Pitucamycin: Structural Merger of a Phenoxazinone with an Epoxyquinone Antibiotic

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Chemical profiling of a *Streptomyces griseus* strain isolated from an old building with moisture damage led to the discovery of two novel phenoxazinones, chandrananimycin D (**1**) and pitucamycin (**2**), along with the known grixazone B (**3**). Pitucamycin represents an unprecedented hybrid molecule composed of a phenoxazinone and an enaminomycin-like epoxyquinone moiety.

The phenoxazinone scaffold is distributed widely in nature and has been isolated repeatedly from a variety of biological sources, including bacteria, fungi, insects, and even Australian marsupials.[1] Notwithstanding their wide occurrence, phenoxazinones appear to be biosynthesized following a common pathway. Upon action of an oxidase, an *o*-aminophenol is converted into its corresponding quinone imine, which then nonenzymatically fuses with another quinone imine or a second *o*-aminophenol molecule.[2,3] Structural variability within the phenoxazinone series of natural products arises primarily from the usage of different *o*-aminophenol precursors that differ in their oxidation state followed by site-specific acetylations and methylations, respectively.[4,5] Further structural modifications are rare and include the nonenzymatic linkage with *N*-acetylcysteine as well as glycosylation.[2,6] Herein we report the isolation and characterization of two new phenoxazinones, chandrananimycin D (**1**) and pitucamycin (**2**). The latter likely results from the unusual linkage of the phenoxazinone core with an epoxyquinone, enaminomycin C (antibiotic 2061B).[7,8]

To evaluate the potential health risk factors of a *Streptomyces griseus* strain growing in an old building with moisture damage, the metabolic profile of the bacterial isolate was investigated. In the course of these studies, a set of antiproliferative phenoxazinones named bezerramycins was isolated recently.[4] In addition to the major components, some minor congeners were detected by TLC analysis, which were missed in our previous study. As these compounds appeared to hold antiproliferative potential, repeat fermentations were carried out on a large scale (200 L). The culture broth was subjected to solid-phase extraction. The eluate was concentrated under reduced pressure and lyophilized. Three compounds (**1**−**3**, Figure 1) that were produced only in minute quantities (6, 2.2, and 1 mg, respectively) were recovered from the lyophilized culture filtrate by methanol extraction and purified by successive column chromatography on silica gel using gradients of $CHCl_3$/MeOH. Selected fractions were combined and subsequently subjected to gel permeation chromatography on Sephadex LH-20 (eluted with MeOH) to yield **1** (6 mg) and **2** (2.2 mg) as orange solids. In addition, one known compound (1 mg) was isolated, which could be identified as grixazone B (**3**) by comparison of its spectroscopic data with those published in the literature.[9]

The UV spectrum of compound **1** showed striking similarities to the UV spectra of various known phenoxazinone-type compounds, such as actinomycins.[1] The molecular formula of compound **1** ($C_{15}H_{12}O_5N_2$) was deduced from HRESIMS (*m*/*z* 299.0677 [M − H]$^-$, calcd 299.0673 for $C_{15}H_{11}O_5N_2$), which pointed to 11 double-bond equivalents. Inspection of the ^{1}H and ^{13}C NMR spectra (Table 1) in combination with DEPT and HSQC spectra revealed the presence of five methines (δ_C 103.4, 112.6, 115.8, 126.7, and 130.4), two methylenes (δ_C 61.8 and 61.8), one NH group (δ_H 9.77), and eight quaternary carbon atoms including two carbonyl groups (δ_C 133.2, 136.1, 140.4, 141.7, 148.3, 149.3, 172.1, and 178.9). The HMBC spectrum provided the crucial information for the presence of a methylene carbon attached to C-8 (Figure 2). The chemical shift of the methylene group (δ_C 61.8) suggested the presence of an attached hydroxy group. The structure of ring A was established by combined analysis of ^{1}H−^{1}H COSY, HSQC, and HMBC spectra. The ^{1}H−^{1}H COSY spectrum revealed the coupling systems H-6/H-7/H-9. Furthermore, the HMBC spectrum showed correlations from H-6 to C-5a, C-8, and C-9a, from H-7 to C-5a, C-9, and C-14, and from H-9 to C-5a, C-7, and C-14 (Figure 2), which led to the assignment of the structure of ring A. The HMBC experiment also allowed for the deduction of the structure of ring C. It showed correlations from the methine proton (H-1) to C-3, C-4a, and C-10a and from the methine proton (H-4) to C-4a and C-10a (Figure 2). Furthermore, **1** has a carbonyl (δ_C 172.1) and a hydroxymethyl group (δ_C 61.8), as substituents of the amine group (NH-11) at C-2. The HMBC correlations (Figure 2) from the protons of NH (H-11) and of the methylene carbon (H-13) to C-12 established this moiety. Compound **1** is structurally related to the previously described chandrananimycin B, which was isolated from a marine *Actinomadura* sp. isolate M048,[10] and was thus named chandrananimycin D. Compound **1** differs from chandrananimycin B in the substitution at C-8; while chandrananimycin B shows a proton at this position, **1** is substituted with a hydroxymethyl residue. The substitution pattern at C-8 of the phenoxazinone core structure is in full agreement with the previously published phenoxazinone-type compounds.[1]

For compound **2** (named pitucamycin, Figure 1) the HRESIMS gave an exact mass at *m*/*z* 425.1143 for [M − H]$^-$ (calcd 425.1103 for $C_{20}H_{17}O_7N_4$), suggesting a molecular formula of $C_{20}H_{18}O_7N_4$, which indicated 14 degrees of unsaturation. The ^{13}C NMR and DEPT spectra showed 20 carbon signals for one methylene (δ_C 62.1), eight methines (δ_C 62.0, 71.1, 72.2, 97.0, 103.0, 115.6, 125.3, and 127.1), and 11 quaternary carbon atoms including three carbonyl groups (δ_C 96.4, 133.6, 139.9, 140.7, 147.2, 148.2, 149.2, 170.1, 173.8, 179.9, and 189.3), respectively. NMR data (^{1}H, ^{13}C, HSQC, ^{1}H−^{1}H COSY, and HMBC) revealed that compound **2** has the same phenoxazinone skeleton as **1**. The main difference between **1** and **2** is that the NH group (H-12) of **2** is linked to another ring (ring D). ^{1}H−^{1}H COSY and HMBC NMR experiments allowed the deduction of the structure of ring D (Figure 2). In the ^{1}H−^{1}H COSY spectrum of **2**, correlations between the protons δ_H 9.09 and 7.07 of NH_2-20 and between the protons δ_H 11.18 and 8.41 of NH_2-21 were observed. Moreover, in the COSY spectrum, the proton (δ_H 4.41) of the methine group at C-17 (δ_C 71.1) showed a correlation to one proton of NH_2-21 (δ_H 8.41). The key HMBC

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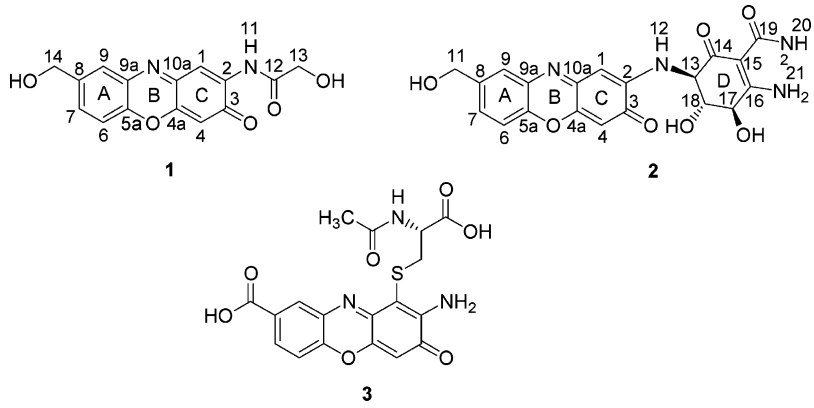


Figure 1. Structures of chandrananimycin D (**1**), pitucamycin (**2**), and grixazone B (**3**).

Table 1. NMR Spectroscopic Data for **1** and **2** (DMSO-d_6)

	1			**2**		
position	δ_H[a] (J in Hz)	δ_C[b]	HMBC[a]	δ_H[c] (J in Hz)	δ_C[d]	HMBC[c]
1	8.24, s	112.6	3, 4a, 10a	6.41, s[e]	97.0	2, 3, 4a, 10a
2		148.3			147.2	
3		178.9			179.9	
4	6.53, s	103.4	4a, 10a	6.41, s[e]	103.0	2, 3, 4a, 10a
4a		149.3			149.2	
5a		141.7			140.7	
6	7.53, d (8.4)	115.8	5a, 8, 9a	7.48, d (8.4)	115.6	5a, 7, 8, 9, 9a
7	7.58, dd (1.9, 8.4)	130.4	5a, 9, 14	7.40, dd (1.9, 8.4)	127.1	5a, 8, 9, 11
8		140.4			133.6	
9	7.79, d (1.8)	126.7	5a, 7, 14	7.61, d (1.8)	125.3	5a, 6, 7, 8, 11
9a		133.2			139.9	
10a		136.1			148.2	
11	9.77, s		1, 3,12	4.56, s	62.1	5a, 6, 7, 8, 9, 9a
12		172.1		6.97, d (8.3)		1, 3, 10a, 13, 14, 18
13	4.09, s	61.8	12	4.29, dd (8.4, 10.2)	62.0	2, 14, 17, 18
14	4.60, s	61.8	8		189.3	
15					96.4	
16					173.8	
17				4.41, d (9.6)	71.1	13, 16, 18
18				3.79, t (10.0)	72.2	13, 17
19					170.1	
20				9.09/7.07, d (4.6, 4.6)		15, 16, 19
21				11.18/8.41, d (5.0, 5.3)		14, 15, 16, 17

[a] Recorded at 500 MHz. [b] Recorded at 125 MHz. [c] Recorded at 600 MHz. [d] Recorded at 150 MHz. [e] Overlapping resonance signals.

correlations from H-13 to C-14, C-17, and C-18, from H-17 to C-13, C-16, and C-18, from H-18 to C-13 and C-17, from H-20 to C-15, C-16, and C-19, and from H-21 to C-14, C-15, C-16, and C-17 established the structure of ring D (Figure 2). The relative configuration of pitucamycin (**2**) was determined from coupling constants of the three methine protons signals, δ_H 4.29 (H-13, dd, $J = 8.4$ and 10.2 Hz), δ_H 4.41 (H-17, d, $J = 9.6$ Hz), and δ_H 3.79 (H-18, t, $J = 10.0$ Hz), and by analysis of the correlations in the NOESY spectrum. The large coupling constant between H-13 and H-18 ($J = 10.0$ Hz) pointed to the axial position of both protons. The *anti* configuration of H-17 and H-18 was deduced also from the coupling constant ($J = 9.6$ Hz). In addition, the strong NOESY correlation between the protons H-13 and H-17 indicated the *syn* configuration (Figure 3).

Compounds **1**−**3** were assayed for their antiproliferative (GI_{50} values) and cytotoxic activities (CC_{50} values) using a number of tumor cell lines (Experimental Section).[4,11] In addition, antimicrobial activities of **1** and **2** were determined in a primary screen by the agar diffusion assay against a series of Gram-positive and Gram-negative bacteria as well as fungi.[12] However, these experiments indicated no antimicrobial activities for **1** and **2** against the organisms evaluated. Comparative antiproliferative and cytotoxic activities of compounds **1**−**3** are summarized in Table 2. While grixazone B (**3**) did not show any significant response, **2** exhibited moderate antiproliferative activities toward the HUVEC and K-562 cell lines (GI_{50} 36.6 and 27.6 μM, respectively) and only weak cytotoxicity toward HeLa cells (CC_{50} 74.8 μM). The most prolific activity was found for **1**, which showed potent antiproliferative activities against a number of cell lines (MCF-7, HUVEC, Hep-G2, RAJI, and HEK-293) with GI_{50} values ranging from 1.3 to 10.3 μM. In stark contrast, the cytotoxicity of **1** was determined as weak (HeLa CC_{50} 90.6 μM). No significant selectivity for tumor (K-562, MCF-7, Hep-G2, RAJI, and HEK-293) and nontumor (HUVEC) cell lines was observed.

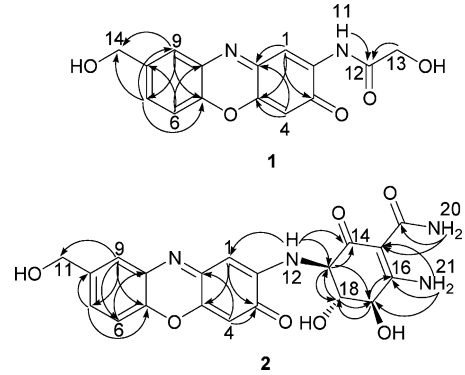


Figure 2. Selected HMBC correlations for **1** and **2**.

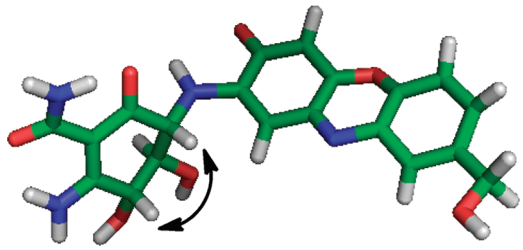


Figure 3. Key NOESY correlation of compound **2**.

While only moderately active in the cancer cell line assays, pitucamycin (**2**) is an intriguing molecule from a structural point of view. The substitution pattern of ring D is strikingly similar to the amide derivative of the epoxyquinone antibiotic enaminomycin C (**5**, Scheme 1), a metabolite previously isolated from a *Streptomyces* sp. strain.[7] The most likely scenario for the formation of **2** would involve a fusion of **5** with the putative precursor 2-amino-8-hydroxymethyl-3*H*-phenoxazin-3-one (**4**) by a nucleophilic oxirane ring opening (Scheme 1). In fact, the observed configuration of C-13 and C-18 of **2** is highly suggestive of an S_N2 mechanism. Epoxides are widespread structural units in natural products. However, there is no precedence for the merger of these two entities. Hydroxy and thiol groups are encountered frequently as nucleophiles in biochemical reactions that involve epoxide cleavage, and respective mechanisms have been either proposed or demonstrated in spiroether formation,[13] enediyne biosynthesis,[14,15] and drug catabolism.[16] The nucleophilic attack of an amine onto an epoxide is less common, but not unprecedented. A corresponding reaction takes place upon binding of the epoxy ketone natural product epoxomicin to the 20S proteasome[17] and may be also implicated in gliotoxin biosynthesis.[18] Thus, on the basis of their structures and co-occurrence, we assume that compounds **1**−**3** share a common pathway involving enzymatic and nonenzymatic steps (Scheme 1). For the formation of grixazone B (**3**), *N*-acetylcysteine is introduced by a nonenzymatic thiol conjugation with *N*-acetylcysteine to the quinone imine (route a), which is produced from 3,4-AHBAL (3-amino-4-hydroxybenzaldehyde) by the *o*-aminophenol oxidase GriF.[2] The quinone imine condensation product **4** could represent the branching point for the formation of **1** and **2**. Chandrananimycin D (**1**) would be formed by enzymatic acetylation and oxygenation (route b), while an unprecedented aminolytic merger of **4** with epoxide **5** would provide **2** (route c) These *N*-functionalizations could, in principle, already take place at the level of 3,4-AHBAL. In this context it should be noted that epoxide **5** was not detected in the culture broth.

In conclusion, we have isolated and fully characterized three minor metabolites (**1**−**3**) from a *S. griseus* strain that partly constituted the microbial flora of an old building with moisture damage. The strong antiproliferative activities of **1** in a range of tumor cell lines (with GI_{50} values down to 1.3 μM) are of interest from a pharmacological point of view, in particular with respect to the low cytotoxicity observed. The structure elucidation of **1**−**3** revealed chandrananimycin D (**1**) and pitucamyin (**2**) to be previously unknown members of the phenoxazinone family of natural products. According to their common heterocyclic nucleus, they share a biogenetic relationship. The proposed intermediate **4** is a versatile building block and could be diversified through various enzymatic and nonenzymatic steps. Herein, we propose the unprecedented merger of the phenoxazinone core with the amide derivative of the epoxyquinone antibiotic enaminomycin C (**5**), giving rise to the unique pitucamycin structure.

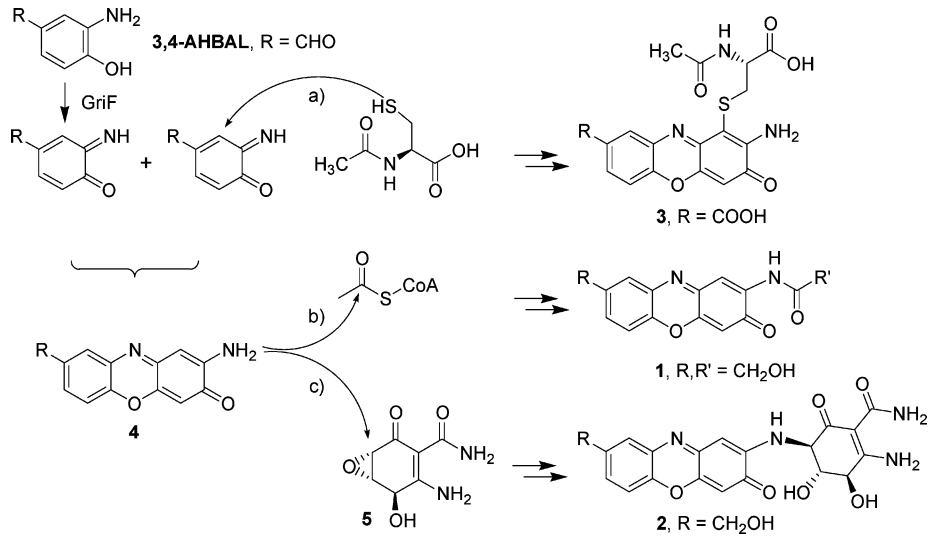
Experimental Section

General Experimental Procedures. Optical rotation was measured using a 0.5 dm cuvette with a JASCO P-1020 polarimeter at 25 °C. UV spectra were recorded on a Varian UV−visible Cary spectrophotometer (Varian, Palo Alto, CA). IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. ^{1}H and ^{13}C NMR, DEPT, and 2D NMR spectra were measured on Bruker Avance III 500 MHz and Bruker Avance III 600 MHz instruments. The chemical shift values (δ) are

Table 2. Antiproliferative (GI_{50}) and Cytotoxic (CC_{50}) Activities (in μM) of Chandrananimycin D (**1**), Pitucamycin (**2**), and Grixazone B (**3**)

compound	K-562 (GI_{50})	HUVEC (GI_{50})	THP-1 (GI_{50})	RAJI (GI_{50})	HEK-293 (GI_{50})	Hep-G2 (GI_{50})	MCF-7 (GI_{50})	HeLa (CC_{50})
1	16.6	5.3	27.6	9.6	10.3	6.6	1.3	90.6
2	27.6	36.6						74.8
3	>76.4	>119.9						>119.9

Scheme 1. Enzymatic and Nonenzymatic Diversification of Phenoxazinones[a]



[a] Model for the formation of **1**−**3** through (a) nucleophilic attack of *N*-acetylcysteine to an *o*-quinone imine; (b) *N*-acetylation; (c) aminolytic oxirane ring opening of **5** and various oxidoreductions.

given in parts per million (ppm), and coupling constants in Hz. ESIMS data were obtained with a triple quadrupole mass spectrometer (Quattro; VG Biotech, Altrincham, Cheshire, UK). Analytical HPLC was performed on an Agilent 1100 Series LC/MSD trap. Columm chromatography was performed on silica gel 60 M (230−400 mesh, Macherey-Nagel, Düren, Germany) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). TLC analysis was performed on silica gel plates (Sil G/UV_{254} 0.20 mm, Macherey-Nagel).

Fermentation. *Streptomyces griseus* (HKI 0545, DSM 41823) was isolated from plaster of an old building (built 1953) with moisture damage and microbial growth in Herne (Germany). A 20 mL amount of liquid organic medium 79 (10 g L^{-1} dextrose, 10 g L^{-1} bactopeptone, 2 g L^{-1} casein hydrolysate, 2 g L^{-1} yeast extract, 6 g L^{-1} NaCl) was inoculated with a 1 mL suspension of the strain and incubated at 28 °C on a rotary shaker for 48 h to yield the preculture. Subsequently, 3 mL of the preculture was transferred to 100 mL of medium 79 (3 × 3 mL of the preculture for each 100 mL Erlenmeyer flask). Then 12 mL of this culture was transferred to 4 L of medium 27 (10 Erlenmeyer flasks with 400 mL of medium 27 each), which was poured into a 300 L scale fermenter filled with 200 L of medium 27 (20 g L^{-1} soybean, 20 g L^{-1} glucose, 5 g L^{-1} NaCl, and 3 g L^{-1} $CaCO_3$). The fermentation was carried out for 5 days with aeration at 30 L min^{-1} and stirring at 200 rpm. The culture filtrate was separated from the mycelium by filtration, subjected to an amberchrom-161 M resin column (20 × 20 cm, Rohm & Hass, Germany), and eluted with MeOH/H_2O (gradient from 10:90 to 90:10 in 35 min). The mycelium and seven fractions of the culture filtrate were lyophilized.

Extraction and Isolation. The lyophilized culture filtrate (8 g) was extracted with MeOH (1 L) and separated by column chromatography on silica gel (normal phase) using a gradient of $CHCl_3$/MeOH as eluent. Fractions were further purified by repeated column chromatography on silica gel (elution with $CHCl_3$/MeOH) and Sephadex LH-20 (elution with MeOH) to yield **1** (6 mg), **2** (2.2 mg), and **3** (1 mg).

Chandrananimycin D (1): orange solid; UV (MeOH) λ_{max} (log ε) 403 (0.56), 242 (0.82), 201 (0.48) nm; IR (film) ν_{max} 3325, 2922, 2380, 2348, 2326, 1593, 1524, 1467, 1018, 783, 629 cm^{-1}; ^1H (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6), Table 1; HRESIMS *m/z* 299.0677 $[M - H]^-$, (calcd 299.0673 for $C_{15}H_{11}O_5N_2$).

Pitucamycin (2): orange solid; $[\alpha]^{26}_D$ −92.6 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 440 (0.21), 275 (0.30), 242 (0.34) nm; IR (film) ν_{max} 3272, 2922, 2852, 2379, 2359, 1585, 1489, 1320, 1188, 1108, 1017, 791, 673 cm^{-1}; ^1H (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6), Table 1; HRESIMS *m/z* 425.1143 $[M - H]^-$ (calcd 425.1103 for $C_{20}H_{17}O_7N_4$).

Biological Assays. Compounds **1**−**3** were assayed for their antiproliferative activities (GI_{50}) against K-562 (human chronic myeloid cells, DSM ACC 10) and HUVEC (vascular endothelium cells, ATCC CRL-1730) as well as for their cytotoxic activities (CC_{50}) against HeLa cells (human cervix carcinoma, DSM ACC 57), as previously described.[4,11] In addition, **1** was tested for its antiproliferative effects against the cell lines THP-1 (human acute monocytic leukemia, DSM ACC 16), RAJI (human Burkitt lymphoma, DSM ACC 319), HEK-293 (human embryonic kidney cells), Hep-G2 (human hepatocellular carcinoma, DSM ACC 180), and MCF-7 (human breast adenocarcinoma, DSM ACC 115). Inhibitory concentrations are provided as 50% inhibition of cell growth (GI_{50}; the concentration needed to reduce the growth of treated cells to 50% of untreated cells) or 50% cytotoxic concentration (CC_{50}; the concentration that kills 50% of treated cells). Antimicrobial activities of **1** and **2** were determined in a primary screen by the agar diffusion assay against *Bacillus subtilis* (6633 B1), *Staphylococcus aureus* (511 B3), *Escherichia coli* (458 B4), *Pseudomonas aeruginosa* (SG137 B7), MRSA *Staphylococcus aureus* (134/93 R9), VRE *Enterococcus faecalis* (1528 R10), *Mycobacterium vaccae* (10670 M4), *Sporobolomyces salmonicolor* (549 H4), *Candida albicans* (H8), and *Penicillium notatum* (JP36 P1).[12]

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Supporting Information Available: NMR spectra of compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Le Roes-Hill, M.; Goodwin, C.; Burton, S. *Trends Biotechnol.* **2009**, *27*, 248–258.
(2) Suzuki, H.; Furusho, Y.; Higashi, T.; Ohnishi, Y.; Horinouchi, S. *J. Biol. Chem.* **2006**, *281*, 824–833.
(3) Barry, C. E.; Nayar, P. G.; Begley, T. P. *Biochemistry* **1989**, *28*, 6323–6333.
(4) Gomes, P. B.; Nett, M.; Dahse, H.-M.; Sattler, I.; Martin, K.; Hertweck, C. *Eur. J. Org. Chem.* **2010**, *2*, 231–235.
(5) Suzuki, H.; Ohnishi, Y.; Horinouchi, S. *J. Bacteriol.* **2007**, *189*, 2155–2159.
(6) Igarashi, Y.; Takagi, K.; Kajiura, T.; Furumai, T.; Oki, T. *J. Antibiot.* **1998**, *51*, 915–920.
(7) Orezzi, G. P. A., E.; Ballabio, M.; Cassinelli, G.; Di Matteo, E.; Garofano, M. L.; Inventi-Solari, A.; Arcamone, E. *Kangshengsu* **1986**, *11*, 474–484.
(8) Itoh, Y.; Haneishi, T.; Arai, M.; Hata, T.; Aiba, K.; Tamura, C. *J. Antibiot.* **1978**, *31*, 838–846.
(9) Ohnishi, Y.; Furusho, Y.; Higashi, T.; Chun, H. K.; Furihata, K.; Sakuda, S.; Horinouchi, S. *J. Antibiot.* **2004**, *57*, 218–223.
(10) Maskey, R. P.; Li, F. C.; Qin, S.; Fiebig, H. H.; Laatsch, H. *J. Antibiot.* **2003**, *56*, 622–629.
(11) Dahse, H. M.; Schlegel, B.; Gräfe, U. *Pharmazie* **2001**, *56*, 489–491.
(12) Huang, X.; Roemer, E.; Sattler, I.; Moellmann, U.; Christner, A.; Grabley, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 3067–3072.
(13) Gallimore, A. R. *Nat. Prod. Rep.* **2009**, *26*, 266–280.
(14) Lin, S.; Horsman, G. P.; Chen, Y.; Li, W.; Shen, B. *J. Am. Chem. Soc.* **2009**, *131*, 16410–16417.
(15) McGlinchey, R. P.; Nett, M.; Moore, B. S. *J. Am. Chem. Soc.* **2008**, *130*, 2406–2407.
(16) Arca, P.; Rico, M.; Brana, A. F.; Villar, C. J.; Hardisson, C.; Suárez, J. E. *Antimicrob. Agents Chemother.* **1988**, *32*, 1552–1556.
(17) Groll, M.; Kim, K. B.; Kairies, N.; Huber, R.; Crews, C. M. *J. Am. Chem. Soc.* **2000**, *122*, 1237–1238.
(18) Gardiner, D. M.; Howlett, B. J. *FEMS Microbiol. Lett.* **2005**, *248*, 241–248.

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