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C-Methylated Flavonoid Glycosides from *Pentarhizidium orientale* Rhizomes and Their Inhibitory Effects on the H1N1 Influenza Virus

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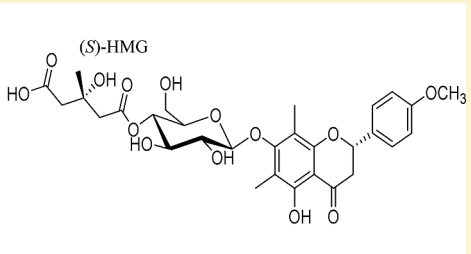
Supporting Information

Pentarhizidium orientale



Matteuorienate D (**1**)

ABSTRACT: Thirteen *C*-methylated flavonoid glycosides (**1**−**13**), along with 15 previously known flavonoids (**14**−**28**), were isolated from rhizomes of *Pentarhizidium orientale*. Among these compounds, matteuorienates D−K (**1**−**8**) were obtained as analogues of matteuorienates A−C (**14**−**16**), which contain a characteristic 3-hydroxy-3-methylglutaryl (HMG) moiety. The structures of **1**−**13** were characterized by spectroscopic analysis and chemical derivatization. The isolates were evaluated for their antiviral activities against influenza virus (H1N1), with compounds **21**, **22**, **23**, **25**, and **26** showing inhibitory effects (IC_{50} of 23.9−30.3 μM) against neuraminidases.

Pentarhizidium orientale (Hook.) Hayata (Onocleaceae) (syn. *Matteuccia orientalis*) is a perennial pteridophyte that is distributed mainly in East Asia. It has been used traditionally as a diuretic and helminthic in Korean folk medicine. Flavonoids, isocoumarins, phthalides, and stilbenes have been reported from *P. orientale*, among which the flavonoids are characterized by *C*-methylation at C-6 and C-8.[1−3] These *C*-methylated flavonoids were reported to exhibit various pharmacological activities; for example, hypoglycemic activity in streptozotocin (STZ)-induced diabetic rats[4] and anti-influenza virus (H1N1) inhibitory effects[5] have been shown. As part of the present investigation on the bioactive compounds from a Korean medicinal plant, the phytochemicals in the 80% aqueous MeOH extract of *P. orientale* rhizome were purified. As a result, flavonoids **1**−**27** and a chromone (**28**) were isolated. Compounds **14**−**28** were identified as matteuorienates A−C (**14**−**16**),[1] matteuorienin (**17**),[1] demethoxymatteucinol 7-*O*-*β*-D-glucoside (**18**),[6] matteucinol 7-*O*-*β*-D-glucoside (**19**),[6] myrciacitrin II (**20**),[7] demethoxymatteucinol (**21**),[4] matteucinol (**22**),[4] matteucin (**23**),[8] farrerol (**24**),[9] methoxymatteucin (**25**),[8] 3′-hydroxy-5′-methoxy-6,8-dimethylhuazhongilexone (**26**),[10] naringenin (**27**),[11] and leptorumol (**28**),[12] by comparison of spectroscopic data with previous literature data. The structural elucidation of the new compounds **1**−**13** and the antiviral activity evaluation of all isolates obtained are described herein.

RESULTS AND DISCUSSION

Compound **1** was isolated as a yellowish powder. Its molecular formula was determined to be $C_{30}H_{36}O_{14}$ from its HRESIMS deprotonated ion peak at *m*/*z* 619.2025 $[M - H]^-$ (calcd for 619.2027). UV absorption bands at 214, 282, and 349 nm suggested that **1** is a flavanone derivative. Its ^1H and ^{13}C NMR data (Table 1) were similar to those of **14**, which suggested that **1** is an analogue of a 5,6,7,8,4′-pentasubstituted flavanone glycoside containing a 3-hydroxy-3-methylglutaryl (HMG) moiety. The HMBC correlation between the methoxy protons (δ_H 3.77) and a carbon at δ_C 159.4 (C-4′) confirmed the location of the methoxy group at C-4′. The glycosidic linkage was located at C-7 and determined by an HMBC correlation between signals of the anomeric proton [δ_H 4.68 (d, J = 7.7 Hz, H-1″)] and C-7 (δ_C 161.2). The HMBC correlation between H-4″ (δ_H 4.60) and C-1‴ (δ_C 169.9) confirmed the HMG group substitution at C-4″ of the sugar moiety. The sugar was confirmed as D-glucose by acid hydrolysis followed by HPLC analysis after arylthiocarbamoyl-thiazolidine derivation.[13] The absolute configuration at C-2 in **1** was identified as (2*S*) due to the negative Cotton effect at 282 nm in the electronic circular dichroism (ECD) spectrum.[14]

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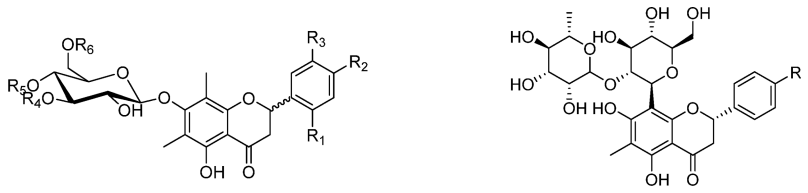
Chart 1



	R_1	R_2	R_3	R_4	R_5	R_6	
1	H	OCH_3	H	H	(*S*)-HMG	H	2*S*
2	H	H	H	H	(*S*)-HMG	H	2*S*
3	H	OCH_3	H	(*S*)-HMG	H	H	2*S*
4	H	H	H	(*S*)-HMG	H	H	2*S*
5	OH	OCH_3	H	H	H	(*S*)-HMG	2*S*
6	OH	OCH_3	H	H	H	(*S*)-HMG	2*R*
7	OH	H	OCH_3	H	H	(*S*)-HMG	2*S*
8	OH	H	H	H	H	(*S*)-HMG	2*S*
9	OH	H	H	H	H	H	2*R*
10	H	H	H	H	H	H	Δ^2

	R
11	H
12	OH
13	OCH_3

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 1−4 in DMSO-d_6 (^{1}H 500 MHz, ^{13}C 125 MHz)

	matteuorienate D (**1**)		matteuorienate E (**2**)		matteuorienate F (**3**)		matteuorienate G (**4**)	
position	δ_C, type	δ_H, (*J* in Hz)	δ_C, type	δ_H, (*J* in Hz)	δ_C, type	δ_H, (*J* in Hz)	δ_C, type	δ_H, (*J* in Hz)
2	77.9, CH	5.57 dd (12.5, 2.9)	78.0, CH	5.65 dd (12.5, 3.0)	77.9, CH	5.57 dd (12.5, 3.0)	78.0, CH	5.65 dd (12.5, 3.0)
3	42.7, CH_2	2.86 dd (17.1, 3.0)	42.3, CH_2	2.92 dd (17.1, 3.1)	42.2, CH_2	2.86 dd (17.1, 3.1)	42.3, CH_2	2.93 dd (17.1, 3.1)
		3.33 m		3.34 m		3.34 m		3.33 m
4	198.5, C		198.2, C		198.5, C		198.3, C	
5	157.9, C		157.9, C		157.8, C		157.8, C	
6	111.7, C		111.3, C		111.3, C		111.4, C	
7	161.2, C		161.2, C		161.1, C		161.1, C	
8	110.6, C		110.2, C		110.2, C		110.2, C	
9	157.2, C		157.1, C		157.2, C		157.1, C	
10	104.9, C		104.9, C		104.9, C		104.9, C	
1′	130.8, C		138.9, C		130.8, C		138.9, C	
2′	128.0, CH	7.46 d (8.7)	126.3, CH	7.53 d (7.3)	128.6, CH	7.46 d (8.7)	126.3, CH	7.54 d (7.3)
3′	114.0, CH	6.99 d (8.8)	128.6, CH	7.44 dd (7.3, 7.3)	114.0, CH	6.99 d (8.8)	128.6, CH	7.44 dd (7.3, 7.3)
4′	159.4, C		128.4, CH	7.38 t (7.3)	159.4, C		128.4, CH	7.38 t (7.3)
5′	114.0, CH	6.99 d (8.8)	128.6, CH	7.44 dd (7.3, 7.3)	114.0, CH	6.99 d (8.8)	128.6, CH	7.44 dd (7.3, 7.3)
6′	128.0, CH	7.46 d (8.7)	126.3, CH	7.53 d (7.3)	128.6, CH	7.46 d (8.7)	126.3, CH	7.54 d (7.3)
1″	103.9, CH	4.68 d (7.7)	103.9, CH	4.69 d (7.7)	103.7, CH	4.73 d (7.7)	103.7, CH	4.74 d (7.7)
2″	74.1, CH	3.44 m	74.1, CH	3.45 m	72.0, CH	3.46 m	72.0, CH	3.47 m
3″	73.5, CH	3.52 t (9.2)	73.5, CH	3.52 m	77.4. CH	4.84 t (9.4)	77.4, CH	4.84 t (9.4)
4″	71.3, CH	4.60 t (9.5)	71.3, CH	4.60 t (9.5)	67.6, CH	3.33 m	67.6, CH	3.34 m
5″	74.4, CH	3.37 m	74.4, CH	3.38 m	76.6, CH	3.20 m	76.6, CH	3.21 m
6″	60.8, CH_2	3.33 m 3.46 m	60.8, CH_2	3.33 m 3.46 m	60.7, CH_2	3.43 m; 3.62 m	60.7, CH_2	3.44 m; 3.62 m
1‴	169.9, C		169.9, C		170.1, C		170.1, C	
2‴	45.9, CH_2	2.62 d (13.8) overlap	45.9, CH_2	2.62 d (13.8) overlap	46.6, CH_2	2.65 d (13.3) overlap	46.7, CH_2	2.65 d (13.3) overlap
3‴	69.2, C		69.2, C		69.3, C		69.3, C	
4‴	45.9, CH_2	2.42 s	45.9, CH_2	2.42 s	45.6, CH_2	2.38 d (14.8) 2.31 d (14.8)	45.6, CH_2	2.36 d (15.3) 2.29 d (14.8)
5‴	173.0, C		173.0, C		173.8, C		173.9, C	
6‴	27.3, CH_3	1.27 s	27.3, CH_3	1.27 s	27.8, CH_3	1.27 s	27.9, CH_3	1.27 s
CH_3-6	8.7, CH_3	2.09 s	8.7, CH_3	2.10 s	8.7, CH_3	2.08 s	8.7, CH_3	2.09 s
CH_3-8	9.3, CH_3	2.07 s	9.3, CH_3	2.10 s	9.3, CH_3	2.06 s	9.3, CH_3	2.09 s
OCH_3-4′	55.2, CH_3	3.77 s			55.2, CH_3	3.77 s		

In turn, absolute configuration at the HMG moiety C-3‴ was determined as (*S*) by a concerted method including amidation, reduction, and acetylation[15] as well as by comparison of the ^{1}H NMR data with a reference value.[16] Thus, compound **1** (matteuorienate D) was characterized as (2*S*)-matteucinol-7-*O*-[4″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside.

Compound **2** gave a molecular formula of $C_{29}H_{34}O_{13}$, as indicated by HRESIMS. The UV, IR, ^{1}H NMR, and ^{13}C NMR data of **2** were similar to those of **1**, but they differed in their B-ring proton couplings. The ^{1}H NMR spectrum of **2** suggested a monosubstituted B-ring substructure [δ_H 7.53 (d, *J* = 7.3 Hz, H-2′, 6′), 7.44 (dd, *J* = 7.3, 7.3 Hz, H-3′, 5′), and 7.38

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Table 2. ^{1}H and ^{13}C NMR Data of Compounds 5−8 in DMSO-d_6 (^{1}H 600 MHz, ^{13}C 150 MHz)

	matteuorienate H (5)		matteuorienate I (6)		matteuorienate J (7)		matteuorienate K (8)	
position	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)	δ_C, type	δ_H, (J in Hz)
2	73.6, CH	5.63 dd (13.3, 2.8)	73.4, CH	5.69 dd (13.8, 2.4)	74.0, CH	5.69 dd (13.1, 2.8)	74.2, CH	5.74 dd (13.1, 2.7)
3	41.5, CH_2	2.73 dd (17.0, 2.8)	41.0, CH_2	2.67 dd (17.2, 2.4) 3.39 overlap	41.3, CH_2	2.81 dd (17.0, 2.8)	41.3, CH_2	2.80 dd (17.1, 2.8)
		3.28 m				3.25 m		3.29 m
4	198.9, C		199.4, C		198.6, C		198.8, C	
5	157.9, C		158.0, C		157.9,[a] C		157.8, C	
6	111.0, C		110.5, C		110.0,[b] C		111.0, C	
7	161.1, C		161.1, C		161.1, C		161.1, C	
8	110.1, C		110.7, C		111.2,[b] C		110.0, C	
9	157.8, C		158.0, C		157.6,[a] C		157.8, C	
10	104.8, C		104.8, C		104.8, C		104.8, C	
1′	117.4, C		117.3, C		125.7, C		124.9, C	
2′	155.7, C		157.2, C		148.1, C		155.0, C	
3′	101.3, CH	6.46 br s	101.7, CH	6.50 d (2.5)	116.3, CH	6.82 d (8.8)	115.8, CH	6.91 d (8.1)
4′	160.3, C		160.5, C		114.3, CH	6.79 dd (9.0, 2.8)	129.3, CH	7.18 m
5′	104.8, CH	6.47 m	104.1, CH	6.41 dd (8.5, 2.5)	152.1, C		118.8, CH	6.85 t (7.5)
6′	127.9, CH	7.36 d (8.2)	127.9, CH	7.32 d (8.6)	112.2, CH	7.03 d (2.9)	126.9, CH	7.44 d (8.6)
1″	104.0, CH	4.62 d (7.6)	104.0, CH	4.62 d (7.7)	104.0, CH	4.63 d (7.7)	104.0, CH	4.60 d (7.7)
2″	73.9, CH	3.29 m	73.9, CH	3.29 m	74.0, CH	3.32 m	74.0, CH	3.28 m
3″	76.0, CH	3.26 m	75.9, CH	3.24 m	76.1, CH	3.25 m	76.1, CH	3.24 m
4″	69.8, CH	3.19 m	70.2, CH	3.15 m	69.8, CH	3.20 m	70.0, CH	3.18 m
5″	73.6, CH	3.30 m	73.6, CH	3.30 m	73.6, CH	3.31 m	73.7, CH	3.31 m
6″	63.1, CH_2	4.04 dd (11.8, 6.0)	63.1, CH_2	3.96 dd (11.8, 7.0)	63.0, CH_2	4.02 dd (11.8, 6.0)	63.0, CH_2	3.96 dd (11.7, 6.5)
		4.20 d (11.5)		4.21 d (11.6)		4.22 d (10.2)		4.23 m
1‴	170.3, C		170.4, C		170.4, C		170.5, C	
2‴	45.4, CH_2	2.61 d (14.1)	46.3, CH_2	2.28 d (13.5)	45.9, CH_2	overlap 2.41 d (13.8)	46.5, CH_2	2.32 s
		2.47 d (14.1)		2.23 d (13.6)				
3‴	68.9, C		68.9, C		68.9, C		68.9, C	
4‴	45.5, CH_2	2.45 d (2.4)	47.5, CH_2	2.12 d (14.7)	48.6, CH_2	2.35 d (14.8)	47.0, CH_2	2.16 d (15.0)
				2.00 m		2.29 d (14.8)		2.03 m
5‴	172.4, C		175.5, C		173.6, C		175.4, C	
6‴	27.2, CH_3	1.18 s	27.9, CH_3	1.04 s	27.3, CH_3	1.14 s	27.7, CH_3	1.05 s
CH_3-6	8.7, CH_3	2.05 s	8.5, CH_3	2.12 s	8.7, CH_3	2.05 s	8.6, CH_3	2.05 s
CH_3-8	9.2, CH_3	2.00 s	9.2, CH_3	2.00 s	9.1, CH_3	2.05 s	9.1, CH_3	2.03 s
OCH_3-4′	55.1, CH_3	3.71 s	55.0, CH_3	3.71 s	55.4, CH_3	3.69 s		

[a,b]Exchangeable signals.

(t, J = 7.3 Hz, 4′)]. The absolute configuration of the HMG group was also determined to be (*S*) using the same method mentioned above. Consequently, compound **2** (matteuorienate E) was identified as (2*S*)-demethoxymatteucinol-7-*O*-[4″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside.

Compound **3** showed the molecular formula $C_{30}H_{36}O_{14}$, the same as that of **1**. The UV, IR, ^{1}H NMR, and ^{13}C NMR data were similar to those of **1**. The location of the HMG moiety was different between **1** and **3**, and it was revealed that compound **3** has an HMG substituent at C-3″ instead of C-4″ due to the HMBC correlation between H-3″ (δ_H 4.84) and C-1‴ (δ_C 170.1). Like compounds **1** and **3**, the spectroscopic data of compound **4** ($C_{29}H_{34}O_{13}$) resembled those of **2**, although the HMBC correlation between H-3″ and C-1‴ indicated a difference in location of the HMG unit. The (*S*)-configurations of **3** and **4** at C-2 and C-3‴ were also identified from the ECD spectra and the same series of reactions mentioned above. Thus, compounds **3** and **4** (matteuorienates F and G) were identified as (2*S*)-matteucinol-7-*O*-[3″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside and (2*S*)-demethoxymatteucinol-7-*O*-[3″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside, respectively.

The molecular formula of **5** was determined to be $C_{30}H_{36}O_{15}$ by HRESIMS. The UV, IR, ^{1}H NMR, and ^{13}C NMR data (Table 2) were similar to those of **14** (Table S1, Supporting Information), except for the proton coupling pattern of the flavonoid B-ring [δ_H 6.46 (br s, H-3′), 6.47 (m, H-5′), and 7.36 (d, J = 8.2 Hz, H-6′)]. From these data, either a 1,3,4-, 1,2,5-, or 1,2,4-trisubstituted aromatic ring system could be implied for the B-ring, and it turned out to be a 1,2,4-trisubstituted ring by analyzing the 2D NMR data. The methoxy moiety was located at C-4′, which was determined by the HMBC correlation between the signals at δ_H 3.71 (OCH_3) and δ_C 160.3 (C-4′). The molecular formula of compound **6** was the same as **5**, $C_{30}H_{36}O_{15}$, which was determined by HRESIMS. The ^{1}H and ^{13}C NMR data were similar, and the planar structure was identical to **5**; however, several differences were observed in the chemical shifts of H-2/H-3 and the aromatic protons on the B-rings of **5** and **6**. This suggested that **6** is a 2-epimer of **5**; this was confirmed from the ECD spectra of these two substances. Compounds **5** and **6** showed negative and positive Cotton effects at 289 nm, respectively, which indicated a (2*S*)-configuration for **5** and a (2*R*)-configuration for **6**. Compound **7** was isolated as a yellowish powder, and its molecular formula was determined to be $C_{30}H_{36}O_{15}$ by HRESIMS. The ^{1}H and ^{13}C NMR data of **7** were similar to those of **5**, with the only difference being that **7** could be assigned a 1,2,5-trisubstituted aromatic ring system [δ_H 6.82 (d, J = 8.8 Hz, H-3′), 6.79 (dd, J = 9.0, 2.8 Hz, H-4′), and 7.03

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(d, J = 2.9 Hz, H-6′)]. Compared with **7**, the molecular formula of **8**, $C_{29}H_{34}O_{14}$, suggested the absence of a methoxy group. Based on the ^{1}H and ^{13}C NMR, the modified pattern of the B-ring and the absence of a methoxy group at C-5′ were observed. The ECD spectra of compounds **7** and **8** showed a negative Cotton effect, indicating that the absolute configuration at C-2 is (*S*).

Experiments for determining the absolute configuration of the HMG moiety in **5** and **6** were not conducted due to their scarce amounts obtained. However, the absolute configuration of the HMG moiety at C-3 was deduced to be (*S*) because the naturally occurring HMG group is known to be in an (*S*)-configuration,[17] and other HMG-containing flavonoid glycosides from this plant (**1**−**4**, **7**, and **8**) were verified as having a (3*S*)-HMG moiety. Accordingly, based on the natural biogenetic pathway of the HMG group, compounds **5** and **6** were identified as (2*S*)-2′-hydroxymatteucinol-7-*O*-[6″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside and (2*R*)-2′-hydroxymatteucinol-7-*O*-[6″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside and were named matteuorienate H and matteuorienate I, respectively. Additionally, compound **7** (matteuorienate J) was characterized as (2*S*)-methoxymatteucinol-7-*O*-[6″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside, while compound **8** (matteuorienate K) was confirmed as (2*S*)-matteucin-7-*O*-[6″-*O*-((*S*)-3-hydroxy-3-methylglutaryl)]-*β*-D-glucopyranoside.

The ^{1}H and ^{13}C NMR spectra of **9** (Table S3, Supporting Information) suggested this compound to be an analogue of **8** without the HMG moiety, which was also supported by the molecular formula of **9**, $C_{23}H_{26}O_{10}$. Comparison of its ECD spectrum with **8** showed a positive Cotton effect at 283 nm in the ECD spectrum of **9**, indicating an (*R*)-configuration at C-2. Therefore, compound **9** was identified as (2*R*)-matteucin-7-*O*-*β*-D-glucopyranoside. The molecular formula of compound **10**, $C_{23}H_{24}O_9$, was obtained by HRESIMS. Its ^{1}H and ^{13}C NMR data were similar to those of matteuorien.[1] The presence of an anomeric proton at δ_H 4.66 (d, J = 7.7 Hz, H-1′) suggested **10** as having a glucose unit, and the position of the glycosidic linkage was identified from the HMBC correlation between δ_H 4.66 (H-1″) and δ_C 159.2 (C-7). The sugar was determined to be D-glucose using the same method mentioned above.[13] Thus, compound **10** was characterized as matteuorien-7-*O*-*β*-D-glucopyranoside.

Compound **11** was obtained as a yellow powder, and its molecular formula was determined to be $C_{28}H_{34}O_{13}$ by HRESIMS. The spectroscopic data of **11** were similar to those of matteuorienin (**17**),[1] but they differed in their B-ring proton coupling. The ^{1}H NMR spectrum of **11** suggested a monosubstituted B-ring substructure [δ_H 7.56 (d, J = 7.4 Hz, H-2′, 6′), 7.45 (dd, J = 7.5, 7.5 Hz, H-3′, 5′), and 7.39 (t, J = 7.3 Hz, H-4′)]. In the tandem mass spectrum, compound **11** showed a series of neutral losses of 120 (m/z 457.1488), 164 (m/z 413.1231) [M − $C_6H_{10}O_4$ − H_2O − H]$^-$, 266 (m/z 311.0912) [M − 120 − $C_6H_{10}O_4$ − H]$^-$, and 284 (m/z 293.0807) [M − $C_6H_{10}O_4$ − H_2O − 120 − H]$^-$, which indicated the compound to be a *C*-glycosylflavonoid with an additional deoxyhexose moiety (Figure S67, Supporting Information). The ^{1}H NMR resonance of an anomeric proton [δ_H 4.70 (d, J = 9.8 Hz, H-1′)] and the HMBC correlation between H-1′ and C-8 (δ_C 103.3) suggested the presence of an 8-C-*β*-glucosyl group (Figure S68 and Table S3, Supporting Information).[18] The position of the interglycosidic linkage was determined as being located on C-2″ by the HMBC cross-peak between δ_H 5.13 (H-1‴) and δ_C 75.5 (C-2″). The deoxyhexose unit was confirmed as L-rhamnose using the HPLC analysis method mentioned above.[13] Compared with **11**, compound **12** was identified as a 4′-hydroxylated analogue, which was suggested by its molecular formula of $C_{28}H_{34}O_{14}$ and the A_2B_2 coupling pattern in the ^{1}H NMR spectrum. The molecular formula of compound **13**, $C_{29}H_{36}O_{14}$, was obtained by HRESIMS. Its ^{1}H and ^{13}C NMR data were similar to those of **12**. The only difference between the two compounds was the presence of a methoxy group [δ_H 3.79; δ_C 55.1] at C-4′. The absolute configuration of compounds **11**−**13** at C-2 was determined as (*S*) via their ECD spectra. Therefore, based on the above evidence, compounds **11**−**13** were identified as (2*S*)-6-methylpinocembrin-8-*C*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside, (2*S*)-6-methylnaringenin-8-*C*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside, and (2*S*)-6-methylisosakuranetin-8-*C*-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside and named matteuorienins B−D, respectively.

During the structural elucidation of the isolates, the ^{1}H and ^{13}C NMR resonances of the flavonoid glycosides (**14**−**20**) were also fully assigned (Tables S1 and S2, Supporting Information). The structures of **14**−**16** were previously reported, but the absolute configurations of their HMG moieties were not verified. These were investigated using the method described above, and it was confirmed that the HMG moieties in compounds **14**−**16** also possess the (*S*)-configuration.

Based on a consideration about previously reported anti-influenza activity of *C*-methylated flavonoids,[5,19,20] several isolated compounds (**1**−**4**, **7**−**9**, **11**−**23**, **25**, **26**, and **28**) from *P. orientale* were screened for their neuraminidase (NA) inhibitory activity of H1N1 influenza virus. Compounds **5**, **6**, and **10** were not investigated due to their scarcity. Compounds **24** and **27** were not examined, because other researchers reported that these compounds have no inhibitory activity on influenza viruses.[21,22] As shown in Figure S70 (Supporting Information), the flavonoid aglycones, such as compounds **21**−**23**, **25**, and **26**, showed more potent inhibitory activities than the glycosylated aglycones. Thus, these aglycones were further investigated to determine their half-maximal inhibitory concentrations (IC_{50}) on the neuraminidase activities of the influenza H1N1 A/PR/8/34 and H9N2A/chicken/Korea/01210/2001 viruses. As indicated in Table 3, compounds **21**−**23**, **25**, and **26**

Table 3. Inhibitory Effects of Selected Compounds on Neuraminidase Activities in the H1N1-Induced CPE Assay[a]

compound	IC_{50} H1N1 (μM)	IC_{50} H9N2 (μM)	EC_{50} (μM)	CC_{50} (μM)
21	30.3 ± 3.0	31.3 ± 5.7	30.7 ± 2.0	77.6 ± 2.6
22	25.2 ± 2.4	27.2 ± 3.2	26.9 ± 1.3	>100
23	23.9 ± 3.0	24.1 ± 1.3	22.9 ± 2.0	>100
25	24.5 ± 1.5	24.6 ± 0.8	23.0 ± 3.4	>100
26	24.4 ± 2.0	23.1 ± 1.7	21.4 ± 2.0	>100
oseltamivir[b]	77.6 ± 9.5 (nM)	19.1 ± 1.3 (nM)	0.6 ± 0.2	N.D.[c]

[a]Compounds **21**−**23**, **25**, and **26** were selected based on the screening data for H1N1 neuraminidase inhibitory activity (Figure S70, Supporting Information). [b]Positive control. [c]Not detected.

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exhibited NA inhibitory activities with IC_{50} values ranging from 23.1 ± 1.7 to 31.3 ± 5.7 μM and were compared with oseltamivir (Hoffman-La Roche Ltd., Basel, Switzerland) as a positive control. Based on the NA results, these compounds were further investigated with cytotoxicity and H1N1-induced cytopathic effect (CPE) assays. The data in Table 3 showed that compound **21** was cytotoxic to MDCK cells (CC_{50}, 77.6 ± 2.6 μM), while the other compounds (**22**, **23**, **25**, and **26**) did not have strong antiproliferative effects at a concentration of 100 μM. Compounds **22**, **23**, **25**, and **26** showed EC_{50} values in the range from 21.4 to 30.7 μM, while the positive control exhibited an EC_{50} of 0.6 μM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2000 polarimeter (JASCO, Easton, MD, USA). All UV and CD spectra were recorded with a Chirascan CD spectrometer (Applied Photophysics, Leatherhead, UK). IR spectra were acquired on a JASCO FT/IR-4200 spectrometer. The NMR spectra were obtained with AVANCE-500, 600, and 850 NMR spectrometers (Bruker, Billerica, MA, USA), equipped with a cryogenic probe (600 and 850 MHz). All HRESIMS data were recorded on a Waters Xevo G2 QTOF mass spectrometer (Waters Co., Milford, MA, USA). Column chromatography was performed with Kieselgel 60 silica gel (40−60 μm, 230−400 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (25−100 μm, Pharmacia, Piscataway, NJ, USA), and Diaion HP20 (200−300 mesh, Mitsubishi Chemical Co., Tokyo, Japan). TLC was performed using Kieselgel 50 F254 coated normal-phase silica gel TLC plates and TLC silica gel 60 RP-18 F254s (Merck). Preparative HPLC was performed with a G-321 pump (Gilson, Middleton, WI, USA) and a G-151 UV detector (Gilson). All solvents were purchased from Daejung Chemicals & Metals Co. Ltd. (Siheung, Korea). The reagents for aldose discrimination (L-cysteine methyl ester hydrochloride and *o*-tolyl isothiocyanate) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Authentic sugars were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Material. *Pentarhizidium orientale* was collected in September 2013 at Ulleung Island, Gyeongsangbuk-do, Korea, and authenticated by S. I. Han (Medicinal Plant Garden of the College of Pharmacy, Seoul National University). A voucher specimen (SNUPT01) of the plant has been deposited at the Herbarium at the Medicinal Plant Garden of the College of Pharmacy, Seoul National University.

Extraction and Isolation. Dried *P. orientale* rhizomes (900 g) were extracted three times with 80% MeOH (4 L × 3, 90 min) at room temperature. After being concentrated in vacuo, the extract (119.9 g) was suspended in H_2O and successively partitioned with CH_2Cl_2 and *n*-BuOH. The CH_2Cl_2 fraction (8.1 g) was fractionated by silica gel column chromatography (CC) and eluted with mixtures of *n*-hexane/EtOAc (ranging from 50:1 to 1:1) followed by $CHCl_3$/MeOH (ranging from 20:1 to 1:1), to afford 14 subfractions (D1−D14). Compounds **21** (5.0 mg) and **22** (4.3 mg) were isolated from subfraction D3 using preparative HPLC (acetonitrile/H_2O, 1:1). Subfraction D5 was subjected to Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1) to afford four subfractions (D51−D54). Compounds **23** (16.2 mg), **27** (3.2 mg), and **28** (2.6 mg) were isolated from subfraction D54 by semipreparative HPLC (45% aqueous acetonitrile). Subfraction D6 was separated into four subfractions (D61−D64) using Sephadex LH-20 and eluted with CH_2Cl_2/MeOH (1:1). Subfraction D64 was further purified using semipreparative HPLC and eluted with 42% aqueous acetonitrile under isocratic conditions to yield compounds **24** (1.0 mg), **25** (2.3 mg), and **26** (24.8 mg).

The *n*-BuOH fraction (67.9 g) was subjected to passage over Diaion HP20 resin using a MeOH/H_2O gradient (0, 30, 70, and 100% MeOH and 100% acetone) to yield five fractions (B0, B30, B70, B100, and BA). Fraction BA was separated using silica gel CC and eluted with CH_2Cl_2/MeOH (ranging from 15:1 to 1:1) to yield seven subfractions (BA1−BA7). Further purification of BA3, BA5, and BA6 was conducted by preparative HPLC with 30% aqueous acetonitrile under isocratic conditions to afford five (BA31−BA35), seven (BA51−BA57), and three (BA61−BA63) subfractions, respectively. Compounds **1** (10.0 mg) and **2** (11.5 mg) were isolated from subfraction BA33 by semipreparative HPLC eluted with a 50% EtOH aqueous solution. Using the same conditions, subfractions BA31, BA32, and BA34 were subjected to semipreparative HPLC to yield compounds **18** (3.1 mg), **19** (3.7 mg), **14** (13.0 mg), **15** (24.0 mg), **3** (6.1 mg), and **4** (6.4 mg). Compound **12** (10.2 mg) was isolated from subfraction BA51 via semipreparative HPLC using 30% aqueous acetonitrile. Subfraction BA53 was purified with semipreparative HPLC (acetonitrile/H_2O, 3:7) to afford compounds **7** (12.6 mg) and **8** (4.7 mg). Compounds **5** (3.6 mg) and **6** (1.1 mg) were isolated from subfraction BA54 by semipreparative HPLC eluted with 29% aqueous acetonitrile. Subfraction BA57 was further purified with Sephadex LH-20 eluted with MeOH to give compound **16** (15.0 mg). Compounds **17** (4.1 mg), **11** (8.2 mg), and **13** (10.2 mg) were isolated from subfraction BA62 by preparative HPLC with 27% aqueous acetonitrile. Fraction B70 was further separated into eight subfractions (B70_1−B70_8) using silica gel CC eluted with $CHCl_3$/MeOH/H_2O (ranging from 25:4:1 to 5:4:1). Subfraction B70_2 was subjected to preparative HPLC (acetonitrile/H_2O, 3:7), yielding five subfractions (B70_21−25). Compounds **9** (4.6 mg) and **20** (10.3 mg) were isolated from subfraction B70_24 by semipreparative HPLC eluted with 20% aqueous acetonitrile.

The aqueous residue (25.4 g) was separated with a Diaion HP20 resin using a MeOH/H_2O gradient (0, 50, and 100% MeOH) to yield three fractions (W0, W50, and W100). Fraction W50 was subjected to passage over a Diaion HP20 resin with a MeOH/H_2O gradient system (0, 25, and 100% MeOH) to afford three subfractions (W50_0, W50_25, and W50_100). Further purification of subfraction W50_100 by RP-MPLC (MeOH/H_2O, ranging from 10 to 100%) afforded 10 subfractions (W50_100_1-W50_100_10). Compound **10** (1.2 mg) was isolated from subfraction W50_100_8 using preparative HPLC and eluted with 25% aqueous acetonitrile.

*Matteuorienate D (**1**):* yellow amorphous powder; $[\alpha]_D^{20}$ −20.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 252 (3.31), 282 (3.62), 349 (3.18); ECD (MeOH) λ_{max} 214 (9.05), 287 (−5.55), 349 (0.98); IR ν_{max} 3393, 2897, 1730 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data, see Table 1; HRESIMS *m*/*z* 619.2025 $[M - H]^-$ (calcd for $C_{30}H_{35}O_{14}$, 619.2027).

*Matteuorienate E (**2**):* yellow amorphous powder; $[\alpha]_D^{20}$ −29.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 250 (3.26), 282 (3.64), 350 (3.14); ECD (MeOH) λ_{max} 216 (5.38), 285 (−4.23), 351 (0.79); IR ν_{max} 3336, 2853, 1730, 1715 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data, see Table 1; HRESIMS *m*/*z* 589.1929 $[M - H]^-$ (calcd for $C_{29}H_{33}O_{13}$, 589.1921).

*Matteuorienate F (**3**):* yellow amorphous powder; $[\alpha]_D^{20}$ −5.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 252 (3.26), 282 (3.54), 351 (3.10); ECD (MeOH) λ_{max} 214 (7.21), 288 (−3.51), 349 (0.98); IR ν_{max} 3335, 2928, 1722, 1715 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data, see Table 1; HRESIMS *m*/*z* 619.2032 $[M - H]^-$ (calcd for $C_{30}H_{35}O_{14}$, 619.2027).

*Matteuorienate G (**4**):* yellow amorphous powder; $[\alpha]_D^{20}$ −16.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 250 (3.26), 282 (3.64), 350 (3.14); ECD (MeOH) λ_{max} 216 (6.08), 285 (−3.40), 350 (0.79); IR ν_{max} 3384, 2882, 1730, 1716 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data, see Table 1; HRESIMS *m*/*z* 589.1923 $[M - H]^-$ (calcd for $C_{29}H_{33}O_{13}$, 589.1921).

*Matteuorienate H (**5**):* yellow amorphous powder; $[\alpha]_D^{20}$ −26.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 252 (3.05), 283 (339), 362 (2.91); ECD (MeOH) λ_{max} 217 (5.84), 289 (−5.18), 352 (1.28); IR ν_{max} 3420, 2853, 1730, 1715 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 2; HRESIMS *m*/*z* 635.1974 $[M - H]^-$ (calcd for $C_{30}H_{35}O_{15}$, 635.1976).

*Matteuorienate I (**6**):* yellow amorphous powder; $[\alpha]_D^{20}$ −10.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 250 (3.34), 284 (3.77), 350 (3.26); ECD (MeOH) λ_{max} 229 (−1.74), 288 (0.91), 361 (−0.06); IR ν_{max} 3446, 2853, 1730, 1715 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 2; HRESIMS *m*/*z* 635.1974 $[M - H]^-$ (calcd for $C_{30}H_{35}O_{15}$, 635.1976).

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Matteuorienate J (7): yellow amorphous powder; $[\alpha]_D^{20}$ −42.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 252 (3.20), 286 (3.55), 350 (3.07); ECD (MeOH) λ_{max} 219 (2.55), 288 (−5.32), 351 (0.38); IR ν_{max} 3446, 2853, 1730, 1715 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 2; HRESIMS *m*/*z* 635.1985 $[M - H]^-$ (calcd for $C_{30}H_{35}O_{15}$, 635.1976).

Matteuorienate K (8): yellow amorphous powder; $[\alpha]_D^{20}$ 4.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 254 (2.94), 285 (3.23), 350 (2.75); ECD (MeOH) λ_{max} 226 (2.48), 279 (−2.13), 350 (0.31); IR ν_{max} 3446, 2853, 1731, 1715 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table 2; HRESIMS *m*/*z* 605.1862 $[M - H]^-$ (calcd for $C_{29}H_{33}O_{14}$, 605.1870).

Matteucin 7-O-β-D-glucoside (9): yellow amorphous powder; $[\alpha]_D^{20}$ 4.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 248 (3.78), 283 (4.34), 361 (3.76); ECD (MeOH) λ_{max} 230 (−0.93), 283 (0.62), 305 (0.33); IR ν_{max} 3274, 2603, 1730, 1715 cm^{-1}; ^1H (850 MHz) and ^{13}C NMR (213 MHz) data, see Table S3, Supporting Information; HRESIMS *m*/*z* 461.1446 $[M - H]^-$ (calcd for $C_{23}H_{25}O_{10}$, 461.1448).

Matteuorien 7-O-β-D-glucoside (10): yellow amorphous powder; $[\alpha]_D^{20}$ 27.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 247 (3.97), 280 (4.41), 349 (3.74); IR ν_{max} 3337, 2927, 1758, 1670, 1640, 1607, 1186 cm^{-1}; ^1H (500 MHz) and ^{13}C NMR (125 MHz) data, see Table S3, Supporting Information; HRESIMS *m*/*z* 443.1337 $[M - H]^-$ (calcd for $C_{23}H_{23}O_9$, 443.1342).

Matteuorienin B (11): yellow amorphous powder; $[\alpha]_D^{20}$ 24.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 257 (3.84), 291 (4.25), 332 (3.83); ECD (MeOH) λ_{max} 222 (9.3), 288 (−5.06), 331 (1.08); IR ν_{max} 3392, 2927, 1729, 1516 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table S3, Supporting Information; HRESIMS *m*/*z* 577.1920 $[M - H]^-$ (calcd for $C_{28}H_{33}O_{13}$, 577.1921).

Matteuorienin C (12): yellow amorphous powder; $[\alpha]_D^{20}$ 24.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 258 (3.91), 291 (4.17), 331 (3.83); ECD (MeOH) λ_{max} 218 (6.7), 290 (−2.95), 335 (0.99); IR ν_{max} 3397, 2930, 1729, 1519 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table S3, Supporting Information; HRESIMS *m*/*z* 593.1874 $[M - H]^-$ (calcd for $C_{28}H_{33}O_{14}$, 593.1870).

Matteuorienin D (13): yellow amorphous powder; $[\alpha]_D^{20}$ 41.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 256 (3.83), 291 (4.25), 331 (3.79); ECD (MeOH) λ_{max} 218 (10.6), 289 (−5.03), 335 (1.69); IR ν_{max} 3397, 2930, 1730, 1518 cm^{-1}; ^1H (600 MHz) and ^{13}C NMR (150 MHz) data, see Table S3, Supporting Information; HRESIMS *m*/*z* 607.2030 $[M - H]^-$ (calcd for $C_{29}H_{35}O_{14}$, 607.2027).

Acid Hydrolysis of Compounds 1−13 and Determination of the Resulting Sugars. The absolute configurations of the sugar moieties in **1−13** were confirmed using the method of Tanaka et al.[13] Compounds (0.5 mg) were hydrolyzed with 0.5 N HCl (0.1 mL) for 2 h at 90 °C and neutralized with NH_4OH. The mixture was dried in vacuo, and the residue was dissolved in pyridine (0.1 mL) containing L-cysteine methyl ester hydrochloride (0.5 mg) and heated at 60 °C for 1 h. Then, a 0.1 mL solution of *o*-tolyl isothiocyanate in pyridine was added to the mixture. The mixture was heated at 60 °C for 1 h, and then the reaction mixture was directly analyzed by HPLC. Analytical HPLC was acquired on an YMC-Triart C_{18} column (4.60 × 250 mm, 5 μm) at 35 °C with a 25% acetonitrile isocratic solvent system for 40 min (0.8 mL/min). The authentic sugars, D-glucose, L-glucose, and L-rhamnose, were subjected to the same derivatization procedure, and all the peaks were detected with a UV detector at 250 nm. The derivatives of D-glucose in **1−10** and L-rhamnose in **11−13** were confirmed by comparison of the retention times with those of the standard sugar. The authentic sugar derivative peaks were recorded at 19.7 (L-glucose), 21.7 (D-glucose), and 37.1 (L-rhamnose) min.

Determination of the Absolute Configuration of HMG in Compounds 1−4, 7, 8, and 14−16. (*S*)-1-Phenylethylamine (2 equiv), triethylamine (Et_3N, Sigma-Aldrich) (3 equiv), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP, Sigma-Aldrich) (1.5 equiv), and 1-hydroxybenzotriazole (HOBt, Sigma-Aldrich) (2 equiv) were added to a solution containing a compound and 0.3 mL of *N*,*N*-dimethylformamide (DMF, Sigma-Aldrich) under ice-cooling. The mixture was stirred at room temperature for 9 h, and the reaction was quenched with diluted aqueous HCl. A yellowish residue was obtained after drying under N_2 gas. The residue was separated using a silica gel Waters Sep-Pak Plus Long column (CH_2Cl_2/MeOH, 30:1, 20:1, 15:1, and 10:1) to obtain amide **A** and was identified by LC-MS analysis.[15] $LiBH_4$ (15 equiv of **A**) was added to the solution containing **A** and THF (0.3 mL) under ice-cooling. The solution was stirred for 24 h at 25 °C, and then the reaction was quenched with dilute aqueous HCl. The resultant mixture was extracted with EtOAc. The resulting extract was separated using a silica gel Waters Sep-Pak Plus Long column ($CHCl_3$/MeOH, 20:1, 15:1, 10:1, and 5:1) to furnish **B**. The product was confirmed by LC-MS analysis and then acetylated with Ac_2O (5 equiv of **A**) in pyridine (30 μL). The reaction mixture was stirred for 24 h at 25 °C, diluted with H_2O, and extracted with EtOAc; a colorless oil **C** was obtained after refining by HPLC. The ^1H NMR spectrum (Figure S69, Supporting Information) of **C** was found to be consistent with that of (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide upon comparison, rather than the (3*S*) isomer that was previously reported.[16] (**1**: 5.1 mg, 8.22 μmol; **2**: 5.3 mg, 8.97 μmol; **3**: 5.1 mg, 8.22 μmol; **4**: 5.1 mg, 8.64 μmol; **7**: 5.6 mg, 8.8 μmol; **8**: 5.3 mg, 8.74 μmol, **14**: 4.2 mg, 6.77 μmol; **15**: 5.8 mg, 9.82 μmol; and **16**: 5.5 mg, 9.34 μmol.)

Neuraminidase Inhibition Assay. This enzyme-inhibition assay was performed as previously reported, with slight modifications.[20] In brief, for the preparation of neuraminidase enzymes, MDCK cells were infected with influenza viruses (H1N1 A/PR/8/34 virus or H9N2 A/chicken/Korea/01210/2001 virus) on a large scale. To inactivate the viral infectivity, the suspension was supplemented with formaldehyde solution at a final concentration of 0.1% at 37 °C and incubated for 30 min. The neuraminidase inhibition assay was performed using a 4-methylumbelliferyl-α-D-*N*-acetylneuraminic acid sodium salt hydrate (4-MU-NANA) (Sigma-Aldrich) as the fluorescent substrate in 32.5 mM 2-(*N*-morpholino)ethanesulfonic acid and 4 mM $CaCl_2$ (pH 6.5) as the enzyme buffer. In general, the assay was performed with the following steps: (1) 10 μL of virus suspension and 10 μL of diluted test compound at various concentrations in the enzyme buffer were added to 96-well plates; (2) after 30 min of incubation at 37 °C, 30 μL of diluted 4-MU-NANA substrate was added to each well; (3) the reaction was maintained at 37 °C for 2 h and quenched by treatment with 150 μL of stop solution (25% EtOH and 0.1 M glycine, pH 10.7); and (4) the fluorescence intensity of the reaction was measured at an excitation wavelength of 360 nm and an emission wavelength of 440 nm with a fluorescence microplate reader (SpectraMax GEMNI XPS, Molecular Devices, Sunnyvale, CA, USA).

Cytopathic Effect Inhibition Assay. Madin-Darby canine kidney (MDCK) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone, Logan, UT, USA) containing 10% fetal bovine serum (HyClone) at 37 °C and 5% CO_2. The cells were seeded onto 96-well plates at 1×10^5 cells per well and incubated for 24 h. Then, the influenza H1N1 A/PR/8/34 virus was incubated with the cell monolayers for 2 h via infection using DMEM containing 0.15 μg/mL trypsin and 5 μg/mL bovine serum albumin. The medium was then replaced with fresh DMEM containing 10 μg/mL trypsin with different concentrations of the test compounds. Virus inhibition was examined with each test compound concentration in triplicate. After 3 days of incubation, the cells were replaced with fresh DMEM and 20 μL of 2 mg/mL MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich) was added to each well and incubated for 4 h at 37 °C. The formazan crystals were dissolved with 100 μL of DMSO, and the absorbance was measured at 550 nm using a microplate reader (VersaMax, Randor, PA, USA).

Cytotoxicity Assay. The cell viability was calculated using an MTT method. Briefly, the MDCK cells were grown on 96-well plates at 1×10^5 cells per well. After 24 h of incubation, the cells were washed with phosphate-buffered saline and replaced with fresh DMEM containing the test compounds at various concentrations. The compounds were tested at each concentration in triplicate. The cultures were incubated for 2 days, and 2 mg/mL MTT solution was added to each well, followed by further incubation for 4 h. The supernatant was removed, and formazan crystals were dissolved in 100 μL of DMSO and measured at a wavelength of 550 nm.

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Statistical Analysis. The data are expressed as the means ± SD of three independent experiments. Statistical analysis of the half-maximal inhibitory concentration (IC_{50}), effective concentration (EC_{50}), and cytotoxic concentration (CC_{50}) was performed using the Sigma Plot Statistical Analysis software (SPCC Inc., Chicago, IL, USA).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00677.

Chemical structures of compounds **14**−**28**; fully assigned ^{1}H and ^{13}C NMR data of compounds **14**−**20**; raw 1D and 2D NMR data of **1**−**13**; scheme and ^{1}H NMR data for determination of absolute configurations of HMG moieties; screening data of neuraminidase inhibitory assay for isolated compounds (PDF)

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Notes

The authors declare no competing financial interest.

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