RESEARCH ARTICLE

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Two New α-Glucosidase Inhibitory Depsidones from the Lichen *Parmotrema cristiferum* (Taylor) Hale

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A bioactivity-guided investigation of the lichen *Parmotrema cristiferum* (Taylor) Hale (Parmeliaceae) led to the isolation of two new depsidones, cristifones A and B (**1** and **2**). The structures of the isolated compounds were identified by spectroscopic methods and comparison with the literature data. Compound **1** showed the initial combined structures of depsidone and depside cores. The two isolated compounds were then evaluated for α-glucosidase inhibition. Compounds **1** and **2** were confirmed as potent, with IC$_{50}$ values of 21.5 and 18.4 μM, respectively. Compound **2** was a non-competitive inhibitor against α-glucosidase, as indicated by the intersect in the second quadrant of each respective plot.

Keywords: *Parmotrema cristiferum*, cristifones A and B, depsidone, depside, α-glucosidase inhibition.

Introduction

Lichens are symbiotic products of algae and fungi that have fascinated the natural products community because of their diverse secondary metabolites, which are unique to the lichen symbiosis.[1–5] Several lichen extracts have been used in folk medicine, and some lichen metabolites have been shown to have antifungal,[6] antimicrobial, antioxidant, and insecticidal[7] properties. Lichen-derived depsidones are thought to be potent α-glucosidase inhibitors,[8–11] as are natural *para*- and *meta*-depsidones.[8,9] The lichen *Parmotrema cristiferum* (Taylor) Hale (Parmelia-

ceae) occurs commonly in high-altitude forests in Vietnam. Previous research on the secondary metabolites of the lichen *P. cristiferum* from Vietnam has indicated that it contained cristiferides A and B, 2,4-dihydroxyphthalide, lecanoric acid, orsellinic acid, 5-chloroorsellinic acid, methyl haematommate, and methyl β-orsellinate.[12] Terpenoids and steroids were also isolated from this lichen, including norreticulatin, anadensin, fusicoauritone, ergosterol, and botulin.[13] Cristiferide A and norreticulatin displayed potent inhibition against α-glucosidase.[12,13] Previous research found that the Vietnamese lichen *P. cristiferum* contains potent α-glucosidase inhibitors. In the search for α-glucosidase inhibitors, we have re-visited the lichen *P. cristiferum* following bioactivity-guided isolation. Two new depsidones, namely cristifones A and B (**1** and **2**), were isolated and structurally elucidated.

These isolated compounds were evaluated for their α-glucosidase inhibition and kinetic study.

Results and Discussion

Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined as $C_{34}H_{24}O_{16}$ by a deprotonated ion peak at *m/z* 687.0988 on the HRESI mass spectrum (calc. for $C_{34}H_{23}O_{16}$, 687.0986). The ^{1}H-NMR (*Table 1*) and HSQC spectra revealed one aldehyde (δ_H 10.57), one hemiacetal (δ_H 6.86), four aromatic methine (δ_H 6.76, 6.61, 6.26, and 6.06, the two latter *meta*-coupled each other), one methylene (δ_H 4.13), and three methyl (δ_H 2.78, 2.50, and 2.11) protons. The JMOD spectrum, in accordance with the HSQC spectrum, exhibited one aldehyde (δ_C 194.7), one methylene (δ_C 20.4), three carboxy ester (δ_C 172.0, 171.4, and 162.8), three methyl (δ_C 24.3, 21.3, and 18.2), four methine (δ_C 118.1, 111.1, 110.3, and 101.5), and 20 quaternary carbons. The spectroscopic data suggested that **1** was a polyketide with four aromatic rings (*Figure 1*). The chemical structure of **1** was identified by HMBCs (*Figure 2*). At first, HMBCs of H_3-9 (δ_H 2.11) to C-1 (δ_C 112.8), C-5 (δ_C 118.1), and C-6 (δ_C 154.3), of H-5 (δ_H 6.76) to C-1, C-3 (δ_C 111.1), C-

4 (δ_C 166.1), and C-8 (δ_C 21.3), and of H-8 (δ_H 10.57) to C-4 (δ_C 194.7), indicating an A-ring chemical structure. 1D NMR data of the A-ring were highly similar to those of previously reported depsidones derived from the *Parmotrema* genus.[3,9] Secondly, the methylene H_2-8' (δ_H 4.13) gave HMBCs to C-2' (δ_C 149.7), C-3' (δ_C 123.7), and C-4' (δ_C 143.4) of the B-ring and to C-4'' (δ_C 152.4) and C-6'' (δ_C 142.5) of the C-ring. This finding indicated a connection between these rings at C-8', Next, in the C-ring, HMBCs of H-3'' (δ_H 6.61) to C-2'' (δ_C 160.1) and C-5'' (δ_C 123.3), and of H_3-8'' (δ_H 2.78) to C-1'' (δ_C 112.4), C-5'', and C-6'' identified connectivity through C-1''-C-6'', The significant downfield signal of H-8'' indicated that it could be adjacent to a carboxy ester group. This chemical shift was reminiscent of the 6-CH_3 groups that can be found in lichen-deriving depsides, i.e., lecanoric acid or gyrophoric acid (recorded in the same deuterated acetone-d_6).[1] Additionally, in the D-ring, HMBCs of H-3''' (δ_H 6.06) to C-1''' (δ_C 104.1) and C-2''' (δ_C 164.4), of H-5''' (δ_H 6.26) to C-1''' and C-3''' (δ_C 101.5), and of H_3-8''' (δ_H 2.50) to C-1''', C-5''' (δ_C 110.3), and C-6''' (δ_C 145.2) defined chemical structure as a D-ring. The long-range HMBC of H_3-8''' to C-4''' (δ_C 154.6) determined the chemical shift of C-4''', The downfield ^{1}H-NMR chemical shift of H_3-8''' and the upfield ^{13}C signal of C-1''' indicated that
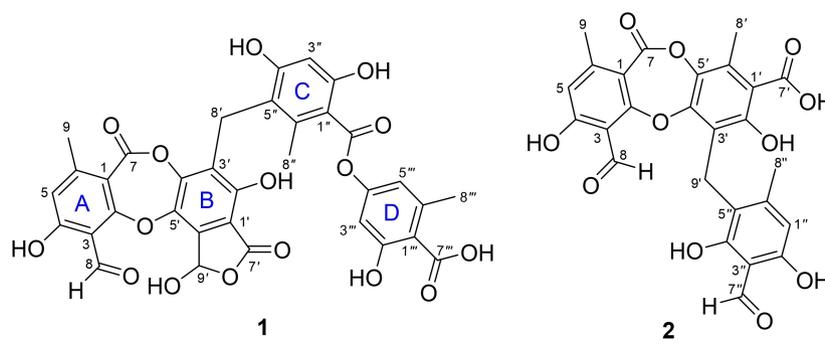
Figure 1. Chemical structures of **1** and **2**.
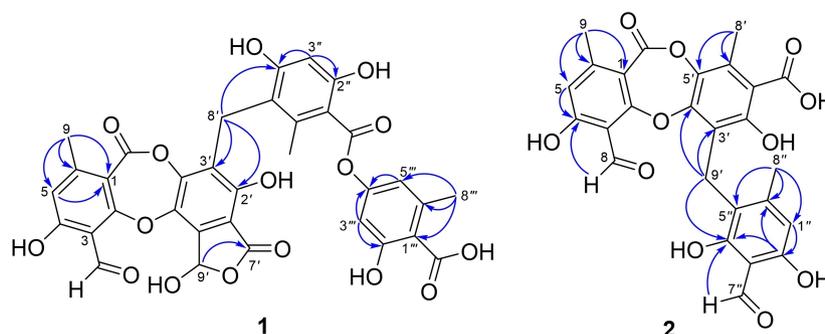
Figure 2. Selected HMBC data of **1** and **2**.

the carboxylic acid group was attached at C-1‴, This is commonly found in the monoaromatic lichen compounds or the A-ring of depsides.[1] The hemiacetal proton H-9′ (δ_H 6.86) appeared in ^{1}H-NMR, but carbon C-9′ could not be found in HSQC and ^{13}C-NMR spectra. The same phenomena were found in similar compounds such as praesorether C from *P. Praesorediosum*,[14] parmosidone F from *P. dilatatum*,[9] and parmetherine D from *P. indicum*[15] due to the minute amount of **1**.

A detailed comparison of NMR data of **1** and parmosidone F[9] provided the complete NMR assignments of the B-ring. Combined, the chemical structure of **1** was elucidated as shown, namely cristifone A (*Figure 1*). Cristifone A (**1**) represents a combined structure of a δ-lactone-bearing *para*-depsidone unit and an orcinol-type depside core, which have not been reported in nature. The biosynthetic mechanism was proposed from the precursors, salazinic acid and lecanoric acid via a Friedel-Crafts acylation (*Scheme S1*). This type of reaction was reported by Pham and co-workers (2022).[11]

Compound **2** was isolated as a white amorphous powder. Its molecular formula was determined to be $C_{26}H_{20}O_{11}$ based on the deprotonated ion at *m/z* 507.0917 [M−H]$^-$ in HRESI mass spectrum (calc. for $C_{26}H_{19}O_{11}{}^-$, 507.0927). The ^{1}H-NMR and HSQC spectra indicated the presence of one chelated hydroxyl (δ_H 12.19), one methylene (δ_H 3.94), two aldehyde (δ_H 10.73 and 10.18), two aromatic methine (δ_H 6.77 and 6.24), and three methyl (δ_H 2.68, 2.47, and 2.46) protons. The JMOD spectrum in combination with the HSQC spectrum indicated the presence of two aldehyde carbonyl (δ_C 194.8 and 194.5), two aromatic methine (δ_C 117.9 and 109.5), three methyl (δ_C 21.9, 21.7, and 15.8), and 16 quaternary (δ_C 166.6, 165.6, 162.4, 162.3, 160.3, 154.0, 150.8, 148.8, 143.8, 132.2, 119.7, 117.7, 114.4, 111.8, 108.9, and 104.5) carbons. The above spectroscopic data indicated that **2** was a depsidone having three aromatic rings.[2,4,7] The ^{13}C-NMR chemical shifts of two aldehyde groups were determined by the signals at δ_C 194.8 and 194.5 in the JMOD and ^{13}C-NMR spectra of **2** (*Figures S11–S13*). These aldehyde groups were also supported by HR-ESI mass data. HMBCs of H$_3$-9 (δ_H 2.47) to C-1 (δ_C 114.4), C-6 (δ_C 154.0), and C-5 (δ_C 117.9), and of H-5 (δ_H 6.77) to C-9 (δ_C 21.9), C-3 (δ_C 111.8), C-1, and C-4 (δ_C 166.6) indicated the connectivity through C-1-C-3-C-4-C-9 in the A-ring (*Figure S1*). In the B-ring, HMBCs of H$_3$-9′ (δ_H 2.68) to C-6′ (δ_C 143.8) and C-5′ (δ_C 132.2), and of H$_2$-8′ (δ_H 3.94) to C-2′ (δ_C 162.4), C-3′ (δ_C 119.7), and C-4′ (δ_C 148.8) defined the structure of the B-ring. The down-

field chemical shift of H$_3$-9′ (δ_H 2.68) and C-2′ indicated that **2** was a parmosidone-type depsidone.[2,3] HMBCs of H$_2$-8′ to C-6″ (δ_C 150.8) and C-4″ (δ_C 162.3) indicated the connection between two B- and C-rings at C-8′, Moreover, HMBCs of H-1″ to C-5″ (δ_C 117.7), C-3″ (δ_C 109.5), and C-8″ (δ_C 21.7), and of H-8″ to C-5″, C-1″ (δ_C 108.9), and C-6″. indicated the structure of the C-ring. In the same deuterated solvent, a detailed comparison of **2** and parmosidone K revealed that they shared the same A- and B-rings. The only difference is in the C-ring with the presence of an additional aldehyde group (δ_H 10.18, H-7″). This was supported by HMBCs of H-7″ to C-3″ and C-4″. All the above spectroscopic data accounted for the $C_{25}H_{19}O_{10}$ moiety. The remaining substituent should be an aldehyde group, supporting the chemical structure of the C-ring. Altogether, compound **2** was elucidated as shown, namely cristifone B.

Compounds **1** and **2** were evaluated for their α-glucosidase inhibition. All isolated compounds showed stronger activity than acarbose (*Table 2*). Depsidones **1** and **2** showed potent α-glucosidase inhibition, with IC$_{50}$ values of 21.5 and 18.4 µM, respectively. This indicated the important role of the depsidone scaffold in α-glucosidase inhibition. The results were consistent with previously reported depsidones from *Parmotrema* lichens.[8–11,15–16] Noteworthy, the difference in α-glucosidase inhibitory activity between **1** and **2** suggested that the phenolic (D-ring) and oxotetrahydrofuranyl moieties in **1** was possibly decrease in inhibitory activity against α-glucosidase.

Compound **2**, which was more active than **1**, was selected to examine the mechanism of α-glucosidase inhibition. Lineweaver-Burk plots gave a group of lines with the same Michaelis constant (K_m) intersecting the y-axis in the second quadrant (*Figure 3A*). An increasing concentration of **2** caused a decrease in the V_{max} of α-glucosidase. The kinetics of enzyme inhibition showed that **2** acted as a non-competitive inhibitor. The comparable values of K_i (20.7±1.8 µM) and K'_i (20.9±1.7 µM) obtained from secondary plots suggested that **2** inhibits α-glucosidase by equally forming enzyme-inhibitor (EI) and enzyme-inhibitor-substrate (EIS) complexes (*Figure 4*).

Conclusions

Two new depsidones, cristifones A and B (**1** and **2**) were isolated from the lichen *Parmotrema cristiferum* (Taylor) Hale (Parmeliaceae) using a bioactivity-guided investigation. Compound **1** represented the first
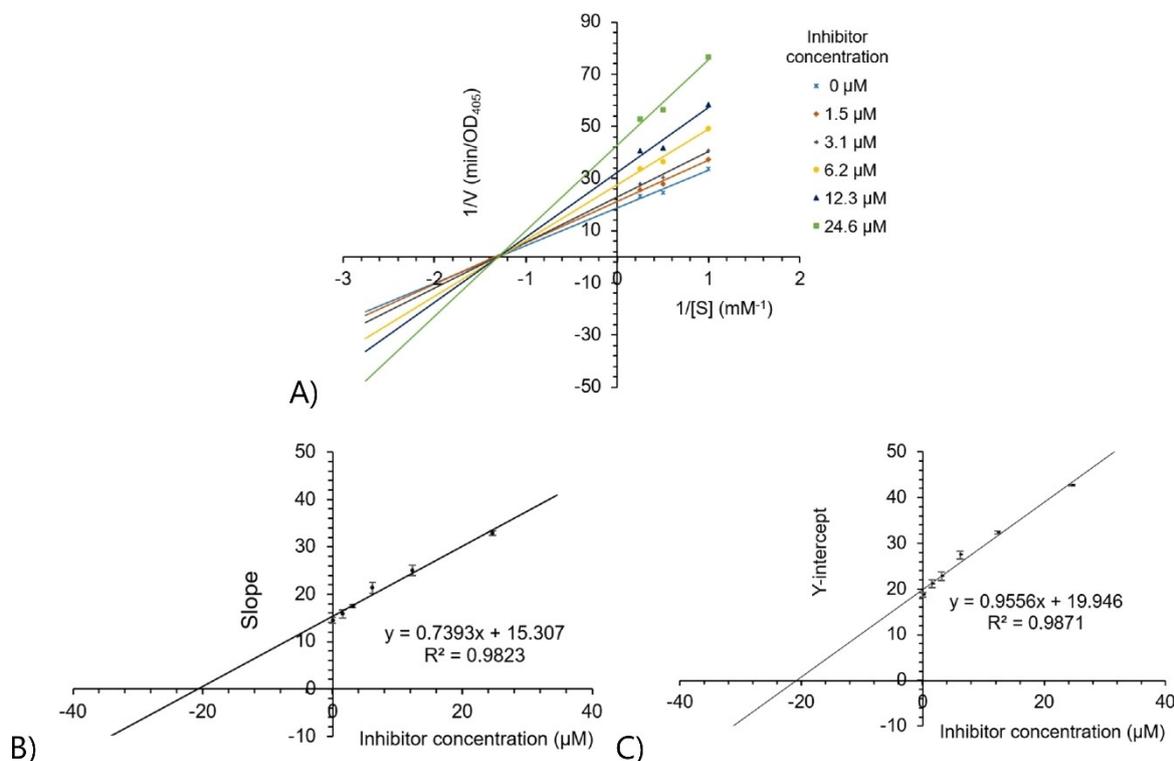
Figure 3. Lineweaver-Burk plot (A) for α-glucosidase inhibition by **2**, the secondary plots of slope *vs.* inhibitor concentration (B), and the secondary plots of y-intercept *vs.* inhibitor concentration (C).
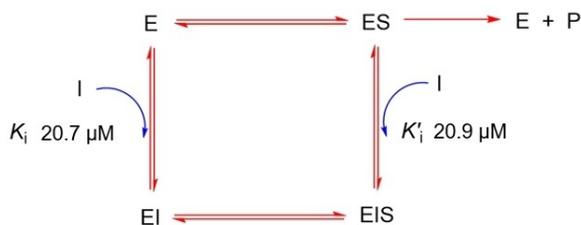
Figure 4. Proposed inhibitory mechanism of **2** against α-glucosidase.

combination of depsidone and depside (lecanoric acid) cores. Both compounds were confirmed as potent α-glucosidase inhibitors with IC_{50} values of 21.5 and 18.4 µM, respectively. Cristifone B (**2**) was selected for further kinetic study, indicating that it was a non-competitive inhibitor against α-glucosidase. This study further clarified the chemical components of the lichen *P. cristiferum* and their α-glucosidase inhibition, which can be useful for the development and utilization of this lichen in the intervention of diabetes.

Experimental Section

General

NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) with TMS as internal standard. HR-ESI-MS was recorded using a MicrOTOF–Q mass spectrometer on an LC-Agilent 1100 LC/MSD Trap spectrometer. Thin-layer chromatography (TLC) was carried out using precoated silica gel 60 F_{254} or 60 RP-18 F_{254S} (Merck). Spots were visualized by applying a 10% H_2SO_4 solution, followed by heating. Gravity column chromatography was performed on the silica gel 60 (0.040–0.063 mm, Himedia). *Saccharomyces cerevisiae* α-glucosidase (E.C 3.2.1.20) and acarbose were obtained from Sigma-Aldrich Co.

Lichen Material

The thallus of lichen *P. cristiferum* was collected in Duc Trong district, Lam Dong province, Vietnam in March 2021. The scientific name of the lichen was determined by Dr. Thi-Phi-Giao Vo from the Faculty of Biology at the Ho Chi Minh University of Science, National University-Ho Chi Minh City. A voucher speci-

men (UE–L006) was deposited in the herbarium of the Department of Organic Chemistry at the Ho Chi Minh University of Education.

Extraction and Isolation

The clean, air-dried, and ground material (520 g) was macerated in AcOEt at room temperature (5 L ×5 times, each for 12 h). The filtrated solution was concentrated under reduced pressure to afford the crude AcOEt extract (185 g). The crude extract was suspended in water and successively liquid-liquid partitioned into hexane, hexane-AcOEt (5:5, *v/v*), and AcOEt to afford the corresponding extracts H (11.7 g), HEA (56.2 g), and EA (88.3 g), respectively. Extract EA (88.3 g) was selected for further analysis due to having the strongest activity toward α-glucosidase (*Table S1*). This extract was applied to silica gel column chromatography (CC) and eluted with a gradient of hexane-AcOEt (10:1 to 1:10, *v/v*) to obtain fractions EA1 (14.1 g), EA2 (12.3 g), EA3 (3.41 g), EA4 (2.43 g), EA5 (4.15 g), EA6 (8.15 g), and EA7 (16.88 g), respectively. These fractions were screened for biological activity, indicating the most active fraction being fraction EA5. This fraction was further subjected to Sephadex LH-20 CC and eluted with MeOH to gain four subfractions (EA5.1–EA5.4). Subfraction EA5.4 (487 mg) was subjected to silica gel CC and eluted with CHCl$_3$–AcOEt–acetone-AcOH (1:2:2:0.01, *v/v/v/v*) to obtain subfractions EA5.4.1 (88 mg), EA5.4.2 (45 mg), EA5.4.3 (101 mg), and EA5.4.4 (67 mg). Subfraction EA5.4.4 was subjected to C$_{18}$ reverse-phase CC and eluted with MeOH–H$_2$O (2:1, *v/v*) to give **1** (2.1 mg) and **2** (3.8 mg).

Cristifone A (**1**). White amorphous powder; IR (neat) cm^{-1}: 3494, 1713, 1426, 1364, 1224, 1050. UV λ_{max} (MeOH) nm (logε): 220 (3.2), 288 (2.7). ^{1}H-NMR (acetone-d_6, 500 MHz) and ^{13}C-NMR (acetone-d_6, 125 MHz) see *Table 1*; HR-ESI-MS *m/z*: [M−H]$^-$ 687.0988 for C$_{34}$H$_{24}$O$_{16}^-$ (calc. 687.0986).

Cristifone B (**2**). White amorphous powder; ^{1}H-NMR (acetone-d_6, 500 MHz) and ^{13}C-NMR (acetone-d_6, 125 MHz) see *Table 1*; HR-ESI-MS *m/z*: [M−H]$^-$ 507.0917 for C$_{26}$H$_{19}$O$_{11}^-$ (calc. 507.0927).

α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity of **1** and **2** was determined using a method adapted from a previous method.[14] Serial concentrations of **1** (0.78–12.50 µg/ mL), **2** (1.56–25.0 µg/mL), and acarbose (25–400 µg/ mL) were prepared by dissolving in DMSO (400 mg/ mL). Sodium phosphate buffer (100 mM, pH 6.8) was used to dissolve the α-glucosidase (0.4 U/mL) and substrate (2.5 mM *p*NPG). The substrate (40 µL) was added to the reaction mixture after the inhibitor (50 µL) was preincubated with α-glucosidase in 96-well plates at 37 °C for 10 min. A mixture without enzyme, sample compound, and acarbose served as blank, while the control contained only DMSO, enzyme, and substrate. The enzymatic reaction was carried out at 37 °C for 30 min and stopped by adding 0.2 M Na$_2$CO$_3$ (130 µL). Absorbance at 410 nm to measure enzyme activity was recorded on a BIOTEK reader. All samples were analyzed in triplicate at five

Table 1. ^{1}H- and ^{13}C-NMR data (500 and 125 MHz) of **1** and **2** in acetone-d_6.

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
1		112.8		114.4
2		160.0		–
3		111.1		111.8
4		166.1		166.6
5	6.76, s	118.1	6.77, s	117.9
6		154.3		154.0
7		162.8		–
8	10.57, s	194.7	10.73, s	194.5
9	2.11, s	21.3	2.47, s	21.9
1′		119.1		104.5
2′		149.7		162.4
3′		123.7		119.7
4′		143.4		148.8
5′		139.8		132.2
6′		135.7		143.8
7′		171.4		165.6
8′	4.13, s	20.4	3.94, s	21.3
9′	6.86, br. s	–	2.68, s	15.8
1″		112.4	6.24, s	108.9
2″		160.1		160.3
3″	6.61, s	113.4		109.5
4″		152.4		162.3
5″		123.3		117.7
6″		142.5		150.8
7″			10.18, s	194.8
8″	2.78, s	18.2	2.46, s	21.7
1‴		104.1		
2‴		154.6		
3‴	6.06, d (2.0)	101.5		
4‴		164.4		
5‴	6.26, s	110.3		
6‴		145.2		
7‴		172.0		
8‴	2.50, s	24.3		
4-OH			12.19, s	

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different concentrations around the IC$_{50}$ values, and the mean values were retained. The inhibition percentage (%) was calculated via the following equation: Inhibition (%) $= [1-(A_{sample}/A_{control})] \times 100$.

Inhibitory Type Assay of **2** *on* α-*Glucosidase*

The inhibition mechanism of α-glucosidase by **2** was determined by Lineweaver-Burk plots, using methods similar to those reported in the literature.[14] The inhibition type was determined using various concentrations of *p*NPG (1.0, 2.0, and 4.0 mM) as a substrate in the presence of different concentrations of **2** (0, 2.3, 4.6, 9.3, and 18.5 μM). The experiments were carried out in three replicates, and the inhibition constants were obtained graphically from secondary plots.

Acknowledgements

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Author Contribution Statement

T.H.D. collected the lichen species and carried out the experiment. T.H.D., N.H.N., and J.S. elucidated the chemical structures and drafted the manuscript. T.G.V. and V.M.D. designed and performed the α-glucosidase inhibition assay and helped in manuscript writing. T.H.D., N.H.N., and J.S. participated in the collection of spectroscopic and spectrometry data. T.N.M.A. provided access to instrumentation and helped in editing the manuscript. N.H.N. and J.S. designed and supervised the work. All authors read and corrected the manuscript.

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