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Design, synthesis and cytotoxic activity of novel spin-labeled rotenone derivatives

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ABSTRACT

Three series of novel spin-labeled rotenone derivatives were synthesized and evaluated for cytotoxicity against four tumor cell lines, A-549, DU-145, KB and KBvin. All of the derivatives showed promising in vitro cytotoxic activity against the tumor cell lines tested, with IC_{50} values ranging from 0.075 to 0.738 μg/mL. Remarkably, all of the compounds were more potent than paclitaxel against KBvin in vitro, and compounds **3a** and **3d** displayed the highest cytotoxicity against this cell line (IC_{50} 0.075 and 0.092 μg/mL, respectively). Based on the observed cytotoxicity, structure–activity relationships have been described.

Cancer is currently the second most important disease leading to death in both developing and developed countries. However, the rising resistance to available chemotherapeutic agents, combined with their adverse side effects and high cost, is driving the search for new alternative anticancer compounds from natural products. Investigation of natural sources not only enhances diversity in the search for new prototype antitumor agents, but also may lead to commercially available marketed products as underscored by the prominent examples of etoposide and vinblastine.[1–5] Natural products and their closely related analogs are thus an important resource for new antitumor agents and are also regularly used as the templates for further sequential chemical modifications and structure optimization.

Rotenone is a naturally occurring flavonoid derived from the roots of cubé (*Lonchocarpus utilis* and *urucu*) or derris (*Derris elliptica*) or from other *Leguminosa* species. It has been used for at least 150 years as a botanical insecticide to control crop pests.[6,7] Its pesticidal activity is attributed to irreversible binding and inactivation of complexes in the mitochondrial electron transport chain. This action can block electron transfer from the complex to ubiquinone, thus, blocking the oxidative phosphorylation process, as well as increasing reactive oxygen species (ROS).[8,9] Other studies have found that rotenone displays anticancer activity by inducing apoptosis[10–12] in cells derived from human B-cell lymphomas,[13] promyelocytic leukemias,[14] and neuroblastomas.[15] In addition, rotenone can inhibit microtubule assembly and arrest cells in mitosis by binding directly to tubulin,[16] and thus, it is a possible scaffold for the design of potent microtubule assembly inhibitors. These findings have made rotenone a very attractive candidate for the clinical treatment of various forms of cancer. However, so far, not much attention has been paid to rotenone as a starting material for further transformations.

As part of an ongoing effort to identify potential antitumor molecules derived from natural products, we have successfully prepared spin-labeled antitumor drugs.[3,17–19] Herein, we report the design, synthesis, and preliminary in vitro cytotoxicity testing of three series of novel spin-labeled rotenones.

The synthetic chemical routes to compounds **3a–d** and **5a–d** are depicted in Scheme 1. Commercially available rotenone (**1**) was first reduced with $NaBH_4$ to yield the intermediate rotenol (**2**) in 85% yield.[20] Moreover, the keto moiety of **1** was converted to an oxime (**4**) by treatment with hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) in pyridine in 70% yield.[21] The intermediates **2** and **4** were then condensed with the appropriate piperidine (pyrroline) nitroxyl acids in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to provide target compounds **3a–d** and **5a–d**, respectively.

Based on previous work,[19,22] as well as the fact that L-amino acids are actively transplanted into mammalian tissues, have good water solubility, and are often used as carrier vehicles for some drugs, we also used an amino acid spacer as a linkage between

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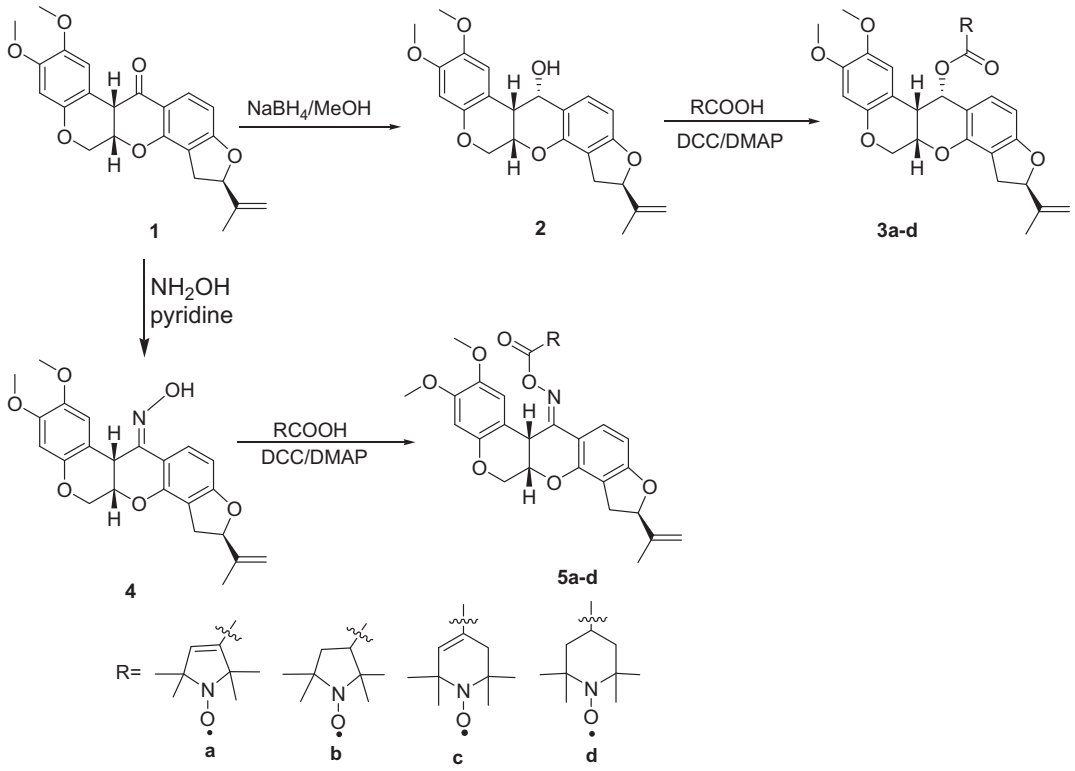


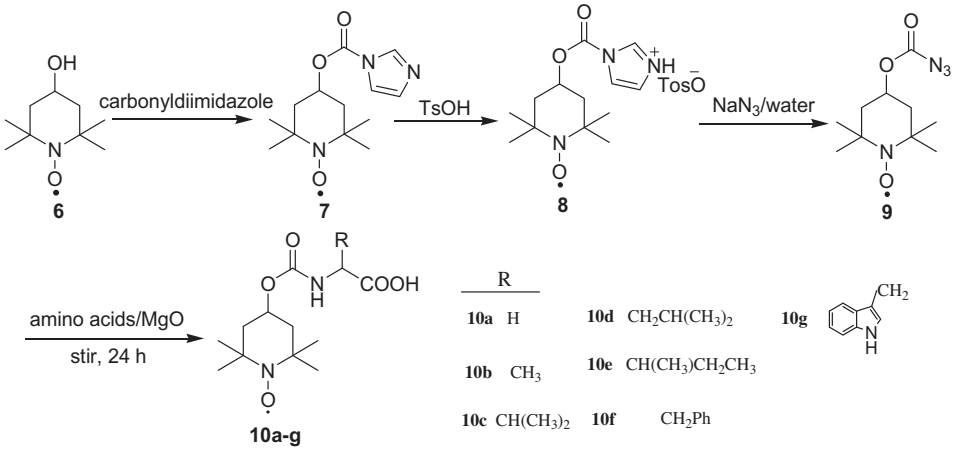
Scheme 1. Synthesis of compounds **3a**–**d** and **5a**–**d**.

rotenone core and the nitroxyl radical moiety. The starting materials [*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl-oxycarbonyl) amino acids **10a–g** for the preparation of the target compounds **11a–g** were synthesized according to our previous procedure as shown in Scheme 2.[19] 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%. Subsequently, the reaction of **6** with *N,N*-carbonyldiimidazole gave *N*-(1-oxyl-2,2,6,6-tetramethyl piperidinyloxycarbonyl)-imidazole (**7**). Without further purification, compound **7** was reacted with *p*-toluenesulfonic acid monohydrate to give its more reactive tosylate (**8**). Compound **8** was instantaneously converted into the corresponding 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 various amino acids in presence of MgO (Scheme 2). The 12-hydroxyl of intermediate **2** was then condensed with **10a–g** to afford compounds **11a–g** by a similar carbodiimide procedure as used in the preparation of **3a–d** (Scheme 3).

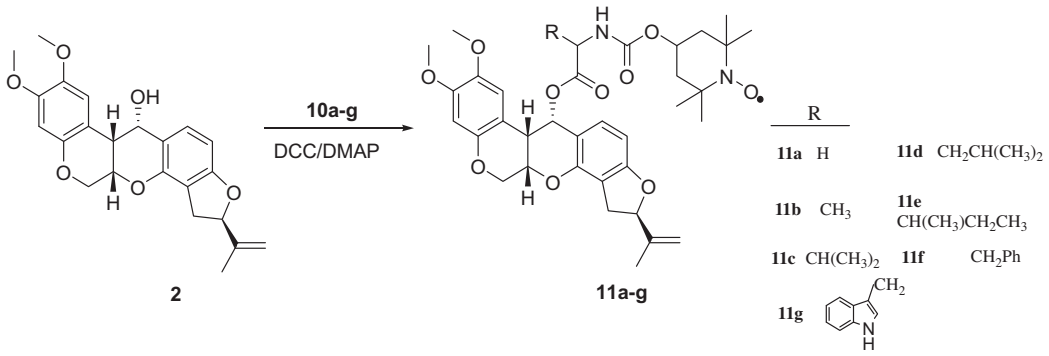
All synthesized target compounds **3a–d**, **5a–d** and **11a–g** were purified by column chromatography, and their structures were confirmed unambiguously from mp, IR, ESR and HRMS analyses.

Target compounds **3a–d**, **5a–d** and **11a–g** 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.[23] Compound **1** and paclitaxel were used as reference compounds. The screening results are shown in Table 1.

Our preliminary investigation showed that **1**-related derivatives are potential lead compounds for new antitumor agents. As



Scheme 2. Synthesis of compounds **10a**–**g**.



Scheme 3. Synthesis of compounds **11a–g**.

Table 1
In vitro cytotoxicity assay against four cancer cell lines

Entry	IC_{50} (μg/mL)			
	A-549	DU-145	KB	KBvin
3a	0.408 ± 0.150	0.223 ± 0.186	0.273 ± 0.138	0.075 ± 0.015
3b	0.533 ± 0.091	0.277 ± 0.170	0.443 ± 0.093	0.241 ± 0.103
3c	0.578 ± 0.062	0.322 ± 0.158	0.427 ± 0.085	0.299 ± 0.045
3d	0.523 ± 0.109	0.266 ± 0.180	0.408 ± 0.142	0.092 ± 0.063
5a	0.738 ± 0.202	0.426 ± 0.030	0.633 ± 0.146	0.620 ± 0.400
5b	0.471 ± 0.038	0.322 ± 0.053	0.560 ± 0.093	0.298 ± 0.045
5c	0.542 ± 0.109	0.358 ± 0.037	0.575 ± 0.066	0.162 ± 0.118
5d	0.651 ± 0.051	0.488 ± 0.122	0.523 ± 0.144	0.358 ± 0.044
11a	0.624 ± 0.111	0.450 ± 0.084	0.510 ± 0.070	0.410 ± 0.054
11b	0.650 ± 0.068	0.417 ± 0.086	0.442 ± 0.030	0.357 ± 0.045
11c	0.615 ± 0.133	0.451 ± 0.059	0.497 ± 0.144	0.381 ± 0.023
11d	0.521 ± 0.238	0.443 ± 0.077	0.483 ± 0.124	0.355 ± 0.024
11e	0.649 ± 0.088	0.396 ± 0.083	0.516 ± 0.092	0.399 ± 0.054
11f	0.713 ± 0.057	0.462 ± 0.082	0.509 ± 0.047	0.383 ± 0.053
11g	0.631 ± 0.021	0.477 ± 0.101	0.565 ± 0.069	0.414 ± 0.046
1	0.901 ± 0.192	0.465 ± 0.082	0.513 ± 0.087	0.595 ± 0.177
Paclitaxel	0.0052 ± 0.0017	0.0037 ± 0.0011	0.0048 ± 0.0009	1.145 ± 0.236

illustrated in Table 1, all new compounds exhibited significant in vitro cytotoxic activity against all tested tumor cell lines, with IC_{50} values ranging from 0.075 to 0.738 μg/mL. Most of the derivatives displayed increased cytotoxic activity compared with the parent compound **1**. Remarkably, all of the compounds also were more potent than paclitaxel against KBvin. With IC_{50} values of 0.075 and 0.092 μg/mL, respectively, compounds **3a** and **3d** showed the greatest cytotoxicity against KBvin, compared to **1** and paclitaxel (IC_{50} 0.595 and 1.145 μg/mL, respectively). Esterification of the C-12-hydroxyl of rotenol with the nitroxides of different ring classes (piperidine, pyrroline) groups (**3a–d**) led to significantly improved cytotoxic activity in comparison to **1**. Conversion of **1** into the oxime esters **5a–d** also resulted in active derivatives, but **5a–d** were generally less potent compared to esters **3a–d**. This finding indicated that the cytotoxic profile of **1**-derivatives may be sensitive to the size and electronic density of the substituents at C-12. Furthermore, the cytotoxicity of these compounds (**3a–d**, **5a–d**) was distinctly correlated with the nitroxide. Also, the ring size and degree of unsaturation did not affect the bioactivity of the target compounds against three (KB, A-549 and DU-145), of the four tested tumor cell lines, which was consistent with the literature.[24] Interestingly, compounds **11a–g**, with an amino acid linker moiety at the C-12 position, showed slightly decreased activity in all the tested cell lines compared to compounds **3a–d**, suggesting a size limitation at position C-12. In our previous paper,[19] we found that using different L-amino acid as linkers markedly affected the biological activity of cytotoxic agents. However, as seen in Table 1, the different substituents at α-carbon of the amino acid in compounds **11a–g** did not lead to obviously different effects on the inhibition of the four tumor cell lines in vitro. As a whole, the introduction of nitroxides into the rotenone molecule potentiated the antitumor activity. Thus, the design and synthesis of these compounds provides valuable information to potentially increase the therapeutic value of rotenone. Synergistic action might also be found against tumor cell lines.

In summary, novel spin-labeling of the rotenone class is a promising direction in antitumor chemotherapy, not only because these compounds exhibit superior cytotoxic activity, but also because they can be monitored by ESR in pharmacological experiments. In this paper, three series of novel spin-labeled derivatives of rotenone were first synthesized successfully, and their antitumor activity was evaluated against for four tumor cell lines using an SRB-assay. The cytotoxic results showed that most of the new spin-labeled compounds exhibited more potent cytotoxicity against A-549, DU-145, KB and KBvin compared to rotenone. Compounds **3a** and **3d** were the most promising derivatives and were selected as lead molecules for further development. More systematic structural modifications will carried out to further clarify these initial interesting findings.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.024.

References and notes

1. Lee, K. H. *Med. Res. Rev.* **1999**, *19*, 569.
2. Lee, K. H. *J. Nat. Prod.* **2004**, *67*, 273.
3. Liu, Y. Q.; Yang, L.; Tian, X. *Curr. Bioact. Compd.* **2007**, *3*, 37.
4. Newman, D. J.; Cragg, G. M.; Snader, K. M. *J. Nat. Prod.* **2003**, *66*, 1022.
5. Danieli, B.; Lesma, G.; Martinelli, M.; Passarella, D.; Silvani, A.; Pyuskyulev, B. *J. Org. Chem.* **1998**, *63*, 8586.
6. Negherbohn, W. O. In *Handbook of Toxicology*; Saunders: Philadelphia, 1959; Vol. III, p 661.
7. Fukami, H.; Nakajima, M. In *Naturally Occurring Insecticides*; Jacobson, M., Crosby, D. G., Eds.; Dekker: New York, 1971; p 71.
8. Radad, K.; Rausch, W. D.; Gille, G. *Neurochem. Int.* **2006**, *49*, 379.
9. Lindahl, P. E.; Oberg, K. E. *Exp. Cell Res.* **1961**, *23*, 228.
10. Wolvetang, E. J.; Johnson, K. L.; Krauer, K.; Ralph, S. J.; Linnane, A. W. *FEBS Lett.* **1994**, *339*, 40.
11. Isenberg, J. S.; Klaunig, J. E. *Toxicol. Sci.* **2000**, *53*, 340.
12. Isenberg, J. S.; Kolaja, K. L.; Ayoubi, S. A.; Watkins, J. B.; Klaunig, J. E. *Carcinogenesis* **1997**, *18*, 1511.
13. Armstrong, J. S.; Hornung, B.; Lecane, P.; Jones, D. P.; Knox, S. J. *Biochem. Biophys. Res. Commun.* **2001**, *289*, 973.
14. Tada, O. S.; Hiraku, Y.; Kawanishi, M.; Kawanishi, S. *Life Sci.* **2003**, *73*, 3277.
15. Chung, W. G.; Miranda, C. L.; Maier, C. S. *Brain Res.* **2007**, *1176*, 133.
16. Srivastava, P.; Panda, D. *FEBS J.* **2007**, *27*, 44788.
17. Wang, J. Z.; Tian, X.; Tsumura, H.; Shimura, K.; Ito, H. *Anti-Cancer Drug Des.* **1993**, *8*, 193.
18. Tian, X.; Zhang, F.-M.; Li, W. G. *Life Sci.* **2002**, *70*, 2433.
19. Liu, Y. Q.; Tian, X. *Synth. Commun.* **2005**, *35*, 2749.
20. VanBrocklin, H. F.; Hanrahan, S. M.; Enas, J. D.; Nandanan, E.; ONeil, J. P. *Nucl. Med. Biol.* **2007**, *34*, 109.
21. Qian, K. D.; Yu, D. L.; Chen, C. H.; Huang, L.; Morris-Natschke, S. L.; Nitz, T. J.; Salzwedel, K.; Reddick, M.; Allaway, G. P.; Lee, K. H. *J. Med. Chem.* **2009**, *52*, 3248.
22. Liu, Y. Q.; Tian, X.; Yang, L.; Zhan, Z. C. *Eur. J. Med. Chem.* **2008**, *43*, 2610.
23. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
24. Jin, Y.; Chen, S. W.; Tian, X. *Bioorg. Med. Chem.* **2006**, *14*, 3062.