Acetylenic Acids Inhibiting Azole-Resistant *Candida albicans* from *Pentagonia gigantifolia*

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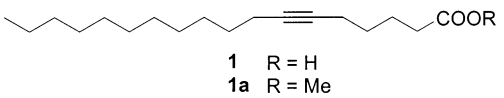
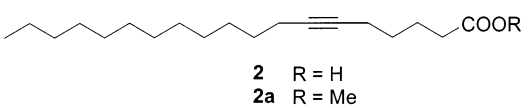
Antifungal bioassay-guided isolation of the ethanol extract of the roots of *Pentagonia gigantifolia* yielded 6-octadecynoic acid (**1**) and the new 6-nonadecynoic acid (**2**). Compounds **1** and **2** inhibited the growth of fluconazole-susceptible and -resistant *Candida albicans* strains. Their antifungal potencies were comparable to those of amphotericin B and fluconazole. Of particular significance is the low cytotoxicity and specific activity of **1** and **2** against *C. albicans*.

Candida albicans, a component of the normal human microflora, is the most common cause of fungal infections, and *Candida* species are the fourth leading cause of nosocomial infection in the United States.[1] Studies have further shown[2] that *C. albicans* can become transiently resistant to azole drugs rapidly after exposure to the antifungal drug fluconazole, which involves inhibition of ergosterol biosynthesis via interaction with the fungal enzyme 14α-lanosterol demethylase.[3] Mechanisms of azole resistance that have been identified include alterations in the gene encoding the target enzyme or overexpression of efflux pump (CDR or MDR) genes.[3,4] The development of fluconazole resistance in *C. albicans* has been well documented for patients with AIDS and recurrent oropharyngeal candidiasis.[2,5] Therefore, there is an urgent need to discover new prototype antibiotics to combat these resistant pathogens.

In our continued search for prototype antifungal agents from higher plants, the ethanol extract of the roots of a Peruvian plant, *Pentagonia gigantifolia* Ducke (Rubiaceae), demonstrated significant antifungal activity against *C. albicans* (IC_{50} < 20 μg/mL). However, no chemical or biological studies have been reported on the plants of the genus *Pentagonia*. A subsequent bioassay-guided fractionation and purification of the active extract led to the identification of two acetylenic acids, 6-octadecynoic acid (**1**) and the new 6-nonadecynoic acid (**2**). The two compounds showed potent antifungal activity against *C. albicans* and a series of fluconazole-resistant *C. albicans* strains. In this paper, we document the isolation, structure identification, and antifungal activity of these two compounds.



1 R = H
1a R = Me



2 R = H
2a R = Me

The ethanol extract of the roots of *P. gigantifolia* was first fractionated by silica gel column chromatography. The most active fraction was further chromatographed on reversed-phase silica gel C_{18} using aqueous methanol to afford compounds **1** and **2** in yields of 0.39% and 0.088%, respectively, of the dried plant material.

Compound **1** was identified as 6-octadecynoic acid (tariric acid) by GC−MS analysis of its methyl ester (**1a**) via a direct library search. The structure of **1** was further supported by its high-resolution ESIMS and ^{1}H and ^{13}C NMR data. Most ^{1}H and ^{13}C NMR signals of **1** were assigned via 2D NMR techniques and are listed in the Experimental Section since there are only low-resolution ^{1}H NMR data (60 MHz) available for this compound.[6]

The molecular formula of compound **2**, $C_{19}H_{32}O_2$, was established by a combination of high-resolution ESIMS and ^{13}C NMR spectra. The ^{1}H and ^{13}C NMR spectra of **2** were in good agreement with those of **1**, displaying characteristic signals for a triple bond and a carboxylic group. The only difference between their ^{13}C NMR spectra is that **2** possessed an additional carbon signal around δ 30.9 (CH_2), while in the ^{1}H NMR spectrum it showed two additional protons in the region δ 1.25−1.37 when the integration area was compared with that of **1**. This indicated that compound **2** is a homologue of **1**. The methyl ester of **2** (**2a**) showed a fragmentation pattern similar to **1a** by GC−MS analysis. The intense fragment ion at *m*/*z* 154 in both **1a** and **2a** was diagnostic of the presence of a triple bond at C-6 position.[7,8] Further support was derived from a DQF-COSY experiment, which clearly identified a coupling network through H-2 (δ 2.38) → H-3 (δ 1.74) → H-4 (δ 1.54) → H-5 (δ 2.18). H-5 was in turn coupled with H-8 (δ 2.13) through a 1,4-long-range coupling (J = 2.2 Hz), which is characteristic of acetylenic compounds. Other 2D NMR techniques, including HMQC and HMBC, were employed to confirm the straight chain structure and facilitate the assignment of its NMR signals. Therefore, the structure of compound **2** was established as 6-nonadecynoic acid.

When examined for antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus* and for antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa*, and *Mycobacterium intracellulare*, compounds **1** and **2** were active only against *C. albicans*. The antifungal potencies of **1** and **2** against *C. albicans* were measured using a modified version of the NCCLS method[9] and comparable to the positive controls of three classes of antifungal drugs, amphotericin B, fluconazole, and flucy-

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Table 1. Antifungal Activity of Compounds **1** and **2** against *Candida albicans* ATCC 90028[a]

	IC_{50}(μg/mL)	MIC (μg/mL)
1	0.38 ± 0.03	1.04 ± 0.45
2	0.33 ± 0.14	0.52 ± 0.23
amphotericin B	0.23 ± 0.06	0.52 ± 0.18
fluconazole	0.18 ± 0.03	0.29 ± 0.00
flucytosine	0.47 ± 0.12	1.04 ± 0.45

[a] Determined in triplicate.

tosine, with minimum inhibitory concentrations (MICs) ranging from 0.29 to 1.04 μg/mL (Table 1). The corresponding methylated products, **1a** and **2a**, were inactive against *C. albicans*. Compounds **1** and **2** were not fungicidal to *C. albicans* and did not show cytotoxicity in assays against human tumor cells (KB, SK-MEL, BT-549, and SK-OV-3) and Vero cells at concentrations of 10 μg/mL.[10]

Compounds **1** and **2** were then evaluated for antifungal activity against a series of *C. albicans* strains isolated from patients during fluconazole therapy with varying degrees of fluconazole resistance (Table 2).[2,11−13] Isolate 17 has been shown to possess several mechanisms of resistance to fluconazole including overexpression (increased mRNA levels) of CDR and MDR efflux pumps in addition to overexpression of the target enzyme of fluconazole, lanosterol 14α-demethylase.[12] Since compounds **1** and **2** were equipotent in the fluconazole-susceptible (isolate 1) and -resistant (isolate 17) strains, it appears that **1** and **2** are not substrates for either the CDR or MDR efflux pumps. In addition, it can be reasoned that the antifungal target of **1** and **2** may not be lanosterol 14α-demethylase. Taking into account the antifungal potency against resistant *C. albicans* strains along with their selectivity and low cytotoxicity, compounds **1** and **2** may be potential antifungal leads for further studies.

Sphingolipid synthesis has been demonstrated to be an antifungal target of sphingofungins,[14−16] viridiofungins,[17] and khafrefungin,[18] which are structurally similar to intermediates of the sphingolipid biosynthesis pathway. Since compounds **1** and **2** are, to some extent, structurally similar to these known sphingolipid synthesis inhibitors, a sphingolipid reversal assay[14,17] was performed to explore their antifungal mechanism of action. The antifungal activity of the two compounds was reversed with addition of the sphingolipid synthesis intermediate, dihydrosphingosine (concentrations at 0, 1.25, 2.5, and 5 μg/mL), to the cell culture. This effect was not observed with the antifungal drugs amphotericin B, fluconazole, or flucytosine (Table 3), which all do not act on sphingolipid synthesis. However, stearylamine, which is structurally similar to dihydrosphingosine but not an intermediate of sphingolipid biosynthesis, also reversed the antifungal activity of compounds **1** and **2** (Table 4). These preliminary results may be helpful in future studies that include the use of these compounds in microarray gene expression experiments to examine their antifungal mechanism of action.

Experimental Section

General Experimental Procedures. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and were uncorrected. UV spectra were measured on a Hewlett-Packard 8453 spectrometer. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer. NMR spectra were recorded with TMS as an internal standard, using a Bruker Avance DRX-400 NMR spectrometer for the ^{1}H, ^{13}C, and 2D NMR spectra. ESI-FTMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. GC−MS was run on a Hewlett-Packard (HP) 5890 Series II Plus gas chromatograph interfaced to a HP 5970B mass selective detector equipped with Wiley Library for search: DB-1 Minibore capillary column (0.18 mm i.d., 20 m), column temperature 170→280 °C (10 °C/min→25 min), He (45 psi). Column chromatography was run using silica gel (40 μm, J. T. Baker) and reversed-phase silica gel (RP-18, 40 μm, J. T. Baker). TLC was performed on silica gel sheets (Alugram Sil G/UV_{254}, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F_{254S}, Merck, Germany). General procedures for antimicrobial and cytotoxicity assays have been described in previous papers.[10,19,20]

Plant Material. The roots of *P. gigantifolia* Ducke were collected by Mr. Manuel Rimachi in Loreto, Peru, in November 1996 and identified by Mr. M. Rimachi and Dr. Sidney McDaniel. A voucher specimen is deposited at the Herbarium of Mississippi State University (MISSA, voucher #IBE-MR 11821).

Extraction and Isolation. Dried roots (500 g) of *P. gigantifolia* were percolated with 95% EtOH (3.5 L × 2). Removal of the solvent under vacuum at 40 °C yielded an EtOH extract (21.5 g; IC_{50} < 20 μg/mL against *C. albicans*). A portion (5.3 g) of the EtOH extract was chromatographed on silica gel (220 g) using a gradient solvent system consisting of $CHCl_3$/MeOH (0% to 100% MeOH, a total of 8 L) to yield several fractions. The fraction (0.93 g) that was eluted with 2% MeOH/$CHCl_3$ and showed strongest activity (IC_{50} < 2 μg/mL) was further chromatographed on reversed-phase silica gel (C_{18}, 84 g) using 85%−90% MeOH (2 L) to give 6-octadecynoic acid (**1**, 480 mg) and 6-nonadecynoic acid (**2**, 108 mg).

6-Octadecynoic acid (1): white powder; mp 49 °C (lit.[21] mp 50−51 °C); UV (MeOH) λ_{max} (log ϵ) 204 (2.73) nm; IR (KBr) ν_{max} 3102, 2916, 2849, 1707, 1692, 1469, 1316, 1264, 1207, 900, 718 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 7.0 Hz, H-18, HMBC/C-16,17), 1.26−1.37 (16H, m, H-10−17), 1.47 (2H, br quint, J = 7.5 Hz, H-9, HMBC/C-7), 1.54 (2H, br quint, J = 7.2 Hz, H-4, HMBC/C-2,3,5,6), 1.74 (2H, br quint, J = 7.6 Hz, H-3, HMBC/C-1,2,4,5), 2.13 (2H, tt, J = 7.1, 2.2 Hz, H-8, HMBC/C-5), 2.18 (2H, tt, J = 7.0, 2.2 Hz, H-5, HMBC/C-3,4,6,7,8), 2.38 (2H, t, J = 7.5 Hz, H-2, HMBC/C-1,3,4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5 (C-18), 18.8 (C-5), 19.1 (C-8), 23.1 (C-17), 24.2 (C-3), 28.8 (C-4), 29.3, 29.5, 29.6, 29.8, 30.0, 30.02, 30.05, 32.3 (C-16), 34.0 (C-2), 79.7 (C-6), 81.3 (C-7), 180.6 (C-1); ESIMS m/z 281.2459 {calcd for [M($C_{18}H_{32}O_2$) + H]$^+$, 281.2475}.

6-Nonadecynoic acid (2): white powder; mp 48 °C; UV (MeOH) λ_{max} (log ϵ) 204 (2.70) nm; IR (KBr) ν_{max} 3114, 2918, 2849, 1705, 1692, 1470, 1317, 1264, 1207, 900, 718 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 7.0 Hz, H-19,

Table 2. Antifungal Activity of Compounds **1** and **2** against Azole-Susceptible and -Resistant *Candida albicans* Strains [IC_{50}/IC_{80}/IC_{95} (μg/mL)][a]

	1	**2**	fluconazole
C. albicans isolate 1[2]	0.45/0.60/0.70	0.25/0.35/0.45	1.00/1.50/2.00
C. albicans isolate 2[2]	0.50/0.65/0.75	0.25/0.30/0.35	1.00/5.00/10.00
C. albicans isolate 5[2]	0.70/1.00/2.00	0.45/0.60/0.80	7.50/10.00/10.00
C. alibicans isolate 8[2]	0.90/1.00/1.50	0.50/0.65/0.80	15.00/20.00/25.00
C. albicans isolate 1[11−13]	0.60/0.90/1.00	0.40/0.50/0.70	0.10/0.20/100.00
C. albicans isolate 17[11−13]	0.65/1.00/1.50	0.45/0.60/0.70	40.00/95.00/NA[b]

[a] Patient isolates during fluconazole therapy: isolate 1, azole-susceptible strain; isolates 2, 5, 8, and 17, azole-resistant strains with increasing azole resistance. [b] Not active at highest test concentration of 100 μg/mL.

Table 3. Antifungal Activity of Compounds **1** and **2** against *Candida albicans* ATCC 90028 with Dihydrosphingosine (IC_{50}, μg/mL)

	dihydrosphingosine (μg/mL)			
	0	1.25	2.5	5.0
1	0.40	0.70	2.0	4.5
2	0.25	1.0	2.0	4.5
amphotericin B	0.30	0.10	0.10	0.10
fluconazole	0.15	0.30	0.50	0.70
flucytosine	0.40	0.20	0.15	0.10

Table 4. Antifungal Activity of Compounds **1** and **2** against *Candida albicans* ATCC 90028 with Stearylamine (IC_{50}, μg/mL)

	stearylamine (μg/mL)			
	0	5	15	30
1	0.40	3.5	15	30
2	0.25	5	15	35
amphotericin B	0.30	0.25	0.40	0.65
fluconazole	0.15	0.65	0.80	0.75
flucytosine	0.40	0.25	0.20	0.25

HMBC/C-17,18), 1.25−1.37 (18H, m, H-10−18), 1.46 (2H, br quint, J = 7.3 Hz, H-9, HMBC/C-7), 1.54 (2H, br quint, J = 7.3 Hz, H-4, HMBC/C-2,3,5,6), 1.74 (2H, br quint, J = 7.6 Hz, H-3, HMBC/C-1,2,4,5), 2.12 (2H, tt, J = 7.1, 2.2 Hz, H-8, HMBC/C-5), 2.17 (2H, tt, J = 7.0, 2.2 Hz, H-5, HMBC/C-3,4,6,7,8), 2.38 (2H, t, J = 7.5 Hz, H-2, HMBC/C-1,3,4); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5 (C-19), 18.8 (C-5), 19.1 (C-8), 23.1 (C-18), 24.2 (C-3), 28.8 (C-4), 29.3, 29.5, 29.6, 29.8, 30.0, 30.1 (×2), 30.2, 32.3 (C-16), 34.0 (C-2), 79.7 (C-6), 81.3 (C-7), 180.3 (C-1); ESIMS m/z 295.2622 {calcd for [M($C_{19}H_{34}O_2$) + H]$^+$, 295.2632}.

Methylation of 1 and 2. A solution of each compound (20 mg) in BF_3/MeOH (14% w/v, 0.6 mL) was heated at 100 °C for 2 min. After cooling, the reaction mixture was diluted with H_2O (1 mL) and then extracted with hexanes (10 mL × 3). The hexane layers were combined, washed with H_2O (5 mL), and dried over Na_2SO_4. Removal of the solvent yielded methylated products **1a** and **2a** (ca. 20 mg each), respectively.

Methyl 6-octadecynoxylate (1a): colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (3H, t, J = 7.0 Hz, H-18), 1.26−1.37 (16H, m, H-10−17), 1.46 (2H, br quint, J = 7.0 Hz, H-9), 1.51 (2H, br quint, J = 7.0 Hz, H-4), 1.72 (2H, br quint, J = 7.5 Hz, H-3), 2.12 (2H, tt, J = 7.1, 2.3 Hz, H-8), 2.17 (2H, tt, J = 7.1, 2.3 Hz, H-5), 2.33 (2H, t, J = 7.5 Hz, H-2), 3.67 (3H, COOMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5 (C-18), 18.8 (C-5), 19.1 (C-8), 23.1 (C-17), 24.5 (C-3), 28.9 (C-4), 29.3, 29.5, 29.6, 29.7, 29.9, 30.0 (×2), 32.3 (C-16), 34.0 (C-2), 51.8 (COO*Me*), 79.7 (C-6), 81.1 (C-7), 174.4 (C-1); GC−MS (t_R = 13.6 min) m/z 263 (1.8) [M − OMe]$^+$, 220 (3.9) [M − CH_2=C(OH)-OMe]$^+$, 154 (53) [CH_2=C=CH−(CH_2)$_4$COOMe]$^+$, 122 (26) [154 − HOMe]$^+$, 94 (95) [122 − CO]$^+$, 80 (100) [154 − CH_2=C(OH)-OMe]$^+$, 67 (48) [94 − (CH_2)$_2$ + H]$^+$.

Methyl 6-nonadecynoxylate (2a): colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, t, J = 7.0 Hz, H-19), 1.24−1.37 (18H, m, H-10−18), 1.45 (2H, br quint, J = 7.0 Hz, H-9), 1.50 (2H, br quint, J = 7.0 Hz, H-4), 1.71 (2H, br quint, J = 7.6 Hz, H-3), 2.11 (2H, tt, J = 7.1, 2.2 Hz, H-8), 2.16 (2H, tt, J = 7.1, 2.2 Hz, H-5), 2.32 (2H, t, J = 7.4 Hz, H-2), 3.65 (3H, COO*Me*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.5 (C-18), 18.9 (C-5), 19.1 (C-8), 23.1 (C-17), 24.5 (C-3), 29.0 (C-4), 29.3, 29.6, 29.8, 30.0, 30.1 (×4), 32.3 (C-16), 34.0 (C-2), 51.9 (COO*Me*), 79.8 (C-6), 81.1 (C-7), 174.4 (C-1); GC−MS (t_R = 16.5 min) m/z 277 (1.6) [M − OMe]$^+$, 234 (4.3) [M − CH_2=C(OH)OMe]$^+$, 154 (62) [CH_2=C=CH−(CH_2)$_4$COOMe]$^+$, 122 (26) [154 − HOMe]$^+$, 94 (95) [122 − CO]$^+$, 80 (100) [154 −CH_2=C(OH)OMe]$^+$, 67 (44) [94 − (CH_2)$_2$ + H]$^+$.

Antifungal Susceptibility Testing. All chemicals were obtained from Sigma Chemical Company (St. Louis, MO) unless otherwise stated. *C. albicans* ATCC 90028 was purchased from ATCC (Rockville, MD). Azole-resistant *C. albicans* strains were kindly provided by Dr. S. W. Redding[11] and Dr. T. C. White.[2,12,13] Antifungal susceptibility tests were performed using a modified version of the NCCLS methods.[9] Compounds were dissolved in DMSO, serially diluted with sterile normal saline, and transferred in duplicate to a flat-bottomed 96-well microplate. Prior to the assay, *C. albicans* growth from a Sabouraud dextrose agar (Difco, Detroit, MI) slant was subcultured in 5 mL of Sabouraud dextrose broth (Difco) at 37 °C for 24 h. The fungal inoculum was prepared by comparison to the 0.5 McFarland standard[9] and diluted in RPMI 1640 without phenol red (2% glucose buffered with MOPS at pH 7.0, Cellgro, Herndon, VA). The fungal inocula were added to the samples, affording a final volume of 200 μL, final initial test concentration of 50 μg/mL, and final inoculum size of approximately 2500 CFU/mL. Growth (saline only), solvent (DMSO only), drug [amphotericin B (ICN Biomedicals, Aurora, OH), fluconazole (Pfizer, Morris Plains, NJ), and flucytosine], and blank (saline + broth) controls were included in each determination. The plates were read at 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Winooski, VT) prior to and after incubation (48 h at 37 °C). Percent growth versus test concentration was calculated and plotted to afford the IC_{50}, IC_{80}, or IC_{95}. The minimum inhibitory concentration (MIC) was defined as the lowest test concentration that allows no detectable growth (100% inhibition). Minimum fungicidal concentrations (MFCs) were determined by removing 5 μL of each duplicate at active test concentration, transferring to Sabouraud dextrose agar, and incubating at 37 °C for 24−48 h. The MFC is defined as the lowest concentration of sample that allows no growth on agar.

Sphingolipid Reversal Assay. The sphingolipid reversal assay[14,17] was performed as above for *C. albicans* ATCC 90028. However, the RPMI broth was supplemented with varying concentrations of DL-dihydrosphingosine or stearylamine (both from Sigma, St. Louis, MO), initially dissolved in a solution of ethanol and methyl β-cyclodextrin. Maximum final test concentrations of ethanol and β-cyclodextrin were 1.56% and 125 μg/mL, respectively, and were not inhibitory to the growth of the fungus.

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References and Notes

(1) Pfaller, M. A.; Jones, R. N.; Messer, S. A.; Edmond, M. B.; Wenzel, R. P. *Diagn. Microbiol. Infect. Dis.* **1998**, *31*, 327−332.
(2) Marr, K. A.; Lyons, C. N.; Rustad, T. R.; Bowden, R. A.; White, T. C. *Antimicrob. Agents Chemother.* **1998**, *42*, 2584−2589.
(3) White, T. C.; Marr, K. A.; Bowden, R. A. *Clin. Microbiol. Rev.* **1998**, *11*, 382−402.
(4) White, T. C.; Holleman, S.; Dy, F.; Mirels, L. F.; Stevens, D. A. *Antimicrob. Agents Chemother.* **2002**, *46*, 1704−1713.
(5) Marr, K. A.; Lyons, C. N.; Ha, K.; Rustad, T. R.; White, T. C. *Antimicrob. Agents Chemother.* **2001**, *45*, 52−59.
(6) Purcell, J. M.; Susi, H. *Anal. Chem.* **1968**, *40*, 571−575.
(7) Pearl, M. B.; Kleiman, R.; Earle, F. R. *Lipids* **1974**, *8*, 627−630.
(8) Valicenti, A. J.; Heimermann, W. H.; Holman, R. T. *J. Org. Chem.* **1979**, *44*, 1068−1073.
(9) National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard M27-A, Vol. 17 (9). National Committee for Clinical Laboratory Standards: Wayne, PA, 1997; 4th ed.
(10) Muhammad, I.; Dunbar, D. C.; Takamatsu, S.; Walker, L. A.; Clark, A. M. *J. Nat. Prod.* **2001**, *64*, 559−562.
(11) Pfaller, M. A.; Rhine-Chalberg, J.; Redding, S. W.; Smith, J.; Farinacci, G.; Fothergill, A. W.; Rinaldi, M. G. *J. Clin. Microbiol.* **1994**, *32*, 59−64.
(12) White T. C. *Antimicrob. Agents Chemother.* **1997**, *41*, 1482−1487.
(13) White T. C. *Antimicrob. Agents Chemother.* **1997**, *41*, 1488−1494.

(14) Zweerink, M. M.; Edison, A. M.; Wells, G. B.; Pinto, W.; Lester, R. L. *J. Biol. Chem*. **1992**, *267*, 25032−25038.
(15) Horn, W. S.; Smith, J. L.; Bills, G. F.; Raghoobar, S. L.; Helms, G. L.; Kurtz, M. B.; Marrinan, J. A.; Frommer, B. R.; Thornton, R. A.; Mandala, S. M. *J. Antibiot.* **1992**, *45*, 1692−1696.
(16) Mandala, A. M.; Frommer, B. R.; Thornton, R. A.; Kurtz, M. B.; Young, N. M.; Gabello, M. A.; Genilloud, O.; Liesch, J. M.; Smith, J. L.; Horn, W. S. *J. Antibiot.* **1994**, *47*, 376−379.
(17) Mandala, A. M.; Thornton, R. A.; Frommer, B. R.; Dreikorn, S.; Kurtz, M. B. *J. Antibiot.* **1997**, *50*, 339−343.
(18) Mandala, A. M.; Thornton, R. A.; Rosenbach, M.; Milligan, J.; Garcia-Calvo, M.; Bull, H. G.; Kurtz, M. B. *J. Biol. Chem*. **1997**, *272*, 32709−32714.
(19) Li, X.-C.; ElSohly, H. N.; Nimrod, A. C.; Clark, A. M. *J. Nat. Prod*. **1999**, *62*, 767−769.
(20) Li, X.-C.; Dunbar, C.; ElSohly, H. N.; Jacob, M. R.; Nimrod, A. C.; Walker, L. A.; Clark, A. M. *J. Nat. Prod*. **2001**, *64*, 1153−1156.
(21) Lumb, P. B.; Smith, J. C. *J. Chem. Soc.* **1952**, 5032−5035.

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