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# Sesquiterpene dimers esterified with diverse small organic acids from the seeds of *Sarcandra glabra*

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ABSTRACT

11 new sesquiterpene dimers, sarglabolides A–K (**1**–**11**), and five known ones were isolated from the seeds of *Sarcandra glabra*. Their structures were elucidated by spectroscopic data analysis and chemical evidence. Sarglabolide A (**1**) was verified to exclusively possess a seventeen-membered macrocyclic ester ring formed by the scaffold of the sesquiterpene dimer and small organic acids, different from the eighteen-membered rings of the other reported analogues. The chiral small organic acid moieties were assigned to L-malic acid, D-malic acid, and D-tartaric acid based on the combination of spectroscopy, chemical derivatization and HPLC analysis. Dimers **1**, **12** and **13** can significantly inhibit NO production in LPS-induced macrophages with $IC_{50}$ values at 3.04, 4.65 and 2.33 μmol/L, respectively.



## 1. Introduction

Lindenane-type sesquiterpene dimers, sometimes featured with an eighteen-membered macrocyclic ester ring under the scaffold, were considered as the characteristic constituents of the plants of Chloranthaceae.[1] Due to the complex structures and potent bioactivities, e.g., endothelial activation, $K^+$ current inhibition, anti-inflammatory, anti-HIV, and cytotoxic activities,[2] these sesquiterpene dimers attracted much attention in the past decades. More than 60 sesquiterpene dimers have been isolated from Chloranthaceae, and most of them comprised the same scaffold constructed by two lindenane moieties via a [4+2] cycloaddition between $\Delta^{15(4),5(6)}$ and $\Delta^{8'(9')}$.[3] In our previous research work, several medicinal plants of Chloranthaceae had been investigated for bioactive ingredients, leading to the isolation of some new lindenane dimers.[2c,4]

*Sarcandra glabra* (Thunb.) Nakai (Chloranthaceae), a subshrub widely growing in China and other East and Southeast Asian countries, was recorded in China Pharmacopeia as a traditional herbal medicine for the treatment of inflammation and traumatic injuries.[5] The early studies revealed the abundance of sesquiterpenes and lindenane dimers in this plant.[6] Recently, some lindenane dimers with novel structures and promising bioactivities were isolated from *S. glabra*[2g,2h] and *Sarcandra hainanensis*,[7] which inspired us to have a deep investigation of the dimers in this plant. As a result, 11 new sesquiterpene dimers, sarglabolides A–K (**1**–**11**), and five known ones (**12**–**16**) were isolated from the seeds of *S. glabra*. Sarglabolide A (**1**) was verified to exclusively possess a seventeen-membered macrocyclic ester ring formed by the scaffold of the sesquiterpene dimer and small organic acids, which was different from the eighteen-membered rings of the other reported analogues. In addition, these dimers contained a variety of small organic acid moieties, including chiral malic acid and tartaric acid, which resulted in a challenge in characterization of their absolute configurations due to the independence of the chiral carbons in organic acid moieties. A combination of spectroscopy, chemical derivatization and HPLC analysis was applied to resolve this problem. The inhibitory effects on NO production in LPS-induced macrophages were evaluated and **1**, **12** and **13** showed excellent results. Herein, we describe the isolation and structural elucidation of the new compounds as well as the assessment of their bioactivities.

## 2. Results and discussion

Sarglabolide A (**1**) was obtained as a white, amorphous powder. Its molecular formula was established as $C_{40}H_{44}O_{14}$ by HRESIMS, which showed the quasimolecular ion peak of **1** $[M+Na]^+$ at *m*/*z* 771.2619 (calcd for $C_{40}H_{44}O_{14}Na$ 771.2623). The $^1H$ NMR and HSQC

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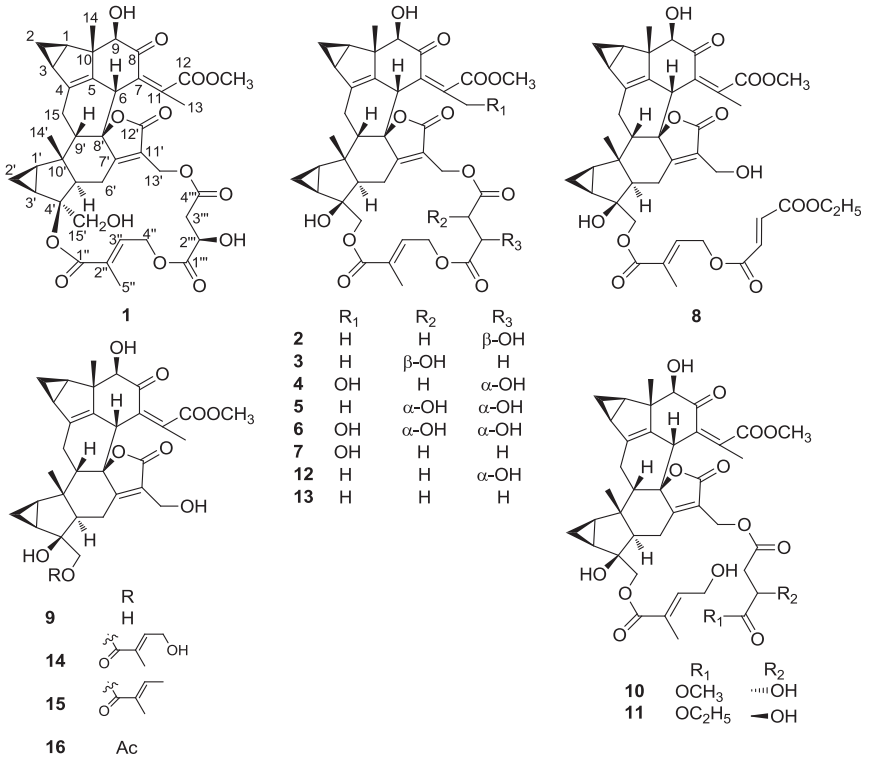
spectra of **1** displayed two pairs of characteristic upfield protons at $\delta_H$ 0.35 (H-2, dd, $J$=7.4, 4.2 Hz), 1.02 (H-2, m), 0.62 (H-2′, m) and 0.85 (H-2′, m), diagnostic of two cyclopropane rings in the lindenane dimers.[3] Disregarding 30 carbons belonging to the core framework of the dimer, 10 carbons were still left, which might account for the existence of a macrocyclic ester ring under the scaffold of **1**. A tiglyl moiety could be recognized at $\delta_H$ 6.73 (H-3″, t, $J$=4.1 Hz), 4.80 (H-4″, ddd, $J$=15.9, 5.0, 1.1 Hz), 5.22 (H-4″, ddd, $J$=15.9, 4.2, 1.5 Hz), and 1.81 ($H_3$-5″, d, $J$=0.9 Hz), which was a common substituent at 15′-OH in the dimers.

Besides $H_2$-4″, another two oxygenated methylene doublets at $\delta_H$ 4.35 (H-13′, d, $J$=12.0 Hz), 5.37 (H-13′, d, $J$=12.0 Hz), 3.73 (H-15′, d, $J$=12.0 Hz) and 4.07 (H-15′, d, $J$=12.0 Hz) were observed, which were ascribed to C-13′ and C-15′ based on the hypothesis of the macrocylic ester ring. Likewise, an oxygenated methine at $\delta_H$ 4.41 (H-2‴, dd, $J$=4.1, 2.9 Hz) and a correlated methylene at $\delta_H$ 2.91 (H-3‴, dd, $J$=17.0, 2.9 Hz) and 2.96 (H-3‴, dd, $J$=17.0, 4.1 Hz) suggested a malic acid moiety probably located at 15′-OH in view of its popularity in these dimers. The $^{13}$C NMR spectrum of **1**, in accord with its molecular formula, showed 40 carbon resonances and was similar to that of shizukaol G (**12**) except for the greatly upfield shifted C-15′ ($\delta_C$ 66.3 in **1**, 72.2 in **12**) and greatly downfield shifted C-4′ ($\delta_C$ 90.0 in **1**, 77.2 in **12**),[8] indicating the possible esterification of 4′-OH, instead of 15′-OH, with 1″-carboxyl. This unprecedented linkage, forming a 17-membered ring, was confirmed by the absence of the correlation from H-15′ to C-1″ in the HMBC spectrum of **1**, which was commonly visible in the analogues. Thus, sarglabolide A (**1**) was found to be the first case that constructed a pendent macrocyclic ester ring by esterification of 4′-OH with 4-hydroxytiglic acid. The complete linkage of the macrocyclic ring was resolved by the key HMBC correlations from H-4″ to C-1‴ and from H-13′ to C-4‴ (Fig. 2). In addition, the orientation of the malic residue was determined according to its $^{13}$C NMR data, which showed the head carbonyl C-1‴ with larger $^{13}$C shift at $\delta_C$ 172.9 and the terminal carbonyl C-4‴ at $\delta_C$ 171.2. The dimerized lindenanes framework of **1** was characterized to be the same as that of **12**, based on the key HMBC correlations from H-15 ($\delta_H$ 2.57 and 2.83) to C-8′ ($\delta_C$ 93.8), C-9′ ($\delta_C$ 55.1) and C-10′ ($\delta_C$ 44.9), and from H-6 ($\delta_H$ 3.92) to C-8′ ($\delta_C$ 93.8). Therefore, the whole planar structure of **1** was obtained as shown in Fig. 1.

Analysis of the ROESY spectrum of **1** revealed its relative configuration (Fig. 2). The correlations of H-1/H-3, H-1/H-9, H-9/H-5′, H-1′/H-5′, H-3′/H-5′, H-1′/H-15′, H-3′/H-15′ and H-5′/H-15′ indicated their co-facial orientation that was arbitrarily assigned as α. As a result, $H_3$-14, H-6, H-9′ and $H_3$-14′ were assigned as β due to the ROESY correlations of $H_3$-14/H-6, H-6/H-9′, and H-9′/$H_3$-14′. Also, the correlation of H-4″ and H-5″ suggested an *E*-form of the double bond in the 4-hydroxytiglic residue. The CD spectrum of **1** was recorded and presented similar data to those of the known analogues. An exciton chirality CD method was applied to resolve the absolute configuration of its scaffold.[2g,2h] Two coupling chromophores in the molecule, *i.e.* the π,π,π-conjugated system (C-7–C-8 and C-11–C-12) and the α,β-unsaturated γ-lactone (C-7′–C-11′–C-12′), generated the split Cotton effects in the CD spectrum of **1**, showing a positive first Cotton effect at 255 nm (Δε +4.15) and a negative second Cotton effect at 221 nm (Δε −26.9). Thus, a right-handed helicity of two coupling chromophores could be derived from this positive exciton chirality (Fig. 3). The orientation of 2‴-OH, however, could not be assigned due to its independence away from the scaffold.

We developed a method involving chemical derivatization and HPLC analysis to determine the absolute configuration of the malic residue. Alkaline hydrolysis of **1** and subsequent acidification afforded a mixture of malic acid and 4-hydroxytiglic acid. In view of the non-optical activity of 4-hydroxytiglic acid, the specific OR ([α] 23 D) of the organic acids mixture in water was measured and basically ascribed to malic acid, showing a value of +2.7° that was close to that of the standard D-malic acid (+4.2°). After esterification in methanol catalyzed by $SOCl_2$, the methyl esters of the organic acids reacted with (*S*)-MTPA chloride to produce (*R*)-MTPA




| | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| **2** | H | H | β-OH |
| **3** | H | β-OH | H |
| **4** | OH | H | α-OH |
| **5** | H | α-OH | α-OH |
| **6** | OH | α-OH | α-OH |
| **7** | OH | H | H |
| **12** | H | H | α-OH |
| **13** | H | H | H |

**Fig. 1.** Structures of sarglabolides A–K (**1**–**11**) and the known dimers (**12**–**16**).

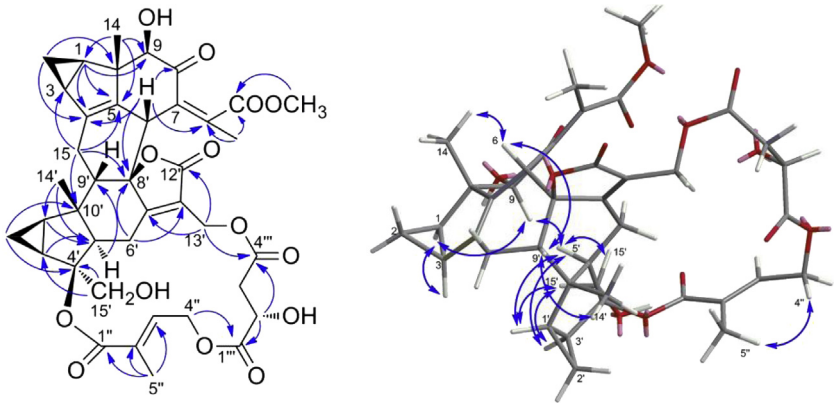



**Fig. 2.** Key HMBC and ROESY correlations of sarglabolide A (**1**).

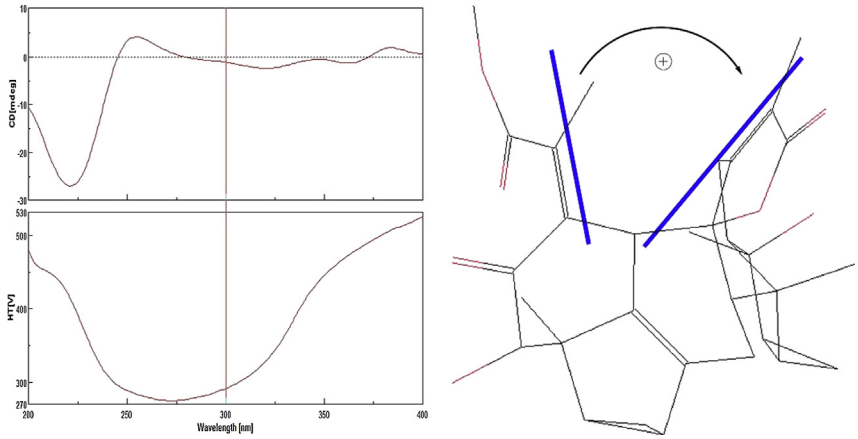



**Fig. 3.** CD spectrum of **1** recorded in MeOH, and the stereoview of the scaffold of **1**. Bold blue lines denote the electronic transition of two dipoles.

esters of dimethyl malate and methyl 4-hydroxytiglate, which were directly analyzed in HPLC. By comparing the retention time of the above (*R*)-MTPA ester of dimethyl malate ($t_R$=6.62 min) with those of the authentic (*R*)-MTPA esters prepared from standard D-malic acid and L-malic acid ($t_R$=6.52 min and 7.72 min, respectively, Fig. 4) in the same manner, the configuration of the malic residue in **1** was established as D-form. It was the first case that the sesquiterpene dimer was esterified with a D-malic acid so far.

Sarglabolides B (**2**) and C (**3**) were considered two isomers of **1** on the basis of their HRESIMS experiments, which afforded the same molecular formula of $C_{40}H_{44}O_{14}$ for them. Analysis of the $^1$H and $^{13}$C NMR spectra of **2** and **3** suggested the close resemblance of their planar structures. In detail, their $^1$H NMR spectra exhibited similar resonance signals and differed only in chemical shifts (Table 1). The characteristic cyclopropane signals at $\delta_H$ 0.32 (H-2 of **2**, dd, *J*=7.4, 4.2 Hz), 0.71 (H-2′ of **2**, td, *J*=8.7, 5.8 Hz), 0.36 (H-2 of **3**,

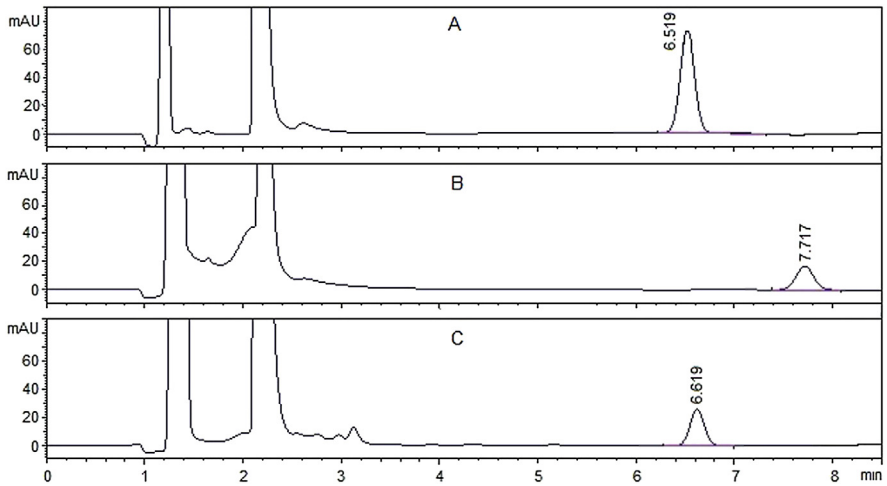



**Fig. 4.** HPLC chromatograms of (*R*)-MTPA esters of dimethyl malates, eluted with an isocratic methanol/water system (65:35, v/v) at 1 mL/min and monitored at 220 nm. A, B and C present the products prepared from standard D-malic acid, standard L-malic acid, and the hydrolyzate of **1**, respectively.

dd, $J$=7.4, 4.2 Hz) and 0.73 (H-2′ of **3**, td, $J$=8.7, 5.8 Hz) suggested their core frameworks of lindenane dimers. A 4-hydroxytiglic acid moiety and a malic acid moiety could be recognized in their $^1$H NMR spectra, respectively, suggestive of macrocyclic ester rings possibly formed in their molecules. The $^{13}$C NMR spectra of **2** and **3** were almost identical except the relatively large distinctions at C-13′ and the carbons corresponding to the malic residues (Table 4). Detailed analysis of the HMBC spectra of **2** and **3** confirmed their planar structures and revealed their close resemblance with **12**. Specifically, **2** possessed the same planar structure as that of **12**, and **3** had a reversed orientation of the malic residue relative to **2** and **12**, which was confirmed by the HMBC correlations from H-13′ ($\delta_H$ 4.76 and 4.85) to C-4‴ (the head carboxyl of malic acid, $\delta_C$ 174.2), and from H-4″ ($\delta_H$ 4.53 and 5.04) to C-1‴ (the terminal carboxyl of malic acid, $\delta_C$ 170.9). On the basis of ROESY and CD experiments, the relative and absolute configurations of the scaffolds of **2** and **3** were established and presented the same results as **1**. Therefore, malic residues were deduced to be the only difference between **2**, **3** and **12**. A series of derivatizations of **2** and **3** and subsequent HPLC analyses, as same as **1**, were conducted and demonstrated that a D-malic residue and an L-malic residue existed in **2** and **3**, respectively, based on the specific OR values of the malic acids hydrolyzed from **2** and **3** (+4.3° for **2** and −6.8° for **3**) and the retention times of their (*R*)-MTPA esters in HPLC (6.62 min for **2** and 7.55 min for **3**). In other words, **2** was an epimer of **12** at C-2‴, and **3** had a reversed linkage of malic residue relative to **12**.

(around $\delta_C$ 20) compared with **12**.[5] The $^1$H NMR of **4**, correspondingly, showed one more oxygenated methylene at $\delta_H$ 4.28 (d, H-13, $J$=15.2 Hz) and 4.35 (d, H-13, $J$=15.2 Hz) and one less methyl located on a double bond, with an overall similarity with **12**. Consequently, **4** was deduced to be a 13- or 5″-oxygenated derivative of **12**. Further analysis of the NMR data of **4**, particularly the key HMBC correlations from H-13 to C-7, C11 and C-12, confirmed this deduction and elucidated its basic structure (Fig. 1), *i.e.*, 13-hydroxyl shizukaol G. A ROESY and a CD experiments revealed the same configuration of the scaffold of **4** as that of **12**. The malic residue proved to be L-form by the aforementioned method ([α]23 $D$=−4.1°, $t_R$=7.60 min). Thus, sarglabolide D (**4**) was identified to be 13-hydroxyl shizukaol G.

Sarglabolide E (**5**) was obtained as a white, amorphous powder. The HRESIMS spectrum of **5** afforded its molecular formula $C_{40}H_{44}O_{15}$, suggestive of another oxygenated derivative of **12**. The $^{13}$C NMR spectrum of **5** also showed one more oxygenated carbon ($\delta_C$ 73.1, C-3‴) and one less upfield carbon (around $\delta_C$ 37) compared with **12**, and the $^1$H NMR of **5** displayed one more oxygenated methine doublet at $\delta_H$ 4.79 (d, H-3‴, $J$=1.5 Hz). Further interpretation of the NMR data, particularly the key HMBC correlations from the additional oxygenated methine proton (H-3‴) to C-1‴ ($\delta_C$ 171.3), C-2‴ ($\delta_C$ 72.9) and C-4‴ ($\delta_C$ 171.3) and the significantly increased shift of C-2‴ ($\delta_C$ 72.9) compared with **12**, defined the overall structure of **5** and determined a hydroxyl was located at C-3‴, namely a tartaric residue replacing the malic residue in **12**,

**Table 1**
$^1$H NMR data of compounds **1**–**4** (500 MHz in $CDCl_3$, $J$ in Hz)

| Position | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 2.09, m | 2.05, m | 2.07, m | 2.08, m |
| 2 | 0.35, dd (7.4, 4.2) | 0.32, dd (7.4, 4.2) | 0.36, dd (7.4, 4.2) | 0.39, dd (7.4, 4.2) |
| | 1.02, m | 1.00, m | 1.02, m | 1.04, td (8.3, 4.6) |
| 3 | 1.87, m | 1.84, m | 1.85, m | 1.86, m |
| 6 | 3.92, d (3.3) | 3.93, d (3.1) | 3.96, d (3.3) | 3.90, d (3.3) |
| 9 | 4.10, s | 3.86, s | 3.86, s | 3.82, s |
| 13 | 1.85, s | 1.87, s | 1.92, s | 4.28, d (15.2) |
| | | | | 4.35, d (15.2) |
| 14 | 1.00, s | 1.02, s | 1.03, s | 1.08, s |
| 15 | 2.57, ddd (16.3, 6.2, 4.0) | 2.57, ddd (16.3, 5.8, 4.1) | 2.58, ddd (16.3, 5.9, 4.0) | 2.55, ddd (16.3, 6.0, 3.8) |
| | 2.83, br.d (16.3) | 2.79, br.d (16.3) | 2.81, br.d (16.3) | 2.85, br. d (16.3) |
| 1′ | 1.70, m | 1.60, m | 1.62, m | 1.63, m |
| 2′ | 0.62, m | 0.71, td (8.7, 5.8) | 0.73, td (8.7, 5.8) | 0.73, td (8.8, 5.9) |
| | 0.85, m | 1.34, m | 1.33, m | 1.32, m |
| 3′ | 1.85, m | 1.38, m | 1.40, m | 1.41, m |
| 5′ | 2.17, dd (13.6, 6.4) | 1.86, m | 1.92, m | 1.94, m |
| 6′ | 2.46, dd (18.8, 6.4) | 2.30, dd (19.0, 6.0) | 2.32, dd (18.7, 6.0) | 2.25, dd (18.8, 6.2) |
| | 3.29, dd (18.8, 13.6) | 2.87, dd (19.0, 13.8) | 2.68, dd (18.7, 13.6) | 2.69, dd (18.8, 13.6) |
| 9′ | 1.99, dd (6.5, 1.5) | 1.84, m | 1.89, m | 1.95, m |
| 13′ | 4.35, d (12.0) | 4.39, d (12.0) | 4.76, d (11.7) | 4.76, d (11.7) |
| | 5.37, d (12.0) | 5.43, d (12.0) | 4.85, d (11.7) | 4.84, d (11.7) |
| 14′ | 0.66, s | 0.80, s | 0.80, s | 0.79, s |
| 15′ | 3.73, d (12.0) | 3.67, d (11.9) | 3.64, d (12.0) | 3.68, d (11.9) |
| | 4.07, d (12.0) | 4.53, d (11.9) | 4.57, d (12.0) | 4.56, d (11.9) |
| 3″ | 6.73, t (4.1) | 6.53, t (5.5) | 6.65, t (5.7) | 6.64, t (5.7) |
| 4″ | 4.80, ddd (15.9, 5.0, 1.1) | 4.59, dd (14.2, 6.6) | 4.53, dd (13.9, 7.3) | 4.54, dd (14.0, 7.5) |
| | 5.22, ddd (15.9, 4.2, 1.5) | 5.47, dd (14.2, 5.5) | 5.04, dd (13.9, 5.2) | 5.04, dd (14.0, 5.2) |
| 5″ | 1.81, d (0.9) | 1.96, br. s | 1.92, br. s | 1.93, br. s |
| 2‴ | 4.41, dd (4.1, 2.9) | 4.40, m | 2.91, dd (17.8, 4.1) | 4.41, t (3.6) |
| | | | 3.16, dd (17.8, 3.1) | |
| 3‴ | 2.91, dd (17.0, 2.9) | 2.87, dd (17.1, 4.8) | 4.35, t (3.4) | 2.91, dd (17.9, 4.3) |
| | 2.96, dd (17.0, 4.1) | 3.32, dd (17.1, 2.6) | | 3.29, dd (17.9, 3.2) |
| $OCH_3$ | 3.79, s | 3.70, s | 3.74, s | 3.76, s |

Sarglabolide D (**4**) was also obtained as a white, amorphous powder. The HRESIMS experiment allowed an accurate molecular formula of $C_{40}H_{44}O_{15}$ to be assigned to **4**, suggestive of an oxygenated derivative of **12**. The $^{13}$C NMR spectrum of **4** showed one more oxygenated carbon ($\delta_C$ 61.1, C-13) and one less upfield carbon

which presented the first case in dimers to date. The relative and absolute configurations of the scaffold of **5** were established on the basis of ROESY and CD experiments, respectively. As for the tartaric residue, the same methodology as the above was applied and demonstrated the tartaric residue in **5** was D-form according to its

**Table 2**
$^1$H NMR data of compounds **5**–**8** (500 MHz in $CDCl_3$ or $CD_3OD$, *J* in Hz)

| Position | **5**[a] | **6**[b] | **7**[a] | **8**[a] |
|---|---|---|---|---|
| 1 | 2.07, m | 1.99, m | 2.06, m | 2.08, m |
| 2 | 0.39, dd (7.3, 4.1) | 0.35, dd (7.1, 4.1) | 0.35, dd (7.3, 4.0) | 0.33, dd (7.3, 4.2) |
| | 1.03, m | 0.99, td (7.8, 4.3) | 1.02, td (7.7, 4.5) | 1.01, m |
| 3 | 1.86, m | 1.94, m | 1.84, m | 1.85, m |
| 6 | 3.95, d (3.1) | 3.89, d (3.4) | 3.90, d (3.3) | 3.92, d (5.7) |
| 9 | 3.83, s | 3.80, s | 3.82, s | 3.93, s |
| 13 | 1.92, s | 4.01, d (14.7) | 4.31, d (15.5) | 1.92, s |
| | | 4.19, d (14.7) | 4.32, d (15.5) | |
| 14 | 1.01, s | 1.15, s | 1.07, s | 1.01, s |
| 15 | 2.56, ddd (16.2, 5.9, 3.9) | 2.53, ddd (16.3, 5.6, 4.1) | 2.56, m | 2.58, ddd (16.4, 6.0, 4.0) |
| | 2.84, br.d (16.2) | 2.85, br.d (16.3) | 2.81, br. d (16.3) | 2.80, br. d (16.3) |
| 1′ | 1.65, m | 1.67, td (8.3, 4.3) | 1.61, m | 1.61, td (8.3, 4.2) |
| 2′ | 0.73, td (8.7, 5.8) | 0.74, td (8.7, 5.5) | 0.73, td (8.8, 6.0) | 0.73, td (8.8, 5.9) |
| | 1.32, m | 1.33, m | 1.32, m | 1.28, m |
| 3′ | 1.42, m | 1.38, m | 1.41, m | 1.50, td (8.9, 3.5) |
| 5′ | 1.98, m | 1.78, m | 1.87, m | 1.88, m |
| 6′ | 2.35, dd (19.0, 6.2) | 2.45, dd (18.9, 6.2) | 2.40, dd (18.8, 6.1) | 2.32, dd (18.3, 6.0) |
| | 2.81, m | 2.90, dd (18.9, 13.8) | 2.73, m | 2.73, dd (18.3, 13.8) |
| 9′ | 1.96, m | 1.82, m | 1.91, m | 1.94, m |
| 13′ | 4.66, d (11.8) | 4.63, d (12.0) | 4.53, d (12.0) | 4.34, d (13.7) |
| | 5.15, d (11.8) | 5.09, d (12.0) | 5.03, d (12.0) | 4.40, d (13.7) |
| 14′ | 0.80, s | 0.85, s | 0.80, s | 0.88, s |
| 15′ | 3.71, d (11.9) | 3.67, d (11.6) | 3.68, d (11.9) | 3.87, d (11.6) |
| | 4.59, d (11.9) | 4.45, d (11.6) | 4.52, d (11.9) | 4.25, d (11.6) |
| 3″ | 6.62, t (5.6) | 6.74, td (5.4, 1.2) | 6.60, t (5.5) | 6.78, td (6.2, 1.3) |
| 4″ | 4.93, dd (14.1, 6.7) | 4.97, dd (14.2, 5.0) | 4.64, dd (14.7, 6.8) | 4.93, br. d (4.3) |
| | 5.07, dd (14.1, 5.3) | 5.04, dd (14.2, 7.3) | 5.05, dd (14.7, 5.0) | |
| 5″ | 1.93, br. s | 1.94, br. s | 1.92, br. s | 1.94, br. s |
| 2‴ | 4.68, d (1.5) | 4.72, d (2.4) | 2.52, m | 6.89, d (16.3) |
| | | | 2.95, m | |
| 3‴ | 4.79, d (1.5) | 4.80, d (2.4) | 2.74, m | 6.88, d (16.3) |
| $OCH_3$ | 3.70, s | 3.66, s | 3.72, s | 3.76, s |
| $OC_2H_5$ | | | | 4.27, q (7.1) |
| | | | | 1.32, t (7.1) |

[a] Measured in $CDCl_3$.
[b] Measured in $CD_3OD$.

specific OR (−6.3° for tartaric acid hydrolyzed from **5**, and −14.2° for standard D-tartaric acid)[9] and $t_R$ (5.89 min for tartaric derivative prepared from **5**, 5.89 min for authentic D-tartaric derivative and 6.07 min for authentic L-tartaric derivative, see supplementary data S39–41), namely, both α-OH at C-2‴ and C-3‴. Therefore, the whole structure of **5** was established as shown in Fig. 1.

Sarglabolide F (**6**), a white, amorphous powder, was assigned a molecular formula of $C_{40}H_{44}O_{16}$ based on the HRESIMS experiment, suggesting a further oxygenated derivative of **4** or **5**. A structural hybrid of **4** and **5** for the structure of **6** could be easily concluded due to the inclusion of both oxygenated methylene in **4** and oxygenated methine in **5**, recognizable at $\delta_H$ 4.01 (d, H-13, *J*=14.7 Hz), 4.19 (d, H-13, *J*=14.7 Hz) and 4.80 (d, H-3‴, *J*=2.4 Hz), and at $\delta_C$ 61.2 (C-13) and 74.2 (C-3‴). A thorough investigation of the NMR data and CD spectrum of **6** confirmed the conclusion and elucidated the exact structure except for the configuration of tartaric residue, which was assigned as D-form, *i.e.*, both α-oriented hydroxyl, by the same method described as the above ([α]23 *D*=−5.1°, $t_R$=5.89 min). As a result, **6** was characterized as a polyhydric dimer as shown in Fig. 1.

Sarglabolide G (**7**) was isolated as a white, amorphous powder, and was assigned a molecular formula of $C_{40}H_{44}O_{14}$ on the basis of HRESIMS. Appearently, **7** was another isomer of **12**. Its $^1$H NMR spectrum, however, suggested much resemblance with **4**. The absence of an oxygenated methine signal, corresponding to H-2‴ in **4**, suggested that **7** might be a 2‴-deoxy derivative of **4**. The $^{13}$C NMR spectrum of **7** showed, likewise, a less oxygenated carbon and a more upfield one compared with **4**. Analysis of the HMBC spectrum confirmed the above deduction based on the correlations from H-2‴ ($\delta_H$ 2.52 and 2.95) to C-1‴ ($\delta_C$ 171.9), C-3‴ ($\delta_C$ 29.3) and C-4‴ ($\delta_C$ 172.1). The relative and absolute configurations of **7** were established by ROESY and CD, respectively. As a result, sarglabolide G (**7**) was determined to be 2‴-deoxysarglabolide D.

Sarglabolide H (**8**) was obtained as a white, amorphous powder. Its molecular formula was assigned as $C_{42}H_{48}O_{14}$ by HRESIMS. The $^1$H NMR spectrum of **8** displayed much complicated resonance signals and had great differences with those of the other dimers. The resonance signals belonging to an ethoxyl and a 4-hydroxytiglyl groups could be easily observed. The $^1$H NMR spectrum also showed two olefinic doublets at $\delta_H$ 6.89 (H-2‴, d, *J*=16.3 Hz) and 6.88 (H-3‴, d, *J*=16.3 Hz), reminiscent of an AB spin system at an *E*-form double bond. The rest of proton signals could be easily assigned to the core framework of a sesquiterpene dimer. Excluding the ethoxyl carbons, the $^{13}$C NMR spectrum of **8** displayed 40 carbon signals, suggestive of the presence of a malic acid-related moiety. Detailed analysis of the $^{13}$C and HMBC spectra, especially the HMBC correlations from H-2‴ ($\delta_H$ 6.89) and H-3‴ ($\delta_H$ 6.88) to C-1‴ ($\delta_C$ 165.0) and C-4‴ ($\delta_C$ 165.0), disclosed that two olefinic carbons replaced the middle two carbons of the malic residue in the corresponding dimers. Apparently, sarglabolide G (**8**) contained a first fumaric residue in its molecule. After the determination of the location of ethoxyl on the basis of a key HMBC correlation from ethoxyl to C-4‴ ($\delta_C$ 165.0), the planar structure of **8** was established as shown in Fig. 1. The ROESY experiment of **8** afforded the same correlations in the scaffold as those of **1**, and suggested an identical relative configuration. Likewise, its absolute configuration was determined to be the same as that of **1** by the similar CD data at 200–260 nm, showing a negative Cotton effect at 217 nm ($\Delta\varepsilon$ −5.69) and a positive Cotton effect at 251 ($\Delta\varepsilon$ +1.82).

Sarglabolide I (**9**), a white, amorphous powder, was assigned a molecular formula of $C_{31}H_{36}O_9$ on the basis of HRESIMS, which, appearently, suggested no pendent macrocyclic ester ring. The

**Table 3**
$^1$H NMR data of compounds **9**–**11** (500 MHz in $CDCl_3$ or $CD_3OD$, *J* in Hz)

| Position | **9**[a] | **10**[b] | **11**[b] |
|---|---|---|---|
| 1 | 2.00, m | 2.07, m | 2.07, m |
| 2 | 0.32, dd (7.3, 4.1) | 0.32, dd (7.3, 4.2) | 0.32, dd (7.3, 4.0) |
| | 0.98, td (7.8, 4.3) | 1.01, m | 1.01, m |
| 3 | 1.93, m | 1.84, m | 1.85, m |
| 6 | 3.94, d (3.3) | 3.93, br. s | 3.93, br. s |
| 9 | 4.04, s | 3.93, s | 3.93, s |
| 13 | 1.85, s | 1.87, s | 1.87, s |
| 14 | 1.03, s | 1.01, s | 1.02, s |
| 15 | 2.53, ddd | 2.57, ddd | 2.58, ddd |
| | (16.4, 6.0, 4.2) | (16.3, 5.8, 4.2) | (16.3, 5.8, 4.2) |
| | 2.83, br. d (16.4) | 2.77, m | 2.78, m |
| 1′ | 1.61, td (8.2, 4.1) | 1.60, m | 1.60, m |
| 2′ | 0.68, td (8.8, 5.5) | 0.72, td (8.8, 5.9) | 0.72, td (8.8, 5.9) |
| | 1.22, m | 1.31, m | 1.32, m |
| 3′ | 1.53, td (8.8, 3.6) | 1.42, m | 1.42, m |
| 5′ | 1.71, dd (13.8, 6.0) | 1.87, m | 1.88, m |
| 6′ | 2.24, dd (18.3, 6.0) | 2.41, dd (18.6, 6.1) | 2.41, dd (18.6, 6.1) |
| | 2.78, dd (18.3, 13.9) | 2.76, dd (18.6, 13.7) | 2.77, dd (18.6, 13.7) |
| 9′ | 1.82, dd (6.1, 1.2) | 1.87, m | 1.89, m |
| 13′ | 4.24, d (13.2) | 4.78, d (12.9) | 4.78, d (12.9) |
| | 4.26, d (13.2) | 4.90, d (12.9) | 4.90, d (12.9) |
| 14′ | 0.91, s | 0.84, s | 0.84, s |
| 15′ | 3.31, d (10.9) | 3.75, d (11.8) | 3.75, d (11.8) |
| | 3.35, d (10.9) | 4.42, d (11.8) | 4.44, d (11.8) |
| 3″ | | 6.79, t (5.5) | 6.80, t (5.5) |
| 4″ | | 4.34, dd (15.0, 5.5) | 4.35, dd (15.0, 5.5) |
| | | 4.40, m | 4.42, m |
| 5″ | | 1.87, br. s | 1.87, br. s |
| 2‴ | | 4.54, dd (6.8, 4.3) | 4.52, dd (6.8, 4.3) |
| 3‴ | | 2.81, dd (16.4, 6.9) | 2.80, dd (16.4, 6.9) |
| | | 2.93, dd (16.4, 4.2) | 2.92, dd (16.4, 4.2) |
| 12-$OCH_3$ | 3.77, s | 3.73, s | 3.73, s |
| 1‴-$OCH_3$ | | 3.79, s | |
| $OC_2H_5$ | | | 4.25, q (7.1) |
| | | | 1.30, t (7.1) |

[a] Measured in $CD_3OD$.
[b] Measured in $CDCl_3$.

NMR data of **9** displayed diagnostic features of the scaffolds of the above dimers. Two pairs of oxygenated methylene doublets at $\delta_H$ 4.26 (1H, d, *J*=13.2 Hz), 4.24 (1H, d, *J*=13.2 Hz), 3.35 (1H, d, *J*=10.9 Hz), and 3.31 (1H, d, *J*=10.9 Hz) were assigned to $H_2$-13′ and $H_2$-15′, respectively. Four methyl singlets at $\delta_H$ 3.77 (3H, s), 1.85 (3H, s), 1.03 (3H, s), and 0.91 (3H, s) corresponded to $-OCH_3$, $CH_3$-13, $CH_3$-14 and $CH_3$-14′, respectively. The characteristic cyclopropane signals, likewise, could be observed at $\delta_H$ 0.32 (H-2, dd, *J*=7.3, 4.1 Hz) and 0.68 (H-2′, td, *J*=8.8, 5.5 Hz). Besides, the easily recognizable H-9 and H-6, similar to the above dimers, were also found at $\delta_H$ 4.04 (H-9, s) and 3.94 (H-6, d, *J*=3.3 Hz). Thus, sarglabolide I (**9**) was deduced to have the structure identical to the scaffolds of the above dimers. Detailed analysis of 1D and 2D NMR data (Tables 3 and 4) confirmed this deduction. A ROESY and a CD experiments were conducted to determine the relative and absolute configurations of **9**, and gave the same results as the above dimers.

Sarglabolide J (**10**) and K (**11**) were also obtained as white, amorphous powders. Their molecular formulae were established to be $C_{41}H_{48}O_{15}$ and $C_{42}H_{50}O_{15}$, respectively, according to their HRESIMS spectra. The $^1$H and $^{13}$C NMR spectra of **10** and **11** exhibited close similarity (Tables 3 and 4). Both of them contained the diagnostic signals of the 4-hydroxytiglic and malic residues. The scaffolds of **10** and **11** could be deduced to be the same as the above dimers from their NMR spectra. An additional oxygenated methyl for **10** at $\delta_H$ 3.79 (3H, s) and an oxygenated ethyl for **11** at $\delta_H$ 4.25 (2H, q, *J*=7.1 Hz) and 1.30 (3H, t, *J*=7.1 Hz) could be recognized in their $^1$H NMR spectra, indicating the main distinction between them. Both **10** and **11** were believed to have opened macrocyclic ester rings due to their molecular formulae and the absent HMBC crosspeaks from H-4″ ($\delta_H$ 4.34 and 4.40 for **10**; $\delta_H$ 4.35 and 4.42 for **11**) to C-1‴ ($\delta_C$ 173.6 for **10**; 173.2 for **11**). Instead, the additional oxygenated methyl and the oxygenated ethyl were found to be esterified with C-1‴ of **10** and **11**, respectively, which were supported by their HMBC correlations. The configurations of both **10** and **11**, including their scaffolds and the malic residues, were established by the aforementioned methodology, showing an L-malic residue for **10** ([α]23 *D*=−2.2°, $t_R$=7.72 min), a D-malic residue for **11** ([α]23 *D*=+2.7°, $t_R$=6.62 min) and the common scaffold for both of them.

Five known sesquiterpene dimers, shizukaol G (**12**),[8] shizukaol B (**13**),[8] sarcandrolide B (**14**),[2h] shizukaol C (**15**),[10] and shizukaol N (**16**)[11] were isolated and identified by comparison of their $^1$H and $^{13}$C NMR data with those of the reported. All the isolates in this research possessed the same scaffold, a Diels–Alder cycloadded product of two lindenanes, and differed in the pendent substituents at C-13′ and C-15′ (C-4′ for **1**), suggesting a common biosynthetic pathway for them. Typically, diverse small organic acids, other than dominant citric acid in the known dimers, were esterified with 13′-OH. The problem in determination of the configurations of those chiral small organic acids (malic acid and tartaric acid) could be thoroughly resolved according to the method developed by us. Additionally, sarglabolide A (**1**) presented a first example to be esterified at OH-4′ and construct a 17-membered ring, other than the eighteen-membered rings in all known analogues. The isolates in this paper also showed great distinction in composition and structural features with the reported dimers from the whole plants of *Sarcandra glabra*, suggestive of significant distributional difference of sesquiterpene dimers in this plant.

As a continuous research on the anti-inflammatory activities of the sesquiterpene dimers in Chloranthaceae,[2c,2d] we conducted the NO release inhibition tests of selective isolates **1**, **2**, **3**, **5**, **10**, **12** and **13**. As a result, compounds **1**, **12** and **13** exhibited significant bioactivities with $IC_{50}$ values at 3.04, 4.65 and 2.33 μmol/L, respectively.

## 3. Experimental section

### 3.1. General experimental procedures

Optical rotations and CD spectra were obtained on a JASCO P-1020 polarimeter and a JASCO 810 polarimeter, respectively. UV and IR spectra were recorded on a Shimadzu UV-2450 spectrometer and a Bruker Tensor 27 spectrometer, respectively. HRESIMS experiments were performed on an Agilent UPLC-Q-TOF (6520B). NMR spectra were recorded in $CDCl_3$ or $CD_3OD$ on a Bruker AV-500 NMR instrument at 500 MHz ($^1$H) and 125 MHz ($^{13}$C). Silica gel (Qingdao Marine Chemical Co., Ltd., China), MCI gel (Mitsubishi Chemical, Japan), ODS (FuJi, Japan), and Sephadex LH-20 (Pharmacia, Sweden) were used for column chromatography. Preparative HPLC was carried out on a Shimadzu LC-6A instrument with an SPD-10A detector and a shim-pack RP-C18 column (20×200 mm, 10 μm). RP-MPLC was carried out on a Quiksep system (H&E Co., Ltd., China). Analytical HPLC was performed on an Agilent 1200 series instrument using a DAD detector and a shim-pack VP–ODS column (150×4.6 mm, 5 μm). The standard L-(−)-malic acid, D-(+)-malic acid and D-(−)-tartaric acid were purchased from J&K Chemical Ltd. All solvents and reagents were of analytical grade.

### 3.2. Plant material

The fresh seeds of *S. glabra* were collected in Ganzhou, Jiangxi province, P. R. China in November 2013. The plant material was authenticated by Prof. Mian Zhang, Department of Medicinal Plants, China Pharmaceutical University. A voucher specimen (No. CSH201311) was deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

**Table 4**
$^{13}$C NMR data of compounds **1**–**11** (125 MHz)

| Position | **1**[a] | **2**[a] | **3**[a] | **4**[a] | **5**[a] | **6**[b] | **7**[a] | **8**[a] | **9**[b] | **10**[a] | **11**[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 26.5 | 26.3 | 26.5 | 26.7 | 26.8 | 26.9 | 26.5 | 26.3 | 26.3 | 26.1 | 26.1 |
| 2 | 16.2 | 16.1 | 16.2 | 16.3 | 16.3 | 16.1 | 16.2 | 16.1 | 16.1 | 16.1 | 16.1 |
| 3 | 25.1 | 24.9 | 25.0 | 25.1 | 25.1 | 25.3 | 25.0 | 25.0 | 25.4 | 24.9 | 24.9 |
| 4 | 142.8 | 142.6 | 142.9 | 143.4 | 143.1 | 143.4 | 143.0 | 142.7 | 143.2 | 142.6 | 142.6 |
| 5 | 132.7 | 132.5 | 132.7 | 131.5 | 131.5 | 133.8 | 132.4 | 132.4 | 133.8 | 132.0 | 132.0 |
| 6 | 41.7 | 41.4 | 41.6 | 42.5 | 42.0 | 43.0 | 42.2 | 41.3 | 42.0 | 41.2 | 41.2 |
| 7 | 131.0 | 131.8 | 131.6 | 132.7 | 133.1 | 135.3 | 131.7 | 131.1 | 134.4 | 132.2 | 132.2 |
| 8 | 201.1 | 201.0 | 201.2 | 202.1 | 201.3 | 203.6 | 201.7 | 200.4 | 202.2 | 200.8 | 200.8 |
| 9 | 80.1 | 80.0 | 80.0 | 80.0 | 79.7 | 80.8 | 80.1 | 80.3 | 81.2 | 80.2 | 80.2 |
| 10 | 51.2 | 51.0 | 51.1 | 50.9 | 50.9 | 52.2 | 50.9 | 51.3 | 52.5 | 51.1 | 51.1 |
| 11 | 147.6 | 147.0 | 147.2 | 148.9 | 146.9 | 148.9 | 148.9 | 147.7 | 147.1 | 146.9 | 147.0 |
| 12 | 170.8 | 170.3 | 170.4 | 168.3 | 170.6 | 169.8 | 168.2 | 168.2 | 173.1 | 171.2 | 171.2 |
| 13 | 20.5 | 20.1 | 20.0 | 61.1 | 19.7 | 61.2 | 61.2 | 20.5 | 20.4 | 20.1 | 20.1 |
| 14 | 15.8 | 15.5 | 15.6 | 15.9 | 15.8 | 16.1 | 15.8 | 15.5 | 15.8 | 15.5 | 15.5 |
| 15 | 25.4 | 25.5 | 25.6 | 25.5 | 25.5 | 26.1 | 25.5 | 25.5 | 26.0 | 25.4 | 25.4 |
| 1′ | 27.7 | 25.7 | 26.0 | 26.0 | 26.2 | 26.7 | 26.0 | 25.7 | 26.6 | 25.7 | 25.7 |
| 2′ | 14.4 | 11.6 | 11.7 | 11.6 | 11.6 | 12.4 | 11.7 | 12.0 | 12.7 | 11.8 | 11.8 |
| 3′ | 26.5 | 28.1 | 28.0 | 28.1 | 28.1 | 28.6 | 28.1 | 28.4 | 29.1 | 28.2 | 28.2 |
| 4′ | 90.0 | 77.4 | 77.5 | 77.4 | 77.4 | 77.5 | 77.3 | 77.6 | 79.5 | 77.6 | 77.6 |
| 5′ | 60.8 | 61.3 | 61.2 | 60.8 | 60.7 | 63.0 | 61.0 | 60.4 | 60.5 | 61.0 | 61.0 |
| 6′ | 23.2 | 24.3 | 23.3 | 23.4 | 24.3 | 25.3 | 23.7 | 22.5 | 23.1 | 23.3 | 23.3 |
| 7′ | 173.5 | 174.6 | 175.3 | 175.3 | 175.0 | 177.2 | 174.6 | 172.3 | 174.2 | 172.4 | 172.4 |
| 8′ | 93.8 | 93.5 | 93.7 | 93.5 | 93.8 | 94.5 | 93.2 | 93.5 | 94.7 | 93.5 | 93.5 |
| 9′ | 55.1 | 55.8 | 55.7 | 55.1 | 55.2 | 57.2 | 55.1 | 55.1 | 57.3 | 55.6 | 55.6 |
| 10′ | 44.9 | 45.2 | 45.3 | 45.4 | 45.3 | 46.1 | 45.3 | 45.0 | 45.8 | 45.2 | 45.1 |
| 11′ | 123.9 | 123.9 | 123.0 | 123.3 | 123.4 | 124.1 | 123.9 | 127.7 | 128.6 | 123.3 | 123.3 |
| 12′ | 171.7 | 171.9 | 171.4 | 174.2 | 171.3 | 173.2 | 171.6 | 171.0 | 171.5 | 171.4 | 171.4 |
| 13′ | 54.4 | 53.4 | 56.5 | 56.2 | 55.6 | 56.0 | 54.5 | 55.2 | 54.4 | 55.8 | 55.8 |
| 14′ | 26.2 | 26.2 | 25.9 | 25.9 | 25.8 | 26.6 | 26.0 | 26.2 | 27.1 | 26.3 | 26.3 |
| 15′ | 66.3 | 72.7 | 72.0 | 72.2 | 72.7 | 73.7 | 72.5 | 71.8 | 69.3 | 72.2 | 72.2 |
| 1″ | 165.3 | 167.3 | 167.2 | 167.3 | 167.1 | 168.9 | 167.3 | 167.4 | | 168.0 | 168.0 |
| 2″ | 129.0 | 129.7 | 130.1 | 130.2 | 130.2 | 130.7 | 129.5 | 130.9 | | 127.6 | 127.6 |
| 3″ | 138.0 | 135.3 | 135.6 | 135.5 | 135.1 | 137.0 | 135.8 | 135.3 | | 142.1 | 142.1 |
| 4″ | 62.8 | 62.0 | 61.6 | 61.6 | 62.3 | 62.4 | 61.6 | 61.9 | | 59.9 | 59.9 |
| 5″ | 12.8 | 13.2 | 12.9 | 12.9 | 13.1 | 12.9 | 13.1 | 13.1 | | 12.8 | 12.8 |
| 1‴ | 172.9 | 173.7 | 170.9 | 171.3 | 171.3 | 172.4 | 171.9 | 165.0 | | 173.6 | 173.2 |
| 2‴ | 67.6 | 67.0 | 37.5 | 66.6 | 72.9 | 74.1 | 28.9 | 134.6 | | 67.4 | 67.4 |
| 3‴ | 38.4 | 37.4 | 66.5 | 37.6 | 73.1 | 74.2 | 29.3 | 133.0 | | 38.7 | 38.8 |
| 4‴ | 171.2 | 170.6 | 174.2 | 171.1 | 171.3 | 172.5 | 172.1 | 165.0 | | 170.4 | 170.4 |
| 12-$OCH_3$ | 52.8 | 52.5 | 52.7 | 52.8 | 52.9 | 52.8 | 52.5 | 52.8 | 53.0 | 52.9 | 53.1 |
| 1‴-$OCH_3$ | | | | | | | | | | 53.1 | |
| $OC_2H_5$ | | | | | | | | 61.6 | | | 62.1 |
| | | | | | | | | 14.2 | | | 14.3 |

[a] Measured in $CDCl_3$.
[b] Measured in $CD_3OD$.

### 3.3. Extraction and isolation

The fresh seeds of *S. glabra* (10 kg) were roughly dried by air and ground. After extraction with 95% EtOH (3 L) under reflux (4×2 h) and successive removal of the solvent under reduced pressure, a brown and odorous crude extract (460 g) was obtained. Then the crude extract was suspended in 2.0 L water and successively extracted by petroleum ether (4×2 L) and ethyl acetate (3×2 L).

The ethyl acetate extract (70 g) was subjected to a silica gel column and eluted with $CH_2Cl_2$/MeOH (50:1, 25:1, 10:1, 0:1, v/v) to afford four fractions (Fr. 1–4). Fr. 2 (27 g) was further applied to a silica gel column using a continuous gradient of petroleum ether/acetone (3:1 to 1:1, v/v) to afford 30 subfractions (Fr. 2.1–30). Frs. 2.16–18 were combined and subjected to an ODS column using a gradient elution of MeOH/$H_2O$ (45–60%), yielding a large amount of **13** (1.0 g). Part of the other eluates were further purified by preparative HPLC to yield **8** (9.0 mg), **15** (17.6 mg) and **16** (4.3 mg). Frs. 2.19–26 were combined and chromatographed on an MCI gel column eluted with 60%, 80% and 100% methanol. The 80% methanol eluate was further run on an LH-20 gel column, and purified by preparative HPLC to afford **2** (17.8 mg), **3** (34.2 mg), **7** (7.0 mg), and **12** (1.0 g). Frs. 2.27–29 were combined and subjected to an ODS column, eluted with 30% and 50% methanol. The eluates were purified by LH-20 gel column and preparative HPLC to yield **1** (30.0 mg), **4** (10.5 mg), **5** (13.8 mg), **10** (70.0 mg) and **11** (8.1 mg). Fr. 3 (12 g) was chromatographed on an MCI gel column and eluted with 40%, 60%, 80% and 100% methanol to obtain four subfractions Fr. 3.1–4. Fr. 3.2 (1.8 g) was run on an ODS column eluted with 50% methanol and further purified by preparative HPLC to yield **6** (8.1 mg), **9** (5.6 mg) and **14** (21.2 mg).

*3.3.1. Sarglabolide A (**1**).* White, amorphous powder; [α]23 D −70.3 (*c* 0.44, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 215 (4.36) nm; CD (MeOH, Δε) $\lambda_{max}$ 383 (+1.92), 363 (−1.29), 321 (−2.36), 255 (+4.15), 221 (−26.9) nm; IR (KBr) $\nu_{max}$ 3448, 2939, 2857, 1739, 1654, 1438, 1377, 1275, 1113, 993 cm$^{-1}$; $^1$H and $^{13}$C NMR spectral data: see Tables 1 and 4; HRESIMS *m/z* 771.2619 $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{14}Na$ 771.2623).

*3.3.2. Sarglabolide B (**2**).* White, amorphous powder; [α]23 D −106.6 (*c* 0.29, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 217 (4.36) nm; CD (MeOH, Δε) $\lambda_{max}$ 348 (+1.06), 306 (−1.79), 250 (+7.95), 209 (−15.5) nm; IR (KBr) $\nu_{max}$ 3446, 2938, 2857, 1738, 1652, 1437, 1378, 1277, 1112, 991 cm$^{-1}$; $^1$H and $^{13}$C NMR spectral data: see Tables 1 and 4; HRESIMS *m/z* 771.2619 $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{14}Na$ 771.2623).

*3.3.3. Sarglabolide C (**3**).* White, amorphous powder; [α]23 D −90.9 (*c* 0.34, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 217 (4.36) nm; CD (MeOH, Δε) $\lambda_{max}$ 384 (+2.65), 364 (−0.55), 347 (+0.83), 250 (+13.7), 211

(−15.2) nm; IR (KBr) $\nu_{max}$ 3446, 2938, 2858, 1738, 1652, 1438, 1378, 1276, 1113, 991 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 1 and 4; HRESIMS *m/z* 771.2620 $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{14}Na$ 771.2623).

*3.3.4. Sarglabolide D (**4**).* White, amorphous powder; [α]23 D −18.5 (*c* 0.35, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 211 (4.30) nm; CD (MeOH, Δε) $\lambda_{max}$ 384 (+2.36), 365 (−1.10), 350 (+0.60), 322 (−1.06), 255 (+4.28), 212 (−3.60) nm; IR (KBr) $\nu_{max}$ 3442, 2939, 2857, 1737, 1653, 1436, 1379, 1274, 1112, 992 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 1 and 4; HRESIMS *m/z* 787.2569 $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{15}Na$ 787.2572).

*3.3.5. Sarglabolide E (**5**).* White, amorphous powder; [α]23 D −51.8 (*c* 0.32, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 216 (4.23) nm; CD (MeOH, Δε) $\lambda_{max}$ 390 (+1.47), 327 (−2.10), 254 (+7.80), 211 (−12.7) nm; IR (KBr) $\nu_{max}$ 3439, 2939, 2855, 1740, 1654, 1433, 1379, 1276, 1119, 993 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 2 and 4; HRESIMS *m/z* 787.2569 $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{15}Na$ 787.2572).

*3.3.6. Sarglabolide F (**6**).* White, amorphous powder; [α]23 D −45.6 (*c* 0.32, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 212 (4.29) nm; CD (MeOH, Δε) $\lambda_{max}$ 391 (+3.56), 348 (+1.79), 320 (−1.23), 257 (+4.54), 212 (−9.00) nm; IR (KBr) $\nu_{max}$ 3440, 2937, 2855, 1739, 1654, 1434, 1379, 1275, 1117, 995 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 2 and 4; HRESIMS *m/z* 803.2515 $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{16}Na$ 803.2522).

*3.3.7. Sarglabolide G (**7**).* White, amorphous powder; [α]23 D −58.3 (*c* 0.53, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 215 (4.29) nm; CD (MeOH, Δε) $\lambda_{max}$ 346 (+0.78), 320 (−1.67), 251 (+10.9), 205 (−16.3) nm; IR (KBr) $\nu_{max}$ 3445, 2939, 2858, 1737, 1654, 1436, 1377, 1272, 1109, 995 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 2 and 4; HRESIMS *m/z* 771.2622 $[M+Na]^+$ (calcd for $C_{40}H_{44}O_{14}Na$ 771.2623).

*3.3.8. Sarglabolide H (**8**).* White, amorphous powder; [α]23 D −39.0 (*c* 0.28, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 213 (4.40) nm; CD (MeOH, Δε) $\lambda_{max}$ 384 (+2.93), 364 (−0.65), 337 (−1.63), 324 (−1.66), 296 (−1.20), 251 (+1.82), 217 (−5.69) nm; IR (KBr) $\nu_{max}$ 3448, 2938, 2855, 1736, 1658, 1436, 1381, 1275, 1116, 997 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 2 and 4; HRESIMS *m/z* 799.2939 $[M+Na]^+$ (calcd for $C_{42}H_{48}O_{14}Na$ 799.2936).

*3.3.9. Sarglabolide I (**9**).* White, amorphous powder; [α]23 D −68.3 (*c* 0.29, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 214 (4.48) nm; CD (MeOH, Δε) $\lambda_{max}$ 378 (+3.89), 355 (+2.52), 319 (−2.24), 254 (+3.41), 220 (−11.3) nm; IR (KBr) $\nu_{max}$ 3442, 2936, 2857, 1737, 1652, 1438, 1380, 1270, 1120, 982 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 3 and 4; HRESIMS *m/z* 575.2254 $[M+Na]^+$ (calcd for $C_{31}H_{36}O_9Na$ 575.2252).

*3.3.10. Sarglabolide J (**10**).* White, amorphous powder; [α]23 D −71.0 (*c* 0.28, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 217 (4.20) nm; CD (MeOH, Δε) $\lambda_{max}$ 366 (−0.51), 351 (+1.03), 296 (−1.22), 253 (+3.13), 213 (−9.83) nm; IR (KBr) $\nu_{max}$ 3439, 2938, 2860, 1734, 1658, 1436, 1377, 1276, 1118, 989 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 3 and 4; HRESIMS *m/z* 803.2886 $[M+Na]^+$ (calcd for $C_{41}H_{48}O_{15}Na$ 803.2885).

*3.3.11. Sarglabolide K (**11**).* White, amorphous powder; [α]23 D −79.3 (*c* 0.39, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 219 (4.31) nm; CD (MeOH, Δε) $\lambda_{max}$ 392 (+2.09), 365 (−2.41), 337 (−2.41), 253 (+4.07), 215 (−11.7) nm; IR (KBr) $\nu_{max}$ 3438, 2938, 2859, 1734, 1658, 1436, 1376, 1276, 1116, 991 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectral data: see Tables 3 and 4; HRESIMS *m/z* 817.3036 $[M+Na]^+$ (calcd for $C_{42}H_{50}O_{15}Na$ 817.3042).

### 3.4. Determination of the absolute configurations of the malic and tartaric residues in 1–6 and 10–11

*3.4.1. Preparation of (R)-MTPA esters of dimethyl malate and dimethyl tartarate.* Compounds **1**–**6** and **10**–**11** (3.0 mg each) were, respectively, dissolved in 100 μL methanol and added to 100 μL 10% $K_2CO_3$ (methanol/water 2: 1, v/v). After hydrolysis at 60 °C for 2 h, the reaction mixtures were blow-dried, then acidified by 0.1 M HCl (500 μL) and extracted with EtOAc (500 μL×3). The aqueous layers were blow-dried and dissolved in methanol (200 μL). A drop of $SOCl_2$ was added to the solution to catalyze the esterification (room temperature, 12 h). After evaporation, the mixed methyl esters of 4-hydroxytiglic acid and malic acid (or tartaric acid) were dissolved in dry pyridine (100 μL) and reacted with (*S*)-MTPA chloride (2 μL) overnight to obtain the final products. The corresponding authentic (*R*)-MTPA esters were also prepared from the commercial D-malic acid, L-malic acid, D-tartaric acid and L-tartaric acid. The $^1H$ NMR data of the authentic (*R*)-MTPA esters of dimethyl malates were in accord with the reported.[8]

(*R*)-MTPA ester of dimethyl D-malate: $^1H$ NMR (500 M, $CDCl_3$) $\delta_H$ 7.62 (2H, m), 7.40–7.42 (3H, m), 5.72 (1H, dd, *J*=8.8, 4.0 Hz), 3.82 (3H, s), 3.64 (3H, s), 3.60 (3H, s), 2.95 (1H, dd, *J*=16.7, 4.0 Hz), 2.88 (1H, dd, *J*=16.7, 8.8 Hz); HRESIMS *m/z* 401.0821 $[M+Na]^+$ (calcd for $C_{16}H_{17}F_3O_7Na$ 401.0819).

(*R*)-MTPA ester of dimethyl L-malate: $^1H$ NMR (500 M, $CDCl_3$) $\delta_H$ 7.58 (2H, m), 7.41–7.43 (3H, m), 5.71 (1H, dd, *J*=9.1, 3.7 Hz), 3.77 (3H, s), 3.70 (3H, s), 3.55 (3H, s), 3.01 (1H, dd, *J*=16.9, 3.7 Hz), 2.93 (1H, dd, *J*=16.9, 9.1 Hz); HRESIMS *m/z* 401.0818 $[M+Na]^+$ (calcd for $C_{16}H_{17}F_3O_7Na$ 401.0819).

(*R*)-MTPA ester of dimethyl D-tartarate: $^1H$ NMR (500 M, $CD_2Cl_2$) $\delta_H$ 7.50–7.53 (4H, m), 7.39–7.47 (6H, m), 6.00 (2H, s), 3.75 (6H, s), 3.38 (6H, s); HRESIMS *m/z* 633.1162 $[M+Na]^+$ (calcd for $C_{26}H_{24}F_6O_{10}Na$ 633.1166).

(*R*)-MTPA ester of dimethyl L-tartarate: $^1H$ NMR (500 M, $CDCl_3$) $\delta_H$ 7.61–7.63 (4H, m), 7.35–7.40 (6H, m), 5.86 (2H, s), 3.57 (6H, s), 3.55 (6H, s); HRESIMS *m/z* 633.1168 $[M+Na]^+$ (calcd for $C_{26}H_{24}F_6O_{10}Na$ 633.1166).

*3.4.2. HPLC analysis of (R)-MTPA esters of dimethyl malate and dimethyl tartarate.* The authentic (*R*)-MTPA ester of dimethyl D-malate and the authentic (*R*)-MTPA ester of dimethyl L-malate were analyzed on an Agilent 1200 series instrument using a DAD detector and a shim-pack VP–ODS column (150×4.6 mm, 5 μm). The running conditions still included a flow rate of 1 mL/min, a column temperature of 25 °C, and a detection wavelength at 220 nm. Their retention times ($t_R$) were 6.52 and 7.72 min, respectively, with isocratic elution of MeOH/$H_2O$ (65:35, v/v). The retention times of the authentic (*R*)-MTPA esters of dimethyl D-tartarate and dimethyl L-tartarate were 5.89 and 6.07 min, respectively, with isocratic elution of MeOH/$H_2O$ (80:20, v/v). The final (*R*)-MTPA esters prepared from **1**–**6** and **10**–**11** were also analyzed under the above conditions.

### 3.5. Inhibitory activity assay on NO production

Raw264.7 cells were seeded into a 96-well plate ($10^5$ cells per well) and pretreated with a range of concentrations of sesquiterpene dimers for 1 h, followed by incubation with or without LPS (100 ng/mL) for 24 h. The supernatant (50 μL) was mixed with an equal volume of Griess reagent in a 96-well plate for 15 min at room temperature before measuring the optical density at 570 nm using

a microplate reader. L-NMMA was used as the reference compound. The screening results were expressed as $IC_{50}$ values.

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## Supplementary data

Supplementary data associated with this article (HRMS, NMR and CD spectra of new compounds, and HPLC chromatograms of prepared compounds) can be found in the online version at http://dx.doi.org/10.1016/j.tet.2015.05.112.

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