Lignan constituents of Tilia amurensis and their biological evaluation on antitumor and anti-inflammatory activities

Ki Hyun Kim, Eunjung Moon, Sun Yeou Kim, Sang Un Choi, Kang Ro Lee

1. Introduction

Cancer, a malignant tumor or a neoplasm, is a generic term for a broad group of diseases that can affect any part of the body via failure of regulation of cell mitosis. The process of cancer development are (1) rapid and abnormal cells division and growth, (2) forming malignant tumors, (3) invasion nearby adjoining parts of the body, and (4) spread to other organs through the lymphatic and/or bloodstream. Cancer is still one of the most threatening disease worldwide affecting human health and quality of life in spite of recent many advances in the knowledge of its molecular biology of induction and progression. Based on the World Health Organization (WHO) 2008 estimates, 7.4 million cancer deaths are estimated to have occurred in 2004, and it is estimated that 8.3 million people more will have died by 2015 without intervention (Olano et al., 2009). The gradual improvement in life expectancy and adoption of cancer-causing behaviors including smoking, physical inactivity, and "westernized" diets are associated with elevated incidence of cancer and mortality from the disease (Jemal et al., 2011). From this fact, we can assume that malignancy will soon be a global problem with its associated burden. Chemotherapy is one of the most frequently used therapeutic modalities for the treatment of cancer, which includes the use of anticancer drugs obtained from natural or synthetic sources. Natural products are the most consistently successful source of drug leads. Approximately 60% of all drugs in clinical trials for the multiplicity of cancer are either natural products or compounds containing pharmacophores derived from natural products, which are composed of various natural product groups including anthracyclines, polyphenols, macrolides, depsipeptides, indolocarbazoles, terpenoids, and lignans (Newman and Cragg, 2007). In the recent decade, renewed interest in investigating natural products has led to the discovery of several important anticancer drugs such as etoposide, etoposide, and teniposide. The three compounds are chemical derivatives of podophyllotoxin, a natural product belonging to the lignan group of natural products that current study concentrates on. Many previous studies have also reported that various

Abbreviations: NMR, nuclear magnetic resonance; HRMS, high resolution mass spectrometry; CD, circular dichroism; IC_{50}, inhibitory concentration; NO, nitric oxide; ESI, electrospray ionization; COSY, correlation spectroscopy; HMBC, heteronuclear multiple bond correlation; NOESY, nuclear Overhauser effect spectroscopy.

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lignans have anticancer properties. The lignans isolated from Schisandra chinensis showed antiproliferative and an anti-apoptotic effects in various cancer cell lines (Min et al., 2008; Vandyke et al., 2007; Park et al., 2009; Hwang et al., 2011). Honokiol, a derivative of Magnoliol officinalis has anti-neoplastic effects on melanoma cells and chondrosarcoma cells (Mannall et al., 2011; Chen et al., 2010). Secoisolariciresinol isolated from Linum usitatissimum, and lignans derived from extra-virgin olive oil also exerted anticancer properties in breast carcinoma cells (Theil et al., 2011; Menendez et al., 2008). Moreover, norhydroguaiaretic acid, a phenolic lignan isolated from Larrea tridentate had also anticancer activity in vitro and in vivo system (Li et al., 2009). Thus, the natural products approach to discovery and development of new lignan derivatives for anticancer drugs is indeed an attractive approach.

_Tilia amurensis_ Rupr. (Tiliaceae) is commonly known as bee tree and widely distributed in countries including Korea, China, and Japan. The flowers of this tree have been applied as a thera- peutic agent for alleviating a fever in Korea, and its leaves have also been used to treat cancer in Korean traditional medicine (Ahn, 2003). A recent pharmacological study revealed the potential of _T. amurensis_ as an anticancer agent by evaluating its cytotoxicity in M1354, SK-OV-3, SK-MEL-2, and HCT-15 cell lines using a SRB bioassay. Among the four fractions, the EtoAc-soluble fraction showed the most significant cytotoxicity against the tested tumor cell lines. The EtoAc-soluble fraction (11.0 g) was subjected to column chromatography on a silica gel column using a gradient of increasing MeOH in CHCl3 from 10% to 50% to give nine fractions (E1–E9). Fraction E2 (530 mg) was applied to column chromatography on a RP-C18 silica gel column with a gradient of increasing MeOH in H2O from 80% to 100% to give three fractions (E21–E23). Fraction E22 (80 mg) was applied to C18 Waters Sep-Pak Vac 6 cc with 100% MeOH and purified by preparative C18 RP HPLC using 40% MeOH to give five fractions (ES1-5ES5). Fraction E533 (84 mg) was applied by preparative C18 RP HPLC using 40% MeOH to obtain compounds 1 (5 mg), 2 (8 mg), 3 (20 mg), 6 (4 mg), and 10 (3 mg). Compound 9 (7 mg) was isolated from fraction E56 (120 mg) through preparative RP HPLC purification using 38% MeOH to yield compounds (ES1-5ES5). The air-dried and pulverized trunk of _T. amurensis_ was collected at Hwacheon-Myun, Hongcheon city, Gangwon-do, Korea, in March 2010. Samples of plant material were identified by one of the authors (K.R. Lee). A voucher specimen (SKKU 2010–03) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

2.3. Extraction, fractionation, and purification methods

The air-dried and pulverized trunk of _T. amurensis_ (6 kg) was extracted twice with 80% aqueous MeOH (2 x 4 h) under reflux, and then filtered. The filtrate was evaporated under vacuum to obtain a MeOH extract (384 g), which was suspended in distilled water and then successively partitioned with n-hexane, CHCl3, EtoAc, and n-BuOH, yielding 12.5, 40.4, 11.0, and 69.3 g residues, respectively. Each fraction was evaluated for its cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines using a SRB bioassay. Among the four fractions, the EtoAc-soluble fraction showed the most significant cytotoxicity against the tested tumor cell lines. The EtoAc-soluble fraction (11.0 g) was subjected to column chromatography on a silica gel column using a gradient of increasing MeOH in CHCl3 from 10% to 50% to give nine fractions (E1–E9). Fraction E2 (530 mg) was applied to column chromatography on a RP-C18 silica gel column with a gradient of increasing MeOH in H2O from 80% to 100% to give three fractions (E21–E23). Fraction E22 (80 mg) was applied to C18 Waters Sep-Pak Vac 6 cc with 100% MeOH and purified by preparative C18 RP HPLC using 40% MeOH to give five fractions (ES1-5ES5). Fraction E533 (84 mg) was applied by preparative C18 RP HPLC using 40% MeOH to obtain compounds 1 (5 mg), 2 (8 mg), 3 (20 mg), 6 (4 mg), and 10 (3 mg). Compound 9 (7 mg) was isolated from fraction E56 (120 mg) through preparative RP HPLC purification using 38% MeOH to yield eight fractions (E71–E78). Compound 7 (6 mg) was obtained from fraction E73 (76 mg) through repeated column chromatography on C18 Waters Sep-Pak Vac 6 cc with 45% MeOH and preparative C18 RP HPLC with 45% MeOH.

2.3.1. Tiliaumires (A)  
Colorless gum; m/z 524.1923, 526.1911; CD (MeOH) 251 (+13.7), 276 (+17.2) nm; 1H and 13C NMR spectra, see Table 1; HR-ESIMS (positive-ion mode) m/z 543.1995 [M+Na]+ (calcld for C29H28NaO14, 543.1995).

2.3.2. Tiliaumires (B)  
Colorless gum; m/z 520.1927, 522.1923, 524.1921; CD (MeOH) 251 (+13.7), 276 (+17.2) nm; 1H and 13C NMR spectra, see Table 1; HR-ESIMS (positive-ion mode) m/z 515.1888 [M+Na]+ (calcld for C29H28NaO14, 515.1883).

2.4. Enzymatic hydrolysis of 1 and 2

A solution of compounds 1 and 2 (each 3 mg) in H2O (3 mL) was individually hydrolyzed with cellulase (from Aspergillus niger, Nagase Biochemical Co., 65 mg) at 37°C for 96 h. Each reaction mixture was extracted with an equal amount of EtOAc (3 ×), and the individual EtoAc extract was evaporated under reduced pressure. The combined EtoAc layers from 1 and 2 were purified by a silica gel Waters Sep-Pak Vac 6 cc (CHCl3-MeOH, 15:1) to give each aglycone, (+)-8-methoxyisolaric-

2.5. Cell cultures

All tumor cell cultures were maintained using RPMI1640 cell growth medium (Gibco, Carlsbad, CA), supplemented with 5% fetal bovine serum (FBS) (Gibco), 100 U/mL penicillin and 100 µg/mL streptomycin. Human tumor cell lines such as

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The cytotoxicity of the compounds against cultured human tumor cell lines was evaluated by the sulforhodamine B (SRB) method (Skehan et al., 1990). Each tumor cell line was incubated for 24 h at 37°C in a humidified incubator with 5% CO2.

2.6. Cytotoxicity assessment

The cytotoxicity of the compounds against cultured human tumor cell lines was evaluated by the sulforhodamine B (SRB) method (Skehan et al., 1990). Each tumor cell line was incubated under standard 96-well flat-bottom microplates and then incubated for 24 h at 37°C in a humidified atmosphere of 5% CO2. The attached cells were then incubated with the serially diluted lignan samples. After continuous exposure to the compounds for 48 h, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4°C for 30 min. Then, the cells were washed again and then solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 570 nm with a microplate reader. The absorbance at 570 nm was measured using a microplate reader. Sodium nitrite was used as a standard to calculate the NO2 acid). After 10 min, the absorbance at 570 nm was measured using a microplate reader. The absorbance at 570 nm was measured using a microplate reader.

2.7. Measurement of nitric oxide production and cell viability

BV-2 cells were plated into a 96-well plate (3 × 104 cells/well). After 24 h, cells were pretreated with compounds 1–10 for 30 min, and then stimulated with 100 ng/ml of LPS for another 24 h. Nitrite, a soluble oxidation product of nitric oxide (NO), was measured in the culture media using the Griess reaction. The supernatant (50 μl) was harvested and mixed with an equal volume of Griess reagent (1% N-1-napthylethylenediamine dihydrochloride in 5% phosphoric acid). The mixture was incubated for 10 min at room temperature. The absorbance at 570 nm was measured using a microplate reader.

The cytotoxicity of the compounds against cultured human tumor cell lines was evaluated by the sulforhodamine B (SRB) method (Skehan et al., 1990). Each tumor cell line was incubated for 24 h at 37°C in a humidified atmosphere of 5% CO2. The attached cells were then incubated with the serially diluted lignan samples. After continuous exposure to the compounds for 48 h, the culture medium was removed from each well and the cells were fixed with 10% cold trichloroacetic acid at 4°C for 1 h. After washing with tap water, the cells were stained with 0.4% SRB dye and incubated for 30 min at room temperature. The cells were washed again and then solubilized with 10 mM unbuffered Tris base solution (pH 10.5). The absorbance was measured spectrophotometrically at 570 nm with a microplate reader.

2.7. Measurement of nitric oxide production and cell viability

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confirmed to be at C-3, C-5, and C-3.

3.3. Tiliamuroside B (Fig. 2). Furthermore, the positions of three methoxy groups were determined by the 1H–1H COSY, HMQC, and HMBC spectra (Fig. 2). Moreover, the NOESY correlations of H-8/H-2, H-7/H-9, and H-8/H-9 indicated the presence of a 1,2,4,5-tetrasubstituted aromatic ring \([\delta_1 H=6.33 (1H, s, H-3)\) and 6.70 (1H, s, H-6)] and a 1',3',4'-trisubstituted aromatic ring system \([\delta_1 H=6.32 (1H, dd, J=2.5, 8.0 Hz, H-6'), 6.61 (1H, d, J=8.0 Hz, H-5'), and 6.81 (1H, d, J=2.5 Hz, H-2')]\). The 1H and 13C NMR spectra (Table 1) of 2 were similar to those of schizandrol A (Ohashi et al., 1994) with apparent differences in their chemical shifts at C-2, C-8, C-1', C-7', and C-8', indicating that compound 2 was the same aryltetralin type lignan as 3 but different in terms of the configuration at C-8, C-7', and C-8'. The structural framework of 2 was confirmed by the analysis of the 1H COSY, HMQC, and HMBC spectra (Fig. 2). The HMBC correlation of H-1' \((\delta_1 4.21)\) to C-9' \((\delta_1 69.6)\) indicated the relative configuration as 7R,13S (Kwon et al., 2010) with high similarity of 1H and 13C NMR data, and enzymatic hydrolysis of 2 with cellulase afforded o-xylene \((\delta_1 d+18.5)\) in H2O. The absolute configuration of 2 was assigned on the basis of the examination of CD spectrum of 2 in combination with the NOESY experiment. The small coupling constant \((J=4.5 Hz)\) of H-7' and H-8' observed in the 1H NMR spectrum of 2, as opposed to the large coupling constant \((J=7.5 Hz)\) between H-7' and H-8' in 3, established that H-7' and H-8' are in the same orientation (Jutiviboonsuk et al., 2005; Ohashi et al., 1994). Moreover, the NOESY correlations of H-8/H-2, H-9/H-2', H-7/H-9', and H-8'/H-9 indicated the relative configuration as 7R,13S (Fig. 3). The CD spectrum of 2 displayed Cotton effects at 240 (\(\Delta c=-13.4)\) and 276 (\(\Delta c=-4.1)\) nm (Fig. 3), which were in good agreement with those of the reported compound, \((-)\)-4-epi-lyoniresinol 3x-O-\(\beta\)-o-glucopyranoside (Ohashi et al., 1994). Furthermore, compound 2 showed high similarity \((1H\) and 13C NMR data at C-2, C-8, C-1', C-7', and C-8' to those of \((-)\)-4-epi-lyoniresinol 3x-O-\(\beta\)-o-glucopyranoside (Ohashi et al., 1994). Thus, the absolute configuration of 2 was established to be 7R,8R,8'R, which was also supported by enzymatic hydrolysis of 2 afforded the aglycone, burselignan.

Fig. 1. Chemical structures of compounds 1–10.

NMR spectrum of 1. This structure was confirmed by analysis of the 1H–1H COSY, HMQC, and HMBC spectra (Fig. 2). Moreover, the presence of a \(\beta\)-o-xylene unit was suggested by its 1H NMR data (Smite et al., 1995; Lee et al., 2001), and enzymatic hydrolysis of 1 with cellulase liberating o-xylene \((\delta_1 d+20.5)\) in H2O. The HMBC correlation from H-1 to C-3, 5-OCH3/C-5, and 3-OCH3/C-3 indicated the xylose unit linked to the oxygen at C-9' (Fig. 2). Furthermore, the positions of three methoxy groups were confirmed to be at C-3, C-5, and C-3', respectively by the HMBC cross-peaks of 3-OCH3/C-3, 5-OCH3/C-5, and 3'-OCH3/C-3', respectively. The absolute configuration of 1 was established on the basis of the examination of CD spectrum of 1 in combination with the NOESY experiment. The observed NOESY correlations of H-8/H-2, H-9/H-2', H-7/H-9', and H-8'/H-9 indicated the relative configuration as 7S,8R,8'R (Fig. 3). The CD spectrum of 1 showed Cotton effects at 244 (\(\Delta c=14.6)\) and 276 (\(\Delta c=5.2)\) nm (Fig. 3) consistent with those of the reported compound, \((+)\)-lyoniresinol 3x-O-\(\beta\)-o-glucopyranoside (Ohashi et al., 1994). Consequently, the absolute configuration of 1 was determined to be 7S,8R,8'R, which was also supported by enzymatic hydrolysis of 1 affording the aglycone, \((+)\)-8-methoxyisolariciresinol. The identity of the aglycone of 1 was performed by the comparison of its 1H NMR, MS, specific rotation, and CD data with those reported in the literature (Raju and Pillai, 1989, 1990). On the basis of above data, compound 1 was assigned as shown in Fig. 1.

3.3. Tiliamuroside B (2)

Tiliamuroside B (2) was isolated as a colorless gum, \([\delta_1 d+20.5=-75.3 (c 0.33, MeOH)]\) with the molecular formula of C25H32O10 based on the positive-ion HR-ESIMS data \((m/z 515.1888 [M+Na]+, \text{calcd for C}_{25}H_{32}NaO_{10}, 515.1893)\). The 1H NMR spectrum (Table 1) showed
(Jutiviboonsuk et al., 2005). On the basis of above data, compound 2 was determined as shown in Fig. 1.

3.4. Biological activities studies

Lignans are a class of secondary metabolites produced by oxidative dimerization of two phenylpropanoid units. Although their molecular backbone only consists of two phenylpropane (C6–C3) units, lignans show an enormous structural diversity. There is a growing interest in lignans and their synthetic derivatives due to its applications in cancer chemotherapy and other various pharmacological effects, particularly anticancer and anti-inflammatory activities (Hirano et al., 1994; Bao-Ning et al., 2004).

In this investigation, the isolated compounds 1–10 from the MeOH extract of T. amurensis were evaluated for their antiproliferative activities against A549, SK-OV-3, SK-MEL-2, and HCT-15 human tumor cell lines using the SRB bioassay (Skehan et al., 1990). The results (Table 2) showed that most of the tested isolates (1–10) had cytotoxicity against above tested cell lines. In compounds 1–5, the same aryl-tetralin type lignans, they showed significant cytotoxicity against all of the cell lines tested with IC50 values of 3.26–29.70 μM. In particular, compounds 2 and 3 exhibited the strongest cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC50 (2): 7.32, 8.89, 7.84, and 6.18 μM, and IC50 (3): 6.90, 5.88, 3.26, and 6.65 μM, respectively). These results indicated that the absence of a methoxy group at C-3 in the aryl-tetralin type lignan seems to increase the cytotoxic activity against the tested cell lines in consideration of above obtained data. Based on this evidence, the pharmacological activity of the well-known anti-tumor agent, podophyllotoxin, which is also the pharmacological precursor for the important anticancer drug etoposide, can be explained by the absence of the methoxy group at C-3 (Canel et al., 2004).

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A549</td>
</tr>
<tr>
<td>1</td>
<td>20.24 ± 0.83(^b)</td>
</tr>
<tr>
<td>2</td>
<td>7.32 ± 0.92</td>
</tr>
<tr>
<td>3</td>
<td>6.90 ± 0.31</td>
</tr>
<tr>
<td>4</td>
<td>21.09 ± 1.84</td>
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<tr>
<td>5</td>
<td>24.33 ± 2.93</td>
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<tr>
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<tr>
<td>7</td>
<td>15.29 ± 2.11</td>
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<tr>
<td>8</td>
<td>22.08 ± 0.23</td>
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<tr>
<td>9</td>
<td>&gt;30.0</td>
</tr>
<tr>
<td>10</td>
<td>23.11 ± 1.25</td>
</tr>
<tr>
<td>Doxorubicin(^c)</td>
<td>0.001 ± 0.001</td>
</tr>
</tbody>
</table>

\(^a\) IC50 value of compounds against each cancer cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth in vitro.

\(^b\) Data are expressed as mean ± SEM of three independent experiments.

\(^c\) Doxorubicin as a positive control.
It was also reported that a lignan glycoside, aviculin, which has the same carbon framework as 2 and 3 with the only difference being its glycosidation of rhamnose, exhibited a potent inhibitory effect on cancer cell invasion through a rat mesothelium monolayer using an MM1 cell line in an in vitro system (Ohashi et al., 2003). The larciresinol-type lignan, compound 6 showed the antiproliferative activity against only the SK-MEL-2 cell line with IC50 value of 13.46 μM. The neolignan, compound 7 showed cytotoxicity against the tested cell lines except for SK-OV-3 cell with IC50 values of 12.29–28.14 μM. In compounds 8–10 in the furuoruran lignan group, compounds 8 and 10 exhibited relatively moderate cytotoxicity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines with IC50 values of 6.15–29.30 μM. However, compound 9 with the methylene dioxy group in the benzene ring exhibited the cytotoxic activity against only the SK-MEL-2 cell line with IC50 value of 12.57 μM. It seems that the presence of the methylene dioxy group decreased the activity against the A549, SK-OV-3, and HCT-15 cell lines in consideration of above obtained data even though this cannot apply to other type of lignans such as podophyllotoxin with the methylene dioxy group. Interestingly, the furuoruran-type lignans, compounds 8–10 exhibited selective cytotoxicity against the SK-MEL-2 cell line (IC50 (8): 6.15 μM, IC50 (9): 12.57 and IC50 (10): 15.21 μM).

It has been known that progression of cancer is associated with inflammatory responses. The expression of inflammatory genes is often negatively correlated with cancer stage and prognosis (Chang et al., 2004; Wang et al., 2006; Galon et al., 2006). Moreover, non-steroidal anti-inflammatory drugs show preventive effects against cancer (Urich et al., 2006). Therefore, cancer and inflammation are related by epidemiology, histopathology, and inflammatory profiles (Rakoff-Nahoum, 2006). On the basis of the expanded understanding that inflammation plays a crucial role in tumor progression, we evaluated the anti-inflammatory activities of the isolate compounds (1–10) in the murine microglia BV-2 cell line. Many investigations about the anti-inflammatory effects of lignans have been announced (Baumgartner et al., 2011; Kou et al., 2011; Guo et al., 2011; Pellegrini et al., 2010). In this study, compounds 8 and 10 significantly reduced the production of LPS-induced pro-inflammatory factor, NO, in BV-2 cells without cell toxicity (Table 3). Compounds 8 and 10 exhibited the inhibitory activity with an IC50 of 15.05 and 34.35 μM. In particular, the activity of 8 was more potent than that of positive control, 1-NMMA, in inhibiting NO production with an IC50 of 15.05 μM. Previously, several studies showed that NO produced in inflamed tissues could contribute to the carcinogenesis process (Oshihama and Bartsch, 1994; Tamir and Tannenbaum, 1996; Liu and Hotchkiss, 1995). Toxicity induced by overproduction of NO, when it becomes chronic, can lead to enhanced cell replication which is a risk factor for many cancers (Armes and Gold, 1990). Moreover, it is known that oxidative damage to DNA by NO may be associated with the development of cancer (Feig et al., 1994; Tamir and Tannenbaum, 1996). Continued exposure to NO lead to accumulation of populations of cells with activated oncogenes or impaired tumor suppressor genes, which induce cell death and mutagenesis. In this study, although compounds 8 and 10 inhibited NO production induced by LPS in BV-2 cells strongly, they did not effectively exert cytotoxicity against cancer cell lines. Moreover, compounds 2 and 3, which had strong cytotoxic activities against cancer cell lines, did not exert significantly the effects on inhibition of NO production. This result demonstrates that the inhibitory activities on NO production may be a different status with cytotoxic effects against cancer cell lines. Therefore, we suggest that the inhibitory activities on NO production of lignans isolated from T. amurenensis may be exerted independently from their cytotoxic properties. However, it is known that various bioactivities of lignans are responsible for their properties on cancer prevention. According to this consideration, we suggest that cytotoxicity of lignans from T. amurenensis against cancer cell lines can be also associated with other bioactivities of lignans (e.g. antioxidant, anti-angiogenic, and anti-apoptotic properties). Further studies will be needed to investigate the precise mechanisms of isolated lignans from T. amurenensis on cytotoxic activities against cancer cell lines and inhibitory activities on NO production.

### Table 3 Inhibitory effect on NO production of compounds 1–10 in LPS-activated BV-2 cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)</th>
<th>Cell viability (%)</th>
<th>Compound</th>
<th>IC50 (μM)</th>
<th>Cell viability (%)</th>
</tr>
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<tbody>
<tr>
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<td>7</td>
<td>73.80</td>
<td>98.2 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td>80.85</td>
<td>96.7 ± 2.8</td>
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<td>15.05</td>
<td>103.5 ± 2.2</td>
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<tr>
<td>3</td>
<td>156.02</td>
<td>97.0 ± 2.1</td>
<td>9</td>
<td>&lt;50.0</td>
<td>88.1 ± 3.7</td>
</tr>
<tr>
<td>4</td>
<td>57.01</td>
<td>97.2 ± 1.9</td>
<td>10</td>
<td>34.35</td>
<td>98.1 ± 3.7</td>
</tr>
<tr>
<td>5</td>
<td>133.74</td>
<td>96.7 ± 3.3</td>
<td>NMMA</td>
<td>16.44</td>
<td>96.7 ± 5.2</td>
</tr>
<tr>
<td>6</td>
<td>53.31</td>
<td>97.6 ± 3.0</td>
<td></td>
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</tr>
</tbody>
</table>

*: IC50 value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells.

: Cell viability after treatment with 20 μM of each compound was expressed as a percentage (%) of the LPS only treatment group. The results are averages of three independent experiments, and the data are expressed as mean ± SD. Statistical comparisons were performed using an one-way ANOVA test with Student’s t-test.

: NMMA as a positive control.

Only p-value < 0.05 was indicated as statistically significant.

### 4. Conclusion

This study deals with the isolation and biological study of 10 lignan derivatives from the most active EtoAc-soluble fraction from MeOH extract of T. amurenensis. This tree T. amurenensis has been used in Korean traditional medicine as an anticancer agent. However, the constituents of this tree responsible for anticancer activity and its underlying mechanism are still unclear. In this study, we isolated and identified 10 lignan derivatives (1–10) including two new lignan glycosides (tillamurorosides A and B) from the EtoAc-soluble fraction through the bioactivity-guided isolation techniques. This study indicates that lignan derivatives are the main component of the trunk of T. amurenensis. With regard to bioactivity, we could confirm the anticancer effects of the lignan derivatives through their bioactive evaluation on antitumor and anti-inflammatory activities.

The present study thus suggests that the lignan derivatives isolated from T. amurenensis may be valuable antitumor agents as contributors to the anticancer activity of this tree and expected to possess beneficial therapeutic potential against various cancers and inflammation-related diseases. This study proves that the use of T. amurenensis as Korean traditional medicine to treat cancer is reasonable because of the anticancer effects found from the isolated lignan derivatives.

### Conflict of Interest

The authors declare that there are no conflicts of interest.
Acknowledgments

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