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Isolation and bioactivity of diterpenoids from the roots of *Salvia grandifolia*

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ABSTRACT

A phytochemical investigation of root extracts of *Salvia grandifolia* led to isolation of six previously unreported diterpenoids, grandifolias A–F, along with eight known compounds. The structures of grandifolias A–F were primarily established by extensive 1D and 2D NMR spectroscopic analyses, as well as HRESIMS data. Their absolute configurations were assigned by their calculated and experimental electronic circular dichroism spectra or by X-ray diffraction analysis. All of the diterpenoids were evaluated for their vasorelaxant effects. Grandifolia B and isograndifoliol both exhibited dose-dependent vasorelaxant effects on rat aortic rings, preconstricted by KCl or norepinephrine, with EC_{50} values of 36.36–74.51 μg/mL.

1. Introduction

Salvia, the largest genus in the family Labiatae, comprises over 900 species, distributed globally (Pan et al., 2010a; Kutrzeba et al., 2009; Choudhary et al., 2012). Dan-Shen, the root of *Salvia miltiorrhiza*, is a famous traditional Chinese medicine widely used and well studied for treatment of cardiovascular diseases (Li et al., 2012; Wang et al., 2008). It was reported that the extract of Dan-Shen and some of its bioactive compounds, such as danshensu and lithospermic acid B, have vasorelaxant effects, which is relevant to its cardiovascular benefit (Wang et al., 2008). *Salvia grandifolia*, the other species of *Salvia*, is endemic to China. The roots of *S. grandifolia* have been used as a surrogate of Dan-Shen to treat cardiovascular diseases in Sichuan and Yunnan Provinces of China for many years. However, research regarding the bioactive constituents of *S. grandifolia* has rarely been reported. To the best of our knowledge, only one paper was reported, which showed that a $CHCl_3$ fraction obtained from a 95% EtOH extract can protect against vascular dysfunction (Xiao et al., 2011). In continuation of this study, the roots of *S. grandifolia* were collected for further investigation. Consequently, six novel diterpenoids and eight known compounds were obtained. The isolation, structural elucidation, plausible biogenetic pathways, and vasorelaxant effects of the diterpenoids are described herein.

2. Results and discussion

2.1. Structural analysis

The $CHCl_3$ fraction of the 95% EtOH extract of the roots of *S. grandifolia* was subjected to column chromatography over silica gel, C-18, and Sephadex LH-20, respectively, with purification using preparative HPLC to yield six novel and eight known diterpenoids (Fig. 1). The relative configurations of a pair of known diterpenoids, castanolide (**7**) and *epi*-castanolide (**8**), were previously reported (Pan et al., 2010b). In this study, the absolute configurations of (5*S*,8*S*,10*R*)-castanolide (**7**) and (5*S*,8*R*,10*R*)-*epi*-castanolide (**8**) were confirmed by X-ray analysis for the first time herein. The other six known compounds were identified by comparison with published data: isograndifoliol (**9**) (Jiang et al., 2013), sugiol (**10**) (Zhang et al., 2013), castanol A (**11**) (Pan et al., 2012), miltipolone (**12**) (Haro and Kakisawa, 1990),

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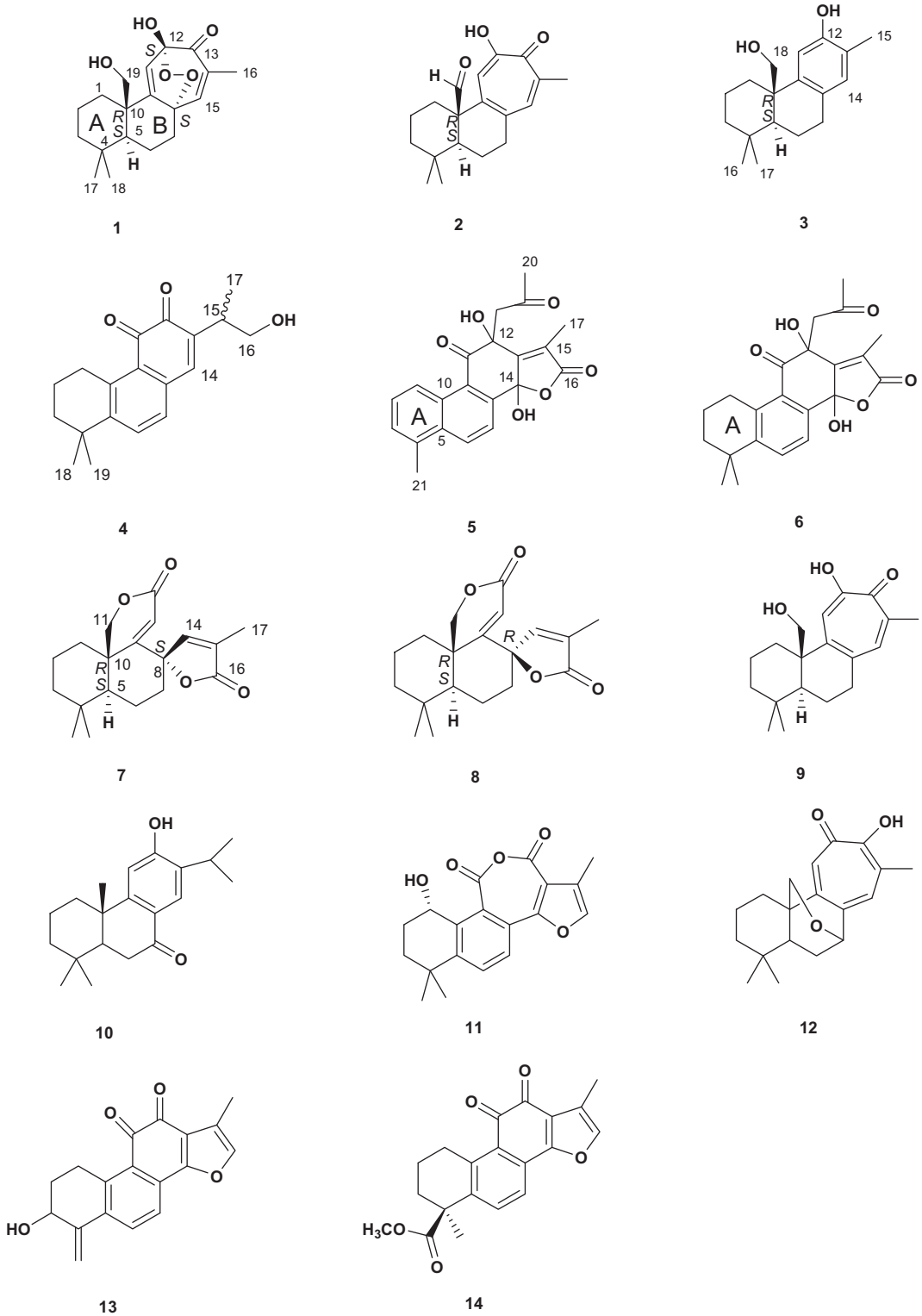

Fig. 1. Structures of compounds **1**–**14**.

hydroxymethylenetanshinquinone (**13**) (Yang et al., 1982), and methyltanshinonate (**14**) (Xue et al., 2000), respectively.

Compound **1**, obtained as a white amorphous powder, had the molecular formula $C_{19}H_{26}O_5$, as established by HRESIMS $[M + Na]^+$ m/z 357.1684, which indicated seven degrees of unsaturation. Its IR spectrum indicated the presence of hydroxyl (3425 cm^{-1}), carbonyl (1692 cm^{-1}), and olefin (1650, 1621 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) showed resonances for three methyl (δ_H 0.89, H_3-17; 0.89, H_3-18; 1.93, H_3-16), one oxymethylene (δ_H 4.16 (d), 3.68 (d), H-19), five methylene (δ_H 2.49 (m), 1.07 (m), H-1; 1.61 (m), 1.41 (m), H-2; 1.37 (m), 1.11 (m), H-3; 1.93 (m), 1.71 (m), H-6; 2.30 (m), 1.85 (m), H-7), and three methine (δ_H 1.31 (dd), H-5; 6.28 (s), H-11; 6.93 (s), H-15) groups. The ^{13}C NMR spectrum displayed a total of 19 carbon

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