Supporting Information

Isolation and cytotoxic activity of selaginellin derivatives and biflavonoids from

*Selaginella tamariscina*

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General experimental procedures

NMR spectra were measured on a Bruker ARX-300 NMR spectrometer with tetramethylsilane (TMS) as the internal reference and chemical shifts are expressed in δ (ppm). HR-ESI-MS spectra were measured on a Bruker Daltonics MicrOTOFQ. IR spectra were recorded on a Bruker IFS-55 spectrophotometer. Purification was performed by Semiprep-HPLC with a Shimadzu SPD-10A apparatus equipped with UV detector under ODS column (i.d. 200 × 10 mm; Dikma Technologies Co., Ltd.). TLC was performed on silica gel GF\textsubscript{254} (10–40 μm; Qingdao Marine Chemical, Inc.). Column chromatography was performed on silica gel (100–200 or 200–300 mesh; Qingdao Marine Chemical, Inc.).

Extraction and isolation

The air-dried *S. tamariscina* (Beauv.) Spring (10.0 kg) plant was extracted three times under reflux with 50% EtOH. Evaporation of the solvent under reduced pressure concentrated the material to 1:1 (1 g herbs/mL) and gave a brown suspension. The suspension was centrifuged for 5000 rev/min (Rcf: 5180 g) to obtain the precipitant (100 g). The precipitant (100 g) was applied to silica gel column chromatography (100-200 mesh, 1000 g, 10×100 cm) with gradient elution [CHCl\textsubscript{3}-MeOH (50:1, 30:1, 20:1, 10:1, 1:1, 10L each)] to give five fractions 1-5. Fraction 4 (CHCl\textsubscript{3}-MeOH 10:1, 10.0 g) was further separated over silica gel column chromatography (200-300 mesh, 500 g, 4×100 cm) and the material was eluted using CHCl\textsubscript{3}-MeOH (30:1, 20:1, 10:1, 5:1, 3:1, 1:1, 1L each) to afford further six fractions (JB-1-01 to JB-1-06). Fraction
JB-1-03 (308 mg) was purified using silica gel column chromatography (200-300
mesh, 5 g, 1 × 65 cm) and eluted with CHCl$_3$-MeOH (20:1, 15:1, 10:1, 7:1, 5:1, 3:1,
1:1, 0.5 L each) to give seven fractions (JB-1-03-001 to JB-1-03-007). Fraction
JB-1-03-003 was compound 1 (24.2 mg). Fraction JB-1-03-004 was compound 3
(32.4 mg). Fraction JB-1-03-001 was purified by semipreparative HPLC (200 × 10
mm, MeOH/H$_2$O 75:25) to yield compounds 2 (2.4 mg, $t_R$: 57 min) and 4 (6.3 mg, $t_R$:
36 min). Fraction JB-1-03-002 was purified by semipreparative HPLC (200 × 10 mm,
MeOH/H$_2$O 62:38) to yield compound 5 (5.3 mg, $t_R$: 28 min). Fraction JB-1-01 (244
mg) was isolated over silica gel column chromatography (200-300 mesh, 5 g, 1 × 65
cm) and eluted with CHCl$_3$-MeOH (20:1, 15:1, 10:1, 7:1, 5:1, 3:1, 1:1, 0.5 L each).
The collected fractions (0.5 L each) were combined on the basis of their TLC profiles
to yield three fractions (JB-1-01-001 to JB-1-01-003). Fraction JB-1-01-001 was
compound 8 (4.2 mg). Fraction JB-1-01-003 was compound 7 (10.3 mg). Fraction
JB-1-01-002 was submitted to Sephadex LH-20 (6.5 × 140 cm eluted with
CHCl$_3$-MeOH 1:1, 0.4 L) to obtain compounds 6 (7.8 mg) and 9 (5.1 mg).

**Cytotoxicity testing**

*Cells and chemicals:* The cells were obtained from the Chinese Academy of Medical
Sciences. Fetal bovine serum (FBS) was obtained from Hao Yang Technology Co.
Ltd. RPMI 1640 medium, MTT and dimethylsulfoxide (DMSO) were purchased from
Sigma Chemical. Cisplatin (99.7%) was obtained from Jiutai Pharmaceutical Co. Ltd.
The purity (> 95%) of compounds 1-9 used for the biological assay *in vitro* was

determined by HPLC.

**MTT assay:** U251, HeLa, MCF-7 cells were cultured in RPMI-1640 medium at 37 °C in 5% CO₂. The media were supplemented with 10% (v/v) heat inactivated fetal bovine serum. The cells were routinely cultured in 96-well tissue culture microplates and the plates were subsequently inoculated with U251, HeLa and MCF-7 cells with 100 μL cultures per well. Samples were diluted in the culture medium at different concentrations and each concentration was repeated in three parallel wells. Samples were cultured at 37 °C in 5% CO₂ for three days. The cells were evaluated using the MTT assay [1] and following cultivation every well was injected with 15 μL of MTT (5 mg/mL) and then maintained for 4 h at 37 °C. The clear supernatant was removed and 150 μL of DMSO was injected into each well and lightly shaken. The drug concentration that achieved 50% inhibition was determined as IC₅₀ (μg/mL) and calculated from three independent assays.

References

For selaginellin M (1)

Figure 1S. $^1$H NMR spectrum of selaginellin M (1) in DMSO-$d_6$.
Figure 2S. $^{13}$C NMR spectrum of selaginellin M (1) in DMSO-$d_6$.
Figure 3S. ESI-TOF-MS spectrum of selaginellin M (1).
Figure 4S. HSQC spectrum of selaginellin M (1) in DMSO-$d_6$.
Figure 5S. HMBC spectrum of selaginellin M (1) in DMSO-$d_6$.

For selaginellin N (2)

Figure 6S. $^1$H NMR spectrum of selaginellin N (2) in DMSO-$d_6$.
Figure 7S. $^{13}$C NMR spectrum of selaginellin N (2) in DMSO-$d_6$.
Figure 8S. ESI-TOF-MS spectrum of selaginellin N (2).
Figure 9S. HSQC spectrum of selaginellin N (2) in DMSO-$d_6$.
Figure 10S. HMBC spectrum of selaginellin N (2) in DMSO-$d_6$. 

Figure 1S. $^1$H NMR spectrum of selaginellin M (1) in DMSO-$d_6$.

Figure 2S. $^{13}$C NMR spectrum of selaginellin M (1) in DMSO-$d_6$. 
Figure 3S. ESI-TOF-MS spectrum of selaginellin M (1).

Figure 4S. HSQC spectrum of selaginellin M (1) in DMSO-\textsubscript{d6}.
Figure 5S. HMBC spectrum of selaginellin M (1) in DMSO-d$_6$.

Figure 6S. $^1$H NMR spectrum of selaginellin N (2) in DMSO-d$_6$. 
Figure 7S. $^{13}$C NMR spectrum of selaginellin N (2) in DMSO-$d_6$.

Figure 8S. ESI-TOF-MS spectrum of selaginellin N (2).
Figure 9S. HSQC spectrum of selaginellin N (2) in DMSO-$d_6$.

Figure 10S. HMBC spectrum of selaginellin N (2) in DMSO-$d_6$. 