Nat. Prod. Bioprospect. (2016) 6:161–166
DOI 10.1007/s13659-016-0095-5

ORIGINAL ARTICLE

Neolignans from *Selaginella moellendorffii*

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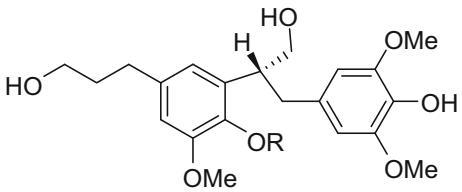
Received: 5 February 2016 / Accepted: 20 March 2016 / Published online: 7 April 2016

Abstract Two new neolignans selaginellol (**1**) and selaginellol 4′-*O*-β-D-glucopyranoside (**2**), together with seven known compounds (**3**–**9**), were isolated from the whole plant of *Selaginella moellendorffii*. The structures of the new isolates were determined through spectroscopic data analysis. Compounds **1**–**9**, as well as compounds **10**–**18** previously isolated from the species, were measured for the activity against platelet aggregation induced by ADP or collagen. Three neoligans (**8**, **11**, and **12**), one flavanone (**14**), and one alkaloid (**16**) showed inhibitory activity against ADP- or collagen-induced platelet aggregation as compared with tirofiban. The dihydrobenzofuran neolignans (**8**, **11**, and **12**) are more potent than the benzofuran neolignan (**13**) and other types of neolignans (**1**–**7**). Glucosidation of the dihydrobenzofuran neolignans (**11** and **12**) is helpful for the activity.

Graphical Abstract Two new neolignans selaginellol (**1**) and selaginellol 4′-*O*-β-D-glucopyranoside (**2**) were isolated from the whole plant of *Selaginella moellendorffii*. Several compounds from this plant showed the activity against platelet aggregation induced by ADP or collagen.



Keywords Selaginellaceae · *Selaginella moellendorffii* · Lignans · Antiplatelet

Jing-Xian Zhuo and Yue-Hu Wang contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s13659-016-0095-5) contains supplementary material, which is available to authorized users.

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1 Introduction

The family Selaginellaceae Willk. includes the single genus *Selaginella* Beauv. *Selaginella* is a nearly worldwide

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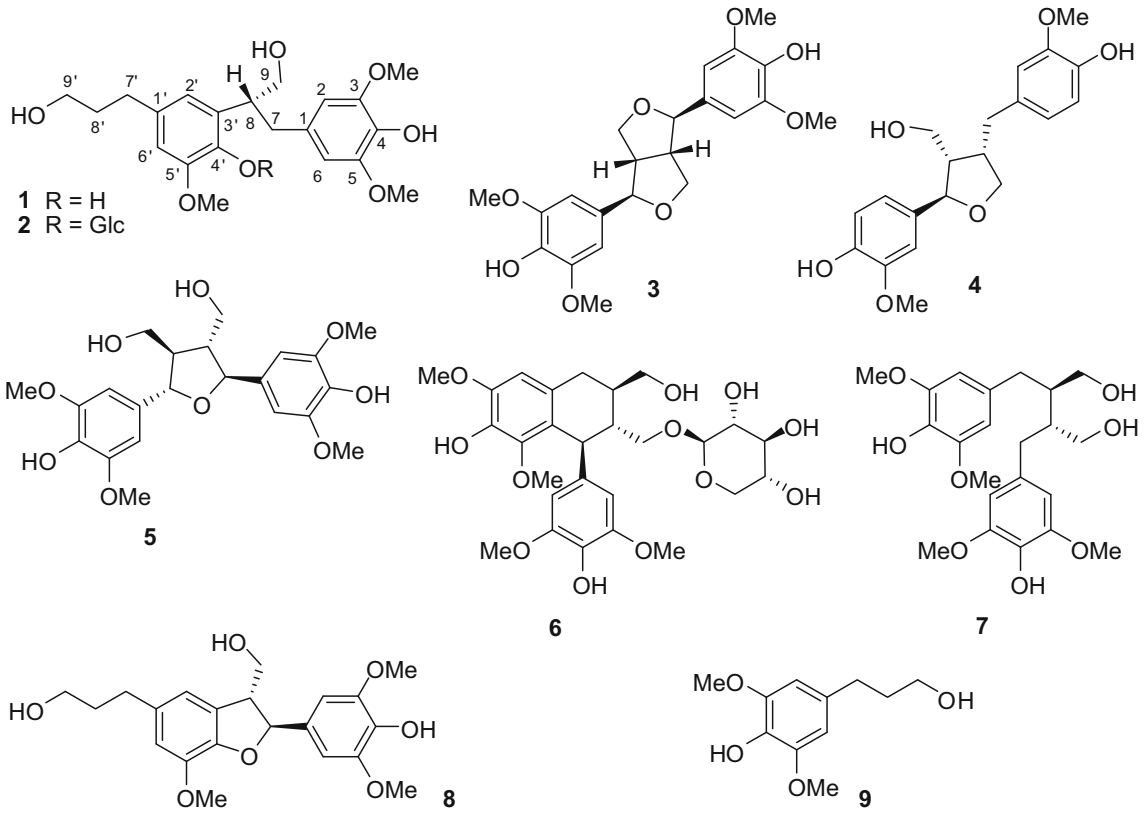

Fig. 1 The chemical structures of **1–9** from *Selaginella moellendorffii*

genus of about 700 species, with 72 of them in China and more than 20 species used in traditional Chinese medicine [1, 2]. Several *Selaginella* species including *S. delicatula* (Desv. ex Poir.) Alston, *S. moellendorffii* Hieron., *S. nipponica* Franch. & Sav., *S. sanguinolenta* (L.) Spring, *S. stauntoniana* Spring, and *S. tamariscina* (P. Beauv.) Spring are used in promotion of blood circulation (Huoxue in Chinese) [1]. Traditional Chinese medicines with the functions of "Huoxue" and/or "Huayu" (removing blood stasis) are claimed to be useful in antiplatelet therapies and the treatment of thrombotic diseases [3, 4]. Previously, a pyrrolidinoindoline alkaloid selaginellic acid with antiplatelet activity was found from the whole plant of *S. moellendorffii* [5, 6]. This result prompted us to further investigate the plant which led to the isolation of nine compounds (**1–9**, Fig. 1) including two new neolignans (**1** and **2**). Compounds **1–9**, as well as those (**10–18**) previously isolated from the plant [5, 7, 8], were evaluated for antiplatelet activity. The structural elucidation of the new compounds and the bioassay results are reported.

2 Results and Discussion

The HRESIMS analysis of selaginellol (**1**) gave an $[M+Na]^+$ ion at *m/z* 415.1729 appropriate for a molecular formula of $C_{21}H_{28}O_7$ requiring eight sites of unsaturation. The IR absorption signals revealed the presence of hydroxy (3428 cm^{-1}) and aromatic (1614, 1518, 1496, and 1461 cm^{-1}) groups. The ^1H NMR data of **1** (Table 1) exhibited three methoxy groups [δ_H 3.82 (3H, s) and 3.70 (6H, s)], and two 1,2,3,5-tetrasubstituted benzene rings [δ_H 6.30 (2H, s); 6.63 (d, $J = 1.6$ Hz) and 6.47 (d, $J = 1.6$ Hz)]. The ^{13}C NMR data of **1** (Table 1) showed the signals for three methoxy groups (δ_C 56.5 × 2 and 56.4), two phenyl rings, five methylenes including two oxygenated ones (δ_C 65.9, 62.2, 37.9, 35.8, and 32.8), and one methine (δ_C 45.4). According to above NMR signal characteristics [8], compound **1** might be a neolignan.

The ^1H–^1H COSY correlations (Fig. 2) exhibited two partial structures from C-7 to C-9 and C-7′ to C-9′. Based on the HMBC correlations (Fig. 2) from H-2 and H-6 to C-4, H_2-7 to C-2 and C-6, H-8 to C-1, H_2-8′ to C-1′, H_2-7′ to C-2′ and C-6′, H-2′ and H-6′ to C-4′, 3-OMe to C-3, 5-OMe to C-5, and 5′-OMe to C-5′, two phenylpropanoid moieties, namely 4-(3-hydroxypropyl)-2,6-dimethoxyphenol and 4-(3-hydroxypropyl)-2-methoxyphenol, were confirmed. The two fragments were linked through C-8-C-3′ by the HMBC correlations from H_2-7 and H_2-9 to C-3′ as well as H-8 to C-2′ and H-2′ to C-8. Therefore, the relative configuration of **1** was elucidated as 3,5,5′-trimethoxy-8,3′-neoligna-4,4′,9,9′-tetraol. The absolute configuration of

Table 1 ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of **1** and **2** in CD_3OD (δ in ppm, J in Hz)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		132.9 (C)		132.5 (C)
2,6	6.30 (s)	107.3 (CH)	6.28 (s)	107.0 (CH)
3,5		148.7 (C)		148.6 (C)
4		134.2 (C)		134.1 (C)
7	3.00 (dd, 13.4, 5.7)	37.9 (CH_2)	3.01 (dd, 14.0, 5.0)	39.6 (CH_2)
	2.88 (dd, 13.4, 9.4)		2.71 (dd, 14.0, 10.0)	
8	3.42 (m)	45.4 (CH)	3.96 (m)	42.7 (CH)
9	3.76 (m)	65.9 (CH_2)	3.76 (dd, 10.6, 4.8)	67.1 (CH_2)
			3.67 (dd, 10.6, 7.9)	
1′		133.7 (C)		140.4 (C)
2′	6.47 (d, 1.6)	122.0 (CH)	6.73 (d, 1.8)	120.2 (CH)
3′		129.3 (C)		138.5 (C)
4′		143.7 (C)		143.5 (C)
5′		148.7 (C)		153.2 (C)
6′	6.63 (d, 1.6)	110.6 (CH)	6.72 (d, 1.8)	111.6 (CH)
7′	2.53 (t, 7.5)	32.8 (CH_2)	2.64 (t, 7.6)	33.1 (CH_2)
8′	1.74 (m)	35.8 (CH_2)	1.82 (m)	35.7 (CH_2)
9′	3.51 (t, 6.4)	62.2 (CH_2)	3.57 (t, 6.2)	62.2 (CH_2)
1″			4.57 (d, 7.5)	105.6 (CH)
2″			3.44 (m)	75.9 (CH)
3″			3.39 (m)	77.8 (CH)
4″			3.38 (m)	71.1 (CH)
5″			3.11 (m)	78.0 (CH)
6″			3.79 (overlapped)	62.4 (CH_2)
			3.70 (overlapped)	
3,5-OMe	3.70 (s)	56.5 (CH_3)	3.70 (s)	56.5 (CH_3)
5′-OMe	3.82 (s)	56.4 (CH_3)	3.80 (s)	56.3 (CH_3)

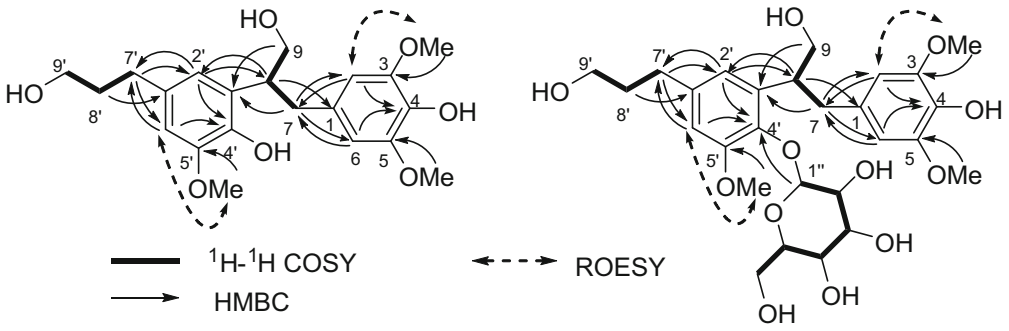

Fig. 2 Key 2D NMR correlations of **1** and **2**

selaginellol (**1**) was elucidated as (8*R*)-3,5,5′-trimethoxy-8,3′-neoligna-4,4′,9,9′-tetraol by comparing its electronic circular dichroism (ECD) spectrum [$\Delta\varepsilon$ −0.12 (273)] with that of a known analogue secodihydrodehydrodiconiferyl alcohol tetraacetate [9].

According to the HREIMS ion at *m/z* 554.2365 $[M]^+$ (calcd for $C_{27}H_{38}O_{12}$, 554.2363), the molecular formula of compound **2** was determined as $C_{27}H_{38}O_{12}$ with nine degrees of unsaturation. The IR absorption signals showed the presence of hydroxy (3425 cm^{-1}) and aromatic (1614,

1518, and 1461 cm^{-1}) groups. The NMR data (Table 1) of **2** were very similar to those of **1**, except that more signals for a β-glucopyranosyl moiety [δ_H 4.57 (d, J = 7.5 Hz); δ_C 105.6, 75.9, 77.8, 71.1, 78.0, and 62.4] were observed. As demonstrated in the ^1H–^1H COSY, HMBC and ROESY correlations (Fig. 2), compound **2** was determined to be the β-glucopyranoside of selaginellol (**1**). The HMBC correlation from H-1″ to C-4′ indicated that the β-glucopyranosyl part was located at C-4′. According to our previously acidic hydrolysis of rel-(7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol 4-*O*-β-D-glucopyranoside (**11**) [8], the sugar in the plant is D-glucose. The absolute configuration of the aglycone was elucidated to be the same as that of selaginellol (**1**) by comparison of its ECD spectrum [Δε −0.37 (273)] with that of **1**. Therefore, compound **2** is selaginellol 4′-*O*-β-D-glucopyranoside.

The known compounds were determined as (−)-syringaresinol (**3**) [10], (−)-lariciresinol (**4**) [11], 7*S*,7′*S*,8*R*,8′*R*-icariol A_2 (**5**) [12], lyoniside (**6**) [13], (−)-8,8′-bisdihydrosiringenin (**7**) [14], (7*S*,8*R*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol (**8**) [8], and dihydrosinapyl alcohol (**9**) [15], by comparing their NMR data (for all known compounds) and optical rotation values (for the neolignans) with those reported in the literature.

All of these compounds (**1**–**9**), along with those previously isolated from the plant, including (7*S*,8*R*)-4,9-dihydroxy-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolignan-9′-oic acid methyl ester (**10**) [8], rel-(7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol 4-*O*-β-D-glucopyranoside (**11**) [8], rel-(7*R*,8*S*)-3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neoligna-4,9,9′-triol 9-*O*-β-D-glucopyranoside (**12**) [8], 3,3′,5-trimethoxy-4′,7-epoxy-8,5′-neolign-7-ene-4,9,9′-triol 9-*O*-β-D-glucopyranoside (**13**) [8], 5-carboxymethyl-7,4′-dihydroxyflavanone 7-*O*-β-D-glucopyranoside (**14**) [7], *N*-selaginelloyl-L-phenylalanine (**15**) [5], paucine 3′-*O*-β-D-glucopyranoside (**16**) [8], paucine (**17**) [8], and N^1-*cis*-*p*-coumaroylagmatine (**18**) [8], were evaluated for the inhibitory activity against platelet aggregation induced by ADP or collagen. As shown in Table 2, compounds **8**, **11**, **12**, **14**, and **16** showed potential inhibitory activity against ADP-induced platelet aggregation with IC_{50} values of 80.84, 35.76, 42.47, 27.70, and 59.19 μM, respectively, as compared with the positive control tirofiban (IC_{50} = 25.32 μM). Compounds **8**, **11**, **12**, and **14** also showed the activity against collagen-induced platelet aggregation with IC_{50} values of 146.70, 31.17, 24.57, and 26.25 μM, respectively, as compared with the positive control tirofiban (IC_{50} = 148.20 μM). The dihydrobenzofuran neolignans (**8**, **11**, and **12**) are more potent than the benzofuran neolignan (**13**) and other types of neolignans (**1**–**7**). Glucosidation of the dihydrobenzofuran neolignans (**11** and **12**) is helpful for the activity as compared the bioassay result of **11** and **12** with that of **8**.

Table 2 The effect of compounds on rabbit platelet aggregation induced by ADP (10 μM) or collagen (2.5 μg/mL)

Compound	ADP (IC_{50} μM)	Collagen (IC_{50} μM)
8	80.84	146.70
11	35.76	31.17
12	42.47	24.57
14	27.70	26.25
16	59.19	>200
Tirofiban (positive control)	25.32	148.20

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were recorded using a JASCO P-1020 Polarimeter (Jasco Corp., Tokyo, Japan). UV spectra were taken on a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). ECD spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics Ltd., Leatherhead, UK). IR spectra were measured on a Bruker Tensor 27 FTIR Spectrometer (Bruker Corp., Ettlingen, Germany) with KBr disks. ^1H and ^{13}C NMR spectra were collected on Bruker Avance 400, DRX-500 or Avance III-600 spectrometers (Bruker Bio-Spin GmbH, Rheinstetten, Germany) with TMS as an internal standard. ESIMS and HRESIMS analyses were carried out on an API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA). HREIMS were carried out on a Waters AutoSpec Premier p776 spectrometer (Waters, Millford, MA, USA). Silica gel G (80–100 and 300–400 mesh, Qingdao Meigao Chemical Co., Ltd., Qingdao, China), C_{18} silica gel (40–75 μm, Fuji Silysia Chemical Ltd., Aichi, Japan), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), and D_{101} macroporous resin (Qingdao Marine Chemical Ltd., Qingdao, China) were used for column chromatography, and silica gel GF_{254} (Qingdao Meigao Chemical Co., Ltd.) was used for preparative TLC as precoated plates. TLC spots were visualized under UV light at 254 nm and by dipping into 5 % H_2SO_4 in alcohol followed by heating. Semipreparative HPLC was performed on an Agilent 1200 series pump (Agilent Technologies, Santa Clara, USA) equipped with a diode array detector and an Agilent Zorbax SB-C_{18} column (5.0 μm, ϕ 9.4 × 250 mm).

3.2 Plant Material

The whole plant of *S. moellendorffii* was collected from Jingxi County of Guangxi Zhuang Autonomous Region in

2008. A voucher specimen (No. JX0801) was identified by one of the authors (Chun-Lin Long) and deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

The air-dried, powdered *S. moellendorffii* plants (15 kg) were exhaustively extracted with MeOH (45 L × 3) at 60 °C. The solvent was removed to give a residue (0.89 kg). The crude extract was subjected to chromatography on a D_{101} macroporous resin column eluted successively with H_2O, 35 % EtOH, and 95 % EtOH to give three portions (I–III), respectively. Portion II (398 g) was subjected to column chromatography (silica gel G; $CHCl_3$/MeOH, 1:0 → 0:1, v/v) to yield six fractions (A–F). Fr. A was subjected to column chromatography (silica gel G; petroleum ether/EtOAc, 15:1 → 0:1, v/v) to yield four fractions (A1–A4). Fr. A1 was purified by column chromatography (silica gel G; $CHCl_3$-acetone, 15:1, v/v) to obtain **9**. Fr. A2 was chromatographed on a Sephadex LH–20 column (MeOH) to give subfractions A2–1 and A2–2. Subfraction A2–1 was subjected to chromatography on a silica gel G column ($CHCl_3$-acetone, 20:1, v/v) and then further purified by semi-preparative HPLC (MeCN/H_2O, 30:70, v/v) to yield **4** (14.9 mg, t_R = 13.099 min). Subfraction A2-2 was purified by preparative TLC ($CHCl_3$/MeOH, 10:1, v/v) to obtain **3** (24.6 mg). Fr. A3 was chromatographed over a C_{18} silica gel column (MeOH/H_2O, 30:70, v/v), a Sephadex LH-20 column (MeOH), and a silica gel G column ($CHCl_3$/MeOH/H_2O, 50:1:0.25), and purified by semi-preparative HPLC (MeOH/H_2O, 40:60, v/v) to obtain **5** (15.5 mg, t_R = 5.864 min). Fr. A4 was chromatographed on a Sephadex LH-20 column (MeOH), a C_{18} silica gel (MeOH/H_2O, 50:50, v/v), and a silica gel G column ($CHCl_3$/MeOH, 60:1, v/v), and purified by semi-preparative HPLC (MeCN/H_2O, 30:70, v/v) to yield **7** (2.0 mg, t_R = 7.716 min), **8** (2.0 mg, t_R = 8.917 min) and **1** (4.0 mg, t_R = 13.652 min). Fr. D was chromatographed on a C_{18} silica gel column (MeOH/H_2O, 30:70, v/v), a Sephadex LH-20 column (MeOH), and a silica gel G column ($CHCl_3$/MeOH/H_2O, 100:10:0.5, v/v), and purified by semi-preparative HPLC (MeOH/H_2O, 40:60, v/v) to yield **2** (7.8 mg, t_R = 12.138 min) and **6** (4.4 mg, t_R = 18.734 min).

*3.3.1 Selaginellol (**1**)*

Pale yellow oil (MeOH); $[\alpha]_D^{24}$ −50.4 (*c* 0.40, MeOH); UV (CH_3OH) λ_{max} (log ε) 280 (3.31), 228 (3.98) nm; ECD Δε (*c* 0.010, MeOH) −0.12 (273), −3.84 (214), +3.63 (197); IR (KBr) ν_{max} 3428, 1614, 1518, 1496, 1461, 1431, 1289, 1217, 1114 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ion ESIMS *m/z* 415 $[M+Na]^+$; positive ion HRESIMS *m/z* 415.1729 $[M+Na]^+$ (calcd for $C_{21}H_{28}O_7Na^+$, 415.1727).

*3.3.2 Selaginellol 4′-O-β-D-glucopyranoside (**2**)*

Pale yellow solid (MeOH); $[\alpha]_D^{22}$ −57.8 (*c* 0.26,MeOH); UV (CH_3OH) λ_{max} (log ε) 274 (3.80) nm; ECD Δε (*c* 0.011, MeOH) −0.37 (273), −6.17 (210), +3.20 (200); IR (KBr) ν_{max} 3425, 1615, 1518, 1461, 1428, 1325, 1216, 1113, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive ion ESIMS *m/z* 577 $[M+Na]^+$; HREIMS *m/z* 554.2365 $[M]^+$ (calcd for $C_{27}H_{38}O_{12}$, 554.2363).

3.4 In Vitro Platelet Aggregation Assay

In vitro platelet aggregation was conducted using the turbidimetric method with a minor modification [16, 17]. Briefly, blood was withdrawn from the carotid artery of New Zealand rabbits,and anticoagulated with 3.8 % sodium citrate (1:9 citrate/blood, v/v) and centrifuged for 15 min at 950 rpm to prepare platelet-rich plasma (PRP) or 10 min at 3000 rpm to obtain platelet-poor plasma (PPP). The platelet concentration was adjusted to 3 × 10^8 platelets/mL. PRP in 270 μL was preincubated at 37 °C for 5 min in the cuvette with 20 μL of sample or vehicle (saline), and then platelet aggregation was induced by 10 μL ADP (10 μM) or collagen (2.5 μg/mL). The maximum platelet aggregation rate was determined within 5 min with continuous stirring at 37 °C using four-channel aggregometer (Beijing Steellex Science Instrument Company, China).

For each compound, five concentrations were chosen and a percentage inhibition-concentration curve was derived. From this curve the IC_{50} value was calculated as the concentration of inhibitor causing a 50 % inhibition of the aggregation using SPSS software.

Acknowledgments This work was funded by the Ministry of Science & Technology of China (2012FY110300), the National Natural Science Foundation of China (3116140345), and the Minzu University of China (YLDX01013, 2015MDTD16C).

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

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