Anti-inflammatory compounds of “Qin-Jiao”, the roots of *Gentiana dahurica* (Gentianaceae)

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**Abstract**

**Ethnopharmacological relevance:** “Qin-Jiao” is a well-known traditional Chinese medicinal (TCM) herb having been used generally for fighting rheumatoid arthritis (RA) since ancient times. The root of *Gentiana dahurica* Fisch (Gentianaceae) is one of the four officially validated “Qin-jiao” as listed in the Chinese Pharmacopoeia. In addition, it is a common Tibetan medicinal herb used for the treatment of tonsillitis, urticaria, and RA, while the flowers have been used as a Mongolian herb for curing cough sore throat and eliminating the phlegm due to its anti-inflammatory effect.

**Aim of the study:** The aim of the study was to characterize the anti-inflammatory compounds in “Qin-jiao”, on the basis of detailed investigation on not only the phytochemical study of *Gentiana dahurica,* but also the bioactive evaluation on compounds obtained presently and previously from different “Qin-jiao” origins and *Gentiana* species.

**Materials and methods:** The ethanol extract of air-dried roots of *Gentiana dahurica* was suspended into H\(_2\)O and extracted with EtOAc and \(n\)-BuOH, successively. Repeated column chromatography (CC) and semi-preparative HPLC were carried out on each of the fractions. The isolated compounds were determined by detailed spectroscopic analysis and acidic hydrolysis. Anti-inflammatory activities of 18 isolates, together with 12 typical compounds obtained previously by our group from the other “Qin-jiao” origins (*Gentiana crassicaulis,* *Gentiana straminea* and *Gentiana rigescens,* were tested by inhibitory effects on LPS-induced NO production in macrophage RAW264.7 cells and TPA-induced cyclooxygenase-2 and -1 (COXs-2/1) production on zebrafish model.

**Results:** A new lignan glycoside (1) was identified, together with 20 known compounds, including 10 iridoid glycosides (2–11), three steroids (12–14), four lignans (15–18), one phenylpropanoid (19) and two triterpenes (20–21). Anti-inflammatory bioassay showed that only compound 21 displayed potential inhibitory effect on NO production \(I_{50}=16.85 \mu \text{M}\), while 20 tested compounds had inhibitory activities on COXs-2/1. Among them, the triterpenoid 21 was the most active compound with an inhibitory value of 78% at a concentration of 30 \(\mu \text{M}\). All the tested compounds showed no cytotoxicity on five human cancer cell lines (40 \(\mu \text{M}\)) and zebrafish (30 \(\mu \text{M}\), except for 21 displaying weak cytotoxicity on human myeloid leukemia HL-60 \(I_{50}=16.43 \mu \text{M}\)).

**Conclusion:** Most of compounds particularly iridoid glycosides from “Qin-jiao” display potential inhibitory effect on COXs-2/1. The results support the historical importance of the well-known TCM herb, “Qin-jiao”, having been commonly used for fighting RA. As major components, the bioactive iridoid glycosides should play important role in the anti-inflammatory effect of “Qin-jiao”. Although further research will be required to evaluate the selective activities of the COXs-2/1 inhibitors, this work validates the medicinal use of “Qin-jiao” and provides information for different “Qin-jiao” origins having different treating effects on RA.

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1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune, and inflammatory disease that primarily attacks the joints, but also the skin, cardiovascular system, lungs, and muscles of human beings. RA is a major cause of disability and affects up to 0.5–1.0%
of the adult population worldwide (Ernest and Gabriel, 2001; Gary, 2003; Gerard et al., 2000). Autoimmune targeting of normal joint proteins results in inflammation, with resultant local release of cytokines, TNF, growth factor, and interleukins, all of which induce cyclooxygenase (COX) expression. In addition, induction of nitric oxide (NO) synthesis has been identified as one of the major responses to inflammatory stimuli in macrophages (Nathan and Hibbs, 1991). COX pathway inhibitors are some of the most frequently prescribed drugs in medicine. The non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used agents in this class (Park et al., 2011).

“Qin-Jiao”, being used for the treatment of rheumatism, arthralgia, stroke, hemiplegia, pains, jaundice, and infantile malnutrition, has been an important traditional Chinese medicinal (TCM) herb for fighting rheumatoid arthritis (RA) since ancient times in China (Cai et al., 2010). In Chinese Pharmacopoeia, the roots from four plants of the genus Gentiana (Gentianaceae) including Gentiana macrophylla Pall., Gentiana crassicaulis Duthie., Gentiana straminea Maxim., and Gentiana dahurica Fisch. are recorded as the original materials of “Qin-Jiao” (Chinese Pharmacopoeia, 2010). To date, the former three, Gentiana macrophylla, Gentiana crassicaulis, and Gentiana straminea have been chemically and biologically investigated by several groups (Lv et al., 2012; Singh, 2008; Tan et al., 1996; Xu et al., 2009a; Liu et al., 1994). The results suggested that “Qin-Jiao” contained mainly iridoid glycosides and the water extracts from roots of Gentiana macrophylla displayed a significant inhibitory effect on acute treatment of rheumatoid rats (Yu et al., 2004). Loganic acid (10), an iridoid glucoside widely existed in Gentiana plants, could inhibit the carrageenan-induced mouse paw edema (María del et al., 1994). Gentiopicroside (2), another major iridoid glucoside, also showed inhibitory effect on inflammatory mediators NO and COX-2 (An and Jin, 2007).

Gentiana dahurica is distributed in most areas of China except for Yunnan province and Northeast area (Li et al., 2006). In addition to be one of the officially validated “Qin-Jiao”, it is a common Tibetan medicine used for fighting RA and the flowers have been used as a Mongolian medicine for curing sore throat, cough and cleaning away lung-heat. Previously, five iridoid glycosides and seven triterpenoids with moderate cytotoxicities were reported from the roots of Gentiana dahurica (Fan et al., 2010). However, its anti-inflammatory constituents have so far not been known. As a part of an ongoing effort to search for new bioactive compounds from Gentiana medicinal plants and to characterize the anti-inflammatory compounds in “Qin-Jiao” (Liu et al., 1994; Xu et al., 2007, 2009a, 2011, 2008, 2009b; Lv et al., 2012), the phytochemical investigation on the roots of Gentiana dahurica was carried on. This led to the isolation of one new lignan glycoside together with 20 known compounds. The anti-inflammatory activities of most of the isolates and 12 typical compounds obtained previously by our group from the other “Qin-Jiao” origins (Gentiana crassicaulis, Gentiana straminea) and Gentiana rigescens, were tested by inhibitory effects on LPS-induced NO production in macrophage RAW264.7 cells and LPS-induced cyclooxygenases-2 and 1 (COXs-2/1) production on zebrafish model, one of the prominent iso-enzyme at sites of inflammation. The structure-activity relationship (SAR) of tested compounds was discussed.

2. Material and methods

2.1. Plant material and compounds tested for anti-inflammatory activities

The roots of Gentiana dahurica were collected in Xining, Qinghai province, PR China and identified by Professor Yang Zeng (School of Life and Geographical Sciences, Qinghai Normal University). A voucher (KUN_551024) specimen has been deposited in Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (CAS).

Thirty compounds were tested for anti-inflammatory activities. These include 18 isolates (1–6, 8, 10, 12–17, and 19–21) reported in the present study. Since compounds 8 and 9 were isolated as a mixture from the title plant, the purified ones for bioassay were obtained previously from Gentiana rhodantha by our group (Xu et al., 2008). In addition, 11 typical iridoid glycosides (22–32) and one chromone glycoside (33) were obtained previously by our group from the other “Qin-Jiao” and related Gentiana species, referring to 6′–O-β-D-glucosylologanic acid (22), qingjaoside A–C (23–25), and 4′–O-β-D-glucosylgentiopicroside (26) from Gentiana crassicaulis (Lv et al., 2012), macrophyllidosides A, E–F (27–29), loganic acid 11-O-β-D-glucopyranosyl ester (30), and macrophyllidoside D (33) from Gentiana straminea (Xu et al., 2009a), and 2-(α-methyl-dihydroxybenzoyl)-sweroside (31) and sweroside (32) from Gentiana rigescens (Xu et al., 2006).

2.2. General experimental techniques

Optical rotations were measured with a HORIBA SEPA-300 high-sensitive polarimeter. IR spectra were measured on a Bio-Rad FTS-135 series spectrometer in dry film. UV spectra were recorded on a Shimadzu UV240A ultraviolet–visible spectrophotometer. ESI-MS and HRESI-MS were run on an API QSTAR Pular-1 spectrometer. NMR spectra measured in DMSO or CD3OD solution and recorded on a Bruker AV400, DRX-500 or Avance III-600 spectrometer at 25 °C, using TMS as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (J) were expressed in Hz. Column chromatography (CC) were carried out over silica gel (200–300 mesh, Qingdao Marine Chemical Factory), Diaion HP20SS (Mitsubishi Chemical Industry, Ltd.), MCI-gel CHP-20P (75–150 μm, Mitsubishi Chemical Industry, Ltd.), Rp-18 (40–63 μm, Merck), and Rp-8 (40–63 μm, Merck). Pre-coated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done under UV light (254 nm and 365 nm) and by spraying the plates with 10% sulfuric acid followed by heating. An Agilent series 1100 (Agilent Technologies) were used for HPLC. An Agilent ZORBAX SB-C18 column 5 μm 143 A column (250 mm × 9.4 mm) were used for semi-preparative HPLC separations. GC analysis was run on Agilent Technologies HP5890 gas chromatography equipped with an H2 flame ionization detector. The column was 30QC2/AC-5 quartz capillary column (30 m × 0.32 mm) with the following conditions: column temperature: 180 °C/280 °C; programmed increase, 3 °C/min; carrier gas: N2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4 μl, split ratio: 1/50.

2.3. Extraction and isolation of compounds

As previously reported on the extraction and isolation of secondary metabolites from Gentianaceous medicinal plants (Geng et al., 2009; Xu et al., 2006), the air-dried roots (2.57 kg) of Gentiana dahurica were extracted with 95% ethanol two times, following with 50% ethanol two times, each time 3 h under reflux at 80 °C. After concentrated in vacuum, the combined extract (147.4 g) was suspended into H2O and partitioned with EtOAc and n-BuOH, successively.

The H2O layer (dry weight 68.9 g) was loaded on a Diaion HP20SS column, eluting with MeOH/H2O (0:1–1:0), to give eight fractions (H1–H8). H1 (13.7 g) was applied to a silica gel column chromatograph (CC), eluting with CHCl3/MeOH/H2O (8:2:0.2–7:3:0.5) to afford compound 10 (35 mg). H2 (3.4 g) was almost the pure compound. H3 (15.9 g) was subjected to CC over...
MCI-gel CHP-20P (MeOH/H2O, 5:95–100:0) and silica gel (CHCl3–MeOH–H2O, 8:2:0:2–6:4:1) to give compound 4 (13.7 mg). H4 (1.5 g) afforded compounds 2 (1.4 g) and 7 (2 mg) over MCI-gel CHP-20P (MeOH/H2O, 1:9:1–10) and silica gel (CHCl3–MeOH–H2O, 8:2:0:2–6:4:1) CC. H5 (1.35 g) was chromatographed repeatedly over MCI-gel CHP-20P (MeOH/H2O, 0:1–1:0), Rp-8 (MeOH/H2O, 5:95:100:0) and silica gel (CHCl3/MeOH–H2O, 8:5:1:5:1–0:1:6:4:1) to yield compounds 1 (5 mg), 2 (45 mg), 3 (8 mg), 5 (5 mg), 6 (10 mg), and a mixture (2 mg) of isomers 8 and 9. H6 (12 g) was separated over MCI-gel CHP-20P (MeOH/H2O, 0:1–1:0) and silica gel (CHCl3/MeOH–H2O, 8:5:1:5:1–0:1:6:4:1) CC. H7 (10 mg) was recrystallized from E12.

2.6. The determination of NO production from RAW264.7

RAW 264.7 cells (2 × 105 cells/well) were stimulated with LPS to produce NO, simultaneously pulsed respectively the testing compounds, and then incubated for 24 h. The equivalent of NO level in the supernatant was determined by the Griess reaction, and was measured with a microplate reader (Bio-Rad, USA) at 570 nm. And the nitrite concentration was determined by referring to a standard curve of sodium nitrite solution.

2.7. Cytotoxicity assay

Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMCC-7721, lung cancer A-549 cells, breast cancer MCF-7, and colon cancer SW480, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA). The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates (Chang et al., 2004). Briefly, adherent cells (100 μl) was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 0.5 × 104–1 × 105 cells/ml. Each tumor cell line was exposed to the test compound dissolved in DMSO in triplicates for 48 h at 37 °C, with DDP and taxol (Sigma, USA) as positive controls. Then, MTT (50 μl) was added to each well, and the tumor cells were incubated for another 4 h at 37 °C. After the supernatant liquor was removed, SDS (200 μl) was added to each well. The optical density was measured at 595 nm on a microplate reader. Cell viability was detected and a cell growth curve was graphed. IC50 values were calculated by Reed and Muench (1938) method.

2.8. Enzyme (cyclooxygenases-2/1) inhibitors screening assay

The zebrafish were processed with TPA (12-O-tetradecanoylphorbol 13-acetate) as model group to induce the expression of COXs-2/1, and then the tested compounds, each 30 μM (Tran et al., 2007), were added separately as the test group. Kit (Amplex® Red reagent) (Batchelor et al., 2003) was used as detection reagent of COXs-2/1 signals in zebrafish. Indomethacin was used as the positive control. Dye group contained dyes without zebrafish, so the signal of this group represented the background values of the dye. The negative control group was the solvent group contained the dye, so this group represented the background value of the test group and could also represent the COXs-2/1 produced by zebrafish normally. Blank control group was used to prove that the solvent did not have harmful effects on the zebrafish, and this group was designed without dye, thus this group represented the background values of the microplate reader. The experiment was carried on 96-well cell culture plates. Each plate design a dye group, a dye group, a negative control group, a model group, a positive control group and seven test groups. Each group processed eight wells, and the zebrafish were seeded four fish per well. The quantitative analysis of signal (S) of COXs-2/1 was carried on a multifunctional microplate reader. The inhibition ratio was calculated by the following equation, and before calculations the S (test group) and S (model group) should minus the background values. The results were shown as X ± SE. Statistical evaluation included one-way analysis of variance followed by...
Dunnett's t-test for multiple comparisons, \( p < 0.05 \) (*) were taken as significant. (Batchelor et al., 2003; Grosser et al., 2002)

\[
\text{COXs-2/1 inhibition ratio(\%)} = \frac{1 - \frac{S_{\text{test group}}}{S_{\text{model group}}}}{1} \times 100\%
\]

3. Results

3.1. Compounds isolated from the title plant

One new lignan glycoside (1) and 20 known compounds were isolated from the roots of Gentiana dahurica (Fig. 1). The known compounds were identified as 10 iridoid glycosides [gentiopicroside (2) (Liu et al., 1994), 6'-O-β-D-glucopyranosyl gentiopicroside (3) (Mpondo and Chula, 1988), scarban G3 (4) (Kakuda et al., 2001), olivieroside C (5) (Takeda et al., 1999), swertiamarin (6) (Luo and Nie, 1993), 1-O-β-D-glucopyranosyl-amplexide (7) (Kakuda et al., 2001), epi-kingaside (8) (Damtoft et al., 1993), kingaside (9) (Damtoft et al., 1993), loganic acid (10) (Calis et al., 1984), and loganin (11) (Calis et al., 1984)], three ecysteryoids [augasterone C (12) (Buděšínský et al., 2008), 20-hydroxyecdysone (13) (Vokáč et al., 1998), and 20-hydroxyecdysone 3-acetate (14) (Buděšínský et al., 2008)], four lignans [syringaresinol-β-D-glucopyranoside (15) (Shahat et al., 2004), liriiodendrin (16) (Gohari et al., 2011), dehydrodiconiferyl alcohol 4,7'-di-O-β-D-glucopyranoside (17) (Deyama et al., 1987), and lariiresolin-4,7'-O-β-D-glucopyranoside (18) (Ullah et al., 2001)], one phenylpropanoid [coniferin (19) (Metzger, 1993), and two triterpenes [2α,3α,24-tri-hydroxyolean-12-en-28-oic acid (20) (Kojima and Ogura, 1986) and roburic acid (21) (Mangoni and Belardini, 1963; Chen et al., 2005)], respectively, by comparison with authentic samples and of their spectroscopic and physical data with those previously reported values.

3.2. Structure elucidation of new compound

Compound 1, a white powder, [α]D = 46.4 (c 0.2, DMSO), had the molecular formula of \( \text{C}_{38}\text{H}_{54}\text{O}_{21} \), as deduced from the HRESI-MS (Calcd. for \( \text{C}_{38}\text{H}_{54}\text{O}_{21}\text{Na} \), \( m/z \) 869.3055), \(^{13}\text{C}-\text{NMR and DEPT data. IR spectrum showed the absorptions for hydroxyl moieties (3407 cm}^{-1} \) and benzene rings (1596–1421 cm}^{-1} \). The \(^{1}\text{H}-\text{NMR spectrum (Table 1) indicated clearly the presence of two sets of 1,3,4-trisubstituted benzene rings [δH 7.02 (1H, br d, J = 8.4 Hz), 6.88 (1H, br s), 6.78 (1H, d, J = 8.4 Hz), and 6.98 (1H, br d, J = 8.4 Hz), 6.82 (1H, br s), 6.68 (1H, d, J = 8.4 Hz)], two methoxys [δH 3.75 (6H, s)], and three anomic protons [δH 4.84 (1H, d, J = 8.4 Hz), 4.95 (1H, d, J = 7.8 Hz), and 4.29 (1H, d, J = 7.8 Hz)]. In addition, proton signals arising from one downfield-shifted benzyl group [δH 4.71 (1H, d, J = 6.0 Hz)] and one benzylic methylene [δH 2.82 (1H, d, J = 11.2 Hz)] were observed. The \(^{13}\text{C}-\text{NMR and DEPT spectra of compound 1 (Table 1) displayed 38 carbon resonances including 12 aromatic carbons (δC 109–150) due to two benzene rings, three aliphatic methines with one oxygen-bearing (δC 81.7), three aliphatic methynes with two oxygen-bearing (δC 72.0 and 58.7), two methoxys (δC 55.7 and 55.6), and 18 aliphatic carbon signals (δC 60.0–103.2) arising from three hexosyl moieties. The above NMR data suggested that compound 1 was a lignan glycoside (Li et al., 2004). Acidic hydrolysis of 1 afforded α-glucose, which was confirmed by GC analysis of the corresponding
trimethylsilylated l-cysteine adducts. The aforementioned data of 1 was similar to those of 7S,8R,8R-(-)-lariciresinol-4,4'-bis-O-β-D-glucopyranoside, a known lignan glycoside obtained from Galium sinaicum (El Gamal et al., 1997), except for a set of additional signals arising from a β-glucopyranosyl moiety. In the HMBC spectrum of 1 (Fig. 2), correlations of H-7 (δH 4.71) with C-1 (δC 137.8), C-2 (δC 109.9), and C-6 (δC 117.9), and of H-7 (δH 2.82) with C-1 (δC 134.7), C-2 (δC 113.0), C-6 (δC 120.4), C-8 (δC 41.9), and C-9 (δC 72.0) confirmed the existence of lariciresinol skeleton. The HMBC correlations of H-C(1′′′) (δH 4.84) of Glc-I with C-4 (δC 145.4), and H-C(1′′′′) (δH 4.95) of Glc-II with C-4′ (δC 144.9) revealed the two glucopyranosyl moieties attached respectively to C-4 and C-4′ of the lariciresinol skeleton. The information was further confirmed by ROESY correlations (Fig. 2) of H-C(1′′′) (δH 4.84) of Glc-I with H-C(5) (δH 6.78), and H-C(1′′′′) (δH 4.95) of Glc-II with H-C(5′) (δH 6.68). Furthermore, the additional third anomeric proton at δH 4.29 [H-C(1′′′′)] (δH 4.95) of Glc-I with C-4′ (δC 144.9) was correlated significantly with C-2′′′′ (δC 80.0). This suggested the existence of the glucopyranosyl unit was linked to the Glc-II C-2′′′′ in 1. The 13C NMR spectrum of 1, the chemical shift of C-2′′′′ of Glc-II was changed obviously by -7 ppm, relative to 7S,8R,8R-(-)-lariciresinol-4,4'-'bis-O-β-D-glucopyranoside, further confirmed the planar structure of 1. The relative configuration of 1 was determined by ROESY spectrum, in which correlations of H-C(8) with H-C(8′), H-C(2), and H-C(6) illustrated that H-C(8) has cis configuration with H-C(8′), but trans configuration with H-C(7). Thus, the absolute configuration of compound 1 has two possibilities, 7S,8R,8R or 7R,8S,8S. Compound 1 ([α]D 26.0 = -46.4 (c 0.2, DMSO) and the known lignan 7S,8R,8R-(-)-lariciresinol-4,4'-'bis-O-β-D-glucopyranoside ([α]D 26.0 = -50.0 (c 0.16, pyridine) had almost the same [α]D value, indicating that they had the same absolute configuration. Therefore, the structure of compound 1 was deduced as 7S,8R,8R-(-)-lariciresinol-4,4'-bis-O-β-D-glucopyranosyl-4'-O-(2-O-β-D-glucopyranosyl)-β-D-glucopyranoside.

### 3.3. Physical and spectroscopic data of new compound

7S,8R,8R-(-)-lariciresinol-4,4'-'bis-O-β-D-glucopyranoside (1): white amorphous powder, [α]D 26.0 = -46.4 (c 0.2, DMSO). UV (MeOH) λmax nm (log ε): 280 (3.84), 249 (3.47), 213 (3.18), 205 (3.15); IR (KBr) νmax cm⁻¹: 3407, 2919, 2882, 1640, 1513, 1451, 1075, 1025 cm⁻¹; ESI-MS (positive): [M+Na]+, 869.3055; found 869.3046 [M+Na]+; 1H (600 MHz) and 13C (150 MHz) NMR spectral data (in DMSO-d6); see Table 1.

### 3.4. Inhibitory effects on NO production and cytotoxicity

Most of isolates (1-6, 8-10, 12-21) were tested for inhibitory effects on LPS-induced NO production in macrophage RAW264.7 cells, together with 12 typical compounds (22-33) existed commonly in “Qin-Jiao” and related Gentiana species (Fig. 3). Among them, only one triterpenoid acid, robic acid (21) showed moderate activity on the inhibition of NO release (IC50 = 16.85 μM). On the cytotoxic assay against five human cancer cell lines (myeloid leukemia HL-60, hepatocellular carcinoma SMCC-7721, lung cancer A-549 cells, breast cancer MCF-7, and colon cancer SW480) by MTT method, all of tested compounds showed no cytotoxicity at a concentration of 40 μM, except for compound 21 showing weak cytotoxicity on human myeloid leukemia HL-60 (IC50 = 16.43 μM).

<table>
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<tr>
<th>Table 1</th>
<th>1H and 13C NMR data for compound 1 (in DMSO-d6).</th>
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* Overlapped with solvent peak.
3.5. Inhibitory effects on COXs-2/1

Compounds 1–6, 8–10, 12–17, and 19–33 were evaluated for their inhibitory effects at a concentration of 30 μM on COXs-2/1 by 12-O-tetradecanoylphorbol 13-acetate (TPA) treated zebrafish model. The results were shown in Table 2. All of the tested compounds showed no cytotoxicity on zebrafish at a concentration of 30 μM, and compared to the positive control, indomethacin (56 ± 1% at a concentration of 0.9 μM), the inhibitory effects of 12 compounds were more than 56% at a concentration of 30 μM. The order of their activities was 21 > 16 > 14 > 8 > 9 > 26 > 2 > 25 > 3 > 5 > 30 > 10. Among them, the triterpenoid, roburic acid (21) and the lignan, liroiodendrin (16) showed the strongest activities with the inhibitory value of 79 ± 5% and 73 ± 4%, respectively. As an exception, the known triterpenoid, 2α,3α,24-trihydroxyolean-12-en-28-oic acid (20) induced the expression of COX-2.

4. Discussion and conclusion

Among pro-inflammatory enzymes, the inducible forms of nitric oxide synthase (NOS) and cyclooxygenase (COX) are responsible for increasing the levels of NO and prostaglandins (PGs), respectively. NO is produced by iNOS in macrophages, hepatocytes and renal cells, under the stimulation of lipopolysaccharide (LPS) and tumor necrosis factor-alpha (TNF-α), while COX is the enzyme that converts arachidonic acid to PGs. Thus, the NO and COXs level are important index of inflammatory response.

Thirty compounds obtained currently or previously from different “Qin-Jiao” and related Gentiana species were tested for anti-inflammatory activities, by inhibitory effects on LPS-induced NO production in macrophage RAW264.7 cells and production of COXs-2/1 on zebrafish model. The tested compounds including 19 iridoid glycosides (2–6, 8–10, 22–32), four lignans (1, 15–17), three edysteroids (12–14), one phenylpropanoid (19), two triterpenes (20–21), and one chromene glycoside (33). Almost all of them showed no cytotoxicity on five human cancer cell lines (40 μM) and zebrafish (30 μM), and only roburic acid (21), with weak cytotoxicity on human myeloid leukemia HL-60 (IC50=16.43 μM), displayed potential inhibitory effect on the NO production. Among the tested compounds, 21 also had the strongest activity on COXs-2/1 with an inhibitory value of 78% at a concentration of 30 μM. These results are similar to those of Cao et al. 2010, who reported that roburic acid from Gentiana macrophylla non-selectively inhibited COX-1 and COX-2 with IC50 values of 5 μM and 9 μM, respectively (Cao et al., 2010). Roburic acid (21), a triterpenoid acid existed widely in all the four validated “Qin-Jiao” origins, has an opening A ring and an oxidized carboxyl group at C-3 as its structural characters, which may be responsible for the docking site access to the COXs.

Table 2

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<th>Compounds</th>
<th>Inhibition (%)a</th>
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<td>19</td>
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<td>22</td>
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<td>23</td>
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<td>15</td>
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<td>54 ± 11</td>
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* Mean ± SE.  
# Indomethacin (0.9 μM).  
* p < 0.05.  
** p < 0.01.  
*** p < 0.001 vs model group.
Iridoid glycosides as the major components in “Qin-Jiao” displayed potential inhibitory effects on COXs-2/1. Of 19 tested compounds, 14 iridoids exhibited COXs-2/1 inhibitory activities, referring to 11 secoiridoid glycosides (2, 3, 5, 8, 9, 23, 24, 26, 28, 31) and 32) and three iridoid glycosides (10, 25, and 30). Among the secoiridoid glycosides, kingiside types (8, 9) existed in Gentiana dahurica showed the strongest inhibitory activities among all tested iridoids. Epi-kingside (8) had better COXs-2/1 inhibitory activity than kingiside (9), suggesting that the configuration of the methyl group at C-8 may affect the activity. Gentiopicroside and its derivatives (2, 3, 5, and 26) generally displayed stronger activities, revealing that the double bond between C-5 and C-6 could enhance inhibitory effect on COXs-2/1. For loganic acid types (10, 22, 25, and 30), qinjiaoside C (6’-O-α-D-xlyloprenosylloganic acid, 25) was the strongest inhibitor on COXs-2/1. However, 6’-O-β-D-glucosylloganic acid (22) with a different terminal sugar moiety showed no inhibitory effect on COXs-2/1, indicating that the composition of the sugar chain should play an important role on their bioactivities.

For four lignans (1, 15–17), a new tetrahydrofuran type lignan (1) from Gentiana dahurica, exhibited inhibitory effect on COXs-2/1 (53 ± 12%), while a known eupomatienoid benzofuran type lignan (17) had no activity. Compound 16, a furanofuran type lignan possessing an additional sugar moiety related to compound 15, exhibited stronger inhibitory effect on COXs-2/1 (73 ± 5%) than 15 (53 ± 6%), indicating that the sugar substitute in the furanofuran lignans could affect the anti-inflammatory activities.

Among these tested ecdysterones, only compound 14, whose hydroxyl group at C-3 was acetylated, displayed inhibitory effect on COXs-2/1. Therefore the appearance of acetyl group may be a key to the inhibitory effect on COXs-2/1 for the ecdysterones. The only isolated chromene glycoside (33) from “Qin-Jiao” (Gentiana macrophylla and Gentiana straminea) also displayed moderate inhibitory effect on COXs-2/1 with the value of 54 ± 11%. Iridoid glycosides as major constituents in “Qin-Jiao” (Gentiana dahurica, Gentiana crassicaulis, and Gentiana straminea) and other related Gentiana species (Gentiana rigescens), showing potential COXs-2/1 inhibitory activities, demonstrated that “Qin-Jiao” fighting RA should be due to the inhibition on production of COXs-2/1. Moreover, besides the major compounds, gentiopicroside (2) and loganic acid (10), most of the minor compounds from “Qin-Jiao” (Gentiana dahurica, Gentiana crassicaulis, and Gentiana straminea), especially the discrepant chemical constituents, also exhibited appreciable influence on COXs-2/1 inhibitory activities. Although further research will be required to evaluate the selective activities of the COXs-2/1 inhibitors, the result provided firstly valuable information for different “Qin-Jiao” origins having different effects on fighting RA.

Acknowledgments

The authors are grateful to the members of the Analytical Group in State Key Laboratory of Phytochemistry and Plant Resources in West China (SKLPPR), Kunming Institute of Botany (KIB), for measurements of all spectra. We thank Prof. Yan Li and Dr. Lin-Mei Kong from SKLPPR, KIB, for testing the cytotoxicity and NO inhibitors screening assays. We would like to acknowledge Hunter Biotechnology Inc for enzyme (cyclooxygenases-2/1) inhibitors screening assays. We also thank Prof. Yang Zeng, Dr. En-De Liu and Dr. Dong Wang for providing the pictures in graphical abstract. This work was supported by Science and Technology Planning Project of Yunnan Province (2010CD106), the 973 Program of Ministry of Science and Technology of PR China (2011CB915503), the State Key Laboratory of Phytochemistry and Plant Resources in West China, Chinese Academy of Sciences (P2010-ZZ03) and The Fourteenth Candidates of the Young Academic Leaders of Yunnan Province (Min XU, 2011CI044).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.03.016.

References


