Fitoterapia 130 (2018) 281–289

Contents lists available at ScienceDirect

Fitoterapia

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

New inhibitors of matrix metalloproteinases 9 (MMP-9): Lignans from *Selaginella moellendorffii*

Yuan Zhu[a,1], Ri-Zhen Huang[b,1], Chun-Gu Wang[a], Xi-Lin Ouyang[a], Xiao-Teng Jing[a], Dong Liang[a,*], Heng-Shan Wang[a,**]

[a] State Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmaceutical Science, Guangxi Normal University, Guilin, Guangxi, People's Republic of China
[b] Pharmaceutical Research Center and School of Chemistry and Chemical Engineering, Southeast University, Nanjing, Jiangsu, People's Republic of China

ARTICLE INFO

Keywords:
Lignans
Selaginella moellendorffii
Matrix metalloproteinase
Migration
Anticancer activity

ABSTRACT

Matrix metalloproteinase 9 (MMP-9) is one of the structurally related zinc-dependent endopeptidases families and provides a new target for cancer therapy owing to its pivotal role in metastatic tumors. In this paper, fourteen lignans, including three novel lignans, named selamoellenin B–D (**1**–**3**), and eleven known lignan derivatives (**4**–**14**) were isolated from the plant of *Selaginella moellendorffii*. Among them, compound **3** is optically active, which was enantiomerically seperated to afford a pair of enantiomers, (−)-**3** and (+)-**3**. Their structures were elucidated by extensive spectroscopic analyses. Their cytotoxic activities were evaluated against four human cancer cell lines. Among them, five compounds (**4**, **5**, **6**, **11** and **13**) exhibited great potent cytotoxicity and their structure-activity relationships were also discussed. All compounds except for 3 lignan analogues with low cytotoxicity were selected for further in vitro enzyme inhibition, surface plasmon resonance (SPR), and molecular docking assays based on the MMPs target. The results shown that, compound **11** have the best inhibitory effect and can be considered as a potential drug candidate targeting at MMP-9 for cancer therapy.

1. Introduction

Cancer is one of a highly lethal disease worldwide, and result about eight million deaths annually, primarily caused by the metastasis, which is initiated by migration and invasion of cancer cells [1]. There has been evidence to suggest that some overexpressed proteolytic enzymes in cancer cell, such as matrix metalloproteinases (MMPs), play critical role in the migration, intravasation, and extravasation [2]. Therefore, the discovery of effective agents to suppress cancer metastasis by inhibition of metastasis associated proteins or signaling pathways are efficient approach to develop a new cancer therapy.

The MMPs, one of the structurally related zinc-dependent endopeptidases families, plays critical roles in composing and decomposing extracellular matrix (ECM) [3]. In particular, MMP-9 has aroused a wide range of research interest owing to its presence in the vicinity of metastatic tumors, and acts as an important precursor to the action of other endopeptidases [4–8]. As a key enzyme for inducing angiogenesis, it can also be induced by some growth factors and regulated by intracellular signaling pathways [9,10]. Therefore, MMP-9 could serve as potent therapeutic targets for antimetastasis drugs and down-regulation the expressions of MMP-9 may also help to improve the therapeutic effect of chemotherapeutic drugs. Recent studies revealed that some naturally occurring phenolic compounds, such as flavonoids [11] and lignans [12], have inhibitory activity against MMP-3 and -9, shown a significant effect on anti-drug resistance. Natural products have been acknowledged to be an important source for antitumor drug discovery and development.

Selaginella is the sole genus in the family *Selaginellaceae*, comprising about 700 species, which is widely distributed in the subtropical regions of the world. Recently, a series of alkynyl phenols, selaginellin A-R and selaginpulvilin A-D, have been isolated from this genus [13,14]. *Selaginella moellendorffii* Hieron, known as “Yan-Bai-Cao” in southwest China, has long been used as ethnic medicine or prescription ingredients for the treatment of hepatitis, gastritis, skin diseases, idiopathic thrombocytopenic purpura (ITP) [15], and urinary tract infection. Previous phytochemical investigation of this species has led to the

* Corresponding author at: State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmaceutical Science, Guangxi Normal University, 15 Yucai Road, Guilin 541004, People's Republic of China.
** Corresponding author.
E-mail addresses: liangdonggxnu@163.com (D. Liang), whengshan@163.com (H.-S. Wang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fitote.2018.09.008
Received 3 July 2018; Received in revised form 16 September 2018; Accepted 17 September 2018
Available online 19 September 2018
0367-326X/

isolation of several flavonoids, biflavonoids, lignans [16,17], and pyrrole alkaloids [18,19]. However, there have been very few pharmacological investigations reported on its anticancer activity. In a continuing search for bioactive constituents from important ethnic medicinal plants in the Karst Mountains of China, phytochemical study on the EtOH extracts of *S. moellendorffii* resulted in three new lignans (**1–3**), and eleven known ones (**4–14**). In this paper, we describe the details of isolation and structure elucidation of new compounds and obtain the results of the anticancer activity in vitro.

2. Experimental

2.1. General experimental procedures

The NMR spectra were recorded in CD_3OD or DMSO-*d6* on a Bruker 400 MHz instrument and a Bruker 500 MHz instrument (Bruker, Karlruhe, Germany), with tetramethylsilane as the internal standard. HRESIMS data were obtained from a Thermo-Scientific Exactive spectrometer. IR spectra (KBr) were recorded on a PerkinElmer Spectrum Two FT-IR spectrometer. Silica gel (200–300 mesh; Qingdao Marine Chemical Factory, China), MCI gel (CHP20, 75–150 μm; Mitsubishi Chemical Corporation, Japan), ODS (50 μm; YMC, Japan), and Sephadex LH-20 gel (Pharmacia Biotech, Sweden) were used for column chromatography. Thin-layer chromatography was carried out on GF254 plates (Qingdao Marine Chemical Factory). Preparative high-performance liquid chromatography (HPLC) was carried out using a Shimadzu LC-6AD (Shimadzu, Japan) instrument with a YMC-Pack ODS-A column (20 mm I.D. × 250 mm, S-5 μm, 12 nm) and an SPD-20A wavelength detector at 210 nm. HPLC solvents were obtained from Tedia Company, A 4.6 mm × 250 mm (5 μm) Daicel Chiralpak OD-H (Daicel, Japan) column was employed for the chiral HPLC preparations of **3a** and **3b**.

2.2. Plant materials

The herb of *S. moellendorffii* was purchased from Jingxi County. Guangxi Zhuang Autonomous Region in September 2016. The plant was identified by Professor Shao-Qing Tang (Guangxi Normal University), and voucher specimen (No. 20160138) was deposited at the School of Life Sciences, Guangxi University.

2.3. Extraction and isolation

The dried whole herb of *S. moellendorffii* (11.95 kg) was extracted four times with 95% EtOH. The EtOH extract was concentrated under vacuum to give a crude extract (810 g), which was suspended in distilled water, and then extracted with petroleum ether (7 × 3 L), EtOAc (7 × 3 L), and *n*-BuOH (7 × 3 L), respectively. The EtOAc -soluble fraction (232 g) was chromatographed on a silica gel column eluted with CH_2Cl_2/MeOH (30:1–1:1) to afford 24 fractions (F1–F24).

Fraction 4 (4.5477 g) was further divided into fifteen subfracions (4_1–4_{15}) by RP-C18 silica gel CC (4 × 60 cm, 400 g) using MeOH-H_2O (from 30% to 80%, then MeOH). $F4_1$ (563.2 mg) was further purified on preparative HPLC (25% MeCN in H_2O, 8 mL/min) to yield **4** (7.9 mg, t_R = 30.8 min), **5** (8.0 mg, t_R = 15.4 min), **6** (5.4 mg, t_R = 45.2 min). A Sephadex LH-20 column using MeOH (100%) was prepared for $F4_2$ (745.0 mg), which finally got two subfractions ($F4_{2-1}$, $F4_{2-2}$). $F4_{2-2}$ (56.2 mg) was further purified on preparative HPLC (27% MeCN in H_2O, 8 mL/min) to yield **1** (14.7 mg, t_R = 56.3 min), **7** (4.5 mg, t_R = 27.8 min), **8** (15.1 mg, t_R = 22.4 min), **3** (6.0 mg, t_R = 39.7 min), respectively. **3** were further separated over a Chiralpak OD-H column with 70% n-hexane-isopropyl alcohol (1.0 mL/min) to afford a pair of enantiomers (−)-**3** (t_R = 23.1 min, 1.0 mg) and (+)-**3** (t_R = 18.2 min, 1.0 mg) (Fig. S3.14, supporting information).

Fraction 5 (26.3766 g) was chromatographed over an MCI gel CC eluted with MeOH/H_2O (from 30% to 80%, then MeOH) to produce 17 subfractions ($F5_1$–5_{17}). $F5_2$ (187.0 mg) was depurated by preparative HPLC (45% MeCN in H_2O, 8 mL/min) to afford **10** (6.0 mg, t_R = 22.2 min) and **11** (5.5 mg, t_R = 34.0 min). A Sephadex LH-20 column using MeOH (100%) was prepared for $F5_3$ (950.0 mg), which finally got 7 subfractions ($F5_{3-1}$–$F5_{3-7}$). $F5_{3-7}$ (32.4 mg) was depurated by preparative HPLC (45% MeCN in H_2O, 8 mL/min) to afford **9** (6.8 mg, t_R = 35.7 min). $F5_5$ (55.2 mg) was depurated by preparative HPLC (45% MeCN in H_2O, 8 mL/min) to afford **14** (10.3 mg, t_R = 47.2 min).

Fraction 6 (9.5318 g) was chromatographed over an MCI gel CC eluted with MeOH/H_2O (from 30% to 80%, then MeOH) to produce 21 subfractions ($F6_1$–6_{21}). $F6_5$ was depurated by preparative HPLC (35% MeCN in H_2O, 8 mL/min) to afford **12** (11.0 mg, t_R = 30.1 min). A Sephadex LH-20 column using MeOH (100%) was prepared for $F6_{11}$ (120.0 mg), which finally afford 5 subfractions ($F6_{11-1}$–$F6_{11-5}$). $F6_{11-4}$ was depurated by preparative HPLC (25% MeCN in acid-H_2O, 8 mL/min) to afford **13** (8.4 mg, t_R = 33.2 min). $F6_{13}$ was depurated by preparative HPLC (45% MeCN in acid-H_2O, 8 mL/min) to afford **2** (14.2 mg, t_R = 29.8 min).

2.3.1. Selamoellennin B (1)

Yellowish powder; UV (MeOH) λ_{max} (log Ɛ) 204, 215, 258, 300 nm; IR νmax (KBr) 3432, 2930. 1616, 1460, 1211, 1112, 532, 455 cm^{-1}。 ^1H (400 MHz) and ^{13}C (100 MHz) NMR data in CD_3OD (Table 1); (+)HR-ESI-MS *m/z* 411.1412 $[M + Na]^+$ (calcd. for $C_{21}H_{24}O_7Na^+$, 411.1414).

2.3.2. Selamoellenin C (2)

White amorphous powder; $[a]_D^{20}$ –13.6 (c 0.985, MeOH); UV (MeOH) λ_{max} 202, 259, 305, 442, 485, 545, 656, 739, 759, 792. CD (MeOH) 220 nm (ΔƐ + 7.5); IR νmax (KBr) 3443, 2941, 1691, 1634, 1504, 1459, 1331, 1245, 1121, 974, 837, 546 cm^{-1}。 ^1H (400 MHz) and ^{13}C (100 MHz) NMR data in DMSO (Table 1); (+)HR-ESI-MS *m/z* 457.1464 $[M + Na]^+$ (calcd. for $C_{22}H_{26}O_9Na^+$, 457.1469).

2.3.3. Selamoellenin D (3)

Pale yellow oil; $[a]_D^{20}$ +7.0 (c 0.064, MeOH); The chiral HPLC separation of **3** revealed a pair of enantiomers, (−)-**3** and (+)-**3**, with a ration of 1 to 1.17. Optical rotations are $[a]_D^{20}$ –48.2 (c 0.062, MeOH) and $[a]_D^{20}$ +48.2 (c 0.062, MeOH), respectively. UV (MeOH) λ_{max} 206, 258, 283, 475, 485, 518, 581, 558, 713; IR vmax (KBr) 3408, 2938, 1613, 1461, 1325, 1213, 1115, 1041, 950,836,710, 650 cm^{-1}。 ^1H (500 MHz) and ^{13}C (125 MHz) NMR data in CD_3OD (Table 1); (−)HR-ESI-MS *m/z* 405.1566 ([M–H];calcd. for $C_{21}H_{25}O_8^-$, 405.1555).

2.4. Cytotoxicity assay

The antitumor activity of the target compounds were evaluated in four human cancer cell lines (HepG2, T24, MGC-803, A549). About 5×10^4 cells/mL of cells, which were in the logarithmic phase, were seeded in each well of 96-well plates and incubated for 12 h at 37 °C in 5% CO_2. Compounds at five different concentrations were then added to the test well and the cells were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. An enzyme labeling instrument was used to read absorbance with 570/630 nm double wavelength measurement. Cytotoxicity was examined on the percentage of cell survival compared with the negative control. The final IC_{50} values were calculated by the Bliss method (n = 5). All of the tests were repeated in triplicate.

2.5. Inhibition of matrix metalloproteinase activity assay

MMPs inhibition assays were carried out in 50 mM Tris/HCl buffer, pH 6.8, 10 mM $CaCl_2$ at 25 °C as described previously [20]. Assays were performed with a fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (13 μM) and human MMPs (nanomolar range concentration) from R&D Systems, except for human MMP-2, MMP-3, and MMP-9 described above. Substrate and enzyme concentrations were kept well below 10% substrate utilization to ensure evaluation of initial rates.