Three Novel Biphenanthrene Derivatives and a New Phenylpropanoid Ester from Aerides multiflora and Their α-Glucosidase Inhibitory Activity

May Thazin Thant 1,2, Boonchoo Sritularak 1,3,*, Nutputsorn Chatsumpun 4, Wanwimon Mekboonsonglarp 5, Yanyong Punpreuk 6 and Kittisak Likhitwitayawuid 1,*

Citation: Thant, M.T.; Sritularak, B.; Chatsumpun, N.; Mekboonsonglarp, W.; Punpreuk, Y.; Likhitwitayawuid, K. Three Novel Biphenanthrene Derivatives and a New Phenylpropanoid Ester from Aerides multiflora and Their α-Glucosidase Inhibitory Activity. Plants 2021, 10, 385. https://doi.org/10.3390/plants10020385

Abstract: A phytochemical investigation on the whole plants of Aerides multiflora revealed the presence of three new biphenanthrene derivatives named aerimultins A–C (1–3) and a new natural phenylpropanoid ester dihydrosinapyl dihydroferulate (4), together with six known compounds (5–10). The structures of the new compounds were elucidated by analysis of their spectroscopic data. All of the isolates were evaluated for their α-glucosidase inhibitory activity. Aerimultin C (3) showed the most potent activity. The other compounds, except for compound 4, also exhibited stronger activity than the positive control acarbose. Compound 3 showed non-competitive inhibition of the enzyme as determined from a Lineweaver–Burk plot. This study is the first phytochemical and biological investigation of A. multiflora.

Keywords: Aerides multiflora; Orchidaceae; α-glucosidase inhibition; biphenanthrene derivatives; phenylpropanoid ester

1. Introduction

Diabetes mellitus (DM) is one of the main causes of global morbidity and mortality [1]. The disease is caused by insufficient insulin secretion and/or action. DM is associated with high blood glucose levels, and type 2 DM is the most common form, covering 90–95% of all diabetes cases [2]. Drugs currently used for treating DM can be classified into several classes following their chemical structures and modes of action, and some have limitations due to their adverse reactions or unpleasant effects [3]. α-Glucosidase is an enzyme located in the small intestine. It is responsible for converting starch and disaccharides into monosaccharides (glucose). Inhibition of this enzyme can significantly reduce postprandial hyperglycemia [4]. α-Glucosidase inhibitors (AGIs) have been widely used in combination with other anti-DM drugs in the management of type 2 DM [5–8].

However, AGIs can cause liver injuries and gastrointestinal side effects [9,10]. There has been a growing interest in developing antidiabetic drugs of botanical origin because they are perceived as possessing fewer undesired effects [11,12]. Several promising AGIs have been reported from some members of the family Orchidaceae, such as Dendrobium tortile [13], Bulbophyllum retususculum [14], and Arundina graminifolia [15].
Aerides is a small genus of epiphytes in the family Orchidaceae. It consists of approximately 21 species that are native to South and South-East Asia [16]. Some Aerides species have been used in traditional medicine. For example, Aerides falcata has been used for boosting the immune system, whereas Aerides odoratum has been known for its antibacterial properties [17]. Phytochemical screening of Aerides odoratum suggested the presence of alkaloids, glycosides, flavonoids, saponins, tannins, terpenoids, steroids, and anthroquinones [18]. Several phenanthrene derivatives have been identified from Aerides rosea [19] and Aerides crispum [20].

Aerides multiflora Roxb. (Figure 1) is commonly known as “The Multi-flowered Aerides” [21] and called “Malai Dang” in Thai [22]. It has several synonyms, including Aerides affinis, Aerides godefroyana, Aerides lobbii, Cleisostoma vacherotiana, and Epidendrum geniculatum [23]. The plant is indigenous to Bangladesh, India, Nepal, Myanmar, Thailand, Malaysia, Philippines, Laos, Cambodia, and Vietnam. A. multiflora has been traditionally used as a tonic [24]. It has also been used to treat cuts and wounds [17,25] and fractured and dislocated bones [26]. In an earlier study, its tubers showed an antibacterial effect in vitro [27]. As a continuation of our investigation of orchids for α-glucosidase inhibitors [28–30], a MeOH extract obtained from the whole plants of Aerides multiflora was evaluated and found to possess strong inhibitory property against the enzyme (82.4 ± 9.5% inhibition at 100 µg/mL). In this communication, we describe our findings on the chemical constituents of this plant and their α-glucosidase inhibitory activity.

Figure 1. Aerides multiflora Roxb.

2. Results and Discussion
2.1. Structural Characterization

A total of 10 polyphenolic compounds were isolated from the MeOH extract of Aerides multiflora through solvent partition and repeated chromatography. They were characterized as three unknown compounds, named aerimultins A-C (1–3) and a new natural product, dihydrosinapyl dihydroferulate (4), together with six known compounds, i.e., 6-methoxycoelonin (5) [31], gigantol (6) [32], imbricatin (7) [33], agrostonin (8) [34], dihydroconiferyl dihydro-3-coumarate (9) [35] and 5-methoxy-9,10-dihydro-phenanthrene-2,3,7-triol (10) [36] (Figure 2).
Compound 1 was isolated as a whitish-brown amorphous solid. It showed a [M+Na]+ at m/z 565.1841 (calculated for C_{32}H_{30}O_{8}Na, 565.1838) in the HR-ESI-MS. The IR spectrum showed absorption bands for hydroxyl (3350 cm⁻¹), aromatic ring (2923, 1605 cm⁻¹), methylene (1462 cm⁻¹) and ether (1221 cm⁻¹) groups. The UV absorptions at 265, 305, and 315 nm were indicative of a dihydrophenanthrene skeleton [37]. The ¹³C NMR and HSQC spectra revealed signals for twenty-four aromatic carbons, plus eight aliphatic carbons representing four methoxy and four methylene groups. The four CH₂ carbons at 29.0 (C-9), 31.4 (C-10), 29.9 (C-9′), and 24.1 (C-10′) displayed HSQC correlations to the protons at δ 2.45 (2H, m, H₂-9) and 2.56 (2H, m, H₂-10) and 2.52 (4H, br s, H₂-9′, H₂-10′), respectively. These NMR signals suggested that 1 should be a dimeric compound consisting of two units of 9,10-dihydrophenanthrene (Table 1). The first unit of 1 (rings A, B, and C) should be derived from methoxycoelonein (5), a dihydrophenanthrene also obtained in this study, because its ¹H and ¹³C NMR properties bore a close resemblance to those of 5 (Table 1). For example, in ring A of the first unit of 1, the proton at C-1 (δ 6.35, 1H, d, J = 2.5 Hz) exhibited HMBC correlation with C-10 (δ 31.4) and NOESY interaction with H₂-10. H-3 (δ 6.46, 1H, d, J = 2.5 Hz) of 1 showed a NOESY cross peak with MeO-4 protons (δ 3.89, 3H, s). The hydroxyl proton at C-2 was observed at δ 8.35 (1H, s). For ring B of 1, the following ¹H NMR signals were found: two one-proton singlets at δ 6.33 (1H, s, H-8) and 7.98 (1H, s, H-5), and a three-proton singlet at δ 3.92 (3H, s, MeO-6) which showed a NOESY cross-peak with H-5. The second unit of 1 (rings A′, B′, and C′) also exhibited ¹H and ¹³C NMR data similar to those of 5 (Table 1). For instance, the ¹H NMR spectrum of 1 exhibited two singlet proton...
Plants 2021, 10, 385

- signals at 6.66 (1H, s, H-8') and 7.93 (1H, s, H-5'), two methoxy groups at δ 3.84 (3H, s, MeO-6') and 3.91 (3H, s, MeO-4'), and two hydroxyl groups at δ 7.44 (s, HO-7') and 8.25 (s, HO-2'). The HMBC spectrum of 1 showed correlation from H-3' to C-1' (δ 133.7) and C-4a' (δ 117.1), and from H-5' to C-4a' (δ 117.1), C-8a' (δ 131.4) and C-7' (δ 145.6). H-8' (δ 6.66, 1H, s, HO-7') displayed HMBC correlation with C-9' (δ 29.9) and NOESY interaction with H-2'. The methoxy protons at C-4' (δ 155.4) and C-6' (δ 146.1) showed NOESY correlations with H-3' and H-5', respectively. However, the second dihydrophenanthrene unit of 1 showed the absence of a H-1' signal, with the signal for H-3' appearing as a singlet at δ 6.65. Moreover, in the 13C NMR spectrum of 1, the signal for C-1' of this unit was downfield shifted and observed as a quaternary carbon at δ 133.7, with HMBC correlations with H-3' (δ 6.65, s), H-2-10' (δ 2.52, br s) and HO-2' (δ 8.25, s). These NMR properties indicated that the structure of 1 consisted of two methoxycoelonin (5) units connected to each other through an ether linkage at C-7 and C-1'. This was also supported by the absence of a hydroxyl proton at C-7 (δ 146.6). Based on the above spectral data, compound 1 was characterized as a new dimeric 9,10-dihydrophenanthrene derivative and given the trivial name aerimultin A.

Table 1. 1H (500 MHz) and 13C-NMR (125 MHz) spectral data of 1 and 5 in acetone-d6.

| Position | 1a (δH (Multiplicity, J in Hz)) | 1b (δC) | HMBC (Correlation with 1H) | 5b (δH (Multiplicity, J in Hz)) | 5c (δC) |
|----------|--------------------------------|---------|---------------------------|--------------------------------|---------|
| 1        | 6.35 (d, J = 2.5 Hz)          | 108.3   | 3, 10, HO-2               | 6.39 (br s)                    | 107.4   |
| 2        | -                             | 157.8   | 1*, 3*, HO-2*             | -                              | 156.5   |
| 3        | 6.46 (d, J = 2.5 Hz)          | 99.1    | 1, HO-2                   | 6.65 (br s)                    | 98.3    |
| 4        | -                             | 158.8   | 3*, MeO-4                 | -                              | 157.7   |
| 4a       | -                             | 115.9   | 1, 3, 5, 10               | -                              | 115.5   |
| 4b       | -                             | 127.8   | 5*, 8, 9                 | -                              | 124.7   |
| 5        | 7.98 (s)                      | 114.3   | -                         | 7.89 (s)                       | 112.2   |
| 6        | -                             | 147.4   | 8, MeO-6                  | -                              | 145.1   |
| 7        | -                             | 146.6   | 5                         | -                              | 144.3   |
| 8        | 6.33 (s)                      | 113.6   | 9                         | 6.69 (s)                       | 114.0   |
| 8a       | -                             | 131.1   | 5, 10                     | -                              | 130.7   |
| 9        | 2.45 (m)                      | 29.0    | 8                         | 2.61 (m)                       | 28.9    |
| 10       | 2.56 (m)                      | 31.4    | 1                         | 2.61 (m)                       | 30.7    |
| 10a      | -                             | 141.6   | 9, 10*                    | -                              | 140.5   |
| 1'       | -                             | 133.7   | 3', 10', HO-2'            | -                              | 140.5   |
| 2'       | -                             | 149.8   | 3', HO-2'                 | -                              | 140.5   |
| 3'       | 6.65 (s)                      | 100.2   | HO-2'                     | -                              | 140.5   |
| 4'       | -                             | 155.3   | 3', MeO-4'                | -                              | 140.5   |
| 4a'      | -                             | 117.1   | 3', 5', 10'               | -                              | 140.5   |
| 4b'      | -                             | 125.2   | 5', 8', 9'                | -                              | 140.5   |
| 5'       | 7.93 (s)                      | 113.4   | -                         | -                              | 140.5   |
| 6'       | -                             | 146.1   | 8', MeO-6', HO-7'         | -                              | 140.5   |
| 7'       | -                             | 145.6   | 5', HO-7'                 | -                              | 140.5   |
| 8'       | 6.66 (s)                      | 114.9   | 9', HO-7'                 | -                              | 140.5   |
| 8a'      | -                             | 131.4   | 5', 10'                   | -                              | 140.5   |
| 9'       | 2.52 (br s)                   | 29.9    | 8'                        | -                              | 140.5   |
| 10'      | 2.52 (br s)                   | 24.1    | -                         | -                              | 140.5   |
| 10a'     | -                             | 133.8   | 9'                        | -                              | 140.5   |
| MeO-4    | 3.89 (s)                      | 56.1    | -                         | 3.86 (s)                       | 55.5    |
| MeO-6    | 3.92 (s)                      | 56.5    | -                         | 3.83 (s)                       | 54.9    |
| MeO-4'   | 3.91 (s)                      | 56.4    | -                         | -                              | 140.5   |
| MeO-6'   | 3.84 (s)                      | 55.8    | -                         | -                              | 140.5   |
| HO-2     | 8.35 (s)                      | -       | -                         | -                              | 140.5   |
| HO-2'    | 8.25 (s)                      | -       | -                         | -                              | 140.5   |
| HO-7'    | 7.44 (s)                      | -       | -                         | -                              | 140.5   |

*a* 1H (500 MHz) and 13C-NMR (125 MHz); *b* 1H (300 MHz) and 13C-NMR (75 MHz); * two-bond coupling.
Compound 2, a brown amorphous solid, exhibited [M+Na]⁺ at m/z 559.1376 (calculated for C₃₂H₃₄O₈Na, 559.1368) in the HR-ESI-MS, corresponding to the molecular formula C₃₂H₃₄O₈. The IR spectrum showed absorption bands due to the presence of hydroxyl (3368 cm⁻¹), aromatic ring (2919, 1587 cm⁻¹) and ether (1259 cm⁻¹) functionalities. The UV absorptions at 265, 315.5 and 370 nm were suggestive of a phenanthrene skeleton [30]. Compound 2 should be a dimeric phenanthrene, as suggested from the ¹H NMR signals for two pairs of ortho-coupled doublets, representing H-9 (δ 7.36, 1H, d, J = 9.5 Hz), H-10 (δ 6.98, 1H, d, J = 9.5 Hz), H-9′ (δ 7.37, 1H, d, J = 9.0 Hz), and H-10′ (δ 6.92, 1H, d, J = 9.0 Hz) (Table 2). The first phenanthrene unit of 2 (rings A, B, and C) exhibited 1H and 13C NMR resonances similar to those of agrostostin (8), a biphenanthrene also isolated from this plant (Table 2). These included three one-proton singlets at δ 6.99 (1H, s, H-3), 7.19 (1H, s, H-8) and 9.24 (1H, s, H-5)), and two methoxy groups at δ 3.96 (3H, s, MeO-6) and 4.22 (3H, s, MeO-4). The proton at C-8 showed HMBC correlation with C-9 (δ 126.5). The protons H-3 and H-5 exhibited three-bond couplings to C-4a (δ 116.2) in the HMBC spectrum. The NOESY correlations of the MeO-4 and MeO-6 protons with H-3 and H-5, respectively, supported the attachment of these methoxy groups at C-4 and C-6. The quaternary carbon at δ 109.3 was assigned as C-1 according to its HMBC cross-peaks with H-3 and H-10. For the second phenanthrene unit (rings A′, B′, and C′), the presence of oxymethylene protons at δ 5.79 (2H, d, J = 1.5 Hz, H₂-11) indicated a phenanthropyran structure [38]. The ¹H NMR spectrum also displayed two sharp one-proton singlets at δ 7.21 (1H, s, H-8′) and a methoxy group at δ 3.95 (3H, s, MeO-6′). The assignments of H-8′ and H-3′ were supported by their HMBC correlations with C-9′ (δ 127.9) and C-1′ (δ 110.2), respectively. The HMBC correlations of C-6′ (δ 144.2) with MeO-6′ protons and H₂-11′ indicated the location of the methoxy group at C-6′. The C-1′ of this second unit showed HMBC correlations with H-3′ and H-10′. The chemical shifts of C-1 (δ 109.3) and C-1′ (δ 110.2) suggested that they were not oxygenated, but, instead, they formed a C=C bridge linking the two monomers [39]. Therefore, it was concluded that 2 had the structure as shown, and the compound was given the trivial name aerimulin B.

Compound 3 was obtained as a brown amorphous solid. Its UV absorptions and IR absorption bands were similar to those of compound 2, indicating a phenanthrene derivative. The HR-ESI-MS exhibited [M+Na]⁺ at m/z 533.1218 (calculated for C₂₉H₂₄O₈Na, 533.1212), suggesting the molecular formula C₂₉H₂₄O₈. However, the ¹³C NMR spectrum showed only 15 carbon signals, suggesting that 3 should be a dimeric phenanthrene with two identical units. Comparison of the ¹H and ¹³C NMR of 3 with those of agrostostin (8) (Table 2) revealed their structural similarity, except for the presence of a hydroxyl group at C-6/C-6′ in 3, instead of a methoxy group. Moreover, the two phenanthrene units were symmetrically linked to each other through a C=C bond between C-1 and C-1′ as supported by the HMBC correlations from C-1/C-1′ to H-3/H-3′, H-10/H-10′ and HO-2/HO-2′ [39]. On the basis of above spectral evidence, the structure of compound 3 was established as shown, and the trivial name aerimulin C was given to the compound.

Compound 4 was obtained as a yellow amorphous solid. The molecular formula was determined as C₂₁H₂₆O₇ by HR-ESI-MS of [M+Na]⁺ at m/z 413.1584 (calculated for C₂₁H₂₆O₇Na, 413.1576). The IR spectrum showed absorption bands for hydroxyl (3432 cm⁻¹), carbonyl ester (2937, 1608 cm⁻¹), carbonyl ester (1723, 1208, 1111 cm⁻¹) and methylene (1455 cm⁻¹) groups. The UV spectrum exhibited maximum absorptions at 280 and 315 nm. The ¹H NMR spectrum (Table 3) exhibited signals for a dihydroferulate structure [δ 2.59 (2H, t, J = 7.5 Hz, H₂-8), 2.81 (2H, m, H₂-7), 3.82 (3H, s, MeO-3), 6.68 (1H, dd, J = 8.1, 1.5 Hz, H-6′), 6.73 (1H, d, J = 8.1 Hz, H-5′), and 6.85 (1H, d, J = 1.5 Hz, H-2′)] [40]. This was confirmed by the HMBC correlations of C-2 (δ 111.8), C-6 (δ 120.6) and C-9 (δ 172.2) with H₂-7 (Table 3). The location of a MeO-3 group was supported by its NOESY correlation with H-2. The ¹H NMR spectrum also showed signals for a dihydrosinapyl structure [δ 1.89 (2H, m, H₂-8′), 2.57 (2H, t, J = 7.5 Hz, H₂-7′), 3.80 (6H, s, MeO-3′, MeO-5′), 4.05 (2H, t, J = 7.5 Hz, H₂-9′), and 6.49 (2H, s, H₂-2′, H-6′)] [41]. The HMBC correlations of C-2′/C-6′ (δ 105.8) and C-9′ (δ 63.2) with H₂-7′ supported the presence of this unit.
Table 2. $^1$H and $^{13}$C-NMR spectral data of 2, 3 and 8 in acetone-d$_6$.

| Position | $\delta$$_H$ (Multiplicity, J in Hz) | $\delta$$_C$ | HMBC (Correlation with $^1$H) | $\delta$$_H$ (Multiplicity, J in Hz) | $\delta$$_C$ | HMBC (Correlation with $^1$H) | $\delta$$_H$ (Multiplicity, J in Hz) | $\delta$$_C$ |
|----------|----------------------------------|------------|-------------------------------|----------------------------------|------------|-------------------------------|----------------------------------|------------|
| 1        | -                                | 109.3      | 3, 10                         | -                                | 108.8      | 3, 10, HO-2                   | -                                | 108.9      |
| 2        | -                                | 155.0      | 3 *                          | -                                | 154.1      | 3 *, HO-2 *                   | -                                | 154.2      |
| 3        | 6.99 (s)                         | 100.0      | -                            | 6.95 (s)                         | 98.8       | HO-2                         | 7.00 (s)                         | 99.2       |
| 4        | -                                | 160.2      | 3 *, MeO-4                    | -                                | 159.4      | 3 *, MeO-4                    | -                                | 159.3      |
| 4a       | -                                | 116.2      | 3, 5, 10                      | -                                | 115.1      | 3, 5, 10                      | -                                | 115.5      |
| 4b       | -                                | 125.8      | 8, 9                         | -                                | 125.3      | 8, 9                         | -                                | 124.9      |
| 5        | 9.24 (s)                         | 109.8      | -                            | 9.19 (s)                         | 112.7      | -                            | 9.26 (s)                         | 111.3      |
| 6        | -                                | 148.5      | 5 *, 8                        | -                                | 145.3      | 8                            | -                                | 147.6      |
| 7        | -                                | 146.0      | 5, 8 *                       | -                                | 144.1      | 5                            | -                                | 145.2      |
| 8        | 7.19 (s)                         | 112.2      | 9                            | 7.19 (s)                         | 111.5      | 9                            | 7.20 (s)                         | 112.3      |
| 8a       | -                                | 128.0      | 5, 10                         | -                                | 126.7      | 5, 10                         | -                                | 127.2      |
| 9        | 7.36 (d, J = 9.5 Hz)             | 126.5      | 8                            | 7.31 (s)                         | 127.2      | 8                            | 7.37 (s)                         | 127.1      |
| 10       | 6.98 (d, J = 9.5 Hz)             | 123.3      | -                            | 6.87 (s)                         | 121.8      | -                            | 6.95 (s)                         | 122.5      |
| 10a      | -                                | 135.4      | 9                            | -                                | 134.6      | 9                            | -                                | 134.6      |
| 1'       | -                                | 110.2      | 3', 10'                      | -                                | 108.8      | 3', 10', HO-2'                | -                                | 108.9      |
| 2'       | -                                | 156.3      | 3' *                         | -                                | 154.1      | 3' *, HO-2' *                 | -                                | 154.2      |
| 3'       | 6.81 (s)                         | 103.1      | -                            | 6.95 (s)                         | 98.8       | HO-2'                         | 7.00 (s)                         | 99.2       |
| 4'       | -                                | 153.7      | 3', 11'                       | -                                | 159.4      | 3', MeO-4'                    | -                                | 159.3      |
| 4a'      | -                                | 113.0      | 3', 10'                      | -                                | 115.1      | 3', 5', 10'                   | -                                | 115.5      |
| 4b'      | -                                | 119.1      | 8', 9', 11'                   | -                                | 125.3      | 8', 9'                       | -                                | 124.9      |
| 5'       | -                                | 120.6      | 11' *                        | 9.19 (s)                         | 112.7      | -                            | 9.26 (s)                         | 111.3      |
| 6'       | -                                | 144.2      | 8', 11', MeO-6'               | -                                | 145.3      | 8'                           | -                                | 147.6      |
| 7'       | -                                | 150.3      | 8' *                         | -                                | 144.1      | 5'                           | -                                | 145.2      |
| 8'       | 7.21 (s)                         | 111.6      | 9'                            | 7.19 (s)                         | 111.5      | 9'                           | 7.20 (s)                         | 112.3      |
| 8a'      | -                                | 126.2      | 10'                          | -                                | 126.7      | 5', 10'                      | -                                | 127.2      |
| 9'       | 7.37 (d, J = 9.0 Hz)             | 127.9      | 8'                           | 7.31 (s)                         | 127.2      | 8'                           | (d, J = 9.0 Hz)                   | 127.1      |
| 10'      | 6.92 (d, J = 9.0 Hz)             | 124.6      | 9'                           | 6.87 (s)                         | 121.8      | -                            | 6.95 (s)                         | 122.5      |
| 10a'     | -                                | 132.2      | 9'                           | -                                | 134.6      | 9'                           | -                                | 134.6      |
| 11'      | 5.79 (d, J = 1.5 Hz)             | 64.8       | -                            | -                                | -          | -                            | -                                | -          |
| MeO-4    | 4.22 (s)                         | 56.1       | -                            | 4.18 (s)                         | 55.0       | -                            | 4.24 (s)                         | 55.3       |
| MeO-6    | 4.06 (s)                         | 56.0       | -                            | -                                | -          | -                            | 4.08 (s)                         | 55.2       |
| MeO-4'   | -                                | -          | -                            | 4.18 (s)                         | 55.0       | -                            | 4.24 (s)                         | 55.3       |
| MeO-6'   | 3.95 (s)                         | 61.3       | -                            | -                                | -          | -                            | 4.08 (s)                         | 55.2       |
| HO-2     | -                                | -          | -                            | 7.54 (s)                         | 7.61 (s)   | -                            | 7.61 (s)                         | -          |
| HO-2'    | -                                | -          | -                            | 7.54 (s)                         | -          | -                            | 7.61 (s)                         | -          |

*H (500 MHz) and $^{13}$C-NMR (125 MHz); $^1$H (300 MHz) and $^{13}$C-NMR (75 MHz); * two-bond coupling.

The NOESY cross-peak between MeO-3'/MeO-5' protons and H-2'/H-6' confirmed the locations of the methoxy groups at C-3'/C-5' (δ 147.7). The two phenylpropanoid units were connected by an ester bond at C-9 and C-9', as determined by HMBC correlation of C-9 (δ 172.2) with H-2'. Based on the above spectroscopic evidence, compound 4 was determined as dihydrosinapyl dihydroferulate. Prior to this study, the natural occurrence of 4 was unknown. However, the compound was earlier synthesized by acylation of the lignins from Arabidopsis thaliana [42].
Table 3. $^1$H (300 MHz) and $^{13}$C-NMR (75 MHz) spectral data of 4 in acetone-$d_6$.

| Position | $\delta_H$ (Multiplicity, $J$ in Hz) | $\delta_C$ | HMBC (Correlation with $^1$H) |
|----------|-------------------------------------|------------|-----------------------------|
| 1        | -                                   | 132.1      | 5, 7 *, 8                    |
| 2        | 6.85 (d, $J = 1.5$ Hz)              | 111.8      | 6, 7                         |
| 3        | -                                   | 147.3      | 5, MeO-3, HO-4               |
| 4        | -                                   | 144.9      | 2, 6, HO-4 *                 |
| 5        | 6.73 (d, $J = 8.1$ Hz)              | 114.8      | HO-4                         |
| 6        | 6.68 (dd, $J = 8.1, 1.5$ Hz)        | 120.6      | 2, 7                         |
| 7        | 2.81 (m)                            | 30.4       | 2, 6, 8 *                    |
| 8        | 2.59 (t, $J = 7.5$ Hz)              | 35.8       | 7 *                          |
| 9        | -                                   | 172.2      | 7, 8 *, 9'                   |
| 1'       | -                                   | 131.7      | 8'                           |
| 2'       | 6.49 (s)                            | 105.8      | 6', 7'                        |
| 3'       | -                                   | 147.7      | 2' *, HO-4', MeO-3'          |
| 4'       | -                                   | 134.2      | 2', 6', HO-4' *              |
| 5'       | -                                   | 147.7      | 6' *, HO-4', MeO-5'          |
| 6'       | 6.49 (s)                            | 105.8      | 2', 7'                        |
| 7'       | 2.57 (t, $J = 7.5$ Hz)              | 31.8       | 2', 6', 8' *, 9'             |
| 8'       | 1.89 (m)                            | 30.4       | 7' *, 9' *                    |
| 9'       | 4.05 (t, $J = 7.5$ Hz)              | 63.2       | 7', 8' *                      |
| MeO-3    | 3.82 (s)                            | 55.3       | -                            |
| MeO-3'   | 3.80 (s)                            | 55.7       | -                            |
| MeO-5'   | 3.80 (s)                            | 55.7       | -                            |
| HO-4     | 7.35 (s)                            | -          | -                            |
| HO-4'    | 6.94 (s)                            | -          | -                            |

* Two-bond coupling.

2.2. Chemotaxonomic Significance

The presence of phenanthrene derivatives in *Aerides multiflora* is in line with the earlier findings in *A. crispum* and *A. rosea* [19,20]. In addition, the chemical profiles of these plants agreed with the conclusion from a molecular phylogenetic analysis that indicated their close relationships [43]. The family Orchidaceae is divided into 5 subfamilies, i.e., Epidendroideae, Orchidoideae, Vanilloideae, Cypripedioideae, and Apostasioideae [44]. So far, dimeric phenanthrenes have been reported from only two subfamilies, i.e., Epidendroideae (the genera *Stanhopea, Bletilla, Pholidota, Pleione, Otochilus, Arundina, Bulbophyllum, Dendrobium, Monomeria, Cymeastra, Agrostophyllum, Liparis, Cyrtopodium, Eria, Eulophia, Cirrhopetalum, Calanthe, Lusia, and Prosthechea*) and Orchidoideae (the genera *Spiranthes* and *Gymnadenia*) [34,39,45–63]. No biphenanthrenes have been found outside these two subfamilies. Interestingly, a previous study revealed a monophyletic relationship between Epidendroideae and Orchidoideae [64]. Thus, from the currently available chemical data, the occurrence of biphenanthrenes could be taken as their chemotaxonomic marker. Nevertheless, additional chemical studies on the other three subfamilies are still needed to verify this postulation.

It should also be noted that the genus *Aerides* belongs to the same clade as *Rhynochostylis* [43]. Both genera have been called “the foxtail orchid” due to the erect or pendent inflorescences of closely packed flowers, and this has sometimes led to confusion. Up to the present, no reports on the secondary metabolites of the latter genus have appeared. Comparative chemical studies, in combination with detailed genetic analysis, may help shed light on the distinction between these two sister genera.

2.3. $\alpha$-Glucosidase Inhibitory Activity

Yeast $\alpha$-glucosidase enzyme was used in this study. In general, $\alpha$-glucosidase enzymes can be obtained from several sources, for example, *Saccharomyces cerevisiae, Rattus norvegicus*, and GANC-human [65]. The enzyme derived from the yeast shows approximately 55% sequence homology with that obtained from mammalian sources [66], and therefore is
widely employed in the investigations of natural compounds for α-glucosidase inhibitory potential [67,68].

All the isolated compounds (1–10) were initially tested for their α-glucosidase inhibitory activity at a concentration of 100 μg/mL. IC₅₀ values were determined for compounds with more than 70% inhibition of the enzyme. As shown in Table 4, all compounds, except for dihydrosinapyl dihydroferulate (4), exhibited stronger activity (IC₅₀ 5.2–266.7 μM) than the drug acarbose (IC₅₀ value of 514.4 ± 9.2 μM). It should be mentioned that biphenanthrenes with α-glucosidase inhibitory activity were isolated from the family Orchidaceae for the first time in this study.

| Compounds                                      | IC₅₀ (μg/mL) | IC₅₀ (μM) |
|------------------------------------------------|-------------|----------|
| Aerimultin A (1)                               | 16.8 ± 1.0  | 30.9 ± 1.9 |
| Aerimultin B (2)                               | 41.8 ± 1.3  | 77 ± 2.5  |
| Aerimultin C (3)                               | 2.7 ± 0.4   | 5.2 ± 0.7 |
| Dihydrosinapyl dihydroferulate (4)             | NA          | NA       |
| 6-Methoxy coelonin (5)                         | 61.2 ± 2.2  | 224.8 ± 7.8 |
| Gigantol (6)                                   | 52.5 ± 1.9  | 191.3 ± 6.8 |
| Imbricatin (7)                                 | 44.9 ± 2.1  | 165.9 ± 7.7 |
| Agrostonin (8)                                 | 20.1 ± 2.5  | 37.2 ± 4.5 |
| Dihydroconiferyl dihydro-p-coumarate (9)       | 88.1 ± 2.9  | 266.7 ± 8.6 |
| 5-Methoxy-9,10-dihydrophenanthrene-2,3,7-triol (10) | 29.7 ± 2.3  | 115.2 ± 9.1 |
| Acarbose                                       | 332.1 ± 5.9 | 514.4 ± 9.2 |

NA = no inhibitory activity.

Overall, the dimeric phenanthrenes (1, 2, 3, and 8) demonstrated higher activity than the monomers (5, 7, and 10), as indicated by their IC₅₀ values (Table 4). Aerimultin C (3) was the most potent α-glucosidase inhibitor, with an IC₅₀ value of 5.2 ± 0.7 μM. Replacing the phenolic groups at C-6 and C-6’ of this compound with methoxy groups reduced the activity by about seven-fold, as can be seen from the increased IC₅₀ value (37.2 ± 4.5 μM) for agrostonin (8). The importance of free OH groups is also supported by the potent activity (IC₅₀ 2.08 ± 0.19 μM) earlier observed for a biphenanthrene (from Dioscorea bulbifera, Dioscoreaceae), the structure of which contains four free phenolic groups [69]. A molecular docking study on flavones with α-glucosidase inhibitory activity has also revealed that replacement of the hydroxyl groups with methoxy groups could lead to loss of activity [70].

Parallel observations were also obtained for the 3-phenylpropyl 3-propionate derivatives (4 and 9). Dihydroconiferyl dihydro-p-coumarate (9) showed appreciable activity (IC₅₀ 266.7 ± 8.6 μM). However, introducing methoxy groups to C-3 and C-5’ of 9 caused a total loss of activity, as seen in dihydrosinapyl dihydroferulate (4). A similar phenomenon, in which the presence of aromatic methoxy groups diminished α-glucosidase inhibitory activity, was earlier reported for p-coumarate esters of long-chain alcohols [71].

A kinetics study was then performed on compound 3 to analyze the mode of enzyme inhibition using various substrate concentrations (0.25–2.0 mM). From the Lineweaver–Burk plot in Figure 3A, the drug acarbose showed the intersection of the lines on the y-axis, indicating competitive type of inhibition. The Kᵢ value of acarbose (190.57 μM) was obtained from the secondary plot by replotting the slopes of the lines against inhibitor concentrations. For compound 3, the increase in concentration (4 and 8 μM) decreased the Vₘₕₜ from 0.10 to 0.035 but did not affect the Kᵢ value (Figure 3B). The results suggested non-competitive inhibition of the enzyme by 3. The Kᵢ value of compound 3 (4.18 μM) was obtained from the secondary plot, as shown in Table 5.
Figure 3. Lineweaver–Burk plots of (A) acarbose and (B) compound 3. The secondary plot of each compound is on the right.

Table 5. Kinetic parameters of α-glucosidase inhibition in the presence of 3.

| Inhibitors  | Dose (µM) | V_max (µOD/min) | K_m (mM) | K_i (µM) |
|-------------|-----------|-----------------|----------|----------|
| None        | -         | 0.10            | 1.22     |          |
| 3           | 8         | 0.035           | 1.20     | 4.18     |
|             | 4         | 0.055           | 1.22     |          |
| Acarbose    | 930       | 0.11            | 6.47     | 190.57   |
|             | 465       | 0.10            | 4.17     |          |

V_max, maximum rate of velocity; K_m, Michaelis constant; K_i, inhibitor constant.

Generally, non-competitive inhibitors have some advantages over competitive inhibitors [72]. Non-competitive inhibitors bind to the allosteric site of the enzyme, and thus do not depend upon the substrate concentration. Moreover, they require lower concentrations than competitive inhibitors to produce the same effect [73]. Compound 3, as a potent non-competitive inhibitor of α-glucosidase, provides a lead structure for the further design and development of AGI drugs.

3. Materials and Methods

3.1. Experimental

Optical rotations were determined with a PerkinElmer Polarimeter 341 (Boston, MA, USA). UV spectra were recorded on a Milton Roy Spectronic 3000 Array spectrophotometer (Rochester, Monroe, NY, USA). IR spectra were obtained with a PerkinElmer FT-IR 1760X spectrophotometer (Boston, MA, USA). Mass spectra were measured using a Bruker MicroTOF mass spectrometer (ESI-MS) (Billerica, MA, USA). NMR spectra were recorded on a Bruker Avance DPX-300FT NMR spectrometer or a Bruker Avance III HD 500 NMR spectrometer (Billerica, MA, USA). Yeast α-glucosidase enzyme and p-nitrophenol-α-D-glucopyranoside were obtained from Sigma Chemical, Inc. (St. Louis, MO, USA), and acarbose was purchased from Fluka Chemical (Buchs, Switzerland). Microtiter plate readings were carried out with a CLARIOstar apparatus (BMGLABTECH, Ortenberg, Germany).
3.2. Plant Material

The plant materials, whole plants of *Aerides multiflora*, were purchased from Chatuchak market in May 2019. Plant identification was performed by Mr. Yanyong Punpreuk, Department of Agriculture, Bangkok, Thailand. A voucher specimen BS-AM-052562 has been deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University.

3.3. Extraction and Isolation

The dried powder from the whole plants of *Aerides multiflora* (6.1 kg) was macerated with MeOH (4 × 18 L). The MeOH extract, at a concentration of 100 µg/mL, showed 82.4 ± 9.5% inhibition of α-glucosidase. This MeOH extract (550 g) was then suspended in water and partitioned with EtOAc and butanol to give an EtOAc extract (201.1 g), a butanol extract (80.8 g), and an aqueous extract (150 g), respectively. The EtOAc extract exhibited 92.9 ± 3.2% inhibition at 100 µg/mL, whereas the others were devoid of activity (<50% inhibition). Therefore, the EtOAc extract was subjected to further investigation.

The EtOAc extract was first fractionated by vacuum liquid chromatography (silica gel, EtOAc–CH₂Cl₂, gradient) to give five fractions (A–E). Fraction B (11.4 g) was further fractionated on a silica gel column (EtOAc–hexane, gradient) to give 3 fractions (BA–BC). Fraction BA (1 g) was separated on Sephadex LH-20 (methanol) to yield fractions BAA, BAB, and BAC. Fraction BAA (200 mg) was further separated by column chromatography (CC, silica gel, EtOAc–CH₂Cl₂, gradient) to give 6-methoxycoelonin (5) (65.4 mg). Fraction BAB (300 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give fractions BAB1 and BAB2. Fraction BAB1 (160.2 mg) was separated by CC (silica gel, acetone–hexane, 3:7) to yield 1 (2.3 mg). Gigantol (6) (14.5 mg) was obtained from fraction BAB2 (100 mg) after purification on Sephadex LH-20 (acetone). Fraction BB (1 g) was separated on Sephadex LH-20 (acetone) to yield BBA and BBB fractions. Fraction BBA (195.8 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give five fractions (A–E). Fraction B (11.4 g) was further fractionated on a silica gel column (EtOAc–hexane, gradient) to give 3 fractions (BA–BC). Fraction BA (1 g) was separated on Sephadex LH-20 (methanol) to yield fractions BAA, BAB, and BAC. Fraction BAA (200 mg) was further separated by column chromatography (CC, silica gel, EtOAc–CH₂Cl₂, gradient) to give 6-methoxycoelonin (5) (65.4 mg). Fraction BAB (300 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give fractions BAB1 and BAB2. Fraction BAB1 (160.2 mg) was separated by CC (silica gel, acetone–hexane, 3:7) to yield 1 (2.3 mg). Gigantol (6) (14.5 mg) was obtained from fraction BAB2 (100 mg) after purification on Sephadex LH-20 (acetone). Fraction BB (1 g) was separated on Sephadex LH-20 (acetone) to yield BBA and BBB fractions. Fraction BBA (195.8 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give five fractions (A–E). Fraction B (11.4 g) was further fractionated on a silica gel column (EtOAc–hexane, gradient) to give 3 fractions (BA–BC). Fraction BA (1 g) was separated on Sephadex LH-20 (methanol) to yield fractions BAA, BAB, and BAC. Fraction BAA (200 mg) was further separated by column chromatography (CC, silica gel, EtOAc–CH₂Cl₂, gradient) to give 6-methoxycoelonin (5) (65.4 mg). Fraction BAB (300 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give fractions BAB1 and BAB2. Fraction BAB1 (160.2 mg) was separated by CC (silica gel, acetone–hexane, 3:7) to yield 1 (2.3 mg). Gigantol (6) (14.5 mg) was obtained from fraction BAB2 (100 mg) after purification on Sephadex LH-20 (acetone). Fraction BB (1 g) was separated on Sephadex LH-20 (acetone) to yield BBA and BBB fractions. Fraction BBA (195.8 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give five fractions (A–E). Fraction B (11.4 g) was further fractionated on a silica gel column (EtOAc–hexane, gradient) to give 3 fractions (BA–BC). Fraction BA (1 g) was separated on Sephadex LH-20 (methanol) to yield fractions BAA, BAB, and BAC. Fraction BAA (200 mg) was further separated by column chromatography (CC, silica gel, EtOAc–CH₂Cl₂, gradient) to give 6-methoxycoelonin (5) (65.4 mg). Fraction BAB (300 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give fractions BAB1 and BAB2. Fraction BAB1 (160.2 mg) was separated by CC (silica gel, acetone–hexane, 3:7) to yield 1 (2.3 mg). Gigantol (6) (14.5 mg) was obtained from fraction BAB2 (100 mg) after purification on Sephadex LH-20 (acetone). Fraction BB (1 g) was separated on Sephadex LH-20 (acetone) to yield BBA and BBB fractions. Fraction BBA (195.8 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give five fractions (A–E). Fraction B (11.4 g) was further fractionated on a silica gel column (EtOAc–hexane, gradient) to give 3 fractions (BA–BC). Fraction BA (1 g) was separated on Sephadex LH-20 (methanol) to yield fractions BAA, BAB, and BAC. Fraction BAA (200 mg) was further separated by column chromatography (CC, silica gel, EtOAc–CH₂Cl₂, gradient) to give 6-methoxycoelonin (5) (65.4 mg). Fraction BAB (300 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give fractions BAB1 and BAB2. Fraction BAB1 (160.2 mg) was separated by CC (silica gel, acetone–hexane, 3:7) to yield 1 (2.3 mg). Gigantol (6) (14.5 mg) was obtained from fraction BAB2 (100 mg) after purification on Sephadex LH-20 (acetone). Fraction BB (1 g) was separated on Sephadex LH-20 (acetone) to yield BBA and BBB fractions. Fraction BBA (195.8 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give five fractions (A–E). Fraction B (11.4 g) was further fractionated on a silica gel column (EtOAc–hexane, gradient) to give 3 fractions (BA–BC). Fraction BA (1 g) was separated on Sephadex LH-20 (methanol) to yield fractions BAA, BAB, and BAC. Fraction BAA (200 mg) was further separated by column chromatography (CC, silica gel, EtOAc–CH₂Cl₂, gradient) to give 6-methoxycoelonin (5) (65.4 mg). Fraction BAB (300 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to give fractions BAB1 and BAB2. Fraction BAB1 (160.2 mg) was separated by CC (silica gel, acetone–hexane, 3:7) to yield 1 (2.3 mg). Gigantol (6) (14.5 mg) was obtained from fraction BAB2 (100 mg) after purification on Sephadex LH-20 (acetone). Fraction BB (1 g) was separated on Sephadex LH-20 (acetone) to yield BBA and BBB fractions. Fraction BBA (195.8 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, gradient) to yield BBA1 and BBA2 fractions. Fraction BBA1 (132.2 mg) was subjected to CC (silica gel, acetone–hexane, 3:7) to produce imbricatin (7) (39 mg) and agrostonin (8) (7 mg). Fraction C (10.5 g) was fractionated on a silica gel column (EtOAc–CH₂Cl₂, gradient) to give 3 fractions (CA–CC). Fraction CB (500 mg) was further separated on Sephadex LH-20 (acetone) to yield CBA and CBB fractions. Fraction CBA (236.9 mg) was further separated by CC (silica gel, EtOAc–hexane, gradient) to give dihydroconiferyl dihydro-p-coumarate (9) (74.1 mg) and 5-methoxy-9,10-dihydrophenanthrene-2,3,7-triol (10) (9.2 mg). Fraction CC (100 mg) was separated on Sephadex LH-20 (acetone) to yield fractions CCA, CCB, and CCC. Fraction CCB (10 mg) was subjected to CC (silica gel, EtOAc–CH₂Cl₂, 0.2: 9.8) to yield 3 (8.8 mg).

Aerimultin A (1): whitish-brown amorphous powder; UV (MeOH) λ\(_{max}\) (log ε) 265 (4.31), 305 (4.2), 315 (4.19); IR: ν\(_{max}\) 3350, 2923, 2850, 1696, 1605, 1462, 1442, 1221, 1201 cm\(^{-1}\); HR-ESI-MS: [M+Na]\(^+\) at m/z 565.1841 (calculated for C\(_{32}\)H\(_{30}\)O\(_8\)Na, 565.1838); \(^1\)H and \(^13\)C NMR data, see Table 1.

Aerimultin B (2): brown amorphous solid; [α] \(_D\) 20 –108 (c 0.005, MeOH); UV (MeOH) λ\(_{max}\) (log ε) 265 (4.67), 315 (4.09), 370 (3.99); IR: ν\(_{max}\) 3368, 2919, 2850, 1736, 1587, 1463, 1259 cm\(^{-1}\); HR-ESI-MS: [M+Na]\(^+\) at m/z 559.1376 (calculated for C\(_{32}\)H\(_{24}\)O\(_8\)Na, 559.1368); \(^1\)H and \(^13\)C NMR data, see Table 2.
Aerimultin C (3): brown amorphous solid; \([\alpha]_D^{20} +67.5 (c 0.008, \text{MeOH})\); UV (MeOH) 
\(\lambda_{\text{max}} (\log \varepsilon) 265 (4.1), 315 (3.42), 355 (3.47), 370 (3.48)\); IR: \(\nu_{\text{max}} 3360, 2921, 2851, 1712, 1588, 1461, 1371 \text{ cm}^{-1}\); HR-ESI-MS: \([M+Na]^+ \text{ at } m/z 533.1218\) (calculated for C\(_{30}\)H\(_{22}\)O\(_8\)Na, 533.1212); \(^1\)H and \(^{13}\)C NMR data, see Table 2.

Dihydrosinapyl dihydroferulate (4): yellow amorphous solid; UV (MeOH) 
\(\lambda_{\text{max}} (\log \varepsilon) 280 (3.76), 315 (3.12)\); IR: \(\nu_{\text{max}} 3432, 2937, 2841, 1723, 1608, 1514, 1455, 1427, 1208, 1111 \text{ cm}^{-1}\); HR-ESI-MS: \([M+Na]^+ \text{ at } m/z 413.1584\) (calculated for C\(_{21}\)H\(_{26}\)O\(_7\)Na, 413.1576); \(^1\)H and \(^{13}\)C NMR data, see Table 3.

3.4. \(\alpha\)-Glucosidase Inhibition Assay

The assays were performed following previous protocols [74]. The liberation of \(p\)-nitrophenol from the substrate \(p\)-nitrophenol-\(\alpha\)-D-glucopyranoside (PNPG) was observed to determine the inhibition of the \(\alpha\)-glucosidase enzyme. Each sample was initially dissolved in 50% DMSO. Then, 0.1 U/mL of \(\alpha\)-glucosidase (40 \(\mu\)L) in phosphate buffer (pH 6.8) was added to each well of a 96-well plate which contained the sample solution (10 \(\mu\)L). The plate was pre-incubated at 37 \(^\circ\)C for 10 min. Then, 2 mM \(p\)-nitrophenol-\(\alpha\)-D-glucopyranoside (50 \(\mu\)L) was added, and the mixture was incubated again at 37 \(^\circ\)C for 20 min. Finally, 1 M Na\(_2\)CO\(_3\) solution (100 \(\mu\)L) was added to terminate the reaction. The absorbance of the mixture was measured at 405 nm using a microplate reader. Two-fold serial dilution was performed for IC\(_{50}\) determination. The drug acarbose was used as the positive control.

The mode of enzyme inhibition of the test compound was determined using the double reciprocal Lineweaver–Burk plot (1/V vs. 1/[S]). The experiment was performed by varying the PNPG concentrations (0.25, 0.5, 1.0, and 2.0 mM) in the absence or presence of compound 3 (4 \(\mu\)M and 8 \(\mu\)M) or acarbose (930 \(\mu\)M and 465 \(\mu\)M). The secondary plot was constructed by replotting the slopes of the lines against inhibitor concentrations, and the K\(_i\) was calculated from the line equation of the plot.

4. Conclusions

In this communication, ten compounds were isolated from Aerides multiflora, including three new compounds, namely, aerimultins A–C (1–3), the new natural product dihydrosoinapyl dihydroferulate (4), and six known compounds (5–10). The structures of the new compounds were established by spectroscopic methods. The findings in this study suggested that biphenantherenes might be taken as a chemotaxonomic marker for the subfamilies Epidendroideae and Orchidoideae within the family Orchidaceae. For the first time, the dimeric phenanthrenes obtained from this plant family were investigated for an \(\alpha\)-glucosidase inhibitory activity. Among the isolates, the biphenantherene aerimultin (3) emerged as the most potent inhibitor, showing much higher potency than the drug acarbose. An enzyme kinetic study on this compound revealed a non-competitive type of inhibition and suggested that it could be a candidate structure for \(\alpha\)-glucosidase inhibitor drug development.

Author Contributions: B.S. conceived, designed, and supervised the research project, as well as prepared and edited the manuscript; M.T.T. performed the experiments and prepared the manuscript; N.C. supervised the \(\alpha\)-glucosidase inhibition assay; W.M. performed the NMR experiments; Y.P. performed the plant collection; K.L. provided comments and suggestions on the preparation of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) 2021.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data presented in this study are available in the article.
Acknowledgments: M.T.T. is grateful to the Graduate School, Chulalongkorn University for a CUASEAN Ph.D. scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Karunaratne, V.; Thadhani, V.M.; Khan, S.N.; Choudhary, M.I. Potent α-glucosidase inhibitors from the lichen Cladonia species from Sri Lanka. J. Natl. Sci. Found. Sri. 2014, 42, 95–98. [CrossRef]

2. Wu, J.H.; Micha, R.; Imamura, F.; Pan, A.; Biggs, M.L.; Ajaz, O.; Mozaffarian, D. Omega-3 fatty acids and incident type 2 diabetes: A systematic review and meta-analysis. Br. J. Nutr. 2012, 107, S214–S227. [CrossRef]

3. Chaudhary, A.; Duvoor, C.; Reddy Dendi, V.S.; Kraleti, S.; Chada, A.; Ravilla, R.; Sasapu, A. Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. Front. Endocrinol. 2017, 8, 1–12. [CrossRef] [PubMed]

4. Nthi, N.X.; Van Kiem, P.; Van Minh, C.; Ban, N.K.; Cuong, N.X.; Tung, N.H.; Kim, Y.H. α-glucosidase inhibition properties of cucurbitane-type triterpene glycosides from the fruits of Monordica charantia. Chem. Pharm. Bull. 2010, 58, 720–724. [CrossRef] [PubMed]

5. Costa, T.M.; Mayer, D.A.; Siebert, D.A.; Micke, G.A.; Alberton, M.D.; Tavares, L.B.B.; De Oliveira, D. Kinetics analysis of the inhibitory effects of alpha-glucosidase and identification of compounds from Gastrodia lipsiense Mycelium. Appl. Biochem. Biotechnol. 2020, 1–14.

6. Hung, H.Y.; Qian, K.; Morris-Natschke, S.L.; Hsu, C.S.; Lee, K.H. Recent discovery of plant-derived anti-diabetic natural products. Nat. Prod. Res. 2012, 29, 580–606. [CrossRef] [PubMed]

7. Nashiru, O.; Koh, S.; Lee, S.Y.; Lee, D.S. Novel α-glucosidase from extreme thermophile Thermus caldophilus GK24. J. Biochem. Mol. Biol. 2001, 34, 347–354.

8. Ernawati, T.; Radji, M.; Hanafi, M.; Munim, A.; Yanuar, A. Cinnamic acid derivatives as α-glucosidase inhibitor agents. Indones. J. Chem. 2017, 17, 151–160. [CrossRef]

9. Yin, Z.; Zhang, W.; Feng, F.; Zhang, Y.; Kang, W. α-Glucosidase inhibitors isolated from medicinal plants. Food Sci. Hum. Well. 2014, 3, 136–174. [CrossRef]

10. Kao, C.C.; Wu, P.C.; Wu, C.H.; Chen, H.H.; Wu, M.S. Risk of liver injury after α-glucosidase inhibitor therapy in advanced chronic kidney disease patients. Sci. Rep. 2016, 6, 18996. [CrossRef]

11. Babu, P.S.; Prabuseenivasan, S.; Ignacimuthu, S. Cinnamaldehyde—A potential antidiabetic agent. Phytomedicine 2007, 14, 15–22. [CrossRef]

12. Liu, M.; Qi, C.; Sun, W.; Shen, L.; Wang, J.; Liu, J.; Zhang, Y. α-Glucosidase inhibitors from the coral-associated fungus Aspergillus terreus. Front. Chem. 2018, 6, 422. [CrossRef]

13. Limpanit, R.; Chuanasa, T.; Likhitwitayawuid, K.; Jongbunprasert, V.; Sritularak, B. α-Glucosidase inhibitors from Dentrobium tortile. Rec. Nat. Prod. 2016, 10, 609–616.

14. Sun, J.; Zhang, Y.; Chen, L.; Zhan, R.; Chen, Y. A new phenanthrene and a new 9,10-dihydrophenanthrene from Bulbophyllum retususculum. Nat. Prod. Res. 2018, 32, 2447–2451. [CrossRef] [PubMed]

15. Auberon, F.; Olatunji, O.J.; Waffo-Teguo, P.; Adekoya, A.E.; Bonte, F.; Merillon, J.M.; Lobstein, A. New glucosyloxybenzyl 2R-benzylmalate derivatives from the underground parts of Arrundina graminifolia (Orchidaceae). Fitoterrapia 2019, 135, 33–43. [CrossRef] [PubMed]

16. Kocyan, A.; de Vogel, E.F.; Conti, E.; Gravendeel, B. Molecular phylogeny of Aerides (Orchidaceae) based on one nuclear and two plastid markers: A step forward in understanding the evolution of the Aeridinae. Mol. Phylogenet. Evol. 2008, 48, 422–443. [CrossRef] [PubMed]

17. Pant, B. Medicinal orchids and their uses: Tissue culture a potential alternative for conservation. Afr. J. Plant Sci. 2013, 7, 448–467. [CrossRef]

18. Akter, M.; Huda, M.K.; Hoque, M.M. Investigation of secondary metabolites of nine medicinally important orchids of Bangladesh. J. Pharmacogn. Phytochem. 2018, 7, 602–606.

19. Cakova, V.; Urbain, A.; Antheaume, C.; Rimlinger, N.; Wehrung, P.; Bonté, F.; Lobstein, A. Identification of phenanthrene derivatives in Aerides rosea (Orchidaceae) using the combined systems HPLC–ESI–HRMS/MS and HPLC–DAD–MS–SPE–UV–NMR. Phytochem. Anal. 2015, 26, 34–39. [CrossRef]

20. Anuradha, V.; Rao, N.P. Aeridin: A phenanthropyran from Aerides crispus. Phytochemistry 1998, 48, 185–186. [CrossRef]

21. Bhowmik, T.K.; Rahman, M.M. In vitro study of medicinally important orchid Aerides multiflora Roxb. from nodal and leaf explants. J. Pharmacogn. Phytochem. 2020, 9, 179–184.

22. Choon, K.K. Management of the Pa Taem Protected Forest Complex to Promote Cooperation for Transboundary Biodiversity Conservation between Thailand, Cambodia and Laos Phase I. Kasetsart University: Bangkok, Thailand, 2004.

23. Schuiteman, A.; Bonnet, P.; Svenssuzska, B.; Barthélémy, D. An annotated checklist of the Orchidaceae of Laos. Nord. J. Bot. 2008, 26, 257–316. [CrossRef]

24. Subedi, A.; Kunwar, B.; Choi, Y.; Dai, Y.; Van Andel, T.; Chaudhary, R.P.; De Boer, H.J.; Gravendeel, B. Collection and trade of wild-harvested orchids in Nepal. J. Ethnobiol. Ethnomed. 2013, 9, 1–10. [CrossRef]

25. Gogoi, K.; Das, R.; Yonzone, R. Present ecological status, diversity, distribution and cultural significance of the genus Aerides Loureiro (Orchidaceae) in Tinsukia District (Assam) of North East India. J. Environ. Ecol. 2012, 30, 649–651.
26. Rao, A.N. Medicinal orchid wellbeing of Arunachal Pradesh. Indian Med. Plants 2004, 1, 1–7.

27. Ghanaksh, A.; Kaushik, P. Antibacterial effect of Aerides multiflora: A study in vitro. J. Orchid Soc. India 1999, 1, 65–68.

28. Intongkaew, P.; Chatsumpun, N.; Supasuteekul, C.; Kitisiripanya, T.; Putalun, W.; Likhitwitayawuid, K.; Sritularak, B. α-Glucosidase and pancreatic lipase inhibitory activities and glucose uptake stimulatory effect of phenolic compounds from Dendrobium formosum. Res. Brus. Farmacogn. 2017, 27, 480–487. [CrossRef]

29. Sarakulwattana, C.; Mekboonsonglarp, W.; Likhitwitayawuid, K.; Rojsitthisak, P.; Sritularak, B. New bisbibenzyl and phenanthrene derivatives from Dendrobium scabriduline and their α-glucosidase inhibitory activity. Nat. Prod. Res. 2020, 34, 1694–1710. [CrossRef] [PubMed]

30. Thant, M.T.; Chatsumpun, N.; Mekboonsonglarp, W.; Sritularak, B.; Likhitwitayawuid, K. New fluorene derivatives from Dendrobium gibsonii and their α-glucosidase inhibitory activity. Molecules 2020, 25, 4931. [CrossRef]

31. Leong, Y.W.; Kong, C.C.; Harrison, L.J.; Powell, A.D. Phenanthrenes, dihydrophenanthrenes and bibenzyls from the orchid Bulbophyllum vaginatum. Phytochemistry 1997, 44, 157–165. [CrossRef]

32. Chen, Y.; Xu, J.; Yu, H.; Qiong, C.; Zhang, Y.; Wang, L.; Liu, Y.; Wang, J. Cytotoxic phenolics from Bulbophyllum odoratissimum. Food Chem. 2008, 107, 169–173. [CrossRef]

33. Simmer, C.; Antheaume, C.; Lobstein, A. Antioxidant biomarkers from Vanda coerulea stems reduce irradiated HaCaT PGE-2 production as a result of COX-2 inhibition. PLoS ONE 2010, 5, 1–9. [CrossRef] [PubMed]

34. Majumder, P.L.; Banerjee, S.; Lahiri, S.; Mukhoti, N.; Sen, S. Dimeric phenanthrenes from two Agrostophyllum species. Phytochemistry 1995, 47, 855–860. [CrossRef]

35. Zhang, X.; Gao, H.; Wang, N.; Yao, X. Phenolic components from Dendrobium nobile. Zhong Cao Yao 2006, 37, 652–655.

36. Leong, Y.W.; Kong, C.C.; Harrison, L.J.; Powell, A.D. Phenanthrene and other aromatic constituents of Bulbophyllum vaginatum. Phytochemistry 1998, 50, 1237–1241. [CrossRef]

37. Estrada, S.; Toscano, R.A.; Mata, R. New Phenanthrene derivatives from Maxillaria densa. J. Nat. Prod. 1999, 62, 1175–1178. [CrossRef]

38. Majumder, P.L.; Sabzabadi, E. Agrostophyllin, a naturally occurring phenanthropyran derivative from Agrostophyllum khasianum. Phytochemistry 1988, 27, 1899–1901. [CrossRef]

39. Liu, L.; Yin, Q.M.; Zhang, X.W.; Wang, W.; Dong, X.Y.; Yan, X.; Hu, R. Bioactivity – guided isolation of biphenanthrenes from Liparis nervosa. Fitoterapia 2011, 82, 15–18. [CrossRef]

40. Beck, J.J.; Kim, J.H.; Campbell, B.C.; Chou, S.C. Fungicidal activities of dihydroferulic acid alkyl ester analogues. J. Nat. Prod. 2007, 70, 779–782. [CrossRef]

41. Zhuo, J.X.; Wang, Y.H.; Su, X.L.; Mei, R.Q.; Yang, J.; Kong, Y.; Long, C.L. Neolignans from Selaginella mollendorffii. Nat. Prod. Bioprospect. 2016, 6, 161–166. [CrossRef]

42. Sibout, R.; Le Bris, P.; Legefe, F.; Cezard, L.; Renault, H.; Lapierre, C. Structural redesigning Arabidopsis lignins into alkali-soluble lignins through the expression of p-coumaroyl-CoA: Monolignol transferase PMT. Plant Physiol. 2016, 170, 1358–1366. [CrossRef]

43. Zhang, G.Q.; Liu, K.W.; Chen, L.J.; Xiao, X.J.; Zhai, J.W.; Li, L.Q.; Liu, Z.J. A new molecular phylogeny and a new genus, Pendulorchis, of the Aerides–Vanda alliance (Orchidaceae: Epidendroideae). Mol. Phylogenetics Evol. 2019, 139, 106540. [CrossRef] [PubMed]

44. Lucca, D.L.; Sa, G.P.; Polastri, L.R.; Ghiraldi, D.M.; Ferreira, N.P.; Chiavelli, L.U.; Pomini, A.M. Biphenanthrene from Stanhopea lietzei (Orchidaceae) and its chemophenetic significance within neotropical species of the Cymbidieae tribe. Biochem. Syst. Ecol. 2020, 89, 104014. [CrossRef]

45. Qian, C.D.; Jiang, F.S.; Yu, H.S.; Shen, Y.; Fu, Y.H.; Cheng, D.Q.; Ding, Z.S. Antibacterial Biphenanthrenes from the fibrous roots of Bletilla striata. J. Nat. Prod. 2015, 78, 939–943. [CrossRef]

46. Guo, X.Y.; Wang, J.; Wang, N.L.; Kitanaka, S.; Liu, H.W.; Yao, X.S. New stilbenoids from Pholidota yunnanensis and their inhibitory effects on nitric oxide production. Chem. Pharm. Bull. 2006, 54, 21–25. [CrossRef]

47. Xu, J.; Yu, H.; Qiong, C.; Zhang, Y.; Liu, Y.; Chen, Y. Two new biphenanthrenes with cytotoxic activity from Bulbophyllum odoratissimum. Fitoterapia 2009, 80, 381–384. [CrossRef] [PubMed]

48. Zhang, G.N.; Zhong, L.Y.; Bligh, S.A.; Guo, Y.L.; Zhang, C.F.; Zhang, M.; Xu, L.S. Bi-bicyclic and bi-tricyclic compounds from Dendrobium thyrsiflorum. Phytochemistry 2005, 66, 1113–1120. [CrossRef]

49. Yang, M.; Cai, L.; Tai, Z.; Zeng, X.; Ding, Z. Four new phenanthrenes from Monomeria barbara Lindl. Fitoterapia 2010, 81, 992–997. [CrossRef]

50. Liu, L.; Li, J.; Zeng, K.W.; Jiang, Y.; Tu, P.F. Five new biphenanthrenes from Cremastra appendiculata. Molecules 2016, 21, 1089. [CrossRef]

51. Auberon, F.; Olutunji, O.J.; Herbette, G.; Raminoson, D.; Antheaume, C.; Soengas, B.; Lobstein, A. Chemical constituents from the aerial parts of Cyrtopodium paniculatum. Molecules 2016, 21, 1418. [CrossRef]

52. Wang, C.; Shao, S.Y.; Han, S.W.; Li, S. Atropisomeric bi (9, 10-dihydro) phenanthrene and phenanthrene/bibenzyl dimers with cytotoxic activity from the pseudobulbs of Pleione bulbocodioides. Fitoterapia 2019, 138, 104313. [CrossRef]

53. Auberon, F.; Olutunji, O.J.; Krissa, S.; Antheaume, C.; Herbette, G.; Bonté, F.; Lobstein, A. Two new stilbenoids from the aerial parts of Arundina graminifolia (Orchidaceae). Molecules 2016, 21, 1430. [CrossRef]
