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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 3062–3068

Synthesis and biological evaluation of new spin-labeled derivatives of podophyllotoxin

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Received 17 October 2005; revised 12 December 2005; accepted 13 December 2005
Available online 9 January 2006

Abstract—In order to find compounds with superior bioactivity and less toxicity, a series of spin-labeled podophyllotoxin derivatives were synthesized and tested for the partition coefficients and cytotoxicity against P-388 and A-549. Furthermore, we also determined antioxidant activities of target molecular in tissues of SD rats by the TBA method. Results revealed that most synthesized compounds showed more significant cytotoxicity against P-388 and A-549 in vitro than VP-16. Among them, **9d** exhibited most potent cytotoxicity against P-388 and A-549 cells (IC_{50} is <0.01 and 0.13 μM, respectively). Also, the antioxidative activities showed that the modified compounds of 4′-demethylepipodophyllotoxin (**9a–d** and **10a–c**) are higher than those of podophyllotoxin series (**8a–d**). The relationship between the cytotoxity and antioxidative activity discussed.

1. Introduction

Etoposide (**1**, VP-16) and teniposide (**2**, VM-26), as semisynthetic derivatives of podophyllotoxin (Fig. 1, **3**), are clinically useful drugs against various cancers, including small cell lung cancer, testicular carcinoma, lymphoma, and Kaposi's sarcoma.[1,2] However, their therapeutic use is often hindered by problems such as acquired drug-resistance and poor water solubility.[3] To obtain better therapeutic agents, etopophos (**4**), NK611 (**5**), as well as GL331 and TOP-53 were developed.[4] Etopophos (**4**), a water-soluble prodrug of **1**, is readily converted in vivo by endogenous phosphatase to the active drug **1** and exhibits pharmacological and pharmacokinetic profiles similar to those of **1**. The bioavailability in vivo was increased from 0.04% to 50% through this prodrug approach.[5] NK611 and GL331 are presently under clinical trial. GL-331 was more potent than **1** as a topo II inhibitor, and more notably, overcame multidrug-resistance in many cancer cells, including **1**-resistant cancer cell lines.[6]

As for mechanism, although **3** is known as an antimicrotubule agent acting at the colchicine binding site on tubulin, **1** and congeners induce a premitotic blockade in the late S stage of the cell cycle because of the inhibition of DNA topoisomerase II (TopII), an enzyme required for the unwinding of DNA during replication. Compound **1** binds to and stabilizes the DNA–protein complex preventing religation of the double-stranded breaks.[7]

Recently, a lot of modifications to podophyllotoxin have been done, podophyllic aldehyde and its analogues were found to show high selectivity against the HT-29 colon carcinoma.[8] 4β-Sulfonamide, (4″-benzamide)-amino, and carbamate derivatives of 4′-demethylepipodophyllotoxin showed strong inhibition to TOP II.[6,9] In addition, 4 or 4′-ester derivatives of **3** have been reported, some results challenging the long-standing structure–activity relationships (SARs), which premises free 4′-phenol group is essential for **1**-related antitumor inhibitions.[10]

The stable nitroxides have a wide range of activities in biology. Some studies have shown that the introduction of nitroxyl moiety can lead to a fast decomposition, higher alkylating, lower carbamoylating activity, better antimelanomic activity, lower general toxicity, and through cell membranes as a transport.[11] Vesselina et al., found that spin-labeled triazenes have a higher

Keywords: Podophyllotoxin; Nitroxides; Antioxidant; Antitumor drugs.
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doi:10.1016/j.bmc.2005.12.025

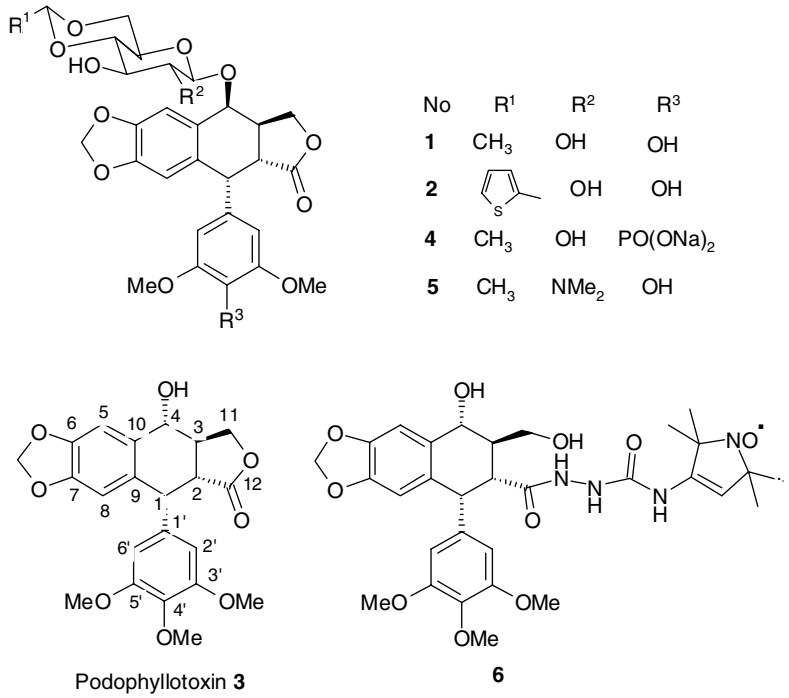


Figure 1. Structure of etoposide, podophyllotoxin, and other analogues.

cytotoxicity to B16 melanoma cells than the non-labeled analogues.[11,12] Stable nitroxyl radicals can be utilized to improve antitumor and antioxidant properties of drugs as well.[13] Recently, it was shown that the nitroxides had a favored effect on the toxicity and anticancer activity of 6-mercaptopurine. This effect might be due in part to antioxidant activity of the nitroxides.[14] Krishna et al. had investigated the relationship between structure and antioxidative activity of nitroxide and pointed out that the six-membered ring nitroxides with the lowest midpoint potential exhibited maximal protection (antioxidant damage).[15]

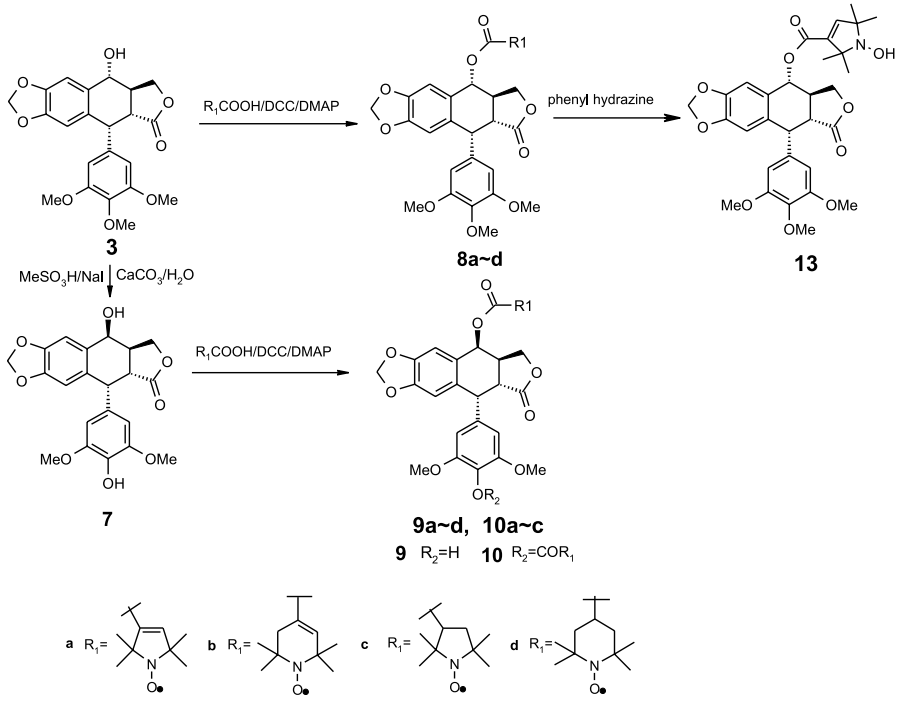
A number of spin-labeled nitroxide derivatives of podophyllotoxin were synthesized in our group, and results showed that these derivatives had significant antitumor activities with a marked decrease in toxicity compared with that of the parent **3** in our previous studies.[16] Among them, GP-11 (Fig. 1, **6**) 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. GP-1 and its congeners were tested against transplanted tumor S180 and HepA, the results showing that the inhibition activity of GP-1 is higher than those of congeners.

Our group has been aiming at the discovery of new antitumor drugs of podophyllotoxin derivatives with improved bioactivities and less toxicity in recent years.[16,17] In order to study the relationship between antitumor and the antioxidative activity of spin-labeled derivatives of podophyllotoxin and its congeners, we reported the synthesis of 4 or (4′)-substituted piperidine (pyrroline) spin-labeled podophyllotoxin and congeners (Scheme 1), in which compounds **8a–d**, **9b–d**, and **10a–c** were first synthesized. Their cytotoxicity against human leukemic (P-388) and human lung carcinoma (A-549) in vitro was evaluated. Also, we determined malondialdehyde (MDA) on liver, lung, and kidney homogenate of SD rats by the TBA method. Furthermore, the partition coefficients were measured and preliminary structure–activity relationships (SARs) were discussed.

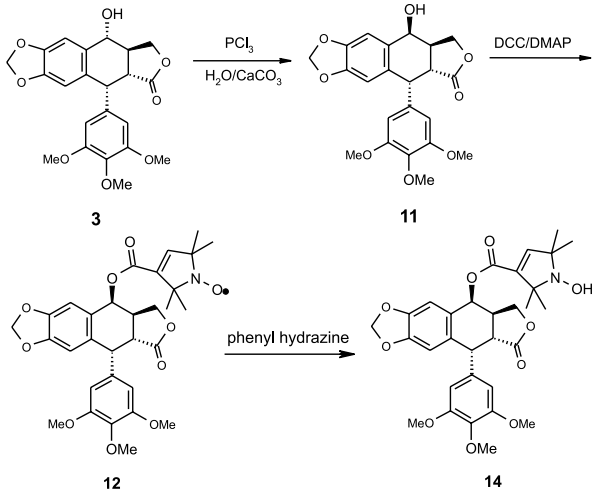
2. Chemistry

The synthetic route to the target compounds is depicted in Scheme 1. Briefly, 4′-demethylepipodophyllotoxin (DMEP, **7**) was prepared stereoselectively from podophyllotoxin **3** through 4′-demethylation, 4-iodination with methanesulfonic acid/sodium iodide, and nucleophilic substitution with water.[18] The nitroxide free radical piperidine (pyrroline) was synthesized according to the literature.[19] Compound **3** or **7** was then condensed with the appropriate nitroxyl acids in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino) pyridine (DMAP) to provide the target compounds **8a–d**, **9a–d**, and **10a–c**. Compound **12** was obtained by a similar procedure for the preparation of **8a**, and **3** was converted into **11** by simple treatment with PCl_3 and barium carbonate (Scheme 2).[20]

In order to confirm the C-4 configuration of final compounds, compounds **8a** and **12** were further reduced with phenyl hydrazine to produce compounds **13** and **14**, their coupling constants between H-3 and H-4 being $J_{3,4}$ = 11.7 and 3.0 Hz, which were in accord with **3** and **7**, respectively. According to the literature,[21] the $J_{3,4}$ of **3** is 10.8 Hz ($\geqslant$10 Hz), which is due to a *trans* relationship between H-3 and H-4, however, $J_{3,4}$ of **7** is about 3.6 Hz (3–5 Hz) because of H-3 being cis to H-4. Besides, we also determined all the compounds' specific rotatory



Scheme 1. Synthesis of compounds **8a**–**d**, **9a**–**d**, **10a**–**c**, and **13**.



Scheme 2. Preparation of compound **12** and **14**.

Table 1. Biology activity of compounds (IC_{50}, μM)

Compound	Cytotoxicity		Antioxidative activity			lg *P*
	P388	A549	Heart	Kidney	Liver	
8a	0.82	0.85	8.76[a]	8.80[b]	7.92[a]	0.070
8b	0.18	8.42	10.47[a]	10.35[b]	13.55[b]	0.129
8c	0.01	6.43	13.39[b]	15.42[b]	15.41[a]	0.188
8d	<0.01	6.86	10.88[b]	10.96[b]	10.59[b]	0.213
9a	0.02	0.30	3.80[b]	7.82[b]	8.98[b]	0.030
9b	0.09	0.46	4.84[a]	5.10[a]	4.19[a]	0.024
9c	0.07	0.90	9.07[b]	5.54[b]	5.50[b]	0.021
9d	<0.01	0.13	5.54[a]	4.96[a]	5.26[b]	0.061
10a	0.34	>10	5.50[b]	3.04[b]	4.26[b]	0.038
10b	0.03	0.28	2.93[b]	5.47[a]	4.31[b]	0.031
10c	0.24	8.19	6.20[a]	5.41[b]	5.54[b]	0.077
12	0.04	0.42	7.56[a]	7.64[b]	7.32[a]	0.070
1	1.18	7.14	82.73[a]	64.63[a]	46.38[a]	0.690
3	0.02	0.02	15.62[a]	15.48[a]	15.36[b]	0.591

[a] $P < 0.01$ compared with control.
[b] $P < 0.05$ compared with control.

power and found that **8a** is −127° and its isomeric compound **12** is −94°, which is consistent with **3** and **11**.[20,22] Based on the above, we determined that the C-4 configuration will not change in the course of forming an ester, which accorded with the mechanism.

3. Results and discussion

The cytotoxicities of **8**–**10** against murine leukemia P-388 and human lung cancer A549 in vitro and antioxidative activity of malondialdehyde (MDA) (liver, lung, and kidney homogenate of SD rats) were tested as well as their partition coefficients lg *P*. The results are summarized in Table 1. Compounds **8a**–**d**, **9a**–**d**, and **10a**–**c** showed a basically stronger inhibition of P-388 and A-549 than that of **1**, and their antioxidative activities on liver were also comparable or superior to that of the heart. Remarkably, to these compounds which exhibited significant inhibitory activity against P-388.

Further, the results in Table 1 revealed their information of preliminary structure–activity relationship (SAR). The bioactivities of **9a**–**d**, including anticancer, antioxidative activities were superior to those of **8a**–**d**. These results were in accord with the general conclusion.[23] The cytotoxicities of 4-β′s substitute were stronger than those of 4-α′s (**12** to **8a**), and bioactivities of the derivatives of **7** (4′-OH and 4-β) were superior to those of **3** (4′-OCH_3 and 4-α). Further, the cytotoxicity of these compounds was distinctly correlated with nitroxide. Also, the size of the ring and whether it was saturated

could not affect the bioactivities of target compounds, obviously, which was consistent with the literature.[24]

The results demonstrated that the glycosidic moiety of VP-16 is not so essential for the antitumor activity and considerable simplification in the sugar structure might be permitted, and the nitroxyl radical moiety could exhibit a beneficially modifying effect on the pharmacological properties of podophyllotoxin **3**, which was shown in our previous studies.[16] Some hypotheses pointed out that etoposide undergoes one-electron oxidation to etoposide–O· catalyzed by both purified myeloperoxidase and myeoperoxidase activity in HL60 cells, suggesting that there may be therapeutic window for the use of etoposide in combination with nutritional antioxidants, the genotoxic effects of etoposide are due mainly to its myeloperoxidase-catalyzed oxidation to phenoxyl radicals and subsequent redox-cycling that may be prevented or reduced by nutritional antioxidants.[25]The cytotoxicity and antioxidative activity of compounds **10a–c** were comparable to those of **9a–d**. The results conflicted with the previous SAR premise that a 4′-hydroxyl is required for tumor inhibition.[23] The mechanism may be similar to that of VP-16, 4′-OH of podophyllotoxin are oxidized to free radical under some matter. At the same time, isomeric compound **12** (4-β substitute) was better than that of **8a** (4-α substitute) both in antioxidative property and cytotoxicity. These results suggested that their cytotoxicity against tumor cells improved with the improvement of antioxidant activity and was in accord with the antioxidant activity. There is the probability that administration of antineoplastic agents such as podophyllotoxin series results in oxidative stress,[25] the production of free radicals and other reactive oxygen species (ROS), ROS slowing the rate of cell proliferation, may interfere with the cytotoxic effects of antineoplastic drugs.[26] On the other hand, from the antioxiditive activity, we found that compounds of 4′-hydroxyl (see **9a–d**) and their 4′-stable nitroxyl radical (see **10a–c**) substitute have similar activity, so we may conclude that, analogous to that of podophyllotoxin, the 4′-free radical substitute bears an analogy with that of 4′-OH in the pharmacological process. In addition, the lg *P* of **9a–c**'s were more near to zero than **8a–d**'s, which means that compounds with 4′-OH substitute would be easier to penetrate cell membrances than that of 4′-OCH_3.

In summary, we believed that spin labeling of podophyllotoxin drugs is a promising direction in antitumor chemotherapy, because they exhibit superior pharmacological properties to those of the parent compounds and also can be monitored by ESR in pharmacological experiments. The target compounds showed more effective cytotoxicity and antioxidative activity than the parent compound podophyllotoxin. Among them, compound **9d** showed the most potent cytotoxicity with IC_{50} <0.01 μM against P-388 and 0.13 μM against A-549, respectively. These compounds may act on tumor cells through an antioxidate way. Furthermore, we may conclude that the compound with a higher antioxidation has higher antitumor activity, which offer more information about the relationship between the antioxidant and anticancer bioactivities and guidance for further research.

4. Experimental

4.1. General methods

Melting points were determined with a Kofler apparatus and are uncorrected. IR spectra were measured on a Nicolet 5DX-FT-IR spectrometer on neat samples placed between KBr plates. ^{1}H NMR spectra were measured on a Varian Mecury-300BB spectrometer with TMS as an internal standard. All chemical shift values are expressed in δ parts per million. Optical rotations were measured on a Perkin Elmer 341 spectrometer. Mass spectra were recorded on a Bruker Daltonics APEX II 49e and VGZAB-HS (70 ev) spectrometer with ESI and FAB source as ionization, respectively. The electron spin resonance (ESR) spectra were obtained from 10^{-5} M methyl alcohol solution, using a Bruker ER200D-SRC spectrometer.

4.2. General preparation of compounds 8a–d, 9a–d and 10a–c

A mixture of the appropriate stable nitroxide acid R_1COOH (1.0 mmol), **3** or **7** (1.0 mmol) and dimethylaminopyridine (DMAP, 40 mg) was stirred in dry dichloromethane (20 ml) for 5 min at room temperature under argon. *N*,*N*-dicyclohexylcarbodiimide (DCC, 208 mg) was added and the reaction mixture was stirred for 3–40 h and monitored by TLC. The reaction mixture was filtered and the filtrate was evaporated. The residue was separated by column chromatography on silica gel with dichloromethane–acetone as an eluant.

4.2.1. 4-α-*O*-(2′,2′,5′,5′-Tetramethyl-3′-carboxypyrroline-1-oxyl)-podophyllic ester (8a). The yield 50.6%; yellow powder. Mp: 119–120 °C; $[\alpha]_D^{25}$ −127 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2931, 2844, 1777, 1713, 1505, 1484, 1461, 1332 (N–O·), 1238, 1127, 929; FABMS *m*/*z*: 580 (M^+); ESR: An = 14.70 G, g_0 = 2.0055; HRESIMS Anal. *m*/*z* calcd for $C_{31}H_{38}N_2O_{10}$: 598.2621. Found: 598.2527. $[M+NH_4]^+$.

4.2.2. 4-α-*O*-(1′-Oxyl-4′-carboxy-2′,2′,6′,6′-tetramethyl-1′,2′,5′,6′-tetrahydropyridine)-podophyllic ester (8b). The yield 74.2%, red powder. Mp: 113–114 °C; $[\alpha]_D^{25}$ −123 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2932, 2852, 1778, 1712, 1505, 1484, 1460, 1328 (N–O·), 1241, 1127, 931; FABMS: *m*/*z* 594 $[M]^+$; ESR: An = 15.28 G, g_0 = 2.0062; HRESIMS Anal. *m*/*z* calcd for $C_{32}H_{40}N_2O_{10}$: 612.2677. Found: 612.2667. $[M+NH_4]^+$.

4.2.3. 4-α-*O*-(2′,2′,5′,5′-Tetramethyl-3′-carboxypyrrolidine-1′-oxyl)-podophyllic ester (8c). The yield 56.0%, yellow powder. Mp: 116–118 °C; $[\alpha]_D^{25}$ −114 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2920, 2859, 1778, 1735, 1505, 1484, 1460, 1326 (N–O·), 1240, 1126, 930; FABMS: *m*/*z* 582 $[M]^+$; ESR: An = 14.76 G, g_0 = 2.0058; HRESIMS Anal. *m*/*z*

calcd for $C_{31}H_{40}N_2O_{10}$: 600.2677. Found: 600.2671. $[M+NH_4]^+$.

4.2.4. 4-α-*O*-(1′-Oxyl-4′-carboxy-2′,2′,6′,6′-tetramethylpiperidine)-podophyllic ester (8d). The yield 52.0%, red powder. Mp: 116–118 °C; $[\alpha]_D^{25}$ −94 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2930, 2850, 1777, 1731, 1588, 1505, 1484, 1459, 1327 (N–O·), 1239, 1126, 930; FABMS: *m/z* 596 $[M]^+$; ESR: An = 15.81 G, g_0 = 2.0063; HRESIMS Anal. *m/z* calcd for $C_{32}H_{42}N_2O_{10}$: 614.2834. Found: 614.2837. $[M+NH_4]^+$.

4.2.5. 4-β-*O*-(2″,2″,5″,5″-Tetramethyl-3″-carboxypyrroline-1″-oxyl)-4′-demethylepipodophyllic ester (9a). The yield 8.4%, yellow powder. Mp: 223–224 °C; $[\alpha]_D^{25}$ −84 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 3395, 2921, 2851, 1777, 1713, 1510, 1483, 1459, 1331 (N–O·), 1233, 1115, 927; FABMS: *m/z* 566 $[M]^+$; ESR: An = 14.62 G, g_0 = 2.0055; HRESIMS Anal. *m/z* calcd for $C_{30}H_{32}NO_{10}Na$: 589.1918. Found: 589.1916. [M+Na]$^+$.

4.2.6. 4-β-*O*-(1″-Oxyl-4″-carboxy-2″,2″,6″,6″-tetramethyl-1″,2″,5″,6″-tetrahydropyridine)-4′-demethylepipodophyllic ester (9b). The yield 5.6%, red powder. Mp: 212–213 °C; $[\alpha]_D^{25}$ −83 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 3368, 2915, 1771, 1707, 1508, 1482, 1461, 1334 (N–O·), 1238, 1113, 925; FABMS: *m/z* 582 $[M+H]^+$; ESR: An = 15.28 G, g_0 = 2.0062; HRESIMS Anal. *m/z* calcd for $C_{31}H_{38}N_2O_{10}$: 598.2621. Found: 598.2633. $[M+NH_4]^+$.

4.2.7. 4-β-*O*-(2″,2″,5″,5″-Tetramethyl-3″-carboxypyrrolidine-1″-oxyl)-4′-demethylepipodophyllic ester (9c). The yield 7.4%, yellow powder. Mp: 214–216°C; $[\alpha]_D^{25}$ −84 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 3403, 2919, 2850, 1776, 1729, 1509, 1484, 1461, 1331 (N–O·), 1229, 1116, 926; FABMS: *m/z* 568 $[M]^+$; ESR: An = 14.76 G, g_0 = 2.0058; HRESIMS Anal. m/z calcd for $C_{30}H_{38}N_2O_{10}$: 586.2521. Found: 586.2527. $[M+NH_4]^+$.

4.2.8. 4-β-*O*-(1″-Oxyl-4″-carboxy-2″,2″,6″,6″-tetramethylpiperidine)-4′-demethylepipodophyllic ester (9d). The yield 56.3%, red powder. Mp: 214–216 °C; $[\alpha]_D^{25}$ −79 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 3356, 2922, 2853, 177, 1728, 1511, 1484, 1460, 1331 (N–O·), 1234, 1118, 930; FABMS: *m/z* 582 $[M]^+$; ESR: An = 15.81 G, g_0 = 2.0063; HRESIMS Anal. *m/z* calcd for $C_{31}H_{40}N_2O_{10}$: 600.2677. Found: 600.2670. $[M+NH_4]^+$.

4.2.9. 4β,4′-*O*-(2″,2″,5″,5″-Tetramethyl-3″-carboxypyrroline-1″-oxyl)-4′-demethylepipodophyllic ester (10a). The yield 34.2%, yellow powder. Mp: 136 °C; $[\alpha]_D^{25}$ −45 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2929, 2855, 1780, 1742, 1506, 1484, 1463, 1354 (N–O·), 1289, 1186, 1130, 928; FABMS: *m/z* 733 $[M+H]^+$; ESR: An = 14.62 G, g_0 = 2.0055; HRESIMS Anal. *m/z* calcd for $C_{39}H_{48}N_3O_{12}$: 750.3233. Found: 750.3233. $[M+NH_4]^+$.

4.2.10. 4β,4′-*O*-(1″-Oxyl-4″-carboxy-2″,2″,6″,6″-tetramethyl-1″,2″,5″,6″-tetrahydropyridine)-4-demethylepipodophyllic ester (10b). The yield 32.7%, red powder. Mp: 134–136 °C; $[\alpha]_D^{25}$ −75 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2928, 2844, 1779, 1708, 1508, 1483, 1453, 1331 (N–O·), 1238, 1126, 928; FABMS: *m/z* 760 $[M]^+$; ESR: An = 15.28 G, g_0 = 2.0062; HRESIMS Anal. *m/z* calcd for $C_{41}H_{52}N_3O_{12}$: 778.3546. Found: 778.3550. $[M+NH_4]^+$.

4.2.11. 4β,4′-*O*-(2″,2″,5″,5″-Tetramethyl-3″-carboxypyrrolidine-1″-oxyl)-4-demethylepipodophyllic ester (10c). The yield 31.6%, red powder. Mp: 194 °C; $[\alpha]_D^{25}$ −80 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2923, 2853, 1778, 1730, 1510, 1484, 1461, 1331 (N–O·), 1231, 1127, 931; FABMS: *m/z* 736 $[M]^+$; ESR: An = 14.76 G, g_0 = 2.0058; HRESIMS Anal. *m/z* calcd for $C_{39}H_{52}N_3O_{12}$: 754.3546. Found: 754.3554. $[M+NH_4]^+$.

4.3. 4-β-*O*-(2′,2′,5′,5′-Tetramethyl-3′-carboxypyrroline-1′-oxyl)-podophyllic ester 12

The yield 37.4%, yellow powder. Mp: 132–134 °C; $[\alpha]_D^{25}$ −94 (*c* 0.5, $CHCl_3$); IR (cm^{-1}): 2931, 2844, 1777, 1713, 1505, 1484, 1461, 1332 (N–O·), 1238, 1127, 929; FABMS: *m/z* 580 $[M]^+$; ESR: An = 14.70 G, g_0 = 2.0055; HRESIMS Anal. *m/z* calcd for $C_{31}H_{38}N_2O_{10}$: 598.2621. Found: 598.2627. $[M+NH_4]^+$.

4.4. Reduction of compounds 8a and 12

To a solution of **8a** and **12** (0.4 ml $CDCl_3$) in nuclear magnetic resonance tube was added one drop of phenyl hydrazine then determined immediately on and 300 M spectrometer, respectively.

4.5. 4-α-*O*-(2′,2′,5′,5′-Tetramethyl-3′-carboxypyrroline-1′hydroxyl)-podophyllic ester 13

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.65 (s, 1H, 5-H), 6.56 (s, 1H, 8-H), 6.40 (s, 2H, 2′6′-H), 5.99 (d, 2H, *J* = 6.9 Hz, –OCH_2O–), 5.96 (d, 1H, $J_{4,3}$ = 11.7 Hz, 4-H), 5.28 (s, 1H, pyrroline 4′-H), 4.60 (m, 1H, 1-H,), 4.38 (m, 1H, 11H-a), 4.24 (m, 1H, 11H-b), 3.81 (s, 3H, 4-OCH_3-H), 3.70 (s, 6H, 3,5-OCH_3- H), 2.90 (m, 2H, 2,3-H), 1.40 (s, 6H, pyrroline 2′-2CH_3), 1.29 (s, 6H, pyrroline 5′-2CH_3).

4.6. 4-β-*O*-(2′,2′,5′,5′-Tetramethyl-3′-carboxypyrroline-1′hydroxyl)-podophyllic ester 14

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.60 (s, 1H, 5-H), 6.56 (s, 1H, 8-H), 6.29 (s, 2H, 2′6′-H), 6.19 (d, 1H, $J_{4,3}$ = 3.0 Hz, 4-H), 5.98 (d, 2H, *J* = 3.3 Hz, –OCH_2O–), 5.28 (s, 1H, pyrroline 4′-H), 4.67 (d, 1H, $J_{1,2}$ = 4.8 Hz, 1-H), 4.38 (m, 1H, 11H-a), 3.97 (m, 1H, 11H-b), 3.84 (s, 3H, 4-OCH_3- H), 3.75 (s, 6H, 3,5-OCH_3-H), 3.22 (dd, 1H, $J_{2,3}$ = 14.1 Hz, $J_{2,1}$ = 4.8 Hz, 2-H), 3.02 (m, 1H, 3-H), 1.39 (s, 3H, pyrroline 2′-CH_3), 1.33 (s, 3H, pyrroline 2′-CH_3), 1.27 (s, 3H, pyrroline 5′-CH_3), 1.26 (s, 3H, pyrroline 5′-CH_3).

5. Biology

5.1. Cell growth inhibition assay

Cells were incubated at 37 °C in a 5% CO_2 atmosphere. The MTT colorimetric assay was used to determine growth inhibition.[27] Cells were plated in 96-well plates

and allowed to attach for 24 h. Spin-labeled derivatives of podophyllotoxin were dissolved in brine. Cells were exposed in triplicate well to derivative concentrations of 0.39–100 μg/ml for 48 h. Then the media were aspirated, and 10 μl of 5 mg/ml MTT solution (dilute in sterile PBS) diluted in serum-free media was added to each well. After 4 h of incubation, the solution was centrifuged for 10 min under 2000 rpm limpid solution extracted carefully, and added 200 μl DMSO, then shaking by oscillator. The absorbance at A_{570} was determined on a plate reader. IC_{50} values were determined from a log plot of percent of control versus concentration.

5.2. In tissues antioxidant activity

The extent of lipid peroxidation was assessed using a modification of the thiobarbituric acid (TBA) assay.[28] Determination of malondialdehyde content (MDA) on liver, heart, and kidney homogenate of SD rats was carried out as 5% and 1% tissue homogenate in 0.9% NaCl solution, respectively. The tissue homogenate 1 ml was incubated with test compounds or vehicle (Me_2SO 2.5 ml/L) at 37 °C for 10 min. Fe^{2+}–ascorbic acid solution was added. After 30 min incubation, MDA was determined by the TBA colorimetric analysis method. The absorbance at λ_{532} was determined on a UV spectrophotometer. IC_{50} values were determined from 50% of control versus concentration. Each compound was assayed twice in quadruplicate.

5.3. Determination of partition coefficients

The partition coefficient P was determined according to the standard method[29] and calculated with the following equation: P = (compound in 1-octanol)/(compound in water).

Acknowledgment

This work was financially supported by NSFG (ZGS033-A43-013).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.12.025.

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