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Serotonin derivatives as inhibitors of β-secretase (BACE 1)

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All serotonin derivatives described here (**1–9**) inhibited BACE 1 in a dose dependent manner. The 50% Inhibition Concentration (IC_{50}) of *N*-cinnamoyl serotonin (**1**) was 86.7 ± 4.0 μM. The peptide conjugation of serotonin derivatives influenced the BACE 1 inhibitory activity. Among serotonin derivatives (**1–8**), introduction of substituents, such as hydroxyl and methoxy groups at the 4′-position decreased the inhibitory activity (*N-p*-coumaroyl serotonin (**2**), *N-p*-methoxy cinnamoyl serotonin (**3**)). With a hydroxylgroup at the 4′-position, and the *meta*-hydroxy function being substituted by a hydroxyl group or methoxy group (*N*-caffeoyl serotonin (**4**), *N*-feruloyl serotonin (**5**)), inhibitory activity was weakened, ($IC_{50} > 400$ μM). BACE 1 inhibitory activity was effected by the substituents of the cinnamic acid moiety. This is the first report on Structure-Activity-Relationships (SAR) for the BACE 1-inhibiting activity of serotonin derivatives. These serotonin derivatives, which have anti-oxidative effects as well are expected to be useful in the study of the mechanisms of Alzheimer's disease.

1. Introduction

Alzheimer's disease (A. D) is a meurodegerative diorder dinically characterized by progressive dementia that inevitably leads to incapacitation and death. Althoug the pathogenesis of AD is complicated and involves in numerous pathways, two major hypotheses are currently under considaration regarding the molecular mechanism, the cholinergic hypothesis and the amyloid cascade hypothesis. Thus, the focus herein is upon inhibitors of select cholinesterase (ChEs) to alleviate cholinergic deficits and improve neurotransmission and β-siteamyloid precursor protein (APP) cleaving enzyme 1 (BACE 1; as party protease, β-secretase, and memapsin 2) inhibitors to preclude formation and accumulation of amyloid β peptide (Aβ)· Pursuant to this, both could then be established as visable therapeutic targets for AD. Furthermore, recent evidence in the field of Alzheimer's disease research has highlighted the importance of the oxidative process in its pathogenesis. Based on laboratory and clinical studies, it appears that reactive oxygen species and reactive nitrogen species that are generated extracellulary and intracellulary by various mechanisms are among the major intermediary risk factors that initiate and promote neuodegeration in idiopathic AD (Prasad et al. 2000, 2002). Thus, attenuation of oxidant stress by antioxidant molecules is a potential therapeutic intervention in AD. In order to have therapeutic potential, the molecular weight of inhibitors should be below 700 Da, because efficacy requires penetration of the blood-brain barrier. For this reason, large peptide-based inhibitors are not proper drug candidates, but the secondary metabolites of plants which have relatively low molecular weight and high lipophilicity might be good BACE 1 inhibitors (Dorrvel 2000; Stachel et al. 2004).

Polyphenols are group of phytochemicals that exhibit a wide range of physiological and therapeutic properties, including anti-allergenic, anti-inflammatory, anti-microbial and antioxidant effects. Phenolic compounds could be a major determinant of the antioxidant potential of food which would therefore act as a natural source of antioxidants (Parr and Bolwell 2000). In the sense, polyphenols might be a good candidate for BACE 1 inhibitors, however, natural product inhibitors have rarely been investigated (Jeou et al. 2003; Choi et al. 2008; Marumoto and Miyazawa 2010). Thus, we focused our interest to antioxidant substances from natural sources.

Serotonin derivatives were identified as the major unique phenolic compounds of safflower seeds, belonging to a family of indole hydroxyl cinnamic acid amides (Sakamura et al. 1978; Sato et al. 1985). These compounds have a number of biological effects, such as, antioxidative activities (Zhang et al. 1997), anti-tyrosinase activity, melanin-production inhibitory activity (Roh et al. 2004), anti-tumor activity (Nagatsu et al. 2000), fibroblasts growth promoting activities (Takii et al. 1999). Among the serotonin derivatives, *N-p*-coumaroyl serotonin and *N*-feruloyl serotonin are commomly detected, while only few other serotonin derivatives have been reported (Park 2008; Yamazaki et al. 2009; Kang et al. 2009).

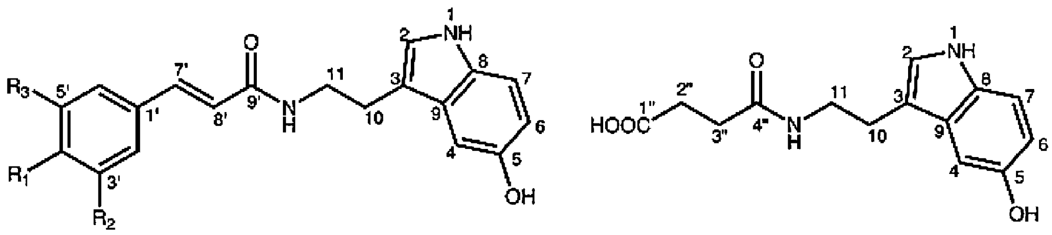
In this study, we synthesized a series of serotonin derivatives (Fig. 1) using cinnamic acid derivatives, and evaluated their antioxidant β−secretase inhibitory effects.

2. Investigations, results and discussion

2.1. *Antioxidant activities of serotonin derivatives 1–9*

The antioxidant activities of compounds **1–9** were evaluated by EC_{50} (50% Effective Concentration) in DPPH radical scavenging activity as shown in Fig. 2.

All serotonin derivatives **1–9** were found to be stronger than BHT in free radical scavenging activity. Therefore, in order to clarify whether the antioxidant activity of serotonin derivatives was due to the cinnamic acid or the serotonin moiety, we evaluated the antioxidant activities of cinnamic acid derivatives.



Bufobutanoic acid (9)

Compound	R_1	R_2	R_3	Compound	R_1	R_2	R_3
N-Cinnamoyl serotonin (**1**)	H	H	H	*N*-Feruloyl serotonin (**5**)	OH	OMe	H
N-p-Coumaroyl serotonin (**2**)	OH	H	H	*N*-Isoferuloyl serotonin (**6**)	OMe	OH	H
N-p-Methoxy-cinnamoyl serotonin (**3**)	OMe	H	H	*N*-3,4Dimethoxy cinnamoyl serotonin (**7**)	OMe	OMe	H
N-Caffeoyl serotonin (**4**)	OH	OH	H	*N*-Sinapoyl serotonin (**8**)	OH	OMe	OMe

Fig. 1: Structure of serotonin derivatives (**1–9**)

Each serotonin derivative showed a stronger antioxidant activity than the cinnamic acid derivatives (Table 1). Furthermore, the EC_{50} value of bufobutanoic acid (**9**) which did not conjugate with the cinnamic acid moiety was 43.6 ± 2.1 µM. These results indicated that the antioxidant activity is strengthened by the serotonin moiety. These results supported previous suggestions (Takii et al. 1999). In the case of cinnamic acid derivatives, the substituents of cinnamic acid derivatives effect antioxidant activity, whereas the substituents of serotonin derivatives appear to be less important. The serotonin moiety effected the radical scavenging activity strongly.

2.2. *β-Secretase inhibitory effects of serotonin derivatives 1–9 and Structure-Activity-Relationships (SAR)*

All the serotonin derivatives **1–9** inhibited BACE 1 in a dose dependent manner (Fig. 3), in particular *N*-cinnamoyl serotonin (**1**) which has no substituents of cinnamic acid. The 50%

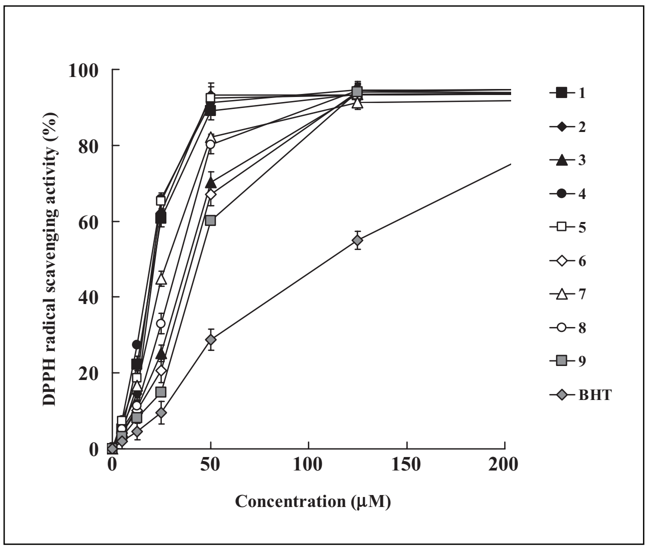


Fig. 2: DPPH radical scavenging activities of serotonin derivatives (**1–9**)

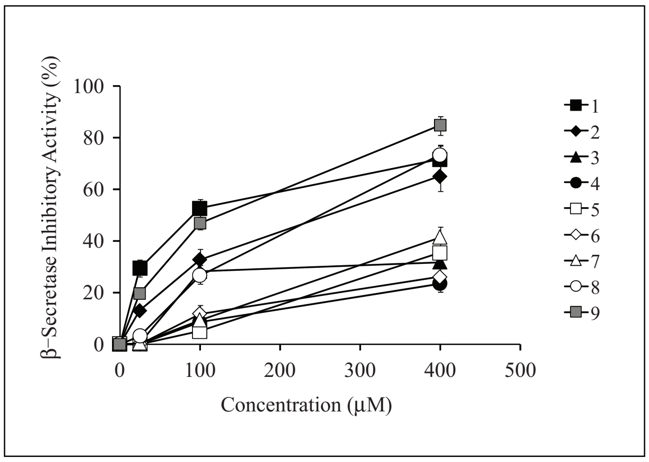


Fig. 3: β–Secretase inhibitory effects of serotonin derivatives (**1–9**)

Table 1: EC_{50} values of serotonin derivatives 1–9 and cinnamic acid derivatives

Compound	Ec_{50}(µM)	Compound	Ec_{50}(µM)
*N-C*innamoyl serotonin (**1**)	20.9 ± 2.3	Cinnamic acid	>250
N-p-Coumaroyl serotonin (**2**)	21.4 ± 1.9	*p*-Coumaric acid	>250
N-p-Methoxy-cinnamoyl serotonin (**3**)	36.8 ± 2.1	*p*-Methoxy cinnamic acid	>250
*N-C*affeoyl serotonin (**4**)	18.9 ± 2.0	Caffeic acid	155.3 ± 3.2
N-Feruloyl serotonin (**5**)	20.2 ± 2.1	Ferulic acid	>250
N-Iso feruloyl serotonin (**6**)	39.2 ± 2.2	Isoferulic acid	>250
N-3,4-Dimethoxy cinnamoyl serotonin (**7**)	30.5 ± 2.4	3,4-Dimethoxy cinnamic acid	>250
N-Sinapoyl serotonin (**8**)	27.2 ± 2.2	Sinapic acid	>250
Bufobutanoic acid (**9**)	43.6 ± 2.1		
BHT*	104.8 ± 2.0		

* reference compound
The results are the means ± SD of three experiments

Table 2: β-Secretase inhibitory activity of serotonin derivatives 1–9

Compound	Concentration (μM)	Inhibition (%)	IC_{50} (μM)	Compound	Concentration (μM)	Inhibition (%)	IC_{50} (μM)
1	25	29.3 ± 3.2		**6**	25	0.0 ± 0.4	
	100	52.5 ± 3.5			100	11.8 ± 3.2	
	400	29.3 ± 5.2	86.7 ± 4.0		400	26.1 ± 4.0	>400
2	25	12.9 ± 1.3		**7**	25	0.4 ± 0.5	
	100	32.7 ± 4.1			100	9.3 ± 0.7	
	400	65.0 ± 5.7	210.8 ± 10.3		400	41.3 ± 2.6	>400
3	25	0.2 ± 0.1		**8**	25	3.2 ± 1.2	
	100	28.2 ± 2.3			100	26.6 ± 3.1	
	400	31.8 ± 2.1	>400		400	73.3 ± 3.9	200.3 ± 6.8
4	25	0 ± 0.2		**9**	25	19.5 ± 0.2	
	100	5.1 ± 1.2			100	46.8 ± 1.0	
	400	35.3 ± 2.1	>400		400	84.6 ± 2.5	110.6 ± 4.0
5	25	0.0 ± 0.2		Cinnamic acid	25	0 ± 1.0	
	100	5.1 ± 1.2			100	0.9 ± 0.5	
	400	35.3 ± 2.1	>400		400	3.2 ± 0.9	>400
				p-Coumaric acid	25	0 ± 1.0	
					100	4.7 ± 2.2	
					400	16.7 ± 3.1	>400
				reference*			0.2 ± 0.01

* Lys-Thr-Glu-lle-Ser-Glu-Val-Asn-(statine)-Val-Ala-Glu-Phe-OH was used as the positive control
The results are the means ± SD of three experiments

Inhibition Concentration (IC_{50}) of **1** was 86.7 ± 4.0 μM. In order to clarify whether the β-secretase inhibitory activity of serotonin derivatives was due to the cinnamic acid or serotonin moiety, we evaluated BACE 1 inhibitory activities of cinnamic acid and *p*-coumaric acid. Since the inhibitory activities of cinnamic acid and *p*-coumaric acid were lower than that of serotonin derivatives, furthermore, bufobutanoic acid which did not have a cinnamic acid moiety showed BACE 1 inhibitory activity (IC_{50} = 110.6 ± 4.0 μM). We assumed that peptide conjugation of serotonin derivatives has effects on BACE 1. Among the serotonin derivatives **1–8**, introduction of substituents, such as hydroxyl and methoxy groups at the 4′-position decreased the inhibitory activity (*N*-*p*-coumaroyl serotonin (**2**), *N*-*p*-methoxy cinnamoyl serotonin (**3**). When a hydroxylgroup was located at the 4′-position, and the *meta*-hydroxy function was substituted by a hydroxyl group or methoxy group (*N*-caffeoyl serotonin (**4**), *N*-feruloyl serotonin (**5**)), inhibitory activities were weakened, (IC_{50} > 400 μM). Furthermore, the introduction of another methoxy group in a *ortho*-position to a hydroxyl group *N*-sinapoyl serotonin (**8**) lead to an increase in the inhibitory activity relatively to *N*-feruloyl serotonin. When a methoxy group was located at the 4′-position, and the *meta*-methoxy function was substituted by a hydroxyl group or methoxy group a (*N*-isoferuloyl serotonin (**6**), *N*-3′, 4′-dimethoxy cinnamoyl serotonin (**7**)) also inhibitory activity was weakened (IC_{50} > 400) (Table 2).

BACE 1 inhibitory activity was effected by the substituents of the cinnamic acid moiety. The results were summarized in Table 2. These serotonin derivatives which have relatively low molecular weight might not be drug candidates themselves since their inhibitory activity is not strong. However, this is the first report on Structure-Activity-Relationships (SAR) for the BACE 1-inhibiting activity of serotonin derivatives. These serotonin derivatives, which have anti-oxidative effects as well as BACE 1-inhibitory activity are expected to be useful in the study of the mechanisms of Alzheimer's disease.

3. Experimental

3.1. Materials

Thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F_{254}, 0.25 mm, Merck, Darmstadt, Germany). Column chromatography was carried out using 70–230 mesh silica gel (Kieselgel 60, Merck, Germany). Melting points (m.p.) were measured on a Yanaco MP-5000D melting-point apparatus. UV spectra were measured on a Hitachi U-1500 spectrophotometer. ^{1}H and ^{13}C NMR data were all obtained on a JEOL ECA-400 (^{1}H: 400 MHz; ^{13}C: 100 MHz), JEOL ECA-500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) and JEOL ECA-700 (^{1}H: 700 MHz; ^{13}C: 175 MHz) in DMSO-d_6 with TMS as internal standard. IR spectra were obtained with a Jasco FT/IR-470 plus Fourier transform infrared spectromoter. The FAB-MS spectrum was obtained with a Jeol Tandem MS station JMS-700TKM. Fluorescence was measured with a MTP-800Lab microplate fluorescence reader. Serotonin hydrochloride, cinnamic acid, *p*-coumaric acid, ferulic acid, caffeic acid, sinapic acid, *p*-methoxy cinnamic acid, 3,4-dimethoxy cinnamic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl), BHT (2,6-di-*tert*-butyl-4-methylphenol), succinic anhydride, piperidine, HOBT (1-hydroxybenzotriazole), tetrakis(triphenylphosphine)palladium morpholine, (0) EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide) were purchased from Wako Pure Chemistry (Osaka, Japan), 3,4-dihydroxy denzaldehyde and dimethylaminopyridine were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Isoferulic acid and all solvents were purchased from Kanto Chemical (Tokyo, Japan).

3.2. Synthesis of serotonin derivatives 1–3, 5–8

Synthesis of serotonin derivatives **1–3**, **5–8** was accomplished according to a published method (Koyama et al. 2006). First, triethylamine (230 μl, 1.65 mmol), 1-hydroxybenzotriazole (223 mg, 1.65 mmol) and EDC (290 μl, 1.65 mmol) were added to a solution of serotonin hydrochloride (350 mg, 1.65 mmol) and cinnamic acid derivatives (1.50 mmol) in dimethylformamide (1.5 ml) and dichloromethane (10 ml). After the mixture was stirred over night at room temperature under nitrogen, it was concentrated *in vacuo*. The residue was treated with water (15 ml) and extracted with ethyl acetate (3 × 10 ml). The organic extract was washed successively with 5% citric acid solution (3 × 20 ml), saturated sodium hydrogencarbonate solution (3 × 30 ml), and brine (50 ml). The extract was dried over Na_2SO_4 and concentrated to yield a solid (yield 64.9%–96.1%).

3.2.1. N-Cinnamoyl serotonin (1)

Violet solid: Yield 95.0%; mp 92–95 °C. IR ν_{max} (KBr): 3292, 1654, 1607, 1531, 798 cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (1H, s, H-1), 8.50 (1H, s, 5-O$\underline{H}$), 8.19 (1H, t, 5.6 Hz, -CON$\underline{H}$), 7.55 (2H, d, 6.8 Hz, H-2′, and H-6′), 7.42 (1H, d, 16.0 Hz, H-7′), 7.38 (3H, m, H-3′, H-4′ and H-5′), 7.12 (1H, d, 8.0 Hz, H-7), 7.05 (1H, s, H-2), 6.85 (1H, d, 2.4 Hz, H-4), 6.63 (1H, d, 16.0 Hz, H-8′), 6.56 (1H, dd, 8.0 Hz, 2.4 Hz, H-6), 3.44 (2H, dt, 5.6 Hz, 6.0 Hz, H-11), 2.79 (2H, t, 6.0 Hz, H-10). ^{13}C NMR (100 MHz, DMSO- d_6) δ 164.8 (C-9′), 150.2 (C-5), 138.4 (C-7′), 135.0 (C-1′), 130.8 (C-8), 129.3 (C-4′), 128.9 (C-2′ and C-6′), 127.9 (C-9), 127.5 (C-3′ and C-5′), 123.1 (C-2), 122.4 (C-8′), 111.6 (C-7), 111.3 (C-6), 110.8 (C-3), 102.2 (C-4), 39.5 (C-11), 25.4 (C-10).

3.2.2. *N-p-Coumaroyl serotonin* (**2**)

Violet solid: Yield 75.0%; mp 192–195 °C. FAB-MS *m/z* 323 $[M+H]^+$. IR ν_{max} (KBr): 3318, 1586, 1512, 830 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6) δ 10.48 (1H, d, 2.3 Hz, H-1), 8.60 (1H, s, 5-O<u>H</u>), 8.06 (1H, t, 5.6 Hz, -CON<u>H</u>), 7.38 (2H, d, 8.6 Hz, H-2′ and H-6′), 7.33 (1H, d, 16.0 Hz, H-7′), 7.12 (1H, d, 8.6 Hz, H-7), 7.05 (1H, d, 2.3 Hz, H-2), 6.85 (1H, d, 2.3 Hz, H-4), 6.79 (2H, d, 8.6 Hz, H-3′ and H-5′), 6.59 (1H, dd, 8.6 Hz, 2.3 Hz, H-6), 6.41 (1H, d, 16.0 Hz, H-8′), 3.42 (2H, dt, 5.6 Hz, 7.8 Hz, H-11), 2.78 (2H, t, 7.8 Hz, H-10). ^{13}C NMR (125 MHz, DMSO- d_6) δ 165.5 (C-9′), 159.0 (C-4′), 150.4 (C-5), 138.7 (C-7′), 131.0 (C-8), 129.4 (C-2′ and C-6′), 128.1 (C-9), 126.2 (C-1′), 123.3 (C-2), 119.1 (C-8′), 115.9 (C-3′ and C-5′), 111.8 (C-7), 111.5 (C-6), 111.0 (C-3), 102.4 (C-4), 40.0 (C-11), 25.6 (C-10).

3.2.3. *N-p-Methoxy cinnamoyl serotonin* (**3**)

Pale yellow solid: Yield 79.2%; mp 178–185 °C. IR ν_{max} (KBr): 3271, 1650, 1601, 1574, 1509, 829 cm^{-1}. ^1H NMR (400 MHz, DMSO- d_6) δ 10.50 (1H, d, 2.3 Hz, H-1), 8.12 (1H, t, 6.0 Hz, -CON<u>H</u>), 7.51 (2H, d, 8.8 Hz, H-2′ and H-6′), 7.39 (1H, d, 15.6 Hz, H-7′), 7.13 (1H, d, 8.8 Hz, H-7), 7.06 (1H, d, 2.3 Hz, H-2), 6.86 (1H, d, 2.0 Hz, H-4), 6.76 (2H, d, 8.8 Hz, H-3′ and H-5′), 6.66 (1H, dd, 8.8 Hz, 2.0 Hz, H-6), 6.50 (1H, d, 15.6 Hz, H-8′), 3.78 (3H, s, 4′-O<u>Me</u>), 3.44 (2H, dt, 6.0 Hz, 7.8 Hz, H-11), 2.79 (2H, t, 7.8 Hz, H-10). ^{13}C NMR (100 MHz, DMSO- d_6) δ 165.1 (C-9′), 160.2 (C-4′), 150.2 (C-5), 138.1 (C-7′), 130.8 (C-8), 129.0 (C-2′ and C-6′), 127.9 (C-9), 127.5 (C-1′), 123.1 (C-2), 119.9 (C-8′), 114.4 (C-3′ and C-5′), 111.6 (C-7), 111.3 (C-6), 110.8 (C-3), 102.2 (C-4), 55.2 (4′-O<u>Me</u>) 39.4 (C-11), 25.4 (C-10).

3.2.4. *N-Feruloyl serotonin* (**5**)

Pale violet solid: Yield 96.1%; mp 110–114 °C. IR ν_{max} (KBr): 3374, 1652, 1589, 1514, 844 cm^{-1}. ^1H NMR (500 MHz, DMSO- d_6) δ 10.49 (1H, d, 2.0 Hz, H-1), 9.42 (1H, s, 4′-O<u>H</u>), 8.61 (1H, s, 5-O<u>H</u>), 8.05 (1H, t, 5.8 Hz, -CON<u>H</u>), 7.34 (1H, d, 15.6 Hz, H-7′), 7.13 (1H, d, 8.6 Hz, H-7), 7.12 (1H, d, 2.0 Hz, H-2′), 7.06 (1H, d, 2.0 Hz, H-2), 6.99 (1H, dd, 8.1 Hz, 2.0 Hz, H-6′), 6.86 (1H, d, 2.3 Hz, H-4), 6.79 (1H, d, 8.1 Hz, H-5′), 6.60 (1H, dd, 8.6 Hz, 2.3 Hz, H-6), 6.46 (1H, d, 15.6 Hz, H-8′), 3.81 (3H, s, 3′-O<u>Me</u>), 3.43 (2H, dt, 5.8 Hz, 7.5 Hz, H-11), 2.78 (2H, t, 7.5 Hz, H-10). ^{13}C NMR (125 MHz, DMSO- d_6) δ 165.5 (C-9′), 150.4 (C-5), 148.4 (C-4′), 148.0 (C-3′), 139.0 (C-7′), 131.0 (C-8), 128.1 (C-9), 126.7 (C-1′), 123.3 (C-2), 121.7 (C-6′), 119.4 (C-8′), 115.8 (C-5′), 111.9 (C-7), 111.5 (C-6), 111.0 (C-3), 110.9 (C-2′), 102.4 (C-4), 55.7 (3′-O<u>Me</u>), 39.6 (C-11), 25.6 (C-10).

3.2.5. *N-Isoferuloyl serotonin* (**6**)

Pale violet solid: Yield 77.0%; mp 99–105 °C. IR ν_{max} (KBr): 3390, 1652, 1584, 1509, 801 cm^{-1}. ^1H NMR (400 MHz, DMSO- d_6) δ 10.47 (1H, d, 2.0 Hz, H-1), 9.16 (1H, s, 3′-O<u>H</u>), 8.59 (1H, s, 5-O<u>H</u>), 8.10 (1H, t, 6.0 Hz, -CON<u>H</u>), 7.27 (1H, d, 15.6 Hz, H-7′), 7.10 (1H, d, 8.0 Hz, H-7), 7.04 (1H, d, 2.0 Hz, H-2), 6.97 (1H, d, 1.2 Hz, H-2′), 6.95 (1H, dd, 8.4 Hz, 1.2 Hz, H-6′), 6.92 (1H, d, 8.4 Hz, H-5′), 6.86 (1H, d, 1.6 Hz, H-4), 6.58 (1H, dd, 8.0 Hz, 1.6 Hz, H-6), 6.39 (1H, d, 15.6 Hz, H-8′), 3.78 (3H, s, 4′-O<u>Me</u>), 3.41 (2H, dt, 6.0 Hz, 7.2 Hz, H-11), 2.77 (2H, t, 7.2 Hz, H-10). ^{13}C NMR (100 MHz, DMSO- d_6) δ 165.1 (C-9′), 150.2 (C-5), 149.1 (C-4′), 146.7 (C-3′), 138.5 (C-7′), 130.8 (C-8), 127.9 (C-9), 127.8 (C-1′), 123.1 (C-2), 120.1 (C-6′), 119.7 (C-8′), 113.3 (C-5′), 112.1 (C-7), 111.6 (C-6), 111.3 (C-3), 110.8 (C-2′), 102.4 (C-4), 55.6 (4′-O<u>Me</u>), 39.4 (C-11), 25.4 (C-10).

3.2.6. *N-3,4-Dimethoxy cinnamoyl serotonin* (**7**)

Pale violet solid: Yield 82.0%; mp 103–105 °C. IR ν_{max} (KBr): 3369, 1654, 1595, 1542, 802 cm^{-1}. ^1H NMR (700 MHz, DMSO- d_6) δ 10.49 (1H, s, H-1), 8.07 (1H, t, 5.6 Hz, -CON<u>H</u>), 7.37 (1H, d, 15.6 Hz, H-7′), 7.15 (1H, d, 1.4 Hz, H-2′), 7.13 (1H, d, 8.7 Hz, H-7), 7.11 (1H, dd, 8.4 Hz, 1.4 Hz, H-6′), 7.07 (1H, s, H-2), 6.98 (1H, d, 8.4 Hz, H-5′), 6.86 (1H, d, 2.2 Hz, H-4), 6.60 (1H, dd, 8.7 Hz, 2.2 Hz, H-6), 6.53 (1H, d, 15.6 Hz, H-8′), 3.80 (3H, s, 3′-O<u>Me</u>), 3.78 (3H, s, 4′-O<u>Me</u>), 3.44 (2H, dt, 5.6 Hz, 7.4 Hz, H-11), 2.79 (2H, t, 7.4 Hz, H-10). ^{13}C NMR (175 MHz, DMSO- d_6) δ 165.4 (C-9′), 150.4 (C-5), 150.2 (C-4′), 149.1 (C-3′), 138.7 (C-7′), 131.0 (C-8), 128.1 (C-9), 128.0 (C-1′), 123.3 (C-2), 121.5 (C-6′), 120.4 (C-8′), 112.0 (C-5′), 111.8 (C-7), 111.5 (C-6), 111.0 (C-3), 110.2 (C-2′), 102.4 (C-4), 55.7 (3′-O<u>Me</u>), 55.6 (4′-O<u>Me</u>), 39.4 (C-11), 25.6 (C-10).

3.2.7. *N-Sinapoyl serotonin* (**8**)

Pale yellow solid: Yield 76.0%; mp. 169–172 °C. IR ν_{max} (KBr): 3308, 1650, 1604, 1512, 800 cm^{-1}. ^1H NMR (400 MHz, DMSO- d_6) δ 10.48 (1H, s, H-1), 8.77 (1H, s, 4′-O<u>H</u>), 8.60 (1H, s, 5-O<u>H</u>), 8.03 (1H, t, 4.7 Hz, -CON<u>H</u>), 7.33 (1H, d, 15.6 Hz, H-7′), 7.12 (1H, d, 8.4 Hz, H-7), 7.06 (2H, s, H-2′ and H-6′), 7.05 (1H, s, H-2), 6.84 (1H, d, 2.4 Hz, H-4), 6.59 (1H, dd, 8.4 Hz, 2.4 Hz, H-6), 6.50 (1H, d, 15.6 Hz, H-8′), 3.78 (6H, s, 3′-O<u>Me</u> and 5′-O<u>Me</u>), 3.42 (2H, dt, 4.7 Hz, 7.2 Hz, H-11), 2.79 (2H, t, 7.2 Hz, H-10). ^{13}C NMR (100 MHz, DMSO- d_6) δ 165.3 (C-9′), 150.2 (C-5), 148.0 (C-3′ and C-5′), 139.1 (C-7′), 137.2 (C-4′), 130.8 (C-8), 127.9 (C-9), 125.4 (C-1′), 123.1 (C-2), 119.6 (C-8′), 111.6 (C-7), 111.3 (C-6), 110.8 (C-3), 105.2 (C-2′ and C-6′), 102.2 (C-4), 55.9 (3′-O<u>Me</u> and 5′-O<u>Me</u>), 39.4 (C-11), 25.4 (C-10).

3.3. *Synthesis of N-caffeoyl serotonin (4)*

First, to a solution of 3,4-dihydroxy benzaldehyde (297 mg, 2.15 mmol) in anh. dimethylformamide (3.0 ml) was added allyl bromide (1.2 equiv. per OH-group) and K_2CO_3 (1.2 equiv.) and NaI (90 mg, 0.60 mmol), the mixture was heated at 60 °C for 2 h. The mixture was treated with 5% HCl solution, and extracted with ethyl acetate (10 ml × 3). The organic extract was washed successively with water (3 × 10 ml) and brine (50 ml). The extract was dried over Na_2SO_4 and concentrated to obtained 3,4-diallyloxybenzaldehyde (459.3 mg, yield 98.0%). Secondly, 3,4-diallyloxybenzaldehyde and malonic acid were dissolved in anh. pyridine (2 ml/mmol aldehyde) containing 3 vol% of piperidine. The mixture was stirred under reflux for 2 h, cooled and poured into ice-cold 2N-HCl. The precipitate was filtered and taken up in EtOAc. The organic layer was washed with 2 N HCl, dried (Na_2SO_4) and concentrated to give 3,4-di-*O*-allylcaffeic acid (453.7 mg, yield 82.6%). Triethylamine (230 µl, 1.65 mmol), 1-hydroxybenzotriazole (223 mg, 1.65 mmol) and EDC (290 µL, 1.65 mmol) were added to a solution serotonin hydrochloride (350 mg, 1.65 mmol) and compound 4′′ (1.50 mmol) in dimethylformamide (1.5 ml) and dichloromethane (10 ml). After the mixture was stirred over night at room temperature under nitrogen, it was concentrated *in vacuo*. The residue was treated with water (15 ml) and extracted with ethyl acetate (3 × 10 ml). The organic extract was washed successively with 5% citric acid solution (3 × 20 ml), saturated sodium hydrogencarbonate solution (3 × 30 ml), and brine (50 ml). The extract was dried over Na_2SO_4 and concentrated to yield a solid (yield 96.3%). The ally protected compound **4** was dissolved in degassed anh. THF (50 ml/mmol) and morpholine (10 equiv. per allyl group to be cleaved) was added $Pd(PPh_3)_4$ (1 mol%). The green mixture was stirred at room temperature (monitored by TLC) and concentrated *in vacuo*. The residue was treated with saturated NH_4Cl solution and extracted with ethyl acetate (3 × 10 ml). The extract was dired over Na_2SO_4 and concentrated to yield a pale yellow solid. The extract was separated by silica gel column chromatography with a hexane-EtOAc (1: 1, v/v) system to give *N*-caffeoyl serotonin (**4**) (422.0 mg, yield 83.3%).
Pale yellow solid: Yield 64.9%; mp 98–102 °C. IR ν_{max} (KBr): 3409, 1647, 1575, 1538, 811 cm^{-1}. ^1H NMR (400 MHz, DMSO- d_6) δ 10.51 (1H, s, H-1), 8.14 (1H, t, 5.2 Hz, -CON<u>H</u>), 7.27 (1H, d, 15.6 Hz, H-7′), 7.14 (1H, d, 8.0 Hz, H-7), 7.07 (1H, s, H-2), 6.98 (1H, d, 2.0 Hz, H-2′), 6.87 (1H, d, 2.0 Hz, H-4), 6.85 (1H, dd, 2.0 Hz, 8.0 Hz, H-6′) 6.77 (1H, d, 8.0 Hz, H-5′), 6.61 (1H, dd, 8.0 Hz, 2.0 Hz, H-6), 6.37 (1H, d, 15.6 Hz, H-8′), 3.43 (2H, dt, 5.2 Hz, 7.6 Hz, H-11), 2.79 (2H, t, 7.6 Hz, H-10). ^{13}C NMR (100 MHz, DMSO- d_6) δ 165.4 (C-9′), 150.2 (C-5), 147.3 (C-4′), 145.6 (C-3′), 139.0 (C-7′), 130.8 (C-8), 127.9 (C-9), 126.5 (C-1′), 123.1 (C-2), 120.4 (C-6′), 118.8 (C-8′), 115.8 (C-5′), 113.9 (C-2′), 111.7 (C-7), 111.3 (C-6), 110.9 (C-3), 102.3 (C-4), 39.5 (C-11), 25.5 (C-10).

3.4. *Synthesis of bufobutanoic acid (9)*

Synthesis of bufobutanoic acid (**9**) was accomplished according to a published method (Somei et al. 2001). Succinic anhydride (150 mg, 1.51 mmol) was added to a solution of serotonin hydrochloride (353.1 mg, 1.66 mmol) in DMF (15.0 ml) and pyridine (1.5 ml) at room temperature and stirring was continued for 24 h. After evaporation under reduced pressure, the residual oil was column chromatographed on SiO_2 with CH_2Cl_2-MeOH-AcOH (10: 1: 0.1, v/v) to give bufobutanoic acid (**9**) (258.4 mg, 62.0%).
Brown oil. Yield 62.0%; IR ν_{max} (KBr): 3294, 1652, 1558, 798 cm^{-1}. ^1H NMR (400 MHz, DMSO- d_6) δ 10.50 (1H, s, H-1), 8.00 (1H, t, 5.2 Hz, -CON<u>H</u>), 7.14 (1H, d, 8.8 Hz, H-7), 7.05 (1H, s, H-2), 6.87 (1H, d, 1.6 Hz, H-4), 6.62 (1H, dd, 8.8 Hz, 1.6 Hz, H-6), 3.30 (2H, dt, 5.2 Hz, 7.6 Hz, H-11), 2.73 (2H, t, 7.6 Hz, H-10). 2.44 (2H, t, 6.8 Hz, H-2′′), 2.35 (2H, t, 6.8 Hz, H-3′′). ^{13}C NMR (100 MHz, DMSO- d_6) δ 174.7 (C-4′′), 172.8 (C-1′′), 150.4 (C-5), 131.0 (C-8), 128.1 (C-9), 123.2 (C-2), 111.8 (C-7), 111.4 (C-6), 111.1 (C-3), 102.4 (C-4), 39.7 (C-11), 30.9 (C-2′′), 30.1 (C-3′′), 25.5 (C-10).

3.5. *DPPH radical scavenging activity*

The scavenging activity of cinnamic acid derivatives for the DPPH radical was monitored according to the method of Gaspar et al. (2009). An amount of 500 µl of a 0.5 mM methanolic DPPH solution was mixed in a cuvette with 500 µl of cinnamic acid derivatives at different concentration levels. These cuvettes were shaken vigorously. The cuvettes were allowed to stand at 27 °C for 30 min, the absorbance was measured at 517 nm using a U-1500 spectrophotometer. The percentage of radical scavenging activity was calculated using the equation:

$$\text{Radical scavenging activity (\%)} = (\text{Control OD} - \text{Sample OD}/\text{Control OD}) \times 100$$

All tests were performed in triplicate. BHT as used as a reference standard for the investigation of radical scavenging activity.

3.6. β-Secretase inhibitory activity

BACE 1 (β-sectretase) assay was carried out using the BACE 1 Assay Kit (Invitrogen, USA) according to the manufactures's instruction. Briefly, a mixture of 10 μl of assay buffer (50 mM sodium acetate, pH 4.5), 10 μl of BACE 1 (recombinant human BACE 1 1.0 U/ml), 10 μl of substrate (750 nM Rh-EVNL-DAEFK-Quencher in 50 mM ammonium bicarbonate), and 10 μl of sample dissolved in 30% DMSO was incubated for 60 min at room temperature in the dark. The mixture was irradiated at 550 nm and the emission intensity at 590 nm was recorded. The inhibition ratio was obtained by the following equation:

$$\text{Inhibition (\%)} = [1 - \{(S - S_0)/(C - C_0)\}] \times 100$$

where C was the fluorescence of the control (enzyme, buffer and substrate) after 60 min of incubation, C_0 was the fluorescence of the control at zero time, S was the fluorescence of the tested samples (enzyme, sample solution and substrate) after incubation, and S_0 was the fluorescence of the tested samples at zero time. To allow for the quenching effect of the samples, the sample solution was added to the reaction mixture C, and any reduction in fluorescence by the sample was then investigated. All data are the mean of three experiments.

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