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Note

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Anti-Respiratory Syncytial Virus Prenylated Dihydroquinolone Derivatives from the Gorgonian-Derived Fungus *Aspergillus* sp. XS-20090B15

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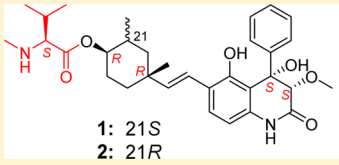
Supporting Information

ABSTRACT: Two new prenylated dihydroquinolone derivatives, 22-*O*-(*N*-Me-L-valyl)aflaquinolone B (**1**) and 22-*O*-(*N*-Me-L-valyl)-21-*epi*-aflaquinolone B (**2**), and two known analogues, aflaquinolones A (**3**) and D (or a diastereomer of D, **4**), were isolated from the mycelia of a gorgonian-derived *Aspergillus* sp. fungus. The structures of the new compounds were elucidated by spectroscopic methods, ECD spectra, Marfey's method, and chemical conversion. Compounds **1** and **2** display an unusual esterification of *N*-Me-L-Val to the side-chain prenyl group. Compound **2** exhibited outstanding anti-RSV activity with an IC_{50} value of 42 nM, approximately 500-fold stronger than that of the positive control ribavirin (IC_{50} = 20 μM), and showed a comparatively higher therapeutic ratio (TC_{50}/IC_{50} = 520).



Anti-RSV	IC_{50} (μM)	TC_{50}/IC_{50}
1	>50	—
2	0.042	520
Ribavirin	20	>150

Human respiratory syncytial virus (RSV) is an enveloped, nonsegmented negative-strand RNA virus of the family Paramyxoviridae. RSV is the most important cause of viral lower respiratory tract infections in infants and young children and the second leading cause of death from respiratory viral infections in the elderly.[1] The only licensed antiviral treatment available today is ribavirin, a guanosine analogue generally administered as a small particle aerosol to immunocompromised patients with lower respiratory tract disease due to RSV.[2] However, the route of administration, the toxicity (risk of teratogenicity), the cost, and the highly variable efficacy limit its use. Given the above, developing effective anti-RSV agents with unique structures is a pressing need. Marine organisms have been proven to produce secondary metabolites that are structurally distinct from those produced by terrestrial organisms due to the special environment in the oceans, such as high salinity, high pressure, low concentration of oxygen, and dark conditions. Undoubtedly, marine organisms, especially marine-derived fungi, represent a frontier source of novel chemical entities for the discovery of new drug candidates including antiviral agents.[3] The most prominent example of antiviral compounds isolated from marine fungi is the hexapeptide halovir A, which exhibited potent inhibition of herpes simplex virus 1 (HSV-1).[4] So far none of natural products isolated from marine-derived fungi have been reported to show promising antiviral activity against RSV.

Recently, we obtained a series of new peptides from a potato-based liquid culture of the fungus *Aspergillus* sp. XS-20090B15 derived from the gorgonian *Muricella abnormaliz* collected from the South China Sea.[5] Unfortunately, these peptides showed no potent biological activities. However, screening of an extract prepared from *Aspergillus* sp. XS-20090B15 cultured on a rice medium revealed anti-RSV activity. Bioassay-guided separation led to the isolation of two new prenylated dihydroquinolone derivatives, 22-*O*-(*N*-Me-L-valyl)aflaquinolone B (**1**) and 22-*O*-(*N*-Me-L-valyl)-21-*epi*-aflaquinolone B (**2**), and two known analogues, aflaquinolones A and D (**3** and **4**). Herein, we report the isolation, structural characterization, and anti-RSV activities of **1**−**4**.

Compound **1** was obtained as a pale yellow, amorphous powder and showed a protonated molecule in the low-resolution positive ESIMS spectrum at m/z 551 $[M + H]^+$. Its molecular formula was determined as $C_{32}H_{42}N_2O_6$ based on HRESIMS data, requiring 13 degrees of unsaturation. The ^1H NMR spectrum (Table 1) exhibited signals for a phenyl group, a pair of *ortho*-coupled aromatic protons indicative of a 1,2,3,4-tetrasubstituted benzene ring, a *trans* olefin unit, and six methyl groups including two methyl groups bound to heteroatoms. The ^{13}C NMR data (Table 1) revealed the presence of two carbonyl carbons (one amide and one ester), 14 aromatic or olefinic carbons (corresponding to the two aromatic rings and one double bond), and four sp^3 carbons bound to O or N atoms. These fragments accounted for 11 of the 13 degrees of unsaturation, requiring two additional rings to be present in **1**. Detailed analysis of the above NMR data as well as 2D NMR

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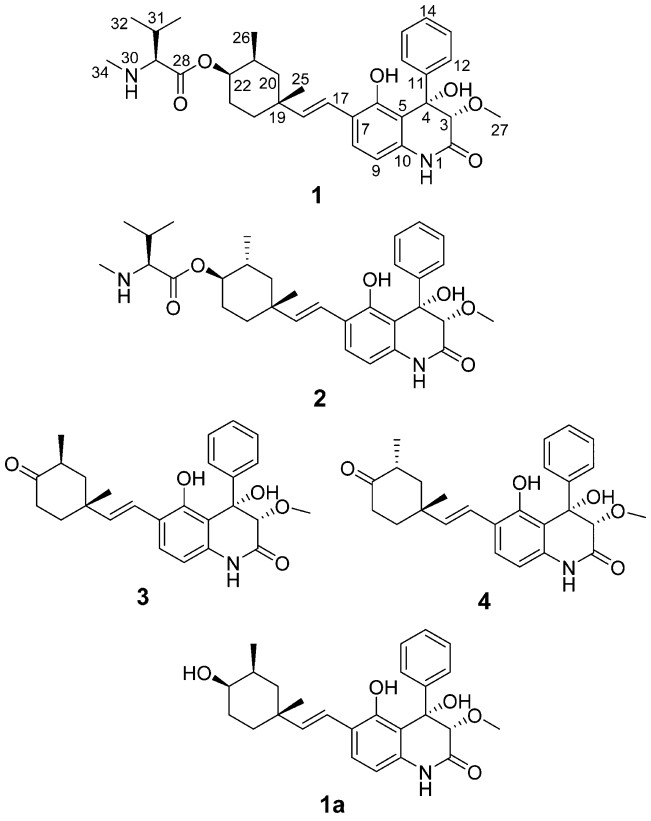

correlations indicated that **1** is a prenylated dihydroquinolone derivative and is structurally related to the known compound aflaquinolone A (**3**).[6] The 4-phenyl-3,4-dihydroquinolin-2-one portion of **1** was the same as that of **3**, as indicated by the HMBC correlations from H-3 to C-2, C-4, C-5, and C-11, from the amide NH proton H-1 to C-5, from the methoxy protons H_3-27 to C-3, and from the aromatic protons H-12/H-16 to C-4 (Figure S19). The terpenoid portion of the side chain in **1** was similar to that of **3**. The main difference was the appearance of ^{1}H and ^{13}C NMR signals for an oxymethine unit (CH-22) in **1** (Table 1) instead of the ketone carbonyl ^{13}C NMR signal in **3**. The continuous sequence of COSY correlations from H-20 to H-24 and the HMBC correlations from H-23 and H_3-26 to C-22 confirmed the connection of the oxymethine unit (CH-22).

Besides the terpenoid portion, one amino acid residue was found in the side chain of **1**. In the ^{1}H and ^{13}C NMR spectra, signals attributed to two methyl groups (δ_H 1.14 (d, J = 6.6 Hz); 1.01 (d, J = 6.6 Hz)), one methyl group bound to the N atom (δ_H 2.71 (brs)), and two methine groups (δ_H 3.65 (m); 2.38 (m)) together with the carbonyl carbon (δ_C 174.5) indicated that an *N*-Me-Val residue was presented in **1**. The COSY and HMBC correlations further confirmed the structure of the *N*-Me-Val residue. The *N*-Me-Val residue was proposed to connect at the C-22 position through an ester bond based on the significant downfield chemical shift of H-22 (δ_H 5.12). This was further confirmed by the hydrolysis product of **1**, the secondary alcohol **1a**, whose chemical shift of H-22 was found at δ_H 3.72 (Figure S17), shifting to the upfield significantly compared with that of **1**.

The relative configuration of **1** was determined by analysis of J values and NOESY data. The observed key NOE correlations from H-3 to H-12/H-16 indicated the cofacial orientation of H-3 and the phenyl ring, while the 3-OCH_3 and 4-OH groups were placed on the opposite face. This assignment was consistent with the corresponding configuration of aflaquinolone A (**3**). As for the terpenoid portion in the side chain, the axial proton at C-20 showed a large *trans*-diaxial-type coupling (12.0 Hz) to H-21, indicating that H-21 must be axially oriented and placing the C-21 methyl group in an equatorial position. The oxymethine signal (H-22) showed very small couplings (brs), indicating a large *trans*-diaxial coupling was clearly absent. Thereby H-22 was placed in an equatorial position, and consequently the *N*-Me-Val residue adopted an axial orientation. The NOESY correlations of H_{ax}-20/19-CH_3, 21-CH_3/19-CH_3, H_{eq}-20/H-17, and H_{eq}-24/H-18 suggested that 19-CH_3 and the disubstituted olefin unit should be equatorial and axial, respectively (Figure 1). On the basis of the above evidence, the relative configurations for the 4-phenyl-3,4-dihydroquinolin-2-one and terpenoid units were determined. However, the stereochemical relationship between these two units could not be correlated based on the NOESY experiment, as no diagnostic NOE cross-peak could be detected between them.

The absolute configuration of **1** was determined by electronic circular dichroism (ECD) data, Marfey's method, and chemical conversion. The absolute configuration of the 4-phenyl-3,4-dihydroquinolin-2-one unit in **1** was designated by comparison of the ECD data with those of known dihydroquinolone derivatives. It was reported that the ECD spectra of dihydroquinolones and the related analogues are affected largely by the absolute configuration of the dihydroquinolone unit, regardless of the configuration of the terpenoid side chain. The ECD spectrum of **1** (Figure 2) showed positive Cotton effects at 224, 280, and 318 nm and a strong negative Cotton effect at 253 nm, which were nearly identical to those observed for aflaquinolone A (**3**).[6] Thus, the 3*S*,4*S*-absolute configuration was assigned to **1**. Hydrolysis of **1** with NaOH−MeOH yielded two fragments, *N*-Me-Val and the secondary alcohol **1a**. The absolute configuration of *N*-Me-Val was determined as L by Marfey's method (Figure S18). Compound **1a** was identified as the known compound aflaquinolone B by the ^{1}H NMR, MS, and specific rotation data (**1a**: $[\alpha]^{25}_D$ +28 (*c* 0.17, MeOH) vs aflaquinolone B: $[\alpha]^{22}_D$ +20 (*c* 0.14, MeOH)), whose absolute configuration of the cyclohexane moiety in the side chain was determined by Mosher's method.[6] Therefore, the absolute configuration of the terpenoid portion in **1** was established as 19*R*,21*S*,22*R*, identical to that of aflaquinolone B. Thus, the full absolute configuration of **1** was determined as 3*S*,4*S*,19*R*,21*S*,22*R*,29*S*. The name 22-*O*-(*N*-Me-L-valyl)-aflaquinolone B is assigned to **1**.

Compound **2** was also obtained as a pale yellow, amorphous powder. The molecular formula of **2** was assigned as $C_{32}H_{42}N_2O_6$ on the basis of HRESIMS data, the same as that of **1**. Detailed analysis of the 1D and 2D NMR data as well as by comparison with the data of **1** (Table 1) indicated that the planar structure of **2** was the same as that of **1**. However, the configuration of the terpenoid portion in **2** slightly differed from that of **1** due to significant changes in ^{1}H and ^{13}C NMR data of the cyclohexane moiety in **2**. Analysis of J values and NOESY data could assign the relative configuration of the cyclohexane portion for **2**. The axial proton at C-20 showed a large *trans*-diaxial-type coupling (13.2 Hz) to H-21, thus indicating that H-21 and the C-21 methyl group were placed axially and equatorially, respectively. The oxymethine proton H-22 showed large *trans*-diaxial couplings with both H-21 (11.6 Hz) and H_{ax}-23 (11.6 Hz), thereby placing H-22 in an axial

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Table 1. NMR Spectroscopic Data (600/150 MHz, $CDCl_3$) for 1 and 2

	1		2	
position	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
1-NH		9.06, brs		9.07, brs
2	166.9, C		167.4, C	
3	83.4, CH	3.67, s	84.2, CH	3.67, s
4	78.2, C		78.9, C	
5	110.2, C		110.7, C	
6	154.0, C		154.8, C	
7	121.8, C		122.6, C	
8	126.4, CH	7.36, d (8.4)	127.2, CH	7.35, d (8.4)
9	106.4, CH	6.41, d (8.4)	107.4, CH	6.41, d (8.4)
10	133.5, C		134.2, C	
11	136.6, C		137.4, C	
12/16	128.2, CH	7.28−7.31, m	128.9, CH	7.29−7.31, m
13/15	125.6, CH	7.28−7.31, m	126.4, CH	7.29−7.31, m
14	128.5, CH	7.28−7.31, m	129.3, CH	7.29−7.31, m
17	121.0, CH	6.60, d (16.8)	119.1, CH	6.57, d (16.2)
18	135.9, CH	6.06, d (16.8)	140.8, CH	6.09, d (16.2)
19	36.2, C		36.0, C	
20eq	39.8, CH_2	1.54, brd (12.0)	44.4, CH_2	1.58, brd (13.2)
20ax		1.36, t (12.0)		1.23, t (13.2)
21	30.5, CH	1.85, m	32.2, CH	1.89, m
22	75.9, CH	5.12, brs	82.0, CH	4.55, td (11.6, 4.2)
23eq	26.7, CH_2	1.74−1.78, m	27.5, CH_2	1.87, m
23ax		1.74−1.78, m		1.63, qd (11.6, 4.2)
24eq	30.7, CH_2	1.67, brd (12.6)	35.3, CH_2	1.48−1.53, m
24ax		1.43, m		1.48−1.53, m
25	31.0, CH_3	1.04, s	22.6, CH_3	1.14, s
26	16.5, CH_3	0.82, d (7.2)	19.3, CH_3	0.85, d (6.0)
27	58.2, CH_3	3.60, s	59.0, CH_3	3.60, s
28	174.5, C		174.5, C	
29	66.3, CH	3.65, m	67.0, CH	3.64, m
31	28.9, CH	2.38, m	29.5, CH	2.35, m
32	18.4, CH_3	1.14, d (6.6)	18.5, CH_3	1.01, d (6.0)
33	17.4, CH_3	1.01, d (6.6)	17.2, CH_3	1.11, d (6.0)
34	31.9, CH_3	2.71, brs	32.6, CH_3	2.70, brs
6-OH		8.20, brs		8.55, brs

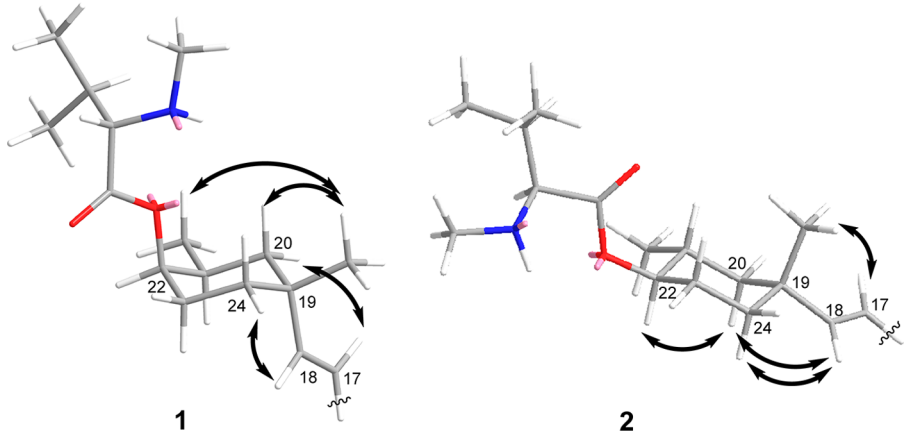

Figure 1. Key NOESY correlations for the side chains of **1** and **2**.

position, and consequently the *N*-Me-Val residue adopted an equatorial orientation. The NOESY correlation of H_{ax}-20/H_{ax}-22 further confirmed the setting of the relative configuration at C-22 (Figure 1). The NOESY correlations of H_{ax}-20/H-18 and H_{ax}-24/H-18 indicated that the disubstituted olefin unit occupied an equatorial orientation and 19-CH_3 adopted an axial orientation. The absolute configuration of 4-phenyl-3,4-dihydroquinolin-2-one unit in **2** could also be assigned as 3*S*,4*S*, as the ECD spectrum of **2** matched closely with that of **1** (Figure 2). Similar to **1**, hydrolysis of **2** with NaOH−MeOH yielded two fragments. The Marfey's analysis showed the *N*-Me-Val residue in **2** was of the L-configuration (Figure S18).

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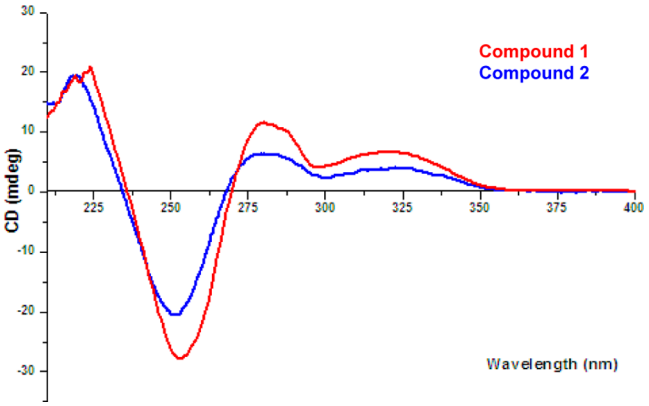

Figure 2. ECD spectra of **1** and **2**.

Unfortunately, the establishment of the absolute configuration of the cyclohexane moiety in the side chain by Mosher's method was unsuccessful because of the limited amount of the secondary alcohol-containing hydrolysate of **2**. However, the configuration at C-19 is presumably set by a terpene cyclase from a biosynthetic standpoint, while it typically would have a preference for one configuration in the same fungal strain. Therefore, the absolute configurations at C-19 in **2** could be tentatively assigned as *R*, identical to that of **1**, but the other possible diastereomer (19*S*,21*S*,22*S*) could not be ruled out. The full absolute configuration of **2** is tentatively proposed as 3*S*,4*S*,19*R*,21*R*,22*R*,29*S*. Compound **2** is named 22-*O*-(*N*-Me-L-valyl)-21-*epi*-aflaquinolone B.

The structure of the known compound **3** was identified as aflaquinolone A on the basis of its NMR, ESIMS, and specific rotation data and by comparison with the data previously reported in the literature.[6] The structure of compound **4** might be aflaquinolone D because its NMR and MS data match well with the published data.[6] Aflaquinolone D was tentatively assigned as 19*S*,21*S*, but the 19*R*,21*R* diastereomer could not be ruled out. Because compounds **1** and **3** have a 19*R* configuration established by Mosher's method along with coupling constant and NOESY data, it seems likely that **4** would have a 19*R* configuration based on biogenetic considerations. Additionally, it would seem to be easier to invert the C-21 configuration, as this stereocenter is α to the ketone carbonyl. Therefore, compound **4** also might be the 3*S*,4*S*,19*R*,21*R* diastereomer of aflaquinolone D, or 21-*epi*-aflaquinolone A.[7]

Compounds **1**−**4** are members of a known general class of prenylated dihydroquinolone derivative fungal metabolites that includes aflaquinolones,[6] aniduquinolones,[8] aspoquinolones,[9] penigequinolones,[10] and yaequinolones.[11] All of these metabolites contain a 3,4-dihydroquinolin-2-one nucleus and a terpenoid side chain. Interestingly, both **1** and **2** have an additional *N*-Me-L-Val residue connected to the terpenoid moiety via an ester bond in the side chain, which represent the first examples of prenylated dihydroquinolone derivatives containing an amino acid residue in the side chain.

Some prenylated dihydroquinolone derivatives have been reported to exhibit diverse bioactivities such as lethality to brine shrimp, cytotoxicity, and antibacterial activity.[6,8−10] In the present study, the antiviral activities of **1**−**4** against RSV were evaluated by a cytopathic effect (CPE) assay. Compound **2** exhibited significant antiviral activity against RSV virus-induced cytopathogenicity in human laryngeal carcinoma (Hep-2) cells with an IC_{50} value of 42 nM (Table 2), approximately 500-fold stronger than that of the positive control ribavirin (IC_{50} = 20 μM). Compound **2** showed a high therapeutic ratio (TC_{50}/IC_{50} = 520). These results suggest that **2** might be a promising drug candidate against RSV. It should be noted that **2** showed more potent anti-RSV activity than **4** (IC_{50} = 6.6 μM), indicating that the additional *N*-Me-L-Val residue may improve the anti-RSV activity. Compounds **1** and **3** exhibited no antiviral activity, suggesting that the configuration of the cyclohexane unit also plays a key role in the anti-RSV activity. In brief, the above findings indicate that both the *N*-Me-L-Val residue and the configuration of the cyclohexane unit in **2** are important for its anti-RSV activity. This is the first report of antiviral activity for prenylated dihydroquinolone derivatives.

Table 2. Antiviral Activities of 1−4 against RSV

compound	IC_{50} (μM)	TC_{50} (μM)	TC_{50}/IC_{50}
1	>50		
2	0.042	22	520
3	>50		
4	6.6	27	4
ribavirin[a]	20	>3000	>150

[a]Ribavirin was used as a positive control.

In summary, two new prenylated dihydroquinolone derivatives, 22-*O*-(*N*-Me-L-valyl)aflaquinolone B (**1**) and 22-*O*-(*N*-Me-L-valyl)-21-*epi*-aflaquinolone B (**2**), were isolated from the gorgonian-derived fungus *Aspergillus* sp. XS-20090B15. In contrast to all known prenylated dihydroquinolone derivatives, compounds **1** and **2** have a terpenoid side chain modified with an *N*-Me-L-Val residue via an ester bond. Compound **2** displayed prominent anti-RSV activity and a high therapeutic ratio. This is the first report of prenylated dihydroquinolone derivatives with potent antiviral activity. Compound **2** might have the opportunity to be further developed as a lead compound for anti-RSV drug discovery.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were obtained on a Beckman DU 640 spectrophotometer. The ECD spectrum was recorded on a JASCO J-810 circular dichroism spectrometer. IR spectra were recorded on a Nicolet-Nexus-470 spectrometer using KBr pellets. NMR spectra were acquired on a JEOL JEM-ECP NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) and an Agilent DD2 500 MHz NMR spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), using TMS as an internal standard. ESIMS and HRESIMS spectra were measured on a Micromass Q-TOF spectrometer. Semipreparative HPLC was performed on a Waters 1525 system using a C_{18} (Kromasil, 5 μm, 250 × 10 mm) column coupled with a Waters 2996 photodiode array detector. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200−300 mesh), Sephadex LH-20 (Amersham Biosciences), and octadecylsilyl silica gel (Unicorn; 45−60 μm) were used for column chromatography. Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin-layer chromatography.

Fungal Material. The isolation and identification of *Aspergillus* sp. XS-20090B15 have been described previously (GenBank HM991281).[5]

Fermentation, Extraction, and Isolation. The fungus *Aspergillus* sp. XS-20090B15 was cultivated in a rice medium (12 g of natural sea salt (from Yangkou saltern, China), 100 g of rice, 0.6 g of peptone, 100 mL of H_2O) in 1 L Erlenmeyer flasks for 35 days at room temperature (rt). The fermented rice substrate (20 flasks) was extracted repeatedly

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with EtOAc (3 × 300 mL for each flask), and the solvent was combined and concentrated *in vacuo* to afford a residue (17 g), which was subjected to silica gel column chromatography (CC) using a step gradient elution with EtOAc–petroleum ether (0–100%) and then with MeOH–$CHCl_3$ (0–100%) to provide six fractions (Fr.1–Fr.6). Fr.3 and Fr.4 exhibited evident antiviral activities against RSV. Fr.3 was subjected to Sephadex LH-20 CC eluting with a mixture of $CHCl_3$–MeOH (v/v, 1:1) and further purified by HPLC eluting with 75% MeOH–(H_2O + 0.2% TFA) to afford **3** (17.2 mg) and **4** (10.5 mg). Fr.4 was subjected to silica gel CC using gradient elution with petroleum ether–EtOAc, then was subjected to an ODS column eluting with 80% MeOH–H_2O, and finally was purified by HPLC eluting with 72% MeOH–(H_2O + 0.2% TFA) to give **1** (6.5 mg) and **2** (3.6 mg).

*22-O-(N-Me-L-valyl)aflaquinolone B (**1**):* pale yellow, amorphous powder; $[\alpha]^{25}_D$ +50 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.8), 235 (3.3), 276 (2.8), 322 (2.7) nm; CD (0.27 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (21.0), 253 (−27.7), 280 (11.5), 318 (6.7) nm; IR (KBr) ν_{max} 3438, 3215, 2860, 1691, 1684, 1636, 1365, 1350, 978 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), Table 1; ESIMS *m*/*z* 551 $[M + H]^+$; HRESIMS *m*/*z* 551.3118 $[M + H]^+$ (calcd for $C_{32}H_{43}N_2O_6$, 551.3116).

*22-O-(N-Me-L-valyl)-21-epi-aflaquinolone B (**2**):* pale yellow, amorphous powder; $[\alpha]^{25}_D$ +15 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.9), 234 (3.3), 278 (2.8), 325 (2.6) nm; CD (0.20 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 222 (20.0), 252 (−20.5), 280 (6.8), 320 (4.5) nm; IR (KBr) ν_{max} 3450, 3222, 2857, 1690, 1684, 1636, 1365, 1345, 977 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), Table 1; ESIMS *m*/*z* 551 $[M + H]^+$; HRESIMS *m*/*z* 551.3111 $[M + H]^+$ (calcd for $C_{32}H_{43}N_2O_6$, 551.3116).

*Aflaquinolone A (**3**):* white, amorphous powder; $[\alpha]^{25}_D$ +16 (*c* 0.20, MeOH) (lit. $[\alpha]^{21}_D$ +14 (*c* 0.19, MeOH)).[6]

*Aflaquinolone D (**4**):* white, amorphous powder; $[\alpha]^{25}_D$ −18 (*c* 0.15, MeOH) (lit. $[\alpha]^{25}_D$ −10 (*c* 0.10, MeOH)).[6]

Hydrolysis of 1 and 2 and Marfey's Analysis of 1 and 2.[12] To a solution of **1** (2.0 mg) or **2** (1.0 mg) in MeOH (0.5 mL) was added a newly prepared NaOH in MeOH solution (1.0 M, pH 9–10). The mixture was stirred at 40 °C for 15–20 min and neutralized with Dowex H^+ resin to pH 7.0 and then filtered. The filtrate of **1** was concentrated and divided into two parts. The major part was purified by HPLC to afford the secondary alcohol-containing portion of **1** (**1a**, 1.1 mg). In addition, the other part was dissolved in H_2O (50 μL), and a 1% solution of FDAA (200 μL) and 1.0 M $NaHCO_3$ (40 μL) were added. The reaction mixture was heated at 45 °C for 1.0 h and then cooled. The mixture was acidified with 2.0 M HCl (20 μL). Separately, the standard amino acids *N*-Me-L-Val and *N*-Me-DL-Val and the hydrolysate of **2** were derivatized with FDAA in the same manner as that of **1**. All FDAA derivatives were analyzed by HPLC using MeCN–(H_2O + 0.2% TFA) as the mobile phase (see Supporting Information).

*Hydrolysis Product of 1 (**1a**):* pale yellow, amorphous powder; $[\alpha]^{25}_D$ +28 (*c* 0.17, MeOH); ^1H NMR ($CDCl_3$, 500 MHz), see Figure S17; ESIMS *m*/*z* 460 $[M + Na]^+$.

Antiviral Activity Assays. The antiviral activities of **1**–**4** against respiratory syncytial virus were determined by the CPE inhibition assay according to established procedures.[13] The assay results were the average mean of three replicate determinations. Ribavirin was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

The NMR and MS spectra of **1** and **2**, the ^1H NMR spectrum of **1a**, HPLC profiles of FDAA derivatives of the hydrolysates of **1** and **2**, and COSY and key HMBC correlations of **1**. These materials are 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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