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Dibenzocyclooctadiene lignans from the fruits of *Schisandra chinensis* and their cytotoxicity on human cancer cell lines

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Abstract

Repeated chromatographic separations of the EtOAc fraction of *Schisandra chinensis* fruits on silica gel, octadecyl silica gel, and Sephadex LH-20 led to the isolation and identification of seven dibenzocyclooctadiene lignans (**1**–**7**). The NMR data reported in the literature for angeloyl gomisin H (**5**) were shown to be incorrect. We unambiguously identified the compounds based on detailed analysis of the 1D and 2D NMR data, especially from HMBC and NOESY experiments. In addition, MTT assays and cell viability experiments verified the cytotoxicity of the isolated dibenzocyclooctadiene lignans against the human cancer cell lines AGS, HeLa, and HT-29.

Keywords: Angeloyl gomisin H, Cytotoxicity, Dibenzocyclooctadiene lignan, Human cancer cells, *Schisandra chinensis*

Introduction

Schisandra chinensis is a deciduous woody vine native to Far East Asia. The fruits, red berries called "Omija" in South Korea, have been used as food as well as a traditional medicine with hepaprotective, cardiovascular, and antibacterial benefits [1, 2]. The Korean word Omija, meaning "five flavors" (sweet, sour, bitter, salty, and spicy), indicates that the fruit has a variety of components that exhibit pharmacological effects. To date, phytochemical studies of *S. chinensis* fruits have led to the isolation of lignans, triterpenoids, monoterpenes, sesquiterpenes, organic acids, and sterols, [3, 4] among which dibenzocyclooctadiene lignans are overwhelmingly the major components [5]. Among its major components, the NMR data reported in the literature for angeloyl gomisin H (**5**) were identified to be a little bit incorrect. Therefore, we unambiguously identified the compound **5** to correct its NMR value based on detail NMR analysis technics especially gHMBC and NOESY. Also, *S. chinensis* extracts and some dibenzocyclooctadiene lignans from this plant have been reported to be cytotoxic to certain cancer cell lines [6–9]. Despites their significant anti-cancer effects, there has been no report concerning their chemical structures relationship between their cytotoxic activity against various cancer cells.

This paper describes the isolation for seven dibenzocyclooctadiene lignans from the fruits of *S. chinensis*, structure determination of the isolation ones, especially angeloyl gomisin H (**5**). In addition, their cytotoxicities were evaluated against several human cancer cell lines (AGS, HeLa, and HT-29), and the relationship of their structure to their activity.

Materials and methods

General experimental procedures

The instruments and chemicals used in these experiments were prepared according to previous studies [10–12].

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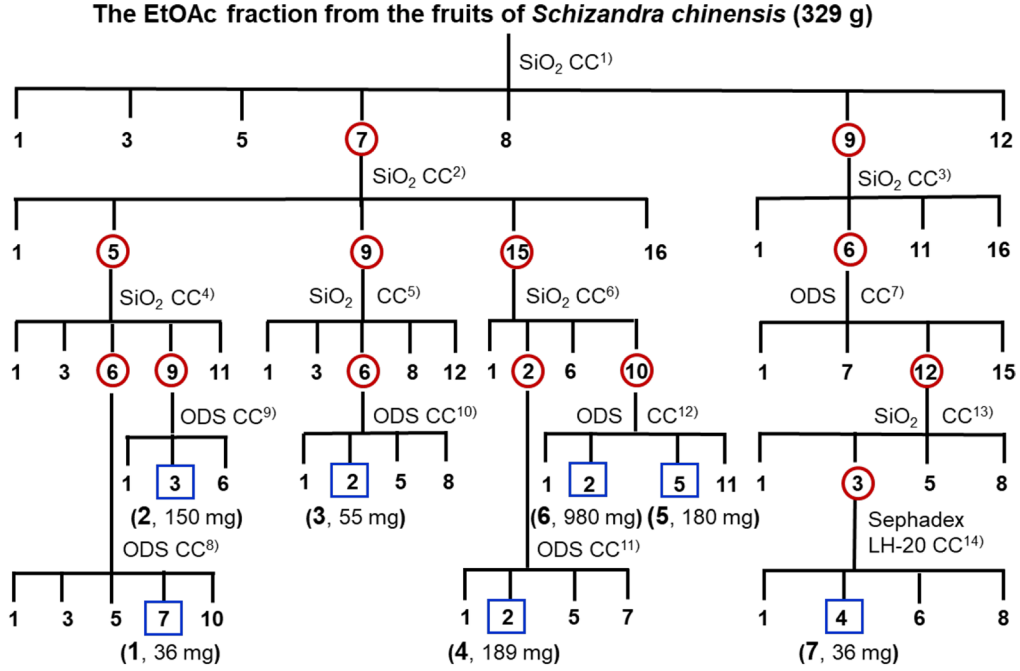
Plant materials

Schisandra chinensis fruits were provided by RDA, Eumseong, Korea, in 2019 and identified by Prof. Dae-Keun Kim, Woo Suk Univ., Jeonju, Korea. A voucher specimen (KHU-NPCL-201904) has been stored in Prof Nam-In Baek's Laboratory.

Extraction and isolation of dibenzocyclooctadiene lignans

The dried fruits of *S. chinensis* (5.4 kg) were soaked in 70% aqueous EtOH (54 L × 3) at room temperature for 24 h. After filtration, the extract was concentrated to afford 1.3 kg of crude material. The obtained concentrate was suspended in H_2O (4.2 L) and sequentially



1) 329 g, Ø 11 x 16cm / *n*-hexane:EtOAc=15:1→10:1→5:1→1:1 (7.8 L of each)

2) 36 g, Ø 7.5 x 17cm / *n*-hexane:EtOAc=5:1 (23.8 L)

3) 27 g, Ø 7.5 x 16cm / *n*-hexane:EtOAc=3:1→2:1→1:1 (5.3 L of each)

4) 3.1 g, Ø 4 x 21 cm / $CHCl_3$:EtOAc=20:1→15:1 (3.3 L)

5) 3.8 g, Ø 4.5 x 17 cm / $CHCl_3$:EtOAc=20:1→15:1→7:1 (2.5 L of each)

6) 6.4 g, Ø 7 x 17 cm / $CHCl_3$:EtOAc=10:1→5:1→1:1 (5.3 L of each)

7) 1.6 g, Ø 5.5 x 9 cm / acetone:H_2O=1:1 (1.3 L)

8) 200 mg, Ø 2.5 x 7 cm / acetone:H_2O=3:2 (600 mL)

9) 380 mg, Ø 3 x 6 cm / acetone:H_2O=1:1 (700 mL)

10) 385 mg, Ø 3 x 10 cm / acetone:H_2O=1:1 (800 mL)

11) 280 mg, Ø 3 x 10 cm / acetone:H_2O=1:1 (900 mL)

12) 1.2 g, Ø 3 x 10 cm / acetone:H_2O=1:1 (1,000 mL)

13) 788 mg, Ø 3 x 16 cm / *n*-Hexane:EtOAc=3:1 (2,000 mL)

14) 112 mg, Ø 1.5 x 55 cm / MeOH (300 mL)

Fig. 1 Isolation of dibenzocyclooctadiene lignans from *Schisandra chinensis* fruit. SiO_2: silica gel; ODS: octadecyl-silica gel; CC: column chromatography

washed with EtOAc (4.2 L × 3) and *n*-BuOH (3.4 L × 3). The partitioned extracts were concentrated to obtain the EtOAc (SCE, 329 g), *n*-BuOH (SCB, 247 g), and H_2O (SCW, 723 g) fractions. SCE (329 g) was applied to a SiO_2 column chromatography (c.c.) (Fig. 1), and the eluate was monitored using TLC and separated into 12 (SCE-1–SCE-12) fractions. Subsequent c.c. separations of fractions 7 (SCE-7) and 9 (SCE-9) using SiO_2, ODS, and Sephadex LH-20 were carried out (Fig. 1) to yield seven purified lignans, **1**–**7**.

Schisandrin A (**1**): colorless solid; $[\alpha]_D^{25}$ +128° (*c* 0.8, MeOH); IR (LiF plate, *ν*) 2947, 1651, 1459 cm^{-1}; EIMS *m*/*z* 416 $[M]^+$; ^1H and ^{13}C NMR spectroscopic data, see Additional file 1: Table S1.

(-)-Gomisin K1 (**2**): colorless solid, $[\alpha]_D^{25}$ −98° (*c* 0.2, $CHCl_3$); IR (LiF plate, *ν*) 3420, 2930, 1582, 1496 cm^{-1}; EIMS *m*/*z* 402 $[M]^+$; ^1H and ^{13}C NMR spectroscopic data, see Additional file 1: Table S2.

Gomisin J (**3**): colorless solid; $[\alpha]_D^{25}$ −38° (*c* 0.6, acetone); IR (LiF plate, *ν*) 3426, 2920, 1583, 1458 cm^{-1}; EIMS *m*/*z* 388 $[M]^+$; ^1H and ^{13}C NMR spectroscopic data, see Additional file 1: Table S3.

Gomisin A (**4**): colorless solid; $[\alpha]_D^{25}$ +71° (*c* 0.7, $CHCl_3$); IR (LiF plate, *ν*) 3332, 2947, 1647 cm^{-1}; EIMS *m*/*z* 416 $[M]^+$; ^1H and ^{13}C NMR spectroscopic data, see Additional file 1: Table S4.

Angeloyl gomisin H (**5**): colorless solid, $[\alpha]_D^{25}$ +17° (*c* 1.1, $CHCl_3$); IR (LiF plate, *ν*) 2953, 1733, 1596, 1457 cm^{-1}; EIMS *m*/*z* 500 $[M]^+$; ^1H and ^{13}C NMR spectroscopic data, see Table 1.

Schisandrin (**6**): colorless solid; $[\alpha]_D^{25}$ +92° (*c* 0.8, $CHCl_3$); IR (LiF plate, *ν*) 3420, 2934, 1594, 1456 cm^{-1}; EIMS *m*/*z* 432 $[M]^+$; ^1H and ^{13}C NMR spectroscopic data, see Additional file 1: Table S5.

Gomisin C (**7**): colorless solid; $[\alpha]_D^{25}$ −132° (*c* 0.6, $CHCl_3$); IR (LiF plate, *ν*) 3502, 2923, 1720, 1595 cm^{-1}; EIMS *m*/*z* 536 $[M]^+$; ^1H and ^{13}C NMR spectroscopic data, see Additional file 1: Table S6.

Cell viability assay

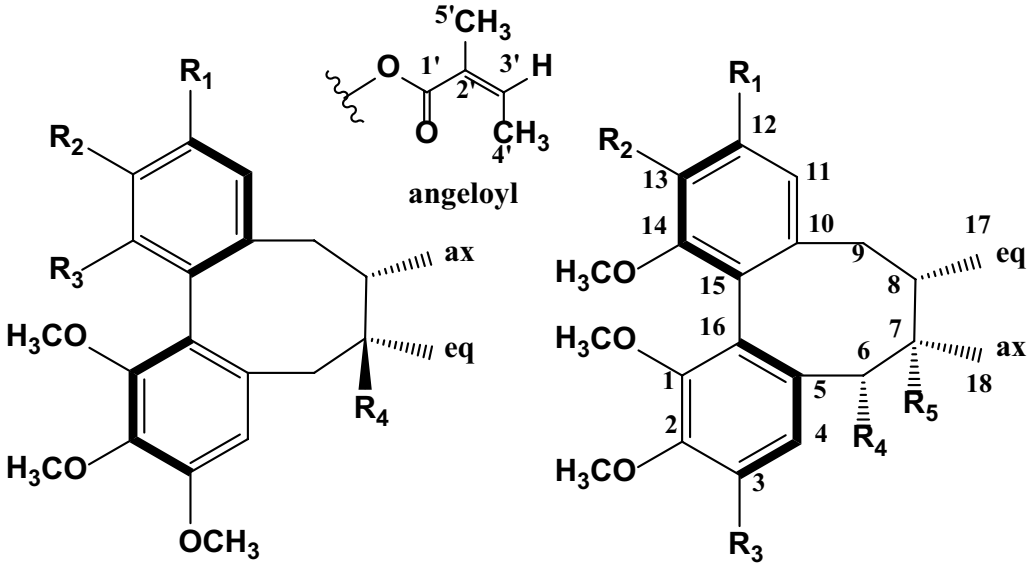
Cell reasonability was dictated by MTT measure as recently portrayed [13]. Cells were seeded at a thickness of 1×10^3 cells/well in a 96-well plate and refined with sans serum DMEM or RPMI-1640 for 16 h. At that point, the cells were treated with sequential groupings of Angeloyl gomisin H, Gomisin A, Gomisin C, Gomisin J, (-)- Gomisin K1, Schisandrin, in different concentration (10, 25, 50 μg/mL) for 24 h. Treatment at every fixation was acted in triplicate. After medicines, the medium was suctioned and cells were washed with PBS. Cells were in this manner hatched with MTT arrangement (5 mg/mL) for 6 h. The supernatant was expelled, and formazan was solubilized in isopropanol and estimated spectrophotometrically at 570 nm. The level of practical cells was assessed in examination with untreated cells. The information shows the mean ± SD of at least three free trials.

Hoechst 33258 and propidium iodide staining

All the cells were seeded onto amplifying instrument coverslip in a 6-well plate until further notice and were treated with IC_{50} union of ginger blends. In the wake of washing twice with PBS, cells were fixed with 4% paraformaldehyde for 15 min. The joined cells were recolored with 500 μL Hoechst 33258 (5 μg/mL) plan and 500 μL propidium iodide (PI, 5 μg/mL) course of action at room temperature for 30 min, exclusively. Apoptotic cells with combined and partitioned centers were overviewed using

Table 1 ^1H and ^{13}C NMR data of angeloyl gomisin H (5) (600 MHz, $CDCl_3$)

No of C	δ_H	δ_C
1		151.70
2		140.31
3		152.57
4	6.52, 1H, s	110.17
5		133.15
6	2.70, 1H, d, *J* = 13.8 Hz 2.30, 1H, d, *J* = 13.8 Hz	40.69
7		72.05
8	1.83, 1H, m	41.94
9	2.67, 1H, br. d, *J* = 13.8 Hz 2.37, 1H, dd, *J* = 13.8, 7.2 Hz	34.29
10		133.91
11	6.66, 1H, s	112.77
12		151.78
13		139.66
14		142.27
15		123.22
16		122.85
17	0.81, 3H, d, *J* = 7.2 Hz	15.90
18	1.20, 3H, s	29.89
1-OMe	3.50, 3H, s	60.63
2-OMe	3.79, 3H, s	60.79
3-OMe	3.83, 3H, s	56.04
12-OMe	3.86, 3H, s	56.04
13-OMe	3.79, 3H, s	60.79
Angeloyl-1′		165.89
Angeloyl-2′		127.63
Angeloyl-3′	5.85, 1H, q, *J* = 7.2 Hz	137.38
Angeloyl-4′	1.72, 3H, d, *J* = 7.2 Hz	15.31
Angeloyl-5′	1.71, 3H, s	20.33



schisandrin A (1):
R_1,R_2,R_3=OCH_3, R_4=H

(-)-gomisin K1 (2):
R_1=OH, R_2, R_3=OCH_3, R_4,R_5=H

gomisin A (4):
R_1,R_2=dioxymethylene, R_3=OCH_3, R_4=OH

gomisin J (3):
R_1,R_3=OH, R_2=OCH_3, R_4,R_5=H

angeloyl gomisin H (5):
R_1,R_2=OCH_3, R_3=angeloyl, R_4=OH

gomisin C (7):
R_1,R_2=dioxymethylene, R_3=OCH_3, R_4=benzoyl, R_5=OH

schisandrin (6):
R_1,R_2,R_3=OCH_3, R_4=OH

Fig. 2 Chemical structures of dibenzocyclooctadiene lignans from *Schisandra chinensis* fruit

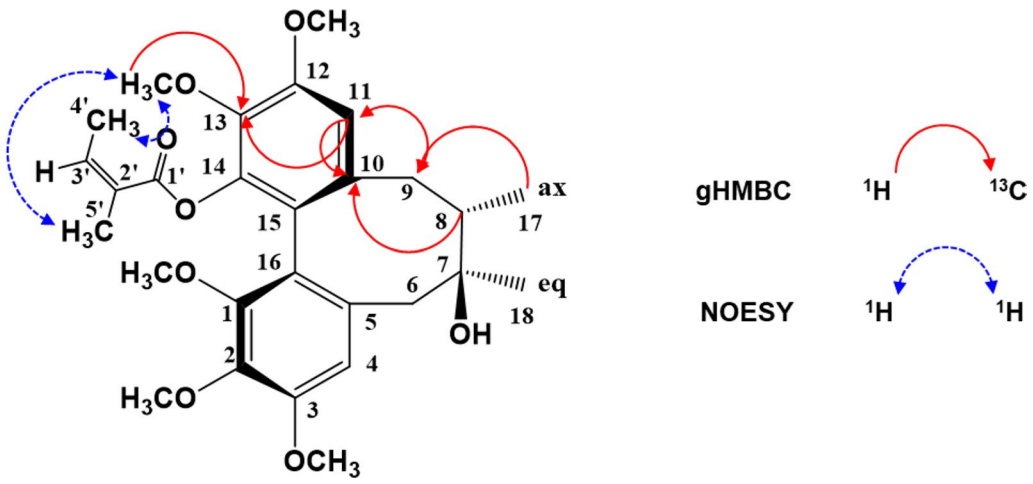


Fig. 3 Selected key gHMBC and NOESY correlations in angeloyl gomisin H (**5**)

a Leica DMLB fluorescence amplifying focal point (Wetzlar, Germany) [14, 15].

Results and discussion

Dried *S. chinensis* fruits were extracted with aqueous EtOH, and the concentrated extract was partitioned into EtOAc, *n*-BuOH, and H_2O. Repeated separations of the EtOAc fraction by SiO_2, ODS, and Sephadex LH-20 c.c. led to the isolation of dibenzocyclooctadiene-type lignans **1**–**7,** identified as schisandrin A (**1**), [16] (-)-gomisin K1 (**2**), [17] gomisin J (**3**), [6] gomisin A (**4**), [6, 17] angeloyl gomisin H (**5**), [2, 17, 18] schisandrin (**6**), [6, 19, 20] and gomisin C (**7**) [17] through detailed analysis of the spectroscopic data from 1D and 2D NMR and IR spectroscopy, FAB/MS, and specific rotation data as well as comparison with the data in the literature (Fig. 2). Of the seven compounds, The NMR data reported in the literatures for angeloyl gomisin H (**5**) were identified to be a little bit incorrect [2, 4, 13, 17, 18].

Compound **5**, a colorless powder, showed UV absorptions at 254 and 365 nm and developed a yellowish color after spraying with 10% H_2SO_4 and heating. Its IR spectrum suggested the presence of carbonyl (1733 cm^{-1}) and conjugated double bonds (1596 and 1457 cm^{-1}). Its molecular weight was determined to be 500 Da from the molecular ion peak *m*/*z* 500 $[M]^+$ in the EIMS. The ^1H NMR spectrum (Table 1) exhibited signals typical of a dibenzocyclooctadiene lignan moiety, two aromatic methines (δ_H 6.66, 1H, s, H-11; δ_H 6.52, 1H, s, H-4), two methylenes with germinal coupling (δ_H 2.70, 1H, d, J=13.8 Hz, H-6a; δ_H 2.30, 1H, d, J=13.8 Hz, H-6b; δ_H 2.67, 1H, br. d, J=13.8 Hz, H-9a; δ_H 2.37, 1H, dd, J=13.8, 7.2 Hz, H-9b), one methine (δ_H 1.83, 1H, m, H-8), two methyl groups (δ_H 1.20, 3H, s, H-18; δ_H 0.81, 3H, d, J=7.2 Hz, H-17), and five methoxy groups (δ_H 3.50, 3H, s, H-1-OMe; δ_H 3.79, 3H, s, H-2-OMe; δ_H 3.83, 3H, s, H-3-OMe; δ_H 3.86, 3H, s, H-12-OMe; δ_H 3.79, 3H, s, H-13-OMe). In addition, one olefin methine (δ_H 5.85, 1H, q, J=7.2 Hz, H-angeloyl-1') and two allylic methyl groups (δ_H 1.72, 3H, d, J=7.2 Hz, H-angeloyl-4'; δ_H 1.71, 3H, s, H-angeloyl-5′) were observed, indicating a 2-methyl-but-2-enoyl substituent [17, 18, 21]. The chemical shifts and coupling patterns confirmed that the organic acid moiety was not tiglic acid but angelic acid because the chemical shifts of the olefin methine proton signal for tiglic acid with the *Z*-configuration and angelic acid with the *E*-configuration are δ_H 6.78 and δ_H 5.88, respectively, and the two methyl carbon signals were also consistent with this assignment (angeloyl, δ_C 21, 16; tigloyl, δ_C 12, 14) [17, 18, 21]. methoxy proton signals for methoxy groups at C-1 or C-14 are usually observed more upfield, δ_H 3.50, compared to those at C-2, C-3, C-12, and C-13, δ_H 3.79 to 3.86 [21]. Therefore, four of the methoxy groups were confirmed to be located at C-2, C-3, C-12, and C-13 and another was present at C-1 or C-14. The position of the hydroxyl group on the cyclooctane ring was determined to be C-7 because CH_3-17 was observed as a doublet, while CH_3-18 was a singlet. Taken together, these results suggest that compound **5** is a dibenzocyclooctadiene lignan with four methoxy groups at C-2, C-3, C-12, and C-13; a hydroxyl and an angeloyl group at C-1 and C-14; and another hydroxyl group at C-7. The two benzene rings are positioned in an *R*-biphenyl configuration [5] (Fig. 2). The ^{13}C NMR spectrum also showed signals indicative of a dibenzocyclooctadiene derivative with a hydroxyl group at C-7, six oxygenated aromatic quaternary carbons (δ_C 152.57, C-3; δ_C 151.78, C-12; δ_C 151.70, C-1; δ_C 142.27, C-14; δ_C 140.31, C-2; δ_C 139.66, C-13), four aromatic quaternary carbons (δ_C 133.91, C-10; δ_C 133.15, C-5; δ_C 123.22, C-15; δ_C 122.85, C-4), two aromatic methines (δ_C 112.77, C-12; δ_C 110.17, C-4), one oxygenated quaternary carbon (δ_C 72.05, C-7), one methine (δ_C 41.94, C-8), two methylenes (δ_C 40.94, C-6; δ_C 34.29, C-9), two methyl carbons (δ_C 29.89, C-18; δ_C 15.90, C-17), and five methoxy carbons (δ_C 60.63, C-1-OMe; δ_C 60.79, C-2-OMe; δ_C 56.04, C-3-OMe; δ_C 56.04, C-12-OMe; δ_C 60.79, C-13-OMe). In addition, signals indicative of an angeloyl moiety, namely, one ester (δ_C 165.89, C-angeloyl-1′), one olefinic quaternary carbon (δ_C 127.63, C-angeloyl-2′), one olefinic methine (δ_C 137.38, C-angeloyl-3′), and two methyl groups (δ_C 20.33, C-angeloyl-5′; δ_C 15.31, C-C-angeloyl-4′), were observed. However, the position of the angeloyl moiety remains undefined. The HMBC experiment offers no solution because it provides long-range correlations via J^2 or J^3, in general. The NOESY experiment alternatively provided proof that the organic acid was on C-1 or C-14. The two allylic methyl proton signals (δ_H 1.72, H-angeloyl-4′; δ_H

Table 2 Inhibitory effects of dibenzocyclooctadiene lignans (1–7) from *Schisandra chinensis* fruit on the growth of AGS, HeLa, and HT29 cells

Compound	IC50 (μM)			
	AGS	Hela	HT29	Raw264.7
1	–	–	–	4.43 ± 0.24
2	–	5.46 ± 0.24	–	7.03 ± 0.22
3	–	6.51 ± 0.26	–	6.54 ± 0.14
4	14.81 ± 1.02	13.76 ± 0.38	–	17.51 ± 0.64
5	12.94 ± 0.12	9.36 ± 0.39	7.94 ± 0.19	15.18 ± 0.91
6	–	–	–	15.42 ± 0.59
7	–	–	–	3.22 ± 0.09

AGS: human stomach adenocarcinoma cells; Hela: human cervical cancer cells; HT29: human colon cancer cells; Raw264.7: Murine macrophage cells

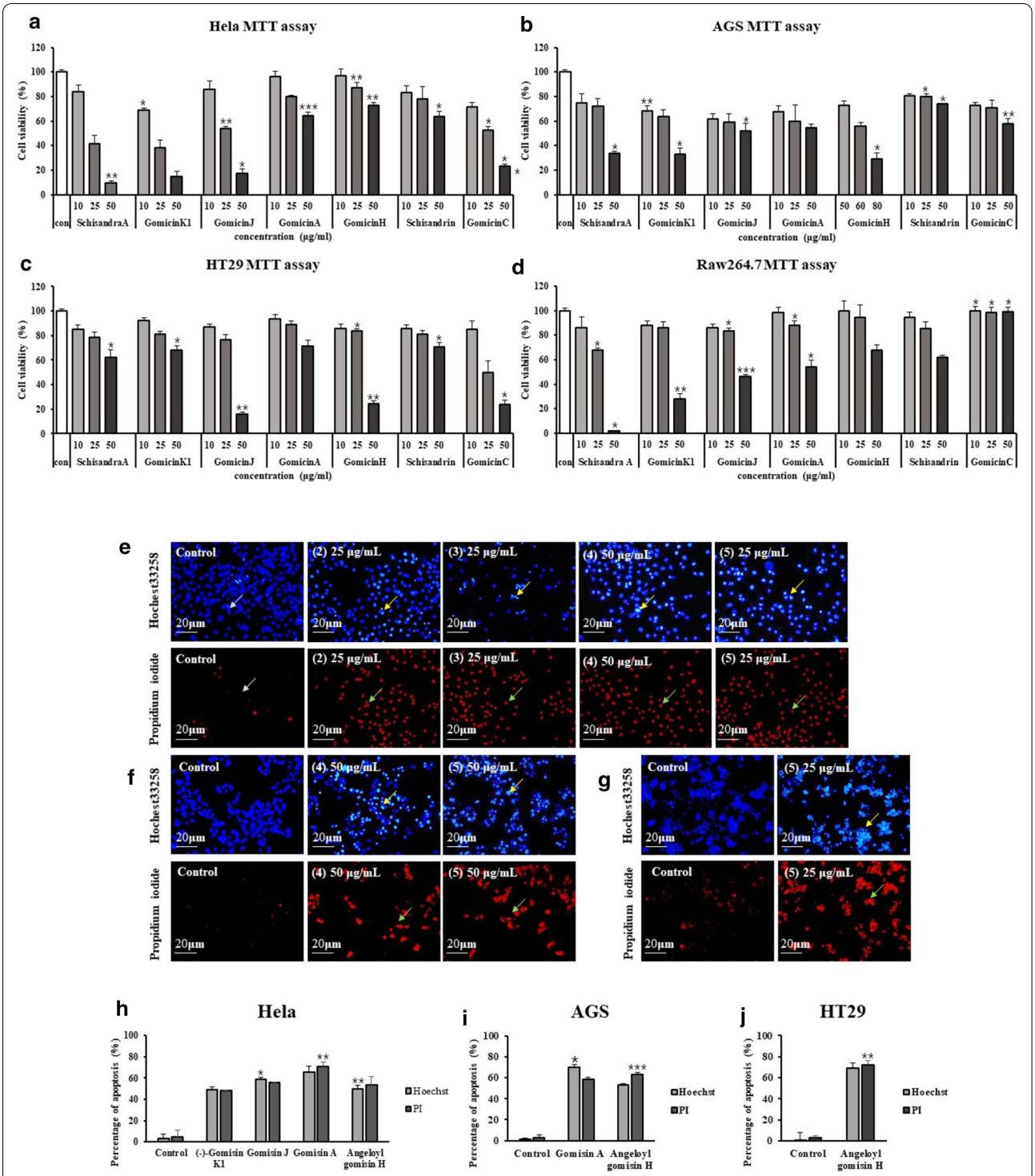


Fig. 4 Cell viability of Hela, AGS, HT29, Raw264.7 cells and cancer cells staining after treatment with different concentration of compounds for 24 h. Cell viability was measured by MTT assay **a** Hela Cell, **b** AGS Cell, **c** HT29, **d** Raw264.7. Effect of dibenzocyclooctadiene lignans from *Schisandra chinensis* fruit on the activation of apoptosis in HeLa (**e**), AGS (**f**), and HT-29 (**g**) cells. Hoechst staining was used to detect nuclear condensation of treated and untreated cells. Propidium iodide (PI) staining was used to measure the cell death caused by treatment with the compounds. The white arrows show the unstained cell, the yellow and green arrows show the necrotic and apoptotic cells. The percentage of apoptosis were showed in **h**, **i** and **j**

1.71, H-angeloyl-5′) of the angeloyl moiety showed NOE correlations with the methoxy proton signal (δ_H 3.79, H-13-OMe), which was already identified as OCH_3-13 from the cross peak between H-11 (δ_H 6.66) and C-13 (δ_C 151.78) in the HMBC spectrum. Ultimately, compound **5** was identified as angeloyl gomisin H (Fig. 3), and all the NMR data were unequivocally assigned.

Schisandra chinensis extract and major components from this plant are previously reported to have an anticancer activity including cytotoxicity on human cancer cell lines [22]. Especially almost compounds which have dibenzocyclooctadiene-type lignans structure, deoxyschisandrin, gomisin A, and *γ*-schisandrin, have significant anti-cancer effect [23–26]. However, there are no reports for the cytotoxicity of dibenzocyclooctadiene-type lignans on gastric (AGS), cervical (HeLa), and colon (HT-29) human cancer cells. Therefore, we evaluated the dibenzocyclooctadiene lignans (**1**–**7**) from *S. chinensis* fruits for the cytotoxicity of the human cancer cells using MTT assay.

Accordingly, to investigate the cytotoxic effects of lignans **1**–**7**, we examined their effects on the viability of AGS, HeLa, HT-29, and RAW 264.7 cells (Table 2 and Fig. 4). The lignans were screened for their cytotoxic effects on RAW 264.7 cells at various concentrations ranging from 10 to 100 μg/mL for 24 h using an MTT assay. Results of MTT assay are presented as the mean ± standard deviation of three independent experiments. The MTT assay showed that compounds **2**–**5**, especially angeloyl gomisin H (**5**), concentration-dependently suppressed the proliferation and viability against three cancer cells. Even though IC_{50} in AGS (22.01 ± 1.87 μM), HeLa (32.68 ± 2.21 μM), and HT29 (156.04 ± 6.71 μM) cells were low relative to those of the well-known and clinically used anticancer compound doxorubicin's IC_{50} value (AGS, 0.25 μM; HeLa, 1.45 ± 0.15 or 3.7 ± 0.3 μM; HT29, 11.39 or 0.75 μM), [27–31] the IC_{50} values are very high in comparison to naturally occurring compounds (baicalein on AGS, 85 μM; galactosyl diglyceride on AGS, 49–83 μM; clausenidin on HT29, 42 μM; quercetin on HT29, 75 μM) [30–33].

Compounds **4**–**6**, which have a hydroxy group at C-7, showed relatively weak toxicity on RAW 264.7 cells compared with lignans without a hydroxyl group at C-7. In comparison, compounds **2**, **3**, and **7**, which have *S*-biphenyl positions, and compound **1**, in which all the hydroxy groups in the benzene ring are substituted by methoxy groups, showed relatively strong toxicity toward RAW 264.7 cells. Additionally, compounds **2** and **3**, which have *S*-biphenyl positions and one or two hydroxyl groups on the benzene ring, showed significant inhibition of HeLa cells. Compounds **4** and **5**, which have relatively low toxicity toward normal cells, suppressed the proliferation and viability of AGS and HeLa cells. In particular, compound **5**, with its angeloyl moiety, exhibited a slightly stronger effect on AGS, HeLa, and HT29 cells than compound **4**. These results indicate that the stereochemistry, the presence of an angeloyl or a hydroxy group at C-7, and the benzene ring could be key factors of dibenzocyclootadiene-type lignans affecting the cytotoxicity against AGS, HeLa, and HT29 cells.

After treatment of the compounds, the cell nuclei were stained with Hoechst 33258 and PI were observed by fluorescence microscopy, respectively. The treated cells exhibited apoptotic morphology, such as cell shrinkage with DNA condensation, high fluorescence, and formation of the apoptotic body. And the IC_{50} value represents the concentration of each compound that inhibits cell activity by 50% (Table 2).

In conclusion, our data reveal that dibenzocyclooctadiene lignans **2**–**5** from *S. chinensis* fruits can be effective candidates as anticancer materials for stomach, cervical, and colon cancers.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13765-020-00524-y.

Additional file 1: Table S1. ^{1}H- and ^{13}C-NMR data of schisandrin A (**1**) (600 MHz, $CDCl_3$). **Table S2.** ^{1}H- and ^{13}C-NMR data of (-)-gomisin K1 (**2**) (600 MHz, $CDCl_3$). **Table S3.** ^{1}H- and ^{13}C-NMR data of gomisin J (**3**) (600 MHz, $CDCl_3$). **Table S4.** ^{1}H- and ^{13}C-NMR data of gomisin A (**4**) (600 MHz, $CDCl_3$). **Table S5.** ^{1}H- and ^{13}C-NMR data of schisandrin (**6**) (600 MHz, $CDCl_3$). **Table S6.** ^{1}H- and ^{13}C-NMR data of gomisin C (**7**) (600 MHz, $CDCl_3$)

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Authors' contributions

S-KC, Y-GL, and N-IB planned the study and wrote the paper. SKC, Y-GL, H-GK, DHY, D-YL, and N-IB isolated lignans. S-KC and Y-GL identified all lignans. RBW and Y-JK evaluated isolated compounds for cytotoxicity. All authors read and approved the final manuscript.

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Availability of data and materials

The data and materials used in this study are available under permission from the corresponding author on reasonable request.

Competing interests

There are no conflicts to declare.

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