Malconenoside A, a New Phenolic Glycoside from Bupleurum malconense

Jie Yan1,*, Qin Luo2,*, Fei Long1 and Mei-Lian Zhao3

Abstract

A new phenolic glycoside, 7-(4-O-β-D-glucopyranosyl)-3-methoxyphenyl-6,8-dihydrofuro[3′,4′:7,8]naphtho[1,2-d][1,3]dioxol-10(7H)-one, named malconenoside A (I), was isolated from Bupleurum malconense Shan et Y. Li. The structure, including absolute configurations, was assigned by using spectroscopic methods and ECD calculation. Biological activities of compound 1 towards human cancer cells (HepG2, BGC-823, and A549) were evaluated. The most sensitive two cell lines were HepG2 and BGC823, in which the IC50 values of compound 1 were 8.70 and 16.94 μM, respectively.

Keywords

Bupleurum malconense, phenolic glycoside, ECD calculation, human cancer cells, cytotoxicity

Results and Discussion

Chaihu is a commonly used traditional Chinese medicine, which was first recorded in the Classic of the Materia Medica (Shen Nong Ben Cao Jing), as top grade. The root of Bupleurum chinense DC. and B. scorzonerifolium Willd. are the major and legal sources of Chaihu recorded in the Pharmacopoeia of People’s Republic of China as a key player of many famous prescriptions, such as Xiao Chaihu Tang, Buzhongyiqi Tang, and Xiaoyao Powder. Bupleurum malconense (Zhu ye chai hu) is 1 source of regional supplies of Chaihu in Sichuan province. There are more than 20 Bupleurum species that can be used for medicine, and hundreds of compounds have been identified from them, such as triterpenoid saponins, lignans, and caffeic acid derivatives.1-7 The medicinal part collected for local medicine is the herb, which is quite different from the root for chaihu described by the Chinese Pharmacopoeia. Bupleurum malconense Shan et Y. Li it used to reduce fever and soothe the liver.1,7,8 In this study, we isolated 1 new phenolic lactone (Figure 1) and, herein, we describe its isolation and structure identification.

1State Key Laboratory of Characteristic Chinese Medicine Resources in Southwest China, Pharmacy College, Chengdu University of TCM, Chengdu, China
2Clinical Lab, Shenzhen University General Hospital, Shenzhen, PR China
3College of Medical Technology, Chengdu University of TCM, Chengdu, PR China

*Jie Yan and Qin Luo contributed equally to this work.

Corresponding Authors:
Jie Yan, State Key Laboratory of Characteristic Chinese Medicine Resources in Southwest China, Pharmacy College, Chengdu University of TCM, Chengdu 611137, PR China.
Email: yanjie@echtcm.edu.cn
Mei-Lian Zhao, College of Medical Technology, Chengdu University of TCM, Chengdu 611137, PR China.
Email: zhaomeilian2009@126.com

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rotation. In addition to the signals of a glucose residue, the remaining resonances were presumed to be of a phenolic derivative. The structure of 1 could be confirmed by detailed interpretation of its 2D NMR data (Figure 2). The COSY spectrum showed cross peaks of H-5/H-6, H-7/H-8, and H-4’/H-5’. The architecture of 1 was assembled by HMBC correlations. HMBC correlations of H-7/C-9, C-1’, C-5’, C-6’, H-8/ C-9, C-10, as well as the correlation of H-12/C-8, C-9, C-10, C-11, and consideration of the downfield chemical shift of C-9 (δC 162.2), indicated the presence of and connection of rings A, B, and C in 1 (Figure 2). Rings B and D linked via C-1 and C-8 from HMBC correlations of H-8/C-1, C-2, C-6 and H-2, H-6/C-8. Information on H-8’/C-3, H-1’’/C-4, and H-7’/C-2’/C-3’ can be used to determine the substitution of methoxy, sugar and C-7 (δC 102.6) substituent methylene. The glucose moiety is connected to C-4, supported by the observed HMBC correlation of H-1’’/C-4. The configuration of the glycosidic bond was assigned as the β-form based on the coupling constant of H-1’’ (J = 7.0 Hz). Furthermore, the ROESY correlations of H-2/H-8’ and H-5/H-1’’ determined the positions of the methoxy and β-D-glucose.

The absolute configuration of 1 was assigned by ECD calculations at the B3LYP/6-31G(d,p) level. The results show that the calculated ECD curve of 3S agree well with the experimental data for 1, allowing the assignment of the absolute configuration of 1 (Figure 3). Therefore, the structure of 1 was assigned as shown; the compound was named malconenoside A (Figure 1).

Considering that Bupleurum species are used for the treatment of cancer, evaluation against human cancer cells (HepG2, BGC-823, and A549) was conducted to determine any potential beneficial effects of 1. We compared the growth inhibitory effects of 1 and 5-FU on human cancer lines originating from liver, gastric, lung and normal human umbilical vein endothelial cells (HUVEC). All cells were exposed to various concentrations (0–80 µM) for 48 h and cell viability was quantified by CCK8 assay. As shown in Figure 4, 1 and 5-FU significantly inhibited the growth of 4 human cell lines in a dose-dependent manner (Figure 4). Compound 1 was more potent than 5-FU (used as positive control). The IC50 values of 1 against these cancer cells were from 8.70 to 65.40 µM (Table 2). The most sensitive 2 cell lines were HepG2 and BGC823, in which the IC50 values of 1 were 8.70 and 16.94 µM respectively. The IC50 could not be determined, even at the high concentration of 80 µM, for normal human umbilical vein endothelial cells, suggesting that 1 exerted acceptable cytotoxicity toward normal cells and high selectivity for cancer cells, especially HepG2 and BGC823 cells.

### Table 1. 1H (600 MHz) and 13C NMR (150 MHz) Data of 1 in Methanol-d4 (δ in ppm, J in Hz).

| No. | δC  | δH  |
|-----|-----|-----|
| 1   | 137.7|     |
| 2   | 114.4| 6.97 (1H, d, 2.0) |
| 3   | 150.4|     |
| 4   | 146.7|     |
| 5   | 117.8| 6.99 (1H, d, 8.4) |
| 6   | 121.4| 6.60 (1H, dd, 8.4, 2.0) |
| 7   | 29.1 | Hα: 3.97 (1H, dd, 22.8, 3.0) Hb: 3.75 (1H, dd, 22.8, 3.0) |
| 8   | 38.5 | 4.90 (1H, m) |
| 9   | 162.2|     |
| 10  | 127.6|     |
| 11  | 174.8|     |
| 12  | 73.1 | Hα: 4.98 (1H, d, 17.3) Hb: 4.92 (1H, d, 17.3) |
| 1’  | 120.9|     |
| 2’  | 146.9|     |
| 3’  | 147.6|     |
| 4’  | 108.6| 6.75 (1H, d, 8.0) |
| 5’  | 122.6| 6.83 (1H, d, 8.0) |
| 6’  | 126.6|     |
| 7’  | 102.6| Hα: 5.87 (1H, d, 1.0) Hb: 5.80 (1H, d, 1.0) |
| 8’  | 56.7 | 3.80 (3H, s) |
| 1’’ | 102.7| 4.81 (1H, d, 7.0) |
| 2’’ | 74.8 | 3.43 (1H, overlap) |
| 3’’ | 77.8 | 3.43 (1H, overlap) |
| 4’’ | 71.3 | 3.34 (1H, overlap) |
| 5’’ | 78.2 | 3.34 (1H, overlap) |
| 6’’ | 62.4 | Hα: 3.82 (1H, dd, 12.0, 2.0) Hb: 3.63 (1H, dd, 12.0, 5.0) |

![Figure 1. Chemical structure of compound 1.](image1.png)

![Figure 2. COSY and key HMBC and ROESY correlations of 1.](image2.png)
Column chromatography was performed on silica gel (200−300 mesh; Qingdao Marine Chemical Inc., China), on C-18 silica gel (40−60 μm; Daiso Co., Japan), MCI gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries, Tokyo, Japan) and on Sephadex LH-20 (Amersham Pharmacia, Sweden). UV spectra were recorded on a Shimadzu UV-2401PC spectrometer. Semi-preparative HPLC was carried out using an Agilent 1200 liquid chromatograph; the column used was a 250 mm × 9.4 mm, i.d., 5 μm, Zorbax SB-C18 and a 250 mm × 4.6 mm, i.d., 5 μm, Daicel Chiralpak IC. NMR spectra were recorded on a Bruker AV-600 spectrometer, with TMS as an internal standard. EI-MS and HR-EI-MS were recorded using an AutoSpec Premier P776 spectrometer, and ESI-MS and HR-ESI-MS with an API QSTAR Pulsar 1 spectrometer.

Plant Material
*B. malconense* Shan et Y. Li was collected from Wenchuan (Sichuan Province, China) in September 2013 and authenticated by Prof. Ying-Fang Wei, Chengdu University of TCM. A voucher specimen (cd20130920024) was deposited at Chengdu University of TCM, Chengdu, China.

Extraction and Isolation
The dried and powdered aerial part of *B. malconense* (10 kg) was soaked in 70% EtOH for 48 h, then percolated. The final extract was evaporated to dryness and extracted with EtOAc. This extract was divided into 7 parts Fr. 1−Fr. 7 by silica gel column chromatography by eluting with a gradient of CHCl3/MeOH (100:1−1:1). Fr. 3 (51 g) was fractionated on a MCI gel CHP 20P column eluting with a gradient of aqueous MeOH (20:80−100:0) to provide eleven portions, Fr. 3.1−Fr. 3.11. Fr. 3.2 (8.4 g) was separated by Sephadex LH-20 (MeOH) chromatography, followed by passage through a RP-18 column (MeOH/H2O, 40:60−50:50), and by semi-preparative HPLC (MeOH/H2O, 25:75) to give compound 1 (2.5 mg, tR = 18.5).

Malconenoside A (1)
Yellowish gum.

[α]D20−13.6 (c 0.022, MeOH); CD (MeOH) Δε202−15.72, Δε237 +5.84, Δε291 +2.38.

UV (MeOH) λmax (log ε) 285 (2.44), 200 (3.53) nm

1H and 13C NMR: Table 1.

ESIMS m/z 537 [M + Na]+.

HRESIMS m/z 537.1374 [M + Na]+ (calcd for C26H26NaO11, 537.1373).

Acid Hydrolysis of Compound 1
Compounds 1 (0.5 mg) was dissolved in 0.5 mL of 6 N HCl and heated at 60 °C for 1.5 h. After cooling, the mixture was extracted with EtOAc. The aqueous layer was concentrated in vacuo followed by TLC examination and optical rotation

**Experimental**

**General**

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**Table 2.** IC_{50} Values for 1 and 5-FU on Different Cell Lines.

| Compounds | HepG2 (μM) | BGC-823 (μM) | A549 (μM) | HUVEC (μM) |
|-----------|------------|--------------|------------|------------|
| 1         | 8.70 ± 1.38 | 16.94 ± 1.38 | 65.40 ± 1.38 | >80        |
| 5-FU (control) | 9.08      | 76.92 ± 1.35 | 69.79 ± 1.35 | >80    |

HepG2: human liver cancer; BGC-823: human gastric cancer; A549: human lung cancer; HUVEC: normal human umbilical vein endothelial cells.

*P < 0.05 (vs. 5-FU group).
IC50 values were calculated using the PrismPad program. Measurement. The optical rotation of the glucose of 1 was [α]D23 + 43.2 (c 0.055, H2O). By comparing of this value with that of D-glucose: [α]D23 + 45.5 (c 0.100, H2O), the glucose in compound 1 was determined to have a D-configuration.

**ECD Calculations**

Molecular Merck force field (MMFF) and DFT/TDDFT calculations were performed with the Gaussian09 program package. Conflex conformational search generated low-energy conformers within a 10 kcal/mol energy, which was finished by software CONFLEX 7. The predominant conformers were optimized by DFT calculation at the B3LYP/6 to 31G (d, p) level with the PCM in MeOH. ECD calculations were conducted at the B3LYP/6 to 31G (d, p) level with the PCM in MeOH. For comparison of the calculated curves and experimental CD spectra, the program SpecDis 1.62 was used.

**Cell Viability**

All cell lines were purchased from the Cell Bank of China Science Academy (Shanghai, China) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 100 U/mL penicillin-streptomycin, and incubated at 37 °C in an atmosphere of 5% CO2. Cell viability was evaluated by a CCK8 assay kit (Dojindo Laboratories, Tokyo, Japan), used according to the manufacturer’s instructions. Exponentially growing cells were seeded at 3 to 8×10^3 cells per well in 96-well culture plates for 24 h. Cells were exposed to increasing concentrations (0–80 μM) of 1 and 5-FU for 48 h. An equal volume of DMSO was used as that of the solvent control. CCK8 solution (10 μL) was added to each well and incubated for another 1 to 4 h. Light absorbance of the solution was measured at 450 nm (Epoch 2, BioTek Instruments, Inc.). The IC50 values were calculated using the PrismPad program.

**Conclusion**

In this paper, one new phenolic glycoside, malconenoside A, was isolated from Bupleurum malconense. It was shown that 1 could inhibit human cancer cells HepG2 and BGC-823, for which the IC50 values were 8.70 and 16.94 μM, respectively. These findings indicate the potential of 1 for its anti-tumor activity.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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**Ethical Approval**

Not applicable, because this article does not contain any studies with human or animal subjects.

**Informed Consent**

Not applicable, because this article does not contain any studies with human or animal subjects.

**ORCID iD**

Jie Yan https://orcid.org/0000-0002-9694-8722

**Trial Registration**

Not applicable, because this article does not contain any clinical trials.

**Supplemental Material**

Supplemental material for this article is available online.

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