BACE1 (β-Secretase) Inhibitory Chromone Glycosides from *Aloe vera* and *Aloe nobilis*

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Key words
- *Aloe vera*
- *Aloe nobilis*
- Liliaceae
- chromone glycosides
- BACE1 (β-secretase) inhibitor
- Alzheimer's disease (AD)

Abstract

Four new chromone glycosides allo-aloeresin D (**2**), *C*-2′-decoumaroyl-aloeresin G (**8**), 2′-*O*-coumaroyl-(*S*)-aloesinol (**9**), 2′-*O*-[*p*-methoxy-(*E*)-cinnamoyl]-(*S*)-aloesinol (**10**) and nine known chromone glycosides (**1**, **3 – 7**, **11 – 13**) were isolated from two *Aloe* spp. plants, *A. vera* and *A. nobilis*. Among them, **1** and **8** showed significant inhibitory activity against BACE1 (β-secretase) with IC_{50} values of 39.0 and 20.5×10^{-6} M, as well as inhibition of $A\beta_{1-42}$ production by 7.4 and 12.3 %, respectively, in B103 neuroblastoma cells at 30 ppm. The preliminary structure-activity relationships of *Aloe* chromone glucosides were also discussed.

Introduction

Alzheimer's disease (AD) has become an increasingly severe medical and social problem, due to the rapid growth of aging people populations in industrialized countries, and even in some developing countries. β-Secretase (BACE1) has been recognized as a valuable target for the treatment of AD. BACE1 inhibitors have promised to be a class of disease disrupting rather than symptom relieving agents [1]. Although many peptides, peptidomimetics and heterocyclic compounds have been designed and evaluated as BACE inhibitors, none of them have been successfully developed as anti-AD agents to date [2], [3]. There are only very few reports on natural products-based BACE inhibitors [4], [5], [6], [7], [8], [9] with IC_{50} s in the 10^{-5} to 10^{-7} M range.

During screening of plant extracts for BACE1 inhibitory activity, *Aloe vera*, a traditional Chinese medicine, was found to potently inhibit β-secretase at 10^{-5} g/mL. Bioassay-guided fractionations of *A. vera* extracts led to the isolation and characterization of eight chromone *C*-glycosides (**1 – 8**) as active components (**Fig. 1**). To elucidate the structure-activity relationship (SAR) of *Aloe* chromone glycosides, we also isolated six chromone *C*-glucosides (**3**, **9 – 13**) from *A. nobilis*, and prepared two hydrolyzed products, **9a** and **13a** (**Fig. 2**). Among these chromone glycosides, **2**, **8**, **9** and **10** are new. The inhibitory activities against BACE1 for all these compounds were evaluated, and preliminary structure activity relationships also discussed.

Materials and Methods

General experimental procedures

Column chromatography: silica gel (100 – 200 mesh, Qingdao Marine Chemical Company); Sephadex LH-20 (Amersham Bioseciences) and RP-18 (40 – 70 μm, YMC CO., Ltd.). Preparative HPLC was performed on a Shimadzu liquid chromatograph LC-9A instrument with UV-VIS spectrophotometric detector (SPD-6AV) at 254 nm using an ODS column (Unicorn ODS, 10 μm, 250 × 20 mm). Melting points were determined with a GERMANY-68 992 apparatus. Optical rotations were measured on a PE-241 digital polarimeter. EI-MS and HR-FAB-MS were obtained on a QB-200 mass spectrometer. ESI-MS measurements were carried out at an Agilent 1100 series LC/MSD Trap SL mass spectrometer. IR spectra were recorded on an IR-47 spectrometer. NMR spectra were recorded on a Varian MERCURY-400 and INOVA-500 spectrometer using TMS as an internal standard, and chemical shifts were given as ppm.

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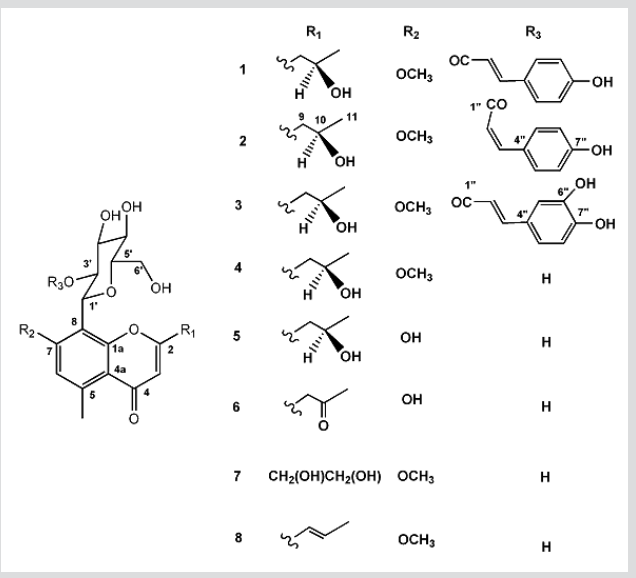

Fig. 1 Chromone glycosides from *A. vera*.

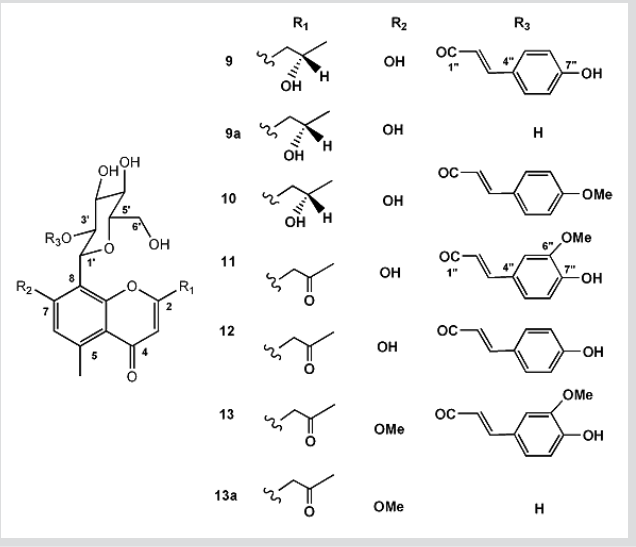

Fig. 2 Chromone glycosides from *A. nobilis* and hydrolysates **9a** and **13a.**

Plant materials

Aloe vera ethanolic extract were purchased from Tong Ren Tang Pharmaceutical Stores in 2003. The quality of the plant extract meets the standards for *Aloe* products in the China Pharmacopoeia 2005 edition, and the original plant was identified by Professor Lin Ma of our institute to be identical to the authenticated sample (No. 18339) of *A. vera* which is deposited at the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical college (CAMS & PUMC), Beijing, China.

Aloe nobilis was collected from the *Aloe* cultivated base of Ruiwangfen, Beijing, P. R. China, in 2004, and identified by Professor Lin Ma. The authenticated sample (No. 21572) of the plant is also deposited at the Herbarium of the Institute of Materia Medica, CAMS & PUMC.

Extraction and isolation

The ethanolic extract of *A. vera* (200.0 g) was subjected to silica gel column chromatography (10 × 150 cm, 2 kg) and eluted with $CHCl_3/CH_3OH/H_2O$ (80 : 20 : 2, 10.0 L) to provide five fractions [Fr. I (0 – 1.2 L,3.5 g), Fr. II (1.3 – 2.7 L, 5.2 g), Fr. III (2.8 – 3.9 L, 2.8 g), Fr. IV (4.0 – 5.7 L, 4.2 g) and Fr. V (5.8 – 10.0 L, 14.6 g)]. Frs. II and IV displayed potent inhibitory activity (64.18% and 60.11%) at 100 μg/mL during the screening for BACE inhibitory activity. Silica gel column chromatography (4.5 × 30 cm, 300.0 g) of Fr. II (5.2 g) eluting with a gradient of $CHCl_3/CH_3OH$ (90 : 10, 80 : 20, 50 : 50, each 4000 mL) afforded three fractions (Fr. II-1 – Fr. II-3). Compounds **3** (840 – 920 mL, 3.0 mg), **8** (1420 – 1520 mL, 5.0 mg) and a mixture of compounds **1** and **2** (220 – 760 mL) were obtained from Fr. II-2 (2.6 g) by silica gel column chromatography (2.5 × 35 cm, 200.0 g) using 15% CH_3OH in chloroform (3000.0 mL) as eluent. The mixture of compounds **1** and **2** was further purified on reversed-phase preparative HPLC [Unicorn ODS, 10 μm, 250 × 20 mm; CH_3OH/H_2O (55 : 45), flow rate 3.0 mL/min] to provide compounds **1** (60.0 mg, t_R = 29.2 min) and **2** (3.0 mg, t_R = 37.8 min). Fr. IV (4.2 g) was subjected to CC over Sephadex LH-20 (2.5 × 80 cm, 100.0 g) with a gradient system of 1 : 0 to 0 : 1 H_2O/CH_3OH (increased by 10% CH_3OH in gradient, each 350 mL), and 11 fractions (Frs. IV-1 – IV-11) were obtained. Fr. IV-8 (233.0 mg) was then chromatographed on a RP-18 column (2 × 25 cm, 40.0 g) with CH_3OH-H_2O (30 : 70, 600 mL) as eluent to afford compounds **4** (200 – 400 mL, 830.0 mg), **7** (60 – 80 mL, 3.4 mg) , **6** (460 – 500 mL, 6.0 mg), and **5** (100 – 160 mL, 10.2 mg), respectively.

Dried leaves (5 kg) of *A. noblis* were segmented and soaked in MeOH (15 L) at room temperature for one week. The resulting extract was filtered and concentrated to dryness under reduced pressure to furnish a brown residue (210 g), which was suspended in water (1 L) and partitioned with petroleum ether (3 × 500 mL), EtOAc (3 × 800 mL), and *n*-BuOH (3 × 800 mL) successively. The EtOAc soluble residue (13.0 g) was subjected to CC over silica gel (4 × 70 cm, 400 g), eluting with a gradient of $CHCl_3/CH_3OH$ (92 : 8, 90 : 10, each 6000 mL), to afford eleven fractions (Frs. I – XI). Fr. IV (943 mg) was then subjected to Sephadex LH-20 (60 g, 2.5 × 50 cm) with MeOH/H_2O (20 : 80, 450 mL) as eluent to afford five subfractions (Fr. IV-1 to Fr. IV-5, the elution volume of each fraction was 90 mL). Fr. IV-3 (120 mg) was separated by reversed-phase preparative HPLC [Unicorn ODS, 10 μm, 250 × 20 mm; CH_3CN/H_2O (27 : 73), flow rate 3.0 mL/min, t_R = 53.8 min] to yield compound **13** (35 mg). Fr. VII (1.6 g) was chromatographed over a Sephadex LH-20 column (60 g, 2.5 × 100 cm), eluting with MeOH/H_2O (20 : 80, 900 mL) to provide eleven fractions (the elution volume of each fraction was nearly about 80 mL). Fr-VII-7 (19 mg) was further purified by reversed-phase preparative HPLC [Unicorn ODS, 10 μm, 250 × 20 mm; CH_3OH/H_2O (55 : 45, 1‰ CF_3COOH, t_R = 58 min), flow rate 3.0 mL/min] to obtain **3** (3 mg). Fr. VII-10 (309 mg) was further purified by preparative TLC (20 × 30 cm, $CHCl_3$: MeOH : H_2O = 85 : 15 : 1) to give **10** (R_f = 0.54, 40 mg), **11** (R_f = 0.47, 140 mg) and **12** (R_f = 0.38, 7.0 mg), respectively. **9** (50 mg) was obtained from Fr. IX (1.7 g) by column chromatography (2.5 × 100 cm, Sephadex LH-20, MeOH, 580 mL) and preparative TLC (20 × 30 cm, $CHCl_3$/MeOH/H_2O = 80 : 20 : 2, R_f = 0.43).

Allo-aloeresin D (**2**): white amorphous powder; m. p. 124 °C; $[\alpha]_D^{16}$: + 49.1 (*c* 0.01, MeOH); UV (MeOH): λ_{max} (log ε) = 211.0 (4.35), 226.0 (4.33), 252.0 (sh); (4.11), 294 (4.13) nm; IR (KBr): ν_{max} = 3361, 1718, 1653, 1599, 1514, 1458, 1383, 1161, 984, 837,

Table 1 ^{1}H NMR and ^{13}C NMR spectral data for compounds **2, 8, 9** and **10***

	2		8		9		10	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	167.4		162.7		167.2		167.2	
3	112.5	6.10 s	109.8	5.95 s	112.1	6.04 s	112.1	6.02 s
4	182.3		182.7		182.2		182.2	
4a	117.2		117.4		117.0		116.9	
5	144.6		144.0		143.5		143.5	
6	112.7	6.74 s	112.8	6.80 s	116.3	6.50 s	115.7	6.50 s
7	162.1		162.5		161.3		160.4	
8	111.8		113.3		110.0		109.9	
1a	159.8		158.8		160.4		160.4	
9	44.6	2.67 d (10.5); 2.86 dd (10.5, 4.0)	124.7	6.20 d (15.6)	44.3	2.75 dd (13.6,5.6); 2.82 dd (14.0,7.6)	44.6	2.75 dd (13.6,5.2); 2.82 dd (13.6,7.2)
10	65.9	4.41 m	139.0	7.03 m	66.7	4.32 m	63.1	4.32 m
11	23.6	1.23 d (6.0)	18.4	1.92 d (6.8)	23.5	1.25 d (6.0)	23.3	1.24 d (6.0)
12	23.7	2.68 s	23.6	2.74 s	23.5	2.58 s	23.5	2.56 s
13	57.0	3.85 s	56.9	3.90 s				
1′	72.8	5.06 d (10.0)	75.1	4.71 d (10.0)	73.0	5.15 d (10.0)	72.2	5.12 d (10.0)
2′	73.4	5.66 d (10.0)	72.4	4.06 t (9.6)	74.0	5.67 t (10.0)	74.0	5.68 t (10.0)
3′	77.7	3.60 m	80.3	3.44 m	77.9		77.9	3.40 – 3.90
4′	72.4	3.48 m	72.3	3.44 m	72.1		72.9	3.40 – 3.90
5′	83.0	3.40 m H-6a′ 3.60 m	82.9	3.25 m H-6a′ 3.64 m	82.8	3.42 – 3.88	82.8	3.40 – 3.90
6′	63.1	H-6b′ 3.90 m	63.0	H-6b′ 3.84 dd (12.0,2.0)	63.1		62.4	
1″	167.6				168.0		168.2	
2″	115.7	5.49 d (12.5)			115.8	6.01 d (16.0)	116.3	6.04 d (16.0)
3″	145.0	6.59 d (12.5)			146.2	7.35 – 7.39	146.6	7.35 – 7.39
4″	127.0				128.3		127.1	
5″, 9″	133.2	6.93 d (8.5)			131.0	7.35 – 7.39	131.1	7.25 d (8.4)
6″, 8″	115.7	6.48 d (8.5)			116.7	6.84 d (8.4)	116.8	6.69 d (8.4)
7″	160.0				161.2		163.2	
7″-OMe					56.1	3.74 s		

* Measured in CD_3OD at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR, respectively, with assignments confirmed by HMQC and HMBC. δ in ppm and J in Hz.

721 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR (CD_3OD): see **Table 1**; HR-ESI-MS: m/z = 557.2012 $[M+H]^+$ (calcd. for $C_{29}H_{32}O_{12}$: 557.2023).

C-2′-Decoumaroyl-aloeresin G (**8**): white amorphous powder; m.p. 119 °C; $[\alpha]_D^{16}$: + 3.08 (*c* 0.33, MeOH); UV (MeOH): λ_{max} (log ε) = 209.0 (3.27), 259.0 (3.01), 308.0 (2.95) nm; IR (KBr): ν_{max} = 3390, 1657, 1626, 1454, 1383, 1122, 1080, 903, 850, 721 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR (CD_3OD): see **Table 1**; HR-ESI-MS: m/z = 393.1592 $[M+H]^+$ (calcd. for $C_{29}H_{32}O_{12}$: 393.1549).

2′-O-Coumaroyl-(S)-aloesinol (**9**): white amorphous powder; $[\alpha]_D^{20}$: –177.3 (*c* 0.105 in MeOH); ^{1}H-NMR and ^{13}C-NMR (CD_3OD), see **Table 1**; HR-negative ESI-MS: m/z = 541.1716 $[M-H]^+$ (calcd. for $C_{28}H_{29}O_{11}$: 541.1710).

*2′-O-[*p*-Methoxy-(*E*)-cinnamoyl]-(S)-aloesinol* (**10**): white amorphous solid; $[\alpha]_D^{20}$: –156.3 (*c* 0.71 in MeOH); ^{1}H-NMR and ^{13}C NMR (CD_3OD): see **Table 1**; HR-positive ESI-MS: m/z = 557.1998 $[M+H]^+$ (calcd. for $C_{29}H_{33}O_{11}$: 557.2023).

Alkaline hydrolysis of *Aloe* chromone glycosides

To a solution of compound **1** (20.0 mg) in MeOH (0.5 mL) was added 10% NaOH-H_2O (0.5 mL). The mixture was stirred at room temperature for 0.5 h, and then 20 mL of H_2O was added and extracted with *n*-BuOH (10 mL × 3). The organic layer was evaporated under reduced pressure and purified by preparative thin-layer chromatography (20 × 30 cm, R_f = 0.3, $CHCl_3/CH_3OH/H_2O$, 75 : 25 : 2) to obtain product **1a** (5.4 mg, R_f = 0.54). Similarly, **2** (1.0 mg) was hydrolyzed to **2a** (< 0.5 mg). Compounds **1a** and **2a** are identical on HPLC analysis (column: 250 × 4.6 mm, Kromasil RP-18, 5 μm; flow rate: 1 mL/min; detector: λ_{max} = 300 nm; mobile phase: CH_3OH/H_2O, 30 : 70; retention times for both **1a** and **2a** are 5.3 min). Compounds **9**, **10, 11** and **13** were hydrolyzed similarly, and (*S*)-aloesinol (**9a**), aloesin (**6**), 7-*O*-methylaloesin (**13a**) were obtained, respectively.

8-C-Glucosyl-7-methoxy-(R)-aloesol (**1a**): amorphous white powder, m.p. 145 °C; $[\alpha]_D^{16}$: –30.1 (*c* 0.24, MeOH); ESI-MS: m/z = 433.2 $[M+Na]^+$; ^{1}H-NMR (500 MHz, CD_3OD): δ = 1.24 (3H, d, J = 6.0 Hz, H-11), 2.58 (2H, dd, J = 9.5, 5.0 Hz, H-9), 2.73 (3H, s, H-12), 3.32 – 3.44 (3H, m, H-3′, H-4′, H-5′), 3.60 (1H, m, H-6′α), 3.84 (1H, m, H-6′β), 3.89 (3H, s, OCH_3-7), 4.12 (1H, t, J = 10.0 Hz, H-2′), 4.32 (1H, m, H-10), 4.94 (1H, d, J = 10.0 Hz, H-1′), 6.03 (1H, s, H-3), 6.86 (1H, s, H-6). Its physical properties and ^{1}H-NMR data

were identical to those of 8-*C*-glucosyl-7-methoxy-(*R*)-aloesol (**4**) [12].

Determination of BACE1 inhibition by an *in vitro* peptide cleavage assay

The ability of the compounds to inhibit the cleavage of APP to Aβ was assessed using a fluorescent resonance energy transfer (FRET) peptide cleavage assay. The FRET peptide substrate Mca-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(Dnp)-Arg-Arg-NH (where Mca is (7-methoxycoumarin-4-yl)-acetyl, and Dnp is 2,4-dinitrophenyl) and recombinant human BACE1 were purchased from R&D Systems, Inc. A commercially available BACE1 inhibitor *N*-benzyloxycarbonyl-Val-Leu-leucinal (*Z*-Val-Leu-Leu-CHO, CALBIOCHEM® 565749) was purchased from EMD Biosciences, Inc. and used as a positive control. The assay was carried out according to the supplier's protocol with modifications. Briefly, assays were performed in triplicate in 96-well black plates with 100 μL of 50 mM sodium acetate buffer (pH 4.0), containing 4 μM substrate, 2 mg/mL recombinant human BACE1 and different concentrations of inhibitors (dissolved in small volumes of DMSO prior to addition to the buffer). The fluorescence intensity was measured using a SpectraMAX Gemini XS plate reader (Molecular Devices Co.) at Ex320/Em405 both at zero time and after 60 min incubation at 25 °C. The inhibition percentage was calculated with the following equation: Inhibition (%) = $[1-(F_S-F_{S0})/(F_C-F_{C0})]\times 100\%$, where F_{S0} and F_S are the fluorescence of samples at zero time and 60 min, and F_{C0} and F_C are the fluorescence of control at zero time and 60 min, respectively. The IC_{50} value was calculated from the non-linear curve fitting of percentage inhibition against inhibitor concentration ([I]) using Prism 3.0 software. All data presented are the mean values of triplicate experiments. The purities of samples were determined by HPLC, and compounds **1 – 13** and the positive control were found to have a purity of more than 99%, and **9a** and **13a** were 93% and 98% pure, respectively.

It is worthwhile to note that the IC_{50} value for our positive control peptide, *N*-benzyloxycarbonyl-Val-Leu-leucinal (CALBIOCHEM® 565749), in the enzyme inhibition assay is higher than its IC_{50} in the cell-based assay, while its IC_{50} determined by us in the cell-based assay is comparable to that reported in literature [21]. The reason for this difference is that the peptide is a competitive inhibitor, and thus its IC_{50} value in the *in vitro* enzyme inhibition assay will vary depending on the concentration of substrate. When the substrate concentration in the *in vitro* assay was reduced from 4 μM to 3 μM, the IC_{50} of the positive control was also reduced from 8.2 μM to 5.5 μM. Since the substrate concentration used in the enzyme inhibition assay is usually higher than that used in the cell-based assay, the *in vitro* IC_{50} for the inhibitor is correspondingly higher.

Effect on Aβ_{1-42} production in B103 neuroblastoma cells

APP_{695}-transfected B103 rat neuroblastoma cells were seeded at a density of 5×10^5 in pi 60 plates and incubated with testing compounds (*ca.* $65-80\times10^{-6}$ M) for 48 h. Then, the media were collected by centrifugation. The amount of Aβ_{1-42} produced was estimated with the "Immunoassay Kit for human β amyloid 1 – 42" from Biosource according to the manual supplied. Briefly, the collected media were transferred to the 96-well plates where Aβ_{1-42} antibody was pre-coated, and they were treated with detection antibody. After 3 h of incubation at room temperature, HRP (horseradish peroxidase) anti-rabbit antibody and stabilized chromogen were added for antigen-antibody reaction. The reaction was stopped by stop solution, and the optical density was read at 450 nm. 1% DMSO was used as a control.

Results and Discussion

The EtOH extract of *A. vera* was separated into five fractions by silica gel column chromatography, and Frs. II and IV exhibited the most potent activity in the BACE inhibition assay. Further bioactivity-guided fractionation of these fractions resulted in the isolation of eight chromone *C*-glycosides (**1 – 8**, see **Fig. 1**), aloeresin D (**1**) [10], allo-aloeresin D (**2**), rebaichromone (**3**) [11], 8-*C*-glucosyl-7-methoxy-(*R*)-aloesol (**4**) [12], 8-*C*-glucosyl-(*R*)-aloesol (**5**) [13], aloesin (**6**) [14] and 8-*C*-glucosyl-7-*O*-methylaloediol (**7**) [13], among which **2** and **8** are new compounds. Chromone **8** was the most potent BACE1 inhibitor with an IC_{50} of 20.5 μM. From the MeOH extract of *A. noblis*, five chromone glycosides (**3, 9 – 13**, see **Fig. 2**) were isolated by HPLC-UV and characterized as 2′-*O*-coumaroyl-(*S*)-aloesinol (**9**), 2′-*O*-(*p*-methoxy-*E*-cinnamoyl)-(*S*)-aloesinol (**10**), 2′-feruloylaloesin (**11**) [13], aloeresin A (**12**) [15] and 2′-feruloyl-7-*O*-methylaloesin (**13**) [16], among which **9** and **10** are identified as new natural chromone *C*-glycosides.

The molecular formula of **2** was established as $C_{29}H_{32}O_{11}$ by HR-ESI-MS (*m/z* = 557.2012 $[M+H]^+$). Its UV spectrum absorption bands are in agreement with the chromone skeleton [17]. Its ^{1}H-NMR and ^{13}C-NMR spectra are similar to those of aloeresin D [12] except for the signals at δ = 5.49 (1H, d, *J* = 12.5 Hz) and 6.59 (1H, d, *J* = 12.5 Hz), indicating the presence of a (*Z*)-*p*-coumaroyl group. The correlations of H-2′ (δ = 7.03) to C-1″ (δ = 167.6) and H-9 (δ = 2.67 and 2.86) to C-2 (δ = 167.4) and C-3 (δ = 112.5) in HMBC supported the attachment of the coumaryl group to C-2′ of the glucose and hydroxylpropyl to C-2 of the chromone. For the assignment of the absolute configuration of the hydroxylpropyl moiety of **2**, it is impossible to differentiate two diastereoisomers of (*R*)- and (*S*)-hydroxylpropyl groups based on their optical rotations, since they exhibited almost the same values [12], [18]. However, it was reported that the C-10 diastereoisomers of **4** exhibited a different optical rotation value {the optical values of the (*S*)-configuration diastereoisomer at C-10 was positive [18] but the (*R*)-configuration diastereoisomer at C-10 was negative [12]} as well as a different retention time (t_R) on HPLC [18]. Thus, the absolute configuration of C-10 was proven to be (*R*)-configuration by comparing the t_R's and optical rotation value of the 2′-deacyl products **1a** ($[\alpha]_D^{16}$: –30.1) with that of **4** ($[\alpha]_D^{30}$: –39.5). From the above evidence, the structure of **2** was identified as 8-[*C*-β-D-[2-*O*-(*Z*)-*p*-coumaroyl]-glucopyranosyl]-2-[(*R*)-2-hydroxypropyl]-7-methoxy-5-methylchromone.

The molecular formula of **8** was assigned as $C_{20}H_{24}O_8$ by its positive HR-ESI-MS (*m/z* = 393.1592 $[M+H]^+$). Comparison of the NMR spectral data of **8** with those of aloeresin G [19] revealed the absence of a (*E*)-*p*-coumaroyl group linked to the 2′-OH of the glucose in **8**. The location of the propenyl group at C-2 was confirmed by the HMBC correlations between H-9 (δ = 6.20), H-10 (δ = 7.03) and C-2 (δ = 162.7). The correlations of H-1′ (δ = 4.71) to C-8 (δ = 113.3) and C-1a (δ = 158.8), H-9 to C-2 in HMBC further confirmed this assignment. From the above evidence, the structure of **8** was elucidated as 2-(*E*)-propenyl-7-methoxy-8-*C*-β-D-glucopyranosyl-5-methylchromone.

The negative HR-ESI-MS of **9** suggested its molecular formula to be $C_{28}H_{29}O_{11}$ (*m/z* = 541.1716 $[M-H]^+$). The ^{1}H- and ^{13}C-NMR spectral data (**Table 1**) of **9** showed key structural features of

a hydroxypropyl, γ-pyrone, 5-Me and 8-*C*-glucoside, which were similar to those of isoaloeresin D [19] and aloeresin D [12], except for the absence of a methyl group linked to 7-OH. The positions of the *p*-coumaroyl group on the sugar and the attachment of glucose and hydroxylisopropyl to the chormone core structure in **9** were assigned based on the correlations of H-2′ (δ = 5.67) to C-1″ (δ = 168.0), H-1′ (δ = 5.15) to C-8 (δ = 110.0) /C-1a (δ = 160.4), and H-9 (δ = 2.75 and 2.82) to C-2 (δ = 167.2) in HMBC. The positive $[\alpha]_D^{20}$ (+ 6.81) of the hydrolyzed product (**9a**) suggested the C-10 configuration to be (*S*). Accordingly, **9** was elucidated as 2′-*O*-coumaroyl-(*S*)-aloesinol.

The HR-positive ESI-MS of **10** suggested its molecular formula to be $C_{29}H_{33}O_{11}$ (*m/z* = 557.1998 $[M+H]^+$). The ^{1}H- and ^{13}C-NMR spectral data (**Table 1**) of **10** were similar to those of **9**, except for one additional methoxy group (δ = 3.74) attached to the aromatic ring of the cinnamoyl group at the C-7″ position, which was determined by its correlation to C-7″ (δ = 163.2) in the HMBC spectrum. The positive $[\alpha]_D^{20}$ (+ 6.94) of the hydrolyzed product (**10a**) suggested the C-10 configuration to be (*S*). From above evidence, **10** was elucidated as 2′-*O*-[*p*-methoxy-(*E*)-cinnamoyl]-(*S*)-aloesinol.

Table 2 BACE inhibitory activities of *Aloe* chromone glycosides

Compounds	Inhibition (%) at 10 μM	Inhibition (%) at 100 μM	IC_{50} (μM)
1	19.2 ± 3.2	71.5 ± 8.5	39.0
4	8.5 ± 1.2	26.8 ± 4.1	
5	20.8 ± 7.7	39.2 ± 4.9	
6	12.2 ± 4.1	37.5 ± 6.0	
8	37.6 ± 4.6	72.0 ± 11.9	20.5
9	17.3 ± 1.8	51.9 ± 5.0	
9a	28.0 ± 6.5	37.2 ± 4.5	
10	23.7 ± 1.1	34.1 ± 2.4	
11	24.9 ± 3.8	36.4 ± 4.0	
12	9.6 ± 2.2	39.5 ± 0.7	
13	20.9 ± 3.7	48.7 ± 2.4	
13a	14.9 ± 3.4	41.0 ± 7.3	
Reference [b]			11.6

[a] The inhibition rates (%), which were calculated as described in Materials and Methods, are mean ± SD of three independent experiments.

[b] *N*-Benzyloxycarbonyl-Val-Leu-leucinal (Z-Val-Leu-Leu-CHO, CALBIOCHEM® 565 749) was used as the positive control.

The BACE1 inhibitory activities of naturally occurring chromone glucosides (**1, 4 – 13**) and two hydrolyzed products (**9a**, **13a**) were tested at two different concentrations (10 and 100 μM), respectively. The most potent compounds **1** and **8** were selected for IC_{50} determinations (**Table 2**).

Preliminary SARs can be drawn for these *Aloe* chromone glycosides:

(1) For chromone *C*-glucosides without C-2′ ester side chains, **4 – 6, 8, 9a** and **13a**, it was found that the C-2 isopropyl side chain substituted either by α-OH, β-OH or keto groups does not significantly affect the activity (**5** vs. **6** or **9a**, **4** vs. **13a**), especially at 100 μM. Although the change of inhibition rates from 10 to 100 μM was different: **6** > **5** > **9a**. However, **8**, with a C-2 propenyl side chain, exhibited the highest activity (IC_{50} 20.5 μM) among all these compounds.

(2) The possible role of the cinnamoyl group at C-2′ of the glucose moiety was also analyzed by comparison of several pairs of compounds (**1** vs. **5**, **9** vs. **9a**, **12** vs. **6**). At 10 μM, attachment of cinnamoyl did not significantly alter the activity; whereas at 100 μM, a cinnamoyl group seems to potentiate the activity with the exception of the activity of **12** that is comparable to that of **6**. For the introduction of additional substitutions at the phenyl ring of C-2′ cinnamoyl group (**9** vs. **10**, **11** vs. **12**), no general trends were observed. Among these esterized chromone glycosides, **1** showed strongest inhibition (IC_{50} 39.0 μM) of BACE1.

Attempts to delineate the inhibition modes of *Aloe* chromone glycosides failed due to unsatisfactory signal to noise ratios during the kinetic determinations of **1** and **8**.

Compounds **1** and **8** were also tested for their ability to reduce the amount of $A\beta_{1-42}$ produced in the APP695-transfected B103 rat neuroblastoma cells (**Fig. 3**), and found to reduce the amount of $A\beta_{1-42}$ by 12.25 % (produced 87.75 ± 6.76 % of a control for **8**) and by 7.36 % (produced 92.64 ± 5.81 % of a control for **1**).

Although these chromone glycosides are only moderately active, their BACE inhibitory activity is confirmed by enzyme inhibition and cell assays, thereby providing another structural motif for the exploitation of natural products based BACE1 inhibitors.

Finally, it should be noted that we have recently reported the isolation of two chromone glycosides from another *Aloe* species plant, *A. arborescens*, with moderate BACE inhibitory activity [20]. One of the two compounds is identical to **13** and another is 7-*O*-methylaloeresin A, and their moderate activities are also confirmed by this study.

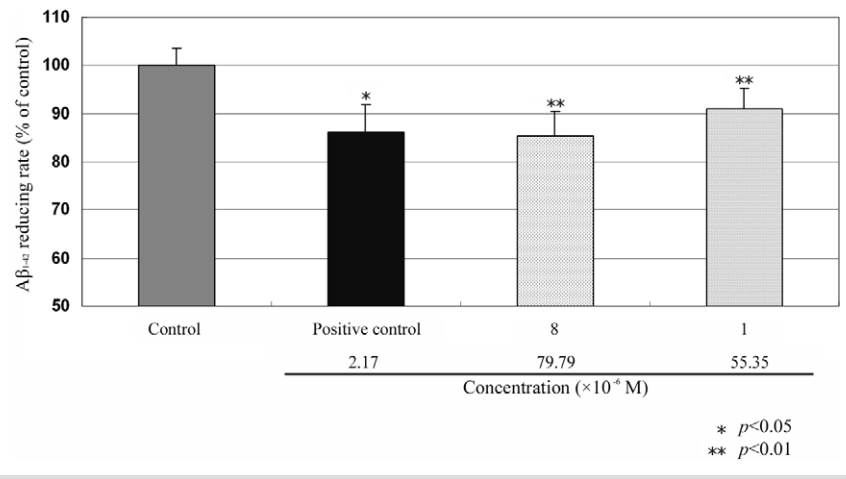

Fig. 3 Inhibitory effect of **8** and **1** on $A\beta_{1-42}$ production in B103 cells. Values are the mean ±SD of triplicate experiments.

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