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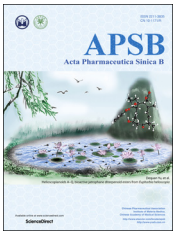

ORIGINAL ARTICLE

Bioactive thionic compounds and aromatic glycosides from *Ligusticum chuanxiong*

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Abstract Three new thionic compounds, (*S*)-2-(2-carboxyl-2-hydroxyethylthio)-ferulic acid (**1**), (*E*)-2-methoxy-4-(3-(methylsulfonyl)prop-1-en-1-yl)phenol (**2**), and thiosenkyunolide C (**3**), together with two new aromatic glycosides (**4** and **5**) were isolated from the rhizome of *Ligusticum chuanxiong* Hort. Two known compounds (**6** and **7**) were also obtained. Their structures were elucidated based on extensive spectroscopic data (UV, IR, 1D and 2D NMR, and HR-ESI-MS). Furthermore the absolute configurations were established by comparison of their calculated and experimental circular dichroism spectra and by a dimolybdenum tetraacetate [$Mo_2(AcO)_4$]-induced circular dichroism procedure. All compounds were evaluated against lipopolysaccharide (LPS)-induced NO production in BV2 cells, and compounds **4** and **5** showed strong inhibitory activities with IC_{50} values of 2.03 and 3.09 μmol/L, respectively (positive control curcumin, IC_{50} = 6.17 μmol/L). In addition, compound **1** showed weak proteintyrosine phosphatase-1B (PTP1B) inhibitory activity.

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1. Introduction

Thionic compounds are common heteroatomic compounds in plants. They have also been detected and isolated from food, fruits and wine[1–6]. Notably, they have been shown to possess various pharmacological activities, such as antifungal, anti-inflammatory, anti-helicobacter, antitumor, and antiparasitic activities, as well as being alternative carbonic anhydrase and neurosteroid-like inhibitors[7–9]. Chuanxiong, a famous traditional Chinese medicine, is derived from the rhizome of *Ligusticum chuanxiong* Hort. After years of research, Chuangxiong has been proven to possess excellent activities for the treatment of cardio-cerebral vascular diseases, inflammatory diseases, and tumor[10–15]. Of its phytochemical constituents, its phenolic acids, alkaloids, and phthalides have been investigated[15–30]. In particular, the phthalides, which are present in a high concentration in Chuanxiong, have been reported to exhibit various bioactivities, such as neuroprotective, anti-inflammatory, antimicrobial, and anti-proliferative effects[15–18]. In a recent report, several thionic phthalides were isolated from Chuanxiong[29]. These novel compounds increased the diversity of the known phthalides in Chuanxiong.

To identify additional novel bioactive thionic compounds, the rhizome of *L. chuanxiong* was investigated. Herein, three new thionic compounds (**1**–**3**, Fig. 1) were successfully isolated. In addition, two new aromatic glycosides (**4** and **5**, Fig. 1) and two known aromatic glycosides (**6** and **7**) were also isolated. Evaluation of their bioactivity showed that compounds **4** and **5** had strong anti-inflammatory activity against LPS-induced NO production in BV2 microglial cells, and compound **1** showed weak PTP1B inhibitory activity.

2. Results and discussion

Compound **1** was obtained as a white, amorphous powder. The molecular formula was calculated to be $C_{13}H_{14}O_7S$ from the positive HR-ESI-MS $[M + H]^+$ ion peak at *m/z* 315.0531 (Calcd. for 315.0533), suggesting the presence of 7 degrees of unsaturation. The ^1H NMR resonances (Table 1) at δ_H 7.44 (1H, d, J = 8.5 Hz, H-6), 6.89 (1H, d, J = 8.5 Hz, H-5), 8.18 (1H, d, J = 16.0 Hz, H-7), and 6.25 (1H, d, J = 16.0 Hz, H-8) suggested a tetrasubstituted benzene ring and a *trans* double bond. In combination with the ^{13}C NMR resonances (Table 1) at δ_C 128.7, 130.2, 148.7, 152.3, 117.4, 123.3, 142.4, 117.8 and 167.8, these data indicated the presence of a tetrasubstituted cinnamic acid group. Additionally, The HR-ESI-MS data indicated one *S* atom was present. In combination with the NMR data [δ_H 3.12 (1H, dd, J = 4.5, 13.0 Hz, H-1′a), 2.97 (1H, dd, J = 8.0, 13.0 Hz, H-1′b), and 3.93 (1H, dd, J = 4.5, 8.0 Hz, H-2′); δ_C 39.7, 69.7, and 173.9], a mercaptopropionic acid moiety was deduced, and it was attached at C-2 based on the correlation of H-1′/C-2 in the HMBC spectrum (Fig. 2). The correlation of H-OCH_3/C-3 confirmed that the methoxy was attached at C-3. Therefore, the planar structure of **1** was determined. The absolute configuration of C-2′ was established by a dimolybdenum tetraacetate $[Mo_2(AcO)_4]$-induced circular dichroism procedure[31]. The diagnostic Cotton effect at approximately 342.5 nm was negative, which indicated the absolute configuration of C-2′ was *S*. Thus, the structure of **1** was confirmed as (*S*)-2-(2-carboxyl-2-hydroxyethylthio)-ferulic acid.

Compound **2** was obtained as a white, amorphous powder. The positive HR-ESI-MS gave a $[M + Na]^+$ ion peak at *m/z* 265.0504 (Calcd. for 265.0505), which was in accordance with an empirical molecular formula of $C_{11}H_{14}O_4S$. The ^1H NMR and ^{13}C NMR spectra of **2** (Table 1) displayed an ABX system [δ_H 7.05 (1H, d, J = 1.5 Hz, H-2), 6.89 (1H, dd, J = 8.0, 1.5 Hz, H-6), and 6.76 (1H, d, J = 8.0 Hz, H-5); δ_C 110.6, 121.8, and 116.4]; a *trans* double bond [δ_H 6.68 (1H, d, J = 16.0 Hz, H-7) and 6.11 (1H, dt, J = 16.0, 7.5 Hz, H-8); δ_C 140.5 and 113.7]; a methylene [δ_H 3.95 (2H, d, J = 7.5 Hz, H-9); δ_C 59.6] and a methyl group [δ_H 2.93 (3H, s, H-11); δ_C 39.6]. Furthermore, a methoxy carbon was

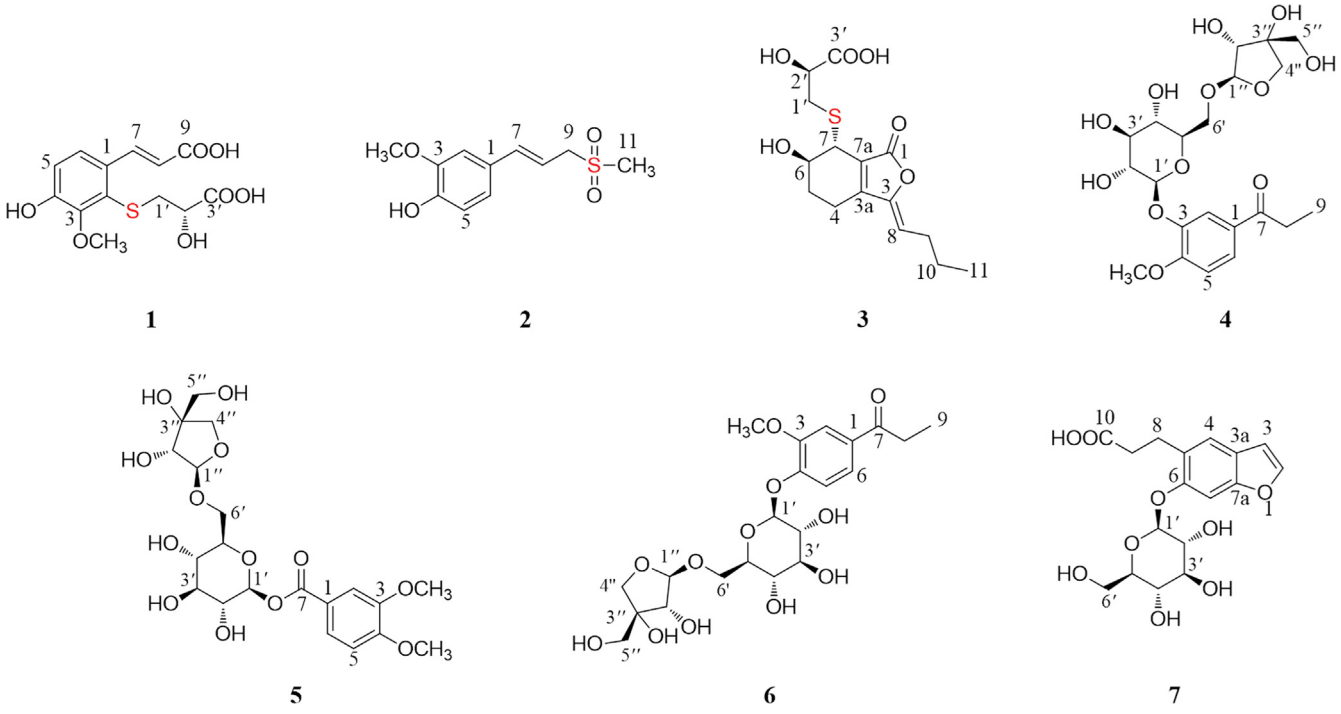

Figure 1 The structures of the isolated compounds **1**–**7**.

Table 1 ^{1}H and ^{13}C NMR data of compounds **1**, **3** (in DMSO-d_6) and **2** (in methanol-d_4).

Position	**1**[a]		**2**[a]		**3**[b]	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		128.7		129.6		168.1
2		130.2	7.05, d (1.5)	110.6		
3		148.7		149.3		147.9
3a						151.6
4		152.3		148.6	2.44, m	16.1
5	6.89, d (8.5)	117.4	6.76, d (8.0)	116.4	1.82, m	22.9
6	7.44, d (8.5)	123.3	6.89, dd (1.5, 8.0)	121.8	4.07, brs	67.5
7	8.18, d (16.0)	142.4	6.68, d (16.0)	140.5	3.52, brs	41.8
7a						124.3
8	6.25, d (16.0)	117.8	6.11, dt (7.5, 16.0)	113.7	5.46, t (8.0)	112.4
9		167.8	3.95, d (7.5)	59.6	2.27, q (8.0)	27.6
10					1.47, m	21.8
11			2.93, s	39.6	0.91, t (7.5)	13.7
1′	2.97, dd (8.0, 13.0); 3.12, dd (4.5, 13.0)	39.7			2.87, dd (7.0, 12.5); 3.05 dd (3.0, 12.5)	37.4
2′	3.93, dd (4.5, 8.0)	69.7			4.11, brs	70.7
3′		173.9				174.0
-OCH3	3.76, s	59.7	3.86, s	56.5		

[a]500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR. [b]600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR.

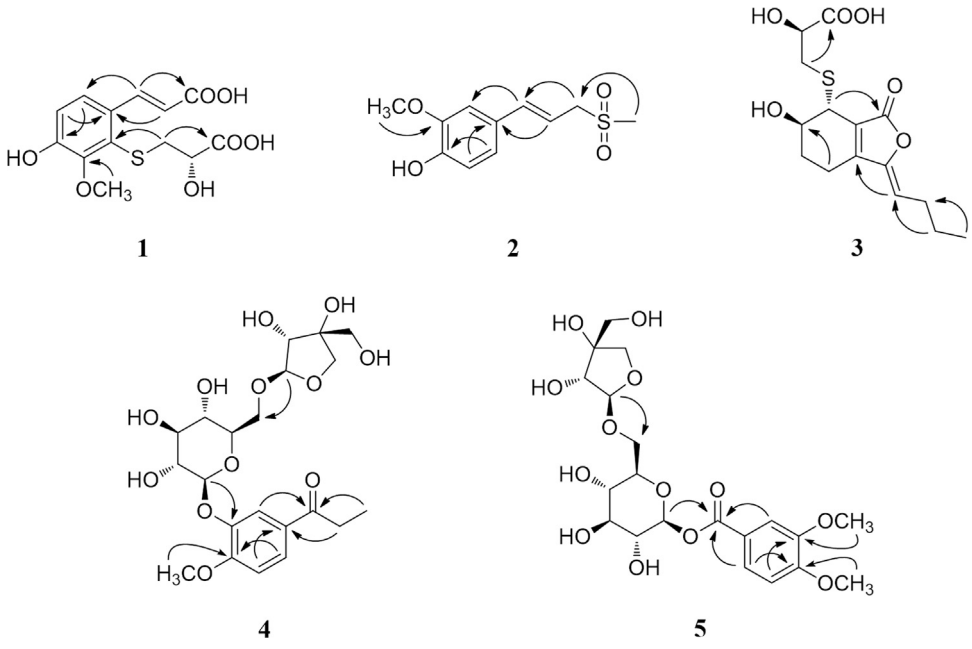

Figure 2 Key HMBC correlations of **1**–**5**.

observed at δ_C 56.5 (3′-OCH_3). A phenylpropanoid group was suggested by the correlations of H-5/C-1, H-6/C-4, H-7/C-2, H-8/C-1, and H-9/C-7 in the HMBC spectrum (Fig. 2). In conjunction with the key correlation of H-11/C-9, a methylsulfonyl moiety was confirmed, and the phenylpropanoid group was attached to the sulfur atom. Therefore, the structure of **2** was established as (*E*)-2-methoxy-4-(3-(methylsulfonyl)prop-1-en-1-yl)phenol.

Compound **3** was obtained as a white, amorphous powder, and its molecular formula was established as $C_{15}H_{20}O_6S$ from its HR-ESI-MS peak at m/z 329.1060 $[M + H]^+$ (Calcd. 329.1053). The bands in the IR spectrum at 3364, 1747 and 1631 cm^{-1} suggested the presence of hydroxy, γ-lactone and diene groups, respectively. The ^{1}H NMR spectrum (Table 1) show a double bond resonance at δ_H 5.46 (1H, t, J = 8.0 Hz, H-8), resonances for five methylene groups at δ_H 2.44 (2H, m, H-4), 1.82 (2H, m, H-5), 2.27 (2H, q, J = 8.0 Hz, H-9), 1.47 (2H, m, H-10), 2.87 (1H, dd, J = 7.0, 12.5 Hz, H-1′a), and 3.05 (1H, dd, J = 3.0, 12.5 Hz, H-1′b), one methyl group at δ_H 0.91 (3H, t, J = 7.5 Hz, H-11), one methine

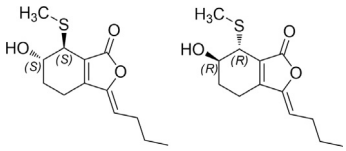

Figure 3 The structures of **3Ja** and **3Jb**.

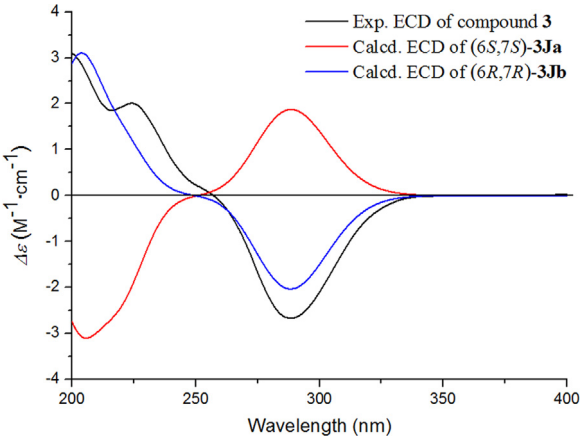

Figure 4 Experimental ECD and calculated ECD spectrum of **3** in MeOH.

resonance at δ_H 3.52 (1H, brs, H-7), and two oxygenated methine resonances at δ_H 4.07 (1 H, brs, H-6) and 4.11 (1H, brs, H-2′). The ^{13}C NMR spectrum (Table 1) showed fifteen carbon resonances, including two carboxyl carbons at δ_C 174.0 (C-3′) and 168.1 (C-1), four olefinic carbons, five methylene carbons, a methine carbon resonance at δ_C 41.8 (C-7), and two oxygenated methine resonances at δ_C 67.5 (C-6) and 70.7 (C-2′). A comparison of the NMR data of **3** with those of the known compound thiosenkyunolide A suggested that these two compounds shared a similar skeleton, except in **3** C-11 bears a methyl group, while in thiosenkyunolide A, that position has a hydroxymethyl moiety[29]. The *trans* configuration of C-6/7 was confirmed by comparing the chemical shifts and coupling constants seen in the ^{1}H and ^{13}C NMR with those of the known compounds thiosenkyunolide A (*trans*) and thiosenkyunolide B (*cis*). Further 2D NMR analysis, including HSQC and HMBC experiments (Fig. 2), confirmed the planar structure of compound **3**.

Using the same method as was used for compound **1**, the absolute configuration of C-2′ in **3** was established by a dimolybdenum tetraacetate [$Mo_2(AcO)_4$]-induced circular dichroism procedure. The diagnostic Cotton effect at approximately 344.5 nm was negative, which indicated the absolute configuration of C-2′ in **3** was *S*[31]. The structure of **3** was simplified to **3Ja** (6*S*,7*S*) and **3Jb** (6*R*,7*R*) for convenience when calculating the ECD spectra (Fig. 3). By comparing the experimental ECD and ECD calculated based on the time-dependent density functional theory (TD-DFT) method at the B3LYP/6–31+G(d,p) level data (Fig. 4), the absolute configuration of **3** was confirmed as 6*R*,7*R*. Finally, the structure of **3** was determined to be (3*Z*,3a*E*)-(6*R*,7*R*,2′*S*)-6-hydroxy-7-(2-carboxyl-2-hydroxyethylthio)-3-butylidene-4,5,6,7-tetrahydrophthalide and was named thiosenkyunolide C.

The molecular formula of compound **4** was confirmed as $C_{21}H_{30}O_{12}$ by analysis of its HR-ESI-MS signal at *m/z* 497.1637 $[M + Na]^+$ (Calcd. 497.1629). A comparison of the NMR data

Table 2 ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **4** and **5** in DMSO-d_6.

Position	**4**		**5**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		129.5		121.2
2	7.60, d (2.0)	114.8	7.49, d (2.0)	112.0
3		146.0		148.4
4		153.0		153.3
5	7.08, d (8.5)	111.7	7.09, d (8.5)	111.1
6	7.63, dd (2.0, 8.5)	123.0	7.65, dd (2.0, 8.5)	123.8
7		198.8		164.3
8	2.97, q (7.0)	30.7		
9	1.06, t (7.0)	8.2		
1′	4.99, d (7.5)	100.0	5.53, d (8.0)	94.7
2′	3.26, overlap	73.1	3.29, overlap	72.4
3′	3.27, overlap	76.6	3.29, overlap	76.3
4′	3.10, m	69.8	3.11, m	69.6
5′	3.50, m	75.5	3.44, overlap	76.2
6′	3.39, dd (7.0, 11.0); 3.85, dd (1.5, 11.0)	67.4	3.42, overlap; 3.85, overlap	67.3
1″	4.76, d (3.0)	109.1	4.79, d (3.5)	109.1
2″	3.66, d (3.0)	75.9	3.74, d (3.0)	75.6
3″		78.8		78.7
4″	3.56, d (9.0); 3.82, d (9.0);	73.3	3.56, d (9.5); 3.83, overlap	73.2
5″	3.31, overlap	63.4	3.30, overlap	62.9
3-OCH3			3.82, s	55.6
4-OCH3	3.84, s	55.8	3.84, s	55.7

(Table 2) of **4** with those of the known compound 4-[*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranosyloxy]-3-methoxypropiophenone suggested that these two compounds shared a similar skeleton except for the location of their sugar moieties[32]. In the HMBC spectrum of **4** (Fig. 2), the correlations of H-1″/C-6′ and H-1′/C-3 suggested that an apiose moiety was attached at C-6′ of the glucose moiety, and the glucose moiety was attached at C-3 of the aglycone. The configurations of the apiose and glucose were determined to both be D by GC analysis after acidic hydrolysis and chiral derivatization. The *β*-anomeric configurations were deduced based on the coupling constants of the anomeric protons (Glc: J = 7.5 Hz; Api: J = 3.0 Hz). Finally, compound **4** was confirmed to be 3-hydroxy-4-methoxypropiophenone 3-*O*-*β*-D-apiofuranosyl-(1→6)-*β*-D-glucopyranoside.

Compound **5** was obtained as a white, amorphous powder, which was found to have a molecular formula of $C_{20}H_{28}O_{13}$ based on its HR-ESI-MS signal at *m/z* 477.1611 $[M + H]^+$. Its ^{1}H NMR (Table 2) spectrum indicated an ABX system involving the benzene ring at δ_H 7.65 (1H, dd, J = 8.5, 2.0 Hz, H-6), 7.49 (1H, d, J = 2.0 Hz, H-2) and 7.09 (1H, d, J = 8.5 Hz, H-5). In addition, the presence of multiple protons between δ_H 3.11 and δ_H 3.85 and two doublets at δ_H 5.53 (1H, d, J = 8.0 Hz, H-1′) and 4.79 (1H, d, J = 3.5 Hz, H-1″) suggested the presence of two sugar moieties. The ^{13}C NMR spectrum (Table 2) showed six aromatic carbons, an ester carbonyl carbon (δ_C 164.3), and two methoxy carbons. The remaining eleven carbons were indicative of an apiose moiety (δ_C 109.1, 78.7, 75.6, 73.2, and 62.9) and a glucose moiety (δ_C 94.7, 76.3, 76.2, 72.4, 69.6, and 67.3). The

HMBC correlations of H-1′/C-7 and H-1′′/C-6′ confirmed that the glucose moiety was attached at C-7 and the apiose was attached at C-6′ of the glucose moiety (Fig. 2). The D-configurations of the glucose and apiose were confirmed through the same method as was used for **4**. The β-anomeric configurations were deduced based on the coupling constants of the anomeric protons (Glc: J = 8.0 Hz; Api: J = 3.5 Hz). In addition, the correlations of H-2/C-7, H-2/C-4, H-5/C-3, H-6/C-2, H-OCH_3 (δ_H 3.82)/C-3, and H-OCH_3 (δ_H 3.84)/C-4 proved the structure of the aglycone moiety as shown. Therefore, the structure of **5** was confirmed as β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl-3,4-dimethoxy-benzoate.

The structures of two known compounds (**6** and **7**) were identified by comparison of their spectroscopic data with those of the known compounds. They were elucidated as 4-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyloxy]-3-methoxypropiophenone[32] and cnidioside A[33].

All compounds were tested for their anti-inflammatory activity against LPS-induced NO production in BV2 cells and for PTP1B inhibitory activity. The results showed that **4** and **5** have strong anti-inflammatory activities with IC_{50} values of 2.03 and 3.09 μmol/L, respectively (positive control curcumin, IC_{50} = 6.17 μmol/L), and compound **1** exhibited a weak PTP1B inhibitory activity with an inhibitory rate of 19.6% at a concentration of 10 μmol/L.

3. Experimental

3.1. General experimental procedures

The optical rotations, UV spectra and ECD spectra were recorded with JASCO P-2000, V650 and J-815 spectrometers (JASCO, Easton, MD, USA), respectively. The infrared spectra were measured on Nicolet 5700 spectrometer (Thermo Scientific, FL, USA). The NMR spectra were recorded with Bruker 500 MHz (Bruker-Biospin, Billerica, MA, USA) and 600 MHz NMR spectrometers (Varian, Inc., Palo Alto, CA, USA). HR-ESI-MS spectra were obtained using an Agilent 6520 HPLC-Q-TOF instrument (Agilent Technologies, Waldbronn, Germany). Preparative HPLC separations were performed using a Shimadzu LC-10AT with an ODS-A column (250 mm × 20 mm, 5 μm; YMC Corp., Kyoto, Japan). An Agilent 1200 series system with an Apollo C_{18} column (250 mm × 4.6 mm, 5 μm; Alltech Corp., KY, USA) was used for HPLC-DAD analysis. An Agilent 7890 A system with a capillary column (HP-5, 60 m × 0.32 mm, with a 1 μm film; Agilent Technologies Inc., CA, USA) was used for GC analysis. Macroporous resin (Diaion HP-20, Mitsubishi Chemical Corp., Tokyo, Japan), RP-C18 (50 μm, YMC Corp., Kyoto, Japan), and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) were used for column chromatography.

3.2. Plant material

The roots of *Ligusticum chuanxiong* Hort. were collected from Pengzhou Town, Sichuan Province, PRC, in June 2013 and identified by professor Lin Ma A voucher specimen (ID-S-2594) was deposited at the Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China.

3.3. Extraction and isolation

The powdered rhizome of *L. chuanxiong* Hort. (100.0 kg) was exhaustively extracted with 80% EtOH at reflux. The solvent was evaporated under reduced pressure, and the residue (23.1 kg) was then successively partitioned with EtOAc and *n*-BuOH. The *n*-BuOH-soluble portion (1300 g) was separated on a macroporous resin (Diaion HP-20) column to give five fractions, A–E, using a gradient elution with H_2O, 15% ethanol, 30% ethanol, 50% ethanol, and 95% ethanol. Fraction C (103.0 g) was chromatographed on an RP-C18 column eluting with H_2O/MeOH (from 100:0 to 0:100) to give 16 fractions (C1–C16) based on HPLC analyses. Fraction C5 was separated by column chromatography over Sephadex LH-20 using H_2O as the eluent and was further purified by preparative HPLC (MeOH/H_2O, 20:80, *v/v*, HOAc, 0.2%) to give **4** (67 mg), **5** (5 mg), **6** (17 mg) and **7** (64 mg). Fraction C8 was separated by column chromatography over Sephadex LH-20 using H_2O as the eluent and was further purified by preparative HPLC (MeOH/H_2O, 30:70, *v/v*, HOAc, 0.2%) to give **1** (17 mg). Fraction D (48.0 g) was chromatographed on an RP-C18 column eluting with H_2O/MeOH (from 95:5 to 0:100) to give 24 fractions (D1–D24) based on HPLC and TLC analyses. Fraction D9 was chromatographed over Sephadex LH-20 with a gradient of increasing MeOH (30%–100%) in H_2O and was further purified by preparative HPLC (MeCN/H_2O, 30:70, *v/v*, HOAc, 0.2%) to give **3** (2 mg).

The EtOAc-soluble portion (5.0 kg) was chromatographed on a silica gel column eluting with petroleum ether/ethyl acetate (from 100:0 to 50:50) to give 10 fractions (Ea–Ej) based on HPLC and TLC analyses. Fraction Ej (179.3 g) was separated on a silica gel column eluting with petroleum ether/ethyl acetate (from 100:1 to 1:10) to give 10 fractions (Ej1–Ej10) based on HPLC and TLC analyses. Fraction Ej5 was chromatographed over Sephadex LH-20 eluting with dichloromethane/MeOH (2:1) and was further purified by preparative HPLC (MeCN/H_2O, 18:82, *v/v*, HOAc, 0.2%) to give **2** (18 mg).

3.3.1. (S)-2-(2-Carboxyl-2-hydroxyethylthio)-ferulic acid (*1*)

White, amorphous powder; $[\alpha]_D^{20}$−7.8 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 203 (4.22), 308 (4.07) nm; IR (KBr) ν_{max} 3282, 2975, 2939, 1692, 1626, 1583, 1477, 1415, 1282, 1193, 1095, 948 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m/z* 315.0531 $[M + H]^+$ (Calcd. for $C_{13}H_{15}O_7S$, 315.0533).

3.3.2. (E)-2-Methoxy-4-(3-(methylsulfonyl)prop-1-en-1-yl)phenol (*2*)

White, amorphous powder; UV (MeOH) λ_{max} (logε) 211 (3.94), 272 (3.79), 299 (3.56) nm; IR (KBr) ν_{max} 3453, 3005, 2943, 1594, 1515, 1465, 1426, 1273, 1238, 1177, 1126, 1031, 974, 880, 822, 798, 777 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m/z* 265.0504 $[M + Na]^+$ (Calcd. for $C_{11}H_{14}O_4SNa$, 265.0505).

3.3.3. Thiosenkyunolide C (*3*)

White, amorphous powder; $[\alpha]_D^{20}$ –32.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 277 (3.90) nm; IR (KBr) ν_{max} 3364, 2961, 1747, 1414, 1242, 1093, 1047, 952 cm^{-1}; CD (MeOH) 224 ($\Delta\varepsilon$ +2.01), 289 ($\Delta\varepsilon$ –2.67) nm; ^1H and ^{13}C NMR data, see Table 1; HR-ESI-MS *m/z* 329.1060 $[M + H]^+$ (Calcd. for $C_{15}H_{21}O_6S$, 329.1053).

*3.3.4. 3-Hydroxy-4-methoxypropiophenone 3-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**4**)*

White, amorphous powder; $[\alpha]_D^{20}$ –68.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 201 (4.09), 224 (3.99), 271 (3.80) nm; IR (KBr) ν_{max} 3409, 2934, 2881, 1735, 1669, 1599, 1515, 1459, 1269, 1174, 930 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 497.1637 $[M + Na]^+$ (Calcd. for $C_{21}H_{30}O_{12}Na$, 497.1629).

*3.3.5. β-D-Apiofuranosyl-(1→6)-β-D-glucopyranosyl-3,4-dimethoxybenzoate (**5**)*

White, amorphous powder; $[\alpha]_D^{20}$ –41.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (logε) 216 (4.17), 275 (3.79) nm; IR (KBr) ν_{max} 3417, 2974, 2924, 1717, 1697, 1609, 1410, 1380, 1240, 1194, 975 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HR-ESI-MS *m/z* 477.1611 $[M + H]^+$ (Calcd. for $C_{20}H_{29}O_{13}$, 477.1603).

3.4. ECD calculations

Briefly, conformational analyses of **3Ja** and **3Jb** were carried out *via* systematic searching in Discovery Studio (version 16.1.0.15350) using the MMFF94 force field. Conformers with Boltzmann distributions over 1% were chosen for ECD calculations. Ground-state geometries were optimized at the B3LYP/6–31+G(d,p) level in the gas phase using the Gaussian 09 program (Gaussian Inc., Wallingford, CT, USA). All quantum computations were performed on an IBM cluster machine located at the High Performance Computing Center of Peking Union Medical College. The energies, oscillator strengths, and rotational strengths (velocity) of the electronic excitations were calculated using TD-DFT methodology at the B3LYP/6–31+G(d,p) level in methanol. The calculated ECD spectra were simulated by the overlapping Gaussian function (half the bandwidth at 1/e peak height, 0.25 eV), and their lowest energy conformers were generated by Boltzmann weighting.

3.5. Anti-inflammatory activity assay

The anti-inflammatory activity was evaluated in microglial BV2 cell, which were purchased from the Cell Culture Center at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. Curcumin was used as a positive control drug. The BV2 microglial cells were cultured at 37 °C (5% CO_2, 100% relative humidity) and maintained in DMEM containing 10% FCS (fetal calf serum). The cells were transferred to 96-well plates and treated with different samples (each compound was tested at three concentrations) after incubation for 24 h. Then, the cells in the 96-well plates were stimulated with LPS (Sigma–Aldrich) for another 24 h. Then, 100 μL aliquots of the supernatants were added to 100 μL of Griess reagent [0.1% (naphthyl)ethylenediamine and 1% sulfanilamide in a 5% H_3PO_4 solution], and the mixture was left to stand at room temperature for 20 min. NO production was measured by recording the concentration of nitrite in the supernatant. The absorbances were measured at 540 nm. Curcumin was used as the positive control drug.

3.6. PTP1B inhibitory activity assay

The pellets of recombinant GST-hPTP1B bacteria were purified on a GST bead column. The dephosphorylation of *para*-nitrophenyl phosphate (*p*-NPP) to *para*-nitrophenol was catalyzed by PTP1B. Enzyme activity using an end-point assay, which intensifies the yellow color, was measured at a wavelength of 405 nm. Compounds were dissolved in 100% dimethyl sulfoxide (DMSO). The DMSO solutions were diluted to 10% DMSO prior to use in the assays. Selected compounds were first evaluated for their ability to inhibit PTPase at a concentration of 10 μmol/L at 30 °C for 10 min in a reaction system with 3 mmol/L *p*-NPP in HEPES assay buffer (pH 7.0). The reaction was initiated by the addition of the enzyme. The reaction was quenched by the addition of 1 mol/L NaOH. A microplate spectrophotometer was used to determine the amount of *p*-nitrophenol present.

3.7. Determination of the absolute configurations of the sugars

Compound **4** (2 mg) was dissolved in 1 mol/L CF_3COOH (14 mL), and then the mixture was heated at 70 °C for 1 h. The mixture was then extracted three times with EtOAc, and the aqueous layer was freeze-dried to obtain the residue. Using a method reported in the literature[34], the residue was dissolved in anhydrous pyridine (2 mL), L-cysteine methyl ester hydrochloride (4 mg) was added, and then the mixture was heated in a water bath (60 °C) for 1 h. After the reaction, the solution was dried under vacuum, *N*-trimethylsilylimidazole (1 mL) was added, and the solution was heated in a water bath (60 °C) for 1 h. The reaction mixture was extracted three times with H_2O/*n*-hexane, and the *n*-hexane layer was analyzed using GC under the following conditions: injection temperature, 300 °C; detector temperature (FID), 300 °C; capillary column, HP-5 (60 m × 0.32 mm, Dikma); initial oven temperature of 200 °C, increased to 260 °C at a rate of 10 °C/min, and the final temperature was maintained for 30 min; and carrier gas, N_2. Compound **5** was also evaluated by the same procedure, and all its sugars were determined to be in the D-configuration.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.apsb.2018.04.002.

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