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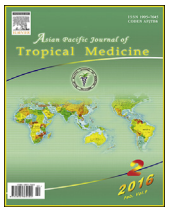

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Anti-Alzheimer's disease potential of coumarins from *Angelica decursiva* and *Artemisia capillaris* and structure-activity analysis

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ABSTRACT

Objective: To use structure-activity analysis to study the anti-Alzheimer's disease (anti-AD) activity of natural coumarins isolated from *Angelica decursiva* and *Artemisia capillaris*, along with one purchased coumarin (daphnetin).

Methods: Umbelliferone, umbelliferone 6-carboxylic acid, scopoletin, isoscopoletin, 7-methoxy coumarin, scoparone, scopolin, and esculetin have been previously isolated; however 2′-isopropyl psoralene was isolated from *Angelica decursiva* for the first time to evaluate their inhibitory effects against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and β-site amyloid precursor protein cleaving enzyme 1 (BACE1) enzyme activity. We scrutinized the potentials of coumarins as cholinesterase and BACE1 inhibitors via enzyme kinetics and molecular docking simulation.

Results: Among the test compounds, umbelliferone 6-carboxylic acid, esculetin and daphnetin exhibited potent inhibitory activity against AChE, BChE and BACE1. Both esculetin and daphnetin have a catechol group and exhibit significant anti-AD activity against AChE and BChE. In contrast, presence of a sugar moiety and methoxylation markedly reduced the anti-AD activity of the coumarins investigated in this study. With respect to BACE1 inhibition, umbelliferone 6-carboxylic acid, esculetin and daphnetin contained carboxyl or catechol groups, which significantly contributed to their anti-AD activities. To further investigate these results, we generated a 3D structure of BACE1 using Autodock 4.2 and simulated binding of umbelliferone 6-carboxylic acid, esculetin and daphnetin. Docking simulations showed that different residues of BACE1 interacted with hydroxyl and carboxylic groups, and the binding energies of umbelliferone 6-carboxylic acid, esculetin and daphnetin were negative (−4.58, −6.25 and −6.37 kcal/mol respectively).

Conclusions: Taken together, our results suggest that umbelliferone 6-carboxylic acid, esculetin and daphnetin have anti-AD effects by inhibiting AChE, BChE and BACE1, which might be useful against AD.

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1. Introduction

Alzheimer's disease (AD) is the major form of dementia and is a deadly neurodegenerative disease that is progressive in nature and develops through a multifactorial process. AD is the fourth leading cause of death in developed countries, and predominates in Europe and USA following cardiovascular disease, cancer, and stroke [1]. The two most common hypotheses used to describe the pathology of AD are known as the 'cholinergic hypothesis' and 'amyloid hypothesis'. The cholinergic hypothesis suggests that AD is caused by a deficiency in the brain levels of the cerebral neurotransmitter acetylcholine (ACh), which is hydrolyzed by acetylcholinesterase (AChE) [2]. Similarly, butyrylcholinesterase (BChE) activity is increased by 40%–90% during the progression of AD [3], and BChE inhibition is considered a potentially important aspect of treating AD. In addition to the AChE and BChE hypotheses, accumulation of amyloid-β peptide (Aβ) in the brain is widely considered to be critically involved in the pathogenesis of AD [4]. Aβ plaques emerge roughly 15 years before the symptoms of AD appear [5], and once AD develops the cognitive decline caused by neuronal damage cannot be reversed, even after Aβ levels in the brain are lowered by immunotherapy [6]. Thus, prevention of Aβ accumulation is considered an important part of preventing AD. Aβ is excised from amyloid-β precursor protein through sequential cleavage by aspartic protease β-secretase 1 (BACE1) [4]. Thus, because BACE1 initiates Aβ processing, inhibition of BACE1 activity may be an effective way to prevent Aβ accumulation [7].

Coumarins are an important class of natural compounds and are used as additives in both foods and cosmetics [8]. Coumarin has been reported to have antibacterial [9], anti-oxidant [10], anti-inflammatory and anticoagulant [11], and anti-AD activities [12]. Coumarins are characterized a fused benzene and α-pyrone ring that serves as the structural nucleus. Importantly, a number of studies have demonstrated the ability of coumarins to inhibit AChE [12–14], and the benzopyrone structural nucleus of coumarins is an essential aspect of hybrid molecules capable of simultaneously inhibiting AChE and AChE-induced β-amyloid aggregation [15]. Likewise, the possibility of numerous chemical substitutions afforded by the structural nucleus makes coumarins interesting molecules for drug discovery.

As part of our continuing efforts to identify compounds from natural resources that inhibit AChE, BChE, and BACE1, we recently found that the methanolic extract of *Angelica decursiva* (*A. decursiva*) (Umbelliferae) fulfills these criteria [16]. This plant is used traditionally as an anti-inflammatory, diuretic, expectorant, and diaphoretic, as well as a remedy for colds, influenza, hepatitis, arthritis, indigestion, coughs, chronic bronchitis, pleurisy, typhoid, headaches, flatulence, fever, colic, travel sickness, rheumatism, bacterial and fungal infections, and diseases of the urinary organs [17,18]. Furthermore, extensive investigations of different species of this genus have been carried out in the last decade, and as a result many classes of compounds have been isolated including different types of coumarin derivatives [19]. In addition, *Artemisia capillaris* (*A. capillaris*) is commonly distributed in sandy areas along the Korean coastline. This plant has been frequently used in the treatment of liver disease, including hepatitis, jaundice, fatty liver and bilious disorder. Infusions of the buds, stems and whole plant of *A. capillaris* have been used in traditional Chinese medicine primarily as a choleric, anti-inflammatory, antipyretic, and diuretic agent for treating epidemic hepatitis [20,21]. This study deals with the anti-AD activities and the structure–activity relationship of coumarins isolated from *A. decursiva* and *A. capillaris*. Specifically, because there is currently no detailed information regarding the molecular interactions between umbelliferone 6-carboxylic acid, esculetin, daphnetin and BACE1, we performed molecular docking analysis and detailed enzyme kinetic analysis in order to investigate the possibility of using compounds umbelliferone 6-carboxylic acid, esculetin and daphnetin as potent anti-AD drug candidates.

2. Materials and methods

2.1. General experimental procedures

The ^{1}H- and ^{13}C-NMR spectra were determined using a JEOL JNM ECP-400 spectrometer (Tokyo, Japan) at 400 MHz for ^{1}H and 100 MHz for ^{13}C in deuterated chloroform ($CDCl_3$). Column chromatography was conducted using silica gel 60 (70–230 mesh, Merck, Darmstadt, Germany). All TLC analyses were conducted using pre-coated Merck Kieselgel 60 F_{254} plates (20 cm × 20 cm, 0.25 mm) and using 50% H_2SO_4 as the spray reagent.

2.2. Chemicals and reagents

Electric-eel acetylcholinesterase (AChE, EC3.1.1.7), horse-serum butyrylcholinesterase (BChE, EC 3.1.1.8), acetyl thiocholine iodide (ACh), butyrylthiocholine chloride (BCh), 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB), quercetin, daphnetin and berberine were purchased from E. Merck, Fluka, or Sigma–Aldrich unless otherwise stated. The BACE1 FRET assay kit (β-secretase) was purchased from Pan Vera Co. (Madison, WI, USA). All chemicals and solvents used for column chromatography were of reagent grade, purchased from commercial sources, and used as received.

2.3. Isolation of coumarins

Umbelliferone, umbelliferone 6-carboxylic acid, scopoletin, isoscopoletin, 7-methoxy coumarin, scoparone, scopolin and esculetin were isolated from *A. decursiva* and *A. capillaris*, according to the method described by Zhao *et al.* [18] and Islam *et al.* [21], respectively. 2′-Isopropyl psoralene was isolated from subfraction-2 of dichloromethane fraction from *A. decursiva*, and identified by spectroscopic evidence including ^{1}H and ^{13}C–NMR, as well as by comparison with spectral published data [22].

2.4. In vitro ChE enzyme assay

The inhibitory activities of the isolated coumarins towards ChE were measured using the spectrophotometric method developed by Ellman *et al.* [23]. ACh and BCh were used as substrates to assay the inhibition of AChE and BChE, respectively. Each reaction mixture consisted of 140 μL sodium phosphate buffer (pH 8.0), 20 μL of test sample solution at a final concentration of 100 μM for all compounds, and 20 μL of either AChE or BChE solution, which were then combined and incubated for 15 min at room temperature. All test samples and positive control (berberine) were dissolved in