

Article

Dianthiamides A–E, Proline-Containing Orbitides from *Dianthus chinensis*

Jin Woo Lee ¹, Jun Gu Kim ¹, Jae Sang Han ¹, Yong Beom Cho ¹, Yu Jin Lee ¹, Dongho Lee ², Dae Hwan Shin ¹, Jin Tae Hong ¹, Mi Kyeong Lee ¹ and Bang Yeon Hwang ^{1,*}

- ¹ College of Pharmacy, Chungbuk National University, Cheongju 28160, Korea; jinu15@hanmail.net (J.W.L.); sossgj@naver.com (J.G.K.); han00768@hanmail.net (J.S.H.); bum94@naver.com (Y.B.C.); yujinli@naver.com (Y.J.L.); dshin@chungbuk.ac.kr (D.H.S.); jinthong@chungbuk.ac.kr (J.T.H.); mkleee@chungbuk.ac.kr (M.K.L.)
- ² Department of Plant Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea; dongholee@korea.ac.kr
- * Correspondence: byhwang@chungbuk.ac.kr; Tel.: +82-43-261-2814

Abstract: Orbitides are plant-derived small cyclic peptides with a wide range of biological activities. Phytochemical investigation of the whole plants of *Dianthus chinensis* was performed with the aim to discover new bioactive orbitides. Five undescribed proline-containing orbitides, dianthiamides A–E (1–5), were isolated from a methanolic extract of *Dianthus chinensis*. Their structures were elucidated by extensive analysis of 1D and 2D NMR and HRESI–TOF–MS as well as ESI–MS/MS fragmentation data. The absolute configuration of the amino acid residues of compounds 1–5 was determined by Marfey’s method. All compounds were tested for their cytotoxic activity, and dianthiamide A (1) exhibited weak activity against A549 cell line with IC₅₀ value of 47.9 μM.

Keywords: *Dianthus chinensis*; Caryophyllaceae; orbitide; structure elucidation; cytotoxic activity



Citation: Lee, J.W.; Kim, J.G.; Han, J.S.; Cho, Y.B.; Lee, Y.J.; Lee, D.; Shin, D.H.; Hong, J.T.; Lee, M.K.; Hwang, B.Y. Dianthiamides A–E, Proline-Containing Orbitides from *Dianthus chinensis*. *Molecules* **2021**, *26*, 7275. <https://doi.org/10.3390/molecules26237275>

Academic Editors: Thomas J. Schmidt, Josphat Matasyoh and Valeria Patricia Sülsen

Received: 2 November 2021
Accepted: 27 November 2021
Published: 30 November 2021

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Dianthus chinensis L. is a perennial herbaceous plant belonging to the Caryophyllaceae family, and is distributed widely in Europe and Eastern Asia. The whole plant of *D. chinensis* is commonly used as a traditional medicine in Korea for treating diuretic, carcinoma, urethritis, and carbuncles [1–6]. Previous investigation on the phytochemical constituents of the genus *Dianthus* led to the isolation of cyclopeptides [4–9], dianthramides [5,10,11], triterpenoidal saponins [1–3,12,13], anthocyanins [14], and pyran-type glycosides [15]. Orbitides, formerly known as Caryophyllaceae-type cyclic peptides, are N-to-C cyclized plant peptides lacking disulfide bonds, which possess 5 to 12 amino acid residues. Orbitides are ribosomally synthesized and post-translationally modified cyclic peptides, which have been discovered in many plants of the families such as Annonaceae, Asteraceae, Caryophyllaceae, Euphorbiaceae, Lamiaceae, Linaceae, Phytolaccaceae, Rutaceae, Schizandraceae, and Verbenaceae. Recently, orbitides have gained increasing attention owed to a wide range of biological activities including cytotoxic, antimalarial, immunomodulatory, and antiproliferative activities [16–19]. The *Dianthus* genus is a rich source of proline-containing orbitides, some of which showed cytotoxic activity against several cancer cell lines [5,6,18,20]. Therefore, we have embarked on a research program for the isolation of new bioactive orbitides from medicinal plant, and five undescribed orbitides, dianthiamides A–E (1–5) (Figure 1), were isolated from a MeOH extract of the whole plants of the *D. chinensis*. Herein, the isolation and structure determination as well as their cytotoxic activity against A549 cell line are described.

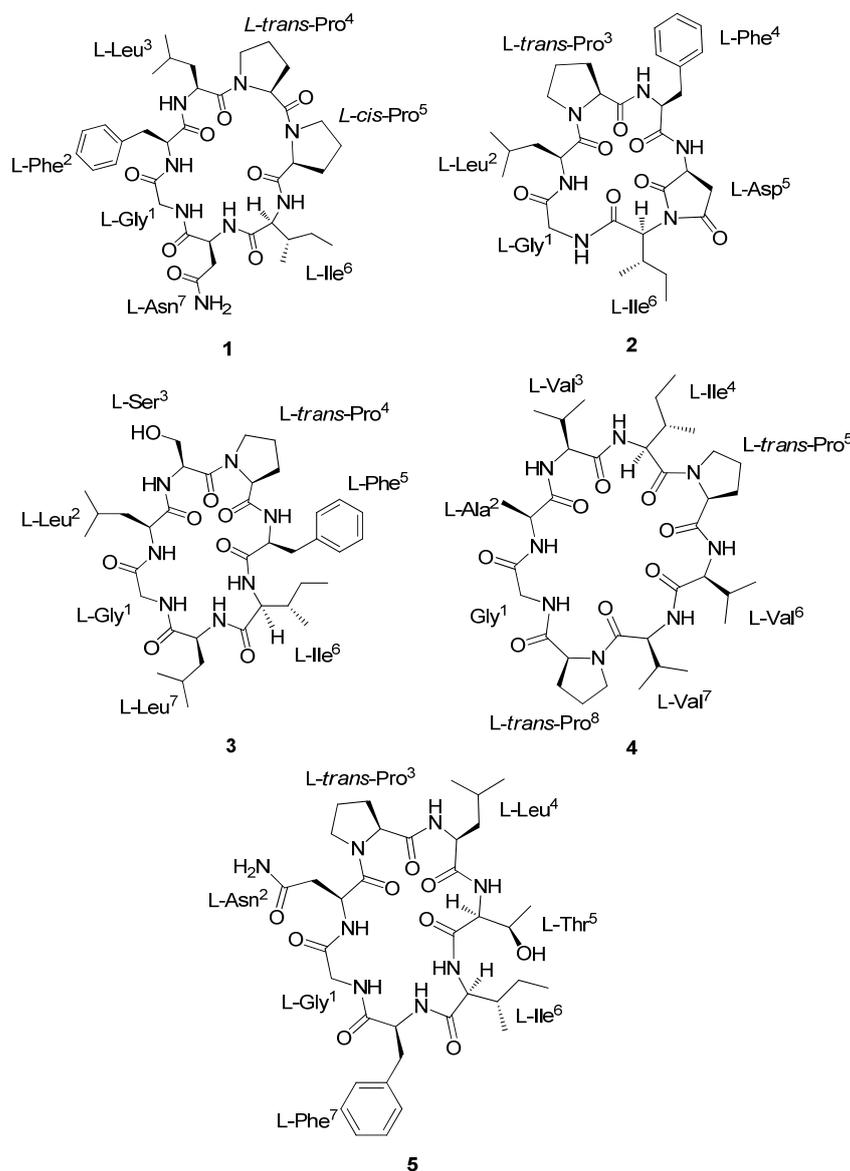


Figure 1. Structures of compounds 1–5.

2. Results and Discussion

Dianthiamide A (**1**) was obtained as a yellow amorphous powder. Its molecular formula of $C_{37}H_{54}N_8O_8$ was determined from the HRESI-TOF-MS data (m/z 739.4144 $[M + H]^+$; calcd for 739.4137). The 1H and ^{13}C NMR in conjunction with HSQC data of **1** displayed the presence of 37 carbon signals assigned to eight amide carbonyl carbons (δ_C 168.8, 169.8, 170.8, 170.9, 171.1, 171.4, 172.3, and 173.4), seven α -amino acid carbons [δ_C 44.4 (CH_2), 48.6 (CH), 49.6 (CH), 56.0 (CH), 59.6 (CH), 60.8 (CH), and 61.6 (CH)], six aromatic carbons [δ_C 126.8, 128.8, (2C), 129.1 (2C), and 138.4], two methines, ten methylenes, and four methyls (Table 1), suggesting that **1** is a heptapeptide. Furthermore, HSQC, HMBC, and COSY spectra showed the identification of seven amino acid residues including phenylalanine (F), glycine (G), isoleucine (I), asparagine (N), leucine (L), and two prolines (P_a and P_b).

In the HMBC and ROESY experiments, the cyclic feature and amino acid sequence of **1** were elucidated by the correlations observed between the amino acid H_{α} and continuous amide group (CONH). Therefore, the linear sequence of **1** was identified as G-F-L- P_a - P_b -I-N. Also, the HMBC correlation from Gly- H_{α} (δ_H 3.40 and 3.55) to Asn-C=O (δ_C 172.3) as well as the ROESY correlation between Gly-NH (δ_H 8.70) and Asn- H_{α} (δ_H 4.18) established the

cyclic heptapeptide as *cyclo*-G-F-L-P_a-P_b-I-N (Figure 2). The amino acid sequence of **1** was further confirmed by analysis of the ESI-MS/MS fragment ions. Presumably, though there were several ring-opening sites, it occurred at two preferred positions at Pro_a⁴-Pro_b⁵ and Ile⁶-Asn⁷, respectively. Each the linear sequences, Pro_b⁵-Ile⁶-Asn⁷-Gly¹-Phe²-Leu³-Pro_a⁴ (b₇P_bP_a) and Asn⁷-Gly¹-Phe²-Leu³-Pro_a⁴-Pro_b⁵-Ile⁶ (b₇NI) was certified by acylium ions (b_nP_bP_a and b_nNI) and after loss of CO (a_nNI) at *m/z* 642 (b₆P_bP_a), 626 (b₆NI), 529 (b₅P_bP_a and b₅NI), 404 (a₄NI), 364 (b₄P_bP_a-H₂O), 319 (b₃NI), and 211 (b₂P_bP_a), corresponding to the successive loss of amino acid residues (Figure S9).

Table 1. ¹H and ¹³C NMR data for dianthiamide A (**1**) (DMSO-*d*₆, 700 MHz, d in ppm, *J* in Hz).

Position	δ _C	δ _H (J in Hz)	Position	δ _C	δ _H (J in Hz)
Gly¹			<i>cis</i>-Pro_b⁵		
C=O	169.8, C		C=O	170.9, C	
NH		8.70 (t, 4.2)	α	60.8, CH	4.55 (d, 7.0)
α	44.4, CH ₂	3.55 (dd, 16.8, 4.2)	β	31.1, CH ₂	2.33 (dd, 11.9, 7.0)
		3.40 (dd, 16.8, 5.6)	γ	22.1, CH ₂	1.92 (m)
Phe²			δ	46.4, CH ₂	1.85 (m)
C=O	171.1, C				1.46 (m)
NH		7.69 (d, 9.1)			3.46 (m)
α	56.0, CH	4.38 (m)	Ile⁶		
β	37.0, CH ₂	3.01 (m)	C=O	171.4, C	
		2.97 (m)	NH		8.49 (d, 7.7)
Phe-1'	138.4, C		α	61.6, CH	3.66 (dd, 9.8, 7.7)
2',6'	129.1, CH	7.15–7.27 (m)	β	35.3, CH	2.05 (m)
3',5'	128.8, CH	7.15–7.27 (m)	γ-CH ₃	16.0, CH ₃	0.90 (t, 7.7)
4'	126.8, CH	7.15–7.27 (m)			
Leu³			γ-CH ₂	25.9, CH ₂	1.50 (m)
C=O	168.8, C				1.15 (m)
NH		7.28 (m)	δ-CH ₃	10.9, CH ₃	0.82 (d, 7.7)
α	48.6, CH	4.48 (m)	Asn⁷		
β	41.0, CH ₂	1.68 (m)	C=O	172.3, C	
		1.19 (m)	NH		7.26 (m)
γ-CH	24.6, CH	1.51 (m)	α	49.6, CH	4.18 (m)
δ-CH ₃	23.6, CH ₃	0.85 (d, 6.3)	β	35.8, CH ₂	3.15 (m)
δ-CH ₃	22.6, CH ₃	0.88 (d, 6.3)			3.05 (m)
<i>trans</i>-Pro_a⁴			C=O	173.4, C	
C=O	170.8, C		NH ₂		7.97 (s)
α	59.6, CH	4.45 (t, 7.0)			7.54 (s)
β	28.5, CH ₂	2.27 (m)			
		1.64 (m)			
γ	25.3, CH ₂	1.96 (m)			
		1.83 (m)			
δ	47.1, CH ₂	3.44 (m)			

The geometry of proline residues was assigned on the basis of the $\Delta\delta_{C\beta-C\gamma}$ values and the presence of a ROE correlations between the proline H α or H δ and the H α of previous amino acid. The $\Delta\delta_{C\beta-C\gamma}$ value (3.2 ppm) of the Pro_a⁴ and the ROE correlation between the H α (δ_H 4.48) of Leu³ and the H δ (δ_H 3.44) of Pro_a⁴ indicated that the amide bond in the Pro_a⁴ was a *trans*. However, the $\Delta\delta_{C\beta-C\gamma}$ value (9.0 ppm) of the Pro_b⁵ and the ROE correlation between the H α (δ_H 4.45) of Pro_a⁴ and the H α (δ_H 4.55) of Pro_b⁵ indicated that the geometry of Pro_b⁵ was a *cis* (Figure 3) [21–23]. The absolute configuration of amino acid residues in **1** were identified as L configuration, which was deduced by acid hydrolysis and Marfey's derivatization, followed by HPLC analysis [24–27]. The *N*- α -(2,4-dinitro-5-fluorophenyl)-L-alaninamide (L-FDAA)-derivatives of **1** gave peaks at *t_R* (min) 15.0 (L-Asp, *m/z* 386), 20.8 (L-Pro, *m/z* 368), 29.5 (L-Leu, *m/z* 384), 29.7 (L-Phe, *m/z* 418),

and 30.2 (L-Ile, m/z 384) (Figure S10). Therefore, dianthiamide A (1) was established as *cyclo*-(Gly¹-L-Phe²-L-Leu³-L-*trans*-Pro^a₄-L-*cis*-Pro^b₅-L-Ile⁶-L-Asn⁷).

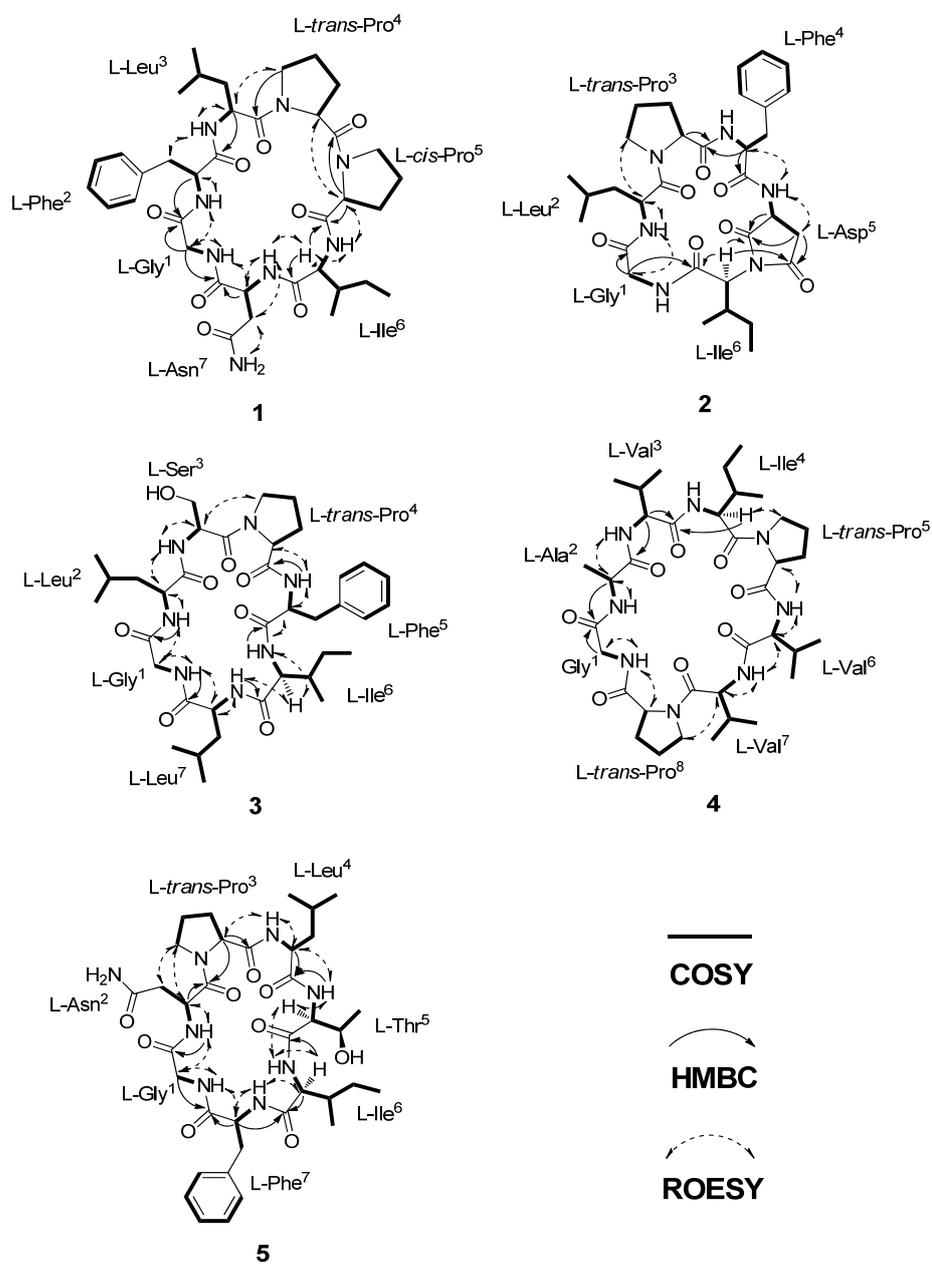


Figure 2. Key COSY, HMBC, and ROESY correlations of 1–5.

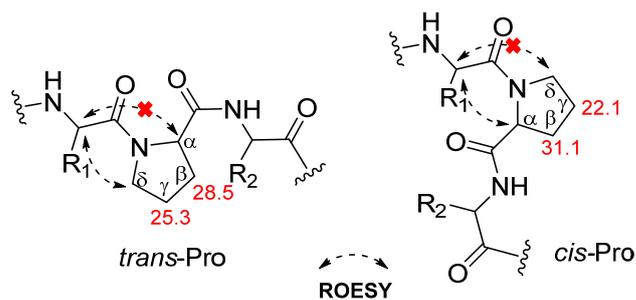


Figure 3. Proline isomerization of 1.

Dianthiamide B (**2**) was isolated as a yellow amorphous powder, the HRESI–TOF–MS data were consistent with the molecular formula $C_{32}H_{44}N_6O_7$ (m/z 647.3161 [$M + Na$] $^+$; calcd for 647.3163). The 1H , ^{13}C and HSQC NMR spectra of **2** showed 32 carbons, consisting of seven amide carbonyl carbons, six α -amino acid carbons, six aromatic carbons, two methines, seven methylenes, and four methyls (Table 2). HSQC, HMBC, and COSY spectra demonstrated the presence of six amino acid residues including aspartic acid (D), phenylalanine (F), glycine (G), isoleucine (I), leucine (L), and proline (P). Moreover, HMBC, COSY, and ROESY spectra indicated that the sequence and connectivity of the hexapeptide was *cyclo*-G-L-P-F-D-I (Figure 2). The HMBC correlations between $H\alpha$ (δ_H 4.19) of Ile⁶ and two carbonyls (δ_C 176.2 and 177.2) of Asp⁵ showed that dehydration of NH-Ile⁶ and COOH-Asp⁵ formed an additional five membered ring system (pyrrolidine-2,5-dione). The amino acid sequence in **2** was further supported by the fragmentation pattern of ESI–MS/MS data, in which the preferred ring-opening of **2** occurred at the amide bond between leucine and proline (Figure S19). The geometry of amide bond of Pro³ residue in **2** was assigned the *trans* configuration, on the basis of the difference of the ^{13}C NMR chemical shift ($\Delta\delta_{C\beta-C\gamma}$ = 3.9 ppm) [21–23] as well as the ROE correlation between the $H\alpha$ (δ_H 4.51) of Leu² and the $H\delta$ (δ_H 3.68 and 3.42) of Pro³ residue. In addition, the absolute configuration of six amino acid residues in **2** were all assigned as L, which was determined by HPLC analysis of the acid hydrolysate after Marfey's derivatization (Figure S20). Therefore, dianthiamide B (**2**) was determined as *cyclo*-(Gly¹-L-Leu²-L-*trans*-Pro³-L-Phe⁴-L-Asp⁵-L-Ile⁶).

Table 2. 1H and ^{13}C NMR data for dianthiamide B (**2**) (DMSO- d_6 , 700 MHz, d in ppm, J in Hz).

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
Gly¹			Phe⁴		
C=O	168.7, C		C=O	170.7, C	
NH		8.68 (m)	NH		8.68 (m)
α	43.3, CH ₂	3.65 (t, 7.0)	α	57.2, CH	3.80 (m)
Leu²			β	33.8, CH ₂	3.33 (m)
C=O	170.7, C				3.30 (m)
NH		6.50 (m)	Phe-1'	139.6, C	
α	48.6, CH	4.51 (m)	2',6'	129.6, CH	7.10–7.30 (m)
β	40.5, CH ₂	1.40 (m)	3',5'	128.6, CH	7.10–7.30 (m)
		1.20 (m)	4'	126.6, CH	7.10–7.30 (m)
γ -CH	24.6, CH	1.62 (m)	Asp⁵		
δ -CH ₃	23.8, CH ₃	0.91 (d, 7.0)	C=O	176.2, C	
δ -CH ₃	21.3, CH ₃	0.93 (d, 7.0)	NH		7.95 (d, 8.4)
trans-Pro³			α	47.3, CH	5.23 (m)
C=O	171.7, C		β	36.5, CH ₂	3.22 (m)
α	61.0, CH	3.92 (t, 7.0)			2.05 (d, 3.5)
β	29.3, CH ₂	1.85 (m)	C=O	177.2, C	
		1.67 (m)	Ile⁶		
γ	25.4, CH ₂	2.01 (m)	C=O	167.0, C	
		1.83 (m)	α	61.5, CH	4.19 (d, 11.9)
δ	47.5, CH ₂	3.68 (m)	β	30.4, CH	2.68 (m)
		3.42 (m)	γ -CH ₃	16.1, CH ₃	0.87 (t, 7.7)
			γ -CH ₂	24.3, CH ₂	1.38 (m)
					0.95 (m)
			δ -CH ₃	10.5, CH ₃	0.79 (d, 7.7)

Dianthiamide C (**3**) was obtained as a yellow amorphous powder, showed a molecular formula of $C_{37}H_{57}N_7O_8$ as determined by its HRESI–TOF–MS data (m/z 750.4165 [$M + Na$] $^+$; calcd 750.4160). The ^{13}C and HSQC NMR data of **3** displayed the presence of 37 carbon signals including seven amide carbonyls, as well as seven α -amino acid carbons, suggesting a heptapeptide (Table 3). Full assignments of 1H and ^{13}C NMR data for each amino acid residue were accomplished by combined analysis of COSY, HSQC, and HMBC spectra and suggested that **3** was composed of seven amino acid such as phenylalanine

(F), glycine (G), isoleucine (I), two leucines (L_a and L_b), proline (P), and serine (S) residue (Table 3). The HMBC and ROESY spectra indicated that the amino acid sequence was *cyclo*-G-L_a-S-P-F-I-L_b (Figure 2), which was further confirmed by ESI-MS/MS fragmentation analysis (Figure S29). The observed $\Delta\delta_{C\beta-C\gamma}$ value (3.2 ppm) of the Pro⁴ and the ROE correlation from the H α (δ_H 4.89) of Ser³ to the H δ (δ_H 3.91 and 3.42) of Pro⁴ revealed that the geometry of Pro⁴ of **3** was a *trans* configuration [21–23]. The absolute configuration of **3** was determined by Marfey's method [24–27], which indicated that all the amino acids were L configuration (Figure S30). Therefore, dianthiamide C (**3**) was confirmed as *cyclo*-(Gly¹-L-Leu²-L-Ser³-L-*trans*-Pro⁴-L-Phe⁵-L-Ile⁶-L-Leu⁷).

Table 3. ¹H and ¹³C NMR data for dianthiamide C (**3**) (DMSO-*d*₆, 700 MHz, d in ppm, J in Hz).

Position	δ_C	δ_H (J in Hz)	Position	δ_C	δ_H (J in Hz)
Gly¹			Phe⁵		
C=O	169.1, C		C=O	170.7, C	
NH		8.91 (t, 4.9)	NH		7.43 (d, 9.8)
α	43.2, CH	4.00 (m)	α	54.6, CH	4.38 (m)
		3.33 (m)	β	38.4, CH ₂	3.21 (dd, 13.3, 3.5)
					2.62 (t, 13.3)
Leu_a²			Phe-1'	138.4, C	
C=O	171.2, C		2',6'	129.4, CH	7.18–7.31 (m)
NH		8.10 (d, 10.5)	3',5'	128.6, CH	7.18–7.31 (m)
α	53.0, CH	4.52 (m)	4'	126.8, CH	7.18–7.31 (m)
β	43.8, CH ₂	1.42 (m)	Ile⁶		
		1.34 (m)	C=O	171.6, C	
γ -CH	24.5, CH	1.55 (m)	NH		7.01 (d, 9.1)
δ -CH ₃	22.9, CH ₃	0.87 (d, 6.3)	α	56.4, CH	4.35 (m)
δ -CH ₃	22.2, CH ₃	0.84 (d, 6.3)	β	37.9, CH	1.71 (m)
Ser³			γ -CH ₃	15.1, CH ₃	0.82 (t, 7.7)
C=O	168.9, C		γ -CH ₂	24.7, CH ₂	1.39 (m)
NH		8.73 (d, 7.0)			1.00 (m)
α	54.2, CH	4.89 (br s)	δ -CH ₃	11.0, CH ₃	0.81 (d, 7.7)
β	61.9, CH ₂	4.24 (m)	Leu_b⁷		
		3.71 (d, 10.5)	C=O	172.3, C	
<i>trans</i>-Pro⁴			NH		8.71 (br s)
C=O	170.5, C		α	54.2, CH	3.87 (m)
α	62.2, CH	3.98 (m)	β	40.1, CH ₂	1.47 (m)
β	29.0, CH ₂	1.97 (m)			1.54 (m)
		1.24 (m)	γ -CH	24.4, CH	1.54 (m)
γ	25.8, CH ₂	1.79 (m)	δ -CH ₃	23.4, CH ₃	0.86 (m)
		1.68 (m)	δ -CH ₃	22.6, CH ₃	0.93 (m)
δ	47.8, CH ₂	3.91 (m)			
		3.42 (m)			

Dianthiamide D (**4**), a yellow amorphous powder, gave the molecular formula C₃₆H₆₀N₈O₈, based on the HRESI-TOF-MS data (*m/z* 755.4448 [M + Na]⁺; calcd 755.4426). Detailed analyses of the 1D and 2D (COSY, HSQC, and HMBC) NMR data revealed that **4** was a octapeptide containing alanine (A), glycine (G), isoleucine (I), two prolines (P_a and P_b) and three valines (V_a, V_b, and V_c) residues (Table 4). The amino acid sequence of **4** was established as *cyclo*-G-A-V_a-I-P_a-V_b-V_c-P_b by analysis of HMBC and ROESY data (Figure 2). This conclusion was also supported by the ESI-MS/MS sequence analysis (Figure S39). The observed $\Delta\delta_{C\beta-C\gamma}$ values of the Pro_a⁵ (2.6 ppm) and Pro_b⁸ (4.0 ppm) and the ROE correlations from the H α of Ile⁴ (δ_H 4.48) to the H δ of Pro_a⁵ (δ_H 3.79 and 3.62), and from the H α of Val_c⁷ (δ_H 4.43) to the H δ of Pro_b⁸ (δ_H 3.76 and 3.53) indicated that the geometry of both Pro_a⁵ and Pro_b⁸ of **4** were *trans* configuration [21–23]. Moreover, the absolute configuration of **4** was assigned by Marfey's method [24–27], which indicated that all the amino acids had L configuration (Figure S40). Therefore, dianthiamide D (**4**) was established as *cyclo*-(Gly¹-L-Ala²-L-Val_a³-L-Ile⁴-L-*trans*-Pro_a⁵-L-Val_b⁶-L-Val_c⁷-L-*trans*-Pro_b⁸).

Table 4. ^1H and ^{13}C NMR data for dianthiamide D (**4**) (DMSO- d_6 , 700 MHz, d in ppm, J in Hz).

Position	δ_{C}	δ_{H} (J in Hz)	Position	δ_{C}	δ_{H} (J in Hz)
Gly ¹			trans-Pro ⁵		
C=O	168.4, C		C=O	170.7, C	
NH		8.97 (t, 4.9)	α	62.0, CH	4.29 (m)
α	43.2, CH	3.84 (dd, 14.7, 4.9)	β	27.6, CH ₂	2.11 (m)
		3.33 (dd, 14.7, 4.2)			1.91 (m)
			γ	25.0, CH ₂	1.89 (m)
					1.79 (m)
Ala ²			δ	48.2, CH ₂	3.79 (m)
C=O	173.1, C				3.62 (m)
NH		7.30 (d, 7.0)			
α	47.2, CH	4.62 (t, 7.0)	Val ⁶		
β	19.6, CH ₃	1.42 (d, 7.0)	C=O	171.6, C	
			NH		8.24 (d, 7.0)
Val ³			α	60.0, CH	3.89 (dd, 7.0, 4.2)
C=O	171.4, C		β	29.3, CH	2.23 (m)
NH		7.99 (d, 4.9)	γ -CH ₃	20.1, CH ₃	0.90 (m)
α	60.8, CH	3.71 (t, 4.9)	γ -CH ₃	18.4, CH ₃	0.85 (d, 5.6)
β	28.7, CH	2.14 (m)	Val ⁷		
γ -CH ₃	19.9, CH ₃	0.92 (d, 6.3)	C=O	169.8, C	
γ -CH ₃	19.0, CH ₃	0.93 (d, 6.3)	NH		7.16 (d, 7.0)
Ile ⁴			α	55.6, CH	4.43 (t, 7.0)
C=O	173.0, C		β	31.1, CH	2.02 (m)
NH		6.70 (d, 7.7)	γ -CH ₃	19.9, CH ₃	0.86 (m)
α	54.7, CH	4.48 (d, 7.7)	γ -CH ₃	18.4, CH ₃	0.75 (d, 5.6)
β	35.9, CH	1.81 (m)			
γ -CH ₃	15.6, CH ₃	0.88 (t, 5.6)	trans-Pro ⁸		
γ -CH ₂	24.3, CH ₂	1.49 (m)	C=O	173.0, C	
		1.08 (m)	α	60.8, CH	4.18 (m)
δ -CH ₃	11.0, CH ₃	0.83 (d, 7.0)	β	29.4, CH ₂	2.08 (m)
					1.74 (m)
			γ	25.4, CH ₂	1.99 (m)
					1.84 (m)
			δ	47.9, CH ₂	3.76 (m)
					3.53 (m)

Dianthiamide E (**5**), a yellow amorphous powder. Its molecular formula of $\text{C}_{36}\text{H}_{54}\text{N}_8\text{O}_9$ was determined from the HRESI-TOF-MS data (m/z 743.4097 $[\text{M} + \text{H}]^+$; calcd for 743.4086). Analysis of 1D and 2D (COSY, HSQC, and HMBC) NMR data (Table 5 and Figure 2) as well as ESI-MS/MS sequence data (Figure S49) demonstrated that the seven amino acid residues were phenylalanine (F), glycine (G), isoleucine (I), asparagine (N), leucine (L), proline (P), and threonine (T). The sequence of these amino acid residues was assigned as *cyclo*-G-N-P-L-T-I-F by the observed HMBC and ROESY data (Figure 2).

The small difference of the $\Delta\delta_{\text{C}\beta\text{-C}\gamma}$ values of the Pro³ (4.4 ppm) and the ROE correlations between the H α of Asn² (δ_{H} 4.79) and the H δ of Pro³ (δ_{H} 3.73 and 3.56) revealed that the geometry of Pro³ of **5** was a *trans* [21–23]. Furthermore, Marfey's analysis assigned L configurations to all the amino acid residues in **5** (Figure S50) [24–27]. Therefore, dianthiamide E (**5**) was established as *cyclo*-(Gly¹-L-Asn²-L-*trans*-Pro³-L-Leu⁴-L-Thr⁵-L-Ile⁶-L-Phe⁷).

Recently, it has been reported that cyclic peptides isolated from the genus *Dianthus* exhibited cytotoxic activity against several cancer cell lines [5,6,18]. Therefore, all isolates were tested for their cytotoxic activity against human non-small cell lung cancer A549 and human stomach adenocarcinoma MKN-28 cells, with docetaxel as a positive control. However, dianthiamide A (**1**) only showed weak activity against A549 cell line with IC₅₀ value of 47.9 μM , and docetaxel was used as a positive control (IC₅₀: 0.08 μM). The other compounds **2–5** were inactive against A549 and MKN-28 cells (IC₅₀: >200 μM).

Table 5. ^1H and ^{13}C NMR data for diantiamide E (5) (DMSO- d_6 , 700 MHz, d in ppm, J in Hz).

Position	δ_{C}	δ_{H} (J in Hz)	Position	δ_{C}	δ_{H} (J in Hz)
Gly ¹			Thr ⁵		
C=O	169.2, C		C=O	170.7, C	
NH		8.01 (m)	NH		7.57 (d, 9.1)
α	43.6, CH ₂	4.02 (m)	α	57.0, CH	4.67 (m)
		3.31 (m)	β	68.7, CH	4.25 (m)
Asn ²			γ -CH ₃	19.4, CH ₃	1.03 (d, 6.3)
C=O	171.0, C		OH		5.26 (d, 6.3)
NH		7.15	Ile ⁶		
α	48.8, CH	4.79 (dd, 14.0, 7.0)	C=O	171.1, C	
β	37.2, CH ₂	2.75 (m)	NH		8.00 (m)
		2.57 (m)	α	59.2, CH	3.95 (t, 5.6)
C=O	172.2, C		β	35.7, CH	1.88 (m)
NH ₂		7.75 (br s)	γ -CH ₃	15.9, CH ₃	0.70 (d, 7.0)
		7.20 (m)	γ -CH ₂	23.9, CH ₂	0.97 (m)
trans-Pro ³			δ -CH ₃	11.8, CH ₃	0.68 (d, 7.0)
C=O	171.5, C		Phe ⁷		
α	62.1, CH	4.11 (t, 7.7)	C=O	171.6, C	
β	29.7, CH ₂	2.20 (m)	NH		7.78 (d, 2.1)
		1.71 (m)	α	54.9, CH	4.41 (m)
γ	25.3, CH ₂	1.92 (m)	β	37.3, CH ₂	3.13 (dd, 14.0, 5.6)
δ	47.5, CH ₂	3.73 (m)			2.84 (dd, 14.0, 9.1)
		3.56 (m)	Phe-1'	138.0, C	
Leu ⁴			2',6'	129.4, CH	7.17–7.29 (m)
C=O	172.2, C		3',5'	128.7, CH	7.17–7.29 (m)
NH		8.39 (br s)	4'	126.8, CH	7.17–7.29 (m)
α	52.4, CH	4.00 (m)			
β	39.2, CH ₂	1.79 (m)			
		1.63 (m)			
γ	25.1, CH	1.54 (m)			
δ -CH ₃	23.7, CH ₃	0.88 (d, 7.0)			
δ -CH ₃	21.3, CH ₃	0.82 (d, 7.0)			

3. Materials and Methods

3.1. Chemicals

HPLC grade acetonitrile was purchased from m Fisher Chemical (Loughborough, UK) and all other chromatographic solvents were purchased from Duksan Pure Chemicals Co., Cheongju, Korea). Paclitaxel as a positive control was obtained from LC Laboratories (Woburn, MA, USA). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

3.2. General Experimental Procedures

Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a JASCO UV-550 spectrophotometer. ECD spectra were obtained on a JASCO J-715 spectrometer, and IR spectra were measured on a JASCO FT-IR 4100 spectrometer (JASCO, Tokyo, Japan). NMR spectra were recorded on a Bruker AVANCE 700 MHz spectrometer (Bruker, MA, USA) using DMSO- d_6 as solvent. ESI-MS and HRESI-TOF-MS were obtained with LCQ Fleet (Thermo Fisher Scientific, San Jose, CA, USA) and maXis 4G mass spectrometers (Bruker, Bremen, Germany), respectively. Column chromatography was performed on silica gel (70–230 mesh, Merck, Darmstadt, Germany) and Lichroprep RP-18 (40–63 μm , Merck, Darmstadt, Germany). MPLC was performed on a Biotage Isolera Prime chromatography system (Biotage, Uppsala, Sweden). Preparative HPLC was performed using Waters HPLC system equipped with two Waters 515 pumps with a 2996 photodiode-array detector (Waters Corporation, Milford, MA, USA) using an YMC J'sphere

ODS-H80 column (4 μm , 150 \times 20 mm, i.d., Kyoto, Japan, flow rate 6 mL/min). TLC was performed using precoated silica gel 60 F₂₅₄ (0.25 mm, Merck, Darmstadt, Germany) plates, and spots were detected by a 10% vanillin-H₂SO₄ in water spray reagent.

3.3. Plant Material

The dried whole plants of *Dianthus chinensis* L. (Caryophyllaceae) were purchased from Kyungdong herbal market in Seoul, Korea, in June 2014. A voucher specimen (CBNU-2014-06-DC) was authenticated by B.Y.H. and deposited at the Herbarium of the College of Pharmacy, Chungbuk National University, Korea.

3.4. Isolation and Purification of Compounds 1–5

The dried and powdered whole plants of *D. chinensis* (3.0 kg) were extracted with MeOH (3 \times 16 L) at room temperature. The extract was evaporated under reduced pressure, and the residue (470 g) was suspended in water and partitioned successively with *n*-hexane (2 \times 1.5 L), CH₂Cl₂ (2 \times 1.5 L), and EtOAc (2 \times 1.5 L). The CH₂Cl₂-soluble fraction (13 g) was separated by MPLC with Lichroprep RP-18 column and eluted with MeOH-H₂O gradient system (10:90 to 100:0) to give eleven fractions (DCC1–DCC11). DCC1 (1.2 g) was separated on a silica gel column and eluted with CH₂Cl₂-MeOH gradient (from 100:0 to 0:100, 400 mL for each step) to obtain seven fractions (DCC1-1–DCC1-7) by MPLC. DCC1-4 (90 mg) was further purified by preparative HPLC (Waters system, YMC J'sphere ODS-H80, 150 \times 20 mm i.d., MeCN-H₂O, 30:70 to 60:40, flow rate 6 mL/min) to yield compound **1** (t_R = 20.1 min, 15 mg). DCC7 (1.0 g) was subjected to silica gel column chromatography and eluted with CH₂Cl₂-MeOH (from 100:0 to 0:100, 400 mL for each step) to give seven fractions (DCC7-1–DCC7-7) by MPLC. DCC7-4 (90 mg) was further purified by preparative HPLC (MeCN-H₂O, 35:65 to 65:35) to yield compound **2** (t_R = 21.4 min, 17 mg). DCC9 (2.2 g) was separated on a silica gel column and eluted with CH₂Cl₂-MeOH gradient (from 100:0 to 0:100, 400 mL for each step) to obtain nine fractions (DCC9-1–DCC9-9) by MPLC. DCC9-7 (130 mg) was further purified by preparative HPLC (MeCN-H₂O, 30:70 to 60:40) to afford compounds **3** (t_R = 18.9 min, 6 mg) and **5** (t_R = 23.1 min, 4 mg). DCC9-9 (90 mg) was further purified by preparative HPLC (MeCN-H₂O, 30:70 to 60:40) to afford compound **4** (t_R = 21.9 min, 5 mg).

3.5. Characterization of Compounds 1–5

Dianthiamide A (**1**, cyclo-(Gly¹-L-Phe²-L-Leu³-L-*trans*-Pro^a₄-L-*cis*-Pro^b₅-L-Ile⁶-L-Asn⁷)), Yellow amorphous powder; $[\alpha]_D^{25}$ -41.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.65) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$) 201 (−10.9), 210 (−6.5), 219 (−7.8) nm; IR ν_{max} (film) 3330, 2944, 1657, 1530, 1454 cm^{−1}; ¹H NMR (700 MHz, DMSO-*d*₆) and ¹³C NMR (175 MHz, DMSO-*d*₆), see Table 1; ESI-MS m/z 739 [M + H]⁺; HRESI-TOF-MS m/z 739.4144 [M + H]⁺ (calcd for C₃₇H₅₅N₈O₈, 739.4137).

Dianthiamide B (**2**, cyclo-(Gly¹-L-Leu²-L-*trans*-Pro³-L-Phe⁴-L-Asp⁵-L-Ile⁶)), Yellow amorphous powder; $[\alpha]_D^{25}$ -20.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (3.70) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$) 201 (−3.2), 205 (−1.5), 217 (−4.2) nm; IR ν_{max} (film) 3312, 2972, 1644, 1530, 1448 cm^{−1}; ¹H NMR (700 MHz, DMSO-*d*₆) and ¹³C NMR (175 MHz, DMSO-*d*₆), see Table 2; ESI-MS m/z 647 [M + Na]⁺; HRESI-TOF-MS m/z 647.3161 [M + Na]⁺ (calcd for C₃₂H₄₄N₆NaO₇, 647.3163).

Dianthiamide C (**3**, cyclo-(Gly¹-L-Leu²-L-Ser³-L-*trans*-Pro⁴-L-Phe⁵-L-Ile⁶-L-Leu⁷)), Yellow amorphous powder; $[\alpha]_D^{25}$ -45.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (3.24) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$) 207 (+1.4), 226 (−8.6) nm; IR ν_{max} (film) 3311, 2944, 1644, 1530, 1462 cm^{−1}; ¹H NMR (700 MHz, DMSO-*d*₆) and ¹³C NMR (175 MHz, DMSO-*d*₆), see Table 3; ESI-MS m/z 750 [M + Na]⁺; HRESI-TOF-MS m/z 750.4165 [M + Na]⁺ (calcd for C₃₇H₅₇N₇NaO₈, 750.4160).

Dianthiamide D (**4**, cyclo-(Gly¹-L-Ala²-L-Val^a₃-L-Ile⁴-L-*trans*-Pro^a₅-L-Val^b₆-L-Val^c₇-L-*trans*-Pro^b₈)), Yellow amorphous powder; $[\alpha]_D^{25}$ -37.8 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.44) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$) 201 (+9.1), 219 (−7.1) nm; IR ν_{max} (film)

3312, 2943, 1644, 1548, 1454 cm^{-1} ; ^1H NMR (700 MHz, $\text{DMSO-}d_6$) and ^{13}C NMR (175 MHz, $\text{DMSO-}d_6$), see Table 4; ESI-MS m/z 755 $[\text{M} + \text{Na}]^+$; HRESI-TOF-MS m/z 755.4448 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{36}\text{H}_{60}\text{N}_8\text{NaO}_8$, 755.4426).

Dianthiamide E (**5**, cyclo-(Gly¹-L-Asn²-L-*trans*-Pro³-L-Leu⁴-L-Thr⁵-L-Ile⁶-L-Phe⁷)), Yellow amorphous powder; $[\alpha]_D^{25}$ -41.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (3.47) nm; ECD (MeOH) λ_{max} ($\Delta\epsilon$) 201 (-5.8) nm; IR ν_{max} (film) 3309, 2942, 1741, 1644, 1548 cm^{-1} ; ^1H NMR (700 MHz, $\text{DMSO-}d_6$) and ^{13}C NMR (175 MHz, $\text{DMSO-}d_6$), see Table 5; ESI-MS m/z 743 $[\text{M} + \text{H}]^+$; HRESI-TOF-MS m/z 743.4097 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{36}\text{H}_{55}\text{N}_8\text{O}_9$, 743.4086).

3.6. Absolute Configuration of Amino Acids in 1–5 Using Marfey's Method

Compounds **1–5** (0.5 mg) were hydrolyzed in 1 mL of 6 N HCl at 105 °C for 12 h. After cooling to room temperature, the hydrolysate was evaporated to dryness and redissolved in 200 μL of water and 1 M NaHCO_3 (20 μL). A solution of *N*- α -(2,4-dinitro-5-fluorophenyl)-L-alaninamide (L-FDAA, Marfey's reagent, Sigma, 100 μL , 1%) in acetone was added to each reaction vial. The reaction mixture was heated at 37 °C for 1 h, quenched by adding 1 N HCl (20 μL), and then dissolved in CH_3CN (800 μL). A volume of 5 μL of the FDAA derivatives were analyzed by LC/MS (YMC UltraHT Pro C₁₈, S-2 μm , 12 nm, 50 \times 2.0 mm, flow rate: 0.2 mL/min) at RT, and monitored by UV absorption at 340 nm. Aqueous CH_3CN containing 0.1% TFA was used as the mobile phase in a gradient mode (10–50% CH_3CN for 0–40 min). From each standard, 50 mM aqueous solution of D- or L-amino acid (Ala, Asp, Phe, Ile, *allo*-Ile, Leu, Asn, Pro, Ser, Thr, *allo*-Thr, and Val) were taken, and 1 M NaHCO_3 (20 μL) and a solution of *N*- α -(2,4-dinitro-5-fluorophenyl)-L-alaninamide (L-FDAA, Marfey's reagent, Sigma, 100 μL , 1%) in acetone was added. The reaction mixture was heated at 37 °C for 1 h, quenched by adding 1 N HCl (20 μL), and then dissolved in CH_3CN (800 μL). A volume of 5 μL of the FDAA derivatives were analyzed by LC/MS (YMC UltraHT Pro C₁₈, S-2 μm , 12 nm, 50 \times 2.0 mm, flow rate: 0.2 mL/min) at RT and monitored by UV absorption at 340 nm. Aqueous CH_3CN containing 0.1% TFA was used as the mobile phase in a gradient mode (10–50% CH_3CN for 0–40 min). The following retention times (min) were observed for the L-FDAA derivatives of the standards, respectively: 19.5 (L-Ala) and 22.7 (D-Ala), 15.0 (L-Asp) and 18.1 (D-Asp), 29.7 (L-Phe) and 33.4 (D-Phe), 29.5 (L-Leu) and 34.4 (D-Leu), 30.3 (L-Ile), 31.0 (L-*allo*-Ile) and 35.2 (D-Ile), 20.8 (L-Pro) and 22.1 (D-Pro), 13.6 (L-Ser) and 14.5 (D-Ser), 14.7 (L-Thr), 15.2 (L-*allo*-Thr) and 18.2 (D-Thr), and 25.4 (L-Val) and 29.6 (D-Val).

3.7. Cytotoxicity Assay

Human non-small cell lung cancer A549 and human stomach adenocarcinoma MKN-28 cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). A549 and MKN-28 cells were cultured as monolayers in RPMI1640 medium containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin at 37 °C in a humidified atmosphere of 5% CO_2 . Growth-inhibitory effect of the isolated compounds on A549 cells and MKN-28 cells was evaluated using MTT assay [28]. Briefly, 5×10^3 cells of A549 cells or MKN-28 cells were seeded in each well of a 96-well plate, respectively, and incubated for 24 h. A549 cells or MKN-28 cells were then treated with various concentrations of compounds **1–5**. The concentration range of the compound tested for the evaluation of the cytotoxic activity was 5–200 μM . After incubation of 48 h, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to each well and the plate was incubated for 4 h. The medium in each well was replaced with dimethyl sulfoxide (DMSO) to dissolve blue formazan crystals. The absorbance at 540 nm was measured using microplate reader (Molecular devices; SpectraMax, CA, USA). All data processing and IC_{50} values were analyzed using GraphPad Prism v.5 (GraphPad Software, La Jolla, CA, USA). Docetaxel was used as a positive control with an IC_{50} value of 0.08 μM on A549 cells [29].

4. Conclusions

We report the isolation and structure determination of five undescribed orbitides, dianthiamides A–E, from the whole plants of *D. chinensis*. The previously reported orbitides isolated from the genus *Dianthus* tend to have five to six amino acid residues [4–9]. All orbitides in this study, however, are characteristic of the presence of six to eight amino acids, while they are featured by containing at least one proline residue. All isolates were tested for their cytotoxic activity, and dianthiamide A (**1**) exhibited weak activity against the A549 cell line. Furthermore, from a chemotaxonomical point of view, it is noteworthy that this finding expands the orbitides diversity in the genus *Dianthus*.

Supplementary Materials: The following are available online, Figures S1–S50: ^1H , ^{13}C -NMR, COSY, HSQC, HMBC, ROESY, ECD, HRESI-MS, ESI-MS/MS spectra, and Marfey's analysis data of new compounds 1–5.

Author Contributions: J.W.L., J.G.K., J.S.H., Y.B.C., and Y.J.L. performed the experiments; D.H.S. and J.T.H. helped with data analysis; D.L. and M.K.L. helped with structure elucidation. J.W.L. and B.Y.H. designed the study and revised the manuscript; All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MIST) (No. 2020R1A2C1008406 and the Medical Research Center Program 2017R1A5A2015541).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data is contained within the article or Supplementary Material.

Acknowledgments: The authors wish to thank the Korea Basic Science Institute for the NMR spec.

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

Sample Availability: Samples of the compounds are not available from the authors.

References

1. Oshima, Y.; Ohsawa, T.; Oikawa, K.; Konno, C.; Hikino, H. Structures of dianosides A and B, analgesic principles of *Dianthus superbus* var. *longicalycinus* herbs. *Planta Med.* **1984**, *50*, 40–43. [[CrossRef](#)]
2. Oshima, Y.; Ohsawa, T.; Hikino, H. Structure of dianosides C, D, E and F, triterpenoid saponins of *Dianthus superbus* var. *longicalycinus* herb. *Planta Med.* **1984**, *50*, 43–47. [[CrossRef](#)] [[PubMed](#)]
3. Oshima, Y.; Ohsawa, T.; Hikino, H. Structures of dianosides G, H and I, triterpenoid saponins of *Dianthus superbus* var. *longicalycinus* herbs. *Planta Med.* **1984**, *50*, 254–258. [[CrossRef](#)] [[PubMed](#)]
4. Wang, Y.C.; Tan, N.H.; Zhou, J.; Wu, H.M. Cyclopeptides from *Dianthus superbus*. *Phytochemistry* **1998**, *49*, 1453–1456. [[CrossRef](#)]
5. Hsieh, P.W.; Chang, F.R.; Wu, C.C.; Wu, K.Y.; Li, C.M.; Chen, S.L.; Wu, Y.C. New cytotoxic cyclic peptides and dianthramide from *Dianthus superbus*. *J. Nat. Prod.* **2004**, *67*, 1522–1527. [[CrossRef](#)] [[PubMed](#)]
6. Hsieh, P.W.; Chang, F.R.; Wu, C.C.; Li, C.M.; Wu, K.Y.; Chen, S.L.; Yen, H.F.; Wu, Y.C. Longicalycinin A, a new cytotoxic cyclic peptide from *Dianthus superbus* var. *longicalycinus* (MAXIM.) WILL. *Chem. Pharm. Bull.* **2005**, *53*, 336–338. [[CrossRef](#)] [[PubMed](#)]
7. Li, F.; Zhang, F.M.; Yang, Y.B.; Yang, X.Q.; Cao, Q.E.; Ding, Z.T. A new cyclopeptide from *Dianthus caryophyllus*. *Chin. Chem. Lett.* **2008**, *19*, 193–195. [[CrossRef](#)]
8. Tong, Y.; Luo, J.G.; Wang, R.; Wang, X.B.; Kong, L.Y. New cyclic peptides with osteoblastic proliferative activity from *Dianthus superbus*. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1908–1911. [[CrossRef](#)] [[PubMed](#)]
9. Han, J.; Huang, M.; Wang, Z.; Zheng, Y.; Zeng, G.; He, W.; Tan, N. Cyclopentapeptides from *Dianthus chinensis*. *J. Pept. Sci.* **2015**, *21*, 550–553. [[CrossRef](#)] [[PubMed](#)]
10. Ponchet, M.; Martin-Tanguy, J.; Marais, A.; Poupet, A. Dianthramides A and B, two N-benzoylanthranilic acid derivatives from elicited tissues of *Dianthus caryophyllus*. *Phytochemistry* **1984**, *23*, 1901–1903. [[CrossRef](#)]
11. Ponchet, M.; Favre-Bonvin, J.; Hauteville, M.; Ricci, P. Dianthramides (N-benzoyl and N-paracoumarylanthranilic acid derivatives) from elicited tissues of *Dianthus caryophyllus*. *Phytochemistry* **1988**, *27*, 725–730. [[CrossRef](#)]
12. Luo, J.G.; Chen, X.; Kong, L.Y. Three new triterpenoid saponins from *Dianthus superbus*. *Chem. Pharm. Bull.* **2011**, *59*, 518–521. [[CrossRef](#)] [[PubMed](#)]
13. Nakano, T.; Sugimoto, S.; Matsunami, K.; Otsuka, H. Dianthosaponins A-F, triterpene saponins, flavonoid glycoside, aromatic amide glucoside and γ -pyrone glucoside from *Dianthus japonicus*. *Chem. Pharm. Bull.* **2011**, *59*, 1141–1148. [[CrossRef](#)] [[PubMed](#)]

14. Terahara, N.; Yamaguchi, M.A. ¹H NMR spectral analysis of the malylated anthocyanins from *Dianthus*. *Phytochemistry* **1986**, *25*, 2906–2907. [[CrossRef](#)]
15. Shimizu, M.; Hayashi, T.; Shimizu, K.; Morita, N. A pyran-type glycoside from *Dianthus superbus* var. *longicalycinus*. *Phytochemistry* **1982**, *21*, 245–247. [[CrossRef](#)]
16. Arnison, P.G.; Bibb, M.J.; Bierbaum, G.; Bowers, A.A.; Bugni, T.S.; Bulaj, G.; Camarero, J.A.; Campopiano, D.J.; Challis, G.L.; Clardy, J.; et al. Ribosomally synthesized and post-translationally modified peptide natural products: Overview and recommendations for a universal nomenclature. *Nat. Prod. Rep.* **2013**, *30*, 108–160. [[CrossRef](#)] [[PubMed](#)]
17. Ramalho, S.D.; Pinto, M.E.F.; Ferreira, D.; Bolzani, V.S. Biologically active orbitides from the Euphorbiaceae family. *Planta Med.* **2018**, *84*, 558–567. [[CrossRef](#)] [[PubMed](#)]
18. Houshdar Tehrani, M.H.; Gholibeikian, M.; Bamoniri, A.; Mirjalili, B.B.F. Cancer treatment by Caryophyllaceae-type cyclopeptides. *Front. Endocrinol.* **2021**, *11*, 600856. [[CrossRef](#)]
19. Rubin, G.M.; Ding, Y. Recent advances in the biosynthesis of RiPPs from multicore-containing precursor peptides. *J. Ind. Microbiol. Biotechnol.* **2020**, *47*, 659–674. [[CrossRef](#)]
20. Gholibeikian, M.; Bamoniri, A.; Houshdar Tehrani, M.H.; Fatemeh Mirjalili, B.B.; Bijanzadeh, H.R. Structure-activity relationship studies of longicalcynin A analogues, as anticancer cyclopeptides. *Chem. Biol. Interact.* **2020**, *315*, 108902. [[CrossRef](#)]
21. Siemion, I.Z.; Wieland, T.; Pook, K.H. Influence of the distance of the proline carbonyl from the β and γ carbon on the ¹³C chemical shifts. *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 702–703. [[CrossRef](#)]
22. Schmidt, G.; Grube, A.; Kock, M. Stylissamides A-D-New proline-containing cyclic heptapeptides from the marine sponge *Stylissa caribica*. *Eur. J. Org. Chem.* **2007**, *24*, 4103–4110. [[CrossRef](#)]
23. Iwasaki, K.; Iwasaki, A.; Sumimoto, S.; Sano, T.; Hitomi, Y.; Ohno, O.; Suenaga, K. Croissamide, a proline-rich cyclic peptide with an N-prenylated tryptophan from a marine cyanobacterium *Symploca* sp. *Tetrahedron Lett.* **2018**, *59*, 3806–3809. [[CrossRef](#)]
24. Zhan, K.X.; Jiao, W.H.; Yang, F.; Li, J.; Wang, S.P.; Li, Y.S.; Han, B.N.; Lin, H.W. Reniochalistatins A-E, cyclic peptides from the marine sponge *Reniochalina stalagmitis*. *J. Nat. Prod.* **2014**, *77*, 2678–2684. [[CrossRef](#)] [[PubMed](#)]
25. Cândido-Bacani, P.D.M.; Figueiredo, P.D.O.; Matos, M.D.F.; Garcez, F.R.; Garcez, W.S. Cytotoxic orbitide from the latex of *Croton urucurana*. *J. Nat. Prod.* **2015**, *78*, 2754–2760. [[CrossRef](#)] [[PubMed](#)]
26. Fukuda, T.; Sudoh, Y.; Tsuchiya, Y.; Okuda, T.; Matsuura, N.; Motojima, A.; Oikawa, T.; Igarashi, Y. Hikiamides A-C, cyclic pentadepsipeptides from *Fusarium* sp. TAMA 456. *J. Nat. Prod.* **2015**, *78*, 797–802. [[CrossRef](#)] [[PubMed](#)]
27. Igarashi, Y.; Yamamoto, K.; Fukuda, T.; Shojima, A.; Nakayama, J.; Carro, L.; Trujillo, M.E. Arthroamide, a cyclic depsipeptide with quorum sensing inhibitory activity from *Arthrobacter* sp. *J. Nat. Prod.* **2015**, *78*, 2827–2831. [[CrossRef](#)] [[PubMed](#)]
28. Jin, Q.; Lee, J.W.; Jang, H.; Lee, H.L.; Kim, J.G.; Wu, W.; Lee, D.; Kim, E.H.; Kim, Y.; Hong, J.T.; et al. Dimeric- and trimeric sesquiterpenes from the flower of *Inula japonica*. *Phytochemistry* **2018**, *155*, 107–113. [[CrossRef](#)] [[PubMed](#)]
29. Jo, M.J.; Lee, Y.J.; Park, C.W.; Chung, Y.B.; Kim, J.S.; Lee, M.K.; Shin, D.H. Evaluation of the physicochemical properties, pharmacokinetics, and in vitro anticancer effects of docetaxel and osthol encapsulated in methoxy poly(ethylene glycol)-*b*-poly(caprolactone) polymeric micelles. *Int. J. Mol. Sci.* **2020**, *22*, 231. [[CrossRef](#)] [[PubMed](#)]