Bioorganic & Medicinal Chemistry Letters 20 (2010) 983–986

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel semisynthetic spin-labeled derivatives of podophyllotoxin with cytotoxic and antioxidative activity

Jia-Qiang Zhang [a], Zhi-Wei Zhang [a], Ling Hui [c], Shi-Wu Chen [a,b,*], Xuan Tian [a,*]

[a] State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, China
[b] School of Pharmacy, Lanzhou University, Lanzhou 730000, China
[c] Experimental Center of Medicine, Lanzhou General Hospital, Lanzhou Command, Lanzhou 730050, China

ARTICLE INFO

Article history:
Received 27 August 2009
Revised 26 November 2009
Accepted 14 December 2009
Available online 22 December 2009

Keywords:
Podophyllotoxin
Nitroxides
Antitumor
Antioxidants

ABSTRACT

A series of novel spin-labeled podophyllotoxin derivatives were synthesized by reacting the corresponding *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyloxy carbonyl)-amino acids with 4β-amino-4′-demethylepipodophyllotoxin. The synthesized derivatives **12a**–**g** were evaluated for the partition coefficients, cytotoxicities in vitro against three tumor cell lines (A-549, HL-60, and RPMI-8226) and antioxidative activities in tissues of SD rats by the TBA method. The vast majority of target compounds have shown superior or comparable activities against A-549, HL-60, and RPMI-8226 compared to VP-16, and they have shown more significant antioxidative activities and superior water solubility than VP-16.

Podophyllotoxin (**1**), as well as its congeners and derivatives exhibit pronounced biological activity mainly as antineoplastic drugs and as strong antiviral agents.[1] The podophyllotoxin derivatives, such as etoposide (VP-16, **2**), teniposide (VM-26, **3**), and etopophos (etoposide phosphate, **4**) have been used as DNA topoisomerase II inhibitors in chemotherapy for various types of cancer.[2,3] Recently, more complex and diverse analogues have been synthesized either to get more potent compounds or to overcome drug resistance.[4] As the results of previous structure–activity relationship studies, it was also found that the *trans*-lactone, 4β-N-substituted and 4′-demethyl moieties of podophyllotoxin were essential to maintain the antineoplastic activity as topoisomerase II inhibitors.[5] In addition, podophyllic aldehyde and its analogues were also found to be a highly selectivity against the HT-29 colon carcinoma. Additional biological studies indicate that these derivatives induce microtubule depolymerization, arrest cells at the G2/M phase of cell cycle, and are able to induce a delayed apoptosis after 48 h of treatment, characterized by caspase-3 activation.[6] In our previous studies, we have synthesized lots of spin-labeled podophyllotoxin derivatives and found that introduction of a stable nitroxyl radical into the molecular of podophyllotoxin or its analogues could increase antitumor activity and marked decrease in toxicity compared with parent compounds.[7–10] Among them, GP-11 (Fig. 1, **5**) was reported as a low immunosuppressive antitumor agent, which increases the mitotic index and results in G2/M, and to a lesser extent, S arrest.[11] Furthermore, L-amino acids are actively transported into mammalian tissue, have good water solubility, and are often used as carrier vehicles for some drugs.

Based upon the above results, as an important part of our program aimed at the discovery and development of bioactive molecules derived from natural product podophyllotoxin,[7–16] in this Letter, a series of novel spin-labeled podophyllotoxin derivatives (**12a**–**g**) were designed, synthesized, and preliminarily evaluated for their cytotoxicities in vitro against three tumor cells (A-549, HL-60, and RPMI-8226) and antioxidative activity in tissues of SD rats. Also, the octanol–water partition coefficients (log *P*) were measured.

The synthesis of nitroxide free radical **10a**–**g** is outlined in Scheme 1. Briefly, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (**6**) was prepared by catalytic oxidation of 4-hydroxy-2,2,6,6-tetramethylpiperidine with sodium tungstate–hydrogen peroxide–EDTA in yield 85%.[17] Following, the reaction of **6** with *N*,*N*′-carbonyldiimidazole proceeds to give *N*-(1-oxyl-2,2,6,6-tetramethyl piperidinyloxycarbonyl)-imidazole (**7**) by the modified method.[18] Compound **7**, without further purification, was further reacted with *p*-toluenesulfonic acid monohydrate to give its higher reactive tosylate (**8**). Compound **8** is instantaneously converted into alkoxycarbonyl azide (**9**) when dissolved in an aqueous solution of sodium azide. Compounds **10a**–**g** were obtained in good yield by reaction of **9** with free amino acids in presence of MgO.[19]

The synthetic route to the target compounds from **1** was depicted in Scheme 2. The intermediate 4β-NH_2-4′-demethylepipodophyllotoxin (**11**) was prepared stereo-selectively from **1**

* Corresponding authors. Tel./fax: +86 931 8915686.
E-mail addresses: chenshwu@gmail.com (S.-W. Chen), xuant@lzu.edu.cn (X. Tian).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.048

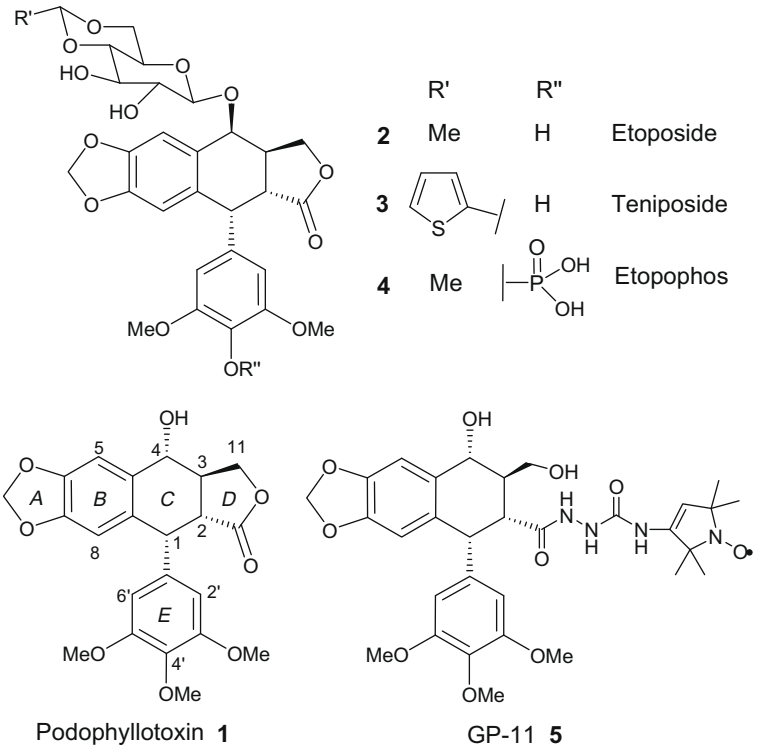
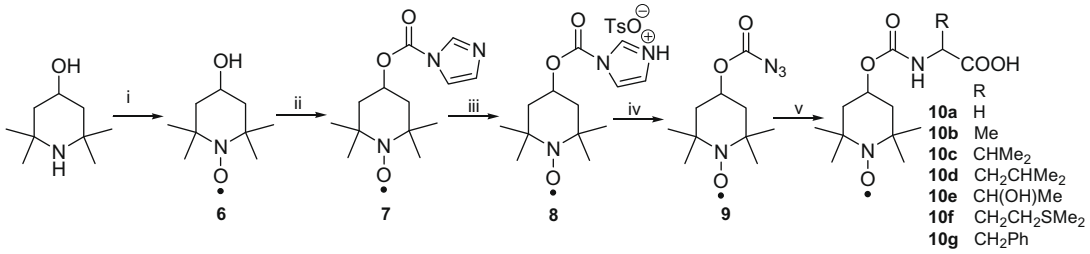
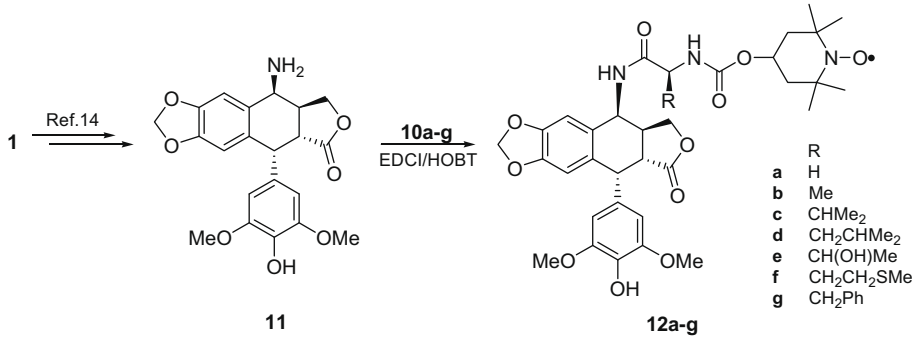


Figure 1. Structures of podophyllotoxin (**1**) and related compounds.



Scheme 1. Reagents: (i) Na_2WO_4/H_2O_2/EDTA; (ii) *N*,*N*′-carbonyl-dimidazole/THF; (iii) TsOH·H_2O; (iv) NaN_3/H_2O; (v) amino acids/MgO.



Scheme 2. Synthesis of compounds **12a**–**g**.

through 4′-demethylation, 4-iodination, azidation, and catalytic hydrogenation according to the previously reported method.[14] Compound **11** was then condensed with the appropriate nitroxide free radical **10a**–**g** in the presence of 1-ethyl-3-(3-dimethyllamino-propyl) carbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole hydrate (HOBT) to provide the target compounds **12a**–**g** in moderate yield.[20]

For the new compounds **12a**–**g**, ESR spectra were recorded to prove the presence of the nitroxide group in the target molecules. The EPR spectra were recorded from freshly prepared 0.3 g/mL chloroform solutions, using a Bruker ER200D-SRC spectrometer at room temperature. The ESR spectra of **12a**–**g** showed triplet with hyperfine coupling constants (a_N) in the range of 15.90–16.28 G due to interaction between an unpaired electron and nitrogen

Table 1
Physical and spectroscopy characters of compounds **12a–g**

Compds	Molecular formula	Mp (°C)	Yield (%)	$[\alpha]_D^{23}$[a]	HRMS (ESI)		IR	ESR[b]		
					Calcd	Found		g	a_N (G)	ΔH (G)
12a	$C_{33}H_{40}O_{11}N_3$	156–158	52	−80	677.2543 ($[M+Na]^+$)	677.2555 ($[M+Na]^+$)	1335(N–O·) 1718(NHCO)	2.0061	16.04	3.02
12b	$C_{34}H_{42}O_{11}N_3$	144–146	47	−59	669.2892 ($[M+H]^+$)	669.2896 ($[M+H]^+$)	1312(N–O·) 1714(NHCO)	2.0060	15.90	2.79
12c	$C_{36}H_{46}O_{11}N_3$	118–120	40	−78	698.3283 ($M+2H]^+$)	698.3285 ($[M+2H]^+$)	1331(N–O·) 1664(NHCO)	2.0060	16.12	3.19
12d	$C_{37}H_{48}O_{11}N_3$	152–154	35	−57	733.3181 ($[M+Na]^+$)	733.3175 ($[M+Na]^+$)	1330(N–O·) 1717(NHCO)	2.0061	16.12	2.80
12e	$C_{35}H_{44}O_{12}N_3$	148–150	37	−78	700.3076 ($[M+2H]^+$)	700.3082 ($[M+2H]^+$)	1333(N–O·) 1713(NHCO)	2.0060	16.01	2.82
12f	$C_{36}H_{46}O_{11}N_3S$	138–140	45	−56	746.3191 ($[M+NH_4]^+$)	746.3200 ($[M+NH_4]^+$)	1330(N–O·) 1717(NHCO)	2.0060	16.02	2.73
12g	$C_{40}H_{46}O_{11}N_3$	142–144	49	−39	745.3205 ($[M+H]^+$)	745.3199 ($[M+H]^+$)	1330(N–O·) 1717(NHCO)	2.0061	16.28	2.72

[a] Concentration is 0.3 g/100 mL in $CHCl_3$.
[b] ESR determined in 10^{-3} M EtOH solution.

Table 2
Biological evaluation of compounds **12a–g** (IC_{50}, μM)

Compounds	Cytotoxic activity			Antioxidative activity			Log P
	A-549[a]	HL-60[a]	RPMI-8226[a]	Liver	Kidney	Heart	
12a	0.21	0.32	0.33	7.47	8.9	7.19	0.11
12b	0.12	0.22	0.26	7.64	7.68	6.39	0.12
12c	0.15	0.24	0.061	8.69	8.88	8.04	0.15
12d	0.19	0.16	0.089	8.85	8.47	ND	0.21
12e	0.21	0.21	0.078	7.12	7.45	8.79	0.10
12f	0.42	0.67	0.48	6.85	7.55	5.89	0.161
12g	0.21	0.21	0.090	6.55	9.63	7.39	0.183
2	0.29	0.42	0.14	43.89	23.77	40.67	0.68

ND = not determined.
[a] Drugs exposure was for 48 h.

nucleus of the aminoxyl radical (g value = 2.006). Further characterization of the final spin-labeled compounds was achieved by infrared spectroscopy, and high revolution mass spectrometry (ESI). Optical rotations and melting points were also determined (Table 1).

Compounds **12a–g** reported here were evaluated in vitro for their cytotoxicities[21] against the following tumor cell lines: A-549 (human lung cancer), HL-60 (human premyelocytic leukemia), and RPMI-8226 (human multiple myeloma). Their antioxidative activities of malondialdehyde (MDA) (liver, heart, and kidney homogenate of SD rats)[22] were also tested. The results obtained are summarized in Table 2. The vast majority of **12a–g** showed comparable or superior inhibition of A-549, HL-60, and RPMI-8226 than VP-16. Remarkably, compounds **12c–e** and **12g** exhibited significant inhibitory activities against RPMI-8226 with the IC_{50} value in the range of 0.06–0.09 μM. Furthermore, all desired compounds showed 3–6-fold more potent antioxidative activities in tissues of liver, heart, and kidney homogenate of SD rats than that of VP-16, with the IC_{50} values in the range of 5.89–9.63 μM. In previous Letter, we found that different L-amino acid as linker markedly affected the activity of podophyllotoxin derivatives, the hydroxyl groups in the amino acid side chains may decrease the antineoplastic activity.[13] However, as seen in Table 2, different alkyl or aryl substituted at α-carbon of L-amino acid did not show obviously different effects on tumor cell and antioxidative activity of this compounds class.

The partition coefficients of **12a–g** were also determined[23] and the results were depicted in Table 2. The log P values of **12a–g** are closer to zero than that of **2**, which means that their water solubility is better than that of VP-16. These results were consistent with our initial design purpose, introduction of amino acid as linkers to podophyllotoxin may increase the aqueous solubility of target compounds and penetrate tumor cell membrane easily.[7,13]

In summary, novel spin-labeling of podophyllotoxin is a promising direction in antitumor chemotherapy, not only because they exhibit superior activities, but also because they can be monitored by ESR in pharmacological experiments. In this work, seven novel spin-labeling amino acid-linked derivatives of podophyllotoxin were designed and synthesized. Compared to VP-16, the vast majority compounds exhibited comparable cytotoxic activity against A-549, HL-60, and RPMI-8226, and more pronounced antioxidative activities in tissues of SD rats and superior water solubility.

Acknowledgment

We thank State Key Laboratory of Applied Organic Chemistry of Lanzhou University for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.12.048.

References and notes

1. Garcia, E. S.; Azambuja, P. *Toxicon* **2004**, *44*, 431.
2. Canela, C.; Moraesb, R. M.; Dayana, F. E.; Ferreira, D. *Phytochemistry* **2000**, *54*, 115.
3. Imbert, T. *Biochimie* **1998**, *84*, 207.
4. Gordaliza, M. *Clin. Transl. Oncol.* **2007**, *9*, 767.

5. Cho, S. J.; Tropsha, A.; Suffness, M.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1996**, *39*, 1383.
6. (a) Gordaliza, M.; Castro, M. A.; Miguel del Corral, J. M.; López-Vázquez, M. L.; García, P. A.; García-Grávalos, M. D.; San Feliciano, A. *Eur. J. Med. Chem.* **2000**, *35*, 691; (b) Castro, M. A.; Miguel del Corral, J. M.; Gordaliza, M.; García, P. A.; Gomez-Zurita, M. A.; García-Grávalos, M. D.; Iglesia-Vicente, J. de la; Gajate, C.; An, F.; Mollinedo, F.; San Feliciano, A. *J. Med. Chem.* **2004**, *47*, 1214.
7. Jin, Y.; Chen, S.-W.; Tian, X. *Bioorg. Med. Chem.* **2006**, *14*, 3062.
8. Liu, Y.-Q.; Tian, X. *Synth. Commun.* **2005**, *35*, 2749.
9. Tian, X.; Zhang, F.-M.; Li, W.-G. *Life Sci.* **2002**, *70*, 2433.
10. Tian, X.; Wang, Y.-G.; Yang, M.-G.; Chen, Y.-Z. *Life Sci.* **1997**, *60*, 511.
11. Wang, J.-Z.; Tian, X.; Tsumura, H.; Shimura, K.; Ito, H. *Anti-Cancer Drug Des.* **1993**, *8*, 193.
12. Chen, S.-W.; Tian, X.; Tu, Y.-Q. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5063.
13. Chen, S.-W.; Xiang, R.; Liu, J.; Tian, X. *Bioorg. Med. Chem.* **2009**, *17*, 3111.
14. Chen, S.-W.; Wang, Y.-H.; Jin, Y.; Tian, X.; Zheng, Y.-T.; Luo, D.-Q.; Tu, Y.-Q. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2091.
15. Chen, S.-W.; Xiang, R.; Tian, X. *Helv. Chim. Acta* **2009**, *92*, 1568.
16. Zhang, F.-M.; Yao, X.-J.; Tian, X.; Tu, Y.-Q. *Molecules* **2006**, *11*, 849.
17. Rozantsev, E. G. *Izv. Akad. Nauk. SSSR Ser. Kim.* **1964**, 2187.
18. Staab, H. A.; Mannschreck, A. *Chem. Ber.* **1962**, *95*, 1284.
19. Hankovszky, H. O.; Hideg, K.; Lex, L. *Synthesis* **1979**, 530.
20. Itoh, Y.; Suzuki, T.; Kouketsu, A.; Suzuki, N.; Maeda, S.; Yoshida, M.; Nakagawa, H.; Miyata, N. *J. Med. Chem.* **2007**, *50*, 5425.
21. Mosmann, T. *J. Immunol. Methods.* **1983**, *65*, 55.
22. Ohkawa, H.; Ohishi, N.; Yaki, K. *Anal. Biochem.* **1979**, *95*, 351.
23. Sosnovsky, G. *Pure. Appl. Chem.* **1990**, *62*, 289.