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Zamamidine C, 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A, and 3,4-dihydromanzamine J *N*-oxide, new manzamine alkaloids from sponge *Amphimedon* sp.

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ABSTRACT

New manzamine alkaloids, zamamidine C (**1**), 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**), and 3,4-dihydromanzamine J *N*-oxide (**3**), have been isolated from an Okinawan marine sponge *Amphimedon* species. The structures and stereochemistries of **1**–**3** were elucidated from the spectroscopic data and chemical derivatization. Zamamidine C (**1**) is a new manzamine alkaloid possessing a second β-carboline ring via an ethylene unit at N-2 of manzamine D, while 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**) is the first manzamine alkaloid possessing an epoxide ring at C-10 and C-11. Zamamidine C (**1**) showed significant antitrypanosomal activity against *Trypanosoma brucei brucei*, the parasite associated with sleeping sickness, and antimalarial activity against *Plasmodium falciparum*, the causative agent of malaria in vitro.

1. Introduction

The manzamine alkaloids have been reported from several genera of marine sponges and are well known to have unique polycyclic ring systems.[1,2] During our search for bioactive metabolites from marine organisms, we recently isolated new manzamine alkaloids, zamamidines A and B, from an Okinawan sponge *Amphimedon* sp. (SS-975).[3] Further investigation of extracts of another lot of the sponge resulted in the isolation of three new manzamine alkaloids, zamamidine C (**1**), 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**), and 3,4-dihydromanzamine J *N*-oxide (**3**). Here we describe the isolation and structural elucidation of **1**–**3**.

2. Results and discussion

Two collections of marine sponges of the genus *Amphimedon* (SS-1251 and SS-264) collected off Kerama Islands, Okinawa, were extracted with MeOH, separately. EtOAc-soluble materials of the extract from the sponge (SS-1251) were subjected to silica gel column chromatographies to yield zamamidine C (**1**, 0.0026% wet weight) and 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**, 0.00028%), while the EtOAc-soluble materials of the extract from the sponge (SS-264) were purified by silica gel columns to give 3,4-dihydromanzamine J *N*-oxide (**3**, 0.0014%).

Zamamidine C (**1**) was obtained as an optically active pale yellow amorphous solid. The ESIMS spectrum of **1** showed the pseudomolecular ion peak at *m*/*z* 747, and the molecular formula of **1** was revealed to be $C_{49}H_{58}N_6O$ by HRESIMS data [*m*/*z* 747.4736 $(M+H)^+$, Δ −1.4 mmu]. UV absorptions [λ_{max} 352 (ε 4100) and 339 nm (4200)] were attributed to a β-carboline chromophore,[4] while the IR absorption indicated the existence of NH and/or OH (3160 cm^{-1}) functionality. The ^1H and ^{13}C NMR spectra disclosed that **1** consists of 10 sp^2 quaternary carbons, 15 sp^2 methines, 2 sp^3 quaternary carbons, 4 sp^3 methines, and 18 sp^3 methylenes (Table 1). Among them, four sp^2 quaternary carbons (δ_C 136.31, 133.70, 127.46, and 107.73), four sp^2 methines (δ_C 121.58, 119.49, 118.18, and 111.20), one sp^3 methine (δ_C 67.22), and two sp^3 methylenes (δ_C 49.52 and 21.35) were ascribed to a 1,2,3,4-tetrahydro-β-carboline ring, while five sp^2 quaternary

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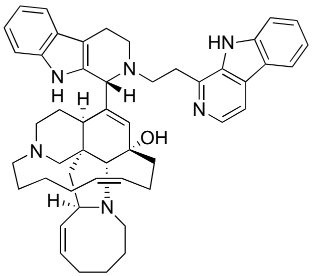

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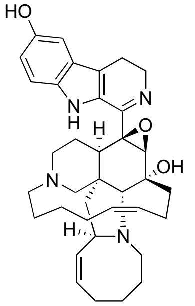

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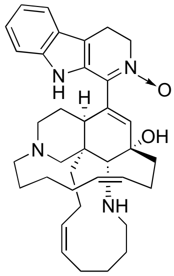


3

carbons (δ_C 145.53, 141.17, 135.61, 129.43, and 122.65) and six sp^2 methines (δ_C 138.59, 128.21, 121.73 120.04, 113.37, and 112.18) were ascribed to a β-carboline ring.

Detailed analyses of the 2D NMR spectra of **1** including ^{1}H–^{1}H COSY, TOCSY, HMQC, HMQC–TOCSY, and HMBC revealed the existence of a characteristic 5/6/6/8/13 ring system for manzamine alkaloids such as manzamine A[5,6] (C-10∼C-20, N-21, C-22∼C-26, N-27, and C-28∼C-36) in addition to a 1,2,3,4-tetrahydro-β-carboline ring (C-1, N-2, C-3∼C-8a, N-9, and C-9a) and a β-carboline ring (C-1′, N-2′, C-3′∼C-8′a, N-9′, and C-9′a) (Fig. 1). HMBC cross-peaks of H-1 to C-10 and H-11 to C-1 indicated that C-1 of the 1,2,3,4-tetrahydro-β-carboline ring was attached to C-10, while HMBC correlations for H-1 to C-11′ and H-10′ to C-1′ suggested that N-2 of 1,2,3,4-tetrahydro-β-carboline ring was connected to C-1′ of a second β-carboline ring via an ethylene unit (Fig. 1).

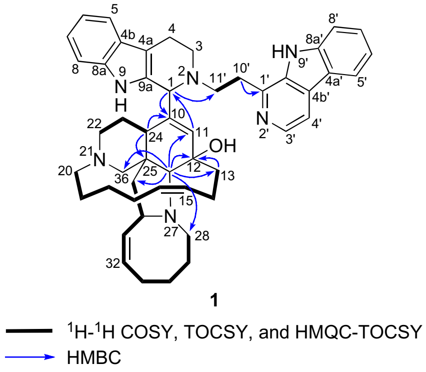
The relative stereochemistry of zamamidine C (**1**) was deduced from analysis of the NOESY spectrum. A chair conformation of a piperidine ring (N-21, C-22∼C-25, and C-36) and a pseudoboat conformation of a cyclohexene ring (C-10∼C-12 and C-24∼C-26) in the *cis* decahydroisoquinoline ring in addition to α-orientation of 12-OH and β-orientation of H-26 were derived from NOESY correlations as shown in Figure 2. NOESY correlations for H-1/H-11 and NH-9′/H-24 suggested that the planes of a 1,2,3,4-tetrahydro-β-carboline ring and a cyclohexene ring were perpendicular to each other, and that a second β-carboline ring was located behind a piperidine ring. Consequently, an orientation of H-1 was assigned as β. Two proton signals for H-22b and H-35b were shifted to higher-field (δ_H −1.61 and −1.68, respectively) as compared with those of manzamine D.[7] These upfield shifts might be explained by anisotropic effect of the second β-carboline plane like zamamidine A.[3] In fact, the stable conformers for **1** obtained from the conformation search carried out by MacroModel program (MMFF94s force-field) were similar to those of zamamidine A.[3] The absolute configuration at C-1 in **1** was assigned as *R* from a positive Cotton effect at 223 nm ($\Delta\varepsilon$ +16.9) on the CD spetrum.[8] Thus, the structure of zamamidine C was elucidated to be **1**.

Table 1
^{1}H and ^{13}C NMR data of zamamidine C (**1**) in CDCl$_3$

Position	δ_H	δ_C
1	4.18	67.22 (d)
3	3.89 (br d, 9.1) 2.70 (td, 7.3, 14.3)	49.52 (t)
4	3.26 (nd) 3.03 (nd)	21.35 (t)
4a	—	107.73 (s)
4b	—	136.31 (s)
5	7.59 (d, 7.4)	118.18 (d)
6	7.17 (m)	119.49 (d)
7	7.18 (m)	121.58 (d)
8	7.34 (d, 7.3)	111.20 (d)
8a	—	127.46 (s)
9a	—	133.70 (s)
10	—	139.80 (s)
11	5.81 (s)	136.79 (d)
12	—	69.21 (s)
	1.48 (nd)	
14	2.08 (2H, nd)	21.53 (t)
15	5.52 (q, 8.0)	128.45 (d)
16	5.41 (m)	131.77 (d)
17	2.16 (nd) 1.66 (nd)	26.26 (t)
18	1.12 (2H, nd)	26.94 (t)
19	1.84 (nd) 1.54 (nd)	31.73 (t)
20	2.15 (nd) 1.90 (nd)	53.80 (t)
22	1.89 (nd) 0.08 (br)	48.32 (t)
23	0.79 (m) 0.64 (br s)	32.11 (t)
24	1.33 (nd)	36.52 (d)
25	—	46.22 (s)
26	3.16 (s)	74.28 (d)
28	2.93 (nd) 2.89 (nd)	50.79 (t)
29	1.80 (nd) 1.17 (nd)	25.34 (t)
30	1.33 (nd) 1.25 (nd)	25.34 (t)
31	2.06 (2H, nd)	28.04 (t)
32	5.77 (m)	134.65 (d)
33	4.80 (t, 9.1)	128.74 (d)
34	3.83 (br s)	54.53 (d)
35	1.55 (nd) −0.05 (nd)	43.00 (t)
36	2.13 (nd) 1.00 (d, 10.8)	67.10 (t)
1′	—	145.53 (s)
3′	8.34 (d, 5.3)	138.59 (d)
4′	7.84 (d, 5.3)	113.37 (d)
4a′	—	129.43 (s)
4b′	—	122.65 (s)
5′	8.09 (d, 7.8)	121.73 (d)
6′	7.22 (m)	120.04 (d)
7′	7.38 (t, 7.5)	128.21 (d)
8′	7.29 (d, 8.0)	112.18 (d)
8a′	—	141.17 (s)
9a′	—	135.61 (s)
10′	3.31 (m) 2.88 (nd)	54.53 (t)
11′	3.67 (nd) 3.32 (nd)	33.17 (t)

nd: Not determined by overlapping.

The molecular formula of 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**) was established to be $C_{36}H_{46}N_4O_3$ by HRESIMS data [*m*/*z* 583.3634 $(M+H)^+$, Δ −1.4 mmu]. IR absorption indicated the existence of NH and/or OH (3180 cm^{-1}) functionality. The ^{1}H and ^{13}C NMR data revealed the presence of 6 sp^2 quaternary carbons, 7 sp^2 methines, 3 sp^3 quaternary carbons, 4 sp^3 methines, and 16 sp^3 methylenes (Table 2). Among them, six sp^2 quaternary carbons (δ_C 157.56, 150.85, 131.93, 127.85, 125.54, and 117.16), three sp^2 methines (δ_C 115.65, 113.00, and 103.48), and two sp^3 methylenes (δ_C 48.40 and 19.10) were ascribed to a 3,4-dihydro-6-hydroxy-β-carboline ring. The $^{1}J_{C/H}$ coupling constant for C-11 and H-11 (175.8 Hz) suggested that C-11 was involved in an epoxide ring.[9]



1

Figure 1. Selected 2D NMR correlations for zamamidine C (**1**).

Inspection of the 2D NMR data of **2** revealed that **2** composed of a manzamine A-type 5/6/6/8/13 ring system (C-10∼C-20, N-21, C-22∼C-26, N-27, and C-28∼C-36) and a 3,4-dihydro-6-hydroxy-β-carboline ring[10] (C-1, N-2, C-3∼C-8a, N-9, and C-9a) (Fig. 3). HMBC cross-peak of H-11 to C-1 revealed that C-1 of the 3,4-dihydro-6-hydroxy-β-carboline ring was attached to C-10. Thus, the gross structure of 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A was elucidated to be **2**.

The relative stereochemistry of 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**) was deduced from analysis of the ROESY spectrum. Relative configurations at C-12, C-24, C-25, C-26, and C-34 were assigned as the same as those of manzamine A on the basis of ROESY correlations as shown in Figure 4. The α-orientations of 3,4-dihydro-6-hydroxy-β-carboline ring and H-11 were derived from a ROESY correlation for NH-9 and H-11.

The molecular formula of 3,4-dihydromanzamine J *N*-oxide (**3**) was established to be $C_{36}H_{48}N_4O_2$ by HRESIMS data [*m*/*z* 569.3870 $(M+H)^+$, Δ +1.4 mmu]. IR absorption indicated the existence of NH and/or OH (3210 cm^{-1}) functionality. The ^{1}H and ^{13}C NMR data revealed the presence of 6 sp^2 quaternary carbons, 9 sp^2 methines, 2 sp^3 quaternary carbons, 2 sp^3 methines, and 17 sp^3 methylenes (Table 3). Though the ^{1}H and ^{13}C NMR data of **3** were close to those of 3,4-dihydromanzamine J,[11] a comparison of the ^{13}C chemical shifts of C-1, C-3 (δ_C 136.86 and 61.39, respectively) in **3** with those (δ_C 159.8 and 48.8, respectively) of 3,4-dihydromanzamine J suggested that **3** was an *N*-oxide form of 3,4-dihydromanzamine J.[11,12] Furthermore, a resulting mixture obtained by treatment of 3,4-dihydromanzamine J *N*-oxide (**3**) with Zn in 1 N HCl under hydrogen atmosphere[12] was purified by HPLC to give manzamines H and L (Scheme 1), whose spectral data including CD spectra were identical with those of natural manzamines H[4] and L,[13] respectively. Thus, the structure of 3,4-dihydromanzamine J *N*-oxide was concluded to be **3**.

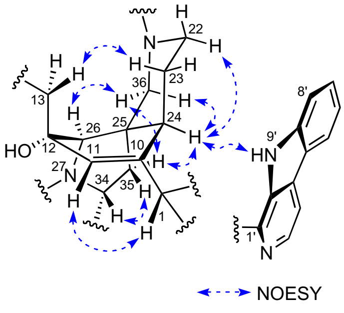


Figure 2. Selected NOESY correlations for partial structure of zamamidine C (**1**).

Table 2
^{1}H and ^{13}C NMR data of 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**) in $CDCl_3$

Position	δ_H	δ_C
1		157.56 (s)
3	3.82 (m) 3.69 (m)	48.40 (t)
4	2.66 (2H, nd)	19.10 (t)
4a	—	117.16 (s)
4b	—	150.85 (s)
5	6.96 (s)	103.48 (d)
6	—	131.93 (s)
7	6.87 (dd, 1.8, 8.6)	115.65 (d)
8	7.15 (d, 8.6)	113.00 (d)
8a	—	125.54 (s)
9a	—	127.85 (s)
10	—	68.18 (s)
11	3.52 (s)	65.12 (d)
12	—	71.05 (s)
13	1.89 (nd) 1.68 (nd)	37.14 (t)
14	2.23 (m) 2.08 (nd)	20.51 (t)
15	5.54 (q, 8.3)	127.58 (d)
16	5.45 (dt, 4.1, 10.8)	132.19 (d)
17	2.43 (m) 1.56 (nd)	25.06 (t)
18	1.36 (nd) 1.16 (m)	26.26 (t)
19	1.91 (m) 1.71 (nd)	25.06 (t)
20	2.51 (m) 2.26 (dd, 5.2, 11.6)	52.99 (t)
22	2.80 (m) 1.68 (nd)	48.69 (t)
23	2.11 (nd) 1.60 (nd)	28.81 (t)
24	2.31 (nd)	35.87 (d)
25	—	46.39 (s)
26	3.15 (s)	73.54 (d)
28	3.32 (m) 3.07 (m)	52.30 (t)
29	1.91 (nd) 1.60 (nd)	29.83 (t)
30	1.36 (2H, nd)	25.06 (t)
31	2.34 (nd) 2.16 (nd)	28.10 (t)
32	5.97 (q, 9.6)	136.93 (d)
33	5.22 (t, 9.6)	128.73 (d)
34	4.60 (brt)	56.02 (d)
35	2.90 (m) 1.60 (nd)	46.10 (t)
36	2.64 (nd) 2.07 (nd)	70.94 (t)

nd: Not determined by overlapping.

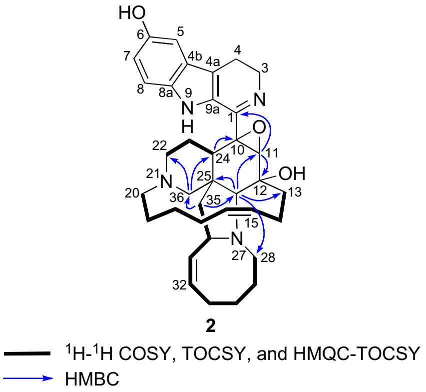


Figure 3. Selected 2D NMR correlations for 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**).

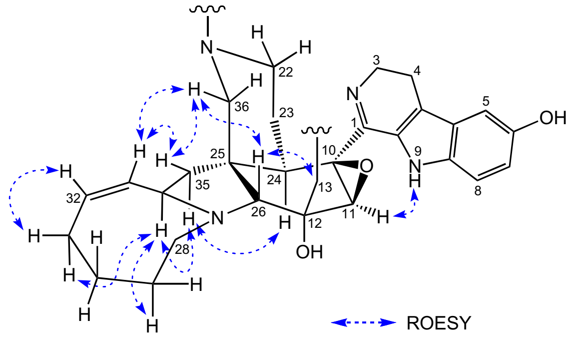
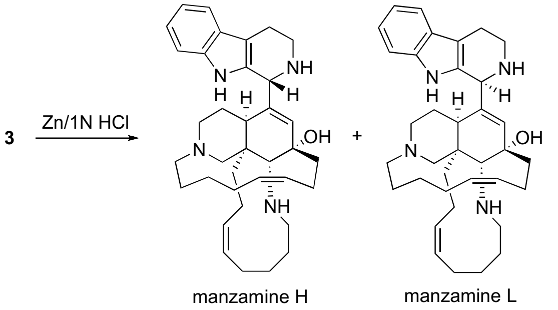


Figure 4. Selected ROESY correlations for partial structure of 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**).



Scheme 1. Conversion of 3,4-dihydromanzamine J *N*-oxide (**3**) to manzamines H and L.

Zamamidine C (**1**) is a new manzamine alkaloid possessing a second β-carboline ring via an ethylene unit at N-2 of manzamine D, while 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**) is the first manzamine alkaloid possessing an epoxide ring at C-10 and C-11. Zamamidine C (**1**), 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**), and 3,4-dihydromanzamine J *N*-oxide (**3**) showed cytotoxicities against P388 murine leukemia (IC_{50}: 14.1, 3.6, and 3.6 μg/mL, respectively), L1210 murine leukemia (IC_{50}: 13.6, 3.9, and 9.0 μg/mL, respectively), and KB human epidermoid carcinoma cells (IC_{50}: 17.7, 7.2, and 6.7 μg/mL, respectively) in vitro. Inhibitory activities of zamamidines A,[3] B,[3] and C (**1**), 3,4-dihydromanzamine J *N*-oxide (**3**), and manzamine A[5,6] against *Trypanosoma brucei brucei* (GUTat 3.1), the parasite associated with sleeping sickness,[14] and *Plasmodium falciparum* (K1 strain), the causative agent of malaria, were examined.[15] Zamamidines A, B, and C (**1**), 3,4-dihydromanzamine J *N*-oxide (**3**), and manzamine A showed inhibitory activities against *T.b. brucei* (IC_{50}: 1.04, 1.05, 0.27, 4.44, and 0.04 μg/mL, respectively), and *P. falciparum* (IC_{50}: 7.16, 12.20, 0.58, 7.02, and 0.97 μg/mL, respectively) in vitro.

Table 3
^{1}H and ^{13}C NMR data of 3,4-dihydromanzamine J *N*-oxide (**3**) in $CDCl_3$

Position	δ_H	δ_C
1	—	136.86 (s)
3	4.37 (ddd, 7.6, 11.7, 14.3) 4.25 (td, 7.3, 14.3)	61.39 (t)
4	3.24 (ddd, 7.3, 11.5, 16.5) 3.16 (td, 7.3, 14.3)	20.03 (t)
4a	—	108.23 (s)
4b	—	137.19 (s)
5	7.49 (d, 7.8)	119.01 (d)
6	7.16 (t, 7.7, 7.3)	120.96 (d)
7	7.21 (t, 7.7, 7.3)	123.48 (d)
8	7.32 (d, 7.8)	111.62 (d)
8a	—	126.00 (s)
9a	—	129.30 (s)
10	—	[a](s)
11	5.88 (s)	134.60 (d)
12	—	66.77 (s)
13	1.52 (nd) 2.04 (nd)	40.69 (t)
14	2.09 (nd) 2.32 (nd)	22.17 (t)
15	5.31 (m)	129.58 (d)
16	5.39 (m)	129.00 (d)
17	2.93 (nd) 1.62 (nd)	28.90 (t)
18	1.52 (nd)	27.28 (t)
19	2.15 (nd) 2.64 (nd)	25.98 (t)
20	2.82 (nd) 2.90 (nd)	53.44 (t)
22	2.88 (nd) 1.89 (nd)	49.23 (t)
23	1.75 (nd) 2.03 (nd)	32.70 (t)
24	2.88 (nd)	41.43 (d)
25	—	45.42 (s)
26	3.70 (br s)	58.73 (d)
28	2.31 (nd) 2.00 (nd)	59.05 (t)
29	1.49 (nd)	24.55 (t)
30	2.11 (nd)	26.08 (t)
31	1.75 (nd) 1.13 (nd)	28.36 (t)
32	5.61 (m)	131.92 (d)
33	5.44 (m)	130.72 (d)
34	1.25 (nd)	29.34 (t)
35	2.55 (nd) 1.57 (nd)	36.71 (t)
36	3.27 (nd) 2.08 (nd)	66.19 (t)

nd: Not determined by overlapping.
[a] Not observed.

3. Experimental section

3.1. General experimental procedures

Optical rotation was recorded on a JASCO P-1030 polarimeter. IR and UV spectra were recorded on JASCO FT/IR-230 and Shimadzu UV-1600PC spectrophotometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA500 spectrometer using 5 mm microcells (Shigemi Co., Ltd.) for $CDCl_3$. ESIMS spectra were recorded on a JEOL JMS-T100LP. The 7.26 and 77.0 ppm resonances of residual $CDCl_3$ were used as internal references for ^{1}H and ^{13}C NMR spectra, respectively.

3.2. Sponge description

Sponges (SS-1251 and SS-264) are light brown mounds. They have a faintly patterned, microhispid surface. The mesohyl is dense and compact and the sponges are springy and compressible. Sponges have a reticulate fiber skeleton. Primary fibers are multispicular, ∼50 μm wide. Secondary fibers form either isodictyal or rectangular meshes and have 1–3 spicules across and are ∼35 μm wide. Spicules are oxeas, small, straight or slightly curved, with short pencil points, 100×5 μm.

3.3. Extraction and isolation

The sponge *Amphimedon* sp. (SS-1251, 0.5 kg, wet weight) collected off the Zamami Island, Okinawa, was extracted with MeOH (2.2 L). The MeOH extract (28.28 g) was partitioned between EtOAc and H_2O (400 mL×2). The EtOAc-soluble fraction (3.08 g) was subjected to a silica gel column ($CHCl_3$/MeOH). The fraction eluted with 90% $CHCl_3$ was separated by a silica gel column (*n*-hexane/acetone/*N*,*N*-diethylamine and then MeOH). The fraction eluted with *n*-hexane/acetone/*N*,*N*-diethylamine (50:50:5) was purified by a silica gel column ($CHCl_3$/MeOH/H_2O) and then a silica gel column (MeCN/MeOH) to yield zamamidine C (**1**, 13.2 mg, 0.0026% wet weight) together with zamamidines A[3] (2.5 mg, 0.00049%) and B[3] (22.5 mg, 0.0044%). The fraction eluted with acetone/*N*,*N*-diethylamine (100:5) was separated by a silica gel column (*n*-hexane/EtOAc and then MeOH) followed by a silica gel column (MeCN/MeOH) to yield 3,4-dihydro-6-hydroxy-10,11-epoxymanzamine A (**2**, 1.4 mg, 0.00028% wet weight).

The sponge *Amphimedon* sp. (SS-264, 0.2 kg, wet weight) collected off the Kerama Islands, Okinawa, was extracted with MeOH (1.2 L). The MeOH extract (17.95 g) was partitioned between *n*-hexane and 90% MeOH aq (300 mL×2). The 90% MeOH layer was evaporated then partitioned between EtOAc and H_2O. The EtOAc-soluble fractions were evaporated under reduced pressure to give a residue (1.31 g), which was subjected to a silica gel column (*n*-hexane/acetone/*N*,*N*-diethylamine and then MeOH/*N*,*N*-diethylamine). The fraction eluted with *n*-hexane/acetone/*N*,*N*-diethylamine (50:50:5) was separated by a silica gel column ($CHCl_3$/MeOH and then $CHCl_3$/MeOH/H_2O), followed by a silica gel column (MeCN/MeOH) to yield 3,4-dihydromanzamine J *N*-oxide (**3**, 2.7 mg, 0.0014% wet weight).

3.4. Zamamidine C (1)

Pale yellow amorphous solid; $[\alpha]_D^{22}$ −54.9 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 352 (ε 4200), 339 (4100), 290 (21,600), 283 sh (17,200), 252 sh (24,500), 229 (60,200), and 203 (45,500) nm; CD (MeOH) λ_{ext} 223 (ε +16.9), 269 (−2.2), and 296 (−2.9) nm; IR (film) ν_{max} 3160, 2920, 2850, 1460, and 740 cm^{-1}; ESIMS *m*/*z* 747 $(M+H)^+$; HRESIMS *m*/*z* 747.4736 [$(M+H)^+$; calcd for $C_{49}H_{59}N_6O$ 747.4750].

3.5. 3,4-Dihydro-6-hydroxy-10,11-epoxymanzamine A (2)

Brown amorphous solid; $[\alpha]_D^{22}$ +35.2 (*c* 0.7, $CHCl_3$); UV (MeOH) λ_{max} 332 (ε 6700), 289 sh (2200), 232 sh (7700), 212 sh (14,700), and 204 (16,000) nm; IR (film) ν_{max} 3180, 2920, 2850, 1460, and 670 cm^{-1}; ESIMS *m*/*z* 583 $(M+H)^+$; HRESIMS *m*/*z* 583.3634 [$(M+H)^+$; calcd for $C_{36}H_{47}N_4O_3$ 583.3648].

3.6. 3,4-Dihydromanzamine J *N*-oxide (3)

Pale yellow amorphous solid; $[\alpha]_D^{23}$ +115.5 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} 354 (ε 11,000), 271 sh (7500), 252 sh (10,000), and 220 (14,000) nm; IR (film) ν_{max} 3210, 2920, 1450, 1320, 1150, and 750 cm^{-1}; ESIMS *m*/*z* 569 $(M+H)^+$; HRESIMS *m*/*z* 569.3870 [$(M+H)^+$; calcd for $C_{36}H_{49}N_4O_2$ 569.3856].

3.7. Reduction of 3,4-dihydromanzamine J *N*-oxide (3)

3,4-Dihydromanzamine J *N*-oxide (**3**, 0.5 mg) was treated with Zn dust (2.0 mg) in 1 N HCl (200 μL) at rt for 5 h. After filtration of the catalyst, the filtrate was made alkaline with 25% NH_4OH, and the free alkaloid was extracted with EtOAc (2 mL×2). The EtOAc-soluble part was purified by a silica gel column (*n*-hexane/acetone), followed by HPLC (Senshu Pak Silica-3301-N, Senshu Scientific Co., Ltd., 8×200 mm; eluent *n*-hexane/*i*-PrOH/Et_3N, 95:5:2; flow rate, 2.0 mL/min, UV detection at 254 nm) to afford manzamines L (0.1 mg, t_R 7.6 min) and H (0.1 mg, t_R 8.4 min).

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