Isolation and Biological Activity of Seven New Drimane-Type Sesquiterpenoids from an Earwig-Associated Aspergillus sp. NF2396

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[ABSTRACT] Drimane-type sesquiterpenoids are widely distributed in fungi. From the ethyl acetate extract of the earwig-derived Aspergillus sp. NF2396, seven new drimane-type sesquiterpenoids, named drimanenoids A-G (1-7), were isolated. Their structures were elucidated by diverse spectroscopic analysis including high-resolution ESI-MS, one- and two-dimensional NMR spectroscopy. Drimanenoids A-F (1-6) are new members of drimane-type sesquiterpenoid esterified with unsaturated fatty acid side chain at C-6. The antibacterial and cytotoxic activity of these new compounds were evaluated. Compound 4 exhibited moderate cytotoxicity against human myelogenous leukemia cell line K562 with IC\textsubscript{50} values of 12.88 ± 0.11 \textmu M.

[KEY WORDS] Fungus; Sesquiterpenoids; Drimane-type; Antibacterial; Cytotoxic

Introduction

Sesquiterpenoids with the drimane skeleton are a class of bioactive metabolites produced by diverse fungi, terrestrial plants and marine animals.\textsuperscript{[1, 2]} Previous research has revealed the presence of a number of rare drimane-type sesquiterpenoid esterified with complex fatty acid side chains, mainly from species of Aspergillus.\textsuperscript{[3-6]} This kind of natural products often displayed a wide range of biological and pharmacological activities, including antibacterial, antifungal, cytotoxic, antifeedant and plant-growth regulatory effects,\textsuperscript{[2, 3, 4]} providing excellent chance for new drug discovery. As part of an ongoing program for the discovery of structurally novel and bioactive natural products from microorganisms isolated from specialized ecological niches,\textsuperscript{[3, 4]} we focused on a fungus strain isolated from an earwig collected from Weifang, Shandong Province of P.R. China. Chemical investigation of the secondary metabolites produced by this strain resulted in the isolation and identification of six new drimane-type sesquiterpenoid esters, drimanenoids A-F (1-6), and one new drimane sesquiterpenoid, drimanenoid G (7) (Figure 1). Drimanenoids A-F (1-6) are new members of drimane-type sesquiterpenoid esters with unsaturated fatty acid moieties at C-6 comprising of one, two or three conjugated olefinic double bonds with a terminal carboxyl or methyl group. The antibacterial activities of these new drimane-type sesquiterpenoids against eight Gram-negative bacteria including Vibrio harveyi, Vibrio alginolyticus, Photobacterium damsela, Ralstonia solanacearum, X. oryzae pv. oryzae, X. campestris pv. Mangiferaeindic., E. coli, and two Gram-positive bacteria including M. luteus and methicillin-resistant S. aureus, and cytotoxic effects of these new natural products against five cell lines including human myelogenous leukemia cell line K562, human gastric carcinoma cell line SGC-7901, human hepatoma carcinoma cell line...
Results and Discussion

The EtOAc extract of the fungal culture was separated by a combination of column chromatography and semi-preparative HPLC purification, resulting in the discovery of seven new drimane-type sesquiterpenoid natural products.

Compound 1 was isolated as white solid. Its molecular formula was assigned to be C_{25}H_{28}O_{2} on the basis of HR-ESI-MS at m/z 413.1569 [M + Na]^+ (calcd 413.1571). The ^1H and ^13C NMR data (Tables 1 and 2). The ^1H and HSQC NMR spectra of 1 displayed the signals of three tertiary methyl groups [δ_{H} 1.08 (s, H_{12}-13), 1.08 (s, H_{12}-14), 0.93 (s, H_{12}-15)], five olefinic protons [δ_{H} 5.83 (br s, H-7), 6.27 (d, J = 15.5 Hz, H-2′), 8.35 (dd, J = 15.5, 11.8 Hz, H-3′), 6.82 (t, J = 11.5 Hz, H-4′), 6.02 (d, J = 11.5 Hz, H-5′)], one oxygenated methylene [δ_{H} 4.88 and 4.80 (both d, J = 12.7 Hz, H-12)], one oxygenated methane [δ_{H} 5.60 (br s, H-6)], and one exchangeable proton [δ_{H} 6.30 (s, OH-9)]. The ^13C NMR and DEPT135 spectra exhibited the presence of 21 carbon signals, which could be assigned to three carbonyl groups [δ_{C} 174.3 (C-11), 164.9 (C-2′)] and six olefinic carbons, three oxygenated carbons, three methyls, and six sp^3 carbons. Comparison of these data with those of the reported compound mono(6-strobiactone-B) ester of (E,E)-2,4-hexadienedioic acid (8) [1] suggested that compound 1 had a similar structure of drimane-type sesquiterpenoid ester with differences on the unsaturated fatty acid side chain. This assumption was evidenced by the HMBC and ^1H-^1H COSY experiments (Figure 2). The major distinction was that 1 possessed (2E, 4Z)-hexadienedioic acid which was inferred from the NOESY correlation of H-4′ with H-2′ and H-5′ (Figure 3) and the relatively small coupling constants (J = 11.5 Hz) between H-4′ and H-5′. The relative configuration of the sesquiterpene core in 1 was determined by NOESY experiment. The NOESY correlations of H-5 with H-6 and OH-9 suggested their locations on the same side, whereas the configuration of C-10 was considered to be on the opposite side by comparison of ^13C chemical shifts (δ_{C} 37.3 for 1 vs. δ_{C} 37.3 for 8) [1]. Thus, the structure of drimaneoid A (1) was elucidated as shown in Figure 1.

Compound 2 was obtained as white solid. The molecular formula of C_{25}H_{28}O_{2} was deduced from its HR-ESI-MS spectrum at m/z 415.1724 [M + Na]^+ (calcd 415.1727). Detailed comparison of the ^1H and ^13C NMR data of 2 with those of compound 8 [1] revealed the only difference was the presence of a CHOH [δ_{H} 5.21 (s), 4.83 (brs, OH)], δ_{C} 97.2] instead of a carbonyl group (δ_{C} 174.7) at C-11 in the five-membered ring in 2, indicating the carbonyl group in compound 8 was reduced. This was convinced by the HMBC correlations from H-12 to C-11, and from H-11 to C-8, C-9, and C-10 (Figure 2). The NOESY correlations of H-5 with H-6 and OH-9 and correlations of H-13 with OH-11 (Figure 3) indicated the relative configuration of 2 as shown in Figure 1.

Compound 3 was isolated as white solid. The HRESIMS (m/z 439.1722 [M + Na]^+ , calcd 439.1727) and 1D NMR data indicated the molecular formula to be C_{28}H_{28}O_{2}. Comparison of the NMR data of 3 with those of known compound 8 revealed that they shared the same drimane-type sesquiterpenoid skeleton except for the unsaturated fatty acid side chain, where six conjugated olefinic protons were observed instead of the four present in 8. The ^1H-^1H COSY correlations of H-3′ with H-2′ and H-4′, and of H-6′ with H-5′ and H-7′ in combination with the HMBC correlations of H-2′ with C-1′, of H-3′ with C-5′, of H-7′ with C-8′, and of H-6 with C-1′ (Figure 2) confirmed the presence of a side chain of (2E,4′E,6′E)-7′-carboxyhepta-2′,4′,6′-diene at C-6 through an ester bond. The relative configuration of 3 was determined by NOESY correlations of H-5 with H-6 and OH-9 (Figure 3) and comparison of ^13C NMR data with those of 1 and 2. Therefore, the structure of 3 was assigned as shown in Figure 1.

Compound 4 was isolated to be a white solid and assigned the molecular formula C_{29}H_{30}O_{3} via the HRESIMS (m/z 443.2037 [M + Na]^+ , calcd 443.2040). The ^1H and ^13C
NMR data for compound 4 indicated a structural similarity to those of 1-3 for the change in the side chain. A different side chain was observed, as suggested by a spin system from H-2′ to H-5′ deduced from the ¹H-¹H COSY spectrum. Furthermore, the HMBC correlations of H-3′, H-6 with C-1′, and of H-4′, H2-8′, OH-7′ with C-6′ in combination with the ¹H-¹H COSY correlations of H-7′ with H3-8′ (Figure 2) supported the (2′E)-7′-hydroxy-6-oxyocta-2′-enoyl side chain at C-6. The NOESY (Figure 3) and ¹³C NMR data suggested the relative configuration of the sesquiterpene core in 4 to be same as those of 1 and 3.

Compound 5 was isolated as white solid. The molecular formula was established to be C₂₄H₃₄O₇ via its HR-ESI-MS (m/z 457.2196 [M + Na]+, calcd 457.2197) and 1D NMR data. Careful analysis of the NMR data for 5 suggested its structure as similar as that of 4, exhibiting an additional methoxy group and two additional olefinic groups in the side chain. These were supported by the ¹H-¹H COSY correlations from H-4′ to H-8′ and HMBC correlations from H-3′ to C-1′, and from H3-8′, OCH₃-7′ to C-7′ (Figure 2), indicating the side chain to be (2′E,4′E)-7′-hydroxy-6-methoxyocta-2′,4′-dienoyl. This moiety was connected to C-6 from the HMBC correlation of H-6 to C-1′. The relative configurations of C-6′ and C-7′ on the side chain were suggested as

Table 1 ¹H NMR data (400 MHz) for compounds 1-7 in DMSO-d₆ (δ in ppm, mult, J in Hz)

<table>
<thead>
<tr>
<th>No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.99 td (13.5, 4.0); 1.87 td (13.5, 3.8); 1.98 td (13.6, 4.0); 1.97 td (13.5, 4.1); 1.97 td (13.3, 4.0); 1.86 m; 1.42 m</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.59 m; 1.49 m; 1.58 m; 1.43 m; 1.60 m; 1.49 m; 1.60 m; 1.48 m; 1.59 m; 1.47 m; 1.59 m; 1.45 m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.35 br d (12.6); 1.23 m; 1.33 m; 1.20 m; 1.35 br d (12.1); 1.22 m; 1.34 m; 1.21 m; 1.34 m; 1.23 m; 1.31 m; 1.18 m</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.03 d (4.7); 2.09 d (4.5); 2.03 d (4.8); 2.01 d (4.8); 2.02 d (4.8); 1.99 d (4.2); 1.24 br s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.60 br s; 5.60 br s; 5.60 br s; 5.55 br s; 5.59 br s; 5.57 t (4.3); 4.04 br s</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

6-OH  | 4.57 d (4.1) |       |         |         |         |         |         |
7-OH  | 5.35 d (6.5) |       |         |         |         |         |         |
9-OH  | 6.30 s; 6.28 s; 6.29 s; 6.26 s; 6.28 s; 4.41 s | 3.52 dd (11.3, 3.6); 3.44 dd (11.3, 3.6) | 10.05 s |         |         |         |         |
11-OH | 4.83 br s | 4.63 t (3.6) |       |         |         |         |         |
12-OH | 4.88 d (12.7); 4.39 d (13.0); 4.88 d (12.7); 4.88 d (12.7); 4.88 d (12.8); 4.14 d (5.3); 2.09 s |       |         |         |         |         |         |
13    | 1.08 s; 1.13 s; 1.07 s; 1.04 s; 1.06 s; 1.05 s; 1.17 s; 1.45 s |       |         |         |         |         |         |
14    | 1.08 s; 1.08 s; 1.08 s; 1.06 s; 1.08 s; 1.05 s; 1.16 s |       |         |         |         |         |         |
15    | 0.93 s; 0.93 s; 0.93 s; 0.91 s; 0.93 s; 0.92 s; 0.92 s |       |         |         |         |         |         |

2′    | 6.27 d (15.5); 6.40 overlap; 6.14 d (15.3); 5.84 d (15.7); 6.02 d (15.3); 6.39 overlap |       |         |         |         |         |         |
3′    | 8.35 dd (15.5, 11.8); 7.33 overlap; 7.28 dd (15.3, 9.3); 6.89 m; 7.24 dd (15.3, 11.0); 7.29 overlap |       |         |         |         |         |         |
4′    | 6.82 d (11.5); 7.31 overlap; 6.89 overlap; 2.38 m; 6.45 dd (15.5, 11.0); 7.31 overlap |       |         |         |         |         |         |
5′    | 6.02 d (11.5); 6.36 overlap; 6.91 overlap; 2.74 m; 6.16 dd (15.5, 7.0); 6.35 overlap | 6.27 d (15.5); 6.40 overlap; 6.14 d (15.3); 5.84 d (15.7); 6.02 d (15.3); 6.39 overlap |       |         |         |         |         |         |
6′    | 7.22 dd (15.3, 9.3); 3.49 m | 7.22 dd (15.3, 9.3); 3.49 m |       |         |         |         |         |

6′-COOH | 12.64 (br s) | 12.63 (br s) |       |         |         |         |         |
6′-OCH₃ | 3.21 s |       |         |         |         |         |         |
7′    | 6.08 d (15.3); 4.03 m; 3.61 m | 6.08 d (15.3); 4.03 m; 3.61 m |       |         |         |         |         |
7′-OH  | 5.34 d (5.3) | 5.34 d (5.3) |       |         |         |         |         |
8′    | 1.15 d (6.9); 1.02 d (6.5) | 1.15 d (6.9); 1.02 d (6.5) |       |         |         |         |         |
8′-COOH | 12.52 (br s) | 12.52 (br s) |       |         |         |         |         |
through analysis of the \( \Delta \delta \) value of chemical shifts for C-6' and C-7' \(^{12}\). The NOESY (Figure 3) data indicated the relative configuration of 5 was same as those of 1, 3 and 4.

Compound 6 was isolated as a white solid and found to have the molecular formula C\(_{21}\)H\(_{30}\)O\(_{7}\) in its HR-ESI-MS spectrum (\( m/z \) 417.1884 [M + Na"], caled 417.1884). The 1D NMR data for 6 were comparable to those of 2 with the difference in the replacement of an oxygenated methine group
with an oxygenated methylene group $[\delta_{H}] 3.52$ (dd, $J = 11.3$, 3.6, H$_2$-11), $3.43$ (dd, $J = 11.3$, 3.6, H$_2$-10), $\delta_C$ 61.6] and an additional hydroxy group $[\delta_{H}] 4.83$ (t, $J = 5.3$, OH) in 6, which could be derived from the ring opening of five-membered ring by reduction. This assumption was evidenced by the HMBC correlations from H$_2$-11 to C-8, C-9 and C-10, and from H$_2$-12 with C-7, C-8 and C-9 (Figure 2). Also the HMBC cross-peak between OH-12 and C-8 were observed. The relative configuration of 6 was determined on the basis of biosynthetic consideration and NOESY data.

Compound 7 was isolated as white solid and had the molecular formula C$_{15}$H$_{20}$O$_5$ on the basis of a HR-ESI-MS peak at m/z 251.1656 [M-H]$^-$. Detailed analysis of the $^1$H, $^1$C, DEPT135 and HSQC NMR data of 7 revealed the structure of 7 was closely related to the reported drim-8-en-6b,7a,11-diol [1], possessing a drimane-type sesquiterpenoid skeleton. The major difference was that 7 contains an additional aldehyde group $[\delta_{H}] 10.05$ (1H, s), $\delta_C$ 194.3] instead of a CH group, which locates at C-11 on the basis of the HMBC correlations from H-11 to C-8 and C-10 (Figure 2). Further comprehensive analysis of the HMBC and $^1$H-$^1$H COSY spectra of 7 allowed the determination of the planar structure. The relative configuration of 7 was deduced from the NOESY correlations of H$_2$-15 with H-5 and H-6, of H-6 with H-5 and H-7, of H-5 with H-7, and of H$_2$-13 with H$_2$-14 (Figure 3). Thus, the structure of 7 was elucidated as shown in Figure 1.

The drimane-type sesquiterpenoid esters have been reported to have antibacterial and cytotoxic activities [3]. The new drimane-type sesquiterpenoid esters 1-4 and 6 were assessed for their antibacterial activities against eight Gram-negative bacteria, V. harveyi, V. owensii, V. alginoleticus, P. damselae and R. solanacearum. Meanwhile, compounds 1-4 and 6 were also evaluated for their ability to inhibit the growth of five carcinoma cells, including K562, SGC-7901, BEL-7402, A549 and Hela cell lines using MTT method. Among them, only compound 4 displayed moderate cytotoxicity against K562 cell with IC$_{50}$ value of 12.88 $\pm$ 0.11 $\mu$M (3.08 $\pm$ 0.05 $\mu$M for the positive drug), while the IC$_{50}$ values of other compounds were more than 40 $\mu$M.

**Experimental section**

**General experimental procedures**

The HR-ESI-MS data of the isolated compounds were acquired on an Agilent 6210 TOF LC-MS instrument (Agilent Technologies Inc., Santa Clara, CA, USA). The optical rotations were recorded in methanol on a Rudolph Autopol IV automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). The UV and IR spectra were measured on a Hitachi U-3000 spectrophotometer and a Nexus 870 FT-IR spectrometer, respectively. The 1D and 2D NMR spectra (including $^1$H, $^1$C, DEPT135, HSQC, HMBC, $^1$H-$^1$H COSY and NOESY) were obtained on a Bruker Avance III 400 MHz NMR spectrometer (400 MHz for $^1$H NMR and 100 MHz for $^13$C NMR).

**Table 3** Antibacterial activities of compounds 1-4 and 6 (inhibition diameters: mm)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ML</th>
<th>MRSA</th>
<th>EC</th>
<th>XOO</th>
<th>XCM</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>4.50 ± 0.40</td>
<td>-</td>
<td>1.00 ± 0.81</td>
<td>2.00 ± 0.00</td>
<td>3.66 ± 0.23</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>4.83 ± 0.23</td>
<td>-</td>
<td>3.83 ± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>4.66 ± 0.23</td>
<td>2.66 ± 0.47</td>
<td>4.83 ± 0.47</td>
<td>5.00 ± 0.23</td>
<td>4.00 ± 1.08</td>
</tr>
<tr>
<td>4</td>
<td>2.66 ± 0.47</td>
<td>4.66 ± 0.47</td>
<td>2.00 ± 0.81</td>
<td>1.00 ± 0.00</td>
<td>1.33 ± 0.94</td>
</tr>
<tr>
<td>6</td>
<td>4.66 ± 0.84</td>
<td>5.00 ± 0.00</td>
<td>3.66 ± 0.94</td>
<td>4.75 ± 0.70</td>
<td>4.50 ± 0.70</td>
</tr>
</tbody>
</table>

ML: Micrococcus luteus, MRSA: methicillin-resistant Staphylococcus aureus, EC: E. coli, XOO: Xanthomonas oryzae pv. oryzae, XCM: Xanthomonas campestris pv. mangiferaeindicae; “-” no activity; “*” positive control: 500 μg per disc; Compounds 1-6: 50 μg per disc.
MHz for $^{13}$C NMR). The chemical shifts of $^1$H and $^{13}$C NMR data were given in ppm and referenced to the solvent signal (DMSO-d$_6$, $\delta_{H}$ 2.50 and $\delta_{C}$ 39.52). Coupling constants ($J$) were reported in Hz. Semi-preparative HPLC was performed on Agilent Technologies 1260 Infinity II instrument equipped with an Agilent Eclipse C18 column (5 mm, 250 mm × 9.4 mm). MPLC fractionation was performed on a Biotage Isolera One using a Biotage SNAP Cartridge C18 column (120 g). Sephadex LH-20 gel (Pharmacia Biotech, Sweden) was used for column chromatography (CC). Precoated glass plates (silica gel GF254, 10–20 μm, Qingdao Marine Chemical Factory, Qingdao, China) were employed for thin layer chromatography (TLC).

Fungus material, Cultivation, Fermentation and Isolation

The fungus strain Aspergillus sp. NF2396 was isolated from an earwig collected from the suburb of Weifang, Shandong Province, P. R. China. The strain was cultivated on potato dextrose agar (PDA) medium at 30 °C for 5 days. Then small agar plugs with mycelia were directly inoculated into 1 L Erlenmeyer flasks containing 400 mL ME liquid medium (consisting of 20 g/L malt extract, 20 g/L sucrose, 1 g/L peptone), which were cultivated at 30 °C for 14 days on a rotary shaker at 140 rpm. After fermentation, the broth was filtered to yield the filtrate and biomass. Then the filtrate was extracted with an equal volume of ethyl acetate (EtOAc) three times. The final extraction solvent was combined and evaporated to dryness under reduced pressure to yield crude extract. Then the crude extract was separated by a medium-pressure liquid chromatography (MPLC) system equipped a Biotage SNAP Ultra C18 120 g column using a linear gradient system from 10% to 100% (v/v) methanol/water to give 7 fractions (A-F). After this, the fractions were fractionated by Sephadex LH-20 chromatography (MeOH) and further purified by semi-preparative HPLC. Compounds 1 (2.2 mg, $t_R$ = 21.0 min), 2 (5.4 mg, $t_R$ = 13.5 min), 3 (6.5 mg, $t_R$ = 22.0 min), 4 (3.1 mg, $t_R$ = 23.5 min) and 6 (2.1 mg, $t_R$ = 9.5 min) were purified from the fraction E by semi-preparative HPLC using 50% MeCN in H$_2$O at a flow rate of 2.2 mL/min. Compound 5 (2.4 mg, $t_R$ = 10.5 min) was purified from the fraction F by semi-preparative HPLC using 62% MeCN in H$_2$O at a flow rate of 2.2 mL/min. Compound 7 (8.5 mg, $t_R$ = 14.5 min) was purified from the fraction D by semi-preparative HPLC using 44% MeCN in H$_2$O at a flow rate of 2.2 mL/min.

Drimanenoid A (1)

white solid; $[\alpha]_{D}^{25} = −198.0$ (c 0.1, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\epsilon$): 267.0 (3.02) nm; CD (1.28 × 10$^{-3}$ M in MeOH) $\lambda_{\text{max}}$ (Δε): 194 (−3.82), 210 (−22.74) nm; IR (KBr) $\nu_{\text{max}}$ 3451, 2924, 1774, 1702, 1677, 1655, 1561, 1459, 1168, 1008, 661 cm$^{-1}$; $^{1}$H-NMR data, see Table 1; $^{13}$C-NMR data, see Table 2; HR-ESI-MS m/z 413.1569 [M + Na]$^+$ (calcd for C$_{23}$H$_{34}$O$_{7}$Na, 413.1571).

Drimanenoid B (2)

white solid; $[\alpha]_{D}^{25} = −64.0$ (c 0.1, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\epsilon$): 266.0 (2.82) nm; CD (1.28 × 10$^{-3}$ M in MeOH) $\lambda_{\text{max}}$ (Δε): 191 (−8.62), 215 (−6.91) nm; IR (KBr) $\nu_{\text{max}}$ 3448, 2931, 1751, 1686, 1655, 1459, 1148, 1025, 645 cm$^{-1}$; $^{1}$H-NMR data, see Table 1; $^{13}$C-NMR data, see Table 2; HR-ESI-MS m/z 415.1724 [M + Na]$^+$ (calcd for C$_{23}$H$_{35}$O$_{7}$Na, 415.1727).

Drimanenoid C (3)

white solid; $[\alpha]_{D}^{25} = −228.0$ (c 0.1, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\epsilon$): 284.0 (3.21), 299.0 (3.19) nm; CD (1.20 × 10$^{-3}$ M in MeOH) $\lambda_{\text{max}}$ (Δε): 192 (−2.68), 195 (−3.04), 210 (−24.74) nm; IR (KBr) $\nu_{\text{max}}$ 3446, 2930, 1794, 1774, 1735, 1655, 1638, 1459, 1008 cm$^{-1}$; $^{1}$H-NMR data, see Table 1; $^{13}$C-NMR data, see Table 2; HR-ESI-MS m/z 439.1722 [M + Na]$^+$ (calcd for C$_{23}$H$_{34}$O$_{7}$Na, 439.1727).

Drimanenoid D (4)

white solid; $[\alpha]_{D}^{25} = −145.0$ (c 0.1, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\epsilon$): 263.0 (2.57) nm; CD (1.19 × 10$^{-3}$ M in MeOH) $\lambda_{\text{max}}$ (Δε): 193 (−11.87), 196 (−1.04), 216 (−33.38) nm; IR (KBr) $\nu_{\text{max}}$ 3423, 2922, 1718, 1701, 1685, 1560, 1458, 1074, 650 cm$^{-1}$; $^{1}$H-NMR data, see Table 1; $^{13}$C-NMR data, see Table 2; HR-ESI-MS m/z 443.2037 [M + Na]$^+$ (calcd for C$_{24}$H$_{36}$O$_{7}$Na, 443.2040).

Drimanenoid E (5)

white solid; $^{1}$H-NMR data, see Table 1; $^{13}$C-NMR data, see Table 2; HR-ESI-MS m/z 457.2196 [M + Na]$^+$ (calcd for C$_{24}$H$_{36}$O$_{7}$Na, 457.2197).

Drimanenoid F (6)

white solid; $[\alpha]_{D}^{25} = −104.0$ (c 0.1, MeOH); UV (MeOH) $\lambda_{\text{max}}$ (log $\epsilon$): 267.0 (3.03) nm; CD (1.27 × 10$^{-3}$ M in MeOH) $\lambda_{\text{max}}$ (Δε): 195 (+ 2.95), 207 (−10.87) nm; IR (KBr) $\nu_{\text{max}}$ 3422, 2956, 1774, 1702, 1655, 1578, 1499, 1080, 650 cm$^{-1}$; $^{1}$H-NMR data, see Table 1; $^{13}$C-NMR data, see Table 2; HR-ESI-MS m/z 417.1884 [M + Na]$^+$ (calcd for C$_{25}$H$_{35}$O$_{7}$Na, 417.1884).

Drimanenoid G (7)

white solid; $^{1}$H-NMR data, see Table 1; $^{13}$C-NMR data, see Table 2; HR-ESI-MS m/z 251.1656 [M-H]$^-$ (calcd for C$_{15}$H$_{20}$O$_{4}$, 251.1653).

Antibacterial Activity Test

Compounds 1-4 and 6 were evaluated for their inhibitory activity against the Gram-negative bacteria including Vibrio harveyi, Vibrio owensi, Vibrio alginolyticus, Photobacterium damsela, Ralstonia solanacearum, Xanthomonas oryzae pv. oryzae, Xanthomonas campestris pv. Mangiferaceae, E. coli, and the Gram-positive bacteria including Micrococcus luteus and methicillin-resistant Staphylococcus aureus (MRSA) according to the previously reported methods [11]. The antibacterial activity was conducted in vitro using the agar diffusion method with 7 mm paper discs with 500 μg of kanamycin coassayed as a positive control. Due to the low amount of the tested compounds, each 7 mm paper disc only contained 50 μg of compound in the test.

Cytotoxic Activity Test

The cytotoxic activity of compounds 1-4 and 6 were screened in vitro towards human myelogenous leukemia cell line K562, human gastric carcinoma cell line SGC-7901, human hepatoma carcinoma cell line BEL-7402, human non-
small cell lung carcinoma cell line A549, and human cervical carcinoma cell line Hela using the previously reported methods\cite{14}. The cis-platinum was used as the positive drug.

**Supplementary data**

Supplementary material related to this article is available at doi:

**Conflicts of interest**

The authors declare no conflicts of interest.

**References**


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