Supporting Information

Bioactive Sesquiterpenes from the Essential Oil of *Thuja orientalis*
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1. Experimental methods

1.1. General experimental procedures. Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco). IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer (Bruker). UV spectra were recorded with a Shimadzu UV-1601 UV-Visible spectrophotometer (Shimadzu). ESIMS and HR-ESIMS spectra were recorded on a Micromass QTOF-2-MS (MicroMass; Waters). Nuclear magnetic resonance (NMR) spectra, including $^1$H-$^1$H COSY, HMOC, HMBC, NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer (Varian) operating at 500 MHz ($^1$H) and 125 MHz ($^{13}$C), with chemical shifts given in ppm (δ). Preparative high performance liquid chromatography (HPLC) used a Gilson 306 pump (Gilson) with a Shodex refractive index detector (Shodex). Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh) and RP-C$_{18}$ silica gel (Merck, 230-400 mesh) were used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia). Merck precoated silica gel F$_{254}$ plates and RP-18 F$_{254s}$ plates (Merck) were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

1.2. Plant materials. The aerial parts of *T. orientalis* (3 kg) were purchased at Kyungdong herbal market, Seoul, Korea, in January 2011, and were identified by one of the authors (K.R.L.). A voucher specimen (SKKU 2011-01) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

1.3. Steam distillation and isolation. The aerial parts of *T. orientalis* (3 kg) were crushed completely and subjected to steam distillation (4 h) in a distillation apparatus. Steam is passed through a vessel containing the plant material-water mixture to yield a condensate. The oils, lighter than water, were separated, dried over anhydrous sodium sulfate and stored in sealed container under N$_2$ and refrigerated (-20 ºC). The essential oils (300 g) were subjected to a silica gel (230-400 mesh) flash column chromatography (900 g, 6 × 85 cm),
eluting with 100% n-hexane (3 L) to give a crude hexane fraction [fraction H (3 L of n-hexane), 100 g]. Fraction H (100 g) was separated over RP-C18 silica gel (230-400 mesh) column chromatography (500 g, 5 × 55 cm) using a solvent system of 100% MeOH (3 L) to give five fractions [H1 – H5; H1 – H4 (each 0.5 L of 100% MeOH) and H5 (1.0 L of 100% MeOH)]. Fraction H3 (4.5 g) was applied to a Sephadex LH-20 column (450 g, 3 × 90 cm) using a solvent system of CH2Cl2-MeOH (1:1, 2 L) to four subfractions (H31 – H34) according to TLC analysis [H31 – H34; H31 (0.4 L of CH2Cl2-MeOH =1:1), H32 (0.3 L of CH2Cl2-MeOH =1:1), H33 (0.8 L of CH2Cl2-MeOH =1:1), and H34 (0.5 L of CH2Cl2-MeOH =1:1)]. Subfraction H33 (1.8 g) was separated over silica gel (230-400 mesh) column chromatography (100 g, 3 × 90 cm) using a solvent system of 100% n-hexane (4 L) to afford 12 subfractions [H33-1 – H33-12; H33-1 – H33-10 (each 0.3 L of 100% n-hexane) and H33-11 – H33-12 (each 0.5 L of 100% n-hexane)]. Subfraction H33-9 (124 mg) was purified by semi-preparative reversed-phase HPLC using a 250 mm × 10 mm i.d., 10 μm, Econosil RP-18 column (Alltech) with a solvent system of MeOH-MeCN (4:1, 800 mL, flow rate; 2 mL/min) to obtain 5 (6 mg), 6 (8 mg), and 7 (30 mg). Subfraction H33-10 (430 mg) was separated over silica gel (230-400 mesh) column chromatography (80 g, 3 × 75 cm) using a gradient solvent system of n-hexane-EtOAc [10:1 (1.5 L) and 5:1 (0.9 L)] to give eight subfractions [H33-10-1 – H33-10-8; H33-10-1 – H33-10-5 (each 0.3 L of n-hexane-EtOAc=10:1) and H33-10-6 – H33-10-8 (each 0.3 L of n-hexane-EtOAc=5:1)]. Subfraction H33-10-3 (140 mg) was purified by semi-preparative normal-phase HPLC using a 250 mm × 10 mm i.d., 5 μm, Apollo Silica column (Alltech) with a solvent system of n-hexane-EtOAc (30:1, 800 mL, flow rate; 2 mL/min) to yield 2 (10 mg). Subfraction H33-10-5 (45 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 95% MeCN (800 mL, flow rate; 2 mL/min) to afford 4 (8 mg). Compound 9 (18 mg) was isolated by separation of semi-preparative reversed-phase HPLC with a solvent system of 90% MeCN.
(800 mL, flow rate; 2 mL/min) from subfraction H33-10-6 (110 mg). Subfraction H33-11 (258 mg) was separated by preparative reversed-phase HPLC using a solvent system of 80% MeOH (2 L, flow rate; 5 mL/min) to give 3 (15 mg) and four other peaks, which provided four subfractions (H33-11-1 – H33-11-4) in sequence. Subfraction H33-11-1 (89 mg) was purified by semi-preparative normal-phase HPLC using a solvent system of n-hexane-EtOAc (14:1, 800 mL, flow rate; 2 mL/min) to afford 1 (15 mg), 10 (9 mg), and 11 (12 mg). Finally, subfraction H33-12 (75 mg) was purified by semi-preparative reversed-phase HPLC with a solvent system of 80% MeOH (800 mL, flow rate; 2 mL/min) to yield 8 (5 mg).

3α-Methoxy-4α-epoxythujopsane (1): Colorless oil; $[\alpha]^{25\text{D}} +7.6$ (c=0.70, CHCl$_3$); IR (KBr) $v_{\text{max}}$ 3060, 1630, 1450, 1384, 1370, 1030, 835 cm$^{-1}$; $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data, see Table 1; ESIMS (positive-ion mode) $m/z$: 251 [M + H]$^+$. HR-ESIMS (positive-ion mode) $m/z$: 251.2015 [M + H]$^+$ (calcd. for C$_{16}$H$_{27}$O$_2$, 251.2011).

Δ$^{3,15}$-4β-Epoxythujopsene (2): Colorless oil; $[\alpha]^{25\text{D}} -25.9$ (c=0.50, CHCl$_3$); IR (KBr) $v_{\text{max}}$ 3090, 2944, 1638, 1452, 1383, 1372, 1030, 885, 670 cm$^{-1}$; $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data, see Table 1; ESIMS (positive-ion mode) $m/z$: 219 [M + H]$^+$. HR-ESIMS (positive-ion mode) $m/z$: 219.1748 [M + H]$^+$ (calcd. for C$_{15}$H$_{23}$O, 219.1749).

Δ$^{3,4}$-Thujopsen-2,15-diol (3): Colorless gum; $[\alpha]^{25\text{D}} +9.2$ (c=0.70, CHCl$_3$); IR (KBr) $v_{\text{max}}$ 3357, 2946, 2833, 1660, 1451, 1115, 1032, 674 cm$^{-1}$; $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data, see Table 2; ESIMS (positive-ion mode) $m/z$: 237 [M + H]$^+$. HR-ESIMS (positive-ion mode) $m/z$: 237.1857 [M + H]$^+$ (calcd. for C$_{15}$H$_{25}$O$_2$, 237.1855).

Thujopsadiene (4): Colorless gum; $[\alpha]^{25\text{D}} +20.4$ (c=0.40, CHCl$_3$); UV (MeOH) $\lambda_{\text{max}}$ (log e) 232 (3.8) nm; IR (KBr) $v_{\text{max}}$ 3080, 2949, 1680, 1401, 1384, 1370, 1030, 885, 670 cm$^{-1}$; $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data, see Table 2; ESIMS (positive-ion mode) $m/z$: 203 [M + H]$^+$. 
2. Biological activity

2.1. In vitro cytotoxicity test. A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each isolated compound against four cultured human tumor cell lines [18]. The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Doxorubicin (purity ≥98%, Sigma) was used as a positive control. The cytotoxicities of doxorubicin against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines were IC$_{50}$ 0.001, 0.002, 0.001, and 0.097 μM, respectively. Tested compounds were demonstrated to be pure as evidenced by NMR and HPLC analyses (purity ≥95%).

2.2. Evaluation of NO regulatory effects of compounds in LPS-activated BV-2 cells. BV-2 microglia cells (3 × 10$^4$ cells/well in 96 well plates) were treated with the samples to be tested for 30 min before exposure to 100 ng/mL of LPS. After 24 h incubation, nitrite in culture medium was measured to assess NO production in BV-2 microglia cells using Griess reaction. The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% N-1-naphtylethylenediamine dihydrochloride in 5% phosphoric acid). After 10 min, the absorbance at 540 nm was measured using a microplate reader. Sodium nitrite was used as a standard to calculate the NO$_2^-$ concentration. Cell viability was assessed by a 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide (MTT) assay. $N^G$-Monomethyl-L-arginine (L-NMMA; purity ≥ 98%, Sigma), a well-known NOS inhibitor, was tested as a positive control.
1S. The $^1$H NMR spectrum of 1 (CDCl$_3$, 500 MHz)

2S. The $^{13}$C NMR spectrum of 1 (CDCl$_3$, 125 MHz)
3S. The DEPT spectrum of 1 (CDCl₃)

4S. The HMQC spectrum of 1 (CDCl₃)
5S. The HMBC spectrum of 1 (CDCl₃)

6S. The NOESY spectrum of 1 (CDCl₃)
7S. The $^1$H NMR spectrum of 2 (CDCl$_3$, 500 MHz)

8S. The $^{13}$C NMR spectrum of 2 (CDCl$_3$, 125 MHz)
9S. The DEPT spectrum of 2 (CDCl₃)

10S. The HMQC spectrum of 2 (CDCl₃)
11S. The HMBC spectrum of 2 (CDCl₃)

12S. The NOESY spectrum of 2 (CDCl₃)
13S. The $^1$H NMR spectrum of 3 (CDCl$_3$, 500 MHz)

14S. The $^{13}$C NMR spectrum of 3 (CDCl$_3$, 125 MHz)