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β-Secretase (BACE-1) inhibitory effect of biflavonoids

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ABSTRACT

Here, we describe amentoflavone-type biflavonoids, which were isolated from natural sources and were found to inhibit β-secretase (BACE-1). The structure–activity relationship was studied, and compounds **1**–**8**, **10**, **17**, and **18** showed BACE-1 inhibitory activity. Among these compounds, 2,3-dihydroamentoflavone **17** and 2,3-dihydro-6-methylginkgetin **18** exhibited potent inhibitory effects with IC_{50} values of 0.75 and 0.35 μM, respectively.

The most common form of dementia is Alzheimer's disease (AD), which now affects over 30 million people worldwide.[1] AD is a neurodegenerative disorder characterized by accumulation and deposition of amyloid β (Aβ) peptides, which are generated from the cleavage of the β-amyloid precursor protein (APP) by consecutive action of β-secretase (BACE-1: β-site APP cleaving enzyme-1) and γ-secretase.[2–4] γ-Secretase affects the Notch cleavage, while β-secretase demonstrates no compensatory mechanism for APP cleavage.[5] The young BACE knockout mice were found to be healthy and fertile.[5] Hence, the discovery of a BACE-1 inhibitor could be an effective and safe therapeutic strategy for AD.

Biflavonoids are well known as constituents of gymnospermous plants and are flavonoid dimers connected by C–C or C–*O*–C bonds. Recently, these plants were found to exhibit anti-influenza,[6,7] anti-inflammatory,[8] and anti-malarial[9] activities.

In this Letter, we report the isolation of biflavonoids from a variety of plants and study their BACE-1 inhibitory activities and structure–activity relationships.

Acetone or $CHCl_3$ extracts of a variety of plants were subjected to silica gel column chromatography, Sephadex LH-20 column chromatography, and HPLC to afford compounds **1**–**21**. All isolated biflavonoids were identified on the basis of their spectroscopic data as well as by comparison with published data. Compounds **1**–**18** were amentoflavone-type biflavonoids with the flavonoid moieties connected by a C3′–C8″ bond. Among them, **17** and **18** were 2,3-dihydro structures (Fig. 1). Amentoflavone **1** and sequoiaflavone **2** were isolated from *Cunninghamia lanceolata*,[10,11] bilobetin **3**, ginkgetin **6**, 7,7″,4′-tri-*O*-methylamentoflavone **12**, sciadopitysin **13**, amentoflavone-7,7″,4′,4‴-tetramethyl ether **16** and 2,3-dihydro-6-methylginkgetin **18** from *Cephalotaxus harringtonia* var. *fastigiata*,[12–14] amentoflavone-7,7″-dimethyl ether **7** from *Cephalotaxus harringtonia* var. *harringtonia*,[15] sotetsuflavone **4**, 4′,7″-di-*O*-methylamentoflavone **9** and kayaflavone **15** from *Torreya nucifera*,[16–18] podocarpusflavone A **5**, podocarpusflavone B **8** and isoginkgetin **10** from *Podocarpus macrophyllus* var. *macrophyl-*

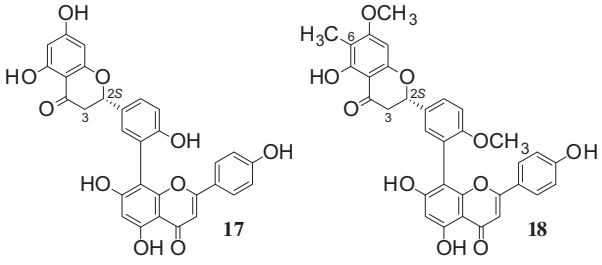

Figure 1. Structures of 2,3-dihydroamentoflavone **17** and 2,3-dihydro-6-methylginkgetin **18**.

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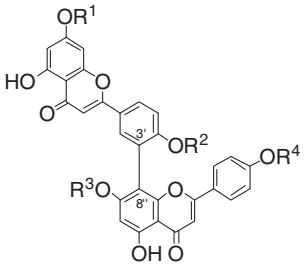
lus,[19–21] 7″,4‴-dimethylamentoflavone 11 and heveaflavone **14** from *Hevea brasiliensis*,[15,22] and 2,3-dihydroamentoflavone **17** from *Cycas revoluta*[10] (Table 1). Robustaflavone **19** with the flavonoid moieties connected by a C3′–C6″ bond was isolated from *Selaginella moellendorffii*,[19] cupressuflavone **20**, with the flavonoid moieties connected by a C8–C8″ bond was isolated from *Cupressus macrocarpa 'Goldcrest'*,[23] and hinokiflavone **21** with the flavonoid moieties connected by a C4′–*O*–C6″ bond was isolated from *Metasequoia glyptostoboides*[20,24] (Fig. 2).

Compounds **1**–**21** were all tested using the BACE-1 FRET assay kit.[25] Several amentoflavone-type biflavonoids showed inhibitory activity, whereas robustaflavone **19**, cupressuflavone **20**, and hinokiflavone **21** did not. Amentoflavone **1** and its monomethoxy analogues **2**–**5** showed strong inhibitory activity with IC_{50} values of 1.54, 1.40, 2.02, 1.58, and 0.99 μM, respectively. Compounds **6**–**8** and **10** showed lower activities than **1**–**5** with IC_{50} values of 4.18, 6.25, 4.21, and 3.01 μM, respectively. The dimethoxy compounds **9** and **11**, trimethoxy compounds **12**–**15**, and tetramethoxy compound **16** exhibited no inhibitory activity. Compound **17**, a 2,3-dihydro analogue of **1**, showed an increase in inhibitory activity, while compound **18** showed the strongest inhibitory activity of BACE-1 among amentoflavone-type biflavonoids (Table 2).

These results indicate that the amentoflavone-type biflavonoids consisting of two apigenin molecules linked at the C3′–C8″ position are important for BACE-1 inhibitory activity. The data also suggest that more than two hydroxyl groups at the R^1–R^4 position are needed for inhibitory activity. The results with compounds **17** and **18** show that the presence of a flavanone moiety in the amentoflavone biflavonoid is advantageous for inhibitory activity. Moreover, the presence of a methyl at the C6 position increases the inhibitory effect.

Some amentoflavone-type biflavonoids exhibited neuroprotective effects on oxidative stress-induced and amyloid β peptide-induced cell death in neuronal cells.[26] In addition, we found that amentoflavone-type biflavonoids have significant BACE-1 inhibitory activity. These results suggest that amentoflavone-type biflavonoids could be multiple targets for the development of novel therapeutic strategies for Alzheimer's disease.

Table 1
Structures of amentoflavone-type biflavonoids **1**–**16**



Compounds **1–16**	R^1	R^2	R^3	R^4
1	H	H	H	H
2	CH_3	H	H	H
3	H	CH_3	H	H
4	H	H	CH_3	H
5	H	H	H	CH_3
6	CH_3	CH_3	H	H
7	CH_3	H	CH_3	H
8	CH_3	H	H	CH_3
9	H	CH_3	CH_3	H
10	H	CH_3	H	CH_3
11	H	H	CH_3	CH_3
12	CH_3	CH_3	CH_3	H
13	CH_3	CH_3	H	CH_3
14	CH_3	H	CH_3	CH_3
15	H	CH_3	CH_3	CH_3
16	CH_3	CH_3	CH_3	CH_3

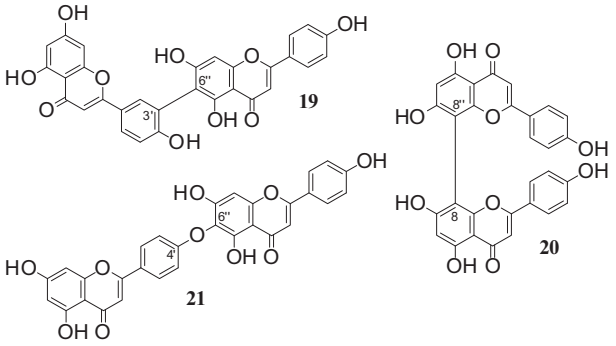


Figure 2. Structures of robustaflavone **19**, cupressuflavone **20** and hinokiflavone **21**.

Table 2
BACE-1 inhibitory assay results for compounds **1–21**

Compound	BACE-1 inhibition IC_{50} (μM)
1	1.54
2	1.40
3	2.02
4	1.58
5	0.99
6	4.18
7	6.25
8	4.21
9	>10.0
10	3.01
11	>10.0
12	>10.0
13	>10.0
14	>10.0
15	>10.0
16	>10.0
17	0.75
18	0.35
19	>10.0
20	>10.0
21	>10.0
β-Secretase inhibitor	0.07

Acknowledgments

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25. BACE-1 assays were performed on 384-well black plates using a BACE-1 FRET assay kit (Invitrogen Co., USA). The assay was carried out according to the supplied manual with modifications. Samples were dissolved in the assay buffer (50 mM sodium acetate, pH 4.5) with DMSO (final concentrations were 10%). 10 μL of test samples, 10 μL of BACE-1 substrate (750 nM Rh-EVNLDAEFK-Quencher, in 50 mM ammonium bicarbonate), and ten microlitre of BACE-1 enzyme (1.0 U/mL) were mixed in the wells, and incubated 60 min in the dark at 25 °C. The fluorescence intensities of the mixtures were measured by fluoroskan ascent (Thermo Scientific) for excitation at 544 nm and emission at 590 nm. The inhibition ratio was calculated by the following equation: inhibition (%) = $[1 - \{(S - S_0) - (B - B_0)/(C - C_0) - (B - B_0)\}] \times 100$, where C was the fluorescence of a control [enzyme, substrate, and assay buffer concentration with DMSO (final concentrations were 10%)] after 60 min of incubation, C_0 was the initial fluorescence of a control [enzyme, substrate, and assay buffer concentration with DMSO (final concentrations were 10%)], B was the fluorescence of a control [substrate and assay buffer concentration with DMSO (final concentrations were 10%)] after 60 min of incubation, B_0 was the initial fluorescence of a control [substrate and assay buffer concentration with DMSO (final concentrations were 10%)], S was the fluorescence of the tested samples (enzyme, sample solution, and substrate) after 60 min of incubation, S_0 was the initial fluorescence of the tested samples (enzyme, sample solution, and substrate). To check the quenching effect of the tested samples, the sample solution was added to reaction mixture C, and any reduction in fluorescence by the sample was investigated. β-Secretase inhibitor (Wako, Japan) was used as a positive control.
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