Isolation and Cytotoxicity Evaluation of the Chemical Constituents from *Cephalantheropsis gracilis*

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**Abstract:** *Cephalantheropsis gracilis* afforded five new compounds: cephalanthrin-A (¹), cephalanthrin-B (²), cephathrene-A (³), cephathrene-B (⁴), methyl 2-(aminocarbonyl)phenylcarbamate (⁵), and 52 known compounds. The structures of the new compounds were determined by spectroscopic analysis. Among the compounds isolated, tryptanthrin (⁶), phaitanthrin A (⁷), cephalinone D (¹⁹), and flavanthrin (³⁰) showed significant cytotoxicity against MCF-7, NCI-H460, and SF-268 cell lines.

**Keywords:** *Cephalantheropsis gracilis*; Orchidaceae; quinazoline; tryptanthrin; indolotryptanthrin; dihydrophenanthrene; cytotoxicity
1. Introduction

The genus *Cephalantheropsis* (also known as *Cephalanceropsis*) belongs to the Orchidaceae family and is comprised of eight species distributed in Southeast Asia. The plant, *Cephalantheropsis gracilis* (Lindl.) Shiu-Ying Hu var. gracilis, is an orchid native to Taiwan and grows in forests at altitudes of 500–1500 m throughout the island [1]. The crude methanol extract of *C. gracilis* showed significant cytotoxicity against human breast carcinoma (MCF-7), lung carcinoma (NCI-H460), and central nervous system carcinoma (SF-268) cell lines in our preliminary screening. In earlier papers, the isolation of indole alkaloids was reported from *C. gracilis*, but they are unlikely to be responsible for such anticancer activity [2,3]. In the course of continuing the search for bioactive molecules from *C. gracilis*, two new quinazolines, cephalanthrin-A (1) and cephalanthrin-B (2), two new dihydrophenanthrenes, cephathrene-A (3) and cephathrene-B (4), and a methyl 2-(aminocarbonyl)phenylcarbamate (5) [4] (Figure 1) as well as 52 known compounds were obtained and identified from a methanol extract (in addition to common long-chain fatty acids, chlorophylls, and steroids). Herein, we describe the structural elucidation of these new compounds and the cytotoxic properties of all compounds identified toward several human cancer cell lines.

![Structures of five new compounds 1–5.](image)

2. Results and Discussion

Cephalanthrin-A (1) was isolated as an optically active white amorphous powder. The molecular formula was determined to be C_{17}H_{12}N_{2}O_{4} from a molecular ion of m/z 308.0794 by HREIMS. In the IR spectrum, a very broad band at 3000 cm\(^{-1}\) and an absorption band at 1652 cm\(^{-1}\) both indicated the presence of a carboxylic acid. The 1D \(^1\)H and \(^13\)C NMR data (Table 1, Figure S1), together with 2D COSY, HMQC, and HMBC spectra, revealed two sets of \(\alpha\)-disubstituted benzene rings, one at δ\(\text{H} 7.59\) (t, \(J = 7.5\) Hz, H-2), 7.77 (d, \(J = 7.5\) Hz, H-4), 7.83 (t, \(J = 7.5\) Hz, H-3), and 8.34 (1H, d, \(J = 7.5\) Hz, H-1);
δC 123.0 (C-12a), 127.4 (C-1), 128.0 (C-2), 128.5 (C-4), 135.2 (C-3), and 148.4 (C-4a). The other was at δH 7.38 (t, J = 7.7 Hz, H-8), 7.51 (t, J = 7.7 Hz, H-9), 7.73 (1H, d, J = 7.7 Hz, H-7), and 8.49 (1H, d, J = 7.7 Hz, H-10); δC 117.2 (C-10), 124.6 (C-7), 127.3 (C-8), 130.8 (C-9), 134.2 (C-6a), and 140.8 (C-10a). The 13C NMR data also indicated the presence of an imino C-5a (δ 161.9) and an amidic C-12 (δ 160.0). These signals appear to be very closely related to indolo[2,1-b]quinazoline-6,12-dione (trypanthrin, 6), except that the carbonyl C-6 is replaced by a saturated quaternary carbon (δ 75.9). NMR spectroscopic data also allowed us to determine the remaining two substituents on C-6 being an –OH group (δ 3.81) and a –CH₂COOH group (δH 3.45, 3.55 (each 1H, d, J = 16.5 Hz, H-1'); δC 43.6 (C-1') and 170.9 (C-2')). The methylene protons adjacent to a quaternary carbon (C-6) could be split due to either chirality or steric hindrance. HMBC correlations from H-1 to C-12, H-7 to C-6 and H-1' to C-5a, C-6 and C-6a, as well as the NOE correlations between H-1' and H-7, supported a structure of 6-hydroxy-6-(carboxymethyl)-trypanthrin for cephalanthrin-A (1).

Table 1. 1H and 13C NMR Spectroscopic Data and HMBC Correlations for Cephalanthrins 1 and 2.

| Position | 1 in Acetone- d₆ (300 MHz/75 MHz) | 2 in CDCl₃ (300 MHz/75 MHz) |
|----------|----------------------------------|-----------------------------|
|          | δH (J in Hz) | δC | HMBC | δH (J in Hz) | δC | HMBC |
| 1        | 8.34 d (7.5) | 127.4 | C-3, -4a, -12 | 7.60 d (7.6) | 126.1 | C-3, -4a, -12 |
| 2        | 7.59 t (7.5) | 128.0 | C-4, -12a | 7.41 t (7.6) | 129.3 | C-4, -12a |
| 3        | 7.83 t (7.5) | 135.2 | C-1, -4a | 7.52 t (7.6) | 131.4 | C-1, -4a |
| 4        | 7.77 d (7.5) | 128.5 | C-2, -12a | 7.72 d (7.6) | 129.7 | C-2, -12a |
| 4a       |              | 148.4 |         |              | 141.9 |         |
| 5a       |              | 161.9 |         |              | 144.7 |         |
| 6        |              | 75.9 |         |              | 184.0 |         |
| 6a       |              | 134.2 |         |              | 120.5 |         |
| 7        | 7.73 d (7.7) | 124.6 | C-6, -9, -10a | 7.83 d (7.6) | 125.5 | C-6, -9, -10a |
| 8        | 7.38 t (7.7) | 127.3 | C-6a, -10 | 7.20 t (7.6) | 124.0 | C-6a, -10 |
| 9        | 7.51 t (7.7) | 130.8 | C-7, -10a | 7.59 t (7.6) | 137.9 | C-7, -10a |
| 10       | 8.49 d (7.7) | 117.2 | C-6a, -8 | 7.23 d (7.6) | 112.3 | C-6a, -8 |
| 10a      |              | 140.8 |         |              | 148.9 |         |
| 12       |              | 160.0 |         |              | 87.4 |         |
| 12a      |              | 123.0 |         |              | 120.5 |         |
| 1'       | 3.45 d (16.5) | 43.6 | C-5a, -6, -6a, -2' | 167.8 |         |         |
| 2'       | 3.55 d (16.5) |         |         |              | 170.9 |         |
| 6-OH     | 3.81 s       |         |         |              |         |         |
| 1'-OCH₃  | 3.71 s       | 53.8 | C-1' |              |         |         |
| 12-OCH₃  | 3.07 s       | 50.6 | C-12 |              |         |         |

Cephalanthrin-B (2), also isolated as an optically active yellow amorphous powder, was determined to have a molecular formula of C₁₈H₁₄N₂O₄. By comparison of the 1H and 13C NMR spectra of 2 (Figure S2) with those of trypanthrin (6) and cephalanthrin-A (1), an oxygenated quaternary carbon (δ 87.4) was shown to replace the carbonyl C-12 to form a 12,12-disubstituted trypanthrin (Table 1). Methoxyl (δH 3.07; δC 50.6) and methoxycarbonyl (δH 3.71; δC 53.8 and 167.8) substituents were also identified, and their presence was confirmed by HMBC correlations from both H-1 and 12-OCH₃ to
C-12 and the NOE correlations between H-1, H-10 and 1'-OCH$_3$, 12-OCH$_3$. Hence, a structure of 12-methoxy-12-(methoxycarbonyl)-tryptanthrin was deduced for cephalanthrin-B (2). Although compound 2 has been synthesized by Cornforth et al. [5], this represents the first isolation of a pure compound from a natural source.

Due to the small specific rotations of compounds 1 ([α]$_D$ +8.0°) and 2 ([α]$_D$ +3.0°), we suspected they might be not optically pure compounds. The configuration of compounds 1 and 2 has not been determined due to the isolation of insufficient amounts of these materials. However, we adopted the similar structure of phaitanthrin A (7) as a model. First, our attempts to synthesize a pair of diastereomeric esters by acylating 7 with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride [(+)-MTPACl] [6], even with acetyl chloride, were unsuccessful. This most likely is due to steric inhibition at the tertiary alcohol, which is present in the tryptanthrin skeleton. We then tried to analyze the C-6 chemical shift behaviors using the chiral shift reagents, tris[3-trifluoroacetyl-D- and L-camphorato]europium(III) [(R)- and (S)-Eu(tfc)$_3$] [7], again to no avail.

Cephathrene-A (3) was isolated as a white amorphous powder with a molecular formula of C$_{17}$H$_{18}$O$_5$ as determined by the molecular ion peak at $m/z$ 302.1153 in HREIMS data. UV absorptions at 267 and 304 nm indicated the presence of a benzene system, and the IR spectrum revealed an OH absorption band at 3404 cm$^{-1}$. From the $^1$H NMR spectrum, two mutually-coupled aromatic protons at δ 6.86 (1H, d, $J = 8.7$ Hz, H-6) and 7.93 (1H, d, $J = 8.7$ Hz, H-5) and a proton at δ 6.63 (1H, s, H-1) suggested the presence of 1,2,3,4-tetrasubstituted and pentasubstituted benzene rings, respectively (Table 2, Figure S3). We also found two mutually coupled aliphatic signals at δ 2.66 (2H, m, H-10) and 2.79 (2H, m, H-9), which were assigned to an ethylene group. HMBC correlations of H-9 with C-4b, C-8, C-8a, C-10, and C-10a, as well as H-10 with C-1, C-4a, C-8a, C-9, and C-10a indicated that the two benzene rings are linked together by the ethylene group. Furthermore, the HMBC correlation between H-5 and C-4a established the existence of a bond between C-4a and C-4b. Thus, compound 3 possessed a 9,10-dihydrophenanthrene skeleton. The HMBC correlations of H-1, H-5, H-6, H-9, and H-10 allowed for the identification of the quaternary aromatic carbons, C-2, C-3, C-4a, C-4b, C-7, C-8, C-8a, and C-10a. We determined the identity of five other substituents, two hydroxyls and three methoxyls. The hydroxyl signal at δ 5.65 showed HMBC correlations with C-6, C-8, and C-7, and the other hydroxyl signal at δ 5.70 showed HMBC correlations with C-1 and C-3, indicating that the two hydroxyl groups are attached to C-7 and C-2, respectively. Whereas the three methoxyl signals at δ 3.75, 3.79, and 3.96 were shown to be located at C-4, C-8, and C-3, respectively, owing to the HMBC correlations of 3-OCH$_3$ with C-3, 4-OCH$_3$ with C-4, and 8-OCH$_3$ with C-8. Additional evidence for the positions of these substituents came from the NOE correlations between 2-OH and H-1, 3-OCH$_3$; H-5 and 4-OCH$_3$; H-1 and H-10; 8-OCH$_3$ and 7-OH, H-9. Therefore, cephathrene-A (3) was assigned the structure 2,7-dihydroxy-3,4,8-trimethoxy-9,10-dihydrophenanthrene.

Cephathrene-B (4) was isolated as a white amorphous powder. The HREIMS showed a molecular ion consistent with the molecular formula C$_{18}$H$_{20}$O$_6$. The spectral data showed a resemblance to compound 3 (Table 2). The $^1$H NMR spectrum (Figure S4) disclosed the presence of two singlet aromatic protons at 6.63 (H-1) and 7.75 (H-5), indicating a hexasubstituted 9,10-dihydrophenanthrene. The regiochemistries of the substituents, two hydroxyls and four methoxyls, were determined by HMQC, HMBC, and NOESY experiments. As in the case of 3, the two hydroxyls at δ 5.59 and 5.70 are located at C-7 and C-2, respectively, whereas three of the four methoxyls at δ 3.75, 3.84, and 3.97
are at C-4, C-8, and C-3, respectively. The remaining methoxyl at $\delta$ 3.93 was thought to be at C-6 due to NOE correlations between H-5 and 4- and 6-OCH$_3$. Thus, the structure of 2,7-dihydroxy-3,4,6,8-tetramethoxy-9,10-dihydrophenanthrene was established for cephathrene-B (4).

Table 2. $^1$H and $^{13}$C NMR Spectroscopic Data and HMBC Correlations for Cephathrenes 3 and 4.

| Position | 3 in CDCl$_3$ (400 MHz/100 MHz) | 4 in CDCl$_3$ (300 MHz/75 MHz) |
|----------|---------------------------------|--------------------------------|
|          | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC |
| 1        | 6.63 s | 109.9 | C-2, -3, -4a, -10 | 6.63 s | 110.1 | C-2, -3, -4a, -10 |
| 2        | 147.6 | 147.7 |
| 3        | 139.0 | 139.0 |
| 4        | 150.5 | 150.4 |
| 4a       | 120.3 | 120.3 |
| 4b       | 125.9 | 124.2 * |
| 5        | 7.93 d (8.7) | 124.2 | C-4a, -7, -8a | 7.75 s | 106.6 | C-4a, -4b, -6, -7, -8a |
| 6        | 6.86 d (8.7) | 112.8 | C-4b, -8 | 145.5 |
| 7        | 147.1 | 137.1 |
| 8        | 143.6 | 143.7 |
| 8a       | 130.8 | 124.3 * |
| 9        | 2.79 m | 22.4 | C-4b, -8, -8a, -10, -10a | 2.73 m | 21.5 | C-4b, -8, -10, -10a |
| 10       | 2.66 m | 29.5 | C-1, -4a, -8a, -9, -10a | 2.64 m | 29.8 | C-1, -4a, -8a, -9 |
| 10a      | 134.5 | 135.2 |
| 2-OH     | 5.70 s | 5.70 s |
| 3-OCH$_3$| 3.96 s | 61.1 | C-3 | 3.97 s | 61.2 | C-3 |
| 4-OCH$_3$| 3.75 s | 60.1 | C-4 | 3.75 s | 60.1 | C-4 |
| 6-OCH$_3$| 3.93 s | 56.3 | C-6 |
| 7-OH     | 5.65 s | 5.59 s |
| 8-OCH$_3$| 3.79 s | 61.3 | C-8 | 3.84 s | 60.8 | C-8 |

* Assignments may be interchangeable.

Compound 5, with molecular formula C$_9$H$_{10}$N$_2$O$_3$, was isolated as a white amorphous powder. It had UV absorptions at 257, 289, and 311 nm, indicative of an aromatic system. The $^1$H NMR spectrum (Figure S5) showed resonances at $\delta$ 7.05 (1H, t, $J$ = 7.8 Hz, H-4), 7.49 (1H, t, $J$ = 7.8 Hz, H-5), 7.83 (1H, d, $J$ = 7.8 Hz, H-3), and 8.38 (1H, d, $J$ = 7.8 Hz, H-6) for an o-disubstituted benzene. The HMBC correlations from H-3 to an amide carbon ($\delta$ 172.0) and from OCH$_3$ ($\delta$ 3.71) to a carbamate carbon ($\delta$ 154.6) and the NOE correlation between H-6 and an amine proton ($\delta$ 11.29) suggested the structure of methyl 2-(aminocarbonyl)phenylcarbamate for 5. The $^1$H and $^{13}$C NMR spectra of 5 and the acylation product of o-aminobenzamide with methyl chloroformate (ClCO$_2$CH$_3$) were identical and further confirmed the structure of 5 (Equation (1)).

\[
\begin{align*}
\text{CONH}_2 + \text{ClCO}_2\text{CH}_3 & \quad \rightarrow \quad \text{CONH}_2 \text{NHCO}_2\text{CH}_3 \\
\end{align*}
\]

Other known compounds were also isolated from C. grilis including 7 quinazolines, tryptanthrin (6) [8], phaitanthrin A (7) [8], phaitanthrin B (8) [8], methylisatoid (9) [8], candidine (10) [8],
1H-quinazoline-2,4-dione (11) [9], and 3-(2-hydroxy-phenyl)-3H-quinazolin-4-one (12) [10]; 15 indole alkaloids, cephalandole A (13) [2], cephalandole B (14) [2], cephalandole C (15) [2], cephalinone A (16) [2], cephalinone B (17) [2], cephalinone C (18) [2], cephalinone D (19) [2], (S)-3-(2-oxopropyl)-3-hydroxyindolin-2-one (20) [2], methyl dioxindole-3-acetate (21) [2], isatan (22) [2], indigo (23) [2], indirubin (24) [2], isatin (25) [2], indole-3-carboxaldehyde (26) [2], and indole-3-carboxylic acid (27) [2]; 2 indolotryptanthrins cephalandole A (28) [3] and cephalandole B (29) [3]; 5 dihydrophenanthrenes, flavanthrin (30) [11], coelonin (31) [11], 6-O-methylcoelonin (32) [12], 2,7-dihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (33) [13], and 2,7-dihydroxy-3,4,6-trimethoxy-9,10-dihydrophenanthrene (34) [11]; 1 lignin, secoisolariciresinol (35) [14]; 1 flavonoid, kaempferol 3-rutinoside (36) [15]; 1 ionol, blumenol A (37) [16]; and 20 benzenoids, 2-aminobenzoic acid (38), methyl 2-aminobenzoate (39), N-cinnamoyltyramine (40), N-p-coumaroyltyramine (41), N-trans-feruloyltyramine (42), dihydroconiferyl dihydro-p-coumarate (43), 4-hydroxybenzaldehyde (44), 1-(4-hydroxy-phenyl)ethanone (45), 4-hydroxyphenyl alcohol (46), 3-(4-hydroxy-phenyl)-propionic acid (47), vanillin (48), vanillic acid (49), 4-hydroxy-3-methoxybenzyl alcohol (50), syringaldehyde (51), 3,5-dimethyl-4-hydroxypropiophenone (52), trans-p-coumaric acid (53), trans-ferulic acid (54), cis-ferulic acid (55), methyl trans-4-hydroxy-3-methoxycinnamate (56), and methylsinapat (57).

All the isolated compounds were subjected to cytotoxic evaluation against MCF-7, NCI-H460, and SF-268 cell lines. Tryptanthrin (6), phaitanthrin A (7), cephalinone D (19), and flavanthrin (30) showed significant cytotoxicity against MCF-7, NCI-H460, and SF-268 cell lines with IC50 values of 7.6–42.9 μM (Table 3).

### Table 3. Cytotoxicity of active compounds toward three cancer lines.

| Compound              | IC50 (μM) MCF-7 | IC50 (μM) NCI-H460 | IC50 (μM) SF-268 |
|-----------------------|----------------|--------------------|-----------------|
| Tryptanthrin (6)      | 9.4 ± 0.3      | 8.5 ± 0.8          | 22.6 ± 1.1      |
| Phaitanthrin A (7)    | 17.8 ± 0.8     | 17.3 ± 1.2         | 42.9 ± 1.0      |
| Cephalinone D (19)    | 7.6 ± 0.7      | 7.8 ± 1.0          | 12.2 ± 1.3      |
| Flavanthrin (30)      | 21.9 ± 1.5     | 22.8 ± 2.3         | 23.0 ± 2.0      |

Values were mean ± SD (n = 3–8); MCF-7 = human breast tumor cell line; NCI-H460 = human lung tumor cell line; SF-268 = human entral nervous system tumor cell line.

3. Experimental Section

3.1. General

Optical rotations were measured on a Jasco DIP-370 digital polarimeter (JASCO, Tokyo, Japan). UV spectra were recorded on an Agilent 8453 spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). IR spectra were recorded on a Nicolet Magna FT-IR spectrophotometer (Nicolet Instrument, Inc., Madison, WI, USA). NMR spectra were recorded on a Bruker Avance 300 (Bruker, Karlsruhe, Germany) and AMX 400 spectrometers (Bruker, Karlsruhe, Germany), and all chemical shifts are given in ppm using tetramethylsilane (δ 0.00) as an internal standard. Mass spectra were obtained on a VG 70-250S spectrometer by a direct inlet system (Micromass Corp., Manchester, UK).
3.2. Plant Material

Whole *Cephalantheropsis gracilis* plants were collected from Pingtung Hsien, Taiwan, in December 2004, as authenticated by Chang-Sheng Kuoh, Department of Biology, National Cheng Kung University, Tainan, Taiwan. A voucher specimen (No: PLW-0401) was deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

3.3. Extraction and Isolation

The dried aerial parts of *C. gracilis* (2.4 kg) were extracted with MeOH (8 L) under reflux 8 times. The combined extracts were concentrated under reduced pressure to produce a dark brown syrup. The syrup was then suspended in H2O and then partitioned with hexane, CHCl3, and EtOAc, successively. The concentrated hexane layer (47 g) was chromatographed on a silica gel column by eluting with a gradient of hexane-Me2CO (10:1 to pure Me2CO) to give six fractions. Fraction 3 was chromatographed on silica gel by elution with hexane-i-Pr2O (1:3 to pure i-Pr2O) to give 48 (9.6 mg). Fraction 4 was chromatographed on silica gel using the same solvent mixture to yield 3 (4.3 mg), 34 (7.0 mg), 52 (0.7 mg), and 57 (1.4 mg). Fraction 5 was chromatographed on silica gel eluting with i-Pr2O (pure i-Pr2O to 30:1 of i-Pr2O-Me2CO to pure Me2CO) to give 4 (6.6 mg), 32 (3.5 mg), and 29 (5 mg).

The CHCl3 extract (30 g) was chromatographed on a silica gel column by eluting with a gradient of hexane-Me2CO (1:2 to pure Me2CO) to yield twelve fractions. Fraction 1 was subjected to chromatography on a silica gel column eluting with a gradient of hexane-Me2CO (10:1 to pure Me2CO) to give 10 (61.8 mg) and 39 (2.2 mg). Fraction 2 was chromatographed on a silica gel column eluting with a gradient of hexane-Me2CO (6:1 to pure Me2CO) to give 7 (39.4 mg), 2 (3.6 mg), 6 (543.1 mg), 13 (8.6 mg), and 17 (5.8 mg). Similarly, fraction 3 was chromatographed with a gradient of hexane-Me2CO (4:1 to pure Me2CO) to give 51 (2.2 mg) and 56 (22.7 mg). Fraction 5 was subjected to chromatography over silica gel eluting with a gradient of hexane-i-Pr2O (1:4 to pure i-Pr2O) to give 14 (20.0 mg) and 24 (48.0 mg). Fraction 6 was further purified on a silica gel column eluting with a gradient of i-Pr2O-MeOH (50:1 to pure MeOH) to give 9 (34.4 mg), 19 (30.0 mg), and 28 (12 mg). Fraction 7 was chromatographed on a silica gel column eluting with a gradient of hexane-CHCl3 (6:1 to pure CHCl3) to give 25 (62.1 mg), 33 (0.7 mg), 5 (2.9 mg), 43 (11.4 mg), 44 (8 mg), and 45 (1.6 mg). Fraction 8 was subjected to chromatography over silica gel eluting with a gradient of CHCl3-Me2CO (30:1 to pure Me2CO) to give 12 (50.4 mg), 26 (2.7 mg), 31 (39.5 mg), 40 (3.4 mg), and 50 (8.3 mg). Fraction 9 was further chromatographed on a silica gel column eluting with a gradient of hexane-EtOAc (15:1 to pure EtOAc) to give 11 (54.1 mg) and 18 (6.2 mg). Fraction 10 was chromatographed on a silica gel column eluting with a gradient of i-Pr2O-MeOH (9:1 to pure MeOH) to give 27 (2.2 mg), 35 (2.3 mg), 42 (103 mg), 46 (1.2 mg), 49 (33.3 mg), and 37 (5.1 mg). Finally, fraction 11 was chromatographed on a silica gel column eluting with a gradient of CHCl3-MeOH (10:1 to pure MeOH) to give 53 (2.1 mg), 54 (37.4 mg), 55 (5.4 mg), and Fraction 12 yielded 23 (328.2 mg) as a pure crystalline material.

The EtOAc extract (20 g) was subjected to column chromatography using Cosmosil 75 C18 and eluted with a gradient of H2O-MeOH (from pure H2O to pure MeOH) to give nine fractions. Fraction 2
was subjected to further chromatography on a Cosm osil 75 C18 column eluting with a gradient of H2O–MeOH (from pure H2O to pure MeOH) to give 21 (3.6 mg) and 47 (12.5 mg). Fraction 3 was chromatographed on a silica gel column eluting with a gradient of i-Pr2O–MeOH (15:1 to pure MeOH) to give 22 (2.2 mg). Fraction 4 was subjected to repeated chromatography on a silica gel column eluting with a gradient of i-Pr2O-MeOH (15:1 to pure MeOH) to give 22 (2.2 mg). Fraction 4 was subjected to repeated chromatography on a silica gel column eluting with a gradient of i-Pr2O–MeOH (10:1 to pure MeOH) to give 1 (1.4 mg), 16 (3.5 mg), 36 (12.6 mg), and 41 (44.5 mg). Fraction 5 was chromatographed on a silica gel column eluting with a gradient of i-Pr2O–MeOH (20:1 to pure MeOH) to give 8 (9.8 mg), 15 (22.2 mg), and 38 (2.9 mg). Fraction 6 was further purified over silica gel eluting with a gradient of CHCl3–MeOH (15:1 to pure MeOH) to give 20 (20.9 mg). Fraction 7 was further chromatographed on a silica gel column eluting with a gradient of i-Pr2O–MeOH (8:1 to pure MeOH) to give 30 (4.0 mg).

Cephalanthrin-A (1). White amorphous powder, mp 212–214 °C; [α]D +8.0° (c 0.07, CH3OH); UV λmax (log ε) CH3OH 206 (4.0), 261 (3.3), 302 (3.0), 314 (3.1), 329 (3.0) nm; IR νmax (KBr) 3000 (br), 1652, 1464 cm−1; EIMS m/z (rel. int.) 308 (M+, 13), 250 (100), 219 (8), 119 (19); HREIMS m/z 308.0794 [M]+ (Calcd for C17H12N2O4, 308.0797).

Cephalanthrin-B (2). Yellow amorphous powder, mp 215–217 °C; [α]D +3.0° (c 0.11, CHCl3); UV λmax (log ε) CHCl3 259 (3.2), 276 (3.0), 316 (2.9), 442 (2.9) nm; IR νmax (KBr) 1754, 1722, 1643, 1607, 1592 cm−1; EIMS m/z (rel. int.) 322 (M+, 2), 291 (5), 263 (100); HREIMS m/z 322.0955 [M]+ (Calcd For C18H14N2O4, 322.0953).

Cephathrene-A (3). White amorphous powder, mp 96–98 °C; UV λmax (log ε) CHCl3 267 (3.3), 304 (3.2) nm; IR νmax (KBr) 3404, 1582, 1483, 1467 cm−1; EIMS m/z (rel. int.) 302 (M+, 100), 255 (35), 184 (10); HREIMS m/z 302.1153 [M]+ (Calcd for C17H18O5, 302.1154).

Cephathrene-B (4). White amorphous powder; UV λmax (log ε) CHCl3: 265 (3.2), 316 (3.0) nm; IR νmax (KBr) 3400, 1606, 1501, 1464 cm−1; EIMS m/z (rel. int.) 332 (M+, 20), 331 (100), 302 (24), 285 (17); HREIMS m/z 332.1262 [M]+ (Calcd For C18H20O6, 332.1260).

Methyl 2-(Aminocarbonyl)phenylcarbamate (5). White amorphous powder, mp 200–202 °C; UV λmax (log ε) CH3OH 212 (2.9), 228 (3.0), 257 (2.9), 289 (2.7), 311 (2.7) nm; IR νmax (KBr) 3417, 3211, 1726, 1686, 1626, 1598, 1531 cm−1; 1H NMR (acetone-d6) δ 3.71 (3H, s, OCH3), 6.99 and 7.74 (each 1H, br s, NH2), 7.05 (1H, t, J = 7.8 Hz, H-4), 7.49 (1H, t, J = 7.8 Hz, H-5), 7.83 (1H, d, J = 7.8 Hz, H-3), 8.38 (1H, d, J = 7.8 Hz, H-6), 11.29 (1H, br s, 1-NH); 13C NMR (acetone-d6) δ 52.2 (OCH3), 119.2 (C-2), 119.6 (C-6), 122.2 (C-4), 129.1 (C-3), 133.4 (C-5), 141.7 (C-1), 154.6 (NC=O), 172.0 (2-C=O); EIMS m/z (rel. int.) 194 (M+, 31), 162 (32), 146 (100), 118 (9); HREIMS m/z 194.0693 [M]+ (calcd for C9H10N2O3, 194.0692).

3.4. Cytotoxicity Assay

The cytotoxicity assay was carried out according to the procedure described in the literature [17].

4. Conclusions

Five new compounds, cephalanthrin-A (1), cephalanthrin-B (2), cephathrene-A (3), cephathrene-B (4), methyl 2-(aminocarbonyl)phenylcarbamate (5), and 52 known compounds were isolated from
Cephalantheropsis gracilis. Cephalinone D (19) showed the strongest cytotoxicity against the tested tumor cell lines, with IC_{50} values ranging from 7.6 to 42.9 μM. The modifications using Cephalinone D as template are being studied in our laboratories, aiming to discover the derivatives with strong anticancer activity.

Supplementary Materials

Supplementary materials can be found at http://www.mdpi.com/1422-0067/16/02/3980/s1.

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Author Contributions

Chi-Fen Chang performed experiments, analyzed data and wrote the paper; Yu-Lin Hsu, Chao-Ying Lee and Chia-Hua Wu performed experiments and analyzed data; Yang-Chang Wu revised the paper; and Ta-Hsien Chuang analyzed data and wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Boufford, D.E.; Hsieh, C.F.; Huang, T.C.; Kuoh, C.S.; Ohashi, H.; Su, H.J. Editorial Committee of the Flora of Taiwan, 2nd ed.; Flora of Taiwan: Taipei, Taiwan, 2000; Volume 5, pp. 795–796.
2. Wu, P.L.; Hsu, Y.L.; Jao, C.W. Indole alkaloids from Cephalantheropsis gracilis. J. Nat. Prod. 2006, 69, 1467–1470.
3. Wu, Y.T.; Hsu, Y.L.; Wu, P.L. Two indolotryptanthrin alkaloids from Cephalantheropsis gracilis. Heterocycles 2008, 75, 1191–1197.
4. Methyl 2-(aminocarbonyl)phenylcarbamate (5) is commercially available (CAS registry number 445285-93-8) from various sources, although no references are available in the scientific literature, according to Chemical Abstracts.
5. Cornforth, S.J.; Hitchcock, P.B.; Rozos, P. Isatin chloride: A phantom. Reactions of 2-(2,2-dichloro-2,3-dihydro-3-oxoindol-1-yl)-3H-indol-3-one. J. Chem. Soc. Perkin Trans. 1996, 1, 2787–2792.
6. Takayama, H.; Matsuda, Y.; Masubuchi, K.; Ishida, A.; Kitajima, M.; Aimi, N. Isolation, structure elucidation, and total synthesis of two new Chimonanthus alkaloids, chimonamidine and chimonanthidine. *Tetrahedron* 2004, 60, 893–900.

7. Ghosh, I.; Zeng, H.; Kishi, Y. Application of chiral lanthanide shift reagents for assignment of absolute configuration of alcohols. *Org. Lett.* 2004, 6, 4715–4718.

8. Jao, C.W.; Lin, W.C.; Wu, Y.T.; Wu, P.L. Isolation, structure elucidation, and synthesis of cytotoxic tryptanthrin analogues from *Phaius mishemensis*. *J. Nat. Prod.* 2008, 71, 1275–1279.

9. Maskey, R.P.; Shaaban, M.; Grun-Wollny, I.; Laatsch, H. Quinazolin-4-one derivatives from *Streptomyces* isolates. *J. Nat. Prod.* 2004, 67, 1131–1134.

10. Deng, K.M.; Wu, X.Y.; Yang, G.J.; Qin, G.W. Alkaloids from *Isatis indigotica*. *Chin. Chem. Lett.* 1997, 8, 237–238.

11. Majumder, P.L.; Pal, S.; Majumder, S. Dimeric phenanthrenes from the orchid *Bulbophyllum reptans*. *Phytochemistry* 1999, 50, 891–897.

12. Majumder, P.L.; Majumder, S.; Sen, S. Triterpenoids from the orchids *Agrostophyllum brevipes* and *Agrostophyllum callosum*. *Phytochemistry* 2003, 62, 591–596.

13. Valencia-Islas, N.A.; Paul, R.N.; Shier, W.T.; Mata, R.; Abbas, H.K. Phytotoxicity and ultrastructural effects of gymnopusin from the orchid *maxillaria densa* on duckweeds (*Lemna pausicostata*) frond and root tissues. *Phytochemistry* 2002, 61, 141–148.

14. Das, B.; Takhi, M.; Srinivas, K.V.N.S.; Yadav, J.S. Phenolics from needles of himalayan *Taxus baccata*. *Phytochemistry* 1993, 33, 1489–1491.

15. Kazuma, K.; Noda, N.; Suzuki, M. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry* 2003, 62, 229–237.

16. Chen, K.S.; Chang, F.R.; Chia, Y.C.; Wu, T.S.; Wu, Y.C. Chemical constituents of *Neolitsea parvigemma* and *Neolitsea konishii*. *J. Chin. Chem. Soc.* 1998, 45, 103–110.

17. Wu, P.L.; Rao, K.V.; Su, C.H.; Kuoh, C.S.; Wu, T.S. Phenanthroindolizidine alkaloids and their cytotoxicity from the leaves of *Ficus septica*. *Heterocycles* 2002, 57, 2401–2408.

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