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•Research article•

Sesquiterpenoids from the leaves of *Sarcandra glabra*

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[ABSTRACT] Sarglanoids A–F, six new sesquiterpenoids belonging to eudesmane (**1**–**5**) and eremophilane (**6**) types, were isolated from the leaves of *Sarcandra glabra*, a famous traditional Chinese medicine (TCM). Their structures including absolute configurations were elucidated through extensive spectroscopic analysis and electronic circular dichroism (ECD) calculations. Compounds **1**–**2** were rare N-containing eudesmane-type sesquiterpenoids. Compound **3** exhibited inhibitory activity against nitric oxide (NO) production in lipopolysaccharides (LPS)-induced RAW 264.7 cells with IC_{50} values at 20.00 ± 1.30 μmol·L^{-1}. These findings provide scientific evidence for sesquiterpenoids as the material foundation of *S. glabra*.

[KEY WORDS] *Sarcandra glabra*; Sesquiterpenoids; N-containing sesquiterpenoids; Anti-inflammatory
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Introuduction

Sarcandra glabra (Thunb.) Nakai (Chloranthaceae), a perennial herb mainly distributed in southern China and Southeast Asia, has been used as traditional Chinese medicines for the treatment of inflammation, traumatic injury, cancer, rheumatism, and diarrhea [1]. It is also used as a kind of herbal tea and food supplement in China to relieve fatigue and stress [2]. In addition to these medicinal and dietary functions, *S. glabra* also has high ornamental values [3]. Current researches indicated that the sesquiterpenoids and their dimers were the main constituents of *S. glabra*, which have attracted increasing attention from organic chemists and pharmacologists due to their structural diversities and significant bioactivities, such as anti-inflammatory [4], anti-HIV [5], antimalarial [6] and cytotoxic activities [7]. Our previous chemical investigations toward *S. glabra* resulted in the isolation of a large array of sesquiterpenoids and their dimers with novel structures and diverse biological activities [8–10]. In a continuing effort for investigating structurally novel and bioactive sesquiterpenoids from *S. glabra*, six new sesquiterpenoids (**1**–**6**, Fig. 1) were obtained from the leaves of *S. glabra*, including five new eudesmane-type sesquiterpenoids, sarglanoids A–E (**1**–**5**), two of which were rare N-containing sesquiterpenoids. Their structures including absolute configurations were elucidated through extensive spectroscopic analysis and electronic circular dichroism (ECD). Compound **3** showed moderate anti-inflammatory activity. Herein, we reported the details of isolation, structure identification, and bioactivity test of these compounds.

Results and Discussion

In this study, the air-dried leaves of *S. glabra* (6.5 kg) collected from Guangxi Province, were extracted with 95% aqueous EtOH. After organic solvent extraction, the dichloromethane fraction (150.0 g) and petroleum ether fraction (207.0 g) were chromatographed on silica gel, Toyoperal HW-40C, MPLC, Sephadex LH-20, followed by preparative HPLC purification to obtain compounds **1**−**6** (Fig. 1).

Sarglanoid A (**1**) was obtained as white amorphous powder, exhibiting a protonated molecular ion peak at *m/z* 246.1489 $[M + H]^+$ (Calcd. 246.1489) by HR-ESI-MS, which was consistent with the molecular formula $C_{15}H_{19}NO_2$ with seven degrees of unsaturation. The IR spectrum (Fig. S11) of **1** implied the presence of an unsaturated lactam carbonyl group (1671 cm^{-1}). The ^1H NMR (Table 1) and HSQC spectra (Fig. S6) of **1** displayed three methyl signals at δ_H 1.89 (3H, s, CH_3-13), 1.72 (3H, s, CH_3-15) and 0.96 (3H, s, CH_3-14), and two olefinic protons at δ_H 6.03 (1H, s, H-9) and 5.37 (1H, s, H-3). In addition, the signal at δ_H 7.21 (1H, s) was identified as an amide proton signal as no correlation peak

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These authors have no conflict of interest to declare.

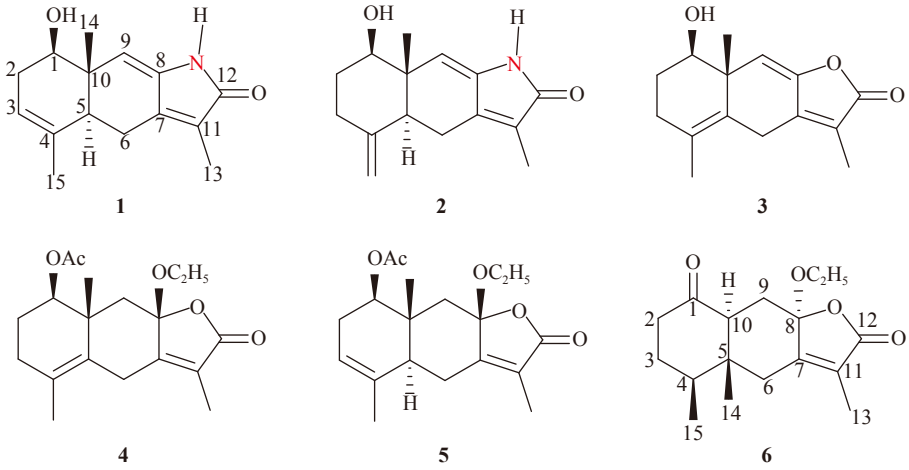

Fig. 1 Structures of compounds 1–6

Table 1 ^{1}H NMR data for compounds 1–6 in $CDCl_3$

No.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[a]	**6**[a]
	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)	δ_H, mult. (*J* in Hz)
1	3.74, br s	3.61, dd (12.0, 4.8)	3.67, d (8.6)	4.82, dd (11.0, 3.5)	4.81 dd (10.2, 6.1)	
2*α*	2.41, m	1.92, m	1.81, m	1.84, m	2.36[c]	2.37, m
2*β*	1.99, m	1.61, m		1.70, m	1.95, m	
3*α*	5.38, s	2.15, m	2.18, m	2.20, m	5.34, s	1.93, m
3*β*		2.38, dd (13.6, 4.4)	2.10, m	2.08, m		1.64[c]
4						2.03, m
5	2.45, m	2.24, d (13.0)			2.92, br s	
6*α*	2.84, d (8.4)	2.67, dd (16.6, 3.9)	3.67, d (18.6)	3.25, s	2.61 ddd (17.8, 7.0, 2.2)	2.62, dd (12.4,1.4)
6*β*	2.36, m	2.52, t (15.0)	3.14, d (18.6)		2.36[c]	2.07, d (12.4)
7						
8*α*						
8*β*						
9*α*	6.03, s	5.96, s	6.04, s	2.27, d (14.6)	2.20 d (14.7)	2.42, m
9*β*				1.84, d (14.6)	1.89 d (14.7)	1.65[c]
12						
13	1.89, s	1.88, s	1.91, s	1.88, s	1.86 d (2.1)	1.83, d (1.4)
14	0.96, s	0.88, s	1.20, s	0.99, s	0.78 s	0.53, s
15	1.72, s	4.93, s 4.70, s	1.68, s	1.66, s	1.70 d (1.3)	1.01, d (6.8)
NH	7.21, s	7.05, s				
1-COC$\underline{H}_3$				2.06, s	2.04, s	
8-OC$\underline{H}_2$CH$_3$				3.37 dd (9.1, 7.0) 3.08 dd (9.1, 7.0)	3.41 dd (9.3, 7.0) 3.19 dd (9.3, 7.0)	3.39 dd (8.9, 7.1) 3.17 dd (8.9, 7.1)
8-OCH$_2$C$\underline{H}_3$				1.10 t (7.0)	1.16 t (7.0)	1.16 t (7.1)

[a]Measured at 500 MHz; [b]Measured at 600 MHz; [c]Overlapped, without designating multiplicity

was observed in HSQC spectrum [11]. The ^{13}C NMR data (Table 2) suggested that **1** possessed a carbonyl carbon (δ_C 172.8), three pairs of double bonds (δ_C 141.0, 136.4, 133.8, 125.7, 120.6, 116.3), along with an oxygenated carbon (δ_C

Table 2 ^{13}C NMR data for compounds **1**–**6** in $CDCl_3$

No.	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[a]	**6**[a]
1	72.5	75.3	73.4	78.6	78.0	209.4
2	33.2	31.9	27.2	23.7	28.7	40.9
3	120.6	33.8	31.7	30.3	120.2	30.9
4	133.8	146.8	128.0	128.6	133.4	42.0
5	45.6	46.6	127.0	127.6	40.0	44.5
6	22.3	22.0	24.2	26.0	24.8	37.2
7	141.0	140.9	147.4	157.7	157.3	156.1
8	136.4	135.9	148.3	106.2	106.7	105.8
9	116.3	116.4	115.0	47.4	40.9	33.0
10	40.5	42.8	41.9	38.1	36.0	53.9
11	125.7	125.3	119.4	124.9	126.0	125.9
12	172.8	172.7	171.8	171.9	171.7	171.3
13	8.5	8.4	8.6	8.5	8.5	8.1
14	13.0	12.8	19.6	23.5	15.0	11.4
15	20.7	108.6	19.4	19.6	20.4	15.0
1-O$\underline{C}$OCH$_3$				170.8	170.9	
1-OCO$\underline{C}$H$_3$				21.4	21.2	
8-O$\underline{C}$H$_2$CH$_3$				59.0	58.9	58.3
8-OCH$_2\underline{C}$H$_3$				15.3	15.4	14.6

[a]Measured at 125 MHz; [b]Measured at 150 MHz

72.5). In the HMBC spectrum, correlations (Fig. 2) were observed from CH_3-13 to C-7, C-8, C-11 and C-12; from CH_3-14 to C-1, C-5, C-9 and C-10; from CH_3-15 to C-3 and C-5; from H-1 to C-2, C-3, C-5 and C-10; and from H-9 to C-5, C-7, C-8. The above data of **1** were similar to those reported for atractylenolactam, which was a rare N-containing eudesmane-type sesquiterpenoid from *Chloranthus fortunei* [11-12]. The differences between them were that C-1 of **1** was substituted by a hydroxyl group and the $\Delta^{4,15}$ terminal double bond of atractylenolactam migrated to $\Delta^{3,4}$ in compound **1** [11], which were confirmed by key HMBC correlations from H-1 to C-2, C-5, C-10 and C-14; and from CH_3-15 to C-3 and C-4. Therefore, the planar structure of **1** was determined as shown in Fig. 1. The relative configuration of **1** was deduced from the analysis of its ROESY spectrum, in which the correlations (Fig. 2) of H-1/H-2α and H-1/H-5 indicated that these protons were α-oriented. Accordingly, the key ROESY correlations between CH_3-14 and H-2β, H-6β revealed the β-orientation of CH_3-14. In order to determine the absolute configuration of **1**, the ECD calculation was carried out. The trends of the calculated ECD spectrum of (1*R*,5*S*,10*S*)-**1** showed good consistency with the measured one (Fig. 3), and the absolute configuration of **1** was thus assigned. Therefore, compound **1** represented the first example of N-containing eudesmane-type sesquiterpenoids found in *S. glabra*.

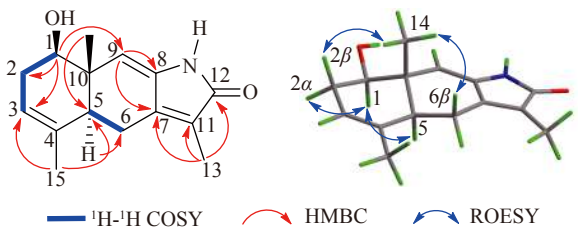

Fig. 2 Key ^{1}H–^{1}H COSY, HMBC and ROESY correlations of compound **1**

Sarglanoid B (**2**) had the same molecular formula of $C_{15}H_{19}NO_2$ as **1** based on its HR-ESI-MS data. The similarity of the ^{1}H and ^{13}C NMR spectra between **2** and **1** suggested the resemblance of their planar structures. Comparing the differences in the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **1** and **2**, revealed that **2** had a $\Delta^{4,15}$ terminal double bond instead of the $\Delta^{3,4}$ double bond in **1**. The analysis of the 2D NMR data (Figs. S15–S17) further confirmed the above conclusions and completed the assignment of the planar structure of **2** (Fig. 1). On the basis of ROESY experiment (Fig. S18), the relative configurations of all chiral centers in **2** were consistent with those of **1**. As illustrated in Fig. S3, the experimental and calculated ECD curves of compound **2** matched well with each other, leading to the the absolute configura-

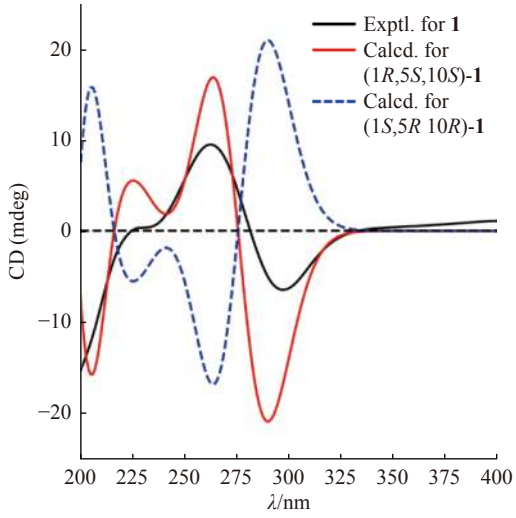

Fig. 3 Experimental and calculated ECD spectra of compound 1

tion assignment of **2** as 1*R*,5*S*,10*S*.

Sarglanoid C (**3**) was obtained as white amorphous powder. The HR-ESI-MS showed a molecular ion peak at *m/z* 247.1329 $[M + H]^+$ (Calcd. for 247.1329) corresponding to the molecular formula $C_{15}H_{18}O_3$, which indicated that **3** was a normal sesquiterpenoid with no nitrogen atom. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **3** were similar to those of **1**, except for the absence of H-3 (δ_H 5.37 for **1**) and H-5 (δ_H 2.45 for **1**) and significant downfield-shift of C-8 ($\Delta\delta_C$ +11.9), which were caused by the migration of $\Delta^{3,4}$ and the replacement of the unsaturated lactam moiety by an unsaturated lactone. These information indicated that **3** was an eudesmane-type sesquiterpenoid with a $\Delta^{4,5}$ double bond (Fig. 1), as an analog of neolitacumone C [13]. The result was also confirmed by the key HMBC correlations (Fig. S1) from H-9 to C-1, C-5, C-7, C-8 and C-10; from CH_3-13 to C-7, C-11 and C-12; from CH_3-14 to C-1, C-5 and C-9; and from CH_3-15 to C-2, C-3, C-4, C-5 and C-10. The relative configuration of **3** was determined by its ROESY experiment (Fig. S27). The absolute configuration of compound **3**, 1*R*,10*R*, was assigned by comparison of its experimental and calculated ECD spectra (Fig. S3). The structure of sarglanoid C (**3**) was thereby identified and shown in Fig. 1.

Sarglanoids D (**4**) and E (**5**) were both obtained as white amorphous powder. They afforded the same molecular formula of $C_{19}H_{26}O_5$ from the HR-ESI-MS data [*m/z* 335.1852 $[M + H]^+$ for **4** and *m/z* 335.1855 $[M + H]^+$ for **5** (Calcd. for 335.1853)]. Their NMR data (Tables 1 and 2) indicated the presence of characteristic ethoxy group [such as δ_H 3.37 (1H, dd, *J* = 9.1, 7.0 Hz), 3.08 (1H, dd, *J* = 9.1, 7.0 Hz) and 1.10 (3H, t, *J* = 7.0 Hz) for **4**] and acetoxy group [δ_C 170.8 ($OCOCH_3$) and δ_H 2.06 (3H, s, $COCH_3$) for **4**] in these two compounds. Data suggested that these two compounds were also eudesmane-type sesquiterpenoids with lactone moiety as 1α-acetoxy-8α-oxyethyl-2-oxo-eudesman-3,7(11)-dien-8,12-olide [14], which were confirmed by HMBC correlations (Fig. 4) from CH_3-13 to C-6, C-7, C-8, C-11 and C-12; from H-1 to C-2, C-3, C-5, C-9 and C-10; from CH_3-15 to C-3, C-4 and C-5. The location of acetoxy group at C-1 and ethoxy group at C-8 was supported by the HMBC correlations from H-1 to ester carbonyl carbon and from oxymethylene proton to C-8, respectively. The differences between **4** and **5** ($\Delta^{4,5}$ for **4**, $\Delta^{3,4}$ for **5**) can be distinguished by the corresponding HMBC correlations from CH_3-15 to C-3, C-4 and C-5. The planar structures of compounds **4** and **5** were thus assigned as shown in Fig. 1. The observed ROESY correlations (Fig. 4) of H-1/H-9α and H-2α, suggesting α-orientation of H-1 in **4**. The ROESY cross-peaks of CH_3-14/H-9β and H-2β, and CH_3-14/methyl of the ethoxy group indicated that CH_3-14 and the ethoxy group were β-oriented. Similarly, the relative configuration of **5** was verified by the ROESY correlations, as shown in Fig. S2. The absolute configurations of compounds **4** and **5** were further established by comparing their experimental ECD spectra with the calculated ECD spectra (Fig. S3). The similarities between the experimental and calculated ECD spectra of **4** and **5** revealed that their absolute configurations were (1*R*,8*S*,10*R*)-**4** and (1*R*,5S,8*S*,10*R*)-**5**, respectively. The structures of **4** and **5** were thus identified (Fig. 1).

Sarglanoid F (**6**) was obtained as yellow oil. Its molecular formula $C_{17}H_{24}O_4$, with six degrees of unsaturation, was determined based on its HR-ESI-MS at *m/z* 293.1748 $[M + H]^+$ (Calcd. 293.1747). The ^1H and ^{13}C NMR data (Tables 1 and 2) of **6** were similar to those of istanbulin A [15]. The only difference was the presence of an ethoxy group [δ_H 3.39 (dd, *J* = 8.9, 7.1 Hz), 3.17 (dd, *J* = 8.9, 7.1 Hz) and 1.16 (1H, t, *J* = 7.1 Hz,)] attached to C-8 in **6** instead of a hydroxyl group at the same position in istanbulin A. This finding was further confirmed by analysis of the HMBC spectrum (Fig. S53). Consequently, the planar structure of **6** was elucidated (Fig. 1). The ROESY correlation (Fig. S2) of H-4/H-10 indicated their syn-orientation, whereas the correlation of CH_3-14/CH_3-15 evidenced that these groups were on the opposite face. Subsequent ECD calculations showed that the calculated ECD spectrum of (4*S*,5*R*,8*R*,10*S*)-**6** coincided well with the experimental ECD spectrum of **6** (Fig. S3), enabling the absolute configuration of **6** to be finally defined. Therefore, compound **6** was identified as the 8-ethoxyl derivative of istan-

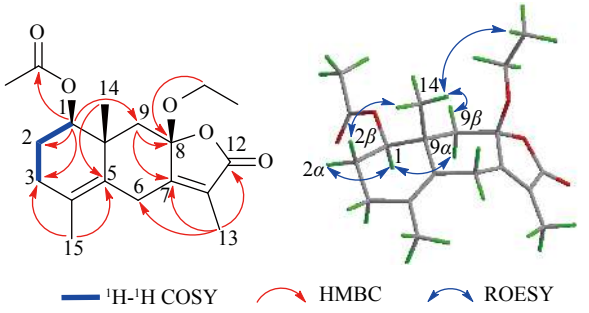

Fig. 4 Key ^1H–^1H COSY, HMBC and ROESY correlations of compound 4

bulin A [15].

As a traditional Chinese herbal medicine, *S. glabra* was often used to treat inflammatory-related diseases, such as bruises, bone fractures and arthritis [4]. Therefore, the anti-inflammatory activities of compounds **1**–**6** were evaluated for their inhibitory effects on NO production in LPS-induced RAW 264.7 cells. As a result, compound **3** exhibited moderate activity with IC_{50} value at 20.00 ± 1.30 μmol·L^{-1} (L-NMMA was used as a positive control, IC_{50} = 41.40 ± 2.30 μmol·L^{-1}), while other compounds showed no significant inhibitory activity at the concentration of 50 μmol·L^{-1}.

Experimental

General experimental procedures

Optical rotation values were measured using a JASCO P-1020 polarimeter. The UV spectra were recorded using a UV-2450 UV/vis spectrophotometer. The ECD spectra were recorded with a JASCO J-1500 spectrometer. The infrared (IR) measurements were performed using a Bruker TENSOR 27 spectrometer. The 1D and 2D spectra were recorded on Bruker Avance III-500 MHz or AV-600 MHz spectrometers using standard pulse sequences in chloroform-*d* with TMS as an internal standard. The HR-ESI-MS data were acquired using an Agilent 6520B UPLC-Q-TOF mass spectrometer. LC-MS analysis was performed on an Agilent 1290 series instrument with a SHIMADZU Shim-pack VP-ODS column (5 μm, 250 mm × 4.6 mm, i.d.). Column chromatography (CC) was carried out using silica gel (100–200 mesh and 200–300 mesh; Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), MCI gel (Mitsubishi Chemical Industries Ltd., Japan), and Toyoperal HW-40C (Tosoh Corporation, Japan). An Agilent 1100 series system with an Agilent ZORBAX Eclipse $XDBC_{18}$ column (5 μm, 150 mm × 4.6 mm, i.d.) was used for HPLC analysis. Preparative HPLC was carried out using a Shimadzu LC-6AD series instrument equipped with a Shim-pack RP-C_{18} column (10 μm, 200 × 20 mm, i.d.). Recycling preparative HPLC was run on a SHIMADZU LC-20A series instrument equipped with a Shim-pack PRC-ODS (H) column (5 μm, 200 mm × 20 mm, i.d.).

Plant material

The leaves of *S. glabra* (Thunb.) Nakai were collected from Guangxi Province in China in September 2018 and identified by Professor ZHANG Mian (China Pharmaceutical University). A voucher specimen (No. YSg201809) was deposited in Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and isolation

Air-dried leaves of *S. glabra* (6.5 kg) were extracted with 95% aqueous EtOH (3 × 20 L, each 3.0 h). The solvent was removed under reduced pressure and yielded a dark green crude extract (796 g). The crude extract was then dissolved in 1.5 L of warm water, extracted with petroleum ether (3 × 3.0 L) and dichloromethane (3 × 3.0 L), successively. The dichloromethane fraction (150.0 g) was subjected to a silica gel CC and eluted with a gradient of CH_2Cl_2/MeOH (1 ∶ 0, 100 ∶ 1, 50 ∶ 1 25 ∶ 1 and 5 ∶ 1, *V*/*V*) to afford five fractions (Frs. A−E) based on TLC analysis. Fr. C (38.5 g) was subsequently subjected to a HW-40C gel eluted with DCM/MeOH (1 ∶ 1) to afford Frs. C-1−C-5. Among them, Fr. C-4 (11.9 g) was subjected to MCI gel, eluted with MeOH/H_2O (40%, 50%, 60%, 70%, 80% and 90%) to afford eight subfractions: Frs. C-4a−C-4h. Then, Fr. C-4e (1.87g) was separated on a Sephadex LH-20 gel with MeOH to yield Frs. C-4e1−C-4e6. Fr. C-4e5 (218.7 mg) was purified by preparative HPLC and recycling preparative HPLC to yield **1** (2.4 mg) and **2** (1.1 mg). Fr. A (16.0 g) was subjected to MCI gel eluted with MeOH/H_2O (40%, 50%, 60%, 70%, 80% and 90%) to give eight subfractions Frs. A-1−8. Fr. A-5 (1.3 g) was separated into eight subfractions Frs. A-5a−A-5h by Sephadex LH-20 gel (MeOH). Fr. A-5f (142.9 mg) was further purified by preparative HPLC to give compound **3** (6.1 mg). Fr. A-6 (1.2 g) was separated on a Sephadex LH-20 gel with MeOH to yield Frs. A-6a−A-6f. Fr. A-6c (454.7 mg) was purified by preparative HPLC to yield **6** (37.8 mg).

The petroleum ether fraction (207.0 g) was subjected to silica gel CC and eluted with a gradient of PE/acetone (9 ∶ 1, 5 ∶ 1, 7 ∶ 3 and 3 ∶ 2, *V*/*V*) to afford three fractions (Frs. a−c). Then, Fr. b (82.2 g) was applied to silica gel CC and eluted with gradient solvents of PE/CH_2Cl_2 (4 ∶ 1, 2 ∶ 1, 1 ∶ 1, 1 ∶ 3, *V*/*V*). Based on the TLC results, four fractions (Frs. b1−b4) were obtained. Fr. b3 (35.1 g) was further isolated by MCI gel eluted with MeOH/H_2O (50%, 60%, 70%, 80% and 90%) to give five subfractions (Frs. b3a−b3f). Fr. b3c (2.0 g) was separated on a Sephadex LH-20 gel with MeOH to yield Frs. b3c1− b3c5. Finally, Fr. b3c2 (640.0 mg) was further purified by preparative HPLC to afford compounds **4** (4.5 mg) and **5** (5.0 mg).

Compound characterization data

Sarglanoid A ***(1)***: white amorphous powder; $[\alpha]_D^{24}$ −4.5 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε): 275 (3.84) nm; ECD (MeOH, Δε) λ_{max} (Δε) 219 (+1.14), 265 (+6.22), 295 (−4.52); IR (KBr) ν_{max} 3420, 2922, 1671, 1384, 1247, 1166, 1026 cm^{-1}; ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2 in the manuscript; HR-ESI-MS *m/z* 246.1489 $[M + H]^+$ (Calcd. for $C_{15}H_{20}NO_2$, 246.1489).

Sarglanoid B ***(2)***: white amorphous powder; $[\alpha]_D^{24}$ +93.8 (*c* 0.113, MeOH); UV (MeOH) λ_{max} (log ε): 275 (4.01) nm; ECD (MeOH, Δε) λ_{max} (Δε) 270 (+22.88); IR (KBr) ν_{max} 3418, 2937, 1659, 1384, 1026 cm^{-1}; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data, see Tables 1 and 2 in the manuscript; HR-ESI-MS *m/z* 246.1488 $[M + H]^+$ (Calcd. for $C_{15}H_{20}NO_2$, 246.1489).

Sarglanoid C ***(3)***: white amorphous powder; $[\alpha]_D^{24}$ +93.8 (*c* 0.113, MeOH); UV (MeOH) λ_{max} (log ε): 275 (4.01) nm; ECD (MeOH, Δε) λ_{max} (Δε) 211 (−4.52), 255 (+8.38), 291 (−13.92) ; IR (KBr) ν_{max} 3423, 2922, 1765, 1644, 1383, 1321, 1069 cm^{-1}; ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data, see Tables 1 and 2 in the manuscript; HR-ESI-MS *m/z* 247.1329 $[M + H]^+$ (Calcd. for $C_{15}H_{19}O_3$, 247.1329).

Sarglanoid D (4): white amorphous powder; $[\alpha]_D^{24}$ −24.1 (*c* 0.075, MeOH); UV (MeOH) λ_{max} (log ε): 213 (3.74) nm; ECD (MeOH, $\Delta\varepsilon$) λ_{max} ($\Delta\varepsilon$) 214 (−54.49), 242 (+10.27), 284 (+5.77) ; IR (KBr) ν_{max} 3423, 2921, 1741, 1679, 1384, 1242, 1104, 1028 cm^{-1}; ^1H (600 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2 in the manuscript; HR-ESI-MS *m/z* 335.1852 $[M + H]^+$ (Calcd. for $C_{19}H_{27}O_5$, 335.1853).

Sarglanoid E (5): white amorphous powder; $[\alpha]_D^{24}$ −51.2 (*c* 0.103, MeOH); UV (MeOH) λ_{max} (log ε): 206 (4.13) nm; ECD (MeOH, $\Delta\varepsilon$) λ_{max} ($\Delta\varepsilon$) 210 (−21.71), 227 (+18.35), 249 (−23.88) ; IR (KBr) ν_{max} 3443, 2922, 1766, 1631, 1445, 1241, 1165, 1112, 1023, 952, 893 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2 in the manuscript; HR-ESI-MS *m/z* 335.1855 $[M + H]^+$ (Calcd. for $C_{19}H_{27}O_5$, 335.1853).

Sarglanoid F (6): yellow oil; $[\alpha]_D^{24}$ −48.9 (*c* 0.185, MeOH); UV (MeOH) λ_{max} (log ε): 219 (3.92) nm; ECD (MeOH, $\Delta\varepsilon$) λ_{max} ($\Delta\varepsilon$) 207 (−11.17), 218 (+4.51), 243 (−42.23), 290 (+2.05) ; IR (KBr) ν_{max} 3436, 2971, 1765, 1711, 1385, 1316, 1279, 1183, 1076, 1025, 976, 950, 885 cm^{-1}; ^1H (500 MHz) and ^{13}C (125 MHz) NMR data, see Tables 1 and 2 in the manuscript; HR-ESI-MS *m/z* 293.1748 $[M + H]^+$ (Calcd. for $C_{17}H_{25}O_4$, 293.1747).

Cell viability assay

RAW 264.7 cells were seeded into 96-well plates (4.0×10^3/well), and incubated at 37 °C for 18 h. The cells were then treated with various concentrations of each compound (**1**−**6**) for 1 h and incubated with LPS (1 μg·mL^{-1}) for another 18 h. Then, 10 μL of MTT (5 mg·mL^{-1} in PBS) was added to the wells and incubated for 4 h. The resultant absorbance was detected on a microplate reader (SpectraMax Plus384, Molecular Devices) at 570 nm.

Inhibition of NO production assay

RAW 264.7 cells were cultured into 96-well plates (6×10^4/well) and pretreated with different concentrations of each compound (**1**-**6**) for 1 h, followed by incubation with LPS (1 μg·mL^{-1}) for 18 h. The supernatant (50 μL) was then mixed with an equal volume of Griess reagent at room temperature for 15 min before measurement of the optical density at 540 nm through a microplate reader. All assays were performed in triplicate. The results showed that the IC_{50} value of compound **3** was 20.00 ± 1.30 μmol·L^{-1}, while those for the other compounds were all > 50 μmol·L^{-1}.

Supplementary Material

Supplementray information can be acquired by e-mail to corresponding author.

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