Cytotoxic Caffeic Acid Derivatives from the Rhizomes of *Cimicifuga heracleifolia*

Soon-Ho Yim1,2, Hyun Jung Kim3, Si-Hwan Park4, Jinmi Kim4, Darren R. Williams4, Da-Woon Jung4, and Ik-Soo Lee1

1College of Pharmacy and Research Institute of Drug Development, Chonnam National University, Gwangju 500-757, Korea, 2GIST Technology Institute, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea, 3College of Pharmacy, Mokpo National University, Jeonnam 534-729, Korea, and 4New Drug Targets Laboratory, School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea

(Received January 20, 2012/Revised May 15, 2012/Accepted May 15, 2012)

Activity profiling of the *n*-BuOH extract from *Cimicifuga heracleifolia* rhizomes led to the identification of three cytotoxic caffeic acid derivatives, carboxymethyl isoferulate (2), cimicifugic acid A (3), and cimicifugic acid B (4) together with a series of structurally related inactive compounds. The extract was separated by time-based fractionation in a gradient HPLC condition, and cytotoxicity of each fraction was evaluated using HCT116 colon cancer cells in vitro. HPLC-hyphenated spectroscopy including LC/NMR and LC/PDA/MS provided structural information for phenolic compounds contained in the extract, and further preparative isolation of active compounds 2-4 was achieved by semi-preparative HPLC. Compounds 2-4 showed cytotoxic activity against cancer cells in a dose-dependent manner at the concentrations of 2.5-40 µM, and western blotting analysis showed that these compounds increased expression of cleaved poly ADP ribose polymerase (PARP), a critical apoptosis marker.

**Key words:** *Cimicifuga heracleifolia*, Activity profiling, Caffeic acid derivatives, Carboxymethyl isoferulate, Cimicifugic acid A, Cimicifugic acid B, Cytotoxicity, Poly ADP ribose polymerase (PARP)

INTRODUCTION

Classical bioassay-guided fractionation has been generally recognized as a tedious and laborious process in the study of natural products chemistry. In the past decade, more effective strategies for tracking biologically and pharmacologically active natural products have been developed, including the improvement of analytical technique, such as HPLC-coupled spectroscopy (Potterat and Hamburger, 2006, 2008). Activity profiling using HPLC has been known as an efficient, miniaturized approach which is directly applicable to mechanistic and cell-based assays (Danzet al., 2001; Potterat et al., 2004; Potterat and Hamburger, 2006; Kim et al., 2008).

In the present investigation for screening anticancer natural products, HPLC-based activity profiling was applied to an *n*-butanol extract from the rhizomes of *Cimicifuga heracleifolia*. Extracts from this plant have been traditionally used as antipyretic, analgesic, anti-inflammatory, and wound healing agents in Asia (Shibata et al., 1975, 1977; Kusano et al., 2001; Li and Yu, 2006). Previous studies suggested several biological effects of *Cimicifuga heracleifolia* extracts, such as anti-inflammation (Zhu et al., 2005), immune-regulation (Zhang et al., 2011), metabolite regulation (Hirabayashi et al., 1995), melanin synthesis inhibition (Sakai et al., 1999), and chondroprotection (Lee et al., 2004). However, anticancer effects and cytotoxic constituents of this plant species have not been extensively studied. With the aid of on-line structural information, HPLC-based activity profiling was carried out to find cytotoxic compounds through a cell-based bioassay using colon cancer cells. We finally obtained three active caffeic acid deriv-
tives; carboxymethylisoferulate (2), cimicifugic acids A (3) and B (4) together with structurally related inactive derivatives including piscidic acid (1), cimicifugic acids E (5) and F (6) (Fig. 1). Piscidic acid, carboxymethyl isoferulate, cimicifugic acids A, B, E, and F have been reported to show various biological activities such as relaxant action on smooth muscle (Noguchi et al., 1998), estrogenic activities (Kruse et al., 1999), and inhibition of neutrophil elastase activity (Loser et al., 2000).

We describe herein the application of activity profiling and structure-activity relationship (SAR) of caffeic acid derivatives obtained from *C. heracleifolia*. Anti-cancer effects of the isolated phenolics (1-6) were tested for anti-proliferative effects on human colon cancer cells (HCT116) in vitro. Western blotting was performed for assessment of cleaved PARP expression to determine apoptotic effects of the active phenolic compounds. Isolation, structural elucidation, and anticancer activity of caffeic acid derivatives from *C. heracleifolia* are reported in detail.

**MATERIALS AND METHODS**

**General experimental procedures**

LC/NMR was performed on a Varian VNMRS 600 MHz NMR spectrometer (1H: 600.006 MHz) hyphenated to a Varian ProStar HPLC system using a 150 μL triple-resonance microflow cryogenic probe. LC/PDA/MS was measured on a Varian 320-MS TQ mass spectrometer coupled to a Varian ProStar HPLC system in negative and positive electrospray ion (ESI) modes. Analytical HPLC was performed on an Agilent HP1100 series, which consists of a degasser, a binary mixing pump, a column oven and a PDA detector, using a Waters SunFire™ (4.6 × 150 mm, 5 μm) column. Semi-preparative HPLC was carried out on a Waters 600E multisolvent delivery system connected with a DECASSIT™ 6342 degasser, using Waters SunFire™ Prep C18 (10 × 250 mm and 19 × 150 mm, 5 μm) columns.

**Plant material**

Cimicifugae Rhizoma was purchased from herbal store, Hansol Pharm. Co., Ltd. in December, 2006. The specimen was identified by Prof. Dong Young Rhyu, Department of Medicinal Plant Resources, Mokpo National University, and deposited in GIST Technology Institute at the Gwangju Institute of Science and Technology (No. GTI Cimicifugae Rhizoma-2006-Bat. 001).

**Preparation of n-BuOH extract**

The dried rhizomes (3.0 kg) from *C. heracleifolia* were extracted with 80% MeOH at room temperature for three times, and the aqueous MeOH extract was concentrated at the reduced pressure. This extract (330.4 g) was suspended in H₂O and successively partitioned with n-hexane, EtOAc, n-BuOH. The n-BuOH layer was evaporated in vacuo to afford an extract (37.0 g) and stored at −20°C.

**LC/PDA/MS method**

HPLC was carried out with H₂O containing 0.1% HCOOH and MeCN. A gradient HPLC condition was as follows; 95% through 35% of H₂O containing 0.1% HCOOH for 35 min (total run time 45 min) at a flow rate of 1.0 mL/min. It was split into 95% for the UV detection (280 nm) and 5% for the MS detection.

**LC/NMR method**

One dimensional ¹H-NMR spectra were obtained both in the stopped-flow mode and continuous-flow mode. HPLC was performed with D₂O containing 0.1% HCOOH and MeCN. For the stopped-flow mode, a gradient HPLC was initiated with 95% of D₂O containing 0.1% HCOOH, followed by 35% of D₂O containing 0.1% HCOOH over 35 min (total run time 45 min), with a flow rate of 1.0 mL/min under 280 nm. The n-butanol extract (50 μL of 100 mg/mL) was injected on to a Waters SunFire™ C18 (4.6 × 150 mm, 5 μm) column. The standard WET1D sequence was used for the pre-saturation of ¹H frequency in the HOD, ACN, and HCOOH. Data were acquired with 9 kHz sweep width using 33 K time domain points with an acquisition time of 1.82 second. Variable numbers of scans (128-512) were used upon the relative concentration of the each compound within the probe