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In silico investigation of cycloartane triterpene derivatives from *Cimicifuga dahurica* (Turcz.) Maxim. roots for the development of potent soluble epoxide hydrolase inhibitors

Nguyen Phuong Thao[a,b,1], Jang Hoon Kim[c,1], Bui Thi Thuy Luyen[d], Nguyen Tien Dat[b], Young Ho Kim[a,*]

[a] College of Pharmacy, Chungnam National University, Daejeon 34134, Republic of Korea
[b] Institute of Marine Biochemistry (IMBC), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Caugiay, Hanoi, Vietnam
[c] Korea Atomic Energy Research Institute, Jeongeup, Jeollabuk-do 580-185, Republic of Korea
[d] Department of Pharmaceutical Industry, Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Hanoi, Vietnam

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ABSTRACT

In our search for natural soluble epoxide hydrolase (sEH) inhibitors from plants, we found that an ethanolic extract of the roots of *Cimicifuga dahurica* (Turcz.) Maxim. significantly inhibits sEH *in vitro*. A phytochemical study on the dichloromethane fraction of *C. dahurica* resulted in the isolation of two new cycloartane triterpenoids (**1** and **6**), together with 13 known cycloartane analogues (**2**–**5** and **7**–**15**). The structures of compounds were determined by spectroscopic methods. All of the triterpenoid derivatives inhibited sEH enzymatic activity in a concentration-dependent manner, and 13 of the tested compounds showed significant activity. Among them, compounds **1**, **3**, **5**, **7**, **9**, and **12** showed the highest levels of inhibitory activity, with IC_{50} values of about 5 μM or less. Kinetic analysis of compounds **1**, **3**, **5**–**9**, **11**, **12**, and **14** revealed that compounds **3**, **6**, **7**, **11**, and **14** were non-competitive; **1**, **5**, **9**, and **12** were mixed-type; and **8** was a competitive inhibitor. Furthermore, *in silico* molecular docking indicated that compounds **3**, **6**–**9**, **11**, **12**, and **14** bound to sEH in a similar manner and had stable binding energies, as calculated by AutoDock 4.2 and processed in a 10,000-ps molecular dynamics simulation to assess the binding stability of compounds **5**, **7**, and **9**.

1. Introduction

Soluble epoxide hydrolase (sEH) is a member of the epoxide hydrolase family and is found primarily in the cytosol and peroxisomes of mammalian tissues, including the liver, kidneys, intestine, and vascular tissues [1–3]. It catalyzes the hydrolysis of epoxyeicosatrienoic acids (EETs) into the corresponding dihydroxyeicosatrienoic acids (DHETs). Accumulating preclinical and epidemiological evidence suggests that the modulation of cytochrome P450 (CYP)-mediated eicosanoid metabolism is a novel therapeutic approach for treating chronic inflammatory and cardiovascular diseases [4]. Over the last several years, the epoxides of arachidonic acid or EETs have been established as lipid mediators with important biological functions. Inflammatory pain can be significantly reduced by increasing EET concentrations through exogenous delivery or by stabilizing EETs via inhibition of sEH. As EETs are converted into inactive DHETs by sEH, sEH inhibitors may be useful in treatment of these diseases [2]. Potent urea-based sEH inhibitors developed in a previous pharmacology study exhibited poor solubility and relatively short durations of action. Therefore, structurally novel compounds possessing sEH inhibitory activity are needed.

The genus *Cimicifuga* is one of the smallest genera in the family Ranunculaceae and consists of about 28 species distributed throughout East Asia, Europe, and North America [5,6]. The dried rhizomes and roots of most *Cimicifuga* species are utilized in traditional Korean, Chinese, and Japanese medicine by Native

Abbreviations: AUDA, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid; COSY, correlated spectroscopy; DHETs, dihydroxyeicosatrienoic acids; EETs, epoxyeicosatrienoic acids; HMBC, heteronuclear multiple bond connectivity; HMQC, heteronuclear multiple quantum coherence; NMR, nuclear magnetic resonance spectroscopy; MD, molecular dynamic; PHOME, 3-phenyl-cyano(6-methoxy-2-naphthalenyl)methyl ester-2-oxiraneacetic acid; sEH, soluble epoxide hydrolase; RMSF, root mean square fluctuation; RMSD, root mean square deviation; ROESY, rotating-frame nuclear overhauser effect correlation spectroscopy.

* Corresponding author.
E-mail address: yhk@cnu.ac.kr (Y.H. Kim).
[1] These authors contributed equally to this work.

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Americans, early American colonists, and in modern medicine [5]. *Cimicifuga dahurica* (Turcz.) Maxim., a species of Cimicifugae rhizome, has been used in cooling and detoxification as well as a antipyretic and analgesic agent for the treatment of some types of headache and toothache in Chinese folk medicine [7], and is included in the Chinese Pharmacopoeia [5,8]. Previous phytochemical investigations of *C. dahurica* led to the isolation of a series of 9,19-cycloartane triterpenes [6,9,10], which exerted growth inhibitory activities against several human tumor cell lines, including HL-60, SMMC-7721, A549, MCF-7, and SW-480 [11–13]. These compounds showed similar effects and induced apoptosis and G2/M cell cycle arrest of hepatoma HepG2 and leukemia HL-60 cell lines. Downregulated expression of Cdc2 and COX-2 contributed to apoptosis and cell cycle arrest in HepG2 cells [13]. However, the sEH inhibitory activity of components from *C. dahurica* is still unknown.

We have been engaged in ongoing efforts to identify new sEH inhibitors from medicinal plants [14,15]. *C. dahurica* has been the subject of extensive investigations of biologically active natural products. This paper describes the isolation and structural elucidation of two new cycloartane triterpenoids (**1** and **6**), together with 13 other known cycloartane analogues (**2**–**5** and **7**–**15**) from the roots of *C. dahurica* (Fig. 1, Section 2.5). We also examined the inhibitory activities of all isolated derivatives on sEH through *in vitro* and docking simulations to investigate interactions between the ligand and enzyme. Molecular dynamics simulations were performed with Gromacs 4.6.5.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were measured using a JASCO P-2000 polarimeter (JASCO, Oklahoma, OK, US). IR spectra were obtained on a Bruker TENSOR 37 FT-IR spectrometer (Bruker, Billerica, MA, USA). The ^{1}H and ^{13}C, HMQC, HMBC, ROESY, and COSY NMR spectra were recorded on JEOL JNM-AL 400 MHz and JEOL ECA 600 MHz spectrometer (JEOL, Peabody, MA, US), chemical shift (δ) are expressed in ppm with reference to the TMS signals. Gas chromatography (GC) spectra were recorded on a Shidmazu-2010 spectrometer (Shimadzu, Kyoto, Japan), SPB-1 capillary (30 m × 0.25 mm and 30 m × 0.32 mm); Mightysil RP-18 GP, Kanto Chemical, 10 × 250 mm. The electrospray ionization (ESI) and the high-resolution electrospray ionization mass spectrometer (HRESIMS) were operated in the positive-ion mode, with sodium iodide being used for mass calibration from an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Micromass, Wythenshawe, UK). Column chromatography (CC) was conducted using on 65–250 or 230–400 mesh silica gel (Sorbent Technologies, Atlanta, GA, USA), porous polymer gel (Diaion® HP-20, 20–60 mesh, Mitsubishi Chemical, Tokyo, Japan), Sephadex™ LH-20 (Supelco, Bellefonte, PA, USA), octadecyl silica (ODS, 50 μm, Cosmosil 140C_{18}-OPN, Nacalai Tesque), and YMC RP-C_{18} resins (30–50 μm, Fuji Silysia Chemical). Analytical thin layer chromatography (TLC) systems were performed on precoated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck) and compounds were visualized by spraying with 10% H_2SO_4 in water and then heating for 1.5–2 min. All procedures were carried out with solvents purchased from commercial sources that were used without further purification.

2.2. Plant material

The roots of *Cimicifuga dahurica* were purchased from the Herbal company, Naemome Dah, Ulsan, Korea in February 2016, and identified by Prof. Young Ho Kim, College of Pharmacy, Chungnam National University. A voucher specimen (CNU-16003) was deposited at the Herbarium of the College of Pharmacy, Chungnam National University.

2.3. Compounds

From the ethanolic extract of *C. dahurica*, 15 compounds (**1–15**) were isolated and structurally elucidated. Stock solutions of tested compounds in DMSO were prepared, kept at −20 °C, and diluted to the final concentration in fresh media before each experiment. For not to affect cell growth, the final DMSO concentration did not exceed 0.5% in all experiments.

2.4. Chemicals and reagents

PHOME, purified recombinant sEH, 14,15-EET, 14,15-DHET, and leukotoxindiol (+/−)9(10)-dihydroxy-octadec-12-enoic acid (9,10-DiHOME) were purchased from Cayman Chemical (Ann Arbor, MI)., AUDA was purchased from Cayman Chemical (Ann Arbor, MI). 6-Methoxy-2-naphtaldehyde (internal standard for fluorometric assays) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Bis-Tris and Greiner 96-well black plates were from Sigma-Aldrich (St. Louis, MO). Information about their isolation method, chemical structure, and purity provided in references listed in main text.

2.5. Extraction and isolation

The fresh roots of *C. dahurica* (2.5 kg) were cut into pieces and extracted with 95% aqueous EtOH (3 × 5.0 L) under ultrasonic agitation at 90 Hz and 40 °C. The ethanol solution was concentrated under vacuum and filtered through a Büchner funnel to give a brown extract (65.3 g), which was suspended in distilled H_2O and successively partitioned with *n*-hexane and CH_2Cl_2 to afford *n*-hexane (9.6 g, A), CH_2Cl_2 (15.2 g, B) fractions, and a H_2O layer (W). The CH_2Cl_2 (B) fraction exerted potent sEH inhibitory activity (75.33 ± 1.15% at a concentration of 25.0 ug/mL), which was greater than in the presence of the ethanol extract (68.02 ± 1.23%) at the same concentration. Thus, CH_2Cl_2 fraction of *C. dahurica* was chosen for further in continuing studies.

The CH_2Cl_2 fraction was partitioned by CC over silica gel and eluted with *n*-hexane-EtOAc (95:5, 80:20, 40:60, v/v) to produce seven fractions (Fractions, B-1–B-7). Fraction B-3 (0.8 g) was chromatographed by Sephadex® LH-20 CC eluted with MeOH-H_2O (95:5, 70:30, 50:50, v/v) to give three sub-fractions (Fractions, B-3.1–B-3.3) and further purified by YMC RP-C_{18} CC using acetone-H_2O (3:2) as the eluent to afford 24-*epi*-acerinol (**2**, 6.8 mg), acerionol (**4**, 4.8 mg), 24-*epi*-7,8-didehydrocimigenol (**11**, 8.9 mg), 25-triepoxy-12β-acetoxy-3β,26-dihydroxy-9,19-cyclolanost-7-ene (**14**, 10.2 mg), and 23-*O*-methylcimiacerogenin B (**15**, 3.5 mg), Next, fraction B-4 (1.1 g) was chromatographed over a silica gel CC eluted with *n*-hexane-EtOAc (2:1) to obtain 25-anhydrocimigenol (**5**, 3.1 mg), 25-anhydro-7,8-didehydrocimigenol (**6**, 3.8 mg), 7,8-didehydrocimigenol (**8**, 13.5 mg), and 24-*epi*-24-*O*-acetyl-7,8-didehydroshengmanol (**13**, 4.0 mg), Similarly, fraction B-5 (1.3 g) was separated by YMC RP-C_{18} CC using acetone-H_2O (2.5:1) and Sephadex™ LH-20 using CH_2Cl_2-MeOH (20:80) as eluent to give 25-*O*-acetyl-7,8-didehydrocimigenol (**7**, 5.6 mg) and 24-*epi*-24-*O*-acetyl-7,8-didehydroshengmanol (**12**, 3.0 mg). Next, fraction B-7 (1.1 g) was chromatographed on a column of Sephadex™ LH-20 and eluted with H_2O in MeOH, successively, to give three sub-fractions (Fractions, B-7.1–B-7.3). Sub-fraction B-7.1 (0.4 g) was separated by YMC RP-C_{18} CC, using MeOH-H_2O (5.5:1) as eluent, and further purified by over silica gel CC, eluted with CH_2Cl_2-acetone (4:1), to obtain 24-*epi*-25-*O*-acetylacerinol (**1**, 3.0 mg)