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# Nortriterpene constituents from *Schisandra sphenanthera*

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ABSTRACT

Nine new highly oxygenated nortriterpenoids, pre-schisanartanins E–J (**1**–**6**) and sphenadilactones D–F (**7**–**9**), together with 17 known ones (**10**–**26**), have been isolated from the acetone extract of the roots and stems of *Schisandra sphenanthera*. The structures of the new metabolites were characterized on the basis of extensive spectroscopic analyses including 1D and 2D NMR experiments. These compounds were all evaluated for their cytotoxicity against HL-60, SMMC-7721, A549, MCF-7, and SW480 tumor cell lines.


## 1. Introduction

*Schisandra* nortriterpenoids are a structurally intriguing group of highly oxygenated, polycyclic, fused heterocyclic natural products isolated from the plants of the genus *Schisandra*.[1] These compounds are further classified into six groups according to different carbon frameworks and oxygenated patterns, including schisanartane, schiartane, 8-norschiartane, 18(13→14)-*abeo*-schiartane, pre-schisanartane, and wuweiziartane skeletons.[2] During the past 10 years, great effort in our group has been devoted to investigate the chemical constituents of the species of *Schisandra*, which resulted in the finding of more than 120 biosynthetically related nortriterpenoids. Some nortriterpenoids showed anti-HIV activities and cytotoxicity,[1,2] such as lancifodilactone G,[3] sphenadilactone A,[4] and pre-schisanartanin A.[5] Consequently, these structurally complex molecules have brought great interest and challenges to the chemists for total synthesis and biological studies.[6,7]

With the aim of discovering more structurally unique and biological natural products from different *Schisandra* species, we reinvestigated the roots and stems of *Schisandra sphenanthera* Rehd et Wils collected in Sichuan province, PR China. Our previous studies on this species led to the isolation and identification of several novel *Schisandra* nortriterpenoids, such as sphenadilactones A and B, and schinalatones A, B, and C.[8–13] Further investigations led to the isolation of a series of 10 new nortriterpenoids pre-schisanartanins E–J (**1**–**6**), and sphenadilactones D–F (**7**–**9**), together with 16 known ones including micrandilactones D (**10**) and E (**11**),[1] lancifodilactones B (**12**) and C (**13**),[14] lancifoldilactone O (**14**),[15] lancifodilactone N (**15**),[16] propindilactones A (**16**) and D (**17**),[17] schirubridilactones A (**18**) and D (**19**),[18] lancifodilactone G (**20**),[3] sphenadilactone A (**21**),[4] henridilactone A (**22**),[19] micranoic acid B (**23**),[20] wuweizilactone acid A (**24**),[11] lancifoic acid A (**25**),[21] and kadsuphilactone A (**26**).[22] In the present paper, we report the isolation, and structure elucidation of these new nortriterpenoids (**1**–**9**) and their biological activities (Fig. 1).

## 2. Results and discussion

Compound **1** was obtained as colorless gum, and the molecular formula was $C_{29}H_{36}O_9$ based on its HRESIMS data (*m*/*z* 551.2247, calcd 551.2257, $[M+Na]^+$). The IR spectrum of **1** showed bands characteristic of hydroxyl and carbonyl groups (3441 and 1759 $cm^{-1}$). Analysis of the $^1H$ NMR and $^{13}C$ NMR spectra of **1** (Tables 1 and 2) showed the presence of one ketone ($\delta_C$ 213.8), two lactone carbonyl carbons ($\delta_C$ 176.2, 171.3), and two pairs of double bonds ($\delta_C$ 148.2, 119.9, and 134.7, 131.1). Above evidence, together with the diagnostic signals of three-membered carbon ring ($\delta_C$ 25.5, s, C-13; $\delta_H$ 0.92, t like, *J*=8.0 Hz, H-17; $\delta_C$ 31.8, d, C-16), suggested that **1** may bear a pre-schisanartane skeleton (7/8/3 consecutive carbon cycle) as same as pre-schisanartanin A.[5] Comparison of the $^{13}C$ NMR data of **1** with those of pre-schisanartanin A suggested the two compounds are structurally similar and the differences may be

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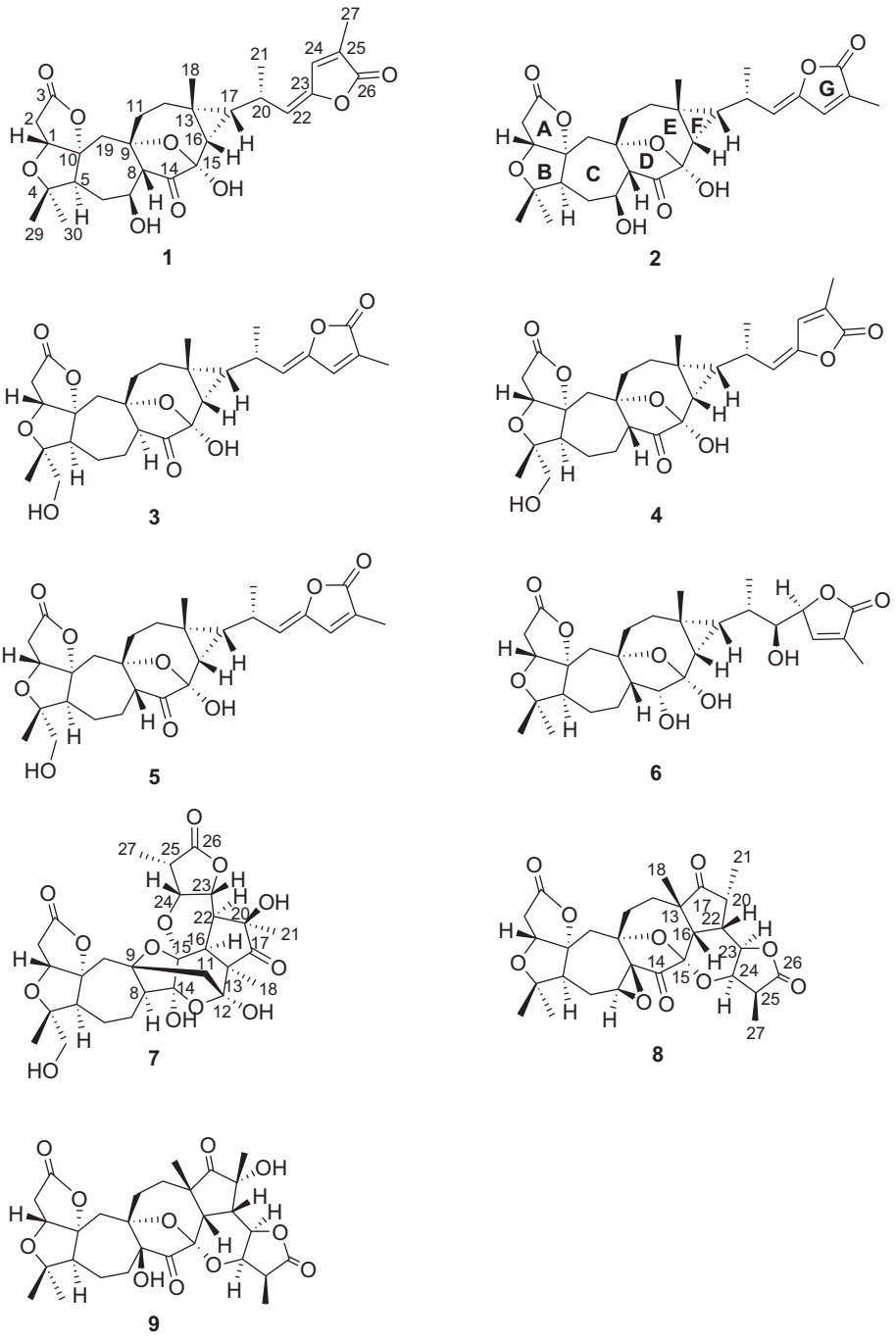



**Fig. 1.** The structures of new compounds **1**–**9**.

deduced by one oxygenated carbon (C-7) and a pair of additional double bond attributed to C-22 and C-23 in **1**. This assignment was supported by $^{1}H-^{1}H$ COSY spin system H-5/H-6/H-7/H-8 and H-17/H-20/H-22, coupled with HMBC correlations from H-22 to C-17, C-21, C-23, and C-24 (Fig. 2). Comprehensive analysis of 2D NMR data enabled the complete planar structure of **1**. Portions of the relative stereochemistry of **1** were assigned by ROESY NMR method (Fig. 3). The ROESY correlations of H-7/H-5, and H-8/H-11β indicated that OH-7 and H-8 were both β-directed. The double bond between C-22 and C-23 were deduced to be *E*-geometry on the basis of the ROESY correlation of H-20 with H-24. Although the σ-bond between C-17 and C-20 could rotate to certain extent, the steric bulk of rings E and F made it fairly fixed as judged by the obvious ROESY correlation of H-17/H-16, H-20/H-12α, which revealed Me-21 was α-orientation, the same as that of the pre-schisanartanin A, which was performed X-ray single crystal structure.[5] The relative stereochemistries of other chiral centers in **1** were found to be the same as those of compound pre-schisanartanin A by the comparison of chemical shifts and analysis of its ROESY spectrum. Therefore, the structure of **1** was determined as shown and given the name as pre-schisanartanin E.

The planar structure of **2** was almost the same as that of **1**, only differing in chemical shifts of double bonds. Comparison of their $^{13}C$ NMR spectra found that the chemical shifts of C-22 ($\delta_C$119.9) and C-24 ($\delta_C$134.7) in **1** were downfield shifted to C-22 ($\delta_C$121.6) and C-24 ($\delta_C$139.2), and the chemical shifts of C-23 ($\delta_C$148.2) and C-25

**Table 1**
$^{13}$C NMR data of compounds **1**–**9** in pyridine-$d_5$, $\delta$ in parts per million [a]

| No. | 1 | 2[a] | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 81.9 (d) | 82.1 (d) | 80.9 (d) | 82.2 (d) | 82.4 (d) | 83.7 (d) | 80.4 (d) | 80.8 (d) | 81.9 (d) |
| 2 | 35.8 (t) | 35.6 (t) | 35.9 (t) | 35.8 (t) | 35.5 (t) | 35.8 (t) | 35.6 (t) | 35.2 (t) | 35.3 (t) |
| 3 | 176.2 (s) | 175.5 (s) | 174.9 (s) | 176.3 (s) | 175.7 (s) | 176.0 (s) | 174.8 (s) | 175.2 (s) | 175.3 (s) |
| 4 | 84.6 (s) | 83.9 (s) | 87.5 (s) | 87.7 (s) | 87.0 (s) | 83.7 (s) | 87.7 (s) | 83.7 (s) | 84.1 (s) |
| 5 | 57.1 (d) | 58.3 (d) | 53.1 (d) | 55.6 (d) | 56.4 (d) | 61.9 (d) | 51.8 (d) | 54.1 (d) | 62.6 (d) |
| 6 | 37.2 (t) | 37.5 (t) | 21.0 (t) | 25.6 (t) | 25.6 (t) | 24.5 (t) | 22.5 (t) | 28.0 (t) | 19.8 (t) |
| 7 | 68.8 (d) | 68.5 (d) | 24.4 (t) | 27.2 (t) | 26.8 (t) | 31.6 (t) | 19.0 (t) | 63.9 (d) | 33.0 (t) |
| 8 | 65.0 (d) | 65.5 (d) | 52.9 (d) | 57.7 (d) | 58.3 (d) | 52.8 (d) | 43.2 (t) | 61.4 (s) | 78.3 (s) |
| 9 | 77.3 (s) | 77.4 (s) | 77.2 (s) | 78.7 (s) | 78.7 (s) | 79.8 (s) | 82.4 (d) | 80.2 (s) | 85.8 (s) |
| 10 | 96.8 (s) | 95.5 (s) | 98.1 (s) | 97.4 (s) | 96.1 (s) | 97.2 (s) | 96.9 (s) | 95.7 (s) | 97.0 (s) |
| 11 | 41.9 (t) | 42.2 (t) | 39.1 (t) | 42.1 (t) | 42.3 (t) | 40.1 (t) | 42.3 (t) | 35.5 (t) | 36.5 (t) |
| 12 | 23.9 (t) | 23.9 (t) | 24.1 (t) | 23.9 (t) | 23.9 (t) | 23.3 (t) | 98.2 (s) | 30.9 (t) | 32.0 (t) |
| 13 | 25.5 (s) | 25.3 (s) | 25.1 (s) | 25.7 (s) | 25.1 (s) | 23.8 (s) | 58.1 (s) | 50.5 (s) | 49.6 (s) |
| 14 | 213.8 (s) | 214.9 (s) | 215.7 (s) | 216.4 (s) | 217.2 (s) | 77.8 (d) | 40.4 (d) | 47.3 (d) | 45.5 (d) |
| 15 | 99.6 (s) | 99.1 (s) | 100.3 (s) | 99.8 (s) | 99.3 (s) | 102.1 (s) | 100.9 (s) | 97.3 (s) | 97.0 (s) |
| 16 | 31.8 (d) | 32.3 (d) | 31.6 (d) | 31.5 (d) | 32.0 (d) | 40.2 (d) | 104.0 (s) | 208.2 (s) | 212.5 (s) |
| 17 | 37.1 (d) | 37.3 (d) | 36.7 (d) | 37.0 (d) | 37.4 (d) | 32.2 (d) | 220.6 (s) | 221.4 (s) | 219.7 (s) |
| 18 | 28.2 (q) | 28.1 (q) | 28.5 (q) | 28.2 (q) | 28.3 (q) | 21.2 (q) | 18.7 (q) | 26.5 (q) | 27.3 (q) |
| 19 | 41.8 (t) | 41.6 (t) | 47.6 (t) | 42.4 (t) | 42.5 (t) | 42.7 (t) | 41.2 (t) | 38.9 (t) | 41.2 (t) |
| 20 | 28.4 (d) | 28.4 (d) | 28.3 (d) | 28.3 (d) | 28.0 (d) | 37.8 (d) | 76.1 (s) | 41.2 (d) | 75.0 (s) |
| 21 | 24.2 (q) | 21.9 (q) | 22.1 (q) | 24.3 (q) | 22.0 (q) | 17.4 (q) | 26.1 (q) | 12.6 (q) | 23.6 (s) |
| 22 | 119.9 (d) | 121.6 (d) | 121.3 (d) | 119.9 (d) | 121.6 (d) | 76.2 (d) | 41.4 (d) | 33.3 (d) | 41.7 (d) |
| 23 | 148.2 (s) | 146.8 (s) | 147.0 (s) | 148.2 (s) | 146.8 (s) | 81.7 (d) | 73.9 (d) | 74.5 (d) | 73.4 (d) |
| 24 | 134.7 (d) | 139.2 (d) | 139.3 (d) | 136.6 (d) | 139.2 (d) | 149.1 (d) | 72.8 (d) | 71.3 (d) | 72.6 (d) |
| 25 | 131.1 (s) | 128.6 (s) | 128.7 (s) | 131.0 (s) | 128.6 (s) | 129.6 (s) | 42.1 (d) | 42.7 (d) | 42.1 (d) |
| 26 | 171.3 (s) | 171.6 (s) | 171.5 (s) | 171.4 (s) | 171.6 (s) | 175.2 (s) | 178.2 (s) | 177.9 (s) | 177.4 (s) |
| 27 | 10.8 (q) | 10.3 (q) | 10.5 (q) | 10.8 (q) | 10.4 (q) | 10.8 (q) | 8.7 (q) | 8.5 (q) | 8.0 (q) |
| 29 | 21.7 (q) | 21.4 (q) | 16.9 (q) | 17.5 (q) | 17.2 (q) | 21.6 (q) | 67.7 (t) | 20.5 (q) | 21.3 (q) |
| 30 | 28.2 (q) | 28.1 (q) | 67.4 (t) | 67.8 (t) | 67.7 (t) | 28.4 (q) | 16.6 (q) | 27.5 (q) | 28.3 (q) |

[a] $^{13}$C NMR data of compound was recorded on a Bruker DRX−500 MHz spectrometer, while data of the other four compounds were recorded on a Bruker DRX−400 MHz spectrometer; assignment were based on DEPT, $^1$H−$^1$H COSY, HSQC, and HMBC experiments.

($\delta_C$131.1) in **1** were upfield shifted to C-23 ($\delta_C$146.8) and C-25 ($\delta_C$128.6) in **2**. In addition, the chemical shift of H-24 [$\delta_H$ 7.74 (br s)] in **1** was upfield shifted to $\delta_H$ 7.04 (d, $J$=1.4) in **2**. Analysis of ROESY spectrum of **2** showed obvious correlation of H-22 with H-24, which suggested the *Z*-geometry of the double bond between C-22 and C-23 (Fig. 3). The relative stereochemistries of other chiral centers in **2** were found to be the same as those of **1** by comparison of the chemical shifts and coupling constants and the analysis of ROESY spectrum. Thus, the structure of **2** was determined and named as pre-schisanartanin F.

Compound **3** was isolated as colorless gum and had the same molecular formula of $C_{29}H_{36}O_9$ as that of **1** and **2**, which was determined by analysis of $^{13}$C and DEPT NMR as well as HRESIMS data ($m/z$ 551.2255; calcd 551.2257, $[M+Na]^+$). The hydroxyl group at C-7 in **1** was absent deduced by the upfield chemical shift of C-7 from $\delta_C$ 68.8 in **1** to $\delta_C$ 24.4 in **3**, and of C-8 from $\delta_C$ 65.0 in **1** to $\delta_C$ 52.9 in **3**. The position of hydroxyl group was deduced to be located at C-30 by the HMBC correlations of $H_2$-30 ($\delta_H$ 3.77, ABd, $J$=11.9; 3.62, ABd, $J$=11.9) with C-4, C-5, and Me-29, which was further supported by the ROESY correlation of $H_2$-30 with H-5. H-8 was assumed to adopt α-orientation by obvious correlations of H-8 with H-5, which also showed obvious difference in chemical shift when compared with that of **1** (Table 2). The *Z*-geometry of the double bond between C-22 and C-23 was deduced by the ROESY correlation of H-22 with H-24, which is the same as that of **2**. Thus, the structure and relative stereochemistry of **3** was established and gave the name of pre-schisanartanin G.

The molecular formula of compound **4** was determined to be $C_{29}H_{36}O_9$ ($m/z$ 551.2266; calcd 551.2257, $[M+Na]^+$), the same as that of **3**. The $^{13}$C NMR spectrum indicated the presence of one ketone group ($\delta_C$ 216.4), two ester carbonyls ($\delta_C$ 171.4 and 176.3), and two oxygenated quaternary carbons at $\delta_C$ 97.4 and 99.8. These diagnostic signals suggested the presence of pre-schisanartane skeleton.[5–11] Comparison of the spectroscopic data of **4** with those of **3** showed the similarities except for the relative stereochemistry of C-8 and the geometry of the double bond between C-22 and C-23. The ROESY correlations of H-8 with H-7β and H-11β determined H-8 to be β-orientated, and the *E*-geometry of the double bond between C-22 and C-23 was deduced by the strong ROESY correlation between H-20β and H-24. Therefore, the structure of **4** was established and named pre-schisanartanin H.

Compound **5** was isolated as colorless gum and the molecular $C_{29}H_{36}O_9$ was deduced from HRESIMS ($m/z$ 551.2265, calcd 551.2257, $[M+Na]^+$). The chemical shift of C-8 ($\delta_C$ 58.3) in **5** was close to that of **4** ($\delta_C$ 57.7), while the chemical shift of the double bonds C-22 ($\delta_C$ 121.6), C-23 ($\delta_C$ 146.8), C-24 ($\delta_C$ 139.2), and C-25 ($\delta_C$ 128.6) in **5** were close to those of **3** [C-22 ($\delta_C$ 121.3), C-23 ($\delta_C$ 147.0), C-24 ($\delta_C$ 139.3), and C-25 ($\delta_C$ 128.7)]. Above evidence, coupled with analysis of its ROESY spectrum, established H-8 in **5** was β-orientated, and the double bond between C-22 and C-23 was *Z*-geometry. Accordingly, the structure of **5** was established and named pre-schisanartanin I.

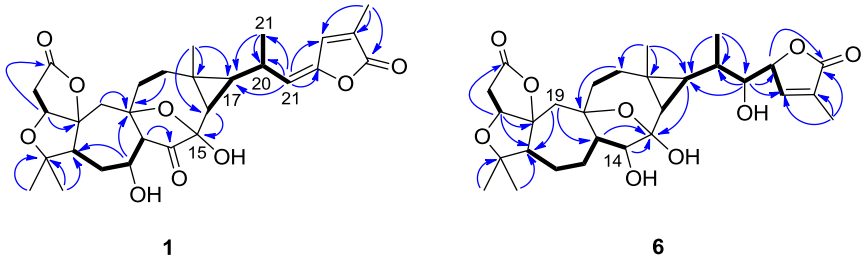
Compound **6** was assigned the molecular formula $C_{29}H_{40}O_9$ by HRESIMS at $m/z$ 555.2565 $[M+Na]^+$ (calcd 555.2570). $^1$H and $^{13}$C NMR showed the diagnostic signal of three-membered carbon ring ($\delta_C$ 23.8, s, C-13; $\delta_H$ 0.86−0.88, H-17; $\delta_C$ 32.2, d, C-16). Analysis of 2D NMR spectra further supported the presence of the unique 7/8/3 consecutive carbon cycle. The existence of two carbonyl groups and a pair of double bond indicated that **6** may be reduction product of **1**. Combined with 2D NMR analysis and molecular formula, the planar structure of **6** was elucidated as showed in Fig. 2. The relative stereochemistries of chiral centers in **6** were assigned according to ROESY spectrum and the spectra of known compound pre-schisanartanin A. The β-orientation of H-14 was deduced by the ROESY correlations of H-14 with H-8β and H-11β. The α-orientation of H-22 was deduced by the ROESY correlations of H-22 with Me-21 and H-17. Thus, the structure of **6** was determined as shown and named as pre-schisanartanin J.

Compound **7** was determined to be $C_{29}H_{36}O_{13}$ by HRESIMS at $m/z$ 615.2060 $[M+Na]^+$ (calcd 615.2053). The $^1$H and $^{13}$C NMR data

**Table 2**
$^1$H NMR data of compounds **1**–**9** in pyridine-$d_5$, $\delta$ in parts per million ($J$ in hertz)[a]

| No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.37 (d, *5.2*) | 4.28–4.31[b] | 4.21 (d, *5.6*) | 4.41 (d, *5.2*) | 4.35 (d, *5.8*) | 4.17 (d, *5.7*) | 4.14 (d, *5.5*) | 4.23 (d, *6.0*) | 4.26 (d, *6.1*) |
| 2α | 2.91 (d, *18.1*) | 2.84 (d, *18.2*) | 2.56 (d, 18.2) | 2.84 (d, *18.2*) | 2.72–2.77[b] | 2.70 (d, *18.0*) | 2.64 (d, *22.0*) | 2.82 (d, *18.5*) | 2.39 (d, *18.5*) |
| 2β | 3.06 (dd, *5.2*, *18.1*) | 3.98 (dd, *5.7*, *18.2*) | 2.80 (dd, *5.7*, *18.2*) | 3.03 (dd, *5.4*, *18.2*) | 3.95 (dd, *5.9*, *18.2*) | 3.10 (dd, *5.6*, *18.0*) | 2.85 (dd, *5.5*, *22.0*) | 3.10 (dd, *6.0*, *18.5*) | 3.05 (dd, *6.0*, *18.5*) |
| 5 | 2.30 (dd, *4.5*, *12.5*) | 2.26 (m) | 2.67 (dd, *1.7*, *13.1*) | 2.77 (dd, *4.2*, *12.9*) | 2.71–2.75[b] | 2.09 (dd, *4.6*, *13.3*) | 2.69 (d, *14.0*) | 2.42 (dd, *3.0*, *14.5*) | 2.19 (dd, *5.0*, *13.5*) |
| 6α | 2.01–2.09 (m) | 2.01–2.05 (m) | 1.71–1.74[b] | 1.56–1.65[b] | 1.61–1.67 (m) | 1.17–1.21[b] | 1.45[b] | 2.16–2.19[b] | 1.84–1.92 (m) |
| 6β | 2.01–2.09 (m) | 2.01–2.05 (m) | 1.71–1.74[b] | 1.56–1.65[b] | 1.47–1.51[b] | 1.46–1.50[b] | 1.21–1.32 (m) | 1.40–1.47 (m) | 1.58–1.64 (m) |
| 7α | 4.62 (dd, *9.7*, *9.7*) | 4.65–4.67 (m) | 1.56–1.59 (m) | 2.03–2.08[b] | 2.07–2.10[b] | 2.14–2.19 (m) | 1.80–1.88 (m) | 3.87 (dd, *6.0*, *6.0*) | 2.10–2.15 (m) |
| 7β | — | — | 1.18–1.24 (m) | 1.90–1.96[b] | 1.91–1.94[b] | 1.70–1.74[b] | 1.96–2.04[b] | — | 1.72–1.77[b] |
| 8 | 2.80 (d, *9.8*)[c] | 2.79–2.81[b,c] | 3.73 (dd, 6.4, 11.2)[d] | 2.55 (dd, *5.3*, *12.8*)[c] | 2.53–2.58[b,c] | 2.21–2.24[b,c] | 3.34 (dd, *7.5*, *15.0*) | — | — |
| 11α | 1.60 (dd, *14.5*, *14.5*) | 1.57–1.60[b] | 1.64–1.71 (m) | 1.54–1.58[b] | 1.54–1.59[b] | 1.47–1.51[b] | 2.93 (d, *17.5*) | 1.79–1.84 (m) | 1.41–1.44[b] |
| 11β | 1.80 (dd, *6.2*, *14.5*) | 1.82–1.89[b] | 1.76–1.82 (m) | 1.76–1.82[b] | 1.83–1.88[b] | 1.66–1.69[b] | 2.23 (d, *17.5*) | 2.02–2.08 (m) | 2.00–2.04[b] |
| 12α | 1.28–1.35 (m) | 1.22–1.27 (m) | 1.40 (dd, 5.9, *13.3*) | 1.26–1.31 (m) | 1.25 (dd, *5.7*, *14.3*) | 1.58–1.62[b] | — | 1.67[b] | 1.48–1.54 (m) |
| 12β | 1.93 (dd, *14.5*, *14.5*) | 2.30–2.35[b] | 2.38 (dd, *13.3*, *13.3*) | 1.90–1.96[b] | 2.33 (dd, *14.3*, *14.3*) | 1.72–1.76[b] | — | 1.91–1.95 (m) | 1.72–1.77[b] |
| 14 | — | — | — | — | — | 4.59 (d, *6.6*) | 2.60 (d, *7.5*) | 2.72 (d, *8.0*) | 2.55 (d, *8.0*) |
| 16 | 1.54[b] | 1.53 (d, *8.8*) | 1.48 (d, *8.9*) | 1.40 (d, *8.9*) | 1.42 (d, *8.8*) | 1.21[b] | — | — | — |
| 17 | 0.94 (dd, *10.1*, *10.1*) | 0.88 (dd, *9.2*, *9.2*) | 0.92 (dd, *10.3*, *10.3*) | 0.88 (dd, *10.0*, *10.0*) | 0.87 (dd, *9.8*, *9.8*) | 0.86–0.88[b] | — | — | — |
| 18 | 0.96 (s) | 0.95 (s) | 1.00 (s) | 0.93 (s) | 0.94 (s) | 0.89 (s) | 1.45 (s) | 0.92 (s) | 1.04 (s) |
| 19α | 2.20 (ABd, *15.8*) | 2.18 (ABd, *15.7*) | 3.03 (ABd, *16.1*) | 2.18 (ABd, *15.9*) | 2.14 (ABd, *15.8*) | 1.92 (ABd, *15.6*) | 1.95 (ABd, *16.5*) | 2.24 (ABd, *16.0*) | 2.10–2.15[b] |
| 19β | 2.35 (ABd, *15.8*) | 2.53 (ABd, *15.7*) | 2.15 (ABd, *16.1*) | 2.29 (ABd, *15.9*) | 2.54 (ABd, *15.8*) | 2.24 (ABd, *15.6*) | 2.19 (ABd, *16.5*) | 2.14 (ABd, *16.0*) | 2.39–2.43[b] |
| 20 | 3.79–3.81 (m) | 4.28–4.31[b] | 4.05–4.11 (m) | 3.75–3.80 (m) | 4.25–4.32 (m) | 1.58–1.60[b] | — | 2.80–2.84[b] | — |
| 21 | 1.53[b] | 1.57 (d, *6.5*) | 1.53 (d, *6.6*) | 1.50 (d, *6.6*) | 1.54 (d, *6.5*) | 1.00 (br s)[b] | 1.51 (s) | 1.31 (d, *8.0*) | 1.34 (s) |
| 22 | 5.78 (d, *10.5*) | 5.18 (d, *11.1*) | 5.26–5.28[b] | 5.77 (d, *10.6*) | 5.17 (d, *11.1*) | 3.47 (d, *8.8*) | 3.03 (d, *10.5*) | 3.40 (dd, *8.5*, *11.0*) | 3.11–3.16 (m) |
| 23 | — | — | — | — | — | 5.25 (br s) | 4.80 (br s) | 4.75 (br s) | 4.68 (br s) |
| 24 | 7.74 (br s) | 7.04 (d, *1.4*) | 7.18 (d, *1.4*) | 7.71 (br s) | 7.05 (d, *1.2*) | 7.18 (d, *1.6*) | 4.94 (br s) | 4.87 (br s) | 4.75–4.76 (m) |
| 25 | — | — | — | — | — | — | 3.15 (m) | 3.20–3.27 (m) | 2.89–2.90[b] |
| 27 | 2.12 (s) | 1.82 (s) | 1.88 (d, 0.9) | 2.09 (s) | 1.83 (s) | 1.84 (s) | 1.61 (d, *9.0*) | 1.62 (d, *7.0*) | 1.10 (d, *7.5*) |
| 29 | 1.08 (s) | 1.04 (s) | 1.17 (s) | 1.18 (s) | 1.15 (s) | 1.00 (br s)[b] | 3.69 (d, *14.5*) 3.49–3.56[b] | 0.96 (s) | 1.12 (s) |
| 30 | 1.13 (s) | 1.10 (s) | 3.77 (ABd, *11.9*) 3.62 (ABd, *11.9*) | 3.72 (ABd, *11.7*) 3.56–3.59[b] | 3.73 (ABd, *11.7*) 3.57 (ABd, *11.7*) | 1.13 (s) | 1.01 (s) | 1.27 (s) | 1.26 (s) |

[a] The assignment were based on DEPT, $^1$H–$^1$H COSY, HSQC, and HMBC experiments.
[b] Overlapped signals.
[c] β-Oriented.
[d] α-Oriented.




**1** **6**

**Fig. 2.** Key correlations of HMBC (H→C) and $^1$H–$^1$H COSY (▬) for **1** and **6**.

showed similarities with those of sphenadilactone A,[4] indicating one ketone ($\delta_C$ 220.6, s, C-17), two ester groups ($\delta_C$ 174.8, s, C-3; $\delta_C$ 178.2, s, C-26), and four methyl groups (Tables 1 and 2). The difference observed was the presence of an additional quaternary carbon ($\delta_C$ 98.2, s) and lack of an oxygenated methine group (C-12), which suggested the existence of a tertiary hydroxyl group. These observations, together with key HMBC correlations of H-14, $H_3$-21, and $H_2$-11 with C-12, indicated that this OH group is positioned at C-12. Thus, the structure of **7** was determined and named sphenadilactone D.

Compound **8** was isolated as white crystals and was assigned the molecular formula $C_{29}H_{34}O_{10}$ through an analysis of its HRESIMS ($m/z$ 565.2049, $[M+Na]^+$). The $^1$H and $^{13}$C NMR data of **8** were very similar to those of schirubridilactone D (**19**).[18] Analysis of its $^1$H–$^1$H COSY, HSQC, and HMBC spectra suggested that compounds **8** and **19** possess the same planar structure. The obvious differences in the $^{13}$C NMR spectrum of **8** with those of **19** were the upfield chemical shift of C-20 and C-22 to $\delta_C$ 41.2 and 33.3 in **8** other than $\delta_C$ 44.8 and 40.1 in **19**, and the upfield chemical shift of Me-21 to $\delta_C$ 12.6 instead of $\delta_C$ 14.7 in **19**. These data indicated that **8** and **19** are a pair of epimers at C-20 position, and the Me-21 in α-orientation in **8** rather than a β-orientation in **19**. This was further confirmed by ROESY correlations of Me-21/H-23 and Me-21/H-24. Therefore, the structure of **8** was determined and named sphenadilactone E.

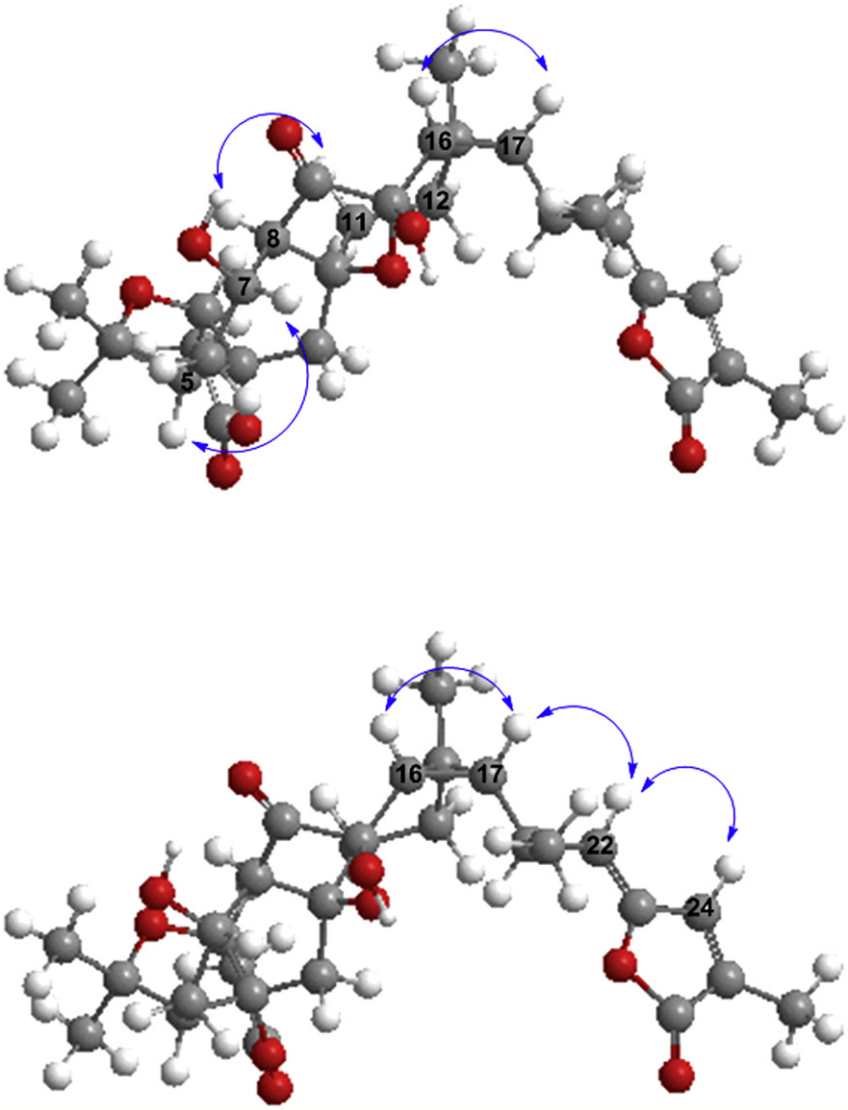



**Fig. 3.** Key ROESY correlations (H⟷H) of **1** and **2**; some protons are hidden for conciseness.

Compound **9** had the molecular formula $C_{29}H_{36}O_{11}$, as derived from HRESIMS (*m*/*z* 583.2151 $[M+Na]^+$, calcd for 583.2155) and $^{13}C$ NMR data. The $^1H$ and $^{13}C$ NMR data of **9** are similar to those of propindilactone B. The differences were one oxygenated methylene ($\delta_C$ 67.5, C-30) and one oxygenated quaternary carbon ($\delta_C$ 75.7, C-22) in propindilactone B[17] were disappeared in **9**. Instead, one methyl ($\delta_C$ 28.3, C-30) and one methine ($\delta_C$ 41.7, C-22) signals were found in **9**. This was further confirmed by analysis of 2D NMR spectra. The relative stereochemistry of **9** was elucidated by its ROESY spectrum and the comparison of the chemical shifts with those of propindilactone B. [17] Therefore, the structure of **9** was established and given the name as sphenadilactone F.

Since some compounds from genus *Schisandra* showed obvious cytotoxicity activity, compounds **1**–**26** were all screened for their cytotoxicity against HL-60 (human promyelocytic leukemia cell line), A549 (lung cancer cell line), SMMC-7721 (human hepatoma cell line), SW480 (human colon adenocarcinoma cell line), MCF-7 (breast cancer cell line) cell lines, using the MTT method with cisplatin as positive control.[23] Among them, kadsuphilactone A (**26**) showed modest cytotoxicity against SMMC-7721, A549, and MCF-7 cell lines with $IC_{50}$ value of 29.9, 32.5, and 24.2 μM, respectively. The other compounds showed not obvious cytotoxicity against the above mentioned cell lines with $IC_{50}$>40 μM.

## 3. Experimental section

### 3.1. General experimental procedures

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV data were obtained on a Shimadzu UV-2401A spectrophotometer. A BioRad FtS-135 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Unless otherwise specified, chemical shifts ($\delta$) were expressed in parts per million with reference to the solvent signals. High-resolution electrospray-ionization (HRESIMS) was performed on a VG Autospec-3000 spectrometer under 70 eV. Column chromatography was performed with silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-$C_{18}$, 9.4 mm×25 cm column. Preparative HPLC was performed on a Shimadzu LC-8A preparative

liquid chromatograph with a Shimadzu PRC-ODS (K) column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% $H_2SO_4$ in EtOH. All solvents including petroleum ether (60–90 °C) were distilled prior to use.

### 3.2. Plant material

The roots and stems of *S. sphenanthera* Rehd et Wils were collected from Sichuan province, People's Republic of China, in September 2007. Voucher specimens (KIB20070926) were deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, and were identified by Prof. Xi-Wen Li.

### 3.3. Extraction and isolation

The air-dried roots and stems of *S. sphenanthera* (4 kg) were extracted with 70% aqueous acetone (4×15 l, 3 days each) at room temperature. The solvent was removed in vacuo to afford a crude extract (270 g), which was dissolved in $H_2O$, and then extracted successfully with petroleum ether and EtOAc. The EtOAc-soluble part (180 g) was purified by CC (on $SiO_2$ with $CHCl_3$/acetone 1→0) to obtain five main fractions (Fr.A–E). Fr.B ($CHCl_3$/acetone 9:1 to 8:2, 38 g) was chromatographed on silica gel column to afford five subfractions B1–B5. Fr.B2 (10 g) was repeatedly chromatographed on C-18 column, and then further purified by semipreparative HPLC (3 ml/min, detector UV $\lambda_{max}$ 230 nm, $CH_3OH/H_2O$, 70:30, and $CH_3CN/H_2O$, 50:50) to yield compounds **8** (5 mg), **12** (15 mg), **13** (3 mg), **15** (9 mg), and **17** (8 mg). Fr.B4 (5 g) was applied to glass column containing Rp-18, eluted with a 50–80% MeOH/$H_2O$ gradient system, to afford three fractions Fr.B4a–Fr.B4c. Compounds **19** (23 mg) and **22** (15 mg) were purified by repeated recrystallization from Fr.B4b (1.2 g) and Fr.B4c (0.8 g), respectively. Fr.C ($CHCl_3$/acetone 9:1 to 7:3, 45 g) was purified by repeated CC, first on Sephadex LH-20 eluted with $CHCl_3$/MeOH (1:1), then on silica gel eluted by petroleum ether/acetone in gradient system to afford fractions C1–C8. Fr.C3 (8 g) was further purified on semipreparative HPLC (3 ml/min, detector UV $\lambda_{max}$ 280 nm, 50% MeOH/$H_2O$) to yield compounds **1** (36.6 min, 5 mg) and **2** (49.6 min, 4 mg). Due to the significant different UV absorption (UV $\lambda_{max}$ 270 nm) from other known nortriterpenoids from the same plant, which demonstrated existence of large conjugated unsaturation system, we found Fr.C5 (10 g) contains constituents owning this kind of UV absorptions. On the guide of this method, we further purified Fr.C5 on semipreparative HPLC (3 ml/min, detector UV $\lambda_{max}$ 280 nm, 47% MeOH/$H_2O$) to get compounds **3** (42.3 min, 5 mg), **4** (35.4 min, 6 mg), **5** (45.7 min, 5 mg), and **6** (28 min, 4 mg), respectively. Fr.C7 (7 g) was repeatedly chromatographed on Rp-18 glass column with MeOH/$H_2O$ gradient elution system 40–70%, to afford four fractions Fr.C7a–Fr.C7d. Compounds **10** (4 mg) and **11** (10 mg) were further purified on semipreparative HPLC (3 ml/min, detector UV $\lambda_{max}$ 230 nm, 55% MeOH/$H_2O$) from Fr.C7b (2.3 g), and compounds **9** (5 mg), **14** (7 mg), **21** (7 mg), and **26** (6 mg) were further purified on semipreparative HPLC (3 ml/min, detector UV $\lambda_{max}$ 230 nm, 50% MeOH/$H_2O$) from Fr.C7c (1.4 g). Fr.D (21 g) was repeatedly chromatographed on silica gel (200–300 mesh) with $CHCl_3$/MeOH gradient elution system (9:1→1:1) to afford four subfractions Fr.D1–Fr.D4. Fr.D1 (4.3 g) was repeatedly chromatographed on Sephadex LH-20 and finally by semipreparative HPLC (3 ml/min, detector UV $\lambda_{max}$ 210 nm, 40% and 50% MeOH/$H_2O$), to yield compounds **7** (5 mg), **18** (2 mg), and **20** (8 mg). Similarly, Fr.D3 (3.8 g) was also purified using the same method to yield compounds **16** (8 mg), **23** (21 mg), **24** (18 mg), and **25** (26 mg).

*3.3.1. Pre-schisanartanin E (**1**).* Colorless gum; $[\alpha]_D^{18}$ +131.8 (*c* 0.11, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 280 (3.93); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3440, 2972, 2946, 1759, 1662, 1456, 1375, 1213, 1081, 1065, 995, 977 $cm^{-1}$; ESIMS *m/z* 551 $[M+Na]^+$; HRESIMS *m/z* 551.2247 $[M+Na]^+$, calcd for $C_{29}H_{36}O_9Na$ *m/z* 551.2257.

*3.3.2. Pre-schisanartanin F (**2**).* Colorless gum; $[\alpha]_D^{17}$ +34.4 (*c* 0.09, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 278 (3.76); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3432, 2928, 1763, 1636, 1628, 1457, 1376, 1232, 1084, 1063, 995, 977 $cm^{-1}$; ESIMS *m/z* 551 $[M+Na]^+$; HRESIMS *m/z* 551.2250 $[M+Na]^+$, calcd for $C_{29}H_{36}O_9Na$ *m/z* 551.2257.

*3.3.3. Pre-schisanartanin G (**3**).* Colorless gum; $[\alpha]_D^{18}$ +38.1 (*c* 0.33, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 278 (3.78); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3435, 2950, 2933, 1763, 1670, 1621, 1455, 1381, 1209, 1072, 1063, 975, 940 $cm^{-1}$; ESIMS *m/z* 551 $[M+Na]^+$; HRESIMS *m/z* 551.2255 $[M+Na]^+$, calcd for $C_{29}H_{36}O_9Na$ *m/z* 551.2257.

*3.3.4. Pre-schisanartanin H (**4**).* Colorless gum; $[\alpha]_D^{18}$ +36.03 (*c* 0.07, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 281 (3.64); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3436, 2933, 2870, 1761, 1621, 1456, 1382, 1204, 1071, 997 $cm^{-1}$; ESIMS *m/z* 551 $[M+Na]^+$; HRESIMS *m/z* 551.2266 $[M+Na]^+$, calcd for $C_{29}H_{36}O_9Na$ *m/z* 551.2257.

*3.3.5. Pre-schisanartanin I (**5**).* Colorless gum; $[\alpha]_D^{18}$ +34.6 (*c* 0.16, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 278 (3.83); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3438, 2936, 2868, 1762, 1621, 1455, 1383, 1207, 1115, 1070, 990, 976 $cm^{-1}$; ESIMS *m/z* 551 $[M+Na]^+$; HRESIMS *m/z* 551.2265 $[M+Na]^+$, calcd for $C_{29}H_{36}O_9Na$ *m/z* 551.2257.

*3.3.6. Pre-schisanartanin J (**6**).* White solid; $[\alpha]_D^{18}$ −46.4 (*c* 0.26, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 210 (3.71); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3440, 2970, 2933, 1757, 1630, 1456, 1384, 1210, 1104, 1063, 1007 $cm^{-1}$; ESIMS *m/z* 551 $[M+Na]^+$; HRESIMS *m/z* 555.2565 $[M+Na]^+$, calcd for $C_{29}H_{40}O_9Na$ *m/z* 555.2270.

*3.3.7. Sphenadilactone D (**7**).* White powder; $[\alpha]_D^{18}$ +91.35 (*c* 0.28, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 209 (2.91); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3462, 2950, 2923, 1773, 1753, 1643, 1384, 1248, 1185, 1057, 611 $cm^{-1}$; ESIMS *m/z* 615 $[M+Na]^+$; HRESIMS *m/z* 615.2060 $[M+Na]^+$, calcd for $C_{29}H_{36}O_{13}Na$ *m/z* 615.2053.

*3.3.8. Sphenadilactone E (**8**).* White crystals; $[\alpha]_D^{18}$ +52.62 (*c* 0.12, $CH_3OH$); UV ($CH_3OH$) $\lambda_{max}$ (log $\varepsilon$) 201 (309); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 2930, 2863, 1773, 1453, 1380, 1238, 1200, 1085, 611 $cm^{-1}$; ESIMS *m/z* 565 $[M+Na]^+$; HRESIMS *m/z* 565.2050 $[M+Na]^+$, calcd for $C_{29}H_{34}O_{10}Na$ *m/z* 565.2049.

*3.3.9. Sphenadilactone F (**9**).* White powder; $[\alpha]_D^{18}$ +20.29 (*c* 0.27, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ (log $\varepsilon$) 196 (3.03); $^1H$ and $^{13}C$ NMR data see Tables 1 and 2; IR (KBr) $\nu_{max}$ 3375, 2943, 2923, 1770, 1765, 1630, 1185, 1047, 994 $cm^{-1}$; ESIMS *m/z* 583 $[M+Na]^+$; HRESIMS *m/z* 583.2151 $[M+Na]^+$, calcd for $C_{29}H_{36}O_{11}Na$ *m/z* 583.2155.

### 3.4. Cytotoxicity assay

The following human tumor cell lines were used: HL-60, MMC-7721, A549, MCF-7, and SW480. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% $CO_2$. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan

formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO).[23] Briefly, 100 μl of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of $1\times10^5$ cells/ml in 100 μl of medium. Each cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μl of 20% SDS/50% DMF after removal of 100 μl of medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The $IC_{50}$ value of each compound was calculated by Reed and Muench's method.[24]

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## Supplementary data

Supplementary data associated with this article can be found in online version, at doi:10.1016/j.tet.2011.11.026.

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