VOL. 56 NO. 10, OCT. 2003 THE JOURNAL OF ANTIBIOTICS pp. 856 — 861

NOTES

New Cytotoxic Bafilomycin C_1-amide Produced by *Kitasatospora cheerisanensis*

SURK-SIK MOON* and WEY-HYUNG HWANG

Department of Chemistry, Kongju National University, Kongju 314-701, Korea

YOUNG RYUN CHUNG

Department of Microbiology, Gyeongsang National University, Jinju 660-701, Korea

JONGHEON SHIN

Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 110-460, Korea

(Received for publication June 12, 2003)

The genus *Kitasatosporia* comprises a group of filamentous and aerobic Gram-positive bacteria and is phenotypically closely related to *Stereptomyces* strains. Characteristics of the genus *Kitasatosporia* are the presence of *meso*-diaminopimelic acid, LL-diaminopimelic acid, and galactose in the cell wall.[1,2] During the course of our search for antifungal metabolites from microbes, we found that methanolic extracts of the culture broth of a new species of the genus *Kitasatosporia* isolated from a soil sample inhibited the growth of plant pathogens, such as *Rhizoctonia solani*, *Botrytis cinerea*, and *Phythium ultimum*. Classification and characteristics of the species have been reported.[3] In this paper, we report the isolation, structure determination, and biological activities of a new bafilomycin derivative **2** designated bafilomycin C_1-amide as well as the known bafilomycins, **1**, **3**, **4**, and **5** from the fermentation broth.

Experimental

Microorganism

The producing microorganism was a newly identified isolate as a strain of *Kitasatospora cheerisanensis* YC75 collected from the Cheerisan mountain located at the southern part of Korea (deposited at the Korean Collection for Type Cultures as KCTC 2395).[3]

Fermentation

A stock culture of *K. cheerisanensis* YC75 was cultured on potato dextrose agar at 28°C for 7 days and two or three loops of the bacteria from the agar was inoculated to a 1-liter Erlenmeyer flask containing tryptic soy broth (200 ml) as a seed medium. The flask was then incubated at 28°C for 3 days on a rotary shaker (180 rpm) and the whole culture broth was transferred into a 5-liter fermenter containing production medium (3 liters) composed of glucose 0.5 g, soluble starch 20 g, beef extract 1 g, soybean flour 25 g, K_2HPO_4 0.5 g, NaCl 2 g, $CaCO_3$ 6 g, and yeast extract 2 g in 1 liter of distilled water. The fermentation was carried out at 28°C for 4 days with agitation at 500 rpm and aeration at 3 liters/minute.

Biological Assays

The paper disc diffusion assay was used for measuring the antifungal activity and the protein-binding sulforhodamine B (SRB) method for the cytotoxicity against cancer cell lines.[4,5]

Analytical Procedures

The UV absorption spectra were obtained on a Waters 996 Photodiode Array Detector during HPLC. The IR spectra were taken in KBr pellets using a Perkin Elmer BX FT-IR spectrometer. The mass spectra were obtained on a JEOL HX110A-HX110A Tandem HR mass spectrometer. The NMR spectra for ^1H and ^{13}C were obtained on a Varian Unity-500 NMR spectrometer at 500 MHz and 125 MHz, respectively. The chemical shifts given in ppm were referenced to solvent peaks: δ_H 3.30 and δ_C 49.0 for methanol-d_4; δ_H 2.05 and δ_C C 29.9 (methyl) for acetone-d_6.

Isolation

The fermentation broth (5 liters, pH 7.2) was centrifuged at 4,000 *g* and cell cake was collected (424 g, wet). The cell cake was extracted with methanol (1 liter×3). The methanolic solutions combined were concentrated to

* Corresponding author: ssmoon@kongju.ac.kr

dryness, which was suspended in methanol (500 ml) and filtered. The filtrate (13 g) was concentrated and suspended in distilled water and chromatographed (Merck, C-18, 43~60 μm, 30×300 mm) under medium-pressure with a continuous gradient elution of water to methanol (10 ml/minute) to obtain 18 fractions (about 200 ml/each fraction). The 15th fraction (methanol-eluate) active against *Rhizoctonia solani* was concentrated to give an orange solid (669 mg), which was applied onto a column of Toyopearl HW-40S (15×950 mm). The column was eluted with methanol to obtain 80 tubes (4 ml/each) and each tube was assayed against *R. solani*. Two groups (12th to 22nd and 23rd to 32nd) of the active fractions pooled were concentrated to obtain orange solids, 223 mg and 80 mg, respectively. Final purification of the active compounds was achieved by reverse-phase HPLC (20×250 mm, Pegasil ODS, Shensu PAK). From the above 223 mg-sample, using continuous gradient of 60% aqueous acetonitrile to 100% acetonitrile for 130 minutes with flow of 7 ml/minute, compound **5** (3.5 mg), **4** (7.0 mg), **2** (4.7 mg), and **3** (20 mg) were eluted in the retention time of 55.5, 65.9, 69.3, and 107.9 minutes, respectively. The above 80-mg sample afforded compound **1** (17 mg) as an orange solid using continuous gradient of 4:1:5 to 2:2:1 acetonitrile/methanol/water for 40 minutes in the retention time of 17.0 minutes.

The compound **2** showed the following physicochemical properties: yellow oil; UV λ_{max} 244.3, 283.3 nm; IR (KBr) 3420, 2923, 2867, 1720, 1684, 1453, 1381, 1249, 1169, 1101, 917 cm^{-1}; (+) HRFAB-MS *m/z* 742.4167 $[M+Na]^+$ (calcd for $C_{39}H_{61}NO_{11}Na$, 742.4142), 643.0, 537.1, 413.0, 175.9; ^1H and ^{13}C NMR data (acetone-d_6) are listed in Table 1.

Results

Isolation

The culture filtrate was extracted with methanol. The filtrate was fractionated on C-18 flash column chromatography. The bioactive fractions were subjected to a series of chromatographic techniques such as size exclusion and prep C-18 HPLC to afford compounds **1**~**5**. Analytical HPLC of the compounds **1**, **5**, **4**, **2**, and **3** showed the retention times of 5.7, 22.8, 28.6, 29.8, and 52.6 minutes, respectively, on a C-18 column (4.6×250 mm, Pegasil ODS, 0.7 ml/minute, 70% aqueous acetonitrile to 100% acetonitrile for 60 minutes).

Structure-Elucidation

The molecular formula of compound **1** was deduced to be $C_{39}H_{60}O_{12}$ as $[M+Na]^+$ was observed at *m/z* 743.4022 (calcd for $C_{39}H_{60}O_{12}Na$, 743.3982) in the positive high resolution FAB mass spectrum. The UV absorption maxima at 245.5 and 284.5 nm and the IR absorptions at 1720, 1686, 1586, 1100 cm^{-1} suggest the presence of a conjugated diene and conjugated diene ester moiety. The ^{13}C NMR spectrum (methanol-d_6) of compound **1** contained thirteen sp^2-carbons including three C=O groups at δ 172.6, 168.1, 167.6, 145.6, 145.5, 143.3, 142.3, 134.8, 134.6, 133.2, 128.8, 126.9, 125.5 ppm. In addition, nine oxygen-bearing sp^3-carbons with a hemiketal functionality were observed at δ 100.3, 84.4, 81.2, 77.7, 77.3, 75.5, 72.0, 60.6, 55.9 ppm. The DEPT experiment showed the presence of eleven methyl groups at 60.6, 55.9, 22.5, 21.8, 20.1, 17.9, 14.7, 14.1, 12.6, 10.5, 7.25 ppm. The former two resonances were readily interpreted as methoxy groups. Combination of extensive 2-D NMR experiments (H,H-COSY, TOCSY, ROSEY, HSQC, and HMBC) provided the total structure of compound **1** which was previously reported as bafilomycin C_1.[6,7]

The molecular formula of compound **2** was determined to be $C_{39}H_{61}NO_{11}$ as $[M+Na]^+$ was observed at *m/z* 742.4167 (calcd for $C_{39}H_{61}NO_{11}Na$, 742.4142) in the positive high resolution FAB mass spectrum. The UV absortion and IR spectra exhibited the characteristic diene and diene ester functionalities as in compound **1**. In addition, Compound **2** showed a quite similar pattern to compound **1** in the ^1H and ^{13}C NMR spectra except the appearance of exchangeable protons at δ 4.05, 4.77, 5.42, 6.74, and 7.32 ppm in the ^1H NMR spectrum in acetone-d_6 of **2**, as shown in Table 1. In particular, two broad signals at 6.74 and 7.32 ppm indicated the presence of amide functionality and comparison of the NMR data of the fumarate moieties suggested assignments of the amide functionality (Figure 1). Thus, the structure of compound **2** was deduced to be an amide form of compound **1** shown in Figure 2 and fully supported by extensive interpretation of 2-D NMR experiments with combination of high resolution FAB mass spectral data. We suggest compound **2** designated as bafilomycin C_1-amide.

The molecular formula of compound **3** was determined to be $C_{44}H_{65}NO_{13}$ as $[M+Na]^+$ was observed at *m/z* 838.4370 (calcd for $C_{44}H_{65}NO_{13}Na$, 838.4354) in the positive high resolution FAB mass spectrum. The UV absorption maxima and IR absorptions were almost the same as those of compounds, **1** and **2**, except a strong IR absorption band at 1620 cm^{-1}. The exchangeable proton

Table 1. ^{1}H and ^{13}C NMR chemical shifts of compounds **1** and **2**[a].

Posotion	1		2	
	δ_H	δ_C	δ_H	δ_C
1		168.1		167.7
2		142.3		141.8
2-OCH_3	3.61 (s)	60.6	3.62 (s)	60.1
3	6.70 (s)	134.8	6.70 (br s)	134.1
4		133.2		132.7
4-CH_3	1.97 (s)	14.1	1.97 (d, 1.0)	14.1
5	5.88 (d, 9.3)	145.6	5.95 (d, 9.3)	145.6
6	2.52 (m)	38.5	2.55 (m)	38.0
6-CH_3	1.05 (d, 6.8)	17.9	1.04 (d, 6.8)	17.7
7	3.22 (m)	81.2	3.30 (m)	80.3
7-OH			4.05 (d, 4.9)	
8	1.84 (m)	41.7	1.88 (m)	41.7
8-CH_3	0.92 (d, 6.8)	22.5	0.91 (d, 6.8)	22.2
9	2.01 (m)	42.5	2.05 (m)	42.2
10		145.5		144.7
10-CH_3	1.87 (s),	20.1	1.92 (s)	20.3
11	5.76 (d, 10.8)	125.5	5.79 (d, 10.8)	125.1
12	6.59 (dd, 14.7, 10.8)	134.6	6.67 (dd, 14.7, 10.8)	134.3
13	5.10 (dd, 14.7, 8.8)	126.9	5.13 (dd, 14.7, 9.3)	126.9
14	3.98 (dd, 8.8, 8.3)	84.4	4.05 (dd, 9.3, 8.3)	83.3
14-OCH_3	3.24 (s)	55.9	3.22 (s)	55.6
15	5.02 (d, 8.30)	77.7	4.95 (dd, 8.3, 1.5)	77.3
16	2.07 (m)	39.4[b]	2.16 (m)	38.2
16-CH_3	0.86 (d, 6.8)	10.5	0.87 (d, 6.8)	10.3
17	4.15 (dd, 10.7, 1.0)	72.0	4.17 (m)	71.5
17-OH			4.77 (d, 4.0)	
18	1.80 (m)	43.7	1.85 (m)	42.9
18-CH_3	0.98 (d, 7.3)	7.3	0.99 (d, 6.8)	7.4
19		100.3		99.7
19-OH			5.42 (d, 2.0)	
20	2.29 (dd, 11.7, 4.9)	40.5	2.33 (dd, 11.7, 4.9)	40.6
	1.31 (t, 11.7)		1.29 (td, 11.7, 2.0)	
21	4.99 (td, 11.7, 4.9)	75.5	5.02 (td, 10.8, 4.9)	75.7
22	1.63 (m)	39.4[b]	1.61 (m)	39.0
22-CH_3	0.83 (d, 6.4)	12.6	0.83 (d, 6.8)	12.5
23	3.58 (dd, 10.3, 2.0)	77.3	3.61 (dd, 10.7, 2.0)	76.4
24	1.92 (m)	29.3	1.93 (m)	28.7
24-CH_3	0.96 (d, 6.8)	21.8	0.95 (d, 6.8)	21.6
25	0.82 (d, 6.8)	14.7	0.81 (d, 6.8)	14.6
1'		167.6		165.3[c]
2'	6.48 (d, 15.9),	128.8	6.67 (d, 15.5)	130.6
3'	6.91 (d, 15.9)	143.3	7.05 (d, 15.5)	137.8
4'		172.6		165.5[c]
4'-NH_2			7.32 (br s)	
			6.74 (br s)	

[a] Measured in methanol-d_4 for **1** and in acetone-d_6 for **2** at 24°C at 500 MHz (^{1}H) and at 125 MHz (^{13}C).
[b] Overlapped.
[c] Interchangeable assignments.

Fig. 1. Chemical shift assignments (δ_H, 6.48~7.58 ppm in italics; δ_C, 128.8~172.6 ppm) for the fumarate moiety A, B, and C of compounds, **1**, **2**, and **3**, respectively.

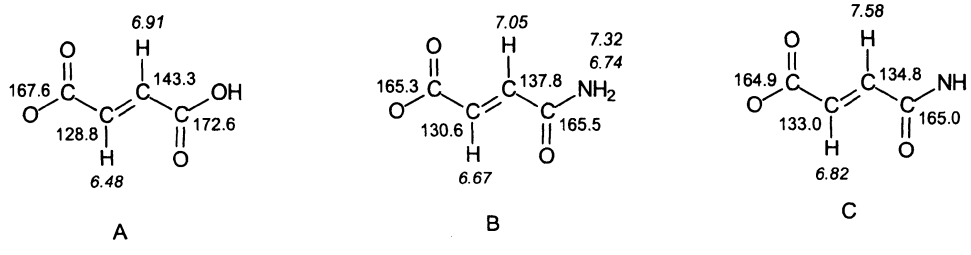

Fig. 2. Structures of compounds **1**~**5** isolated from *Kitasatospora cheerisanensis*.

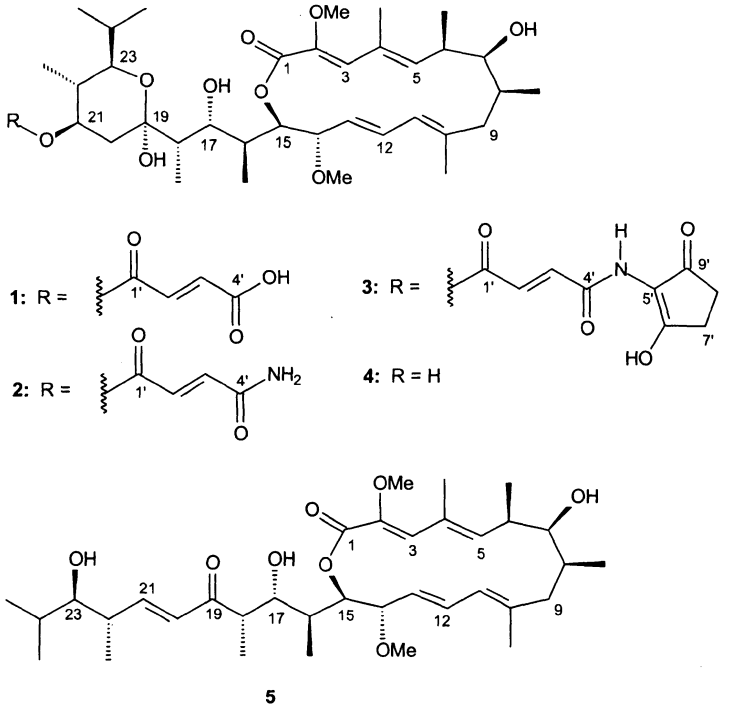

Table 2. Cytotoxicity (IC_{50}, ng/ml) of compounds against human tumor cell lines.

Cell line[a]	Compound				
	1	**2**	**3**	**4**	Adriamycin
A-549	0.03	1.08	0.18	1.86	170
SK-OV-3	0.02	0.91	0.09	1.64	530
SK-Mel-2	0.008	0.20	0.02	1.93	260
XF-498	0.007	0.28	0.03	0.81	840
HCT-15	0.02	0.32	0.02	1.70	960

[a] Cell lines: A-549, non-small cell lung cancer; SK-OV-3, ovarian cancer; SK-Mel-2, melanoma; XF-498, central nerve system cancer; and HCT-15, colon cancer.

signals at 6.74 and 7.32 ppm of compound **2** disappeared in compound **3**. The methine signals in the fumarate moiety showed remarkable difference in both ^{1}H and ^{13}C NMR spectra, whose chemical shifts are depicted in Figure 1. In addition, newly found was the signals for 3-hydroxy-2-cyclopentenone-2-yl group. Thus, the structure of compound **3** was determined to be bafilomycin B_1.[6,7]

The molecular formula of compound **4** and **5** was determined to be $C_{35}H_{58}O_9$ and $C_{35}H_{56}O_8$, respectively, as $[M+Na]^+$ was observed at *m/z* 645.3986 (calcd for $C_{35}H_{58}O_9Na$, 645.3979) and *m/z* 627.3871 (calcd for $C_{35}H_{56}O_8Na$, 627.3873) in the positive high resolution FAB mass data, respectively. UV and IR absortion spectrum confirmed the presence of the same 16-membered macrolide lactone as in compounds **1**~**3**. The structures of compound **4** and **5** were determined to be bafilomycin A_1 and bafilomycin D, respectively, from the interpretation of extensive 2-D NMR experiments with combination of high resolution FAB mass spectral data and by comparison of the ^{1}H and ^{13}C NMR assignments with literature values.[6~8]

Biological Activity

Cytotoxicity was measured against several human tumor

Table 3. Antifungal activity (inhibition zone, mm) of compounds against various plant-pathogens[a].

Test fungus	Compound			
	1	2[b]	3	4
Rhizoctonia soliani	12	NT	10	2
Botrytis cinerea	15	NT	6	8
Sclerotinia. sclerotiorum	6	NT	5	6
Botryosphaeria. dothidea	10	NT	5	0
Collectotrichum .cingulata	10	NT	10	0
Magnaporthe griesa	8	NT	6	2
Phythium ultimum	10	NT	6	5

[a] Each compound (250 μg) was loaded onto each paper disc, which was placed on a PDA medium of test fungi. After incubation for 5 days, the inhibitory zone outside the paper disc was measured in mm.
[b] NT: not tested because of insufficient material.

cellular models using SRB method.[4,5] All the compounds tested were extremely cytotoxic against non-small cell lung cancer; ovarian cancer; melanoma; central nervous system cancer, and colon cancer cell lines in the range of 0.008 to 1.93 ng/ml (Table 2). Compound **1** (bafilomycin C_1) was the most potent in cytotoxicity. Compound **2** (bafilomycin C_1-amide) was cytotoxic at the concentrations of 0.20~1.08 ng/ml, which are over 100-fold more potent than adriamycin. The presence of the fumarate moiety at C21 seems to attribute to higher cytotoxcity.

The antifungal activity of compounds **1**, **3** and **4** was measured against plant pathogens (Table 3). Compound **1** showed the strongest activity. As in the case of cytotoxicity, the fumarate functionality seems to attribute to higher antifungal activity.

Discussion

The bafilomycins, a group of 16-membered macrolide lactones, have been isolated from several *Sterptomyces* species[8~14] and *Kitasatosporia setae*.[15,16] This macrolide lactone moiety has been found in some other metabolites such as hygrolidins,[17] L-155,175,[18] leucanicidins,[19] PD 118,576s,[20,21] and micromonospolides.[22,23] These macrolides show a variety of biological activities such as antifungal, antiparasitic, and antitumor activity as well as inhibitory activity against Na^+, K^+ activated ATPase[24] or gastrulation of the starfish embryo. Bafilomycin A_1 inhibits cell growth through apoptosis.[25~28] Bafilomycin C_1 is the most potent in antibacterial activity[9] and bafilomycin B_1 in nematode development inhibitory activity among natural bafilomycins.[12] The absolute stereochemistry of bafilomycin A_1 has been established by the X-ray crystallographic analysis and NMR spectroscopy.[29,30] Those remarkable biological properties have stimulated great interest in synthesis.[31,32] Structure-activity relationship of bafilomycin A_1 analogues shows that the parent bafilomycin A_1 is most potent in the vacuolar ATPases than any of the new analogues.[33]

Bafilomycins (**1~5**) were purified from the fermentation of a new isolate *Kitasatospora cheerisanensis*. Compound **2** (bafilomycin C_1-amide) has not been yet reported, although it is not clear whether **2** is derived from **3** (bafilomycin B_1) during isolation. Compounds (**1~4**) tested showed potent cytotoxicity against human cancer cell lines, which was significantly dependent on the substitutional change at 21-OH. Compound **1** (bafilomycin C_1) was the most potent. The fumarate ester functionality at C21 leads to more significant cytotoxicity (**1**, **2**, and **3** *vs* **4**) while change of functionality from carboxylic acid to amide at the fumarate moiety resulted in decrease in cytotoxicity as shown between **1** and **2**. Compound **1** was the most potent against plant pathogens tested. It is noteworthy that the fumarate ester moiety in micromonospolides does not affect the activity of gastrulation of starfish embryos.[23] Structural modification at C21-OH would give valuable structure-activity information.

Acknowledgements

This work was supported in part by the Korea Science and Engineering Foundation (Grant No. 96-0402-01-01-03). We thank Drs. CHONG OCK LEE and SANG UN CHOI (Korea Research Institute of Chemical Technology, Taejon) for the SRB cytotoxicity assay and Dr. JONGSHIN YOO (Korea Basic Science Institute, Taejon) for mass spectra.

References

1) OMURA, S.; Y. IWAI, Y. TAKAHASHI, K. KOJIMA, K. OTOGURO & R. OIWA: Type of diaminopimelic acid different in aerial and vegetative mycelia of setamycin-producing actinomycete KM-6054. J. Antibiotics 34: 1633~1634, 1981
2) OMURA, S.; Y. TAKAHASHI, Y. IWAI & H. TANAKA: *Kitasatosporia*, a new genus of the order *Actinomycetales*. J. Antibiotics 35: 1013~1019, 1982
3) CHUNG, Y. R.; K. C. SUNG, H. K. MO, D. Y. SON, J. S. NAM, J. CHUN & K. S. BAE: *Kitasatospora cheerisanensis* sp. Nov., a new species of the genus *Kitasatospora* that produces an antifungal agent. Int. J. Syst. Bacteriol. 49: 753~758, 1999
4) SKEHAN, P.; R. STORENG, D. SCUDIERO, A. MONKS, J. MCMAHON, D. VISTICA, J. T. WARREN, H. BOKESCH, S. KENNY & M. R. BOYD: New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst.

82: 1107~1112, 1990

5) RUBINSTEIN, L. V.; R. H. SHOEMAKER, K. D. PAULL, R. M. SIMON, S. TOSINI, P. SKEHAN, D. A. SCUDIERO, A. MONKS & M. R. BOYD: Comparison of *in vitro* anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. J. Natl. Cancer Inst. 82: 1113~1118, 1990

6) HENSENS, O. D.; R. L. MONAGHAN, L. HUANG & G. ALBERS-SCHONBERG: Structure of the sodium and potassium ion activated adenoinetriphosphatase L-681.110. J. Am. Chem. Soc. 105: 3672~3679, 1983

7) WERNER, G.; H. HAGENMAIER, K. ALBERT & H. KOHLSHORN: The structure of the bafilomycins, a new group of macrolide antibiotics. Tetrahedron Lett. 24: 5193~5196, 1983

8) KRETSCHMER, A.; M. DORGERLOH, M. DEEG & H. HAGENMAIER: The structures of novel insecticidal macrolides: bafilomycins D and E, and oxohygrolidin. Agric. Biol. Chem. 49: 2509~2511, 1985

9) WERNER, G.; H. HAGENMAIER, H. DRAUTZ, A. BAUMGARTNER & H. ZAHNER: Metabolic products of microorganisms. 224. Bafilomycins, a new group of macrolide antibiotics: production, isolation, chemical structure and biological activity. J. Antibiotics 37: 110~117, 1984

10) MEYER, M.; W. KELLER-SCHIERLEIN, H. DRAUTZ, W. BLANK & H. ZAHNER: Isolation of bafilomycin-A1-21-O-(α-L-rhamnopyranoside). Structure determination by chemical correlation with bafilomycin A_1 and leucanicidin. Helv. Chim. Acta 68: 83~94, 1985

11) HUANG, L.; G. ALBERS-SCHONBERG, R. L. MONAGHAN, K. JAKUBAS, S. S. PONG, O. D. HENSENS, R. W. BURG, D. A. OSTLIND, J. CONROY & E. O. STAPLEY: Discovery, production and purification of the Na^+, K^+ activated ATPase inhibitor, L-681,110 from the fermentation broth of *Streptomyces* sp. MA-5038. J. Antibiotics 37: 970~975, 1984

12) LACEY, E.; J. H. GILL, M. L. POWER, R. W. RICKARDS, M. G. OSHEA & J. M. ROTHSCHILD: Bafilolides, potent inhibitors of the motility and development of the free-living stages of parasitic nematodes. Int. J. Parasitol. 25: 349~357, 1995

13) FRANDBERG, E.; C. PETERSSON, L. N. LUNDGREN & J. SCHNURER: *Streptomyces halstedii* K122 produces the antifungal compounds bafilomycin B_1 and C_1. Canadian J. Microbiol. 46: 753~758, 2000

14) UYEDA, M.; K. I. KONDO, A. ITO, K. YOKOMIZO & Y. KIDO: A new antiherpetic agent produced by *Streptomyces* sp. strain no. 758. J. Antibiotics 48: 1234~1239, 1995

15) OTOGURO, K.; A. NAKAGAWA & S. OMURA: Setamycin, A 16-membered macrolide antibiotic identification and nematocidal activity. J. Antibiotics 41: 250~252, 1988

16) OMURA, S.; K. OTOGURO, T. NISHIKIORI, R. OIWA & Y. IWAI: Setamycin, a new antibiotic. J. Antibotics 34: 1253~1256, 1981

17) SETO, H.; I. TAJIMA, H. AKAO, K. FURIHATA & N. OTAKE: The structure of a new antibiotic, hygrolidin. Terahedron Lett. 23: 2667~2670, 1982

18) GOETZ, M. A.; P. A. MCCORMICK, R. L. MONAGHAN, D. A. OSTLIND, O. D. HENSENS, J. M. LIESCH & G. ALBERS-SCHONBERG: L-155,175: A new antiparasitic macrolide fermentation, isolation and structure. J. Antibiotics 38: 161~168, 1985

19) ISOAGI, A.; S. SAKUDA, S. MATSUMOTO, M. OGURA, K. FURIHATA, H. SETO & A. SUZUKI: The structure of leucanicidin, a novel insectividal macrolide produced by *Sterptomyces halstedii*. Agric. Biol. Chem. 48: 1379~1381, 1984

20) HATFIELD, G.; R. W. WOODARD & J.-K. SON: Isolation and structure determination of new macrolide antibiotics. J. Nat. Prod. 55: 753~759, 1992

21) WILTON, J. H.; G. C. HOKANSON & J. C. FRENCH: PD 118,576: a new antitumor macrolide antibiotic. J. Antibiotics 38: 1449~1452, 1985

22) OHTA, E.; S. OHTA, N. K. KUBOTA, M. SUZUKI, T. OGAWA, A. YAMASAKI & S. IKEGAMI: Micromonospolide A, a new macrolide from *Micromonospora* sp. Tetrahedron Lett. 42: 4179~4181, 2001

23) OHTA, E.; N. K. KUBOTA, S. OHTA, M. SUZUKI, T. OGAWA, A. YAMASAKI & S. IKEGAMI: Micromonospolides A~C, new macrolides from *Micromonospora* sp. Tetrahedron 57: 8463~8467, 2001

24) BOWMAN, E J.; A. SIEBERS & K. ALTENDORF: Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. Proc. Natl. Acad. Sci. USA 85: 7972~7976, 1988

25) KINOSHITA, K.; T. WARITANI, M. NOTO, K. TAKIZAWA, Y. MINEMOTO, Y. NISHIKAWA & S. OHKUMA: Bafilomycin A_1 induces apoptosis in PC12 cells independently of intracellular pH. FEBS Lett. 398: 61~66, 1996

26) OHKUMA, S.; S. SHIMIZU, M. NOTO, Y. SAI, K. KINOSHITA & H. TAMURA: Inhibition of cell growth by bafilomycin A_1, a selective inhibitor of vacuolar H(+)-ATPase. In Vitro Cell. Dev. Biol. 29A: 862~866, 1993

27) NISHIHARA, T.; S. AKIFUSA, T. KOSEKI, S. KATO, M. MURO & N. HANADA: Specific inhibitors of vacuolar type H(+)-ATPases induce apoptotic cell death. Biochem. Biophys. Res. Commun. 212: 255~262, 1995

28) MANABE, T.; T. YOSHIMORI, N. HENOMATSU & Y. TASHIRO: Inhibitors of vacuolar-type H(+)-ATPase suppresses proliferation of cultured cells. J. Cell. Physiol. 157: 445~452, 1993

29) BAKER, G. H.; P. J. BROWN, R. J. J. DORGAN, J. R. EVERETT, S. V. LEY, A. M. Z. SLAWIN & D. J. WILLIAMS: A conformational study of bafilomycin A_1 by X-ray crystallography and NMR techniques. Tetrahedron Lett. 28: 5565~5568, 1987

30) O'SHEA, M. G.; R. W. RICKARDS, J. M. ROTHSCHILD & E. LACEY: Absolute configurations of macrolide antibiotics of the bafilomycin and leucanicidin groups. J. Antibiotics 50: 1073~1077, 1997

31) TOSHIMA, K.; H. YAMAGUCHI, T. JYOJIMA, Y. NOGUCHI, M. NAKATA & S. MATSUMURA: Total synthesis of bafilomycin A_1. 2: The assemblage and completion of the synthesis. Tetrahedron Lett. 37: 1073~1076, 1996

32) SCHEIDT, K. A.; T. D. BANNISTER, A. TASAKA, M. D. WENDT, B. M. SAVALL, G. J. FEGLEY & W. R. ROUSH: Total synthesis of (−)-bafilomycin A(1). J. Am. Chem. Soc. 124: 6981~6990, 2002

33) GAGLIARDI, S.; P. A. GATTI, P. BELFIORE, A. ZOCCHETTI, G. D. CLARKE & C. FARINA: Synthesis and structure-activity relationships of bafilomycin A_1 derivatives as inhibitors of vacuolar H^+-ATPase. J. Med. Chem. 41: 1883~1893, 1998