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Schinalactone A, a New Cytotoxic Triterpenoid from *Schisandra sphenanthera*

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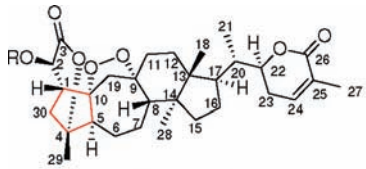
ABSTRACT



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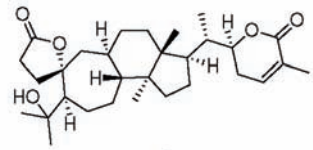
1a R = Ac



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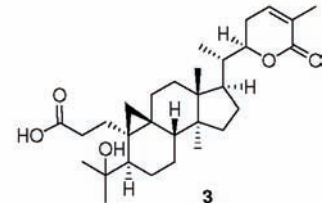

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A new cytotoxic triterpenoid, schinalactone A (1), together with two new biogenetically related compounds, schinalactones B (2) and C (3), has been isolated from the roots and stems of *Schisandra sphenanthera*. Their structures were elucidated on the basis of extensive spectroscopic analysis. Compounds 1 and 2 showed significant cytotoxicity against PANC-1 cell lines with IC_{50} values of 5.9 and 4.1 μM, respectively. A plausible biosynthetic pathway of 1 was also postulated.

During the past 10 years, our group has made great efforts in the study of the chemical constituents and biological activities of the genus *Schisandra*, resulting in the discovery of more than 100 highly oxygenated nortriterpenoids with nine unprecedented carbon skeletons.[1]

Those unusual ring assemblies highly oxygenated structures have brought great interest and challenges to chemists for total synthesis and biogenetic studies.[2] The previous work carried out by our group on nortriterpenoids of *Schisandra sphenanthera* Rehd et Wils led to finding a series of novel nortriterpenoids with complicated polycyclic systems.[3] In a continuing search for structurally unique and biogenetically interesting triterpenoids, the chemical constituents of *S. sphenanthera* have been reinvestigated. A new cytotoxic triterpenoid, schinalactone A (**1**), together with two new biogenetically related

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(1) (a) Xiao, W. L.; Li, R. T.; Huang, S. X.; Pu, J. X.; Sun, H. D. *Nat. Prod. Rep.* **2008**, *25*, 871–891. (b) Luo, X.; Chang, Y.; Zhang, X. J.; Pu, J. X.; Gao, X. M.; Wu, Y. L.; Wang, R. R.; Xiao, W. L.; Zheng, Y. T.; Lu, Y.; Chen, G. Q.; Zheng, Q. T.; Sun, H. D. *Tetrahedron Lett.* **2009**, *50*, 5962–5964. (c) Huang, S. X.; Li, R. T.; Liu, J. P.; Lu, Y.; Chang, Y.; Lei, C.; Xiao, W. L.; Yang, L. B.; Zheng, Q. T.; Sun, H. D. *Org. Lett.* **2007**, *9*, 2079–2082. (d) Huang, S. X.; Yang, J.; Huang, H.; Li, L. M.; Xiao, W. L.; Li, R. T.; Sun, H. D. *Org. Lett.* **2007**, *9*, 4175–4178. (e) Li, R. T.; Han, Q. B.; Zheng, Y. T.; Wang, R. R.; Yang, L. M.; Lu, Y.; Sang, S. Q.; Zheng, Q. T.; Zhao, Q. S.; Sun, H. D. *Chem. Commun.* **2005**, *23*, 2936–2938. (f) Xiao, W. L.; Yang, L. M.; Gong, N. B.; Wu, L.; Wang, R. R.; Pu, J. X.; Li, X. L.; Huang, S. X.; Zheng, Y. T.; Li, R. T.; Lu, Y.; Zheng, Q. T.; Sun, H. D. *Org. Lett.* **2006**, *8*, 991–994. (g) Xiao, W. L.; Zhu, H. J.; Shen, Y. H.; Li, R. T.; Li, S. H.; Sun, H. D.; Zheng, Y. T.; Wang, R. R.; Lu, Y.; Wang, C.; Zheng, Q. T. *Org. Lett.* **2005**, *7*, 2145–2148. (h) Xiao, W. L.; Li, R. T.; Li, S. H.; Li, X. L.; Sun, H. D.; Zheng, Y. T.; Wang, R. R.; Lu, Y.; Wang, C.; Zheng, Q. T. *Org. Lett.* **2005**, *7*, 1263–1266. (i) Xiao, W. L.; Lei, C.; Ren, J.; Liao, T. G.; Pu, J. X.; Pittman, C. U., Jr.; Lu, Y.; Zheng, Y. T.; Zhu, H. J.; Sun, H. D. *Chem.—Eur. J.* **2008**, *14*, 11584–11592. (j) Lei, C.; Pu, J. X.; Huang, S. X.; Chen, J. J.; Liu, J. P.; Yang, L. B.; Ma, Y. B.; Xiao, W. L.; Li, X. N.; Sun, H. D. *Tetrahedron* **2009**, *65*, 164–170.

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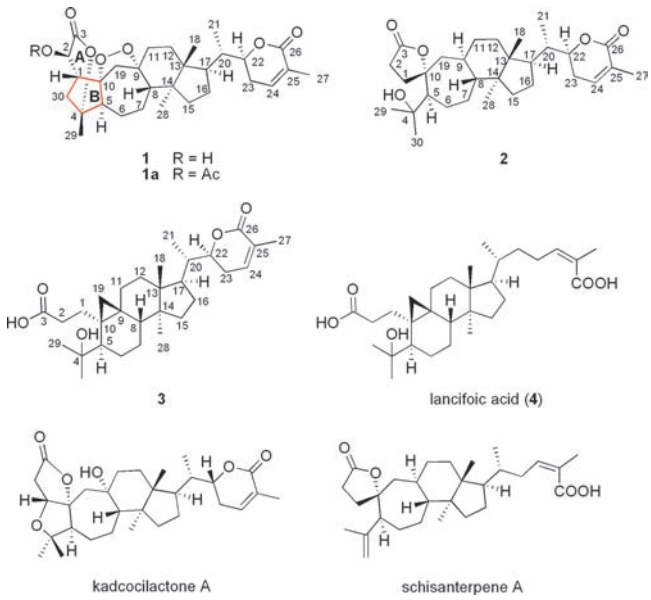
triterpenoids, schinalactones B (**2**) and C (**3**), and one known compound, lancifoic acid A (**4**), had been isolated from the roots and stems of this plant. Among them, **1** possessed a rearranged cycloartane type triterpenoid skeleton with a novel five-membered carbon ring featuring C-30 connected to C-1 (ring B) and showed significant cytotoxicity against SK-BR-3 and PANC-1 cell lines with IC_{50} values of 5.2 and 5.9 μM, respectively, which was one of the most significant cytotoxic triterpenoids isolated from the *Schisandra* species. In this paper, the isolation, structure elucidation, and biological activities of these new compounds are described.



Schinalactone A (**1**) was obtained as a white, amorphous powder, and the molecular formula $C_{30}H_{42}O_7$ was assigned through its HRESIMS (*m/z* 537.2839, $[M + Na]^+$), requiring 10 degrees of unsaturation. The ^1H and ^{13}C NMR spectra of **1** revealed the presence of two lactone carbonyl carbons and a pair of olefinic carbons. Apart from three degrees of unsaturation occupied by one double bond and two carbonyls, the remaining seven degrees of unsaturation indicated that **1** should possess a heptacyclic system. Comparison of the ^1H and ^{13}C NMR data of **1** with those of kadcoccilactone A[4] suggested that they were similar and might both be derived from cycloartane type triterpenoids and differed mainly in ring A and ring B. In **1**, a six-membered lactone

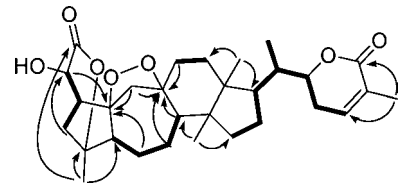


Figure 1. Selected HMBC (H→C) and $^1H-^1H$ COSY (−) correlations of **1**.

ring (ring A) was assigned on the basis of observations from HMBC (Figure 1) correlations of H-2 with C-1, C-3 and C-10, and of H-1 with C-2 and C-3. Furthermore, weak HMBC correlation from H_3-29 (δ_H 1.30, 3H, s) to C-3 (δ_C 172.4) also suggested that the C-3 connected to C-4 (δ_C 90.6) through an oxygen bridge. In addition, a five-membered carbon ring fused system (ring B) was deduced from HMBC correlations from H-5 to C-1, C-10, and C-30, from H-1 to C-4 and C-30, and from H_2-30 to C-4 and C-5, coupled with $^1H-^1H$ COSY (Figure 1) correlations of H-1 with H-2 and H_2-30. Further evidence supporting fusion of rings A and B was provided by the presence of a spin system (H_2-30/H-1/H-2) as deduced from the COSY spectrum and HMBC correlation from H-2 to C-30. From the above deduction, coupled with the molecular formula and degrees of unsaturation, there must be one more ring. From the HMBC spectrum, two oxygenated quaternary carbons C-9 and C-10 were assigned on the basis of correlations from H-7, H-8, H-11, and H-19 to C-9 and from H-2, H-6, and H-19 to C-10, respectively. Meanwhile, the positive FAB mass spectrum of **1** revealed a moderately strong fragment peak at *m/z* 465 $[M + H - H_2O - O_2]^+$, indicating the cleavage of a peroxyl group. Moreover, an AB doublet for the C-19 methylene group resonances occurred at relatively low field region (δ_H 2.88, 1H, d, $J = 12.2$ Hz; δ_H 2.26, 1H, d, $J = 12.2$ Hz) due to the effect of the peroxyl bridge.[5] These data indicated that the peroxyl bridge was located between C-9 and C-10. Consequently, the planar structure of **1** was assigned by comprehensive analysis of 2D NMR data, including HSQC, HSQC-TOCSY, $^1H-^1H$ COSY, and HMBC experiments. The planar structure of **1** was further confirmed by comparing the ^{13}C NMR chemical shift differences between **1** and its acetate derives (**1a**) (Scheme 1) as well. Chemical shift differences ($\Delta\delta_C = \delta_C$ **1**$-$**1a**) between **1** and **1a** of C-1 ($\Delta\delta_C$ +2.0 ppm), C-3 ($\Delta\delta_C$ +5.3 ppm), C-4 ($\Delta\delta_C$ −0.9 ppm), and C-30 ($\Delta\delta_C$ −0.4 ppm) indicated that these carbons were proximal to C-2.

With the gross structure of **1** in hand, the relative stereochemistry of **1** was readily assigned by ROESY correlations and modeling in Chem3D 10.0 (Cambridge Soft, Inc.) as shown in Figure 2. Correlations between H_2-19β (δ_H

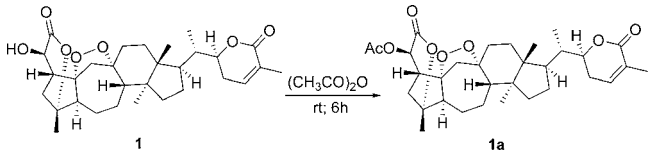
(2) (a) Tang, Y. F.; Zhang, Y. D.; Dai, M. J.; Luo, T. P.; Deng, L. J.; Chen, J. H.; Yang, Z. *Org. Lett.* **2005**, *7*, 885–888. (b) Zhang, Y. D.; Tang, Y. F.; Luo, T. P.; Shen, J.; Chen, J. H.; Yang, Z. *Org. Lett.* **2006**, *8*, 107–110. (c) Fischer, D.; Theodorakis, E. A. *Eur. J. Org. Chem.* **2007**, *25*, 4193–4196. (d) Krishnan, K. S.; Smitha, M.; Suresh, E.; Radhakrishnan, K. V. *Tetrahedron* **2006**, *62*, 12345–12350.

(3) (a) Xiao, W. L.; Huang, S. X.; Wang, R. R.; Zhong, J. L.; Gao, X. M.; He, F.; Pu, J. X.; Lu, Y.; Zheng, Y. T.; Zheng, Q. T.; Sun, H. D. *Phytochemistry* **2008**, *69*, 2862–2866. (b) Xiao, W. L.; Yang, L. M.; Li, L. M.; Pu, J. X.; Huang, S. X.; Weng, Z. Y.; Lei, C.; Liu, J. P.; Wang, R. R.; Zheng, Y. T.; Li, R. T.; Sun, H. D. *Tetrahedron Lett.* **2007**, *48*, 5543–5546. (c) Xiao, W. L.; Pu, J. X.; Chang, Y.; Li, X. L.; Huang, S. X.; Yang, L. M.; Li, L. M.; Lu, Y.; Zheng, Y. T.; Li, R. T.; Zheng, Q. T.; Sun, H. D. *Org. Lett.* **2006**, *8*, 1475–1478. (d) Xiao, W. L.; Pu, J. X.; Chang, Y.; Li, X. L.; Huang, S. X.; Yang, L. M.; Li, L. M.; Lu, Y.; Zheng, Y. T.; Li, R. T.; Zheng, Q. T.; Sun, H. D. *Org. Lett.* **2006**, *8*, 4669.

(4) Gao, X. M.; Pu, J. X.; Huang, S. X.; Lu, Y.; Lou, L. G.; Li, R. T.; Xiao, W. L.; Chang, Y.; Sun, H. D. *J. Nat. Prod.* **2008**, *71*, 1182–1188.

(5) (a) Chen, G. F.; Li, Z. L.; Chen, K.; Tang, C. M.; He, X.; Pan, D. J.; Mcphail, D. R.; Mcphail, A. T.; Lee, K. H. *Tetrahedron Lett.* **1990**, *31*, 3413–3416. (b) Zhou, T. X.; Zhang, H. P.; Zhu, N. Y.; Chiu, P. *Tetrahedron* **2004**, *60*, 4931–4936.

Scheme 1. Esterification of Schinalactone A (**1**)



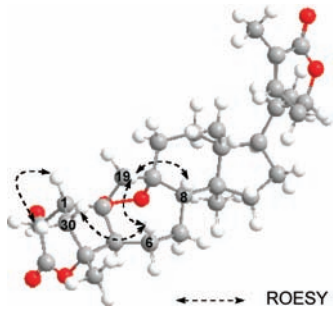


Figure 2. Key ROESY correlations of **1**.

2.88) with H-6β and H-8β clearly showed that these protons were on the same face. Thus, the peroxyl bridge in **1** was deduced to be in the α-orientation. The lactone ring between C-3 and C-4 adopted an α-orientation, which was deduced from strong correlations of H_2-30/H-1 and H-30β/H-6β. On the contrary, correlation of H-30β/H-6β in the ROESY spectrum might have disappeared when the lactone ring (ring A) adopted the β-orientation on C-4. In addition, a broad singlet at δ_H 4.85 ppm (H-2) in the ^{1}H NMR spectrum indicated that the dihedral angle between H-1 and H-2 should be 90°, so that the hydroxyl at C-2 should have adopted a β-orientation. H-22 has strong correlation with H-16β in ROESY spectrum, indicating that H-22 was in the α-orientation. The relative configuration of other stereocenters in **1** was the same as kadcoccilactone A. On the basis of the above deduction, the carbon backbone of **1** was unambiguously established.

Schinalactone B (**2**) was deduced to have the molecular formula $C_{30}H_{46}O_5$ from its HRESIMS at *m*/*z* 509.3251 ([M + Na]$^+$, calcd 509.3243), requiring 8 degrees of unsaturation. On analysis of its ^{1}H and ^{13}C NMR spectra, features similar to those of schisanterpene A[6] were evident, but a pair of double bonds between C-4 and C-30 in schisanterpene A was absent in **2**, which instead exhibited a tertiary alcohol (δ_C 72.7). As a result, **2** was pentacyclic and belonged to 3,4;9,10-*seco*-cycloartane-type triterpenoids. The oxygen atom of the spiro-ring on C-10 was in the α-orientation, which was determined by ROESY correlations (between H-2β and H_2-19 and H-1β and H_3-29). H-22 has strong correlation with H-16β in the ROESY spectrum, indicating that H-22 was in the α-orientation.

Schinalactone C (**3**) was isolated as colorless needles and exhibited a [M + Na]$^+$ ion peak at *m*/*z* 509.3245 in the HRESIMS, associated with a molecular formula of $C_{30}H_{46}O_5$. The presence of a three-membered ring as suggested by characteristic signals in the ^{1}H and ^{13}C NMR spectra (δ_C 22.6, s, C-9; δ_C 27.5, s, C-10; δ_C 31.5, t, C-19, and δ_H 0.58, ABd, J = 5.5 Hz, and 0.79, ABd, J = 5.5 Hz, H_2-19) indicated that **3** belonged to the cycloartane skeleton triterpenoid family (Table 1). The NMR data of **3** were very similar to those of lancifoic acid A (**4**),[7] which belongs to 3,4-*seco*-cycloartane-type triterpenoids, with the main difference being that **3** is pentacyclic whereas **4** is tetracyclic. There is a six-membered α,β-unsaturated lactone ring in **3**, which was supported by ^{1}H−^{1}H COSY and HMBC correlations. H-22 has strong correlation with H-16β in ROESY spectrum, indicating that H-22 was on α-orientation. The other relative stereochemistry centers were identical to those of **4**.

The co-occurrence of **1** with various 3,4-*seco*- or 3,4;9,10-*seco*-cycloartane triterpenoids (**2**−**4**) within the same plant raises interesting questions about the biogenesis of **1**. Biogenetically, **1** may be produced from schizandronic acid,

Table 1. ^{13}C NMR Data of Compounds **1**−**3** in Pyridine-d_5[a]

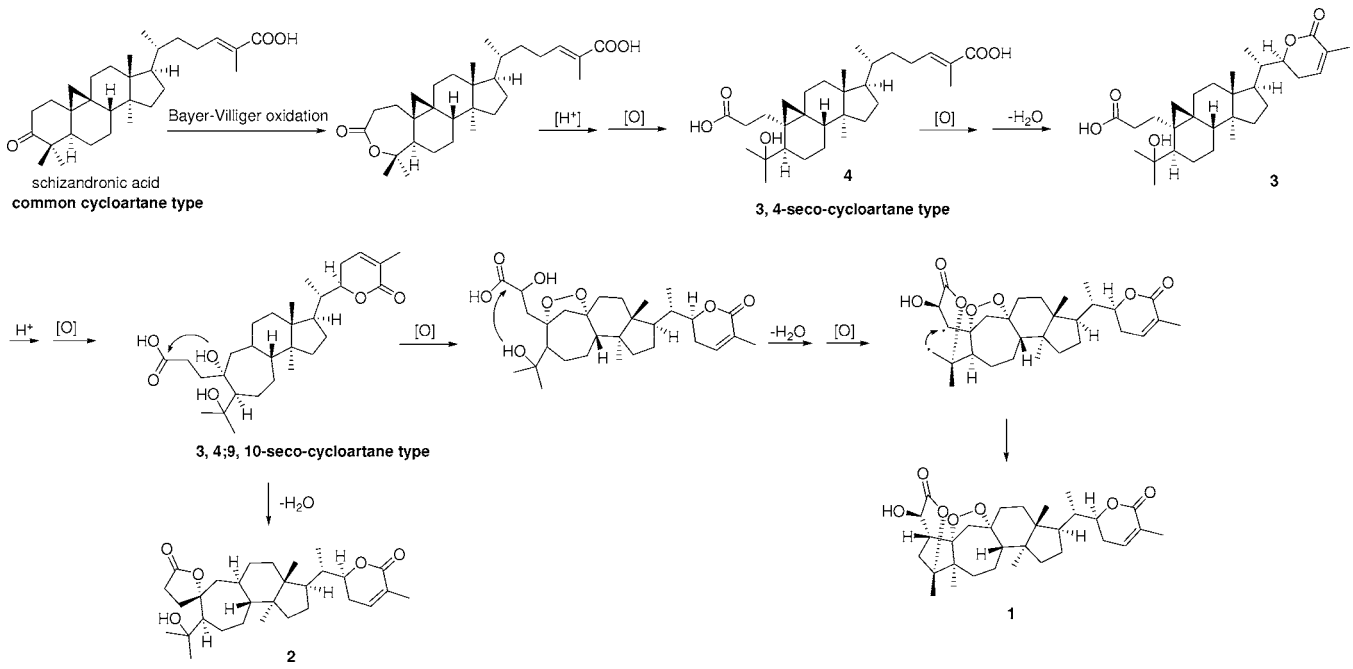
	1	1a	2	3
1	48.5 (d)	46.5 (d)	29.1 (t)	31.1 (t)
2	67.4 (d)	67.8 (d)	30.3 (t)	32.0 (t)
3	172.4 (s)	167.1 (s)	177.5 (s)	174.8 (s)
4	90.6 (s)	91.5 (s)	72.7 (s)	75.1 (s)
5	59.7 (d)	59.2 (d)	55.7 (d)	45.9 (d)
6	27.1 (t)	27.0 (t)	25.6 (t)	25.5 (t)
7	26.7 (t)	26.6 (t)	33.5 (t)	26.2 (t)
8	48.7 (d)	48.7 (d)	31.3 (d)	48.8 (d)
9	86.9 (s)	87.2 (s)	47.9 (d)	22.6 (s)
10	91.8 (s)	91.5 (s)	92.2 (s)	27.5 (s)
11	35.5 (t)	35.2 (t)	26.7 (t)	26.7 (t)
12	30.6 (t)	30.5 (t)	32.9 (t)	33.2 (t)
13	45.7 (s)	45.6 (s)	46.1 (s)	45.5 (s)
14	48.2 (s)	48.2 (s)	48.8 (s)	48.8 (s)
15	33.6 (t)	33.5 (t)	31.9 (t)	36.3 (t)
16	26.6 (t)	26.4 (t)	33.4 (t)	27.1 (t)
17	46.6 (d)	46.1 (d)	47.1 (d)	48.4 (d)
18	14.1 (q)	14.0 (q)	14.7 (q)	18.5 (q)
19	57.2 (t)	56.5 (t)	52.1 (t)	31.5 (t)
20	39.5 (d)	39.5 (d)	39.5 (d)	39.4 (d)
21	13.6 (q)	13.5 (q)	13.5 (q)	13.1 (q)
22	80.4 (d)	80.4 (d)	80.4 (d)	80.5 (d)
23	23.8 (t)	23.6 (t)	23.6 (t)	23.6 (t)
24	140.2 (d)	140.1 (d)	140.1 (d)	140.2 (d)
25	128.0 (s)	128.0 (s)	128.0 (s)	128.0 (s)
26	166.3 (s)	166.3 (s)	166.3 (s)	166.3 (s)
27	17.2 (q)	17.1 (q)	17.2 (q)	17.3 (q)
28	18.0 (q)	17.9 (q)	16.7 (q)	19.8 (q)
29	21.6 (q)	21.4 (q)	31.4 (q)	32.0 (q)
30	35.6 (t)	36.0 (t)	29.7 (q)	26.7 (q)

[a] Data for compounds **1**, **1a**, and **3** were recorded at 125 MHz, data for compound **2** were recorded at 100 MHz, and assignments were based on HSQC, COSY, HMBC, and ROESY experiments.

(6) Xiao, P. G.; Zhu, Y.; Yang, J. S.; Wang, L. W. *Faming Zhuanli Shenqing Gongkai Shuomingshu* **2002**, CN 1335308A.

(7) Xiao, W. L.; Tian, R. R.; Pu, J. X.; Li, X.; Wu, L.; Lu, Y.; Li, S. H.; Li, R. T.; Zheng, Y. T.; Zheng, Q. T.; Sun, H. D. *J. Nat. Prod.* **2006**, *69*, 277–279.

Scheme 2. Hypothetical Biogenetic Pathway of **1**



after many steps of oxidation, ring-opening, esterification, and possibly a free-radical mechanism to form the five-membered carbon ring (ring B),[8] as shown in Scheme 2.

Table 2. Cytotoxic Activity of Compounds **1**−**4**[a]

compounds	HL-60	SMMC-7721	A-549	SK-BR-3	PANC-1
1	>40	>40	17.7	5.2	5.9
1a	8.2	>40	36.6	34.3	>40
2	>40	>40	>40	23.5	4.1
3	>40	>40	>40	>40	>40
4	>40	>40	>40	>40	>40
cis-platin	1.6	13.6	11.8	19.9	14.4

[a] Results are expressed as IC_{50} values in micrometers, data were obtained from triplicate experiments, and *cis*-platin was used as positive control.

Compounds **1**−**4** were all tested for their cytotoxicity against HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 human cancer cell lines. Among them, compounds **1** and **2** showed strong cytotoxicity against PANC-1 cell lines with IC_{50} values of 5.9 and 4.1 μM, respectively. Compound **1** also showed significant cytotoxicity against SK-BR-3 cell lines with an IC_{50} value of 5.2 μM (Table 2).

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Supporting Information Available: Detailed method of cytotoxicity test, experimental section, and 1D and 2D NMR spectra, UV, IR, optical rotation, and ^{1}H NMR data assignment of compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Li, L. M.; Li, G. Y.; Ding, L. S.; Yang, L. B.; Zhao, Y.; Pu, J. X.; Xiao, W. L.; Han, Q. B.; Sun, H. D. *J. Nat. Prod.* **2008**, *71*, 684–688.