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Phenolic Glycosides from the Twigs of *Salix glandulosa*

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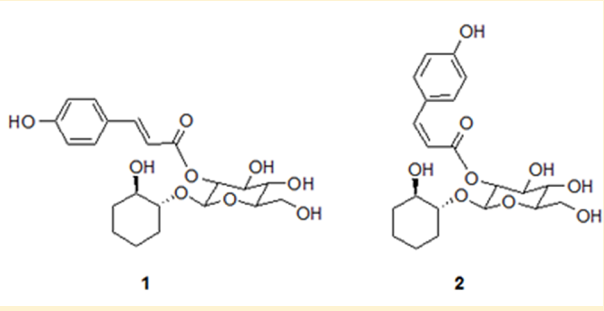
Supporting Information



ABSTRACT: As a part of an ongoing search for bioactive constituents from Korean medicinal plants, the phytochemical investigations of the twigs of *Salix glandulosa* afforded 12 new phenolic glycosides (**1**−**12**) and a known analogue (**13**). The structures of **1**−**13** were characterized by a combination of NMR methods (^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, and HMBC), chemical hydrolysis, and GC/MS. The absolute configuration of **13** [(1*R*,2*S*)-2-hydroxycyclohexyl-2′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside] was determined for the first time. Compounds **1**−**3**, **6**, and **7** exhibited inhibitory effects on nitric oxide production in lipopolysaccharide-activated murine microglial cells (IC_{50} values in the range 6.6−20.5 μM).

The genus *Salix* (willow) includes about 400 species of deciduous trees and shrubs, and extracts of their bark have been used in folk medicine for the treatment of fever, pain, and inflammation.[1] Several species in this genus are known to contain salicin, which is a precursor of a potent nonsteroidal anti-inflammatory drug (NSAID), acetylsalicylic acid (aspirin). Previous phytochemical investigations on the genus *Salix* have led to reports on phenolic compounds, flavonoids, terpenoids, and lignans, which have been associated with biological activities including cytotoxic, neuroprotective, and antiplasmodial activities.[2−6] *Salix glandulosa* Seemen (= *S. chaenomeloides* Kimura, Salicaceae), also called "Korean King Willow", is distributed in Korea, Japan, and mainland China, reaching up to 20 m in height. Although several salicin derivatives and flavonoids were isolated from this plant,[7] it was decided that an additional investigation was merited.

In a continuing search for bioactive constituents from Korean medicinal plants,[8−11] a MeOH extract of *S. glandulosa* twigs was investigated, and 12 new phenolic glycosides (**1**−**12**) were isolated, along with a previously known analogue (**13**). The structures of **1**−**13** were characterized by the use of NMR methods (^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, and HMBC), chemical hydrolysis, and GC/MS. The absolute configuration of **13** was determined for the first time in this study. All the isolates (**1**−**13**) were evaluated for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, a microglial cell line. Herein, the isolation and structural elucidation of compounds **1**−**13**, along with their inhibitory effects on NO production of isolates (**1**−**13**), are reported.

RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless gum, and the molecular formula $C_{21}H_{28}O_9$ was determined from the pseudomolecular ion peak $[M + Na]^+$ at m/z 447.1636 (calcd for $C_{21}H_{28}O_9Na$, 447.1631) in the HRESIMS. The IR spectrum of **1** displayed absorptions characteristic of hydroxy (3390 cm^{-1}) and carbonyl (1696 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** showed the presence of a 1,4-disubstituted aromatic ring [δ_H 7.46 (2H, d, J = 8.4 Hz, H-2″ and H-6″) and 6.81 (2H, d, J = 8.4 Hz, H-3″ and H-5″)], a *trans*-double bond [δ_H 7.65 (1H, d, J = 15.9 Hz, H-7″) and 6.36 (1H, d, J = 15.9

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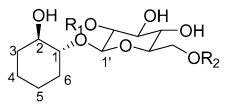
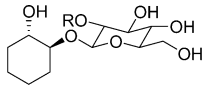
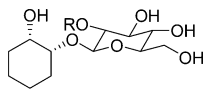


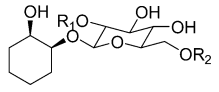
	R_1	R_2
1	*trans*-*p*-coumaroyl	H
2	*cis*-*p*-coumaroyl	H
3	H	*trans*-*p*-coumaroyl
4	H	*cis*-*p*-coumaroyl
5	H	4-hydroxybenzoyl
1a	H	H



	R
6	*trans*-*p*-coumaroyl
7	*cis*-*p*-coumaroyl
6a	H



	R
8	*cis*-*p*-coumaroyl
13	*trans*-*p*-coumaroyl



	R_1	R_2
9	*trans*-*p*-coumaroyl	H
10	*cis*-*p*-coumaroyl	H
11	H	*trans*-*p*-coumaroyl
12	H	*cis*-*p*-coumaroyl

Hz, H-8″)], an anomeric proton [δ_H 4.60 (1H, d, J = 8.2 Hz, H-1′)], and four methylenes [δ_H 1.92 and 1.22 (each 1H, m, H-3), 1.92 and 1.14 (each 1H, m, H-6), and 1.61 and 1.19 (each 2H, m, H-4 and H-5)]. The ^{13}C NMR spectrum of **1** revealed 19 peaks for 21 carbons including a carbonyl carbon (δ_C 168.6), a 1,4-disubstituted aromatic ring [δ_C 161.5, 131.3 (×2), 127.3 and 117.0 (×2)], and a double bond (δ_C 147.0 and 115.3) signals.

The ^{1}H and ^{13}C NMR data of **1** (Tables 1 and 2) were found to be very similar to those of grandidentatin (**13**)[12] with major differences in the downfield shift of C-1 and C-2 (δ_C 85.1 and 74.0 for **1**; δ_C 80.0 and 71.5 for **13**, respectively), indicating **1** to be a stereoisomer of **13**. The planar structure of **1** was elucidated through 2D NMR analysis (^{1}H−^{1}H COSY, HMQC, and HMBC; see Figure 1). Alkaline hydrolysis of **1** afforded *trans*-*p*-coumaric acid and (1*R*,2*R*)-*trans*-1,2-cyclohexanediol-1-*O*-*β*-D-glucopyranoside (**1a**), with the latter compound identified by comparing the optical rotation, HRFABMS, and ^{13}C NMR data with previously reported values.[13−15] The configuration of the 1,2-cyclohexanediol moiety of **1a** was assigned as *trans*, from the more downfield shifted carbon signal of C-1 (δ_C 84.5, 1*R* form; δ_C 86.8, 1*S* form; δ_C 84.4, **1a**) when compared with *cis*-analogues (δ_C 79.7, 1*R* form; δ_C 80.2, 1*S* form).[13−15] In turn, the 1*R* configuration of **1a** was deduced from the more upfield shifted anomeric proton signal (δ_H 4.35; δ_H 4.37, **1a**) than that of the 1*S* compound (δ_H 4.46) in the same NMR solvent.[16] The enzymatic hydrolysis of **1a** gave (1*R*,2*R*)-*trans*-1,2-cyclohexanediol and D-glucose {$[\alpha]_D^{25}$ +62.1 (*c* 0.05, H_2O)}. The former compound was identified by comparing the optical rotation {$[\alpha]_D^{20}$ −39 (*c* 1.6, H_2O), 1*R*,2*R* form; $[\alpha]_D^{20}$ +39 (*c* 1.6, H_2O), 1*S*,2*S* form; $[\alpha]_D^{25}$ −38.7 (*c* 0.10, H_2O), (1*R*,2*R*)-*trans*-1,2-cyclohexanediol from **1a**} with an authentic sample,[17] and the sugar was confirmed by GC/MS analysis.[9] Thus, the structure of **1** was established as (1*R*,2*R*)-2-hydroxycyclohexyl-2′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside, and this substance was named *trans*-glanduloidin A.

The ^{1}H and ^{13}C NMR data of **2** were quite similar to those of **1** except for the chemical shifts of H-7″ and H-8″, and their small J values [δ_H 6.86 (1H, d, J = 13.3 Hz, H-7″) and 5.78 (1H, d, J = 13.3 Hz, H-8″)] were indicative of a *cis*-configuration. A full NMR analysis (Tables 1 and 2) supported the structure of **2** (*cis*-glanduloidin A) as (1*R*,2*R*)-2-hydroxycyclohexyl-2′-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound **3** (*trans*-glanduloidin B) gave the molecular formula $C_{21}H_{28}O_9$, and inspection of its ^{1}H and ^{13}C NMR data revealed the structure of **3** to be closely related to that of **1**. The major differences were downfield shifts of C-6′ [δ_H 4.59 (1H, dd, J = 11.9, 2.1 Hz, H-6′a) and 4.24 (1H, dd, J = 11.9, 6.3 Hz, H-6′b); δ_C 64.8], which indicated that the *trans*-*p*-coumaroyl moiety is located at C-6′. The HMBC correlation of H-6′/C-9″ (δ_C 169.2) corroborated the location of the *trans*-*p*-coumaroyl moiety (Supporting Information). The absolute configuration of **3** was determined as 1*R*,2*R* by the same method as described for **1**. Thus, **3** was established as (1*R*,2*R*)-2-hydroxycyclohexyl-6′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside.

Inspection of the ^{1}H and ^{13}C NMR data of **4** suggested this compound to be very similar structurally to **3**. The chemical shift of H-7″ and the smaller J value [δ_H 6.88 (1H, d, J = 12.6 Hz)] coupled with that of H-8″ [δ_H 5.81 (1H, d, J = 12.6 Hz)] indicated that the *p*-coumaroyl moiety is in the *cis*-form. The ^{1}H−^{1}H COSY, HMQC, and HMBC spectra of **4** (*cis*-glanduloidin B) confirmed the structure as (1*R*,2*R*)-2-hydroxycyclohexyl-6′-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound **5** (glanduloidin C) was obtained as a colorless gum. The molecular formula was determined to be $C_{19}H_{26}O_9$ from the $[M + Na]^+$ pseudomolecular ion peak in the positive-ion HRESIMS. The ^{1}H and ^{13}C NMR spectra were similar to those of 6′-*O*-4-hydroxybenzoylgrandidentin,[1] except for the shifted signals of C-1, C-2, and C-1′ (δ_C 85.1, 74.2, and 103.1, respectively), indicating that **5** is a stereoisomer of 6′-*O*-4-hydroxybenzoylgrandidentin. The HMBC cross-peak of H-6′/C-7″ confirmed the 4-hydroxybenzoyl moiety to be located at C-6′ (Figure 1). Alkaline hydrolysis afforded **1a** and 4-hydroxybenzoic acid, which was identified by the comparison of spectroscopic data with an authentic sample. Thus, the structure of **5** was determined as (1*R*,2*R*)-2-hydroxycyclohexyl-6′-*O*-4″-hydroxybenzoyl-*β*-D-glucopyranoside.

The ^{1}H and ^{13}C NMR data of **6** resembled those of **1**, but there were slight differences in the signals of C-1 [δ_H 3.45 (1H, overlap); δ_C 83.8] and C-1′ [δ_H 4.78 (1H, d, J = 7.7 Hz); δ_C 102.6], suggesting that **6** is a stereoisomer of **1**. The planar structure of **6** was established through ^{1}H−^{1}H COSY, HMQC, and HMBC NMR spectroscopic analysis (Supporting Information). Alkaline hydrolysis of **6** gave *trans*-*p*-coumaric acid and (1*S*,2*S*)-*trans*-1,2-cyclohexanediol-1-*O*-*β*-D-glucopyranoside (**6a**), which was identified by comparing its optical rotation, HRFABMS, and ^{13}C NMR data with previously reported data.[13−15] The absolute configuration at C-1 and C-2 was corroborated by the downfield shifted anomeric proton (δ_H 4.50) of **6a**.[16] Enzymatic hydrolysis of **6a** afforded (1*S*,2*S*)-*trans*-1,2-cyclohexanediol, identified by the positive optical rotation {$[\alpha]_D^{25}$ +40.8 (*c* 0.10, H_2O)},[17] and D-glucose, with the sugar confirmed by GC/MS analysis.[9] Therefore, the structure of **6** (*trans*-glanduloidin D) was established as (1*S*,2*S*)-2-hydroxycyclohexyl-2′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound **7** (*cis*-glanduloidin D) was found to possess a *cis*-*p*-coumaroyl moiety instead of a *trans*-*p*-coumaroyl moiety as in **6**. The NMR data of these two compounds were very similar to one another. In the ^{1}H NMR spectrum, a smaller J value was observed between H-7″/H-8″ (12.6 Hz), indicating the double bond at C-7/C-8 to have a *cis*-configuration. Analysis of the 1D and 2D NMR data confirmed the structure of **7** as (1*S*,2*S*)-2-hydroxycyclohexyl-2′-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound **8** (*cis*-grandidentatin) was isolated as a colorless gum with the same molecular formula of $C_{21}H_{28}O_9$ as **1**. Inspection of the ^{1}H and ^{13}C NMR data revealed the chemical shifts of C-1 and C-2 of **8** (δ_H 3.83 and 3.63; δ_C 80.0 and 71.6)

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Table 1. ^{1}H NMR [ppm, mult, (J in Hz)] Data of Compounds 1−13 in CD_3OD (700 MHz)

position	1	2	3	4	5	6	7	8	9	10	11	12	13
1	3.39, overlap	3.37, overlap	3.41, overlap	3.38, overlap	3.42, overlap	3.45, overlap	3.45, overlap	3.83, overlap	3.85, overlap	3.83, overlap	3.75, overlap	3.70, overlap	3.85, overlap
2	3.37, overlap	3.37, overlap	3.42, overlap	3.40, overlap	3.41, overlap	3.41, overlap	3.40, overlap	3.63, overlap	3.70, overlap	3.70, overlap	3.77, overlap	3.77, overlap	3.63, overlap
3a	1.92, m	1.89, m	1.97, m	1.95, m	1.95, m	1.83, m	1.81, m	1.62, m	1.67, m	1.67, m	1.76, m	1.76, m	1.62, m
3b	1.22, m	1.22, m	1.25, m	1.23, m	1.22, m	1.25, m	1.29, m	1.52, m	1.45, m	1.45, m	1.50, m	1.51, m	1.53, m
4a	1.61, m	1.61, m	1.69, m	1.69, m	1.67, m	1.61, m	1.59, m	1.50, m	1.57, m	1.57, m	1.60, m	1.59, m	1.54, m
4b	1.19, m	1.17, m	1.24, m	1.24, m	1.23, m	1.22, m	1.20, m	1.21, m	1.26, m	1.26, m	1.26, m	1.26, m	1.23, m
5a	1.61, m	1.61, m	1.69, m	1.69, m	1.67, m	1.61, m	1.59, m	1.40, m	1.57, m	1.57, m	1.60, m	1.61, m	1.40, m
5b	1.19, m	1.17, m	1.24, m	1.24, m	1.23, m	1.22, m	1.20, m	1.20, m	1.26, m	1.26, m	1.19, m	1.18, m	1.20, m
6a	1.92, m	1.92, m	2.06, m	2.04, m	2.04, m	2.00, m	1.95, m	1.79, m	1.86, m	1.85, m	1.93, m	1.88, m	1.80, m
6b	1.14, m	1.11, m	1.34, m	1.30, m	1.32, m	1.36, m	1.35, m	1.36, m	1.53, m	1.52, m	1.51, m	1.48, m	1.37, m
1′	4.60, d (8.2)	4.53, d (7.7)	4.41, d (7.7)	4.38, d (7.7)	4.23, d (7.7)	4.78, d (7.7)	4.73, d (8.4)	4.52, d (8.4)	4.79, d (7.7)	4.75, d (8.4)	4.41, d (8.4)	4.38, d (7.7)	4.59, d (8.4)
2′	4.81, dd (9.7, 8.2)	4.78, dd (9.8, 7.7)	3.24, dd (8.4, 7.7)	3.22, dd (9.1, 7.7)	3.25, dd (9.1, 7.7)	4.81, dd (9.0, 7.7)	4.78, dd (9.1, 8.4)	4.82, overlap	4.82, dd (8.7, 7.7)	4.80, t (8.4)	3.26, dd (9.1, 8.4)	3.24, overlap	4.83, overlap
3′	3.61, dd (9.7, 8.8)	3.56, dd (9.8, 7.1)	3.39, overlap	3.37, overlap	3.41, overlap	3.59, t (9.0)	3.55, t (9.1)	3.57, t (9.1)	3.59, t (8.7)	3.54, t (8.4)	3.38, t (9.1)	3.36, overlap	3.61, dd (9.1, 8.4)
4′	3.40, dd (9.7, 8.8)	3.39, overlap	3.37, overlap	3.32, overlap	3.41, overlap	3.40, t (9.0)	3.37, overlap	3.40, overlap	3.40, dd (9.1, 8.7)	3.40, overlap	3.34, overlap	3.27, overlap	3.40, overlap
5′	3.37, overlap	3.33, overlap	3.58, ddd (9.0, 6.3, 2.1)	3.54, ddd (9.8, 6.3, 2.1)	3.62, ddd (9.8, 6.3, 2.1)	3.31, ddd (9.0, 5.7, 2.3)	3.29, overlap	3.32, overlap	3.32, ddd (9.1, 5.6, 2.1)	3.32, overlap	3.52, ddd (9.1, 6.3, 2.1)	3.47, ddd (9.1, 6.3, 2.1)	3.34, overlap
6′a	3.88, dd (11.9, 2.3)	3.87, dd (11.9, 2.1)	4.59, dd (11.9, 2.1)	4.51, dd (11.9, 2.1)	4.67, dd (11.9, 2.1)	3.88, dd (11.9, 2.3)	3.88, dd (11.9, 2.1)	3.87, dd (11.9, 2.8)	3.88, dd (11.9, 2.1)	3.88, dd (11.9, 2.1)	4.46, dd (11.9, 2.1)	4.44, dd (11.9, 2.1)	3.87, dd (11.9, 2.1)
6′b	3.69, dd (11.9, 5.8)	3.68, dd (11.9, 5.6)	4.24, dd (11.9, 6.3)	4.22, dd (11.9, 6.3)	4.34, dd (11.9, 6.3)	3.70, dd (11.9, 5.7)	3.69, dd (11.9, 5.6)	3.69, dd (11.9, 5.6)	3.70, dd (11.9, 5.6)	3.69, dd (11.9, 5.6)	4.35, dd (11.9, 6.3)	4.28, dd (11.9, 6.3)	3.69, dd (11.9, 5.6)
2″/6″	7.46, d (8.4)	7.66, d (8.4)	7.47, d (8.4)	7.66, d (8.4)	7.93, d (8.4)	7.47, d (8.6)	7.67, d (8.4)	7.69, d (8.5)	7.47, d (8.5)	7.69, d (8.4)	7.45, d (8.4)	7.68, d (8.4)	7.45, d (8.4)
3″/5″	6.81, d (8.4)	6.72, d (8.4)	6.80, d (8.4)	6.76, d (8.4)	6.82, d (8.4)	6.80, d (8.4)	6.73, d (8.4)	6.74, d (8.5)	6.80, d (8.5)	6.73, d (8.4)	6.80, d (8.4)	6.75, d (8.4)	6.78, d (8.4)
7″	7.65, d (15.9)	6.86, d (13.3)	7.68, d (15.4)	6.88, d (12.6)		7.66, d (15.9)	6.86, d (12.6)	6.90, d (12.8)	7.67, d (15.9)	6.88, d (13.3)	7.63, d (16.1)	6.87, d (12.6)	7.66, d (15.9)
8″	6.36, d (15.9)	5.78, d (13.3)	6.36, d (15.4)	5.81, d (12.6)		6.37, d (15.9)	5.82, d (12.6)	5.81, d (12.8)	6.39, d (15.9)	5.83, d (13.3)	6.34, d (16.1)	5.78, d (12.6)	6.34 d (15.9)

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Table 2. ^{13}C NMR Data of Compounds 1−13 in CD_3OD (175 MHz)

carbon	1	2	3	4	5	6	7	8	9	10	11	12	13
1	85.1	84.9	85.9	85.5	85.1	83.8	83.4	80.0	80.0	81.2	81.7	81.7	80.1
2	74.0	74.0	74.4	74.3	74.2	73.9	74.0	71.6	71.6	71.6	70.6	70.5	71.8
3	33.5	33.5	33.6	33.5	33.6	33.6	33.5	31.0	31.0	31.6	31.5	31.5	31.0
4	24.8[a]	24.8[a]	24.9[a]	24.9[a]	24.9[a]	24.4	24.3	24.0	22.7[a]	22.8[a]	22.8	23.2	23.8
5	25.0[a]	25.0[a]	25.0[a]	25.3[a]	25.3[a]	24.8	24.7	22.0	23.2[a]	23.6[a]	25.2	25.2	21.8
6	31.4	31.2	31.6	31.5	31.4	32.2	32.0	29.0	30.4	30.2	30.2	30.2	29.0
1′	101.4	101.3	103.6	103.4	103.1	102.6	102.3	101.1	102.4	102.3	104.5	104.5	101.3
2′	75.5	75.2	74.9	74.8	74.9	75.8	75.4	75.2	75.9	75.5	75.5	75.5	75.5
3′	76.2	76.2	77.9	77.9	77.9	76.5	76.3	76.2	76.4	76.4	78.0	78.0	76.3
4′	71.7	71.9	71.9	71.8	71.9	71.8	71.9	71.8	71.9	72.0	72.1	72.0	71.8
5′	78.2	78.3	75.7	75.6	75.8	78.1	78.1	78.2	78.2	78.2	75.6	75.5	78.2
6′	62.7	62.7	64.8	64.5	64.9	62.8	62.7	62.7	62.8	62.8	64.8	64.6	62.7
1″	127.3	127.2	127.3	127.7	122.3	127.4	127.4	127.5	127.4	127.4	127.2	127.7	126.6
2″/6″	131.3	134.1	131.4	134.0	133.2	131.4	134.0	134.1	131.4	134.1	131.3	134.0	131.4
3″/5″	117.0	116.3	117.0	116.0	116.3	117.0	116.2	116.0	117.0	116.2	117.0	116.0	117.5
4″	161.5	161.5	161.6	160.4	163.8	161.4	161.0	160.8	161.6	161.0	161.6	160.3	163.0
7″	147.0	145.5	147.1	145.7	168.1	147.0	145.4	145.9	147.1	145.7	146.9	145.4	147.3
8″	115.3	116.1	114.9	116.3		115.5	116.4	116.4	115.5	116.5	115.1	116.4	114.7
9″	168.6	167.6	169.2	168.1		168.8	167.8	167.5	168.9	167.7	169.2	168.2	168.9

[a]Exchangeable peaks.

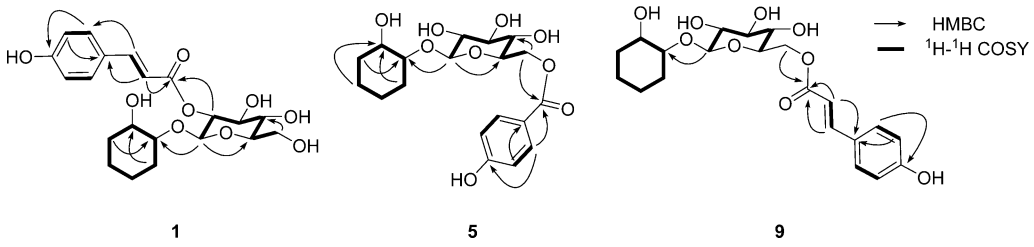

Figure 1. HMBC and ^{1}H−^{1}H COSY correlations of 1, 5, and 9.

to be quite different from those of 1−7 (δ_H 3.37−3.45; δ_C 83.4−85.9 and 73.9−74.4), suggesting that the stereochemistry of the 1,2-cyclohexanediol unit of 8 is in the *cis*-form.[13−15] Acid hydrolysis of 8 gave D-glucose from the aqueous phase and a mixture of *cis*-*p*-coumaric acid and *cis*-1,2-cyclohexanediol from the organic phase, which were identified by co-TLC using authentic samples. The *cis*-configuration of 1,2-cyclohexanediol was determined directly from the zero optical rotation of the organic phase, because *p*-coumaric acid is optically inactive.[18] The absolute configuration of 8 at C-1 was determined from the chemical shift of the anomeric carbon in the ^{13}C NMR spectrum. In both the *trans* and *cis* forms of 1,2-cyclohexanediol-1-*O*-*β*-D-glucopyranoside, the chemical shifts of the anomeric carbon of the 1*R* forms occur more upfield shifted than those of the 1*S* forms.[13−15] Compound 8 showed a more upfield shifted anomeric carbon signal (δ_C 101.1) than that of 10 (δ_C 102.3) (see below), which was assigned as a C-1 and C-2 diastereomer of 8. Thus, compound 8 was assigned with a 1*R* configuration and was established as (1*R*,2*S*)-2-hydroxycyclohexyl-2′-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside.

The ^1H and ^{13}C NMR data of 9 resembled those of 6, but had more upfield shifted signals of C-1 and C-2 (δ_C 80.0 and 71.6, respectively), indicating the presence of the same *cis*-1,2-cyclohexanediol moiety as 8. However, the anomeric carbon chemical shift of 9 (δ_C 102.4) was more downfield than that of 1 (δ_C 101.4), suggesting that 9 has a 1*S* configuration.[13−15] Thus, 9 (*trans*-glanduloidin E) was established as (1*S*,2*R*)-2-hydroxycyclohexyl-2′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside.

The spectroscopic data of 10 were observed to be very similar to those of 9, but showed a smaller *J* value between H-7″ and H-8″ (13.3 Hz) in the ^1H NMR spectrum, suggesting that compound 10 possesses a *cis*-*p*-coumaroyl moiety. A full NMR analysis including all 2D NMR data confirmed the structure of 10 (*cis*-glanduloidin E) as (1*S*,2*R*)-2-hydroxycyclohexyl-2′-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound 11 (*trans*-glanduloidin F) gave the same molecular formula and similar ^1H and ^{13}C NMR data to 9, except for the downfield shift of H-6′ (δ_H 4.46 and 4.35) and C-6′ (δ_C 64.8), indicating the *trans*-*p*-coumaroyl moiety to be located at C-6′. The cross-peak of H-6′ with C-9″ in the HMBC spectrum corroborated the location of the *trans*-*p*-coumaroyl moiety. A downfield shifted signal of C-1′ (δ_C 104.5) in 11 supported the 1*S* configuration, as in 9. Therefore, the structure of 11 was determined as (1*S*,2*R*)-2-hydroxycyclohexyl-6′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound 12 showed very similar ^1H and ^{13}C NMR spectroscopic data to those of 11. The major difference observed was the smaller *J* value of the olefinic protons ($J_{H7'',H8''}$ = 12.6 Hz), showing that 12 contains a *cis*-*p*-coumaroyl moiety. Thus, the structure of 12 (*cis*-glanduloidin F) was determined as (1*S*,2*R*)-2-hydroxycyclohexyl-6′-*O*-*cis*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound 13 was isolated as a colorless gum having the molecular formula $C_{21}H_{28}O_9$. The ^1H and ^{13}C NMR data of 13

were identical with those of grandidentatin, which was reported earlier without any determination of the absolute configuration at the *cis*-1,2-cyclohexanediol moiety.[12] The more upfield shifted signal of C-1′ (δ_C 101.3) of **13** confirmed the 1*R* configuration as **8**. The full NMR assignment of **13** was performed through extensive 1D and 2D NMR, and the structure of **13** was established as (1*R*,2*S*)-2-hydroxycyclohexyl-2′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside.

The antineuroinflammatory effects of the compounds (**1**−**13**) isolated from *S. glandulosa* were evaluated via measurement of NO levels using the bacterial endotoxin lipopolysaccharide in the murine microglia BV-2 cell line. The inhibitory activities of the isolated compounds on NO production were expressed as 50% inhibition concentration (IC_{50}). As shown in Table 3,

Table 3. Inhibitory Effect on NO Production of Compounds 1−3, 6, and 7 in LPS-Activated BV-2 Cells

compound	IC_{50} (μM)[a]	cell viability (%)[b]
1	20.5	107.2 ± 1.86
2	6.6	102.5 ± 1.15
3	14.8	100.7 ± 5.26
6	15.5	93.6 ± 1.61
7	18.1	96.2 ± 3.90
L-NMMA[c]	24.3	99.8 ± 4.29

[a]The IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells. [b]The cell viability was expressed as a percentage (%) of the LPS-only treatment group. The results are averages of three independent experiments, and the data are expressed as mean ± SD. [c]Positive control.

compounds **1**−**3**, **6**, and **7** all exhibited inhibition of NO production, with IC_{50} values of 20.5, 6.6, 14.8, 15.8, and 18.1 μM, respectively, in LPS-induced BV-2 cells, and did not show any cellular toxicity. Their activities were more potent than that of the positive control, N^G-monomethyl-L-arginine (L-NMMA), which inhibited NO production with an IC_{50} value of 24.3 μM. Active compounds (**1**−**3**, **6**, and **7**) all possess a *trans*-1,2-cyclohexanediol unit, and the compounds of *cis*-1,2-cyclohexanediol derivatives (**8**−**13**) were inactive, suggesting that the *trans*-1,2-cyclohexanediol unit is important for the inhibitory activity on NO production.

The cytotoxic activity of the isolates (**1**−**13**) was determined against four human cancer cell lines (A549, SK-OV-3, A498, HCT15), but none of the compounds were found to be active (IC_{50} >10 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. Infrared spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded using an Agilent 8453 UV−visible spectrophotometer. NMR spectra were recorded on a Bruker AVANCE III 700 NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C). HRESIMS and HRFABMS were obtained on a Waters SYNAPT G2 and JEOL JMS700 mass spectrometer, respectively. Silica gel 60 (Merck, 230−400 mesh) and RP-C_{18} silica gel (Merck, 230−400 mesh) were used for column chromatography (CC). Preparative HPLC was performed using a Gilson 306 pump with a Shodex refractive index detector. TLC was performed using Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates.

Plant Material. Twigs of *S. glandulosa* were collected in Gunwi, Korea, in March 2013, and the plant was identified by one of the authors (K.R.L.). A voucher specimen (SKKU-NPL 1304) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The twigs of *S. glandulosa* (10.0 kg) were extracted with 80% aqueous MeOH under reflux and filtered. The filtrate was evaporated under reduced pressure to obtain a MeOH extract (630 g), which was suspended in distilled H_2O and successively partitioned with *n*-hexane, $CHCl_3$, EtOAc, and *n*-butanol, yielding 7, 13, 65, and 100 g of each residue, respectively. The EtOAc-soluble fraction (20 g) was separated over a silica gel column ($CHCl_3$−MeOH−H_2O, 4:1:0.1) to give 11 fractions (E1−E11). Fraction E3 (2.5 g) was separated on an RP-C_{18} silica gel column with 50% aqueous MeOH to yield seven subfractions (E3a−E3g). Fraction E3c (600 mg) was chromatographed on a silica gel column ($CHCl_3$−MeOH−H_2O, 6:1:0.1) to yield six subfractions (E3ca−E3cf). Fraction E3cc (150 mg) was purified by preparative HPLC [23% CH_3CN(aq)] to yield compounds **5** (5 mg), **6** (10 mg), and **7** (5 mg). Fraction E3d (90 mg) was purified by preparative HPLC [25% CH_3CN(aq)] to give compounds **9** (4 mg) and **10** (4 mg). Fraction E3e (90 mg) was purified by preparative HPLC [27% CH_3CN(aq)] to yield compounds **1** (10 mg), **2** (6 mg), **3** (4 mg), **4** (4 mg), **8** (4 mg), and **13** (5 mg). Compounds **11** (4 mg) and **12** (4 mg) were obtained by purification of fraction E3f (70 mg) using preparative HPLC [28% CH_3CN(aq)].

*trans-Glanduloidin A (**1**):* colorless gum; $[\alpha]_D^{25}$ −64.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3390, 2940, 1696, 1600, 1156, 1084, 988 cm^{-1}; UV (MeOH) λ_{max} (log ε) 309 (3.02), 231 (2.99) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1636 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*cis-Glanduloidin A (**2**):* colorless gum; $[\alpha]_D^{25}$ −59.2 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3389, 2941, 1698, 1601, 1154, 1081, 990 cm^{-1}; UV (MeOH) λ_{max} (log ε) 290 (3.10), 231 (3.11) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1625 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*trans-Glanduloidin B (**3**):* colorless gum; $[\alpha]_D^{25}$ −49.1 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3390, 2942, 1696, 1599, 1155, 1083, 987 cm^{-1}; UV (MeOH) λ_{max} (log ε) 308 (3.01), 231 (2.99) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1631 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*cis-Glanduloidin B (**4**):* colorless gum; $[\alpha]_D^{25}$ −50.7 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3389, 2940, 1698, 1600, 1153, 1080, 990 cm^{-1}; UV (MeOH) λ_{max} (log ε) 288 (3.11), 230 (3.12) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1631 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*Glanduloidin C (**5**):* colorless gum; $[\alpha]_D^{25}$ −52.1 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3390, 2943, 1696, 1601, 1153, 1084, 992 cm^{-1}; UV (MeOH) λ_{max} (log ε) 254 (3.5) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 421.1478 $[M + Na]^+$ (calcd for $C_{19}H_{26}O_9Na$, 421.1475).

*trans-Glanduloidin D (**6**):* colorless gum; $[\alpha]_D^{25}$ −24.8 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3390, 2942, 1695, 1599, 1156, 1083, 987 cm^{-1}; UV (MeOH) λ_{max} (log ε) 307 (3.08), 230 (3.08) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1631 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*cis-Glanduloidin D (**7**):* colorless gum; $[\alpha]_D^{25}$ −21.2 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3389, 2941, 1698, 1603, 1152, 1081, 990 cm^{-1}; UV (MeOH) λ_{max} (log ε) 287 (3.10), 229 (3.11) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1628 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*cis-Grandidentatin (**8**):* colorless gum; $[\alpha]_D^{25}$ −10.0 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3389, 2941, 1696, 1601, 1156, 1084, 988 cm^{-1}; UV (MeOH) λ_{max} (log ε) 288 (3.00), 231 (3.10) nm; ^{1}H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1630 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

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*trans-Glanduloidin E (**9**):* colorless gum; $[\alpha]_D^{25}$ −19.2 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3389, 2941, 1698, 1601, 1154, 1081, 990 cm^{-1}; UV (MeOH) λ_{max} (log ε) 309 (3.11), 230 (3.03) nm; ^1H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1628 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*cis-Glanduloidin E (**10**):* colorless gum; $[\alpha]_D^{25}$ −20.9 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3390, 2942, 1696, 1599, 1155, 1083, 987 cm^{-1}; UV (MeOH) λ_{max} (log ε) 288 (3.02), 231 (2.99) nm; ^1H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1629 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*trans-Glanduloidin F (**11**):* colorless gum; $[\alpha]_D^{25}$ −12.3 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3389, 2940, 1699, 1601, 1153, 1082, 990 cm^{-1}; UV (MeOH) λ_{max} (log ε) 307 (3.11), 230 (3.12) nm; ^1H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1626 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*cis-Glanduloidin F (**12**):* colorless gum; $[\alpha]_D^{25}$ −13.5 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3389, 2940, 1698, 1600, 1153, 1080, 990 cm^{-1}; UV (MeOH) λ_{max} (log ε) 288 (3.12), 230 (3.14) nm; ^1H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1629 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

*(1R,2S)-2-Hydroxycyclohexyl-2′-O-trans-p-coumaroyl-β-D-glucopyranoside (**13**):* colorless gum; $[\alpha]_D^{25}$ −15.7 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3390, 2939, 1699, 1600, 1155, 1080, 990 cm^{-1}; UV (MeOH) λ_{max} (log ε) 308 (3.12), 231 (3.08) nm; ^1H (CD_3OD, 700 MHz) and ^{13}C NMR (CD_3OD, 175 MHz) data, see Tables 1 and 2; positive HRESIMS *m*/*z* 447.1628 $[M + Na]^+$ (calcd for $C_{21}H_{28}O_9Na$, 447.1631).

Alkaline Hydrolysis of 1−7. Compounds **1**−**7** (each 2.0 mg) were individually hydrolyzed using 0.1 N KOH (3 mL) and stirred at room temperature for 24 h. The reaction mixture was neutralized with Dowex HCR W2 (H^+ form), and the resin was removed by filtration. A portion of the reaction product was partitioned between $CHCl_3$−H_2O (1 mL each). From the H_2O layer, (1*R*,2*R*)-*trans*-1,2-cyclohexanediol-1-*O*-*β*-D-glucopyranoside (**1a**, 1.0 mg each from **1**−**5**) and (1*S*,2*S*)-*trans*-1,2-cyclohexanediol-1-*O*-*β*-D-glucopyranoside (**6a**, 1.0 mg each from **6** and **7**) were obtained, and from the $CHCl_3$ layer, *trans*-*p*-coumaric acid (0.3 mg each from **1**, **3**, and **6**), *cis*-*p*-coumaric acid (0.3 mg each from **2**, **4**, and **7**), and 4-hydroxybenzoic acid (0.4 mg from **5**) were afforded.

*(1R,2R)-trans-1,2-Cyclohexanediol-1-O-β-D-glucopyranoside (**1a**):* colorless gum; $[\alpha]_D^{25}$ −16.2 (*c* 0.05, MeOH); ^1H NMR (CD_3OD, 700 MHz) *δ* 4.37 (1H, d, *J* = 8.1 Hz, H-1′), 3.87 (1H, brd, *J* = 11.4 Hz, H-6′a), 3.68 (1H, brd, *J* = 11.4 Hz, H-6′b), 3.45 (1H, overlap, H-1), 3.44 (1H, overlap, H-2), 3.38 (1H, m, H-3′), 3.32 (1H, m, H-4′), 3.31 (1H, m, H-5′), 3.23 (1H, overlap, H-2′), 2.08, 2.00 (each 1H, m, H-3a and H-6a), 1.72 (2H, m, H-4a and H-5a), 1.32, 1.29 (each 1H, m, H-3b and H-6b), 1.28 (2H, m, H-4b and H-5b); ^1H NMR (pyridine-d_5, 700 MHz) *δ* 4.98 (1H, d, *J* = 7.7 Hz, H-1′), 4.54 (1H, dd, *J* = 11.6, 2.6 Hz, H-6′a), 4.34 (1H, dd, *J* = 11.6, 5.7 Hz, H-6′b), 4.24 (1H, t, *J* = 7.7 Hz, H-3′), 4.23 (1H, dd, *J* = 8.5, 7.7 Hz, H-4′), 4.01 (1H, t, *J* = 7.7 Hz, H-2′), 4.00 (1H, ddd, *J* = 8.5, 5.7, 2.6 Hz, H-5′), 3.78 (1H, m, H-1), 3.74 (1H, m, H-2), 2.20, 2.12 (each 1H, m, H-3a and H-6a), 1.54 (2H, m, H-4a and H-5a), 1.45, 1.43 (each 1H, m, H-3b and H-6b), 1.11 (2H, m, H-4b and H-5b); ^{13}C NMR (CD_3OD, 175 MHz) *δ* 103.0 (C-1′), 84.7 (C-1), 78.1 (C-3′ and C-5′), 75.0 (C-2′), 74.3 (C-2), 71.7 (C-4′), 62.8 (C-6′), 33.9, 31.5 (C-3 and C-6), 25.3, 25.1 (C-4 and C-5); ^{13}C NMR (pyridine-d_5, 175 MHz) *δ* 103.5 (C-1′), 84.4 (C-1), 78.5 (C-5′), 78.3 (C-3′), 74.7 (C-2′), 73.1 (C-2), 71.5 (C-4′), 62.5 (C-6′) 33.1, 30.9 (C-3 and C-6), 24.3, 23.9 (C-4 and C-5); positive HRFABMS *m*/*z* 301.1262 $[M + Na]^+$ (calcd for $C_{12}H_{22}O_9Na$, 301.1263).

*(1S,2S)-trans-1,2-Cyclohexanediol-1-O-β-D-glucopyranoside (**6a**):* colorless gum; $[\alpha]_D^{25}$ −23.4 (*c* 0.05, MeOH); ^1H NMR (CD_3OD, 700 MHz) *δ* 4.50 (1H, d, *J* = 7.7 Hz, H-1′), 3.87 (1H, brd, *J* = 11.4 Hz, H-6′a), 3.68 (1H, brd, *J* = 11.4 Hz, H-6′b), 3.48 (1H, m, H-2), 3.40 (1H, overlap, H-1), 3.38 (1H, overlap, H-3′), 3.30 (1H, overlap, H-4′), 3.29 (1H, overlap, H-5′), 3.23 (1H, dd, *J* = 9.2, 7.7 Hz, H-2′), 2.20, 1.98 (each 1H, H-3a and H-6a), 1.70 (2H, m, H-4a and H-5a), 1.31 (2H, m, H-3b and H-6b), 1.29 (2H, m, H-4b and H-5b); ^1H NMR (pyridine-d_5, 700 MHz) *δ* 5.24 (1H, d, *J* = 8.0 Hz, H-1′), 4.54 (1H, dd, *J* = 11.7, 2.5 Hz, H-6′a), 4.40 (1H, dd, *J* = 11.7, 5.1 Hz, H-6′b), 4.23 (1H, t, *J* = 8.5 Hz, H-4′), 4.22 (1H, t, *J* = 8.5 Hz H-3′), 4.08 (1H, dd, *J* = 8.5, 8.0 Hz H-2′), 3.96 (1H, ddd, *J* = 8.5, 5.2, 2.6 Hz, H-5′) 3.81 (1H, m, H-2), 3.73 (1H, m, H-1), 2.22, 2.11 (each 1H, H-3a and H-6a), 1.52 (2H, m, H-4a and H-5a), 1.51, 1.47 (each 1H, m, H-3b and H-6b), 1.13 (2H, m, H-4b and H-5b); ^{13}C NMR (CD_3OD, 175 MHz) *δ* 106.0 (C-1′), 87.4 (C-1), 78.2 (C-3′), 78.1 (C-5′), 75.8 (C-2′), 75.4 (C-2), 71.7 (C-4′), 62.9 (C-6′), 33.9, 33.5 (C-3 and C-6), 25.5, 25.2 (C-4 and C-5); ^{13}C NMR (pyridine-d_5, 175 MHz) *δ* 106.4 (C-1′), 86.9 (C-1), 78.6 (C-5′), 78.5 (C-3′), 75.9 (C-2′), 74.8 (C-2), 71.4 (C-4′), 62.6 (C-6′), 33.1, 32.5 (C-3 and C-6), 24.6, 24.1 (C-4 and C-5); positive HRFABMS *m*/*z* 301.1263 $[M + Na]^+$ (calcd for $C_{12}H_{22}O_9Na$, 301.1263).

Enzymatic Hydrolysis of 1a and 6a. Compounds **1a** and **6a** (each 1.0 mg) with 1 mL of H_2O and 10 mg of *β*-glucosidase (almonds, Sigma) was stirred for 48 h at 37 °C. The H_2O solution was then extracted with $CHCl_3$ three times, and the $CHCl_3$ extract was evaporated in vacuo to yield (1*R*,2*R*)-*trans*-1,2-cyclohexanediol (from **1a**) and (1*S*,2*S*)-*trans*-1,2-cyclohexanediol (from **6a**) as a white powder, which was identified by comparing the optical rotation {$[\alpha]_D^{20}$ −39 (*c* 1.6, H_2O), 1*R*,2*R*-authentic sample; $[\alpha]_D^{20}$ +39 (*c* 1.6, H_2O), 1*S*,2*S*-authentic sample; $[\alpha]_D^{25}$ −38.7 (*c* 0.10, H_2O), (1*R*,2*R*)-*trans*-1,2-cyclohexanediol from **1a**; $[\alpha]_D^{25}$ +40.8 (*c* 0.10, H_2O), (1*S*,2*S*)-*trans*-1,2-cyclohexanediol from **6a**} with an authentic sample (Sigma Chemical Co. Ltd., St. Louis, MO, USA).[17]

Acid Hydrolysis of 1−13. Compounds **1**−**13** (each 1.0 mg) were individually hydrolyzed by 1 N HCl (1.0 mL) under reflux conditions for 2 h. The reaction mixtures of **8**−**13** were extracted with $CHCl_3$, and the organic layers evaporated under reduced pressure to yield a mixture of *cis*-1,2-cyclohexanediol and *p*-coumaric acid (0.6 mg), identified by co-TLC with the standard sample (Sigma) and zero optical rotation. The sugars were obtained from the aqueous layers of **1**−**13** by neutralization through an Amberlite IRA-67 column.

Determination of Absolute Configuration of Glucose in Compounds 1−13. The sugars obtained from the hydrolysis of **1**−**13** were dissolved in anhydrous pyridine (0.5 mL) followed by adding L-cysteine methyl ester hydrochloride (2 mg) (Sigma). The mixture was stirred at 60 °C for 90 min and trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) (Sigma) for 2 h. Each mixture was partitioned between *n*-hexane and H_2O (1 mL each), and the organic layer (1 *μ*L) was analyzed by GC/MS.[9] D-Glucose {$[\alpha]_D^{25}$ +62.1 (*c* 0.05, H_2O)} was detected by co-injection of the hydrolysate with standard silylated samples, giving a single peak at 9.715 min. Authentic samples (Sigma) treated in the same way showed a single peak at 9.731 min.

Measurement of NO Production and Cell Viability. BV-2 cells were maintained in DMEM supplemented with 5% FBS and 1% penicillin−streptomycin. To measure the NO production, BV-2 cells were plated onto a 96-well plate (3×10^4 cells/well). After 24 h, the cells were pretreated with each compound for 30 min and stimulated with 100 ng/mL LPS for 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture medium using the Griess reaction. The supernatant was harvested and mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% *N*-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid). After 10 min, the absorbance at 540 nm was measured using an Emax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Sodium nitrite was used as a standard to calculate the nitrite concentration. The cell viability was measured using the MTT assay. L-NMMA (Sigma), a known NO synthase inhibitor, was used as a positive control.[19]

Cytotoxicity Assays. A sulforhodamine B bioassay was used to determine the cytotoxicity of each compound isolated against four cultured human tumor cell lines.[20] The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small-cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (malignant melanoma), and HCT-15

(colon adenocarcinoma). Doxorubicin was used as a positive control. The cytotoxicities of doxorubicin against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines were IC_{50} 0.0014, 0.0217, 0.0025, and 0.1077 μM, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR and HRMS data of **1**−**13**, **1a**, and **6a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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