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A biphenyl derivative from the twigs of *Chaenomeles speciosa*

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ABSTRACT

In our continuing search for bioactive constituents of Korean medicinal sources, we investigated an 80% MeOH extract of the twigs of *Chaenomeles speciosa*. Column chromatographic purification of the $CHCl_3$ fraction resulted in the isolation of a new biphenyl derivative (**1**), along with four known biphenyl compounds (**2**–**5**) and six triterpenes (**6**–**11**). The chemical structure of the new compound was determined on the basis of spectroscopic analyses including 1D and 2D NMR data. Among isolates, compound **3** exhibited potent cytotoxic activities against SK-OV-3, SK-MEL-2, and XF498 cell lines (IC_{50} = 5.91, 4.22, and 6.28 μM, respectively). Also, Compounds **9** and **10** showed strong anti-neuroinflammatory activities (IC_{50} 2.38, and 6.70 μM, respectively).

1. Introduction

Microglia are a type of neuronal cell that plays an immune function in the central nervous system (CNS). However, if they are uncontrolled and activated chronically, they contribute to operating various proinflammatory factors such as nitric oxide (NO), tumor necrosis factor alpha (TNF-α) and prostaglandin E_2 (PGE_2) [1,2]. These factors cause inflammation of the CNS and eventually lead to neurological diseases such as Parkinson's disease [3]. Recently, it has been reported that neurotrophic factor can prevent LPS-induced neuroinflammation by inhibiting JNK signaling in neurons [4]. This means that neurotrophic factor may be potential targets of neuroinflammatory therapy in central nervous system (CNS) disorders, and therefore need to focus on finding new compounds that regulate the integration of neurotrophic factors. As part of our continuing search for bioactive constituents that exhibit various activities such as anti-inflammatory, anti-cancer and neuroprotective effects from Korean medicinal resources, we found that the MeOH extract from the twigs of *Chaenomeles speciosa* (Sweet) Nakai showed inhibitory effect on NO levels in lipopolysaccharide (LPS)-stimulated murine microglia BV2 cells in Preliminary screening test.

C. speciosa is a deciduous shrub belonging to the Rosaceae family and distributed in Korea, and China [5,6]. *C. speciosa* has been used in traditional medicine. The fruit of this plant has been used for the treatment of rheumatoid arthritis, hepatitis, and common cold [7,8]. Previous studies have suggested that terpenoids, flavonoids, and tannins from the fruit of *C. speciosa* associated with anti-inflammatory, antimicrobial, and antioxidant effects [8–10]. Recently, several isolated components, such as triterpenoids and lignan glycosides, from the twigs of same genus plant, *Chaenomeles sinensis,* have reported that they exhibit good cytotoxic and anti-inflammatory activity [11,12]. In a process of searching for more active substances from another *Chaenomeles* genus plants, we found that the $CHCl_3$ layer of a *C. speciosa* MeOH extract showed strong cytotoxicity against A549, SK-OV-3, SK-MEL-2, and XF498 cells and inhibitory to produce NO in lipopolysaccharide (LPS)-stimulated murine microglia BV2 cells. The chemical investigation of the extracts from the twigs of *C. speciosa* resulted in the isolation of a new biphenyl compound (**1**), along with ten known ones (**2**–**11**). The chemical structures of the isolated compounds were elucidated by extensive NMR data (^1H and ^{13}C NMR, ^1H-^1H COSY, HSQC, and HMBC), optical rotation and MS analysis. The isolated compounds (**1**–**11**) were evaluated for their cytotoxic, anti-neuroinflammatory, and NGF-potentiating activities. Herein, we report the isolation and structural elucidation of bioactive compounds from the twigs of *C. speciosa* and their biological activities.

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2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco, Easton, MD, USA). Infrared (IR) spectra were recorded on a Bruker IFS-66/S Fourier-transform IR spectrometer (Bruker, Karlsruhe, Germany). Ultraviolet (UV) spectra were recorded with a Shimadzu UV-1601 UV–Visible spectrophotometer (Shimadzu, Tokyo, Japan). HRFABMS was conducted on a Waters SYNAPT G2 (Milford, MA, USA). NMR spectra were recorded on a Bruker AVANCE III 700 NMR spectrometer at 700 MHz (^{1}H) and 175 MHz (^{13}C). The preparative high performance liquid chromatography (HPLC) system had a Gilson 306 pump (Middleton, WI, USA) with a Shodex Refractive Index Detector (New York, NY, USA). Column chromatography was performed with silica gel 60 (70–230 and 230–400 mesh; Merck, Darmstadt, Germany) and RP-C_{18} silica gel (Merck, 230–400 mesh). LPLC was performed over a LiChroprep Lobar-A RP-C_{18} column (Merck, 240 mm × 10 mm i.d.) equipped with a FMI QSY-0 pump. Merck precoated silica gel F_{254} plates and reversed-phase (RP)-18 F_{254s} plates (Merck) were used for thin-layer chromatography (TLC). Spots were detected on TLC under UV light or by heating after spraying the samples with anisaldehyde-sulfuric acid.

2.2. Plant material

The twigs of *C. speciosa* were collected from Suwon, Korea, in June 2014. Samples of plant material were identified by one of the authors (K.R. Lee). A voucher specimen (SKKU-NPL-1403) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

2.3. Extraction and isolation

The twigs of *C. speciosa* (7 kg) were extracted with 80% MeOH (3 × 4 L every 3 days) under reflux, and filtered. The methanol extract (398 g) was suspended in distilled water (2.4 L) and then successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, yielding 28, 25, 19, and 81 g of residue, respectively. Each layer was tested for cytotoxicity against A549, SK-OV-3, SK-MEL-2, and XF498 cells using a SRB assay. The $CHCl_3$-soluble fraction showed the most significant cytotoxic activity against the tested tumor cell lines. In our screening test, the active $CHCl_3$-soluble fraction also strongly inhibited NO production in LPS-stimulated BV-2 microglial cells. The $CHCl_3$ fraction (20 g) was separated over a silica gel (230–400 mesh, 100 g) column with a solvent system of $CHCl_3$/MeOH (20:1 → 1:1, gradient system) to obtain eight fractions (A-H). Fraction B (1.4 g) was chromatographed on a RP-C_{18} silica gel column with 80% aqueous MeOH to give six subfractions (A1–A6). Fraction A1 (48 mg) was purified by semi-preparative HPLC (2 mL/min, 50% aqueous CH_3OH) to yield compounds **3** (2 mg), **4** (3 mg), and **5** (2 mg). Fraction B (4.2 g) was chromatographed on a RP-C_{18} silica gel column with 80% aqueous MeOH to give nine subfractions (B1–B9). Fraction B1 (270 mg) was separated by a Lobar-A RP-C_{18} column with 50% aqueous MeOH and further purified by semi-preparative HPLC (2 mL/min, 35% aqueous CH_3CN) to yield compound **1** (5 mg). Fraction B2 (120 mg) was separated by a Lobar-A RP-C_{18} column with 70% aqueous MeOH and further purified by semi-preparative HPLC (2 mL/min, 35% aqueous CH_3CN) to yield compound **2** (6 mg). Fraction B6 (137 mg) was purified by semi-preparative HPLC (2 mL/min, 90% aqueous CH_3OH) to yield compound **8** (3 mg). Fraction B8 (540 mg) was purified by semi-preparative HPLC (2 mL/min, 90% aqueous CH_3OH) to yield compounds **7** (3 mg), **9** (3 mg), and **11** (3 mg). Fraction C (1.0 g) was chromatographed on a RP-C_{18} silica gel column with 80% aqueous MeOH to give 5 subfractions (C1–C5). Fraction C4 (90 mg) was purified by semi-preparative HPLC (2 mL/min, 85% aqueous CH_3OH) to yield compound **6** (3 mg).

2.3.1. Chaenomin B (*1*)

Yellowish gum; $[\alpha]_D^{25}$ − 3.1 (*c* 0.30, $CHCl_3$); IR (KBr): ν_{max} = 3400, 2944, 2830, 1746, 1690, 1647, 1488, 1362, 1249, 1032, 758 cm^{-1}; UV (MeOH) λ_{max} (log ε) 205 (1.3), 262 (0.3), 288 (0.2) nm ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data, see Table 1; HR-FAB-MS (negative mode): *m/z* 453.1543 [M − H − H_2O]$^-$ (calcd. for $C_{25}H_{25}O_8$, 453.1549).

2.4. Cytotoxicity assessment

The cytotoxicity of the compounds against cultured human tumor cell lines was evaluated by the SRB method. The assays were performed at the Korea Research Institute of Chemical Technology. Each tumor cell line was seeded in standard 96-well flat-bottomed microplates and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. The attached cells were incubated with serially diluted samples. Following 48 h of continuous exposure to the compounds, the culture medium was removed, 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). Absorbance was measured spectrophotometrically at 520 nm using a microtiter plate reader. Etoposide (≥98%; Sigma Chemical Co., St. Louis, MO, USA) was used as a positive control. Etoposide showed IC_{50} values against A549 (non-small cell lung adenocarcinoma), SK-OV-3 (malignant ovarian ascites), SK-MEL-2 (skin melanoma), and XF498 (human CNS solid tumor) of 0.92, 1.75, 0.19, and 0.23 μM, respectively.

Table 1
^{1}H and ^{13}C NMR data of **1** in $CDCl_3$. (δ in ppm, 700 MHz for ^{1}H and 175 MHz for ^{13}C).[a]

Position	**1**	
	δ_H	δ_C
1		131.1
2	6.74 (d, 2.0)	111.3
3		144.2
4		132.1
5		148.5
6	6.64 (d, 2.0)	106.4
1′		123.4
2′		157.6
3′	6.46 (d, 2.0)	104.9
4′		160.5
5′	6.47 (dd, 8.0, 2.0)	99.2
6′	7.16 (d, 8.0)	131.2
1″		128.4
2″	6.88 (d, 1.8)	109.9
3″		147.1
4″		146.6
5″	6.88 (d, 8.0)	114.8
6″	6.90 (dd, 8.0, 1.8)	121.1
7″	4.92 (d, 8.1)	76.4
8″	3.98 m	78.5
9″	3.82 m, 3.51 m	61.7
5-OCH_3	3.84 s	56.3
2′-OCH_3	3.73 s	55.8
4′-OCH_3	3.77 s	55.6
3″-OCH_3	3.85 s	56.4

[a] *J* values are in parentheses and reported in Hz; the assignments were based on ^{1}H-^{1}H COSY, HSQC, and HMBC experiments.