Full Paper

Semi-Synthesis and Biological Evaluation of 1,2,3-Triazole-Based Podophyllotoxin Congeners as Potent Antitumor Agents Inducing Apoptosis in HepG2 Cells

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A series of 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxin congeners were synthesized by employing click chemistry and further evaluated for their antitumor activity by MTT assay. Among them, six congeners (**10**, **11**, **12**, **13**, **22**, and **24**) exhibited approximately 100-fold more potent inhibitory activity against four tumor cell lines (HepG2, MKN-45, NCI-H1993, and B16) than etoposide as positive control. Docking studies on binding in the ATPase domain of topoisomerase II revealed perfect docking of four congeners in the active site. Furthermore, the podophyllotoxin congeners **10**, **11**, **12**, and **13** induced cell cycle arrest of HepG2 cells at the G_2/M phase in a concentration-dependent manner, assessed by flow cytometric analysis, highlighting that they exert their antitumor activity *via* HepG2 cell apoptosis.

Keywords: Antitumor activity / Cell cycle / Docking study / G_2/M phase / Podophyllotoxin congeners

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Supporting information available online

Introduction

Podophyllotoxin (**1a**, Fig. 1), a well-known naturally occurring aryltetralin lignan isolated from the root of *Podophyllum hexandrum*, possesses potent antiproliferative activity against several tumor cell lines and has also been used as specific antimicrotubule agent acting at the colchicine binding site of tubulin [1–5]. However, severe toxicity and gastrointestinal side effects of podophyllotoxin limited its application as an antitumor drug in cancer chemotherapy. Hence, with podophyllotoxin as lead compound, chemical disconnections and structural modifications were undertaken to develop novel semi-synthetic congeners, leading to the discovery of potent and low-cytotoxic podophyllotoxin-based drugs. These drugs, including etoposide (**1b**) [6–8] and teniposide (**1c**) [9, 10], were widely used in the clinic for the treatment of malignancies such as small-cell lung cancer, Kaposi's sarcoma, lymphoma, glioblastoma multiforme, and leukemia [11–14]. Structural modification of the podophyllotoxin scaffold resulted in an essential change in the mechanism of action: from the antimicrotubule activity of podophyllotoxin to the anti-topoisomerase II (Topo-II) activity of etoposide [15, 16]. The anti-microtubule agent podophyllotoxin promotes cell death by interfering with the function of the mitotic spindle and induces cell apoptosis by promoting mitotic arrest, while the Topo-II inhibitor etoposide inhibits DNA Topo-II by stabilizing the covalent Topo-II–DNA cleavable complex.

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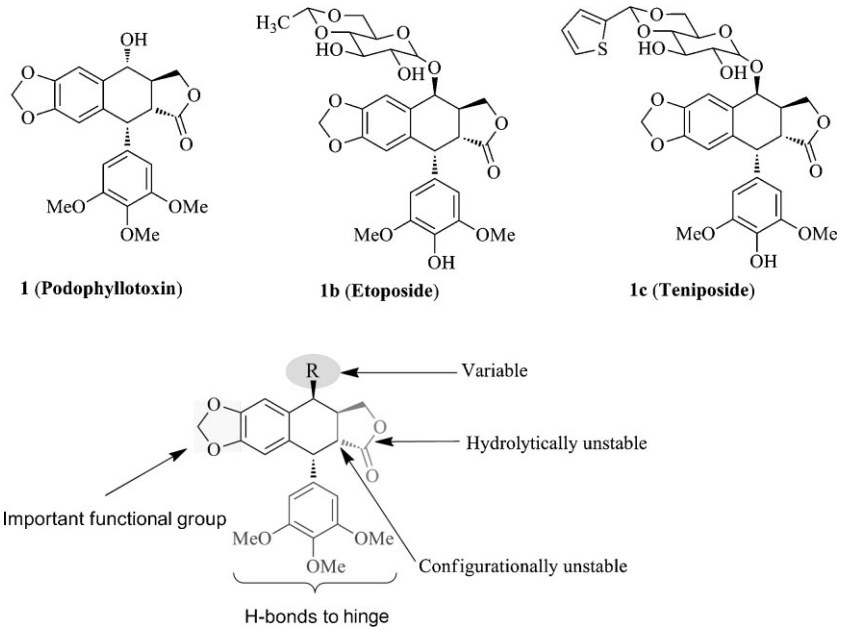


Figure 1. Structures of several podophyllotoxin derivatives.

Although etoposide is widely used in the clinic, many studies have focused on its side effects, such as potential drug resistance, metabolic inactivation, myelosuppression, poor water solubility, and even induction of secondary tumors [17, 18]. Hence, structural modifications of etoposide have been developed, thus exploring for more effective congeners. Previous studies have proven that the *trans*-lactone, 4β-substituted, and 4′-demethyl moieties were crucial for the antitumor activity [19–21]. Therefore, the preparation of 4β-substituted podophyllotoxin congeners might be an effective strategy to improve the anti-Topo-II and antitumor potency [19–21]. According to the reported works, podophyllotoxin linked with different aliphatic side chains by an 1,2,3-triazole ring exhibited significant binding affinity to Topo-II, which gave us the inspiration to focus on other substitutions at this position of the scaffold.

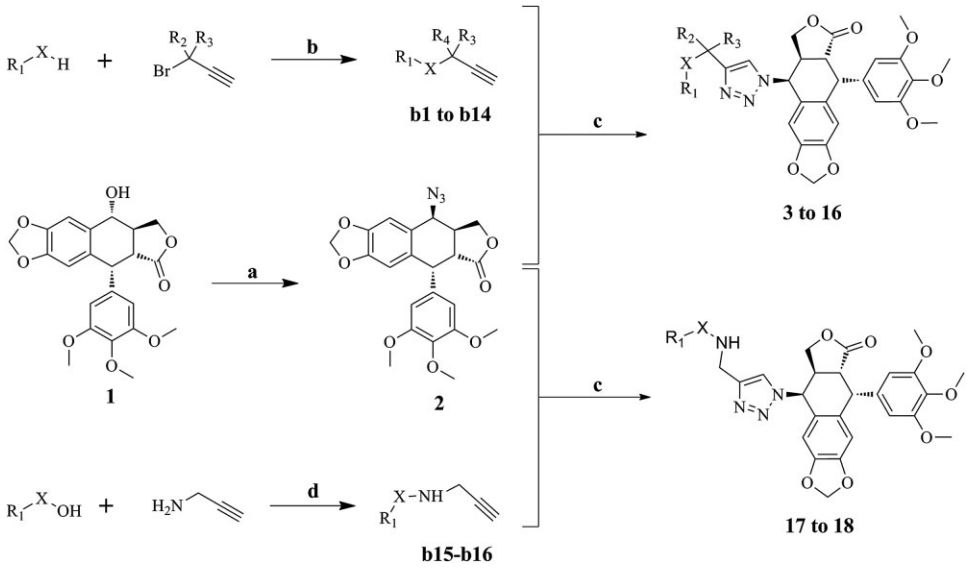
In this study, a series of 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxin congeners with various aromatic substituents were synthesized by click chemistry. Twenty-seven derivatives were subsequently screened against four human tumor cells, and most of these compounds showed comparable or superior antiproliferative activity compared to etoposide. The docking results of binding in the ATPase domain of Topo-II revealed perfect docking of the four congeners in the active site. Additionally, the podophyllotoxin congeners **10**, **11**, **12**, and **13** concentration-dependently induced cell cycle arrest of HepG2 cells at the G_2/M phase, highlighting that they exert their antitumor potency *via* HepG2 cell apoptosis.

Results and discussion

Chemistry

The synthesis of 4β-(4-aryl)-1,2,3-triazole-1-yl)podophyllotoxin congeners is illustrated in Scheme 1. Congeners **3** and **4** were synthesized according to the previously reported method [22]. The key intermediate, 4β-azidopodophyllotoxin **2** was obtained by treating podophyllotoxin **1** with TFA and NaN_3 in dichloromethane, with good yield. The *O*-propargylated (thio)phenols **b1**–**b14** were prepared through the condensation of propargyl bromide or 3-bromo-3-dimethyl-1-butyne with commercially available (thio)phenols in the presence of KOH as base and NaI as a valid catalyst. The acylation of the appropriate benzoic acid with propargyl amine led to the corresponding *N*-(prop-2-yn-1-yl)-benzamides (**b15** and **b16**) by employing dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP). Finally, the condensation of **2** with **b1**–**b16** afforded the target congeners though click chemistry, with excellent yields. The click chemistry was accomplished using $CuSO_4 \cdot 5H_2O$, sodium ascorbate, and *t*-butyl ammonium bromide (TBAB) in THF/water (1:1). Click chemistry in this study enabled a modular approach to generate the pharmacophores by utilizing the collection of reliable chemical reactions and provided near-perfect properties including high yields, few byproducts and mild conditions [23, 24].

The intermediates **c2**–**c11** were obtained by aldol condensation of **c1** with appropriate benzaldehydes using 50% KOH

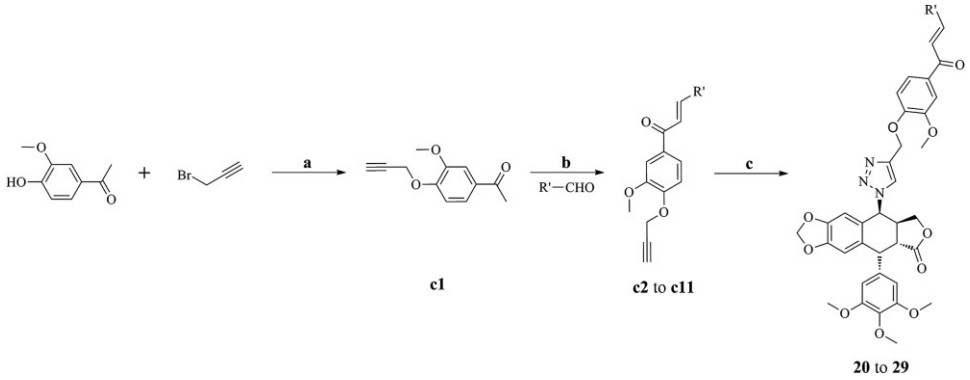


Scheme 1. Synthesis of 4β-[(4-aryl)-1*H*-1,2,3-triazol-1-yl)podophyllotoxin congeners. Reagents and conditions: (a) NaN_3, trifluoroacetic acid, CH_2Cl_2, 24 h, 95.0%; (b) KOH, NaI, acetone, 25°C, 8 h, 95.0%; (c) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, TBAB, THF/H_2O (1:1 v/v); (d) 2-propynylamine, DCC, DMAP, CH_2Cl_2, r.t., 12 h, 80.0%.

and methanol [25]. Subsequent cycloaddition of **c2–c11** with **2** was achieved by the above-mentioned synthesis methods (Scheme 2). At this stage, all 27 congeners were fully characterized by NMR, MS, and HPLC before being submitted to biological screening, and the analysis results are summarized in Table 1.

Biological evaluation

The anticancer efficacy of the 27 compounds was evaluated by measuring their antiproliferative activity on well-established cancer cells, namely, HepG2, MKN-45, NCI-H1993, and B16, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Etoposide was selected as a reference. The results are summarized in Table 2. From the collected data, compounds **11**, **12**, and **13** were shown to be highly potent against all the cell lines. Similarly, **10** exhibited high *in vitro* anticancer activity, and comparable cytotoxic effects were observed for **22** and **24**. Among the six representative congeners, compound **11** containing a biphenyl ring showed the highest antiproliferative activity against the four cell lines (especially an IC_{50} value of 150 nM at 48 h against HepG2 cells, which was 100-fold more active than the positive controls). The introduction of a sulfur atom in place of the oxygen atom showed a great improvement in inhibitory potency (**12** *vs.* **5**). Importantly, the attachment of a fluorine atom in the phenyl moiety (e.g., **24**) was optimal for the



Scheme 2. Synthesis of 4β-[(4-chalcone)-1*H*-1,2,3-triazol-1-yl)podophyllotoxin congeners. Reagents and conditions: (a) NaH, DMF, 25°C, 12 h, 98.0%; (b) 50% KOH (aq.), CH_3OH, r.t., 24 h, 70.0%.

Table 1. Structural profiles of the podophyllotoxin congeners.

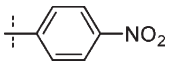
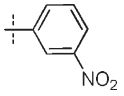
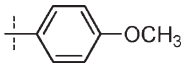
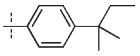
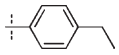
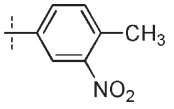
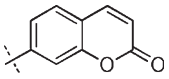
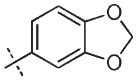
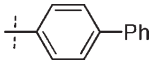
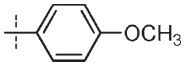
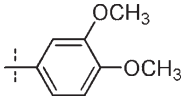
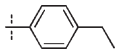
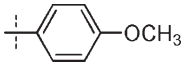
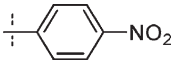
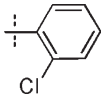
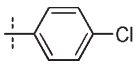
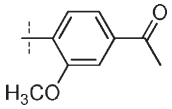
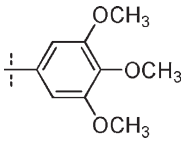
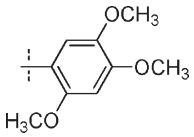
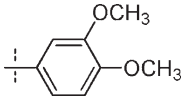
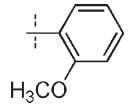
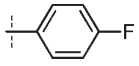
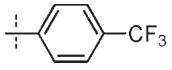
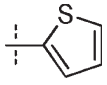
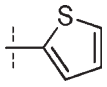
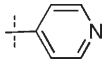
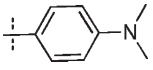
Compounds	X	R_1	R_2	R_3	R′
3	O		H	H	
4	O		H	H	
5	O		H	H	
6	O		H	H	
7	O		H	H	
8	O		H	H	
9	O		H	H	
10	O		H	H	
11	O		H	H	
12	S		H	H	
13	S		H	H	
14	O		CH_3	CH_3	
15	O		CH_3	CH_3	
16	O		CH_3	CH_3	
17			H	H	
18			H	H	

Table 1. (*continued*)

Compounds	X	R_1	R_2	R_3	R′
19	O		H	H	
20	O		H	H	
21	O		H	H	
22	O		H	H	
23	O		H	H	
24	O		H	H	
25	O		H	H	
26	O		H	H	
27	O		H	H	
28	O		H	H	
29	O		H	H	

chalcone-containing analogues. In addition, two methoxyl groups at the *meta* or *para* position of the phenyl ring were also tolerated as **22** displayed distinct increases in antitumor activity with an average IC_{50} value of 1.58 μM.

As demonstrated in the biological evaluation, an electron-donating group (EDG; e.g., **5**, **7**, **10**, **12**, **13**) at the *meta* or *para* position of the aromatic ring tended to possess much better activity than the corresponding electron-withdrawing group (EWG; e.g., **3**, **4**, **8**, and **9**). However, the placement of the amide bond between the triazole and the phenyl ring showed negative effects on the antitumor potency (**17** and **18** were almost fivefold less potent than podophyllotoxin). However, it was found that replacement of the hydrogen atoms on the linker between the triazole ring and the phenyl ring with the

Table 2. IC_{50} values of various podophyllotoxin congeners against selected tumor cells.

Compounds	IC_{50} at 48 h (μM)[a]				IC_{50} at 72 h (μM)			
	HepG2	MKN-45	NCI-H1993	B16	HepG2	MKN-45	NCI-H1993	B16
Etoposide[b]	>10.00	6.34 ± 0.89	7.93 ± 0.77	>10.00	1.85 ± 0.15	6.05 ± 0.65	4.40 ± 0.25	1.35 ± 0.15
3	3.87 ± 0.23	6.10 ± 0.98	59.60 ± 2.34	30.00 ± 1.87	3.21 ± 0.21	3.40 ± 0.19	37.80 ± 2.22	3.60 ± 0.18
4	1.05 ± 0.04	0.99 ± 0.01	54.50 ± 4.78	10.00 ± 1.89	0.87 ± 0.01	0.84 ± 0.01	15.80 ± 2.87	0.98 ± 0.02
5	0.98 ± 0.04	1.05 ± 0.06	10.70 ± 1.26	2.85 ± 0.67	0.80 ± 0.01	0.91 ± 0.06	4.00 ± 0.56	1.05 ± 0.04
6	19.00 ± 1.89	7.62 ± 0.87	64.00 ± 4.76	66.60 ± 4.56	8.35 ± 0.78	5.93 ± 0.66	19.70 ± 1.89	32.00 ± 2.67
7	4.18 ± 0.56	4.26 ± 0.65	8.25 ± 0.89	>80.00	3.16 ± 0.16	4.10 ± 0.24	5.20 ± 0.33	>80.00
8	3.80 ± 0.19	3.11 ± 0.22	4.13 ± 0.34	4.53 ± 0.55	2.88 ± 0.17	2.76 ± 0.17	3.68 ± 0.22	5.75 ± 0.43
9	7.78 ± 0.77	1.45 ± 0.13	6.92 ± 0.55	4.35 ± 0.65	4.17 ± 0.61	1.23 ± 0.11	6.63 ± 0.56	2.10 ± 0.17
10	0.25 ± 0.01	0.93 ± 0.04	0.85 ± 0.05	2.93 ± 0.32	0.20 ± 0.01	0.64 ± 0.02	0.50 ± 0.05	0.30 ± 0.02
11	0.15 ± 0.01	0.22 ± 0.01	0.24 ± 0.03	0.54 ± 0.09	0.13 ± 0.02	0.13 ± 0.01	0.17 ± 0.01	0.40 ± 0.04
12	0.26 ± 0.02	0.13 ± 0.02	0.49 ± 0.05	2.52 ± 0.33	0.14 ± 0.01	0.31 ± 0.02	0.40 ± 0.02	0.27 ± 0.01
13	0.31 ± 0.08	0.44 ± 0.04	1.45 ± 0.12	0.90 ± 0.35	0.23 ± 0.01	0.36 ± 0.04	0.85 ± 0.04	0.68 ± 0.04
14	0.18 ± 0.01	0.31 ± 0.02	0.29 ± 0.05	0.57 ± 0.01	0.17 ± 0.01	0.26 ± 0.01	0.26 ± 0.01	0.27 ± 0.01
15	1.72 ± 0.09	1.72 ± 0.09	3.00 ± 0.14	2.50 ± 0.34	1.11 ± 0.33	0.92 ± 0.04	2.33 ± 0.15	2.19 ± 0.48
16	3.14 ± 0.19	3.14 ± 0.78	2.04 ± 0.43	3.35 ± 0.12	2.64 ± 0.67	3.30 ± 0.23	1.61 ± 0.11	5.16 ± 0.78
17	8.55 ± 0.98	4.20 ± 0.98	7.25 ± 0.98	8.25 ± 0.65	6.75 ± 0.99	3.23 ± 0.15	5.80 ± 0.67	3.70 ± 0.34
18	4.85 ± 0.56	3.10 ± 0.81	3.90 ± 0.56	4.10 ± 0.45	4.25 ± 0.87	1.70 ± 0.16	3.17 ± 0.11	2.30 ± 0.24
19	9.88 ± 1.00	>10	8.73 ± 0.75	8.72 ± 0.99	4.99 ± 0.66	6.03 ± 0.67	5.09 ± 0.23	5.60 ± 0.44
20	2.80 ± 0.09	1.05 ± 0.02	1.63 ± 0.07	2.48 ± 0.32	2.57 ± 0.43	0.92 ± 0.06	1.26 ± 0.15	2.02 ± 0.17
21	>80.00	>10.00	>10.00	>80.00	20.87 ± 1.04	>10.00	>10.00	>20.00
22	1.61 ± 0.23	1.01 ± 0.05	2.14 ± 0.07	1.56 ± 0.09	0.67 ± 0.04	0.69 ± 0.04	0.67 ± 0.06	1.14 ± 0.17
23	9.65 ± 0.98	6.80 ± 0.66	>10.00	>20.00	8.48 ± 1.07	5.16 ± 0.97	6.67 ± 1.02	8.09 ± 0.91
24	0.81 ± 0.04	0.69 ± 0.04	0.85 ± 0.11	0.53 ± 0.01	0.53 ± 0.05	0.48 ± 0.02	0.49 ± 0.05	0.55 ± 0.07
25	2.39 ± 0.11	2.34 ± 0.22	8.20 ± 0.99	3.45 ± 0.45	2.17 ± 0.44	2.24 ± 0.41	2.45 ± 0.43	1.98 ± 0.19
26	2.20 ± 0.15	2.33 ± 0.19	4.37 ± 0.76	2.18 ± 0.88	2.27 ± 0.34	1.78 ± 0.31	1.95 ± 0.33	2.63 ± 0.22
27	1.64 ± 0.11	1.04 ± 0.08	2.23 ± 0.19	1.01 ± 0.02	1.09 ± 0.14	0.85 ± 0.11	1.00 ± 0.30	0.93 ± 0.06
28	11.95 ± 1.01	>10.00	>10.00	>10.00	4.33 ± 0.89	3.21 ± 0.93	3.27 ± 0.76	6.29 ± 0.87
29	2.26 ± 0.44	0.91 ± 0.04	2.04 ± 0.17	2.93 ± 0.22	2.15 ± 0.42	1.03 ± 0.04	0.98 ± 0.15	1.93 ± 0.12

[a] Half-maximum inhibitory concentration.
[b] Positive control.

methyl groups led to a moderate increase in inhibitory activity (methyl in **14** and **16**, hydrogen in **3** and **7**). **21** and **23** showed weaker antiproliferative activity than the corresponding compounds **22** and **24**, because of an *ortho*-methoxyl group close to the double bond of α,β-unsaturated ketone. Additionally, the replacement of the phenyl ring by a pyrimidine group could reduce the inhibitory potency (e.g., **28** with all IC_{50} values >10.0 μM at 48 h).

Docking study

To study the molecular interaction and affinity of binding of the podophyllotoxin congeners, all the ligands were docked in the ATPase domain of Topo-II, which has been proven to be an active binding site of etoposide (see the data in the Supporting Information) [26, 27]. The high-ranked (by computational scoring) compounds **10**–**13** are depicted in Fig. 2. The scaffold occupied the active site of the enzyme, deeply located inside the cavity and surrounded by the residues, and formed hydrogen bond (H-bond) interactions with several residues. Compound **10** possessed three H-bonds with Gly164, Asn163, and Arg162 and two H-bonds with Ala167 and Asn91, while **11** exhibited four strong H-bonds with Arg98, Thr215, Ser149, and Asn91. Compound **12** (hydrophobic interaction with Arg98, and Thr215) and **13** (five H-bonds with residues: one methoxyl group with Gly164, Asn163, and Arg162; S and N atom with Ala167 and Asn150, respectively) also showed strong affinity to the receptor.

Cell cycle analysis

The cell cycle of a eukaryotic cell is a crucial checkpoint for chemotherapeutic drugs killing tumor cells, involving a variety of fundamental processes such as cell division and duplication (replication), etc. [28]. As shown in Table 3, all HepG2 cells were accumulated in the G_2/M phase, with a concomitant decrease of cells in the G_1 and S phases, demonstrating that the congeners **10**–**13** dose-dependently blocked the progression of the cell cycle in the G_2/M phase. Importantly, compound **11**, superior to the other compounds (**10**, **12**, and **13**), significantly induced apoptosis of the HepG2 cells in a dose-dependent manner (Fig. 3), highlighting that the four congeners exerted their antitumor potency by inducing cell apoptosis.

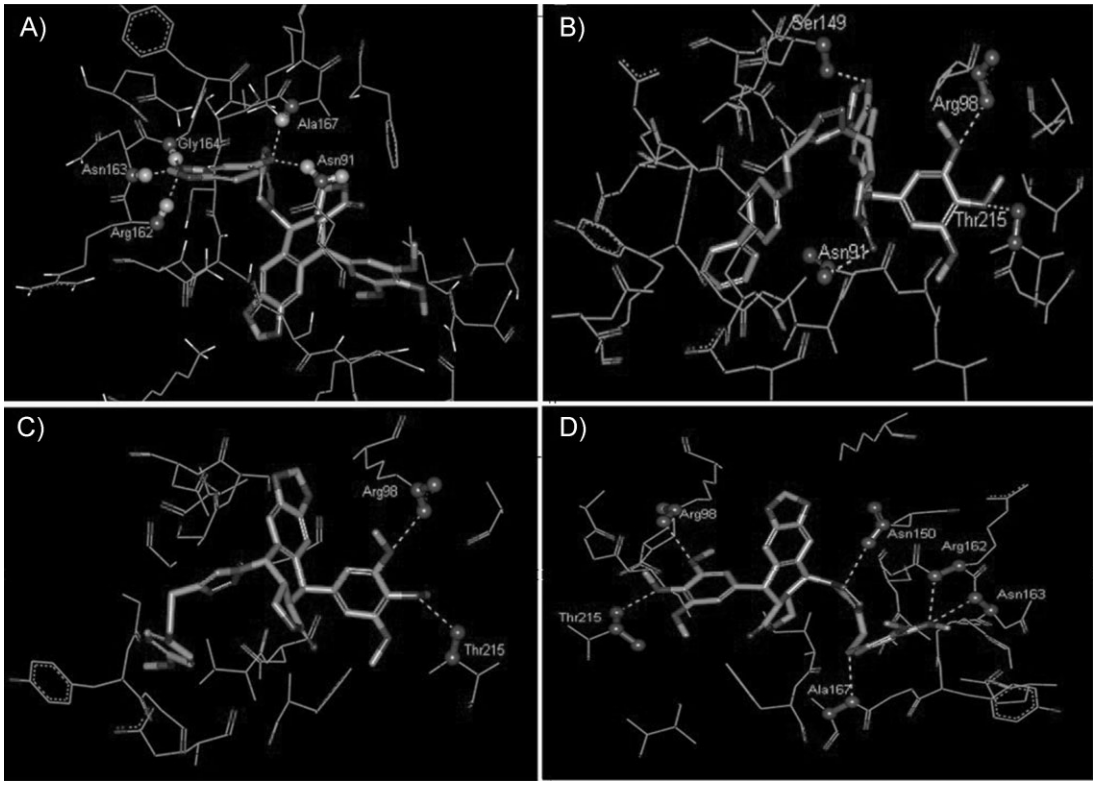


Figure 2. Docking models of podophyllotoxin congeners in the ATPase domain of Topo-II. Interactions of compounds **10** (A), **11** (B), **12** (C), and **13** (D) with residues within the ATPase domain of Topo-II.

Table 3. Effects of four podophyllotoxin congeners on the cell cycle distribution in HepG2 cells.

μM	Compounds	% G1	% G2	% S	Compounds	% G1	% G2	% S
0	10	50.66	15.60	33.74	11	50.66	15.60	33.74
0.1		53.77	17.33	28.90		41.06	39.53	19.41
0.2		20.88	63.77	15.34		1.83	70.85	27.32
0.4		0.43	95.64	3.93		0.24	94.95	7.34
0	12	50.66	15.60	33.74	13	50.66	15.60	33.74
0.1		17.63	57.18	25.19		49.66	21.00	29.34
0.2		25.14	49.62	25.25		11.25	63.03	25.72
0.4		0.50	94.66	4.84		0.64	85.89	13.46

The cells were exposed to various concentrations of podophyllotoxin derivatives for 24 h, and then the DNA content was analyzed by flow cytometry.

Conclusion

In summary, a series of 1,2,3-triazole-based podophyllotoxin congeners were synthesized and evaluated for their antitumor activity against four tumor cell lines. Among them, compound **11**, featuring a biphenyl motif substituted at the 4-position of the 1,2,3-triazole ring, exhibited the most potent antitumor activity. Meanwhile, **10**, **12**, and **13** also exerted strong anticancer potency. The docking study revealed perfect docking of the four congeners in the active site of the ATPase domain. Further, the four congeners **10**–**13** were validated as specific G_2/M phase blockers by cell cycle analysis.

Experimental

Chemistry

Melting points were recorded on an SGW X-4 micro melting point apparatus. ^{1}H NMR spectra were recorded on a Varian spectrometer (Varian, Palo Alto, CA, USA) model Gemini 400; chemical shifts are expressed in ppm, relative to internal tetra-

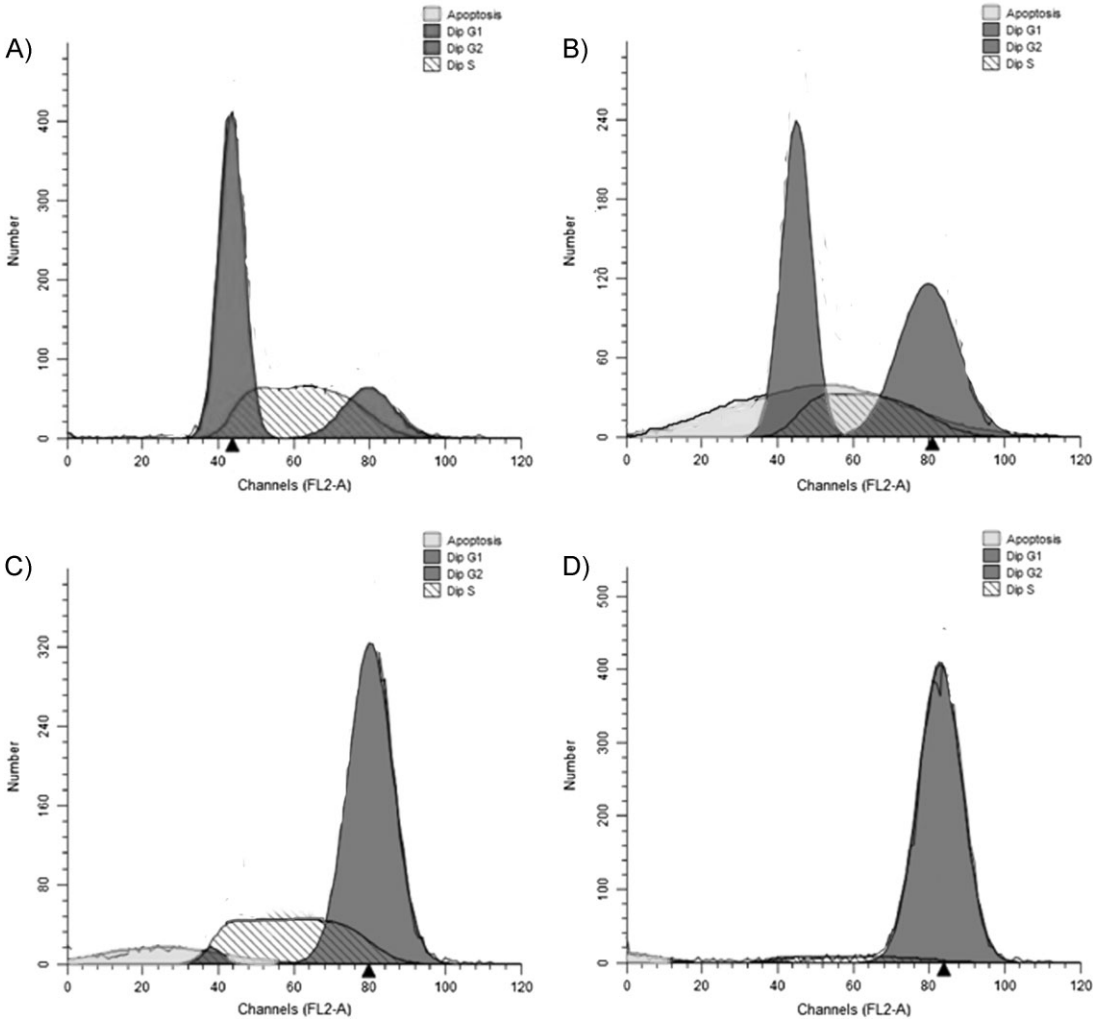


Figure 3. Flow cytometric analysis showing DNA histograms of HepG2 cells. (A) Control; (B) treated with 0.1 μM **11**; (C) treated with 0.2 μM **11**; (D) treated with 0.4 μM **11**.

methylsilane (TMS), where TMS (δ) = 0.00 ppm. The multiplicity of the signal is indicated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, defined as all multipeak signals where overlap or complex coupling of signals makes definitive descriptions of peaks difficult. Reactions were monitored by analytical TLC on 0.20 mm silica gel F_{254} plates (Qingdao Ocean Chemical Factory, Shandong, China). Low-resolution mass spectra were obtained by a Q-TOF Premier mass spectrometer utilizing electrospray ionization (ESI; Micromass, Manchester, UK). HPLC was performed using a photodiode array detector (Waters, Milford, MA, USA) and the chromatographic column was an Atlantis C_{18} (150 mm × 4.6 mm, id 5 μm) (Waters, Milford, Ireland) at a flow rate of 1 mL/min for 30 min.

*General procedure for the synthesis of 4′-O-demethyl-4-azido-4-desoxypodophyllotoxin **2***

Of TFA/CH_2Cl_2 (1:1), 4 mL was slowly added to a stirred solution of podophyllotoxin (**1**) (400.0 mg, 0.96 mmol), sodium azide (305.8 mg, 4.70 mmol), and 10 mL CH_2Cl_2 in an ice bath. The mixture was stirred overnight at room temperature. Water was added and the mixture was extracted with CH_2Cl_2, washed with brine and dried over anhydrous Na_2SO_4, followed by evaporation to afford a brown solid. The crude was purified by silica gel (300–400 mesh) column chromatography using petroleum ether/ethyl acetate (4:1) to yield a brown solid (422.5 mg, 96.0%).

*General procedure for the preparation of chalcones **c2–c11***

To a solution of 1-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)ethanone (**c1**) (2.45 mmol) and the appropriate benzaldehydes (4.90 mmol) in methanol was added 40 mL of 50% KOH aqueous solution. The reaction mixture was stirred under nitrogen atmosphere at room temperature for 24 h. After completion, the mixture was neutralized with 1 N HCl, and the formed solid was collected by filtration. The crude was purified through recrystallization with methanol to yield the product.

General procedure for click chemistry

To a solution of 4′-*O*-demethyl-4-azido-4-desoxypodophyllotoxin (**2**) (0.22 mmol) in THF/water (1:1, 4 mL) was added $CuSO_4 \cdot 5H_2O$ (0.22 mmol), sodium ascorbate (0.5 mmol), and TABA (0.5 mmol). The mixture was stirred at 60°C for 20 h. After completion, the mixture was diluted with water and extracted with ethyl acetate, washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The crude was purified by silica gel column chromatography (300–400 mesh) with petroleum ether/ethyl acetate (1:1) to yield the solid products **3**–**29**.

*Podophyllotoxin **1***

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (s, 1H), 7.11 (s, 1H), 6.60 (s, 1H), 6.36 (s, 2H), 5.98 (d, 2H, J = 8.0 Hz), 4.77 (d, 1H, J = 8.8 Hz), 4.60 (t, 2H, J = 8.4 Hz), 4.08 (t, 1H, J = 8.8 Hz), 3.80 (s, 3H), 5.76 (s, 3H), 2.82–2.77 (m, 2H). MS (ESI), m/z: 437.41 $[M+Na]^+$.

*4β-N$_3$-Desoxypodophyllotoxin **2***

Yield 62.14%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (s, 1H), 6.65 (s, 1H), 6.24 (s, 2H), 5.99 (d, 2H, J = 5.2 Hz), 4.78 (d, 1H, J = 3.6 Hz), 4.61 (d, 1H, J = 5.2 Hz), 4.33–4.27 (m, 2H), 3.79 (s, 3H), 3.70 (s, 6H), 3.23 (d, 1H, J = 5.2 Hz), 2.95–2.92 (m, 1H). MS (ESI), m/z: 462.22 $[M+Na]^+$.

*4β-[(4-((4-Nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **3***

Yield 57.04%; yellow solid; mp 119–120°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (s, 1H), 7.87 (s, 1H), 7.34–7.32 (m, 2H), 6.68 (s, 1H), 6.67 (s, 1H), 6.33 (s, 2H), 6.05 (d, 2H, J = 7.2 Hz), 5.25 (s, 2H), 4.79 (d, 1H, J = 5.2 Hz), 4.45 (t, 1H, J = 7.2 Hz), 4.15–4.10 (m, 1H), 3.82 (s, 3H), 3.77 (s, 6H), 3.34–3.25 (m, 2H), 3.03 (m, 1H, J = 9.2 Hz). MS (ESI), m/z: 639.56 $[M+Na]^+$.

*4β-[(4-((3-Nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **4***

Yield 68.24%; yellow solid; mp 120–123°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, 1H, J = 8.0 Hz), 7.82 (m, 1H), 7.46 (t, 1H, J = 8.4Hz), 7.37–7.31 (m, 2H), 6.70 (s, 1H), 6.62 (s, 1H), 6.32 (s, 2H), 6.13 (d, 1H, J = 4.8 Hz), 6.02 (d, 2H, J = 6.4 Hz), 5.24 (s, 2H), 4.76 (d, 1H, J = 5.2 Hz), 4.43–4.41 (m, 1H), 3.80 (s, 3H), 3.77–3.74 (m, 6H), 3.36–3.25 (m, 2H), 3.05–3.00 (m, 1H). MS (ESI), m/z: 639.52 $[M+Na]^+$.

*4β-[(4-(4-Methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **5***

Yield 51.33%; white solid; mp 124–125°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.18 (m, 1H), 6.66 (s, 2H), 6.61–6.60 (m, 3H), 6.37–6.28 (m, 2H), 6.10 (d, 2H, J = 9.6 Hz), 4.78 (s, 1H), 4.40 (s, 1H), 3.82–3.74 (m, 12H), 3.29 (s, 2H), 3.29 (s, 2H), 2.97 (s, 1H). MS (ESI), m/z: 624.57 $[M+Na]^+$.

*4β-[(4-(4-(Tert-pentyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl]-4-desoxypodophyllotoxin **6***

Yield 46.47%; white solid; mp 122–124°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00–6.91 (m, 2H), 6.69 (s, 1H), 6.69 (s, 1H), 6.31 (s, 2H), 6.03–5.98 (m, 3H), 5.15 (s, 2H), 4.78 (s, 1H), 4.01 (s, 1H), 3.82 (s, 3H), 3.78 (s, 6H), 3.27 (s, 2H), 3.04 (s, 1H), 1.55 (q, 2H, J = 7.2 Hz), 0.84–0.82 (m, 6H), 0.69–0.65 (t, 3H, J = 7.2 Hz). MS (ESI), m/z: 664.64 $[M+Na]^+$.

*4β-[(4-(4-Ethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **7***

Yield 60.45%; colorless oil; mp 123–125°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H), 7.11 (s, 1H), 6.90 (s, 1H), 6.87 (s, 1H), 6.65 (s, 1H), 6.62 (s, 1H), 6.32 (s, 2H), 6.10 (d, 1H, J = 3.6Hz), 6.03–6.00 (m, 2H), 5.15 (s, 2H), 4.75 (d, 1H, J = 4.8 Hz), 4.40 (s, 1H), 3.82 (s, 3H), 3.77 (s, 6H), 3.23 (t, 2H, J = 6.0 Hz), 3.06–3.01 (m, 1H), 3.80 (s, 3H), 3.77 (s, 6H), 3.25–3.22 (m, 2H), 3.06–3.01 (m, 1H), 2.60 (q, 2H, J = 7.2 Hz), 1.21 (t, 3H, J = 7.6 Hz). MS (ESI), m/z: 622.59 $[M+Na]^+$.

*4β-[(4-(4-Methyl-3-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **8***

Yield 55.77%; yellow solid; mp 123–124°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.20 (s, 2H), 6.67 (s, 1H), 6.69 (s, 1H), 6.31 (s, 2H), 6.04 (d, 2H, J = 7.2 Hz), 5.30 (s, 2H), 4.78 (s, 1H), 4.42 (s, 1H), 3.82 (s, 3H), 3.77 (s, 6H), 3.28 (s, 2H), 3.00 (s, 1H), 2.54 (s, 3H). MS (ESI), m/z: 653.21 $[M+Na]^+$.

*4β-[(4-((2-Oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **9***

Yield 45.65%; white solid; mp 122–125°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.60 (s, 1H), 7.33–7.26 (m, 2H), 6.64 (s, 1H), 6.67 (s, 1H), 6.39 (s, 2H), 6.03 (d, 2H, J = 10.0 Hz), 5.30 (s, 2H), 4.76 (s, 2H), 4.39 (s, 1H), 3.81 (s, 3H), 3.76 (s, 6H), 3.24 (s, 2H), 3.06 (s, 1H). MS (ESI), m/z: 662.59 $[M+Na]^+$.

*4β-[(4-(Benzo[d][1,3]dioxol-5-yloxy)methyl)-1H-1,2,3-triazol-1-yl]-4-desoxypodophyllotoxin **10***

Yield 51.43%; white solid; mp 112–114°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.52 (m, 2H), 7.43–7.39 (m, 1H), 7.03 (d, 1H, J = 8.0 Hz), 6.62 (d, 1H, J = 10.4 Hz), 6.31 (s, 1H), 6.01–5.97 (m, 2H), 5.21 (s, 2H), 4.75 (d, 1H, J = 4.0 Hz), 4.41 (m, 1H), 3.81 (s, 3H), 3.76–3.73 (m, 6H), 3.32–3.23 (m, 2H), 3.08–3.04 (m, 1H). MS (ESI), m/z: 638.07 $[M+Na]^+$.

*4β-[(4-(([1,1′-Biphenyl]-4-yloxy)methyl)-1H-1,2,3-triazol-1-yl]-4-desoxypodophyllotoxin **11***

Yield 46.67%; colorless oil; mp 126–130°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68–7.52 (m, 2H), 6.71–6.62 (m, 3H), 6.63–6.62 (d, 2H, J = 8.4 Hz), 6.40–6.33 (m, 1H, J = 6.0 Hz), 6.32–6.31 (m, 2H), 6.10 (d, 1H, J = 4.0 Hz), 6.05–6.01 (m, 4H), 5.92 (s, 2H), 5.10 (s, 2H), 4.75 (d, 1H, J = 5.2 Hz), 4.42–4.39 (m, 1H), 4.30 (t, 1H, J = 6.4 Hz), 4.14–4.09 (m, 1H), 3.82–3.74 (m, 9H), 3.28–3.19 (m, 2H), 3.04–3.01 (m, 1H). MS (ESI), m/z: 670.36 $[M+Na]^+$.

*4β-[(4-((4-Methoxyphenyl)thio)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **12***

Yield 53.76%; white solid; mp 124–126°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.53 (m, 1H), 7.43–7.39 (m, 2H), 6.82 (s, 2H), 6.64–6.31 (m, 2H), 6.62 (s, 1H), 6.65–6.30 (m, 2H), 5.29 (s, 2H), 4.73–4.70 (d, 1H, J = 10.0 Hz), 4.60 (s, 1H), 3.84–3.74 (m, 12H), 3.25 (s, 1H), 2.98–2.82 (m, 1H). MS (ESI), m/z: 640.37 $[M+Na]^+$.

*4β-[4-(3,4-Dimethoxyphenyl)thio)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin **13***

Yield 67.87%; white solid; mp 127–130°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.21 (m, 3H), 6.74 (s, 1H), 6.62 (s, 1H), 6.61 (s, 1H), 6.28 (s, 2H), 5.98–5.95 (m, 3H), 4.69 (s, 2H), 4.31 (s, 1H), 3.85–3.84

(m, 6H), 3.81(s, 3H), 3.75 (s, 6H), 3.32–3.19 (m, 2H), 2.78 (s, 1H). MS (ESI), *m/z*: 670.43 $[M+Na]^+$.

4β-[(4-(2-(4-Ethylphenoxy)propan-2-yl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***14***

Yield 59.41%; white solid; mp 126–127°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32–7.27 (m, 3H), 6.63 (s, 1H), 6.68–6.61 (m, 1H), 6.30–6.28 (m, 2H), 6.03–5.97 (m, 3H), 4.77 (s, 1H), 4.40 (s, 1H), 3.82 (s, 3H), 3.76 (s, 6H), 3.24 (s, 2H), 2.99–2.97 (m, 1H), 1.27–1.18 (m, 9H). MS (ESI), *m/z*: 650.79 $[M+Na]^+$.

4β-[(4-(2-(4-Methoxyphenoxy)propan-2-yl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***15***

Yield 65.12%; white solid; mp 135–139°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73–7.54 (m, 4H), 6.62 (s, 1H), 6.68 (d, 1H, J = 5.2 Hz), 6.31 (s, 2H), 6.02 (d, 2H, J = 6.0 Hz), 4.41 (s, 1H), 4.30 (m, 1H, J = 6.4 Hz), 3.87–3.80 (m, 12H), 3.24 (s, 2H), 2.01–1.99 (m, 1H), 1.47 (s, 6H). MS (ESI), *m/z*: 652.52 $[M+Na]^+$.

4β-[(4-(2-(3-Nitrophenoxy)propan-2-yl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***16***

Yield 71.90%; white solid; mp 139–141°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65–7.43 (m, 4H), 6.62 (d, 2H, J = 8.0 Hz), 6.31 (s, 2H), 6.07–6.02 (m, 3H), 4.75 (d, 1H, J = 4.8 Hz), 4.41 (s, 1H), 3.87 (s, 3H), 3.71 (s, 6H), 3.25 (s, 2H), 3.06–3.03 (m, 1H), 1.36 (s, 6H). MS (ESI), *m/z*: 667.54 $[M+Na]^+$.

4β-[((2-Chlorobenzamide)methyl)-H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***17***

Yield 66.67%; white solid; mp 131–135°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 2H), 7.41 (s, 2H), 6.65 (s, 1H), 6.66 (s, 1H), 6.30 (s, 2H), 6.03 (d, 2H, J = 9.6 Hz), 4.87 (s, 1H), 4.86 (s, 2H), 4.38 (t, 1H, J = 7.0 Hz), 4.15–4.09 (m, 2H), 3.82 (s, 3H), 3.76 (s, 6H), 3.25 (s, 2H), 3.00 (m, 1H). MS (ESI), *m/z*: 655.51 $[M+Na]^+$.

4β-[((4-Chlorobenzamide)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***18***

Yield 59.88%; white solid; mp 134–139°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 2H), 7.41 (s, 2H), 6.65 (s, 1H), 6.66 (s, 1H), 6.30 (s, 2H), 6.03 (d, 2H, J = 9.6 Hz Hz), 4.77 (s, 2H), 4.79 (d, 1H, J = 5.2 Hz), 4.45 (s, 1H), 3.81 (s, 3H), 3.76 (s, 6H), 3.27 (s, 2H), 3.04 (s, 1H). MS (ESI), *m/z*: 655.51 $[M+Na]^+$.

4β-[((4-(4-Acetyl-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl))]-4-desoxypodophyllotoxin ***19***

Yield 75.44%; white solid; mp 145–150°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, 2H, J = 7.2 Hz), 7.33 (s, 1H), 7.10 (s, 1H), 6.64 (s, 1H), 6.69 (s, 1H), 6.30 (d, 2H), 6.10 (d, 1H, J = 4.4 Hz), 6.03 (d, 2H, J = 10.4 Hz), 5.34 (s, 2H), 4.74 (d, 1H, J = 5.2 Hz), 4.40 (s, 1H), 3.91 (s, 3H), 3.82 (s, 3H), 3.77 (s, 6H), 3.50 (d, 1H, J = 5.2 Hz), 3.25 (t, 1H, J = 4.8 Hz), 2.57 (s, 3H). MS (ESI), *m/z*: 666.59 $[M+Na]^+$.

4β-[(4-((2-Methoxy-4-(3-(3,4,5-trimethoxyphenyl)-acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***20***

Yield 66.33%; yellow solid; mp 122–124°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.13 (s, 2H), 7.56 (t, 1H, J = 7.6 Hz), 7.45 (d, 1H, J = 15.6 Hz), 7.34 (s, 1H), 7.16 (d, 1H, J = 7.6 Hz), 6.88 (s, 2H), 6.70 (s, 1H), 7.05 (s, 1H), 7.03 (s, 1H), 6.30 (s, 1H), 6.02 (d, 2H, J = 10.8 Hz), 5.37 (s, 2H), 4.74 (d, 1H, J = 3.6 Hz), 4.40 (s, 1H), 3.94–3.90 (m, 9H), 3.86 (s, 3H), 3.81–3.73 (m, 9H), 3.26 (s, 1H), 3.03 (d, 1H, J = 5.2 Hz). MS (ESI), *m/z*: 844.56 $[M+Na]^+$.

4β-[(4-((2-Methoxy-4-(3-(2,4,5-trimethoxyphenyl)-acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl]-4-desoxypodophyllotoxin ***21***

Yield 60.67%; yellow solid; mp 135–139°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, 1H, J = 15.6 Hz), 7.65 (t, 1H, J = 8.0 Hz), 7.51 (s, 1H), 7.34 (s, 1H), 7.11 (s, 1H), 7.64 (s, 1H), 7.59 (s, 1H), 6.63 (d, 1H, J = 5.2 Hz), 6.31 (s, 2H), 6.09 (d, 1H, J = 3.6 Hz), 6.02 (d, 2H, J = 9.6 Hz), 5.35 (s, 1H), 5.27 (s, 1H), 4.74 (d, 1H, J = 4.4 Hz), 4.40 (d, 1H, J = 6.4 Hz), 3.95–3.67 (m, 21H), 3.28 (d, 2H, J = 8.4 Hz), 3.04 (t, 1H, J = 8.8 Hz). MS (ESI), *m/z*: 844.59 $[M+Na]^+$.

4β-[(4-((4-(3-(3,4-Dimethoxyphenyl)acryloyl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl]-4-desoxypodophyllotoxin ***22***

Yield 65.23%; yellow solid; mp 140–144°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, 1H, J = 15.6 Hz), 7.64 (t, 1H, J = 8.8 Hz), 7.61 (s, 1H), 7.36 (d, 1H, J = 6.8 Hz), 7.19 (d, 1H, J = 3.6 Hz), 7.15 (s, 1H), 7.05 (d, 1H, J = 8.8 Hz), 6.90 (t, 1H, J = 8.0 Hz), 6.64 (s, 1H), 6.60 (s, 1H), 6.31 (s, 2H), 6.09 (s, 1H), 6.02 (d, 2H, J = 10.4 Hz), 5.36 (s, 2H), 4.74 (d, 1H, J = 4.4 Hz), 4.40 (s, 1H), 3.96–3.70 (m, 18H), 3.40–3.24 (m, 1H), 3.03 (t, 1H, J = 4.0 Hz). MS (ESI), *m/z*: 814.66 $[M+Na]^+$.

4β-[4-((2-Methoxy-4-(3-(2-methoxyphenyl)-acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***23***

Yield 68.55%; white solid; mp 139–141°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, 1H, J = 16. 0 Hz), 7.66–7.62 (m, 3H), 7.40 (d, 1H, J = 2.4 Hz), 7.02–6.94 (m, 2H), 6.78–6.74 (m, 1H), 6.64 (s, 1H), 6.69 (t, 1H, J = 5.2 Hz), 6.30 (s, 2H), 6.09 (s, 1H), 6.02 (d, 2H, J = 9.0 Hz), 5.35 (s, 2H), 4.75 (d, 1H, J = 4.4 Hz), 4.40 (s, 1H), 3.97–3.81 (m, 8H), 3.76 (s, 6H), 3.27 (m, 2H), 3.02 (t, 1H, J = 8.4 Hz). MS (ESI), *m/z*: 784.59 $[M+Na]^+$.

4β-[(4-(4-(3-(4-Fluorophenyl)acryloyl)-2-methoxyphenoxy)methyl]-4-desoxypodophyllotoxin ***24***

Yield 71.12%; yellow solid; mp 145–150°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, 1H, J = 15.6 Hz), 7.67–7.63 (m, 3H), 7.55 (s, 2H), 7.12 (t, 2H, J = 8.4 Hz), 6.64 (s, 1H), 6.69 (s, 1H), 6.30 (s, 2H), 6.09 (s, 1H), 6.02 (d, 2H, J = 10.4 Hz), 5.36 (d, 2H, J = 10.4 Hz), 4.75 (d, 1H, J 4.4 Hz), 4.40 (s, 1H), 3.94–3.76 (m, 12H), 3.26 (s, 1H), 3.02 (d, 1H, J = 9.2 Hz). MS (ESI), *m/z*: 772.63 $[M+Na]^+$.

4β-[4-((2-Methoxy-4-(3-(4-(trifluoromethyl)phenyl)-acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl]-4-desoxypodophyllotoxin ***25***

Yield 67.33%; white solid; mp 131–135°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, 1H, J= 16.0 Hz), 7.83–7.60 (m, 6H), 7.35 (s, 1H), 7.16 (d, 1H, J = 8.4 Hz), 6.65 (s, 1H), 6.60 (s, 1H), 6.30 (s, 2H), 6.10 (d, 1H, J = 4.4 Hz), 6.02 (d, 2H, J = 10.4 Hz), 5.36 (s, 2H), 4.74 (d, 1H, J = 4.8 Hz), 4.40 (s, 1H), 3.87–3.95 (m, 3H), 3.81 (s, 3H), 3.76 (s, 6H), 3.26–3.23 (m, 2H), 3.04–3.03 (d, 1H, J = 5.2 Hz). MS (ESI), *m/z*: 822.58 $[M+Na]^+$.

4β-[(4-((2-Methoxy-4-(3-(thiophen-2-yl)acryloyl)phenoxy)-methyl)-1H-1,2,3-triazol-1-yl)-]-4-desoxypodophyllotoxin ***26***
Yield 60.98%; yellow solid; mp 124–126°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, 1H, J = 15.6 Hz), 7.65–7.61 (m, 2H), 7.42 (d, 1H, J = 5.2 Hz), 7.42–7.32 (m, 2H), 7.14–7.08 (m, 2H), 6.64 (s, 1H), 6.69 (s, 1H), 6.30 (s, 2H), 6.09 (d, 1H, J = 4.4 Hz), 6.01 (d, 2H, J = 9.2 Hz), 5.33 (s, 2H), 4.74 (d, 1H, J = 4.8 Hz), 4.40 (t, 1H, J = 8.0 Hz), 3.93 (s, 3H), 3.81 (s, 3H), 3.75 (s, 6H), 3.31–3.23 (m, 2H), 3.04 (d, 1H, J = 8.8 Hz). MS (ESI), m/z: 760.40 $[M+Na]^+$.

4β-[(4-((2-Methoxy-4-(3-(thiophen-3-yl)acryloyl)phenoxy)-methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***27***
Yield 66.09%; yellow solid; mp 134–139°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, 1H, J = 15.6 Hz), 7.63–7.58 (t, 3H, J = 8.0 Hz), 7.44–7.43 (d, 1H, J = 4.8 Hz), 7.38–7.34 (m, 2H), 7.13 (d, 1H, J = 8.4 Hz), 6.63 (s, 1H), 6.69 (s, 1H), 6.30 (s, 2H), 6.09 (d, 1H, 4.4 Hz), 6.01 (d, 2H, J = 10.0 Hz), 5.31 (s, 2H), 3.90 (s, 3H), 3.81 (s, 3H), 3.79 (s, 6H), 3.29–3.17 (m, 2H), 3.04–2.99 (m, 1H, J = 9.2 Hz). MS (ESI), m/z: 760.43 $[M+Na]^+$.

4β-[(4-((2-Methoxy-4-(3-(pyridin-4-yl)acryloyl)phenoxy)-methyl)-1H-1,2,3-triazol-1-yl]-4-desoxypodophyllotoxin ***28***
Yield 60.48%; white solid; mp 136–140°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 2H), 7.40–7.33 (m, 1H), 7.10–7.08 (d, 2H, J = 8.4 Hz), 6.64 (s, 1H), 6.69 (s, 1H), 6.30 (s, 2H), 6.08 (d, 1H, J = 4.4 Hz), 6.03 (s, 1H), 6.00 (s, 1H), 5.33 (s, 2H), 4.73 (d, 1H, J = 5.2 Hz), 4.40 (s, 1H), 3.93 (s, 3H), 3.82 (s, 3H), 3.76 (s, 6H), 3.25 (s, 2H), 3.02–2.97 (m, 1H). MS (ESI), m/z: 756.78 $[M+Na]^+$.

4β-[4-((4-(3-(4-(Dimethylamino)phenyl)acryloyl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)]-4-desoxypodophyllotoxin ***29***
Yield 65.97%; brown solid; mp 146–150°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, 1H, J = 15.6Hz), 7.55–7.54 (m, 2H), 7.40–7.33 (m, 1H), 7.08 (d, 1H, J = 8.4 Hz), 6.64 (s, 1H), 6.63 (s, 1H), 6.30 (s, 1H), 6.08 (d, 1H, J = 3.6 Hz), 6.03 (d, 2H, J = 10.8 Hz), 5.33 (s, 2H), 4.73 (d, 1H, J = 5.2 Hz), 4.40 (s, 1H), 3.94–3.91 (m, 3H), 3.82 (s, 3H), 3.76 (s, 6H), 3.28–3.25 (m, 2H), 3.02–2.97 (m, 1H), 1.24–0.86 (m, 6H). MS (ESI), m/z: 797.46 $[M+Na]^+$.

Antiproliferative activity

MTT assays were carried out using HepG2, MKN-45, NCI-H1993, and B16 tumor cell lines. The four cell lines were maintained in RPMI 1640 medium containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in a 95% air/5% CO_2 humidified atmosphere at 37°C. The cells were seeded into 96-well culture plates at a density of 1×10^4 cells/well with 500 μL culture medium. The cells were pretreated with the compounds **3**–**29**, washed with PBS and incubated in 10 μL MTT (5 mg/mL) dissolved in DMSO for 4 h. The optical density was recorded on an ELISA reader at 570 nm. Cell growth was calculated by subtracting the mean OD value of the respective blank from the mean OD value of the experimental set.

Cell cycle analysis

The HepG2 cell line was used by incubating the cells at 4×10^5 cells/well with the tested compounds (0.1, 0.2, and 0.4 μM) for 24 h and then harvesting by trypsinization. The cells were washed twice with ice-cold PBS. After centrifugation for 3 min, the cells were fixed in 70% ethanol at 4°C in the dark. The cells were then incubated with RNAase (0.1 mg/mL) at 37°C for 30 min and stained with propidium iodide (2 mg/mL) for 30 min in the dark. Finally, flow cytometric analysis was performed with a FACSCalibur flow cytometer (Becton-Dickinson, San Jose, CA, USA) with excitation at 488 nm and emission at 630 nm. The percentage of cell cycle distribution was determined using the MODFIT software (Becton-Dickinson).

Molecular docking

The simulation system was built based on the X-ray crystal structure of the human Topo-II ATPase-AMP-PNP complex, which was obtained from the Protein Data Bank (PDB – 1ZXM; www.rcsb.org) and further modified for docking calculations. Water molecules were removed and H atoms were added to the structure. 3D structures of the podophyllotoxin derivatives were generated and optimized by the Discovery Studio 2.1 package (Accelrys, San Diego, CA, USA). The receptor-grid files were carried out using a grid-receptor generation program using default settings after ensuring that the ligands and the protein are in correct form. The GOLD program in the Discovery Studio software was used to perform the docking simulations, which allows full flexibility of the ligand.

The structures of the synthesized compounds were drawn in chem3D with standard lengths and angles. The Gasteiger–Huckel charge, with a distance-dependent dielectric function, and AM1 docking calculations were applied for the minimization of the molecules. To modify the structure of Topo-II, missing atoms, bonds, and contacts were checked, hydrogen atoms were added to the enzyme structure, and water molecules were removed. Intercalation models were optimized using the CHARMm forcefield with the added parameters. After performing the docking simulation, the scores of the docked conformers were ranked and the best four compounds (**10**–**13**) were speculated regarding the detailed binding modes in the cavity.

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References

[1] A. D. Kinghorn, M. F. Balandrin, in *The Human Medicinal Agents from Plants*, American Chemical Society, Washington, DC **1993**, p. 170.

[2] T. Imbert, *Biochemie* **1998**, *80*, 207–222.

[3] D. M. Reddy, J. Sriniva, G. Chashoo, A. K. Saxena, H. M. Kumar, *Eur. J. Med. Chem.* **2011**, *46*, 1983–1991.

[4] A. Kamal, B. A. Kumar, P. Suresh, N. Shankaraiah, M. S. Kumar, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 350–353.

[5] A. Knmal, P. Suresh, A. Mallareddy, B. A. Kumar, P. V. Reddy, P. Raju, J. R. Tamboli, T. B. Shaik, N. Jain, S. V. Kalivendi, *Bioorg. Med. Chem.* **2011**, *19*, 2349–2358.

[6] A. M. Wilstermann, R. P. Bender, M. Godfrey, S. Choi, C. Anklin, D. B. Berkowitz, N. Osheroff, D. E. Graves, *Biochemistry* **2007**, *46*, 8127–8225.

[7] K. H. Lee, S. A. Beers, M. Mori, Z. Wang, Y. Kuo, L. Li, S. Liu, Y. Cheng, F. Han, Y. Cheng, *J. Med. Chem.* **1990**, *33*, 1364–1368.

[8] H. Stahelin, A. Von Wartburg, *Prog. Drug Res.* **1989**, *33*, 169–173.

[9] K. J. Chavez, X. H. Feng, J. A. Flanders, E. Rodriguez, F. C. Schroeder, *J. Nat. Prod.* **2011**, *74*, 1293–1297.

[10] B. You, B. Trabchand, P. Girard, C. Falandry, B. Ribba, S. Chabaud, P. J. Souquet, C. F. Isabelle, T. L. Veronique, C. Fournel, M. Tod, F. Freyer, *Lung Cancer.* **2008**, *62*, 261–272.

[11] C. M. Das, P. E. Zage, P. Taylor, D. Aguilera, E. A. Wolff, D. Lee, V. Gopalakrishnan, *Eur. J. Cancer* **2010**, *46*, 2771–2780.

[12] J. H. Li, Y. Chen, J. S. Wang, W. Kong, Y. H. Jin, *Chem. Res. Chin. Univ.* **2008**, *24*, 597–602.

[13] A. Longhi, E. Mariani, J. J. Kuehn, *Eur. J. Integr. Med.* **2009**, *1*, 27–33.

[14] L. S. Thiago, E. S. Costa, D. V. Lopes, I. B. Otazu, A. E. Nowill, F. A. Mendes, D. M. Portilho, J. G. Abreu, C. S. Mermelstein, A. Orfao, M. I. D. Rossi, R. Borojevic, *Biomed. Pharmacother.* **2010**, *64*, 63–72.

[15] S. L. Pitts, M. J. Jablonksy, M. Duca, D. Dauzonne, C. Monneret, P. B. Adrimondo, C. Anklin, D. E. Graves, N. Oscheroff, *Biochemistry* **2011**, *50*, 5058–5066.

[16] D. Passarellla, A. Giardini, B. Petretto, F. Gabriele, A. Sacchetti, A. Silvani, C. Ronchi, G. Cappelletti, D. Caetelli, C. Blorlak, B. Danieli, *Med. Chem.* **2008**, *16*, 6269–6285.

[17] J. Shah, J. R. Chen, D. Chow, *Int. J. Pharm.* **1995**, *113*, 103–111.

[18] K. Kobayashi, M. J. Ratain, *Cancer Chemother. Pharmacol.* **1994**, *34*, S64–S68.

[19] M. Gordaliza, M. Angels-Castro, J. M. Miguel del Corral, M. L. López-Vázquez, P. A. García, A. San Feliciano, M. D. García-Grávalos, H. Broughton, *Tetrahedron* **1997**, *53*, 15743–15760.

[20] M. A. Castro, J. M. Corral, M. Gordaliza, P. A. Carcia, M. A. Zurita, A. S. Feliciano, *Bioorg. Med. Chem.* **2007**, *15*, 1670–1678.

[21] D. Imperio, T. Pirali, U. Galli, F. Pagliai, L. Cafici, P. L. Canonico, G. Sorba, A. A. Genazzani, G. C. Tron, *Bioorg. Med. Chem.* **2007**, *15*, 6748–6757.

[22] W. H. Binder, R. Sachsenhofer, *Macromol. Rapid Commun.* **2007**, *28*, 15–54.

[23] P. B. Reddy, S. K. Agrawal, S. Singh, B. A. Bhat, A. K. Saxena, H. M. Kumar, G. N. Qazi, *Chem. Biodivers.* **2008**, *5*, 1792–1802.

[24] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

[25] P. Boeck, C. B. Falcao, P. C. Leal, R. A. Yunes, V. C. Filho, E. C. Santos, B. R. Bergmann, *Bioorg. Med. Chem.* **2006**, *14*, 1538–1545.

[26] D. Leroy, A. V. Kajava, C. Frei, S. M. Gasser, *Biochemistry* **2001**, *40*, 1624–1634.

[27] A. K. Patel, S. Patel, P. K. Naik, A. T. Vihar, P. K. Distt, *Curr. Res. Biol. Sci.* **2010**, *2*, 13–23.

[28] A. C. Nagy, D. Battogtokh, K. C. Chen, B. Novak, J. J. Tyson, *Biophys. J.* **2006**, *90*, 4361–4379.

