## Supporting Information to:

# Evaluation of Cytotoxic Activity of *Schisandra chinensis* Lignans

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## *S. chinensis* Lignans Separation

*Analytical HPLC*: Agilent 1100 UV/VIS DAD (Agilent), Supelcosil ABZ+Plus (15 cm × 4.6 mm, 3 μm; Supelco), gradient elution ACN:$H_2O$, starting from 30:70 (v/v) to 100:0 (v/v) in 20. min. Flow rate 1 mL.min$^{-1}$, 40 °C. Detection λ= 254 nm.
*Preparative HPLC*: LCP 4100 (Ecom), manual injection 500 μL, Supelcosil ABZ+Plus (25 cm × 21.2 mm, 5 μm; Supelco). Gradient of MeCN:$H_2O$, flow rate 20 mL.min$^{-1}$, 35 °C, detection λ= 254 nm.
*Semipreparative HPLC*: Agilent 1100 UV/VIS DAD (Agilent), Supelcosil ABZ+Plus (25 cm × 10 mm, 5 μm; Supelco). Flow rate 5 mL.min$^{-1}$, 40 °C. Mobile phase composition, gradient elution: fraction C - gradient of ACN:$H_2O$ (v/v): 0. min 50:50, 4. min 60:40, 15. min 70:30, 16. min 80:20, 25. min 80:20; fraction D: gradient of ACN:$H_2O$ (v/v): 0. min 55:45, 23. min 65:35; fraction E: gradient of MeOH:ACN:$H_2O$ (v/v): 0. min 25:35:40, 19. min 15:50:35; fraction H: gradient of ACN:$H_2O$ (v/v): 0. min 40:60, 20. min 90:10; fraction I: gradient of MeOH:ACN:$H_2O$ (v/v): 0. min 50:50, 11. min 100:0.

## Cytotoxic activity assay on BY-2 cells

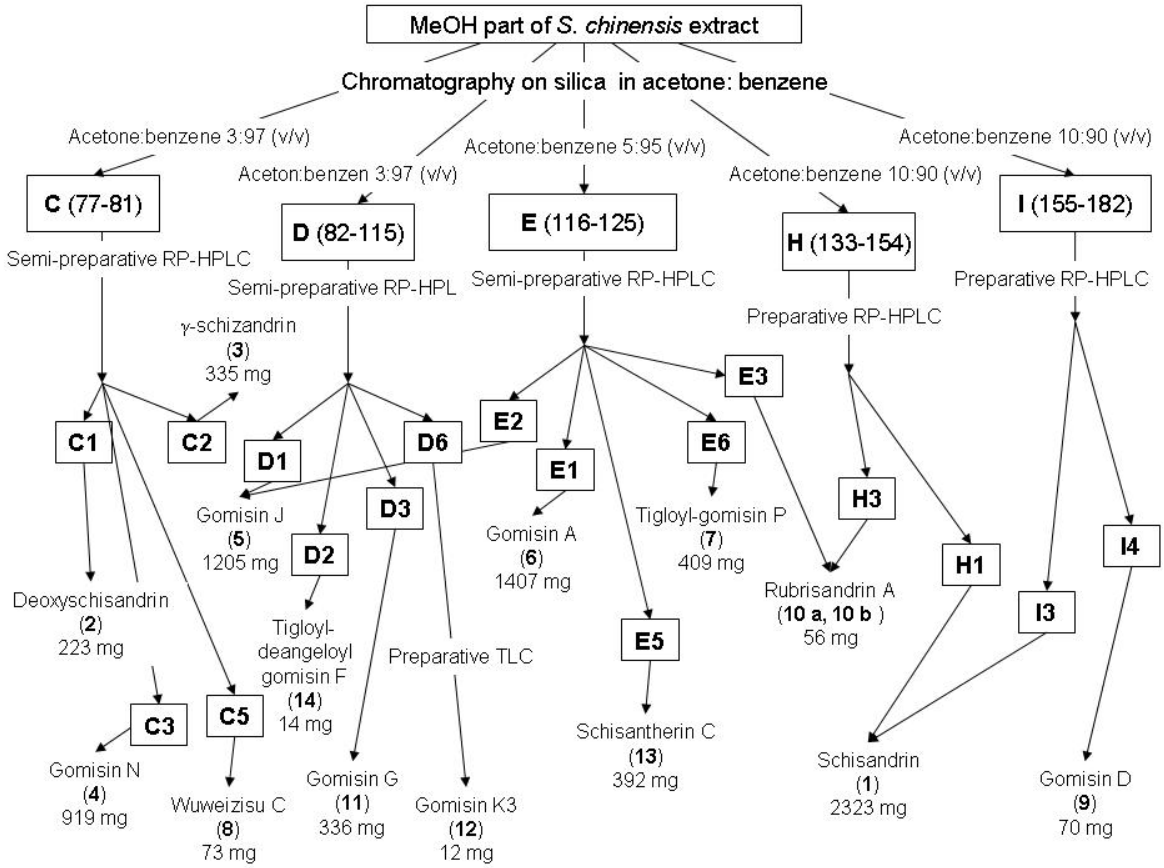
The assay was carried out on a *Nicotiana tabaccum BY-2* cell line originally developed by Nagata et al. cultivated in Murashihe and Skoog cultivation medium [13] as modified by Nagata [14]. All the compounds were tested at 250 μM concentrations. Briefly: selected lignans (250 μM) without/with camptothecin (Sigma; 95 % purity according to HPLC, 50 μM) in the form of DMSO stock solutions were added to culture in exponential growth phase. Each concentration was cultivated in three independent series on an orbital shaker at 27±1 °C and 130 rpm in the dark in 100 mL Erlenmeyer flasks. The samples were collected in strictly defined time intervals in sterile conditions and evaluated using methods of fluorescence microscopy. Cell viability was determined using double staining with propidium iodide (PI) and fluorescein diacetate (FDA). Sample (20 μL) of cell suspension culture was adjusted to 50 μL with cultivation medium and incubated at room temperature with FDA (2.4 μmol.L$^{-1}$) and PI (30 μmol.L$^{-1}$). The percentage of viable and dead cells was determined by counting using a fluorescent microscope (Olympus AX 70) equipped with broad-spectrum excitation. From each set, 10 random fields were evaluated. Nuclear architecture and mitotic changes were determined using fluorescent probe Hoechst 33258 (1.8 μmol.L$^{-1}$). Cells were fixed

using PEM buffer containing formaldehyde (4 %, w/w) to avoid the creation of artifacts. One thousand nuclei of each preparation were evaluated using a fluorescent microscope (Olympus AX 70) equipped with broad-spectrum excitation; nuclear and apoptotic, as well as mitotic changes were expressed as a percentage of total cells. For apoptosis determination, a combination of Hoechst 33342 (9.3 $\mu mol.L^{-1}$) and PI (2.4 $\mu mol.L^{-1}$) was used. Cells in a volume of 1 mL were treated at room temperature for 30 min with Hoechst 33342 and PI. The number of apoptotic cells was evaluated using a fluorescent microscope (Olympus AX 70) equipped with broad-spectrum excitation.

## Antiproliferative activity assay on LoVo cells

Carcinoma cells were seeded ($1\times10^4$ cells/mL) in 96-well tissue plates for 24 h, and then treated with various concentrations of lignans for 24, 48 and 72 h. Cellular growth was evaluated by the MTT reduction assay. Podophyllotoxin (Sigma, 95 %) was used as a control. A solution of MTT (5 mg/mL in PBS) was added to each well and incubated for another 4 h at 37 °C. The resulting MTT-formazan product was dissolved by the addition of 150 µL of DMSO. Absorbance was measured with a microplate reader (Titertek Multiscan) at 570 nm and at a reference wavelength of 630 nm. For each assay, five different experiments were performed in triplicate. The results were statistically evaluated by Student's t-test.

**Scheme 1S**


MeOH part of S. chinensis extract
Chromatography on silica in acetone: benzene
Acetone:benzene 3:97 (v/v)
Aceton:benzen 3:97 (v/v)
Acetone:benzene 5:95 (v/v)
Acetone:benzene 10:90 (v/v)
Acetone:benzene 10:90 (v/v)
C (77-81)
D (82-115)
E (116-125)
H (133-154)
I (155-182)
Semi-preparative RP-HPLC
Semi-preparative RP-HPL
Semi-preparative RP-HPLC
Preparative RP-HPLC
Preparative RP-HPLC
γ-schizandrin
(3)
335 mg
C1
C2
D1
D6
E2
E3
E1
E6
H3
H1
I4
I3
D3
D2
E5
Gomisin J
(5)
1205 mg
Gomisin A
(6)
1407 mg
Tigloyl-gomisin P
(7)
409 mg
Rubrisandrin A
(10 a, 10 b )
56 mg
Deoxyschisandrin
(2)
223 mg
Tigloyl-
deangeloyl
gomisin F
(14)
14 mg
Preparative TLC
C3
C5
Schisantherin C
(13)
392 mg
Gomisin N
(4)
919 mg
Wuweizisu C
(8)
73 mg
Gomisin G
(11)
336 mg
Gomisin K3
(12)
12 mg
Schisandrin
(1)
2323 mg
Gomisin D
(9)
70 mg