New and bioactive polyketides from Hawaiian marine-derived fungus Trichoderma sp. FM652

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ABSTRACT
Two new sorbicillinoid derivatives (1 and 2), together with ten other related compounds (3–12) were isolated from a Hawaiian marine fungal strain Trichoderma sp. FM652. The structures of compounds 1 and 2, including the absolute configuration, were elucidated by extensive analysis of NMR spectroscopy, HRESIMS and electronic circular dichroism (ECD) data. Compounds 6–12 exhibited significant anti-proliferative activity against ovarian cancer cell line A2780, with the IC50 values ranging from 0.5 to 8.07 μM. Moreover, compounds 1, 7 and 8 showed significant inhibition against NF-κB with IC50 values of 13.83, 24.40 and 14.63 μM, respectively. Compounds 6, 9 and 12 also demonstrated moderate inhibitory activity against S. aureus and methicillin resistant S. aureus with the MIC values in the range of 10–40 μg/mL.

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1. Introduction
Many drugs currently used in clinics are either obtained directly from natural sources or modified from natural products. On the other hand, only 27% of the current approved drugs are synthetic compounds despite great investments to the field of...
synthetic drug discovery (Newman and Cragg 2016). Therefore, unfolding new natural sources to discover novel compounds for their biological potentials is still a good approach for new drug discovery. Because of the biodiversity of the underexplored kingdom fungi, fungi are a great source for novel and biologically active natural product drug discovery.

As part of our continuing search for new secondary metabolites and biologically active compounds from Hawaiian fungi (Zaman et al. 2020a; 2020b; 2021), we isolated a fungal strain *Trichoderma* sp. FM652 (Genbank accession #OK626586) from the offshore sea sediments collected near the Hanauma Bay, Hawaii. *Trichoderma* species are well known for their production of polyketides, sorbicillin derivatives, etc. with variety of biological activities such as e phytotoxic, antibacterial, antioxidant and antifungal activities (Pang et al. 2018). A crude methanolic extract of FM652 was active against human ovarian cancer cell line A2780 at 20 μg/mL. The crude sample was also evaluated for their antibacterial activity, which inhibited gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* at 80 μg/mL. From a large scale culture of FM652, we isolated 12 secondary metabolites (Figure 1), including two new sorbicillinoid derivatives (1 and 2), together with 10 other related known compounds (3–12). Herein, we report the isolation, structural elucidation, using HR-ESIMS, NMR spectral

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**Figure 1.** Structure of compounds 1–12.
interpretation, and ECD analysis, and the biological evaluation of compounds 1–12 from *Trichoderma* sp. FM652.

2. Results and discussion

Compound 1 was isolated as a brown powder. The molecular formula, C_{14}H_{20}O_{5}, was determined by HRESIMS based on the [M + H]^{+} peak at m/z 269.13682 (calcd for C_{14}H_{21}O_{5}^{+}, 269.13890), requiring five degrees of unsaturation. Comprehensive analysis of the ^{1}H, DEPTQ and HSQC NMR spectra (Table S1, Supplementary material) displayed 14 carbon resonances ascribable to four quaternary carbons including two carboxyls (δ_{C} 177.4, 199.9), three methyl groups (δ_{C} 19.0, 20.3, 25.1), two methylenes (δ_{C} 29.4, 34.7), five methines including one oxygenated (δ_{C} 80.5) and four olefinic ones (δ_{C} 127.9, 130.6, 140.6, 143.0). Two spin systems, −CH_{2}−CH_{2}− and −CH=CH−CH=CH−CH_{3} (Figure S4, Supplementary material), were demonstrated in the COSY spectrum. In the HMBC spectrum (Figure S4, Supplementary material), H3-13 showed correlations to C-1, C-2 and C-3, and H3-14 correlated to C-3 and C-4. Also, H-3 exhibited HMBC correlations to C-1, C-2, C-4, C-5, C-13 and C-14. Furthermore, the HMBC correlations from H-8 to C-6, C-7, C-9 and C-10, H-9 to C-7, C-8, C-10 and C-11, and H2-6 to C-5 and C-7, enabled us to connect the two spin systems together as the side chain of 1, which was similar like vertinolide (4) (Wrobel and Ganem 1983). Based on the molecular formula and the chemical shift of C-4 (δ_{C} 86.3), C-1 must be connected to C-4 through an oxygen bridge to form a γ-lactone. Finally, the HMBC correlations from H3-14 and C-3 to C-5, and C-13 to C-4 confirmed the connectivity of the side chain to the γ-lactone at C-4. Hydration of the double bond at 2,3-positions of 4 could yield 1. Hence, the planar structure of compound 1 as shown in Figure 1.

The four olefinic proton resonances at δ_{H} 6.09 (H-8), 7.21 (H-9), 6.29 (H-10), and 6.32 (H-11) with the coupling constants of J_{H-10, H-11} = 15 Hz and J_{H-8, H-9} = 15 Hz, indicated the presence of two *trans* (*E*) double bonds. The presence of two conjugated *trans* (*E*) double bonds was also confirmed by ROESY, in which clear NOE correlations were found between H-8 and H-10, and between H-9 and H-11. Furthermore, H3-13 and H3-14 may have different orientations as they didn’t show any ROESY correlation between them. To confirm it, we ran 1D NOE. When H3-13 was irradiated, no NOE found with H3-14 and vice versa (Figure S8, Supplementary material).

To reinforce the relative stereochemical assignment, we undertook DFT calculations of NMR chemical shifts followed by statistical analysis using DP4+ (Marcarino et al. 2022). The four possible diastereoisomers were generated by changing the configurations at C-2 and C-3 whilst keeping the remaining stereocenter fixed as 4S (Figure S9, Supplementary material). After systematic conformational searches using MMFF, the resulting structures were optimized at the B3LYP/6-31G* level and the GIAO isotropic shielding constants were computed at the PCM/mPW1PW91/6-31+G**/B3LYP/6-31G* level. It was shown that the relative energies computed with PCM might be biased towards conformations featuring intramolecular hydrogen bonding interactions (IHB), and for that reason the computed chemical shifts were Boltzmann averaged with the Gibbs free energies computed at the SMD/M06-2X/6-31G* level (Zanardi et al. 2020). The DP4+ calculations suggested 1b to be the most likely structure
(DP4+ > 99%) (Grimblat et al. 2015) (Table S2 and Figure S10, Supplementary material), fully consistent with the NOE and ROESY experiments discussed above.

Once the relative configuration of 1 was settled, we carried out TDDFT-ECD calculations to determine its absolute configuration (Pescitelli and Bruhn 2016). Each B3LYP/6-31G* optimized structure of 1b was submitted to ECD calculations at the PBE0/def2-SVP level, and the resulting spectra were Boltzmann-averaged using the relative energies refined at the SMD/M06-2X/6-31G+ level. The calculated ECD spectrum for (2S,3S,4S)-1 showed good agreement with the experimental ECD collected for the natural product (Figure S11, Supplementary material). Therefore, the absolute configuration of 1 was determined as a vertinolide derivative, shown in Figure 1, and it was given a trivial name, 2,3-dihydro 2-hydroxy vertinolide.

Compound 2 was obtained as dark yellow powder and its molecular formula was determined as C14H16O4 by HRESIMS based on the [M + H]+ peak at m/z 249.11248 (calcd for C14H17O4+, 249.11268), requiring seven degrees of unsaturation. The detailed analysis of 1H, 13C and HSQC NMR spectra (Table S1, Supplementary material) confirms the presence of 14 carbon resonances ascribable to six nonprotonated carbons including two carbonyls (δC 199.3, 204.1), three methyl groups (δC 14.4, 17.6, 27.9), five olefinic methines (δC 117.4, 130.9, 136.7, 140.9, 143.6). After analysing the 2D NMR (Figure S17, Supplementary material), it was confirmed that the planner structure of 2 was determined to be the same as the previously reported compound (S,Z)-2-hydroxy-6-((2E,4E)-1-hydroxyhexa-2,4-dien-1-ylidene)-2,4-dimethylcyclohex-4-ene-1,3-dione (Han et al. 2020). However, compound 2 had a different absolute configuration (2R) because it showed a negative optical rotation (½a/C138 25D 7.58 (c 0.002, MeOH)) while the reported one exhibited a positive optical rotation (½a/C138 25D +5.8 (c 0.1, MeOH)) (Han et al. 2020). This was also proved by the ECD calculation, where there was a characteristic positive cotton effect at 310 nm for compound 2R, which is consistent with the calculated ECD for 2R configuration (Figure S20, Supplementary material). Hence, the structure of compound 2 was determined as shown, which was given a trivial name (-)-trichodermatone.

Besides the new compounds 1 and 2, nine known hexaketide compounds with a sorbyl chain, 5-hydroxyvertinolide (3) (Andrade et al. 1997), vertinolide (4) (Wrobel and Ganem 1983), 2,3-dihydrosorbicillin (5) (Harned and Volp 2011), sorbicillin (6) (Cram 1948), isobisvertinol (7) (Koyama et al. 2007), bisvertinol (8) (Zhang et al. 2019), trichodimerol (9) (Andrade et al. 1992), sorbiquinol (10) (Andrade et al. 1996) and bislongiquinolide (11) (Andrade et al. 1997), and one curvularin derivative α,β-dehydrocurvularin (12) (Greve et al. 2008) were also isolated. The structures of these known compounds were determined based on comparisons of their NMR and HRESIMS data with previously reported ones.

Compounds 1–12 were evaluated for their antiproliferative activity against A2780 human ovarian cancer cells (Wang et al. 2020). All the compounds showed significant activities with the IC50 values ranging from 0.5 to 47.4 μM (Table S3, Supplementary material). Compounds 6–12 exhibited more significant antiproliferative activity (0.5-8.07 μM) than compounds 1–5, among which compound 7 demonstrated the highest activity with an IC50 value of 0.5 μM. When evaluated in a mammalian cell-based assay designed to monitor TNF-α-induced NF-κB activity (Zaman et al. 2020a), compounds 1,
and 8 were found to mediate inhibitory responses with IC₅₀ values of 13.83, 24.40 and 14.63 μM, respectively (Table S3, Supplementary material). Compounds 1–12 were also evaluated for their cytotoxicity against the human embryonic kidney cells 293 (HEK 293) using the same conditions as the NF-κB assay, and no cytotoxicity was observed at a concentration of 50 μM. Thus, in the absence of a cytotoxic response, inhibition of TNF-α-induced NF-κB activity suggests the potential of mediating a cancer chemopreventive response.

Compounds 1–12 were further evaluated for their antibacterial activity against S. aureus, methicillin resistant S. aureus and Bacillus subtilis (Table S4, Supplementary material) (Zaman et al. 2021). Compounds 6, 9 and 12 were active against both S. aureus and methicillin resistant S. aureus with the MIC values ranging from 10 to 80 μg/mL; and compound 10 and 12 also showed inhibition against Bacillus subtilis with MIC values of 80 and 20 μg/mL, respectively.

4. Conclusion

Trichoderma species are well known for their production of pyranone, sorbicillinoid, hexaketide, dodecaketide, carotane derivatives, cyclopentenones etc (Matsumoto et al. 1999; Macias et al. 2000; Abdel-Lateff et al. 2009). This study chemically investigated the marine-derived fungal strain Trichoderma sp. FM652, which led to the isolation of two new sorbicillinoid derivatives (1–2), together with ten other related compounds (3–12). Compounds 6–12 showed substantial antiproliferative activity (0.5–8.07 μM). Compounds 1, 7 and 8 demonstrated inhibitory responses to TNF-α-induced NF-κB activity with IC₅₀ values of 13.83, 24.40 and 14.63 μM, respectively. Compounds 6, 9 and 12 were also active against both S. aureus, methicillin resistant S. aureus and Bacillus subtilis with the MIC values ranging from 10 to 80 μM. These results provide a basis for considering these compounds as potential leads for further research.

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Disclosure statement

The authors declare no competing financial interest.

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