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Short communication

NO inhibitory constituents as potential anti-neuroinflammatory agents for AD from *Blumea balsamifera*

Jun Ma [a], Quanhui Ren [a], Bangjian Dong [a], Zhaoyu Shi [a], Jie Zhang [b], Da-Qing Jin [c], Jing Xu [a,*], Yasushi Ohizumi [d], Dongho Lee [e], Yuanqiang Guo [a,*]

[a] State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy, and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300350, People's Republic of China
[b] Key Laboratory for Green Processing of Chemical Engineering of Xinjiang Bingtuan, School of Chemistry and Chemical Engineering, Shihezi University, Shihezi 832003, People's Republic of China
[c] School of Medicine, Nankai University, Tianjin 300071, People's Republic of China
[d] Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
[e] Department of Biosystems and Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Republic of Korea

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ABSTRACT

Our continuous search for new nitric oxide (NO) inhibitory substances as anti-neuroinflammatory agents for AD resulted in the isolation of one new labdane diterpenoid and three new guaiane sesquiterpenoids, as well as ten known compounds from *Blumea balsamifera*. Their structures were elucidated by NMR spectroscopic data analysis and the time-dependent density functional theory (TDDFT) electronic circular dichroism (ECD) calculations. The anti-neuroinflammatory effects were examined by inhibiting NO release in LPS-induced murine microglial BV-2 cells. The possible mechanism of NO inhibition of some bioactive compounds was also investigated using molecular docking, which revealed the interactions of bioactive compounds with the iNOS protein.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder with a high incidence and has been regarded as a major threat to the health of elderly persons [1,2]. Patients with AD usually have characteristic symptoms, such as progressive cognitive decline, psychobehavior disturbances, and memory impairment, all of which worsen the life quality severely and bring about huge burden to both their families and the society [2,3]. In consideration of the severity, AD has been subjected to extensive studies and several drugs have been approved by FDA to treat this disease. However, the application of these drugs have only provided symptomatic treatment and improvement for AD patients [4,5]. Thus, to treat AD effectively, new therapeutical agents to prevent or/and treat are needed urgently. Although there have been several hypotheses on the etiology of AD, accumulating evidence has revealed that the initiation and progress of AD have a close relation to neuroinflammation and anti-neuroinflammatory compounds may be potentially useful for the treatment of AD [6–10].

Blumea balsamifera (L.) DC, belonging to the Asteraceae plant family, is a perennial herb or subshrub distributed mainly in Southeast Asia including China, Malaysia, Thailand, Vietnam, and Philippines [11]. This plant has a strong aromatic fragrance and its leaves have been used as a flavoring ingredient and a healthy tea for cough and gas pain [12,13]. In addition, the whole plant or its leaves have been used as a folk medicine to treat eczema, dermatitis, beriberi, lumbago, menorrhagia, rheumatism, and skin injury, and as an insecticide [12–14]. The major constituents of *B. balsamifera* have been reported to be sesquiterpenoids and flavonoids, which displayed antitumor, antifungal, plasmin inhibitory, anti-obesity, free-radical-scavenging, and nitric oxide (NO) inhibitory effects [13–24]. In our search for new NO inhibitory substances as anti-neuroinflammatory agents for AD [25], considerable attention has been given to the occurrence of bioactive compounds with anti-neuroinflammatory effects, since active compounds of this type are expected to be potentially useful for the treatment of neuroinflammation and related chronic neurodegenerative diseases [6,10]. In our ongoing phytochemical investigation to obtain new NO inhibitory compounds as potential

* Corresponding authors.
E-mail addresses: xujing611@nankai.edu.cn (J. Xu), victgyq@nankai.edu.cn (Y. Guo).

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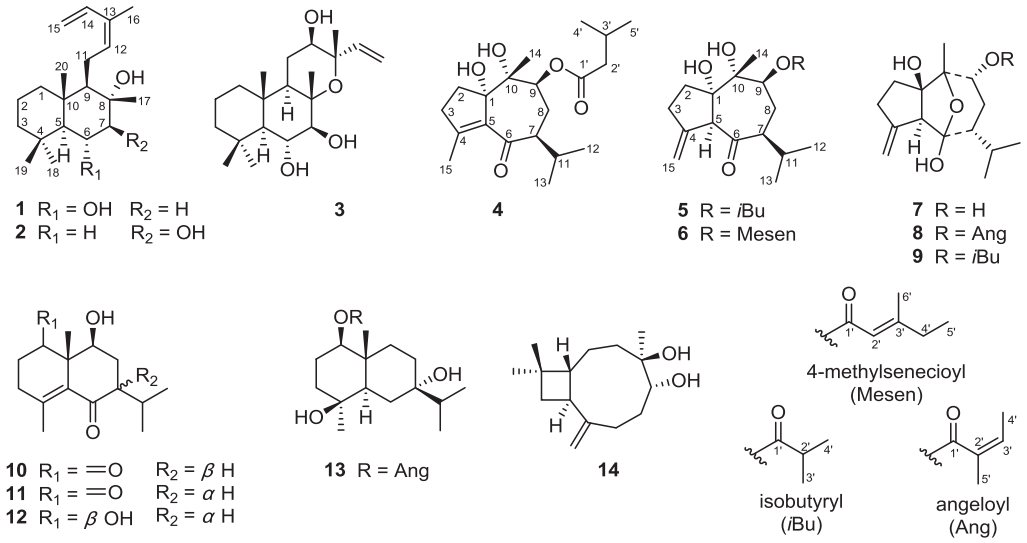

Fig. 1. Structures of compounds **1**–**14** from *B. balsamifera*.

anti-neuroinflammatory agents for AD, the plant *B. balsamifera* used both as a healthy tea and a traditional medicine, evoked our great interest.

Our purpose in this study was to investigate the chemical and biological profiles of *B. balsamifera* including the chemical structures, anti-neuroinflammatory activities, and interactions with the iNOS protein. In this study, one new labdane diterpenoid (**1**) and three new guaiane sesquiterpenoids (**4**–**6**), as well as ten known compounds (**2**, **3**, and **7**–**14**), were isolated from the aerial parts of *B. balsamifera* (Fig. 1). Their structures were established on the basis of extensive analysis of nuclear magnetic resonance (NMR) spectroscopic data and the time-dependent density functional theory (TDDFT) electronic circular dichroism (ECD) calculations. The biological peculiarities of anti-neuroinflammation, namely NO inhibitory effects, and the interactions of bioactive compounds with the iNOS protein were also investigated. Details of phytochemical investigation, NO inhibitory effects, and the binding affinities with the iNOS protein are described herein.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured in CH_2Cl_2 using an InsMark IP120 automatic polarimeter (Shanghai InsMark Instrument Technology Co., Ltd., Shanghai, People's Republic of China). Infrared (IR) spectra were taken on a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany) with KBr disks. One dimension (1D) and two dimension (2D) NMR spectra were recorded on a Bruker AV 400 instrument (Bruker Group, Fallanden, Switzerland, 400 MHz for ^1H and 100 MHz for ^{13}C) with tetramethylsilane (TMS) as an internal standard. Electrospray ionization mass spectrometry (ESIMS) spectra were acquired on a Thermo Finnigan LCQ-Advantage mass spectrometer (Finnigan Co., Ltd., San Jose, CA, USA). High resolution (HR)-ESIMS were recorded by ACQUITY UPLC I-Class MS (Waters Co., Ltd., Manchester, UK). High performance liquid chromatography (HPLC) purifications were performed on a P3000 pump (Beijing Chuangxintongheng Science & Technology Co., Ltd., Beijing, People's Republic of China), equipped with a Shodex RI-102 detector (Showa Denko Co., Ltd., Tokyo, Japan) and a YMC-pack ODS-AM (20 × 250 mm) column (YMC Co., Ltd., Kyoto, Japan). Medium pressure liquid chromatography (MPLC) was run on a P0100 pump with an ultraviolet (UV) detector (Huideyi Co., Beijing, People's Republic of China) and a column (40 × 400 mm) filled by octadecylsilyl (ODS, 50 μm, YMC Co., Ltd.). Thin-layer chromatography (TLC) was carried out with glass precoated silica gel GF_{254} plates (Yantai Jiangyou Silica Gel Development Co., Yantai, People's Republic of China). Spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH (1:9, v/v) followed by heating. Silica gel was used for column chromatography (100–200 mesh, Qingdao Haiyang Chemical Group Co., Ltd., Qingdao, People's Republic of China). Chemical reagents for isolation were of analytical grade and purchased from Tianjin Chemical Reagent Co. (Tianjin, People's Republic of China). The deuterated chloroform for NMR measurement was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Biological reagents were from Sigma-Aldrich Co. (St. Louis, MO, USA). The BV-2 cell line was from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, People's Republic of China).

2.2. Plant material

The aerial parts of *B. balsamifera* were purchased in July 2014 from Bozhou Materia Medica Market, Anhui province, People's Republic of China. The botanical identification was made by one of the authors (Y. Guo), and a voucher specimen (No. 20140715) was deposited at the laboratory of the Research Department of Natural Medicine, College of Pharmacy, Nankai University, People's Republic of China.

2.3. Extraction and isolation

The aerial parts of *B. balsamifera* (7.0 kg) were extracted with MeOH (3 × 56 L) under reflux. The organic solvent was evaporated to obtain a crude extract (700 g). This crude extract was suspended in H_2O (0.7 L) and then successively partitioned with petroleum ether (6 × 0.7 L) and ethyl-acetate (6 × 0.7 L) to give the petroleum ether-soluble portion (137 g) and ethyl-acetate-soluble portion (216 g).

The petroleum ether-soluble portion (137 g) was subjected to a silica gel column chromatography (silica gel, 1.0 kg; column, 9 × 70 cm), using a gradient of acetone in petroleum ether (100: 0, 100: 1, 100: 3, 100: 5, 100: 7, 100: 10, 100: 14, 100: 20, and 100: 30, 21 L for each gradient elution), to give eight fractions (F_1–F_8) based on TLC analysis. Fraction F_6 was subjected to MPLC over octadecylsilyl (ODS) eluting with a step gradient from 60 to 90%