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Forsythoneosides A–D, Neuroprotective Phenethanoid and Flavone Glycoside Heterodimers from the Fruits of *Forsythia suspensa*

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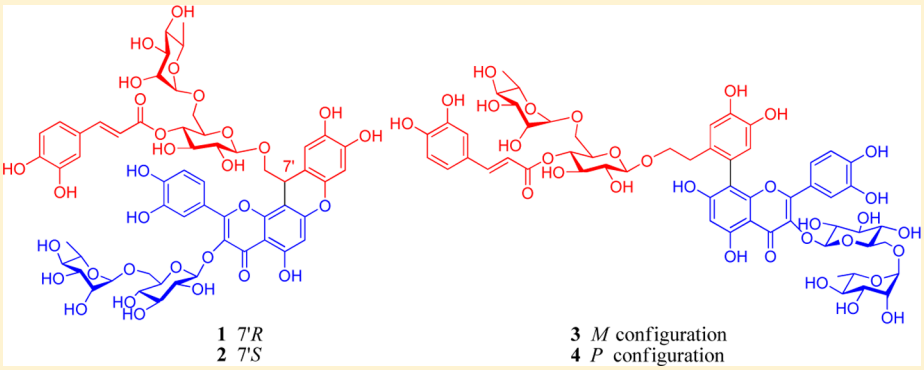
Supporting Information



ABSTRACT: Forsythoneosides A–D (**1**–**4**), four unusual adducts of a flavonoid unit fused to a phenylethanoid glycoside through a pyran ring or carbon–carbon bond, and four new phenylethanoid glycosides (**5**–**8**) were isolated from the fruits of *Forsythia suspensa*, together with nine known compounds. The structures of **1**–**8**, including their absolute configurations, were elucidated by spectroscopic data as well as experimental and calculated electronic circular dichroism analysis. Compounds **2** and **4** inhibited PC12 cell damage induced by rotenone, and increased cell viability from 53.9 ± 7.1% to 70.1 ± 4.0% and 67.9 ± 5.2% at 0.1 μM, respectively.

Forsythia suspensa (Thunb.) Vahl grows in northern mainland China, and its fruits, known as Fructus Forsythiae, are used widely as a traditional Chinese medicine to treat inflammation, pyrexia, ulcers, gonorrhea, and erysipela.[1,2] Over the past few decades, a series of phenylethanoid glycosides, lignans, flavonoids, alkaloids, and triterpenoids have been reported from species of this genus, and a few have been found to exhibit antioxidant, neuroprotective, cytotoxic, anti-inflammatory, antibacterial, and antiviral effects.[3−10]

Of particular interest for this study was the *n*-BuOH-soluble portion of the fruits of *F. suspensa* obtained from a 75% EtOH extract, which exhibited neuroprotective effects and could inhibit rotenone-induced damage of pheochromocytoma (PC12) cells, increasing the cell viability from 54.2 ± 2.8% to 78.5 ± 5.0% at a concentration of 10 μg/mL (Table S1, Supporting Information). Through bioactivity-guided isolation, four heterodimers of phenylethanoid glycoside and flavonoid units, i.e., forsythoneosides A–D (**1**–**4**), and four new phenylethanoid glycosides (**5**–**8**) were isolated from the fruits of *F. suspensa*, together with nine known compounds. Reported herein are the isolation and structure elucidation of compounds **1**–**8** and the evaluation of compounds **1**–**4** as potential neuroprotective agents in inhibiting cell damage in PC12 cells.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow, amorphous powder. The molecular formula, $C_{56}H_{62}O_{31}$, was established by the protonated molecular ion peak at *m*/*z* 1231.3354 $[M + H]^+$ (calcd for $C_{56}H_{63}O_{31}$, 1231.3353) in the HRESIMS, indicating 26 degrees of unsaturation. The IR spectrum showed absorption bands attributed to hydroxy groups (3371 cm^{-1}), carbonyl groups (1693 cm^{-1}), and aromatic rings (1601 and 1519 cm^{-1}). Analysis of the ^1H NMR spectrum (Table 1) of **1** revealed the presence of two sets of ABX system aromatic protons at δ_H 7.92 (1H, d, *J* = 2.5 Hz), 7.10 (1H, d, *J* = 8.5 Hz), and 7.95 (1H, dd, *J* = 8.5, 2.5 Hz) and δ_H 7.15 (1H, d, *J* = 2.5 Hz), 6.85 (1H, d, *J* = 8.5 Hz), and 7.02 (1H, dd, *J* = 8.5, 2.5 Hz), three singlet aromatic protons at δ_H 7.12 (1H, s), 6.70 (1H, s), and 6.52 (1H, s), and two olefinic protons at δ_H 7.64 (1H, d, *J* = 16.0 Hz) and 6.33 (1H, d, *J* = 16.0 Hz). In addition, there were many aliphatic proton signals between δ_H 5.10 and 1.20, including a methylene signal at δ_H 3.98 (1H, dd, *J* = 10.5, 7.5 Hz) and 3.87 (1H, m), a methine signal at δ_H 4.64 (1H, dd, *J* = 7.5, 4.0 Hz), and four anomeric protons at δ_H 5.10 (1H, d, *J* = 7.5 Hz), 4.48 (1H, d, *J* = 8.0 Hz), 4.74 (1H, br s), and 4.70

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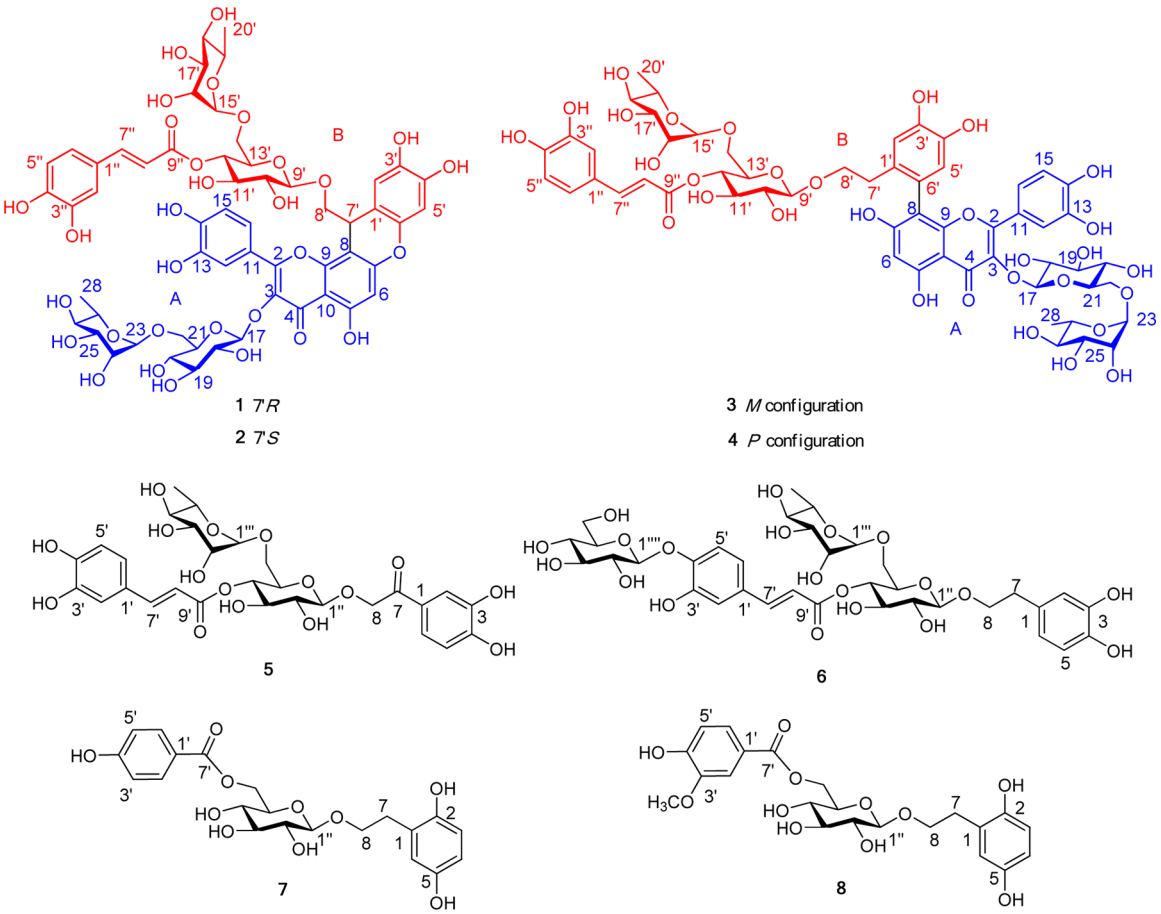
Chart 1



(1H, br s). After acid hydrolysis, the sugar units of **1** were confirmed to be D-glucose and L-rhamnose by GC analysis of the trimethylsilyl L-cysteine derivatives of these compounds (Figure S1, Supporting Information). The ^{13}C NMR and HSQC spectra of **1** revealed 56 carbon resonances, including 19 quaternary carbons, 32 methines, three methylenes, and two methyls. In addition to a caffeoyl unit (nine carbons) and four hexoses (24 carbons), the remaining 23 carbons could be assigned to a flavonol aglycone and a phenylethanoid moiety. A careful comparison of the 1D NMR signals of **1** with the corresponding data of rutin[11] and forsythoside A,[12] two main components isolated from the fruits of *F. suspensa*, suggested the presence of a rutin moiety and phenylethanoid glycoside moiety in **1**. The connectivity of these two moieties was established by analyzing the HMBC spectrum (Figure 1). Correlations from H-7′ at δ_H 4.64 (dd, J = 7.5, 4.0 Hz) (monomer B) to C-7, C-8, and C-9 (monomer A) were observed, suggesting that the C-7′ of the phenylethanoid unit is connected to the C-8 of the rutin moiety by a C−C bond. Two singlet aromatic protons of the phenylethanoid moiety at δ_H 7.12 (1H, s) and 6.70 (1H, s) in the ^{1}H NMR spectrum suggested that a tetrasubstituted aromatic ring is present in the phenylethanoid moiety in **1**. Furthermore, the presence of a downfield carbon signal at δ_C 147.3 and the remaining one degree of unsaturation implied that an ether bond is formed between C-6′ and C-7. Therefore, **1** possesses a pyran ring, which consists of [C-7−C-8−C-7′−C-1′−C-6′−O], formed by the fusion of the phenylethanoid glycoside moiety to the flavonol moiety. The ECD spectrum of **1** showed λ_{max} (nm) ($\Delta\varepsilon$) values of 367 (+4.45), 274 (−1.02), and 217 (−2.44). The 7′*R* configuration was determined by comparing the experimental and calculated ECD data.[13] Accordingly, the structure of **1** was assigned as shown, and this compound was accorded the trivial name forsythoneoside A.

Compound **2** was obtained as a yellow, amorphous powder and exhibited the same IR, UV, and HRMS characteristics as **1**. The NMR spectroscopic data of **2** were similar to those of **1**, except for subtle shifts in the glycosyl chain protons in the ^{1}H NMR spectrum. A careful analysis of the 1D and 2D NMR spectra of **2** suggested this compound to have the same planar structure as **1** (Tables 1 and 2). The ECD spectrum of **2** exhibited an opposite Cotton effect of that observed for **1** (Figure S26, Supporting Information), and **2** was accorded the trivial name forsythoneoside B.

According to this spectroscopic data analysis, **1** and **2** are a pair of diastereoisomers with different configurations at C-7′. The absolute configurations of C-7′ in **1** and **2** were established by comparing the experimental and calculated ECD data. Considering that the flexible glycosyl chains and caffeoyl moiety far from the chiral center C-7′ have no significant influence on the Cotton effect at approximately 275 and 370 nm, which are peaks attributed to the electronic transition from the benzopyran and flavonol groups, a simplified structure of **1** was used for conformational analysis (Figure 2). After scanning for stable conformations of **1a** and **1b** using the MMFF94 molecular mechanics force field, the preferred conformers were optimized using the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d) level (Table S2,

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Table 1. ^{1}H NMR Spectroscopic Data for 1−4 in Acetic Acid-d_4 (δ in ppm, J in Hz)

position	1[a]	2[a]	3[b]	4[b]
6	6.52, s	6.53, s	6.51, s	6.51, s
12	7.92, d (2.5)	7.93, d (2.5)	7.74, d (1.8)	7.67, d (1.8)
15	7.10, d (8.5)	7.10, d (8.5)	6.81, d (8.4)	6.83, d (8.4)
16	7.95, dd (8.5, 2.5)	7.81, dd (8.5, 2.5)	7.29, dd (8.4, 1.8)	7.33, dd (8.4, 1.8)
17	5.10, d (7.5)	5.20, d (7.5)	5.04, d (7.8)	5.05, d (7.2)
18	3.76, m	3.75, m	3.76, m	3.72, m
19	3.75, m	3.76, m	3.73, m	3.70, m
20	3.60, m	3.64, m	3.60, m	3.59, m
21	3.53, m	3.58, m	3.48, m	3.50, m
22a	3.86, m	3.83, m	3.79, m	3.79, m
22b	3.54, m	3.56, m	3.49, m	3.49, m
23	4.70, br s	4.76, br s	4.65, d (1.8)	4.65, br s
24	3.85, m	3.88, m	3.80, m	3.81, m
25	3.80, m	3.78, m	3.78, m	3.78, m
26	3.50, m	3.48, m	3.51, m	3.52, m
27	3.61, m	3.56, m	3.53, m	3.58, m
28	1.20, d (6.0)	1.17, d (6.0)	1.19, d (6.0)	1.20, d (6.0)
2′	7.12, s	7.15, s	7.00, s	7.05, s
5′	6.70, s	6.66, s	6.83, s	6.81, s
7′	4.64, dd (7.5, 4.0)	4.67, dd (7.5, 4.0)	2.64, m	2.69, m
8′a	3.98, dd (10.5, 7.5)	4.10, dd (10.5, 4.0)	3.84, m	3.87, m
8′b	3.87, m	3.78, m	3.60, m	3.63, m
9′	4.48, d (8.0)	4.50, d (8.0)	4.20, d (7.8)	4.14, d (7.8)
10′	3.56, m	3.55, m	3.41, m	3.44, m
11′	3.84, m	3.77, m	3.72, m	3.70, m
12′	4.98, t (10.0)	5.04, t (10.0)	4.93, t (9.6)	4.93, t (9.6)
13′	3.39, m	3.69, m	3.50, m	3.53, m
14′a	3.65, d (8.5)	3.70, d (8.5)	3.63, d (8.4)	3.72, d (8.4)
14′b	3.51, m	3.47, m	3.50, m	3.55, m
15′	4.74, br s	4.70, br s	4.79, d (1.8)	4.79, br s
16′	3.91, m	3.85, m	3.98, m	3.98, m
17′	3.82, m	3.67, m	3.85, m	3.86, m
18′	3.48, m	3.43, m	3.48, m	3.49, m
19′	3.65, m	3.63, m	3.67, m	3.71, m
20′	1.12, d (6.0)	1.10, d (6.0)	1.16, d (6.0)	1.19, d (6.0)
2″	7.15, d (2.5)	7.15, d (2.5)	7.14, d (1.8)	7.15, d (1.8)
5″	6.85, d (8.5)	6.85, d (8.5)	6.85, d (8.4)	6.86, d (8.4)
6″	7.02, dd (8.5, 2.5)	7.02, dd (8.5, 2.5)	7.02, dd (8.4, 1.8)	7.01, dd (8.4, 1.8)
7″	7.64, d (16.0)	7.64, d (16.0)	7.63, d (16.2)	7.64, d (16.2)
8″	6.33, d (16.0)	6.33, d (16.0)	6.31, d (16.2)	6.32, d (16.2)

[a]Recorded at 500 MHz. [b]Recorded at 600 MHz.

Supporting Information). The calculated electronic circular dichroism (ECD) spectra of **1a** and **1b** were generated by a Boltzmann weighting of their lowest energy conformers with different populations. The overall pattern of the calculated ECD spectra of **1a** and **1b** was in agreement with the experimental data obtained for **2** and **1** (Figure 3), respectively. Therefore, the absolute configurations of C-7′ in forsythoneoside A and B were determined to be *R* and *S*, respectively.

Compound **3** was obtained as a yellow, amorphous powder. The molecular formula, $C_{56}H_{64}O_{31}$, was established by the protonated molecular ion peak (m/z 1233.3502 [M + H]$^+$) in the HRESIMS, indicating 25 degrees of unsaturation. The IR spectrum showed absorption bands attributed to hydroxy groups (3394 cm^{-1}), carbonyl groups (1694 cm^{-1}), and aromatic rings (1603 and 1514 cm^{-1}). On comparing the key NMR data of **3** with those of **1** (Tables 1 and 2), their close resemblance indicated that **3** was a heterodimer of rutin and phenylethanoid glycoside, making it structurally similar to **1**. In the ^{1}H NMR spectrum of **3**, a typical β-methylene signal of a phenylethanol unit at δ_H 2.64 (m) indicated that the C-7′ position is not substituted. There were two singlet aromatic protons at δ_H 7.00 (s) and δ_H 6.83 (s), illustrating the presence of a tetrasubstituted aromatic ring in the phenylethanol unit of **3**. Focusing on the ^{13}C NMR resonance of C-6′, the chemical shift at δ_C 125.1 was distinctly different from that of **1**, revealing that C-6′ of the phenylethanol unit is linked to C-8 of ring A of rutin via a C−C bond. This result was confirmed by the HMBC spectrum (Figure 1), in which a key correlation between H-5′ and C-8 was observed. The ECD spectrum of **3** showed the following values at λ_{max} (nm) ($\Delta\varepsilon$): 342 (+0.83), 264 (+5.52), and 225 (−13.02). The *M* configuration was confirmed based on the experimental and calculated ECD data. Thus, **3** was accorded the trivial name forsythoneoside C.

Compound **4** was obtained as a yellow, amorphous powder. Similar NMR data (Tables 1 and 2) and Cotton effects opposite those of **3** at approximately 220, 270, and 350 nm in the ECD spectrum (Figure S48, Supporting Information) suggested that **4** is a diastereoisomer of **3**, with the opposite axial chirality at C-8−C-6′. Compound **4** was accorded the trivial name forsythoneoside D.

The absolute configurations of **3** and **4** were established by the same method applied to **1** and **2**. Similarly, simplified structures of **3** were used for the conformational analysis (Figure 2). A systematic conformational analysis and optimization were performed for **3a** and **3b** using the same method applied to **1a** and **1b** (Table S3, Supporting Information). A comparison of the theoretically calculated and experimental ECD curves (Figure 4) permitted the assignment of the absolute configurations of *M* and *P* to **3** and **4**, respectively.

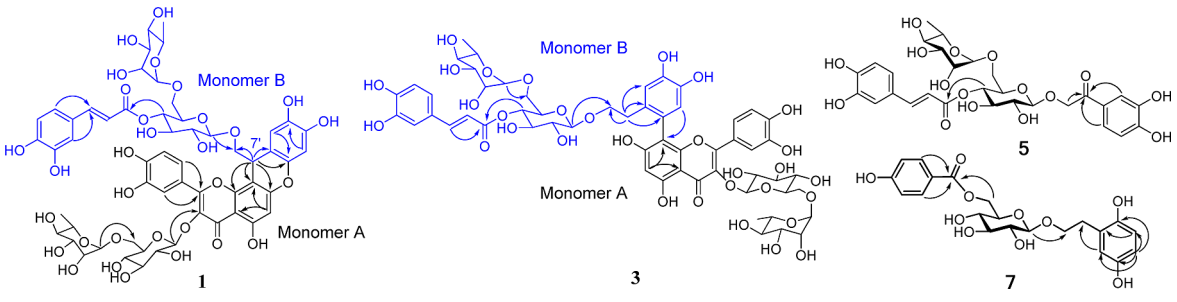

Figure 1. Key HMBC correlations of **1**, **3**, **5**, and **7**.

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Table 2. ^{13}C NMR Spectroscopic Assignments for 1−5 in Acetic Acid-d_4 and 6−8 in DMSO-d_6 (δ in ppm)

position	1[a]	2[a]	3[b]	4[b]	position	5[a]	6[a]	7[a]	8[a]
2	161.1	161.4	160.7	161.1	1	130.0	129.0	124.8	124.8
3	138.7	138.6	138.0	138.2	2	118.0	115.5	147.6	147.6
4	181.5	181.5	181.5	181.9	3	147.5	144.9	115.4	115.4
5	162.7	162.7	163.4	163.6	4	153.9	143.6	113.3	113.3
6	102.6	102.6	102.3	102.4	5	118.2	116.4	149.6	149.6
7	161.5	161.6	164.5	164.5	6	125.5	119.4	117.0	117.0
8	103.6	103.9	110.6	110.7	7	199.1	35.1	30.7	30.7
9	156.8	157.0	157.1	157.3	8	73.8	70.3	68.6	68.6
10	110.0	110.1	107.9	108.2	1′	130.0	128.5	120.3	120.5
11	125.0	125.2	125.1	125.3	2′	117.5	115.0	131.4	112.3
12	119.5	120.2	120.4	120.4	3′	147.7	146.9	115.3	147.3
13	147.0	147.0	146.6	146.7	4′	150.6	147.6	162.0	151.5
14	151.5	151.2	151.1	151.2	5′	118.6	115.9	115.3	115.2
15	118.6	118.6	118.2	118.4	6′	125.5	120.7	131.4	123.6
16	126.9	126.3	125.7	126.1	7′	149.7	145.0	165.4	165.4
17	107.6	107.0	107.7	107.9	8′	116.9	115.7		
18	77.4	77.3	77.5	77.7	9′	170.3	165.6		
19	79.7	79.8	79.8	79.8	1″	105.7	102.8	103.0	103.0
20	72.4	72.4	72.2	72.7	2″	76.7	72.8	73.4	73.4
21	78.3	78.5	78.1	78.4	3″	77.3	73.9	76.5	76.5
22	70.6	70.0	70.5	70.8	4″	73.7	71.2	70.0	70.2
23	103.6	103.6	103.7	103.8	5″	76.8	73.4	73.7	73.8
24	73.8	73.8	73.7	73.9	6″	69.7	66.8	63.6	64.0
25	74.3	74.3	74.3	74.5	1‴	103.3	100.5		
26	75.9	75.8	75.9	76.1	2‴	73.7	70.2		
27	71.3	71.3	71.3	71.6	3‴	74.3	70.5		
28	19.8	19.7	19.7	19.9	4‴	75.9	71.8		
1′	115.8	115.6	134.2	134.5	5‴	71.4	68.4		
2′	119.0	119.3	120.1	120.4	6‴	19.7	17.8		
3′	144.0	143.7	147.9	147.9	1⁗		101.5		
4′	147.7	147.7	146.0	146.2	2⁗		73.2		
5′	106.7	106.7	121.8	122.1	3⁗		75.8		
6′	147.3	147.7	125.1	125.2	4⁗		69.7		
7′	36.3	36.6	36.2	36.3	5⁗		77.2		
8′	77.4	77.7	73.5	73.5	6⁗		60.7		
9′	105.6	106.3	105.6	105.9	OCH_3-3′				55.3
10′	76.8	76.9	76.4	76.7					
11′	77.5	77.5	77.3	77.5					
12′	73.9	74.1	73.8	73.9					
13′	76.3	76.2	76.5	76.8					
14′	69.6	69.8	69.3	69.8					
15′	103.2	103.4	103.1	103.5					
16′	73.7	73.6	73.7	73.5					
17′	74.2	74.3	74.3	74.3					
18′	75.8	75.8	75.8	76.1					
19′	71.3	71.4	71.2	71.5					
20′	19.6	19.6	19.6	19.8					
1″	130.0	130.0	130.0	130.3					
2″	117.6	117.6	117.5	117.8					
3″	147.6	147.6	147.7	147.8					
4″	150.6	150.6	150.6	150.7					
5″	118.6	118.5	118.6	118.8					
6″	125.6	125.6	125.6	125.7					
7″	149.9	149.9	149.8	149.9					
8″	116.9	117.0	116.9	117.3					
9″	170.5	170.5	170.4	170.5					

[a]Recorded at 125 MHz. [b]Recorded at 150 MHz.

Compound **5** was obtained as a yellow powder, and its molecular formula was determined to be $C_{29}H_{34}O_{16}$ based on the sodiated molecular ion peak observed at m/z 661.1739 $[M + Na]^+$ in the HRESIMS. The ^{1}H NMR spectrum of **5** (Table

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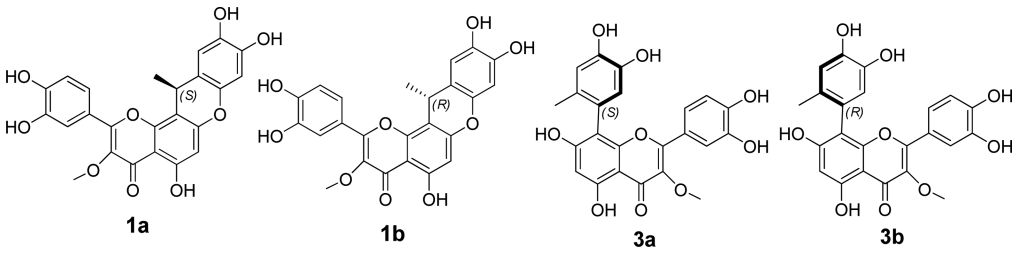

Figure 2. Structures of **1a**, **1b**, **3a**, and **3b**.

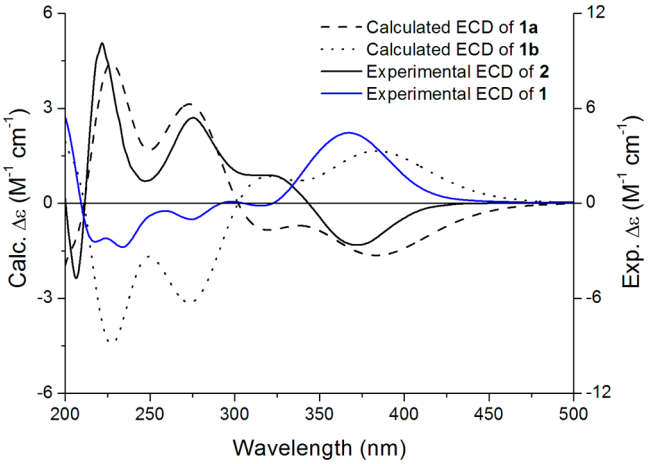

Figure 3. Experimental ECD spectra of **1** and **2** and calculated ECD spectra of **1a** and **1b** in MeOH.

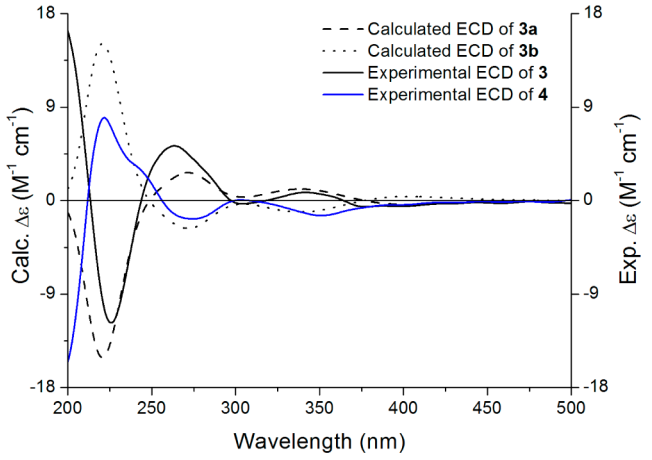

Figure 4. Experimental ECD spectra of **3** and **4** and calculated ECD spectra of **3a** and **3b** in MeOH.

3) exhibited two ABX system aromatic rings [δ_H 7.50 (1H, d, J = 2.0 Hz), 7.47 (1H, dd, J = 8.0, 2.0 Hz), 7.13 (1H, d, J = 2.0 Hz), 7.03 (1H, dd, J = 8.0, 2.0 Hz), 6.92 (1H, d, J = 8.0 Hz), and 6.85 (1H, d, J = 8.0 Hz)], a set of typical *trans*-double-bond resonances [δ_H 7.64 (1H, d, J = 16.0 Hz), and 6.33 (1H, d, J = 16.0 Hz)], and a methylene group [δ_H 5.13 (1H, d, J = 16.0 Hz) and 4.96 (1H, d, J = 16.0 Hz)]. Additionally, a glucopyranosyl anomeric proton signal at δ_H 4.48 (1H, d, J = 8.0 Hz) and a rhamnopyranosyl anomeric proton signal at δ_H 4.83 (1H, br s) were observed in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of **5** (Table 2) exhibited 29 carbon signals, of which 12 could be assigned to a glucose unit and rhamnose unit, with the remaining 17 carbons assigned to a caffeoyl moiety and a C-6−C-2 moiety. The above data demonstrated

Table 3. ^{1}H NMR Spectroscopic Data for 5 in Acetic Acid-d_4 6−8 in DMSO-d_6 (δ in ppm, J in Hz)[a]

position	5	6	7	8
2	7.50, d (2.0)	6.60, d (2.0)		
3			6.55, d (8.5)	6.55, d (8.5)
4			6.40, dd (8.5, 2.5)	6.40, dd (8.5, 2.5)
5	6.92, d (8.0)	6.61, d (7.5)		
6	7.47, dd (8.0, 2.0)	6.47, d (7.5)	6.49, d (2.5)	6.49, d (2.5)
7		2.67, m	2.71, m	2.71, m
8a	5.13, d (16.0)	3.81, m	3.79, m	3.81, m
8b	4.96, d (16.0)	3.60, m	3.57, m	3.57, m
2′	7.13, d (2.0)	7.15, s	7.78, d (8.5)	7.38, br s
3′			6.80, d (8.5)	
5′	6.85, d (8.0)	7.10, br s	6.80, d (8.5)	6.81, d (8.0)
6′	7.03, dd (8.0, 2.0)	7.10, br s	7.78, d (8.5)	7.44, dd (8.0, 2.0)
7′	7.64, d (16.0)	7.53, d (16.0)		
8′	6.33, d (16.0)	6.40, d (16.0)		
1″	4.48, d (8.0)	4.30, d (7.5)	4.25, d (7.5)	4.25, d (7.5)
2″	3.74, m	3.08, m	3.00, m	3.00, m
3″	3.92, m	3.58, m	3.17, m	3.17, m
4″	5.08, t (10.0)	4.67, t (10.0)	3.20, m	3.17, m
5″	3.65, m	3.44, m	3.43, m	3.44, m
6″a	3.80, m	3.50, m	4.48, d (12.0)	4.55, d (12.0)
6″b	3.62, m	3.32, m	4.18, dd (12.0, 6.0)	4.13, dd (12.0, 6.5)
1‴	4.83, br s	4.49, br s		
2‴	4.03, m	3.57, m		
3‴	3.89, m	3.38, m		
4‴	3.54, m	3.12, m		
5‴	3.75, m	3.33, m		
6‴	1.22, d (6.0)	1.03, d (6.0)		
1⁗		4.76, d (7.0)		
2⁗		3.27, m		
3⁗		3.26, m		
4⁗		3.14, m		
5⁗		3.31, m		
6⁗a		3.70, m		
6⁗b		3.44, m		
OCH_3-3′				3.67, s

[a]Recorded at 500 MHz.

that the structure of **5** is similar to that of forsythoside A,[12] except for the resonances of the methylene (CH_2-7) in

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forsythoside A being replaced by that of a carbonyl in **5** from the ^{1}H and ^{13}C NMR spectra (Tables 2 and 3). The structure was confirmed by the correlations of H-8a at δ_H 5.13, H-8b at δ_H 4.96, H-2 at δ_H 7.50, and H-6 at δ_H 7.47 with C-7 at δ_C 199.1 in the HMBC spectrum and by the downfield shift of H-2, H-6, and H-8 compared with those of forsythoside A. From the above analysis, **5** was characterized as 2-(3,4-dihydroxyphenyl)-2-oxo-ethyl-*O*-*α*-L-rhamnopyranosyl-(1→6)-(4-*O*-caffeoyl)-*β*-D-glucopyranoside.

Compound **6** ($C_{35}H_{46}O_{20}$) showed NMR spectroscopic features similar to those of forsythoside A, but with an additional glucose unit. The correlation of H-1‴″ at δ_H 4.76 with the C-4′ in the HMBC spectrum confirmed the location of the glucose unit at C-4′. Based on the above analysis, **6** was defined as forsythoside A 4′-*O*-*β*-D-glucopyranoside.

Compound **7** gave the molecular formula $C_{21}H_{24}O_{10}$ (*m*/*z* 459.1276 for $C_{21}H_{24}NaO_{10}$ in the HRESIMS) and exhibited hydroxy (3350 cm^{-1}), carbonyl (1697 cm^{-1}), and phenyl (1608 and 1513 cm^{-1}) group absorptions in the IR spectrum. The ^{1}H NMR spectrum (Table 3) of **7** showed seven aromatic proton signals at δ_H 7.78 (2H, d, *J* = 8.5 Hz), 6.80 (2H, d, *J* = 8.5 Hz), 6.55 (1H, d, *J* = 8.5 Hz), 6.49 (1H, d, *J* = 2.5 Hz), and 6.40 (1H, dd, *J* = 8.5, 2.5 Hz), revealing the presence of an ABX system aromatic ring and an AA′BB′ system aromatic ring. In addition, two pairs of methylene proton signals at δ_H 3.79 (1H, m), 3.57 (1H, m), and 2.71 (2H, m) and a glucopyranosyl anomeric proton at δ_H 4.25 (1H, d, *J* = 7.5 Hz) were observed in the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum (Table 2), aside from the six carbon signals of a glucose unit, the remaining 15 carbon signals could be attributed to a 4-hydroxybenzoyl group and a phenylethanoid moiety. Subsequently, the location of the 4-hydroxybenzoyl group was determined to be at C-6″ by the characteristic correlation of H-6″ with C-7′ in the HMBC spectrum (Figure 1). Furthermore, the substituted pattern of the aromatic ring in the phenylethanoid moiety was defined as a 2,5-dioxygenated aromatic ring by the HMBC correlations of H-4 with C-2, C-3, C-5, and C-6 and that of H-7 with C-2 and C-6. The correlation of H-1″ with C-8 indicated that the linkage of the glucose unit is at C-8. On the basis of the above data, **7** was defined as 2-(2,5-dihydroxyphenyl)-ethyl-*O*-(6-*O*-*p*-hydroxybenzoyl)-*β*-D-glucopyranoside.

The molecular formula of compound **8** was determined to be $C_{22}H_{26}O_{11}$ from the sodiated molecular ion peak (*m*/*z* 489.1367 [M + Na]$^+$) in the HRESIMS. The UV, IR, and NMR spectroscopic data of **8** were similar to those of **7** except that the resonances for a 4-hydroxy-3′-methoxybenzoyl moiety in **8** replaced those for a 4-hydroxybenzoyl unit in **7**. This substitution pattern was confirmed by the ^{1}H NMR and HMBC spectra. The ^{1}H NMR spectrum of **7** showed an ABX system aromatic ring at δ_H 7.44 (1H, dd, *J* = 8.0, 2.0 Hz), 7.38 (1H, br s), and 6.81 (1H, d, *J* = 8.0 Hz) and a methoxy group at δ_H 3.67 (3H, s). In combination with the correlations from H-6′ to C-7″ and from OCH_3-3″ to C-3″ in the HMBC spectrum, **8** was elucidated as 2-(2,5-dihydroxyphenyl)-ethyl-*O*-(6-*O*-vanilloyl)-*β*-D-glucopyranoside.

The structures of nine known compounds were also identified by comparing their spectroscopic data to those found in the literature. The known compounds isolated were forsythoside A,[12] calceolarioside A,[14] calceolarioside C,[15] forsythoside F,[16] angoroside A,[17] poliumoside,[18] rutin,[19] kaempferol-3-*O*-*β*-D-glucopyranoside-7-*O*-*α*-L-rhamnopyranoside,[20] and kaempferol-3-*O*-*β*-D-(2″-*O*-*β*-D-glucopyranosyl-6″-*O*-*α*-L-rhamnopyranosyl)glucopyranoside.[21]

A literature search revealed that no heterodimer consisting of phenylethanoid glycoside and flavonoid glycoside has been previously reported. The most intriguing structural feature of **1** and **2** is the presence of a pyran ring formed by the fusion of phenylethanoid glycoside and flavonoid glycoside. From a biosynthetic perspective, the most reasonable precursors of these compounds may be proposed to be rutin and forsythoside A, two primary components isolated from the fruits of *F. suspensa*. The formation of these adducts could be rationalized by alkylation and/or free radical reactions by oxidase enzymes, including peroxidase and laccase systems, known to be radical generators in plants.[22] A biogenetic pathway for these compounds is provided in Scheme S1 (Supporting Information). Interestingly, forsythoneosides A–D (**1**–**4**), bearing certain glucose moieties, could be synthesized using forsythoside A and rutin as starting materials via laccase and peroxidase under mild conditions instead of the synthesis of their aglycones followed by glucosidation.

Considering the bioactivity screening results obtained for the extract, the neuroprotective activities of **1**–**17** were tested. Compounds **1**–**4** exhibited significant effects in a dose-dependent manner against PC12 cells that were injured by rotenone (Table 4), particularly **2** and **4**, increasing the cell viability from 53.9 ± 7.1% for the model to 70.1 ± 4.0% and 67.9 ± 5.2% at 0.1 *μ*M, respectively, using the coenzyme Q10 as a positive control (cell viability of 59.0 ± 2.2%, at 10 *μ*M). In addition, forsythoside A, calceolarioside C, and rutin showed moderate neuroprotective activities, which increased the cell viability from 53.9 ± 7.1% for the model to 60.4 ± 5.5%, 56.2 ± 4.8%, and 67.9 ± 6.0% at 10 *μ*M, respectively. The other compounds exhibited no discernible neuroprotective activities at the concentrations tested.

Table 4. Neuroprotective Effects of Compounds 1–4 against Rotenone-Induced Injury in PC12 Cells (10, 1, and 0.1 *μ*M means ± SD, *n* = 6)[a]

	rotenone (4 *μ*M)		
group	10 *μ*M	1 *μ*M	0.1 *μ*M
control	100.0 ± 8.3	100.0 ± 3.9	100.0 ± 1.4
model	53.9 ± 7.1$^{\#\#\#}$	53.9 ± 7.1$^{\#\#\#}$	53.9 ± 7.1$^{\#\#\#}$
1	81.1 ± 4.1***	59.2 ± 3.3	55.8 ± 7.8
2	91.2 ± 3.6***	75.0 ± 4.8***	70.1 ± 4.0**
3	93.2 ± 2.1***	64.0 ± 4.6	54.3 ± 9.3
4	96.2 ± 1.9***	75.5 ± 2.5***	67.9 ± 5.2*

[a]$^{\#\#\#}p < 0.001$ vs control, ***$p < 0.001$ vs model, **$p < 0.01$ vs model, *$p < 0.1$ vs model.

EXPERIMENTAL SECTION

General Experimental Procedures. The optical rotations were measured on a JASCO P-2000 polarimeter. UV spectra were detected on a JASCO V650 spectrometer. ECD spectra were recorded with a JASCO J-815 spectrometer. Infrared (IR) spectra were recorded on a Nicolet 5700 spectrometer using an FT-IR microscope transmission method. ^{1}H NMR (500 and 600 MHz), ^{13}C NMR (125 and 150 MHz), and 2D NMR spectra were recorded with Varian 500 and 600 MHz NMR spectrometers using TMS as internal standard, and the values are given in ppm. High-resolution electrospray ionization mass spectrometry (HRESIMS) was performed on an Agilent 1100 series LC/MSD ion trap mass spectrometer. Preparative HPLC was performed using a Shimadzu LC-10AT instrument with an SPD-10A

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detector, using a YMC-Pack ODS-A column (250 mm × 20 mm, 5 μm; YMC Corp., Kyoto, Japan). HPLC-DAD analysis was performed using an Agilent 1200 series system with an Apollo C_{18} column (250 mm × 4.6 mm, 5 μm; Alltech Corp., Lexington, KY, USA). GC analysis was performed using an Agilent 7890A series system with a capillary column, HP-5 (60 m × 0.25 mm, with a 0.25 μm film; Dikma Technologies Inc., Beijing, People's Republic of China). Column chromatography was performed on macroporous resin (Diaion HP-20, Mitsubishi Chemical Corp., Tokyo, Japan), RP-C_{18} (50 μm, YMC Corp.), and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden).

Plant Material. The fruits of *F. suspensa* were collected in Yuncheng City, Shanxi Province, People's Republic of China, in December 2011. The plant material was identified by L. Ma (Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing). A voucher specimen (ID-S-2597) has been deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing.

Extraction and Isolation. Air-dried deseeded fruits of *F. suspensa* (90.0 kg) were extracted with 75% EtOH (600 L) under reflux three times and filtered. After the filtrate was evaporated under reduced pressure, the residue (LQ1, 12.6 kg) was suspended in water (10 L) and successively partitioned with petroleum ether (LQ2), EtOAc (LQ3), and *n*-BuOH (LQ4). The *n*-BuOH-soluble portion was further suspended in water (1:10) to yield an aqueous layer (LQ7). The aqueous layer was concentrated and subjected to macroporous adsorption resin (HP-20) column chromatography eluted with H_2O (LQ8), 15% ethanol (LQ9), 30% ethanol (LQ10), 50% ethanol (LQ11), and 95% ethanol (LQ12). The 30% ethanol solution (460 g) was chromatographed over Sephadex LH-20 eluting with H_2O–MeOH in a mixture gradient to yield five fractions (fractions A–E) and forsythoside F (200 mg).

Fraction A (67 g) was subjected to passage over an RP-C_{18} column and eluted with H_2O–MeOH (from 100:0 to 0:100) in a gradient to yield 22 fractions (fractions A1–A22). Fraction A13 was chromatographed over Sephadex LH-20 eluting with gradient mixtures of H_2O–MeOH (from 100:0 to 80:20) to yield fractions A13-1–A13-22. Fraction A13-7 was purified using reversed-phase preparative HPLC with MeOH–H_2O (28:72) as the mobile phase to yield **6** (9 mg). Fraction A13-21 was purified using reversed-phase preparative HPLC with MeOH–H_2O (28:72) as the mobile phase to yield **7** (4 mg) and **8** (5 mg). Fraction B (35 g) was chromatographed over RP-C_{18} with a gradient of H_2O–MeOH (from 100:0 to 0:100) to yield 20 fractions (fractions B1–B20). Fraction B9 was chromatographed over Sephadex LH-20 eluting with H_2O–MeOH (from 100:0 to 70:30) in gradient to yield fractions B9-1–B9-15. Fraction B9-5 was purified using reversed-phase preparative HPLC with MeOH–H_2O (35:65) as the mobile phase to yield kaempferol-3-*O*-β-D-(2″-*O*-β-D-glucopyranosyl-6″-*O*-α-L-rhamnopyranosyl)glucopyranoside (18 mg). Fraction B16 was purified using reversed-phase preparative HPLC with MeOH–H_2O (33:67) as the mobile phase to yield angoroside A (16 mg) and poliumoside (10 mg). Fraction B19 was purified using reversed-phase preparative HPLC with MeOH–H_2O (30:70) as the mobile phase to yield forsythoside F (90 mg). Fraction C (116 g) was separated over Sephadex LH-20 (H_2O–MeOH from 90:10 to 60:40) to yield fractions C1–C14. Fraction C7 was purified by preparative HPLC with MeOH–H_2O (30:70) to afford kaempferol-3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (12 mg). Fraction C7 was purified by preparative HPLC with MeOH–H_2O (35:65) to afford forsythoside A (110 mg) and calceolarioside C (34 mg). Fraction D (52 g) was chromatographed over Sephadex LH-20 with a gradient of H_2O–MeOH (from 80:20 to 50:50) to yield 39 fractions (fractions D1–D39). Fractions D21 and D24 were purified by preparative HPLC (MeOH–H_2O, 30:70) to afford **3** (25 mg) and **4** (20 mg), respectively. Fraction D22 was purified by preparative HPLC with MeOH–H_2O (28:72) to afford **5** (28 mg). Fraction D30 was purified by preparative HPLC with MeOH–H_2O (34:66) to afford calceolarioside A (10 mg). Fraction E (45 g) was subjected to Sephadex LH-20 (MeOH–H_2O from 30% to 60%) column chromatography to obtain 43 fractions (fractions E1–E43). Fraction E26 was purified by preparative HPLC (MeOH–H_2O, 38:62) to afford **1** (30 mg) and **2** (25 mg).

*Forsythoneoside A (**1**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −58.5 (*c* 0.10, EtOH); UV (MeOH) λ_{max} (log ε) 334 (4.47), 274 (4.37), 255 (4.37), 202 (4.78) nm; ECD (EtOH) λ_{max} ($\Delta\varepsilon$) 367 (+4.45), 315 (−0.165), 274 (−1.02), 217 (−2.44) nm; IR ν_{max} 3371, 1693, 1601, 1519, 1448 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 1 and 2; HRESIMS *m*/*z* 1231.3354 $[M + H]^+$ (calcd 1231.3353 for $C_{56}H_{63}O_{31}$).

*Forsythoneoside B (**2**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −140.0 (*c* 0.10, EtOH); UV (MeOH) λ_{max} (log ε) 334 (4.49), 273 (4.39), 255 (4.40), 202 (4.79) nm; ECD (EtOH) λ_{max} ($\Delta\varepsilon$) 375 (−2.62), 323 (+1.74), 275 (+5.39), 222 (+10.09) nm; IR ν_{max} 3376, 1695, 1601, 1517, 1446 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 1 and 2; HRESIMS *m*/*z* 1231.3323 $[M + H]^+$ (calcd 1231.3353 for $C_{56}H_{63}O_{31}$).

*Forsythoneoside C (**3**):* yellow, amorphous powder; $[\alpha]_D^{25}$ +19.9 (*c* 0.09, EtOH–H_2O, 1:1); UV (MeOH) λ_{max} (log ε) 335 (4.42), 277 (4.43), 250 (sh), 202 (4.88) nm; ECD (EtOH–H_2O, 1:1) λ_{max} ($\Delta\varepsilon$) 342 (+0.83), 264 (+5.52), 225 (−13.02); IR ν_{max} 3394, 1694, 1603, 1514, 1445 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 1 and 2; HRESIMS *m*/*z* 1233.3502 $[M + H]^+$ (calcd 1233.3510 for $C_{56}H_{65}O_{31}$).

*Forsythoneoside D (**4**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −8.0 (*c* 0.09, EtOH–H_2O, 1:1); UV (MeOH) λ_{max} (log ε) 336 (4.41), 276 (4.44), 251 (sh), 201 (5.00) nm; ECD (EtOH–H_2O, 1:1) λ_{max} ($\Delta\varepsilon$) 351 (−1.50), 273 (−1.82), 220 (+8.96); IR ν_{max} 3393, 1693, 1604, 1514, 1447 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 1 and 2; HRESIMS *m*/*z* 1233.3504 $[M + H]^+$ (calcd 1233.3510 for $C_{56}H_{65}O_{31}$).

*2-(3,4-Dihydroxyphenyl)-2-oxo-ethyl-O-α-L-rhamnopyranosyl-(1→6)-(4-O-caffeoyl)-β-D-glucopyranoside (**5**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −185.6 (*c* 0.11 MeOH); UV λ_{max} (MeOH) λ_{max} (log ε) 324 (4.15), 204 (4.44) nm; IR ν_{max} 3338, 1685, 1596, 1522, 1451 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 2 and 3; HRESIMS *m*/*z* 661.1739 $[M + Na]^+$ (calcd for $C_{29}H_{34}NaO_{16}$, 661.1745).

*Forsythoside A 4′-O-β-D-glucopyranoside (**6**):* yellow, amorphous powder; $[\alpha]_D^{25}$ −59.8 (*c* 0.12 MeOH); UV (MeOH) λ_{max} (log ε) 321 (4.36), 289 (4.27), 216 (4.80) nm; IR ν_{max} 3392, 1701, 1608, 1509, 1441 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 2 and 3; HRESIMS *m*/*z* 809.2468 $[M + Na]^+$ (calcd for $C_{35}H_{46}NaO_{20}$, 809.2480).

*2-(2,5-Dihydroxyphenyl)-ethyl-O-(6-O-p-hydroxybenzoyl)-β-D-glucopyranoside (**7**):* brown, amorphous powder; $[\alpha]_D^{25}$ −100.7 (*c* 0.10 MeOH); UV (MeOH) λ_{max} (log ε) 257 (4.00) nm; IR ν_{max} 3350, 1697, 1608, 1513, 1452 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 2 and 3; HRESIMS *m*/*z* 459.1276 $[M + Na]^+$ (calcd for $C_{21}H_{24}NaO_{10}$, 459.1267).

*2-(2,5-Dihydroxyphenyl)ethyl-O-(6-O-vanilloyl)-β-D-glucopyranoside (**8**):* brown, amorphous powder; $[\alpha]_D^{25}$ −74.6 (*c* 0.07 MeOH); UV (MeOH) λ_{max} (log ε) 293 (3.99), 263 (4.67) nm; IR ν_{max} 3335, 1702, 1598, 1515, 1455 cm^{-1}; ^1H and ^{13}C NMR spectrum, see Tables 2 and 3; HRESIMS *m*/*z* 489.1367 $[M + Na]^+$ (calcd for $C_{22}H_{26}NaO_{11}$, 489.1373).

Determination of the Absolute Configuration of Sugar. Compound **1** (5 mg) was dissolved in 0.1 N HCl (5 mL) and refluxed for 3 h. The mixture was concentrated under vacuum, and the residue was suspended in H_2O and extracted three times with EtOAc. The aqueous layer was evaporated under a vacuum, repeatedly diluted with H_2O, and evaporated under a vacuum to produce a neutral residue. The residue was dissolved in fresh anhydrous pyridine (1.0 mL). L-Cysteine methyl ester hydrochloride (2 mg) was added, and the reaction mixture was incubated at 60 °C for 2 h. Then, *N*-trimethylsilylimidazole (0.2 mL) was added to the mixture, which had been dried using a current of nitrogen. The reaction mixture was incubated at 60 °C for 2 h and partitioned between *n*-hexane and H_2O (2 mL each). The *n*-hexane extract was subjected to GC analysis under the following conditions: capillary column, HP-5 (60 m × 0.25 mm, with a 0.25 μm film, Dikma); detection, FID; detector temperature,

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280 °C; injection temperature, 250 °C; initial temperature, 200 °C, then raised to 280 °C at a rate of 10 °C/min; the final temperature was maintained for 35 min; carrier, N_2 gas. D-Glucose and L-rhamnose were confirmed by comparing the retention time of their derivatives with the original sugar derivatized in a similar manner, which exhibited retention times of 27.9 and 22.3 min, respectively (Figure S1, Supporting Information).

Neuroprotective Effects of the Compounds. The PC12 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5% horse serum and 5% fetal bovine serum. Then, 100 μL of cells with an initial density of 5×10^4 cells/mL was seeded in each well of a poly-L-lysine-coated, 96-well culture plate and precultured for 24 h at 37 °C under a 5% CO_2 atmosphere. The medium was then replaced by different fresh medium including the control (complete medium), the model (complete medium with 4 μM rotenone), and the sample (the test compounds with different drug concentrations, 10, 1, and 0.1 μM, were added to the aforementioned model medium), and the cells were cultured for 48 h. Then, 10 μL of MTT (0.5 mg/mL) was added to each well. After incubation for 4 h, the medium was removed, and 100 μL of DMSO was added to dissolve the formazan crystals. The optical density (OD) of the PC12 cells was measured on a microplate reader at 550 nm.[23] The cell viability (%) of each sample was calculated with the following formula:

$$\text{Cell viability}(\%) = \text{OD}_{(\text{model or sample})}/\text{OD}_{\text{control}} \times 100$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00372.

UV, IR, NMR, and MS spectra for compounds **1**–**8** (PDF)

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Notes

The authors declare no competing financial interest.

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