Bioactive Iridoid Glucosides from the Fruit of *Gardenia jasminoides*

Yang Yu,[†] Zuo-lei Xie,[∥] Hao Gao,[‡,⊥] Wei-wei Ma,[†] Yi Dai,[‡,⊥] Ying Wang,[‡,⊥] Yi Zhong,[*,§] and Xin-sheng Yao[*,†,‡]

Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China, Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, People's Republic of China, Department of Biological Science and Biotechnology, Tsinghua University, Beijing 100084, People's Republic of China, Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University, Guangzhou 510632, People's Republic of China, and Joekai Biotech. Co., Ltd., Beijing 100020, People's Republic of China

Received March 20, 2009

Seven new iridoid glucosides, 6″-*O*-*trans*-sinapoylgenipin gentiobioside (**1**), 6″-*O*-*trans*-*p*-coumaroylgenipin gentiobioside (**2**), 6″-*O*-*trans*-cinnamoylgenipin gentiobioside (**3**), 6′-*O*-*trans*-*p*-coumaroylgeniposide (**4**), 6′-*O*-*trans*-*p*-coumaroylgeniposidic acid (**5**), 10-*O*-succinoylgeniposide (**6**), and 6′-*O*-acetylgeniposide (**7**), two new monoterpenoids, 11-(6-*O*-*trans*-sinapoylglucopyranosyl)gardendiol (**8**) and 10-(6-*O*-*trans*-sinapoylglucopyranosyl)gardendiol (**9**), and three known ones, 6′-*O*-*trans*-sinapoylgeniposide (**10**), geniposide (**11**), and 10-*O*-acetylgeniposide (**12**), were isolated from the fruit of *Gardenia jasminoides*. The structures of these compounds were elucidated on the basis of 1D and 2D NMR spectra analyses. Furthermore, short-term memory assays on an A*β* transgenic drosophila model showed that compounds **4** and **6**−**12** can improve the short-term memory capacity to varying degrees, with compounds **4** and **7** being the most active ones, suggesting that these compounds may have a potential antagonism effect against Alzheimer's disease.

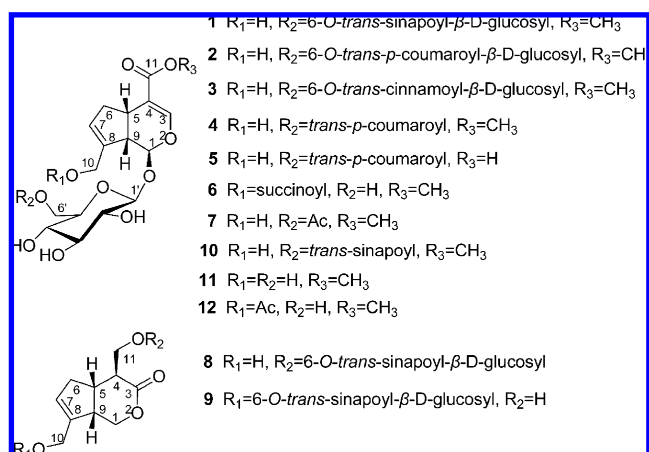
Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by a progressive cognitive decline associated with impairment in activities of daily living and behavioral disturbances throughout the disease course.[1] As the pathogenesis of AD is complicated, there is no ideal drug for preventing or treating AD.[2] Currently available drugs, such as AchE inhibitors and the NMDA (*N*-Me D-aspartate) receptor antagonist, improve the symptoms of AD but do not halt or reverse the pathophysiological process.[3]

According to the amyloid hypothesis,[4] the accumulation of A*β* in the brain is the primary cause leading to AD pathogenesis.[5] The extract of *Gardenia jasminoides* showed potential enhancement of short-term learning/memory abilities in human A*β*42 transgenic flies. It suggested that the components of *G. jasminoides* might have antagonism effects against AD.

The fruit of *G. jasminoides* (Rubiaceae) is widely used as a traditional Chinese medicine in many Asian countries for its cholagogue, diuretic, antiphlogistic, and antipyretic effects.[6] A number of iridoid glucosides,[7−11] monoterpenes,[12,13] flavonoids,[14] and crocetin[15,16] have been reported from *G. jasminoides*. Our recent studies led to the discovery of nine new and three known glucosides (**1**−**12**). Their biological activities were evaluated by using a transgenic fly AD model. In the drosophila AD model, it is found that the human A*β*42 peptide expressed in the drosophila brain can induce pathological phenotypes resembling AD.[4] The expression of A*β*42 led to the formation of diffused amyloid deposits, age-dependent learning defects, and extensive neurodegeneration, which makes it an excellent tool in potential pharmaceutical drug finding.[4]

Results and Discussion

The 60% (v/v) EtOH extract of the dried fruit of *G. jasminoides* was subjected to column chromatography over D101 macroporous adsorptive resins, silica gel, RP-18, Toyopearl HW-40, and HPLC



to afford glycosides **1**−**12**. Each glycoside showed a positive reaction to the Molisch reagent. Structural elucidation of the 12 glycosides was achieved by extensive 1D and 2D NMR spectroscopic analyses.

Compound **1** was obtained as a yellow, amorphous powder. HRESIMS gave a quasimolecular ion peak at *m/z* 779.2369 [M + Na]$^+$, corresponding to the molecular formula $C_{34}H_{44}O_{19}$. The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) showed four olefinic proton and six olefinic carbon signals, including a set of *trans*-double-bond signals [*δ* 7.63 (1H, d, *J* = 15.8 Hz, H-3‴), 6.43 (1H, d, *J* = 15.8 Hz, H-2‴), 147.3 (C-3‴), 115.7 (C-2‴)]. Proton signals at *δ* 6.91 (2H, s, H-5‴, 9‴) and 3.88 (6H, s, 6‴, 8‴-OCH$_3$), together with carbon signals at *δ* 107.0 (C-5‴, 9‴), 126.6 (C-4‴), 140.0 (C-7‴), 149.4 (C-6‴, 8‴), and 56.9 (6‴, 8‴-OCH$_3$), suggested the presence of a symmetrical 1,3,4,5-tetrasubstituted benzene ring. Furthermore, the HMBC correlations observed at H-3‴ (*δ* 7.63)/C-4‴ (*δ* 126.6), C-5‴, 9‴ (*δ* 107.0), and C-1‴ (*δ* 169.1), and those at H-2‴ (*δ* 6.43)/C-1‴ (*δ* 169.1) and C-4‴ (*δ* 126.6), revealed the presence of a *trans*-sinapoyl moiety. After acid hydrolysis and derivatization of **1** by the method of Hara,[17] GC analysis revealed the presence of D-glucose. The procedure was described in refs 18 and 19. Additionally, the signals of the two glucosyl units were assigned on the basis of the analysis of the information of the ^{1}H−^{1}H COSY and TOCSY experiments. The HMBC correlations observed at H-6′ (*δ* 4.11, 3.76)/C-1″ (*δ* 105.0) and H-1″ (*δ* 4.40)/C-6′ (*δ* 70.2) suggested that the sugar chain was glucopyranosyl-

* To whom correspondence should be addressed. Tel: +86-20-85225849. Fax: +86-20-85221559. E-mail: tyaoxs@jnu.edu.cn; zhongyi@cshl.edu.
[†] Shenyang Pharmaceutical University.
[∥] Joekai Biotech. Co., Ltd.
[‡] College of Pharmacy, Jinan University.
[⊥] Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Jinan University.
[§] Tsinghua University.

10.1021/np900176q CCC: $40.75 © 2009 American Chemical Society and American Society of Pharmacognosy
Published on Web 08/03/2009

Table 1. NMR Spectroscopic Data (methanol-d_4) of Compounds **1**−**3**

	1		**2**		**3**	
pos.	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	98.8	5.13, d (7.8)	98.8	5.14, d (7.8)	98.8	5.15, d (7.8)
3	153.3	7.45, d (0.8)	153.4	7.47, brs	153.4	7.47, brs
4	112.3		112.3		112.4	
5	36.6	3.13, m	36.7	3.16, m	36.7	3.13, m
6	39.7	2.79, dd (16.1, 8.3)	39.7	2.79, dd (16.0, 8.4)	39.8	2.79, dd (16.4, 8.7)
		2.12, dd (16.1, 8.2)		2.14, dd (16.0, 8.0)		2.15, dd (16.4, 8.5)
7	129.0	5.82, brs	129.0	5.83, brs	129.0	5.83, brs
8	144.8		144.8		144.8	
9	47.0	2.70, t (7.7)	46.9	2.70, t (7.8)	47.0	2.70, t (7.7)
10	61.5	4.31, o[a]	61.5	4.31, o	61.5	4.29, o
		4.20, brd (13.4)		4.20, brd (13.3)		4.20, brd (14.0)
11	169.5		169.6		169.6	
11-OCH$_3$	51.7	3.68, s	51.7	3.69, s	51.7	3.69, s
1′	100.6	4.71, d (7.9)	100.6	4.71, d (7.9)	100.6	4.71, d (7.9)
2′	74.8	3.25, m	74.8	3.25, m	74.8	3.25, m
3′	77.8	3.40, m	77.8	3.40, m	77.8	3.40, m
4′	71.7	3.28, m	71.7	3.26, m	71.8	3.28, m
5′	77.5	3.54, m	77.6	3.53, m	77.6	3.54, m
6′	70.2	4.11, dd (12.0, 1.6)	70.1	4.10, dd (12.0, 1.5)	70.1	4.10, dd (12.0, 1.5)
		3.76, dd (12.0, 7.1)		3.74, dd (12.0, 7.1)		3.74, dd (12.0, 7.1)
1″	105.0	4.40, d (7.9)	105.0	4.40, d (7.9)	105.0	4.40, d (7.7)
2″	75.1	3.21, m	75.1	3.21, m	75.1	3.21, m
3″	77.8	3.38, m	77.8	3.38, m	77.8	3.38, m
4″	71.6	3.35, m	71.6	3.34, m	71.6	3.35, m
5″	75.4	3.52, m	75.4	3.51, m	75.4	3.52, m
6″	64.7	4.53, dd (12.0, 1.8)	64.7	4.53, dd (12.0, 1.9)	64.9	4.54, dd (12.0, 1.9)
		4.30, o		4.29, m		4.33, o
1‴	169.1		169.2		168.5	
2‴	115.7	6.43, d (15.8)	115.0	6.36, d (15.7)	118.7	6.58, d (15.8)
3‴	147.3	7.63, d (15.8)	146.9	7.64, d (15.8)	146.6	7.73, d (15.8)
4‴	126.6		127.2		135.8	
5‴, 9‴	107.0	6.91, s	131.3	7.47, d (8.6)	129.3	7.62, m
6‴, 8‴	149.4		116.8	6.81, d (8.6)	130.0	7.41, m
7‴	140.0		161.3		131.6	7.41, m
6″, 8″-OCH$_3$	56.9	3.88, s				

[a] "o" means peaks overlap with other signals.

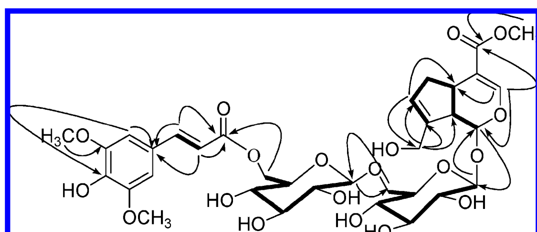


Figure 1. Key HMBC (→) and COSY (−) correlations of **1**.

(1→6)-glucopyranoside, i.e., a gentiobiosyl moiety. The β-configuration was established due to the coupling constants of the anomeric proton signals at δ 4.71 (1H, d, J = 7.9 Hz, H-1′) and 4.40 (1H, d, J = 7.9 Hz, H-1″).

The remaining ^{1}H and ^{13}C NMR signals were similar to those of genipin by comparing their spectroscopic data with reported data.[13] It suggested that **1** should be an iridoid glycoside with a *trans*-sinapoyl substituent. The partial structures of the genipin, *trans*-sinapoyl, and gentiobiosyl moieties were confirmed by ^{1}H−^{1}H COSY, HSQC, and HMBC spectra (Figure 1). The gentiobiosyl moiety was attached at C-1 of genipin, due to the HMBC correlations observed at H-1 (δ 5.13)/C-1′ (δ 100.6) and H-1′ (δ 4.71)/C-1 (δ 98.8). Furthermore, the linkage of the sinapoyl to the gentiobiosyl group was established at C-6″ by the downfield shift of C-6″ (δ 64.7), as well as the HMBC correlations at H-6″ (δ 4.53, 4.30)/C-1‴ (δ 169.1). Thus, the structure of **1** was elucidated as 6″-*O*-*trans*-sinapoylgenipin gentiobioside (**1**).

Compound **2** was obtained as a yellow, amorphous powder. It had a molecular formula of $C_{32}H_{40}O_{17}$ by analysis of its HRESIMS spectrum. The ^{1}H and ^{13}C NMR data of **2** were similar to those of **1**, showing the absence of two methoxy groups, suggesting that **2** had a *trans*-*p*-coumaroyl substituent at C-6″. Therefore, the structure of **2** was elucidated as 6″-*O*-*trans*-*p*-coumaroylgenipin gentiobioside (**2**).

Compound **3**, obtained as a yellow, amorphous powder, had a molecular formula of $C_{32}H_{40}O_{16}$ established by HRESIMS. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **1**, except that a *trans*-cinnamoyl moiety in **3** replaced the *trans*-sinapoyl unit in **1**. Similarly, the linkage of the cinnamoyl to the gentiobiosyl unit was established at C-6″ (δ 64.9) by correlations between the H-6″ (δ 4.54, 4.33) and the ester carbonyl signal at δ 168.5 (C-1‴) in its HMBC spectrum. Therefore, the structure of **3** was elucidated as 6″-*O*-*trans*-cinnamoylgenipin gentiobioside (**3**).

Compound **4** was obtained as a pale yellow gum with a molecular formula of $C_{26}H_{30}O_{12}$. The ^{1}H and ^{13}C NMR data of **4** were similar to those of **2**, showing the absence of a glucosyl moiety, suggesting that **4** should be geniposide with a *trans*-*p*-coumaroyl substituent. Thus, the structure of **4** was elucidated as 6′-*O*-*trans*-*p*-coumaroylgeniposide (**4**).

Compound **5**, a pale brown, amorphous powder, had a molecular formula of $C_{25}H_{28}O_{12}$ by analysis of its HRESIMS spectrum. The ^{1}H and ^{13}C NMR data of **5** were similar to those of **4**, showing the absence of a methoxy group at C-11. Therefore, the structure of **5** was elucidated as 6′-*O*-*trans*-*p*-coumaroylgeniposidic acid (**5**).

Compound **6** was obtained as a yellow, amorphous powder. The molecular formula of **6**, $C_{21}H_{28}O_{13}$, was deduced from its HRESIMS spectrum. The ^{1}H and ^{13}C NMR data of **6** were similar to those of **4**, except that a succinoyl group in **6** replaced the coumaroyl group in **4**. The ^{1}H and ^{13}C NMR data of **6** showed the presence of two methylene groups [δ 2.60 (2H, m, H-3″), 2.46 (2H, m, H-2″), 33.1 (C-3″), 31.6 (C-2″)], a carboxylic acid carbonyl [δ 181.8 (C-4″)],

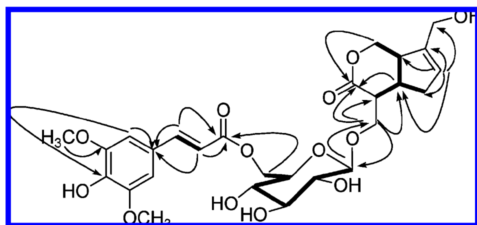


Figure 2. Key HMBC (→) and COSY (−) correlations of **8**.

and an ester carbonyl [δ 175.3 (C-1″)]. The HMBC correlations at H-2″/C-1″, C-4″ and H-3″/C-4″, C-1″ suggested the presence of a succinoyl group. In addition, the attachment of the succinoyl group was indicated to be at C-10 (δ 63.8) by the HMBC correlation between H-10 (δ 4.78) and the ester carbonyl of the succinoyl group (δ 175.3). Therefore, the structure of **6** was assigned as 10-*O*-succinoylgeniposide (**6**).

Compound **7** was obtained as a yellow, amorphous powder, and its elemental composition was determined to be $C_{19}H_{26}O_{11}$ by HRESIMS. The ^{1}H and ^{13}C NMR data of **7**, however, lacked the signals of the coumaroyl in **4** and instead showed signals characteristic of an acetyl group [δ 2.02 (3H, s, H-2″), 20.7 (C-2″), 172.7 (C-1″)]. The acetylation position was established at C-6′ due to the HMBC correlations between the carbonyl C-1″ (172.7) and H-6′ (δ 4.35, 4.25). Consequently, the structure of **7** was deduced to be 6′-*O*-acetylgeniposide (**7**).

Compound **8** was purified as a brown, amorphous powder with a molecular formula of $C_{27}H_{34}O_{13}$, as determined by HRESIMS analysis. The ^{1}H and ^{13}C NMR data of **8** showed the presence of a sinapoyl group and a β-D-glucosyl unit. The remaining signals arising from the aglycone moiety were deduced as gardendiol by comparing the observed and reported[20] NMR data and confirmed by the correlations of $^{1}H-^{1}H$ COSY and HMBC spectra (Figure 2). The relative configuration was established from the NOE correlations at H-5/H-1β and H-9/H-11 in the ROESY experiment. The linkage between the glucosyl and the aglycone moiety at C-11 was deduced by the HMBC correlations at H-1′ (δ 4.39)/C-11 (δ 69.2) and H-11 (δ 3.98)/C-1′ (δ 105.1). Furthermore, the HMBC correlations at H-6′ (δ 4.52, 4.35)/C-1″ (δ 169.0) indicated that the sinapoyl group was attached at C-6′ through an ester bond. The CD spectrum of **8** showed positive Cotton effects at 195.6 nm (Δε +6.86) and 216.0 nm (Δε −5.48), suggesting the 4*S* absolute configuration.[20] Therefore, the structure of **8** was elucidated as 11-(6-*O*-*trans*-sinapoylglucopyranosyl)gardendiol (**8**).

Compound **9** was a brown, amorphous powder with a molecular formula of $C_{27}H_{34}O_{13}$. The ^{1}H and ^{13}C NMR data of **9** showed signals due to 6′-*O*-sinapoyl glucopyranosyl and gardendiol moieties. In the HMBC spectrum, the anomeric proton H-1′ (δ 4.31) showed a long-range correlation with the oxygenated methylene carbon at δ 67.7 (C-10), indicating that the 6′-*O*-sinapoyl glucosyl was linked to C-10 of the aglycone. Thus, **9** was determined as 10-(6-*O*-*trans*-sinapoylglucopyranosyl)gardendiol (**9**).

In addition, the structures of three known compounds were identified as 6′-*O*-*trans*-sinapoylgeniposide (**10**),[10] geniposide (**11**),[21] and 10-*O*-acetylgeniposide (**12**)[22] by comparing their spectroscopic data with reported data.

The biological activities of the isolated compounds from *G. jasminoides* were evaluated by using the human Aβ42 transgenic fly AD model. The activities of compounds **1**−**12** were indicated by the performance index (PI); see Figure 3. Compounds **4** and **6**−**12** showed activities of short-term memory enhancement in AD flies to varying degrees, with compounds **4** and **7** being the most active.

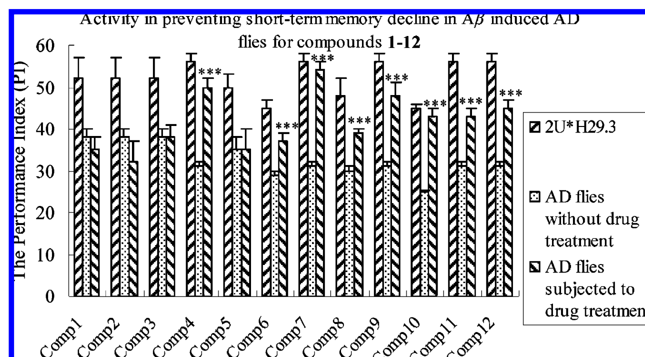


Figure 3. Performance index (PI) of AD flies fed with compounds **1**−**12**. $N = 8$ for all groups. Each value is the mean ± SE (SE refers to standard error), *** $P < 0.001$, compared with AD flies without drug group.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a JASCO V-550 UV/vis spectrometer. CD spectra were measured with a JASCO J810-150S spectrometer. IR spectra were obtained using a JASCO FT/IR-480 plus spectrometer. ESIMS spectra were taken on a Finnigan LCQ Advantage MAX mass spectrometer. HRESIMS spectra were acquired using a Micromass Q-TOF and an Agilent 6210 LC/MSD TOF mass spectrometers. 1D and 2D NMR spectra were measured with a Bruker AV-400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). HPLC analysis was performed on a Dionex HPLC system equipped with a Dionex P-680 quaternary pump, a PDA-100 diode-array detector (DAD), a TCC-100 oven, and an ASP-100 autosampling system (Dionex, USA) using a reversed-phase (RP) C_{18} column (5 μm, 4.6 × 250 mm; Purospher STAR). Preparative HPLC was carried out on a Varian instrument equipped with UV detectors (Varian, USA) and a reversed-phase (RP) C_{18} column (5 μm, 20 × 250 mm; Purospher STAR). Column chromatography was performed using silica gel (200−300 mesh, Qingdao), macroporous adsorptive resins D101 (250−300 μm, Tianjin), Sephadex LH-20 (Amersham Biosciences), ODS (60−80 μm, Merck), and Toyopearl HW-40 (Toyo Soda MFG). TLC was performed on precoated silica gel plates (silica gel GF_{254}, 1 mm, Merck).

Plant Material. The fruit of *G. jasmonoides* was purchased from Guangzhou Qingping Medical Material Market, China, in April 2007 and identified by Professor Danyan Zhang, Guangzhou Chinese Medicine University. A voucher specimen was deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, China.

Extraction and Isolation. Dried fruit (8.0 kg) of *G. jasmonoides* was cut into small pieces and refluxed with 60% (v/v) EtOH (×3, 2 h each). The 60% EtOH extract was concentrated in vacuo to afford a dark brown residue, which was dissolved in H_2O and subjected to column chromatography over D101 eluted with an EtOH−H_2O gradient. The 50% (v/v) EtOH eluate was separated over a silica gel column eluted with a $CHCl_3$−MeOH gradient. Fraction 7 ($CHCl_3$−MeOH, 9:1, eluant) was separated by column chromatography over ODS eluted with an MeOH−H_2O gradient. Subfraction 7-2 (MeOH−H_2O, 5:5, eluant) was chromatographed over an open Toyopearl HW-40 column eluted with an MeOH−H_2O gradient (2:8, 4:6, 6:4). The subfraction 7-2-2 (MeOH−H_2O, 4:6, eluant) was further purified by preparative RPHPLC (MeOH−H_2O, 4:6) to afford compounds **6** (6.0 mg), **7** (28.9 mg), **11** (45.0 mg), and **12** (22.0 mg). The subfraction 7-2-3 (MeOH−H_2O, 6:4, eluant) was further purified by preparative RPHPLC (MeOH−H_2O, 5:5) to give compounds **8** (11.6 mg) and **9** (15.9 mg). Compounds **10** (35.7 mg) and **4** (28.5 mg) were obtained from the successive purification over Toyopearl HW-40 and preparative RPHPLC (MeOH−H_2O, 4:6) from subfraction 7-3 (MeOH−H_2O, 7:3, eluant), respectively. Fraction 9 ($CHCl_3$−MeOH, 8:2, eluant) was separated by column chromatography over ODS eluted with an MeOH−H_2O gradient. Compounds **1** (140.0 mg), **3** (120.0 mg), **5** (40.0 mg), and **2** (300.0 mg) were obtained from the successive purification over Toyopearl HW-40 and preparative RPHPLC (MeOH−H_2O, 4:6) from subfraction 9-2 (MeOH−H_2O, 5:5, eluant).

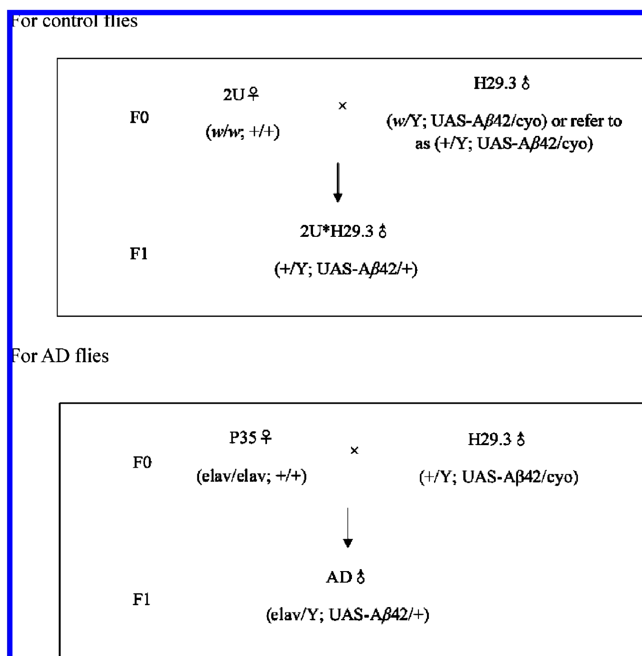
Bioassays. Fly Stock. w^{1118} (*isoCJ1*) was an isogenic line used as a control in all of the experiments. In our lab we named this stock "2U" for convenience. Expression of Aβ42 (UAS-Aβ42; referred to as H29.3) was driven by a ubiquitous neuronal expressing Gal4 line,

Table 2. NMR Spectroscopic Data (methanol-d_4) for Compounds **4**−**9**

pos.	**4**		**5**		**6**		**7**		**8**		**9**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	98.8	4.98, d (8.1)	98.8	4.97, d (7.9)	98.2	5.15, d (8.0)	98.8	5.16, d (8.0)	70.0	4.47, dd (11.1, 5.4)	70.1	4.57, dd (11.3, 5.8)
										4.09, t (11.4)		4.05, t (11.4)
3	153.3	7.48, brs	153.0	7.44, brs	153.3	7.50, brs	153.3	7.51, d (1.2)	176.4		176.8	
4	112.5		114.9		112.6		112.6		46.5	2.85, m	47.8	2.60, m
5	36.9	3.13, m	37.2	3.14, m	36.4	3.19, m	36.8	3.16, m	37.9	2.71, m	37.0	2.66, m
6	39.7	2.71, m	39.9	2.76, m	39.9	2.83, dd (16.4, 8.2)	39.8	2.82, dd (16.4, 8.3)	40.0	2.76, brd (16.3)	39.9	2.75, dd (16.5, 8.9)
		1.91, ddt (16.1, 8.5, 1.9)		1.96, m		2.10, ddt (16.4, 8.0, 2.0)		2.08, ddt (16.4, 8.0, 2.0)		2.37, brd (16.4)		2.30, brd (16.8)
7	128.8	5.75, brs	128.9	5.75, brs	131.2	5.85, brs	128.7	5.81, brs	129.1	5.64, brs	131.7	5.76, brs
8	144.9		144.9		139.7		145.0		142.4		139.2	
9	46.5	2.69, m	46.7	2.68, t (6.6)	47.1	2.72, t (7.4)	46.8	2.71, t (7.6)	47.0	3.13, m	47.5	3.18, m
10	61.6	4.24, m	61.6	4.23, m	63.8	4.78, brd (12.2)	61.4	4.30, brd (14.3)	60.1	4.00, o	67.7	4.28, brd (12.3)
								4.19, brd (14.3)				4.18, brd (12.3)
11	169.4		169.0		169.5		169.5		69.2	3.98, o	60.9	3.78, m
												3.74, m
11-OCH_3	51.8	3.67, s			51.7	3.71, s	51.7	3.71, s				
1′	100.5	4.73, d (7.9)	100.6	4.73, d (7.8)	100.3	4.71, d (8.0)	100.7	4.70, d (7.9)	105.1	4.39, d (7.9)	103.6	4.31, d (7.7)
2′	74.8	3.27, m	74.8	3.26, m	74.7	3.22, m	74.7	3.23, m	75.0	3.22, m	75.1	3.24, m
3′	77.7	3.43, m	77.7	3.42, m	77.9	3.36, m	77.7	3.38, m	77.9	3.39, m	78.0	3.40, m
4′	71.9	3.37, m	71.8	3.38, m	71.5	3.27, m	71.5	3.31, m	71.7	3.38, m	71.7	3.39, m
5′	75.7	3.56, m	75.7	3.56, m	78.4	3.28, m	75.6	3.46, m	75.5	3.52, m	75.5	3.53, m
6′	64.4	4.42, o[a]	64.4	4.44, dd (11.6, 1.6)	62.7	3.85, dd (12.0, 1.8)	64.3	4.35, dd (11.9, 2.2)	64.6	4.52, dd (12.0, 2.2)	64.5	4.52, dd (12.1, 1.7)
				4.39, dd (11.5, 6.1)		3.63, dd (12.0, 5.6)		4.25, dd (12.1, 5.9)		4.35, dd (11.8, 5.8)		4.38, dd (11.9, 5.7)
1″	169.0		171.0		175.3		172.7		169.0		169.0	
2″	115.0	6.29, d (15.9)	115.9	6.31, d (15.9)	31.6	2.46, m	20.7	2.02, s	115.9	6.43, d (15.9)	115.8	6.42, d (15.8)
3″	146.8	7.59, d (15.9)	146.8	7.60, d (16.0)	33.1	2.60, m			147.3	7.63, d (15.9)	147.4	7.62, d (15.9)
4″	127.1		127.1		181.8				126.7		126.6	
5″, 9″	131.2	7.42, d (8.6)	131.2	7.44, d (8.2)					107.1	6.92, s	107.1	6.89, s
6″, 8″	116.9	6.80, d (8.6)	116.9	6.80, d (8.1)					149.5		149.5	
7″	161.3		161.3						139.6		139.2	
6″, 8″-OCH_3									57.0	3.88, s	57.0	3.86, s

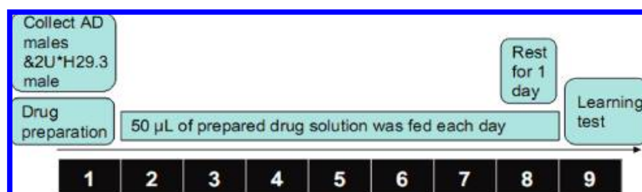
[a] "o" means peaks overlap with other signals.

e*lav-GAL4*^{c155} (P35). A behavioral assay was made from the first generation of the cross between P35 × H29.3. For details see below:



Fly Culture. All flies were reared at 23 °C, 42% RH (relative humidity). On the first day, newly born 2U*H29.3 male flies and AD male flies were selected and put into vials (each vial contains about 120 flies). Those flies were kept at 28 °C, 42% RH during the drug feeding process. The flies were transferred to new vials after 4 h of drug feeding from day 2 to day 8. All flies were kept at 28 °C, 42% RH until 1 h before the Pavlovian olfactory learning assay.

Drug Feeding Schedule. Drugs were prepared on the first day of eclosion, and the drug feeding was implemented on day 2. The test compounds were dissolved in DMSO as stock solutions (0.076% m/v). Each stock solution was diluted 100 times with sucrose solution (4%) and used in the treatment group. DMSO (15 μL) was also diluted 100 times with sucrose solution (4%) and used as the control. For each PI, two vials of flies were fed with 50 μL of the resulting solution for 7 days (e.g., from day 2 to day 8). Because some flies died of natural and other causes, about 100 flies were left in each vial when the Pavlovian olfactory learning assay was performed on day 9.



Pavlovian Olfactory Learning. The procedure is described in refs 23 to 25. Briefly, for all behavior assays, during one training session, a group of about 100 flies was exposed sequentially to two odors [*n*-octanol and methylcyclohexanol at a concentration of 0.1% (v/v)] for 60 s with a 45 s rest interval after each odor presentation. During exposure to the first odor, flies were simultaneously subjected to electric shock (1.5 s pulses with 3.5 s intervals, 60 V). To measure "immediate memory" (also referred to as "learning"), flies were allowed to choose between the two odors for 2 min at the "T maze" choice point, after which they were trapped in the collection arms, anesthetized, and counted. The performance index (PI) for each odor-shock pair (PI_{OCT} or PI_{MCH}) was calculated by subtracting the number of flies making the incorrect choice from those making the correct one, dividing by the total number of flies, and multiplying by 100; the PI is the average of PI_{OCT} and PI_{MCH}. PI = 0 represents a 50:50 distribution, which means the flies cannot remember one odor with electric shock, whereas PI = 100 represents 100% of flies avoiding the shock-paired odor by running into the other T-maze arm. Note: The learning assay was carried out in a dark room at 25 °C, 70% RH. Flies were moved into the dark room 1 h before the assay, so that the flies could get familiar with the environment.

Statistical Analyses. Data were analyzed and graphs were also plotted with the program Microsoft Excel 2007. Two-tail, equal variance Student's *t* test was used. The results are described in Figure 3.

6″-*O*-*trans*-Sinapoylgenipin gentiobioside (1): yellow, amorphous powder; $[\alpha]^{18}_{D}$ −22.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 203.5 (4.70), 238.0 (4.60), 327.5 (4.40) nm; IR (KBr) ν_{max} 3406, 1703, 2927, 1616, 1076 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 1; HR-ESI-Q-TOF-MS *m/z* 779.2369 [M + Na]$^+$ (calcd for $C_{34}H_{44}O_{19}Na$, 779.2374).

6″-*O*-*trans*-*p*-Coumaroylgenipin gentiobioside (2): yellow, amorphous powder; $[\alpha]^{18}_{D}$ −27.0 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 203.0 (3.95), 232.0 (4.02), 314.5 (4.06) nm; IR (KBr) ν_{max} 3406, 1703, 1631, 1516, 1273, 1076 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 1; HR-ESI-Q-TOF-MS *m/z* 719.2149 [M + Na]$^+$ (calcd for $C_{32}H_{40}O_{17}Na$, 719.2163).

6″-*O*-*trans*-Cinnamoylgenipin gentiobioside (3): pale yellow gum; $[\alpha]^{18}_{D}$ −21.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 203.0 (4.06), 217 (3.94), 239.5 (3.80), 277.5 (3.85) nm; IR (KBr) ν_{max} 3418, 2915, 1710, 1634, 1286, 1080 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 1; HR-ESI-Q-TOF-MS *m/z* 703.2224 [M + Na]$^+$ (calcd for $C_{32}H_{40}O_{16}Na$, 703.2214).

6′-*O*-*trans*-*p*-Coumaroylgeniposide (4): pale yellow gum; $[\alpha]^{18}_{D}$ −16.0 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 203.5 (4.06), 313.5 (3.86) nm; IR (KBr) ν_{max} 3405, 2923, 1693, 1630, 1272, 1079 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 2; HR-ESI-Q-TOF-MS *m/z* 557.1641 [M + Na]$^+$ (calcd for $C_{26}H_{30}O_{12}Na$, 557.1635).

6′-*O*-*trans*-*p*-Coumaroylgeniposidic acid (5): pale brown, amorphous powder; $[\alpha]^{18}_{D}$ −23.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 204.0 (4.01), 230.0 (3.99), 314.0 (4.00) nm; IR (KBr) ν_{max} 3405, 2923, 1693, 1630, 1272, 1079 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 2; HR-ESI-TOF-MS *m/z* 543.1475 [M + Na]$^+$ (calcd for $C_{25}H_{28}O_{12}Na$, 543.1478).

10-*O*-Succinoyl geniposide (6): yellow, amorphous powder; $[\alpha]^{18}_{D}$ +15.0 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 202.5 (3.99), 238.0 (4.01) nm; IR (KBr) ν_{max} 3414, 2927, 1700 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 2; HR-ESI-Q-TOF-MS *m/z* 511.1400 [M + Na]$^+$ (calcd for $C_{21}H_{28}O_{13}Na$, 511.1428).

6′-*O*-Acetylgeniposide (7): yellow, amorphous powder; $[\alpha]^{18}_{D}$ +10.0 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 203.5 (3.59), 237.5 (3.70) nm; IR (KBr) ν_{max} 3439, 2926, 1645 cm^{-1}; ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 2; HR-ESI-Q-TOF-MS *m/z* 453.1394 [M + Na]$^+$ (calcd for $C_{19}H_{26}O_{11}Na$, 453.1373).

11-(6-*O*-*trans*-Sinapoylglucopyranosyl)gardendiol (8): brown, amorphous powder; $[\alpha]^{23.2}_{D}$ +10.2 (*c* 0.5, MeOH). UV (MeOH) λ_{max} (log ε) 204.0 (4.67), 240.5 (4.43), 328.5 (4.43) nm; IR (KBr) ν_{max} 3424, 2925, 1635, 1388, 1115 cm^{-1}; CD $\Delta\epsilon_{195.6\ nm}$ 6.86, $\Delta\epsilon_{216.0\ nm}$ −5.48 (*c* 0.5, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 2; HR-ESI-Q-TOF-MS *m/z* 589.1913 [M + Na]$^+$ (calcd for $C_{27}H_{34}O_{13}Na$, 589.1897).

10-(6-*O*-*trans*-Sinapoylglucopyranosyl)gardendiol (9): brown, amorphous powder; $[\alpha]^{23.2}_{D}$ +13.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 203.5 (4.63), 241.0 (4.45), 328.5 (4.44); IR (KBr) ν_{max} 3416, 2926, 1710, 1628, 1079 cm^{-1}; CD $\Delta\epsilon_{193.9\ nm}$ 9.26, $\Delta\epsilon_{215.7\ nm}$ −6.86 (*c* 0.5, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) and ^{13}C NMR (methanol-d_4, 100 MHz), see Table 2; HR-ESI-Q-TOF-MS *m/z* 589.1848 [M + Na]$^+$ (calcd for $C_{27}H_{34}O_{13}Na$, 589.1897).

Acknowledgment. This work was supported by grants from the National Natural Science Foundation of People's Republic of China (No. 30701047) and the Key Project of the Chinese Ministry of Education (No. 208173), the National Basic Research Projects (973 program) of the Ministry of Science and Technology of China (2006CB500806 and 2009CB941301), the Tsinghua-Yue-Yuen Medical Sciences Fund, and the Project of Beijing Municipal Science & Technology Plan (Z07000200540705).

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Scarpini, E.; Scheltens, P.; Feldman, H. *Lancet Neurol.* **2003**, *2*, 539–547.
(2) Wang, S.; Jiang, N.; Zhou, W. X.; Zhang, Y. X. *Zhongguo Xinyao Zazhi* **2007**, *16*, 831–836.
(3) Mathis, C. A.; Lopresti, B. J.; Klunk, W. E. *Nucl. Med. Biol.* **2007**, *34*, 809–822.
(4) Iijima, K.; Liu, H. P.; Chiang, A. S.; Hearn, S. A.; Konsolaki, M.; Zhong, Y. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 6623–6628.
(5) Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353–356.
(6) Ni, H. Y.; Zhang, Z. H.; Fu, H. Z. *Zhongguo Zhongyao Zazhi* **2006**, *31*, 538–541.
(7) Machida, K.; Onodera, R.; Furuta, K.; Kikuchi, M. *Chem. Pharm. Bull.* **1998**, *46*, 1295–1300.
(8) Machida, K.; Takehara, E.; Kobayashi, H.; Kikuchi, M. *Chem. Pharm. Bull.* **2003**, *51*, 1417–1419.
(9) Chang, W. L.; Wang, H. Y.; Shi, L. S.; Lai, J. H.; Lin, H. C. *J. Nat. Prod.* **2005**, *68*, 1683–1685.
(10) Zhou, X. Q.; Bi, Z. M.; Li, P.; Tang, D.; Cai, H. X. *Chin. Chem. Lett.* **2007**, *12*, 1221–1223.
(11) Fu, X. M.; Chou, G. X.; Wang, Z. T. *Helv. Chim. Acta* **2008**, *91*, 646–653.
(12) Zhao, W. M.; Xu, J. P.; Qin, G. W.; Xu, R. S. *Phytochemistry* **1994**, *37*, 1079–1081.
(13) Chen, Q. C.; Youn, U. J.; Min, B. S.; Bae, K. H. *J. Nat. Prod.* **2008**, *71*, 995–999.
(14) Wang, G. L.; Zhao, S. J.; Chen, D. C. *Zhongguo Xinyao Zazhi* **1999**, *24*, 38–40.
(15) Pfister, S.; Meyer, P.; Steck, A.; Pfander, H. *J. Agric. Food Chem.* **1996**, *44*, 2612–2615.
(16) Carmona, M.; Zalacain, A.; Sanchez, A. M.; Novella, J. L.; Alonso, G. L. *J. Agric. Food Chem.* **2006**, *54*, 973–979.
(17) Dai, Y.; Zhou, G. X.; Kurihara, H.; Ye, W. C.; Yao, X. S. *J. Nat. Prod.* **2006**, *69*, 1022–1024.
(18) Gao, H.; Wang, Z.; Yao, Z. H.; Wu, N.; Dong, H. J.; Li, J.; Wang, N. L.; Ye, W. C.; Yao, X. S. *Helv. Chim. Acta* **2008**, *91*, 451–459.
(19) Gao, H.; Wang, Z.; Yang, L.; Yu, Y.; Yao, Z. H.; Wang, N. L.; Zhou, G. X.; Ye, W. C.; Yao, X. S. *Magn. Reson. Chem.* **2008**, *46*, 630–637.
(20) Zhao, W. M.; Xu, J. P.; Qin, G. W.; Xu, R. S. *Phytochemistry* **1994**, *37*, 1079–1081.
(21) Guvenalp, Z.; Kilic, N.; Kazaz, C.; Kaya, Y.; Demirezer, L. O. *Turk. J. Chem.* **2006**, *30*, 515–523.
(22) Boros, C. A.; Stermitz, F. R. *J. Nat. Prod.* **1990**, *53*, 1055–1147.
(23) Tully, T.; Quinn, W. G. *J. Comp. Physiol. A* **1985**, *157*, 263–277.
(24) Tully, T.; Preat, T.; Boynton, S. C.; Del Vecchio, M. *Cell* **1994**, *79*, 35–47.
(25) Yin, J. C.; Wallach, J. S.; Del Vecchio, M.; Wilder, E. L.; Zhou, H.; Quinn, W. G.; Tully, T. *Cell* **1994**, *79*, 49–58.

NP900176Q