Eumitrins F-H: three new xanthone dimers from the lichen *Usnea baileyi* and their biological activities

Van-Kieu Nguyena,b, Hoai-Vu Nguyen-Sia,b, Asshaima Paramita Devic,d, Pakarapon Poonsukkhoe, Ek Sangvichien, Thanh-Nha Tranf, Hioki Yusukeg, Tohru Mitsunagah and Warinthorn Chavasiric

aInstitute of Fundamental and Applied Sciences, Duy Tan University, Ho Chi Minh City, Vietnam; bFaculty of Natural Sciences, Duy Tan University, Da Nang, Vietnam; cCenter of Excellence in Natural Products Chemistry, Department of Chemistry, Faculty of Science, Chulalontorn University, Pathumwan, Bangkok, Thailand; dProgram of Biotecnology, Faculty of Science, Chulalontorn University, Bangkok, Thailand; eLichen Research Unit and Lichen Herbarium, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand; fDepartment of Enviromental Engineering, Thu Dau Mot University, Binh Duong, Vietnam; gGraduate School of Natural Science and Technology, Gifu University, Gifu, Japan; hFaculty of Applied Biological Sciences, Gifu University, Gifu, Japan

**ABSTRACT**

The lichen *Usnea baileyi* is a fruticose lichen belonging to the *Usnea genus*. It is well known as a rich source of natural xanthone dimers and possesses various bioactivities. Nevertheless, the chemical investigation on this type of lichen is still rare as most of researches reported its components without structural elucidation. Herein, in the continuous study on this type of lichen, we further isolate xanthone dimers from the dichloromethane extract and explore three new xanthone dimers, eumitrins F–H (1–3). Their structures were elucidated unambiguously by spectroscopic analyses, including high resolution electrospray ionisation mass spectrometry (HRESIMS), 1D and 2D nuclear magnetic resonance spectroscopy (1D and 2D NMR), and DP4 probability. All compounds were evaluated for their enzyme inhibition against α-glucosidase, tyrosinase, and antibacterial activity. They revealed moderate antimicrobial and weak tyrosinase inhibition. For α-glucosidase inhibition, compound 3 displayed the most significant inhibitory against α-glucosidase possessing an IC₅₀ value of 64.2 μM.

**ARTICLE HISTORY**

Received 17 August 2021
Accepted 19 December 2021

**KEYWORDS**

Lichen; *Usnea baileyi*; dimeric xanthone; α-glucosidase; enzyme inhibitory; antibacterial

**CONTACT**

Van-Kieu Nguyen nguyenvankieu2@duytan.edu.vn

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2021.2023143.

© 2022 Informa UK Limited, trading as Taylor & Francis Group
1. Introduction

The *Usnea baileyi*, a fruticose lichen of the genus *Usnea*, is generally found in tropical forests (Nguyen et al. 2020). It has been proved to contain usnic acid, salazinic acid and eumitrin A3 as major components (Din et al. 2010; Nguyen et al. 2021). This type of lichen presents various bioactivities, such as antioxidant (Nguyen et al. 2019) and antibacterial (Santiago et al. 2010; Sinha and Biswas 2011). Although various secondary metabolites, such as triterpenoids, depsides, depsidones, aliphatic acids, dibenzofurans, and dimeric xanthones have been reported (Yang et al. 1973; Ramesh et al. 1994; Din et al. 2010; Duong et al. 2017, 2021; Nguyen et al. 2017; Bui et al. 2020, Devi et al. 2020; Huynh et al. 2020; Tuong et al. 2020). Many dimeric xanthones, including eumitrin A3, B2 from *Usnea baileyi* (Din et al. 2010) and eumitrin U, X, or Y from other lichen sources were reported without structural elucidation (Bungartz et al. 2004; Giralt and Eix 2010; Lendemer et al. 2012). Thus, the isolation and elucidation of this structural series is interesting. In our previous reports, the phytochemical investigation of *U. baileyi* led to the isolation and structural elucidation of bailexanthone (Nguyen et al. 2018) and eumitrins C–E (Nguyen et al. 2020). Continuing the search for new xanthone dimers from *U. baileyi*, our current study reports the isolation, structural elucidation, and evaluation of α-glucosidase, tyrosinase, and antibacterial activity of three new xanthone dimers: eumitrins F–H (1–3).

2. Results and discussion

Continuing chromatographic fractionation of the dichloromethane extract of *U. baileyi* led to the purification of three new compounds (1–3).

Compound 1 was obtained as yellow powder. The HRESIMS of 1 showed a sodiated ion peak at m/z 633.1942, appropriate to a molecular formula of C\textsubscript{32}H\textsubscript{34}O\textsubscript{12}. However, the $^1$H NMR analysis displayed apparently 16 protons signal. Thus, one proton was concluded to be present as an aliphatic hydroxy group. The above characteristic data determined 1 to be a homodimeric xanthone that was similar to those of bailexanthone and eumitrin C from same lichen biosources (Nguyen et al. 2018, 2020). The $^1$H NMR spectrum of 1 revealed the occurrence of one chelated hydroxy group ($\delta_{\text{H}}$ 12.21), two ortho aromatic protons ($\delta_{\text{H}}$ 7.48, d, $J = 8.4$ Hz and 6.58, d, $J = 8.4$ Hz), one oxymethine proton ($\delta_{\text{H}}$ 3.89, 1H, d, $J = 6.8$ Hz), one methoxy proton ($\delta_{\text{H}}$ 3.84, 3H, s), one
doublet methyl proton (\(\delta_H 1.11\), 3H, d, \(J = 6.8\) Hz) and six protons in the range of 1.20–3.50 ppm including two methines (\(\delta_H 3.40\) and 2.06 ppm) and two methylene protons (\(\delta_H 2.24, 1.83, 1.75, 1.29\) ppm).

The \(^{13}\)C NMR, in agreement with the HSQC spectra of 1, suggesting the presence of 16 carbon signals, consisting of one conjugated ketone carbon (\(\delta_C 198.7\)), one ester carbonyl carbon (\(\delta_C 170.1\)), two aromatic methine carbons (\(\delta_C 141.1\) and 107.6), one methoxy group (\(\delta_C 53.3\)), three methine carbons (\(\delta_C 36.0, 46.4,\) and 74.0), two methylene carbons (\(\delta_C 27.3\) and 21.5), one methyl group (\(\delta_C 17.7\)) and five quaternary carbons (\(\delta_C 159.5, 157.3, 117.8, 106.8\) and 85.6).

The relative configuration of 1 was recognised by the general analysis of \(^1\)H NMR (Table S1) and NOESY correlations (Figure S1). The coupling constant of H-5 (\(\delta_H 3.89\), d, \(J = 6.8\) Hz) was inconsistent with the corresponding value reported for secalonic acid A (\(J = 11.3\) Hz) (Le Pogam and Boustie 2016) or ergochrome B-D (\(J = 10.8\) Hz) (Nguyen et al. 2018). In addition, the NOESY correlation between protons H-5 (\(\delta_H 3.89\)) and H3–11 (\(\delta_H 1.11\); Figure S1) designated the anti-facial of H-5 and H-6. Therefore, the relative assignment of C-5 and C-6 was supported the assigned 5\(S, 6R\) configuration by the comparison of the NMR data with those of bailexanthone (Nguyen et al. 2018), eumitrin C (Nguyen et al. 2020), and blennolide G, dimeric secalonic B and its C-5 epimer (Zhang et al. 2008). The anti-facial orientation of ester group at \(\delta_H 3.84\) (H3–13) and the methine proton at \(\delta_H 3.40\) (H-8a) with H-5 and H3–11 was supported by the disappearance of NOESY correlation of H-5 or H3–11 to H-8a or H3–13 that further determined the interestingly cis-decalin orientation of 1 the same as in bailexanthone (Nguyen et al. 2018). However, the coupling constants of H-8a (\(\delta_H 3.40,\) s) were inconsistent with those of bailexanthone (Nguyen et al. 2018), leading to the definition of the equatorial position of H-8a. Furthermore, a negative \(n\pi^*\) ECD band (299 nm, \(\Delta\varepsilon = -5.6\); Figure S2) determined the S configuration of 10a/10a’ S and also indicated the production of the other chiral centres based on the relative stereochemistry, consistent with literature data (Zhang et al. 2008). Thus, the \((5S, 6R, 8aS, 10aS)\) absolute configuration of 1, eumitrin F, was endorsed, as presented in Figure 1.
Compound 2 was obtained as a yellow powder. The HRESIMS of 2 showed a sodiated molecular ion peak at m/z 633.1922, consistent with a molecular formula of C_{32}H_{34}O_{14}. The 1H NMR spectrum showed 30 protons, confirming the presence of two additional aliphatic hydroxyl groups. The exhaustive analysis of the 2D NMR spectra indicated a similar monomer subunit A as in 1 with the quaternary carbon C-2' as a linkage site to the other monomer subunit. However, the methine, methoxy ester and oxymethine protons were shifted upfield to \delta_H 2.99 (H-8a'), 3.68 (H_3-13') and 3.73 (H-5'), respectively, in comparison to those of 1. In this case, the coupling constant of the proton H-5' (\delta_H 3.73, 1H, d, J = 10.4 Hz) defined its axial orientation and thus determined a 5'S configuration, identical to the structure of eumitrin C of the subunit B (Nguyen et al. 2020). Moreover, the syn facial orientation of the methyl group, methine and oxymethine protons was suggested by the NOESY correlation from H-5' to both H_3-11' and H-8a'. In addition, the coupling constants of H-8a' (\delta_H 2.99, dd, J = 11.6, 4.4 Hz) in 2 indicated the axial position of H-8a', identical to those of eumitrin C (Nguyen et al. 2020). Furthermore, the 10a'R configuration was further supported by the positive n-π* ECD band (311 nm, \Delta\varepsilon = +9.4; Figure S2). Thus, the (S,S,6R,8aS,10aS,5'S,6'R,8'R,10a'R) absolute configuration of 2, namely eumitrin G, was determined, as shown in Figure 1.

Compound 3 was isolated as a light yellow amorphous. The molecular formula C_{34}H_{32}O_{14}Na was established by the sodiated ion peak at m/z 687.1673 (calc. for C_{34}H_{34}O_{14}Na^+, 687.1690). The spectral comparison of 3 with those of 1 and 2 indicated their closely related molecular formula; however, differences in the 1H and 13C NMR spectra indicated the replacement of an aliphatic hydroxyl group located at C-5' (in 1 and 2) by an acetoxycarbonyl, supported by the HMBC correlations of both the methyl at \delta_H 1.82 (H_3-15') and methine proton at \delta_H 5.44 (H-5') to carbon C-14' (\delta_C 169.2). Moreover, one methyl group was shifted downfield to \delta_H 2.08 (H_3-11') demonstrating its aromatic nature and placing it at C-3', further suggested by the HMBC correlations of proton H_3-11' to C-2' (\delta_C 111.4), C-3' (\delta_C 150.5) and C-4' (\delta_C 115.8), consistent with the appearance of a singlet aromatic proton at \delta_H 6.50 (H-2'). The COSY spectrum, as well as the full set of 2J and 3J correlations in the HMBC spectrum revealed the H-5'/H_2-6'/H_2-7'/H-8' spin system and established the hexahydroxanthone scaffold of the first monomer (subunit B). In addition, the analysis of the 1H and 13C NMR spectra revealed a similar gross structure of eumitrin A2, except for the lack of the H-8a' methine proton and the occurrence of an aromatic proton at \delta_H 7.30 that indicated the appearance of a Δ8(9) double bond. Thus, the relative configuration of C-5' and C-10' were determined as similar as those of eumitrin A2, a major xanthone dimer isolated from the same biosource (Yang et al. 1973; Nguyen et al. 2018).

The second monomer, subunit A, was very similar to those of 1 and 2, the most noticeable spectroscopic difference indicating the occurrence of an enolic moiety at C-8 and C-8a positions from the intense downfield shifts of C-8 and C-8a (\delta_C values of 179.7 and 100.4 vs 21.5 and 46.4 ppm, respectively) compared to the subunit of 1. The HMBC correlations of both H-2' and H-3 (with respective \delta_H values of 6.50 and 7.27) to C-4', led to the indicated of C-2,C-4' linkage between subunits A and B. Furthermore, the anti-orientation of H_3-11 and the hydroxyl group at C-5 was determined by the NOESY correlation between H-5 (\delta_H 4.18) and H_3-11 (\delta_H 1.19). Additionally, the
anticlockwise manner of the two benzoyl chromophores of 3 as a R could be assumed from the negative exciton couplet centred at around 240 nm (Ola et al. 2014; Li et al. 2016). However, the ECD spectroscopy does not provide enough information for the assignment of the relative configuration at C-5, C-6, and C-10. Due to the NOESY correlation between H-5 and H3-11, the two diastereomer candidates (3a and 3b) were suggested (Figure 2), which would be assigned through the DP4 parameter for three conformers of 3a and five of 3b that displayed the Boltzmann distribution above 10%. As seen in Table S2, all 3a conformers show very low probabilities in both DP4+ (0.39%, 0.39% and 0.42%) and J-DP4 (2.15, 2.14 and 2.20) columns, suggesting the low probability of 3a being the proper candidate for 3. In contrast, conformers 3b1 and 3b2 show the highest DP4 probabilities among the five candidates (46.03% and 46.35% from DP4+; 37.11% and 37.75% from J-DP4, respectively), suggesting their ability to be the proper structures of 3. Besides, the 3a (ave.) and 3b (ave.) average NMR peaks were also assigned to DP4+ and J-DP4 calculations. The probabilities in Table S2 show a similar trend to the separate conformer calculations, indicating that the candidate 3b (with DP4+ and J-DP4 percents as 92.76% and 83.59%, respectively) would be the proper structure for 3. This determined stereochemistry was further confirmed by the negative n-π* ECD band (306 nm, Δε = −5.1; Figure S2) opposite to those of eumitrin A2 (Nguyen et al. 2018), or usneaxanthone D (Tuong et al. 2019). Thus, the structure of 3, namely eumitrin H, was established.

In the previously reported biological activities of the related skeletons, secalonic acid and eumitrin derivatives revealed good activity on antimicrobial (Zhang et al. 2008) and cytotoxicity (Tuong et al. 2019). Unfortunately, xanthone dimers isolated from U. baileyi exhibited weak or no cytotoxic activity (Nguyen et al. 2018; Nguyen et al. 2020). In this study, compounds 1–3 were evaluated for their antimicrobial activity and enzyme inhibition against tyrosinase and α-glucosidase (Table S3). Most of these compounds exhibited weak antimicrobial activity, except for compound 1 showing medium activity against E. coli and B. subtilis (62.5 μg/mL for each bacterium). In addition, 2, having the same co-structure as 1, expressed good activity against B. subtilis (62.5 μg/mL) (Table S3). These results are similar to those of secalonic acid analogs reported in the previous research (Bao et al. 2013). For enzyme inhibition, all of tested compounds exhibited weak active against both tyrosinase and α-glucosidase, except for compound 3. This compound not only showed good activity against α-glucosidase
but also exhibited better activity than acarbose (IC$_{50}$ 93.6 μM) with an IC$_{50}$ value of 64.2 μM.

### 3. Experimental

#### 3.1. General experimental procedures

The 1D and 2D NMR spectra were acquired using a Bruker Advance (400/100 MHz for $^1$H/$^{13}$C NMR, respectively) spectrometer. The residual solvent signal (CDCl$_3$: $\delta_H = 7.26$, $\delta_C = 77.16$) was used to reference the chemical shifts. HRESIMS data were measured using a Bruker MicroTOF Q-II mass spectrometer. The column chromatography was performed by using (0.040–0.063 mm, Himedia)-silica gel. TLC analysis were accomplished on silica gel 60 F$_{254}$ or silica gel 60 RP-18 F$_{254}$S plates (Merck), and 10% H$_2$SO$_4$ solution was used to visualise the spots after heating.

#### 3.2. Lichen material

The whole thalli of *Usnea baileyi* (Parmeliaceae) were collected and identified as stated in our previous report (Nguyen et al. 2020).

#### 3.3. Extraction and isolation

In this work, we continue our study of further fractionation of the three fractions DCM2.1-3. The description for extraction and isolation were reported in (Nguyen et al. 2020). DC2.2.2 (450.9 mg) was designated for further isolation by silica gel chromatographic column eluted with n-hexane/CH$_2$Cl$_2$/MeOH (3:7:0.1) to yield compounds 1 (5.6 mg) and 2 (6.7 mg). Moreover, the fraction DCM2.2.3 (385.7 mg) was applied to silica gel chromatographic column with n-hexane/CH$_2$Cl$_2$/EtOAc/MeOH (6:4:2:0.1) as mobile phase to obtain compounds 3 (2.2 mg).

#### 3.3.1. Eumitrin F (1)

Yellow, amorphous solid; $[\alpha]^{25}_D = -104.4$ (c 0.02, MeOH); ECD (0.1, CHCl$_3$) $\lambda$(Δε) 205 (+25.9), 215 (+0.6), 234 (+18.8), 252 (+17.4), 299 (-5.6), 357 (-4.0); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta_H$ 12.21 (2H, s, 1-OH, 1$_0$-OH), 7.48 (2H, d, $J = 8.4$ Hz, H-3, 3$_0$), 6.58 (2H, d, $J = 8.4$ Hz, H-4, 4$_0$), 3.89 (2H, d, $J = 6.8$ Hz, H-5, 5$_0$), 3.84 (6H, s, H-13, 13$'_0$), 2.24 (2H, m, H-a8, a8'), 2.06 (2H, m, H-6, 6'), 1.83 (2H, m, H-a7, a7'), 1.75 (2H, m, H-b8, b8'), 1.29 (2H, m, H-a7, a7'), 1.11 (6H, d, $J = 6.8$ Hz, H-11, 11$'_0$). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta_C$ 198.7 (C-9, 9$'_0$), 170.1 (C-12, 12$'_0$), 159.5 (C-1, 1$'_0$), 157.3 (C-4a, 4a$'_0$), 141.1 (C-3, 3$'_0$), 117.8 (C-2, 2$'_0$), 107.6 (C-4, 4$'_0$), 106.8 (C-9a, 9a'), 85.6 (C-10a, 10$'_0$), 74.0 (C-5, 5$'_0$), 53.3 (C-13, 13$'_0$), 46.4 (C-8a, 8a'), 36.0 (C-6, 6$'_0$), 27.3 (C-7, 7$'_0$), 21.5 (C-8, 8$'_0$), 17.7 (C-11, 11$'_0$). HRESIMS m/z: [M + Na]$^+$ 633.1942 for C$_{32}$H$_{34}$O$_{12}$Na$^+$ (calcd. 633.1948).

#### 3.3.2. Eumitrin G (2)

Yellow, amorphous solid; $[\alpha]^{25}_D = -68.4$ (c 0.02, MeOH); ECD (0.1, CHCl$_3$) $\lambda$(Δε) 205 (+25.9), 217 (+2.8) 231 (+17.1), 251 (+11.4), 311 (+9.4), 358 (-2.8); $^1$H NMR (CDCl$_3$, 400 MHz) $\delta_H$ 12.19 (1H, s, 1-OH), 11.88 (1H, s, 1$_0$-OH), 7.49 (1H, d, $J = 8.0$ Hz, H-3$'_0$), 7.47...
(1H, d, J = 7.6 Hz, H-3), 6.62 (1H, d, J = 8.8 Hz, H-4'), 6.58 (1H, d, J = 8.4 Hz, H-4), 3.89 (1H, d, J = 6.8 Hz, H-5), 3.83 (3H, s, H-13), 3.73 (1H, d, J = 10.4 Hz, H-5'), 3.68 (3H, s, H-13'), 3.39 (1H, m, H-8a), 2.99 (1H, dd, J = 11.6, 4.4 Hz, H-8a'), 2.20 (1H, m, H-a8), 2.17 (2H, m, H-a8'), 2.07 (1H, m, H-6), 1.96 (1H, m, H-a7'), 1.84 (1H, m, H-6'), 1.80 (1H, m, H-a7), 1.73 (1H, m, H-b8), 1.29 (1H, m, H-b7'), 1.23 (1H, m, H-a7'), 1.11 (6H, d, J = 6.6 Hz, H-11, 11'). 13C NMR (CDCl3, 100 MHz) δC 198.7 (C-9), 197.4 (C-9'), 170.2 (C-12), 169.3 (C-12'), 159.6 (C-1), 159.0 (C-1'), 159.0 (C-4a'), 157.3 (C-4a), 141.0 (C-3), 140.4 (C-3'), 117.8 (C-2), 117.6 (C-2'), 107.5 (C-4), 107.4 (C-4'), 107.6 (C-9a'), 106.8 (C-9a), 87.6 (C-10'), 85.6 (C-10a), 80.3 (C-5'), 74.0 (C-5), 53.3 (C-13), 53.0 (C-13'), 51.2 (C-8a'), 46.3 (C-8a), 36.0 (C-6), 34.3 (C-6'), 31.2 (C-7), 27.2 (C-7), 21.4 (C-11). HRESIMS m/z: [M + Na]+ 633.1922 for C32H34O12Na+ (calcd. 633.1948).

3.3.3. Eumitrin H (3)

Yellow, amorphous solid; [α]25° D +264.8 (c 0.02, MeOH); ECD (0.1, CHCl3) k (De) 207 (-7.0), 222 (+2.3), 244 (-2.8), 282 (+2.6), 306 (-5.1), 370 (-6.8); 1H NMR (CDCl3, 400 MHz) δH 12.01 (1H, s, 1-OH), 11.60 (1H, s, 1-OH), 7.30 (1H, brs, H-8'), 7.27 (1H, d, J = 8.0 Hz, H-3), 6.61 (1H, d, J = 8.0 Hz, H-4), 6.50 (1H, s, H-3'), 5.44 (1H, brs, H-5'), 4.18 (1H, d, J = 8.0 Hz, H-5), 3.78 (3H, s, H-13'), 3.70 (3H, s, H-13), 2.54 (1H, d, J = 16.4, 4.0 Hz, H-b7), 2.40 (1H, m, H-6'), 2.08 (3H, s, H-11'), 2.01 (2H, m, H-6'), 1.19 (3H, d, J = 6.4 Hz, H-11). 13C NMR (CDCl3, 100 MHz) δC 188.1 (C-9), 184.9 (C-9'), 179.7 (C-8), 171.4 (C-12'), 169.9 (C-12), 169.2 (C-14'), 162.3 (C-1'), 159.5 (C-1), 157.5 (C-4a), 156.2 (C-4a'), 150.5 (C-3'), 141.7 (C-8'), 140.3 (C-3), 129.1 (C-8a'), 117.9 (C-2), 115.8 (C-4'), 111.4 (C-2'), 108.0 (C-4'), 107.2 (C-9a), 105.8 (C-9a'), 100.4 (C-8a), 85.0 (C-10a), 81.0 (C-10'), 71.5 (C-5), 66.3 (C-5'), 53.8 (C-13'), 53.7 (C-13), 32.8 (C-7), 28.7 (C-6), 23.6 (C-6'), 22.0 (C-7'), 21.3 (C-11'), 20.5 (C-15'), 17.7 (C-11). HRESIMS m/z: [M + Na]+ 687.1673 for C34H34O14Na+ (calcd. 687.1690).

3.4. Tyrosinase activity

The tyrosinase inhibitory activity was performed using 96 well micro plate (Larik et al. 2017) with modification. Compounds were prepared in 10% DMSO in buffer and two fold dilution was done to obtain various concentration. 50 μL of sample solution in buffer were placed in 96 well plate, then 50 μL tyosinase enzyme from mushroom (250 U/mL) was added and the mixture were incubated for 5 minutes. 50 μL of 5 mM L-tyrosine was added later as a substrate the mixtures then incubated further for 30 minutes. The reaction was measured at 490 nm. Kojic acid was used as positive control. The concentration range of pure compounds used for the activity was 0–200 μg/mL. Percent of tyrosinase inhibition was calculated from the following Equation (1) (Larik et al. 2017) and IC50 was determined for each sample in triplicate at several concentrations (200, 100, 50, 25, 12.5, and 6.25 μM).

%Tyrosinase inhibition = \[ \frac{([\Delta A \text{ control} - \Delta A \text{ sample}])}{([\Delta A \text{ control}])} \times 100 \] (1)

Where "ΔA control" was the absorbance value at 490 nm without the test sample and "ΔA sample" was the absorbance value with mixture contained the sample.
3.5. α-Glucosidase activity

The α-glucosidase assay was tested according to Ramadhan and Phuwapraisirisan (2015). The measuring absorbance at 405 nm using ALLSHENG micro plate reader AMR-100 machine to determine the enzymatic activity. For each compound, the experiment was evaluated in triplicate at several concentrations (200, 100, 50, 25, 12.5, and 6.25 μM) to achieve the IC50 value, that was also further identified the mean values and standard deviation.

3.6. Antimicrobial activity

The minimum inhibitory concentration (MIC) for each compound was determined by the broth micro-dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2015) (Patel et al. 2015).

Compounds 1-3 were evaluated for their antimicrobial activities against Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and Candida albicans (ATCC 10231) by microbroth dilution method in 96-well culture plates. The test microorganisms were incubated at 37°C for 24 h in Mueller-Hinton broth and the microbial suspensions were adjusted to 0.5 McFarLand unit. The inoculum was then diluted 100 times and 100 μL of inoculum was added to 96-well.

Stock solution of each compound was dissolved in 100% dimethylsulphoxide (DMSO) to a stock solution of 1 mg/mL. The compound was further two fold diluted in DMSO and tested at final concentrations between 500 to 0.98 μg/mL. Chloramphenicol was used as a positive control. The growth was observed after 24 hours of incubation using visual reaction by addition colour of iodonitrotetrazolium (INT), wherereading +: growth colour pink (growth) and reading −: growth colour yellow (no growth).

3.7. NMR calculation

The two candidates structures of 3, denoted 3a and 3b, were elucidated by the probabilities 13C NMR assignment, with DP4+ and J-DP4 theories applied (Grimblat et al. 2015; Grimme 2019). The conformer sampling for 3a and 3b were performed in CHCl3 solvent, utilised CREST code (Grimme 2019; Pracht et al. 2020), within 1.0 kcal/mol energy window. The collected conformers then were calculated Boltzmann distribution (pi) employed the (Equation (2)), where Ei or Ej is the total energy of the selected conformer, and RT value is 0.593 kcal/mol at room temperature.

\[ p_i = \frac{\exp(-E_i/RT)}{\sum_{j=1}^{all} \exp(-E_j/RT)} \]  

(2)

Only the conformers that have pi were above 10% would be sent to the NMR peak prediction. The NMR calculations for the 3 conformers were performed by ORCA code code (Neese et al. 2020), employed PBE0/pcSseg-2//B97-3c/def2-mTZVP level of theory.

The PBE0/pcSseg-2//B97-3c/def2-mTZVP level of theory was employed, and the calculation was performed by ORCA code, version 4.2.1. The B97-3c/def2-mTZVP
functional/basis set was applied to the geometry optimisation, and the PBE0/pcSseg-2 for NMR calculation, as Folmsbee and Semenov suggested (Semenov and Krivdin 2020; Folmsbee and Hutchison 2021). Two probability theories (DP4+ and J-DP4) were applied to verify the candidate structures because of a difference in DFT theory used in NMR calculation in this research (compared to mPW1PW91 functional in DP4 theory).

4. Conclusions

In this study, the fractionation of dichloromethane extract from lichen Usnea baileyi led to the isolation of three new compounds (1-3). Their structures were elucidated as xanthone dimers namely eumitrin F-H by using HRESIMS and 1D and 2D NMR along with DP4 parameters. Their enzyme inhibition against α-glucosidase, tyrosinase, and antimicrobial activity were evaluated, exhibiting good activity against α-glucosidase (3), moderate inhibition against B. subtilis and E. coli (1, 2; and 1, respectively).

Supplementary material

Supplementary material relating to this article is available online.

Acknowledgement

V.-K. Nguyen would like to thank N. Dangphui for the authentification of the lichen material.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was financially supported by the Thailand Research Fund via Directed Basic Research Grant (Grant no. DBG6180029), the 90th anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) and the Overseas Research Experience Scholarship for Graduate Student.

ORCID

Hioki Yusuke (http://orcid.org/0000-0002-7068-9654

References


