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# Molecular mechanisms of antiproliferative effects induced by Schisandra-derived dibenzocyclooctadiene lignans (+)-deoxyschisandrin and (−)-gomisin N in human tumour cell lines

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ABSTRACT

A different behavior of the two dibenzocyclooctadiene lignans (+)-deoxyschisandrin (**1**) and (−)-gomisin N (**2**), from *Schisandra chinensis* fruits, was observed against two human tumour cell lines, (2008 and LoVo). These lignans inhibited cell growth in a dose-dependent manner on both cell lines, but inducing different types of cell death. In particular, (+)-deoxyschisandrin (**1**) caused apoptosis in colon adenocarcinoma cells (LoVo) but not in ovarian adenocarcinoma cells (2008), while (−)-gomisin N (**2**) induced apoptosis on both the cell lines used. Mitochondrial-mediated pathway was not involved in apoptotic stimuli. Both compounds caused G2/M phase cell growth arrest correlated with tubulin polymerization.



## 1. Introduction

Natural products have played a crucial role especially on the field of anticancer compounds in last decades [1]. The induction of apoptotic cell death is considered to be an important mechanism of action of possible anti-cancer drugs. Many tumors are able to suppress or completely block apoptotic processes, allowing them to survive despite to the undergoing genetic and morphologic transformations [2]. Therefore, substances targeting Bcl2 and p53 proteins or corresponding genes, compounds affecting caspases or interacting with inhibitor of apoptosis (IAP) proteins, could bring advance into anticancer therapy [2,3]. Several lignan derivatives, such as the semisynthetic podophyllotoxin-related compounds etoposide or teniposide, are well known for their antitumor activity [1,4–6], but, despite several papers dealing with antiproliferative activities of *Schisandra* lignans [5,7], only relatively few studies have evaluated the possible mechanisms of action and the effects on apoptosis of dibenzocyclooctadiene derivatives [8].

Dibenzocyclooctadiene lignans are the main group of bioactive compounds contained in the fruit of the well-known medicinal plant *Schisandra chinensis* (Turcz.) Baill. (Schisandraceae) and up to date, more than 40 derivatives have been isolated from this source [9]. These lignans possess a unique structure of dibenzocyclooctadiene and are categorized into two series on the basis of their stereochemistry: with *S*- or *R*-biphenyl configuration. In addition, their cyclooctene ring exhibits a twist-boat-chair (TBC) or a twist-boat (TB) conformation. The structures of these lignans are complex because of substitution patterns, chiral centers and stereoisomery [7]. In previously published papers, some *Schisandra* lignans were studied for their potential effects against tumor cell lines. In particular schizandrin B was able to enhance doxorubicin induced apoptosis in hepatic carcinoma and breast tumor cell lines [10]. Lignans isolated from *S. sphenathera* were studied for

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their antiproliferative and estrogenic effects [11], derivatives from *S. neglecta* were evaluated for their cytotoxic properties against tumor cell lines [12,13].

The various biological activities of the dibenzocyclooctadiene lignans have been extensively investigated and recently, gomisin A, gomisin G, schisandrin, and schisanhenol were reported to possess activity by inhibiting Epstein-Barr virus early antigen (EBV-EA) activation, reversing P-glycoprotein-mediated multi-drug resistance (Pgp-MDR) in cancer cells, and enhancing doxorubicin-induced apoptosis in human hepatic cancer cells [7]. A different mechanism of action was identified for two of these derivatives, gomisin N and gomisin A, against different cell lines [10], and some structure-activity relationships of several dibenzocyclooctadiene lignans able to overcome the MDR in a lung cancer cell line were also reported [14].

In our previous work, we assessed the antiproliferative activity of two *Schisandra* lignan derivatives, (+)-deoxyschisandrin (**1**) and (−)-gomisin N (**2**) isolated from *S. chinensis*, in different *in vitro* models [5]. (+)-deoxyschisandrin (**1**) and (−)-gomisin N (**2**) present similar chemical structure (with the exception of methylenedioxy substituted **1** and two free phenolic groups substituted **2**, and +/− optical rotation) and in this paper we report results showing the different behavior in assays on two human tumor cell lines. In particular, the ability to induce apoptosis, the capability to modify cell cycle progression and the interaction with tubulin were evaluated. Significant differences between **1** and **2** were observed, showing that small changes in the chemical structure of dibenzocyclooctadiene lignans may induce modification of the antiproliferative activity.

## 2. Experimental

### 2.1. General experimental procedures

#### 2.1.1. Chemicals

(+)-deoxyschisandrin (**1**) and (−)-gomisin N (**2**) were isolated from fruits of *S. chinensis* as previously described [5]. Information on the structure was obtained on the basis of both 1D and 2D NMR experiments; the stereospecifity was determined using NMR and CD analysis (data not shown), purity of isolated compounds was >98% on the basis of NMR and HPLC assays. Compounds, dissolved in DMSO at 10 mg/mL and stored at 4 °C, were diluted with growth medium before each experiment and used immediately.

#### 2.1.2. Cell lines

Two human cell lines were used: 2008 cells derived from ovary carcinoma and maintained in RPMI 1640 medium; LoVo cells derived from colon-rectal adenocarcinoma and grown in HAM's F-12 medium [31]. All culture media were supplemented with 10% heat-inactivated FBS and 1% antibiotics (Biochrom KG Seromed). All cells were maintained at 37 °C in humidified atmosphere containing 5% $CO_2$.

#### 2.1.3. Antiproliferative activity assay

Cells were seeded ($1 \times 10^4$ cells/mL) in 96-well tissue plates (Falcon Plymouth, England) and, following overnight incubation, exposed for 24, 48 and 72 h to different concentrations of the lignans **1** and **2** (from 25 to 250 μM) and to podophyllotoxin (from 1 to 20 μM), used as a positive control. Cell viability was assessed by MTT assay [32]: 20 μL of MTT solution (5 mg/mL in PBS) was added to each well 4 h before the end of the treatment, and plates were incubated at 37 °C. Then, the culture medium was discarded and the pigment produced was dissolved in DMSO (150 μL/well). Absorbance was measured on a micro-culture plate reader (Titertek Multiscan) using 570 nm and 630 nm as test and reference wavelength, respectively.

#### 2.1.4. Apoptosis assay

Cells were seeded ($1 \times 10^5$ cells/mL) in 25 mL flasks (Falcon) and after 24 h treated with various concentrations of lignans **1** and **2** or podophyllotoxin for 72 h. Then, cells and supernatants were collected and centrifuged. Pellets were re-suspended in Hepes buffer with Annexin V (2.5 μL; Invitrogen Molecular Probes, Oregon, USA) and Propidium Iodide (PI; 3.5 μL; Sigma-Aldrich, St. Louis USA). Stained cells were incubated for 15 min in dark and on ice and then analyzed by flow cytometry (Epics XL, Beckmann Coulter). Cells negative for both Annexin V and PI are viable, cells Annexin V positive /PI negative are in early stage of apoptosis, and cells Annexin V positive /PI positive stained are necrotic or in late stage of apoptosis.

#### 2.1.5. Mitochondrial membrane potential assay

Cells were seeded ($1 \times 10^5$ cells/mL) in 6-well plates, after 24 h incubation treated with different concentrations of lignans for 72 h, then washed and incubated with Rhodamine 123 (10 μM; Sigma-Aldrich) for 15 min in dark at room temperature. The samples were analyzed by flow cytometry. Rhodamine 123, a cell-permeable cationic probe, is able to highlight the depolarization of mitochondrial membrane potential ($\Delta\Psi m$). A depolarization of $\Delta\Psi m$, resulting from apoptotic signals, triggers a loss of Rhodamine 123 from mitochondria.

#### 2.1.6. Cell cycle analysis

Cells were seeded ($1 \times 10^5$ cells/mL) in 6-well plates and, after 24 h incubation, treated for 72 h with different concentrations of lignans. Cells were detached, centrifuged, permeated

**Table 1**
Growth inhibition effect of (+)-deoxyschisandrin, (−)-gomisin N and podophyllotoxin on two human cancer cell lines. Each value represents means ± standard deviation (SD) of three independent experiments.

| | $IC_{50}$ (μM) | | | | | |
|---|---|---|---|---|---|---|
| | LoVo | | | 2008 | | |
| | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| **(+)-Deoxyschisandrin(1)** | 198.5 ± 36.7 | 79.6 ± 3.6 | 54.3 ± 13.6 | 195.4 ± 28.9 | 156.3 ± 18.2 | 72.6 ± 3.7 |
| **(−)-Gomisin N(2)** | 173.2 ± 52.1 | 97.5 ± 17.1 | 68.5 ± 10.0 | 169.7 ± 15.9 | 129.2 ± 10.9 | 118.0 ± 13.2 |
| **Podophyllotoxin** | 21.3 ± 1.6 | 13.6 ± 1.8 | 4.1 ± 1.3 | 14.3 ± 1.6 | 11.8 ± 1.3 | 3.2 ± 0.6 |