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New Lignans from the Leaves and Stems of *Schisandra chinensis* and Their Anti-HIV-1 Activities†

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Six new lignans (**1**—**6**), as well as five known ones (**7**—**11**) were isolated from the leaves and stems of *Schisandra chinensis*. The structures of **1**—**6** were established on the basis of spectroscopic methods including 1D- and 2D-NMR techniques and CD experiments. Compound **1** was the first example of naturally occurring *N*-containing lignans featuring a nicotinoyl group. All the new compounds were evaluated for their anti-HIV-1 activities and showed EC_{50} values in the range 17.89—138.23 μg/mL.

Keywords Schisandraceae, *Schisandra chinensis*, lignan, anti-HIV-1

Introduction

The family Schisandraceae contains the genera *Schisandra* and *Kadsura*. Several species of this family have been reported to exhibit beneficial pharmacological effects, including enhancement of the cholinergic nervous system, antihepatitis, and antioxidant and detoxificant activities.[1-3] Since the 1970's, plants of the genus *Schisandra* have been a hot topic in the field of medicinal chemistry and drug discovery[1-3] and have come to the foreground of interest of phytochemical research due to the discovery of series of bioactive lignans[2,4] and novel nortriterpenoids.[5,6] *Schisandra chinensis* (Turcz.) Baill, which is endemic to the northeast of China, Korea, Japan, and the far east of Russia, is the most famous species in the genus *Schisandra*.[1] Its fruit has long been used as sedative and tonic agent in case of physical exhaustion and to prevent fatigue in traditional Chinese medicine.[3] Our previous studies on *S. chinensis* collected in the Yabuli mountain area of Heilongjiang province in China have led to the isolation of various types of *schinortriterpenoids*.[7,8] Further studies led to the isolation of six new lignans (**1**—**6**) and five known ones (**7**—**11**) from the EtOAc-soluble portion of the extract (Figure 1). Most notably, nicotinoylgomisin Q (**1**) was the first example of naturally occurring *N*-containing lignans possessing a nicotinoyl group. Described in this paper are the isolation, structure elucidation, and anti-HIV-1 activities of the new compounds.

Experimental

General experimental procedures

Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV data were obtained on a Shimadzu UV2401PC spectrophotometer. Experimental CD spectra were measured on a Chirascan instrument. A Bruker Tensor-27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D- and 2D-NMR spectra were recorded on Bruker AM-400, DRX-500, and AVANCE III-600 spectrometers. Unless otherwise specified, chemical shifts (δ) were expressed in parts per million with reference to the solvent signals. ESIMS were performed on Waters Xevo TQ-S. HRESIMS were performed on Waters AutoSpec Premier P776. HRESIMS was performed on an API QSTAR Pulsar i spectrometer. Column chromatography was performed with silica gel (200—300 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40—63 μm, Merck, Darmstadt, Germany), MCI gel (75—150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 gel (40—70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden). Semipreparative HPLC was performed on an Agilent 1200 liquid chromatograph with a Zorbax

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† Dedicated to Professor Chengye Yuan and Professor Li-Xin Dai on the occasion of their 90th birthdays.

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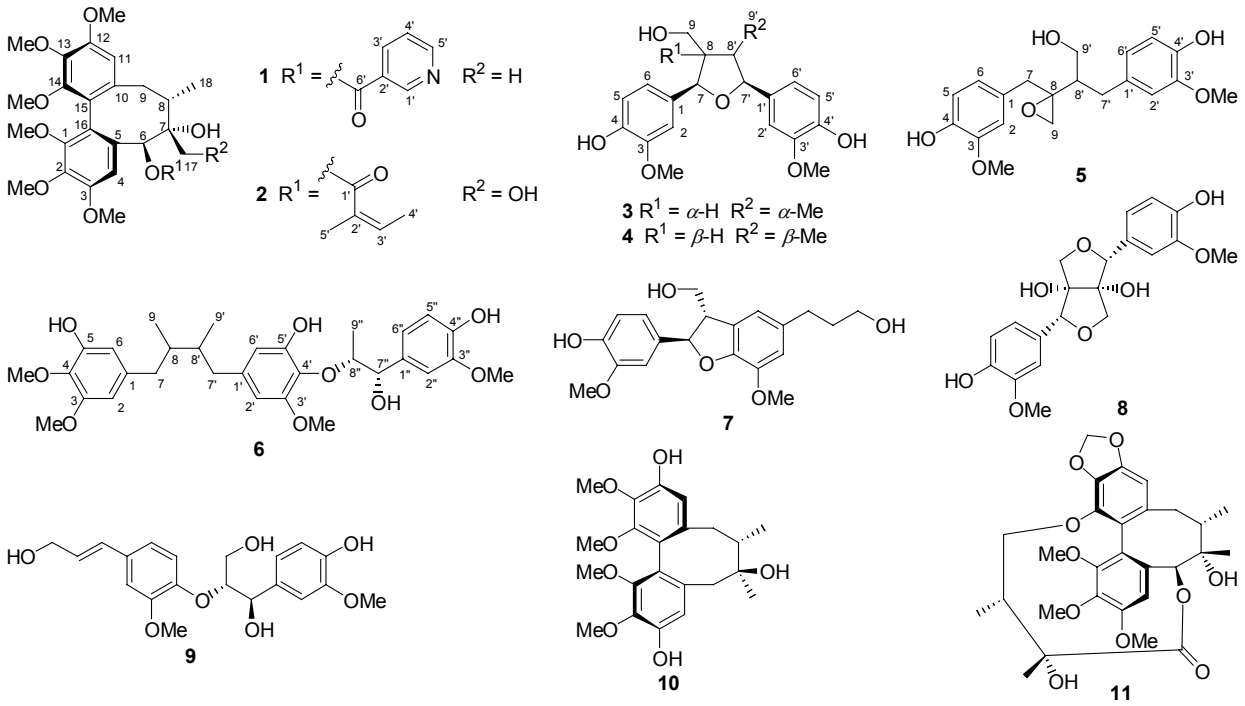

Figure 1 The structures of compounds **1**–**11**.

SB-C18, 9.4 mm × 25 cm 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 ℃) were distilled prior to use.

Plant material

The leaves and stems of *Schisandra chinensis* were collected from Yabuli mountain area of Heilongjiang Province, People's Republic of China, in August 2010. Voucher specimens (KIB 20100813) 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.

Extraction and isolation

The air-dried and powdered leaves and stems (10 kg) were extracted with 70% aqueous Me_2CO (10 L×3, 3 d each) at room temperature, and concentrated under reduced pressure to give crude extract (1.6 kg), which was partitioned between H_2O and EtOAc. The EtOAc part (438 g) was separated by silica gel CC with a gradient elution of $CHCl_3$/Me_2CO (1∶0, 9∶1, 8∶2, 7∶3, 6∶4, 1∶1, 0∶1, *V*∶*V*) to furnish seven fractions A—G, and each fraction was decolorized on MCI gel and eluted with 90% MeOH-H_2O.

Fraction D (19 g) was separated over Rp-18 CC with a MeOH/H_2O (3∶7, 4∶6, 5∶5, 6∶4, 7∶3, 8∶2, 1∶0, *V*∶*V*) gradient to give seven fractions, D1—D7. Fraction D4 (2.6 g) was separated over Sephadex LH-20 CC, eluting with $CHCl_3$/MeOH (1∶1, *V*∶*V*) to afford three fractions, D41—D43. Fraction D43 (163 mg) was separated over silica gel CC, eluting with a $CHCl_3$/Me_2CO (15∶1, 10∶1, 5∶1, 1∶1, 0∶1, *V*∶*V*) gradient to give five fractions, D431—D435. Fraction D431 (46 mg) was further purified by semipreparative HPLC [3 mL/min, detector UV λ_{max} 210 nm, *V*(MeOH)∶*V*(H_2O)=50∶50] to afford **1** (2 mg), **2** (4 mg), **10** (3 mg), and **11** (25 mg). Fraction D432 (21 mg) was purified by semipreparative HPLC [3 mL/min, detector UV λ_{max} 210 nm, *V*(MeCN)∶*V*(H_2O)=28∶72] to afford **3** (2 mg). Fraction D433 (69 mg) was further separated by silica gel CC eluting with a $CHCl_3$/*i*-PrOH (90∶1, 80∶1, 70∶1, *V*∶*V*) gradient to afford three fractions, D4331—D4333. Fraction D4332 (58 mg) was purified by semipreparative HPLC [3 mL/min, detector UV λ_{max} 210 nm, *V*(MeCN)∶*V*(H_2O)=22∶78] to afford **4** (2 mg), **5** (3 mg), and **7** (28 mg). Fraction D434 (26 mg) was purified by semipreparative HPLC [3 mL/min, detector UV λ_{max} 210 nm, *V*(MeCN)∶*V*(H_2O)=50∶50] to afford **6** (2 mg), **8** (8 mg), and **9** (4 mg).

Nicotinoylgomisin Q (1) Yellow gum; $[\alpha]_D^{26}$ −78 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε): 217 (3.78), 250 (3.23) nm; CD (*c* 0.02, MeOH) ($\Delta\varepsilon$) 252 (−16.49), 236 (−18.16), 218 (+11.46) nm; ^{1}H and ^{13}C NMR data (CD_3OD, 600 and 150 MHz), see Table 1; positive ESIMS *m/z* 576 $[M+Na]^+$; IR (KBr) ν_{max}: 3441, 2937, 1725, 1629, 1597, 1461, 1406, 1271, 1197, 1128, 1104 cm^{-1}; positive HRESIMS *m/z* 576.2194 $[M+Na]^+$ (calcd for $C_{30}H_{35}NO_9Na$, 576.2209).

17-Hydroxyangeloylgomisin Q (2) Yellow gum; $[\alpha]_D^{26}$ −14 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.00) nm; CD (*c* 0.01, MeOH) ($\Delta\varepsilon$): 253 (−20.45), 236 (−16.06), 216 (+34.60) nm; ^{1}H and ^{13}C NMR data (CD_3OD, 600 and 150 MHz), see Table 1; IR (KBr) ν_{max}: 3462, 2940, 1712, 1597, 1491, 1460, 1406, 1383, 1331, 1268, 1234, 1197, 1160, 1127, 1044, 1007 cm^{-1}; posi-

tive ESIMS *m/z* 569 $[M+Na]^+$; HREIMS *m/z* 546.2463 $[M]^+$(calcd for $C_{29}H_{38}O_{10}$, 546.2465).

(7*R*,7'*S*,8*R*,8'*S*)-3,3'-Dimethoxy-7,7'-epoxylignan-4,4',9-triol (3) Yellow gum; $[\alpha]_D^{27}$ +5.3 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε): 205 (4.03), 230 (3.52), 281 (3.13) nm; CD (*c* 0.01, MeOH) ($\Delta\varepsilon$) 235 (+1.59), 208 (+2.62), 198 (–3.28) nm; ^1H and ^{13}C NMR data (CD_3OD 400 and 125 MHz), see Table 2; IR (KBr) ν_{max}: 3426, 2956, 2930, 1609, 1517, 1463, 1432, 1383, 1275, 1237, 1208, 1158, 1122, 1032, 822 cm^{-1}; positive ESIMS *m/z* 383 $[M+Na]^+$; HREIMS *m/z* 360.1575 $[M]^+$ (calcd for $C_{20}H_{24}O_6$, 360.1573).

(7*R*,7'*S*,8*S*,8'*R*)-3,3'-Dimethoxy-7,7'-epoxylignan-4,4',9-triol (4) Yellow gum; $[\alpha]_D^{27}$ +47 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 205 (3.95), 231 (3.44), 281 (3.02) nm; CD (*c* 0.01, MeOH) ($\Delta\varepsilon$) 235 (+4.49), 207 (+11.14), 196 (–9.86) nm; ^1H and ^{13}C NMR data (CD_3OD, 600 and 150 MHz), see Table 2; IR (KBr) ν_{max}: 3427, 2965, 2931, 1611, 1516, 1453, 1431, 1384, 1275, 1237, 1208, 1159, 1122, 1068, 1035, 824 cm^{-1}; positive ESIMS *m/z* 383 $[M+Na]^+$; HREIMS *m/z* 360.1573 $[M]^+$ (calcd for $C_{20}H_{24}O_6$, 360.1573).

3,3'-Dimethoxy-8,9-epoxylignan-4,4',9'-triol (5) Yellow gum; $[\alpha]_D^{23}$ –49 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 204 (3.95), 226 (3.45), 282 (3.08) nm; ^1H and ^{13}C NMR data (CD_3OD, 400 and 100 MHz), see Table 2; IR (KBr) ν_{max}: 3443, 2935, 1631, 1516, 1453, 1384, 1273, 1237, 1154, 1124, 1033 cm^{-1}; positive ESIMS *m/z* 383 $[M+Na]^+$; HREIMS *m/z* 360.1567 $[M]^+$ (calcd for $C_{20}H_{24}O_6$, 360.1573).

(7"*S*,8"*R*)-4",5,5'-Trihydroxy-3,3',3',4-tetramethoxy-4',8'-oxy-8,8'-sesquineolignan-7'-ol (6) Yellow gum; $[\alpha]_D^{27}$ –21 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.28), 278 (2.97) nm; CD (*c* 0.01, MeOH) ($\Delta\varepsilon$) 241 (–3.70), 212 (–5.65), 204 (+5.03) nm; ^1H and ^{13}C NMR data (CD_3OD, 600 and 150 MHz), see Table 3; IR (KBr) ν_{max}: 3425, 2957, 2935, 1612, 1592, 1512, 1462, 1431, 1378, 1350, 1272, 1237, 1203, 1159, 1099, 1059, 1034 cm^{-1}; positive ESIMS *m/z* 579 $[M+Na]^+$; HREIMS *m/z* 556.2670 $[M]^+$(calcd for $C_{31}H_{40}O_9$, 556.2672).

Results and Discussion

Compound **1** was obtained as a yellow gum. It showed a pseudomolecular ion peak at *m/z* 576 $[M+Na]^+$ in positive ESIMS, indicating that it might be a *N*-containing compound. The positive HRESIMS (*m/z* 576.2194 $[M+Na]^+$) further confirmed this deduction and assigned a molecular formula of $C_{30}H_{35}NO_9$ (calcd for $C_{30}H_{35}NO_9Na$, 576.2209). The IR spectrum showed absorption bands of OH (3441 cm^{-1}) and aromatic (1597 and 1461 cm^{-1}) groups, and the UV spectrum displayed absorption bands at 217 and 250 nm. The ^{13}C and DEPT NMR spectra (Table 1) exhibited 30 carbon atoms, including two methyls (δ_C 19.4 and 29.2), six methoxy groups (δ_C 56.6, 56.7, 60.6, 60.7, 61.2, and 61.5), one methylene (δ_C 37.7), two sp^3 methines (δ_C 44.0 and 87.2), two quaternary carbon (δ_C 73.6 and 164.7), and 17 carbons corresponding to three aromatic rings (δ_C 108.7 – 154.9). The ^1H-^1H COSY correlations of H-9a/H-8/Me-18 and the HMBC correlations from H-4 (δ_H 6.92, s) to C-5 (δ_C 132.8), C-6 (δ_C 87.2), and C-16 (δ_C 123.6) and from H-11 (δ_H 6.85, s) to C-9 (δ_C 37.7), C-13 (δ_C 140.9), and C-15 (δ_C 123.7) were observed (Figure 2). This evidence implied that **1** was dibenzocyclooctadiene lignan.[9] Comparison of the ^1H and ^{13}C NMR spectroscopic data of the lignan part of **1** and those of benzoylgomisin Q, suggested that the lignan part in **1** was strikingly similar to that of benzoylgomisin Q.[10] Careful analysis of the 2D NMR data of **1** could readily allow us to determine the planar structure of the lignan part as shown in Figure 1. The remaining moiety containing one N- and six C-atoms could be established to be a nicotinoly group due to the existence of characteristic signals for nicotinoly group in the ^1H NMR spectrum including δ_H 8.66 (brd, J=4.3 Hz, 1H), 8.49 (brs, 1H), 7.73 (brd, J=7.5 Hz, 1H), and 7.40 (dd, J=7.5, 4.3 Hz, 1H).[11] The nicotinoyl group was located at C-6 (δ_C 87.2), as supported by the HMBC correlation from H-6 (δ_H 5.85, s) to C-6' (δ_C 164.7).

The configuration of the biphenyl groups in dibenzocyclooctadiene lignans were elucidated on the basis of the circular dichroism (CD) spectra.[12] The CD spectrum of **1** showed two negative Cotton effects at 252 and 236 nm and a positive Cotton effect at 218 nm, suggesting that **1** had an *S*-biphenyl configuration, as in benzoylgomisin Q.[10,12] With the axial chirality confirmed, a ROESY experiment was adopted to elucidate the absolute configurations of the remaining stereogenic centers in **1** (Figure 3). The ROESY correlations from H-11 to H-9b (δ_H 2.29, d, J=13.8 Hz) and H-8 (δ_H 2.10–2.15, m), from H-8 to Me-17 (δ_H 1.34, s), and from H-4 to H-6, indicated that H-8, H-9b, and Me-17 were β-oriented and H-6 was α-oriented.[13] These conclusions were compatible with **1** being a dibenzocyclooctadiene lignan with a twisted boat/chair conformation possessing 6*S*, 7*S*, and 8*S* absolute configurations. Thus, compound **1** was established as shown and named nicotinoylgomisin Q.

Compound **2** was assigned a molecular formula of $C_{29}H_{38}O_{10}$, by HREIMS at *m/z* 546.2463 $[M]^+$ (calcd for $C_{29}H_{38}O_{10}$, 546.2465). Its ^1H and ^{13}C NMR spectroscopic data (Table 1) closely resembled those of angeloylgomisin Q.[14,15] Differences resulted from the signal due to the presence of an oxygenated methylene (δ_C 66.8; δ_H 3.71, 3.28) and the absent of a methyl in **2**. In addition, the oxygenated methylene could be located at C-7 (δ_C 75.3), which was supported by that the oxygenated methylene showed HMBC correlations to C-6 (δ_C 78.6) and C-7 (δ_C 75.3) (Figure 2). The *S*-biphenyl configuration of the biphenyl group in **2** was determined by its CD spectrum. Therefore, compound **2** was determined as shown and named 17-hydroxyangeloylgomisin Q.

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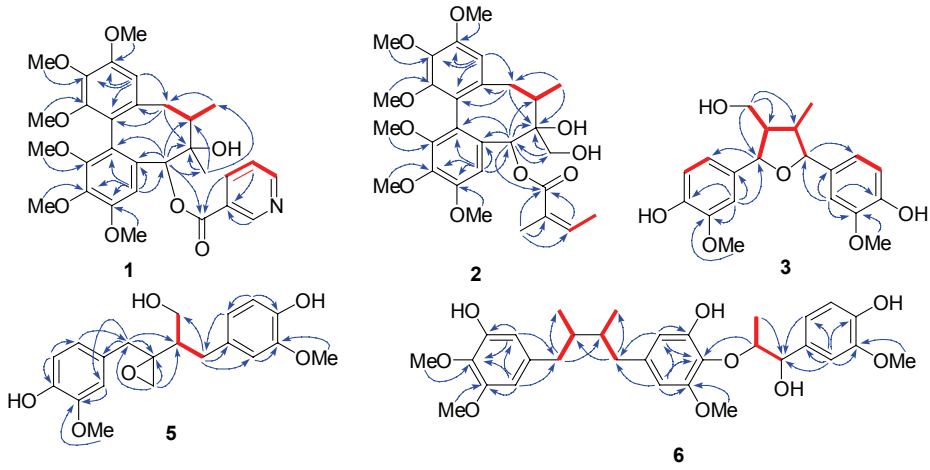

Figure 2 Key HMBC (H→C) and ^{1}H-^{1}H COSY (—) correlations of **1**—**3**, **5**, and **6**.

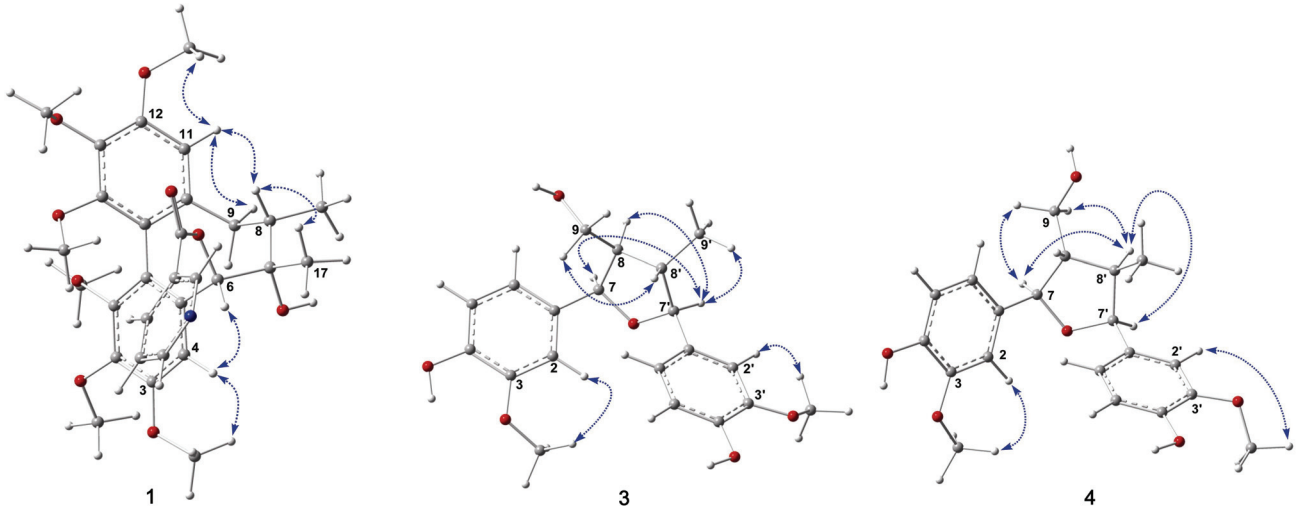

Figure 3 Key ROESY correlations of **1**, **3**, and **4**.

Compound **3** had a molecular formula of $C_{20}H_{24}O_6$ as determined by HREIMS (*m*/*z* 360.1575 $[M]^+$; calcd for $C_{20}H_{24}O_6$, 360.1573). Its ^{1}H NMR spectrum (Table 2) exhibited signals corresponding to two oxybenzyl methines (δ_H 5.15, d, J=8.4 Hz; δ_H 4.37, d, J=9.1 Hz), two sp^3 methines (δ_H 2.34—2.37, m; δ_H 2.03—2.09, m), and one methyl (δ_H 1.12, d, J=6.6 Hz), indicating that **3** was an asymmetric tetrahydrofuran lignan.[16,17] Meanwhile, the ^{1}H NMR spectrum showed six aromatic protons as two ABX systems, one at δ_H 6.97 (d, J=1.4 Hz), 6.78 (d, J=8.1 Hz), and δ_H 6.94 (dd, J=8.1, 1.6 Hz) and another at δ_H 7.08 (d, J=1.5 Hz), 6.82 (d, J=8.1 Hz), and 6.86 (dd, J=8.1, 1.5 Hz), which suggested the existence of two 1,3,4-trisubstituted benzene rings. Side-by-side comparison of the NMR spectroscopic data of **3** and (7*S*,7'*R*,8*S*,8'*R*)-4,4'-dihydroxy-3,3',5'-trimethoxy-7,7'-epoxylignan, demonstrated that the major difference in the tetrahydrofuran moiety between them was replacement of a methyl in (7*S*,7'*R*,8*S*,8'*R*)-4,4'-dihydroxy-3,3',5'-trimethoxy-7,7'-epoxylignan by an oxygenated methylene (δ_C 63.8) in **3**.[18] This difference was further supported by the HMBC correlations of **3** from H_2-9 (δ_H 3.28, overlapped; 3.21, dd, J=10.8, 6.5 Hz) to C-7 (δ_C 83.0), C-8 (δ_C 54.6), and C-8' (δ_C 46.4) (Figure 2). In the ROESY spectrum, the NOE correlations from H-7' to H-7, H-8, and Me-9' and from H-8' to H_2-9, suggested that H-7, H-7', H-8, and Me-9' were cofacial and H-8 and H_2-9 were on the other side (Figure 3). The CD spectrum of **3** displayed two positive Cotton effects at 208 and 235 nm, reverse to those of (7*S*,7'*R*,8*S*,8'*R*)-4,4'-dihydroxy-3,3',5'-trimethoxy-7,7'-epoxylignan,[18] indicating that **3** possessed the 7*R* and 7'*S* configurations. Consequently, compound **3** was established as shown and named (7*R*,7'*S*,8*R*,8'*S*)-3,3'-dimethoxy-7,7'-epoxylignan-4,4',9-triol on the basis of IUPAC recommendations for the nomenclature of lignans.[19]

The NMR spectroscopic data of **4** (Table 2) implied that it was an isomer of **3**. Detailed comparison of their NMR spectroscopic data showed that H-7, H-8, H-7', H-8', and H-9' and C-7', C-8, C-9, and C-9' in **4** were shifted by [$\Delta\delta$=δ(**4**)–δ(**3**)] $\Delta\delta_H$ –0.43, +0.37, +1.13,

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (methanol-d_4) of **1** and **2**

Position	**1**		**2**	
	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	151.6, C		152.6, C	
2	143.1, C		142.6, C	
3	153.1, C		152.9, C	
4	113.1, CH	6.92, s	112.8, CH	6.89, s
5	132.8, C		133.5, C	
6	87.2, CH	5.85, s	78.6, CH	6.09, s
7	73.6, C		75.3, C	
8	44.0, CH	2.10−2.15, m	40.3, CH	1.89−1.94, m
9a	37.7, CH_2	2.64, dd (13.8, 10.0)	37.8, CH_2	2.65, dd (13.7, 9.8)
9b		2.29, d (13.8)		2.17, d (13.7)
10	139.1, C		138.6, C	
11	108.7, CH	6.85, s	108.7, CH	6.68, s
12	154.9, C		154.6, C	
13	140.9, C		140.9, C	
14	152.9, C		151.8, C	
15	123.7, C		123.8, C	
16	123.6, C		123.8, C	
17a	29.2, CH_3		66.8, CH_2	3.71, d (11.2)
17b		1.34, s		3.28, overlapped
18	19.4, CH_3	1.22, d (7.1)	19.2, CH_3	1.11, d (7.1)
1'	151.1, CH	8.49, brs	167.2, C	
2'	127.4, C		128.5, C	
3'	139.0, CH	7.73, brd (7.5)	141.5, CH	5.96, q (7.1)
4'	125.3, CH	7.40, dd (7.5, 4.3)	15.9, CH_3	1.76, d (7.1)
5'	154.4, CH	8.66, brd (4.3)	20.5, CH_3	1.29, s
6'	164.7, C			
OMe-1	60.6, CH_3	3.23, s	61.0, CH_3	3.40, s
OMe-2	61.5, CH_3	3.86, s	61.3, CH_3	3.85, s
OMe-3	56.7, CH_3	3.96, s	56.4, CH_3	3.93, s
OMe-12	56.6, CH_3	3.97, s	56.5, CH_3	3.85, s
OMe-13	60.7, CH_3	3.31, overlapped	60.8, CH_3	3.75, s
OMe-14	61.2, CH_3	3.43, s	60.8, CH_3	3.49, s

+0.61, and –0.41 and $\Delta\delta_C$ –2.8, +1.8, –3.6, and –7.2, along with the coupling constant of H-7' with H-8' reducing from 9.1 Hz in **3** to 4.4 Hz in **4**. In addition, H-7 (δ_H 4.72, d, J=9.5 Hz), H-7' (δ_H 5.50, d, J=4.4 Hz), H-8' (δ_H 2.66−2.68, m), and H_2-9 (δ_H 3.71−3.75, m; 3.56, dd, J=11.0, 4.3 Hz) were cofacial, which was supported by the ROESY correlations (Figure 3). The CD spectrum of **4** exhibited two positive Cotton effects at 207 and 235 nm, consistent with those of **3**. Thus, compound **4** was established as shown and named (7*R*,7'*S*,8*S*,8'*R*)-3,3'-dimethoxy-7,7'-epoxylignan-4,4',9-triol.

The molecular formula of **5** was determined to be $C_{20}H_{24}O_6$ by HREIMS m/z 360.1567 $[M]^+$(calcd for $C_{20}H_{24}O_6$, 360.1573). It showed characteristic NMR spectroscopic data (Table 2) for dibenzylbutane analogues with two methoxy and two hydroxy groups substituted at the aromatic rings.[20] Full assignments of all proton and carbon signals revealed that **5** and secoisolariciresinol were structurally similar,[21] except for H_2-7 (δ_H 2.90, s), H_2-9 (δ_H 2.41, d, J=4.7 Hz; 2.30, d, J=4.7 Hz), C-8 (δ_C 61.4), and C-9 (δ_C 51.7) in **5**. Considering the fact that the hydroxymethyl group at C-8 in secoisolariciresinol was replace by a special methylene at δ_C 51.7, along with the aforementioned abnormal chemical shifts,[22] it could be concluded that **3** contained an 8,9-oxirane group. The formation of an epoxy ring between C-8 and C-9 could also be deduced by the HMBC correlations from H_2-7 to C-8 and C-9, from H_2-9 to C-7 and C-8, and from H-8' (δ_H 1.85−1.89, m) to C-8 and C-9 (Figure 2). Since the C−C bonds from C-7 to C-7' could rotate randomly, the relative configuration could not be determined by their ROESY correlations. As a reslut, compound **5** was established as shown and named 3,3'-dimethoxy-8,9-epoxylignan-4,4',9'-triol.

Compound **6** had a molecular formula of $C_{31}H_{40}O_9$, as indicated by HREIMS (calcd for $C_{31}H_{40}O_9$, 556.2672). The NMR spectra of **6** (Table 3) displayed signals attributable to one trisubstituted and two tetrasubstituted aromatic rings, four methoxy groups, three methyls, two methylenes, and four sp^3 methines. This observation implied that **6** was a sesquineolignan with four methoxy and three hydroxy substituents, which was structurally similar to (7"*S*,8*S*,8'*R*,8"*R*)-4,4"-dihydroxy-3,3',3",5'-tetramethoxy-4',8"-oxy-8,8'-sesquineolignan-7"-ol.[18] Detailed comparison of their NMR spectroscopic data suggested that the differences between them were due to the substitution at C-4, C-5, and C-5'. A hydroxy group at C-4, an aromatic proton at C-5, and a methoxy group at C-5' in (7"*S*,8*S*,8'*R*,8"*R*)-4,4"-dihydroxy-3,3',3",5'-tetramethoxy-4',8"-oxy-8,8'-sesquineolignan-7"-ol were replaced by a methoxy group (δ_C 61.0) and two hydroxy groups in **6**, respectively, which was supported by the HMBC correlations from methoxy protons at δ_H 3.77 to C-4 (δ_C 135.6), from H-6 (δ_H 6.35, d, J=1.8 Hz) to C-5 (δ_C 151.2), and from H-6' (δ_H 6.39, d, J=1.7 Hz) to C-5' (δ_C 152.0) (Figure 2). The 7",8"-*erythro* configuration was deduced by the small coupling constant of H-7" with H-8" (3.0 Hz).[23] In addition, the CD spectrum of **6** showed a negative

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Table 2 ^{1}H and ^{13}C NMR data (methanol-d_4) of **3**–**5**

Position	3		4		5	
	δ_C, type[a]	δ_H (J in Hz)[d]	δ_C, type[b]	δ_H (J in Hz)[e]	δ_C, type[a]	δ_H (J in Hz)[c]
1	132.5, C		135.7, C		129.4, C	
2	116.2, CH	6.97, d (1.4)	110.9, CH	7.03, d (1.8)	114.7, CH	6.68, overlapped
3	148.8, C		149.1, C		148.8, C	
4	147.0, C		147.4, C		146.2, C	
5	111.8, CH	6.78, d (8.1)	116.0, CH	6.80, overlapped	116.0, CH	6.74, d (8.1)
6	120.8, CH	6.94, dd (8.1, 1.6)	120.4, CH	6.86, dd (8.1, 1.8)	123.7, CH	6.63, brd (8.1)
7	83.0, CH	5.15, d (8.4)	82.7, CH	4.72, d (9.5)	38.9, CH_2	2.90, s
8	54.6, CH	2.34－2.37, m	56.4, CH	2.68－2.73, m	61.4, C	
9a	63.8, CH_2	3.28, overlapped	60.2, CH_2	3.71－3.75, m	51.7, CH_2	2.41, d (4.7)
9b		3.21, dd (10.8, 6.5)		3.56, dd (11.0, 4.3)		2.30, d (4.7)
1'	133.3, C		132.7, C		133.3, C	
2'	115.9, CH	7.08, d (1.5)	110.8, CH	6.95, brs	113.7, CH	6.67, overlapped
3'	149.1, C		148.7, C		148.7, C	
4'	147.6, C		146.5, C		146.1, C	
5'	112.1, CH	6.82, d (8.1)	115.9, CH	6.80, overlapped	115.8, CH	6.70, overlapped
6'	120.8, CH	6.86, dd (8.1, 1.5)	119.8, CH	6.81, brd (8.1)	122.6, CH	6.60, brd (8.1)
7'	89.3, CH	4.37, d (9.1)	86.5, CH	5.50, d (4.4)	34.3, CH_2	2.59, d (7.4)
8'	46.4, CH	2.03－2.09, m	41.9, CH	2.66－2.68, m	49.2, CH	1.85－1.89, m
9'	16.7, CH_3	1.12, d (6.6)	9.5, CH_3	0.71, d (6.9)	63.0, CH_2	3.55－3.60, m
OMe-3	56.5, CH_3	3.85, s	56.4, CH_3	3.90, s	56.3, CH_3	3.80, s
OMe-3'	56.5, CH_3	3.89, s	56.3, CH_3	3.87, s	56.3, CH_3	3.79, s

[a] Recorded at 400 MHz. [b] Recorded at 600 MHz. [c] Recorded at 100 MHz. [d] Recorded at 125 MHz. [e] Recorded at 150 MHz.

Table 3 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data (methanol-d_4) of **6**

Position	δ_C, type	δ_H (J in Hz)	Position	δ_C, type	δ_H (J in Hz)
1	139.3, C		8'	39.9, CH	1.80, overlapped
2	105.4, CH	6.31, d (1.8)	9'	16.8, CH_3	0.89, d (3.1)
3	154.4, CH		1"	133.4, C	
4	135.6, C		2"	111.2, CH	7.01, brs
5	151.2 C		3"	148.8, C	
6	110.9, CH	6.35, d (1.8)	4"	146.8, C	
7	40.1, CH_2	2.72, dt (13.3, 5.5)	5"	115.8, CH	6.77, overlapped
8	40.0, CH	1.80, overlapped	6"	120.4, CH	6.77, overlapped
9	16.7, CH_3	0.88, d (3.1)	7"	75.9, CH_2	4.83, d (3.0)
1'	139.5, C		8"	83.7, CH	4.33, dq (6.4, 3.0)
2'	105.5, CH	6.33, d (1.8)	9"	14.0, CH_3	1.11, d (6.4)
3'	154.6, C		OMe-3	56.3, CH_3	3.81, s
4'	133.1, C		OMe-4	61.0, CH_3	3.77, s
5'	152.0, C		OMe-3'	56.4, CH_3	3.81, s
6'	110.9, CH	6.39, d (1.7)	OMe-3"	56.3, CH_3	3.86, s
7'	40.1, CH_2	2.27－2.30, m			

Cotton effect at 241 nm, along with the small coupling constant of $J_{H7"\text{-}H8"}$, indicating that **6** had 7"*S*,8"*R*-configuration.[18,23,24] Owing to the free rotation of C－C bonds from C-7 to C-7', the relative configuration could not be elucidated by their ROESY correlations. Thus, compound **6** was established as shown and named (7"*S*,8"*R*)-4",5,5'-trihydroxy-3,3',3",4-tetramethoxy-4', 8"-oxy-8,8'-sesquineolignan-7"-ol.

Five known ligans were identified by comparison of their spectroscopic data with those reported in the lit-

erature as dihydrodehydrodiconiferyl alcohol (**7**),[25] prinsepiol (**8**),[26] 7*R*,8*R*,7'*E*-7',8'-didehydro-4,7,9,9'-tetrahydroxy-3-methoxy-8-*O*-4'-neolignan (**9**),[27] marlignan L (**10**),[28] and gomisin D (**11**).[29]

Compounds **1**—**6** were tested for their potencies in preventing the cytopathic effects of HIV-1 in C8166 cells. Cytotoxicity was measured in parallel with the determination of antiviral activity with AZT as a positive control (EC_{50}=0.00135 μg/mL and CC_{50}=1317.41 μg/mL) by using the method previously reported.[30] Compound **1** showed anti-HIV-1 activity with EC_{50} value of 17.89 μg/mL and a therapeutic index (TI) more than 11.18 (Table 4).

Table 4 Anti-HIV-1 activities of compounds **1**—**6**

Compound	CC_{50}/(μg•mL^{-1})	EC_{50}/(μg•mL^{-1})	TI (CC_{50}/EC_{50})
1	>200	17.89	>11.18
2	>200	93.43	>2.14
3	>200	116.48	>1.72
4	117.95	23.81	4.95
5	>200	117.02	>1.71
6	107.37	138.23	>0.78
AZT	1317.41	1.35×10^{-3}	975859

Conclusions

In summary, six new lignans together with five known ones were isolated from the leaves and stems of *Schisandra chinensis* collected in the Yabuli mountain area of Heilongjiang province in China. The structures of the new compounds were established by 1D- and 2D-NMR techniques and CD experiments. Most notably, nicotinoylgomisin Q (**1**) was the first example of naturally occurring *N*-containing lignans featuring a nicotinoyl group. All the new compounds were evaluated for their anti-HIV-1 activities and compound **1** showed anti-HIV-1 activity with EC_{50} value of 17.89 μg/mL and a TI more than 11.18

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