Isolation, Structure, and Antibacterial Activity of Phaeosphenone from a *Phaeosphaeria* sp. Discovered by Antisense Strategy

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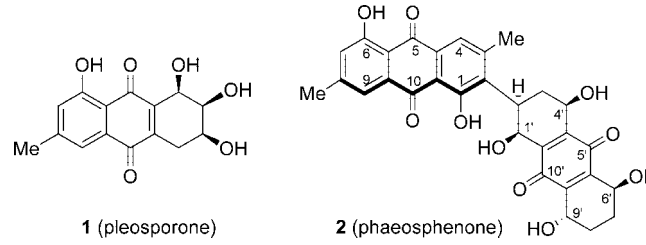
Ribosomal protein S4 (RPSD), a part of the ribosomal small subunit, is one of the proteins that is a part of the ribosomal machinery and is a potential new target for the discovery of antibacterial agents. Continued screening of microbial extracts using antisense-sensitized rpsD *Staphylococcus aureus* strain led to the isolation of a new dimeric compound, phaeosphenone (**2**). Compound **2** showed broad-spectrum antibacterial activity against Gram-positive bacteria, exhibiting MIC values ranging from 8 to 64 μg/mL. Phaeosphenone showed the highest sensitivity for *Streptococcus pneumoniae* (8 μg/mL) and inhibited the growth of *Candida albicans* with an MIC of 8 μg/mL. Phaeosphenone showed a modest selectivity for the inhibition of RNA synthesis over DNA and protein synthesis in *S. aureus*.

Drug-resistant bacteria continue to emerge at an alarming rate, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, and remain a serious threat to human lives.[1] The current antibiotics target fewer than 25 molecular targets. Protein synthesis is one of the global targets that has led to a number of highly effective antibacterial agents.[2,3] Protein synthesis is catalyzed by the ribosome, which is comprised of two asymmetric macromolecular units, the large (50S) and small (30S) subunits. The large subunit consists of two rRNAs, 23S and 5S, and 34 unique ribosomal proteins (r-proteins), L1−L34.[4−6] The small subunit is composed of 16S rRNA and 21 r-proteins, S1−S21.[4−6] The catalytic power of the protein synthesis resides in the ribosome itself. Ribosomal proteins are known to help the ribosome to maintain its quaternary structure. Most of the drugs that are in clinical use today bind not only to rRNA but also to one or more ribosomal proteins, and the inhibition of protein synthesis is the result of interferences of either of the bindings. Alteration of the rRNA binding to r-proteins inactivates the protein synthesis function. Therefore, selective alteration of the conformation of a particular r-protein, or inhibiting the synthesis of an r-protein, would result in the loss of function and may lead to inhibition of the bacterial protein synthesis. Small ribosomal protein S4 is one of those proteins. It is encoded by the *rpsD* gene in both Gram-positive and Gram-negative bacteria, conserved across bacterial species, and essential for bacterial growth.[7,8]

We recently reported the design and application of a two-plate whole-cell differential sensitivity screening assay for the discovery of new antibiotics using an antisense-sensitized *S. aureus* strain with reduced expression of the *fabF/H* gene and thus reduced production of FabF/H proteins.[9,10] This led to the discovery of platensimycin and platencin, two novel and potent inhibitors of FabF and FabF/H enzymes with *in vivo* antibiotic activities.[11−14] A similar two-plate assay with a reduced expression of the *rpsD* gene by antisense was developed and used for screening of natural product extracts. This screening strategy led to the identification of lucensimycins A and B[15] from a strain of *Streptomyces lucensis* MA7349, coniothyrione from a fungal strain of *Coniothyrium cerealis* MF7209,[16] and pleosporone (**1**) from an unidentified Ascomycete.[17] Continued screening of microbial extracts led to the discovery of a fungal strain tentatively identified as a *Phaeosphaeria* sp., which showed good activity in the two-plate screening assay. Bioassay-guided fractionation of the acetone extract led to the isolation of a new compound named herein phaeosphenone (**2**), which showed the requisite differential sensitivity against *rpsD* strain, selectivity for RNA synthesis inhibition, and modest Gram-positive antibacterial activity. The isolation, structure elucidation, and biological activity of **2** are herein described.



The fungal strain MF7026 was isolated from granitic stone collected near Madrid, Spain. Sequence analysis of the internal transcribed spacer (ITS) regions of the rDNA indicated that the fungus was an unidentified species of *Phaeosphaeria* I. Miyake (Pleosporales, Ascomycota). The strain was grown in a liquid medium containing both defined and complex sources of nutrients and was extracted with an equal volume of acetone. The acetone extract was subjected to a two-step isolation procedure employing Amberchrome and reversed-phase HPLC, affording **2** (3.2 mg/L) as a yellow oil.

HRESI-FTMS of **2** produced a protonated molecular ion *m*/*z* 547.1585 [M + H] that analyzed for the molecular formula $C_{30}H_{26}O_{10}$ possessing 18 degrees of unsaturation. The UV spectrum showed absorption maxima at λ_{max} 286 and 420 nm, indicative of a highly conjugated system. The IR spectrum displayed absorption bands for conjugated ketone (ν_{max} 1659 and 1635 cm^{-1}) and OH groups (3410 cm^{-1}). The ^{13}C NMR spectrum in CD_3OD and in C_5D_5N exhibited signals for 30 carbons. A number of ^{1}H resonances recorded in CD_3OD significantly overlapped but were nicely dispersed in C_5D_5N. The DEPT spectrum displayed 13 signals, including two methyl groups, three methylenes, and eight methines (including four oxymethines). Also 12 sp^2 carbons, including three proton-bearing carbons and two oxygenated carbons appearing at δ_C 160.0 and 162.2, four quinone-type conjugated ketone groups, and two olefinic methyl groups appearing at δ_C 22.2 and 22.7 were present. The HMBC correlations of H_3-11 (δ_H 2.17) to C-7 (δ_C 124.2), C-8 (δ_C 148.2), and C-9 (δ_C 120.7); H-7 (δ_H 7.00) to C-6 (δ_C 162.2), C-9 (δ_C 120.7), C-5a (δ_C 113.9), and C-11 (δ_C 22.2); H-9 (δ_H 7.55) to C-7 (δ_C 124.2), C-5a (δ_C 113.9), C-10 (δ_C 185.3),

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and C-11 (δ_C 22.2); H-12 (δ_H 2.91) to C-2 (δ_C 138.1), C-3 (δ_C 148.6), and C-4 (δ_C 121.8); and H-4 (δ_H 7.74) to C-2 (δ_C 138.1), C-5 (δ_C 184.6), C-10a (δ_C 114.2), and C-12 (δ_C 22.7) confirmed a 1,6-dihydroxy-3,8-dimethylanthraquinone ring system. COSY and TOCSY experiments established two isolated spin systems for the remainder of the molecule. One of the spin systems involved H-1′, H-2′, H-3′, and H-4′, and the other involved H-6′, H-7′, H-8′, and H-9′. These two spin systems were connected to each other by HMBC correlations. For example, H-2′ (δ_H 5.04) showed HMBC correlations to C-10′a (δ_C 146.8); H-3′ (δ_H 3.22) to C-4′a (δ_C 144.1); H-7′ (δ_H 3.15/3.33) to C-5′a (δ_C 144.0); and H-8′ (δ_H 2.99/3.14) to C-9′a (δ_C 142.7); these plus two remaining carbonyl carbons (δ_C 190.0 and 190.7) allowed the assembly of the 1,4,6,9-tetrahydroxyoctahydroanthraquinone moiety, which also accounted for the remaining seven degrees of unsaturation. The connectivity of the two structural units at C-2 and C-2′ was established by HMBC correlations of H-2′ (δ_H 5.04) to C-1 (δ_C 160.0), C-2 (δ_C 138.1), and C-3 (δ_C 148.6) and of H-1′ (δ_H 4.83) to C-2 (δ_C 138.1).

The relative configuration at four oxygenated methines and C-2′ was determined by measurements of the vicinal coupling constants and NOE. Both H-6′ and H-9′ appeared as a doublet of doublet of doublets with J = 6.5, 5.0, and 2.0 Hz. The lack of a large (~10 Hz) coupling and the presence of ~6 Hz coupling indicated that these protons are equatorially disposed in a pseudo-chair conformation. H-2′ appeared as a doublet of doublet of doublets showing only small couplings (J = 1.3 and 3.5 Hz) with both methylene protons at C-3′ and a large coupling (J = 8.1 Hz) with H-1′. Both protons at C-3′ also showed small couplings (J = 3.3 and 3.9 Hz) with H-4′. These vicinal couplings indicated that H-2′ and H-4′ must be equatorial in a slightly flattened pseudo-chair conformation, which will allow ~50−60° dihedral angles between the methylene protons and H-2′ and H-4′. The 8.1 Hz coupling between H-2′ and H-1′ suggested that H-1′ must be axially oriented, which is consistent with the observation of a weak NOESY correlation (CD_3OD) with H-3′$_\alpha$ (δ_H 2.79). The anthraquinone moiety was axially oriented at C-2′. H-1′ and H-4′ exhibited homoallylic coupling (J = 2.5 Hz). Similar homoallylic coupling was also observed between H-6′ and H-9′ (J = 2.0 Hz). The NOESY correlation of H-2′ to H-12 indicated they were on the same side of the molecule. Thus, the structure and relative configuration of **2** at C-1′, C-2, C-4′, C-6′, and C-9′ must be as shown.

A substructure search in SciFinder did not result in meaningful hits. While many anthraquinone monomers and dimers have been reported as natural products, the monomeric dimethyl dihydroxy anthraquinone of **2** has not been reported as a natural product but was reported by synthesis.[18] It is surprising that few octahydro anthraquinones have been reported either from nature or by synthesis. The octahydro tetrahydroxy tricyclic quinone portion of the molecule appears to be unprecedented. Species of *Phaeosphaeria* have been reported to produce rousselianone A as an antifungal agent,[19] gibberellins and phaeoside,[20] and phaeosphaeride A, an inhibitor of STAT3-dependent signaling.[21]

In the antisense *rpsD*-sensitized two-plate differential sensitivity assay, phaeosphenone (**2**) showed higher sensitivity for the sensitized strain than the control strain. It showed a zone differential of 3−4 mm with zone sizes of 16.0 and 11.8 mm at 500 μg/mL against the antisense and the control strains, respectively, in a dose−response assay with 2-fold dilution starting from 500 μg/mL to 31.25 μg/mL. At the lowest concentration, a 7 mm faint zone of clearance was observed in the antisense plate, but no zone of clearance was observed in the control plate, suggesting a MDC (minimum detection concentration) of 31.25−62.5 μg/mL. Compound **2** inhibited the growth of wild-type *S. aureus* strains (MIC of 32−64 μg/mL). It showed an MIC_{80} (inhibition of 80% growth) of 16−32 μg/mL (Table 1). Phaeosphenone showed similar activity against *Streptococcus pneumoniae* (MIC 64 μg/mL) when tested in Mueller Hinton broth medium containing 2.5% lysed horse blood

Table 1. Antibacterial Activities of Phaeosphenone (**2**)

strain[a]	phenotype	strain #	MIC (μg/mL)[b]
S. aureus	meth^s	ATCC 29213	32 (16)
S. aureus	meth^s	MB2865	64 (32)
S. aureus (+50% human serum)	meth^s	MB2865	>64
S. pneumonieae[c]	pen^s, quin^s, mac^s	CL2883	32
S. pneumonieae[d]	pen^s, quin^s, mac^s	CL2883	8
E. faecalis	van^s, mac^R	CL8516	64
B. subtilis	Wt	MB964	16
H. influenzae	Amp^s, quin^s, mac^s	MB4572	>64
E. coli	Wt	MB2884	>64
C. albicans	Wt	MY1055	8

[a] All strains were tested in cation-adjusted Mueller Hinton broth (CAMHB) medium, unless mentioned otherwise, under National Committee for Clinical Laboratory Standards (NCCLS) guidelines. [b] MIC (minimum inhibitory concentration); the numbers in parentheses show the concentration of compound that inhibits 80% of cell growth. [c] CAMHB +2.5% lysed horse blood medium. [d] Isosensitet medium.

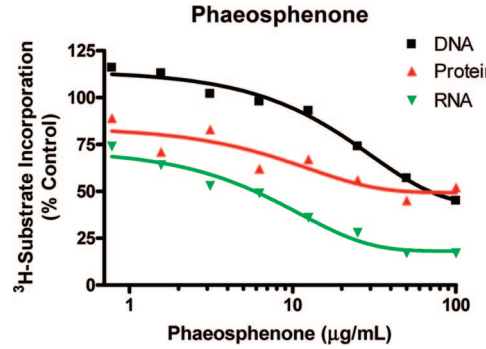

Figure 1. Macromolecular synthesis inhibition by phaeosphenone (**2**).

and showed 2−4-fold better activity (MIC 8 μg/mL) when *S. pneumoniae* was grown in isosensitet medium. Activity against other Gram-positive bacteria such as *Enterococcus faecalis* and *Bacillus subtilis* was similar to that observed for *S. aureus* (MIC 16−32 μg/mL). Compound **2** did not inhibit the growth of Gram-negative bacteria such as *Haemophilus influenzae* and *Escherichia coli*. It inhibited the growth of *Candida albicans* at 8 μg/mL, indicating lack of selectivity for the bacterial strains over fungal strains. Interaction or binding of inhibitors with RPSD protein is expected to show inhibition of protein synthesis. Compound **2**, like monomeric tetrahydroxy anthraquinone pleosporone (**1**),[17] showed preference for the inhibition of *S. aureus* RNA synthesis (IC_{50} 6 μg/mL) over protein and DNA synthesis (Figure 1). The preferential inhibition of RNA synthesis over protein synthesis is not clear and suggests that phaeosphenone likely has another yet unknown mode of action in addition to the weak interaction with RPSD protein.

In summary, we have isolated phaeosphenone (**2**), a new dimeric compound consisting of an anthraquinone and an octahydro anthraquinone. It was isolated from an unidentified species of *Phaeosphaeria* using an antisense differential sensitivity strategy. It showed broad-spectrum Gram-positive antibacterial activity that was similar to pleosporone (**1**), another anthraquinone with higher sensitivity exhibited for *S. pneumoniae* in isosensitet medium. Unlike pleosporone, **2** did not show activity against Gram-negative organisms.

Experimental Section

General Experimental Procedures. HP1100 was used for analytical HPLC. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were recorded in MeOH on a Beckman DU-70 spectrophotometer. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer. HRESI-FTMS were obtained on a Finnigan LTQ-FT spectrometer using electrospray ionization. The NMR spectra

were recorded on a Varian INOVA 500 FT-NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C in pyridine-d_5.

Producing Organism. The producing organism (MF7026) was isolated from a sample of granitic stone collected in La Cabrera (Madrid, Spain) using a direct plating technique described by Ruibal et al.[22] It was identified as *Phaeosphaeria* sp. (Pleosporales, Ascomycetes). The isolate failed to produce spores in agar culture stage. Sequence analysis of the internal transcribed spacers (ITS) of the rDNA indicated 94% similarity with GenBank sequences from *Phaeosphaeria nodorum* (E. Müll.) Hedjar (AF181708, AF817684) and *P. avenaria* (G.F. Weber) O.E. Erikss. (AF196988). On the basis of the results from ITS sequencing, the fungus was characterized as an unidentified species of the genus *Phaeosphaeria*.

Fermentation of *Phaeosphaeria* sp. (MF7026). Plugs from potato dextrose agar (PDA, Difco) cultures were preserved in vials containing sterile glycerol (10%) at 80 °C. Seed flasks were prepared by transferring four agar plugs into 250 mL Erlenmeyer flasks containing 60 mL of seed medium of the following composition in g·L^{-1} distilled H_2O (corn steep powder, 2.5; tomato paste, 40.0; oat flour, 10.0; glucose, 10.0; $FeSO_4 \cdot 7H_2O$, 0.01; $MnSO_4 \cdot 4H_2O$, 0.01; $CuCl_2 \cdot 2H_2O$, 0.0025; $CaCl_2 \cdot 2H_2O$, 0.001; H_3BO_3, 0.00056; $(NH_4)_6MoO_{24} \cdot 4H_2O$, 0.00019; $ZnSO_4 \cdot 7H_2O$, 0.01). The pH was adjusted to 6.8 before autoclaving. The seed flasks were incubated for 5 days at 22 °C at 220 rpm on a gyratory shaker prior to inoculation of the production medium. Two milliliter aliquots of the seed culture were used to inoculate 250 mL unbaffled Erlenmeyer flasks containing 50 mL of MOF production medium consisting of g·L^{-1} (mannitol, 75 g; oat flour, 15 g; yeast extract, 5 g; L-glutamic acid, 4 g; MES, 16.2 g, pH = 6). Production flasks were incubated at 22 °C in a gyratory shaker at 220 rpm for 14 days.

Extraction and Isolation. Twenty flasks (~1 L) were harvested, pooled, and extracted with 1 L of acetone by shaking on a platform shaker for 60 min. The extract was concentrated to remove most of the acetone and charged on an Amberchrome column. The column was eluted with a linear gradient using aqueous acetone at a flow rate of 10 mL/min, affording one active fraction weighing 37.4 mg that eluted at 80% aqueous acetone. Half of the active fraction was further chromatographed by semipreparative reversed-phase HPLC using an Atlantis C_{18} (10 × 250 mm) column eluting for 40 min with a gradient of 20−95% aqueous CH_3CN containing 0.1% TFA at a flow rate of 5 mL/min. Lyophilization of the fraction eluting at 16 min afforded 1.6 mg (3.2 mg/L) of **2** as a yellow oil.

Phaeosphenone (2): amorphous powder; $[\alpha]^{25}_D$ +170 (*c* 0.1,CH_3OH); UV (CH_3OH) λ_{max} 286 (ϵ 9391) 420 (ϵ 4586) nm; IR (ZnSe) ν_{max} 3410, 1659, 1635, 1615, 1384, 1276, 1202, 1140, 1071, 756, 724 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 12.83 (1H, s, OH), 7.74 (1H, s, H-4), 7.55 (1H, brd, J = 1.6 Hz, H-9), 7.00 (1H, brd, J = 1.6 Hz, H-7), 5.04 (1H, ddd, J = 8.0, 3.5, 1.5 Hz, H-2′), 4.85 (1H, td, J = 3.5, 2.5 Hz, H-4′), 4.83 (1H, dd, J = 8.0, 2.5 Hz, H-1′), 4.44 (1H, ddd, J = 6.5, 5.0, 2.0 Hz, H-6′), 4.42 (1H, ddd, J = 6.5, 5.0, 2.0 Hz, H-9′), 3.73 (1H, ddd, J = 19.2, 3.5, 1.5 Hz, H-3′), 3.33 (1H, dddd, J = 19.5, 9.0, 6.5, 2.5 Hz, H-7′), 3.22 (1H, td, J = 3.5, 19.2 Hz, H-3′), 3.15 (1H, m, H-7′), 3.14 (1H, m, H-8′), 2.99 (1H, tdd, J = 3.0, 5.0, 19.5 Hz, H-8′), 2.91 (3H, s, H_3-12), 2.17 (3H, s, H_3-11); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.50 (1H, s, H-4), 7.40 (1H, brd, J = 1.6 Hz, H-9), 6.95 (1H, brd, J = 1.6 Hz, H-7), 4.44 (1H, ddd, J = 8.0, 3.5, 1.3 Hz, H-2′), 4.26 (1H, td, J = 3.5, 2.4 Hz, H-4′), 4.20 (1H, dd, J = 8.0, 2.3 Hz, H-1′), 3.95 (1H, m, H-6′), 3.95 (1H, m, H-9′), 3.09 (1H, ddd, J = 19.2, 3.5, 1.3 Hz, H-3′), 2.79 (1H, td, J = 3.2, 19.2 Hz, H-3′), 2.77 (1H, m, H-7′), 2.72 (3H, s, H_3-12), 2.71 (1H, m, H-7′), 2.71 (1H, m, H-8′), 2.63 (1H, m, H-8′), 2.37 (3H, s, H_3-11); ^{13}C NMR (C_5D_5N, 125 MHz) δ 190.7[b] (C-10′), 190.0[b] (C-5′), 185.3 (C-10), 184.6 (C-5), 162.2 (C-6), 160.0 (C-1), 148.8 (C-10′a), 148.6 (C-3), 148.2 (C-8), 144.1 (C-4′a), 144.0 (C-5′a), 142.7 (C-9′a), 138.1 (C-2), 133.0[a] (C-4a), 130.5[a] (C-9a), 124.2 (C-7), 121.8 (C-4), 120.7 (C-9), 114.2 (C-10a), 113.9 (C-5a), 73.6 (C-1′), 68.8 (C-4′), 68.7 (C-6′), 68.7 (C-9′), 41.6 (C-2′), 32.3 (C-3′), 30.8 (C-7′), 30.0 (C-8′), 22.7 (C-12), 22.2 (C-11); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 190.9[b] (C-10′), 190.1[b] (C-5′), 185.6 (C-10), 185.0 (C-5), 162.5 (C-6), 160.0 (C-1), 149.4 (C-3), 149.2 (C-8), 146.6 (C-10′a), 143.9 (C-4′a), 143.5 (C-5′a), 142.7 (C-9′a), 137.6 (C-2), 133.2[a] (C-4a), 131.0[a] (C-9a), 124.4 (C-7), 122.1 (C-4), 120.8 (C-9), 114.4 (C-10a), 114.0 (C-5a), 73.4 (C-1′), 68.9 (C-4′), 68.7 (C-6′), 68.7 (C-9′), 41.4 (C-2′), 31.4 (C-3′), 29.9 (C-7′), 29.3 (C-8′), 22.2 (C-12), 22.0 (C-11); HRESI-FTMS *m/z* 547.1585 (calcd for $C_{30}H_{26}O_{10}$+H: 547.1605).

Two-Plate Differential Sensitivity RPSD Assay. *Staphyloccus aureus* cells (RN450) carrying plasmid S1-782B bearing antisense to RPSD (*rpsD* AS-RNA strain) or vector (control strain) were inoculated from a frozen vial source into a tube containing 3 mL of Miller's LB broth (Invitrogen) plus 34 μg/mL chloramphenicol. Tubes were incubated at 37 °C at 220 rpm for 18−20 h and kept at room temperature until use. Miller's LB broth was supplemented with 1.2% Select agar (Invitrogen), 0.2% glucose, 15 μg/mL chloramphenicol, and 12 mM xylose (only for the antisense strain). The OD_{600} of the culture was measured and 1:1000 of OD 3.0 inoculated. Next, 100 mL was poured into each NUNC plate, well-caster templates were placed into the agar, and the agar was allowed to solidify. Then, 20 μL of test samples was added to the wells, the plates were incubated at 37 °C for 18 h, and zones of inhibition were measured. MDC (minimum detection concentration) values were determined by 2-fold serial dilution.

Antibiotic Assay (MIC). The MIC (minimum inhibitory concentration) against each of the strains was determined as previously described.[23] Cells were inoculated at 105 colony-forming units/mL followed by incubation at 37 °C with a 2-fold serial dilution of compounds in the growth medium for 20 h. MIC is defined as the lowest concentration of an antibiotic inhibiting visible growth.

Macromolecular Synthesis Inhibition. The assay was performed as previously described.[11,24] Briefly, mid-log (A_{600} = 0.5−0.6) *S. aureus* growth was incubated at an increasing concentration of each inhibitor at 37 °C for 20 min with 1 μCi/mL 6-[^{3}H]-thymidine, 1 μCi/mL 5,6-[^{3}H]uracil, or 5 μCi/mL 4,5-[^{3}H]leucine, to measure DNA, RNA, and protein synthesis, respectively. The reaction was stopped by addition of 10% trichloroacetic acid, and the cells were harvested using a glass fiber filter (Perkin-Elmer Life Sciences, 1205-401). The filter was dried, and cells were counted with scintillation fluid.

Supporting Information Available: Copies of ^{1}H, ^{13}C, COSY, TOCSY, HSQC, HMBC, and NOESY data of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Klevens, R. M.; Morrison, M. A.; Nadle, J.; Petit, S.; Gershman, K.; Ray, S.; Harrison, L. H.; Lynfield, R.; Dumyati, G.; Townes, J. M.; Craig, A. S.; Zell, E. R.; Fosheim, G. E.; McDougal, L. K.; Carey, R. B.; Fridkin, S. K. *J. Am. Med. Assoc.* **2007**, *298*, 1763–1771.

(2) Poehlsgaard, J.; Douthwaite, S. *Nat. Rev. Microbiol.* **2005**, *3*, 870–881.

(3) Singh, S. B.; Barrett, J. F. *Biochem. Pharmacol.* **2006**, *71*, 1006–1015.

(4) Ramakrishnan, V. *Cell* **2002**, *108*, 557–572.

(5) Culver, G. M. *Biopolymers* **2003**, *68*, 234–249.

(6) Ogle, J. M.; Carter, A. P.; Ramakrishnan, V. *Trends Biochem. Sci.* **2003**, *28*, 259–66.

(7) Grundy, F. J.; Henkin, T. M. *J. Bacteriol.* **1991**, *173*, 4595–602.

(8) Forsyth, R. A.; Haselbeck, R. J.; Ohlsen, K. L.; Yamamoto, R. T.; Xu, H.; Trawick, J. D.; Wall, D.; Wang, L.; Brown-Driver, V.; Froelich, J. M.; Kedar, G. C.; King, P.; McCarthy, M.; Malone, C.; Misiner, B.; Robbins, D.; Tan, Z.; Zhu, Z.-Y.; Carr, G.; Mosca, D. A.; Zamudio, C.; Foulkes, J. G.; Zyskind, J. W. *Mol. Microbiol.* **2002**, *43*, 1387–1400.

(9) Young, K.; Jayasuriya, H.; Ondeyka, J. G.; Herath, K.; Zhang, C.; Kodali, S.; Galgoci, A.; Painter, R.; Brown-Driver, V.; Yamamoto, R.; Silver, L. L.; Zheng, Y.; Ventura, J. I.; Sigmund, J.; Ha, S.; Basilio, A.; Vicente, F.; Tormo, J. R.; Pelaez, F.; Youngman, P.; Cully, D.; Barrett, J. F.; Schmatz, D.; Singh, S. B.; Wang, J. *Antimicrob. Agents Chemother.* **2006**, *50*, 519–526.

(10) Singh, S. B.; Phillips, J. W.; Wang, J. *Curr. Opin. Drug Discovery Dev.* **2007**, *10*, 160–166.

(11) Wang, J.; Soisson, S. M.; Young, K.; Shoop, W.; Kodali, S.; Galgoci, A.; Painter, R.; Parthasarathy, G.; Tang, Y.; Cummings, R.; Ha, S.; Dorso, K.; Motyl, M.; Jayasuriya, H.; Ondeyka, J.; Herath, K.; Zhang, C.; Hernandez, L.; Alloco, J.; Basilio, Á.; Tormo, J. R.; Genilloud, O.; Vicente, F.; Pelaez, F.; Colwell, L.; Lee, S. H.; Michael, B.; Felcetto, T.; Gill, C.; Silver, L. L.; Hermes, J.; Bartizal, K.; Barrett, J.; Schmatz, D.; Becker, J. W.; Cully, D.; Singh, S. B. *Nature* **2006**, *441*, 358–361.

(12) Wang, J.; Kodali, S.; Lee, S. H.; Galgoci, A.; Painter, R.; Dorso, K.; Racine, F.; Motyl, M.; Hernandez, L.; Tinney, E.; Colletti, S.; Herath, K.; Cummings, R.; Salazar, O.; Gonzalez, I.; Basilio, A.; Vicente, F.; Genilloud, O.; Pelaez, F.; Jayasuriya, H.; Young, K.; Cully, D.; Singh, S. B. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 7612–7616.

(13) Singh, S. B.; Jayasuriya, H.; Ondeyka, J. G.; Herath, K. B.; Zhang, C.; Zink, D. L.; Tsou, N. N.; Ball, R. G.; Basilio, A.; Genilloud, O.;

Diez, M. T.; Vicente, F.; Pelaez, F.; Young, K.; Wang, J. *J. Am. Chem. Soc.* **2006**, *128*, 11916−20 and–15547.
(14) Jayasuriya, H.; Herath, K. B.; Zhang, C.; Zink, D. L.; Basilio, A.; Genilloud, O.; Diez, M. T.; Vicente, F.; Gonzalez, I.; Salazar, O.; Pelaez, F.; Cummings, R.; Ha, S.; Wang, J.; Singh, S. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 4684–4688.
(15) Singh, S. B.; Zink, D. L.; Huber, J.; Genilloud, O.; Salazar, O.; Diez, M. T.; Basilio, A.; Vicente, F.; Byrne, K. M. *Org. Lett.* **2006**, *8*, 5449–5452.
(16) Ondeyka, J. G.; Zink, D.; Basilio, A.; Vicente, F.; Bills, G.; Diez, M. T.; Motyl, M.; Dezeny, G.; Byrne, K.; Singh, S. B. *J. Nat. Prod.* **2007**, *70*, 668–670.
(17) Zhang, C.; Ondeyka, J.; Zink, D.; Basilio, A.; Vicente, F.; Collado, J.; Platas, G.; Huber, J.; Dorso, K.; Motyl, M.; Byrne, K.; Singh, S. B. Bioorg. Med. Chem. (published online April 12, 2008).
(18) Balenovic, K.; Poje, M. *Tetrahedron Lett.* **1975**, *3427*, 28.
(19) Xiao, J. Z.; Kumazawa, S.; Tomita, H.; Yoshikawa, N.; Kimura, C.; Mikawa, T. *J Antibiot. (Tokyo)* **1993**, *46*, 1570–1574.
(20) Kenmoku, H.; Sugai, T.; Yajima, H.; Sassa, T. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2418–2420.
(21) Maloney, K. N.; Hao, W.; Xu, J.; Gibbons, J.; Hucul, J.; Roll, D.; Brady, S. F.; Schroeder, F. C.; Clardy, J. *Org. Lett.* **2006**, *8*, 4067–4070.
(22) Ruibal, C.; Platas, G.; Bills, G. *Mycol. Progr.* **2005**, *4*, 23–38.
(23) Kodali, S.; Galgoci, A.; Young, K.; Painter, R.; Silver, L. L.; Herath, K. B.; Singh, S. B.; Cully, D.; Barrett, J. F.; Schmatz, D.; Wang, J. *J. Biol. Chem.* **2005**, *280*, 1669–1677.
(24) Onishi, H. R.; Pelak, B. A.; Gerckens, L. S.; Silver, L. L.; Kahan, F. M.; Chen, M. H.; Patchett, A. A.; Galloway, S. M.; Hyland, S. A.; Anderson, M. S.; Raetz, C. R. *Science* **1996**, *274*, 980–982.

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