Seven chromanoid norbisabolane derivatives from the marine-alga-endophytic fungus *Trichoderma asperellum* A-YMD-9-2

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**Abstract**

An examination of the endophytic fungus *Trichoderma asperellum* A-YMD-9-2 obtained from the marine red alga *Gracilaria verrucosa* led to the isolation of seven new chromanoid norbisabolane derivatives, trichobisabolins I–L (1–4) and trichaspsides C–E (5–7). Their structures and relative configurations were established on the basis of spectroscopic techniques, mainly including 1D/2D NMR and MS, and the absolute configuration of 1 was assigned by X-ray crystallographic analysis using Cu Kα radiation. All of these isolates feature a 1,9-epoxy ring system, and 5–7 represent the second occurrence of norbisabolane aminoglycosides. Compounds 1–7 exhibited potent inhibition of several marine phytoplankton species.

1. Introduction

Among the marine-derived filamentous fungi, those of algicolous origin are the major contributors of new secondary metabolites [1]. To date, more than 400 new compounds, including terpenes, polyketides, alkaloids, peptides and so on, with various bioactivities, such as antimicrobial, antiprotrozoal, antioxidative, enzyme-modulatory, and cytotoxic properties, have been characterized from them [2–4]. Although only some ten algicolous *Trichoderma* strains have been chemically investigated, they have contributed over fifty new compounds [5–11]. Terpenes make up the largest family of new secondary metabolites from algicolous *Trichoderma*, and their number approximates to forty [5–11]. It is worth mentioning that rarely-occurring norterpenes have been encountered in them more than once [9–11], which diversified the terpenes from *Trichoderma* to some extent. In our continuous survey on the chemical diversity of marine algicolous *Trichoderma* species, *T. asperellum* A-YMD-9-2 obtained from the inner tissue of a marine red alga was examined. Efforts on it led to the isolation and identification of seven new chromanoid norbisabolane derivatives, trichobisabolins I–L (1–4) and trichaspsides C–E (5–7) (Fig. 1). Herein, their isolation, structure elucidation, and bioactivity are described in detail.

2. Experimental

2.1. General

1D and 2D NMR spectra were obtained on a Bruker Avance III 500 NMR spectrometer (Bruker Corp., Billerica, MA, USA). Low and high resolution EI mass spectra were measured on an Autospec Premier P776 mass spectrometer (Waters Corp., Milford, MA, USA). IR spectra were recorded on a Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Optical rotations, UV, and ECD spectra were determined on a Chirascan CD spectrometer (Applied Photophysics Ltd., Surrey, UK). High performance liquid chromatography (HPLC) separation was operated on an Agilent 1260 HPLC system using an Eclipse SB-C18 (5 μm, 9.4 × 250 mm) column (Agilent Technologies Inc., Santa Clara, CA, USA). Column chromatography (CC) was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), RP-18 (AAG12SS0, YMC Co. Ltd., Kyoto, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden). Thin-layer chromatography (TLC) was carried out with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co., Qingdao, China).
2.2. Fungal material and fermentation

Following a previous procedure [12], the endophytic *Trichoderma asperellum* A-YMD-9-2 was isolated from the inner tissue of the marine red alga *Gracilaria verrucosa* collected from the Yangma Island, Yantai, China in August 2016. This fungal strain was identified by morphology and by analysis of the ITS regions of its rDNA, whose sequence data have been deposited at GenBank with the accession number MH819724. The large-scale fermentation was performed statically at room temperature for 40 days in 200 × 1 L Erlenmeyer flasks, each containing 50 g rice, 0.6 g peptone, 50 mL pure water, and 50 mL natural seawater from the coast of Yantai, China.

2.3. Extraction and isolation

At the end of the above fermentation, the whole cultures were filtered to obtain mycelia, which were dried, homogenized, and then exhaustively extracted with CH2Cl2 and MeOH (1:1, v/v). The concentrated extract was partitioned between EtOAc and H2O to give an EtOAc-soluble extract (202.1 g). The filtrate was extracted with EtOAc and then evaporated under reduced pressure to afford an extract (10.3 g). Due to the identical TLC profiles, the two extracts were combined and subjected to silica gel CC with step-gradient solvent systems consisting of petroleum ether (PE)/EtOAc (50:1 to 0:1) and then CH2Cl2/MeOH (10:1 to 0:1) to give eight fractions (Fr. 1–8). Fr. 2 eluted with PE/EtOAc (5:1) and was further purified by CC on RP-18 (MeOH/H2O, 7:3) and Sephadex LH-20 (MeOH) as well as semi-preparative HPLC (ACN/H2O, 2:3 to 3:2) to afford 2 (2.0 mg), 3 (1.0 mg), and 4 (0.8 mg). Fr. 3 eluted with PE/EtOAc (2:1) and was further purified by CC on RP-18 (MeOH/H2O, 3:2) and silica gel (PE/EtOAc, 6:1) to yield 1 (4.6 mg). Fr. 7 eluted with (CH2Cl2/MeOH, 5:1) and was further purified by CC on RP-18 (MeOH/H2O, 7:3) and Sephadex LH-20 (MeOH) and preparative TLC (CH2Cl2/MeOH, 7:1) to obtain 5 (2.8 mg), 6 (3.4 mg), and 7 (2.2 mg).

| Table 1 |
| --- |
| **1H and 13C NMR data for 1 (in CDCl3 and CD2OD).** |
| | **1** (in CDCl3) | **1** (in CD2OD) |
| δ (J in Hz) | δ (J in Hz) |
| **1** | 67.7, CH | 69.2, CH |
| 2 | 124.5, CH | 125.2, CH |
| 3 | 140.2, C | 141.5, C |
| 4 | 68.8, CH | 69.1, CH |
| 5a | 33.1, CH2 | 34.3, CH2 |
| 5b | 1.51, br d (13.7) | 1.47, br d (13.6) |
| 6 | 32.6, CH | 34.0, CH |
| 7 | 31.0, CH | 32.6, CH |
| 8a | 33.9, CH2 | 34.5, CH2 |
| 8b | 1.20, br d (15.0) | 1.31, ddd (13.7, 2.1, 1.8) |
| 9 | 73.5, CH | 72.1, CH |
| 10a | 44.8, CH2 | 46.2, CH2 |
| 10b | 1.46, ddd (14.5, 2.4, 2.3) | 1.41, ddd (13.9, 5.0, 4.8) |
| 11 | 68.4, CH | 66.8, CH |
| 12 | 22.6, CH3 | 23.5, CH3 |
| 13 | 19.5, CH3 | 19.8, CH3 |
| 14 | 21.1, CH3 | 21.2, CH3 |

| Table 2 |
| --- |
| **13C NMR data for 2-6 (in CDCl3, δ in ppm).** |
| **Position** | 2 | 3 | 4 | 5 | 6 |
| **1** | 153.7, C | 154.2, C | - | 68.5, CH | 73.1, CH |
| 2 | 117.2, CH | 117.2, CH | 117.2, CH | 121.4, CH | 123.7, CH |
| 3 | 137.4, C | 137.3, C | 137.4, C | 141.0, C | 138.5, C |
| 4 | 121.9, CH | 121.6, CH | 121.7, CH | 31.6, CH2 | 26.0, CH2 |
| 5 | 127.2, CH | 127.2, CH | 129.2, CH | 24.3, CH2 | 23.3, CH2 |
| 6 | 124.3, C | 124.6, C | 124.3, C | 39.2, CH | 39.3, CH |
| 7 | 29.3, CH | 29.5, CH | 27.6, CH | 32.0, CH | 26.7, CH |
| 8 | 38.3, CH2 | 37.9, CH2 | 35.2, CH2 | 34.1, CH | 40.6, CH |
| 9 | 76.8, CH | 73.7, CH | 72.2, CH | 69.7, CH | 70.1, CH |
| 10 | 44.8, CH2 | 44.4, CH2 | 44.2, CH2 | 44.3, CH2 | 43.6, CH2 |
| 11 | 67.5, CH | 64.8, CH | 67.5, CH | 70.1, CH | 75.8, CH |
| 12 | 23.7, CH3 | 23.9, CH3 | 23.3, CH3 | 19.3, CH3 | 22.7, CH3 |
| 13 | 20.5, CH3 | 20.5, CH3 | 23.9, CH3 | 19.7, CH3 | 19.7, CH3 |
| 14 | 21.1, CH3 | 21.1, CH3 | 20.8, CH3 | 23.8, CH3 | 23.8, CH3 |
| 15 | 94.4, CH | 98.1, CH |
| 16 | 53.8, CH | 53.0, CH |
| 17 | 74.0, CH | 74.7, CH |
| 18 | 71.6, CH | 72.0, CH |
| 19 | 71.7, CH | 71.4, CH |
| 20 | 62.2, CH2 | 62.3, CH2 |
| 21 | 172.3, C | 173.1, C |
| 22 | 23.4, CH3 | 23.2, CH3 |

2.4. Spectral data of new compounds

**Trichobisabolin I (1):** Colorless crystals; mp 152-154°C; [α]D20 109 (c 0.29, MeOH); IR (KBr) νmax 3409, 2962, 2931, 2917, 2877, 2853, 1437, 1378,1318, 1302, 1204, 1150, 1128, 1067, 1031, 1007, 978, 812 cm−1. 1H and 13C NMR data, Table 1; EIMS m/z (%) 240 [M+] (5), 222 (15), 204 (13), 196 (36), 135 (49), 119 (46), 109 (100), 95 (78); HREIMS m/z 240.1720 [M+]1, calc for C14H22O3s, 240.1725.

**Trichobisabolin J (2):** Colorless oil; [α]D20 +43 (c 0.050, MeOH); IR
1H and 13C NMR data for Trichobisabolin L (4): Colorless oil; [α]20° + 111 (c 0.067, MeOH); IR (KBr) v max 3417, 2927, 1648, 1448, 1382, 1122, 1027, 739 cm −1; 1H and 13C NMR data, Tables 2 and 3; EIMS m/z (%) 220 [M]+, calcd for C41H70O2, 220.1463.

Trichobisabolin K (3): Colorless oil; [α]20° + 68 (c 0.13, MeOH); IR (KBr) v max 3443, 2923, 1635, 1383, 1119, 743 cm −1; 1H and 13C NMR data, Tables 2 and 3; EIMS m/z (%) 220 [M]+, calcd for C41H70O2, 220.1463.

Trichaspide C (5): Colorless oil; [α]20° + 70 (c 0.21, MeOH); IR (KBr) v max 3147, 2927, 1648, 1448, 1382, 1122, 1027, 739 cm −1; 1H and 13C NMR data, Tables 2 and 3; EIMS m/z (%) 220 [M]+, calcd for C41H70O2, 220.1463.
and 13C NMR data, Tables 2–4; EIMS m/z (%) 427 [M]+ (5), 253 (4), 237 (7), 223 (44), 204 (100), 189 (22), 165 (18), 138 (27), 121 (80), 114 (43), 95 (45), 59 (41), 44 (52); HREIMS m/z 427.2574 [M]+, calcld for C22H37NO7, 427.2570.

Trichaspside D (6): Colorless oil; [α]20^0 + 46 (c 0.18, MeOH); IR (KBr) v_max 3407, 2923, 1654, 1438, 1382, 1032, 739 cm⁻¹; 1H and 13C NMR data, Tables 2–4; EIMS m/z (%) 427 [M]+ (6), 253 (7), 237 (3), 223 (59), 204 (100), 189 (28), 165 (20), 138 (32), 121 (98), 114 (48), 95 (41), 60 (34), 44 (40); HREIMS m/z 427.2562 [M]+, calcld for C22H37NO7, 427.2570.

Trichaspside E (7): Colorless oil; [α]20^0 + 95 (c 0.14, MeOH); IR (KBr) v_max 3385, 2922, 1660, 1544, 1447, 1374, 1121, 1025 cm⁻¹; 1H and 13C NMR data, Table 4; EIMS m/z (%) 427 [M]+ (4), 253 (4), 237 (5), 223 (49), 204 (86), 189 (19), 165 (19), 138 (21), 121 (100), 114 (43), 95 (53), 59 (43), 44 (57); HREIMS m/z 427.2577 [M]+, calcld for C22H37NO7, 427.2570.

2.5. X-ray crystallographic analysis

All crystallographic data were obtained on a Bruker Smart-1000 CCD diffractometer equipped with a graphite-monochromatic Cu-Kα radiation (λ = 1.54178 Å) at 293(2) K. These data were corrected for absorption through the program SADABS [13]. The structure was solved by direct methods with the SHELXTL software package [14], and all non-hydrogen atoms were refined anisotropically. The hydrogen atoms were located by geometrical calculations, and their positions and thermal parameters were fixed during the structure refinement. The structure was refined by full-matrix least-squares techniques [15].

Crystal data for 1: C14H24O3, F.W. = 240.33, Monoclinic space group, P21/c, unit cell dimensions a = 10.3320 (2) Å, b = 10.3320 (2) Å, c = 11.3997 (5) Å, V = 1053.88 (5) Å³, α = β = 90°, γ = 120°, Z = 2, d_calc = 1.136 Mg/m³, crystal dimensions 0.34 × 0.20 × 0.22 mm, μ = 0.622 mm⁻¹, F(000) = 396. The 1612 measurements yielded 1534 independent reflections after equivalent data were averaged, and Lorentz and polarization corrections were applied. The final refinement gave R₁ = 0.0331 and wR₂ = 0.0870 [I > 2σ(I)]. The absolute structure parameter was 0.03 (3). These data have been deposited at the Cambridge Crystallographic Data Centre, with deposition No. CCDC 1865405, which can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: + 44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

2.6. Bioassay

The inhibition of the marine phytoplankton Chattonella marina, Heterosigma akashiwo, Karlodinium veneficum, and Prorocentrum donghaiense and the marine-derived pathogenic bacteria Vibrio para-haemolyticus, V. anguillarum, V. harveyi, V. splendidus, and Pseudoalteromonas citrea by 1–7 were assayed as described previously [6,9].

3. Results and discussion

Trichosibalin J (1) was obtained as colorless crystals. Its molecular formula was determined as C14H20O2 by HREIMS (m/z 240.1720 [M]+, calcld for C14H20O2, 240.1725), requiring three degrees of unsaturation. The IR absorption band at 3409 cm⁻¹ along with the EIMS fragmental ion peak at m/z 202, the IR absorption band at 3430 cm⁻¹ suggested the presence of a hydroxyl group. The 1H and 13C NMR spectra (Tables 2 and 3) together with DEPT and HSQC data delineated signals characteristic of three methyl groups (C-12, C-13, and C-14), two sp³ methylenes (C-8 and C-10), three sp² groups (C-7, C-9, and C-11) and three sp³ methines (C-2, C-4, and C-5), and three sp² quaternary carbons (C-1, C-3, and C-6). Of those, the signals for three sp³ methines and three sp² quaternary carbons indicated the presence of a 1,3,6-trisubstituted phenyl group (ring A) [16], confirmed by the COSY correlation between H-4 and H-5 and HMBB correlations from H-2 to C-1, C-4, and C-6, from H-4 to C-2 and C-6, and from H-5 to C-1 and C-3 (Fig. 2). An oxygen atom and a methyl group were attached to C-1 and C-3, respectively, as supported by the deshielded signal for C-1 and HMBB correlations from H-14 to C-2, C-3, and C-4. The remaining NMR signals resembled to those of 1, sugested the linkage from C-1 to C-16 through an oxygen atom. However, the deshielded NMR signal of C-9 suggested its linkage to C-1,

CD3OD) demonstrated the presence of a trisubstituted vinyl group, which extended to C-4 via C-1, C-6, and C-5 as evidenced by the COSY correlations of H-2/H-1/H-6/H-5/H-4 (Fig. 2). HMBB correlations from H-4 to C-3 and from H-14 to C-2, C-3, and C-4 established the connectivity of ring A. On the other hand, COSY correlations of H-13/H-7/H-8/H-9/H-10/H-11/H-12 indicated the linkage from C-13 to C-12, which was attached to C-6 of ring A via C-7 as seen from the HMBB correlations from H-13 to C-6, C-7, and C-8. The presence of ring B was supported by the HMBB correlation from H-9 to C-1, as determined in CDCl₃. Combined with the molecular formula, the deshielded signals of C-4 and C-11 suggested that they were hydroxylated, respectively. The above information confirmed the planar structure of 1, which was validated by other HMBB correlations (Fig. 2). The relative configuration was determined by the NOE correlations of H-13 with H-1, H-6, and H-9 (Fig. 3), and the absolute configuration was assigned to be 1S, 4S, 6R, 7S, 9R, and 11S by a single-crystal X-ray diffraction experiment using Cu Kα radiation (Fig. 4).

Trichosibalin J (2) was isolated as a colorless oil. The molecular formula C14H20O2 was established on the basis of HREIMS (m/z 220.1459 [M]+, calcld for C14H20O2, 220.1463), implying five degrees of unsaturation. In combination with the EIMS fragmental ion peak at m/z 202, the IR absorption band at 3430 cm⁻¹ suggested the presence of a hydroxyl group. The 1H and 13C NMR spectra (Tables 2 and 3) together with DEPT and HSQC data delineated signals characteristic of three methyl groups (C-12, C-13, and C-14), two sp³ methylenes (C-8 and C-10), three sp² groups (C-7, C-9, and C-11) and three sp³ methines (C-2, C-4, and C-5), and three sp² quaternary carbons (C-1, C-3, and C-6). Of those, the signals for three sp³ methines and three sp² quaternary carbons indicated the presence of a 1,3,6-trisubstituted phenyl group (ring A) [16], confirmed by the COSY correlation between H-4 and H-5 and HMBB correlations from H-2 to C-1, C-4, and C-6, from H-4 to C-2 and C-6, and from H-5 to C-1 and C-3 (Fig. 2). An oxygen atom and a methyl group were attached to C-1 and C-3, respectively, as supported by the deshielded signal for C-1 and HMBB correlations from H-14 to C-2, C-3, and C-4. The remaining NMR signals resembled to those of 1, which suggested the linkage from C-1 to C-16 through an oxygen atom. However, the deshielded NMR signal of C-9 suggested its linkage to C-1,
supported by the comparable NMR data with those of 1. Additionally, H-7 and H-9 were located on the same face of ring B by their NOE correlations (Fig. 3), which also indicated them to be axial. The similar splitting pattern and coupling constants of H-9, H-10a, H-10b, and H-11 required the same relative configuration between H-9 and H-11.

Trichobisabolin K (3) was purified as a colorless oil. A molecular formula of C_{14}H_{20}O_{2}, the same as that of 2, was deduced by interpretation of HREIMS data (m/z 220.1461 [M]+, calcd for C_{14}H_{20}O_{2}, 220.1463). The carbon signals were not detected in the $^{13}$C NMR spectrum due to the low concentration, but most of them (Table 2) were pointed out by HSQC and HMBC correlations. These $^{13}$C NMR signals along with the $^1$H NMR data (Table 3) resembled those of 2, except for the relatively small coupling constant between H-7 and H-8b. Thus, 4 was also speculated to be a stereoisomer of 2, supported by the COSY and HMBC correlations as shown in Fig. 2. In the NOESY spectrum, H-9 displayed a correlation with H-13 rather than H-7 (Fig. 3), which suggested the cofacial property of H-9 and C-13. The similar coupling constants of H-9 and H-11 with H-10a and H-10b allowed their identical relative configuration between H-9 and H-11.

Trichobisabolin L (4) was isolated as a colorless oil. A molecular formula of C_{14}H_{20}O_{2}, the same as that of 2, was deduced by interpretation of HREIMS data (m/z 220.1461 [M]+, calcd for C_{14}H_{20}O_{2}, 220.1463). The carbon signals were not detected in the $^{13}$C NMR spectrum due to the low concentration, but most of them (Table 2) were pointed out by HSQC and HMBC correlations. These $^{13}$C NMR signals along with the $^1$H NMR data (Table 3) resembled those of 2, except for the relatively small coupling constant between H-7 and H-8b. Thus, 4 was also speculated to be a stereoisomer of 2, supported by the COSY and HMBC correlations as shown in Fig. 2. In the NOESY spectrum, H-9 displayed a correlation with H-13 rather than H-7 (Fig. 3), which suggested the cofacial property of H-9 and C-13. The similar coupling constants of H-9 and H-11 with H-10a and H-10b to those of 1 and 2 allowed their identical relative configuration between H-9 and H-11.

Trichobisabolin K (3) was purified as a colorless oil. A molecular formula of C_{14}H_{20}O_{2}, the same as that of 2, was deduced by interpretation of HREIMS data (m/z 220.1461 [M]+, calcd for C_{14}H_{20}O_{2}, 220.1463). The carbon signals were not detected in the $^{13}$C NMR spectrum due to the low concentration, but most of them (Table 2) were pointed out by HSQC and HMBC correlations. These $^{13}$C NMR signals along with the $^1$H NMR data (Table 3) resembled those of 2, except for the relatively small coupling constant between H-7 and H-8b. Thus, 4 was also speculated to be a stereoisomer of 2, supported by the COSY and HMBC correlations as shown in Fig. 2. In the NOESY spectrum, H-9 displayed a correlation with H-13 rather than H-7 (Fig. 3), which suggested the cofacial property of H-9 and C-13. The similar coupling constants of H-9 and H-11 with H-10a and H-10b to those of 1 and 2 allowed their identical relative configuration between H-9 and H-11.
correlations from H-12 to C-10 and C-11, from H-13 to C-6, C-7, and C-8, from H-14 to C-2, C-3, and C-4, and from H-15 to C-11 and C-19 supported the identical planar structure with that of 5, corroborated by the COSY correlations as shown in Fig. 2. The NOE correlations of H-6 with H-1 and H-13 and of H-7 with H-9 allowed H-9 to be opposite to H-1, H-6, and C-13 (Fig. 3). As was the case for 5, the relative configuration at C-11 of 6 was not assigned.

Trichaspide E (7) was isolated as a colorless oil. HREIMS (m/z 427.2577 [M]+, calcd for C22H37NO7, 427.2570) analysis gave the molecular formula C22H37NO7, the same as that of 6. Its 1H and 13C NMR data (Table 4) highly resembled those of 6, except for the signals around C-11. Thus, 7 was proposed to be a C-11 epimer of 6, validated by the COSY and HMBC correlations as shown in Fig. 2. The relative configurations among H-1, H-6, H-9, and C-13 were further evidenced to be the same as those of 6 by the NOE correlations of H-6 with H-1 and H-13 and of H-7 with H-9 (Fig. 3).

Bisabolane sesquiterpenes have been discovered in a broad spectrum of plants, animals, and fungi of terrestrial and marine origin [9,16,18–20]. To date, more than 40 new bisabolane derivatives have been isolated and identified from the marine-derived fungi, mainly including those of the genera Aspergillus, Penicillium, Trichoderma, and Verticillium [9,10,16,20–25]. It is worth mentioning that most of them are phenolic derivatives, and norbisabolanes only accounts for a low proportion. Although they possess diverse substitution and cyclization types, a 1,9-epoxy ring system has rarely occurred in the molecules. Thus, the discovery of compounds 1–7 greatly diversifies the structures of norbisabolane sesquiterpenes.

Compounds 1–7 were assayed for growth inhibition of four marine phytoplankton species (Chattonella marina, Heterosigma akashiwo, Karlodinium veneficum, and Prorocentrum donghaiense) [9]. The results (Table 5) showed that 1–4 were more active to C. marina, K. veneficum, and P. donghaiense than 1 and 5–7. A structure-activity relationship analysis reveals that the phenyl group in 2–4 may contribute to their inhibitory ability, but the isomerization at C-9 and/or C-11 of 2–7 only has slight influences on their activities. In addition, the antibacterial activity of 1–7 against the marine-derived pathogens Vibrio para-haemolyticus, V. anguillarum, V. harveyi, V. splendidus, and Pseudoalteromonas citrea was also evaluated [6], but they only exhibited weak inhibition of two or more of the bacteria tested.

4. Conclusion

Chemical investigation towards the marine algicolous fungus T. asperellum A-YMD-9-2 has resulted in the characterization of seven new chromanoid norbisabolane derivatives (1–7). Among them, 2–4 feature a phenyl group that is widespread in marine-derived bisabolane sesquiterpenes, and 5–7 represent the second occurrence of norbisabolane aminoglycosides. These isolates greatly add to the molecular diversity of natural norbisabolane sesquiterpenes. Compounds 1–7 showed potent inhibition one or more marine phytoplankton species tested, and the high activities of 2–4 were deduced to profit from their phenyl group.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.04.014.

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Table 5

| Compound | IC50 (µg/mL) | Inhibitory zone diameter (mm) at 40 µg/disk |
|----------|-------------|------------------------------------------|
|          | C. marina   | H. akashiwo | K. veneficum | P. donghaiense | V. anguillarum | V. harveyi | V. parahaemolyticus | V. splendidus | P. citreus |
| 1        | 11          | 4.6         | 12           | 23           | 6.1          | 6.0        | 0              | 0           | 0          |
| 2        | 12          | 4.3         | 1.3          | 5.7          | 6.5          | 7.0        | 7.5             | 7.0          | 8.0        |
| 3        | 3.3         | 9.2         | 1.5          | 6.8          | 6.3          | 7.1        | 7.0             | 7.0          | 7.1        |
| 4        | 0.93        | 7.8         | 2.7          | 4.9          | 6.6          | 7.1        | 7.2             | 7.0          | 8.1        |
| 5        | 6.7         | 2.9         | 6.6          | 10           | 6.1          | 6.1        | 7.0             | 6.2          | 0          |
| 6        | 5.4         | 5.8         | 8.4          | 14           | 6.2          | 6.2        | 6.5             | 6.3          | 0          |
| 7        | 3.7         | 6.9         | 9.4          | 12           | 6.7          | 6.3        | 7.0             | 6.5          | 0          |
| KClO4     | 0.46        | 0.98        | 0.89         | 1.9          | 26           | 29         | 26              | 27           | 30         |
Chloramphenicol |         |             |              |              |              |            |                 |              |            |
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