NOTE

Four new lignans obtained from the leaves of *Schisandra cauliflora* and their effect on skeletal muscle cell proliferation

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Abstract

Plants of the *Schisandra* genus are commonly used in folk medicinal remedies. Some *Schisandra* species and their lignans have been reported to improve muscle strength. In the present study, four new lignans, named schisacaulins A–D, together with three previously described compounds ananonin B, alismoxide, and pregomisin were isolated from the leaves of *S. cauliflora*. Their chemical structures were determined by extensive analyses of HR-ESI–MS, NMR, and ECD spectra. Schisacaulin D and alismoxide significantly stimulated skeletal muscle cell proliferation by increasing the number of fused myotubes and expression of myosin heavy chain (MyHC) which may be good candidates for the treatment of sarcopenia.

Graphical Abstract
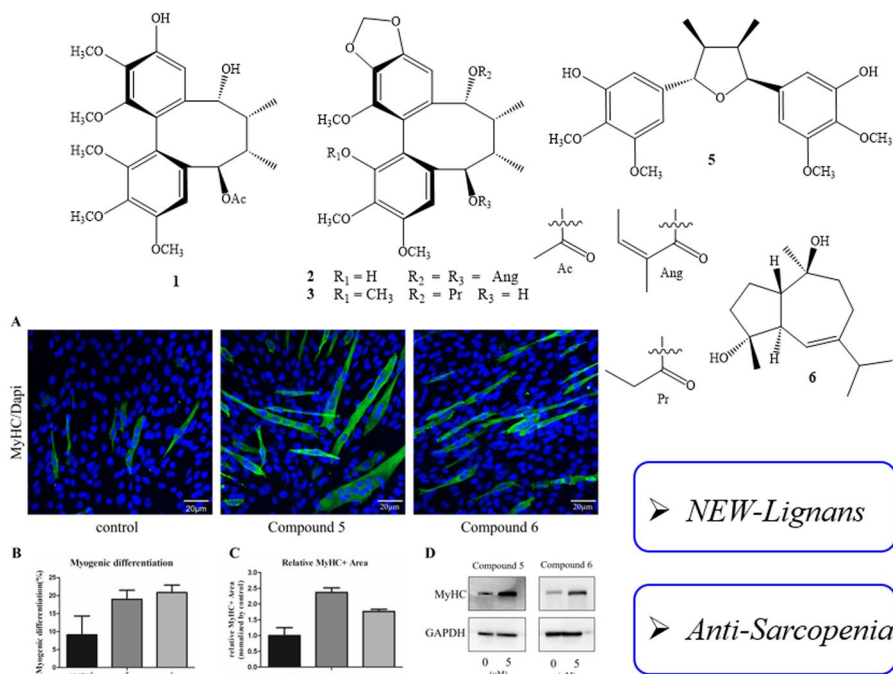
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Introduction

Indigenous plants of the *Schisandra* genus are commonly used in folk medicinal remedies. Various parts, such as the roots, leaves, stems, and fruits, have different effects and uses and are used mainly for their hepato-protective and anti-inflammatory properties and promoting the circulatory and digestive systems [1]. Phytochemical investigations of the *Schisandra* genus have resulted in the isolation of numerous lignans and triterpenoids with unique skeletal frameworks (e.g., dibenzocyclooctadiene lignans and schinortriterpenoids) [2–4]. *Schisandra* plants are of particular interest due to their unique structural diversity and broad therapeutic application in traditional medicine [5–8]. *S. cauliflora* was recently identified as a new species belonging to the *Schisandra* genus [9]. This plant is an evergreen liana that is endemic to several provinces in northern Vietnam and rarely found outside of this region [9]. Therefore, a study of the phytochemical properties of *S. cauliflora*, which has been poorly investigated, could provide valuable information concerning its health benefits and reveal new bioactive compounds.

The aging population is increasing worldwide, and this trend is accompanied by increased incidences of chronic age-related diseases, such as sarcopenia, which is characterized by progressive loss of muscle mass, strength, and function [10]. There is no Food and Drug Administration- or European Medicines Agency-approved treatment for this disease, but natural products are being explored as candidates. For example, *Schisandra* species and their lignans have been reported to improve muscle strength [11, 12]. Thus, herein, we report the identification of four new lignans and three known compounds obtained from the leaves of *S. cauliflora*. Their effects on skeletal muscle cell proliferation and differentiation were also evaluated.

Materials and methods

General experimental procedures

Melting point was taken on a Mel-Temp 3.0 apparatus (Thermo Fisher Scientific, Waltham, Massachusetts, United States). UV spectrum was obtained on a Jasco V-630 spectrophotometer (JASCO, Tokyo, Japan). IR spectrum was recorded on a Spectrum Two FT-IR Spectrometer (PerkinElmer, Waltham, Massachusetts, United States). ECD spectra were measured on a ChiraScan (Applied Photophysics, Surrey, United Kingdom). Optical rotations were obtained on a Jasco

P2000 polarimeter (JASCO, Tokyo, Japan). HR-ESI–MS was performed on an Agilent 6530 Accurate-Mass Q-TOF LC/MS (Agilent Technologies, Santa Clara, California, United States). NMR spectra were measured on a Bruker Avance III 500 MHz (Bruker BioSpin, 76,275 Ettlingen, Germany). Column chromatography was performed using silica gel (40–63 μm, Merck KGaA, 64,271 Darmstadt, Germany) or ODS (150 μm, YMC, Kyoto, Japan) resins. Thin-layer chromatography was carried out on pre-coated plates. Semi-preparative HPLC was acquired on an Agilent 1260 infinity II system, including binary pump, autosampler, DAD detector (monitoring at 205, 230, 254, and 280 nm), fraction collector, YMC J'sphere ODS-H80 column (20×250 mm, 4 μm), and running flow rate of 3 mL/min.

Plant material

The leaves of *Schisandra cauliflora* were collected at the Tam Dao National Park, Vinh Phuc province, Vietnam in April 2022. Its scientific name was identified by botanist Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. Voucher specimen (No. NCCT-P147) was kept at the Institute of Ecology and Biological Resources, VAST.

Extraction and isolation

Dried powdered leaves of *S. cauliflora* (4 kg) were extracted with methanol at room temperature three times (10 L of methanol and 60 min in an ultrasonic bath each time). The methanol residue (320 g) was well mixed with water and succesively partitioned with *n*-hexane and dichloromethane. The dichloromethane soluble fraction (82 g) was roughly separated on a silica gel column, eluting with *n*-hexane/acetone (stepsiwe, 40/1, 20/1, 10/1, 5/1, 3/1, and 1/1, v/v) to obtain six fractions, SC1 (3.4 g), SC2 (8.2 g), SC3 (21.5 g), SC4 (17.0 g), SC5 (9.5), and SC6 (7.8 g). Fraction SC3 (21.5 g) was loaded on a silica gel column, and eluted with *n*-hexane/ethyl acetate (5/1, v/v) to give five fractions, SC3A-SC3E. Fraction SC3A was chromatographed on a reversed-phase C-18 (RP-18) column, eluting with acetone/water (2/1, v/v) and then purified by semi-preparative HPLC using 40% acetonitrile in water to give compound **6** (10.1 mg, t_R 42.9 min). Fraction SC3C was separated by a RP-18 column, eluting with methanol/water (3/1, v/v) to give two fractions, SC3C1 and SC3C2. Fraction SC3C2 was purified by semi-preparative HPLC using 60% acetonitrile in water to give compound **4** (12.0 mg, t_R 42.5 min). Fraction SC3D was also chromatographed on a RP-18 column, eluting with methanol/water (3/1, v/v) to give three fractions, SC3D1–SC3D3. Fraction SC3D2 was purified by semi-preparative HPLC using 60% acetonitrile

in water to give compound **2** (5.1 mg, t_R 56.8 min). Fraction SC4 (17.0 g) was separated on a silica gel column, eluting with dichloromethane/methanol (10/1, v/v) to give four fractions, SC4A–SC4D. Fraction SC4A was separated on a RP-18 colum, eluting with acetone/water (1.8/1, v/v) to give three fractions, SC4A1–SC4A3. Fraction SC4A1 was purified by semi-preparative HPLC using 65% acetonitrile in water to give compound **3** (12.8 mg, t_R 40.6 min). Fraction SC4A3 was also purified by semi-preparative HPLC using 65% acetonitrile in water to give compound **7** (6.5 mg, t_R 42.8 min). Fraction SC4C was first separated on a RP-18 column, eluting with acetone/water (1.8/1, v/v) and then futher purified by semi-preparative HPLC using 50% acetonitrile in water to give compound **1** (10.0 mg, t_R 42.5 min). Fraction SC4D was chromatographed on a silica gel column, eluting with n-hexane/dichloromethane/methanol (2/1/0.1, v/v/v) to give two fractions, SC4D1 and SC4D2. Fraction SC4D2 was purified by semi-preparative HPLC using 55% acetonitrile in water to give compound **5** (24.0 mg, t_R 40.8 min) (Fig. 1).

Schisacaulin A (**1**)

Yellow amorphous powder; m.p. 131–133 °C; $[\alpha]_D^{25} = +41$ (c 0.1, MeOH); UV (MeOH) λ_{max}: 215, 290 nm; IR (KBr) v: 3460, 2939, 2880, 1736, 1620, 1104 cm^{-1}; ECD (0.3 mg/mL in MeOH) $\theta_{(\lambda)}$: $+3.63_{(216)}, -2.28_{(251)}$ mdeg; HR-ESI–MS: m/z 511.1720 [M + ^{35}Cl]$^-$ (calcd. for [$C_{25}H_{32}O_9{}^{35}$Cl]$^-$, 511.1740, $\Delta = -3.9$ ppm), m/z 513.1690 [M + ^{37}Cl]$^-$ (calcd.

for [$C_{25}H_{32}O_9{}^{37}$Cl]$^-$, 513.1710, $\Delta = -3.9$ ppm); ^{1}H-NMR (500 MHz, CDCl$_3$) and ^{13}C-NMR (125 MHz, CDCl$_3$) spectral data are given in Table 1.

Schisacaulin B (**2**)

Yellow amorphous powder; m.p. 110–112 °C; $[\alpha]_D^{25} = +33$ (c 0.1, MeOH); UV (MeOH) λ_{max}: 217, 290 nm; IR (KBr) v: 3454, 2940, 2879, 1733, 1620, 1595, 1105 cm^{-1}; ECD (1.0 mg/mL in MeOH) $\theta_{(\lambda)}$: $+17.88_{(213)}, -6.45_{(248)}$ mdeg; HR-ESI–MS m/z 617.2135 [M + ^{35}Cl]$^-$ (calcd. for [$C_{32}H_{38}O_{10}{}^{35}$Cl]$^-$, 617.2159, $\Delta = -3.9$ ppm), m/z 619.2145 [M + ^{37}Cl]$^-$ (calcd. for [$C_{32}H_{38}O_{10}{}^{37}$Cl]$^-$, 619.2129, $\Delta = +2.6$ ppm); ^{1}H-NMR (500 MHz, CDCl$_3$) and ^{13}C-NMR (125 MHz, CDCl$_3$) spectral data are given in Table 1.

Schisacaulin C (**3**)

Yellow amorphous powder; m.p. 115–117 °C; $[\alpha]_D^{25} = +36$ (c 0.1, MeOH); UV (MeOH) λ_{max}: 218, 290 nm; IR (KBr) v: 3441, 2955, 2926, 1715, 1615, 1107 cm^{-1}; ECD (0.5 mg/mL in MeOH) $\theta_{(\lambda)}$: $+7.01_{(225)}, -3.89_{(244)}$ mdeg; HR-ESI–MS m/z 523.1726 [M + Cl]$^-$ (calcd. for [$C_{26}H_{32}O_9{}^{35}$Cl]$^-$, 523.1740, $\Delta = -2.7$ ppm), m/z 525.1706 [M + ^{37}Cl]$^-$ (calcd. for [$C_{26}H_{32}O_9{}^{37}$Cl]$^-$, 525.1710, $\Delta = -0.8$ ppm); ^{1}H NMR (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectral data are given in Table 1.
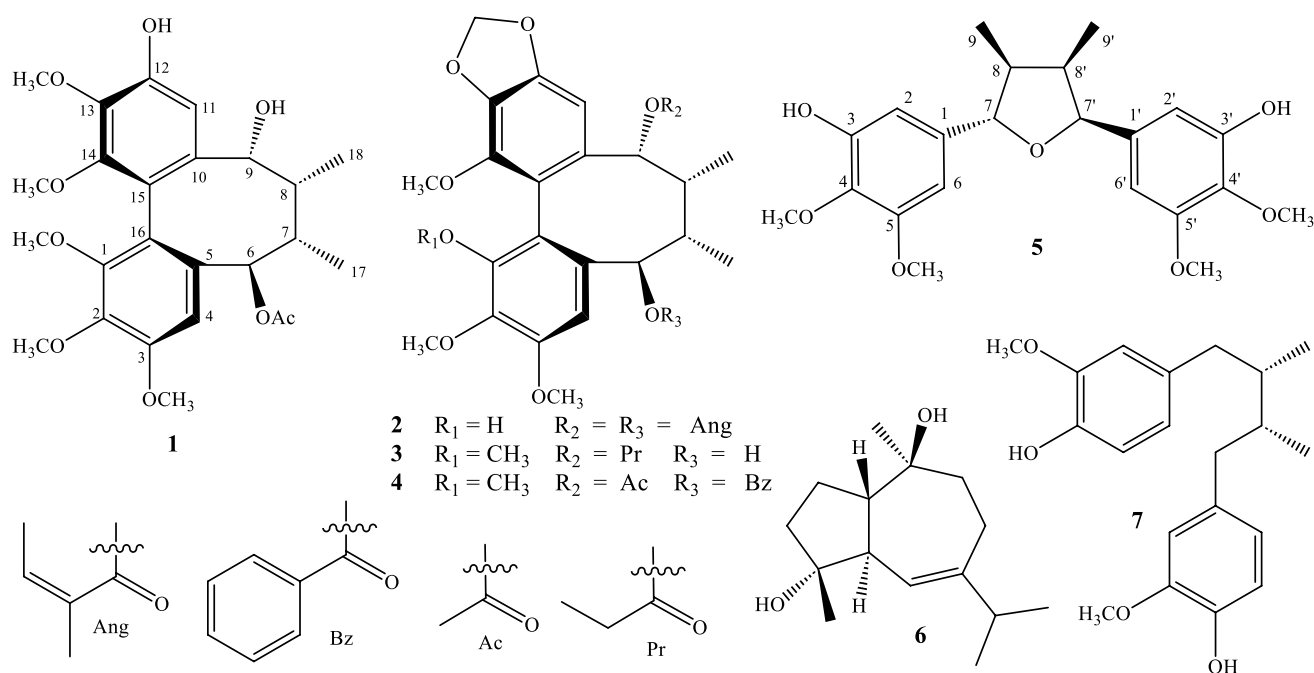

Fig. 1 Compounds **1**–**7** isolated from the leaves of *S. cauliflora*

Table 1 ^{1}H NMR and ^{13}C NMR spectral data of compounds **1–3**

No	1		2		3	
	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult., J in Hz)	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult., J in Hz)	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult., J in Hz)
1	152.2	–	147.5	–	151.7	–
2	142.5	–	134.9	–	141.1	–
3	152.9	–	150.7	–	152.2	–
4	111.4	6.76 (s)	107.3	6.54 (s)	109.9	6.55 (s)
5	131.0	–	131.6	–	133.5	–
6	81.2	5.73 (d, 9.0)	80.9	5.82 (d, 8.0)	81.3	4.48 (br)
7	38.2	2.00 (m)	38.7	2.17 (m)	41.0	1.91 (m)
8	41.5	2.08 (m)	38.7	2.30 (m)	38.5	2.19 (m)
9	80.0	4.65 (dd, 10.0, 4.0)	80.9	5.67 (d, 4.0)	79.5	5.75 (d, 4.5)
10	138.4	–	134.5	–	135.3	–
11	109.1	6.47 (s)	102.9	6.53 (s)	102.6	6.46 (s)
12	148.9	–	148.9	–	149.2	–
13	138.5	–	136.2	–	136.2	–
14	151.2	–	141.8	–	141.8	–
15	119.6	–	119.7	–	120.0	–
16	122.5	–	116.3	–	122.0	–
17	17.5	0.91 (d, 7.0)	ND	0.97 (d, 7.0)	17.0	0.98 (d, 7.0)
18	14.0	1.05 (d, 7.0)	ND	1.09 (d, 7.5)	ND	0.96 (d, 7.0)
1-OCH$_3$	60.0	3.66 (s)	–	–	60.2	3.57 (s)
2-OCH$_3$	60.8	3.89 (s)	60.4	3.86 (s)	60.6	3.88 (s)
3-OCH$_3$	56.0	3.89 (s)	55.9	3.88 (s)	56.1	3.90 (s)
13-OCH$_3$	60.8	3.95 (s)	–	–	59.6	3.89 (s)
14-OCH$_3$	60.5	3.68 (s)	59.5	3.78 (s)	–	–
OCH$_2$O	–	–	101.1	5.95 (d, 1.5) 5.92 (d, 1.5)	101.3	5.99 (d, 1.5) 5.98 (d, 1.5)
1′	170.0	–	166.9	–	173.6	–
2′	21.0	1.78 (s)	127.9	–	27.2	1.81 (m)
3′			138.3	5.93 (q, 7.0)	8.7	0.84 (t, 7.5)
4′			15.6	1.84 (d, 7.0)		
5′			19.9	1.52 (s)		
1″			166.9	–		
2″			127.4	–		
3″			139.0	5.85 (q, 7.0)		
4″			15.6	1.86 (d, 7.0)		
5″			20.2	1.32 (s)		

Measured at ^{a}CDCl$_3$, ^{b}125 MHz, ^{c}500 MHz, NDNot observed in the ^{13}C-NMR spectrum due to low intensity

Schisacaulin D (5)

Yellow amorphous powder; m.p. 122–124 °C; $[\alpha]_D^{25} = +52$ (c 0.1, MeOH); UV (MeOH) λ_{max}: 207, 271 nm; IR (KBr) ν: 3434, 2940, 2870, 1612, 1519, 1110 cm^{-1}; ECD (1.0 mg/mL in MeOH) $\theta_{(\lambda)}$: +6.38$_{(216)}$, +4.49$_{(238)}$ mdeg; HR-ESI–MS m/z 439.1521 [M + Cl]$^-$ (calcd. for [C$_{22}$H$_{28}$O$_7$^{35}Cl]$^-$, 439.1529, $\Delta = -1.8$ ppm), m/z 441.1480 [M + ^{37}Cl]$^-$ (calcd. for [C$_{22}$H$_{28}$O$_7$^{37}Cl]$^-$, 441.1499, $\Delta = -4.3$ ppm); ^{1}H NMR (500 MHz, CDCl$_3$) and ^{13}C NMR (125 MHz, CDCl$_3$) spectral data are given in Table 2.

Cell culture and differentiation

C2C12 murine myoblast cells (ATCC, CRL-1772) were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with fetal bovine serum (FBS) and 1% penicillin–streptomycin for 48 h until the confluency reached 100%. Culture media were changed to differentiation media containing DMEM with 2% horse serum (HS) and 1% antibiotics every day for next 3 days.

Table 2 ^{1}H NMR and ^{13}C NMR spectral data of compound **5**

No	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult., J in Hz)
1	139.4	–
2	105.8	6.61 (s)
3	149.2	–
4	134.8	–
5	152.5	–
6	101.7	6.54 (s)
7	85.7	4.59 (d, 9.0)
8	43.3	2.40 (m)
9	12.0	1.02 (d, 6.5)
1′	136.9	–
2′	105.6	6.54 (s)
3′	149.0	–
4′	134.2	–
5′	152.3	–
6′	102.0	6.54 (s)
7′	84.7	5.37 (d, 4.5)
8′	47.4	2.43 (m)
9′	9.4	0.62 (d, 6.5)
4-OMe	60.9	3.88 (s)
5-OMe	55.9	3.83 (s)
4′-OMe	60.9	3.88 (s)
5′-OMe	55.9	3.87 (s)

Measured at ^{a}CDCl$_3$, ^{b}125 MHz, ^{c}500 MHz

Cell viability assay and proliferation activity

The skeletal muscle cell toxic effect of the compound from *S. cauliflora* on C2C12 cells was measured by Cell Counting Kit (CCK) assay. When the cell confluency reaches 100%, the medium was replaced with fresh culture medium (DMEM), and 5 μM of the compounds from *S. cauliflora* was treated. CCK reagents were treated after 24 h, and cell viability was measured at the 450 nm absorbance following incubation of 2 h. Cell toxicity of the compounds was calculated by comparing to non-treated cells. Myoblast proliferation activity was evaluated by treating these compounds during differentiation periods. When cell confluency reaches 70%, the medium was replaced with a differentiation medium (DMEM containing 2% Horse serum) and the compounds were treated for 6 days. Finally, six days after the differentiation induction, cell proliferation activity was measured by CCK assay and calculated by comparison with the non-treated cells.

Myotube immunocytochemistry

Cells were washed in PBS and fixed in 4% paraformaldehyde for 30 min at room temperature and permeabilized in ice-cold 100% methanol for 19 min at −20 °C. They were incubated with blocking buffer including goat serum and incubated with primary antibody (MyHC, MAB4470, R&D systems, 1:200) overnight at 4 °C. Following incubation with secondary antibody (goat anti-mouse alexafluo 488, Ab150117, Abcam, 1:2000) for 1 h at room temperature. DAPI solution is used for staining nuclei. Stained cells were visualized using the ZEISS LSM 710 microscope (ZEISS, Germany), and myogenic differentiation (number of MyHC + nuclei/ total number of nuclei) was assessed using Image J software. Three pictures per sample are visualized and analyzed for these experiments.

Protein extraction and western blot

Cells are washed in PBS and lysed with RIPA buffer (R4100-010, Gendepot) containing protease inhibitor and phosphatase inhibitor cocktail (p3300, Gendepot). Proteins are harvested following incubation in ice for 30 min and centrifugation at 13000 rpm for 20 min. Bradford assay was used for determining protein concentration. Total proteins were separated by SDS-PAGE and transferred to 0.45 μm nitrocellulose membrane (10,600,003, Cytiva). The membranes were blocked with 5% bovine serum albumin in TBS-T (Tris-buffered saline with 0.1% Tween 20) and incubated overnight at 4℃ with primary antibody (MyHC, 1:1000). Following the wash procedure with TBS-T, it was incubated in secondary antibodies which are conjugated with horse-radish peroxidase for 1 h at room temperature. Protein bands were detected with chemiluminescent substrate (ECL western, Thermo scientific, 32,106) using Fusioncapt advance software.

Results and discussion

Compound **1** was obtained as a yellow amorphous powder. Molecular formula of **1** was determined to be $C_{25}H_{32}O_9$ based on chlorine adductive ion peaks at *m/z* 511.1720 $[M + ^{35}Cl]^-$ (calcd. for $[C_{25}H_{32}O_9{}^{35}Cl]^-$, 511.1740), *m/z* 513.1690 $[M + ^{37}Cl]^-$ (calcd. for $[C_{25}H_{32}O_9{}^{37}Cl]^-$, 513.1710) in the HR-ESI–MS. The ^{1}H NMR and HSQC spectra of **1** indicated two olefinic protons [δ_H 6.76 and 6.47 (each, 1H, s)], two carbinol protons [δ_H 5.73 (1H, d, $J = 9.0$ Hz) and 4.65 (1H, dd, $J = 10.0$ and 4.0 Hz)], five methoxy groups [δ_H 3.95, 3.89, 3.89, 3.68, and 3.66 (each, 3H, s)], and three methyl groups [δ_H 1.78 (3H, s), 1.05 (d, $J = 7.0$ Hz) 0.91 (d, $J = 7.0$ Hz)]. The ^{13}C NMR and HSQC spectra of **1** revealed 25 carbon signals, which were classified into 11 non-protonated carbons, 6 methine carbons, and 8 methyl carbons. Of these, the carbonyl (δ_C 170.0) and methyl (δ_C 21.0/ δ_H 1.78) signals were assigned to an acetyl group. Five methoxy groups were observed at δ_C 60.8, 60.8, 60.5, 60.0, and 56.0. The remaining 18

carbons, including 12 olefinic carbons and 6 saturated carbons, suggested a lignan compound. Moreover, HMBC correlations between H-6 and C-4/C-5/C-16, and between H-9 and C-10/C-11/C-15, and the COSY cross-peaks of H-6/H-7/H-8/H-9 indicated a dibenzocyclooctadiene lignan, which occurs naturally in the *Schisandra* genus (Fig. 2) [4]. The HMBC correlations between H-11 and C-12/C-9 and chemical shift values of C-9 and C-12 indicated the presence of a hydroxy group bound to these carbons. Meanwhile, the HMBC correlation between H-4 and C-6, and between H-6 and carbonyl carbon C-1′, indicated an acetoxy group at C-6. Therefore, five methoxy groups were located at all five remaining positions in the two benzene rings, i.e., at C-1, C-2, C-3, C-13, and C-14. The presence of a methoxy group at C-3 was also confirmed by a ROESY correlation between H-4 and 3-OCH₃, and this group clearly differed from the presence of a hydroxy group at C-12 (none of

ROESY correlations between H-11 and any methoxy protons was observed). Dibenzocyclooctadiene lignans can display two stable conformations of the cyclooctadiene ring: *twist-boat–chair* and *twist-boat* forms [13, 14]. Of these, a ROESY correlation between H-11 and H-8 indicated a *twist-boat–chair* conformation of the cyclooctadiene ring and *axial* orientation of H-8 (Figure S33), similar to previously reported dibenzocyclooctadiene lignans isolated from *Kadsura induta* [15]. The ROESY correlations between H-8 and H-9, and between H-9 and H-11 indicated *equatorial* orientation of H-9. The ROESY correlations between H₃-17 and H-4 indicated *axial* orientation of the methyl group C-17. The ROESY correlations between H₃-17 and H-6, and between H-4 and H-6 indicated *equatorial* orientation of H-6. The absolute configuration of **1** was elucidated via ECD analysis (Fig. 3). A positive Cotton effect at 216 nm (+3.63 mdeg) and a negative Cotton effect at 251 nm (-2.28
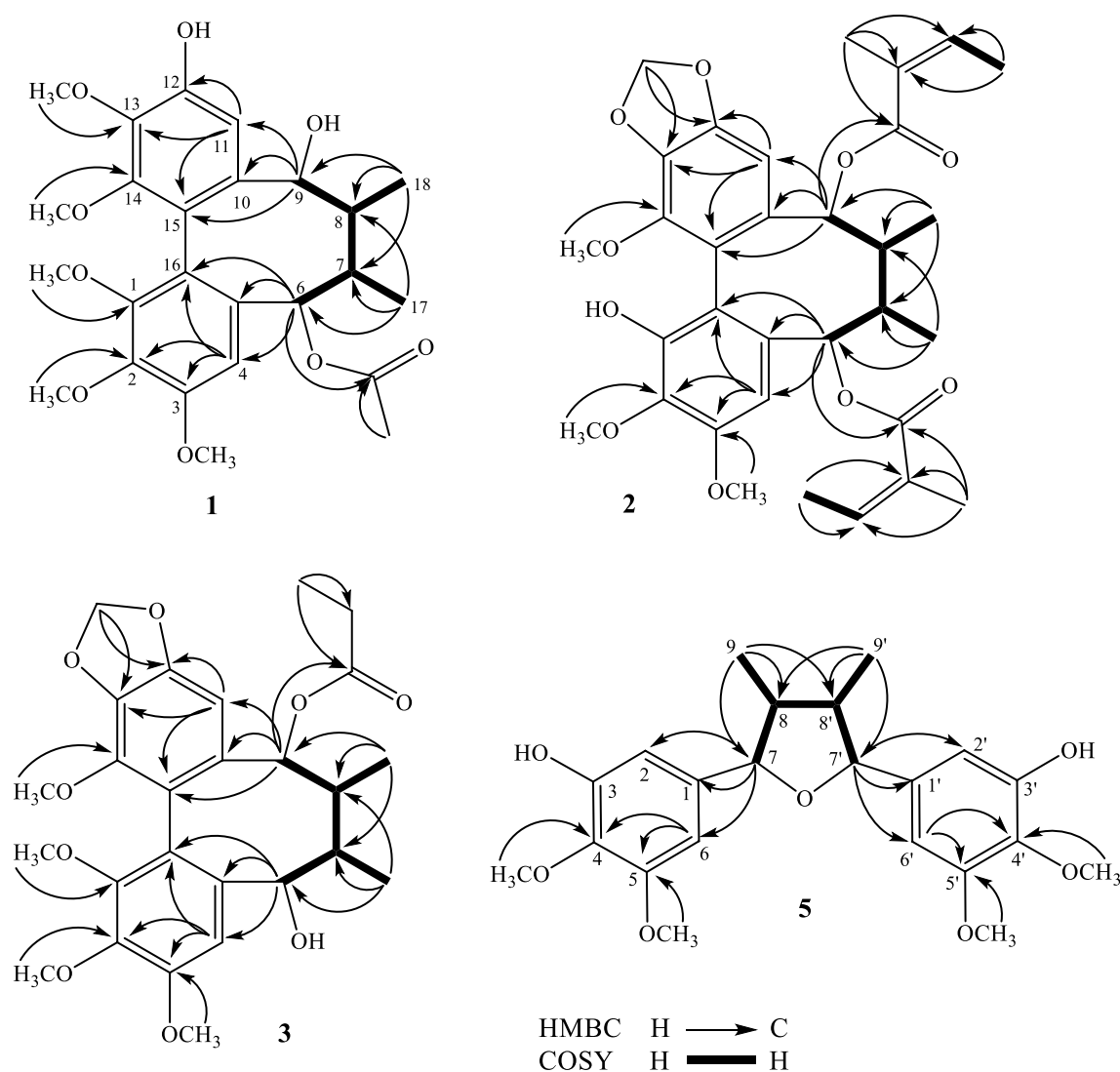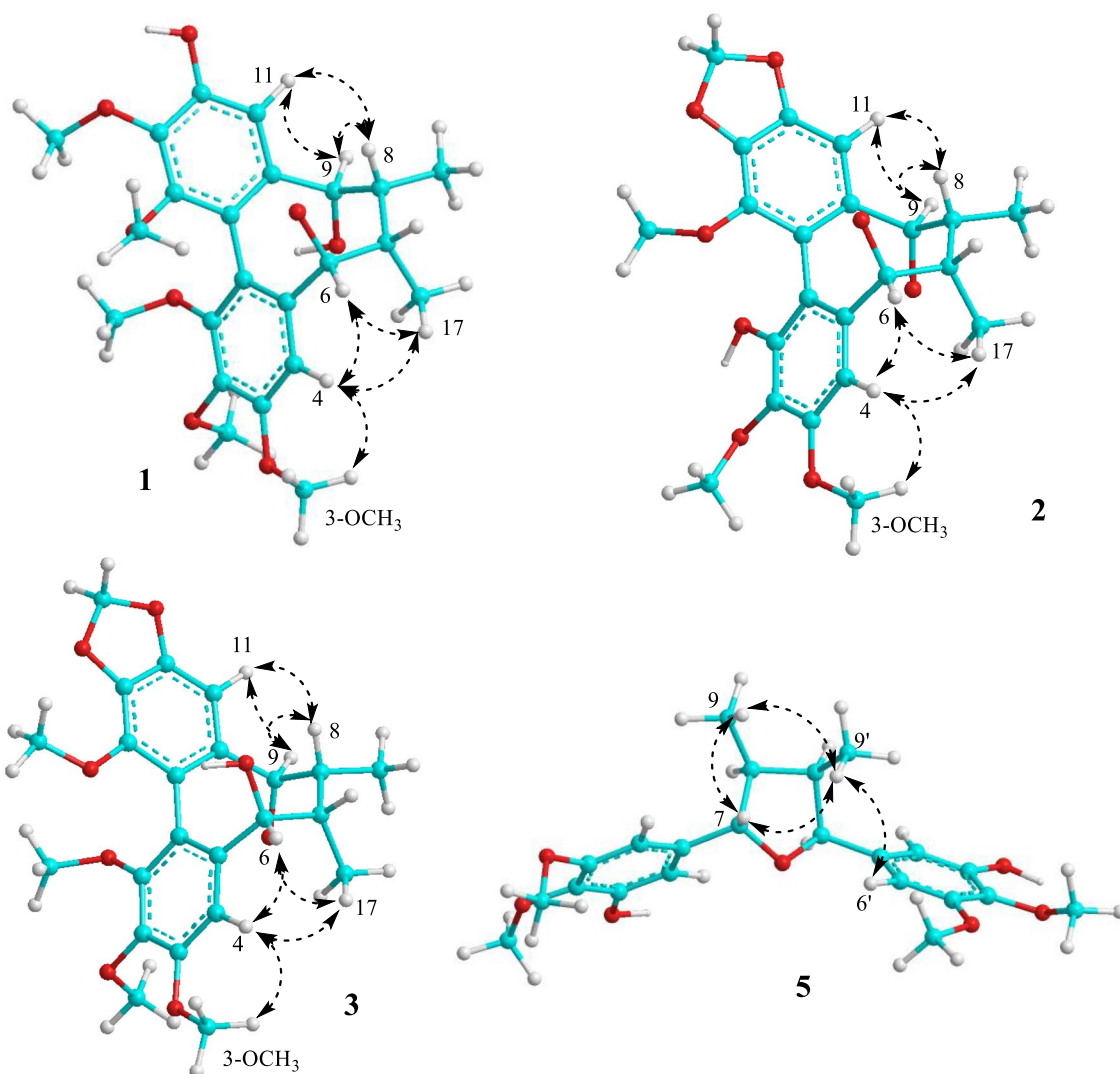

Fig. 2 Key COSY and HMBC correlations of compounds **1–3** and **5**

Fig. 3 Key ROESY correlations of compound **1–3** and **5**

mdeg) in the ECD spectrum of **1** (Figure S8) confirmed the *S*-configuration of the biphenyl system as previously reported for dibenzocyclooctadiene lignans [13]. According to the Cahn–Ingold–Prelog rule, the absolute configuration of the C–C biphenyl structure of **1** was established as the *S*-configuration, and other chiral centers had the 6*S*, 7*S*, 8*R*, and 9*R* configurations. Consequently, the structure of **1** was completely elucidated and the compound named schisacaulin A.

The HR-ESI–MS of **2** displayed chlorine adductive ion peaks at *m/z* 617.1235 [M + ^{35}Cl]$^-$ (calcd. for [$C_{32}H_{38}O_{10}{}^{35}Cl$]$^-$, 617.2159) and *m/z* 619.2145 [M + ^{37}Cl]$^-$ (calcd. for [$C_{32}H_{38}O_{10}{}^{37}Cl$]$^-$, 619.2129) which indicated the molecular formula of **2** to be $C_{32}H_{38}O_{10}$. On the other hand, the HR-ESI–MS of **3** showed chlorine adductive ion peaks at *m/z* 523.1726 [M + Cl]$^-$ (calcd. for [$C_{26}H_{32}O_9{}^{35}Cl$]$^-$, 523.1740), *m/z*

525.1706 [M + ^{37}Cl]$^-$ (calcd. for [$C_{26}H_{32}O_9{}^{37}Cl$]$^-$, 525.1710) which indicated the molecular formula of **3** to be $C_{26}H_{32}O_9$. Similar to **1**, the ^{1}H and ^{13}C NMR spectra of **2** and **3** indicated that they all shared a dibenzocyclooctadiene lignan framework, but **2** and **3** displayed signals corresponding to different substituted groups. Of these, the NMR spectra of **2** contained signals corresponding to three methoxy groups, one dioxymethylene group, and two angeloyl (Ang) groups (Table 1). The HMBC correlations between H-6 and Ang C-1′, and between H-9 and Ang C-1″, confirmed the location of two angeloyl groups at C-6 and C-9. The HMBC correlations between H-11 and C-9/C-12/C-13, and between dioxymethylene protons and C-12/C-13, indicated dioxymethylene group at C-12 and C-13. Three methoxy groups were located at C-2, C-3, and C-14, which were confirmed by HMBC correlations between 2-OCH$_3$ and C-2, 3-OCH$_3$ and C-3, and 14-OCH$_3$ and C-14. The NMR

spectra of **3** displayed signals corresponding to four methoxy groups, one dioxymethylene group, and one propionyl (Pr) group (Table 1). The HMBC correlation between H-9 and Pr C-1′/C-11 indicated that a propionyl group was located at C-9. The HMBC correlations between H-11 and C-12/C-13, and between dioxymethylene protons and C-12/C-13, demonstrated a dioxymethylene group linkage at C-12 and C-13. The assignment of four methoxy groups at all four remaining positions of the two benzene rings (i.e., C-1, C-2, C-3, and C-14) was confirmed by HMBC correlations between 1-OCH$_3$ and C-1, 2-OCH$_3$ and C-2, 3-OCH$_3$ and C-3, and 14-OCH$_3$ and C-14. Compounds **1**–**3** also possessed similar ROSEY correlations, including H-11/H-8, H-11/H-9, H$_3$-17/H-4, H$_3$-17/H-6, and H-4/H-6, as described in the above structural elucidation of **1** (Fig. 3), indicating identical relative configurations. Additionally, the absolute configurations of **2** and **3** were also similar to that of **1** according to ECD analysis and the Cahn–Ingold–Prelog rule. All of these compounds displayed positive Cotton effects at approximately 220 nm ($+17.88$ mdeg at 213 nm for **2** and $+7.01$ mdeg at 225 nm for **3**) and negative Cotton effects at approximately 240 nm (-6.45 mdeg at 248 nm for **2** and -3.89 mdeg at 244 nm for **3**) (Figures S16 and S24), which indicated the *S*-configuration of the biphenyl system [13]. The structures of **2** and **3** were thus completely established, and the compounds were named schisacaulin B and schisacaulin C, respectively.

Compound **5** was isolated as a yellow amorphous powder. The HR-ESI–MS of **5** revealed chlorine adductive ion peaks at *m/z* 439.1521 [M + Cl]$^-$ (calcd. for [C$_{22}$H$_{28}$O$_7$^{35}Cl]$^-$, 439.1529), *m/z* 441.1480 [M + ^{37}Cl]$^-$ (calcd. for [C$_{22}$H$_{28}$O$_7$^{37}Cl]$^-$, 441.1499), indicating a molecular formula of C$_{22}$H$_{28}$O$_7$. The ^{1}H NMR spectrum of **5** had signals corresponding to four olefinic protons [δ_H 6.61 (1H, s) and 6.54 (overlapped 3H, s)], two oxygenated methine protons [δ_H 5.37 (1H, d, *J* = 4.5 Hz) and 4.59 (1H, d, *J* = 9.0 Hz)], and two methyl groups [δ_H 1.02 and 0.62 (each 3H, d, *J* = 6.5 Hz)]. The ^{13}C-NMR spectrum of **5** contained signals corresponding to 22 carbons, which were classified by HSQC into eight non-protonated carbons (all olefinic carbons), eight methine groups (four olefinic and four saturated methine groups), four methoxy groups, and two methyl groups. Except for four methoxy groups, the presence of 18 carbons, including 12 olefinic and 6 saturated carbons suggested that **5** was a lignan compound. An aliphatic chain of **5** was established by COSY cross-peaks of H-7/H-8/H$_3$-9 and H-7′/H-8′/ H$_3$-9′ and by HMBC correlations between H$_3$-9/H$_3$-9′ and C-8/C-8′. Signals corresponding to 12 aromatic carbons, including four methine (each appeared to be singlet in the ^{1}H-NMR spectrum) and eight non-protonated carbons, suggested the presence of two asymmetric pyrogallol moieties. The HMBC correlations between methoxy protons and carbons,

including 4-OCH$_3$ and C-4, 5-OCH$_3$ and C-5, 4′-OCH$_3$ and C-4′, and 5′-OCH$_3$ and C-5′ confirmed four methoxy groups at C-4, C-5, C-4′, and C-5′, and hence two hydroxy groups were assigned at C-3 and C-3′ based on their ^{13}C chemical shifts (δ_{C-3} 149.2 and $\delta_{C-3'}$ 149.0). The HMBC correlations between H-7 and C-1/C-2/C-6, and between H-7′ and C-1′/C-2′/C-6′, confirmed two pyrogallol moieties connected to an aliphatic chain by C-1/C-7 and C-1′/C-7′ linkages. Although correlations either between H-7′ and C-7 or between H-7 and C-7′ were not evident in the HMBC spectra, the ^{13}C chemical shifts of C-7 (δ_C 85.7) and C-7′ (δ_C 84.7), and nine indices of hydrogen deficiency from the molecular formula of **5** (C$_{22}$H$_{28}$O$_7$), indicated the presence of an ether bridge between C-7 and C-7′. The low intensities of the abovementioned correlations were attributed to the flexibility of the tetrahydrofuran ring, as in the furano-lignan heilaohuguosu S reported previously [16]. Due to the partially overlapping signals between H-8 (δ_H 2.40) and H-8′ (δ_H 2.43), ROESY evidence from those protons could not be used to elucidate the relative configuration of **5**. In contrast, the ROESY correlations between H-7 and both H$_3$-9 and H$_3$-9′, and between H$_3$-9′ and H-2′/ H-6′, indicated close spatial proximity of H-7/H$_3$-9/H$_3$-9′ and H$_3$-9′/H-2′/H-6′ (Fig. 3). Finally, positive Cotton effects at wavelengths of 216 ($+6.38$ mdeg) and 238 nm ($+4.49$ mdeg) in the ECD spectrum of **5** (Figure S32) indicated the 7*S*, 8*S*, 8′*R*, and 7′*S* absolute configurations, which are resemble those of (+)-chicanine (7*S*, 8*S*, 8′*R*, and 7′*S*: [θ]$_{(\lambda\ nm)}$ +9670$_{(221)}$, +24,665$_{(236)}$; [17]) but are opposite those of (-)-chicanine (7*R*, 8*R*, 8′*S*, and 7′*R*: [θ]$_{(\lambda\ nm)}$ -9000$_{(221)}$, +24,000$_{(236)}$ [17]). Therefore, the structure of **5** was completely established and the compound named schisacaulin D.

Three other known compounds were identified to be ananonin B (**4**), alismoxide (**6**), and pregomisin (**7**), whose NMR data were in good agreement with published data [18–20].

Compounds **1** and **3**–**7** were evaluated for skeletal muscle cell proliferation activity. First, the compounds were examined for their effect on cell viability. At a concentration of 5 μM, the compounds did not show significant cytotoxic effect on C2C12 cells by CCK assay (Fig. 4). Compounds **5** and **6** increased cell proliferation, and these compounds were selected for further evaluation of the expression of myosin heavy chain (MHC), which is a final product of the differentiation of myoblasts and the main component of myotubes. When myoblasts differentiate, they form myotubes and multinuclear fused myotubes. Therefore, the effect of these two compounds on MHC expression on differentiating C2C12 would be revealed if they promote muscle differentiation and regeneration. Our results indicated that both **5** and **6** increase the number of fused myotubes and expression of

Fig. 4 Effects of **1, 3–7** on the viability and proliferation of C2C12 cells. **A** Cell viability results of compounds **1** and **3–7** on C2C12 cells for 24 h by CCK assay. **B** Cell proliferation results of the compounds 1 and 3–7 on differentiating C2C12 cells for 3 days by CCK assay. The data were expressed as mean $\pm$ SEM
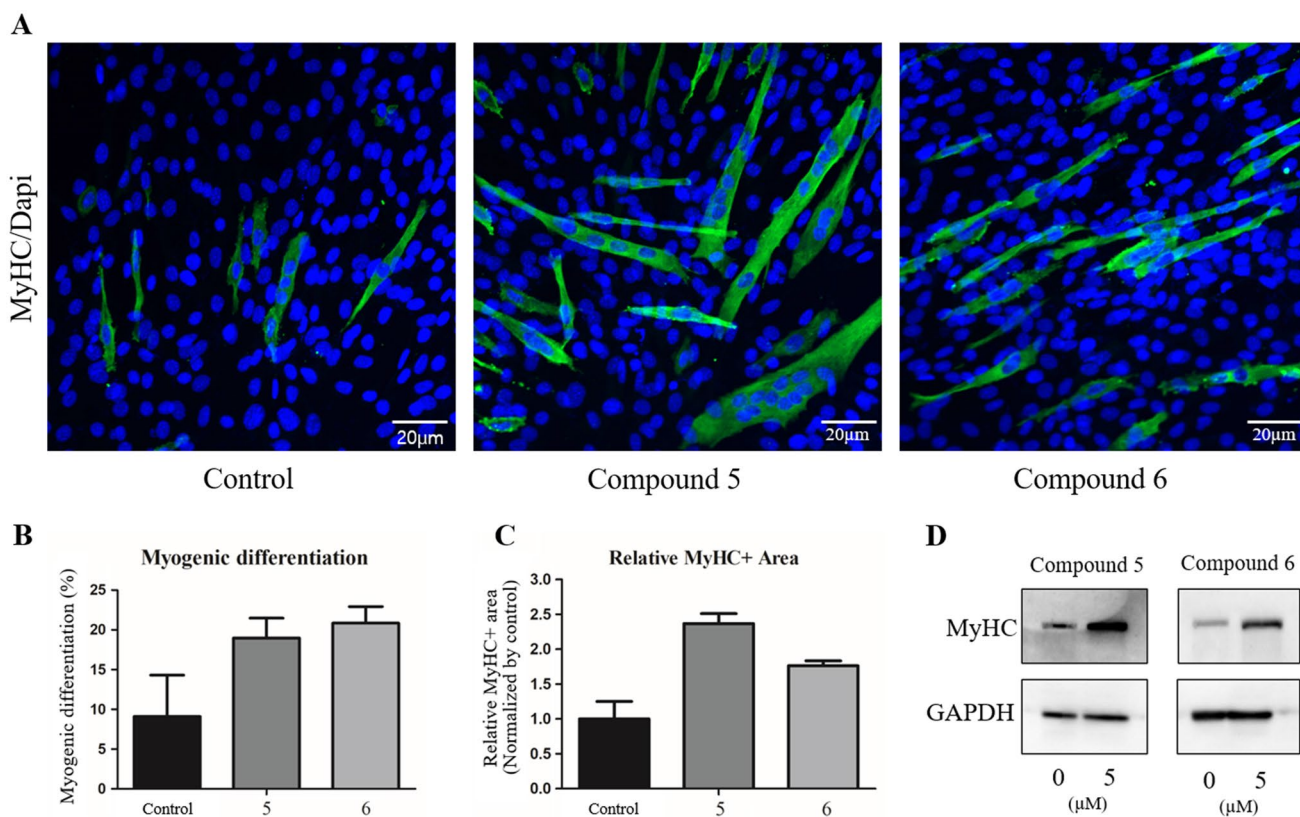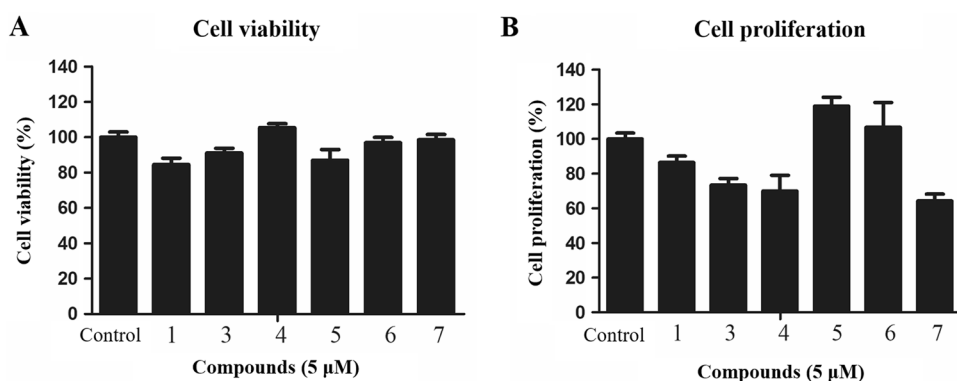
Fig. 5 Effects of compounds **5** and **6** on myogenic differentiation. Compounds **5** and **6** (5 uM) were treated during the differentiation of C2C12 ($n=3$; representative of 3 biological replicates per group). **A** Representative images and quantification results of MyHC immunostaining ($n=3$, Scale bar, 20um). **B** The percent of myogenic differentiation is calculated by (Number of MyHC positive nuclei)/(Total nuclei). **C** MyHC positive area is measured by image J Fiji and normalized by non-treated samples. **D** Western blotting analysis of MyHC protein expression in compound-treated groups

MHC (Fig. 5). Although there was an insufficient amount of compound **2** for bioassay evaluation, the structures and activities of **1–7** suggested that dibenzocyclooctadiene and dibenzylbutane lignans (**1–4**, and **7**) are weak activities, while furanoid lignan (**5**) and sesquiterpene (**6**) promote skeletal muscle cell proliferation. Herein, we confirmed that compounds **5** and **6** promote myoblast cell proliferation and contribute to myogenic differentiation. Therefore, compounds **5** and **6** might be candidates for the treatment of sarcopenia.

Conclusions

A phytochemical study of the leaves of *S. cauliflora* resulted in the discovery of four new lignans (three dibenzocyclooctadiene-type and one furanoid-type, named schisacaulin A–D) together with three known compounds (ananonin B, alismoxide, and pregomisin). Their chemical structures and absolute configurations were determined via HR-ESI–MS, NMR, and ECD analyses. Among the isolated compounds, schisacaulin D and alismoxide significantly stimulated skeletal muscle cell proliferation by increasing the number of fused myotubes and the expression of MHC. These compounds may be good candidates for the treatment of sarcopenia.

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