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New diterpene pyrone-type compounds, metarhizins A and B, isolated from entomopathogenic fungus, *Metarhizium flavoviride* and their inhibitory effects on cellular proliferation

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ABSTRACT

To date, entomopathogenic fungi have not been extensively examined by natural product chemists. In this study, we isolated novel pyrone diterpene-type compounds, metarhizins A (**1**) and B (**2**), from methanol extracts of entomopathogenic fungus, *Metarhizium flavoviride*. They showed potent and selective antiproliferative activity against both insect and human cancer cell lines. These results indicate that metarhizins A (**1**) and B (**2**) can be used as novel lead compounds for anti-cancer agents and probes for cell cycle regulation. To further investigate the structural requirements for this inhibitory activity, we synthesized many metarhizin derivatives and evaluated their antiproliferative activities.

1. Introduction

Entomopathogenic fungi infect insects through the cuticle, grow as hyphal bodies or hyphae in the hemocoel, and cause host death by nutritional destruction of tissues and producing toxic metabolites and pathogenic enzymes.[1,2] The fruiting bodies of some of these fungi have been used as crude tonics and antitussives.[3] Some biologically active compounds with insecticidal, antituberculous, and immunosuppressive activities have been isolated from them.[4,5] However, to date entomopathogenic fungi have not been extensively studied by natural product chemists.

We focused on entomopathogenic fungi as a resource for novel biologically active compounds and studied the diversity of their secondary metabolites.[6,7] After screening entomopathogenic fungi for toxic effects on the insect cell line, *Drosophila* S2 cells, a methanol extract of mycelium of *Metarhizium flavoviride*, was found to potently inhibit cell proliferation. *M. flavoviride* has been used for insect pest control.[8] Pyrone diterpene-type compounds, viridoxins A (**4**) and B (**5**), were isolated from this fungus as potent insect toxins.[9]

This paper reports on the isolation and structural elucidation of novel pyrone diterpene-type compounds, metarhizins A (**1**) and B (**2**), from *M. flavoviride*, and their antiproliferative activities on both insect and human cancer cell lines (Fig. 1). Additionally, we synthesized many metarhizin derivatives, evaluated their antiproliferative activities, and discuss the structural requirements for this activity.

2. Results and discussion

2.1. Isolation

M. flavoviride was cultivated in Czapek-Dox medium supplemented with peptone and yeast extract. The mycelial cake of culture broth (38.6 L) was extracted three times with methanol at room temperature to yield the extract (89.8 g), which inhibited (IC_{50} 1–3 μg/mL) *Drosophila* S2 cell proliferation. Bioactivity-guided fractionation of the ethyl acetate solubles (9.86 g) in the extract by chromatography over a silica gel column and GPC column yielded metarhizins A (**1**) (45 mg) and B (**2**) (90 mg) and a known 3-desacyl compound **3** (244 mg).[10]

2.2. Structural elucidation

HREIMS (*m*/*z* 542.3607 [M^+]), ^1H, and ^{13}C NMR spectra indicated the molecular formula of **1** as $C_{33}H_{50}O_6$. The ^{13}C NMR spectrum of **1** showed the presence of two ester carbonyl, eight olefinic, two

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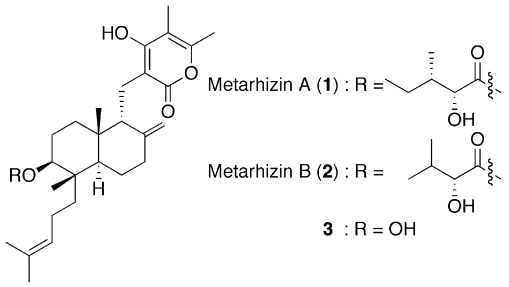


Figure 1. Structures of metarhizins A (**1**) and B (**2**), and **3**.

oxymethine, two quaternary, three methine, eight methylene, and eight methyl carbons (Table 1). ^{1}H–^{1}H COSY revealed that C-1–C-2–C-3, C-5–C-6–C-7, C-9–C-19, C-11–C-12–C-13, and C-2″–C-3″(–C-6″)–C-4″–C-5″ were connected. In the HMBC spectrum, the correlations for H_3-17 to C-3, C-4, and C-5; H_3-20 to C-1, C-5, C-9, and C-10; H_2-18 to C-7 and C-9; and H_2-7 and H-9 to C-8 indicated a substituted decalin skeleton (Fig. 2). HMBC correlations were observed for H-5 and H_3-17 to C-11; H_3-15 and H_3-16 to C-13 and C-14, suggesting that a prenyl group was attached to C-11. The existence of a 2-hydroxy-3-methylpentanoyloxy group at C-3 was indicated by the correlations for H-3 and H-2″ to C-1″. The correlational peaks for H-19 to C-2′, C-3′, and C-4′; H_3-7′ to C-4′, C-5′, and C-6′; and H_3-8′ to C-5′ and C-6′ suggested that a 5,6-dimethyl-4-hydroxy-α-pyrone moiety was attached to C-19. The maximum UV absorption of **1** at 292 nm (ε 8300) supported the existence of an α-pyrone ring,[11] although compound **1** seemed to be in equilibrium between two tautomers, 4-hydroxy-α-pyrone and 2-hydroxy-γ-pyrone. Therefore, the planar structure of **1** was deduced to be a diterpene pyrone-type compound.

Table 1
^{13}C and ^{1}H NMR spectral data of metarhizins A (**1**) and B (**2**)[a]

	Metarhizin A (**1**)		Metarhizin B (**2**)	
	^{13}C	^{1}H	^{13}C	^{1}H
1	34.2	1.26–1.41[b] (1H, m), 1.96–2.04[c] (1H, m)	34.2	1.25–1.41[f] (1H, m), 1.95–2.03[g] (1H, m)
2	24.6	1.83–1.88 (1H, m), 1.88–1.96 (1H, m)	24.6	1.76–1.86[h] (1H, m), 1.87–1.95 (1H, m)
3	76.6	5.15 (1H, dd, *J*=11.0, 5.0 Hz)	76.6	5.13 (1H, dd, *J*=10.9, 5.1 Hz)
4	40.4		40.3	
5	39.7	1.96–2.04[c] (1H, m)	39.6	1.95–2.03[g] (1H, m)
6	23.1	1.26–1.41[b] (1H, m), 1.52–1.57 (1H, m)	23.1	1.25–1.41[f] (1H, m), 1.52–1.57 (1H, m)
7	31.5	2.21–2.25 (1H, m), 2.84 (1H, td, *J*=13.5, 5.5 Hz)	31.5	2.20–2.24 (1H, m), 2.83 (1H, td, *J*=13.5, 5.4 Hz)
8	149.4		149.4	
9	55.5	2.66 (1H, dd, *J*=11.4, 4.6 Hz)	55.5	2.65 (1H, dd, *J*=11.4, 4.2 Hz)
10	37.9		37.9	
11	38.2	1.26–1.41[b] (2H, m)	38.1	1.25–1.41[f] (2H, m)
12	22.1	1.74–1.82[d] (1H, m), 2.06–2.11[e] (1H, m)	22.1	1.76–1.86[h] (1H, m), 2.04–2.10 (1H, m)
13	125.1	5.02–5.06 (1H, m)	125.1	5.01–5.04 (1H, m)
14	131.0		131.0	
15	17.6	1.54 (3H, s)	17.5	1.53 (3H, s)
16	25.7	1.62 (3H, s)	25.8	1.61 (3H, s)
17	18.4	0.95 (3H, s)	18.4	0.94 (3H, s)
18	110.1	4.68 (1H, br s), 4.73–4.75 (1H, m)	110.1	4.66 (1H, br s), 4.74 (1H, br s)
19	22.9	2.98 (1H, dd, *J*=13.1, 4.5 Hz), 3.23 (1H, dd, *J*=13.1, 11.7 Hz)	22.8	2.96 (1H, dd, *J*=13.0, 4.4 Hz), 3.22 (1H, dd, *J*=13.0, 11.5 Hz)
20	23.3	1.04 (3H, s)	23.3	1.03 (3H, s)
2′	165.4		165.4	
3′	103.6		103.6	
4′	166.0		166.0	
5′	107.3		107.3	
6′	155.1		155.1	
7′	11.0	1.92 (3H, s)	11.0	1.91 (3H, s)
8′	17.2	1.98 (3H, s)	17.2	1.98 (3H, s)
1″	174.9		174.5	
2″	73.9	4.53 (1H, d, *J*=3.9 Hz)	76.0	4.34 (1H, d, *J*=4.7 Hz)
3″	39.3	2.06–2.11[e] (1H, m)	32.8	2.28–2.34 (1H, m)
4″	26.5	1.41–1.50 (1H, m), 1.74–1.82[d] (1H, m)	19.3	1.16 (3H, d, *J*=6.9 Hz)
5″	12.1	0.98 (3H, t, *J*=7.4 Hz)	17.4	1.14 (3H, d, *J*=6.8 Hz)
6″	14.3	1.14 (3H, d, *J*=6.8 Hz)		

[a] Frequency: 600 MHz for ^{1}H and 150 MHz for ^{13}C in pyridine-d_5.
[b] These signals are overlapped.
[c] These signals are overlapped.
[d] These signals are overlapped.
[e] These signals are overlapped.
[f] These signals are overlapped.
[g] These signals are overlapped.
[h] These signals are overlapped.

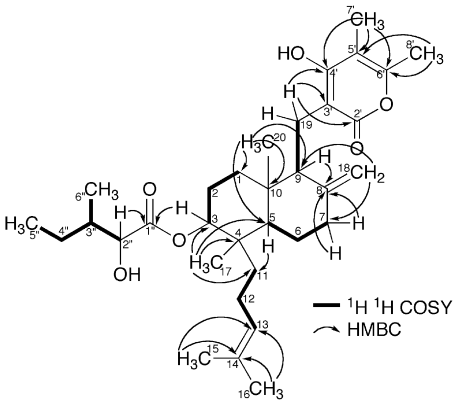
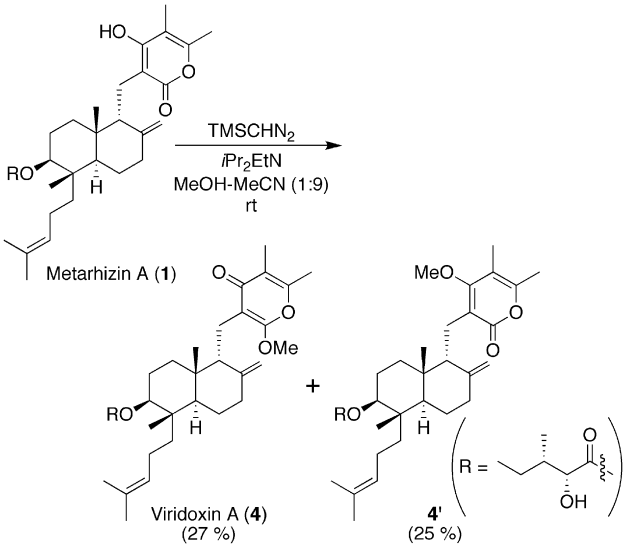


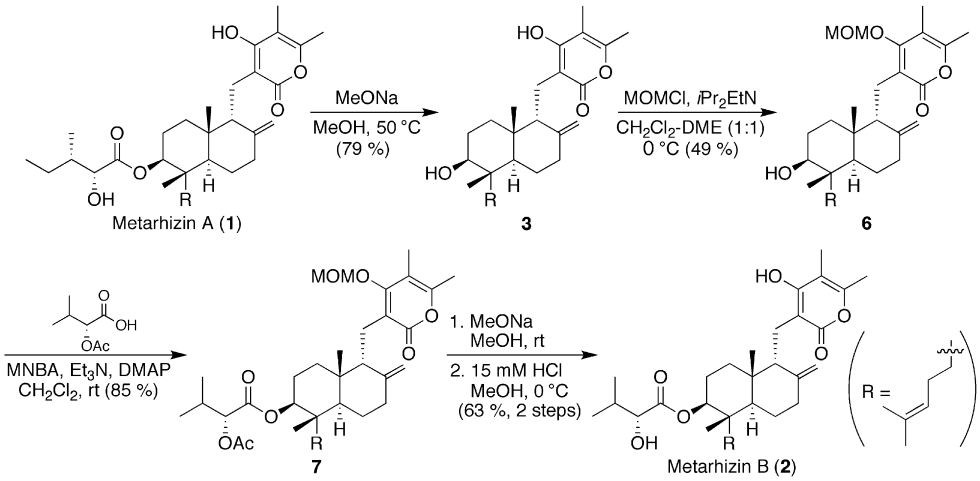
Figure 2. Planar structure of metarhizin A (**1**).

The HREIMS of **2** (*m*/*z* 528.3451 [M$^+$]) gave the molecular formula, $C_{32}H_{48}O_6$, which differs from that of **1** by a methylene unit. The ^{1}H and ^{13}C NMR spectra of **2** were nearly identical to those of **1**, although compound **2** bore a methyl group (δ_C 17.5, δ_H 1.18 (3H, d, *J*=7.1 Hz)) instead of methyl (δ_C 12.4, δ_H 0.99 (3H, t, *J*=7.5 Hz)) and methylene (δ_C 26.8, δ_H 1.38–1.54 (1H, m) and 1.75–1.88 (1H, m)) groups in **1**. As a result, we concluded that **2** had a 2-hydroxy-3-methylbutanoyloxy group at C-3 instead of the 2-hydroxy-3-methylpentanoyloxy group in **1**.

Compound **3** showed a molecular ion peak at *m*/*z* 428.2927 in HREIMS, suggesting that its molecular formula is $C_{27}H_{40}O_4$. The ^{1}H



Scheme 1. Conversion of metarhizin A (**1**) into viridoxin A (**4**).



Scheme 2. Conversion of metarhizin A (**1**) into metarhizin B (**2**).

and ^{13}C NMR spectra of **3** lacked the signals of a 2-hydroxy-3-methylpropanoyloxy group compared to those of **1**, indicating that compound **3** was a known 3-desacyl derivative previously reported as BR-050.[10]

2.3. Determination of relative and absolute configurations

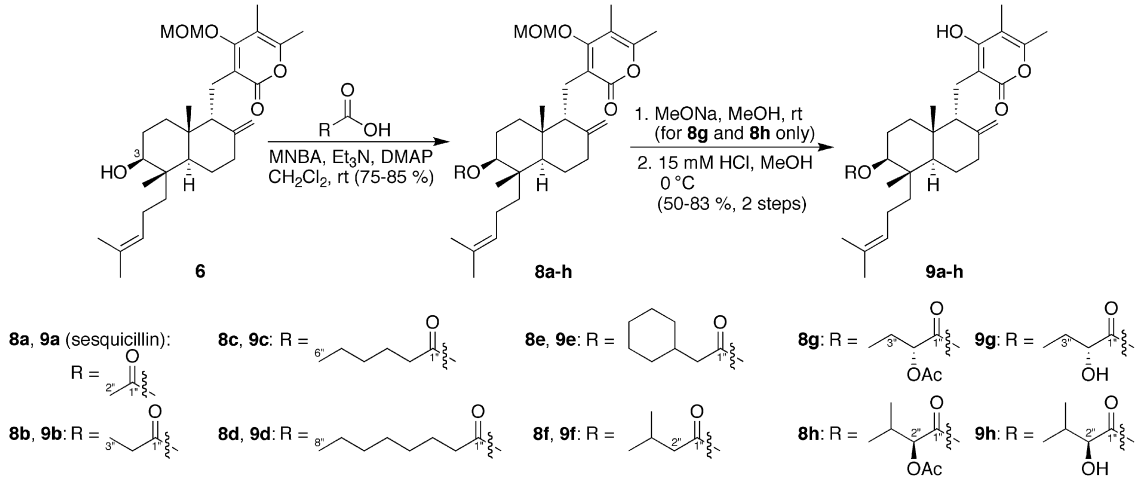
To determine the absolute configurations of metarhizin A (**1**), we converted **1** into viridoxin A (**4**) (Scheme 1).[9] Treating **1** with trimethylsilyldiazomethane in the presence of DIPEA[12] afforded **4** and its α-pyrone-type isomer **4′** due to the tautomerism between α- and γ-pyrone. All of the spectral data on synthetic **4**, including the specific rotation (natural $[\alpha]_D$ −36.5 (*c* 1.51, $CHCl_3$),[9] synthetic $[\alpha]_D$ −26.7 (*c* 0.18, $CHCl_3$)), were identical to those of its natural compound. From these results, the absolute configuration of metarhizin A (**1**) was determined to be 3*S*, 4*S*, 5*R*, 9*R*, 10*R*, 2″*R*, and 3″*S*.

The absolute configuration of metarhizin B (**2**) was determined by converting metarhizin A (**1**) into **2** (Scheme 2). Methanolysis of **1** by sodium methoxide afforded **3**, which showed the same specific rotation ($[\alpha]_D$ −25.7 (*c* 0.19, MeOH)) as that of natural **3** ($[\alpha]_D$ −32.1 (*c* 0.38, MeOH)). The enol in the pyrone ring was protected by MOM group to give α-pyrone **6** in moderate yield, along with a minor γ-pyrone-type isomer, that was easily degraded. Esterification of **6** with (*R*)-2-acetoxy-3-methylbutanoic acid[13] in the presence of 2-methyl-6-nitrobenzoic anhydride (MNBA)[14] produced **7**. Finally, deacetylation of **7** by sodium methoxide and the subsequent deprotection of the MOM group under weak acidic condition (15 mM HCl) allowed us to obtain metarhizin B (**2**). All of the spectral data on synthetic **2**, including the specific rotation (natural $[\alpha]_D$ −35.3 (*c* 0.27, pyridine), synthetic $[\alpha]_D$ −41.4 (*c* 0.30, pyridine)) were identical to those of its natural compound, indicating that the absolute configuration of **2** was 3*S*, 4*S*, 5*R*, 9*R*, 10*R*, and 2″*R*. The fact that ^{1}H and ^{13}C NMR spectra of 2″*S* isomer (**9h**), which is described later in Scheme 3, were different from those of **2** supported the 2″*R* configuration of **2**.

2.4. Inhibitory activity on cell proliferation

We evaluated the inhibitory activities of **1**–**3** on *Drosophila* S2 cells (Table 2). Although compound **3** had only a weak effect, metarhizins A (**1**) and B (**2**) potently inhibited (IC_{50} 23 and 65 nM, respectively) insect cell proliferation, indicating that compounds **1** and **2** may play an important role in the entomopathogenicity of *M. flavoviride*.

The antiproliferative activities of **1**–**3** on four human cancer cell lines were also evaluated (Table 2). Metarhizins A (**1**) and B (**2**)



Scheme 3. Syntheses of 3-acyl group-modified metarhizin derivatives.

Table 2
Antiproliferative activities of metarhizins A (**1**) and B (**2**), and compound **3**

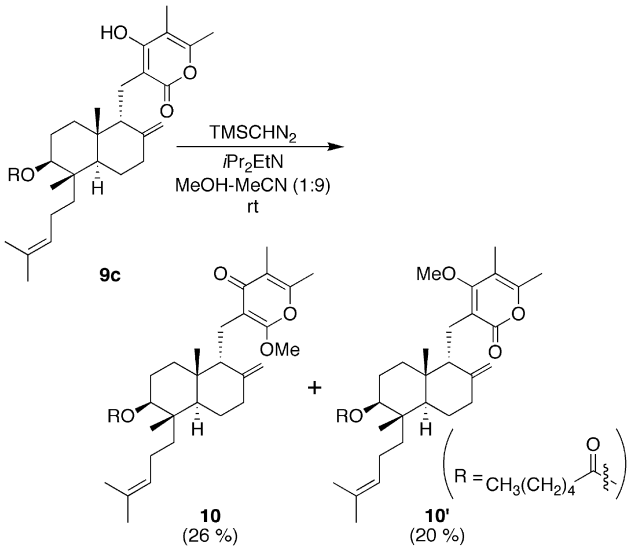
Compound	IC_{50} (μM)				
	S2	K562	THP-1	A549	HCT116
	Drosophila	Leukemia	Leukemia	Lung cancer	Colon cancer
Metarhizin A (**1**)	0.0093	0.043	4.4	0.039	0.031
Metarhizin B (**2**)	0.017	0.086	8.2	0.093	0.067
3	1.3	4.9	24	3.3	33
Doxorubicin	0.065	0.026	0.073	0.065	0.053
Cisplatin	94	>1	31	21	35.2

potently inhibited the proliferation of K562, A549, and HCT116 cells (IC_{50} 43–93 nM), but minimally affected THP-1 cells. This implied that the activities of **1** and **2** were cell type-specific. On the other hand, compound **3** scarcely affected all four cell lines, indicating that a 3-acyl group is essential for this antiproliferative activity.

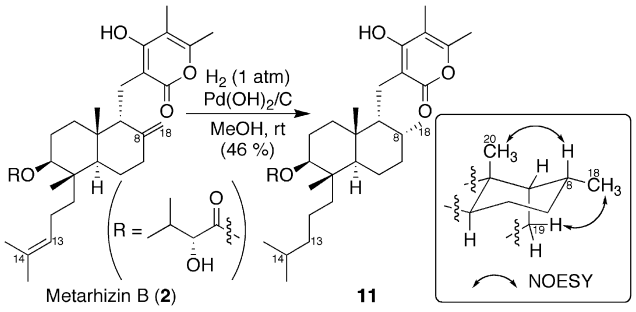
2.5. SAR studies of metarhizin derivatives

To reveal the structural requirements of metarhizins to inhibit cellular proliferation, we synthesized and evaluated several derivatives, particularly 3-acyl group-modified compounds, based on the method of converting **1** into **2**. In Scheme 3, MOM-ether **6** was acylated with acyl chlorides or carboxylic acid and MNBA to give **8a**–**h**. Subsequent deacetylation (if necessary) and removal of the MOM group afforded metarhizin derivatives **9a**–**h**. To find how many carbons in the 3-acyl group are crucial for activity, compounds **9a**–**d**, which bear C2, C3, C6, and C8 acyl groups, respectively, were synthesized. Compound **9a** was previously isolated as sesquicillin from fungus *Acremonium* sp.[15] Cyclohexaneacetate **9e**, with bulkiness added to the acyl group, was also synthesized. Compounds **9f**, **9g** (des-2″-hydroxyl and des-3″-methyl analogs, respectively), and **9h** (2″-epimer) were synthesized to determine the effects of 2″-hydroxyl and 3″-methyl groups on metarhizin B activity (**2**).

In addition to viridoxin A (**4**) and **4′** described in Scheme 1, we synthesized pyrone ring-methylated analogs **10** and **10′** based on 3-hexyl compound **9c** (Scheme 4). Catalytic hydrogenation of **2** resulted in the generation of **11**, in which the absolute configuration at C-8 was determined to be *R* by the correlations for H-8–H_3-20 and H_3-18–H-19 in the NOESY spectrum (Scheme 5).



Scheme 4. Syntheses of **10** and **10′**.



Scheme 5. Synthesis of **11**.

Table 3
Antiproliferative activities of metarhizins A (**1**) and B (**2**), and compound **3**

Compound	IC_{50} (μM)		
	K562	A549	HCT116
	Leukemia	Lung cancer	Colon cancer
Metarhizin A (**1**)	0.043	0.039	0.031
Metarhizin B (**2**)	0.086	0.093	0.067
Sesquicillin (**9a**)	0.46	0.63	0.70
9b	0.45	0.62	0.69
9c	0.27	0.22	0.23
9d	0.093	0.16	0.20
9e	0.095	0.16	0.15
9f	0.15	0.25	0.34
9g	0.35	0.52	0.15
9h	0.26	0.35	0.37
Viridoxin A (**4**)	0.017	0.044	0.043
4′	n.d.[a]	7.0	3.5
10	0.048	0.13	0.10
10′	n.d.[a]	3.7	3.3
11	n.d.[a]	1.0	1.1

[a] Not determined.

The antiproliferative activities of the metarhizin derivatives described above were evaluated on K562, A549, and HCT116 cells (Table 3). Due to the effects of **9a**–**d**, compounds bearing a longer acyl chain at C-3 showed more potent activity, although the inhibitory activity seemed to be saturated at the C8 acyl chain. This indicates that the lipophilicity of the 3-acyl group is crucial for these compounds to inhibit cell proliferation. The activity of cyclohexylacetate **9e** was almost equal to that of **9d**, indicating that the shape of the 3-acyl group is not important. In studies comparing metarhizin B (**2**) and its analogs **9f**–**h**, the 2″-hydroxyl and 3″-methyl groups were not critical for the inhibitory activity. The activities of 4-methoxy-α-pyrones **4′** and **10′** almost disappeared, while those of 2-methoxy-γ-pyrones **4** and **10** were maintained. These results suggest that the γ-pyrone ring is required for antiproliferative activity and that the active form of metarhizins and their analogs may be the γ-pyrone-type tautomer. The much weaker inhibitory activity of **11** compared to metarhizin B (**2**) indicated that either of the 8,18- or 13,14-double bond was critical.

3. Conclusions

To date, several pyrone diterpene-type compounds with various biological activities, including cytotoxic, antimicrobial, insecticidal, and immunosuppressive activities have been isolated from filamentous fungi.[9–11,15,16] Sesquicillin (**9a**), equivalent to the 3-acetyl analog of metarhizins, was previously reported to inhibit cell proliferation via G1 phase arrest in the human breast cancer line, MCF-7.[17] Although the mechanisms of metarhizins A (**1**) and B (**2**)

may be the same as sesquicillin (**9a**), these compounds have much more potent antiproliferative activity, indicating that metarhizins can be used as novel lead compounds for anti-cancer agents and as probes for cell cycle regulation. In addition, the isolation of pyrone diterpene-type compounds including **1** and **2** indicates that entomopathogenic fungi are promising resources of chemically and biologically novel compounds.

4. Experimental section

4.1. General

Analytical TLC was performed on silica gel 60 F_{254}. Column chromatography was carried out on silica gel 60 (70–230 mesh). ^{1}H NMR spectra were recorded at 600 MHz, 500 MHz, or 400 MHz. ^{13}C NMR spectra were recorded at 150 MHz, 125 MHz, or 100 MHz. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (δ_H 0.00) and residual solvent signals (δ_C 77.0, 128.0, and 123.5 for $CDCl_3$, C_6D_6, and pyridine-d_5, respectively) as internal standards.

4.2. Organism and culture conditions

The strain F-778 was isolated from the field cricket, Teleogryllus emma Ohmachi et Matsuura (Orthoptera: Gryllidae) collected at Tsukuba, Ibaraki on October 19, 1989. Based on its conidiogenous structures and conidial morphology, the fungus was identified as *M. flavoviride* Gams & Rozsypal *var. flavoviride* by one of the authors, M. Shimazu. This strain was cultured in a Czapek-Dox medium (sucrose 3%, $NaNO_3$ 0.3%, K_2HPO_4 0.1%, $MgSO_4\cdot 7H_2O$ 0.05%, KCl 0.05%, $FeSO_4\cdot 7H_2O$ 0.001%) supplemented with bacto peptone 0.5% and yeast extract 0.2% at 28 °C for 170 h on a rotary shaker at 150 rpm.

4.3. Isolation of 1–3

The mycelia of *M. flavoviride*, which was obtained from the cultured broth (38.6 L), were extracted three times with methanol (9 L) at room temperature to give the extract (89.8 g). This extract was partitioned with ethyl acetate and water to yield ethyl acetate solubles (9.86 g). The ethyl acetate solubles were chromatographed over SiO_2, and the column eluted with *n*-hexane–ethyl acetate mixtures with increasing polarity to afford hexane–ethyl acetate (2:1) eluent (fraction A, 2.15 g) and hexane–ethyl acetate (1:1) eluent (fraction B, 1.00 g). Fraction A was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give metarhizins A (**1**) (45 mg) and B (**2**) (90 mg). Fraction B was also subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give **3** (244 mg).

Metarhizin A (**1**): colorless amorphous solid; $[\alpha]_D$ −45.2 (*c* 1.01, MeOH); ^{1}H NMR and ^{13}C NMR data are shown in Table 1; EIMS *m*/*z* 542 $[M]^+$, 426, 410, 299, 258, 243, 201, 153 (100%); HREIMS *m*/*z* 542.3570 (542.3607 calcd for $C_{33}H_{50}O_6$).

Metarhizin B (**2**): colorless amorphous solid; $[\alpha]_D$ −32.9 (*c* 0.857, MeOH); $[\alpha]_D$ −35.3 (*c* 0.27, pyridine); ^{1}H NMR and ^{13}C NMR data are shown in Table 1; EIMS *m*/*z* 528 $[M]^+$, 411, 395, 299, 258, 243, 201, 153 (100%); HREIMS *m*/*z* 528.3463 (528.3451 calcd for $C_{32}H_{48}O_6$).

4-Hydroxy-3-(((1*R*,4a*R*,5*S*,6*S*,8a*R*)-6-hydroxy-5,8a-dimethyl-2-methylene-5-(4-methylpent-3-enyl)-decahydronaphthalen-1-yl)-methyl)-5,6-dimethyl-2*H*-pyran-2-one (**3**): colorless amorphous solid; $[\alpha]_D$ −32.1 (*c* 0.380, MeOH); ^{1}H NMR (600 MHz, pyridine-d_5) δ 5.17 (1H, t, *J*=7.0 Hz), 4.76 (1H, br s), 4.70 (1H, br s), 3.84 (1H, dd, *J*=11.1, 4.1 Hz), 3.30–3.35 (1H, m), 3.09–3.13 (1H, m), 2.99–3.05 (1H, m), 2.71 (1H, dd, *J*=11.0, 4.6 Hz), 2.24–2.27 (1H, m), 2.09–2.20 (3H, m), 1.91–2.06 (4H, m), 1.99 (3H, s), 1.95 (3H, s), 1.63–1.70 (1H, m), 1.65 (3H, s), 1.56 (3H, s), 1.37–1.50 (3H, m), 1.12 (3H, s), 1.07 (3H, s); ^{13}C NMR (150 MHz, pyridine-d_5) δ 169.4, 166.3, 154.1, 150.4, 130.5, 126.1, 109.4, 109.0, 102.2, 73.2, 56.1, 41.7, 39.5, 38.34, 38.28, 35.0, 32.0, 28.8, 25.8, 23.7, 23.6, 23.0, 22.3, 18.0, 17.6, 17.2, 11.2; EIMS *m*/*z* 428 $[M]^+$, 395, 299, 257, 153 (100%); HREIMS *m*/*z* 428.2947 (428.2927 calcd for $C_{27}H_{40}O_4$).

4.4. Conversion of metarhizin A (1) into viridoxin A (4) and 4′

To a solution of **1** (14.6 mg, 27 μmol) in MeOH–MeCN (1:9) (2.0 mL) were added *N*,*N*-diisopropylethylamine (20 μL) and 10% tetramethylsilyldiazomethane in hexane (60 μL). After being stirred for 2.5 h at room temperature, the reaction mixture was evaporated off. The residue was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give viridoxin A (**4**) (4.0 mg, 7.2 mmol, 27%) and its α-pyrone-type isomer **4′** (3.8 mg, 6.8 μmol, 25%).

Viridoxin A (**4**): colorless amorphous; $[\alpha]_D$ −26.7 (*c* 0.18, $CHCl_3$); other spectral data are identical with the literature data.[9]

(1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Methoxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl (2*R*,3*S*)-2-hydroxy-3-methylpentanoate (**4′**): colorless amorphous solid; $[\alpha]_D$ −31.4 (*c* 0.164, MeOH); ^{1}H NMR (600 MHz, C_6D_6) δ 5.18–5.23 (1H, m), 5.18 (1H, dd, *J*=11.5, 4.4 Hz), 4.68–4.71 (1H, m), 4.59–4.62 (1H, m), 4.23 (1H, dd, *J*=5.1, 3.0 Hz), 3.18 (3H, s), 2.86 (1H, d, *J*=5.2 Hz), 2.78 (1H, dd, *J*=12.8, 11.7 Hz), 2.63 (1H, dd, *J*=12.8, 4.2 Hz), 2.57–2.62 (1H, m), 2.48 (1H, dd, *J*=11.7, 4.2 Hz), 2.23–2.32 (1H, m), 2.02–2.14 (2H, m), 1.77–1.89 (4H, m), 1.69–1.75 (1H, m), 1.70 (3H, s), 1.67 (3H, s), 1.60–1.66 (1H, m), 1.57 (3H, s), 1.46–1.52 (1H, m), 1.40 (3H, s), 1.25–1.40 (5H, m), 0.96 (3H, s), 0.93 (3H, d, *J*=6.9 Hz), 0.90 (3H, t, *J*=7.4 Hz), 0.86 (3H, s); ^{13}C NMR (150 MHz, C_6D_6) δ 175.4, 167.7, 164.4, 156.1, 148.5, 131.4, 124.9, 114.4, 110.7, 108.0, 77.9, 73.0, 59.8, 55.5, 40.3, 39.5, 39.1, 38.2, 37.7, 34.2, 31.2, 26.3, 25.9, 24.5, 23.1, 22.95, 22.93, 22.2, 18.3, 17.6, 16.8, 13.5, 12.0, 10.1; EIMS *m*/*z* 556 $[M]^+$, 542, 425, 409, 343, 257, 167 (100%); HREIMS *m*/*z* 556.3752 (556.3764 calcd for $C_{34}H_{52}O_6$).

In the similar procedure, compounds **10** (yield 26%) and **10′** (20%) were prepared from **9c**.

(1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((2-Methoxy-5,6-dimethyl-4-oxo-4*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl hexanoate (**10**): colorless oil; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.18–5.22 (1H, m), 5.14–5.17 (1H, m), 4.66 (1H, br s), 4.44 (1H, br s), 3.79 (3H, s), 2.85 (1H, dd, *J*=12.9, 4.3 Hz), 2.77 (1H, dd, *J*=12.9, 11.4 Hz), 2.62–2.70 (1H, m), 2.36 (2H, t, *J*=7.1 Hz), 2.30 (1H, dd, *J*=11.4, 4.3 Hz), 2.27–2.300 (1H, m), 2.16–2.20 (1H, m), 2.02–2.16 (2H, m), 1.95–2.02 (1H, m), 1.96 (3H, s), 1.92 (3H, s), 1.82–1.89 (2H, m), 1.70 (3H, s), 1.58–1.70 (3H, m), 1.66 (3H, s), 1.34–1.45 (4H, m), 1.22–1.31 (4H, m), 1.02 (3H, s), 0.99 (3H, s), 0.83 (3H, t, *J*=7.1 Hz); ^{13}C NMR (125 MHz, pyridine-d_5) δ 179.7, 173.1, 162.9, 155.3, 149.4, 131.0, 125.3, 118.5, 109.7, 103.1, 75.9, 56.1, 55.5, 40.4, 39.4, 38.1, 37.9, 34.7, 34.3, 31.4, 31.1, 25.9, 25.2, 24.6, 23.4, 23.1, 22.6, 22.2, 20.5, 18.6, 17.6, 16.5, 14.0, 10.2; EIMS *m*/*z* 540 $[M]^+$, 525, 509 , 425, 409, 343, 167 (100%); HREIMS *m*/*z* 540.3823 (540.3815 calcd for $C_{34}H_{52}O_5$).

(1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Methoxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl hexanoate (**10′**): colorless oil; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.19–5.23 (1H, m), 5.13–5.16 (1H, m), 4.67 (1H, br s), 4.51 (1H, br s), 3.68 (3H, s), 2.85 (1H, dd, *J*=12.8, 11.7 Hz), 2.77 (1H, dd, *J*=12.8, 4.1 Hz), 2.64–2.71 (1H, m), 2.41 (1H, dd, *J*=11.7, 4.1 Hz), 2.38 (2H, t, *J*=7.2 Hz), 2.21–2.29 (1H, m), 2.16–2.21 (1H, m), 2.04–2.13 (1H, m), 1.89–1.99 (2H, m), 1.94 (3H, s), 1.72 (3H, s), 1.82–1.89 (2H, m), 1.71 (3H, s), 1.63–1.70 (2H, m), 1.55–1.61 (1H, m), 1.67 (3H, s), 1.31–1.44 (4H, m), 1.22–1.31 (4H, m), 1.02 (3H, s), 0.99 (3H, s), 0.84 (3H, t, *J*=7.0 Hz); ^{13}C NMR (125 MHz, pyridine-d_5) δ 173.1, 168.4, 165.0, 156.3, 148.8, 131.1, 125.2, 114.0, 110.4, 108.9,

75.7, 60.4, 55.9, 40.3, 39.4, 38.1, 37.8, 34.7, 34.2, 31.4, 31.2, 25.8, 25.1, 24.5, 23.2, 22.9, 22.8, 22.5, 22.1, 18.5, 17.6, 17.1, 14.0, 10.3; EIMS *m*/*z* 540 $[M]^+$, 425, 409, 343, 257, 167 (100%); HREIMS *m*/*z* 540.3832 (540.3815 calcd for $C_{34}H_{52}O_5$).

4.5. Hydrolysis of metarhizin A (1) into 3

To a solution of **1** (482 mg, 0.89 mmol) in MeOH (2.0 mL) was added 28% sodium methoxide in MeOH (4.0 mL, 20.7 mmol). After being stirred for 15 h at 50 °C, the reaction mixture was neutralized with Dowex 50w (H^+ form). The solvent was evaporated, and the residue was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, MeOH) to give **3** (300 mg, 0.70 mmol, 79%).

Synthetic **3**: $[\alpha]_D$ −25.7 (*c* 0.19, MeOH); other spectral data were identical with those of the natural **3**.

4.6. 3-(((1*R*,4a*R*,5*S*,6*S*,8a*R*)-6-Hydroxy-5,8a-dimethyl-2-methylene-5-(4-methylpent-3-enyl)-decahydronaphthalen-1-yl)methyl)-4-(methoxymethoxy)-5,6-dimethyl-2*H*-pyran-2-one (6)

To a solution of **3** (300 mg, 0.70 mmol) in CH_2Cl_2–DME (1:1) (2.0 mL) were added *N*,*N*-diisopropylethylamine (250 μL, 1.43 mmol) and chloromethyl methyl ether (53 μL, 0.70 mmol). After being stirred for 1 h at 0 °C, the reaction mixture was poured into saturated ammonium chloride solution and extracted with EtOAc three times. The combined organic layer was washed with H_2O and brine, dried over sodium sulfate, and evaporated. The residue was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give **6** (162 mg, 0.34 mmol, 49%).

Compound **6**: colorless amorphous solid; ^{1}H NMR (600 MHz, pyridine-d_5) δ 5.73 (1H, br s), 5.21–5.26 (1H, m), 5.10 (1H, d, *J*=6.0 Hz), 5.08 (1H, d, *J*=6.0 Hz), 4.68–4.70 (1H, m), 4.55 (1H, br s), 3.86 (1H, dd, *J*=11.0, 4.1 Hz), 3.61 (3H, s), 3.06 (1H, dd, *J*=13.1, 11.6 Hz), 2.89 (1H, dd, *J*=13.1, 4.4 Hz), 2.71 (1H, td, *J*=13.6, 5.4 Hz), 2.55 (1H, dd, *J*=11.6, 4.4 Hz), 2.13–2.30 (3H, m), 2.00–2.11 (2H, m), 1.91–1.99 (3H, m), 1.93 (3H, s), 1.77 (3H, d, *J*=0.7 Hz), 1.68 (3H, s), 1.63–1.67 (1H, m), 1.63 (3H, s), 1.38–1.47 (3H, m), 1.08 (3H, s), 1.07 (3H, s); ^{13}C NMR (150 MHz, pyridine-d_5) δ 166.4, 164.8, 156.0, 149.4, 130.7, 125.8, 114.9, 110.2, 108.9, 99.6, 72.8, 57.9, 55.6, 41.6, 39.3, 38.15, 38.14, 35.0, 31.5, 28.6, 25.8, 23.47, 23.44, 23.3, 22.2, 18.1, 17.6, 17.1, 10.9; EIMS: *m*/*z* 472 $[M]^+$, 427, 409, 197, 153, 45 (100%).; HREIMS: *m*/*z* 472.3171 $[M]^+$ (472.3189 calcd for $C_{29}H_{44}O_5$).

4.7. ((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) (*R*)-2-acetoxy-3-methylbutanoate (7)

To a solution of **6** (7.9 mg, 17 μmol) in CH_2Cl_2 (1 mL) were added triethylamine (30 μL), DMAP (3.0 mg), and 2-methyl-6-nitrobenzoic anhydride[14] (51 mg) at 0 °C. After being stirred for 10 min, (*R*)-2-acetoxy-3-methylbutanoic acid[13] (13 mg, 26 μmol) was added to the mixture, which was then stirred for 4 h. The reaction mixture was poured into saturated ammonium chloride solution and extracted with EtOAc three times. The combined organic layer was washed with saturated sodium bicarbonate solution and brine, dried over sodium sulfate, and evaporated. The residue was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give **7** (8.7 mg, 14 μmol, 85%).

Compound **7**: colorless oil; $[\alpha]_D$ −38.0 (*c* 0.872, pyridine); ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.24–5.30 (1H, m), 5.17 (1H, dd, *J*=10.2, 5.0 Hz), 5.11 (1H, d, *J*=5.9 Hz), 5.07 (1H, d, *J*=5.9 Hz), 5.06 (1H, d, *J*=4.6 Hz), 4.68 (1H, br s), 4.54 (1H, br s), 3.59 (3H, s), 2.97 (1H, dd, *J*=12.8, 11.7 Hz), 2.78 (1H, dd, *J*=12.8, 4.2 Hz), 2.68 (1H, td, *J*=13.4, 5.4 Hz), 2.52 (1H, dd, *J*=11.7, 4.2 Hz), 2.12–2.33 (3H, m), 2.11 (3H, s), 1.94 (3H, s), 1.78–1.93 (2H, m), 1.77 (3H, s), 1.71 (3H, s), 1.69 (3H, s), 1.50–1.58 (3H, m), 1.31–1.45 (5H, m), 1.07 (3H, d, *J*=6.9 Hz), 1.04 (3H, d, *J*=6.9 Hz), 1.00 (3H, s), 0.96 (3H, s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 170.5, 169.5, 166.4, 164.8, 156.1, 148.8, 131.0, 125.1, 114.7, 110.5, 109.0, 99.7, 77.4, 77.0, 57.9, 55.3, 40.3, 39.4, 37.8, 37.7, 34.1, 31.2, 30.3, 25.8, 24.3, 23.3, 23.1, 22.9, 22.1, 20.4, 18.6, 18.5, 17.7, 17.6, 17.1, 10.9; EIMS *m*/*z* 614 $[M]^+$, 569, 409, 197, 153 (100%); HREIMS *m*/*z* 614.3832 (614.3819 calcd for $C_{36}H_{54}O_8$).

In the similar procedure, compounds **8a** (yield 78%), **8b** (85%), **8c** (79%), **8d** (75%), **8e** (85%), **8f** (75%), **8g** (85%), and **8h** (77%) were prepared from the corresponding carboxylic acid.

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) acetate (**8a**): colorless oil; ^{1}H NMR (400 MHz, pyridine-d_5) δ 5.16–5.20 (1H, m), 5.06–5.13 (1H, m), 5.09 (1H, d, *J*=5.9 Hz), 5.07 (1H, d, *J*=5.9 Hz), 4.67 (1H, br s), 4.53 (1H, br s), 3.58 (3H, s), 2.98 (1H, dd, *J*=12.8, 11.6 Hz), 2.80 (1H, dd, *J*=12.8, 4.4 Hz), 2.64–2.72 (1H, m), 2.51 (1H, dd, *J*=11.6, 4.4 Hz), 2.18–2.30 (2H, m), 2.01–2.08 (1H, m), 2.04 (3H, s), 1.94 (3H, s), 1.78–1.93 (4H, m), 1.77 (3H, s), 1.68 (3H, s), 1.65 (3H, s), 1.54–1.64 (1H, m), 1.31–1.38 (4H, m), 1.02 (3H, s), 0.95 (3H, s); ^{13}C NMR (100 MHz, pyridine-d_5) δ 170.5, 166.4, 164.8, 156.0, 148.8, 131.2, 125.0, 114.7, 110.5, 109.0, 99.7, 75.9, 57.9, 55.4, 40.3, 39.5, 38.1, 37.8, 34.3, 31.2, 25.7, 24.4, 23.3, 23.2, 22.9, 22.1, 21.0, 18.4, 17.4, 17.1, 10.9; EIMS *m*/*z* 514 $[M]^+$, 409, 197, 153 (base); HREIMS *m*/*z* 514.3281 (514.3294 calcd for $C_{31}H_{46}O_6$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) propionate (**8b**): colorless oil; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.17–5.20 (1H, m), 5.12 (1H, dd, *J*=8.6, 7.1 Hz), 5.10 (1H, d, *J*=6.0 Hz), 5.07 (1H, d, *J*=6.0 Hz), 4.68 (1H, br s), 4.54 (1H, br s), 3.59 (3H, s), 2.98 (1H, dd, *J*=12.9, 11.7 Hz), 2.79 (1H, dd, *J*=12.9, 4.3 Hz), 2.66–2.73 (1H, m), 2.52 (1H, dd, *J*=11.7, 4.3 Hz), 2.35 (2H, q, *J*=7.6 Hz), 2.18–2.29 (2H, m), 2.01–2.08 (1H, m), 1.94 (3H, s), 1.86–1.96 (2H, m), 1.80–1.84 (2H, m), 1.77 (3H, s), 1.69 (3H, s), 1.65 (3H, s), 1.56–1.63 (1H, m), 1.31–1.45 (4H, m), 1.12 (3H, t, *J*=7.6 Hz), 1.03 (3H, s), 0.97 (3H, s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 173.8, 166.4, 164.8, 156.0, 148.9, 131.2, 125.1, 114.7, 110.5, 109.0, 99.7, 75.7, 57.9, 55.4, 40.4, 39.6, 38.2, 37.8, 34.3, 31.3, 28.0, 25.8, 24.5, 23.3, 23.2, 22.9, 22.1, 18.5, 17.5, 17.2, 10.9, 9.5; EIMS *m*/*z* 528 $[M]^+$, 409, 373, 327, 258, 197, 153 (base); HREIMS *m*/*z* 528.3454 (528.3451 calcd for $C_{32}H_{48}O_6$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) hexanoate (**8c**): colorless oil; ^{1}H NMR (400 MHz, pyridine-d_5) 5.18–5.22 (1H, m), 5.12–5.17 (1H, m), 5.10 (1H, d, *J*=6.0 Hz), 5.08 (1H, d, *J*=6.0 Hz), 4.68 (1H, br s), 4.55 (1H, br s), 3.59 (3H, s), 2.98 (1H, dd, *J*=12.8, 11.5 Hz), 2.79 (1H, dd, *J*=12.8, 4.2 Hz), 2.66–2.75 (1H, m), 2.53 (1H, dd, *J*=11.5, 4.2 Hz), 2.37 (2H, t, *J*=7.4 Hz), 2.18–2.31 (2H, m), 2.01–2.12 (1H, m), 1.94 (3H, s), 1.83–2.00 (4H, m), 1.77 (3H, s), 1.70 (3H, s), 1.67 (3H, s), 1.55–1.73 (3H, m), 1.31–1.44 (4H, m), 1.19–1.31 (4H, m), 1.03 (3H, s), 0.99 (3H, s), 0.83 (3H, t, *J*=6.9 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 173.1, 166.4, 164.8, 156.0, 148.9, 131.2, 125.0, 114.7, 110.5, 109.0, 99.7, 75.6, 57.9, 55.4, 40.3, 39.4, 38.1, 37.8, 34.7, 34.3, 31.4, 31.2, 25.8, 25.1, 24.5, 23.3, 23.2, 22.9, 22.5, 22.1, 18.6, 17.5, 17.1, 14.0, 10.9; EIMS *m*/*z* 570 $[M]^+$, 454, 409, 373, 327, 299, 258, 197, 185, 153 (base); HREIMS *m*/*z* 570.3912 (570.3920 calcd for $C_{35}H_{54}O_6$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) octanoate (**8d**): colorless oil; ^{1}H NMR (400 MHz, pyridine-d_5) 5.14–5.22 (2H, m), 5.10 (1H, d, *J*=6.0 Hz), 5.09 (1H, d, *J*=6.0 Hz), 4.69 (1H, br s), 4.55 (1H, br s), 3.60

(3H, s), 3.00 (1H, dd, *J*=12.8, 11.6 Hz), 2.80 (1H, dd, *J*=12.8, 4.4 Hz), 2.66–2.75 (1H, m), 2.53 (1H, dd, *J*=11.6, 4.4 Hz), 2.39 (2H, t, *J*=7.3 Hz), 2.18–2.33 (2H, m), 2.02–2.12 (1H, m), 1.93 (3H, s), 1.82–1.99 (4H, m), 1.76 (3H, s), 1.70 (3H, s), 1.67 (3H, s), 1.55–1.73 (3H, m), 1.19–1.44 (12H, m), 1.03 (3H, s), 0.99 (3H, s), 0.83 (3H, t, *J*=7.0 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 173.2, 166.5, 164.8, 156.0, 148.9, 131.2, 125.0, 114.7, 110.5, 109.0, 99.7, 75.6, 57.9, 55.4, 40.3, 39.5, 38.1, 37.8, 34.8, 34.3, 31.8, 31.2, 29.3, 29.2, 25.8, 25.5, 24.5, 23.3, 23.2, 22.9, 22.8, 22.1, 18.6, 17.5, 17.1, 14.2, 10.9; EIMS *m*/*z* 598 [M]$^+$, 454, 409, 373, 327, 299, 258, 197, 185, 153 (base); HREIMS *m*/*z* 598.4212 (598.4233 calcd for $C_{37}H_{58}O_6$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) cyclohexylacetate (**8e**): colorless oil; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.17–5.21 (1H, m), 5.12–5.16 (1H, m), 5.15 (1H, d, *J*=5.9 Hz), 5.08 (1H, d, *J*=5.9 Hz), 4.69 (1H, br s), 4.55 (1H, br s), 3.60 (3H, s), 2.99 (1H, dd, *J*=12.9, 11.7 Hz), 2.80 (1H, dd, *J*=12.9, 4.4 Hz), 2.66–2.74 (1H, m), 2.54 (1H, dd, *J*=11.7, 4.4 Hz), 2.18–2.30 (2H, m), 2.26 (2H, d, *J*=7.1 Hz), 2.02–2.10 (1H, m), 1.82–1.97 (5H, m), 1.94 (3H, s), 1.71–1.79 (2H, m), 1.77 (3H, s), 1.71 (3H, s), 1.67 (3H, s), 1.51–1.65 (4H, m), 1.31–1.42 (4H, m), 1.16–1.26 (2H, m), 1.04 (3H, s), 1.02–1.10 (1H, m), 1.00 (3H, s), 0.93–0.98 (2H, m); ^{13}C NMR (125 MHz, pyridine-d_5) δ 172.4, 166.4, 164.8, 156.0, 148.9, 131.1, 125.0, 114.7, 110.5, 109.0, 99.7, 75.6, 57.9, 55.4, 42.6, 40.3, 39.5, 38.1, 37.8, 35.3, 34.3, 33.2, 33.1, 31.3, 26.4, 26.33, 26.30, 25.8, 24.5, 23.4, 23.2, 22.9, 22.1, 18.6, 17.6, 17.2, 10.9; EIMS *m*/*z* 596 [M]$^+$, 409, 373, 327, 299, 258, 197, 153 (base); HREIMS *m*/*z* 596.4084 (596.4077 calcd for $C_{37}H_{56}O_6$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) 3-methylbutanoate (**8f**): colorless oil; ^{1}H NMR (400 MHz, pyridine-d_5) δ 5.17–5.23 (1H, m), 5.12 (1H, m), 5.09 (1H, d, *J*=6.0 Hz), 5.07 (1H, d, *J*=6.0 Hz), 4.68 (1H, br s), 4.54 (1H, br s), 3.59 (3H, s), 2.99 (1H, dd, *J*=13.0, 11.6 Hz), 2.80 (1H, dd, *J*=13.0, 4.4 Hz), 2.69 (1H, td, *J*=13.6, 5.3 Hz), 2.52 (1H, dd, *J*=11.6, 4.4 Hz), 2.04–2.25 (6H, m), 1.94 (3H, s), 1.78–1.93 (4H, m), 1.77 (3H, s), 1.69 (3H, s), 1.66 (3H, s), 1.57–1.63 (1H, m), 1.33–1.42 (4H, m), 1.03 (3H, s), 0.98 (3H, s), 0.93 (6H, d, *J*=6.5 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 172.4, 166.4, 164.8, 156.0, 148.9, 131.2, 125.0, 114.7, 110.5, 109.0, 99.7, 75.6, 57.9, 55.4, 43.8, 40.3, 39.5, 38.1, 37.8, 34.3, 31.3, 26.0, 25.8, 24.5, 23.3, 23.2, 22.9, 22.4, 22.3, 22.1, 18.6, 17.6, 17.2, 10.9; EIMS *m*/*z* 556 [M]$^+$, 511, 409, 258, 197, 153 (100%); HREIMS *m*/*z* 556.3754 (556.3764 calcd for $C_{34}H_{52}O_6$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) (2*R*)-2-acetoxybutanoate (**8g**): colorless oil; ^{1}H NMR (400 MHz, pyridine-d_5) δ 5.22–5.29 (1H, m), 5.13–5.17 (2H, m), 5.09 (1H, d, *J*=6.2 Hz), 5.07 (1H, d, *J*=6.2 Hz), 4.67 (1H, br s), 4.53 (1H, br s), 3.59 (3H, s), 2.97 (1H, dd, *J*=13.0, 11.6 Hz), 2.77 (1H, dd, *J*=13.0, 4.2 Hz), 2.67 (1H, td, *J*=13.0, 6.8 Hz), 2.51 (1H, dd, *J*=11.6, 4.2 Hz), 2.15–2.30 (2H, m), 2.05–2.15 (1H, m), 2.10 (3H, s), 1.92–1.97 (1H, m), 1.94 (3H, s), 1.81–1.92 (3H, m), 1.77 (3H, s), 1.70 (3H, s), 1.68 (3H, s), 1.30–1.60 (7H, m), 1.01 (3H, t, *J*=7.4 Hz), 1.00 (3H, s), 0.95 (3H, s); ^{13}C NMR (100 MHz, pyridine-d_5) δ 170.4, 170.1, 166.4, 164.7, 156.1, 148.8, 131.1, 125.1, 114.7, 110.5, 109.0, 99.7, 77.0, 73.9, 57.9, 55.3, 40.3, 39.4, 37.8, 37.7, 34.1, 31.2, 25.8, 24.8, 24.3, 23.3, 23.1, 22.9, 22.1, 20.4, 18.4, 17.6, 17.1, 10.9, 9.5; EIMS *m*/*z* 600 [M]$^+$, 555, 409, 153 (100%); HREIMS *m*/*z* 600.3647 (600.3662 calcd for $C_{35}H_{52}O_8$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-(Methoxymethoxy)-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) (2*S*)-2-acetoxy-3-methylbutanoate (**8h**): colorless oil; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.14–5.26 (2H, m), 5.08–5.11 (1H, m), 5.09 (1H, d, *J*=5.9 Hz), 5.07 (1H, d, *J*=5.9 Hz), 4.67 (1H, br s), 4.53 (1H, br s), 3.59 (3H, s), 2.97 (1H, dd, *J*=12.9, 11.8 Hz), 2.79 (1H, dd, *J*=12.9, 4.3 Hz), 2.69 (1H, td, *J*=13.4, 5.3 Hz), 2.51 (1H, dd, *J*=11.8, 4.3 Hz), 2.29–2.37 (1H, m), 2.12 (3H, s), 2.05–2.28 (3H, m), 1.94 (3H, s), 1.79–1.95 (3H, m), 1.77 (3H, s), 1.69 (3H, s), 1.67 (3H, s), 1.57 (1H, m), 1.30–1.48 (5H, m), 1.08 (3H, d, *J*=7.0 Hz), 1.04 (3H, d, *J*=7.0 Hz), 1.01 (3H, s), 0.95 (3H, s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 170.7, 169.5, 166.4, 164.8, 156.1, 148.8, 131.2, 124.9, 114.7, 110.5, 109.0, 99.7, 77.5, 77.1, 57.9, 55.4, 40.3, 39.3, 37.8, 37.7, 34.2, 31.2, 30.4, 25.8, 24.4, 23.3, 23.1, 22.8, 22.0, 20.4, 18.9, 18.6, 17.7, 17.2, 17.1, 10.9; EIMS *m*/*z* 614 [M]$^+$, 569, 409, 153 (100%); HREIMS *m*/*z* 614.3799 (614.3819 calcd for $C_{36}H_{54}O_8$).

4.8. ((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) (*R*)-2-acetoxy-3-methylbutanoate (metarhizin B (2))

To a solution of **7** (5.5 mg, 8.9 μmol) in MeOH (2.0 mL) was added 28% sodium methoxide in MeOH (10 μL). After being stirred for 2 h at room temperature, the reaction mixture was neutralized with Dowex 50w (H^+ form). The solvent was evaporated off, and the residue was resolved in 15 mM hydrogen chloride solution in MeOH (1.0 mL) at 0 °C. After being stirred for 3 h, this solution was poured into saturated sodium bicarbonate solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give **2** (3.0 mg, 5.6 μmol, 63% from **7**).

Synthetic **2**: $[\alpha]_D$ −41.4 (*c* 0.30, pyridine); other spectral data were identical with those of the natural **2**.

In similar procedure, compounds **9g** (yield 64%) and **9h** (77%) were prepared from **8g** and **8h**, respectively.

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) (2*R*)-2-hydroxybutanoate (**9g**): colorless amorphous solid; ^{1}H NMR (400 MHz, pyridine-d_5) δ 5.13 (1H, dd, *J*=8.9, 7.0 Hz), 5.01–5.06 (1H, m), 4.74 (1H, br s), 4.67 (1H, br s), 4.50 (1H, dd, *J*=6.9, 5.0 Hz), 3.23 (1H, dd, *J*=13.0, 11.2 Hz), 2.97 (1H, dd, *J*=13.0, 4.5 Hz), 2.80–2.88 (1H, m), 2.66 (1H, dd, *J*=11.2, 4.5 Hz), 2.21–2.26 (1H, m), 1.88–2.14 (6H, m), 1.98 (3H, s), 1.91 (3H, s), 1.76–1.84 (2H, m), 1.61 (3H, s), 1.50–1.60 (1H, m), 1.54 (3H, s), 1.25–1.44 (4H, m), 1.15 (3H, t, *J*=7.4 Hz), 1.03 (3H, s), 0.94 (3H, s); ^{13}C NMR (100 MHz, pyridine-d_5) δ 174.8, 165.9, 165.3, 155.0, 149.3, 130.9, 125.1, 110.1, 107.3, 103.6, 76.5, 72.3, 55.5, 40.4, 39.7, 38.2, 38.0, 34.3, 31.6, 28.5, 25.9, 24.6, 23.4, 23.2, 22.9, 22.2, 18.5, 17.6, 17.3, 11.1, 10.0; EIMS *m*/*z* 514 [M]$^+$, 411, 395, 329, 299, 258, 243, 153 (100%); HREIMS *m*/*z* 514.3286 (514.3294 calcd for $C_{31}H_{46}O_6$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) (2*S*)-2-hydroxy-3-methylbutanoate (**9h**): colorless amorphous solid; ^{1}H NMR (400 MHz, pyridine-d_5) δ 5.16 (1H, dd, *J*=8.8, 7.1 Hz), 5.08–5.12 (1H, m), 4.74 (1H, br s), 4.66 (1H, br s), 4.35 (1H, d, *J*=4.2 Hz), 3.22 (1H, dd, *J*=13.0, 11.4 Hz), 2.96 (1H, dd, *J*=13.0, 4.5 Hz), 2.80–2.89 (1H, m), 2.64 (1H, dd, *J*=11.4, 4.5 Hz), 2.30–2.38 (1H, m), 2.21–2.26 (1H, m), 2.05–2.17 (1H, m), 1.90–2.04 (3H, m), 1.97 (3H, s), 1.91 (3H, s), 1.75–1.84 (2H, m), 1.64 (3H, s), 1.53–1.62 (1H, m), 1.55 (3H, s), 1.31–1.44 (3H, m), 1.24–1.31 (1H, m), 1.18 (3H, d, *J*=6.8 Hz), 1.13 (3H, d, *J*=6.8 Hz), 1.03 (3H, s), 0.97 (3H, s); ^{13}C NMR (100 MHz, pyridine-d_5) δ 174.5, 165.9, 165.3, 155.0, 149.3, 130.9, 125.0, 110.1, 107.2, 103.6, 76.4, 76.3, 55.5, 40.3, 39.4, 38.0, 37.8, 34.1, 32.6, 31.5, 25.8, 24.5, 23.3, 23.0, 22.8, 22.0, 19.6, 18.7, 17.7, 17.1, 16.9, 10.9; EIMS *m*/*z* 528 [M]$^+$, 426, 410, 395, 329, 299, 258, 243, 153 (100%); HREIMS *m*/*z* 528.3444 (528.3451 calcd for $C_{32}H_{48}O_6$).

4.9. ((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) acetate (sesquicillin (9a))

Compound **8a** (6.1 mg, 11.9 μmol) was resolved in 15 mM hydrogen chloride solution in MeOH (1.0 mL) at 0 °C. After being stirred for 3 h, this solution was poured into saturated sodium bicarbonate solution and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give **9a** (2.8 mg, 6.0 μmol, 50%). Data for **9a**: colorless amorphous solid; ^{1}H NMR (400 MHz, pyridine-d_5) δ 5.02–5.12 (2H, m), 4.73 (1H, br s), 4.66 (1H, br s), 3.20 (1H, dd, *J*=13.0, 11.5 Hz), 2.96 (1H, dd, *J*=13.0, 4.6 Hz), 2.80–2.88 (1H, m), 2.64 (1H, dd, *J*=11.5, 4.6 Hz), 2.20–2.26 (1H, m), 2.03–2.14 (1H, m), 2.02 (3H, s), 1.98 (3H, s), 1.81–1.96 (3H, m), 1.91 (3H, s), 1.71–1.78 (2H, m), 1.65 (3H, s), 1.56 (3H, s), 1.50–1.58 (1H, m), 1.24–1.42 (4H, m), 1.03 (3H, s), 0.93 (3H, s); ^{13}C NMR (100 MHz, pyridine-d_5) δ 170.3, 165.9, 165.3, 154.9, 149.3, 130.9, 125.1, 110.1, 107.3, 103.6, 76.2, 55.5, 40.3, 39.7, 38.2, 38.0, 34.3, 31.6, 25.9, 24.6, 23.4, 23.1, 22.9, 22.1, 21.1, 18.5, 17.5, 17.3, 11.1; EIMS *m*/*z* 470 [M]$^+$, 329, 299, 258, 243, 153 (100%); HREIMS *m*/*z* 470.3034 (470.3032 calcd for $C_{29}H_{42}O_5$).

In the similar procedure, compounds **9b** (yield 82%), **9c** (82%), **9d** (62%), **9e** (74%), and **9f** (77%) were prepared from **8b**–**f**, respectively.

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) propionate (**9b**): colorless amorphous solid; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.04–5.11 (2H, m), 4.75 (1H, br s), 4.67 (1H, br s), 3.21 (1H, dd, *J*=13.0, 11.7 Hz), 2.96 (1H, dd, *J*=13.0, 4.6 Hz), 2.80–2.87 (1H, m), 2.65 (1H, dd, *J*=11.7, 4.6 Hz), 2.33 (2H, q, *J*=7.5 Hz), 2.20–2.26 (1H, m), 2.04–2.14 (1H, m), 1.98 (3H, s), 1.81–1.96 (3H, m), 1.91 (3H, s), 1.75–1.80 (2H, m), 1.66 (3H, s), 1.57 (3H, s), 1.52–1.59 (1H, m), 1.24–1.43 (4H, m), 1.10 (3H, t, *J*=7.5 Hz), 1.04 (3H, s), 0.94 (3H, s); ^{13}C NMR (500 MHz, pyridine-d_5) δ 173.7, 165.9, 165.3, 155.0, 149.4, 130.9, 125.2, 110.1, 107.2, 103.6, 75.9, 55.5, 40.3, 39.6, 38.2, 37.9, 34.2, 31.5, 28.0, 25.8, 24.5, 23.3, 23.0, 22.8, 22.0, 18.4, 17.4, 17.1, 10.9, 9.5; EIMS *m*/*z* 484 [M]$^+$, 329, 299, 258, 243, 201, 153 (100%); HREIMS *m*/*z* 484.3176 (484.3189 calcd for $C_{30}H_{44}O_5$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) hexanoate (**9c**): colorless amorphous solid; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.07–5.13 (2H, m), 4.74 (1H, br s), 4.67 (1H, br s), 3.22 (1H, dd, *J*=13.0, 11.6 Hz), 2.97 (1H, dd, *J*=13.0, 4.6 Hz), 2.81–2.88 (1H, m), 2.66 (1H, dd, *J*=11.6, 4.6 Hz), 2.35 (2H, t, *J*=7.3 Hz), 2.22–2.26 (1H, m), 2.07–2.15 (1H, m), 1.83–2.01 (3H, m), 1.98 (3H, s), 1.91 (3H, s), 1.77–1.81 (2H, m), 1.67 (3H, s), 1.59–1.68 (2H, m), 1.58 (3H, s), 1.53–1.59 (1H, m), 1.18–1.44 (8H, m), 1.05 (3H, s), 0.97 (3H, s), 0.82 (3H, t, *J*=7.2 Hz); ^{13}C NMR (500 MHz, pyridine-d_5) δ 173.1, 165.9, 165.3, 155.0, 149.4, 130.9, 125.2, 110.1, 107.2, 103.6, 75.8, 55.5, 40.3, 39.6, 38.2, 37.9, 34.7, 34.2, 31.5, 31.4, 25.8, 25.1, 24.5, 23.3, 23.0, 22.8, 22.5, 22.0, 18.5, 17.5, 17.1, 14.0, 10.9; EIMS *m*/*z* 526 [M]$^+$, 426, 410, 393, 299, 258, 243, 201, 153 (100%); HREIMS *m*/*z* 526.3635 (526.3658 calcd for $C_{33}H_{50}O_5$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) octanoate (**9d**): colorless amorphous solid; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.08–5.13 (2H, m), 4.75 (1H, br s), 4.67 (1H, br s), 3.22 (1H, dd, *J*=13.1, 11.3 Hz), 2.97 (1H, dd, *J*=13.1, 4.5 Hz), 2.81–2.89 (1H, m), 2.66 (1H, dd, *J*=11.3, 4.5 Hz), 2.37 (2H, t, *J*=7.3 Hz), 2.22–2.26 (1H, m), 2.07–2.15 (1H, m), 1.85–2.04 (3H, m), 1.98 (3H, s), 1.91 (3H, s), 1.78–1.86 (2H, m), 1.60–1.73 (2H, m), 1.68 (3H, s), 1.54–1.60 (1H, m), 1.59 (3H, s), 1.14–1.44 (12H, m), 1.05 (3H, s), 0.98 (3H, s), 0.83 (3H, t, *J*=7.0 Hz); ^{13}C NMR (500 MHz, pyridine-d_5) δ 173.1, 165.9, 165.3, 155.0, 149.4, 130.9, 125.2, 110.1, 107.2, 103.6, 75.8, 55.5, 40.3, 39.6, 38.2, 37.9, 34.8, 34.2, 31.8, 31.5, 29.3, 29.2, 25.8, 25.5, 24.6, 23.3, 23.1, 22.9, 22.8, 22.1, 18.5, 17.5, 17.1, 14.2, 11.0; EIMS *m*/*z* 554 [M]$^+$, 426, 410, 395, 393, 329, 299, 258, 243, 153, 149 (100%); HREIMS *m*/*z* 554.3937 (554.3971 calcd for $C_{35}H_{54}O_5$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) cyclohexylacetate (**9e**): colorless amorphous solid; ^{1}H NMR (500 MHz, pyridine-d_5) δ 5.07–5.13 (2H, m), 4.75 (1H, br s), 4.67 (1H, br s), 3.22 (1H, dd, *J*=13.0, 11.6 Hz), 2.97 (1H, dd, *J*=13.0, 4.6 Hz), 2.81–2.89 (1H, m), 2.66 (1H, dd, *J*=11.6, 4.6 Hz), 2.18–2.25 (1H, m), 2.24 (2H, d, *J*=7.2 Hz), 2.05–2.13 (1H, m), 1.91–2.03 (2H, m), 1.98 (3H, s), 1.76–1.92 (4H, m), 1.91 (3H, s), 1.67–1.74 (2H, m), 1.68 (3H, s), 1.50–1.62 (4H, m), 1.58 (3H, s), 1.23–1.43 (4H, m), 1.13–1.23 (2H, m), 1.02–1.10 (1H, m), 1.05 (3H, s), 0.98 (3H, s), 0.89–0.97 (2H, m); ^{13}C NMR (500 MHz, pyridine-d_5) δ 172.3, 165.9, 165.3, 155.0, 149.4, 130.9, 125.1, 110.1, 107.2, 103.6, 75.8, 55.5, 42.6, 40.3, 39.5, 38.1, 37.9, 35.3, 34.2, 33.1, 33.0, 31.5, 26.4, 26.3, 26.2, 25.8, 24.6, 23.3, 23.0, 22.8, 22.0, 18.6, 17.6, 17.1, 11.0; EIMS *m*/*z* 552 [M]$^+$, 410, 395, 393, 329, 299, 258, 243, 153 (100%); HREIMS *m*/*z* 552.3787 (552.3815 calcd for $C_{35}H_{52}O_5$).

((1*S*,2*S*,4a*R*,5*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a-dimethyl-6-methylene-1-(4-methylpent-3-enyl)decahydronaphthalen-2-yl) 3-methylbutanoate (**9f**): colorless amorphous solid; ^{1}H NMR (400 MHz, pyridine-d_5) δ 5.03–5.12 (2H, m), 4.74 (1H, br s), 4.67 (1H, br s), 3.21 (1H, dd, *J*=13.1, 11.4 Hz), 2.96 (1H, dd, *J*=13.1, 4.6 Hz), 2.80–2.89 (1H, m), 2.64 (1H, dd, *J*=11.4, 4.6 Hz), 2.21–2.28 (1H, m), 2.22 (2H, d, *J*=6.7 Hz), 2.07–2.20 (2H, m), 1.98 (3H, s), 1.85–2.02 (3H, m), 1.91 (3H, s), 1.75–1.82 (2H, m), 1.66 (3H, s), 1.57 (3H, s), 1.50–1.58 (1H, m), 1.26–1.44 (4H, m), 1.04 (3H, s), 0.96 (3H, s), 0.92 (6H, d, *J*=6.5 Hz); ^{13}C NMR (100 MHz, pyridine-d_5) δ 172.4, 165.9, 165.3, 155.0, 149.4, 130.9, 125.1, 110.1, 107.2, 103.6, 75.8, 55.5, 43.8, 40.3, 39.5, 38.1, 37.9, 34.2, 31.5, 25.9, 25.8, 24.5, 23.3, 23.0, 22.8, 22.4, 22.3, 22.0, 18.5, 17.5, 17.1, 10.9; EIMS *m*/*z* 512 [M]$^+$, 410, 395, 329, 299, 258, 243, 201, 153 (100%); HREIMS *m*/*z* 512.3499 (512.3502 calcd for $C_{32}H_{48}O_5$).

4.10. ((1*S*,2*S*,4a*R*,5*R*,6*R*,8a*R*)-5-((4-Hydroxy-5,6-dimethyl-2-oxo-2*H*-pyran-3-yl)methyl)-1,4a,6-trimethyl-1-(4-methylpentyl)decahydronaphthalen-2-yl) (2*R*)-2-hydroxy-3-methylbutanoate (11)

Metarhizin B (**2**) (10.9 mg, 20.5 μmol) and 20% Pd(OH)$_2$ on carbon (6.3 mg) in MeOH (2.0 mL) were stirred at room temperature for 9 h under hydrogen atmosphere. After filtration, the filtrate was evaporated. The residue was subjected to recycle preparative HPLC (column, JAIGEL-GS310 (ϕ 20.0 mm×500 mm); solvent, EtOAc) to give **11** (5.0 mg, 9.4 μmol, 46%). Data for **11**: colorless amorphous solid; ^{1}H NMR (600 MHz, pyridine-d_5) δ 5.19 (1H, dd, *J*=8.8, 7.2 Hz), 4.35 (1H, d, *J*=4.8 Hz), 2.94 (1H, dd, *J*=14.8, 5.9 Hz), 2.71 (1H, dd, *J*=14.8, 4.3 Hz), 2.28–2.35 (1H, m), 2.23–2.28 (1H, m), 2.16–2.19 (1H, m), 2.08–2.15 (1H, m), 2.01 (3H, s), 1.91 (3H, s), 1.78–1.86 (2H, m), 1.72–1.77 (1H, m), 1.23–1.51 (9H, m), 1.22 (3H, d, *J*=6.8 Hz), 1.15–1.23 (1H, m), 1.15 (3H, d, *J*=6.8 Hz), 1.13 (3H, d, *J*=6.8 Hz), 1.11 (3H, s), 0.99–1.05 (2H, m), 0.96 (3H, s), 0.75 (3H, d, *J*=6.6 Hz), 0.73 (3H, d, *J*=6.6 Hz); ^{13}C NMR (150 MHz, pyridine-d_5) δ 174.5, 165.1, 165.0, 155.0, 107.2, 106.4, 76.7, 76.1, 51.5, 40.2, 39.8, 39.4, 38.7, 37.9, 34.3, 32.8, 31.1, 30.2, 27.5, 24.6, 23.6, 22.9, 22.5, 22.0, 20.4, 20.3, 19.2, 19.1, 18.1, 17.3, 17.2, 11.2; EIMS *m*/*z* 532 [M]$^+$, 415, 399, 329, 301, 275, 261, 153 (100%); HREIMS *m*/*z* 532.3762 (532.3764 calcd for $C_{32}H_{52}O_6$).

4.11. Assay for cell proliferation in K562 cells

Human leukemia K562 cells were maintained at 37 °C (5% CO_2) in tissue culture dishes filled with a growth medium (an RPMI1640

medium with 10% fetal bovine serum, 25 μg/mL penicillin, and 50 μg/mL streptomycin; designated RPMI). For the assay for cell growth, K562 cells were incubated in a 12-well plate, each well containing 1 mL of RPMI ($3–5\times10^4$ cells/mL) in the presence or absence of drugs. After 3 days, 50 μL of Alamar Blue was added to each well, and after 1–2 h incubation at 37 °C (5 CO_2), 150 μL of each of the sample solutions was transferred into a 96-well plate, and absorbance at 570 nm (reference at 595 nm) was measured. A cell number was given as percentage of the control absorbance.

4.12. Assay for cell proliferation in A549, THP-1, and HCT116 cells

A549, THP-1, and HCT116 cells were maintained at 37 °C (5% CO_2) in tissue culture dishes filled with a growth medium (an RPMI1640 medium with 10% fetal bovine serum, 1% Antibiotic–Antimycotic (GIBCO), and 2 mM L-glutamic acid; designated RPMI). For the assay for cell growth, cells were incubated in a 96-well plate, each well containing 50 μL of RPMI (2.5×10^3 cells/mL for A549 and HCT116; 2.0×10^4 cells/mL for THP-1) in the presence or absence of drugs. After 3 days, 10 μL of MTT reagent was added to each well, and after 1 h incubation at 37 °C (5% CO_2), absorbance at 450 nm was measured. A cell number was given as percentage of the control absorbance.

4.13. Assay for cell proliferation in S2 cells

S2 cells were maintained at 37 °C (5% CO_2) in tissue culture dishes filled with a growth medium (an Schneider's medium with 20% fetal bovine serum, 1% Antibiotic–Antimycotic (GIBCO); designated RPMI). For the assay for cell growth, cells were incubated in a 96-well plate, each well containing 100 μL of RPMI (2.0×10^4 cells/mL) in the presence or absence of drugs. After 3 day, 10 μL of MTT reagent was added to each well, and after 4 h incubation at 37 °C (5% CO_2), absorbance at 450 nm was measured. A cell number was given as percentage of the control absorbance.

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