Bioorganic & Medicinal Chemistry Letters xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Mariannamides A and B, new cyclic octapeptides isolated from *Mariannaea elegans* NBRC102301

Kan'ichiro Ishiuchi[a,*], Dai Hirose[b], Taishi Kondo[a], Kohei Watanabe[b], Kazuyoshi Terasaka[a], Toshiaki Makino[a]

[a] *Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-Dori, Mizuho-ku, Nagoya 467-8603, Aichi, Japan*
[b] *School of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi 274-8555, Chiba, Japan*

ARTICLE INFO

Keywords:
Mariannaea elegans NBRC102301
Mariannamides A-B
Cyclic peptides
Sirtuin 1

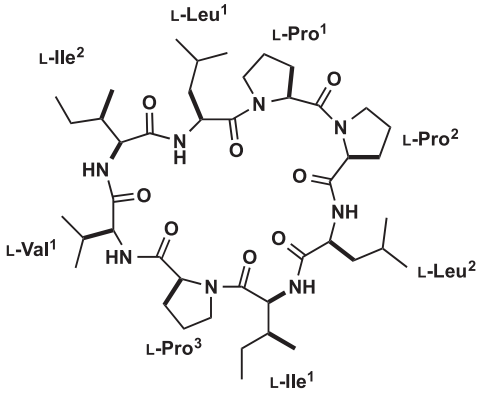
ABSTRACT

Two new cyclic octapeptides, mariannamides A (**1**) and B (**2**), have been isolated from *Mariannaea elegans* NBRC102301, a *Pinus densiflora*-derived filamentous fungus. Their structures were elucidated to be *cyclo*-(L-Leu[1]-L-Pro[1]-L-Pro[2]-L-Leu[2]-L-Ile[1]-L-Pro[3]-L-Val[1]-L-Ile[2]) and *cyclo*-(L-Leu[1]-L-Pro[1]-L-Pro[2]-L-Leu[2]-L-Ile[1]-L-Pro[3]-L-Val[1]-L-Val[2]) based on spectroscopic data and Marfey's method. Mariannamide A (**1**) promoted mRNA expression of sirtuin 1 (SIRT1) in C2C12 cells, a mouse skeletal muscle myoblast cell line, and showed the antimicrobial activity against *Escherichia coli* and *Cryptococcus neoformans*.

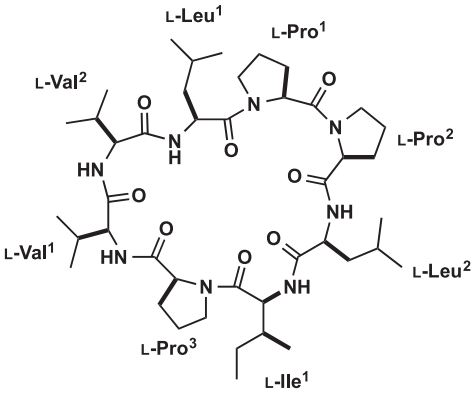
The genus *Mariannaea* belongs to the Nectriaceae, and the species of this genus can be isolated from soil, dead plant material, and insect larva.[1,2] Currently, the following 15 species are accepted in the genus; *M. aquaticola*, *M. camptospora*, *M. catenulata*, *M. chlamydospora*, *M. cinerea*, *M. dimorpha*, *M. elegans*, *M. fusiformis*, *M. humicola*, *M. lignicola*, *M. macrochlamydospora*, *M. pinicola*, *M. punicea*, *M. samuelsii*, *M. superimposita*.[3] There are few reports regarding the chemical components of *Mariannaea* fungi. Only three polyketides, marianins A and B from *M. camptospora* TAMA 118,[4] and mariannaeapyrone from *M. elegans* UR 742,[5] have ever been isolated. Additionally, Mihara *et al.* reported a glycosphingolipid, Glc1-6Galβ1-Cer from *M. elegans* JCM12789.[6] We were interested in the metabolites produced by *Mariannaea* genus fungi and their biological profiles, and began to survey the chemical constituents of *M. elegans* NBRC102301. As a result, two new cyclic octapeptides, mariannamides A (**1**) and B (**2**), were isolated. According to a review paper by Zhou *et al.*, 293 fungal cyclic peptides excluding cyclodipeptides have been discovered over the past 50 years, of them, octapeptides only occupy 4.4% (13 compounds).[7] Activation of sirtuin 1 (SIRT1), an NAD^+-dependent deacetylase, in the muscle tissue causes deacetylation of peroxisome proliferator-activated receptor (PPAR)-γ coactivator (PGC)-1α, a transcriptional coactivator binding to PPARγ, to improve the mitochondrial function such as the oxygen consumption and the heat-conservation abilities, and is a promising strategy for treating diseases related to aging.[8] Resveratrol is known to extend life span and impact mitochondrial function and metabolic homeostasis by activating SIRT1.[8] Recently, the number of reports about other natural products enhancing SIRT1 activity are increasing.[9] Herein, we describe the isolation and structural elucidation of **1** and **2** and provide evaluations of their SIRT1 activities in C2C12 cells, a mouse skeletal muscle myoblast cell line, and their antimicrobial activities.

* Corresponding author.
E-mail address: ishiuchi@phar.nagoya-cu.ac.jp (K. Ishiuchi).

https://doi.org/10.1016/j.bmcl.2019.126946
Received 21 September 2019; Received in revised form 24 December 2019; Accepted 29 December 2019



1



2

The culture broth of *M. elegans* NBRC102301 was extracted using ethyl acetate (EtOAc). The EtOAc-soluble fraction was subjected to a silica gel column, an ODS column, and C_{18} HPLC to obtain mariannamides A (**1**) and B (**2**).[10] The molecular formula of mariannamide A (**1**)[11] was elucidated to be $C_{44}H_{74}N_8O_8$ by HRESIMS [*m/z* 865.5535 $(M+Na)^+$, Δ + 0.8 mmu]. Five amide protons observed in the ^{1}H NMR spectrum (DMSO-d_6) indicated that **1** is a peptidic compound (Table 1). ^{1}H and ^{13}C NMR data and the HSQC spectrum of **1** revealed 44 carbon signals due to eight carbonyl groups, 13 sp^3 methine groups, 13 sp^3 methylene groups, and ten sp^3 methyl groups. Among these, eight sp^3 methine carbons (δ_C 65.7, 60.9, 60.2, 59.4, 59.1, 54.5, 53.9, and 49.7) were ascribed to α-carbons of amino acid residues, which indicated that **1** is a cyclic octapeptide.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of mariannamide A (**1**) in DMSO-d_6.

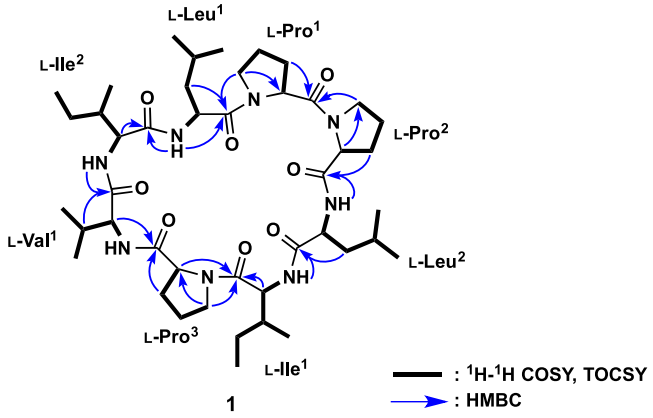
position	δ_H	δ_C	position	δ_H	δ_C
Leu^1			Ile^1		
α	4.42 (1H, nd)	49.7	α	4.42 (1H, nd)	53.9
β	1.79 (1H, nd)	40.6	β	1.54 (1H, nd)	37.9
	1.12 (1H, nd)		γ	1.43 (1H, m)	23.7
γ	1.54 (1H, nd)	24.8		0.97 (1H, m)	
Me	0.88 (3H, nd)	21.4	δ	0.79 (3H, t 7.0 Hz)	10.7
Me	0.84 (3H, nd)	23.4	β-Me	0.89 (3H, nd)	14.7
CO		168.9	CO		171.1
NH	7.59 (1H, d 7.0 Hz)		NH	7.39 (1H, d 9.5 Hz)	
Pro^1			Pro^3		
α	4.02 (1H, dd 9.0, 3.5 Hz)	59.1	α	3.96 (1H, dd 8.0, 8.0 Hz)	60.9
β	2.23 (1H, nd)	27.7	β	2.17 (1H, nd)	29.1
	1.70 (1H, nd)			1.77 (1H, nd)	
γ	1.91 (1H, m)	24.5	γ	2.01 (1H, nd)	24.7
	1.82 (1H, nd)			1.82 (1H, nd)	
δ	3.35 (2H, nd)	46.6	δ	3.59 (2H, m)	47.7
CO		170.2	CO		173.1
Pro^2			Val		
α	4.46 (1H, nd)	60.2	α	3.03 (1H, dd 10.5, 7.0 Hz)	65.7
β	2.21 (1H, nd)	30.9	β	2.65 (1H, m)	27.2
	2.04 (1H, nd)		Me	0.84 (3H, nd)	19.6
γ	1.82 (1H, nd)	21.7	Me	0.82 (3H, nd)	19.3
	1.49 (1H, nd)		CO		172.0
δ	3.37 (2H, nd)	46.2	NH	8.91 (1H, d 7.0 Hz)	
CO		170.4			
			Ile^2		
Leu^2			α	3.87 (1H, dd 7.5, 7.5 Hz)	59.4
α	4.07 (1H, m)	54.5	β	1.55 (1H, nd)	36.5
β	1.75 (1H, nd)	40.2	γ	1.37 (1H, m)	24.6
	1.56 (1H, nd)			1.10 (1H, nd)	
γ	1.56 (1H, nd)	24.6	δ	0.81 (3H, nd)	10.7
Me	0.89 (3H, nd)	22.3	β-Me	0.82 (3H, nd)	15.4
Me	0.82 (3H, nd)	21.5	CO		170.6
CO		171.4	NH	8.54 (1H, d 7.5 Hz)	
NH	7.94 (1H, d 7.0 Hz)				

nd: *J*-values were not determined because of overlapping with other signals.

2D NMR correlations of ^{1}H–^{1}H COSY, TOCSY, and HMBC spectra revealed that **1** possesses three proline, one valine, two leucine, and two isoleucine residues (Fig. 1). The connectivities of amino acid residues were elucidated by HMBC spectral analysis. The HMBC correlations for Ile^1-NH (δ_H 7.59) to Pro^2-CO (δ_C 171.4) and Leu^2-NH (δ_H 7.94) to Pro^2-CO (δ_C 170.4) revealed amide linkages of Ile^1 and Pro^2 through Leu^2. Furthermore, HMBC cross peaks of Pro^1-Hδ (δ_H 3.35) to Leu^1-CO (δ_C 168.9), Leu^1-NH (δ_H 7.59) to Pro^2-CO (δ_C 170.6), Ile^2-NH (δ_H 8.54) to Val-CO (δ_C 172.0), and Val-NH (δ_H 8.91) to Pro^3-CO (δ_C 173.1) indicated sequences of Pro^1-Leu^1-Pro^2-Ile^2-Val-Pro^3. Finally, HMBC correlations for Pro^2-Hδ (δ_H 3.37) to Pro^1-CO (δ_C 170.2) and Pro^3-Hδ (δ_H 3.59) to Ile^1-CO (δ_C 171.1) revealed amide linkages between Pro^1 and Pro^2 and between Ile^1 and Pro^3. Thus, the planar structure of **1** was elucidated to be *cyclo*-(Leu^1-Pro^1-Pro^2-Leu^2-Ile^1-Pro^3-Val^1-Ile^2) (Fig. 1).

The geometries of the amide bonds of Leu^1-Pro^1, Pro^1-Pro^2, and Ile^1-Pro^3 were empirically assigned from the $\delta_{C\gamma}$ and $\Delta\delta_{C\beta\text{-}C\gamma}$ values of prolines.[13] These values (Pro^1 $\delta_{C\gamma}$ 24.5 ppm, $\Delta\delta_{C\beta\text{-}C\gamma}$ 3.2 ppm; Pro^2 $\delta_{C\gamma}$ 21.7 ppm, $\Delta\delta_{C\beta\text{-}C\gamma}$ 9.2 ppm; and Pro^3 $\delta_{C\gamma}$ 24.7 ppm, $\Delta\delta_{C\beta\text{-}C\gamma}$ 4.4 ppm) indicated that the geometries are *trans* (Leu^1-Pro^1), *cis* (Pro^1-Pro^2), and *trans* (Ile^1-Pro^3). The Marfey's analysis[14] of the hydrolysates of **1** revealed that the absolute configurations of the amino acids were all in the L-form. The difference between L-Ile and L-*allo*-Ile was distinguished by HPLC analysis of the 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) derivatives[15] of the hydrolysate of **1**, which revealed that the two Ile residues were both L-Ile.

The molecular formula of mariannamide B (**2**)[12] was elucidated to be $C_{43}H_{72}N_8O_8$ by the HRESIMS [*m/z* 851.5378 $(M+Na)^+$, Δ + 0.7 mmu], and it indicated that the difference between **2** and **1** was



1

Fig. 1. Selected 2D correlations for mariannamide A (**1**).

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of mariannamide B (**2**) in DMSO-d_6.

position	δ_H	δ_C	position	δ_H	δ_C
Leu^1			Ile^1		
α	4.41 (1H, nd)	49.7	α	4.42 (1H, nd)	53.8
β	1.80 (1H, nd)	40.6	β	1.54 (1H, nd)	37.8
	1.13 (1H, ddd 12.5, 9.0, 3.0 Hz)		γ	1.43 (1H, m)	23.8
γ	1.55 (1H, nd)	24.9		0.97 (1H, m)	
Me	0.88 (3H, nd)	21.5	δ	0.78 (3H, t 7.5 Hz)	10.6
Me	0.84 (3H, nd)	23.4	β-Me	0.87 (3H, nd)	14.8
CO		168.9	CO		171.1
NH	7.56 (1H, d 7.5 Hz)		NH	7.39 (1H, d 9.5 Hz)	
Pro^1			Pro^3		
α	4.02 (1H, dd 9.0, 3.5 Hz)	59.1	α	3.96 (1H, dd 8.0, 8.0 Hz)	60.9
β	2.23 (1H, nd)	27.7	β	2.17 (1H, nd)	29.1
	1.70 (1H, nd)			1.78 (1H, nd)	
γ	1.91 (1H, m)	24.6	γ	2.01 (1H, nd)	24.7
	1.81 (1H, nd)			1.81 (1H, nd)	
δ	3.35 (2H, nd)	46.6	δ	3.59 (2H, m)	47.7
CO		170.2	CO		173.1
Pro^2			Val^1		
α	4.46 (1H, nd)	60.2	α	3.04 (1H, dd 10.0, 6.5 Hz)	65.7
β	2.21 (1H, nd)	30.8	β	2.67 (1H, m)	27.2
	2.04 (1H, nd)		Me	0.84 (3H, nd)	19.6
γ	1.82 (1H, nd)	21.7	Me	0.83 (3H, nd)	19.3
	1.49 (1H, nd)		CO		172.1
δ	3.37 (2H, nd)	46.2	NH	8.90 (1H, d 7.0 Hz)	
CO		170.4			
			Val^2		
Leu^2			α	3.84 (1H, dd 7.0, 7.0 Hz)	60.5
α	4.08 (1H, m)	54.4	β	1.80 (1H, nd)	30.3
β	1.75 (1H, nd)	40.2	Me	0.87 (3H, nd)	19.2
	1.56 (1H, nd)		Me	0.84 (3H, nd)	18.1
γ	1.56 (1H, nd)	24.5	CO		170.7
Me	0.89 (3H, nd)	22.3	NH	8.54 (1H, d 7.0 Hz)	
Me	0.82 (3H, nd)	21.5			
CO		171.4			
NH	7.94 (1H, d 7.5 Hz)				

nd: *J*-values were not determined because of overlapping with other signals.

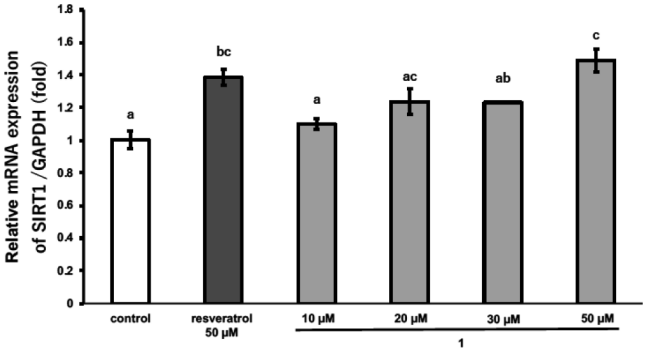


Fig. 3. The effect of mariannamide A (**1**) on mRNA expression levels of SIRT1 in C2C12 cells. GAPDH was used as the reference gene. The data are presented as mean ± S.E.M. (n = 3). Different letters indicate significant differences as determined by Turkey's multiple comparison procedure (P < 0.05).

only due to a methyl group. ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1** except for the L-Ile^2 residue of **1** (Table 2). Comparison of the HSQC spectra of **1** and **2** revealed that a methine cross peak (δ_H 1.55, δ_C 36.5) at the β-position in L-Ile^2 of **1** was shifted to δ_H 1.80 and δ_C 30.3 in **2** and that methylene cross peaks (δ_H 1.37, 1.10, δ_C 24.6) at the γ-position in L-Ile^2 of **1** was disappeared in **2** (Figs. S6 and S13). ^{1}H–^{1}H COSY and HMBC analyses revealed that this newly observed methine formed a valine residue (Fig. 2). Furthermore, HMBC correlations for Leu^1-NH (δ_H 7.56) to Val^2-CO (δ_C 170.7) and Val^2-NH (δ_H 8.54) to Val^1-CO (δ_C 173.1) revealed the sequence of -Val^1-Val^2-Leu^1-. Thus, the planar structure of **2** was elucidated to be *cyclo*-(Leu^1-Pro^1-Pro^2-Leu^2-Ile^1-Pro^3-Val^1-Val^2) (Fig. 2). These values (Pro^1 $\delta_{C\gamma}$ 24.6 ppm, $\Delta\delta_{C\beta\text{-}C\gamma}$ 3.1 ppm; Pro^2 $\delta_{C\gamma}$ 21.7 ppm, $\Delta\delta_{C\beta\text{-}C\gamma}$ 9.1 ppm; and Pro^3 $\delta_{C\gamma}$ 24.7 ppm, $\Delta\delta_{C\beta\text{-}C\gamma}$ 4.4 ppm) indicated that the geometries of the amide bonds of Leu^1-Pro^1, Pro^1-Pro^2, and Ile^1-Pro^3 were *trans*, *cis*, and *trans*, respectively.[13] The absolute configurations of the amino acid residues were elucidated to all be the L-form by the Marfey's method.[14] Finally, the stereoisomer of the Ile residue was assigned to be L-Ile via HPLC analysis of the GITC derivative[15] of **2**.

We tested the effect of **1** on the mRNA expression levels of SIRT1 in C2C12 cells using qRT-PCR.[16,17,19] As shown in Fig. 3, mRNA expression by 50 μM of **1** was detected at 1.5-fold higher level compared with control (DMSO) group with statistical significance, and this activity was as the same level of resveratrol used as positive control. To our

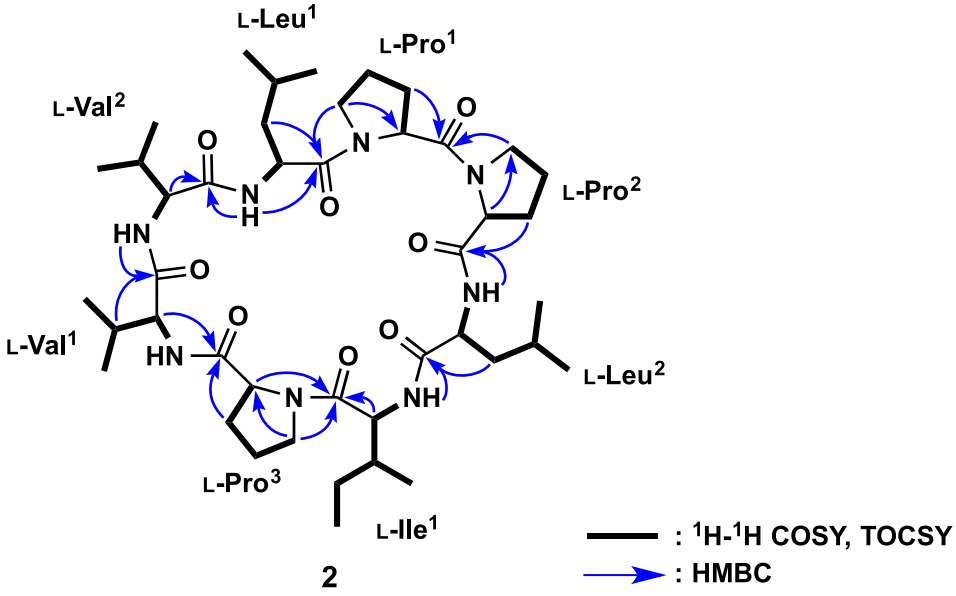


Fig. 2. Selected 2D correlations for mariannamide B (**2**).

knowledge, this is the first report of the cyclic peptide activating SIRT1. Furthermore, the antimicrobial activities of mariannamides A (**1**) and B (**2**) against bacteria (*Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Bacillus subtilis*, and *Enterococcus faecalis*) and fungi (*Candida auris, C. guilliermondii, C. parapsilosis, C. glabrata, C. albicans* and *Cryptococcus neoformans*) were evaluated.[18] Mariannamide A (**1**) showed the inhibition against only *E. coli* and *C. neoformans* with diameters of the inhibition zone of 9.4 ± 0.4 mm and 7.7 ± 0.2 mm, respectively. Compound **2** did not show any antimicrobial activities against above all the microbials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was partly supported by Grant-in Aid for Young Scientists B (No. 17K15467 to K.I.) from Japan Society for the Promotion of Science (JSPS).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126946.

References

1. Gräfenhan T, Schroers HJ, Nirenberg HI, Seifert KA. *Stud Mycol.* 2011;68:79–113.
2. Tang L, Hyun M, Yun Y, Suh D, Kim S, Sung G. *Mycobiology.* 2012;40:14–19.
3. Hu DM, Wang M, Cai L. *Mycol Prog.* 2017;16:271–283.
4. Fukuda T, Sudho Y, Tsuchiya Y, Okuda T, Fujimori F, Igarashi Y. *J Nat Prod.* 2011;74:1327–1330.
5. Fabian K, Anke T, Sterner O. *Z Naturforsch.* 2001;56:106–110.
6. Tani Y, Nakamura K, Sawa R, et al. *Biosci Biotechnol Biochem.* 2013;77:754–759.
7. Wang X, Lin M, Xu D, Lai D, Zhou L. *Molecules.* 2017;22:2069.
8. Lagouge M, Argmann C, Gerhart-Hines Z, et al. *Cell.* 2006;127:1109–1122.
9. (a) Wan HF, Li JX, Liao HT, et al. *Mol Med Rep.* 2019;19:555–562;
   (b) Sun P, Yin JB, Liu LH, et al. *Biosci Rep.* 2019:39 in press;
   (c) Yang S, Lee W, Lee BS, et al. *Am J Clin Med.* 2019;47:613–633;
   (d) Li K, Zhai M, Jiang L, et al. *Oxid Med Cell Longev.* 2019;9:6746907;
   (e) Peng XP, Li XH, Li Y, Huang XT, Luo ZQ. *Int Immunopharmacol.* 2019;70:520–529.
10.. *Extraction and isolation procedure of mariannamides A (**1**) and B (**2**)*: *Mariannaea elegans* NBRC102301 was cultured on a PDA plate at 25 °C for 4 days. Ten 0.3-cm squared pieces of the grown mycelia were inoculated in 20 Erlenmeyer flasks (300 mL) containing 100 mL of potato dextrose broth medium, and shaken at 180 rpm at 25 °C for 7 days. The broth (2 L) was extracted with EtOAc (1 L × 3) to give EtOAc-soluble materials (415 mg). The EtOAc-soluble fraction was subjected to a silica gel column ($CHCl_3$/MeOH/H_2O, 1:0:0 → 6:4:1). A fraction eluted with $CHCl_3$/MeOH (20:1 and 10:1) was separated using a C_{18} column (MeOH/H_2O, 0:1 → 1:0). The fraction eluted with MeOH/H_2O (1:0) was further purified using a C_{18} HPLC (COSMOSIL 5C_{18}-AR-II, 20 mm I.D. × 250 mm, Nacalai Tesque, Kyoto, Japan, solvent of MeOH/H_2O, 65:35, flow rate of 8.0 mL/min, detection at 220 nm) to afford mariannamides A (**1**, 6.5 mg) and B (**2**, 5.1 mg).
11.. *Mariannamide A (**1**)*: a colorless amorphous solid; $[\alpha]_D^{30}$ −108 (*c* 1.0, MeOH); IR (ATR) ν_{max} 3356, 3248, 2959, 2874, 1651, 1616, 1520, and 1454 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; ESIMS *m/z* 865 $[M+Na]^+$; HRESIMS *m/z* 865.5535 $[M+Na]^+$ (calcd for $C_{44}H_{74}N_8O_8Na$, 865.5527).
12.. *Mariannamide B (**2**)*: a colorless amorphous solid; $[\alpha]_D^{30}$ −111 (*c* 1.0, MeOH); IR (ATR) ν_{max} 3399, 3291, 2963, 2874, 1651, 1616, 1520, and 1454 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; ESIMS *m/z* 851 $[M+Na]^+$; HRESIMS *m/z* 851.5378 $[M+Na]^+$ (calcd for $C_{43}H_{72}N_8O_8Na$, 851.5370).
13. (a) Dorman DE, Bovey FA. *J Org Chem.* 1973;38:1719–1722;
   (b) Dorman DE, Bovey FA. *J Org Chem.* 1973;38:2379–2383;
   (c) Siemion IZ, Wieland T, Pook KH. *Angew Chem Int Edit.* 1975;14:702–703.
14. Marfey P. *Carlsberg Res Commun.* 1984;49:591–596.
15. (a) Nimura N, Toyama A, Kinoshita T. *J Chromatogr, A.* 1984;316:547–552;
   (b) Hess S, Gustafson KR, Milanowski DJ, Alvira E, Lipton MA, Pannell LKJ. *Chromatgr, A.* 2004;1035:211–219;
   (c) Takada K, Ninomiya A, Naruse M, et al. *J Org Chem.* 2013;78:6746–6750.
16.. *Cell culture*: The C2C12 cell line (derived from a mouse skeletal muscle myoblast) was obtained from RIKEN BioResource Research Center (BRC, Tsukuba, Japan). C2C12 cells were grown at 37 °C in a Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO, USA) containing 10% fetal bovine serum (Sigma), 100 units/mL Penicillin, and 100 μg/mL streptomycin (both from Nacalai Tesque) under a humidified atmosphere containing 5% CO_2 in air up to 80% confluent state. Media were replaced with those after the cell detachment by treating with 0.25% trypsin (Sigma), 0.02% EDTA·2Na. The C2C12 cells were seeded in 24-well plates at 2 × 10^5 cells/well and cultured up to 90% confluent. Media were replaced with DMEM containing 2% horse serum (Thermo Fisher Scientific, Walthem, MA, USA), 100 units/mL penicillin, 100 μg/mL streptomycin, and cultured to induce differentiation for two days. DMSO, resveratrol (50 μM, Fujifilm Wako Pure Chemical, Osaka, Japan), **1** (10, 20, 30, or 50 μM), or **2** (10, 20, 30, or 50 μM) was added in the culture media and incubated for 24 h.
17.. *Gene expression analysis by quantitative reverse transcription-polymerase chain reaction (qRT-PCR)*: Total RNA was prepared using RNAiso Plus (Takara Bio, Kusatsu, Japan). First-strand cDNAs for qRT-PCR were synthesized from 500 ng of the total RNA using PrimeScript RT Master Mix (Perfect Real Time) (Takara Bio). SIRT1 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) amplification was performed with StepOne™ or StepOnePlus™ real-time PCR system (Thermo Fisher Scientific) using SYBR® Select Master Mix (Thermo Fisher Scientific) according to the manufacturer's instructions. The specific primers used were: SIRT1_Fw (TCCTGTGAAAGTGATGACGATGA); SIRT1_Rv (ACAAAAGTATATGGACCTATCCGC) GAPDH_Fw (CAAGATTGTCAGCAATGCATCC); and GAPDH_Rv (CCTTCCACAATGCCAAAGTTG). The GAPDH primer pair was used as an internal control. PCR condition was as follows: precycling at 95 °C for 10 min; followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The expression level of mRNA was analyzed by ΔΔCt method. The experiments were performed in triplicate for each compound.
18.. *Antimicrobial assay*: The antimicrobial activity of the compounds was evaluated using the disc diffusion method. The fungal cultures (*C. neoformans* strain IFM 40215, *C. guilliermondii* strain TIMM 0260, *C. parapsilosis* strain IFM 40218, *C. glabrata* strain IFM 40217, *C. albicans* strain IFM 40213, and *C. auris* strain JCM 15448) were pre-cultured on YPD agar (10 g of yeast extract, 20 g of peptone, 20 g of glucose, 15 g of agar, and 1 L of distilled water) at 37 °C for 24 h. The fungal cells on the pre-cultured plate were suspended in Müller Hinton broth. The bacterial cultures (*E. coli* strain U5-41, MRSA strain N315, *B. subtilis* strain HA 101, and *E. faecalis* strain JCM 7783) were inoculated in Müller Hinton broth and incubated at 37 °C for 24 h. The turbidity of the fungal and the bacterial suspensions were adjusted to 0.5 of the McFarland standard. Sterile filter paper discs (6 mm) were impregnated with 20 μL (2 μg) of each compound. The discs were put on the Müller Hinton agar spread with the fungal suspension. After incubation at 37 °C for 24 h, the inhibition zones, including the diameter of the disc were measured. The discs without the compounds (5% DMSO) were used as a negative control. The experiments were performed in triplicate for each compound.
19.. *Statistical analysis*: All the data are expressed as mean ± standard error of the means (S.E.M). Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison procedure. $P < 0.05$ was considered as statistically significant.