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Nakinadines B–F: new pyridine alkaloids with a β-amino acid moiety from sponge *Amphimedon* sp.

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Abstract

Five new 3-alkylpyridine alkaloids with a β-amino acid moiety, nakinadines B–F (**1**–**5**), have been isolated from an Okinawan marine sponge *Amphimedon* sp. (SS-1059), and the structures and stereochemistry were elucidated by spectroscopic data. Nakinadines B (**1**) and C (**2**) showed modest cytotoxicity.

Keywords: Sponge; Alkaloid; Pyridine; β-Amino acid; Nakinadines B–F

1. Introduction

Marine sponges are a rich source of bioactive secondary metabolites with unprecedented skeletons. A number of 3-alkylpyridine alkaloids have been isolated from marine sponges of several genera.[1] Most of them possess a long aliphatic chain with a various nitrogen-containing terminus,[2] some of which have dimeric or polymeric structures of 3-alkylpyridine.[3] During our continuing search for bioactive substances from marine sponges,[4] we previously isolated cytotoxic pyridine alkaloids from sponges of the genera *Theonella*,[5] *Nyphates*,[6] *Amphimedon*,[7] and *Cribrochalina*.[8] More recently, new 3-alkylpyridine alkaloids with a β-amino acid moiety, nakinadines B–F (**1**–**5**), have been isolated together with nakinadine A[4] (**6**) from an Okinawan marine sponge *Amphimedon* sp. (SS-1059). Here we describe the isolation and structural elucidation of nakinadines B–F (**1**–**5**).

2. Results and discussion

The sponge *Amphimedon* sp. (SS-1059) collected off Nakijin, Okinawa was extracted with MeOH. EtOAc-soluble materials of the MeOH extract were subjected to a silica gel column ($CHCl_3$/MeOH), in which a fraction eluted with $CHCl_3$/MeOH (7:3) was purified by C_{18} HPLC (MeOH/H_2O) to afford nakinadines B (**1**, 0.004%, wet weight) and C (**2**, 0.005%). The fraction eluted with $CHCl_3$/MeOH (9:1) in the silica gel column was further purified by an amino silica gel column ($CHCl_3$/MeOH) followed by C_{18} HPLC (MeOH/H_2O) to afford nakinadines D (**3**, 0.001%, wet weight), E (**4**, 0.002%), and F (**5**, 0.001%) together with nakinadine A[4] (**6**, 0.001%).

Nakinadine B (**1**) was revealed to have the molecular formula, $C_{27}H_{40}N_2O_2$, by HRESIMS [*m*/*z* 423.2998 $(M-H)^-$, Δ −0.3 mmu]. Aromatic proton signals [H-2 and H-6, δ_H 8.43; H-4, δ_H 7.48; H-5, δ_H 7.18; H-25 and H-29, δ_H 7.33 (2H); H-26 and H-28, δ_H 7.26 (2H); H-27, δ_H 7.20] in the ^{1}H NMR spectrum and five sp^2 carbon signals (C-2, δ_C 149.8; C-3, δ_C 138.4; C-4, δ_C 135.8; C-5, δ_C 123.2; C-6, δ_C 147.1) and four sp^2 carbon signals (C-24, δ_C 135.9; C-25 and C-29, δ_C 128.7 (2C); C-26 and C-28, δ_C 128.2 (2C); C-27, δ_C 127.3) in the ^{13}C NMR spectrum

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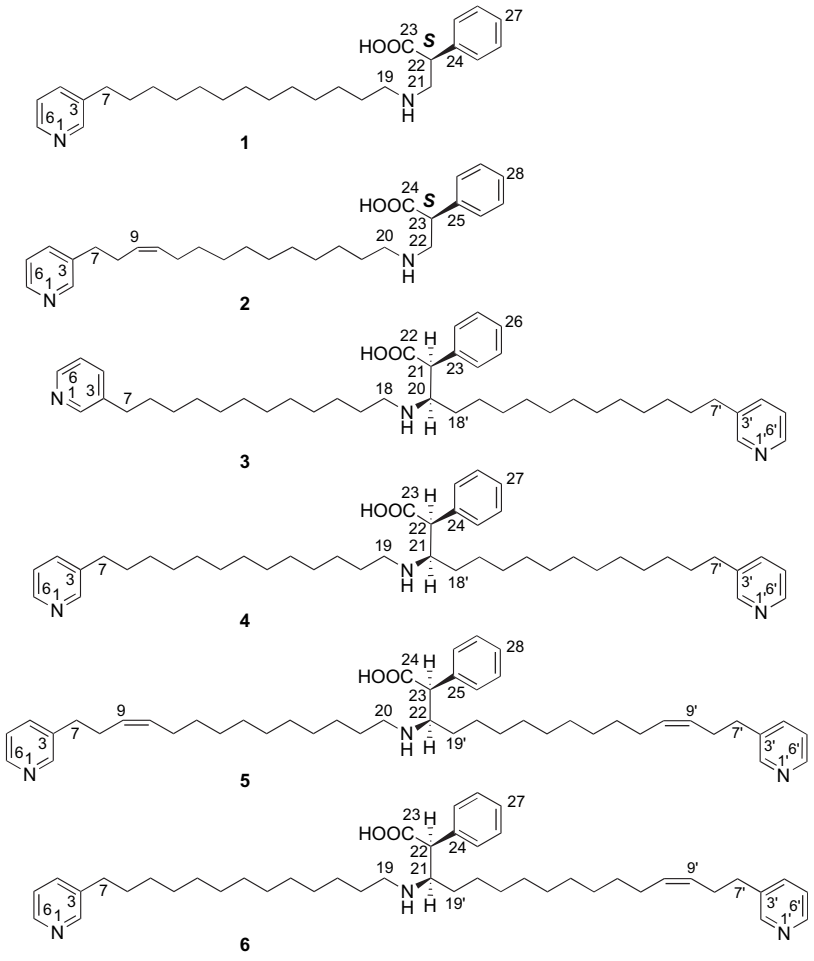

Stereochemistries for **3-6** denote relative one.

suggested that **1** possessed a 3-alkylpyridine ring and a phenyl group. The ^{13}C NMR data disclosed the presence of a carbonyl carbon (δ_C 176.6). IR absorptions at 3400−2600 and 1733 cm^{-1} indicated the presence of carboxylic acid functionality. The ^{1}H and ^{13}C NMR data suggested that CH_2-19 (δ_H 2.89 (2H); δ_C 47.9) and CH_2-21 (δ_H 3.47 and 2.89; δ_C 51.1) were adjacent to a nitrogen atom.

The gross structure of **1** was elucidated by analyses of 2D NMR data in $CDCl_3$ (Fig. 1). The ^{1}H−^{1}H COSY and TOCSY spectra revealed connectivities of five structural fragments, **a** (C-2 to C-6), **b** (C-7 to C-8), **c** (C-18 to C-19), **d** (C-21 to C-22), and **e** (C-25 to C-29). HMBC correlations from H-7 to C-2, C-3, and C-4 suggested the connectivity of C-7 to C-3. The connectivity of C-19 to C-21 through a nitrogen atom was implied by HMBC cross-peaks for H_2-19 to C-21. HMBC cross-peaks for H-22 to C-23 and C-25, and H-29 to C-24 indicated that a carboxyl and a phenyl group were both attached to C-22.

The connection of C-8 to C-18 via a long alkyl chain was elucidated on the basis of fragmentation patterns of the ESIMS/MS spectrum of **1** (Fig. 2). Thus, the gross structure of nakinadine B was elucidated to be **1**.

To assign the absolute configuration at C-22, the carboxy group at C-22 in **1** was converted into the (*S*)- and (*R*)-phenylglycine methyl ester (PGME) amides. The $\Delta\delta$ [δ (*S*-PGME amide)−δ (*R*-PGME amide)] values obtained from the ^{1}H NMR spectra of the PGME amides suggested that the absolute configuration at C-22 in **1** was *S* (Fig. 3).[9]

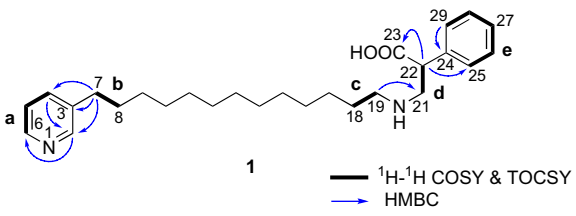

Figure 1. Selected 2D NMR correlations for nakinadine B (**1**).

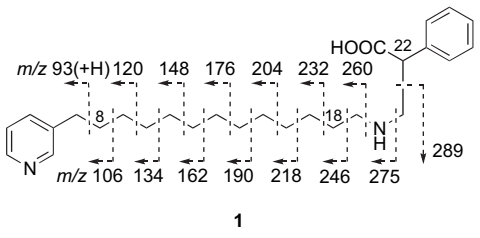

Figure 2. Fragmentation patterns of nakinadine B (**1**) in ESIMS/MS [parent ion; at *m/z* 425 $(M+H)^+$].

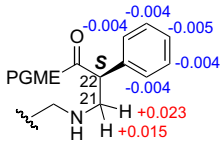

Figure 3. $\Delta\delta$ values [$\Delta\delta$ (in ppm) $\delta_S-\delta_R$] obtained for (*S*)- and (*R*)-PGME amides of nakinadine B (**1**).

Nakinadine C (**2**) was revealed to have the molecular formula, $C_{28}H_{40}N_2O_2$, by HRESIMS [*m/z* 437.3163 $(M+H)^+$, Δ −0.6 mmu]. ^1H and ^{13}C NMR data of **2** were similar to those of nakinadine B (**1**), except for the presence of signals due to a double bond in **2**. *Z*-Geometry of an olefin at C-9 was assigned from chemical shifts of allylic carbons [C-8, δ_C 28.7; C-11, δ_C 26.7].[10] The $^1H-^1H$ COSY, TOCSY, and HMBC spectra revealed connectivities from a β-substituted pyridine ring to C-12 and C-19 to C-30 (Fig. 4). The fragmentation patterns obtained from ESIMS/MS spectrum of **2** disclosed connectivities from C-12 to C-19 (Fig. 5). The $\Delta\delta$ values obtained from the ^1H NMR spectra of the PGME amides of **2** showed the same pattern as those of **1**, suggesting that the absolute configuration at C-23 of **2** was *S*. Thus, the stereostructure of nakinadine C was elucidated to be **2**.

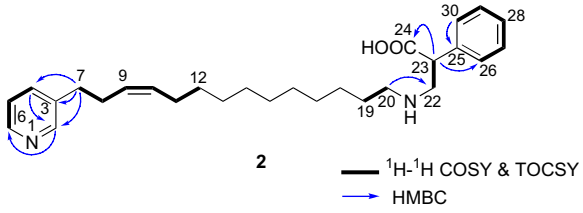

Figure 4. Selected 2D NMR correlations for nakinadine C (**2**).

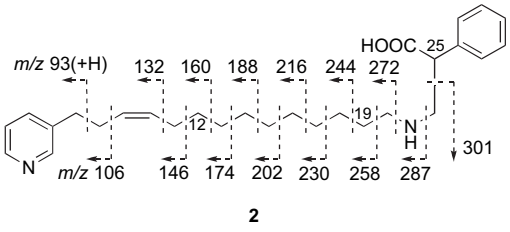

Figure 5. Fragmentation patterns of nakinadine C (**2**) in ESIMS/MS [parent ion; at *m/z* 437 $(M+H)^+$].

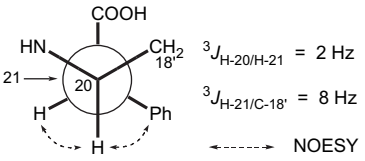

Figure 7. Rotation model for C-20 to C-21 of nakinadine D (**3**).

Nakinadine D (**3**) was revealed to have the molecular formula, $C_{43}H_{65}N_3O_2$, by HRESIMS [*m/z* 656.5179 $(M+H)^+$, Δ +2.4 mmu]. ^1H and ^{13}C NMR data of **3** were similar to those of nakinadine A (**6**), except for lack of signals due to a double bond in **6**. Analysis of the ESIMS/MS spectrum of **3** revealed connectivities from two pyridine rings to β-amino acid moiety (Fig. 6). Thus, the gross structure of nakinadine D was assigned as **3**.

The relative stereochemistry of **3** was deduced from NOESY correlations and *J*-values[11] as shown in Figure 7. A *gauche* relation for H-20 and H-21 was deduced from the NOESY cross-peak for H-20/H-21 and $^3J_{H-20/H-21}$ (2 Hz). The NOESY cross-peaks for H-20/H-24 and H-20/H-25 suggested a *gauche* relation for H-20 and a phenyl group. A large coupling constant ($^3J_{H-21/C-18'}$=8 Hz), which was obtained from the HETLOC spectrum of **3**, indicated an *anti* relation for H-21 and C-18′ (Fig. 7).

The spectral data of nakinadine E (**4**) were almost identical with those of nakinadine D (**3**), except for the molecular weight derived from ESIMS [*m/z* 670 $(M+H)^+$]. The molecular formula of **4**, $C_{44}H_{67}N_3O_2$, revealed by HRESIMS [*m/z* 670.5315 $(M+H)^+$, Δ +0.3 mmu] suggested that the carbon chain of **4** was longer by one methylene unit as compared with that of **3**. The fragmentation patterns obtained from ESIMS/MS data of **4** (Fig. 8) also supported the proposed structure of nakinadine E (**4**). The relative stereochemistry at C-21 and C-22 of **4** was elucidated to be the same as those of **3** on the basis of NOESY correlations (H-21 to H-22, H-25, and H-26) and *J*-values ($^3J_{H-21/H-22}$=2 Hz and $^3J_{H-22/C-18'}$=8 Hz) of **4**. Thus, the structure of nakinadine E was elucidated to be **4**.

The spectral data of nakinadine F (**5**) were similar to those of nakinadine A (**6**). The molecular formula, $C_{46}H_{67}N_3O_2$, revealed from HRESIMS [*m/z* 694.5313 $(M+H)^+$, Δ +0.2 mmu] and the ^1H and ^{13}C NMR spectra indicated that

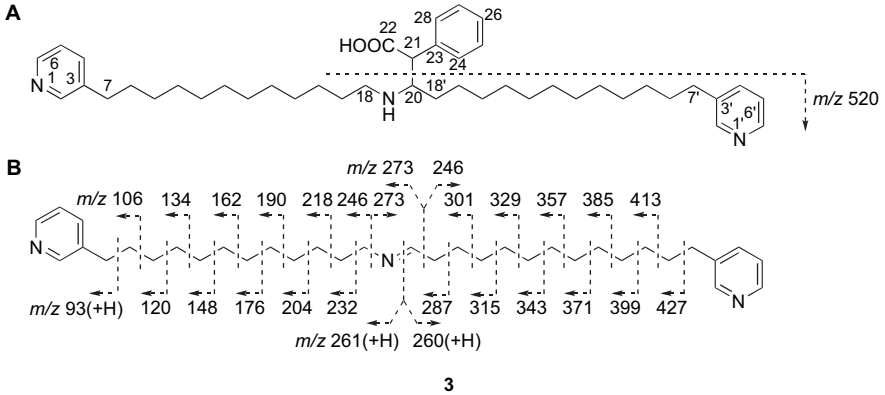

Figure 6. Fragmentation patterns of nakinadine D (**3**) in ESIMS/MS [parent ion; (A) at *m/z* 656 $(M+H)^+$, (B) at *m/z* 520 $(M-C_8H_9O_2+H)^+$].

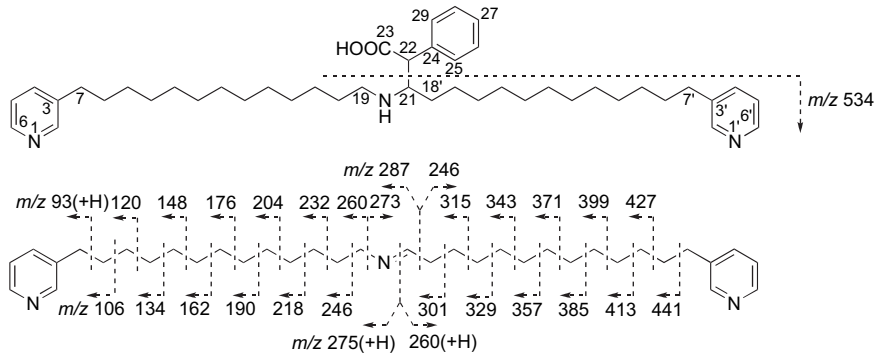

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Figure 8. Fragmentation patterns of nakinadine E (**4**) in ESIMS/MS [parent ion; (A) at *m/z* 670 $(M+H)^+$, (B) at *m/z* 534 $(M-C_8H_9O_2+H)^+$].

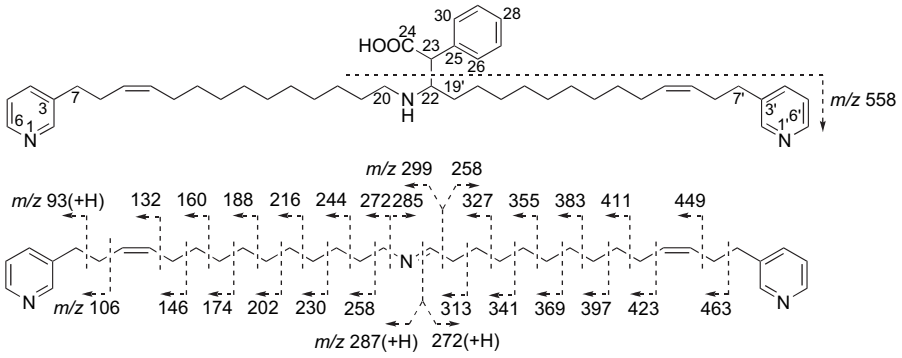

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Figure 9. Fragmentation patterns of nakinadine F (**5**) in ESIMS/MS [parent ion; (A) at *m/z* 694 $(M+H)^+$, (B) at *m/z* 558 $(M-C_8H_9O_2+H)^+$].

the carbon chain of **5** was shorter by one methylene unit as compared with that of **6**, and **5** had two *Z* double bonds. Analysis of the ESIMS/MS spectrum of **5** revealed connectivities from two pyridine rings to β-amino acid moiety including position of two double bonds (Fig. 9). The relative stereochemistry of a β-amino acid moiety in **5** was elucidated to be the same as those of **3** on the basis of NOESY correlations (H-22 to H-23, H-26, and H-27) and *J*-values ($^3J_{H\text{-}22/H\text{-}23}$=2 Hz and $^3J_{H\text{-}23/C\text{-}19'}$=8 Hz) of **5**. Thus, the structure of nakinadine F was assigned as **5**.

Nakinadines B (**1**) and C (**2**) showed cytotoxicity against L1210 murine leukemia (IC_{50}, 3.0 and 5.0 μg/mL, respectively) and KB human epidermoid carcinoma cells (IC_{50}, 7.0 and >10 μg/mL, respectively) in vitro, while nakinadines D (**3**), E (**4**), and F (**5**) did not show such activity (IC_{50}>10 μg/mL).

3. Experimental

3.1. General

IR and UV spectra were recorded on a JASCO FT/IR-230 and a Shimadzu UV-1600PC spectropolarimeters. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer using 2.5 mm micro cells (Shigemi Co., Ltd). The 7.26 and 77.0 ppm resonances of residual $CDCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. ESI mass spectra were obtained on a JEOL JMS-700TZ spectrometer.

3.2. Sponge description

The sponge *Amphimedon* sp. (SS-1059; order Haplosclerida; family Niphatidae) was collected off Nakijin, Okinawa and kept frozen until used. The sponge is thick walled tube with a dense internal skeleton. Specimen has a central oscule and canal. Sponge has a reticulate fiber skeleton, close meshed, centrally cored with spicules. Primary meshes are slightly elongate. Primary fibers are multispicular, approximately 60 μm wide cored by approximately 5 spicules. Secondary fibers are occasionally unispicular, 40 μm wide cored by 1–2 spicules. Spicules are oxeas, 105×5 μm. Exterior surface is smooth, shiny.

3.3. Extraction and isolation

The sponge (SS-1059, 0.2 kg, wet weight) was extracted with MeOH (1.0 L×3), and the extract was partitioned between EtOAc and H_2O. The EtOAc-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH, 10:0 to 0:10) to give alkaloidal fractions. The fraction eluted with $CHCl_3$/MeOH (7:1) was purified by reversed-phase HPLC (Luna 5u phenyl–hexyl,

250×10 mm; eluent, MeOH/H_2O, 85:15; flow rate, 1.5 mL/min; UV detection at 263 nm) to afford nakinadines B (**1**, 0.0037% wet weight, t_R 19 min) and C (**2**, 0.0045%, t_R 21 min). The fraction eluted with $CHCl_3$/MeOH (9:1) was separated by an amino silica gel column chromatography ($CHCl_3$/MeOH, 10:0 to 0:10) followed by reversed-phase HPLC (Luna 5u phenyl–hexyl, 250×10 mm; eluent, MeOH/H_2O, 89:11; flow rate, 1.0 mL/min; UV detection at 263 nm) to afford nakinadines D (**3**, 0.0011%, t_R 12 min), E (**4**, 0.0022%, t_R 15 min), F (**5**, 0.0022%, t_R 18 min), and A (**6**, 0.0011%, t_R 12 min).

3.3.1. Nakinadine B (*1*)

Colorless oil; UV (MeOH) λ_{max} 257 (ε 3100), 263 (3300), and 270 (2900) nm; IR (film) ν_{max} 3360, 3190, 2910, and 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.43 (2H, m), 7.48 (m), 7.33 (2H, m), 7.26 (2H, m), 7.20 (m), 7.18 (m), 4.10 (br s), 3.47 (br s), 2.89 (3H, br s), 2.59 (2H, m), 1.60 (2H, m), 1.0–1.4 (20H, m); ^{13}C NMR ($CDCl_3$) δ 176.6 (s), 149.8 (d), 147.1 (d), 138.4 (s), 135.9 (s), 135.8 (d), 128.7 (2C, d), 128.2 (2C, d), 127.3 (d), 123.2 (d), 51.1 (t), 50.8 (d), 47.9 (t), 33.0 (d), 29–30 (11C, t), 26.8 (t); ESIMS (neg.) *m/z* 423 $(M-H)^-$; HRESIMS (neg.) *m/z* 423.2998 $(M-H)^-$, Δ −0.3 mmu.

3.3.2. (S)-PGME amide of nakinadine B (*1*)

To an ice cooled DMF solution (1 mL) of **1** (1.0 mg) and (*S*)-PGME (1.0 mg) were added PyBOP® (benzotriazole-1-yl-oxytrispyrrolidinephosphoniumhexafluorophosfate) (2.0 mg), HOBt (1-hydroxybenzotriazole) (1.0 mg), and *N*-methylmorpholine (50 μL), and stirring was continued at rt for 18 h. After addition of 5% HCl, the mixture was extracted with EtOAc (2.5 mL). The extract was washed with satd $NaHCO_3$ aq and brine, and then concentrated in vacuo. The residue was subjected to a silica gel column ($CHCl_3$/MeOH, 10:0 to 0:10) to give the (*S*)-PGME amide of **1** (0.6 mg). (*S*)-PGME amide of nakinadine B (**1**); colorless amorphous solid; ^1H NMR ($CDCl_3$) δ 8.42 (2H, m), 7.85 (NH, d), 7.47 (m), 7.36 (5H, m), 7.31 (2H, m), 7.29 (2H, m), 7.24 (m), 7.18 (m), 5.50 (m), 3.79 (m), 3.66 (3H, s), 3.24 (m), 2.95 (3H, m), 2.58 (2H, m), 1.0–1.8 (22H, m); ESIMS (pos.) *m/z* 572 $(M+H)^+$.

3.3.3. (R)-PGME amide of nakinadine B (*1*)

The (*R*)-PGME amide of **1** (0.5 mg) was obtained from **1** (1.0 mg) according to the same procedure as described for the preparation of (*S*)-PGME amide of **1**. (*R*)-PGME amide of nakinadine B (**1**); colorless amorphous solid; ^1H NMR ($CDCl_3$) δ 8.42 (2H, m), 7.85 (NH, d), 7.47 (m), 7.34 (5H, m), 7.32 (2H, m), 7.29 (2H, m), 7.24 (m), 7.18 (m), 5.51 (m), 3.76 (m), 3.66 (3H, s), 3.21 (m), 2.94 (3H, m), 2.58 (2H, m), 1.0–1.8 (22H, m); ESIMS (pos.) *m/z* 572 $(M+H)^+$.

3.3.4. Nakinadine C (*2*)

Colorless oil; UV (MeOH) λ_{max} 258 (ε 3200), 263 (3300), and 269 (3000) nm; IR (film) ν_{max} 3350, 3200, 2920, and 1733 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.44 (2H, m), 7.49 (m), 7.33 (2H, m), 7.25 (2H, m), 7.20 (m), 7.18 (m), 5.37 (2H, m), 4.11 (br s), 3.49 (br s), 2.86 (br s), 2.82 (2H, br s), 2.65 (2H, t, J=7.4 Hz), 2.35 (2H, dt, J=7.4 and 6.7 Hz), 1.92 (2H, dt, J=7.1 and 6.7 Hz), 1.61 (2H, br s), 1.0–1.4 (14H, m); ^{13}C NMR ($CDCl_3$) δ 176.7 (s), 149.9 (d), 147.2 (d), 137.3 (s), 135.8 (s), 135.8 (d), 131.4 (d), 128.7 (2C, d), 128.2 (2C, d), 127.7 (d), 127.3 (d), 123.2 (d), 51.1 (t), 50.8 (d), 47.9 (t), 33.0 (t), 29–30 (7C, t), 28.7 (t), 27.2 (t), 26.7 (t); ESIMS (neg.) *m/z* 437 $(M+H)^+$; HRESIMS (pos.) *m/z* 437.3163 $(M+H)^+$, Δ −0.6 mmu.

3.3.5. (S)-PGME amide of nakinadine C (*2*)

The (*S*)-PGME amide of **2** (0.4 mg) was obtained from **2** (1.0 mg) according to the same procedure as described for the preparation of (*S*)-PGME amide of **1**. (*S*)-PGME amide of nakinadine C (**2**); colorless amorphous solid; ^1H NMR ($CDCl_3$) δ 8.42 (2H, m), 7.85 (NH, d), 7.47 (m), 7.36 (5H, m), 7.31 (2H, m), 7.29 (2H, m), 7.24 (m), 7.18 (m), 5.53 (m), 5.34 (2H, m), 3.79 (m), 3.69 (3H, s), 3.24 (m), 2.95 (3H, m), 2.61 (2H, m), 2.31 (2H, m), 1.95 (2H, m), 1.0–1.8 (16H, m); ESIMS (pos.) *m/z* 584 $(M+H)^+$.

3.3.6. (R)-PGME amide of nakinadine C (*2*)

The (*R*)-PGME amide of **1** (0.5 mg) was obtained from **2** (1.0 mg) according to the same procedure as described for the preparation of (*S*)-PGME amide of **1**. (*R*)-PGME amide of nakinadine C (**2**); colorless amorphous solid; ^1H NMR ($CDCl_3$) δ 8.42 (2H, m), 7.85 (NH, d), 7.47 (m), 7.34 (5H, m), 7.32 (2H, m), 7.29 (2H, m), 7.24 (m), 7.18 (m), 5.54 (m), 5.34 (2H, m), 3.76 (m), 3.66 (3H, s), 3.21 (m), 2.94 (3H, m), 2.61 (2H, m), 2.31 (2H, m), 1.95 (2H, m), 1.0–1.8 (16H, m); ESIMS (pos.) *m/z* 584 $(M+H)^+$.

3.3.7. Nakinadine D (*3*)

Colorless oil; UV (MeOH) λ_{max} 258 (ε 4700), 263 (5000), and 270 (4400) nm; IR (film) ν_{max} 3200, 2853, and 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.44 (4H, m), 7.49 (2H, m), 7.32 (4H, m), 7.20 (2H, m), 7.19 (m), 3.84 (br s), 2.98 (br s), 2.82 (m), 2.72 (m), 2.60 (4H, t, J=7.8 and 7.5 Hz), 1.1–1.7 (42H, m); ^{13}C NMR ($CDCl_3$) δ 176.0 (s), 149.6 (2C, d), 146.8 (2C, d), 136.9 (2C, s), 135.8 (s), 135.5 (2C, d), 129.4 (2C, d), 128.2 (2C, d), 127.0 (d), 122.9 (2C, d), 59.5 (d), 51.9 (d), 44.9 (t), 32.7 (2C, t), 30.8 (2C, t), 28–30 (17C, t), 26.9 (t), 26.6 (t); ESIMS (pos.) *m/z* 656 $(M+H)^+$; HRESIMS (pos.) *m/z* 656.5179 $(M+H)^+$, Δ +2.4 mmu.

3.3.8. Nakinadine E (*4*)

Colorless oil; UV (MeOH) λ_{max} 259 (ε 4900), 263 (5100), and 269 (4500) nm; IR (film) ν_{max} 3200, 2853, and 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.41 (4H, m), 7.50 (2H, m), 7.33 (4H, m), 7.21 (2H, m), 7.20 (m), 3.87 (br s), 3.05 (br s), 2.85 (m), 2.72 (m), 2.61 (4H, m), 1.0–1.8 (44H, m); ^{13}C NMR ($CDCl_3$) δ 176.0 (s), 149.6 (2C, d), 146.8 (2C, d), 136.9 (2C, s), 135.8 (s), 135.5 (2C, d), 129.4 (2C, d), 128.2 (2C, d), 127.0 (d), 122.9 (2C, d), 59.5 (d), 51.9 (d), 44.9 (t), 32.7 (2C, t), 30.8 (2C, t), 28–30 (18C, t), 26.9 (t), 26.6 (t); ESIMS (pos.) *m/z* 670 $(M+H)^+$; HRESIMS (pos.) *m/z* 670.5315 $(M+H)^+$, Δ +0.3 mmu.

*3.3.9. Nakinadine F (**5**)*

Colorless oil; UV (MeOH) λ_{max} 258 (ε 4900), 264 (5200), and 270 (4600) nm; IR (film) ν_{max} 3200, 2853, and 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.40 (4H, m), 7.49 (2H, m), 7.31 (4H, m), 7.20 (2H, m), 7.18 (m), 5.38 (4H, m), 3.84 (br s), 3.01 (br s), 2.80 (m), 2.70 (m), 2.65 (2H, m), 2.60 (2H, m), 2.35 (4H, m), 1.0–1.7 (36H, m); ^{13}C NMR ($CDCl_3$) δ 176.0 (s), 149.6 (2C, d), 146.8 (2C, d), 136.9 (2C, s), 135.8 (s), 135.5 (2C, d), 131.1 (2C, d), 129.4 (2C, d), 128.2 (2C, d), 127.4 (2C, d), 127.0 (d), 122.9 (2C, d), 59.5 (d), 51.9 (d), 44.9 (t), 32.7 (2C, t), 28.4 (2C, t), 28–30 (14C, t), 26.9 (3C, t), 26.6 (t); ESIMS (pos.) *m/z* 694 $(M+H)^+$; HRESIMS (pos.) *m/z* 694.5313 $(M+H)^+$, Δ +0.2 mmu.

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