New Cytotoxic Cyclic Peptides and Dianthramide from *Dianthus superbus*

Pei-Wen Hsieh,[†] Fang-Rong Chang,[†] Ching-Chung Wu,[†] Kuen-Yuh Wu,[‡] Chien-Ming Li,[‡] Su-Li Chen,[†] and Yang-Chang Wu*,[†]

Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung 807, Taiwan, and Division of Environmental Health and Occupational Medicine, National Health Research Institute, Kaohsiung 807, Taiwan, Republic of China

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Four new cyclic peptides, dianthins C−F (**1**−**4**), and a new dianthramide, 4-methoxydianthramide B (**5**), were isolated from the MeOH extract of the traditional Chinese medicinal plant *Dianthus superbus*. The sequences of cyclic peptides **1**−**4** were elucidated as cyclo(Gly[1]-Pro[2]-Phe[3]-Tyr[4]-Val[5]-Ile[6]-), cyclo(Gly[1]-Ser[2]-Leu[3]-Pro[4]-Pro[5]-Ile[6]-Phe[7]-), cyclo(Gly[1]-Pro[2]-Ile[3]-Ser[4]-Phe[5]-Val[6]-), and cyclo(Gly[1]-Pro[2]-Phe[3]-Val[4]-Phe[5]-) on the basis of ESI tandem mass fragmentation analysis, chemical evidence, and extensive 2D NMR methods. The conformation of compound **1** was established as an α-helix by CD analysis. Furthermore, compounds **3** and **5** showed cytotoxicities toward the Hep G2 cancer cell line with IC_{50} values of 2.37 and 4.08, respectively.

The genus *Dianthus* includes over 300 species, which are mainly distributed in temperate and cold regions of the northern hemisphere. Two species (*D. palinensis* and *D. pygmaeus*) and two varieties (*D. superbus* var. *longicalysinus* and *D. superbus* var. *taiwanensis*) grow in Taiwan.[1] In previous studies, triterpene saponins,[2,3] cyclic peptides,[4] dianthramides,[5,6] dihydropyrans,[7,8] and anthocyanins[9] were isolated from this genus. The species *D. superbus* is used as a traditional Chinese diuretic and in the treatment of urethritis, carbuncles, and carcinoma.[4] This material was imported from mainland China and used as an ingredient of a complex mixture. As part of our research for bioactive constituents of Caryophyllaceae, we previously investigated the extract of *Drymaria diandra* and isolated four new cyclic peptides, diandrine A−D. Diandrine A exhibited selective inhibition against collagen-induced platelet aggregation.[10] In a continuing investigation of the plants of this family, four new cyclic peptides, dianthin C−F (**1**−**4**), a new dianthramide, 4-methoxydianthramide B (**5**), along with a known compound, aurantiamide benzoate, were isolated from *D. superbus*. The isolation, structural elucidation, and cytotoxicities of these new compounds are reported herein.

Results and Discussion

The methanolic extracts of *D. superbus* were partitioned between *n*-hexane and 80% aqueous MeOH, and the latter extract was separated to yield five new compounds (Figure 1), dianthins C−F (**1**−**4**) and 4-methoxydianthramide B (**5**), together with a known compound, aurantiamide benzoate. The known compound was identified by comparison of spectroscopic data with those reported.[11]

Dianthin C (**1**) was obtained as a pale yellow powder. The NMR spectra of **1** (Table 1) showed five amide N−H signals [δ 7.56 (s), 8.96 (d), 9.16 (t), 9.40 (d), 10.05 (d)] and six carbonyls (δ 173.1, 172.4, 172.1, 171.4, 170.9, and 170.9), which indicated that **1** might belong to the peptide class of compounds. A negative ninhydrin test indicated its cyclic nature. Absorptions at 3304, 1642, and 1516 cm^{-1} in the IR were characteristic of amide, carbonyl, and aromatic functions, respectively. The molecular weight of 676 was obtained from the ESI mass spectrum, which showed the protonated molecular ion $[M + H]^+$ at *m*/*z* 677 and the sodium adduct at *m*/*z* 699, respectively. On the basis of HRFABMS data, the molecular formula was defined as $C_{36}H_{48}N_6O_7$. Analysis of 2D NMR data (HMBC,

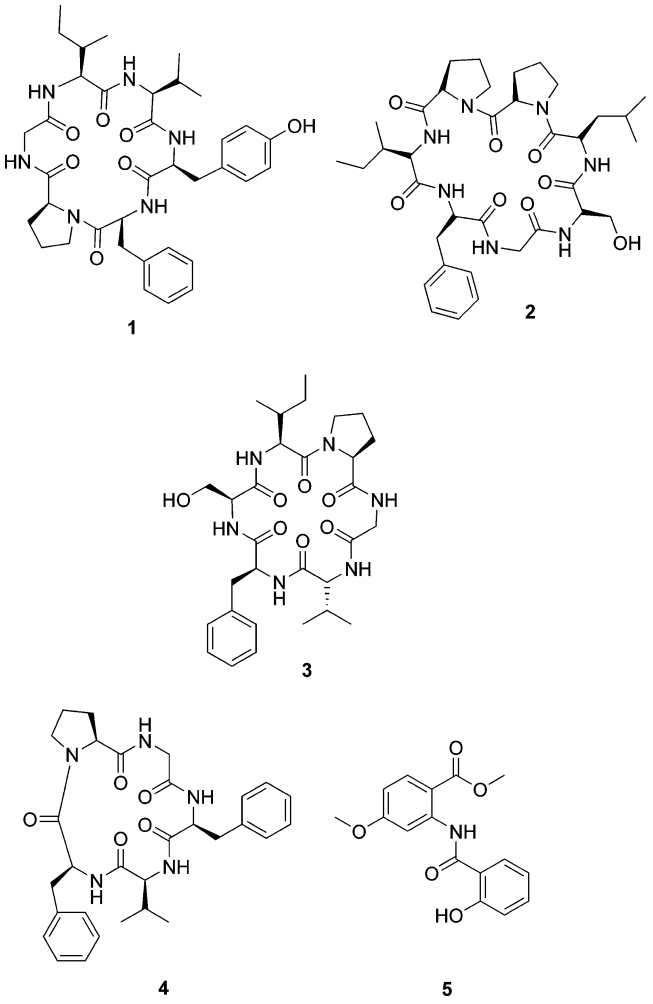

Figure 1. Structures of dianthins C−F (**1**−**4**) and 4-methoxydianthramide B (**5**).

* To whom correspondence should be addressed. Phone: 886-7-3121101, ext. 2197. Fax: 886-7-3114773. E-mail: yachwu@kmu.edu.tw.
† Kaohsiung Medical University.
‡ National Health Research Institute.

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1** in C_5D_5N[a]

		δ_H (mult., *J* in Hz)	δ_C (mult.)	ROESY (δ_H)
Gly^1	C=O		170.9 (s)	
	NH	9.16 (t, 5.2)		3.44, 4.68, 5.30
	α	3.44 (ddd, 14.0, 5.2, 3.6)	44.3 (t)	9.16
		4.68 (dd, 14.0, 7.2)		8.96, 9.16
Pro^2	C=O		172.4 (s)	
	α	3.91 (d, 7.6)	61.8 (d)	6.89, 9.40
	β	2.22 (dd, 11.6, 6.0)	29.9 (t)	
		1.00 (m)		
	γ	1.22 (m)	21.7 (t)	
	δ	3.32 (m)	46.6 (t)	
		3.55 (m)		5.30
Phe^3	C=O		171.4 (s)	
	NH	9.40 (d, 8.4)		3.33, 3.91, 4.74, 5.30
	α	5.30 (m)	55.4 (d)	3.55, 9.16, 9.40
	β	3.33 (dd, 11.8, 3.4)	38.3 (t)	
		3.73 (dd, 11.8, 5.2)		
	Ar	7.10−7.36 (m)	138.9 (s)	
			129.6 (d)	
			128.7 (d)	
			126.7 (d)	
Tyr^4	C=O		170.9 (s)	
	NH	10.05 (d, 2.0)		3.43, 4.74, 4.91, 7.57
	α	4.74 (m)	55.0 (d)	6.89, 9.40, 10.05
	β	3.01 (dd, 12.4, 10.3)	37.6 (t)	6.89
		3.43 (t, 12.4)		6.89, 10.05
	Ar		126.1 (s)	
		6.98 (d, 8.4)	131.1 (d)	4.91
		6.89 (d, 8.4)	116.4 (d)	3.01, 3.43, 3.91, 4.74
			158.1 (s)	
Val^5	C=O		173.1 (s)	
	NH	7.57 (d, 8.8)		4.60, 4.91, 10.05
	α	4.91 (t, 8.8)	58.3 (d)	2.40, 7.57, 10.05
	β	2.40 (sextet, 5.6)	31.6 (d)	1.09, 1.24, 4.91
	γ	1.09 (d, 6.8)	19.1 (q)	2.40
		1.24 (d, 6.8)	19.2 (q)	2.40
Ile^6	C=O		172.1 (s)	
	NH	8.96 (d, 6.8)		1.12, 2.10, 4.60, 4.68
	α	4.60 (t, 6.8)	61.4 (d)	1.12, 2.10, 7.57, 8.96
	β	2.10 (m)	36.3 (d)	
	γ	2.29 (t, 7.6)	25.5 (t)	
		1.12 (d, 6.8)	16.1 (q)	4.60, 8.96
	δ	0.84 (t, 7.6)	11.4 (q)	4.60

[a] All assignments were confirmed by DEPT, HMQC, and HMBC.

TOCSY, and ROESY) and ESIMS^3 data demonstrated that the amino acid residues are Gly, Pro, Val, Try, Ile, and Phe. The sequence of the amino acid residues was deduced from HMBC and ROESY data. The connectivity was further confirmed by ESIMS^3 analysis (Figure 2). As shown, the collisional induced decomposition (CID) experiment on the $[M + H]^+$ ion of **1** gave preferential ring opening at the Tyr^4−Phe^3 amide bond and gave relative B ions (a peptide fragmented at a single peptide bond retaining the positive charge at the N-terminus) of peptide fragments. The ion at *m/z* 514 could be attributed to Val^5-Ile^6-Gly^1-Pro^2-Phe^3 and was followed by the subsequent losses of Val^5, Ile^6, and Gly^1. A second series of main peaks at *m/z* 579, 564, and 550 were assigned to Phe^3-Tyr^4-Val^5-Ile^6-Gly^1. Thus the structure of **1** was established as cyclo(Gly^1-Pro^2-Phe^3-Tyr^4-Val^5-Ile^6). Finally, the configuration of each amino acid residue was assigned as L, which was deduced by acid hydrolysis and Marfey's analysis of the individual amino acids.[12,13] The ROESY cross-peaks (Phe^3-Hα/Pro^2-Hδ) and the difference of ^{13}C NMR chemical shifts of Pro^2 ($\Delta\delta_{C\beta-C\gamma}$ = 8.6 ppm) provided evidence that the amide bond in the Pro residue is *cis*.[14,15]

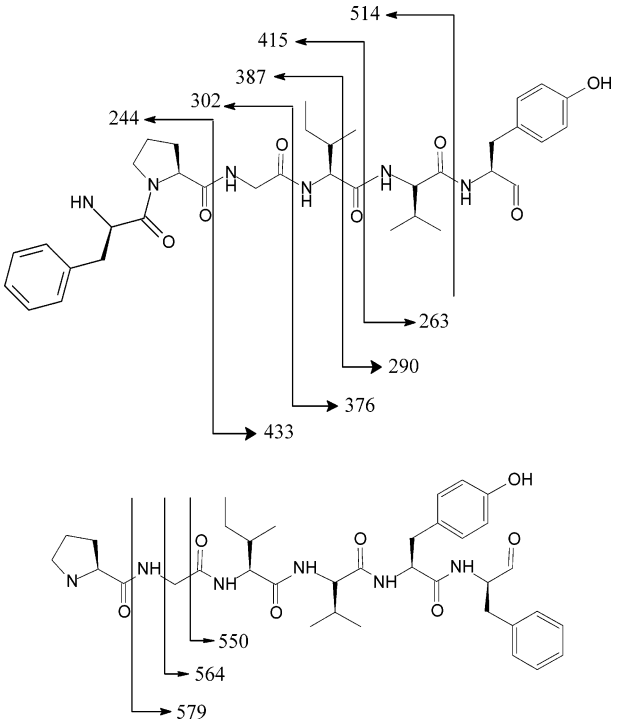

Figure 2. Dianthin C (**1**) ESIMS^3 fragment analysis.

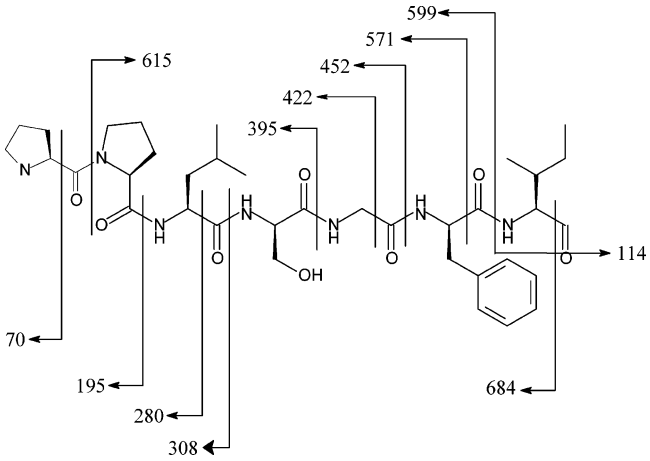

Figure 3. Dianthin D (**2**) ESIMS^3 fragment analysis.

Dianthin D (**2**) was obtained as a pale yellow powder. The molecular weight of 711 for **2** was obtained from the ESI mass spectrum, which showed the protonated molecular ion $[M + H]^+$ at *m/z* 712 and the sodium adduct ion at *m/z* 734. IR absorptions at 3391, 1636, and 1602 cm^{-1} were assigned to amide, carbonyl, and aromatic functions, respectively. The ^{1}H and ^{13}C NMR spectra of **2** showed five amide N−H signals at δ 10.37 (t), 8.83 (d), 8.19 (d), 8.06 (d), and 7.49 (d) and seven carbonyls (δ 172.3, 171.9, 171.8, 171.7, 171.4, 170.1, and 169.5), which indicated that it might be a heptapeptide. The molecular formula, $C_{36}H_{53}N_7O_8$, was determined by HRFABMS ($[M + H]^+$ at *m/z* 712.4045, calcd 712.4034). All amino acid residues and the peptide sequence were deduced from the ESIMS^3 (Figure 3) and ^{13}C NMR data as Gly, Ser, Leu, Pro × 2, Ile, and Phe. The CID experiment on the $[M + H]^+$ ion of **2** afforded preferential ring opening at the Ile^6−Pro^5 amide bond and gave relative B ions of the peptide fragments. The A ion fragment at *m/z* 684 could be attributed to $[M + H - C{=}O]^+$, followed by the subsequent losses of Ile^6, Phe^7,

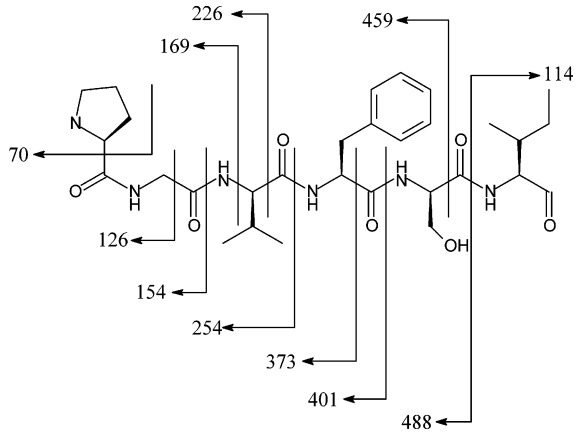

Figure 4. Dianthin E (**3**) ESIMS^3 fragment analysis.

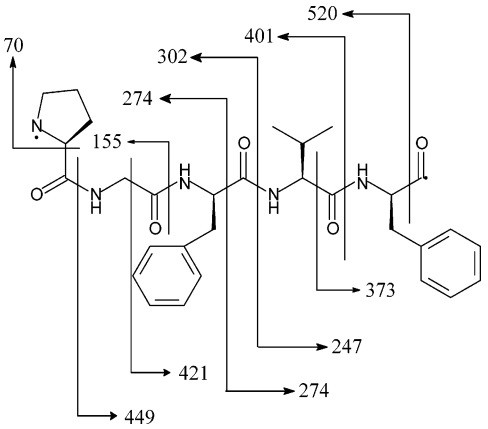

Figure 5. Dianthin F (**4**) ESIMS^3 fragment analysis.

Gly^1, Ser^2, and Leu^3, which displayed the corresponding ions at *m/z* 599, 452, 395, 308, and 195. Furthermore, the main peak at *m/z* 615 was assigned to Ile^6-Phe^7-Gly^1-Ser^2-Leu^3-Pro^4, and thus the structure of **2** was established as cyclo(Gly^1-Ser^2-Leu^3-Pro^4-Pro^5-Ile^6-Phe^7). The ^{13}C NMR chemical shifts of Pro^{4}Cγ and Pro^{5}Cγ were at δ 25.2 and 25.7, respectively, which suggested the *trans* geometry of the amide bonds in both Pro residues in **2**.[14,15] Finally, the configuration of each amino acid residue in **2** was assigned as L by Marfey's analysis.[12,13]

Dianthin E (**3**) was obtained as a pale yellow powder. The IR absorptions at 3300, 1642, and 1527 cm^{-1} were assigned to amide, carbonyl, and aromatic functions, respectively. The ^{1}H and ^{13}C NMR spectra of **3** showed five amide signals, δ 8.97 (d), 8.92 (d), 8.99 (d), 8.68 (br d), and 8.26 (br s), together with six carbonyls (δ 173.2, 172.5, 171.9, 171.8, 171.2, and 168.6), which indicated that it might be a hexapeptide. The ESI mass spectrum showed the pseudomolecular ion at *m/z* 601 and the [M + Na]$^+$ ion at *m/z* 623, respectively, and HRFABMS gave a pseudomolecular ion peak at *m/z* 601.3340 (calcd for $C_{30}H_{45}N_6O_7$, 601.3349), which confirmed the molecular formula as $C_{30}H_{44}N_6O_7$. The amino acid residues and sequence of the peptide were deduced from the ESIMS^3 experiments (Figure 4) and ^{13}C NMR data as Gly, Val, Ser, Pro, Ile, and Phe. The CID experiment on the [M + H]$^+$ ion of **3** provided preferential ring opening at the Ile^5−Pro^6 amide bond and gave relative B ions of peptide fragments. The fragment ion at *m/z* 488 could be attributed to Pro^6-Gly^1-Val^2-Phe^3-Ser^4, subsequent losses of Ser^4, Phe^3, and Val^2 corresponding to ion peaks at *m/z* 401, 254, and 154. Therefore, the structure of **3** was elucidated as cyclo(Gly^1-Pro^2-Ile^3-Ser^4-Phe^5-Val^6-). The amide bond in the Pro residue in **3** has *trans* geometry, according to the ^{13}C NMR chemical shift of Pro^{6}Cγ.[14,15] Finally, each amino acid residue configuration of **3** has the L configuration on the basis of the Marfey's analysis.[12,13]

Dianthin F (**4**) was obtained as a pale yellow powder. As with compounds **1**−**3**, the IR spectrum of **4** displayed amide, carbonyl, and aromatic function absorptions at 3362, 1668, 1647, and 1538 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR spectra of **4** showed five amide signals, together with five carbonyls (see Experimental Section), which suggested a heptapeptide. The ESI mass spectrum showed the pseudomolecular ion at *m/z* 548 and the [M + Na]$^+$ ion at *m/z* 570, respectively, which indicated that the molecular formula is $C_{30}H_{37}N_5O_5$. The CID experiment on the [M + H]$^+$ ion of **4** provided the preferential ring opening at the Phe^3−Pro^2 amide bond and gave relative B ions of peptide fragments. The fragments were assigned as the subsequent losses of Phe^3, Val^4, and Phe^5, corresponding to the ion peaks at *m/z* 401, 302, and 155 (Figure 5). Therefore, the structure of **4** was elucidated as cyclo(Gly^1-Pro^2-Phe^3-Val^4-Phe^5). The amide bond in the Pro residue in **4** was assigned the *cis* geometry by the ^{13}C NMR chemical shift of Pro^{6}Cγ.[14,15] Moreover, each amino acid residue configuration of **4** was assigned as L on the basis of the Marfey's analysis.[12,13]

Establishing the peptide secondary structure/conformation was important to understand the bioactivity of the dianthins, and techniques such as IR, CD (circular dichroism), and NMR were used to resolve this question.[16] CD spectroscopy is widely used for the structural determination of peptides because of its sensitivity to conformations.[17] In general, an α-helix gives rise to two negative bands at 222 and 208 nm of almost equal intensity and a strong positive band at ca. 192 nm.[18,19] The spectrum for a β-sheet shows a negative Cotton effect near 217 nm and a positive maximum near 198 nm.[20] Furthermore, the spectrum for a β-turn exhibits negative Cotton effects near 195 and 208 (sh) nm and positive ones near 230 and 270 nm.[20] The CD spectrum of **1** exhibited negative Cotton effects at ca. 216 and 208 nm and a positive deflection near 195 nm, which implied an α-helix in **1**. Furthermore, when the pH values were changed (pH 2.0 and pH 9.0, respectively), the shapes of the spectra were similar. The results indicated that conformation of **1** is stable between pH 2.0 and 9.0. The CD spectra of compounds **2** and **3** showed negative bands at 220 and 214 nm, and both gave positive maxima below 190 nm that did not match either the α-helix, β-turn, or β-sheet. However, these data suggested that **2** and **3** possibly possess the L_{+2} helix conformation, which exhibits a minimum, a maximum, and a zero crossing at 219, 190, and 207 nm, respectively.[21] Similarly, when the pH was changed, the spectra of compounds **2** and **3** were unchanged. Moreover, the CD spectrum of **4** showed a negative Cotton effect at 206 nm, accompanied by a shoulder at 213 nm that was similar to a right-handed 3_{10}-helix;[22] however, the CD spectrum of **4** did not show the low-amplitude positive Cotton effect at 195 nm. Noteworthily, the CD spectrum showed negative bands at 228 and 206 nm in the pH 9.0 solution, which suggested the conformation of **4** should be an α-helix. Simultaneously, the CD shape was inverted (positive bands at 225 and 204 nm) while the pH was altered to 2.0. The CD variations with negative and positive curves for pH 2.0 and pH 9.0, respectively, at 250 nm may be due to the changes of phenylalanine conformations. However, the detailed mechanism is unclear.

4-Methoxydianthramide B (**5**) was obtained as a white powder. The IR absorptions at 3248, 1612, and 1529 cm^{-1}

suggested amide, carbonyl, and aromatic functions, respectively. The ^{1}H spectrum of **5** showed two aromatic ring systems, an ABX system at δ 9.36 (d), 8.10 (d), and 6.93 (dd), as well as a 1,2-disubstituted benzene ring with peaks at δ 8.41 (dd), 7.43 (t), 7.05 (d), and 7.03 (t). Additionally, two *O*-methyl signals appeared at δ 3.98 and 3.81. EIMS gave [M$^+$] at *m/z* 301. Furthermore, two strong fragment ions at *m/z* 167 and 135 were attributable to two parts derived from methyl 2-amino-4-methoxybenzoate and 2-hydroxybenzamide, respectively. In comparison with the chemical and physical data of dianthramide B,[5] the structure of **5** was established as shown (Figure 1) and named 4-methoxydianthramide B.

The cytotoxicities of compounds **1**, **2**, **3**, and **5** were evaluated against the cell lines of human hepatocellular carcinoma Hep G2 and Hep 3B, human breast carcinoma MCF-7 and MDA-MB-231, and human lung carcinoma A-549. Compounds **3** and **5** showed selective activities to the Hep G2 cancer cell line with IC_{50} values of 2.37 and 4.08 μg/mL, respectively. However, compounds **1**, **2**, **3**, and **5** exhibited no effect on the growth inhibition of the Hep 3B, MCF-7, MDA-MB-231, and A-549 cell lines.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 digital polarimeter. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer, and IR spectra were measured on a Hitachi 260-30 spectrophotometer. CD spectra were measured on a Jasco J-810 spectrometer. NMR (400 and 500 MHz, using C_5D_5N as solvent) spectra were obtained on a Varian NMR spectrometer (Unity Plus 400 and Unity INOVA-500) or a Bruker AMX-400 NMR spectrometer. ESIMS^n was obtained on an API 3000 (Applied Biosystems). Low-resolution EIMS were recorded on a Quattro GC/MS spectrometer having a direct inlet system. High-resolution FABMS were recorded on a Finnigan/Thermo Quest MAT 95XL spectrometer. High-resolution EIMS were collected on a JEOL JMS SX/SX 102A spectrometer. Shimadzu LC-10AT pumps, a SPD-10A UV−Vis detector, and Hypersil ODS 5 μm (250 × 4.6 mm i.d.) and preparative ODS 5 μm (250 × 21.2 mm i.d.) columns were employed for HPLC.

Plant Material. *D. superbus* was purchased from a traditional Chinese medicine store (Yi-Chen Co.) in Kaohsiung in 2001 and identified by Dr. Hsin-Fu Yen (National Museum of Natural Science, Taichung, Taiwan). The samples were authenticated and deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Taiwan (KMU-DS-001).

Extraction and Isolation. The air-dried plant (250 g) of *D. superbus* was extracted with MeOH at room temperature. The MeOH extract (23 g) was partitioned between *n*-hexane−80% MeOH/H_2O to yield *n*-hexane and MeOH extracts. These were evaporated to give dark green viscous residues. The MeOH extract (15 g) was further separated on Sephadex LH-20 with 80% MeOH/H_2O to give eight fractions (A−H). Fraction B (2.3 g) was further separated using a Dianion LH-20 column eluted with a gradient of H_2O, 30% MeOH/H_2O, 50% MeOH/H_2O, 70% MeOH/H_2O, and MeOH (each 1000 mL) to yield five subfractions. The subfraction B-4 was further purified by an RP-18 (LiChroprep, 40−63 mm, Merck) column and HPLC (MeCN/H_2O, 30:70, flow rate 3.6 mL/min, detection at 220 nm) to give **2** (1.2 mg) and **3** (5.6 mg). Fraction C was further separated using a Sephadex LH-20 column to afford five fractions. Subfraction C-3 was purified by preparative reverse-phase HPLC (MeCN/H_2O, 30:70, flow rate 3.6 mL/min, detection at 220 nm) to obtain **4** (1.2 mg). Fraction E (1.2 g) was further separated using an RP-18 column (eluting with H_2O, 90% MeCN/H_2O, 70% MeCN/H_2O, 50% MeCN/H_2O, and 100% MeCN) to give five subfractions. Subfraction E-2 was separated by an RP-18 column and purified by preparative HPLC (MeCN/H_2O, 33:67, flow rate 3.5 mL/min, detection at 210 nm) to yield **1** (8.3 mg). Subfraction E-3 was purified by preparative reverse-phase HPLC (MeCN/H_2O, 36:64, flow rate 3.5 mL/min, detection at 210 nm) to obtain **5** (4.2 mg). Subfraction E-5 was purified by preparative reverse-phase HPLC (MeCN/H_2O, 40:60, flow rate 3.5 mL/min, detection at 210 nm) to yield aurantiamide benzoate (2.1 mg).

Dianthin C (1): pale yellow powder; $[\alpha]^{21}_D$ −50° (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 226 (3.80), 250 (3.41), 255 (3.43), 261 (sh, 3.37), 277 (3.28) nm; CD (*c* 2.7 × 10^{-4} M, MeOH) 260 (−0.49), 255 (−0.16), 216 (−6.92), 208 (−6.85), 194 (4.93) nm; CD (*c* 1.8 × 10^{-4} M, pH 2.0 MeOH/HCl) 262 (−0.18), 256 (0.55), 217 (−4.20), 212 (−4.05), 208 (−4.33), 194 (4.58) nm; CD (*c* 1.8 × 10^{-4} M, pH 9.0 MeOH/20% NaOH) 260 (−0.52), 254 (−0.14), 214 (−3.85), 209 (−3.70), 208 (−7.46), 198 (1.41) nm; IR (KBr) ν_{max} 3304, 2954, 2921, 2849, 1642, 1516, 1457, 1233, 1064, 1030 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (100 MHz, C_5D_5N), see Table 1; ESIMS (full scan) *m/z* 700 (82, [M + Na]$^+$), 678 (100, [M + H]$^+$); ESIMS/MS *m/z* 678 (87, [M + H]$^+$), 650 (46), 632 (20), 579 (24), 564 (21), 550 (22), 514 (80), 486 (17), 465 (13), 434 (10), 415 (85), 387 (75), 319 (36), 302 (100), 270 (24), 263 (11), 245 (26), 217 (30), 70 (11); HRFABMS (postive ion) *m/z* 677.3655 (calcd for $C_{36}H_{49}N_6O_7$, 677.3662).

Dianthin D (2): pale yellow powder; $[\alpha]^{21}_D$ −19.6° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (3.98), 253 (3.66) nm; CD (*c* 1.7 × 10^{-4} M, MeOH) 261 (−0.04), 220 (−1.01), 202.5 (0.01), 192 (1.93) nm; CD (*c* 2.0 × 10^{-4} M, pH 2.0 MeOH/HCl) 260 (−0.14), 256 (0.27), 215 (−6.98), 210 (−7.55), 208 (−7.46) 205 (−7.62) nm; CD (*c* 2.0 × 10^{-4} M, pH 9.0 MeOH/20% NaOH) 258 (−0.46), 254 (0.27), 216 (−7.92, sh), 211 (−8.50), 208 (−8.28), 205 (−8.42) nm; IR (KBr) ν_{max} 3391, 2921, 2852, 1636, 1602, 1442, 1368, 1349, 1029 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) δ 10.37 (1H, t, *J* = 3.2 Hz), 8.83 (1H, d, *J* = 8.0 Hz), 8.54 (1H, d, *J* = 3.2 Hz), 8.19 (1H, d, *J* = 6.4 Hz), 8.06 (1H, d, *J* = 8.8 Hz), 7.49 (1H, d, *J* = 7.6 Hz), 7.36 (2H, d, *J* = 6.8 Hz), 7.25−7.14 (3H, m), 5.24 (1H, m), 4.69 (1H, t, *J* = 8.4 Hz), 4.61 (1H, br. d, *J* = 10.4 Hz), 4.44 (1H, m), 4.11 (1H, dd, *J* = 15.6, 6.0 Hz), 3.96 (1H, m), 3.83 ∼ 3.55 (4H, m), 3.05 (1H, dd, *J* = 12.0, 14.4 Hz), 2.73 (1H, dd, *J* = 11.6, 4.8 Hz), 2.52 (1H, m), 2.32 (1H, m), 2.14 ∼ 1.64 (8H, m), 1.50 (1H, m), 1.30 (1H, m), 1.09 (3H, d, *J* = 6.8 Hz), 0.94 (3H, t, *J* = 7.2 Hz), 0.91 (3H, d, *J* = 2.8 Hz), 0.89 (3H, d, *J* = 2.4 Hz); ^{13}C NMR (100 MHz, C_5D_5N)[23] δ 172.3 (s), 171.9 (s), 171.8 (s), 171.7 (s), 171.4 (s), 170.1 (s), 169.5 (s), 138.3 (Phe-Ar, s), 129.2 (Phe-Ar, d, C × 2), 128.9 (Phe-Ar, d, C × 2), 127.1 (Phe-Ar, s), 64.5 (SerC_β, t), 61.6 (ProC_α, d), 61.4 (ProC_α, d), 60.3 (IleC_α, d), 56.5 (PheC_α, d), 55.0 (SerC_α, d), 50.0 (LeuC_α, d), 47.7 (ProC_δ, t), 46.8 (ProC_δ, t), 44.8 (GlyC_α, t), 41.1 (LeuC_β, t), 38.4 (PheC_β, t), 36.4 (IleC_β, d), 31.7 (ProC_β, t), 29.9 (ProC_β, t), 28.9 (LeuC_γ, t), 26.1 (ProC_γ, t), 25.7 (ProC_γ, t), 25.2 (IleC_γ, t), 23.4 (LeuC_δ, q), 22.3 (LeuC_δ, q), 16.3 (IleC_γ, q), 11.1 (IleC_δ, q); ESIMS (full scan) *m/z* 734 (58, [M + Na]$^+$), 712 (96), 394 (100); ESIMS/MS *m/z* 712 (57, [M + H]$^+$), 684 (100), 615 (10), 599 (62), 571 (74), 502 (20), 452 (21), 422 (8), 395 (28), 323 (27), 308 (77), 280 (27), 212 (35), 195 (19), 183 (29), 86 (46), 70 (10); HRFABMS (postive ion) *m/z* 712.4045 (calcd for $C_{36}H_{54}N_7O_8$, 712.4034).

Dianthin E (3): pale yellow powder; $[\alpha]^{21}_D$ −30.5° (*c* 0.02, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (3.59), 250 (3.29), 255 (3.29), 261 (sh, 3.25), 329 (3.02) nm; CD (*c* 3.8 × 10^{-4} M, MeOH) 261 (0.10), 251 (0.12), 214 (−6.78), 204 (sh, −5.75), 192 (0.15), 190.5 (1.02) nm; CD (*c* 1.2 × 10^{-4} M, pH 2.0 MeOH/HCl) 267 (0.22), 253 (0.08), 222 (−0.96), 210 (−0.52), 204 (−0.63) nm; CD (*c* 1.2 × 10^{-4} M, pH 9.0 MeOH/20% NaOH) 253 (−0.21), 228 (−1.37, sh), 217 (−1.83), 205 (−1.17, sh) nm; IR (KBr) ν_{max} 3300, 2959, 2925, 1642, 1527, 1453, 1055, 1033 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) and ^{13}C NMR (100 MHz, C_5D_5N), see Table 2; ESIMS (full scan) *m/z* 623 (98, [M + Na]$^+$), 601 (100, [M + H]$^+$); ESIMS/MS *m/z* 601 (19, [M + H]$^+$), 583 (33), 573 (27), 555 (24), 538 (27), 488 (13), 484 (43), 469 (15), 459 (9), 442 (30), 425 (18), 401 (34), 387 (44), 373 (47), 330 (19), 313 (33), 302 (18), 254 (37), 226 (43), 197 (17), 169 (100), 155 (21), 126 (3), 70 (5); HRFABMS (postive ion) *m/z* 601.3340 (calcd for $C_{30}H_{45}N_6O_7$, 601.3349).

Dianthin F (4): pale yellow powder; $[\alpha]^{21}_D$ −16.0° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (3.82), 229 (sh, 3.55), 260

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **3** in C_5D_5N[a]

		δ_H (mult., J in Hz)	δ_C (mult.)	ROESY (δ_H)
Gly[1]	C=O		168.6 (s)[b]	
	NH	8.03 (br d, 4.4)		4.52
	α	4.52 (ddd, 14.8, 6.8, 4.4)	43.3 (t)	
		4.82 (br d, 14.8)		4.53, 4.26
Pro[2]	C=O		172.5 (s)[b]	
	α	4.53 (dd, 8.0, 4.4)	62.2 (d)	4.82
	β	2.15 (m)	30.1 (t)	
		1.66 (m)		
	γ	2.06 (m)	25.1 (t)	
	δ	3.19 (m)	46.7 (t)	4.53, 4.26, 1.66
		4.26 (m)		4.82, 3.19, 2.06
Ile[3]	C=O		171.9 (s)[b]	
	NH	9.12 (d, 4.8)		5.03, 4.63
	α	4.32 (dd, 6.4, 4.8)	61.1 (d)	2.03, 1.27, 1.04, 0.61
	β	2.03 (m)	35.6 (d)	
	γ	1.27 (m)	25.0 (t)	
		1.04 (br dd, 6.0, 14.6)		
		0.61 (d, 6.8)	15.4 (q)	
	δ	0.59 (t, 7.2)	11.3 (q)	
Ser[4]	C=O		171.8 (s)[b]	
	NH	8.72 (s)		9.12
	α	5.03 (m)	55.6 (d)	
	β	4.45 (dd, 12.8, 3.6)	62.7 (d)	4.45
		4.63 (dd, 9.2, 3.6)		9.12, 4.63
Phe[5]	C=O		171.2 (s)[b]	
	NH	8.73 (d, 12.0)		
	α	5.33 (td, 12.0, 4.0)	55.6 (d)	3.86
	β	3.34 (dd, 14.0, 4.0)	37.6 (t)	3.86
		3.86 (dd, 14.0, 4.0)		5.33
	Ar	7.10−7.36 (m)	139.1 (s)	
			129.5 (d)	
			128.6 (d)	
			126.6 (d)	
Val[6]	C=O		173.2 (s)[b]	
	NH	8.79 (d, 10.0)		
	α	4.95 (dd, 10.0, 7.0)	58.3 (d)	2.89, 1.11, 1.16
	β	2.89 (sextet, 7.2)	30.6 (d)	8.79
	γ	1.11 (q, 6.8)	18.6 (q)	
		1.16 (d, 6.8)	20.4 (q)	

[a] All assignments were confirmed by DEPT, TOCSY, and HMQC. [b] Assignments may be interchanged.

Table 3. Cytotoxicities of Compounds **1**, **2**, **3**, and **5**

	cancer line (IC_{50}, μg/ml)				
compound	Hep G2	Hep 3B	MCF-7	A-549	MDA-MB-231
1	17.17	>20	>20	>20	>20
2	>20	>20	>20	>20	>20
3	2.37	>20	>20	>20	>20
5	4.08	16.02	>20	>20	>20
doxorubicin	0.19	0.31	1.21	0.19	0.73

(sh, 3.19), 282 (sh, 3.08) nm; CD (*c* 2.7 × 10^{-4} M, MeOH) 240 (0.24), 225 (−0.93), 213 (−3.91), 206 (−4.83), 196 (−1.56) nm; CD (*c* 1.0 × 10^{-4} M, pH 2.0 MeOH/HCl) 251 (−0.21), 228 (0.18), 212 (0.02), 206 (0.06) nm; CD (*c* 1.0 × 10^{-4} M, pH 9.0 MeOH/20% NaOH) 252 (0.01), 226 (−0.42), 213 (−0.33), 205 (−0.41) nm; IR (KBr) ν_{max} 3362, 2926, 2855, 1668, 1648, 1554, 1538, 1452, 1376, 1077, 1054, 1029 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) *δ* 9.94 (1H, s), 9.73 (1H, br s), 9.46 (1H, d, *J* = 7.6 Hz), 7.39 (2H, d, *J* = 7.2 Hz), 7.30−7.14 (6H, m), 1.10 (3H, d, *J* = 6.4 Hz), 1.00 (3H, d, *J* = 6.4 Hz), 0.91 (3H, d, *J* = 2.8 Hz), 0.89 (3H, d, *J* = 2.4 Hz); ^{13}C NMR (125 MHz, C_5D_5N)[23] *δ* 175.4 (s), 173.9 (s), 173.2 (s), 171.8 (s), 170.7 (s), 138.3 (Phe-Ar, s), 137.1 (Phe-Ar, s), 130.3 (Phe-Ar, d, C × 2), 130.0 (Phe-Ar, d, C × 2), 129.4 (Phe-Ar, d, C × 2), 129.3 (Phe-Ar, d, C × 2), 127.8 (Phe-Ar, s), 127.6 (Phe-Ar, s), 61.4 (Pro$C_α$, d), 60.5 (Val$C_α$, d), 56.2 (Phe$C_α$, d), 55.5 (Phe$C_α$, d), 47.6 (Pro$C_δ$, t), 43.1 (Gly$C_α$, t), 39.0 (Phe$C_β$, t), 38.8 (Phe$C_β$, t), 32.0 (Val$C_β$, t), 30.5 (Pro$C_β$, t), 23.5 (Pro$C_γ$, t), 20.1 (Val$C_γ$, q), 19.8 (Val$C_γ$, q); ESIMS (full scan) *m/z* 586 (46, $[M + K]^+$), 570 (40, $[M + Na]^+$), 548 (100, $[M + H]^+$); ESIMS/MS *m/z* 548 (14, $[M + H]^+$), 520 (32), 503 (9), 449 (13), 421 (17), 401 (49), 373 (54), 302 (100), 274 (17), 247 (12), 219 (8), 172 (13), 155 (35), 70 (3).

4-Methoxydianthramide B (5): white powder; UV (MeOH) λ_{max} (log ϵ) 214 (4.21), 251 (4.44), 278 (4.22), 311 (sh, 4.01) nm; IR (KBr) ν_{max} 3248, 2921, 1696, 1612, 1597, 1530, 1470, 1434, 1256, 1226, 1123, 1076, 1021, 753 cm^{-1}; ^{1}H NMR (400 MHz, C_5D_5N) *δ* 12.67 (1H, s, NH), 9.37 (1H, d, *J* = 2.6 Hz, H-3), 8.42 (1H, dd, *J* = 1.8, 7.8 Hz, H-6′), 8.10 (1H, d, *J* = 8.8 Hz, H-6), 7.45 (1H, td, *J* = 8.0, 1.8 Hz, H-4′), 7.01 (1H, d, *J* = 8.0 Hz, H-3′), 7.04 (1H, td, *J* = 8.0, 1.8 Hz, H-5′), 6.95 (1H, dd, *J* = 2.6, 8.8 Hz, H-5); ^{13}C NMR (100 MHz, C_5D_5N) *δ* 168.0 (s, *C*OOCH$_3$), 165.0 (s, NHC=O), 164.5 (s, C-4), 157.9 (s, C-2′), 144.3 (s, C-2), 133.7 (d, C-4′), 133.6 (d, C-6), 132.6 (s, C-6′), 121.1 (s, C-1′), 121.1 (d, C-5′), 112.1 (d, C-3′), 111.2 (d, C-5), 108.6 (d, C-3), 108.1 (s, C-1), 55.7 (q, OCH$_3$), 51.6 (q, COO*C*H$_3$); EIMS *m/z* 301 (13, $[M]^+$), 207 (5), 167 (65), 135 (100), 92 (5), 77 (19); HREIMS *m/z* 301.0956 (calcd for $C_{16}H_{15}NO_5$, 301.0950).

Hydrolysis and Derivatization of 1−4 (Marfey's Procedure).[12,13] Compounds **1**−**4** (0.1 mg) were each dissolved in 6 N HCl (0.5 mL) in a sealed tube and heated at 130 °C for 16 h. After cooling, the water was evaporated and the mixture redissolved in 100 μL of water and treated with FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alaninamide, 1% in acetone, 250 μL) and 1 M $NaHCO_3$ (300 μL). The mixture was heated at 50 °C for 1 h. HPLC analysis (Hypersil 5μ-ODS, 250 × 4.6 mm; MeCN (0.01% TFA)/H_2O (0.1% TFA) (0 to 30%) and UV detection at 340 nm of FDAA-derived amino acid standards established the configuration of the constituent amino acids (except tyrosine). All amino acids were found to be the L form.

Electrospray Ionization Tandem Mass Spectrometry. Each cyclic peptide was directly infused into the mass spectrometer at a flow rate of 10 μL/min to acquire full scan and product ion mass spectra. A Q1 full scan spectrum was first conducted to obtain their corresponding protonated molecular ions. Their product ion scan spectrum was further acquired by transmitting the protonated molecular ion via Q1 and scanning for products resulting from fragmentations in the collision cell. The electrospray voltage at the spraying needle was optimized at 4500 V. The TurboIonSpray source was operated with nitrogen as the nebulizing (set to 10), curtain (set to 10). Low-energy collision-activated dissociation (CAD) experiments were performed using nitrogen (CAD gas valve set to 4) as collision gas, and a collision energy of 40 eV was used.

Cytotoxicity Assays. Compounds were assayed for cytotoxicity against Hep G2, Hep 3B, A549, MCF-7, and MDA-MB-231 cells using the MTT method. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 5000−10 000 cells per well with tested compounds added from DMSO-diluted stock. After 3 days in culture, attached cells were incubated with MTT (0.5 mg/mL, 1 h) and subsequently solubilized in DMSO. The absorbency at 550 nm was then measured using a microplate reader. The IC_{50} is the concentration of agent that reduced cell growth by 50% under the experimental conditions.

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