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Short communication

Synthesis of 4β-triazole-podophyllotoxin derivatives by azide–alkyne cycloaddition and biological evaluation as potential antitumor agents

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ABSTRACT

A representative synthetic process of derivatizing the natural product podophyllotoxin utilizing the copper-catalyzed azide–alkyne cycloaddition (CuAAC) is described including molecular design, reaction optimization and X-ray structure confirmation. Evaluation of cytotoxicity against human cancer cell lines (Hela, K562 and K562/A02) using MTT assay proves that these triazole derivatives have good antitumor activities. High activities toward the drug resistant K562/A02 cell line reveal promising future for these derivatives. The rarely prepared 1,5-disubstituted triazole isomers, which would be omitted by the "click chemistry", were found to have superior cytotoxicities to that of the 1,4-disubstituted isomers.

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1. Introduction

Podophyllotoxin, an aryl tetralin lignan, is able to inhibit tubulin by binding with its colchicine domain [1]. Several podophyllotoxin derivatives such as etoposide, teniposide are in clinical use as antineoplastic agents. They block the DNA topoisomerase II by stabilizing enzyme–DNA complex [2–5]. However, their high toxicity, low water solubility, acquired drug-resistance and gastrointestinal disturbances have limited their application in cancer chemotherapy. Numerous structure modifications had been performed since the 1950s. Among them, NK611, NPF, GL-311 and TOP53 are presently under clinical trials [6–8]. Since 1,2,3-triazole ring is a widespread functional group in drugs [9,10], it is intriguing to attach 1,2,3-triazoles to podophyllotoxin parent nucleus.

The click chemistry of copper-catalyzed Huisgen 1,3-dipolar azide–alkyne cycloaddition (CuAAC) forming 1,2,3-triazole has exhibited significant advantages with respect to high chemoselectivities and mild reaction conditions [11–15]. During the process, 1,4-disubstituted-1,2,3-triazoles are commonly formed, whereas 1,5-disubstituted isomers can be selectively prepared *via* ruthenium catalysis [16,17] and the reactions without metal catalysis result into mixtures of 1,4- and 1,5-disubstituted isomers. Previously, 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxins reported by Kumar [18] showed promising antitumor activity which revealed significant outlook for further in-depth research. In this paper, a classic structure modification on podophyllotoxin is described including methodology research, structure confirmation of isomers, further chemical synthesis, and biological evaluation.

2. Results and discussion

2.1. Chemistry

To find out the most compatible reaction conditions for synthesizing this series of natural product derivatives, a wide range of reaction parameters were tested by altering the catalyst and the additive as well as the temperature and the solvent in a test reaction of 4β-azido-podophyllotoxin and methyl propiolate (Table 1).

As can be clearly seen in Table 1, the CuAAC reactions resulted in regioselective products. Single 1,4-disubstituted-1,2,3-triazole was formed in entries 1–7. 2,6-Lutidine was a beneficial additive for CuI compared to other basic additives while L-ascorbic acid was

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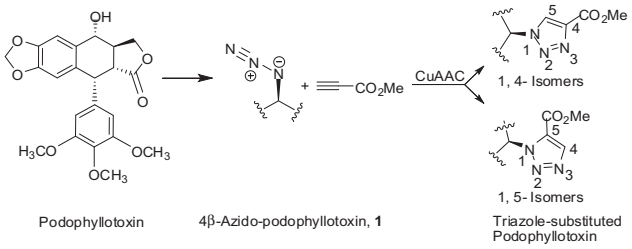
Table 1
Screening of the reaction conditions for the cycloaddition reaction between 4β-azido-podophyllotoxin and methyl propiolate.



Entry	Catalyst	Additive	Solvent	*t* (h)	*T* (°C)	Yield[a] (%)	
						1,4-Isomers	1,5-Isomers
1	$CuSO_4·5H_2O$	L-Ascorbic acid	*t*-BuOH/H_2O (1:1)	2	25	70[b]	0
2	$CuSO_4·5H_2O$	L-Ascorbic acid	*t*-BuOH/H_2O (1:1)	2	50	95[b]	0
3	$CuSO_4·5H_2O$	L-Ascorbic acid	*t*-BuOH/H_2O (1:1)	2	75	65[b]	0
4	$CuSO_4·5H_2O$	L-Ascorbic acid	THF/H_2O (1:1)	2	25	0	0
5	$CuSO_4·5H_2O$	L-Ascorbic acid	DMF/H_2O (1:1)	2	25	80[b]	0
6	$CuSO_4·5H_2O$	L-Ascorbic acid	DMSO/H_2O (1:1)	2	25	83[b]	0
7	CuI	2,6-Lutidine	$CHCl_3$	12	0	90[b]	0
8	CuI	Et_3N	$CHCl_3$	12	0	30[b]	0
9	CuI	Pyridine	$CHCl_3$	12	0	20	17
10	CuI	L-Ascorbic acid	*t*-BuOH/H_2O (1:1)	12	25	0	0
11	$CuSO_4·5H_2O$	2,6-Lutidine	*t*-BuOH/H_2O (1:1)	2	25	15	10
12	CuI	None	PEG_{400}/H_2O (1:1)	2	25	95	0
13	None	None	CH_2Cl_2	6	25	15	12

[a] Isolated yield (%) after column chromatography.
[b] Multiple side products were detected without 1,5-disubstituted products.

necessary for $CuSO_4$ to generate the active Cu(I). Later, the solvent PEG_{400} (entry 12) was found to greatly enhance the reaction rate in CuI catalyzed reaction without any additive [19]. Entries 2, 7, 12 were picked out as candidate conditions for further synthesis (Table 2).

1,5-Disubstituted triazole podophyllotoxins obtained by accident was found to have comparative or even superior anticancer activity. Therefore, the traditional thermal condition without any catalyst was applied and the obtained mixture of the 1,4-disubstitued and the 1,5-disubstituted triazole podophyllotoxins were subjected to column separation. In most cases, ratio of two regioisomers formed were approximately 1:1 (Table 3). X-ray crystallography confirmed that the product formed through the CuAAC reaction was the 1,4-disubstituted triazole podophyllotoxins (Fig. 1) [20].

2.2. Pharmacology

These derivatives were evaluated for cytotoxicity against human cancer cell lines HeLa, K562, K562/A02, using the MTT assay *in vitro* with comparison to the parent compound VP-16, podophyllotoxin and ADM. Most of 1,4-disubstituted triazole podophyllotoxins were demonstrated to have significant antitumor activity (Table 2, IC_{50} values were found at the range of 10^{-6}–10^{-8} mol/L while the IC_{50} values of VP-16 is in 10^{-6} magnitude). Some derivatives exhibited high cytotoxicities toward the drug resistant K562/A02 leukemic cell line, whereas VP-16 was not active. Among them, compound **3g** showed excellent cytotoxicities, more than 40 times more cytotoxic than VP-16 in HeLa, K562 and K562/A02. In the structure-activity analysis, free carboxylic acids in R greatly reduce the cytotoxicity (compounds **3b** and **3c**) while esters and amides are well tolerated (compounds **3a** and **3g**). Secondary amine attached to an aromatic ring in R (compounds **3d** and **3f**) is another promising functionality for future development of analogs. Meanwhile, the 1,5-disubstituted products had comparative or even superior anticancer activity *in vitro* (Table 3). Up to 20 folds of enhancement on cytotoxicity was observed compared to their 1,4-disubstituted counterparts. Although Wang et al. has previously published a short report on the synthesis and antitumor activities of a few 1,5-disubstituted triazole podophyllotoxins [21], to the best of our knowledge, this is the first time that a systematic comparison on antitumor activities of two regioisomers is being made. Previous studies indicate that VP-16 and VM-26, which have a free hydroxyl group at E-ring, are inhibitors of topoisomerase II [2–5], whereas podophyllotoxin targets microtubule [22]. Same as podophyllotoxin, the derivatives we prepared do not have free hydroxyl groups at E-ring, and in our preliminary studies they also target microtubule [23].

3. Conclusion

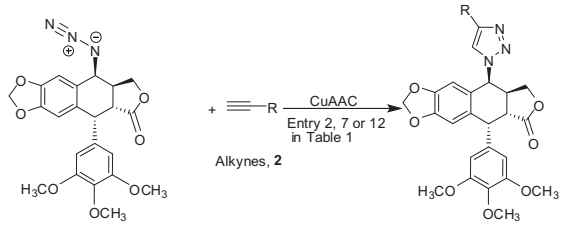
In conclusion, CuAAC reactions showed a broad and profound perspective for structural modification on natural products. In this communication, nine new 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxins were prepared and evaluated for cytotoxicity against human cancer cell lines. Some of these derivatives exhibit excellent activities, even toward the multidrug-resistant cell line. Meanwhile, the superior activities of 1,5-disubstitued triazole podophyllotoxins prove the importance of developing methodology leading to the less accessible regioisomers. In order to get 1,5-disubstituted triazoles, the traditional method without any catalyst are worth trying. Further synthesis of 1,4- and 1,5-disubstituted triazole podophyllotoxins and *in vivo* tests will be reported soon.

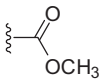
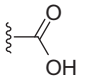
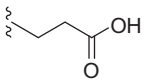
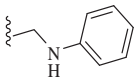
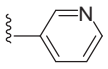
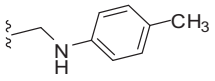
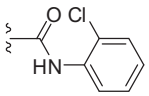
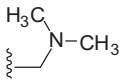
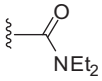
4. Experimental

4.1. Materials and methods

All materials and reagents were obtained from commercial sources and used without further purification unless stated. Podophyllotoxin (98% purity) was purchased from Qingze Corporation of Nanjing in China. CH_2Cl_2 was redistilled over P_2O_5. Melting points were determined on an electric X-4 digital visual melting point

Table 2
Synthesis of triazole derivatives.[a]



Comp. **3**	R	Yield %[b]	IC_{50} (μmol/L)		
			Hela	K562	K562/A02
3a		95	1.12	4.15	2.069
3b		65	>10	5.4	8.87
3c		60	>10	>10	>10
3d		95	2.36	5.73	5.96
3e		93	35.3	>10	>10
3f		80	0.845	0.47	0.52
3g		60	0.082	0.053	0.059
3h		85	>10	>10	>10
3i		57	>10	>10	>10
VP-16			6.27	2.11	151.69
Podophyllotoxin			0.10	0.03	0.17
ADM			0.43	0.36	19.21

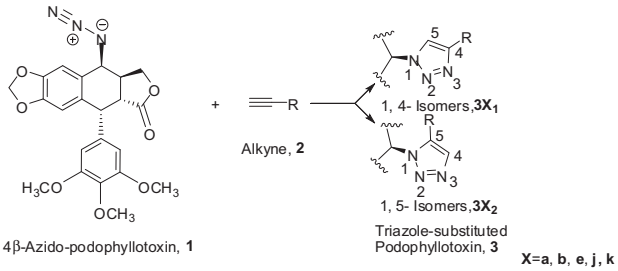
[a] Reaction conditions from Table 1 (entries 2, 7 or 12) were applied.
[b] Isolated yield.

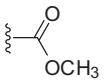
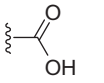
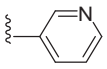
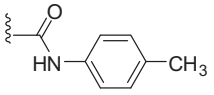
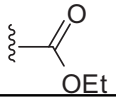
apparatus. The ^{1}H NMR spectra were obtained using a BRUKER ARX-300 instrument (300 MHz) with tetramethylsilane (TMS) as the internal standard, and the multiplicity were marked as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Mass spectra data were obtained on Agilent 6210 TOP-MS and are reported as *m*/*z*.

4.2. General procedure for the CuAAC reactions

1 equiv of 4β-azido-podophyllotoxin (44 mg, 0.1 mmol), 10 equiv of alkynes (1.0 mmol), 10 mol% L-ascorbic acid (2 mg) and

Table 3
Synthesis of 4β-triazole-podophyllotoxin derivatives with no catalyst[a] and comparison on cytotoxicities of two regioisomers against human cancer cell lines.



Entry	Alkynes	R	Yield %[b]		IC_{50} (μmol/L)		
					Hela	K562	K562/A02
1	**2a**		**3a$_1$**	30%	1.12	4.15	2.069
			3a$_2$	25%	0.42	0.40	0.60
2	**2b**		**3b$_1$**	25%	>10	5.4	8.87
			3b$_2$	20%	n.d.	n.d.	n.d.
3	**2e**		**3e$_1$**	30%	35.3	>10	>10
			3e$_2$	27%	1.46	0.39	1.23
4	**2j**		**3j$_1$**	18%	>100	>100	>100
			3j$_2$	15%	>10	5.1	19.8
5	**2k**		**3k$_1$**	34%	3.53	4.25	2.21
			3k$_2$	17%	0.92	0.85	0.69

[a] Reaction condition: ethanol, 80 °C.
[b] Total isolated yields (%) were calculated after column chromatography; The ratio of isomers were determined by HPLC.

4 mol% $CuSO_4 \cdot 5H_2O$ (1.0 mg) or 1 equiv of 2,6-lutidine (11 mg, 0.1 mmol) and 5 mol% CuI (1.0 mg) were added into a reaction vial charged with a magnetic stir bar. *t*-BuOH/H_2O (5.0 ml, 1:1) were used to dissolve the reactants. After stirring for 2 h at corresponding temperature, the reaction mixture was quenched with water (15 ml), and extracted with ethyl acetate (2 × 20 ml). The combined organic layers were washed with water (2 × 10 ml), and then the solvent was removed under reduced pressure. The residue was dissolved in acetone and purified on silica gel to afford the product.

4.3. General procedure for the traditional 1,3-dipolar cycloaddition

1 equiv of 4β-azido-podophyllotoxin (44 mg, 0.1 mmol), 1.2 equiv of alkynes (0.12 mmol) were added into ethanol (5.0 ml) and stirred for 8 h at 80 °C. The workup was similar to the procedure described for the CuAAC Reactions.

4.4. Product characterization

4.4.1. Synthesis of 4β-(4-methoxy-carbonyl-1,2,3-triazole)-podophyllotoxin (*3a$_1$*) by CuAAC

Yield: 95%. White solid. Mp 212 °C. $[\alpha]_D^{25}$ −85 (*c* 0.35 $CHCl_3$). IR (KBr) *ν* 1772, 1762 (C=O), 1603 (N=N), 1555, 1484 (C=C). ^{1}H NMR ($CDCl_3$) *δ*/ppm 7.85 (s, 1H, triazole−H), 6.64 (s, 1H), 6.33 (s, 2H),

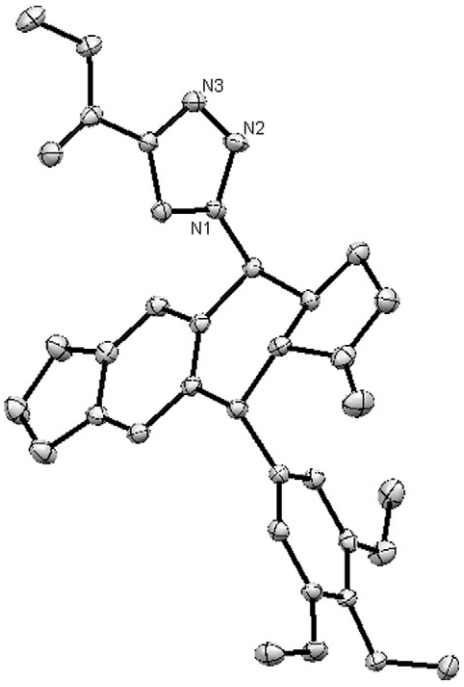


Fig. 1. X-ray structure of 4β-(4-methoxy-carbonyl-1,2,3-triazole)-podophyllotoxin [20]. (Hydrogen atoms are omitted for clarity).

6.61 (s, 1H), 6.18–6.19 (d, 1H, J = 3.9 Hz), 6.04 (d, 1H, J = 1.1 Hz, OCH_2O), 6.01 (d, 1H, J = 1.1 Hz, OCH_2O), 4.75–4.77 (d, 1H, J = 5.0 Hz), 4.37–4.46 (m, 1H), 4.04–4.16 (m, 1H), 3.92 (s, 3H), 3.81 (s, 3H), 3.77 (s, 6H), 3.02–3.36 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 30.9, 37.0, 41.3, 43.6, 56.4, 59.2, 60.8, 67.3, 102.1, 108.3, 108.7, 110.7, 123.9128.0, 133.5, 134.0, 137.7, 139.9, 148.3, 149.7, 152.8, 160.8, 172.7. HR-ESI-MS calculated for $C_{26}H_{25}N_3NaO_9$ $[M + Na]^+$ 546.1483, found 546.1446.

*4.4.2. Synthesis of 4β-(5-methoxy-carbonyl-1,2,3-triazole)-podophyllotoxin (**3a$_2$**) by the traditional 1,3-dipolar cycloaddition*

Yield: 25%. White solid. Mp 164–165 °C. $[\alpha]_D^{25}$ −87 (c 0.41 $CHCl_3$). IR (KBr) ν 1775, 1760 (C=O), 1605 (N=N), 1559, 1481 (C=C). ^{1}H NMR ($CDCl_3$) δ/ppm 8.23 (s, 1H, triazole–H), 6.76–6.78 (d, 1H, J = 5.3 Hz), 6.63 (s, 1H), 6.47 (s, 1H), 6.36 (s, 2H), 5.99 (d, 1H, J = 1.2 Hz, OCH_2O), 5.93 (d, 1H, J = 1.2 Hz, OCH_2O), 4.81–4.83 (d, 1H, J = 5.1 Hz), 4.30–4.36 (m, 1H), 4.02 (s, 3H), 3.80–3.85 (m, 1H), 3.82 (s, 3H), 3.78 (s, 6H), 3.00–3.35 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 30.9, 37.0, 41.3, 43.6, 56.4, 59.2, 60.8, 67.3, 102.2, 108.3, 108.7, 110.7, 123.8, 128.0, 133.5, 134.0, 137.8, 140.1, 148.4, 149.8, 152.9, 160.8, 172.6. HR-ESI-MS calculated for $C_{26}H_{25}N_3NaO_9$ $[M + Na]^+$ 546.1483, found 546.1460.

*4.4.3. Synthesis of 4β-(4-carboxyl-1,2,3-triazole)-podophyllotoxin (**3b$_1$**) by CuAAC*

Yield: 65%. White solid. Mp 177–179 °C. $[\alpha]_D^{25}$ −81 (c 0.31 $CHCl_3$). IR (KBr) ν 3510 (OH), 1768, 1752 (C=O), 1611 (N=N), 1559, 1443 (C=C). ^{1}H NMR ($CDCl_3$) δ/ppm 8.30 (s, 1H, 5′-H), 8.05 (s, 1H, triazole–H), 6.80 (d, 1H, J = 5.3 Hz, 4-H), 6.66 (s, 1H, 5-H), 6.60 (s, 1H, 8-H), 6.37 (s, 2H, 2′,6′-H), 6.02 (d, 1H, J = 1.3 Hz, OCH_2O), 5.99 (d, 1H, J = 1.3 Hz, OCH_2O), 4.82 (d, 1H, J = 5.1 Hz, 1-H), 4.35–4.45 (m, 1H, 11-H-a), 4.04–4.16 (m, 1H, 11-H-b), 3.92 (s, 3H, $-OCH_3$), 3.77 (s, 6H, 3′,5′-OCH_3), 3.02–3.36 (m, 2H, 2,3-H). ^{13}C NMR ($CDCl_3$) δ 36.8, 40.2, 43.5, 56.3, 56.5, 60.4, 67.6, 102.1, 108.6, 109.2, 110.3, 123.7, 126.1, 130.0, 133.7, 135.7, 137.0, 147.5, 148.1, 152.6, 162.1, 174.0. HR-ESI-MS calculated for $C_{25}H_{23}N_3NaO_9$ $[M + Na]^+$ 532.1327, found 532.1354.

*4.4.4. Synthesis of 4β-(4-(2-carboxyl)-ethyl-1,2,3-triazole)-podophyllotoxin(**3c**) by CuAAC*

Yield: 60%. Yellow solid. Mp 151–153 °C. $[\alpha]_D^{25}$ −79 (c 0.36 $CHCl_3$). IR (KBr) ν 3455 (OH), 1773, 1752 (C=O), 1605 (N=N), 1556, 1446 (C=C). ^{1}H NMR ($CDCl_3$) δ/ppm 7.09 (s, 1H, triazole–H), 6.62 (s, 1H, Ar–H), 6.59 (s, 1H, Ar–H), 6.30 (s, 2H, 2′,6′-H), 6.03–6.04 (d, 1H, J = 3.2 Hz, 4-H), 6.00 (d, H, J = 1.1 Hz, OCH_2O), 5.98 (d, H, J = 1.1 Hz, OCH_2O), 4.72–4.74 (d, 1H, J = 4.7 Hz, 1-H), 4.32–4.39 (m, 1H, 1-H-a), 4.08–4.15 (m, 1H, 1-H-b), 3.80 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′,5′-OCH_3), 3.13–3.21 (m, 2H, 2,3-H), 3.98–3.03 (t, 2H, J = 7.1 Hz), 2.75–2.80 (t, 2H, J = 6.8Hz). ^{13}C NMR ($CDCl_3$) δ 30.8, 33.1, 37.1, 41.5, 43.6, 56.2, 58.6, 60.5, 67.4, 101.9, 108.2, 108.8, 110.3, 122.5, 124.8, 133.2, 134.5, 137.4, 146.3, 148.0, 149.3, 152.7, 173.6, 176.1. HR-ESI-MS calculated for $C_{27}H_{27}N_3NaO_9$ $[M + Na]^+$ 560.1640, found 560.1641.

*4.4.5. Synthesis of 4β-(4-anilino-methylene-1,2,3-triazole)-podophyllotoxin (**3d**) by CuAAC*

Yield: 95%. Mp 165–166 °C. $[\alpha]_D^{25}$ −82 (c 0.35 $CHCl_3$). IR (KBr) ν 3320 (N–H), 1766 (C=O), 1601 (N=N), 1553, 1480 (C=C), 1475. ^{1}H NMR ($CDCl_3$) δ/ppm 8.02 (1H, s, N–H), 7.40 (1H, s, triazole–H), 7.00 (d, 1H, J = 10.5 Hz, Ar–H), 6.88 (d, 2H, J = 7.9 Hz, Ar–H), 6.70 (s, 1H, 5-H), 6.65 (d, 2H, J = 8.6 Hz, Ar–H), 6.38 (s, 2H, 2′,6′-H), 6.18 (s, 1H, 8-H), 5.98 (d, 1H, J = 1.3 Hz, OCH_2O), 5.96 (d, 1H, J = 1.3, OCH_2O), 5.78 (s, 1H, 4-H), 4.38–4.60 (m, 4H, 1-H, 11-H-a, $-CH_2-$), 3.85–3.91 (m, 1H, 11-H-b), 3.78 (s, 6H, 3′,5′-OCH_3), 3.60 (m, 2H, 2,3-H). This compound has been characterized before [18].

*4.4.6. Synthesis of 4β-(4-pyridyl-1,2,3-triazole)-podophyllotoxin (**3e$_1$**) by CuAAC*

Yield: 93%. Yellow solid. Mp 139–141 °C. $[\alpha]_D^{25}$ −85 (c 0.41 $CHCl_3$). IR (KBr) ν 1768 (C=O), 1601 (N=N), 1588, 1528 (C=C), 1472. ^{1}H NMR ($CDCl_3$) δ/ppm 8.75 (d, 1H, J = 7.3 Hz), 8.07 (s, 1H, triazole–H), 7.93 (dd, 1H, J = 2.2, 7.8 Hz), 7.77 (d, 1H, J = 7.8 Hz), 7.43 (m, 1H), 6.98 (d, 1H, J = 8.2 Hz), 6.63 (s, 1H), 6.51 (s, 1H), 6.42 (s, 2H), 5.96 (d, 1H, J = 1.3 Hz, OCH_2O), 5.90 (d, 1H, J = 1.3 Hz, OCH_2O), 4.65 (d, 1H, J = 3.4 Hz), 4.42 (m, 1H), 4.09 (m, 1H), 3.82 (s, 3H), 3.78 (s, 6H), 3.16–3.25 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 30.9, 41.6, 43.7, 56.4, 58.9, 60.8, 67.5, 102.0, 108.2, 108.9, 110.5, 120.5, 120.3, 123.3, 124.4, 133.3, 134.2, 137.3, 137.6, 148.2, 148.3, 149.3, 149.6, 149.6, 152.8, 173.0, HR-ESI-MS calculated for $C_{29}H_{27}N_4O_7$ $[M + H]^+$ 543.1874, found 543.1890.

*4.4.7. Synthesis of 4β-(5-pyridyl-1,2,3-triazole)-podophyllotoxin (**3e$_2$**) by the traditional 1,3-dipolar cycloaddition*

Yield: 27%. Yellow solid. Mp 145–147 °C. $[\alpha]_D^{25}$ −81 (c 0.37 $CHCl_3$). IR (KBr) ν 1766 (C=O), 1605 (N=N), 1585, 1550 (C=C), 1481. ^{1}H NMR ($CDCl_3$) δ/ppm 8.56 (d, 1H), 8.20 (dd, 1H, J = 1.7, 7.6 Hz), 7.85 (s, 1H, triazole–H), 7.85 (dd, 1H, J = 1.2, 7.6 Hz), 7.27 (m, 1H), 6.69 (s, 2H), 6.34 (s, 2H), 6.19 (d, 1H, J = 8.3 Hz), 6.04 (d, 1H, J = 1.2 Hz, OCH_2O), 5.98 (d, 1H, J = 1.2 Hz, OCH_2O), 4.77 (d, 1H, J = 5.0 Hz), 4.46 (m, 1H), 4.10 (m, 1H), 3.85 (s, 3H), 3.78 (s, 6H), 3.20–3.38 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 30.9, 41.8, 43.8, 56.4, 58.9, 61.5, 67.5, 103.2, 108.6, 108.9, 110.5, 120.7, 120.4, 123.4, 124.8, 134.4, 134.6, 137.8, 138.7, 148.6, 148.9, 149.3, 149.8, 149.3, 152.9, 173.4. HR-ESI-MS calculated for $C_{29}H_{27}N_4O_7$ $[M + H]^+$ 543.1874, found 543.1882.

*4.4.8. Synthesis of 4β-(4-(4-methylbenzenamino)-methylene-1,2,3-triazole)-podophyllotoxin (**3f**) by CuAAC*

Yield: 80%. White solid. Mp 153–154 °C. $[\alpha]_D^{25}$ −85 (c 0.42 $CHCl_3$). IR (KBr) ν 3365 (N–H), 1767 (C=O), 1611 (N=N), 1558, 1550 (C=C), 1475. ^{1}H NMR ($CDCl_3$) δ/ppm 7.18 (s, 1H, 5-H), 7.02–6.94 (m, 2H), 6.61

(s, 1H, 8-H), 6.58 (d, 2H, J = 10.6 Hz, Ar–H), 6.31 (s, 2H, 2′,6′-H), 6.08 (s, 1H, 4-H), 5.97 (d, 1H, J = 1.3 Hz, OCH_2O), 5.93 (d, 1H, J = 1.3 Hz, OCH_2O), 4.76 (s, 1H, 1-H) 4.43 (s, 2H, $-CH_2-$), 4.38 (m, 1H, 11-H-a), 3.85–3.91 (m, 1H, 11-H-b), 3.82 (s, 3H, 4′-OCH_3), 3.78 (s, 6H, 3′,5′-OCH_3), 3.18 (m, 2H, 2,3-H). This compound has been characterized before [18].

4.4.9. Synthesis of 4β-[4-(2-chloro-phenyl-amino-carbonyl)-1,2,3-triazole]-podophyllotoxin (**3g**) by CuAAC

Yield: 60%. White solid. Mp 167–168 °C. $[\alpha]_D^{25}$ −82 (*c* 0.37 $CHCl_3$). IR (KBr) ν 3360 (N–H), 1770, 1650 (C=O), 1605 (N=N), 1557, 1550 (C=C), 1452. ^{1}H NMR ($CDCl_3$) δ/ppm 8.32 (s, 1H, triazole–H), 7.82 (s, 1H, N–H), 7.74 (dd, 1H, J = 3.3, 7.0 Hz), 7.77 (d, 2H, J = 3.3 Hz), 7.34 (m, 1H), 6.82 (d, 1H, J = 8.4 Hz), 6.63 (s, 1H), 6.54 (s, 1H), 6.38 (s, 2H), 5.99 (d, 1H, J = 1.4 Hz, OCH_2O), 5.96 (d, 1H, J = 1.4 Hz, OCH_2O), 4.83 (d, 1H, J = 3.4 Hz), 4.45 (m,1H), 4.09 (m,1H), 3.82 (s, 3H), 3.83 (s, 6H), 3.11–3.20 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 30.6, 37.0, 41.2, 42.7, 56.4, 60.8, 67.2, 102.2, 108.3, 108.5, 110.5, 117.9, 120.0, 124.9, 126.9, 128.8, 130.3, 130.9, 133.9, 134.5, 135.0, 138.4, 143.0, 148.0, 148.5, 152.9, 157.6, 172.6. HR-ESI-MS calculated for $C_{31}H_{28}ClN_4O_8$ $[M + H]^+$ 619.1590, found 619.1574.

4.4.10. Synthesis of 4β-(4-(N,N-dimethylamino)methylene-1,2,3-triazole)-podophyllotoxin (**3h**) by CuAAC

Yield: 85%. White solid. Mp 172–174 °C. $[\alpha]_D^{25}$ −79 (*c* 0.35 $CHCl_3$). IR (KBr) ν 1769 (C=O), 1603 (N=N), 1562, 1495 (C=C). ^{1}H NMR ($CDCl_3$) δ/ppm 7.19 (1H, s, triazole–H), 6.62 (1H, s, Ar–H), 6.60 (1H, s, Ar–H), 6.31 (2H, s, 2′,6′-H), 6.07–6.09 (1H, d, J = 4.1Hz, 4-H), 6.01 (d, 1H, d, J = 1.2 Hz, OCH_2O), 5.98 (d, H, J = 1.2 Hz, OCH_2O), 4.72–4.72 (d, 1H, J = 4.9 Hz, 1-H), 4.38–4.41 (m, 1H, 1-H-a), 3.79–3.83 (m, 1H, 11-H-b), 3.81 (s, 1H, 4′-OCH_3), 3.76 (s, 6H, 3′,5′-OCH_3), 3.57 (s, 2H, $-CH_2-N-$), 2.26 (s, 6H, $-N-CH_3$). ^{13}C NMR ($CDCl_3$) δ 32.2, 37.2, 41.7, 43.7, 56.3, 58.4, 60.7, 62.2, 67.5, 102.0, 108.2, 108.9, 110.4, 121.1, 125.0, 133.1, 134.4, 137.5, 148.1, 148.3, 149.3, 152.8, 173.3. HR-ESI-MS calculated for $C_{27}H_{31}N_4O_7$ $[M + H]^+$ 523.2187, found 523.2195.

4.4.11. Synthesis of 4β-(4-(N,N-diethylamino)carbonyl-1,2,3-triazole)-podophyllotoxin (**3i**) by CuAAC

Yield: 57%. White solid. Mp 180–181 °C. $[\alpha]_D^{25}$ −87 (*c* 0.38 $CHCl_3$). IR (KBr) ν 1775, 1765 (C=O), 1601 (N=N), 1560, 1483 (C=C). ^{1}H NMR ($CDCl_3$) δ/ppm 7.83 (s, 1H, triazole–H), 6.64 (s, 1H, 5-H), 6.59 (s, 1H, 8-H), 6.32 (s, 2H, 2′,6′-H), 6.03 (d, 1H, J = 1.1 Hz, OCH_2O), 6.03 (d, 1H, J = 1.1 Hz, OCH_2O), 4.83 (d, 1H, J = 4.9 Hz), 4.45 (t, 1H, J = 7.2 Hz), 4.09 (m, 1H), 4.00 (q, 2H, J = 7.1 Hz, NCH_2), 3.83 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 3.50 (q, 2H, J = 7.1 Hz, NCH_2), 3.11–3.20 (m, 2H), 1.38 (t, 3H, J = 7.1 Hz, CH_3), 1.22 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ 19.1, 36.4, 37.0, 43.6, 44.9, 45.0, 56.3, 60.7, 67.6, 102.1, 108.2, 108.8, 110.6, 124.1, 126.0, 133.3, 134.2, 137.5, 143.3, 148.2, 149.6, 152.8, 159.8, 172.9. HR-ESI-MS calculated for $C_{29}H_{33}N_4O_8$ $[M + H]^+$ 565.2293, found 565.2279.

4.4.12. Synthesis of 4β-[5-(4-methylphenylamino-carbonyl)-1,2,3-triazole]-podophyllotoxin (**3j$_2$**) by the traditional 1,3-dipolar cycloaddition

Yield: 15%. White solid. Mp 184–185 °C. $[\alpha]_D^{25}$ −79 (*c* 0.40 $CHCl_3$). IR (KBr) ν 3375, 1770, 1762 (C=O), 1608 (N=N), 1560, 1520 (C=C), 1481. ^{1}H NMR δ/ppm 8.87 (s, 1H, N–H), 7.95 (s, 1H, triazole–H), 7.59 (d, 2H, J = 7.8 Hz), 6.93 (d, 2H, J = 7.8 Hz), 6.68 (s, 1H), 6. 40 (s, 2H), 6.29 (s, 1H), 6.02 (d, 1H, J = 1.4 Hz, OCH_2O), 5.98 (d, 1H, J = 1.4 Hz, OCH_2O), 5.89 (d, 1H, J = 8.1 Hz), 4.59 (m, 2H), 4.10 (m, 1H), 3.85 (s, 3H), 3.81 (s, 6H), 3.50–3.65 (m, 2H), 2,19 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 21.0, 31.0, 41.2, 43.5, 56.4, 59.2, 60.8, 67.2, 102.1, 108.2, 108.5, 110.5, 120.0, 123.9, 126.6, 129.8, 133.6, 134.0, 134.6, 137.6, 143.5, 148.1, 149.6, 152.8, 157.4, 172.6. HR-ESI-MS calculated for $C_{32}H_{31}N_4O_8$ $[M + H]^+$ 599.2136, found 599.2123.

4.4.13. Synthesis of 4β-(4-ethoxy-carbonyl-1,2,3-triazole)-podophyllotoxin (**3k$_1$**) by CuAAC

Yield: 73%. White solid. Mp 177–178 °C. $[\alpha]_D^{25}$ −86 (*c* 0.33 $CHCl_3$). IR (KBr) ν 1772, 1760 (C=O), 1608 (N=N), 1561, 1466 (C=C). ^{1}H NMR δ/ppm 8.24 (s, 1H, triazole–H), 6.75 (s, 1H), 6.54–6.55 (d, 1H, J = 3.8 Hz), 6.44 (s, 2H), 6.99 (d, 1H, J = 1.3 Hz, OCH_2O), 5.80 (d, 1H, J = 1.3 Hz, OCH_2O), 4.71–4.77 (d, 1H, J = 2.0 Hz), 4.69 (m, 1H), 4.25–4.33 (m, 2H, OCH_2), 4.19–4.24 (m, 1H), 3.85 (s, 6H, OCH_3), 3.83 (s, 3H, OCH_3), 3.63–3.79 (m, 2H), 1.34 (t, 3H, J = 7.3 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ14.3, 24.2, 37.0, 41.3, 43.6, 56.4, 59.2, 60.4, 67.3, 102.1, 108.7, 109.7, 110.7, 123.9, 127.9, 133.4, 133.5, 137.7, 140.4, 148.3, 149.8, 152.6, 160.4, 172.7. HR-ESI-MS calculated for $C_{27}H_{27}N_3NaO_9$ $[M + Na]^+$ 560.1640, found 560.1619.

4.4.14. Synthesis of 4β-(5-ethoxy-carbonyl-1,2,3-triazole)-podophyllotoxin (**3k$_2$**) by the traditional 1,3-dipolar cycloaddition

Yield: 17%. White solid. Mp 135–136 °C. $[\alpha]_D^{25}$ −82 (*c* 0.37 $CHCl_3$). IR (KBr) ν 1769, 1761 (C=O), 1601 (N=N), 1555, 1484 (C=C), 1456. ^{1}H NMR δ/ppm 7.91 (s, 1H, triazole–H), 6.69 (s, 1H), 6.38 (s, 2H), 6.44 (s, 2H), 6.25 (s, 1H), 6.00 (d, 1H, J = 1.2 Hz, OCH_2O), 5.97 (d, 1H, J = 1.2 Hz, OCH_2O), 5.92 (d, 1H, J = 4.8 Hz), 4.57 (d, 1H, J = 2.5 Hz,), 4.43–4.54 (m, 2H, OCH_2), 4.36–4.43 (m, 2H, OCH_2), 3.83 (s, 3H, OCH_3), 3.80 (s, 6H, OCH_3), 3.50–3.67 (m, 2H), 1.45 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR ($CDCl_3$) δ 14.4, 24.1, 36.7, 40.5, 42.3, 56.8, 59.3, 60.4, 67.2, 101.4, 108.6, 109.8, 110.8, 124.0, 127.5, 131.6, 133.4, 137.7, 140.5, 147.9, 150.2, 152.2, 162.4, 173.9; HR-ESI-MS calculated for $C_{27}H_{27}N_3NaO_9$ $[M + Na]^+$ 560.1640, found 560.1614.

4.5. Cell culture and cytotoxic assay

All the cell lines were supplied by the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. Tumor cells were maintained in RPM11640 medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml) under humidified air with 5% CO_2 at 37 °C. Exponentially growing cells were seeded into 96-well tissue culture-treated plates and precultured for 1 day. The test compounds at various concentrations were added, and the cells were incubated for additional 2 days. The cytotoxic activity was measured by MTT assay [24], and the IC_{50} values were obtained from dose–response curves. VP-16, Podophyllotoxin and ADM were used as the positive control.

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References

[1] I. Jardine, in: J.M. Cassady, J. Douros (Eds.), Anticancer Agents Based on Natural Product Models, Academic Press, New York, 1980, p. 319.
[2] K.H. Lee, Y. Imakura, M. Haruna, S.A. Beers, L.S. Thurston, H.J. Dai, C.H. Chen, S.Y. Liu, Y.C. Cheng, J. Nat. Prod. 52 (1989) 606.
[3] K.H. Lee, S.A. Beers, M. Mori, Z.Q. Wang, Y.H. Kuo, L. Li, S.Y. Liu, J.Y. Chang, F.S. Han, Y.C. Cheng, J. Med. Chem. 33 (1990) 1364.
[4] Z.Q. Wang, Y.H. Kuo, D. Schnur, J.P. Bowen, S.Y. Liu, F.S. Han, J.Y. Chang, Y.C. Cheng, K.H. Lee, J. Med. Chem. 33 (1990) 2660.
[5] T.L. Macdonald, E.K. Lehenert, J.T. Loper, K.C. Chow, W.E. Ross, in: DNA Topoisomerase in Cancer, Oxford University, New York, 1991, p. 119.
[6] T. Terada, K. Fujimoto, M. Nomura, J. Yamashita, K. Wierzba, R. Yamazaki, J. Shibata, Y. Sugimoto, Y. Yamada, T. Kobunai, S. Takeda, Y. Minami, K. Yoshida, H. Yamaguchi, J. Med. Chem. 36 (1993) 1689.
[7] Z. Xiao, Y.D. Xiao, J. Feng, A. Golbraikh, A. Tropsha, K.H. Lee, J. Med. Chem. 45 (2002) 2294.

[8] R. Tawa, M. Takami, Y. Imakura, K.H. Lee, H. Sakuri, Bioorg. Med. Chem. Lett. 7 (1997) 489.
[9] V.P. Krivopalov, O.P. Shkurko, Russ. Chem. Rev. 74 (2005) 339.
[10] E.A. Shafran, V.A. Bakulev, Yu.A. .Rozin, Yu.M. .Shafran, Chem. Heterocycl. Compd. 44 (2008) 1040.
[11] T.R. Chan, R. Hilgraf, K.B. Sharpless, V.V. Fokin, Org. Lett. 6 (2004) 2853.
[12] J.M. Wang, L.J. Li, Chin. J. Org. Chem. 29 (2009) 13.
[13] P.M.E. Gramlich, C.T. Wirges, A. Manetto, T. Carell, Angew. Chem. Int. Ed. 47 (2008) 8350.
[14] C.D. Hein, X.M. Liu, D. Wang, Pharm. Res. 25 (2008) 2216.
[15] W.H. Binder, R. Sachsenhofer, Macromol. Rapid Commun. 29 (2008) 952.
[16] L. Zhang, X. Chen, P. Xue, H.H.Y. Sun, I.D. Williams, K.B. Sharpless, V.V. Fokin, G. Jia, J. Am. Chem. Soc. 127 (2005) 15998.
[17] B.C. Boren, S. Narayan, L.K. Rasmussen, V.V. Fokin, J. Am. Chem. Soc. 130 (2008) 8923.
[18] B.A. Bhat, P.B. Reddy, S.K. Agrawal, A.K. Saxena, H.M.S. Kumar, G.N. Qazi, Eur. J. Med. Chem. 43 (2008) 2067.
[19] D. Kumar, V.B. Reddy, R.S. Varma, Tetrahedron Lett. 50 (2009) 2065.
[20] S. Zuo, H. Chen, Y. Lu, B. Cao, D. Liu, Acta Crystallogr. Sect. E: Struct. Rep. Online E65 (2009) o3257.
[21] C.N. Cao, Y.Y. Chen, Y.G. Wang, Y.Z. Chen, Chin. Chem. Lett. 10 (1999) 1003.
[22] J.D. Loike, C.F. Brewer, H. Sternlicht, W.J. Gensler, S.B. Horwitz, Cancer Res. 38 (1978) 2688.
[23] Related research will be reported soon.
[24] J.M. Jin, Y.J. Zhang, C.R. Yang, J. Nat. Prod. 67 (2004) 5.