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Terpenoids from *Glechoma hederacea* var. *longituba* and Their Biological Activities

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ABSTRACT

Glechoma hederacea var. *longituba* (common name: ground-ivy) has been used for the treatment of asthma, bronchitis, cholelithiasis, colds, and inflammation. In the present study, three new sesquiterpene glycosides (**1**–**3**), two new diterpene glycosides (**4**–**5**), and four known compounds (**6**–**9**) were isolated from its MeOH extract. Structure elucidation was performed for the five new compounds (**1**–**5**) using 1D and 2D NMR, HRESIMS, ECD calculation, and chemical methods. All the isolates (**1**–**9**) were assessed for their anti-neuroinflammatory activity on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 cells, nerve growth factor (NGF) secretion stimulation activity in C6 glioma cells, and cytotoxic activity against four human cancer cell lines (A549, SK-OV-3, SK-MEL-2, and HCT15). Compounds **2** and **5**–**7** exhibited inhibitory effects on NO production with IC_{50} values of 52.21, 47.90, 61.61, and 25.35 μM, respectively. Compound **5** also exhibited a significant stimulating effect on NGF secretion (122.77 ± 8.10%). Compound **9** showed potent cytotoxic activity against SK-OV-3 (IC_{50} 3.76 μM) and SK-MEL-2 (IC_{50} 1.48 μM) cell lines, while **7** displayed a strong cytotoxic activity against SK-MEL-2 (IC_{50} 9.81 μM) cell line.

Keywords: *Glechoma hederacea* var. *longituba*; Terpenoid; Anti-neuroinflammation; Neurotrophic effect; Cytotoxicity

INTRODUCTION

Glechoma hederacea var. *longituba* is a perennial plant in the family Labiatae. It is commonly known as ‘ground ivy’ and ‘gill over the ground’ and is widely distributed in Korea, Japan, and China. This plant has been used as Korean traditional medicine for treating asthma, bronchitis, cholelithiasis, colds, and inflammation [1,2]. Previous studies have shown that *G. hederacea* var. *longituba* contains phytochemicals such as monoterpenoids, sesquiterpene lactones, lignans, flavonoids, phenolic compounds that show anti-inflammatory, cytotoxic, and/or cytoprotective effects [3-7]. However, bioactive terpenoids of *G. hederacea* var. *longituba* with anti-neurodegenerative effects remain large unknown. In this study, nine terpenoids (**1**–**9**) including five new compounds (**1**–**5**) were isolated and characterized from *G. hederacea* var. *longituba* (Figure 1). Structures of these compounds were established by 1D and 2D NMR and HR-ESIMS, comparison of experimental and calculated ECD data, and hydrolysis. Herein, the isolation and structural elucidation of the isolated compounds (**1**–**9**) and assessment for their anti-neuroinflammatory activity on NO production in lipopolysaccharide (LPS)-activated BV-2 cells, NGF secretion-stimulation activities in C6 glioma cells, and cytotoxic activities are described.

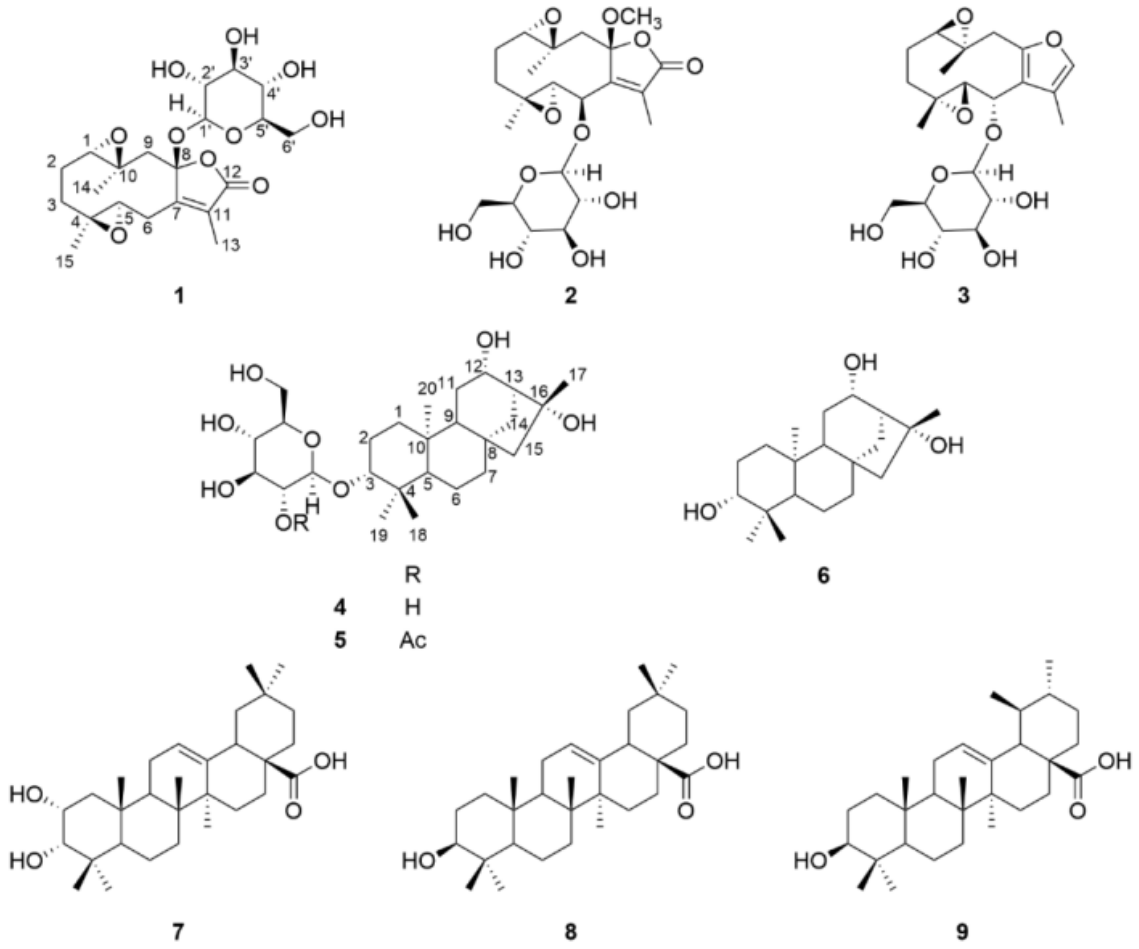

Figure 1: Chemical structures of compounds **1–9**.

RESULTS AND DISCUSSION

Compound **1** was purified as a colorless gum with a molecular formula of $C_{21}H_{30}O_{10}$ (*m/z* 465.1737 $[M + Na]^+$, calcd for $C_{21}H_{30}O_{10}Na$, 465.1737). The ^1H NMR spectrum of **1** displayed three methyl groups [δ_H 1.89 (3H, d, *J* = 1.4 Hz), 1.28 (3H, s), and 1.13 (3H, s)], two oxygenated methines [δ_H 3.45 (1H, dd, *J* = 8.8, 5.9 Hz), and δ_H 2.99 (1H, dd, *J* = 10.9, 1.1 Hz)], and a glucopyranosyl unit [δ_H 4.20 (1H, d, *J* = 7.8Hz), 3.78 (1H, dd, *J* = 11.9, 2.2 Hz), 3.59 (1H, dd, *J* = 11.9, 5.8 Hz), 3.31 (1H, overlap), 3.26 (1H, dd, *J* = 9.6, 9.0 Hz), 3.23 (1H, dd, *J* = 9.2, 7.8 Hz), and 3.05 (1H, ddd, *J* = 9.6, 5.8, 2.2 Hz)]. The ^{13}C NMR spectrum of **1** showed 21 carbon signals, including four oxygenated carbons (δ_C 70.0, 61.4, 60.5, and 57.9), a lactone moiety (δ_C 172.6, 159.0, 131.2, and 109.2), and a glucopyranosyl moiety (δ_C

97.2, 78.7, 78.1, 74.5, 71.4, and 62.5) (Table 1). Comparison of these ^{1}H and ^{13}C NMR spectra of **1** with those of 1*β*,10*α*;4*α*,5*β*-diepoxy-8*β*-hydroxy-glechoman-8,12-olide (DHG) indicated that **1** could be the glucopyranosyl-DHG [8]. The HMBC cross peak H-1' (δ_H 4.20) to C-8 (δ_C 109.2) suggested a glucopyranosyl unit located at C-8 (Figure 2A). The NOESY spectrum of **1** showed cross peaks between CH_3-14, H-3a/H-2a; H-3a, H-6a/CH_3-15 indicating their *α*-orientation, whereas the NOESY cross peaks of H-1, H-1'/H-9b; H-3b,H-6b/H-5 suggested their *β*-orientation (Figure 2A). The absolute configuration of **1** was implied based on comparison of the calculated ECD spectra of **1a** (aglycone of **1**) and experimental ECD spectra of **1** (Figure 2B). The experimental ECD spectrum of **1** showed a negative Cotton effect at 230 nm and a positive Cotton effect at 260 nm, which showed a similarity with those of calculated ECD spectrum of 1*S*,4*S*,5*R*,8*S*,10*R*-(**1a**). Finally, D-glucopyranosyl moiety was identified by GC/MS analysis of a chiral derivatization product of sugar obtained by enzyme hydrolysis of **1** [9,10]. The retention time of glucopyranose (11.3 min) corresponded to that of the standard D-glucopyranose (11.3 min) (Supporting Information; Figure S8). Thus, structure of **1** was determined as (1*S*,4*S*,5*R*,8*S*,10*R*)-1,10;4,5-diepoxy-8-*O*-*β*-D-glucopyranosyl glechoman-8,12-olide.

Table 1: ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data of compounds **1** and **2** in methanol-d_4

Position	**1**		**2**	
	δ_C	δ_H multi (*J* in Hz)	δ_C	δ_H multi (*J* in Hz)
1	70.0	2.99 dd (10.9, 1.1)	70.7	2.98 d (10.1)
2a	24.2	1.49 m	23.7	1.50 m
2b		2.00 dt (14.2, 3.3)		1.98 dt (13.9, 3.3)
3a	37.8	2.23 dt (13.3, 3.3)	38.2	1.41 td (13.4, 4.1)
3b		1.35 td (13.6, 4.0)		2.25 dt (13.2, 3.3)
4	61.4		62.3	
5	60.5	3.45 dd (8.8, 5.9)	65.7	3.52 d (7.5)
6a	29.2	2.56 dd (15.6, 8.9)	76.6	4.79 d (7.5)
6b		3.53 ddd (15.5, 5.7, 1.3)		

7	159.0		151.4	
8	109.2		110.9	
9a	46.8	3.01 d (15.2)	45.3	3.01 d (15.0)
9b		2.16 d (15.2)		2.08 d (15.0)
10	57.9		57.9	
11	131.2		136.9	
12	172.6		171.9	
13	9.8	1.89 d (1.4)	10.2	2.01 s
14	17.6	1.13 s	17.2	1.08 s
15	17.0	1.28 s	17.7	1.29 s
1'	97.2	4.20 d (7.8)	103.1	4.42 d (7.7)
2'	74.5	3.23 dd (9.2, 7.9)	75.4	3.27 overlap
3'	78.1	3.31 overlap	78.4	3.37 overlap
4'	71.4	3.26 dd (9.6, 9.0)	71.9	3.27 overlap
5'	78.7	3.05 ddd (9.7, 5.8, 2.2)	78.6	3.32 overlap
6'a	62.5	3.78 dd (11.9, 2.2)	63.3	3.90 dd (11.9, 2.3)
6'b		3.59 dd (11.9, 5.8)		3.64 dd (11.9, 6.8)
8-OCH_3			51.3	3.29 s

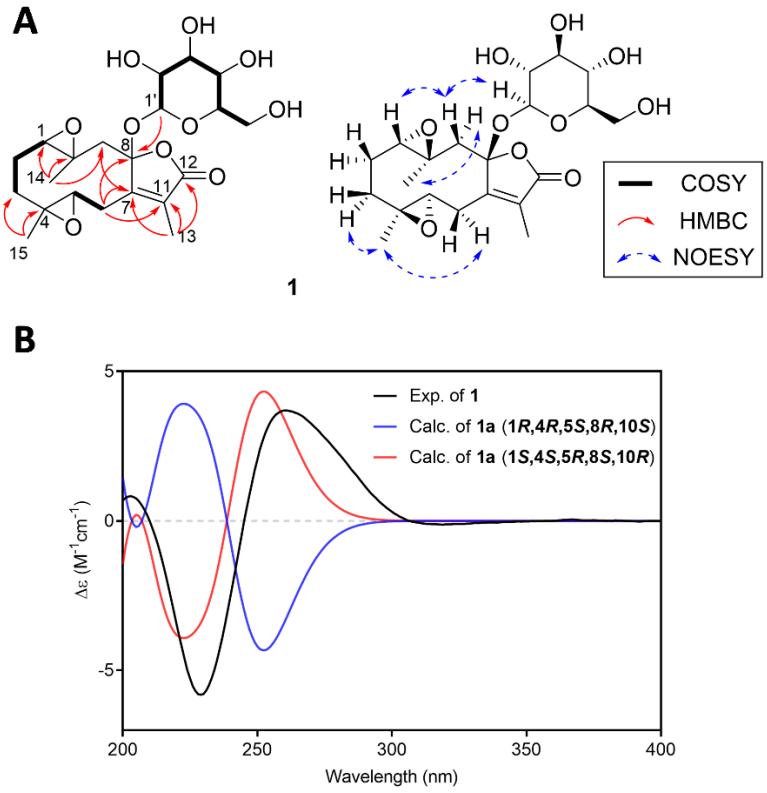

Figure 2: Structure elucidation of **1**. (A) Key COSY, HMBC, and NOESY correlations of **1**. (B) Comparison of calculated ECD data **1a** and experimental ECD spectrum of **1**.

Compound **2** was obtained as a colorless gum. Its molecular formula $C_{22}H_{32}O_{11}$ was determined based on HRESIMS data (*m/z* 495.1839 $[M + Na]^+$, calcd for $C_{22}H_{32}O_{11}Na$, 495.1842). The ^1H NMR spectrum of **2** displayed three methyl groups [δ_H 2.01 (3H, s), 1.29 (3H, s), and 1.08 (3H, s)], three oxygenated methines [δ_H 4.79 (1H, d, J = 7.5 Hz), δ_H 3.52 (1H, d, J = 7.5 Hz), and δ_H 2.98 (1H, d, J = 10.1 Hz)], a methoxy group [δ_H 3.29 (3H, s)] and a glucopyranosyl unit [δ_H 4.42 (1H, d, J = 7.7Hz), 3.90 (1H, dd, J = 11.9, 2.3 Hz), 3.64 (1H, dd, J = 11.9, 6.8 Hz), 3.37 (1H, overlap), 3.32 (1H, overlap), 3.27 (1H, overlap), and 3.27 (1H, overlap)]. The ^{13}C NMR spectrum of **2** showed 22 carbon signals including five oxygenated carbons (δ_C 76.6, 70.7, 65.7, 62.3, and 57.9), a lactone moiety (δ_C 171.9, 151.4, 136.9, and 110.9), a methoxy carbon (δ_C 51.3), and a glucopyranosyl unit (δ_C 103.1, 78.8, 78.6, 75.4, 71.9, and 63.3) (Table 1). These NMR spectra of **2** were similar to those of substolide A except for signals of methoxy group attached to C-8 and of a monossacharide at C-6 in **2** [11]. The planar structure of **2** was established based on 2D NMR spectroscopic data (COSY, HSQC, and HMBC). The HMBC correlation H-1' (δ_H 4.42) to C-6 (δ_C 76.6) suggested a glucopyranosyl unit located at C-6. Also, the HMBC correlations from 8-OCH_3 (δ_H 3.29) to C-8 (δ_C 110.9) indicated that an additional methoxy group was located at C-8 (Figure 3). The relative configuration of **2** was established based on NOESY data analysis. The NOESY correlations of H-1/H-9b and H-9b/8-OCH_3 indicated that H-1, H-9b, and 8-OCH_3 were β-oriented. The NOESY correlations of CH_3-14, H-3a/H-2a; H-6/H-3a/CH_3-15 suggested that H-6, CH_3-14, and CH_3-15 were α-oriented (Figure 3). The ECD spectrum of **2** showed a positive Cotton effect 257 nm, which was consistent with that of **1** (Figure S19, Supporting Information). This similarity suggested that the absolute configuration of aglycone of **2** was identical to that of **1**. Therefore, the absolute configuration of **2** was proposed as 1*S*,4*S*,5*R*,6*R*,8*S*,10*R*. Enzyme hydrolysis and following sugar identification were performed using the same methods as those used for **1**. As a result, monosaccharide of

2 was identified as D-glucose (Figure S17, Supporting Information). Thus, the structure of **2** was established as (1*S*,4*S*,5*R*,6*R*,8*S*,10*R*)-1,10;4,5-diepoxy-6-*O*-*β*-D-glucopyranosyl-8-methoxy glechoman-8,12-olide.

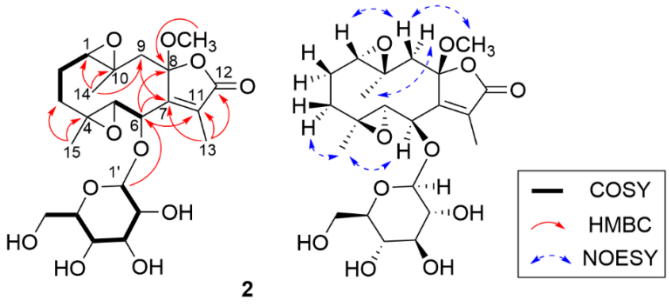

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Figure 3: Key COSY, HMBC, and NOESY correlations of **2**.

Compound **3** was obtained as a colorless gum. The HRESIMS spectrum of **3** provided a molecular formula $C_{21}H_{30}O_9$ (*m/z* 449.1789 $[M + Na]^+$, calcd for $C_{21}H_{30}O_9Na$, 449.1788). The ^1H NMR data of **3** exhibited three methyl groups [δ_H 2.02 (3H, d, *J* = 1.2 Hz), 1.06 (3H, s), and 0.95 (3H, s)], three oxygenated methine [δ_H 4.62 (1H, d, *J* = 6.0 Hz), δ_H 3.15 (1H, d, *J* = 10.4 Hz), and δ_H 3.67 (1H, overlap)], an olefinic proton [δ_H 7.25 (1H, d, *J* = 1.2 Hz)], and a glucopyranosyl unit [δ_H 4.23 (1H, d, *J* = 7.8Hz), 3.88 (1H, dd, *J* = 11.9, 2.3 Hz), 3.67 (1H, dd, *J* = 11.9, 6.1 Hz), 3.29 (1H, overlap), 3.28 (1H, overlap), 3.24 (1H, overlap), and 3.21 (1H, overlap)]. The ^{13}C NMR spectrum of **3** showed 21 carbon signals for five oxygenated carbons (δ_C 74.5, 68.7 (×2), 61.6, and 61.4), a furan moiety (δ_C 151.2, 138.7, 124.5, and 119.6), and a glucopyranosyl unit (δ_C 101.7, 78.2 (×2), 75.3, 72.0, and 63.2) (Table 2). These NMR spectra of **3** were similar to those of glechomafuran, except for the signals assignable to H-6/C-6 (δ_H 4.62/ δ_C 74.5) and those indicative of the presence of a glucopyranosy residue in **3** [12]. The COSY correlation between H-5 (δ_H 3.59) and H-6 (δ_H 4.62) and the HMBC cross peak of H-1' (δ_H 4.23) to C-6(δ_C 74.5) and between H-6 and C-7 (δ_C 119.6) confirmed the location of the glucopyranosyl group at C-6 (Figure 4A). The relative configuration of **3** was established

using NOESY spectrum (Figure 4A). The NOESY correlations of CH_3-14, H-3b/H-2b; H-6/H-3b/CH_3-15 suggested that H-6, CH_3-14, and CH_3-15 were positioned in the same orientation. The absolute configuration of **3** was confirmed by comparing calculated ECD spectrum of **3a** (aglycone of **3**) with experimental ECD spectrum of **3**. The experimental ECD of **3** displayed positive Cotton Effects at 217 and 243 nm, which was similar to those of 1*R*,4*R*,5*S*,6*S*,10*S*–(**3a**) (Figure 4B). Enzyme hydrolysis and sugar identification were performed and monosaccharide of **3** was identified as D-glucopyranose (Supporting Information; Figure S27). Thus, the structure of **3** was determined as (1*R*,4*R*,5*S*, 6S,*10S*)-1,10;4,5-diepoxy-6-*O*-*β*-D-glucopyranosyl glechomafuran.

Table 2: ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR data of compound **3** in methanol-d_4

Position	**3**	
	δ_C	δ_H multi (*J* in Hz)
1	68.7	3.15 d (10.4)
2a	23.6	1.99 m
2b		1.49 m
3a	38.5	1.41 td (13.3, 5.0)
3b		2.26 ddd (13.3, 3.9, 2.7)
4	61.6	
5	68.7	3.67 overlap
6	74.5	4.62 d (6.0)
7	119.6	
8	151.2	
9a	37.8	2.80 d (14.3)
9b		3.19 m
10	61.4	
11	124.5	
12	138.7	7.25 d (1.2)
13	8.4	2.02 d (1.2)
14	18.1	0.95 s
15	17.3	1.06 s
1'	101.7	4.23 d (7.8)
2'	75.3	3.21 overlap
3'	78.2	3.29 overlap
4'	72.0	3.28 overlap
5'	78.2	3.24 overlap
6'a	63.2	3.88 dd (11.9, 2.3)
6'b		3.67 dd (11.9, 6.1)

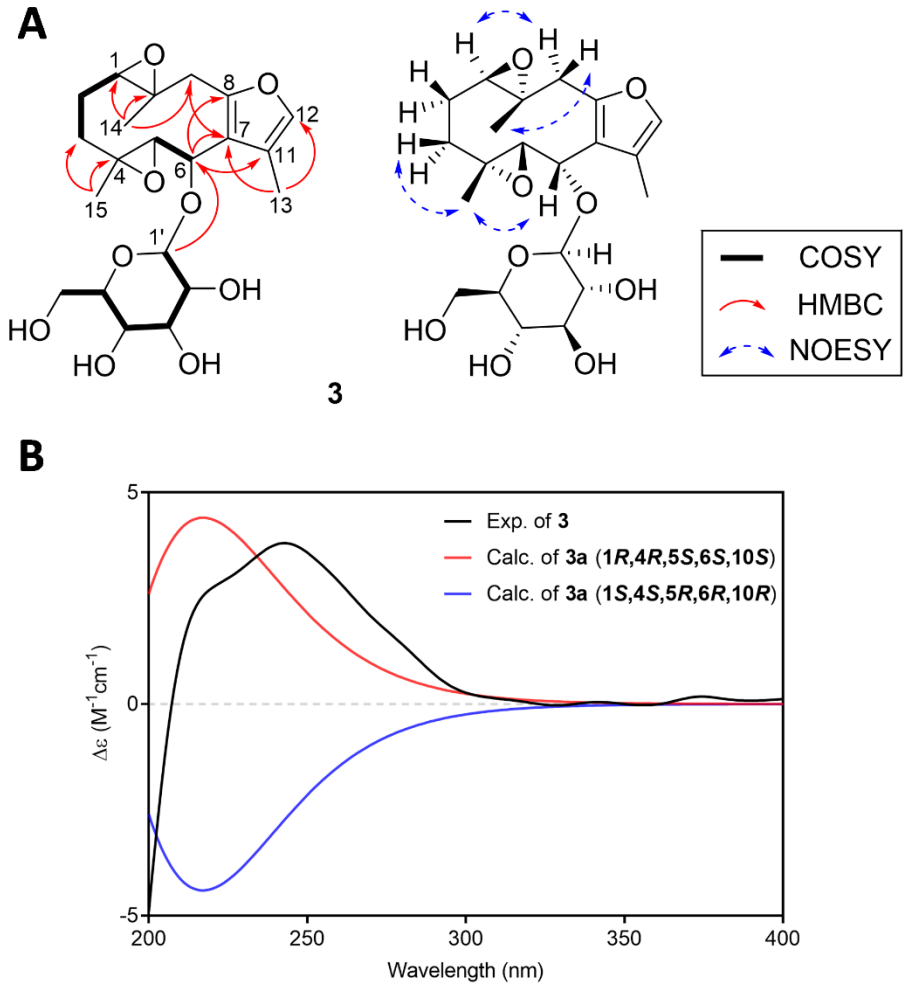

Figure 4: Structure elucidation of **3**. (A) Key COSY, HMBC, and NOESY correlations of **3**. (B) Comparison of calculated and experimental ECD spectra of **3**.

Compound **4** was purified as a colorless gum. The HRESIMS spectrum of **4** gave a molecular formula of $C_{26}H_{44}O_8$ according to a $[M + Na]^+$ ion at *m*/*z* 507.2935 (calcd for $C_{26}H_{44}O_8Na$, 507.2934). The ^1H NMR spectrum of **4** displayed four methyl groups [δ_H 1.46 (3H, s), 1.06 (3H, s), 1.04 (3H, s), and 0.83 (3H, s)], two oxygenated methines [δ_H 3.97 (1H, ddd, *J* = 11.8, 6.7, 3.6 Hz) and 3.33 (1H, overlap)], and a glucopyranosyl unit [δ_H 4.33 (1H, d, *J* = 7.8Hz), 3.86 (1H, dd, *J* = 11.7, 2.4 Hz), 3.68 (1H, dd, *J* = 11.7, 5.7 Hz), 3.37 (1H, t, *J* = 9.0 Hz), 3.31 (1H, overlap), 3.23 (1H, ddd, *J* = 9.6, 5.7, 2.4 Hz), and 3.18 (1H, dd, *J* = 9.1, 7.8 Hz)]. The ^{13}C NMR spectrum of **4** displayed 26 carbon signals including two

oxygenated carbons (δ_C 85.8, and 72.4), an oxygenated quaternary carbon (δ_C 79.5), and a glucopyranosyl unit (δ_C 101.9, 78.3, 77.7, 75.2, 71.9, and 63.0) (Table 3). These ^{1}H and ^{13}C NMR spectra of **4** were similar to those of *ent*-kauran-3*α*,12*α*,16*α*-triol except for an additional glucopyranosyl unit [13]. The location of glucopyranosyl unit at C-3 was confirmed by HMBC cross peak from H-1' (δ_H 4.21) to C-3 (δ_C 85.8), as well as by the deshielding and shielding effects of the glucopyranosyl substituent on C-3 and C-2, respectively, whose resonances were found at δ_C 79.3 and 27.7, respectively, in the corresponding aglycone (Figure 5A). The relative configuration of **4** was determined as 3*α*, 12*α*, 16*α* based on NOESY data analysis and comparison of their ^{1}H and ^{13}C NMR with reported values except for those signals attributed to a glucopyranosyl unit in **4** (Figure 5B).[13] Finally, D-glucopyranose unit in **4** was confirmed by GC/MS analysis as described above (Supporting Information; Figure S36) [10]. Thus, the structure of compound **4** was identified as *ent*-kauran-3*α*,12*α*,16*α*-triol 3-*O*-*β*-D-glucopyranoside.

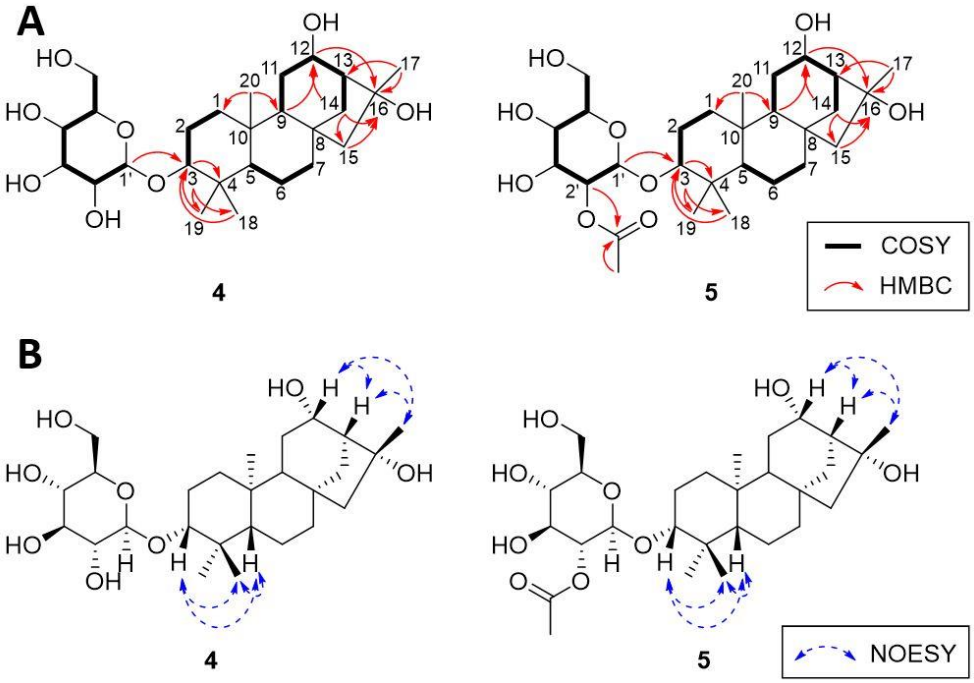

Figure 5: 2D NMR data of **4** and **5**. (A) Key COSY and HMBC correlations of **4** and **5**. (B) Key NOESY correlations of **4** and **5**.

Table 3: ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR Data of Compounds **4** and **5** in methanol-d_4

Position	**4**		**5**	
	δ_C	δ_H multi (J in Hz)	δ_C	δ_H multi (J in Hz)
1	39.7	1.90, 0.90 m	39.8	1.91, 0.89 m
2	24.1	1.79, 1.68 m	24.0	1.77, 1.48 m
3	85.8	3.33, overlap	85.5	3.31, overlap
4	39.2		39.3	
5	56.9	0.84 dd (11.9, 1.3)	56.8	0.82 dd (11.7, 1.2)
6	21.2	1.59, 1.37 m	21.2	1.59, 1.36 m
7	42.1	1.67, 1.50 m	42.2	1.68, 1.49 m
8	45.7		45.8	
9	59.0	1.10 d (8.9) m	59.0	1.10 d (8.8) m
10	39.7		39.6	
11	28.7	1.87, 1.56 m	28.8	1.86, 1.57 m
12	72.4	3.97 ddd (11.8, 6.7, 3.6)	72.4	3.98 ddd (11.8, 6.7, 3.6)
13	56.0	2.02 m	56.1	2.02 t (4.0)
14	38.4	1.83, 1.71 m	38.5	1.83, 1.72 m
15	59.3	1.57, 1.54 m	59.4	1.56 m
16	79.5		79.6	
17	26.3	1.46 s	26.4	1.47 s
18	29.0	1.04 s	29.0	1.01 s
19	17.1	0.83 s	16.9	0.73 s
20	18.4	1.06 s	18.5	1.04 s
1'	101.9	4.33 d (7.8)	99.5	4.52 d (8.0)
2'	75.2	3.18 dd (9.1, 7.8)	75.7	4.69 dd (9.6, 8.0)
3'	78.3	3.37 t (9.0)	76.3	3.54 t (9.3)
4'	71.9	3.31 overlap	72.0	3.39 t (9.3)
5'	77.7	3.23 ddd (9.6, 5.7, 2.4)	78.5	3.28 ddd (9.7, 5.7, 2.2)
6'a	63.0	3.86 dd (11.7, 2.4)	62.9	3.88 dd (11.8, 2.2)
6'b		3.68 dd (11.7, 5.7)		3.71 dd (11.8, 5.7)
2'-OAc			172.0	
			21.2	2.03 s

Compound **5** was obtained as a colorless gum. Based on its HRESIMS and NMR data, its molecular formula was determined as $C_{28}H_{46}O_9$. The ^{1}H NMR spectrum of **5** showed four

methyl groups [δ_H 1.47 (3H, s), 1.04 (3H, s), 1.01 (3H, s), and 0.73 (3H, s)], two oxygenated methines [δ_H 3.98 (1H, ddd, *J* = 11.8, 6.7, 3.6 Hz), and 3.31 (1H, overlap)], and a (2'-*O*-acetyl)glucopyranosyl unit [δ_H 4.69 (1H, dd, *J* = 9.6, 8.0 Hz), 4.52 (1H, d, *J* = 8.0 Hz), 3.88 (1H, dd, *J* = 11.8, 2.2 Hz), 3.71 (1H, d, *J* = 11.8, 5.7 Hz), 3.54 (1H, t, *J* = 9.3 Hz), 3.39 (1H, t, *J* = 9.3Hz), and 3.28 (1H, ddd, *J* = 9.7, 5.7, 2.2 Hz)]. The ^{13}C NMR spectrum of **5** displayed 28 carbon signals including two oxygenated carbons (δ_C 85.5, and 72.4), an oxygenated quaternary carbon (δ_C 79.6), a glucopyranosyl unit (δ_C 99.5, 75.7, 76.3, 72.0, 78.5, and 62.9), and an acetyl group carbon (δ_C 172.0, and 21.2) (Table 3). The ^{1}H and ^{13}C NMR spectra of compound **5** were similar to those of compound **4** except for the presence of acetyl group signals. The HMBC correlation from H-2' (δ_H 4.52) to the carbonyl carbon at δ_C 172.0 (C-2") located the acetyl group at C-2' (Figure 5A). Moreover, by the shielding effect of the acetyl group on C-1' and C-3', whose chemical shifts were observed at δ_C 99.5 and 76.3, respectively. The relative configuration of **5** was determined as 3*α*,12*α*,16*α* using the same method as described for **4** (Figure 5B). Finally, D-glucopyranoside was confirmed by LC/MS analysis for monosaccharide derivatives obtained by derivatization reaction after acid hydrolysis of **5** [14]. The retention time of glucopyranose (13.8 min) was corresponded with standard D-Glc (13.8 min) (Supporting Information; Figure S44). Thus, the structure of compound **5** was established as *ent*-kauran-3*α*,12*α*,16*α*-triol 3-*O*-*β*-D-(2'-*O*-acetyl)-glucopyranoside.

By comparing the NMR and MS data with those reported in literature, the four known compounds **6**–**9** were identified as *ent*-kauran-3*α*,12*α*,16*α*-triol (**6**) [13], 3-epimaslinic acid (**7**) [15], oleanolic acid (**8**) [16], and ursolic acid (**9**) [17].

To find potential anti-neuroinflammatory, neurotrophic, and cytotoxic secondary metabolites from *G. hederacea* var. *longituba*, the isolated compounds (**1**–**9**) were evaluated

for these biological activities. The anti-neuroinflammatory activity of all isolates (**1**–**9**) was assessed by measuring nitric oxide (NO) production levels in the LPS-stimulated murine microglia BV-2 cells (Table 4). Compounds **2** and **5**–**7** significantly reduced NO levels, with IC_{50} values of 52.21, 47.90, 61.61, 25.35 μM, respectively. Interestingly, of the two diterpene glucosides (**4** and **5**) that were structurally similar except for C-2' functionality (**4**, -OH; **5**, -OAc) of the glucopyranosyl group, only compound **5** showed NO inhibitory activity. This suggests that the presence of an acetyl group at C-2' might play an important role in NO inhibitory activity.

Table 4: Inhibitory Effects of Compounds **1**–**9** on NO Production in LPS-Activated BV-2 Cells

Compound	IC_{50} (μM)[a]	Cell viability (%)[b]
1	119.48	71.22 ± 2.49
2	52.21	68.86 ± 3.27
3	292.32	97.02 ± 5.57
4	>500	103.11 ± 4.17
5	47.90	106.26 ± 3.71
6	61.61	95.29 ± 5.25
7	25.35	104.88 ± 4.74
8	>500	101.17 ± 3.89
9	104.25	97.34 ± 3.97
L-NMMA[c]	55.75	97.84 ± 2.05

[a]IC_{50} value of each compound was defined as the concentration (10 μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells

[b]Cell viability after treatment with 10 μM of each compound was determined by MTT assay and is expressed in percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD

[c]L-NMMA as positive control.

Compounds **1**–**9** were also tested for their abilities to stimulate NGF secretion in C6 glioma cells (Table 5). Compound **5** exhibited a significant stimulating effect on the secretion of NGF (122.77 ± 8.10%), whereas other compounds did not. Similar to results of their NO

inhibitory activities, compound **5** showed a stronger stimulating effect on the secretion of NGF than compound **4** (76.56 ± 6.51%).

Table 5: Effects of Compounds **1**–**9** on NGF Secretion in C6 Cells

Compound	NGF secretion[a]	Cell viability (%)[b]
1	82.87 ± 8.63	93.47 ± 9.87
2	97.01 ± 11.40	103.47 ± 2.80
3	80.06 ± 3.46	101.18 ± 5.56
4	76.56 ± 6.51	85.46 ± 11.23
5	122.77 ± 8.10	125.51 ± 2.37
6	85.51 ± 6.68	119.35 ± 11.92
7	76.50 ± 7.21	121.92 ± 7.43
8	88.40 ± 9.04	120.92 ± 6.78
9	87.55 ± 6.62	100.33 ± 9.68
6-shogaol[c]	116.85 ± 14.91	99.22 ± 3.75

[a]C6 cells were treated with 10 μM of compounds. After 24 h, the content of NGF secretion in C6-conditioned media was measured by ELISA. The level of secreted NGF cells is expressed as percentage of the untreated control. The data shown represent the means ± SD of three independent experiments performed in triplicate

[b]Cell viability after treatment with 10 μM of each compound was determined by MTT assay and is expressed in percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD

[c]6-shogaol as positive control.

Cytotoxic effects of compounds **1**–**9** against four human cancer cell lines [non-small-cell lung adenocarcinoma (A549), malignant ovarian ascites (SK-OV-3), skin melanoma (SK-OV-2), and colon adenocarcinoma (HCT15)] were also investigated using sulforhodamine B (SRB) bioassay (Table 6). Compound **9** showed selective cytotoxic activities against SK-OV-3 and SK-MEL-2 cell lines, with IC_{50} values of 3.76 and 1.48 μM, respectively. Compound **7** also displayed a cytotoxic activity against SK-MEL-2 cell line, with an IC_{50} value of 9.81 μM.

Table 6: Cytotoxic activities of Compounds against Four Cultured Human Cancer Cell Lines in the SRB bioassay.

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT15
7	>10	>10	9.81	>10
9	>10	3.76	1.48	>10
Etoposide[b]	1.21	2.27	2.04	2.38

[a]50% inhibitory concentration; the concentration of compound that caused a 50% inhibition in cell growth.

[b]Etoposide as a positive control.

Conclusion

Nine terpene derivatives, including three new sesquiterpene glycosides (**1**–**3**), two new diterpene glycosides (**4** and **5**), a known diterpene (**6**), and three known triterpenes (**7**–**9**) were isolated from $CHCl_3$-, EtOAc-, and *n*-BuOH-soluble layers of 80% MeOH extract of *G. hederacea* var. *longituba*. The structures of these compounds were established by 1D and 2D NMR and HRESIMS, comparison of experimental and calculated ECD data, and hydrolysis. All isolated compounds were evaluated for anti-neuroinflammatory activity, neurotrophic effect, and cytotoxicity. Compounds **2** and **5**–**7** show significantly reduced NO levels. Compound **5** exhibited neurotrophic effect. Interestingly, compounds **4** and **5** showed differences in anti-neuroinflammatory activity and neurotrophic effect according to C-2' functional group of the glucopyranosyl group. Compounds **7** and **9** showed selective cytotoxic activities against human cancer cell lines. This study suggests that the active tepenoids (**2**, **5–7**, and **9)** from *G. hederacea* var. *longituba* would be potential new drug candidates.

EXPERIMENTALS

General Experimental Procedures. Optical rotations were measured on a Jasco P-2000 polarimeter using methanol solvent. High Resolution ESI Mass Spectrometer data were recorded on a Waters SYNAPT G2 mass spectrometer. ECD spectra were garnered with a JASCO J-1500 CD spectrometry (JASCO, Easton, MD, USA). NMR spectra were recorded on a Bruker AVANCE III 700 NMR spectrometer at 700 MHz (^{1}H) and 175 MHz (^{13}C). To practice LC-MS analysis, an Agilent 1200 Series high-performance liquid chromatography (HPLC) furnished with a diode array detector and a 6130 Series ESIMS spectrometry connected to an analytical Kinetex C_{18} 100 Å column (100 mm × 2.1 mm, 5 μm; flow rate 0.3 mL/min; Phenomenex, Torrance, CA, USA) was utilized. Agilent 7820A GC system equipped with a 5977B mass selective detector system was controlled by a qualitative navigator version B.08.00 software. Preparative HPLC was performed using a Gilson 321 pump with a Shodex Refractive Index Detector, an INNO column C18 5μm column (250 × 10 mm), a HAISIL 100 silica 5μm column (250×10 mm)., and a Lux 5μm Cellulose-1 column (250 × 4.6 mm). Silica gel 60 (Merck, 70-230 mesh and 230–400 mesh), RP-C_{18} silica gel (Merck, 230–400 mesh), Sephadex LH-20 (Parmacia Co., Japan), and Diaion HP-20 (Mitsubishi Chemical Co., Japan) were used for column chromatography. Merck precoated silica gel F_{254} plates and reversed-phase (RP)-18 F_{254s} plates (Merck) were used for thin-layer chromatography (TLC). Spots of compounds on TLC were detected under UV light or by heating after dipping in anisaldehyde-sulfuric acid.

Plant material. Aerial parts of *G. hederacea* var. *longituba* were purchased from Hongcheon, Gangwon-do, Korea, in May 2016. The plant material was identified by Kang

Ro Lee. A voucher specimen of this material (SKKU-NPL-1412) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and Isolation. The dried aerial parts of *G. hederacea* var. *longituba* (3 kg) were extracted with 80% MeOH (each 12 L × 1day, 3 times) at room temperature and filtered. The filtrate was evaporated in vacuo to yield an 80% MeOH extract (400 g). The 80% MeOH extract was suspended in distilled H_2O (2.4 L) and then successively partitioned with hexanes (2.4 L × 3), $CHCl_3$ (2.4 L × 4), EtOAc (2.4 L × 3), *n*-BuOH (2.4 L × 3) to yield 11 g, 16 g, 14 g, and 37 g, respectively. The $CHCl_3$ soluble layer (13 g) was subjected to a silica gel column ($CHCl_3$-MeOH, 20:1 → 1:1) to give five fractions (C1-C5). Fraction C2 (3.0 g) was applied to an RP-C_{18} silica gel column (50% MeOH) to give 12 subfractions (C2a-C2l). Compound **6** (4 mg) was yielded by purifying subfraction C2h (32 mg) using a semipreparative HPLC (65% MeCN). Subfraction C2j (155 mg) was purified by semipreparative HPLC (70% MeCN) to yield compound **7** (3 mg). Compounds **8** (5 mg) and **9** (6 mg) were obtained by purification of subfraction C2k (238 mg) using a semipreparative HPLC (100% MeOH). Fraction C3 (1.3 g) was applied to an RP-C_{18} silica gel column (60% MeOH) to yield nine subfractions (C3a-C3i). Subfraction C3a (266 mg) was fractionated by with a silica gel column ($CHCl_3$-MeOH, 20:1) to give three subfractions (C3a1-C3a3). C3a1 (62 mg) was purified by semipreparative HPLC (25% MeCN) to obtained compound **5** (3 mg). The EtOAc soluble layer (9.5 g) was chromatographed over a silica column ($CHCl_3$-MeOH, 30:1 → 1:1) to give six fractions (E1-E6). Fraction E4 (1.1 g) was applied to an RP-C_{18} silica column (40% MeOH) to yield six subfractions (E4a-E4f). Subfraction E4d (274 mg) was subjected to a silica column ($CHCl_3$-MeOH-H_2O, 6:1:0.1) and further purified by semipreparative HPLC (39% MeOH) to obtain compound **4** (4 mg). The *n*-BuOH soluble layer (34 g) was subjected to Diaion HP-20 (H_2O → MeOH) to yield six fractions (B1-B6). Fraction B4 (4.1 g) was applied to a silica gel

column ($CHCl_3$-MeOH-H_2O, 6:1:0.1 → 1:1:0.1) to give five subfractions (B1a-B1e). Subfraction B1a (484 mg) was chromatographed over an RP-C_{18} silica gel column (40% MeOH) to yield five subfractions (B1a1-B1a5). Subfraction B1a1 was purified by semipreparative HPLC (26% MeCN) to obtain compounds **1** (4 mg) and **2** (5 mg). Subfraction B1a3 (66 mg) was purified by semipreparative HPLC (26% MeCN) to obtain compound **3** (4 mg).

*(1S,4S,5R,8S,10R)-1,10;4,5-Diepoxy-8-O-β-D-glucopyranosyl glechoman-8,12-olide (**1**).* Colorless gum; $[\alpha]^{25,D}$ +228.0 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 230 (–5.8), 260 (+3.7) nm; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 465.1737 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_{10}Na$, 465.1737).

*(1S,4S,5R,6R,8S,10R)-1,10;4,5-Diepoxy-6-O-β-D-glucopyranosyl-8-methoxy glechoman-8,12-olide (**2**).* Colorless gum; $[\alpha]^{25,D}$ +24.6 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 217 (+1.4), 257 (+3.1) nm; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 495.1839 $[M + Na]^+$ (calcd for $C_{22}H_{31}O_{11}Na$, 451.1599).

*(1R,4R,5S,6S,10S)-1,10;4,5-Diepoxy-6-O-β-D-glucopyranosyl glechomafuran (**3**).* Colorless gum; $[\alpha]^{25,D}$ –28.0 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 217 (+2.5), 243 (+3.8) nm; ^1H and ^{13}C NMR data, see Table 2; HRFABMS *m/z* 449.1789 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_9Na$, 449.1788).

*ent-Kauran-3α,12α,16α-triol 3-O-β-D-glucopyranoside (**4**).* Colorless gum; $[\alpha]^{25,D}$ –94.6 (*c* 0.1, MeOH); ^1H and ^{13}C NMR data, see Table 3; HRFABMS *m/z* 507.2935 $[M + Na]^+$ (calcd for $C_{26}H_{44}O_8Na$, 507.2934).

*ent-Kauran-3α,12α,16α-triol 3-O-β-D-(2'-O-acetyl)glucopyranoside (**5**).* Colorless gum; $[\alpha]^{25,D}$ –24.6 (*c* 0.1, MeOH); ^1H and ^{13}C NMR data, see Table 3; HRFABMS *m/z* 549.3031 $[M + Na]^+$ (calcd for $C_{28}H_{46}O_9Na$, 549.3040).

Enzyme Hydrolysis of Compounds 1–3. Compounds **1** (0.8 mg), **2** (0.7 mg), and **3** (0.8 mg) were dissolved in water (2 mL) and then hydrolyzed with *β*-glucosidase (20 mg, from *Almonds*, Sigma) at 37℃ for 48 h. The reaction mixture was extracted with $CHCl_3$ (3 times), and the aqueous layer was evaporated in vacuo to obtain monosaccharide.

Acid Hydrolysis of Compounds 4 and 5. Each compound (**4**, 1.0 mg; **5**, 0.8 mg) was hydrolyzed with 1N HCl (1 mL) under reflux for 1.5 h. The hydrolysate was extracted with $CHCl_3$ (3 times), and a monosaccharide residue was obtained from aqueous layer.

Sugar analysis using LC/MS. The monosaccharide (**5**, 0.3 mg) obtained by hydrolysis was dissolved in pyridine (0.5 mL), which was then added with L-cysteine methyl ester hydrochloride (2 mg). The reactant was stirred at 60℃ for 1 h. Then *O*-tolyl isothiocyanate (30 μL) was added and stirred at 60℃ for 1 h. The reaction mixture was analyzed without purification by LC/MS analysis. The monosaccharide of **5** was detected at 13.8 min, the same detection time (13.8 min) as D-glucopyranoside.

Sugar analysis using GC/MS. Monosaccharides (**1**, 0.4 mg; **2**, 0.3 mg; **3**, 0.4 mg; **4**, 0.4 mg) obtained by hydrolysis were dissolved in pyridine (0.5 mL) and, added L-cysteine methyl ester hydrochloride (2 mg). Reaction mixtures were then stirred at 60℃ for 2 h. After adding 1-trimethylsilyl imidazole (0.1 mL), the reaction mixture was allowed to react again at 60℃ for 2 h. The reactant was suspended in distilled H_2O (1 mL) and then partitioned with *n*-hexane (1 mL). The *n*-hexane soluble layer was analyzed using GC/MS analysis. All monosaccharides were detected at 11.3 min, the same detection time (11.3 min) as D-glucopyranoside.

Cytotoxic activity, NO Production, NGF Secretion, and Cell Viability Assays. The biological activity assays were performed for all compounds isolated using methods described in Supporting Information S1.

Computational analysis. All conformers of **1** and **3** used in this study were found using

the Macromodel (version 2019-2, Schrödinger LLC) module with "Mixed torsional/Low-mode sampling" in the MMFF force field. The searches were implemented in the gas phase with a 15 kJ/mol energy window limit and 10,000 maximum number of steps to explore all potential conformers. The Polak−Ribiere Conjugate Gradient (PRCG) method was utilized to minimize conformers with 10,000 iterations and a 0.001 kJ (mol Å)$^{-1}$ convergence threshold on the Root Mean Square (RMS) gradient. All the conformers of **1** and **3** within 1 kJ/mol and 3 kJ/mol, respectively, of each global minimum were subjected to geometry optimization using the Gaussian 16 package (Gaussian Inc.) in the gas phase at B3LYP/6-31G(d) level and proceeded to calculation of excitation energies, oscillator strength, and rotatory strength at B3LYP/SVP level in the Polarizable Continuum Modle (PCM, methanol). The ECD spectra were Boltzmann-weighted and generated using SpecDis software (Version 1.71) [18] with a σ/γ value of 0.30eV.

Supporting Information

HRESIMS and 1D and 2D NMR data of compounds **1**–**5**, experimental ECD data of **1**–**3**, comparison with standard samples and monosaccharides of **1**–**5**, Coordinates of the conformers, Cytotoxic activity, NO Production, NGF Secretion, and Cell Viability Assays.

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