Phytochemistry xxx (2017) 1–6

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Lignans from the fruits of *Schisandra chinensis* (Turcz.) Baill inhibit proprotein convertase subtilisin/kexin type 9 expression

Pisey Pel [a], Hee-Sung Chae [a], Piseth Nhoek [a], Woojin Yeo [b], Young-Mi Kim [a], Young-Won Chin [a, *]

[a] College of Pharmacy and Integrated Research Institute for Drug Development, Dongguk University-Seoul, 32, Dongguk-lo, Goyang-si, Gyeonggi-do 10326, Republic of Korea
[b] College of Liberal Arts and Sciences, University of Iowa, Iowa City, IA 52242, USA

ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form
31 December 2016
Accepted 18 January 2017
Available online xxx

Keywords:
Schisandra chinensis
Schisandraceae
Proprotein convertase subtilisin/kexin type 9 (PCSK9)
Lignan
Schinlignan D
(+)-schisandrol B

ABSTRACT

Bioactivity-guided fractionation of the fruits of *Schisandra chinensis*, using the proprotein convertase subtilisin-kexin type 9 (PCSK9) mRNA expression screening assay, led to isolation of two previously unknown lignans, 14-tigloylschinlignan D and *rel*-(7*R*, 8*R*, 7′*R*, 8′*R*)-manglisin E, along with 28 known compounds. All structures were established by NMR spectroscopic data as well as CD and MS analysis. All isolates were tested for their inhibitory activities on the mRNA expression of PCSK9. Of the tested compounds, four of the compounds *rel*-(7*R*, 8*R*, 7′*R*, 8′*R*)-manglisin E, (−)-schisandrin C, schinlignan D, and (+)-schisandrol B potently inhibited PCSK9 mRNA expression with IC_{50} values of 3.15, 3.85, 0.36, and 1.10 μM, respectively. Furthermore, schinlignan D and (+)-schisandrol B were found to suppress PCSK9 protein expressions and schinlignan D deemed to increase low density lipoprotein receptor expression.

1. Introduction

Dyslipidemia is associated with cardiovascular disease (CVD), the leading cause of death in the Western world (Turakhia and Tseng, 2007). It is characterized by increased triglyceride (TG) or low-density lipoprotein (LDL) levels and by declined high-density lipoprotein (HDL) levels (Miller et al., 2011). Among them, elevated LDL-cholesterol (LDL-C) levels have been treated with statin therapy to inhibit hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is involved in the synthesis of cholesterol (Tobert, 2003). Despite the efficacy of statin therapy, some patients with familial hypercholesterolemia (FH), an inherited autosomal dominant disorder characterized by extremely high levels of LDL-C and premature atherosclerosis (Strong and Rader, 2010; Collins et al., 2003), still face substantial residual risks associated with high levels of LDL-C because statin therapies were not entirely successful in lowering LDL-C levels (Raal et al., 2012; Raal and Santos, 2012).

Proprotein convertase subtilisin-kexin type 9 (PCSK9) impedes the function of LDL receptors involved in LDL-C uptake from blood into cells and thus reduces LDL-C levels, leading to high levels of LDL-C. Therefore, inhibition of PCSK9 was proposed as a new strategy to overcome inappropriate statin therapy in FH, and several PCSK9 inhibitors are under clinical trials. In 2015, two antibody-based PCSK9 inhibitor drugs were approved by the USFDA (Zimmerman, 2015). However, these two drugs are administered by subcutaneous injections and have high treatment costs (Zimmerman, 2015). Therefore, orally available small molecules may be advantageous as an alternative method of treatment. So far, several naturally occurring molecules such as berberine and curcumin have been shown to inhibit PCSK9 mRNA expression (Pagliaro et al., 2015).

During an initial bioassay screen to monitor PCSK9 mRNA expression in HepG2 cell lines, the hexane-soluble extract of the fruits of *Schisandra chinensis* (Turcz.) Baill (Schisandraceae) was found to be active in inhibiting PCSK9 mRNA expression. Therefore, this study was undertaken to identify the chemical constituents responsible. *S. chinensis* mainly grows in northeastern China, Korea, Japan (Duan et al., 2011), and eastern parts of Russia (Liu et al.,

* Corresponding author.
E-mail address: f2744@dongguk.edu (Y.-W. Chin).

http://dx.doi.org/10.1016/j.phytochem.2017.01.010
0031-9422/

2013), and it has been used as a tonic and sedative herbal medicine (Zhang et al., 2012). In addition, the fruits of *S. chinensis* have been widely used as a dietary supplement to treat dyspnea, chronic cough (Chen et al., 2011), diaphoresis, spontaneous diaphoresis, nocturnal dysentery, amnesia, and insomnia (Sun et al., 2013). Pharmacological investigations on the fruits of *S. chinensis* established that its extract or individual constituents possessed anti-asthmatic, anti-gastric ulcer (Zhang et al., 2012), antioxidant (Šmejkal et al., 2010), anti-hepatotoxic, anti-viral (Dilshara et al., 2013), anti-inflammatory (Dilshara et al., 2013; Seo et al., 2004), anti-HIV effect (Park et al., 2009a, b), anti-hepatitis (Park et al., 2012), and anticancer (Yang et al., 2011) properties. In the present study, two new lignans and 28 known compounds were isolated, and their inhibitory activities against PCSK9 mRNA expression were assessed.

2. Results and discussion

Compound **1** was obtained as a colorless powder, and its molecular formula was determined to be $C_{27}H_{32}O_8$ from the quasi-molecular ion peak $[M+Na]^+$ at *m/z* 507.1973 in the high-resolution electrospray ionization mass spectrometry (HRESIMS). Its ^{1}H-NMR data displayed signals for two aromatic rings at δ_H 6.67 (1H, s, H-11) and δ_H 6.85 (1H, s, H-4), resonances for a methylenedioxy group at δ_H 6.03 (1H, s, H-19a) and δ_H 6.02 (1H, s, H-19b), resonances for a tigloyl moiety at δ_H 6.81 (1H, q, 5.7 Hz, H-22), 1.70 (3H, s, H-23), and 1.69 (3H, d, 5.7 Hz, H- 24), resonances for two methylenes at δ_H 2.70 (1H, d, 13.7, Heq-6a), 2.65 (1H, d, 14.1 Hz, Hax-9a), 2.40 (1H, dd, 14.1, 7.5 Hz, Heq-9b), and 2.35 (1H, d, 13.7 Hz, Hax-6b), and a resonance for a methine at δ_H 1.88 (1H, m, Hax-8), suggesting the similarity of compound **1** to schinlignan D (Xue et al., 2015), except for a different substituent at C-14 and its location.

In compound **1**, the location of a tigloyl moiety was indirectly deduced at C-14 by observing heteronuclear multiple bond coherence (HMBC), ^{1}H-^{1}H-COSY, and NOE correlations. The methylenedioxy group was found to be located between C-12 and C-13 of ring B from the HMBC correlations of δ_H 6.02 and 6.03 (H-19a and 19b) to δ_C 137.6 (C-13) and δ_C 147.6 (C-12), and δ_H 6.67 (H-11) to δ_C 137.6 (C-13), 147.6 (C-12), 123.5 (C-15), 133.0 (C-10), and 33.8 (C-9). Additionally, the HMBC correlation of H-11 to C-9 and sequential ^{1}H-^{1}H-COSY correlations of H-9/H-8/H-18 supported that C-9 was connected to ring B. In the case of ring A, the observed HMBC correlations of H-2 to C-2 and H-3 to C-3 enabled the linkage of two methoxy groups at 3.83 (H-2) and 3.89 (H-3) to C-2 and C-3, respectively, and additional HMBC correlations of δ_H 6.58 (H-4) to δ_C 152.4 (C-3), 140.0 (C-2), 132.3 (C-5), 122.8 (C-16), and 40.4 (C-6) confirmed the location of ring A with these methoxy groups. The position of the remaining methoxy group was deduced to C-1 of ring A from the NOE correlation between δ_H 3.46 (1-OCH$_3$) and 3.83 (2-OCH$_3$) and the HMBC correlation of δ_H 3.46 to δ_C 151.8 (C-1), suggesting the presence of three methoxy groups in ring A.

Based on these data, the tigloyl group could possibly be placed at either C-7 or C-14. When the tigloyl group was attached to C-7, the chemical shift of C-7 should appear at lower field than the present value; however, the chemical shift of C-7 was not shifted to the down-field region (Takeya et al., 1994). Consequently, the location of the tigloyl group was assigned to being of C-14. Finally, HMBC correlations of H-8 to C-6 and C-17 enabled the construction of the overall structure of compound **1** as shown in Fig. 1. The absolute configuration of the biphenyl part in **1** was established as having a *R* configuration by comparison of the observed CD spectroscopic data [220 (−0.98), 238 (0.60), and 252 (0.72) nm] with published values (Xue et al., 2015; Blunder et al., 2010). The coupling constants (14.1 and 7.5 Hz) of H-9 and NOE correlations between H-11/H-9ax/H-18eq indicated that H-8 and H-18 were positioned at axial and equatorial positions (Fig. 2). Moreover, the observed NOE correlations of H-8/H-17 and H-17/H-18 supported that H-17 was in an equatorial orientation. Taken together, this compound was assigned the structure **1**, and named 14-tigloylschinlignan D.

Compound **2** was obtained as a colorless oil, whose molecular formula was determined to be $C_{21}H_{26}O_6$ from the quasimolecular ion peak $[M+Na]^+$ at *m/z* 397.1608 in the HRESIMS. Its ^{1}H-NMR data of **2** displayed resonances for five aromatic protons at δ_H 6.98 (1H, brs, H-2), 6.90 (2H, brs, H-5 and H-6), 6.70 (1H, d, 1.6 Hz, H- 6′), and 6.57 (1H, d, 1.6 Hz, H-2′), resonances for four methine groups at δ_H 4.48 (1H, d, 8.5 Hz, H-7), 4.46 (1H, s, d, 8.5 Hz, H-7′), and 2.31 (2H, m, H-8 and H-8′), and resonances for two methyl groups at δ_H 1.05 (3H, d, 6.6 Hz, H-9′) and 1.00 (3H, d, 6.6 Hz, H-9), suggesting the structural similarity of compound **2** to manglisin E (Ding et al., 2014). This inference was supported by the observed HMBC and ^{1}H-^{1}H-COSY correlations. HMBC correlations of δ_H 6.98 (H-2) and δ_C 146.5 (C-3), 145.0 (C-4), 119.0 (C-6), and 87.3 (C-7) indicated that the 1,3,4-trisubstituted benzene ring (ring A) was attached to C-7 of the butane-1,4-diol (Fig. 3). The remaining aromatic ring (ring B) with two methoxy groups and a hydroxyl group was also linked to C-7′ of the butane-1,4-diol substructure, as shown in Fig. 1, based on the HMBC correlations of δ_H 6.70 (H-6′) and δ_C 148.9 (C-5′), 134.6 (C-4′), 138.7 (C-1′), 102.4 (C-2′), and 87.3 (C-7′). Sequential correlations of δ_H 4.48 (H-7) to 2.31 (H-8 and H-8′), 2.31 (H-8 and H-8′) to 1.00 (H-9), and 1.05 (H-9′) and 2.31 (H-8 and H-8′) to 4.46 (H-7′) in the ^{1}H-^{1}H COSY spectroscopic data supported that the methyl groups were attached to the butane-1,4-diol at C-8 and C-8′, respectively. However, the chemical shifts and coupling constants of H-7 (δ_H 4.48, $J_{7,8}$ = 6.9 Hz) and H-7′ (δ_H 4.46, $J_{7',8'}$ = 6.2 Hz) in **2** were slightly different from published values [H-7 (δ_H 5.11, $J_{7,8}$ = 8.4 Hz), H-7′ (δ_H 4.36, $J_{7',8'}$ = 9.0 Hz) in manglisin E], perhaps implying that configurations in compound **2** were different compared to those of manglisin E (Ding et al., 2014). Therefore, NOESY and 1D-NOE difference experiments were carried out to resolve the relative configurations of compound **2**. NOE correlations were observed between H-7 and H-9, as well as at H-7′and H-9′ while no NOE correlations were observed between H-9 and H-9′. This suggested that H-7 and H-9 were in the same orientation, whereas H-7′and H-9′ had a different orientation as shown in Fig. 3. Further analysis of the coupling constants of H-7 (6.9 Hz) and H-7′ (6.2 Hz) supported the relative configurations of compound **2** as shown in Fig. 3 (Wu and Cremer, 2003). Based on interpretations of the overall data, compound **2** was assigned as *rel*-(7*R*, 8*R*, 7′*R*, 8′*R*)-manglisin E.

Known structures **3**–**30** were identified by comparison of their spectroscopic data with the literature as (−)-gomisin M_1 (**3**) (Xue et al., 2015), (−)-schisandrin C (**4**) (Park et al., 2009a, b), (−)-schisandrin B (**5**) (Ikeya et al., 1982), (−)-neglschisandrin E (**6**) (Chen et al., 2013a, b), (−)-gomisin L_1 (**7**) (Ikeya et al., 1982), (−)-gomisin L_2 (**8**) (Ikeya et al., 1982), (−)-rubrisandrin B (**9**) (Chen et al., 2006), schinlignan D (**10**) (Xue et al., 2015), (+)-schilignan E (**11**) (Xue et al., 2015), (+)-schisandrol B (**12**) (Ikeya et al., 1982), (+)-schisandrol A (**13**) (Cao et al., 2010), (+)-tigloylgomisin H (**14**) (Taguchi and Ikeya, 1978), (+)-angeloylgomisin H (**15**) (Lei et al., 2007), (−)-angeloylgomisin Q (**16**) (Ikeya et al., 1979), (−)-tigloylgomisin Q (**17**) (Miller et al., 2011), (−)-schisantherin A (**18**) (Cheng et al., 2009), (−)-tigloylgomisin P (**19**) (Raal et al., 2012), (−)-angeloylgomisin P (**20**) (Chen et al., 2013a, b), dimethyl-malate (**21**) (Brimble et al., 2011), methyl-malate (**22**) (Baker et al., 2014), butyl-1-methyl malate (**23**) (Vereshchagin et al., 1989), 1,5-dibutyl-1′-methyl citrate (**24**) (Vereshchagin et al., 1989), 1-butyl-1′,5′-dimethyl citrate (**25**) (Vereshchagin et al., 1989), 2-hydroxy-5-methyl ester (**26**) (Utaka et al., 1986), (+)-schisandrin A (**27**) (Duan et al., 2011), (+)-14-tigloylgomisin K_3 (**28**) (Li et al., 2008),

Please cite this article in press as: Pel, P., et al., Lignans from the fruits of *Schisandra chinensis* (Turcz.) Baill inhibit proprotein convertase subtilisin/kexin type 9 expression, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2017.01.010