Published in final edited form as:
Med Chem Res. 2014 November ; 23(11): 4926–4931. doi:10.1007/s00044-014-1042-9.

Synthesis of novel spin-labeled podophyllotoxin derivatives as potential antineoplastic agents: Part XXV

Liu Yang,
Environmental and Municipal Engineering School, Lanzhou, Jiaotong University, Lanzhou 730000, People's Republic of China

Xiang Nan,
School of Pharmacy, Lanzhou University, Lanzhou 730000, People's Republic of China

Wen-Qun Li,
School of Pharmacy, Lanzhou University, Lanzhou 730000, People's Republic of China

Mei-Juan Wang,
School of Pharmacy, Lanzhou University, Lanzhou 730000, People's Republic of China

Xiao-Bo Zhao,
School of Pharmacy, Lanzhou University, Lanzhou 730000, People's Republic of China

Ying-Qian Liu,
School of Pharmacy, Lanzhou University, Lanzhou 730000, People's Republic of China

Zhi-Jun Zhang, and
School of Pharmacy, Lanzhou University, Lanzhou 730000, People's Republic of China

Kuo-Hsiung Lee
Natural Products Research Laboratories, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599, USA

Ying-Qian Liu: yqliu@lzu.edu.cn; Kuo-Hsiung Lee: khlee@email.unc.edu

Abstract

A series of novel spin-labeled 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxin derivatives (**17a**–**h**) were firstly designed and synthesized with significant regioselectivity by employing Cu(I) catalyzed click approach, and evaluated for cytotoxicity against four human tumor cell lines (A-549, DU145, KB, and KBvin). Among them, compound **17h** displayed the highest cytotoxic activity against the tumor cell lines tested. Significantly, compound **17h** showed superior cytotoxic activity compared with etoposide (IC_{50} 6.30 to>10 μM), a clinically available anticancer drug. Significant activity toward the drug resistant KBvin cell line revealed promising future for compound **17h** as a new generation of epipodophyllotoxin-derived antitumor clinical trial candidate.

Correspondence to: Ying-Qian Liu, yqliu@lzu.edu.cn; Kuo-Hsiung Lee, khlee@email.unc.edu.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

Keywords

Podophyllotoxin; Click chemistry; Cytotoxic activity; Spin-labeled

Introduction

Podophyllotoxin (**1**, PPT), a naturally occurring aryltetralin lignan, has been used as a lead compound in the development of new potent anticancer drugs (Garcia and Azambuja, 2004). Its powerful cytotoxic properties have been attributed by its binding to tubulin during mitosis and thus inhibiting microtubule assembly (Jardine, 1980). The extensive chemical modifications of **1** led to two anticancer drugs, etoposide (**2**) and teniposide (**3**), as well as etopophos (**4**), a water-soluble prodrug of **2** (Stahelin and von Wartburg, 1989; Canetta *et al.*, 1982; Joel 1996). These compounds are currently used as drugs, alone or in association, in clinical cancer chemotherapy against small cell lung cancer, acute leukemia, lymphoma, testicular carcinoma, and Kaposi's sarcoma. Notably, these two structural modifications also led to a change in the mechanism of action. While PPT acts as antimicrotubule agent, **2** and **3** function as topoisomerase II (topo II) inhibitors (Canela *et al.*, 2000; Imbert, 1998). However, the therapeutic use of **2** and **3** is often hindered by problems such as acquired drug-resistance and poor water solubility (Liu *et al.*, 2008). To get more potent analogs and to overcome drug-resistance, recently some nonsugar substituted analogs, particularly *N*-linked congeners, exhibit superior pharmacological properties to etoposide, and consequently, several newer-generation clinical candidates, including NPF (**5**), GL-331 (**6**), and TOP-53 (**7**), have emerged through C-4 modification as alternatives to overcome the drawbacks of etoposide (Cragg and Newman, 2004; Lee, 1999; Liu *et al.*, 1989). These successful examples imply that C-4 substitution plays an important role in the activity profiles of **1**-analogs, and that optimization of this compound class through rational C-4 modification is quite feasible. Both a composite pharmacophore model and comparative molecular field analysis also further demonstrated that the C-4 molecular area could accommodate considerable structural diversity (Cho *et al.*, 1996).

Recently, the applications of click chemistry are increasingly interest in all aspects of drug discovery, ranging from initial lead identification through combinatorial chemistry and target-templated in situ chemistry, to proteonmics and DNA research, using bioconjugation reaction. The copper(I)-catalyzed 1,2,3-trizole formation from azides and terminal acetylenes is a particularly powerful linking reaction, in addition to be passive linkers, 1,2,3-triazole ring is a widespread functional group in drugs (Kolb and Sharpless, 2003). Accordingly, it is intriguing to attach 1,2,3-triazoles to podophyllotoxin parent nucleus and has generated various potent aniline, phenol, thiophenol, and carbohydrate-based 1,2,3-triazole derivatives, some of which exhibited significant antitumor activity (Bhat *et al.*, 2008; Reddy *et al.*, 2008a, b; Chen *et al.*, 2011, 2012). Additionally, a recent docking studies revealed that 1,2,3-triazole derivatives with various substituents in triazolemoiety showed better binding ability to topoisomerase II enzyme than etoposide (Reddy *et al.*, 2011). From this standpoint, logic-based design utilizing click chemistry could be advantageous.

In our previous studies, we have introduced a stable nitroxyl radical into different positions in the PPT skeleton and proved that the resulting analogs can exhibit significant antitumor activity against several mouse transplantable tumors with remarkably decreased toxicity (Jin *et al.*, 2006; Liu and Tian, 2005; Tian *et al.*, 1997, 2002). Especially, GP-11 (**8**) is a typical example which has promise to be a new antitumor drug, GP-11 has been found which could increased the mitotic index and resulted in G2/M phase, and to a lesser extent, S arrest (Wang *et al.*, 1993).

Inspired by the growing impact of click chemistry on drug discovery as well as our previous studies, we introduced the nitroxyl radical moiety into the molecule of podophyllotoxin at its C-4 via 1,2,3-triazol spacer as a part of our drug discovery program. Herein, a series of novel spin-labeled 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives (**17a**–**h**) were firstly designed, synthesized, and evaluated for their in vitro cytotoxic activity against four tumor cell lines (A-549, DU145, KB, and KBvin) (Fig. 1).

Results and discussion

Chemistry

As illustrated in Scheme 1, the starting materials, *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl-oxycarbonyl) amino acids **14a**–**h**, for the preparation of the target compounds **17a–h** were synthesized according to our previous procedure (Zhang *et al.*, 2010; Liu *et al.*, 2012; Hankovszky *et al.*, 1979). Briefly, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl **10** was prepared by catalytic oxidation of 4-hydroxy-2,2,6,6-tetramethylpiperidine **9** with sodium tungstate-hydrogen peroxide-EDTA in yield 85 %. Following, the reaction of compound **10** with *N*,*N*′-carbonyldiimidazol proceeds to give *N*-(1-oxyl-2,2,6,6-tetramethyl piperidinyloxycarbonyl)-imidazole **11** by the modified method. Compound **11**, without further purification, was reacted with *p*-toluenesulfonic acid monohydrate to give its higher reactive tosylate **12**. Compound **12** is instantaneously converted into alkoxycarbonyl azide **13** when dissolved in an aqueous solution of sodium azide. Compounds **14a**–**h** were obtained in good yield by reaction of **13** with free amino acids in presence of MgO. As illustrated in Scheme 2, 4β-Azido podophyllotoxin **15** was obtained in excellent yield by treating podophyllotoxin **1** with hydrazoic acid (HN_3) in the presence of $BF_3{\cdot}OEt_2$ (Tian *et al.*, 1997). Compound **15** was allowed to react with propargyl alcohol in the presence of $CuSO_4{\cdot}5H_2O$, sodium ascorbate in t-butyl alcohol, and water (1:2) at room temperature to selectively get 4β-[4-(4-methylol)-1,2,3-triazol-1-yl] podophyllotoxin **16**. Compound **16** was then condensed with the appropriate nitroxide free radical **14a**–**h** in the presence of 1,3-diisopropylcarbodiimide (DIPC), 4-dimethylaminopyridine (DMAP) to provide the target compounds **17a**–**h** in moderate yields. Synthesized target compounds **17a**–**h** were characterized by melting point, ESR, IR, and HRMS spectral analyses.

Biology

Target compounds **17a**–**h** were evaluated for in vitro cytotoxic activity against four different tumor cell lines, KB (nasopharyngeal), A-549 (lung), DU-145 (prostate), and KBvin (an MDR KB subline), using a sulforhodamine B colorimetric assay with triplicate experiments (Skehan *et al.*, 1990). Etoposide was used as reference compound. The screening results are

shown in Table 1. Remarkably, compound **17h** exhibited significant inhibitory activities against A549, DU-145, and KB with the IC_{50} value in the range of 5.49–5.6.30 μg/mL. Also, compound **17h** displayed more potent cytotoxic activity against KBvin than etoposide (IC_{50}>10 μg/mL). Surprisingly, all other compounds only displayed weak cytotoxicity against the four human tumor cell lines. However, the reason is not clear to our current knowledge and has to be subject of further investigations.

Experimental

General

Melting points were determined in Kofler apparatus and were uncorrected. IR spectra were measured on a Nicolet 5DX-FT-IR spectrometer on neat samples placed between KBr plates. Mass spectra were recorded on a Bruker Daltonics APEXII49e spectrometer with ESI source as ionization. The electron spin resonance (ESR) spectra were obtained from 10^{-3} M ethanol solution using a Bruker ER200D-SRC spectrometer. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AM-400 spectrometer using TMS as reference (Bruker Company, USA). The *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-amino acids **14a**–**h** for the experiments were prepared by following a modified previous procedure as shown in Scheme 1 (Zhang *et al.*, 2010; Liu *et al.*, 2012; Hankovszky *et al.*, 1979).

Synthesis of 4β-azido podophyllotoxin (15)—To a solution of Podophyllotoxin **1** (10 g, 24 mmol) in appropriate, anhydrous dichloromethane was added hydrazoic acid (25 mL, C = 1.04 M, benzene) with stirring. $BF_3{\cdot}Et_2O$ (4.5 mL) was slowly drop into the mixture so as to keep temperature below −10 °C. Through TLC analysis, when most of the starting material had been consumed, pyridine (4.5 mL) and distilled water (80 mL) were added. The separated organic phase was washed successively by distilled water, 5 % hydrochloric acid, distilled water, 2 % sodium bicarbonate, distilled water, then dried over anhydrous sodium sulfate, and evaporated on rotary evaporator. The residue was recrystallized in acetone–methanol to afford a white solid. Yield 75 %. mp: 110–112 °C; IR(KBr) ν/cm^{-1}: 3430 (NH_2); 1 776 (Lactone); 1588, 1550, 1484 (aromatic C=C); 935 (OCH_2O), ^{1}H NMR($CDCl_3$) δ: 6.84 (s, 1H, H-5), 6.47 (s, 1H, H-8), 6.36 (s, 2H, H-2′, 6′), 5.97 (s, 2H, OCH_2O), 4.58 (d, J = 5.1 Hz, 1H, H-1), 4.32 (m, 2H, H-11), 4.25 (d, J = 4.0 Hz, H-4), 3.80 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′,5′-OCH_3), 3.32 (q, 1H, H-2), 2.92–2.63 (m, 1H, H-3), 1.83 (d, 2H, 4-NH_2).

Synthesis of 4β-[4-(4-methylol)-1,2,3-triazol-1-yl] podophyllotoxin (16)—A mixture of propargyl alcohol (30.6 mg, 0.55 mmol), $CuSO_4{\cdot}5H_2O$ (138 mg, 0.55 mmol), and sodium ascorbate (181 mg, 0.92 mmol) in *t*-butyl alcohol and water (1:2, 25 mL) was added 4β-azido podophyllotoxin **15** (200 mg, 0.46 mmol). The reaction mixture was stirred for 6 h at room temperature. After completion, the reaction mixture was diluted with 50 mL of water and extracted with chloroform (3 × 30 mL). The combined extracts were washed with saturated salt water, dried over anhydrous sodium sulfate, and evaporated on rotary evaporator. The residue was chromatographed on a silica gel column and eluted with ethyl acetate–petroleum ether to afford a white powder 80 mg, yield 35 %. mp: 160–162 °C; IR (KBr) cm^{-1}: 3384, 2926, 1763, 1611, 1516, 1483, 1232, 1109, 1033, 997, 928; ^{1}H NMR

($CDCl_3$) *δ*: 7.25 (s, 1H), 6.66 (s, 1H), 6.62 (s, 1H), 6.32 (s, 2H), 6.06 (d, *J* = 4.89 Hz, 1H), 6.03 (s, 1H), 6.01 (s, 1H), 4.76 (d, *J* = 4.77 Hz, 1H), 4.42–4.39 (t, J = 6.79 Hz,1H), 3.77 (s, 6H), 3.74 (s, 2H), 3.28–3.17 (m, 2H), 3.09–3.05 (dd, *J* = 8.57 Hz, 4.88 Hz, 1H); ESI–MS: 482 [M +1].

General procedure of synthesis of 17a–h—Compound **16** (0.4 mmol) and appropriate nitroxide free radical **14a**–**h** (0.48 mmol) were dissolved in appropriate anhydrous dichloromethane at room temperature, and to this solution, 1,3-diisopropylcarbodiimide (DIPC 0.19 mL, 1.2 mmol) and 4-dimethylaminopyridine (DMAP 97.6 mg, 0.8 mmol) were added at 0 °C and then warm to room temperature and left overnight. The product was washed with 0.1 M HCl (3 × 20 mL) and then washed with saturated salt water; the organic layer was dried over anhydrous sodium sulfate and evaporated on rotary evaporator. The residue was chromatographed on a silica gel column and eluted with ethyl acetate–petroleum ether to afford **17a**–**h**.

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-glycinemethyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17a): Yield: 55 %; red powder; mp: 126–129 °C; IR (cm^{-1}) 3370, 2973, 2939, 1778, 1713, 1420, 1361 (N–O·), 934; ESR: An = 16.04G, g_0 = 2.0061; HRMS (ESI) 773. 2866 for $[M + Na]^+$ (calcd 773.2879 for $C_{37}H_{44}O_{12}N_5$).

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-alaninemethyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17b): Yield: 62 %; red powder; mp: 128–131 °C; IR (cm^{-1}) 3343, 2975, 2939, 1779, 1711, 1422, 1360 (N–O·), 934; ESR: An = 15.90G, g_0 = 2.0060; HRMS (ESI) 787. 3063 for $[M + Na]^+$ (calcd 787.3035 for $C_{38}H_{46}O_{12}N_5$).

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-prolinemethyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17c): Yield: 60 %; red powder; mp: 142–146 °C; IR (cm^{-1}) 3343 2973, 2939, 1780, 1702, 1419, 1365 (N–O·), 929; ESR: An = 16.12G, g_0 = 2.0060; HRMS (ESI) 813. 3173 for $[M + Na]^+$ (calcd 813.3192 for $C_{40}H_{48}O_{12}N_5$).

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-phenylalaninemethyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17d): Yield: 56 %; red powder; mp: 127–131 °C; IR (cm^{-1}) 3343, 2972, 2936, 1779, 1715, 1420, 1388 (N–O·), 933; ESR: An = 16.12G, g_0 = 2.0061; HRMS (ESI) 863. 3356 for $[M + Na]^+$ (calcd 863.3348 for $C_{44}H_{50}O_{12}N_5$).

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-methioninemethyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17e): Yield: 55 %; red powder; mp: 115–119 °C; IR (cm^{-1}) 3334, 2972, 2936, 1779, 1712, 1421, 1360 (N–O·), 935; ESR: An = 16.01G, g_0 = 2.0060; HRMS (ESI) 847. 3081 for $[M + Na]^+$ (calcd 847.3069 for $C_{40}H_{50}O_{12}SN_5$).

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-isoleucinemethyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17f): Yield: 55 %; red powder; mp: 121–126 °C; IR (cm^{-1}) 3364, 2969, 2936, 1780, 1714, 1420, 1360 (N–O·), 934; ESR: An = 16.02G, g_0 = 2.0060; HRMS (ESI) 829. 3525 for $[M + Na]^+$ (calcd 829.3035 for $C_{41}H_{52}O_{12}N_5$).

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-tryptophanmetheyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17g): Yield: 39 %; red powder; mp: 148–154 °C; IR (cm^{-1}) 3354, 2971, 2936, 1777, 1714, 1507, 1422, 1361 (N–O·), 932; ESR: An = 16.28G, g_0 = 2.0061; HRMS (ESI) 902.3442 for $[M + Na]^+$ (calcd 902.3457 for $C_{46}H_{51}O_{12}N_6$).

4β-[{4-[N-(1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl)-L-valine methyl]}-1,2,3-triazol-1-yl] podophyllotoxin (17h): Yield: 45 %; red powder; mp: 122–125 °C; IR (cm^{-1}) 3352, 2969, 2935, 1780, 1713, 1420, 1361 (N–O·), 934; ESR: An = 16.04G, g_0 = 2.0061; HRMS (ESI) 793. 3543 for $[M + H]^+$ (calcd 793.3529 for $C_{40}H_{50}O_{12}N_5$).

Conclusion

In conclusion, a series of novel 4β-[(*N*-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives were synthesized by employing click chemistry protocol, and their structural information was extensively characterized by using IR, ESR, and HRMS. Also, their cytotoxic activity was evaluated against four tumor cell lines (A549, DU-145, KB, and KBvin) in vitro by using an SRB-assay. Especially, compound **17h** displayed more potent cytotoxic activity against KBvin than etoposide. The cytotoxic results indicated that compound **17h** may be a good lead for further structural modification.

Acknowledgments

This work was supported financially by the Young Scholars Science Foundation of Lanzhou Jiaotong University (2011011); the National Natural Science Foundation of China (30800720, 31371975); the Fundamental Research Funds for the Central Universities (lzujbky-2013-69); and Partial support was also supplied by NIH grant CA177584 from the National Cancer Institute awarded to K.H. Lee. We thank for the support of Taiwan Department of Health Cancer Research Center of Excellence (DOH-100-TD-C-111-005).

References

Bhat BA, Reddy PB, Agrawal SK, Saxena AK, Sampath KHM, Qazi GN. Studies on novel 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxins as potential anticancer agents. Eur J Med Chem. 2008; 43:2067–2072. [PubMed: 17988764]

Canela C, Moraesb RM, Dayana FE, Ferreira D. Podophyllotoxin. Phytochemistry. 2000; 54(115–120):12.

Canetta R, Hilgard P, Florentine S, Bedogni P, Lenaz L. Current development of podophyllotoxins. Cancer Chemother Pharmacol. 1982; 7:93–98. [PubMed: 7044596]

Chen H, Zuo S, Wang XC, Tang XW, Zhao M, Lu YL, Chen LT, Liu J, Liu YF, Liu DL, Zhang S, Li T. Synthesis of 4β-triazole-podophyllotoxin derivatives by azide alkyne cycloaddition and biological evaluation as potential antitumor agents. Eur J Med Chem. 2011; 46:4709–4714. [PubMed: 21821321]

Chen J, Ma L, Zhang R, Tang J, Lai H, Wang J, Wang G, Xu Q, Chen T, Peng F, Qiu J, Liang X, Cao D, Ran Y, Peng A, Wei Y, Chen L. Semi-synthesis and biological evaluation of 1,2,3-triazole-based podophyllotoxin congeners as potent antitumor agents inducing apoptosis in HepG2 cells. Arch Pharm Chem Life Sci. 2012; 345:945–956.

Cho SJ, Tropsha A, Suffness M, Cheng YC, Lee KH. Three-dimensional quantitative structure-activity relationship study of 4′-*O*-demethyl-epipodophyllotoxin analogs using the modified CoMFA/q2-GRS approach. J Med Chem. 1996; 39:1383–1395. [PubMed: 8691468]

Cragg GM, Newman DJ. A Tale of two tumor targets: topoisomerase I and tubulin. The Wall and Wani contribution to cancer chemotherapy. J Nat Prod. 2004; 67:232–244. [PubMed: 14987065]

Garcia ES, Azambuja P. Podoplhyllotoxin: distribution, sources, applications and new cytotoxic derivatives. Toxicon. 2004; 44:431–459. [PubMed: 15302525]

Hankovszky HO, Hideg K, Lex L. Synthesis. 1979; 1:530–531.

Imbert T. Discovery of podophyllotoxins. Biochimie. 1998; 84:207–222. [PubMed: 9615861]

Jardine, I. Anticancer agent based on natural product models. Cassady, JM.; Douros, J., editors. Academic Press; New York: 1980. p. 319-351.

Jin Y, Chen SW, Tian X. Synthesis and biological evaluation of new spin-labeled derivatives of podophyllotoxin. Bioorg Med Chem. 2006; 14:3062–3068. [PubMed: 16406792]

Joel S. The clinical pharmacology of etoposide. An update. Cancer Treatment Rev. 1996; 22:179–221.

Kolb HC, Sharpless KB. The growing impact of click chemistry on drug discovery. Drug Discov Today. 2003; 8:1128–1137. [PubMed: 14678739]

Lee KH. Novel antitumor agents from higher plants. Med Res Rev. 1999; 19:569–596. [PubMed: 10557371]

Liu YQ, Tian X. Synthesis of novel spin-labeled derivatives of podophyllotoxin as potential antineoplastic agents. Synth Commun. 2005; 35:2749–2758.

Liu SY, Hwang BD, Haruna M, Imakura Y, Lee KH, Cheng YC. Podophyllotoxin analogs: effects on DNA topoisomerase II, tubulin polymerization, human tumor KB cells, and their VP-16-resistant variants. Mol Pharmacol. 1989; 36:78–82. [PubMed: 2546053]

Liu YQ, Liu YQ, Tian X, Yang L. Synthesis of novel derivatives of bispodophyllotoxin as potential antineoplastic agents—part XVI. Nat Prod Res. 2008; 22:285–291. [PubMed: 18322843]

Liu YQ, Ohkoshi E, Li LH, Yang L, Lee KH. Design, synthesis and cytotoxic activity of novel spin-labeled rotenone derivatives. Bioorg Med Chem Lett. 2012; 22:920–923. [PubMed: 22204911]

Reddy PB, Agrawal SK, Singh S, Bhat BA, Saxena AK, Sampath KHM, Qazi GN. Synthesis and biological evaluation of 4β-[(4-substituted)-1,2,3-triazol-1-yl]podophyllotoxins as potential anticancer agents. Chem Biodiver. 2008a; 5:1792–1802.

Reddy PB, Paul DV, Agrawal SK, Saxena AK, Sampath KHM, Qazi GN. Design, synthesis, and biological testing of 4β-[(4-substituted)-1,2,3–triazol-1-yl] podophyllotoxin analogues as antitumor agents. Arch Pharm Chem Life Sci. 2008b; 341:126–131.

Reddy DM, Srinivas J, Chashoo G, Saxena AK, Sampath KHM. 4β-[(4-Alkyl)-1,2,3-triazol-1-yl]podophyllotoxins as anti-cancer compounds: design, synthesis and biological evaluation. Eur J Med Chem. 2011; 46:1983–1991. [PubMed: 21477899]

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990; 82:1107–1112. [PubMed: 2359136]

Stahelin H, von Wartburg A. From podophyllotoxin glucoside to etoposide. Prog Drug Res. 1989; 33:169–267. [PubMed: 2687938]

Tian X, Wang YG, Yang MG, Chen YZ. Synthesis and antitumor activity of spin labeled derivatives of podophyllotoxin. Life Sci. 1997; 60:511–517. [PubMed: 9042385]

Tian X, Zhang FM, Li WG. Antitumor and antioxidant activity of spin labeled derivatives of podophyllotoxin (GP-1) and congeners. Life Sci. 2002; 70:2433–2443. [PubMed: 12150207]

Wang JZ, Tian X, Tsumura H, Shimura K, Ito H. Antitumor activity of a new low immunosuppressive derivative of podophyllotoxin (GP-11)and its mechanisms. Anti-Cancer Drug Des. 1993; 8:193–205.

Zhang ZW, Zhang JQ, Hui L, Chen SW, Tian X. First synthesis and biological evaluation of novel spin-labeled derivatives of deoxypodophyllotoxin. Europ J Med Chem. 2010; 45:1673–1677.

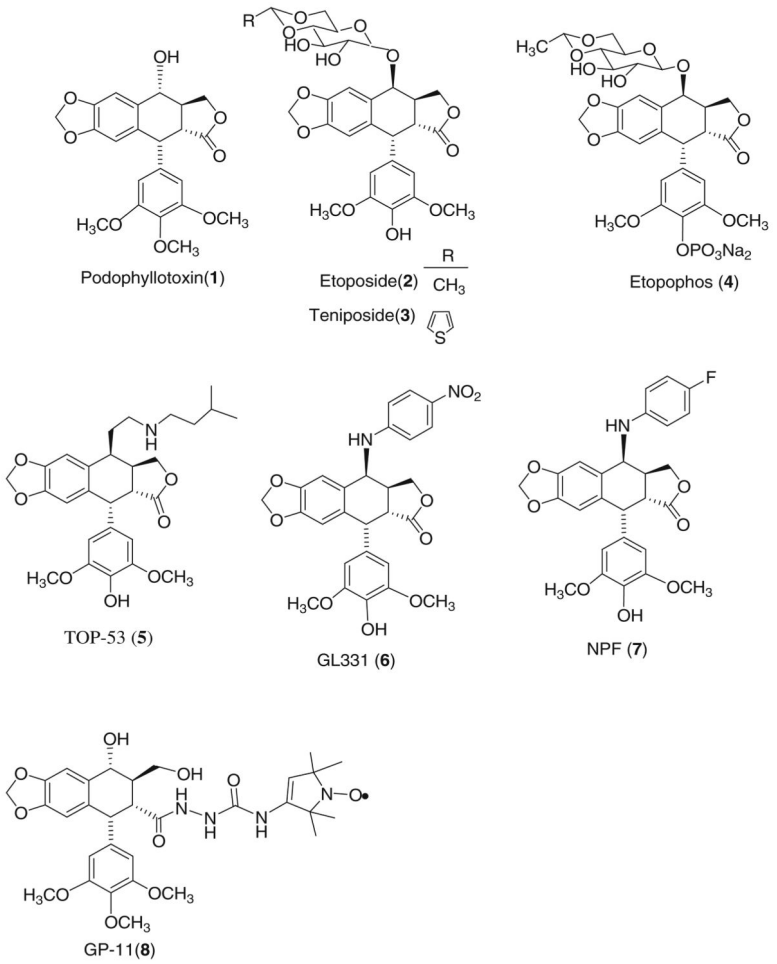
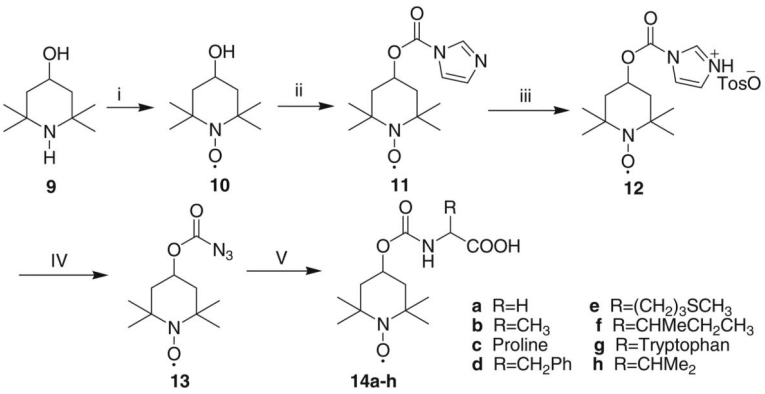


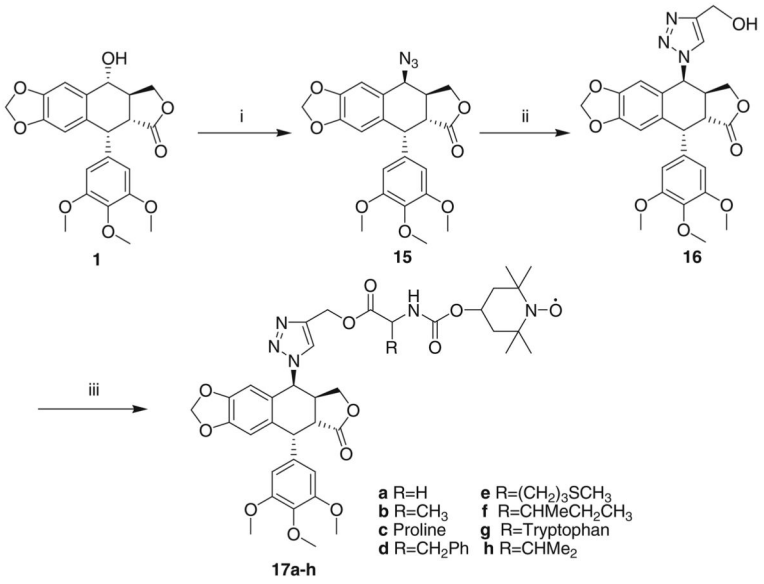
Fig. 1.
Structures of podophyllotoxin derivatives



Scheme 1.

Synthesis of *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyloxycarbonyl) amino acids **14a**–**h**

Reagents and conditions: (i) Na_2WO_4/H_2O_2/EDTA; (ii) N,N′-carbonyldimidazole/THF; (iii) p-toluenesulfonic acid monohydrate; (iv) NaN_3/water, stir; (v) amino acids/MgO, stir, 24 h.



Scheme 2.

Synthesis of target compounds **3a–f**

Reagents and conditions: (i) $HN_3/BF_3{\cdot}Et_2O$, CH_2Cl_2; (ii) Propargyl alcohol, $CuSO_4$,Sodium ascorbate, H_2O: t-BuOH=2:1; (iii) DIPC/DMAP, dry CH_2Cl_2

Table 1

In vitro cytotoxic assay against four tumor cell lines

Entry	IC_{50} (μg/mL)			
	A549	**DU-145**	**KB**	**KBvin**
17a	>10	>10	>10	>10
17b	>10	>10	>10	>10
17c	>10	>10	>10	>10
17d	>10	>10	>10	>10
17e	>10	>10	>10	>10
17f	>10	>10	>10	>10
17g	>10	>10	>10	>10
17h	5.91 ± 0.53	5.49 ± 0.42	5.71 ± 0.11	6.30 ± 0.41
Etoposide	1.51 ± 0.25	1.19 ± 0.12	2.28 ± 0.19	>10