3, 38.6, 30.9, 23.4. HRMS (ESI) 563.2863 for $[M+H]^+$ (calcd. 563.2393 for $C_{31}H_{34}N_2O_8$).

*4.1.2.6. 4β-N-[5-{(Pyrrolidin-1-yl)methyl)furan-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5f**).* Yield: 70%; white powder solid; mp: 220–221 °C; $[\alpha]_D^{25}$ − 66° (c 0.1 CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6.30 (s, 1H), 6.24 (s, 2H), 6.18 (d, *J* = 3.0 Hz, 2H), 5.91 (dd, *J* = 5.5, 1.3 Hz, 2H), 4.51 (d, *J* = 5.2 Hz, 1H), 3.93–3.85 (m, 2H), 3.79 (s, 3H), 3.72 (s, 6H), 3.60 (d, *J* = 14.9 Hz, 1H), 3.41–3.23 (m, 2H), 2.82–2.69 (m, 1H), 2.63 (s, 4H), 1.82 (dd, *J* = 6.6, 3.4 Hz, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.4, 152.5, 147.7, 147.3, 137.1, 135.7, 132.3, 131.6, 110.1, 108.7, 108.4, 108.2, 101.3, 68.4, 60.7, 56.2, 54.8, 46.6, 43.7, 41.2, 38.6, 30.9, 23.5. HRMS (ESI) 577.3030 for $[M+H]^+$ (calcd. 577.2550 for $C_{32}H_{36}N_2O_8$).

*4.1.2.7. 4β-N-[5-{(4-Methylpiperazin-1-yl)methyl)furan-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5g**).* Yield: 73%; white powder solid; mp: 211–212 °C; $[\alpha]_D^{25}$ − 61° (c 0.1 CH_3CN); ^1H NMR (400 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.36 (s, 1H), 6.27 (s, 2H), 6.20 (d, *J* = 7.4 Hz, 2H), 5.93 (d, *J* = 6.1 Hz, 2H), 4.51 (d, *J* = 5.2 Hz, 1H), 4.29–4.20 (m, 2H), 3.93–3.85 (m, 2H), 3.76 (s, 6H), 3.66–3.60 (m, 1H), 3.58 (d, *J* = 5.2 Hz, 2H), 3.31 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.83–2.71 (m, 1H), 2.59 (s, 8H), 2.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 153.2, 147.6, 147.2, 146.3, 133.9, 132.4, 131.8, 131.2, 110.1, 108.5, 108.4, 107.9, 101.2, 68.4, 56.5, 56.4, 54.9, 54.7, 54.7, 46.7, 43.5, 41.3, 38.6. HRMS (ESI) 592.2984 for $[M+H]^+$ (calcd.592.2659 for $C_{32}H_{37}N_3O_8$).

*4.1.2.8. 4β-N-[5-{(4-Methylpiperazin-1-yl)methyl)furan-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5h**).* Yield: 73%; white powder solid; mp: 214–215 °C; $[\alpha]_D^{25}$ − 55° (c 0.1 CH_3CN); ^1H NMR (400 MHz, $CDCl_3$) δ 6.46 (s, 1H), 6.36 (s, 1H), 6.26 (s, 2H), 6.23–6.17 (m, 2H), 5.93 (dd, *J* = 5.2, 1.3 Hz, 2H), 4.53 (d, *J* = 5.3 Hz, 1H), 4.32–4.21 (m, 2H), 3.93–3.86 (m, 2H), 3.79 (s, 3H), 3.73 (s, 6H), 3.69–3.61 (m, 1H), 3.59 (d, *J* = 5.3 Hz, 2H), 3.41–3.29 (m, 2H), 2.82–2.69 (m, 1H), 2.62 (s, 8H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 153.2, 152.5, 147.6, 147.3, 137.1, 135.6, 132.4, 131.6, 110.1, 110.0, 108.5, 108.4, 108.3, 101.3, 68.4, 60.7, 56.2, 54.7, 54.5, 54.2, 46.7, 43.7, 41.2, 38.6. HRMS (ESI) 606.3324 for $[M+H]^+$ (calcd.606.2815 for $C_{33}H_{39}N_3O_8$).

*4.1.2.9. 4β-N-[5-{(4-Ethylpiperazin-1-yl)methyl)furan-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5i**).* Yield: 71%; white powder solid; mp: 222–224 °C; $[\alpha]_D^{25}$ − 56° (c 0.1 CH_3CN); ^1H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6.32 (s, 1H), 6.25 (s, 2H), 6.22–6.15 (m, 2H), 5.91 (d, *J* = 4.6 Hz, 2H), 4.50 (d, *J* = 5.2 Hz, 1H), 4.33–4.18 (m, 2H), 3.93–3.82 (m, 2H), 3.75 (s, 4H), 3.66–3.52 (m, 2H), 3.30 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.81–2.71 (m, 1H), 2.65–2.49 (m, 8H), 2.44 (q, *J* = 7.1 Hz, 2H), 1.09 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 153.2, 147.6, 147.2, 146.3, 133.9, 132.4, 131.7, 131.1, 110.1, 109.9, 108.5, 108.4, 107.9, 101.2, 68.4, 56.4, 54.9, 54.7, 52.3, 52.2, 46.6, 43.5, 41.3, 38.6, 11.6. HRMS (ESI) 606.2809 for $[M+H]^+$ (calcd.606.2815 for $C_{33}H_{39}N_3O_8$).

*4.1.2.10. 4β-N-[5-{(4-Ethylpiperazin-1-yl)methyl)furan-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5j**).* Yield: 70%; white powder solid; mp: 226–227 °C; $[\alpha]_D^{25}$ − 59° (c 0.1 CH_3CN); ^1H NMR (400 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.35 (s, 1H), 6.26 (s, 2H), 6.23–6.16 (m, 2H), 5.93 (dd, *J* = 5.0, 1.4 Hz, 2H), 4.53 (d, *J* = 5.2 Hz, 1H), 4.34–4.20 (m, 2H), 3.96–3.85 (m, 2H), 3.79 (s, 3H), 3.73 (s, 5H), 3.64 (d, *J* = 5.2 Hz, 1H), 3.62–3.55 (m, 2H), 3.32 (dd, *J* = 13.7, 5.2 Hz, 1H), 2.82–2.71 (m, 1H), 2.68–2.49 (m, 8H), 2.46 (t, *J* = 7.0 Hz, 2H), 1.10 (*t*, *J* = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.46, 153.17, 152.46, 147.65, 147.30, 137.08, 135.70, 132.43, 131.62, 110.11, 109.89, 108.53, 108.45, 108.31, 101.30, 68.48, 60.75, 56.25, 54.99, 54.71, 52.42, 52.24, 46.69, 43.71, 41.28, 38.70, 14.39. HRMS (ESI) 620.2986 for $[M+H]^+$ (calcd.620.2972 for $C_{34}H_{41}N_3O_8$).

*4.1.3. General procedure for the synthesis of compounds **5k**–**p***

A mixture of the appropriate intermediate (1.5 mmol), **3** (1.0 mmol), and glacial acetic acid (60 μL) was stirred in dry MeOH (15 mL) for 12 h at room temperature. Then $NaBH_4$ (4 mmol) was added and the mixture was stirred for 4 h at 0 °C.The reaction mixture was neutralized with 1 M HCl, and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic fractions were evaporated. The residue was separated by column chromatography on silica gel with petroleum ether-acetic ether to afford compounds **5k**–**p**.

*4.1.3.1. 4β-N-[{Furan-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5k**).* Yield: 75%, white powder solid; mp 201–203 °C; $[\alpha]_D^{25}$ − 73° (c 0.1, CH_3CN); ^1H NMR (400 MHz, $CDCl_3$) δ 6.45–6.47 (m, 1H), 6.38–6.40 (m, 1H), 6.25–6.27 (m, 1H), 6.43 (s, 1H), 6.30 (s, 1H), 6.25 (s, 2H), 5.93 (dd, *J* = 5.2, 1.3 Hz, 2H), 4.51 (d, *J* = 5.2 Hz, 1H), 4.24–4.27 (m, 2H), 3.75–3.78 (m, 1H) 3.88–3.94 (m, 2H), 3.75 (s, 6H), 3.27–3.34 (m, 1H), 2.69–2.81 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 153.1, 147.1, 147.5, 146.2, 142.2, 133.9, 132.3, 131.2, 131.8, 110.7, 110.2, 108.5, 108.1, 101.6, 69.0, 57.1, 55.5, 47.4, 44.3, 42.2, 39.5. HRMS (ESI) 480.1648 for $[M+H]^+$(calcd. 480.1658 for $C_{26}H_{25}NO_8$).

*4.1.3.2. 4β-N-[{Furan-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5l**).* Yield: 78%, white powder solid; mp 205–206 °C; $[\alpha]_D^{25}$ − 67° (c 0.1, CH_3CN); ^1H NMR (400 MHz, $CDCl_3$) δ 6.45–6.47 (m, 1H), 6.38–6.40 (m, 1H), 6.25–6.27 (m, 1H), 6.43 (s, 1H), 6.30 (s, 1H), 6.25 (s, 2H), 5.93 (dd, *J* = 5.2, 1.3 Hz, 2H), 4.51 (d, *J* = 5.2 Hz, 1H), 4.24–4.27 (m, 2H), 3.75–3.78 (m, 1H), 3.88–3.94 (m, 2H), 3.78 (s, 3H), 3.72 (s, 6H), 3.24–3.38 (m, 1H), 2.74–2.88 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4, 153.4, 152.4, 147.6, 147.3, 142.2, 137.2, 135.7, 132.4, 131.6, 110.5, 110.1, 108.4, 108.3, 107.9, 101.3, 68.4, 60.7, 56.2, 54.8, 43.7, 41.3, 38.7. HRMS (ESI) 494.1808 for $[M+H]^+$ (calcd. 494.1815 for $C_{27}H_{27}NO_8$).

*4.1.3.3. 4β-N-[5-{Methylfuran-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5m**).* Yield: 72%, white powder solid; mp 211–212 °C; $[\alpha]_D^{25}$ − 58° (c 0.1, CH_3CN); ^1H NMR (400 MHz, $CDCl_3$) δ 6.44 (s, 1H), 6.32 (s, 1H), 6.24 (s, 2H), 6.13 (d, *J* = 3.0 Hz, 2H), 5.94 (dd, *J* = 5.2, 1.3 Hz, 2H), 4.50 (d, *J* = 5.2 Hz, 1H), 4.25–4.27 (m, 2H), 3.93 (d, *J* = 3.1 Hz, 1H), 3.80–3.88 (m, 2H), 3.75 (s, 6H), 3.32 (dd, *J* = 13.8, 5.2 Hz, 1H), 2.34 (s, 3H), 2.34–2.84 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.4, 153.5, 152.5, 147.7, 147.4, 142.3, 137.2, 135.6, 133.9, 131.6, 110.2, 110.1, 108.3, 108.0, 107.9, 101.4, 68.4, 60.7, 56.3, 54.7, 43.5, 41.3, 38.7, 13.6. HRMS (ESI) 494.1841for $[M+H]^+$ (calcd. 494.1815 for $C_{27}H_{27}NO_8$).

*4.1.3.4. 4β-N-[5-{Methylfuran-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5n**).* Yield: 65%, white powder solid; mp 213–214 °C; $[\alpha]_D^{25}$ − 63° (c 0.1, CH_3CN); ^1H NMR (400 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.34 (s, 1H), 6.25 (s, 2H), 6.03 (d, *J* = 3.2 Hz, 2H), 5.95 (dd, *J* = 5.2, 1.3 Hz, 2H), 4.52 (d, *J* = 5.2 Hz, 1H), 4.27–4.30 (m, 2H), 3.93 (d,

J = 2.7 Hz 1H), 3.80–3.88 (m, 2H), 3.80 (s, 3H), 3.75 (s, 6H), 3.32 (dd, J = 13.8, 5.2 Hz, 1H), 2.34 (s, 3H), 2.34–2.84 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.5, 151.9, 150.5, 147.7, 146.2, 142.3, 137.1, 135.7, 133.9, 131.6, 110.2, 110.1, 108.4, 108.0, 107.9, 101.4, 68.5, 60.7, 56.3, 54.7, 43.5, 41.3, 38.1, 13.6. HRMS (ESI) 508.2005 for $[M+H]^+$ (calcd. 508.1971 for $C_{28}H_{29}NO_8$).

*4.1.3.5. 4β-N-[5-{Nitrofuran-2-yl}methyl]-amido-4′-demethyl-4-desoxy-podophyllotoxin (**5o**).* Yield: 68%, yellow powder solid; mp 222–223 °C; $[\alpha]_D^{25}$ − 78° (c 0.1, CH_3CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 3.8 Hz, 1H), 7.04 (d, J = 3.8 Hz, 1H), 6.56 (s, 1H), 6.53 (s, 1H), 6.33 (s, 2H), 5.94 (dd, J = 5.2, 1.3 Hz, 2H), 4.71 (d, J = 5.2 Hz, 1H), 4.67–4.28 (m, 2H), 3.97–4.05 (m, 2H), 3.89 (d, J = 3.2 Hz 1H), 3.79 (s, 6H), 3.56 (dd, J = 13.8, 5.2 Hz, 1H), 3.04–3.18 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9,152.1,152.4,148.4,147.5, 134.2, 132.6, 132.6, 131.0, 128.2, 110.8, 108.3, 108.6, 101.6, 67.8, 67.7, 56.5, 56.5, 43.9, HRMS (ESI) 525.1545 for $[M+H]^+$ (calcd. 525.1509 for $C_{26}H_{24}N_2O_{10}$).

*4.1.3.6. 4β-N-[5-{Nitrofuran-2-yl}methyl]-amido-4-desoxy-podophyllotoxin (**5p**).* Yield: 66%, yellow powder solid; mp 229–230 °C; $[\alpha]_D^{25}$ − 73° (c 0.1, CH_3CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (d, J = 3.9 Hz, 1H), 6.43 (d, J = 3.9 Hz, 1H), 6.51 (s, 1H), 6.26 (s, 1H), 6.16 (s, 2H), 5.84 (dd, J = 5.2, 1.3 Hz, 2H), 4.46 (d, J = 5.2 Hz, 1H), 4.27–4.18 (m, 2H), 3.85–3.93 (m, 2H), 3.80 (d, J = 2.9 Hz, 1H), 3.71 (s, 3H), 3.65 (s, 6H), 3.21 (dd, J = 13.8, 5.2 Hz, 1H), 2.69–2.82 (m, 1H). ^{13}C NMR (75 MHZ, $CDCl_3$) δ 174.9, 152.1, 152.4, 148.4, 147.5, 134.2, 132.6, 132.6, 131.0, 128.2, 110.8, 108.6, 108.3, 101.6, 67.8, 67.7, 56.5, 56.5, 43.9. HRMS (ESI) 539.1710 for $[M+H]^+$ (calcd. 539.1666 for $C_{27}H_{26}N_2O_{10}$).

4.2. Biological evaluation

4.2.1. Cell viability assay

HeLa, K562, and K562/A02 cells were cultured in RPMI-1640 containing 10% FBS, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. The cells were seeded at a density of 5 × 10^3 cells/well in 96-well plates and allowed to attach for 24 h. The thiazolyl blue tetrazolium blue (MTT) assay was performed to quantify cell viability following treatment with the synthetic compounds or reference compound etoposide (VP-16) [25]. After 48 h, 20 μL MTT (5 mg/mL) solution was added for 4 h at 37 °C. Then, the supernatant was discarded and dimethylsulfoxide (DMSO) was added to dissolve the formazan product. The intensity was measured at a wavelength of 490 nm.

4.2.2. Cell growth curves

We used the K562 and K562/A02 cell lines for cell growth curves. The cells were cultured on 96-well plates with **5p** or etoposide at varying concentrations of 3, 6, and 12 μM on Day 1. This was followed by examination of their inhibitory effects on cell survival recorded for each plate every 24 h. The assessment was performed using a modification of the MTT assay. Following the addition of 5 mg/mL MTT, samples were cultured for a further 4 h. After the removal of culture medium, followed by the addition of DMSO, absorption at 490 nm was measured.

4.2.3. Morphological analysis with fluorescence microscopy

Apoptosis is one of the major pathways leading to cell death, and is associated with classical morphologies, including chromatin condensation and nuclear fragmentation. To evaluate the apoptotic activity of **5p**, we performed nuclear staining with the DNA-binding dye, Hoechst-33342. Briefly, K562/A02 cells were plated into 6-well plates (1 × 10^5 cells in 3 mL) and treated with 3.0, 6.0, or 12.0 μM of **5p** for 48 h; 6.0 μM etoposide was used as a positive control. Cells were collected by centrifugation at 1000 × g for 5 min, washed with ice-cold PBS and then incubated with Hoechst-33342 (10 μg/mL) for 15 min in the dark, then placed on slides, and observed under a fluorescence microscope (excitation 346 nm, emission 460 nm; NIKON TE2000-E). Apoptotic cells were identified by condensation of chromatin and fragmentation of nuclei. Pictures were obtained using a video camera Q-imaging (Burnaby, BC, Canada).

4.2.4. Apoptosis detection using Annexin V/PI staining

Apoptosis can be detected by translocation of phosphatidylserine to the cell surface using an Annexin V-FITC antibody. K562/A02 cells were seeded into 6-well plates (1 × 10^5 cells/well), incubated overnight, treated with 3.0, 6.0, or 12.0 μM of **5p** for 48 h; 6.0 μM etoposide was used as the positive control. To assess apoptosis, cells were washed with ice-cold PBS (pH 7.4). Annexin V-FITC and PI staining were performed using an Annexin V-FITC kit (Beyotime Co., Ltd). Annexin V-FITC and PI fluorescence were monitored using a Beckman Coulter flow cytometer. Ten thousand events were collected per sample. Data were analyzed using FlowJo analysis software.

4.2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR)

Since we determined that compound **5p** was the most effective among the podophyllotoxin derivatives with respect to abrogation of MDR, we decided to evaluate *MDR-1* expression by RT-PCR. K562 and K562/A02 cells were seeded on 6-well plates at a density of 1 × 10^5 cells/well in 10% FCS/RPMI-1640, and varying amounts of **5p** were added 24 h later; cells were then cultured for another 48 h. Total RNA was extracted by Trizol on Day 3 and absorbance was measured in an RNA/DNA calculator at 260 and 280 nm cDNA was synthesized from 2 μg of total RNA using TIANScript RT Kit reverse transcription reagents (TIANEN BIOTECH, RT120420, Beijing). Amplification of *MDR-1* was performed under the following cycling conditions: 94 °C for 3 min and then 30 cycles of 94 °C for 40 s, 55 °C for 40 s, 72 °C for 1 min, finally 72 °C for 10 min, followed by cooling to 4 °C by Program Temp Control System (ASTSC. PC-818s. Japan). Primers for *MDR-1* (157bp product) amplification were 5′-CCC ATA ATT GCA ATA GCA GC-3′ and 5′-GTT CAA ACT TCT GCT CCT CA-3′, while 5′-GTG GGG CGC CCC AGC CAC CA -3′ and 5′-CTT CCT TAA TGT CAC GCA CGA TTT C-3′ were for *β-actin* (540bp) PCR target products were then separated electrophoretically on a 5% agarose DNA gel and stained with Gold View. The mRNA levels were normalized to the levels of *β-actin*.

Acknowledgments

This work has been financially supported by National S & T Major Special Project on Major New Drug Innovation (2011ZX09307-002-01 and 2013ZX09508104), PUMC Youth Fund (33320140074), the Fundamental Research Funds for the Central Universities and Program for Innovative Research Team in IMPLAD (IT1305).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.006.

References

[1] S.B. Kaye, Curr. Opin. Oncol. 10 (1998) S15–S19.
[2] T. Imbert, Biochimie 80 (1998) 207–222.
[3] M. Gordaliza, P.A. Garcia, J.M. del Corral, M.A. Castro, M.A. Gomez-Zurita, Toxicon 44 (2004) 441–459.
[4] K.R. Hande, Eur. J.Cancer 34 (1998) 1514–1521.

[5] A.K. Mukherjee, S. Basu, N. Sarkar, A.C. Ghosh, Curr. Med. Chem. 8 (2001) 1467–1486.
[6] H.K. Lee, K.H. Wang, J. Food Drug Anal. 3 (1995) 209–232.
[7] D. Passarella, B. Peretto, R. Blasco y Yepes, G. Cappelletti, D. Cartelli, C. Ronchi, J. Snaith, G. Fontana, B. Danieli, J. Borlak, Eur. J. Med. Chem. 45 (2010) 219–226.
[8] A. Kamal, A. Mallareddy, P. Suresh, V. Lakshma Nayak, R.V. Shetti, N. SankaraRao, J.R. Tamboli, T.B. Shaik, M.V. Vishnuvardhan, S. Ramakrishna, Eur. J. Med. Chem. 47 (2012) 530–545.
[9] J.F. Liu, C.Y. Sang, X.H. Xu, L.L. Zhang, X.Yang, L.Hui, J.B. Zhang, S.W. Chen, 64 (2013) 621–628.
[10] A. Ogundaini, M. Farah, P. Perera, G. Samuelsson, L. Bohlin, J. Nat. Prod. 59 (1996) 587–590.
[11] E.L. Baldwin, N. Osheroff, Curr. Med. Chem. 5 (2005) 363–372.
[12] A. Jordan, J.A. Hadfield, N.J. Lawrence, A.T. McGown, Med. Res. Rev. 18 (1998) 259–296.
[13] Y. You, Curr. Pharm. Des. 11 (2005) 1695–1717.
[14] M. Zhao, M. Feng, S.F. Bai, Y. Zhang, Bi W. Chao, H. Chen, Chin. Chem. Lett. 20 (2009) 901–904.
[15] T. Ai, S.Y. Shi, L.T. Chen, L. Li, B. Cao, Y. Gao, H. Chen, J. Zhou, Chin. Chem. Lett. 24 (2013) 37–40.
[16] H. Chen, S. Zuo, X. Wang, X. Tang, M. Zhao, Y. Lu, L. Chen, J. Liu, Y. Liu, D. Liu, Eur. J. Med. Chem. 46 (2011) 4709–4714.
[17] A. Kamal, N. Laxman, G. Ramesh, Bioorg. Med. Chem. Lett. 10 (2000) 2059–2062.
[18] K.H. Lee, Y. Imakura, M. Haruna, S.A. Beers, L.S. Thurston, H.J. Dai, C.H. Chen, S.Y. Liu, Y.C. Cheng, J. Nat. Prod. 52 (1989) 606–613.
[19] 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, Y.C. Cheng, J. Med. Chem. 33 (1990) 1364–1368.
[20] Z.Q. Wang, Y.H. Kuo, D. Schnur, J.P. Bowen, S.Y. Liu, F.S. Han, J.Y. Chang, Y.C. Cheng, K.H. Lee, J. Med. Chem. 33 (1990) 2660–2666.
[21] T.L. Macdonald, E.K. Lehenert, J.T. Loper, K.C. Chow, W.E. Ross, DNA Topoisomerase in Cancer, Oxford University, New York, 1991, p. 119.
[22] Related Research Will be Reported Soon.
[23] J.D. Loike, C.F. Brewer, H. Sternlicht, W.J. Gensler, S.B. Horwitz, Cancer Res. 38 (1978) 268.
[24] D. Mahadevan, Leuk. Res. 30 (2006) 1077–1078.
[25] J.M. Jin, Y.J. Zhang, C.R. Yang, J. Nat. Prod. 67 (2004) 5–9.