Accepted Manuscript

Conjugates of podophyllotoxin and norcantharidin as dual inhibitors of topoisomeraseII and protein phosphatase 2A

Zhen-Bo Tang, Yao-Zhang Chen, Jie Zhao, Xiao-Wen Guan, Yong-Xin Bo, Shi-Wu Chen, Ling Hui

PII: S0223-5234(16)30579-7

DOI: 10.1016/j.ejmech.2016.07.031

Reference: EJMECH 8749

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 13 January 2016

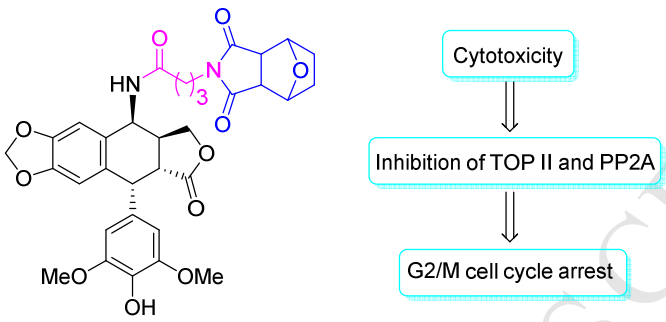
Revised Date: 15 July 2016

Accepted Date: 16 July 2016

Please cite this article as: Z.-B. Tang, Y.-Z. Chen, J. Zhao, X.-W. Guan, Y.-X. Bo, S.-W. Chen, L. Hui, Conjugates of podophyllotoxin and norcantharidin as dual inhibitors of topoisomeraseII and protein phosphatase 2A, *European Journal of Medicinal Chemistry* (2016), doi: 10.1016/j.ejmech.2016.07.031.

Conjugates of podophyllotoxin and norcantharidin as dual inhibitors of topoisomerase□ and protein phosphatase 2A

Zhen-Bo Tang, Yao-Zhang Chen, Jie Zhao, Xiao-Wen Guan, Yong-Xin Bo, Shi-Wu Chen*, Ling Hui*



Conjugates of podophyllotoxin and norcantharidin as dual inhibitors of topoisomerase Ⅱ and protein phosphatase 2A

Zhen-Bo Tang [a], Yao-Zhang Chen [c], Jie Zhao [a], Xiao-Wen Guan [a], Yong-Xin Bo [a], Shi-Wu Chen [a,*], Ling Hui [b,d,*]

[a] *School of Pharmacy, Lanzhou University, Lanzhou 730000, China*

[b] *Experimental Center of Medicine, General Hospital of Lanzhou Military Command, Lanzhou 730050, China*

[c] *Department of Parmaceutics, Gansu Provincial Hospital of TCM, Lanzhou 730050, China*

[d] *Key Laboratory of Stem Cells and Gene Drug of Gansu Province, General Hospital of Lanzhou Military Command, Lanzhou 730050, China*

Abstract: A series of novel conjugates of podophyllotoxin and norcantharidin was designed using association strategy, and synthesized by coupling 4′-demethylepi-podophyllotoxin with *N*-amino acid norcantharimides, and their cytotoxicitiy was evaluated against four human tumor cell lines (A-549, HepG2, HeLa and HCT-8) and normal human diploid fibroblast line WI-38. These compounds exhibited potent cytotoxic effects on tumor cell lines, whereas it was less toxic to WI-38 cells than anticancer drug VP-16 or its parent compound norcantharidin. Furthermore, conjugates **7a**, **7c**, **7f**, **7j**, **7k** and **7l** displayed excellent PP2A inhibition activity with IC_{50} values of 0.49–9.52 μM. The most potent compound **7l** also exhibited topoisomerase Ⅱ inhibition activity. In addition, compound **7l** induced cell-cycle arrest in the G2/M phase in HepG2 by regulating levels of cyclinB1 and cdc2.

Key words: podophyllotoxin, cantharidin, TOP-Ⅱ, PP2A, cell-cycle arrest

* Corresponding author.

For Prof. Shi-Wu Chen: *e-mail*, chenshw@lzu.edu.cn; Tel./fax, 86-931-8915686.

For Prof. Ling Hui: *e-mail*, zyhuil@hotmail.com; Tel./fax, 86-931-8994582.

1. Introduction

In tumor therapy, anticancer drugs based on a single biological target or pathway show good efficacy, and reduce the risk of side effects. However, cancer is a complex disease with multiple causes. In many cases, when a particular target or pathway is completely blocked, the tumor cells will compensate for this change by another potential pathway, and the therapeutic effect disappears [1]. For each specific tumour type, effective chemotherapy treatment depends on the use of a suitable drug combination. Such drug combinations consist of two or more single agents or drugs joined together either directly or indirectly via a chemical linker [2].

Podophyllin, an ethanolic extract of *Podophyllum peltatum* L. or *P. emodi* Wall, is a good source of the aryltetralin-type lignan, podophyllotoxin (PPT, **1**). The latter compound, as well as its congeners and derivatives exhibit pronounced biological activity mainly as strong antiviral agents and as antineoplastic drugs [3]. The podophyllotoxin derivatives etoposide (VP-16, **3**), etopophos (etoposide phosphate), and teniposide (VM26, **4**) are thus successfully utilized in the treatment of a variety of malignant conditions [4]. The cytotoxic mechanism of VP-16 is the inhibition of topoisomerase Ⅱ (TOP Ⅱ), unlike the parent compound which inhibits mitosis by interfering with tubulin. VP-16 induces cell death by enhancing the TOP II-mediated DNA cleavage through the stabilization of the transient DNA/TOP II cleavage complex [5]. The clinical success of VP-16 and VM26 has triggered the search for compounds with a similar mechanism of action but without their shortcoming such as poor water-solubility and acquired drug-resistance. Continuing research on PPT is

currently focused on structure optimization to generate derivatives with superior pharmacological profiles and broader therapeutic scope. Tafluposide (F11782) and F14512 are two potential drug candidates based on PPT recently applied in clinical trials. F11782 is a dual inhibitor of TOP I and Ⅱ which impairs the binding of the enzyme to DNA, but does not stabilize the cleavage complex [6]. The spermine-conjugated 4'-demethy-4-epipodophyllotoxin (DMEP, **2**) derivative F14512 is a TOP Ⅱ poison that inhibits the polyamines transport system to target preferentially tumor cells [7].

Cantharidin (CTD, **5**), the principle active ingredients of *Epicanta gorhami* or *Mylabris spp* (blister beetles), is active in vitro against several tumor cell lines, including cervical, hepatoma, ovarian, laryngocarcinoma, colon, osteocarcinoma, and leukamia cell lines. However its applications are limited by its severe nephrotoxic and inflammatory side effects, and it has mainly been used to treat the gastrointestinal tract, the ureter, and the kidney [8]. Norcantharidin (NCTD, **6**, Fig. 1), the demethylated analogue of cantharidin, is active in vitro against several tumor cell lines and appears to cause less nephrotoxic and inflammatory side effects [9]. Recently, a series of bioactive analogues of CTD or NCTD have been synthesized in an attempt to reduce its toxicity and thereby increase its potential use [10–14]. The anticancer activity of CTD and NCTD is thought to come from the inhibition of serine/threonine protein phosphatase 1 (PP1) and/or protein phosphatase 2A (PP2A) [15–17]. These two phosphatases are known to be involved in many different cellular processes, including repairing DNA damage, cell cycle progression, and apoptosis

[18,19].

Recently, we have reported some conjugates of podophyllotoxin together with 5-FU [20–22], d4T [23,24] and dichloroplatinum [25], which show better antitumor or antiviral activities than the parent compound. In our continuing efforts to find new compounds with potent activities and low toxicity based on a natural product, we report herein a series of conjugates of DMEP together with NCTD, and their cytotoxicities in vitro against four human tumor cell lines, together with a human lung fibroblast cell line. The most potent compound **7l** was further evaluated for its effect on inhibition of PP2A and TOP Ⅱ, cell cycle progression, and interaction with CT DNA.

2. Results and discussion

2.1 Chemistry

In a previous publication [26], amino acid substituted norcantharimides were synthesized by a modified Gabriel synthesis, by reaction of NCTD and amino acids in the presence of equivalents anhydrous Et_3N in anhydrous toluene in a thick-walled glass pressure vessel for 24 h. However, this reaction was time consuming and dangerous. We synthesized cantharimides **6a-l** with high yield via a melting method as the procedure outlined in Scheme 1. Briefly, the mixture of NCTD [27] and appropriate α-amino acids, or *β*-alanine, or *γ*-aminobutyric acid, or 4-aminobenzoic acid were heated to melting and reacted for another 40–60 mins under N_2. The above mixture was then cooled to room temperature and recrystallized to provide the corresponding intermediate **6a-l**.

The synthesis of conjugates **7a-l** was carried out according to the procedure outlined in Scheme 2. Firstly, the intermediate 4β-amino-4′-demethyl-4-deoxylpodophyllotoxin was obtained from DMEP as described in previous publication [24]. Compounds **7a-l** were obtained through reaction of the intermediate 4β-amino-4′-demethyl-4-deoxypodophyllotoxin with the corresponding **6a-l** under 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) in the presence of *N*-hydroxybenzotriazole (HOBT) in dichloromethane. The structures of target compounds **7a-l** were identified by IR, ^{1}H NMR spectra, ^{13}C NMR spectra and high resolution mass spectrometry (HRMS).

Furthermore, the octanol-water partition coefficients of **7a-l** were also determined to evaluate their water-solubility [28]. The results are shown in Table 1. As expected, compounds **7a-l** were more soluble in water than VP-16.

2.2 Biological activity

*2.2.1 Cytotoxicities of compounds **7a-7l***

The in vitro cytotoxicity of compounds **7a-7l** were evaluated against a panel of four human cancer cell lines (lung carcinoma A-549, hepatic carcinoma HepG2, cervical carcinoma HeLa and human colorectal adenocarcinoma HCT-8 cells) together with the human lung fibroblast cell line WI-38, using VP-16 and NCTD as reference compounds. The screening procedure was based on the standard MTT method [29], and the IC_{50} values are summarized in Table 1.

The conjugates **7a-7l** were generally more potent than VP-16 and NCTD in their cytotoxicity to these four cell lines (Table 1). In the group of compounds, these

compounds were most effective in HepG2 cells, and had lowest potency in HCT-8 cells, although the order of potency varied in each cell line. The IC_{50} values for compound **7l**, which is the most potent conjugate in the group compounds, were 4.57±0.21, <0.01, 4.33±0.12, and 3.48±0.45 μM for the A-549, HepG2, HeLa, and HCT-8 tumor cells, respectively. Whereas compound **7l** was less toxic than VP16 and NCTD to the normal human lung fibroblast cell WI-38, the IC_{50} values for compound **7l**, VP16 and NCTD were >100, 17.6±3.29 and 26.73±2.28 μM for WI-38, respectively.

2.2.2 PP2A inhibition activities

Serine/threonine protein phosphatases (S/T-PPs) are thought to be cancer suppressive target, since the inhibition of the S/T-PPs can lead to an increased phosphorylation and hence the activation of substrate kinases [18]. Cantharidin and its analogues have been of considerable interest as potent inhibitors of the PP1 and PP2A S/T-PPs. However, cantharimides with non-polar or acidic amino acid residues are only poor inhibitors of PP1 and PP2A [26], or even the norcantharimides have shown to be devoid of protein phosphatase inhibition [30,31]. To determine whether conjugates **7a–7l** inhibit PP1 and PP2A, we evaluated their ability to directly inhibit PP1 and PP2A in vitro assay, NCTD was included as internal standards to ensure the relative validity of our protocol [32].

All conjugates were initially screened at a dose of 100 μM. In the case of PP1 inhibition, none of the conjugates reported herein displayed any noteworthy activity (data not shown). Otherwise, conjugates **7a**, **7c**, **7f**, **7j**, **7k** and **7l** display excellent

potency on inhibition of PP2A, their IC_{50} value were shown in Table 2. Compound **7l** possess the most potent PP2A inhibition with IC_{50} values of 0.49 μM. These results indicate that compound **7l** shows the selectivity for PP2A over PP1, which is consistent with previous results with the cantharimides [15,26].

To further confirm and analyze the cellular effects related to the alteration of PP2A activity by evaluating the effect of compound **7l**. The HepG2 cells were incubated with different concentration **7l** for 24 h, and a phosphopeptide substrate was measured [33]. PP2A activity was reduced to approximately 73%, 32% and 27% of the control activity at 1 μM, 2 μM and 5 μM, respectively (Figure 2). PP2A activity was decreased by **7l** exposure in a concentration-dependent manner.

In view of the differential cytotoxicity, inhibition of PP2A and solubility, compound **7l** has the most potential as an anticancer drug, and we choose the HepG2 cell line to further investigate the mechanism of its cytotoxic effect.

*2.2.3 Compound **7l** inhibits Topo Ⅱ*

In a previous paper it was demonstrated the ability of DMEP derivates can inhibit the relaxation activity of TOP Ⅱ [34]. Thus, we investigate the effect of **7l** on the catalytic activity of TOP Ⅱ by evaluated the effect of compound **7l** on topoisomerase II-mediated DNA relaxation [35]. The supercoiled DNA was converted into relaxed forms by the enzyme (TOP Ⅱ) as shown in Figure 3 (lane 2). At 50 μM concentration **7l** exerts a partial inhibitory effect (lane 3), as demonstrated by the increase of supercoiled DNA and by the concurrent decrease in relaxed products. Nevertheless, by increasing the concentration up to 100 μM, a complete inhibition is

observed for **7l** (lane 4), but only a partial effect on TOP Ⅱ relaxation for VP-16 (lane 5). Taking into account that VP-16 exerts the lower antiproliferative capacity, these data could suggest the contribution of the inhibition on TOP Ⅱ to the cytotoxicity.

*2.2.4 Compound **7l** induces cell cycle arrest in G2/M phase by effect on cyclin B1 and cdc2*

Podophyllum derivates including VP-16 [29,34], and cantharidin [36] inhibit cancer cells though G2/M cell cycle arrest. Thus, we investigated the effect of **7l** on cell cycle progression by means of fluorescence-activated cell sorting analysis of HepG2 cells stained with propidium iodide. Treatment with **7l** led to a dose-dependent accumulation of cells in the G2/M phase with a concomitant decrease in the population of G1 phase cells (Figure 4). After exposure to 0.5 μM (Fig. 4B) and 1 μM (Fig. 4C) **7l** for 24 h, 27.6% and 58.6%, respectively, of the cells were in G2/M phase, compared with 9.4% in untreated cultures (Fig. 4A). These results demonstrated that **7l** also induces cell cycle arrest in G2/M phase.

During normal cell cycle, the progression of cells from the G2 to M phase is triggered by activation of the cyclin B1-dependent cdc2 kinase [37]. We therefore investigated the expression levels of cyclin B1 and cdc2 in HepG2 cells following treatment with **7l** (0, 0.5, 1 μM) for 24 h. Western blot analysis revealed a marked increase in cyclin B1 and cdc2 protein levels in **7l**-treated HepG2 cells compared with the control (Figure 5). Furthermore, the effect of **7l** treatment on these cell-cycle regulators was also time-dependant. These results indicate that the **7l**-induced G2/M cell cycle arrest involves cell-cycle regulators cdc2 and cyclin B1 in a dose-dependent

manner.

*2.2.5 Interaction of **7l** with CT DNA*

DNA is an important pharmacological target of antitumor drugs. It is therefore essential to the development of effective chemotherapeutic agents to explore their interaction with DNA. A binding assay of compound **7l** with CT DNA was performed by monitoring changes in the fluorescence spectroscopy emission pattern of compound **7l** (1 μM, excited at 264 nm) with increasing concentrations of CT DNA (0, 1, 2, 3, 4, and 5 μM) in pH 7.2 PBS [38]. Even though no appreciable change in the position of the charge transfer band of the complex was observed upon addition of DNA, the fluorescence intensity of the complex decreases progressively with increasing concentration of DNA, suggesting that compound **7l**-based emission is quenched when it is bound to DNA (Figure 6).

3. Conclusion

In summary, a series of novel conjugates of PPT and NCTD show promising in vitro cytotoxicities against a panel of human cancer cell lines and less toxicities on human normal cell line WI-38. Especially, the most potential conjugate **7l** which inhibits TOPⅡ and PP2A, interacts with CT DNA, and induces cell cycle arrest in the G2/M phase involving cell-cycle regulators cyclin B1 and cdc2. These results suggest that conjugate **7l** is a dual inhibitor of TOPⅡ and PP2A with potential for further development as anticancer agent.

4. Experimental Methods

4.1. Chemistry

All starting materials and regents were purchased commercially and used without further purification, unless otherwise stated. All reactions were monitored by thin layer chromatograph (TLC) on silica gel GF_{254} (0.25 mm thick). Column chromatography (CC) was performed on Silica Gel 60 (230-400 mesh, Qingdao Ocean Chemical Ltd., China). Melting points were determined in Kofler apparatus and were uncorrected. IR spectra were measured on a NicoLET iS5 spectrometer on neat samples placed between KBr plates. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Mecury-600BB spectrometer with TMS as an internal standard, all chemical shift values are reported as ppm. Mass spectra were recorded on a Bruker Dalton APEX Ⅱ 49e and Esquire 6000 (ESI-ION TRAP) spectrometer with ESI source as ionization, respectively.

4.1.1. General procedure of synthesis of ***6a-l***

A mixture of NCTD (336 mg, 2.0 mmol), appropriate α-amino acids, or *β*-alanine, or *γ*-aminobutyric acid, or 4-aminobenzoic acid (2.0 mmol) were heated to melt and further reacted for 40–60 mins under N_2. The above mixture was then cooled to room temperature and recrystallized to provide the corresponding intermediate **6a-l**.

*4.1.1.1 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)acetic acid (**6a**)*

Yield: 76%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.63 (s, 2 H), 4.00 (s, 2 H), 3.10 (s, 2 H), 1.60–1.58 (m, 4 H).

*4.1.1.2 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)propanoic acid (**6b**)*

Yield: 72%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 5.43–5.42 (m, 1 H), 4.76–4.32 (m, 2 H), 2.96–2.92 (s, 2 H), 1.87–1.84 (m, 2 H), 1.63–1.60 (m, 2 H), 1.58 (d, J = 7.2 Hz,

3 H).

4.1.1.3 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-3-methylbutanoic acid ***(6c)***

Yield: 70%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.85 (d, J = 4.2 Hz, 1 H), 4.78 (d, J = 3.6 Hz, 1 H), 4.25 (d, J = 10.2 Hz, 1 H), 2.95–2.90 (m, 3 H), 1.88–1.86 (m, 2 H), 1.63–1.60 (m, 2 H), 1.10 (d, J = 6.6 Hz, 3 H), 0.78 (d, J = 6.6 Hz, 3 H).

4.1.1.4 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-4-methylpentanoic acid ***(6d)***

Yield: 51%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.75 (d, J = 4.2 Hz, 2 H), 4.73–4.71 (m, 1 H), 2.95−1.92 (m, 2 H), 1.98−1.93 (m, 1 H), 1.88−1.83 (m, 2 H), 1.64−1.61 (m, 3 H), 1.37−1.36 (m, 1 H), 0.92 (d, J = 6.6 Hz, 3 H), 0.90 (d, J = 6.6 Hz, 3 H).

4.1.1.5 (2S,3S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-3-methylpentanoic acid ***(6e)***

Yield: 65%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.87 (d, J = 4.8 Hz, 1 H), 4.79 (d, J = 4.8 Hz, 1 H), 4.34 (d, J = 11.4 Hz, 1 H), 2.95−2.88 (m, 2 H), 1.90−1.87 (m, 2 H), 1.63−1.58 (m, 4 H), 1.28−1.24 (m, 1 H), 1.06 (d, J = 6.0 Hz, 3 H), 0.83 (t, J = 7.2 Hz, 3 H).

4.1.1.6 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-4-(methylthio) butanoic acid ***(6f)***

Yield: 68%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.85–4.84 (m, 1 H), 4.76 (t, J = 5.4 Hz, 1 H), 2.98−2.93 (m, 2 H), 2.58–2.33 (m, 4 H), 2.08 (s, 3 H), 1.88−1.82 (m, 2

H), 1.65−1.59 (m, 2 H).

*4.1.1.7 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-2-phenylacetic acid (**6g**)*

Yield: 70%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.46–7.43 (m, 2 H), 7.38–7.36 (m, 3 H), 5.76 (s, 1 H), 4.88 (d, *J* = 5.4 Hz, 1 H), 4.67 (d, *J* = 4.8 Hz, 1 H), 2.98−2.95 (m, 2 H), 1.86−1.80 (m, 2 H), 1.64−1.60 (m, 2 H).

*4.1.1.8 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-3-phenylpropanoic acid (**6h**)*

Yield: 64%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.25–7.19 (m, 3 H), 7.12–7.10 (m, 2 H), 4.97–4.92 (m, 1 H), 4.71 (d, *J* = 7.2 Hz, 1 H), 4.67 (d, *J* = 6.6 Hz, 1 H), 3.61–3.56 (m, 1 H), 3.44–3.37 (m, 1 H), 2.80 (d, *J* = 10.8 Hz, 1 H), 2.68 (d, *J* = 10.2 Hz, 1 H), 1.86−1.75 (m, 2 H), 1.62−1.51 (m, 2 H).

*4.1.1.9 (2S)-3-(1H-benzo[d]imidazol-2-yl)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)propanamide (**6i**)*

Yield: 56%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.12 (s, 1 H), 7.48 (d, *J* = 7.8 Hz, 1 H), 7.32 (d, *J* = 7.8 Hz, 1 H), 7.18 (t, *J* = 7.2 Hz, 1 H), 7.06 (d, *J* = 7.8 Hz, 1 H), 7.02 (d, *J* = 1.8 Hz, 1 H), 5.04–5.01 (m, 1 H), 4.74 (d, *J* = 4.8 Hz, 1 H), 4.68 (d, *J* = 4.8 Hz, 1 H), 3.72–3.68 (m, 1 H), 3.57–3.52 (m, 1 H), 2.76 (d, *J* = 6.6 Hz, 1 H), 2.61 (d, *J* = 6.6 Hz, 1 H), 1.84−1.77 (m, 2 H), 1.57−1.48 (m, 2 H).

*4.1.1.10 4-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)benzoic acid (**6j**)*

Yield: 66%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.80 (d, *J* = 9.0 Hz, 2 H), 7.31 (d, *J* = 9.6 Hz, 2 H), 4.98 (s, 2 H), 4.67 (d, *J* = 4.8 Hz, 1 H), 3.04−3.01 (m, 2 H),

1.94−1.92 (m, 2 H), 1.69−1.68 (m, 2 H).

*4.1.1.11 3-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)propanoic acid (**6k**)*

Yield: 74%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.82 (d, *J* = 4.2 Hz, 1 H), 4.71 (d, *J* = 4.2 Hz, 1 H), 3.75−3.72 (m, 2 H), 2.86 (t, *J* = 7.2 Hz, 2 H), 2.56 (t, *J* = 6.6 Hz, 2 H), 1.85−1.82 (m, 2 H), 1.60−1.58 (m, 2 H).

*4.1.1.12 4-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)butanoic acid (**6l**)*

Yield: 70%; ^{1}H NMR (600 MHz, DMSO-d_6) δ 4.82 (d, *J* = 4.8 Hz, 1 H), 4.77 (d, *J* = 4.2 Hz, 1 H), 3.53−3.50 (m, 2 H), 2.84−2.81 (m, 2 H), 2.17–2.11 (m, 2 H), 1.98−1.92 (m, 2 H), 1.85−1.82 (m, 2 H), 1.61−1.60 (m, 2 H).

*4.1.2. General procedure of synthesis of **7a-l***

A mixture of 4β-amino-4-deoxylpodophyllotoxin (210 mg , 0.53 mmol), **6a-l** (0.5 mmol), EDC·HCl (115 mg, 0.6 mmol) and HOBt (81 mg, 0.6 mmol)) was stirred in dried CH_2Cl_2 (5 ml) for 1 h at 0℃, then stirred for overnight at room temperature. After complete reaction, the mixture was washed by H_2O and then the extract was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography, using CH_2Cl_2/acetone 20:1 as the eluent to afford pure compounds **7a-l**.

*4.1.2.1 2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR,9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7]naphtho[2,3-d][1,3]dioxol-5-yl)acetamide (**7a**)*

Yield: 68%; mp: 201−203 ℃; $[\alpha]_D^{23}$ −78° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3348, 2953, 1775, 1705, 1613, 1516, 1484, 1393, 1229, 1112, 1036, 999, 938; ^{1}H NMR

(600 MHz, $CDCl_3$) δ 6.75 (s, 1 H), 6.50 (s, 1 H), 6.40 (d, *J* = 7.2 Hz, 1 H), 6.27 (s, 1 H), 5.97 (d, *J* = 5.4 Hz, 2 H), 5.24−5.21 (m, 1 H), 4.79 (d, *J* = 4.8 Hz, 1 H), 4.61 (d, *J* = 4.8 Hz, 1 H), 4.53 (d, *J* = 4.8 Hz, 1 H), 4.33 (t, *J* = 8.4 Hz, 1 H), 4.25 (d, *J* = 16.2 Hz, 1H), 4.16 (d, *J* = 16.2 Hz, 1 H), 3.82 (t, *J* = 9.6 Hz, 1 H), 3.75 (s, 6 H), 2.99−2.96 (m, 2 H), 2.92–2.90 (m, 1 H), 2.80 (dd, *J* = 14.4, 4.8 Hz, 1 H), 1.84−1.79 (m, 2 H), 1.63−1.61 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.3, 176.2, 174.5, 165.5, 148.2, 147.4, 146.4 (2 C), 133.9, 132.4, 130.2, 128.5, 109.9, 109.1, 107.7 (2 C), 101.5, 79.8 (2 C), 68.6, 56.3 (2 C), 49.9 (2 C), 49.8, 48.0, 43.5, 41.6, 36.8, 28.4, 28.2; LRMS(ESI) *m/z* 629.1861 ($[M+Na]^+$, 100%); HRMS (ESI) 629.1731 for $[M+Na]^+$ (calcd 629.1742 for $C_{31}H_{30}N_2O_{11}Na$).

*4.1.2.2 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR, 9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl)propanamide (**7b**)*

Yield: 52%; mp: 169−170 ℃; $[\alpha]_D^{23}$ –92° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3379, 2943, 1774, 1704, 1614, 1515, 1484, 1391, 1228, 1114, 1035, 998, 931; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.71 (s, 1 H), 6.48 (s, 1 H), 6.27 (s, 2 H), 5.99−5.98 (m, 2 H), 5.91 (d, *J* = 7.8 Hz, 1 H), 5.43 (s, 1 H), 5.31−5.27 (m, 1 H), 4.76−4.74 (m, 3 H), 4.54 (d, *J* = 4.8 Hz, 1 H), 4.34 (t, *J* = 8.4 Hz, 1 H), 3.94 (t, *J* = 9.9 Hz, 1 H), 3.76 (s, 6 H), 2.96−2.90 (m, 3 H), 2.81 (dd, *J* = 14.4, 4.8 Hz, 1 H), 1.87−1.85 (m, 2 H), 1.63−1.61 (m, 2 H), 1.59 (d, *J* = 7.2 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.6, 176.3, 174.6, 168.1, 148.3, 147.5, 146.4 (2 C), 133.9, 132.3, 130.3, 128.8, 110.0, 109.0, 107.7 (2 C), 101.5, 80.2, 80.0, 68.5, 56.4 (2 C), 49.6 (2 C), 49.5, 49.4, 48.0, 43.5, 41.4,

36.6, 30.9, 28.4, 28.1, 13.7; MS-ESI: 643.2007 ($[M+Na]^+$, 100%); HRMS (ESI) 643.1887 for $[M+Na]^+$ (calcd 643.1898 for $C_{32}H_{32}N_2O_{11}Na$).

*4.1.2.3 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR, 9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl)-3-methylbutanamide (**7c**)*

Yield: 57%; mp: 163−165 ℃; $[\alpha]_D^{23}$ −78° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3368, 2966, 1775, 1705, 1613, 1505, 1484, 1386, 1228, 1114, 1037, 999, 930; ^1H NMR (600 MHz, $CDCl_3$) δ 6.68 (s, 1 H), 6.53 (d, *J* = 7.2 Hz, 1 H), 6.50 (s, 1 H), 6.28 (s, 2 H), 5.98 (d, *J* = 3.6 Hz, 2 H), 5.30 (s, 1 H), 5.18−5.15 (m, 1 H), 4.86 (d, *J* = 4.2 Hz, 1 H), 4.78 (d, *J* = 3.6 Hz, 1 H), 4.56 (d, *J* = 4.8 Hz, 1 H), 4.36 (t, *J* = 8.4 Hz, 1 H), 4.25 (d, *J* = 10.2 Hz, 1 H), 3.85 (t, *J* = 9.9 Hz, 1 H), 3.76 (s, 6 H), 2.96−2.90 (m, 3 H), 2.84 (dd, *J* = 14.4, 4.8 Hz, 1 H), 2.77–2.74 (m, 1 H), 1.89−1.86 (m, 2 H), 1.62-1.60 (m, 2 H), 1.11 (m, 3 H), 0.79−0.77 (m, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.4, 177.2, 174.5, 168.0, 148.3, 147.4, 146.4 (2 C), 134.0, 132.5, 130.3, 128.7, 110.1, 108.9, 107.7 (2 C), 101.5, 79.6 (2 C), 68.7, 62.0, 56.4 (2 C), 49.6, 49.3, 48.2, 43.5, 41.6, 37.0, 28.5, 28.4, 26.3, 20.6, 19.0; MS-ESI: 671.2226 ($[M+Na]^+$, 100%); HRMS (ESI) 649.2382 for $[M+H]^+$ (calcd 649.2392 for $C_{34}H_{37}N_2O_{11}$).

*4.1.2.4 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR,9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7]naph tho[2,3-d][1,3]dioxol-5-yl)-4-methylpentanamide (**7d**)*

Yield: 52%; mp: 171−173 ℃; $[\alpha]_D^{23}$ −96° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3378, 2959, 1775, 1700, 1614, 1506, 1484, 1388, 1228, 1114, 1037, 999, 933; ^1H NMR

(600 MHz, $CDCl_3$) δ 6.71 (s, 1 H), 6.48 (s, 1 H), 6.27 (s, 2 H), 6.04 (d, *J* = 8.4 Hz, 1 H), 5.98 (s, 2 H), 5.43 (s, 1 H), 5.30−5.25 (m, 1 H), 4.76−4.71 (m, 3 H), 4.53 (d, *J* = 4.8 Hz , 1 H), 4.34 (t, *J* = 4.4 Hz, 1 H), 3.89 (t, *J* = 9.9 Hz, 1 H), 3.76 (s, 6 H), 2.96−2.88 (m, 3 H), 2.82 (dd, *J* = 14.4, 5.4 Hz, 1 H), 1.98−1.93 (m, 1 H), 1.88−1.83 (m, 2 H), 1.64−1.61 (m, 3 H), 1.37−1.36 (m, 1 H), 0.93−0.89 (m, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.8, 176.7, 174.6, 168.2, 148.3, 147.5, 146.3 (2 C), 133.9, 132.3, 130.3, 128.8, 110.0, 109.0, 107.6 (2 C), 101.5, 80.2, 80.0, 68.5, 56.3 (2 C), 52.8, 49.5 (2 C), 48.0, 43.5, 41.5, 36.7, 35.6, 28.5, 28.1, 24.8, 23.1, 21.1; MS-ESI: 685.1986 ($[M+Na]^+$, 100%); HRMS (ESI) 685.2354 for $[M+Na]^+$ (calcd 685.2368 for $C_{35}H_{38}N_2O_{11}$ Na).

*4.1.2.5 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR, 9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl)-3-methylpentanamide (**7e**)*

Yield: 53%; mp: 177−179 ℃; $[\alpha]_D^{23}$ –74° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3463, 2966, 1775, 1704, 1615, 1506, 1484, 1385, 1227, 1114, 1037, 999, 931; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.68 (s, 1 H), 6.60 (d, *J* = 7.2 Hz, 1 H), 6.51 (s, 1 H), 6.28 (s, 2 H), 5.98 (d, *J* = 5.4 Hz, 2 H), 5.43 (s, 1 H), 5.16−5.13 (m, 1 H), 4.87 (d, *J* = 4.8 Hz, 1 H), 4.79 (d, *J* = 4.8 Hz, 1 H), 4.58−4.56 (m, 1 H), 4.38 (t, *J* = 4.5 Hz, 1 H), 4.33 (d, *J* = 11.4 Hz, 1 H), 3.83 (t, *J* = 9.9 Hz, 1 H), 3.77 (s, 6 H), 2.95−2.88 (m, 3 H), 2.84 (dd, *J* = 14.4, 4.8 Hz, 1 H), 2.61−2.59 (m, 1 H), 1.90−1.87 (m, 2 H), 1.63−1.59 (m, 3 H), 1.28−1.24 (m, 1 H), 1.06 (d, *J* = 6.0 Hz, 3 H), 0.83 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.7, 177.2, 174.6, 168.3, 148.3, 147.4, 146.4 (2 C), 133.9, 132.5,

130.3, 128.6, 110.1, 108.9, 107.7 (2 C), 101.5, 79.6 (2 C), 68.8, 61.2, 56.4 (2 C), 49.6, 49.3, 48.3, 43.5, 41.6, 37.1, 31.6, 28.5, 28.4, 24.9, 16.5, 10.1; MS-ESI: 685.1986 ($[M+Na]^+$, 100%); HRMS (ESI) 685.2362 for $[M+Na]^+$ (calcd 685.2368 for $C_{35}H_{38}N_2O_{11}$ Na).

*4.1.2.6 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR, 9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl)-4-(methylthio)butanamide (**7f**)*

Yield: 67%; mp: 158−160 ℃; $[\alpha]_D^{23}$–88° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3371, 2917, 1775, 1705, 1613, 1505, 1484, 1386, 1227, 1113, 1037, 999, 931; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.71 (s, 1 H), 6.48 (s, 1 H), 6.27 (s, 2 H), 5.98 (d, *J* = 3.6 Hz, 2 H), 5.95 (d, *J* = 8.4 Hz, 1 H), 5.28−5.26 (m, 1 H), 4.87−4.84 (m, 1 H), 4.76 (t, *J* = 5.4 Hz, 1 H), 4.54 (d, *J* = 5.4 Hz, 1 H), 4.34 (t, *J* = 8.4 Hz, 1 H), 3.91 (t, *J* = 10.2 Hz, 1 H), 3.75 (s, 6 H), 2.98−2.87 (m, 3 H), 2.81 (dd, *J* = 14.4, 5.4 Hz, 1 H), 2.59−2.33 (m, 4 H), 2.08 (s, 3 H), 1.88−1.82 (m, 2 H), 1.65−1.59 (m, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.8, 176.7, 174.6, 167.4, 148.3, 147.5, 146.4 (2 C), 133.9, 132.3, 130.3, 128.7, 110.0, 109.0, 107.7 (2 C), 101.6, 80.2, 80.1, 68.4, 56.4 (2 C), 53.1, 49.6, 49.5, 48.0, 43.5, 41.4, 36.6, 28.4, 28.1, 25.9 (2 C), 15.4; MS-ESI: 703.1952 ($[M+Na]^+$, 100%); HRMS (ESI) 703.1923 for $[M+ Na]^+$ (calcd 703.1932 for $C_{34}H_{36}N_2O_{11}$ SNa).

*4.1.2.7 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR, 9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl)-2-phenylacetamide (**7g**)*

Yield: 63%; mp: 184−185 ℃; $[\alpha]_D^{23}$ –28° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3416,

2912, 1775, 1707, 1613, 1504, 1484, 1387, 1228, 1113, 1037, 1000, 941; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46−7.40 (m, 2 H), 7.38−7.34 (m, 3 H), 6.72 (s, 1 H), 6.45 (s, 1 H), 6.26 (s, 2 H), 5.97 (d, *J* = 6.0 Hz, 2 H), 5.76 (s, 1 H), 5.24−5.22 (m, 1 H), 4.89 (d, *J* = 5.4 Hz, 1 H), 4.67 (d, *J* = 4.8 Hz, 1 H), 4.49 (d, *J* = 4.2 Hz, 1 H), 4.38 (t, *J* = 8.7 Hz, 1 H), 4.10 (t, *J* = 10.2 Hz, 1 H), 3.76 (s, 6 H), 2.98−2.92 (m, 3 H), 2.65 (dd, *J* = 13.8, 4.8 Hz, 1 H), 1.86−1.80 (m, 2 H), 1.64−1.60 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.5, 176.2, 174.4, 166.7, 148.3, 147.5, 146.4 (2 C), 134.0, 133.1, 132.6, 130.2, 129.3, 129.2 (2 C), 129.1 (2 C), 128.2, 110.1, 108.9, 107.8, 107.7, 101.6, 79.6, 79.4, 68.9, 58.3, 56.4 (2 C), 49.9 (2 C), 48.9, 43.5, 41.8, 37.2, 28.5, 28.4; MS-ESI: 705.1467 ($[M+Na]^+$, 100%); HRMS (ESI) 705.2040 for $[M+ Na]^+$ (calcd 705.2055 for $C_{37}H_{34}N_2O_{11}Na$).

*4.1.2.8 (2S)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR, 9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7] naphtho[2,3-d][1,3]dioxol-5-yl)-3-phenylpropanamide (**7h**)*

Yield: 56%; mp: 175−177 °C; $[\alpha]_D^{23}$ –124° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3387, 2955, 1775, 1704, 1613, 1505, 1484, 1387, 1228, 1113, 1037, 999, 931; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.24 (d, *J* = 11.4 Hz, 2 H), 7.20 (d, *J* = 10.2 Hz, 1 H), 7.12 (d, *J* = 10.2 Hz, 2 H), 6.71 (s, 1 H), 6.48 (s, 1 H), 6.27 (s, 2 H), 5.98 (d, *J* = 4.2 Hz, 2 H), 5.95 (d, *J* = 8.4 Hz, 1 H), 5.43 (s, 1 H), 5.32−5.29 (m, 1 H), 4.96−4.93 (m, 1 H), 4.71 (d, *J* = 4.8 Hz, 1 H), 4.68 (d, *J* = 4.8 Hz, 1 H), 4.54 (d, *J* = 4.8 Hz, 1 H), 4.35 (t, *J* = 8.4 Hz, 1 H), 3.91 (t, *J* = 9.9 Hz, 1 H), 3.77 (s, 6 H), 3.58 (dd, *J* = 14.4, 5.4 Hz, 1 H), 3.43−3.38 (m, 1 H), 2.92−2.90 (m, 1 H), 2.80−2.67 (m, 3 H), 1.85−1.78 (m, 2 H),

1.56−1.25 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.9, 176.1, 174.6, 167.2, 148.3, 147.5, 146.4 (2 C), 136.4, 134.0, 132.2, 130.3, 128.8, 128.7 (2 C), 128.6 (2 C), 127.0, 110.0, 109.0, 107.7 (2 C), 101.5, 80.1, 79.9, 68.4, 56.4 (2 C), 55.1, 49.3 (2 C), 47.9, 43.5, 41.4, 36.6, 33.0, 28.3, 28.1; MS-ESI: 719.2307 ($[M+Na]^+$, 100%); HRMS (ESI) 697.2389 for $[M+H]^+$ (calcd 697.2392 for $C_{38}H_{37}N_2O_{11}$).

*4.1.2.9 (2S)-3-(1H-benzo[d]imidazol-2-yl)-2-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR,9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo- 5, 5a,6,8,8a,9-hexahydrofuro[3',4':6,7]naphtho[2,3-d][1,3]dioxol-5-yl)propanamide (**7i**)*

Yield: 58%; mp: 195−196 ℃; $[\alpha]_D^{23}$ −106° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3384, 2910, 1773, 1701, 1616, 1506, 1483, 1389, 1227, 1112, 1036, 998, 932; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (s, 1 H), 7.50 (d, *J* = 7.8 Hz, 1 H), 7.34 (d, *J* = 7.8 Hz, 1 H), 7.9 (t, *J* = 7.2 Hz, 1 H), 7.08 (t, *J* = 7.2 Hz, 1 H), 7.04 (d, *J* = 1.8 Hz, 1 H), 6.72 (s, 1 H), 6.46 (s, 1 H), 6.26 (s, 2 H), 6.00 (d, *J* = 7.8 Hz, 1 H), 5.99 (s, 2 H), 5.43 (s, 1 H), 5.30−5.26 (m, 1 H), 5.04−5.01 (m, 1 H), 4.74 (d, *J* = 4.8 Hz, 1 H), 4.69 (d, *J* = 4.8 Hz, 1 H), 4.46 (d, *J* = 5.4 Hz, 1 H), 4.30 (t, *J* = 8.4 Hz, 1 H),, 3.81−3.77 (m, 1 H), 3.76 (s, 6 H), 3.70 (dd, *J* = 15.0, 6.0 Hz, 1 H), 3.57−3.52 (m, 1 H), 2.88−2.84 (m, 1 H), 2.76 (d, *J* = 6.6 Hz, 1 H), 2.62 (d, *J* = 6.6 Hz, 1 H), 2.50 (dd, *J* = 14.4, 4.8 Hz, 1 H), 2.18−1.77 (m, 2 H), 1.63 (s, 1 H), 1.57−1.48 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.0, 176.4, 174.5, 167.4, 148.3, 147.5, 146.4 (2 C), 136.0, 133.9, 132.2, 130.3, 128.7, 127.0, 122.6, 122.4, 119.8, 118.2, 111.4, 110.7, 110.0, 109.0, 107.6 (2 C), 101.5, 79.9, 79.8, 68.4, 56.4 (2 C), 54.9, 49.5, 49.4, 47.8, 43.5, 41.3, 36.6, 28.4, 28.1, 23.1; MS-ESI: 758.2207 ($[M+Na]^+$, 100%); HRMS (ESI) 758.2310 for $[M+Na]^+$

(calcd 758.2320 for $C_{40}H_{37}N_3O_{11}Na$).

*4.1.2.10 4-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR,9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7]naphtho [2,3-d][1,3]dioxol-5-yl)benzamide (**7j**)*

Yield: 72%; mp: 220−221 ℃; $[\alpha]_D^{23}$ –64° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3469, 2961, 1774, 1712, 1609, 1503, 1483, 1383, 1228, 1113, 1036, 999, 929; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (d, *J* = 9.0 Hz, 2 H), 7.32 (d, *J* = 8.4 Hz, 2 H), 6.79 (s, 1 H), 6.64 (d, *J* = 7.8 Hz, 1 H), 6.53 (s, 2 H), 6.30 (s, 2 H), 5.98 (d, *J* = 7.8 Hz, 1 H), 5.50 (s, 1 H), 5.43–5.40 (m, 1 H), 4.98 (s, 2 H), 4.58 (d, *J* = 4.8 Hz, 1 H), 4.45 (t, *J* = 8.1 Hz, 1 H), 3.83 (t, *J* = 9.6 Hz, 1 H), 3.77 (s, 6 H), 3.05−2.97 (m, 4 H), 1.93−1.91 (m, 2 H), 1.69−1.67 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.0, 175.9, 174.4, 166.4, 148.4, 147.6, 146.4 (2 C), 134.9, 134.0, 133.1, 132.7, 130.1, 128.6, 127.9 (2 C), 126.5 (2 C), 110.0, 109.1, 107.7 (2 C), 101.7, 79.5 (2 C), 69.1, 56.4 (2 C), 50.0 (2 C), 48.6, 43.6, 41.8, 37.3, 28.6, 28.5; MS-ESI: 691.1542 ($[M+Na]^+$, 100%); HRMS (ESI) 691.1887 for $[M+Na]^+$ (calcd 691.1898 for $C_{36}H_{32}N_2O_{11}$ Na).

*4.1.2.11 4-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR,9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7]naphth o[2,3-d][1,3]dioxol-5-yl)butanamide (**7k**)*

Yield: 65%; mp: 238−240 ℃; $[\alpha]_D^{23}$ –85° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3344, 2955, 1770, 1691, 1612, 1524, 1480, 1396, 1224, 1116, 1032, 996, 920; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.81 (s, 1 H), 6.51 (s, 1 H), 6.27 (s, 2 H), 6.20 (d, *J* = 7.2 Hz, 1 H), 5.98 (d, *J* = 7.2 Hz, 2 H), 5.21−5.18 (m, 1 H), 4.82 (d, *J* = 4.2 Hz, 1 H), 4.72 (d, *J*

= 4.2 Hz, 1 H), 4.56 (d, *J* = 4.2 Hz, 1 H), 4.38−4.35 (m, 1 H), 3.85−3.82 (m, 1 H), 3.81−3.73 (m, 8 H), 2.91−2.85 (m, 4 H), 2.58−2.55 (m, 2 H), 1.86−1.82 (m, 2 H), 1.60−1.58 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.1, 177.0, 174.6, 169.8, 148.3, 147.5, 146.4 (2 C), 133.9, 132.5, 130.3, 128.7, 110.0, 109.2, 107.6 (2 C), 101.6, 79.1, 79.0, 68.9, 56.3 (2 C), 49.8 (2 C), 48.2, 43.5, 41.6, 37.0, 35.2, 34.1, 28.5 (2 C); MS-ESI: 643.1505 ($[M+Na]^+$, 100%); HRMS (ESI) 643.1895 for $[M+ Na]^+$ (calcd 643.1898 for $C_{32}H_{32}N_2O_{11}$ Na).

*4.1.2.12 5-(1,3-dioxooctahydro-2H-4,7-epoxyisoindol-2-yl)-N-((5S,5aS,8aR,9R)-9-(4-hydroxy-3,5-dimethoxyphenyl)-8-oxo-5,5a,6,8,8a,9-hexahydrofuro[3',4':6,7]naphtho [2,3-d][1,3]dioxol-5-yl)pentanamide (**7l**)*

Yield: 68%; mp: 178−179 °C; $[\alpha]_D^{23}$ –82° (c 0.5, $CHCl_3$); IR (KBr cm^{-1}) 3350, 2952, 1773, 1698, 1613, 1516, 1484, 1404, 1228, 1113, 1036, 999, 940; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.81 (s, 1 H), 6.52 (s, 1 H), 6.31−6.29 (m, 3 H), 5.99−5.97 (m, 2 H), 5.26−5.23 (m, 1 H), 4.83 (d, *J* = 4.8 Hz, 1 H), 4.78 (d, *J* = 4.2 Hz, 1 H), 4.59 (d, *J* = 4.2 Hz, 1 H), 4.42−4.38 (m, 1 H), 3.93−3.89 (m, 1 H), 3.78 (s, 6 H), 3.53−3.50 (m, 2 H), 2.98−2.95 (m, 2 H), 2.89−2.87 (m, 2 H), 2.17−2.12 (m, 2 H), 1.98−1.92 (m, 2 H), 1.85−1.82 (m, 2 H), 1.62−1.60 (m, 2 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.7, 177.5, 174.6, 171.9, 148.2, 147.5, 146.4 (2 C), 133.9, 132.4, 130.3, 129.1, 110.0, 109.0, 107.7 (2 C), 101.5, 79.2 (2 C), 69.0, 56.4 (2 C), 49.8 (2 C), 48.0, 43.6, 41.7, 38.0, 37.1, 32.8, 28.5 (2 C), 23.7; MS-ESI: 657.1684 ($[M+Na]^+$, 100%); HRMS (ESI) 657.2043 for $[M+ Na]^+$ (calcd 657.2055 for $C_{33}H_{34}N_2O_{11}Na$).

*4.1.3. logP of **7a-l** determination*

The partition coefficient *P* was determined according to the standard method [36] and calculated with the following equation: *P* = (compound in 1-octanol)/(compound in water).

4.2 Biological evaluation

4.2.1 Cytotoxicity assays

Cells were incubated at 37 □ in a 5% CO_2 atmosphere. The MTT assay was used to determined growth inhibition. The synthetic compounds **7a-l** and reference compounds VP16 and norcantharidin were dissolved in saline for five concentrations (0.01–100 μM). The A-549, HepG2, HeLa, HCT-8 and WI-38 cells were seeded in 96-well plates at a density of 5×10^3 cells/well in 150 μL of the RPMI1640 medium with 10% heat-inactivated FBS and allowed to attach for 4–6 h, and then incubated with the indicated concentrations of compound for 48 h. The media was aspirated, and 10 μL of 5 mg/mL MTT solution (dilute in sterile PBS) diluted in 100 μL serum-free media was added to each well. After 4 h of incubation, the solution was discarded and then added 100 μL DMSO, 96-well plates were shaken on an oscillator for 10 min. The absorbance at 490 nm was determined on a plate reader. IC_{50} values were determined from a log plot of percent of control versus concentration.

4.2.2 Protein phosphatase inhibition

Inhibition of protein phosphatase 1 and 2A activity was performed according to previously reported assay [12,27]. Protein phosphatase PP1 was purchased from New England Biolabs (Beijing) LTD., and PP2A was purchased from Merck Millipore Corporation (USA). A serine/threonine PP assay was used in which free phosphate ion

released from a substrate phosphopeptide (Lys-Arg-pThr-Ile-Arg) was quantified by colorimetric analysis using the Malachite Green method. PP1, PP2 and substrate used in the assay were 30 mU/well, 0.6 mU/well and 200 μM, respectively. The reaction was initiated by addition of substrate (5 μL) to a mixture containing enzyme (5 μL), reaction buffer (10 μL; 50 mM Tris-HCl, pH 7.0, 100 μM $CaCl_2$) and test compound (10 μL, 100 μM), producing a total reaction volume of 30 μL/well and incubated at room temperature for 60 min. Reactions were halted via addition of a malachite green solution (50 μL), and the absorbance readings were taken at 650 nm after 10 min development time. Enzyme dilutions were made with buffer containing 20 mM MOPS, pH 7.5, 0.15 M NaCl, 60 mM 2-mercaptoethanol, 1 mM $MgCl_2$, 2 mM EGTA, 0.1 mM $MnCl_2$, 1mM DTT, 10% glycerol and 0.1 mg/mL serum albumin. For each of the enzymatic assays, the enzyme and the inhibitor were incubated at 37 ˚C for 10 min prior to the addition of the substrate. Samples were blanked against wells containing enzyme and buffer only. Initial inhibitor dilutions were made in DMSO and subsequent dilutions were made in distilled deionised H_2O. A dose-response curve of percentage enzyme activity versus drug concentration was produced from which an IC_{50} value was calculated indicating the concentration of drug required to inhibit enzyme activity by 50%. Data represents the mean IC_{50} of three independent experiments performed in duplicate.

*4.2.3 PP2A inhibition of **7l** in HepG2 cells*

PP2A activity was assayed according to the PP2A phosphatase-activity assay kit obtained from Promega Corporation (V2460, Madison, WI) protocol [33]. The PP2A

phosphatase-activity assay kit contains the chemically synthesized phosphopeptide, RRAT (pT) VA, a peptide substrate that is compatible with PP2A. PP2A dephosphorylates the phosphopeptide substrate, allowing the free phosphate to react with the molybdate dye mixture and show the green color.

The HepG2 cells were incubated in six-well plates. After treatment with compound **7l** for 24 h, the cells were collected and lysed, and endogenous phosphates were removed. The prepared enzyme samples were added to premixes containing 5 μL of 1 mM phosphopeptide substrate, after 30 min of incubation at 37°C, 50 μL of molybdate dye-additive mixture was added to stop the reaction. The absorbance was measured at 600 nm after 15 min, and PP2A activity was obtained using the phosphate standard curve.

4.2.4 DNA topoisomerase-Ⅱ inhibition assay

Topoisomerase-Ⅱ activity was determined using a kit (Topogen Inc., USA, Cat No. 2000H). The reaction was run in microcentrifuge tube that contained supercoiled pBR322 plasmid DNA 250 ng/μL and topoisomerase-Ⅱ (4 units) in assay buffer (A 0.1 volume and B 1volume). In each reaction 2 μL sample was added and the volume was made up to 20 μL with water and then incubated for 60 mins at 37℃. The reaction was terminated by the addition of 2 μL of 10% SDS. Each sample tube was treated with proteinase K and extracted once with chloroform/isoamyl alcohol (24:1). Products were resolved by 1% agarose gel electrophoresis in TAE buffer (40 mM tris-acetate, pH8.0, and 1 mM EDTA) and stained with 0.5 mg/mL ethidium bromide [35].

4.2.5 Analysis of cell cycle by flow cytometry

The cell cycle was analyzed by flow cytometry. Firstly, HepG2 cells were treated with different concentrations of compound **7l** (0, 0.5, and 1 μM) for 24 h. After incubation, a total of (1–5)×10^5 cells were harvested from the treated and untreated samples. The cells were washed twice with PBS and fixed in 70% ice-cold ethanol for at least overnight. The sample was concentrated by removing the ethanol and then cells were then washed three times with PBS, staining the cellular DNA with fluorescent solution (1% (v/v) Triton X-100, 0.01% RNase, 0.05% PI) for 15 min in darkness. The cell cycle distribution was then detected by flow cytometry (COULTER EPICS XL, USA).

4.2.6 Western blotting anaylsis

HepG2 cells were seeded into culture flasks. After 24 h incubation, cells were treated with **7l** (0, 0.5, and 1 μM), respectively. For total cell protein extracts, cells were washed and lysed in lysis buffer (50 mM Tris-HCl (pH 7.5), 1% NP-40, 2 mM EDTA, 10 mM NaCl, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM DTT, 0.1% SDS and 1 mM phenyl methyl sulfonyl fluoride). Total proteins were obtained by centrifuging (12,000 g for 20 min at 4 □). The protein concentrations were determined by using Bradford method. The Western blotting assay was performed as described previously [29]. For Western blot analysis, equal amounts of proteins (30 μg) were separated on 10% or 12% SDS-PADE gels and transferred to polyvinylidine difluoride (PVDF) membranes (Millipore Corporation, USA). The blot was blocked in blocking buffer (5% non-fat dry milk in TBST) for 2 h at room temperature, and

then incubated with dilute solution (1:500~1:1000) of cdc2 or CyclinB1 antibodies (BioLegend, US) in blocking buffer overnight at 4□. The blot was then incubated with appropriate secondary antibody (ZSGB-BIO, Beijing, China) (1:5000~1:10000 dilution), *β*-Actin (ZSGB-BIO, Beijing, China) was used as a loading control. The protein bands were visualized using the Gel Imaging System (ChemDoc-It610, UVP, USA).

4.2.7 Fluorescence titrations

The binding of the compound **7l** to CT DNA was performed by monitoring the changes in the emission spectral pattern of compound **7l** in pH 7.2 PBS by increasing the concentrations of CT DNA. After addition of indicated amount of DNA (0–5 μM) to the compound **7l** (1 μM), the resulting solution was allowed to equilibrate for 5 min at 25 □, excited at 318 nm followed by recording the emission spectral changes in the range of 280–400 nm. The appropriate concentration samples of CT DNA in PBS were also determined as blank.

Acknowledgments

We gratefully acknowledge the financial support from the National Natural Science Foundation of China (Grant No. 21372110, 81372177 and 21302079).

References

[1] E. Frei III, A. Elias, C. Wheeler, P. Richardson, W. Hryniuk, The relationship between high-dose treatment and combination chemotherapy: the concept of summation dose intensity, Clin. Cancer Res. 4 (1998) 2027–2037.

[2] G. J. Peters, C. L. Van der Wilt, C. J. A. Van Moorsel, J. R. Kroep, A. M. Bergman, S. P. Ackland, Basis for effective combination cancer chemotherapy with antimetabolites, Pharmacol. Ther. 87 (2000) 227–253.

[3] T.F. Imbert, Podophyllotoxin, Biochimie 80 (1998) 207–222.

[4] M. Gordaliza, P.A. García, J.M.M. del Corral, M.A. Castro, M.A. Gómez-Zurita, Podophyllotoxin: distribution, sources, applications and new cytotoxic derivatives. Toxicon 44 (2004) 441–459.

[5] E.L. Baldwin, N. Osheroff, Etoposide, topoisomerase II and cancer. Curr. Med. Chem.: Anti-Cancer Agents 5 (2005) 363–372.

[6] A. Kruczynski, J.-M. Barret, B. van Hille, N. Chansard, J. Astruc, Y. Menon, C. Duchier, L. Créancier, B. T. Hill, Decreased nucleotide excision repair activity and alterations of Topoisomerase IIα are associated with the in vivo resistance of a P388 leukemia subline to F11782, a novel catalytic inhibitor of Topoisomerases I and II, Clin. Cancer Res. 10 (2004) 3156–3168.

[7] A. Kruczynski , I. Vandenberghe, A. Pillon, S. Pesnel, L. Goetsch, J.M. Barret, Y. Guminski, A. Le Pape, T. Imbert, C. Bailly, N. Guilbaud, Preclinical activity of F14512, designed to target tumors expressing an active polyamine transport system. Invest. New Drug 29 (2011) 9–21.

[8] A. Y. Leung, Traditional toxicity documentation of Chinese Materia Medica-an overview, *Toxicol. Pathol*. **2006**, *34*, 319–326.

[9] O. Kadioglu, N. S. Kermani, G. Kelter, U. Schumacher, H.-H. Fiebig, H. J. Greten, T. Efferth, Pharmacogenomics of cantharidin in tumor cells. Biochem. Pharmacol. 87 (2014) 399–409.

[10] H. L. K. Stanton, H. C. Chung, S. L. Wing, C. Jien, Y. L. Fung, S. M.W. Raymond, Y. M. C. Gregory, B. S. L. Paul, W. T. L. Thomas, W. Y. Y. Michael, C. O. T. Johnny, S. C. C. Albert, Synthesis and structure evaluation of a novel cantharimide and its cytotoxicity on SK-Hep-1 hepatoma cells, Bioorg. Med. Chem. Lett. 17 (2007) 1155–1159.

[11] T. A. Hill, S. G. Stewart, S P. Ackland, J. Gilbert, B. Sauer, J. A. Sakoff, A. McCluskey, Norcantharimides, synthesis and anticancer activity: Synthesis of new norcantharidin analogues and their anticancer evaluation, Bioorg. Med. Chem. 15 (2007) 6126–6134.

[12] A. Thaqi, J. L. Scott, J. Gilbert, J. A. Sakoff, A. McCluskey, Synthesis and biological activity of *Δ*-5,6-norcantharimides: importance of the 5,6-bridge, Eur. J. Med. Chem. 45 (2010) 1717–1723.

[13] C.-B. Yeh, C.-J. Su, J.-M. Hwang, M.-C. Chou, Therapeutic effects of cantharidin analogues without bridging ether oxygen on human hepatocellular carcinoma cells, Eur. J. Med. Chem. 45 (2010) 3981–3985.

[14] M. Tarleton, J. Gilbert, J. A. Sakoff, A. McCluskey, Synthesis and anticancer activity of a series of norcantharidin analogues, Eur. J. Med. Chem. 54 (2012) 573−581.

[15] A. McCluskey, M. C. Bowyer, E. Collins, A. T. R. Sim, J. A. Sakoff, M. L.

Baldwin, Anhydride Modified Cantharidin Analogues: Synthesis, inhibition of protein phosphatases 1 and 2A and anticancer activity, Bioorg. Med. Chem. Lett. 10 (2000) 1687–1690.

[16] M. E. Hart, A. R. Chamberlin, C. Walkom, J. A. Sakoff, A. McCluskey, Modified norcantharidins: synthesis, protein phosphatases 1 and 2A inhibition, and anticancer activity, Bioorg. Med. Chem. Lett. 14 (2004) 1969–1973.

[17] T. A. Hill, S. G. Stewart, B. Sauer, J. Gilbert, S. P. Ackland, J. A. Sakoff, A. McCluskey, Heterocyclic substituted cantharidin and norcantharidin analogues—synthesis, protein phosphatase (1 and 2A) inhibition, and anti-cancer activity, Bioorg. Med. Chem. Lett. 17 (2007) 3392–3397.

[18] A. McClusky, A. T. R. Sim, J. A. Sakoff, Serine-threonine protein phosphatase inhibitors: Development of potential therapeutic strategies, J. Med. Chem. 45 (2002) 1151–1175.

[19] J. L. Li, Y. C. Cai, X. H. Liu, L. J. Xian, Norcantharidin inhibits DNA replication and induces apoptosis with the cleavage of initiation protein cdc6 in HL-60 cells, Anti-cancer Drug 17 (2006) 307–314.

[20] S.-W. Chen, X. Tian, Y.-Q. Tu, Synthesis and cytotoxic activity of novel derivatives of 4′-demethylepipodophyllotoxin, Bioorg. Med. Chem. Lett. 14 (2004) 5063–5066.

[21] S.-W. Chen, R. Xiang, J. Liu, X. Tian, Synthesis and biological evaluation of novel conjugates of podophyllotoxin and 5-FU as antineoplastic agents, Bioorg. Med. Chem. 17 (2009) 3111–3117.

[22] W.-T. Huang, J. Liu, J.-F. Liu, L. Hui, Y.-L. Ding, S.-W. Chen, Synthesis and biological evaluation of conjugates of deoxypodophyllotoxin and 5-FU as inducer of caspase-3 and -7, Eur. J. Med. Chem. 49 (2012) 48–54.

[23] S.-W. Chen, R. Xiang, X. Tian, Synthesis of new conjugates of modified podophyllotoxin and stavudine, Helve. Chim. Acta 92 (2009), 1568–1574.

[24] S.-W. Chen, Y.-H. Wang, Y. Jin, X. Tian, Y.-T. Zheng, D.-Q. Luo, Y.-Q. Tu, Synthesis and anti-HIV-1 activities of novel podophyllotoxin derivatives, Bioorg. Med. Chem. Lett. 17 (2007) 2091–2095.

[25] X. Liu, L.-L. Zhang, X.-H. Xu, L. Hui, J.-B. Zhang, S.-W. Chen, Synthesis and anticancer activity of dichloroplatinum(II) complexes of podophyllotoxin, Bioorg. Med. Chem. Lett. 23 (2013) 3780–3784.

[26] A. McCluskey, C. Walkom, M. C. Bowyer, S. P. Ackland, E. Gardiner, J. A. Sakoff, Cantharimides: A new class of modified cantharidin analogues inhibiting protein phosphatases 1 and 2A, Bioorg. Med. Chem. Lett. 11 (2001) 2941–2946.

[27] J. Zhao, X.-W. Guan, S.-W. Chen, L. Hui, Synthesis and biological evaluation of norcantharidin derivatives as protein phosphatase-1 inhibitors, Bioorg. Med. Chem. Lett. 25 (2015) 363–366.

[28] G. Sosnovsky, The quest for a predicitive design of anticancer drugs. Pure Chem. 62 (1990) 289−294.

[29] J.-F. Liu, C.-Y. Sang, W.-W. Qin, J. Zhao, L. Hui, Y.-L. Ding, S.-W. Chen, Synthesis and evaluation of the cell cycle arrest and CT DNA interaction properties of 4β-amino-4′-*O*-demethyl-4-deoxypodophyllotoxins, Bioorg. Med.

Chem. 23 (2013) 6948−6955.

[30] M. J. Robertson, C. P. Gordon, J. Gilbert, A. McCluskey, J. A. Sakoff, Norcantharimide analogues possessing terminal phosphate esters and their anti-cancer activity, Bioorg. Med. Chem. 19 (2011) 5734–5741.

[31] B. E. Campbell, M. Tarleton, C. P. Gordon, J. A. Sakoff, J. Gilbert, A. McCluskey, R. B. Gasser, Norcantharidin analogues with nematocidal activity in *Haemonchus contortus*, Bioorg. Med. Chem. Lett. 21 (2011) 3277–3281.

[32] V. Gupta, A.K. Ogawa, X. Du, K.N. Houk, R.W. Armstrong, A model for binding of structurally diverse natural product inhibitors of protein phosphatases PP1 and PP2A. J. Med. Chem 40 (1997) 3199–3206.

[33] Y. Sun, Q. Zheng, Y.-T. Sun, P. Huang, Z.-L. Guo, L.-H. Xu, Microcystin-LR induces protein phosphatase 2A alteration in a human liver cell line, Environ. Toxicol. 29 (2014) 1236–1244.

[34] C. Q. Hu, D. Q. Xu, W. T. Du, S. J. Qian, L. Wang, J. S. Lou, Q. J. He, B. Yang, Y. Z. Hu, Novel 4β-anilino-podophyllotoxin derivatives: design synthesis and biological evaluation as potent DNA-topoisomerase II poisons and anti-MDR agents, Mol. BioSyst. 6 (2010) 410−420.

[35] S. C. Michael, F. Nikolas, P. Daniele, N. G. Aída, M. G. Ornella, P. Ingemar, D.V. Lisa, A. H. Serkos, Synthesis and biological evaluation of novel tamoxifen analogues, Bioorg. Med. Chem. 21 (2013) 4120−4131.

[36] W. Li, L. Xie, Z. Chen, Y. Zhu, Y. Sun, Y. Miao, Cantharidin, a potent and selective PP2A inhibitor, induces an oxidative stress-independent growth

inhibition of pancreatic cancer cells through G2/M cell-cycle arrest and apoptosis, Cancer Sci. 101 (2010) 1226−1233.

[37] N. Asli, O. Ozlem, B.T. Neslihan, D. Beyhan, Cyclin A and cyclin B1 overexpression in differentiated thyroid carcinoma, Med. Oncol. 29 (2012) 294−300.

[38] N. Kumari, B. K. Maurya, R. K. Koiri, S. K. Trigun, S. Saripella, M. P. Coogan, L. Mishra, Cytotoxic activity, cell imaging and photocleavage of DNA induced by a Pt(II) cyclophane bearing 1,2 diamino ethane as a terminal ligand, Med. Chem. Commun. 2 (2011) 1208–1216.

Figure Capture:

Figure 1. Structures of podophyllotoxin (**1**), DMEP (**2**), etoposide (**3**), teniposide (**4**), cantharidin (**5**), and norcantharidin (**6**)

Figure 2. Compound **7l** inhibits PP2A in HepG2 cells. Data are presented as fold change in protein expression relative to control. The results are expressed as the mean ± SD; *P<0.05, **P< 0.001 *vs* control.

Figure 3. Effect of compound **7l** on topoisomerase II-mediated DNA relaxation. Lane 1: pBR322 plasmid DNA; Lane 2: Topo-□ control (Topo-□+DNA); Lane 3: 50 μM of **7l** +DNA + Topo-□; Lane 4: 100 μM of **7l** +DNA + Topo-□; Lane 5: 100 μM of VP16 +DNA + Topo-□.

Figure 4. Effects of **7l** on cell cycle progression. (A) Control HepG2 cells; (B) HepG2 cells treated with 0.5 μM **7l** for 24 h; (C) HepG2 cells treated with 1 μM **7l** for 24 h.

Figure 5. Western blotting analysis of G2/M cell-cycle regulators expression of cyclin B1, cdc2 treated with **7l** in HepG2 cells for 24h.

Figure 6. Effects of compound **7l** on CT DNA. Fluorescence spectra of **7l** (1 μM) by increasing concentrations of CT DNA (0–5 μM).

Table 1. The cytotoxicity (IC_{50}, μM)[a,b] and log*P* of compounds **7a-l**

Compound	Tumor cell				Normal cell	Log*P*
	A549	HepG2	HeLa	HCT-8	WI-38	
7a	5.47±0.78	0.14±0.04	1.65±0.64	17.1±0.94	33.5±2.11	0.13
7b	44.5±2.14	13.7±1.54	15.1±1.48	16.9±1.64	35.6±2.59	0.62
7c	6.73±0.48	1.62±0.86	8.3±0.69	7.81±0.24	44.4±3.21	1.48
7d	8.11±0.59	10.5±1.46	>100	21.4±2.51	21.9±2.16	1.83
7e	10.1±0.97	3.21±0.55	2.52±0.49	23.6±1.99	65.2±1.44	1.91
7f	6.12±0.24	7.04±0.14	2.82±0.89	12.3±3.46	30.4±3.26	0.93
7g	7.03±0.68	1.98±0.33	28.5±2.24	15.3±2.55	26.7±4.01	1.99
7h	14.1±1.32	5.53±0.92	16.0±3.05	11.6±1.64	29.6±2.68	2.27
7i	12.0±0.98	6.84±0.32	2.35±0.52	15.2±0.97	72.4±3.31	1.69
7j	3.42±0.12	0.10±0.03	2.61±0.88	3.12±0.26	46.5±1.57	2.06
7k	6.21±0.45	8.42±0.24	10.8±3.27	17.2±2.19	>100	0.41
7l	4.57±0.21	<0.01	4.33±0.12	3.48±0.45	>100	0.68
VP16	29.7±1.64	5.21±0.75	13.5±1.83	10.0±0.97	17.6±3.29	2.74
NCTD	12.2±1.31	24.7±1.56	12.4±3.21	28.4±2.23	26.7±2.28	---

[a] IC_{50} values are presented as the means ± SD of triplicate experiments.

[b] MTT method

Table 2. Inhibition of protein phosphatases 2A by compounds **7a**–**7l** [a]

Compound	PP2A inhibition IC_{50} (μM)	Compound	PP2A inhibition IC_{50} (μM)
7a	0.68	**7h**	>100
7b	>100	**7i**	58.3
7c	1.12	**7j**	3.63
7d	>100	**7k**	9.52
7e	75.7	**7l**	0.49
7f	1.29	**NCTD**	0.48
7g	>100		

[a] Average of three experiments in triplicate.

Figure 1.

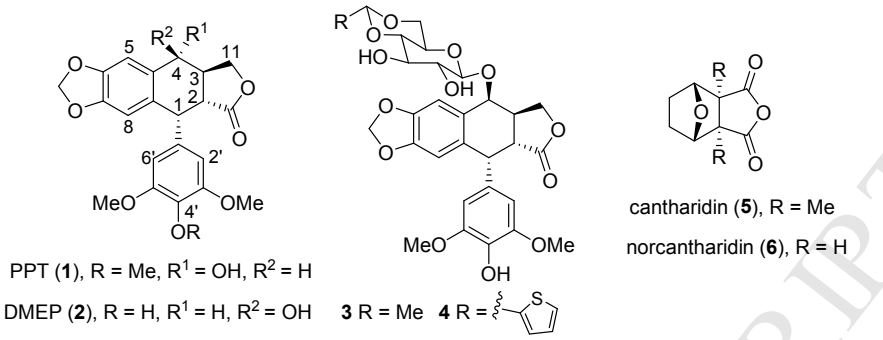


Figure 1. Structures of podophyllotoxin (**1**), DMEP (**2**), etoposide (**3**), teniposide (**4**), cantharidin (**5**), and norcantharidin (**6**)

Figure 2.

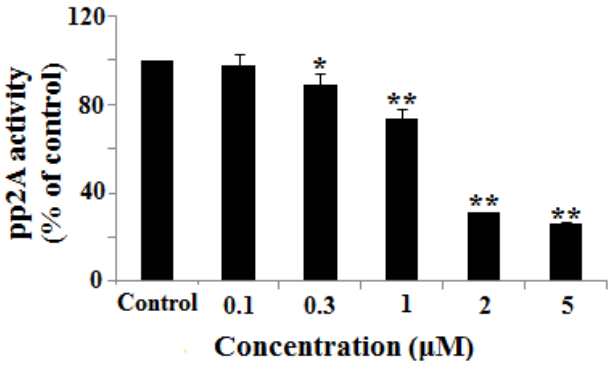


Figure 2. Compound **7l** inhibits PP2A in HepG2 cells. Data are presented as fold change in protein expression relative to control. The results are expressed as the mean ± SD; *P<0.05, **P< 0.001 *vs* control.

Figure 3.

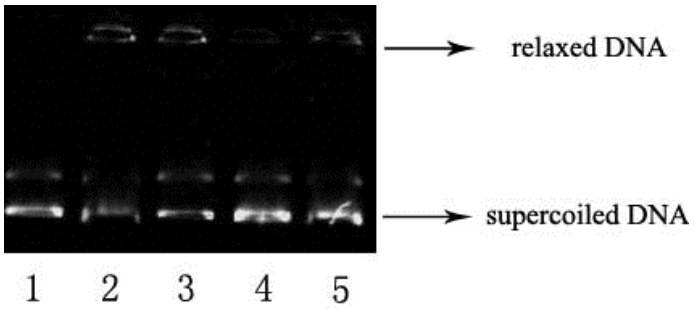


Figure 3. Effect of compound **7l** on topoisomerase II-mediated DNA relaxation. Lane 1: pBR322 plasmid DNA; Lane 2: Topo-□ control (Topo-□+DNA); Lane 3: 50 μM of **7l** +DNA + Topo-□; Lane 4: 100 μM of **7l** +DNA + Topo-□; Lane 5: 100 μM of VP16 +DNA + Topo-□.

Figure 4.

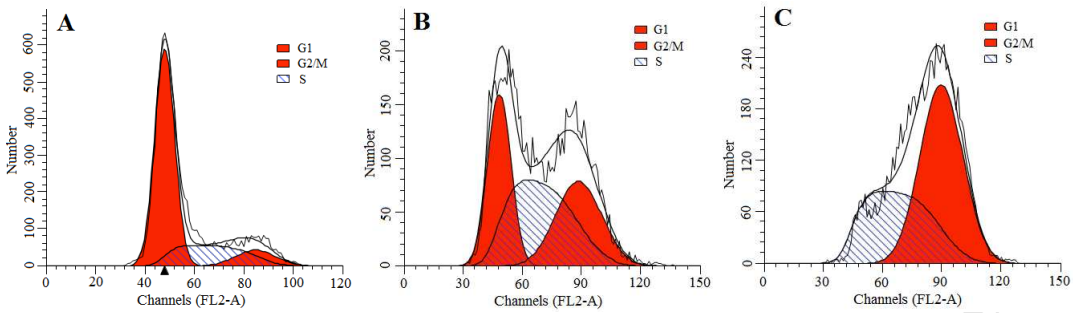


Figure 4. Effects of **7l** on cell cycle progression. (A) Control HepG2 cells; (B) HepG2 cells treated with 0.5 μM **7l** for 24 h; (C) HepG2 cells treated with 1 μM **7l** for 24 h.

Figure 5.

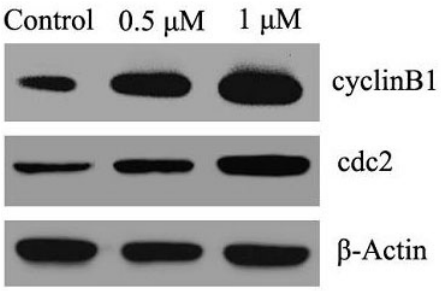


Figure 5. Western blotting analysis of G2/M cell-cycle regulators expression of cyclin B1, cdc2 treated with **7l** in HepG2 cells for 24h.

Figure 6.

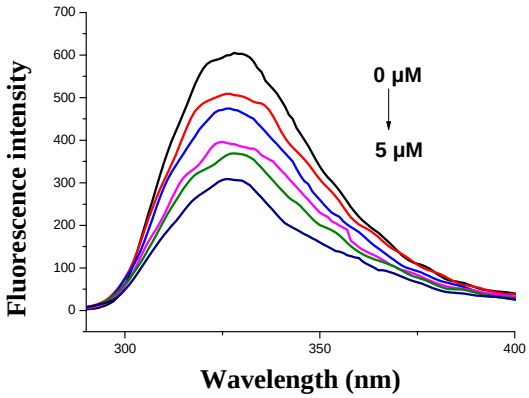
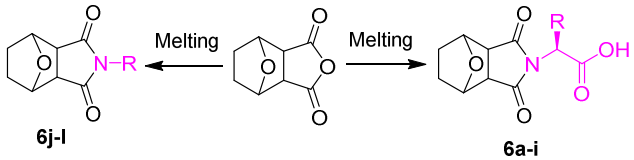
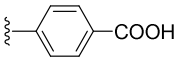
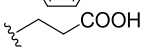
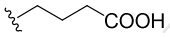


Figure 6. Effects of compound **7l** on CT DNA. Fluorescence spectra of **7l** (1 μM) by increasing concentrations of CT DNA (0–5 μM).

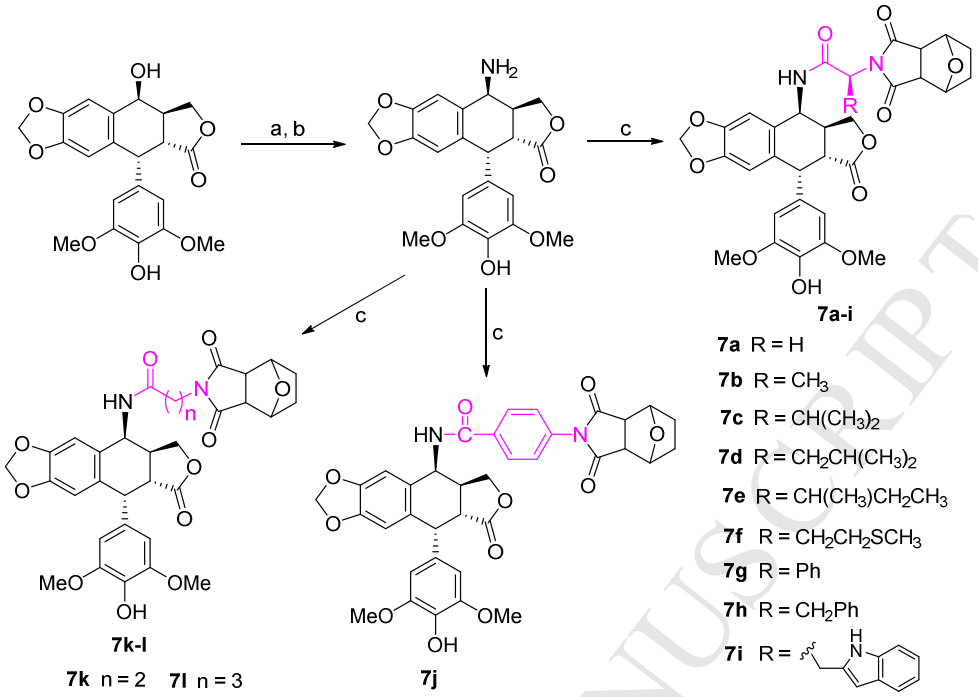
Scheme 1



6a R = H
6b R = -CH_3
6c R = -$CH(CH_3)_2$
6d R = -$CH_2CH(CH_3)_2$
6e R = -$CHCH_3CH_2CH_3$
6f R = -$CH_2CH_2SCH_3$
6g R =-Ph
6h R = -CH_2Ph
6i R = -$CH_2C_8H_6N$
6j R = 
6k R = 
6l R = 

Scheme 1. Synthesis of compounds **6a-l**. Reagents and conditions: 160–170℃, N_2, 40–60 mins.

Scheme 2



Scheme 2. Synthesis of compounds **7a-l**. Reagents and conditions: (a) CH_2Cl_2, HN_3/ $BF_3 \cdot Et_2O$, -15℃; (b) 4 MPa H_2, 10% Pd-C, EtOAc; (c) **6a-l**, EDCI, HOBt, CH_2Cl_2, rt.

Research highlights:

1. 12 conjugates of podophyllotoxin and norcantharidin were synthesized.
2. Most compounds exploited potent cytotoxicities compared with VP-16.
3. Compound **7l** inhibits TOP□ and PP2A.
4. **7l** induces accumulation of cells in G2/M phase in HepG2 cells.
5. **7l** regulates expression of cyclin B1, cdc2 in HepG2 cells.
6. **7l** is bound to CT DNA.