NOTE

A New Depsidone from *Teloschistes flavicans* and the Antileukemic Activity

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Abstract: Lichens produce a variety of secondary metabolites that could be potential sources of pharmaceutically useful chemicals. However, only a limited number of lichen metabolites have been investigated for their biological significance. The objective of this study was to identify the potential compounds responsible for the antileukemic activity of lichen *Teloschistes flavicans*. Among three fractions (*n*-hexane, EtOAc, and MeOH-H₂O), the ethyl acetate (EtOAc) fraction of *T. flavicans* methanolic extract showed the strongest inhibition in the HL-60 cell line. Additionally, the EtOAc fraction was further purified to obtain a new depsidone, 2,7-dichloro-3,8-dimethoxy-1,6,9-trimethyl-11*H*-dibenzo[*b,e*][1,4]dioxepin-11-one, named as flavicansone, along with rhizonic acid, parietin, and vicanicin. Flavicansone demonstrated the most significant inhibitory action against cell proliferation among the four isolated compounds.

Key words: *Teloschistes flavicans*, depsidone, HL-60 cell, antileukemic activity

1 Introduction

Lichens are an association between fungi (mycobionts) and photoautotrophic algal partners (photobionts), with approximately 18,500 different lichen species available worldwide¹. Lichens can survive in extreme environmental conditions, adapting to extreme temperatures, drought, inundation, salinity, high concentrations of air pollutants, and nutrient-poor and highly nitrified environments. Across centuries, lichens have been used for various purposes, particularly, as dyes, perfumes, and remedies in folk medicines².

Lichens synthesize a variety of secondary metabolites. Many of these compounds, including depsides, depsidones, usnic acids, dibenzofuranes, xanthenes, anthraquinones, pulvinic acid derivatives, and aliphatic acids, are unique with respect to those obtained from higher plants and have revealed interesting biological and pharmacological activities^{3,4}. The secondary metabolites of lichens exhibit antimicrobial, antioxidant, anti-inflammatory, cytotoxic, analgesic, antipyretic, and antiviral properties⁵⁻⁸.

Indonesia is one of the very few countries in the world endowed with rich biodiversity. About 40,000 endemic

plant species and various lichens have been found in Indonesia. Based on the ethnopharmacological background, lichens are utilized to treat inflammatory diseases in Indonesia⁹. As traditional herbal medicines, lichens provide an interesting, largely unexplored source for the development of potential new drugs. Therefore, further investigation must be undertaken to elucidate their clinical advantages. In this study, the secondary metabolites of *Teloschistes flavicans* were isolated and their antileukemic activity was evaluated.

2 Experimental Procedures

2.1 General experimental procedures

All solvents and reagents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan) and used without further purification. HRESIMS (High-resolution electrospray ionization mass spectrometry) spectra were obtained using the Waters UPLC-MS (ultraperformance liquid chromatography-tandem mass spectrometer) system (Aquity UPLC XevoQTof). Proton nuclear magnetic

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resonance (^1H NMR) (400 MHz and 600 MHz) and ^{13}C NMR (100 MHz and 150 MHz) spectra were recorded using a JEOL ECX 400 and Bruker Biospin AVANCE III 600 spectrometer with tetramethylsilane as an internal standard. Column chromatography (CC) was performed on silica gel (Wakogel® 60 N, 38–100 μm , FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Thin-layer chromatography (TLC) plates precoated with Silica gel 70F254 TLC Plate-Wako (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were evaluated using a UV lamp (254 nm).

2.2 Plant materials

In June 2018, *T. flavicans* was collected from the trees in Rejang Lebong, Bengkulu Province, Indonesia. The plant material was identified and deposited in Herbarium Bogoriense, Indonesian Institute of Sciences (LIPI) (specimen number 20362018).

2.3 Extraction and isolation

Fresh lichen *T. flavicans* (1,230 g) was macerated with methanol (7 $\times$ 2 L) for 4 days at room temperature. The extract was filtered, and the filtrate was evaporated *in vacuo* to yield the methanol extract (95.34 g). The methanolic extract of *T. flavicans* (61.0 g) was suspended in water and partitioned successively with *n*-hexane, ethyl acetate (EtOAc) to yield the *n*-hexane fraction (6.4 g), EtOAc fraction (22.0 g), and methanol-water (MeOH- H_2O) fraction (4.8 g), respectively. Subsequently, the EtOAc fraction (22.0 g) was subjected to silica gel CC (300 g), eluted with *n*-hexane–EtOAc (10/0 to 0/10), EtOAc–acetone (10/0 to 0/10), and acetone–MeOH (10/0 to 0/10), in a stepwise manner to obtain 6 subfractions (T1–T6), T1 (33.9 mg), T2 (0.2304 g), T3 (0.552 g), T4 (1.938 g), T5 (10.27 g), and T6 (3.075 g). T1 was further purified by silica gel CC, eluted using *n*-hexane: EtOAc 9/1 to 1/9, to obtain compound 1 (10.2 mg). Subfraction T3 was subjected to silica gel CC, eluted by *n*-hexane: EtOAc 9/1 to 1/9 to obtain T3.1 – T3.5. Further purification of T3.3 was performed on Sephadex LH-20 CC and eluted with MeOH to yield compound 2 (8.4 mg). Moreover, compound 3 (5.6 mg) was obtained from T4 over Sephadex LH-20 CC with CHCl_3 –MeOH 3/7. A portion of T5 was subjected to silica gel CC, eluted using *n*-hexane: EtOAc 9/1 to 1/9 to provide six subfractions T5.1 – T5.6. Later, T5.5 was subjected to CC and eluted using *n*-hexane: EtOAc 9/1 to 1/9 to afford compound 4 (6.8 mg).

2.3.1 Rhizonic acid (1)

Yellowish crystal ^1H NMR (400 MHz, CDCl_3): δ 2.09 (3H, s, H-7), 2.44 (3H, s, H-9), 3.91 (3H, s, H-8), 5.16 (1H, s, OH), 6.20 (1H, s, H-5), 12.05 (1H, s, COOH). ^{13}C NMR (100 MHz, CDCl_3): δ 7.8 (C-7), 24.2 (C-9), 51.9 (C-8), 105.3 (C-5), 108.6 (C-1), 110.6 (C-3), 140.3 (C-6), 158.1 (C-4), 163.2 (C-2), 172.7 (C-10). HRESIMS: m/z 197.0797 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{10}\text{H}_{13}\text{O}_4$, 197.0814).

2.3.2 Parietin (2)

Yellowish powder ^1H NMR (400 MHz, CDCl_3): δ 2.45 (3H, s, H-16), 3.94 (3H, s, H-15), 6.68 (1H, d, $J = 2.3$ Hz, H-7), 7.08 (1H, s br, H-2), 7.36 (1H, d, $J = 2.3$ Hz, H-5), 7.62 (1H, s br, H-4), 12.12 (1H, s, OH), 12.32 (1H, s, OH). ^{13}C NMR (100 MHz, CDCl_3): δ 22.3 (C-16), 56.2 (C-15), 106.8 (C-2), 108.3 (C-4), 110.3 (C-13), 113.7 (C-12), 121.4 (C-5), 124.6 (C-7), 133.3 (C-11), 135.3 (C-14), 148.5 (C-6), 162.6 (C-8), 165.3 (C-1), 166.6 (C-3), 182.1 (C-10), 190.9 (C-9). HRESIMS: m/z 285.0755 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{16}\text{H}_{13}\text{O}_5$, 285.0763).

2.3.3 Vicanicin (3)

Yellowish powder ^1H NMR (400 MHz, CDCl_3): δ 2.30 (3H, s, H-8'), 2.42 (3H, s, H-9), 2.49 (3H, s, H-7'), 2.50 (3H, s, H-8), 3.77 (3H, s, O- CH_3), 6.20 (1H, s, OH). ^{13}C NMR (100 MHz, CDCl_3): δ 10.4 (C-8'), 10.8 (C-9), 14.8 (C-8), 18.6 (C-7'), 60.5 (C-9'), 114.5 (C-3), 115.4 (C-6'), 118.7 (C-6), 122.5 (C-3'), 125.3 (C-5'), 126.9 (C-5), 138.2 (C-1'), 142.1 (C-2'), 146.6 (C-1), 152.1 (C-4'), 153.5 (C-4), 159.6 (C-2), 162.9 (C-7). HRESIMS: m/z 383.0432 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{18}\text{H}_{17}\text{O}_5\text{Cl}_2$, 383.0453).

2.3.4 2,7-Dichloro-3,8-dimethoxy-1,6,9-trimethyl-11H-dibenzo[*b,e*][1,4]dioxepin-11-one (4) (Flavicansone)

Yellowish powder ^1H NMR (600 MHz, CDCl_3): δ 2.45 (3H, s, H-8'), 2.48 (3H, s, H-7'), 2.54 (3H, s, H-8), 3.84 (3H, s, O- CH_3), 3.86 (3H, s, O- CH_3), 6.71 (1H, s, H-3). ^{13}C NMR (150 MHz, CDCl_3): δ 10.9 (C-8'), 15.1 (C-8), 18.5 (C-7'), 56.6 (C-9), 60.3 (C-9'), 101.9 (C-3), 119.4 (C-5'), 119.5 (C-5), 122.0 (C-3'), 127.0 (C-6), 130.3 (C-6'), 138.7 (C-1), 142.9 (C-2), 143.4 (C-1'), 152.7 (C-4), 158.2 (C-4'), 159.4 (C-2'), 162.8 (C-7). HRESIMS: m/z 383.0441 $[\text{M} + \text{H}]^+$ (calcd. for $\text{C}_{18}\text{H}_{17}\text{O}_5\text{Cl}_2$, 383.0453).

2.4 Cell culture

HL-60 cells (DS Pharma Biomedical Co., Ltd., Osaka, Japan) were cultured in RPMI 1640 medium (FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotics, penicillin-streptomycin (Gibco®, Life Technologies, Thermo Fisher Scientific Inc., MA, USA). The cells were maintained at 37°C under a humidified atmosphere of 5% CO_2 .

2.5 Antileukemic activity

CCK-8 assay: HL-60 cells were obtained from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) and cultured in RPMI 1640 medium (FUJIFILM Wako Pure Chemical Industries, Ltd., Osaka, Japan), supplemented with 10% heat-inactivated FBS and 1% antibiotics, penicillin-streptomycin (Gibco®, Life Technologies, Thermo Fisher Scientific Inc., MA, USA). The cells were maintained at 37°C under a humidified atmosphere of 5% CO_2 . Cell counting kit-8 (CCK-8) was purchased from Dojindo Molecular Technolo-

gies, Inc. (Kumamoto, Japan). The cells (2.5×10^4 cells/mL) were seeded in 96-well plates. After 24 h, sample solutions were added. Following a 48-h incubation, CCK-8 solution (10 μ L) was added and the plates were incubated for an additional 4 h. Visible absorption (490 nm) was measured using a microplate reader^{10, 11}.

3 Results and Discussion

3.1 Antileukemic activity of fractions

The cytotoxicity of these fractions (*n*-Hex, EtOAc, and MeOH-H₂O) was evaluated *in vitro* on human leukemia HL-60 cells using the CCK-8 assay. Cells in the rapid growth phase were exposed to the tested fractions for 48 h. The 100 μ g/mL EtOAc fraction inhibited cell proliferation, demonstrating cell viability of 34.1%. However, the cell viability demonstrated by the *n*-Hex and MeOH-H₂O fractions on HL-60 cells was 43.8% and 96.5%, respectively (Fig. 1).

3.2 Structure elucidation of isolated compounds

Based on the antileukemic activity on HL-60 cells, the EtOAc fraction showed the strongest inhibition among the three fractions (*n*-Hex, EtOAc, and MeOH-H₂O). Therefore, the EtOAc fraction was further purified to obtain a new depsidone (4) and three known compounds, rhizonic acid (1)¹², parietin (2)¹³, and vicanicin (3)³ (Fig. 2).

Compound 4 was obtained as a yellowish amorphous powder, and its molecular formula was C₁₈H₁₆O₅Cl₂ based on HRESIMS analysis: *m/z* 383.0441 [M + H]⁺ (calcd. 383.0452). The ¹H NMR spectrum revealed the resonances of three methyl groups at δ_H 2.45 (3H, s), δ_H 2.48 (3H, s), and δ_H 2.54 (3H, s), and two methoxy groups at δ_H 3.84 (3H, s) and δ_H 3.86 (3H, s). One aromatic proton at δ_H 6.71 (1H,

Rhizonic acid (1)

Parietin (2)

Vicanicin (3)

Flavicansone (4)

Fig. 2 Structures of isolated compounds from *Teloschistes flavicans*.

s) was also observed. The ¹³C NMR spectrum exhibited a total of 18 carbon resonances, comprising 3 methyl groups, 2 methoxy groups, 1 protonated aromatic carbon, and 11 unprotonated aromatic carbons. The HMQC correlations revealed that δ_H 2.45, δ_H 2.48, and δ_H 2.54 were methyl protons at C-8' (δ_C 10.9), C-7' (δ_C 18.5), and C-8 (δ_C 15.1), respectively. The HMBC correlations observed between an aromatic proton H-3 (δ_H 6.71) and C-1 (δ_C 138.7), C-2 (δ_C 142.9), C-4 (δ_C 152.7), and C-5 (δ_C 119.5) confirmed the substitution pattern of the A ring. Furthermore, the HMBC interaction from a methoxy group H-9 (δ_H 3.86) to C-4 (δ_C 152.7) was observed. A long-range HMBC correlation between H-8 (δ_H 2.54) and C-1 (δ_C 138.7) suggested the presence of the depsidone scaffold. The HMBC correlations of H-7' (δ_H 2.48) to C-6' (δ_C 130.3), C-1' (δ_C 143.4) and a quaternary carbon C-5' (δ_C 119.4) indicated the presence of a chlorine atom at position C-5'. Other HMBC correlations formed second identical aromatic ring C as shown in Fig. 3. An unassigned ester carbonyl carbon at C-7 (δ_C 162.8) was maintained. Conclusively, the ester carbonyl carbon (C-7)

Fig. 1 Antiproliferative effects of EtOAc, *n*-hexane and MeOH-H₂O fractions of the *Teloschistes flavicans* on human leukemia HL-60 cells (means $\pm$ SEMs, *n* = 6).

Fig. 3 The HMBC correlations of flavicansone (4).

was attached at the C-1 position, with the ester group forming a bridge between C-1 in the A ring and C-2' in the C ring. Likewise, C-2 in the A ring and C-1' in the C ring were linked by an oxygen atom. All above arguments determined the structure of compound 4 as 2,7-dichloro-3,8-dimethoxy-1,6,9-trimethyl-11*H*-dibenzo[*b,e*][1,4]dioxepin-11-one. Based on the literature survey, the isolated compound 4 was an unreported novel compound, which we named flavicansone.

3.3 Antileukemic activity of isolated compounds

All four isolated compounds (1 - 4) were evaluated for their antileukemic activity against HL-60 cells (Fig. 4). This investigation only estimated the antileukemic activity among the four isolated compounds. Parietin (2) and rhizonic acid (1) showed low inhibitory activity on HL-60 cells. However, vicanicin (3) and flavicansone (4), which comprised of a depsidone skeleton, exhibited higher cytotoxicity against HL-60 cells. Depsidone-based derivatives are a class of typical metabolites, possessing a tricyclic core bearing a central seven-membered lactone¹⁴. Flavicansone (4) showed moderate activity, with an IC_{50} value of 58.18 μ M. In contrast, reportedly the IC_{50} values of 5-fluorouracil and quercetin are 9.5 μ M and 61.1 μ M, respectively^{15, 16}.

Flavicansone (4) was the strongest among the four tested compounds. Flavicansone (4) presented a similar molecular structure with vicanicin (3). The structural differences included functional groups at the C-3 and C-4 positions in vicanicin (3) and flavicansone (4). The hydroxyl moiety at C-3 in vicanicin (3) was methoxylated at C-3 in flavicansone (4). The methyl substituent at C-4 in vicanicin (3) was lost in flavicansone (4). This feature could have affected the antileukemic activity of depsidone compounds during this investigation.

4 Conclusions

In conclusion, we presented a phytochemical investigation of *T. flavicans* and isolated a new depsidone, 2,7-dichloro-3,8-dimethoxy-1,6,9-trimethyl-11*H*-dibenzo[*b,e*][1,4]dioxepin-11-one (4), as well as three known compounds, rhizonic acid (1), parietin (2), and vicanicin (3). Furthermore, the antileukemic activities of isolated compounds were evaluated. Flavicansone (4) showed the strongest activity among the four tested compounds. To the best of our knowledge, no previous report has presented depsidone 2,7-dichloro-3,8-dimethoxy-1,6,9-trimethyl-11*H*-dibenzo[*b,e*][1,4]dioxepin-11-one (4), which we named flavicansone.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Fig. 4 Antiproliferative effects of isolated constituents of the *Teloschistes flavicans* on human leukemia HL-60 cells (means $\pm$ SEMs, $n = 6$). 1: rhizonic acid; 2: parietin; 3: vicanicin; 4: flavicansone.

Supporting Information

This material is available free of charge via the Internet at <http://dx.doi.org/jos.69.10.5650/jos.ess20209>

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