Bafilomycins Produced by an Endophytic Actinomycete *Streptomyces* sp. YIM56209†

Zhiguo Yu,[1,2] Li-Xing Zhao,[3] Cheng-Lin Jiang,[3] Yanwen Duan,[2] Lily Wong,[4] Kristopher C. Carver,[4] Linda A. Schuler,[4] and Ben Shen[1,5,6]*

[1]Division of Pharmaceutical Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53705, USA; [2]Hunan Engineering Research Center of Combinatorial Biosynthesis and Natural Product Drug Discovery, Changsha, Hunan 410329, China; [3]Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, China; [4]Department of Comparative Biosciences, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA; [5]University of Wisconsin National Cooperative Drug Discovery Group, University of Wisconsin-Madison, Madison, Wisconsin 53705, USA; and [6]Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53705, USA

TITLE RUNNING HEAD: New Bafilomycins from *Streptomyces* sp. YIM56209

*To whom correspondence should be addressed: Ben Shen, Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, 777 Highland Ave., Madison, WI 53705-2222, USA. Tel: (608) 263-2673, Fax: (608) 262-5345, E-mail: bshen@pharmacy.wisc.edu

†Dedicated to the late Professor C. Richard "Dick"/"Hutch" Hutchinson for his exceptional contributions to natural product biosynthesis, engineering, and drug discovery.

Supplementary Information

EXPERIMENTALS

Cytotoxicity test. The cytotoxicities of bafilomycins were tested on two cell lines, human lung carcinoma (A-549) and human colon carcinoma (HT-29). Both of cell lines were maintained as previously reported.[1] Cells were harvested by trypsinization using 0.25% trypsin and 0.1% EDTA and then counted in a ViCell XR coulter in duplicate, before and after dilution for assay plating. Cell plating, compound handling, and assay set up were performed as previously reported.[1] Calcein AM (acetoxymethyl ester) reagent (30 μL, 1M) was added and the cells were incubated for 30 min at 37°C. Plates were read for emission by using a fluoresce filter (excitation 485 nm, emission 535 nm). An equal volume (30 μL) of cell titer-glo reagent (Promega, Madison, WI, USA) was added and incubated for 10 min at room temperature with gentle agitation to lyse the cells. Each plate was re-read for luminescence to confirm the inhibition observed in the fluorescent Calcein AM assay. The data represent the mean of three experiments performed in triplicate and are expressed as means ± standard deviation (SD).

Reference 1: Li, W., Ju, J., Rajski, S. R., Osada, H. & Shen, B. Characterization of the tautomycin biosynthetic gene cluster from Streotomyces spiroverticllatus unveiling new insights into dialkylmaleic anhydride and polyketide biosynthesis. *J. Biol. Chem.* **283**, 28607-28617 (2008).

High-throughput in-cell western analysis. Human breast cancer (MCF-7) cells were plated at 1.5 x 10^4 cells/well in 384-well CellBIND® microplates (#3683; Corning Inc., Corning, NY). After 24-48 h of serum-starvation, cells were incubated at 37 °C in the presence of vehicle or bafilomycins for 1 h prior to addition of PRL (400 ng/mL; 16 nM) or EGF (120 ng/mL; 18.75 nM) stimulation for 15 min. Cells were then fixed for 4 h with Prefer fixative (#411; Anatech Ltd, Battle Creek, MI), permeabilized for 30 min using 1% Triton in 1xPBS, and blocked overnight at 4 °C using SEA BLOCK blocking buffer (#37527; Pierce Biotechnology, Rockford, IL). Cells were incubated with primary antibody (p-ERK1/2, 1:2500) for 2 h and secondary antibody (IRDye 800CW, 1:10000) for 1 h at 25 °C with gentle shaking. p-ERK1/2 signals were detected at 800 nm and quantified using the Odyssey® Infrared Imaging System (LI-COR Biosciences, Lincoln, NE). Cell-permeable red fluorescent nucleic acid-staining SYTO® dyes were used to control for cell number within each well. Briefly, cells were incubated with SYTO 62 (1:25000) for 30 min at 37°C, and emission was detected at 700 nm using the Odyssey® Infrared Imaging System. All liquid handling was performed using a BioMek® FX Laboratory Automation Workstation (Beckman Coulter, Fullerton, CA). IC_{50} values were derived using Prism v4.00 (GraphPad Software, Inc., San Diego, CA), and represent the means of at least 2 experiments performed in triplicate.

Table S1 ^{1}H NMR data of **1** and **2** in comparison with bafilomycin D (**3**)

Position	9-Hydroxybafilomycin D (**1**) in $CDCl_3$	29-Hydroxybafilomycin D (**2**) in $CDCl_3$	Bafilomycin D (**3**) in $CDCl_3$
	δ (^{1}H) [p.p.m.] *J* in Hz	δ (^{1}H) [p.p.m.] *J* in Hz	δ (^{1}H) [p.p.m.] *J* in Hz
1	–	–	–
2	–	–	–
3	6.63 (1H, s)	6.60 (1H, s)	6.64 (1H, s)
4	–	–	–
5	5.73 (1H, d, 9.0)	5.81 (1H, d, 9.0)	5.76 (1H, d, 9.0)
6	2.54 (1H, m)	2.54 (1H, m)	2.52 (1H, m)
7	3.41 (1H, d, 7.0)	3.43 (1H, d, 7.0)	3.30 (1H, d, 7.0)
8	2.31 (1H, m)	1.91 (1H, m)	1.91 (1H, m)
9	3.85 (1H, d, 10.5)	2.30 (1H, m), 2.10 (1H, m)	2.12 (1H, m), 1.99 (1H, m)
10	–	–	–
11	6.02 (1H, d, 10.5)	5.90 (1H, d, 11.0)	5.81 (1H, d, 11.0)
12	6.51 (1H, dd, 14.5, 11.0)	6.64 (1H, dd, 15.0, 11.0)	6.48 (1H, dd, 15.0, 10.5)
13	5.29 (1H, dd, 15.0, 9.5)	5.23 (1H, dd, 15.0, 9.5)	5.17 (1H, dd, 15.0, 9.0)
14	3.81 (1H, dd, 8.5, 8.0)	3.81 (1H, dd, 8.5, 8.0)	3.82 (1H, dd, 8.5, 8.0)
15	5.05 (1H, d, 8.5)	5.05 (1H, d, 9.0)	5.06 (1H, d, 8.5)
16	2.06 (1H, m)	2.07 (1H, m)	2.06 (1H, m)
17	3.77 (1H, m)	3.76 (1H, m)	3.76 (1H, m)
18	2.97 (1H, m)	2.97 (1H, m)	2.98 (1H, m)
19	–	–	–
20	6.28 (1H, d, 16.0)	6.29 (1H, d, 16.0)	6.28 (1H, d, 15.5)
21	6.90 (1H, dd, 15.5, 8.0)	6.90 (1H, dd, 16.0, 8.0)	6.91 (1H, dd, 16.0, 8.5)
22	2.54 (1H, m)	2.52 (1H, m)	2.52 (1H, m)
23	3.18 (1H, t, 6.0)	3.18 (1H, t, 6.0)	3.18 (1H, t, 6.0)
24	1.72 (1H, m)	1.73 (1H, m)	1.72 (1H, m)
25	0.92 (3H, d, 7.0)	0.93 (3H, d, 7.0)	0.92 (3H, d, 7.0)
26	1.98 (3H, s)	1.98 (3H, s)	1.98 (3H, s)
27	1.07 (3H, d, 7.0)	1.07 (3H, d, 7.0)	1.07 (3H, d, 6.5)
28	0.95 (3H, d, 7.0)	0.97 (3H, d, 7.0)	0.95 (3H, d, 7.5)
29	2.03 (3H, s)	4.58 (1H, d, 12.5), 4.07 (1H, d, 12.5)	1.91 (3H, s)
30	0.95 (3H, d, 7.0)	0.95 (3H, d, 7.0)	0.94 (3H, d, 6.5)
31	1.21 (3H, d, 7.0)	1.21 (3H, d, 7.0)	1.21 (3H, d, 7.0)
32	1.08 (3H, d, 6.5)	1.08 (3H, d, 6.5)	1.08 (3H, d, 7.0)
33	0.95 (3H, d, 7.0)	0.95 (3H, d, 7.0)	0.94 (3H, d, 6.5)
2-OMe	3.68 (3H, s)	3.67 (3H, s)	3.68 (3H, s)
14-OMe	3.22 (3H, s)	3.21 (3H, s)	3.22 (3H, s)

Table S2 ^{13}C NMR data of **1** and **2** in comparison with bafilomycin D (**3**)

Position	9-Hydroxybafilomycin D (**1**) in $CDCl_3$	29-Hydroxybafilomycin D (**2**) in $CDCl_3$	Bafilomycin D (**3**) in $CDCl_3$
1	166.7	166.4	166.7
2	141.8	141.6	141.7
3	133.2	132.6	133.2
4	133.4	133.2	133.0
5	141.0	142.1	142.6
6	37.2	36.6	37.1
7	80.0	81.4	81.5
8	38.8	40.6	40.1
9	77.2	36.5	41.6
10	147.2	143.7	143.1
11	125.5	128.1	125.5
12	133.3	132.0	133.1
13	130.5	129.5	127.3
14	83.4	83.0	83.6
15	76.7	76.2	76.6
16	38.8	38.3	38.8
17	72.9	72.2	72.9
18	46.6	46.4	46.5
19	203.4	203.0	203.4
20	129.6	129.3	129.6
21	148.9	148.5	148.8
22	40.3	40.0	40.3
23	80.0	79.8	80.0
24	31.2	30.9	31.2
25	17.0	16.8	17.1
26	14.2	14.1	14.2
27	17.4	17.0	17.7
28	18.2	22.0	22.2
29	18.8	63.1	20.2
30	10.9	10.6	11.0
31	10.6	10.2	10.5
32	16.9	16.6	16.9
33	19.9	19.7	19.9
2-OMe	60.5	60.1	60.4
14-OMe	56.0	55.8	55.9

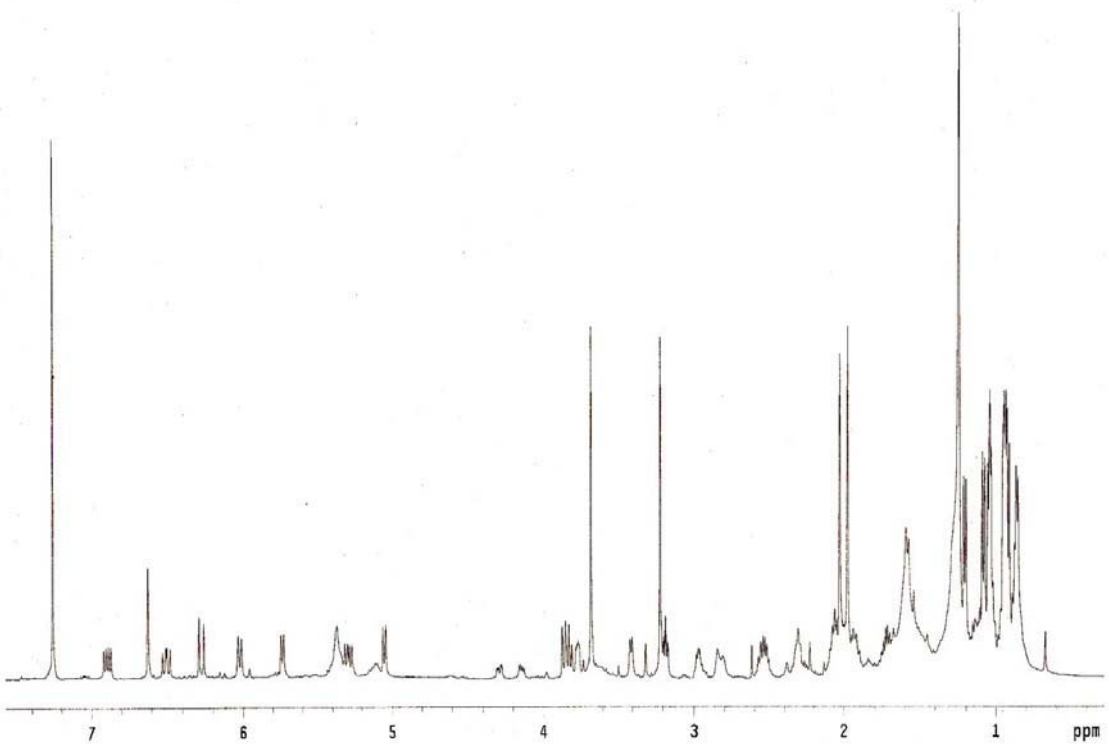

Figure S1 ^{1}H NMR spectrum of **1** (500 MHz, $CDCl_3$)

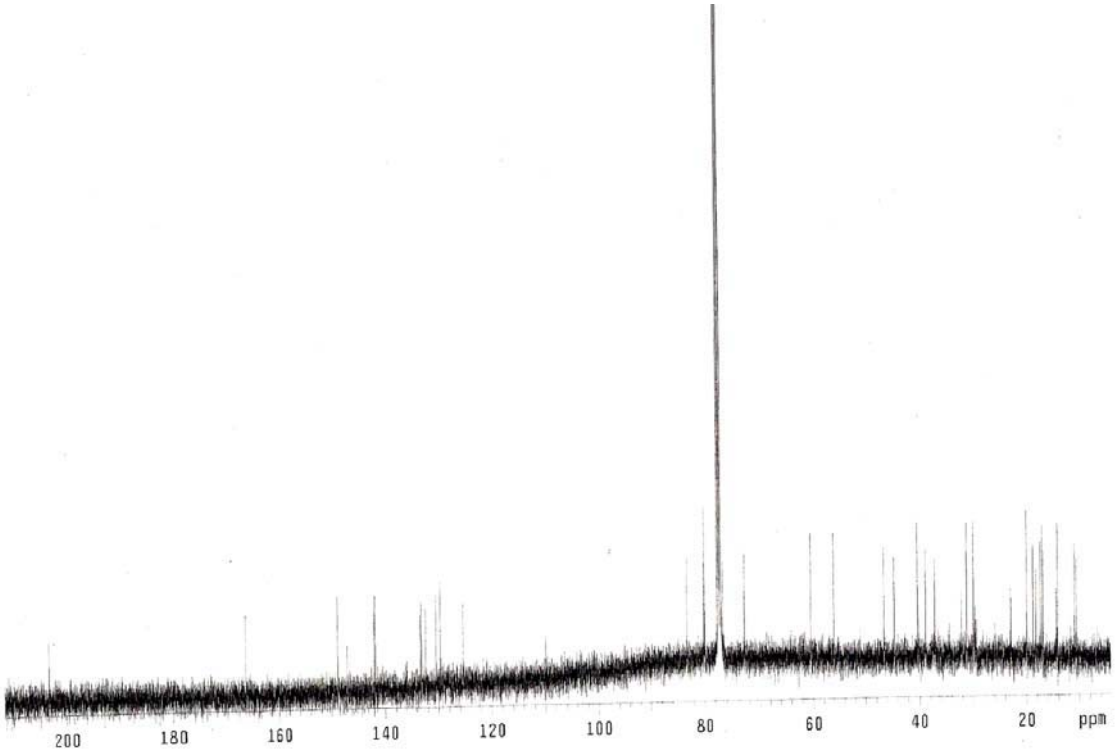

Figure S2 ^{13}C NMR spectrum of **1** (125 MHz, $CDCl_3$)

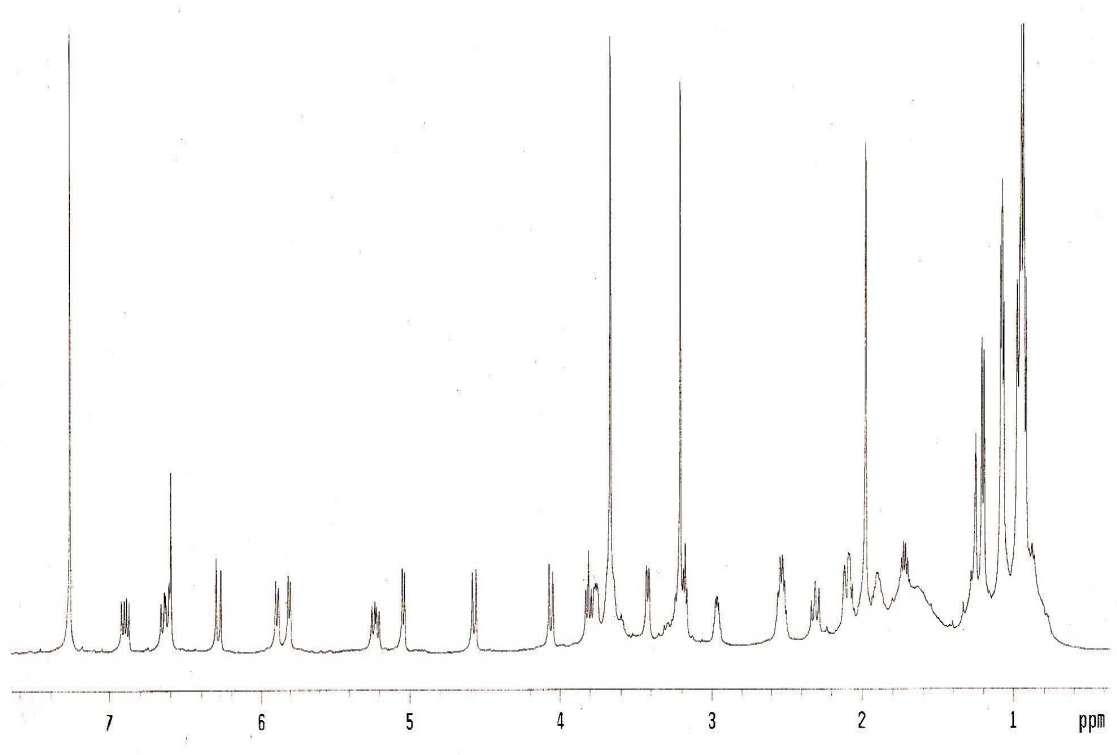

Figure S3 ^{1}H NMR spectrum of **2** (500 MHz, $CDCl_3$)

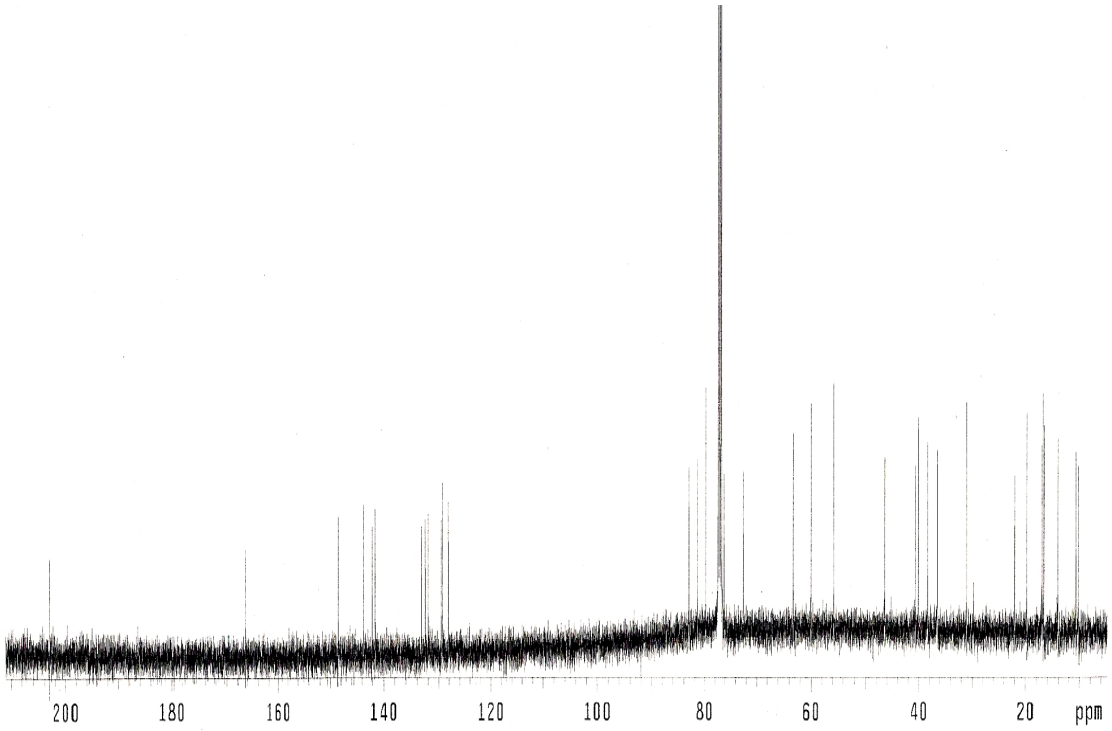

Figure S4 ^{13}C NMR spectrum of **2** (125 MHz, $CDCl_3$)