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Tryptophan-derived Alkaloids from *Hedera rhombea* Fruits and Their Butyrylcholinesterase Inhibitory Activity

Manh Tuan Ha[1,2], Se Eun Park[3], Jeong Ah Kim[4], Mi Hee Woo[1], Jae Sue Choi[3], and Byung Sun Min[1,*]

[1]*College of Pharmacy, Drug Research and Development Center, Daegu Catholic University, Gyeongbuk 38430, Republic of Korea*
[2]*College of Pharmacy, Thanh Do University, Hanoi, Vietnam*
[3]*Department of Food and Life Science, Pukyong National University, Busan 48513, Republic of Korea*
[4]*College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea*

Abstract – Alzheimer's disease (AD) is the most common age-related neurodegenerative disease in industrialized countries. It is estimated that about 47 million people living with dementia and the number of cases will be tripled by 2050. However, the exact mechanism of AD is not known, and full therapy has still not been found. Various tryptophan-derived alkaloids have been reported as promising agents for the treatment of AD. In the present study, a series of tryptophan-derived alkaloids were isolated and characterized from the methanol extract of *Hedera rhombea* fruit. Based on the analysis of their observed and reported spectroscopic data, their structures were identified as *N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan (**1**), *N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan (**2**), *N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (**3**), and *N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (**4**). These compounds were screened for anti-Alzheimer activity via their inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes *in vitro.* As a result, compounds **3** and **4** showed moderate BChE inhibition with IC_{50} values of 86.9 and 78.4 μM, respectively, compared to those of the positive control [berberine (IC_{50} = 11.5 μM)]. However, all four compounds did not show significant inhibition of the AChE enzyme. This is the first time, the AChE and BChE inhibitory activities of these tryptophan-derived alkaloids were investigated and reported.
Keywords – *Hedera rhombea*, Araliaceae, tryptophan-derived alkaloids, AChE, BChE, Alzheimer

Introduction

Alzheimer's disease (AD) is a chronic neurological disorder characterized by memory impairment, confusion, impaired judgment, personality change, disorientation, and loss of language skills.[1,2] AD affects about 40% of people over 80 and produces devastating problems for the AD patients themselves and a very high economic burden for their families and society.[3] It is estimated that about 47 million people living with dementia and the number of cases will be tripled by 2050, especially in developing countries.[4] Unfortunately, the exact mechanism of AD is not known, and full therapy has still not been found.[5] To date, there are about 20 hypotheses referring to this disease, where tau-protein aggregation, *β*-amyloid deposition, oxidative stress, and cholinergic dysfunction are the common ones.[4] Actually, most of the anti-AD drugs in clinical trials failed to demonstrate the disease-modifying ability to eliminate the accumulation of *β*-amyloid or tau-proteins in the brain.[6] According to the cholinergic hypothesis, low levels of acetylcholine (ACh) resulting from neuronal death are associated with cognitive and memory deterioration.[4] ACh is considered to play a role in the pathology of AD and is a neurotransmitter inhibited primarily by two types of cholinesterases (ChEs) including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE).[2] To date, the cholinergic hypothesis has been widely accepted, of which ChEs inhibitors increase the availability of acetylcholine in central cholinergic synapses and are considered the most effective Alzheimer's disease treatment agent.[7] However, four approved drugs for the management of AD symptoms include three AChE inhibitors (rivastigmine, donepezil, and galantamine) and N–methyl-D-aspartate receptor antagonist (memantine) that only provide palliative care and are of limited and

*Author for correspondence
Byung Sun Min, College of Pharmacy, Drug Research and Development Center, Daegu Catholic University, Gyeongbuk 38430, Republic of Korea.
Tel: +82-53-850-3613; E-mail: bsmin@cu.ac.kr

temporal efficacy.[2]

Hedera rhombea Bean, an evergreen vine, belongs to the Araliaceae family, which is widely distributed in Korea, China, and Japan.[8] *H. rhombea* has traditionally been used to treat hemorrhage, chronic catarrh, jaundice, lithiasis, and convulsion.[9] The previous phytochemical investigations of *H. rhombea* revealed that this plant contained megastigmane glucosides, neolignanes, saponins, polyacetylenes, quinic acid derivatives, triterpenoids, fatty acids, flavonoids, tryptophan-derived alkaloids, and phenolic compounds.[8,10] Our recent study demonstrated that the polyacetylene (falcarindiol) and tryptophan-derived alkaloids isolated from *H. rhombea* fruit act as potent dual α-glucosidase and PTP1B inhibitors.[10] In addition, tryptophan-derived alkaloids have been reported as promising agents for the treatment of AD. Various tryptophan metabolites modulate several metalloproteinases regulating brain *β*-amyloid peptide levels under normal and pathological conditions such as AD via interacting with the aryl hydrocarbon receptor.[3] Chalupova et al. reported that a combination of tacrine and tryptophan led to the development of a new family of heterodimers as multi-target agents with the potential to treat AD. Recently, a family of huprineY-tryptophan heterodimers displayed a potential inhibition of cholinesterase and neuronal nitric oxide synthase enzymes, with effect against *β*-amyloid and potential ability to cross the blood-brain barrier.[2] Therefore, in this study, with the series of tryptophan-derived alkaloids isolated from *H. rhombea* fruit, we set out to investigate their anti-AD activity via inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In addition, the structure-activity relationships in the AChE and BChE inhibitory assays were observed and discussed.

Experimental

General experimental procedures – Jasco P-1020 polarimeter was used to measure optical rotations. The NMR spectra were recorded using a Varian Unity Inova 400 MHz spectrometer and a Bruker 500 MHz spectrometer using TMS as the internal standard. The packing material for the molecular sieve column chromatography (CC) was Sephadex LH-20 (Pharmacia Company). Silica gel 60 (Merck, 230-400 mesh) and reversed-phase RP C-18 silica gel (Merck, 75 mesh) were used for CC. Thin-layer chromatography (TLC) was performed using Merck precoated silica gel F_{254} plates and RP C-18 F_{254s} plates, and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3-5 minutes.

Plant material – The fruits of *Hedera rhombea* Bean (Araliaceae) were collected from the Seogwipo-si of Jeju-do, Republic of Korea, and authenticated by Prof. Byung Sun Min, College of Pharmacy, Daegu Catholic University, Republic of Korea. The voucher specimen (CUD-2285-1) was deposited at the Herbarium of the College of Pharmacy, Daegu Catholic University, Republic of Korea.

Extraction and isolation – The dried powder of *H. rhombea* fruits (11 kg) was extracted with methanol (15 L× 4) under reflux and then filtered. The combined extracts were concentrated under reduced pressure to obtain a crude extract (4 kg), which was dissolved in water (3L) and then successively partitioned with CH_2Cl_2, EtOAc, and *n*-butanol to give CH_2Cl_2, EtOAc, and *n*-butanol fractions, respectively. The EtOAc fraction (85 g) was chromatographed on a silica gel column using a stepwise gradient of CH_2Cl_2–MeOH (100:1 to 0:1, v/v) to yield 17 fractions (E1–E17). Fraction E11 (12.5 g) was further fractionated by column chromatography (CC) on silica gel eluted with a mixture of CH_2Cl_2–MeOH (10:1, v/v) to yield 12 fractions (E11.1–E11.12). Fraction E11.4 (2.5 g) was subjected to Sephadex LH-20 CC eluted with a mixture of MeOH–H_2O (1:1, v/v) to yield eight fractions E11.4.1–E11.4.8. Fraction E11.4.6 (450 mg) was separated using an RP C-18 silica gel column eluted with a mixture of MeOH–H_2O (1:1, v/v) to yield compounds **1** (18.5 mg) and **2** (5.0 mg). Fraction E5 (1.3 g) was subjected to silica gel CC eluted with a mixture of CH_2Cl_2–MeOH (20:1, v/v) to yield nine fractions E5.1–E5.9. Fraction F5.3 (523 mg) was separated using a silica gel column with CH_2Cl_2–acetone (8:1, v/v) as the mobile phase to yield compound **3** (5.5 mg). Fraction E5.6 (0.6 g) was purified using an RP C-18 silica gel column and eluted with a mixture of MeOH–H_2O (1.5:1, v/v) to give compound **4** (7.0 mg).

***N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan (1)** – Yellow powder; $[\alpha]_D^{20}$ –17.5 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3380, 3100, 1710, 1646, 1580, 1510, 1430, 1280, 1206 cm^{-1}; UV (MeOH) λ_{max} (log ε) 290 (3.05), 310 (2.92) nm. ^1H NMR (500 MHz in methanol-d_4) δ (ppm): 7.57 (1H, d, J = 8.0 Hz, H-4), 7.42 (1H, d, J = 15.5 Hz, H-7′), 7.36 (2H, d, J = 8.0 Hz, H-2′, 6′), 7.30 (1H, d, J = 8.0 Hz, H-7), 7.08 (1H, s, H-2), 7.05 (1H, t, J = 8.0 Hz, H-5), 6.97 (1H, t, J = 8.0 Hz, H-6), 6.76 (2H, d, J = 8.0 Hz, H-3′, 5′), 6.44 (1H, d, J = 15.5 Hz, H-8′), 4.86 (1H, overlap, H-9), 3.40 (1H, dd, J = 14.5, 5.0 Hz, H-8a), 3.23 (1H, dd, J = 14.5, 8.0 Hz, H-8b); ^{13}C NMR (500 MHz in methanol-d_4) δ (ppm): 175.4 (C-10), 169.1 (C-9′), 160.7 (C-4′), 142.5 (C-7′), 138.1 (C-7a), 130.8 (C-2′, 6′), 129.0 (C-3a), 127.8 (C-1′), 124.5 (C-2), 122.5 (C-5), 119.9 (C-6), 119.4

(C-4), 116.8 (C-3′, 5′), 112.3 (C-7), 118.2 (C-8′), 111.2 (C-3), 55.0 (C-9), 28.8 (C-8).

***N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan (2)** – Yellow powder; $[\alpha]_D^{20}$ –18.3 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3370, 3110, 1710, 1650, 1586, 1520, 1418, 1280, 1210 cm^{-1}; UV (MeOH) λ_{max} (log ε) 290 (2.85), 318 (2.70) nm. ^{1}H NMR (500 MHz in methanol-d_4) δ (ppm): 7.61 (1H, d, J = 8.0 Hz, H-4), 7.40 (1H, d, J = 15.5 Hz, H-7′), 7.35 (1H, d, J = 8.0 Hz, H-7), 7.12 (1H, s, H-2), 7.10 (1H, t, J = 8.0 Hz, H-5), 7.03 (1H, t, J = 8.0 Hz, H-6), 7.00 (1H, d, J = 2.0 Hz, H-2′), 6.92 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.78 (1H, d, J = 8.0 Hz, H-5′), 6.44 (1H, d, J = 15.5 Hz, H-8′), 4.87 (1H, overlap, H-9), 3.44 (1H, dd, J = 14.5, 5.0 Hz, H-8a), 3.26 (1H, dd, J = 14.5, 8.0 Hz, H-8b); ^{13}C NMR (125 MHz in methanol-d_4) δ (ppm): 175.4 (C-10), 169.2 (C-9′), 148.9 (C-4′), 146.8 (C-3′), 142.9 (C-7′), 138.1 (C-7a), 129.0 (C-3a), 128.4 (C-1′), 124.5 (C-2), 122.5 (C-5), 122.3 (C-6′), 119.9 (C-6), 119.5 (C-4), 118.2 (C-8′), 116.6 (C-5′), 115.3 (C-2′), 112.3 (C-4), 111.2 (C-3), 55.0 (C-9), 28.8 (C-8).

***N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (3)** – Yellow powder; $[\alpha]_D^{20}$ –19 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3386, 3110, 1714, 1650, 1586, 1510, 1420, 1270, 1210 cm^{-1}; UV (MeOH) λ_{max} (log ε) 290 (2.65), 320 (2.55) nm ^{1}H NMR (400 MHz in methanol-d_4) δ (ppm); 7.51 (1H, d, J = 8.0 Hz, H-4), 7.44 (1H, d, J = 15.5 Hz, H-7′), 7.33 (2H, d, J = 8.0 Hz, H-2′, 6′), 7.32 (1H, d, J = 8.0 Hz, H-7), 7.05 (1H, s, H-2), 7.07 (1H, t, J = 8.0 Hz, H-5), 6.99 (1H, t, J = 8.0 Hz, H-6), 6.76 (2H, d, J = 8.0 Hz, H-3′, 5′), 6.45 (1H, d, J = 15.5 Hz, H-8′), 4.87 (1H, overlap, H-9), 3.60 (3H, s, -OCH_3-10), 3.34 (1H, dd, J = 14.5, 5.0 Hz, H-8a), 3.23 (1H, dd, J = 14.5, 8.0 Hz, H-8b); ^{13}C NMR (100 MHz in methanol-d_4) δ (ppm): 174.0 (C-10), 168.9 (C-9′), 160.5 (C-4′), 142.5 (C-7′), 138.7 (C-7a), 130.6 (C-2′, 6′), 128.6 (C-3a), 127.5 (C-1′), 124.3 (C-2), 122.4 (C-5), 119.8 (C-6), 119.1 (C-4), 116.6 (C-3′, 5′), 112.3 (C-7), 117.7 (C-8′), 110.6 (C-3), 55.0 (C-9), 52.6 (-OCH_3-10), 28.6 (C-8).

***N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (4)** – Yellow powder; $[\alpha]_D^{20}$ –21.5 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3380, 3100, 1710, 1646, 1580, 1520, 1420, 1274, 1200 cm^{-1}; UV (MeOH) λ_{max} (log ε) 292 (2.80), 308 (2.68) nm ^{1}H NMR (400 MHz in methanol-d_4) δ (ppm): 7.52 (1H, d, J = 8.0 Hz, H-4), 7.38 (1H, d, J = 15.5 Hz, H-7′), 7.30 (1H, d, J = 8.0 Hz, H-7), 7.07 (1H, t, J = 8.0 Hz, H-5), 7.06 (1H, s, H-2), 6.99 (1H, t, J = 8.0 Hz, H-6), 6.99 (1H, d, J = 2.0 Hz, H-2′), 6.88 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.75 (1H, d, J = 8.0 Hz, H-5′), 6.42 (1H, d, J = 15.5 Hz, H-8′), 4.87 (1H, overlap, H-9), 3.64 (3H, s, -OCH_3-10), 3.34 (1H, dd, J = 14.5, 5.0 Hz, H-8a), 3.24 (1H, dd, J = 14.5, 8.0 Hz, H-8b); ^{13}C NMR (100 MHz in methanol-d_4) δ (ppm): 174.1 (C-10), 169.0 (C-9′), 148.8 (C-4′), 146.6 (C-3′), 142.9 (C-7′), 138.0 (C-7a), 128.7 (C-3a), 128.1 (C-1′), 124.3 (C-2), 122.4 (C-5), 122.2 (C-6′), 119.8 (C-6), 119.1 (C-4), 117.7 (C-8′), 116.9 (C-5′), 115.1 (C-2′), 112.3 (C-4), 110.7 (C-3), 55.0 (C-9), 52.7 (-OCH_3-10), 28.6 (C-8).

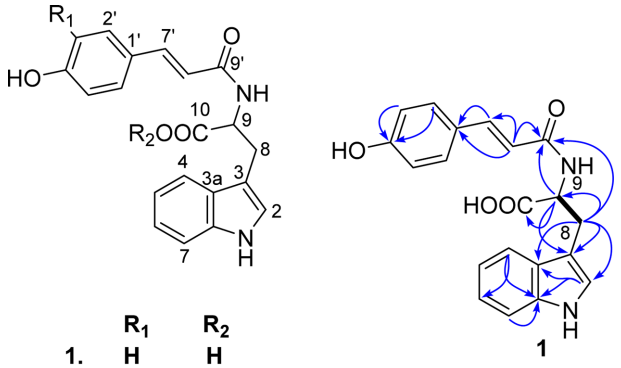
AChE and BChE inhibition assays – The AChE and BChE inhibition assays were performed as described previously.[12] ACh and BCh were used as substrates to examine the inhibition of AChE and BChE, respectively. All tested samples and the positive control (berberine) were dissolved in 10% analytical grade dimethyl sulfoxide (DMSO). The solution added with 100 mM sodium phosphate buffer (pH 8.0) 140 μL, each sample 20 μL, and AChE (0.36 U) or BChE (0.36 U) 20 μL, respectively, in 96 well plates, was incubated for 15 min at room temperature, and then the reaction mixture was adjusted to 200 μL adding 10 μL DTNB [5,5'-dithiobis(2- nitrobenzoic acid)] and the substrate, 10 μL of ACh or BCh, in 96-well plate. After 15 min, the yellow 5-thio-2-nitrobenzoate anion formed by the reaction between DTNB and thiocholine resulting from the hydrolysis of ACh or BCh was measured at 412 nm. The reaction was performed in triplicate and recorded in 96-well microplates using a microplate reader [VERSA max (Molecular Devices, Sunnyvale, CA, USA)]. The cholinesterase inhibition activity was expressed as the IC_{50} value (μg/mL) which is the concentration inhibiting the hydrolysis of ACh and BCh by 50%. The cholinesterase inhibition rate (%) was calculated by the following equation: % ChE inhibition = (1 – S / E) × 100, where E and S were the enzyme activities with and without the tested samples, respectively.

Statistics – All results are expressed as the mean ± standard error of the mean (SEM) of at least four independent experiments. Statistical significance was analyzed using one-way ANOVA and Duncan's test (Systat Inc., Evanston, IL, USA), and was noted at $p < 0.05$.

Results and discussion

The dried powder of *H. rhombea* fruit was extracted using MeOH to obtain a crude extract, which was suspended in distilled water and then partitioned successively with CH_2Cl_2, EtOAc, and *n*-butanol. The EtOAc fraction was separated over repeated CC (silica gel, RP C-18, and Sephadex LH-20) to give four tryptophan-derived alkaloids (**1**–**4**) (Fig. 1).

N-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan (**1**) was obtained as a yellow powder with a negative optical



	R_1	R_2
1.	H	H
2.	OH	H
3.	H	CH_3
4.	OH	CH_3

Fig. 1. Chemical structures of tryptophan-derived alkaloids **1**–**4**, and key HMBC correlations of **1**.

rotation. The UV spectrum of **1** showed absorption maxima at 290 and 310 nm. The IR spectrum of **1** exhibited typical absorption bands at 3380 (OH), 3100 (NH), 1710 (C=O), 1650 and 1580 (amido), 1510 (CH in benzene), and 1430 (CN in indole ring) cm^{-1}. The ^1H and ^{13}C NMR spectra of **1** revealed the presence of one tryptophan moiety including signals of one 1,2-disubstituted benzene ring [δ_H/δ_C 7.57 (1H, d, $J = 8.0$ Hz, H-4)/119.4, 7.30 (1H, d, $J = 8.0$ Hz, H-7)/112.3, 7.05 (1H, t, $J = 8.0$ Hz, H-5)/122.5, 6.97 (1H, t, $J = 8.0$ Hz, H-6)/119.9], one sp^3 methine [δ_H/δ_C 4.86 (1H, overlap, H-9)/55.0], one sp^2 methine [δ_H/δ_C 7.08 (1H, s, H-2)/124.5], one methylene group [δ_H/δ_C 3.40 (1H, dd, $J = 14.5$, 5.0 Hz, H-8a), and 3.23 (1H, dd, $J = 14.5$, 8.0 Hz, H-8b)/28.8], one carbonyl group [δ_C 175.4 (C-10)], and three quaternary carbons [138.1 (C-7a), 129.0 (C-3a), and 111.2 (C-3)].[13] This observation was confirmed by the HMBC correlations from H-2 (δ_H 7.08) to C-3a (δ_C 129.0)/ C-7a (δ_C 138.1), from H_2-8 (δ_H 3.40 and 3.23) to C-3a (δ_C 129.0)/C-3 (δ_C 111.2)/ C-2 (δ_C 125.2), and from H-9 (δ_H 4.87) to C-10 (δ_C 175.4)/C-3 (δ_C 111.2) (Fig.1). In addition, typical ^1H and ^{13}C NMR signals of one *p*-coumaroyl group at δ_H 7.42 (1H, d, $J = 15.5$ Hz, H-7′), 7.36 (2H, d, $J = 8.0$ Hz, H-2′, 6′), 6.76 (2H, d, $J = 8.0$ Hz, H-3′, 5′), and 6.44 (1H, d, $J = 15.5$ Hz, H-8′) and δ_C 169.1 (C-9′), 160.7 (C-4′), 142.5 (C-7′), 130.8 (C-2′, 6′), 127.8 (C-1′), 116.8 (C-3′, 5′), and 118.2 (C-8′) were observed in the ^1H and ^{13}C NMR spectra of **1**. The key HMBC cross-peak from H-9 (δ_H 4.87) to C-9′ (δ_C 169.1) indicated that one amide moiety was formed between the *p*-coumaric acid and the tryptophan. Based on the above analysis and the comparison with literature data,[13] the structure of **1** was determined as *N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan (**1**).

N-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan (**2**) was obtained as a yellow powder. The ^1H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the replacement of the *p*-coumaroyl group in **1** by one caffeoyl group at δ_H 7.40 (1H, d, $J = 15.5$ Hz, H-7′), 7.00 (1H, d, $J = 2.0$ Hz, H-2′), 6.92 (1H, dd, $J = 8.0$, 2.0 Hz, H-6′), 6.78 (1H, d, $J = 8.0$ Hz, H-5′), and 6.44 (1H, d, $J = 15.5$ Hz, H-8′); δ_C 169.2 (C-9′), 148.9 (C-4′), 146.8 (C-3′), 142.9 (C-7′), 128.4 (C-1′), 122.3 (C-6′), 118.2 (C-8′), 116.6 (C-5′), and 115.3 (C-2′). Thus, The intensive analysis of the 1D and 2D NMR data of **2** including COSY, HMQC, and HMBC analyses led to the elucidation of the structure of **2** was determined to be *N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan (**2**, caffeoyltryptophan).[14]

N-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (**3**) was obtained as a yellow powder. The ^1H and ^{13}C NMR spectra of **3** closely resembled those of **1**, except for the additional signals of one methoxy group at δ_H/δ_C 3.60 (3H, s, -OCH_3-10)/52.6. The HMBC correlation from OCH_3-10 (δ_H 3.60) to C-10 (δ_C 174.0) indicated that the methoxy group was attached at C-10. The intensive analysis of the 1D and 2D NMR data of **3** including COSY, HMQC, and HMBC analyses led to the elucidation of the structure of **3** as *N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (**3**).[15] Similar like that, the structure of compound **4** [*N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester] was similar to that of **2** except for the additional methoxy group at C-10.[15]

The anti-AD potential of the isolated tryptophan-derived alkaloids (**1-4**) was evaluated by their inhibition of AChE and BChE using ACh and BCh as substrates, respectively, and the results are expressed as IC_{50} values (Table 1). As a result, compounds **3** and **4** showed moderate BChE inhibition with IC_{50} values of 86.9 and 78.4 μM, respectively, compared to those of the positive control [berberine ($IC_{50} = 11.5$ μM)]. However, all four compounds (**1-4**) did not show significant inhibition of AChE enzyme. Similar structure-activity relationships were observed in the BChE assay for compounds **1-4**. The structures of compounds **3** and **4** were significantly different from compounds **1** and **2** in the ester group (R_2OOC) at position 10 (Fig. 1). Compounds **3** and **4** showed BChE inhibition while compounds **1** and **2** did not. This observation suggested that the presence of the ester group (R_2OOC) was needed for the BChE inhibition of the tryptophan-derived alkaloids **1-4**.

In the previous reports, some plant extracts or compounds showed potent and selective inhibitory activity of BChE.[1,5] AChE inhibition currently is the most used therapeutic treatment for the symptoms of AD.[5] However,

Table 1. Cholinesterase inhibitory activities of the tryptophan-derived alkaloids **1**-**4** isolated from *H. rhombea* fruit

Compounds	AChE[a]	BChE[a]
	IC_{50} (μM ± SEM)	IC_{50} (μM ± SEM)
1	> 100	> 100
2	> 100	> 100
3	> 100	86.91 ± 1.70
4	> 100	78.47 ± 1.86
Berberine[a]	0.60 ± 0.01	11.50 ± 0.37

[a]Positive control.

not only AChE participates in the cholinergic regulation of the central nervous system in humans, but also BChE, which can hydrolyze Ach. In severe AD, levels of AChE and choline acetyltransferase are decreased by as much as 90% compared with normal, while the level of BChE increases.[16] This fact has suggested that BChE may be a new approach to affecting the progression of AD.[17] Based on our study as well as the previously reported anti-AD activity of tryptophan-derived alkaloids,[3,11] the synthesis of novel tryptophan-derived alkaloids with dual inhibitory effects on AChE and BChE is an attractive and necessary research direction.

In conclusion, four tryptophan-derived alkaloids including *N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan (**1**), *N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan (**2**), *N*-[4′-hydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (**3**), and *N*-[3′,4′-dihydroxy-(*E*)-cinnamoyl]-L-tryptophan methyl ester (**4**) were isolation from *H. rhombea* fruit. Their anti-AD potential was investigated via inhibiting of AChE and BChE. Compounds **3** and **4** showed moderate BChE inhibition, while all these tryptophan-derived alkaloids did not show significant inhibition of AChE enzyme. To the best of our knowledge, this is the first investigation of the AChE and BChE inhibitory activities of tryptophan-derived alkaloids isolated from *H. rhombea* fruit.

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References

(1) Orhan, I.; Şener, B.; Choudhary, M.; Khalid, A. *J. Ethnopharmacol.* **2004**, *91*, 57-60.
(2) Mezeiova, E.; Hrabinova, M.; Hepnarova, V.; Jun, D.; Janockova, J.; Muckova, L.; Prchal, L.; Kristofikova, Z.; Kucera, T.; Gorecki, L.; Chalupova, K.; Kunes, J.; Hroudova, J.; Soukup, O.; Korabecny, J. *Bioorg. Med. Chem. Lett.* **2021**, *43*, 128100.
(3) Maitre, M.; Klein, C.; Patte-Mensah, C.; Mensah-Nyagan, A. G. *Prog. Neurobiol.* **2020**, *190*, 101800.
(4) Khaw, K. Y.; Murugaiyah, V.; Khairuddean, M.; Tan, W. N. *Nat. Prod. Sci.* **2018**, *24*, 88-92.
(5) Heo, J. H.; Eom, B. H.; Ryu, H. W.; Kang, M. G.; Park, J. E.; Kim, D. Y.; Kim, J. H.; Park, D.; Oh, S. R.; Kim, H. *Sci. Rep.* **2020**, *10*, 21695.
(6) Aguzzi, A.; O'connor, T. *Nat. Rev. Drug Discov.* **2010**, *9*, 237-248.
(7) Hung, T. M.; Thuong, P. T.; Nhan, N. T.; Mai, N. T. T.; Quan, T. L.; Choi, J. S.; Woo, M. H.; Min, B. S.; Bae, K.;H. *Nat. Prod. Sci.* **2011**, *17*, 108-112.
(8) Yamazoe, S.; Hasegawa, K.; Shigemori, H. *Phytochemistry.* **2007**, *68*, 1706-1711.
(9) Kim, K. S.; Choi, Y. H.; Lee, I. R.; Kwon, B. M.; Lee, S. H.. *Arch. Pharm. Res.* **1997**, *20*, 191-193.
(10) Ha, M. T.; Lee, T. H.; Kim, C. S.; Prajapati, R.; Kim, J. A.; Choi, J. S.; Min, B. S. *Phytochemistry* **2022**, *197*, 113100.
(11) Chalupova, K.; Korabecny, J.; Bartolini, M.; Monti, B.; Lamba, D.; Caliandro, R.; Pesaresi, A.; Brazzolotto, X.; Gastellier, A. J.; Nachon, F.; Pejchal, J.; Jarosova, M.; Hepnarova, V.; Jun, D.; Hrabinova, M.; Misik, J.; Muckova, L.; Jost, P.; Soukup, O.; Benkova, M.; Setnicka, V.; Habartova, L.; Ulissi, E.; Valis, M.; Nepovimova, E.; Bolognesi, M. L.; Kuca, K. *Eur. J. Med. Chem.* **2019**, *168*, 491-514.
(12) Nugroho, A.; Choi, J. S.; Seong, S. H.; Song, B. M.; Park, K. S.; Park, H. J. *Nat. Prod. Sci.* **2018**, *24*, 259-265.
(13) Murata, M.; Okada, H.; Homma, S. *Biosci. Biotech. Biochem.* **1995**, *59*, 1887-1890.
(14) Morishita, H.; Takai, Y.; Yamada, H.; Fukuda, F.; Sawada, M.; Iwahashi, H.; Kido, R. *Phytochemistry* **1987**, *26*, 1195-1196.
(15) Berti, F.; Navarini, L.; Colomban, S.; Forzato, C. *Molecules* **2020**, *25*, 1704.
(16) Giacobini, E. *Int. J. Geriatr. Psychiatry.* **2003**, *18*, S1-S5.
(17) Kulhánková, A.; Cahlíková, L.; Novák, Z.; Macáková, K.; Kuneš, J.; Opletal, L. *Chem. Biodivers.* **2013**, *10*, 1120-1127.

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