# Arixanthomycins A−C: Phylogeny-Guided Discovery of Biologically Active eDNA-Derived Pentangular Polyphenols

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Ⓢ *Supporting Information*

ABSTRACT: Soil microbiomes are a rich source of uncharacterized natural product biosynthetic gene clusters. Here we use short conserved biosynthetic gene sequences (natural product sequence tags) amplified from soil microbiomes as phylogenetic markers to correlate genotype to chemotype and target the discovery of novel bioactive pentangular polyphenols from the environment. The heterologous expression of an environmental DNA-derived gene cluster (the ARX cluster), whose ketosynthase beta (KS_β) sequence tag was phylogenetically distinct from any known KS_β sequence, led to the discovery of the arixanthomycins. Arixanthomycin A (**1**) exhibits potent antiproliferative activity against human cancer cell lines.
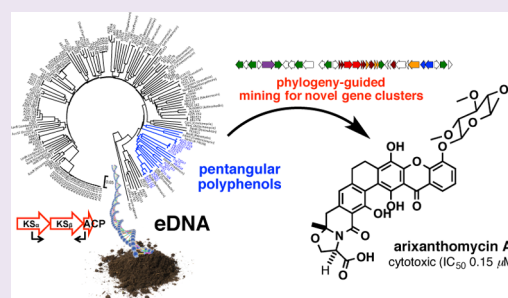
Microbially based natural products discovery programs have traditionally relied on the random screening of a pool of novel biodiversity (i.e., new microbial species) to secure a source of structurally novel small molecules.[1] As most bacteria in the environment have not yet been cultured, libraries of DNA extracted directly from environmental samples (environmental DNA, eDNA) represent large reservoirs of unexplored natural product biosynthetic gene clusters that could serve as new sources of additional bioactive small molecules.[2,3] Advances in modern sequencing technologies, bioinformatics analysis tools, and environmental DNA cloning methods should allow for a movement in natural products discovery away from a random microbe screening approach to a more systematic sequence-based screening approach that will allow for the functional examination of gene clusters that remain hidden in the environment.[4,5] In its simplest version, we envision that this sequence-based approach will use highly conserved biosynthetic genes as phylogenetic markers to identify novel gene clusters that can serve as starting points for the discovery of novel bioactive natural products. Here, we report the use of a single gene phylogeny-based mining approach to guide the discovery of arixanthomycins A−C (**1**−**3**, Chart 1), new pentangular polyphenols that exhibit potent *in vitro* antiproliferative activity.

Pentangular polyphenols are a subfamily of aromatic polyketides (PK) that use C24 to C30 polyacetate precursors.[6] Among characterized biosynthetic pathways, these are the longest polyketide chains to be generated by minimal type II polyketide synthases (min-PKSs).[7] Known pentangular polyphenols exhibit a wide range of biological activities, including antibacterial, antiproliferative, and antifungal activities.[8,9] In comparison to the structural diversity reported for many other aromatic PK subfamilies, pentangular polyphenols appear to be underrepresented among known natural products. Their larger size should theoretically afford the ability to generate more

Chart 1
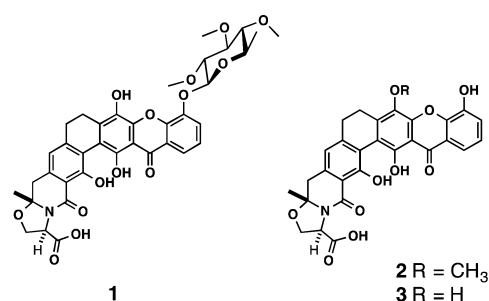
diverse folding patterns, skeletal rearrangements, and tailoring modifications than is seen in the biosynthesis of the smaller aromatic PKs. We reasoned that the relative dearth of known pentangular structures, yet high structural potential of this biosynthetic subgroup, would make pentangular polyphenols a rewarding target for phylogeny-guided natural product discovery approaches.

Our search for novel pentangular polyphenols began by PCR amplifying and sequencing ketosynthase beta (KS_β) genes from archived soil eDNA libraries. The KS_β gene encodes a subunit of the min-PKS that is responsible for producing the nonreduced polyketide, which is used as an intermediate in aromatic polyketide biosynthesis. KS_β genes have been shown to be robust phylogenetic markers for structural differences in the natural products encoded by type II PKS gene clusters.[5,10] When eDNA-derived KS_β amplicons were mapped onto a phylogenetic tree of functionally characterized KS_β genes, a
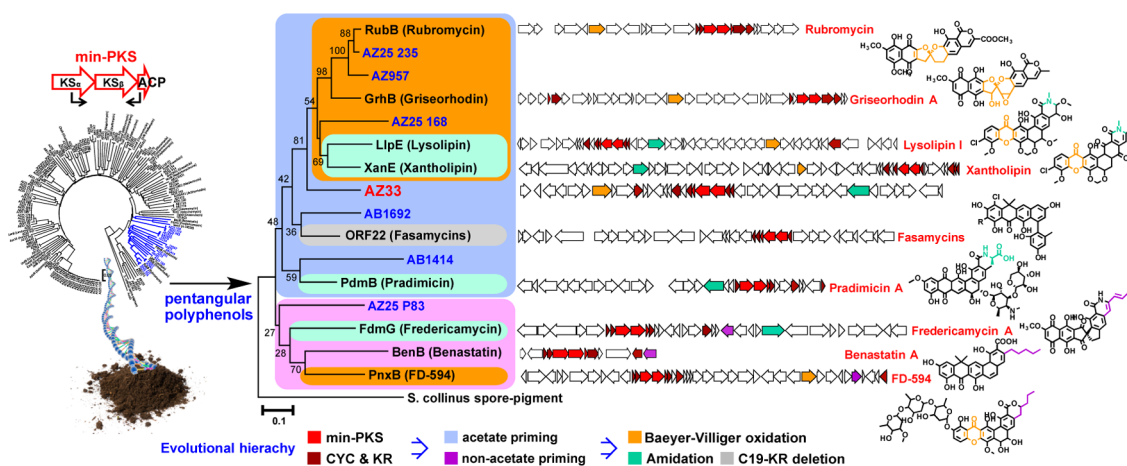
Figure 1. Maximum likelihood subtree (1,000 bootstrap replicates) of KS$_\beta$ genes that clade with known pentangular polyphenol KS$_\beta$ gene sequences. Based on a comparison of chemical structures and gene cluster contents, biosynthetic relationships in the KS$_\beta$ tree have been grouped into color-coded boxes. Natural pentangular polyphenol structural diversification appears to be dictated by four major driving forces: (i) start unit selection, (ii) initial cyclization pattern, (iii) oxidative rearrangement of the core skeleton, and (iv) functionalization of the terminal carboxylic acid. On the basis of this analysis, we hypothesized that phylogenetic comparison of eDNA-derived KS$_\beta$ sequence tags to KS$_\beta$ genes from functionally characterized gene clusters should be sufficient to enable detailed structural predictions (chemotype) of metabolites encoded by eDNA-derived gene clusters (genotype). The eDNA-derived KS$_\beta$ sequence AZ33 is shown in red.

number of amplicons were found to fall into the well-defined pentangular polyphenol clade, suggesting the presence of pentangular polyphenol biosynthetic gene clusters in our archived eDNA libraries (Figure 1). A close inspection of pentangular polyphenol KS$_\beta$ gene phylogeny indicates that its divergence correlates strongly with the detailed differences in the functional output of their respective gene clusters (Figure 1).[11−18] In other words, this single gene can predict not only the general class of a type II PKS gene cluster (e.g., anthracycline, tetracycline, angucycline, pentangular polyphenol etc.) but also many of the tailoring details encoded within a type II PKS gene cluster.

The strong correlation between final structural differences and KS$_\beta$ gene sequence phylogeny enabled us to prioritize eDNA-derived pentangular polyphenol gene clusters for heterologous expression experiments based solely on our initial PCR screening. The eDNA-derived KS$_\beta$ sequences of initial interest to us were those that fell into the subclade characterized by the presence of a Baeyer−Villiger oxidase. This enzyme is often used for carbon skeleton remodeling and thus is responsible for introducing significant structural and biological activity diversity into the pentangular polyphenol family (e.g., rubromycin, griseorhodin, lysolipin, and xantholipin).[11,12,14,18] Among the four eDNA-derived KS$_\beta$ sequences that fell into this subclade, AZ25_235 and AZ957 showed high sequence identity to KS$_\beta$ sequences from rubromycin biosynthesis (96% and 91%, respectively), suggesting that the eDNA gene clusters from which they arise likely encode either rubromycin itself or simple derivatives of this structure.[12] In contrast, the AZ33 KS$_\beta$ sequence appears to define a new branch within the Baeyer−Villiger oxidase containing subclade, suggesting that AZ33 was likely associated with the biosynthesis of a novel pentangular polyphenol subclass.

The AZ33 gene cluster was recovered on three overlapping cosmid clones (AZ1076, AZ33, and AZ378) from the archived Arizona desert eDNA library from which the AZ33 KS$_\beta$ sequence tag was amplified (Figure 2). To permit heterologous expression studies, these three overlapping clones were assembled into one bacterial artificial chromosome (BAC-
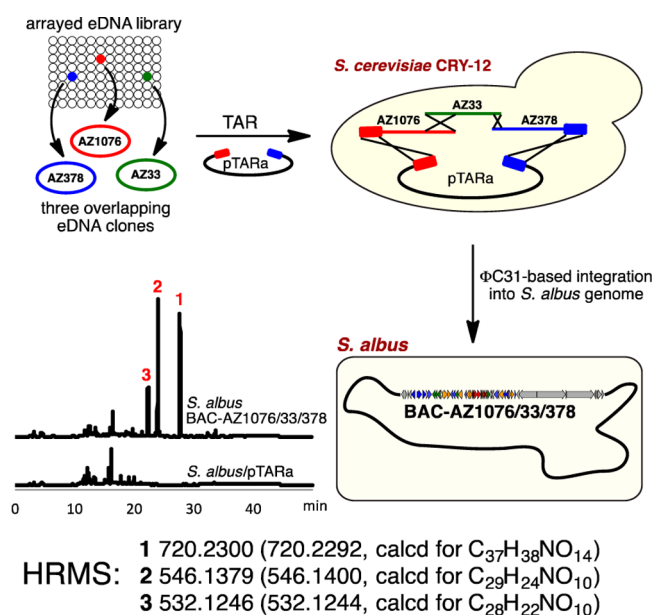
Figure 2. TAR reassembly of three overlapping eDNA clones carrying the ARX pentangular polyphenol pathway and heterologous expression of BAC-AZ1076/33/378.

AZ1076/33/378) by transformation-associated recombination (TAR) in yeast (Figure 2).[19,20] The correct assembly of the AZ33 cluster was confirmed by *de novo* sequencing of BAC-AZ1076/33/378. This BAC construct was then transferred into *Streptomyces albus* via intergenic conjugation and integrated into the genome by ΦC 31 integrase to generate the strain *S. albus* BAC-AZ1076/33/378. For natural product isolation studies, this strain was grown at 30 °C in R5A media for 7 days with shaking, and the resulting cultures were extracted with ethyl acetate. *S. albus* BAC-AZ1076/33/378 culture broth extract showed a potent antiproliferative activity against human carcinoma cell lines (Table 1), and LC−MS analysis of the extract showed the presence of three gene cluster-specific molecules. Each molecule was predicted by HRMS to have a

Table 1. Anticancer, Antifungal, and Antibacterial Activities of 1−3

		crude[a]	1	2	3
anticancer[b]	HCT-116	1.1	0.15	5.14	25.42
	WiDr	2.5	0.83	>50	>50
	MDA-MB-231	1.4	0.25	9.36	>50
antifungal[c]	S. cerevisiae	>50	>50	>50	>50
antibacterial[c]	MRSA USA300		1.6	25	>50
	B. subtilis RM125		3.1	12.5	>50
	VRE EF16		50	>50	>50
	E. coli DRC39		>50	>50	>50

[a]IC$_{50}$ in μg/mL. [b]IC$_{50}$ in μM. [c]MIC in μg/mL.

molecular formula that was distinct from any previously isolated pentangular polyphenol natural product (Figure 2). On the basis of the potent crude extract bioactivity, the bioinformatics, and chemical novelty, we chose to carryout a detailed structure analysis of the clone specific metabolites produced by *S. albus* BAC-AZ1076/33/378.

The three clone specific metabolites were purified from large-scale cultures (1 L) broth extracts using C$_{18}$ reversed-phase HPLC to yield compounds **1** (1.9 mg L^{-1}), **2** (4.6 mg L^{-1}), and **3** (4.0 mg L^{-1}). Compounds **1−3** were named arixanthomycins A, B, and C, respectively. The planar structures and relative configurations of arixanthomycins A−C (**1−3**) were determined by a combination of HRESIMS and 1D and 2D NMR data including ^{1}H, ^{13}C, COSY, HMQC, HMBC, and NOESY (Figure 3; Supporting Information includes a detailed structure elucidation discussion).
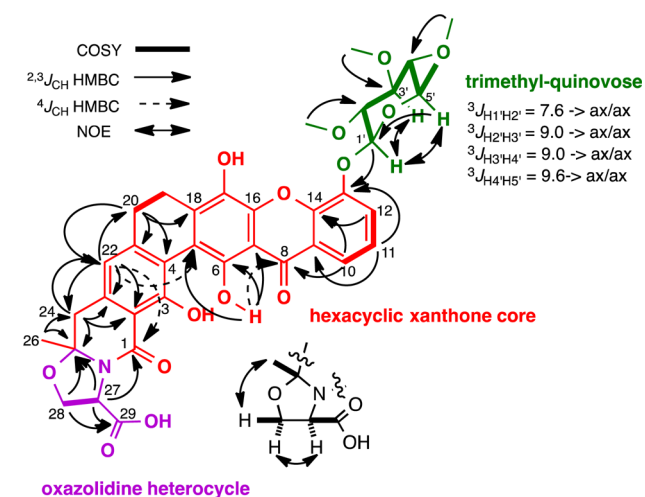
Figure 3. 2D NMR data used to define the structure of arixanthomycin A (**1**). The configurations shown for the oxazolidine ring and sugar moieties are relative.

The arixanthomycins feature a hexacyclic xanthone core (Figure 3, red) that is predicted to arise from a Baeyer−Villiger oxidative rearrangement of a pentangular polyphenol precursor as would be expected from the position of the AZ33 KS$_{\beta}$ gene sequence in the KS$_{\beta}$ phylogenetic tree. However, as we anticipated from the position of the AZ33 KS$_{\beta}$ gene sequence on a new branch in the phylogenetic tree, the arixanthomycins differ from known members of the xanthone family pentangular polyphenols by a number of key features. These differences include the presence of the carboxylated oxazolidine ring (Figure 4, G ring), the oxidation state of the D ring, the
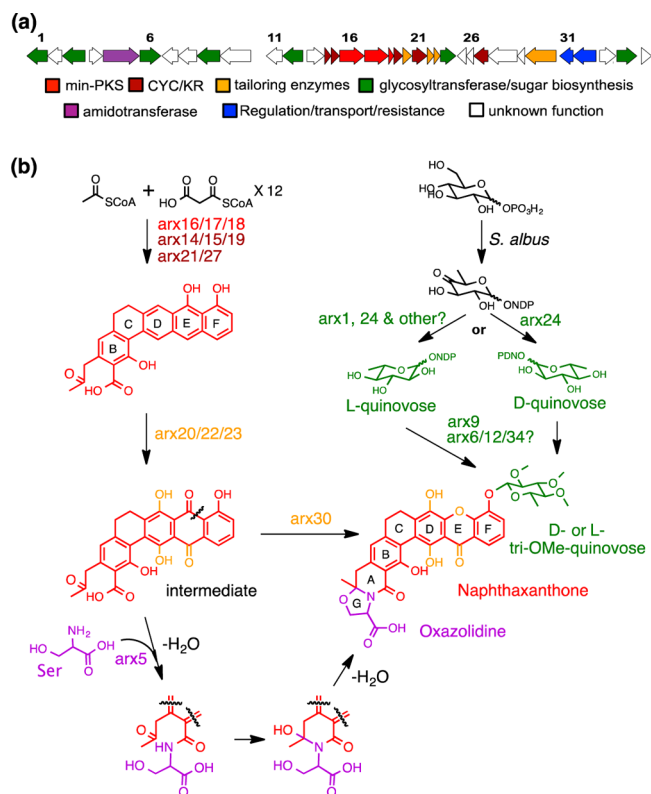
Figure 4. (a) ARX (arixanthomycin) gene cluster (GenBank accession no. KF931341). (b) Proposed biosynthetic scheme for arixanthomycin A (**1**) based on gene function prediction defined by sequence homology and domain analysis.

oxidation state of the F ring, and the glycosylation at C-13 (Figure 4, green). The oxazolidine ring likely arises from the coupling of a serine to the terminal polyketide carboxylic acid (Figure 4). The kigamicins and cervinomycins are the only members of the pentangular polyphenol family that possess an oxazolidine G ring.[21−23] In these known metabolites, the oxazolidine rings would appear to arise from the incorporation of an ethanolamine and therefore the carboxylate is not present. While sugars are seen on some pentangular polyphenols,[23−25] no previous reports of a C-13 glycosylation appear in the literature.

Arixanthomycins A−C (**1−3**) were assayed for antiproliferative, antibacterial, and antifungal activities. The arixanthomycins were found to be most active in antiproliferative assays (Table 1). Arixanthomycin A (**1**) shows a particularly potent antiproliferative activity against colon (HCT-116 and WiDr) and breast (MDA-MB-231) cancer cell lines (Table 1). Arixanthomycins B (**2**) and C (**3**) are significantly less active in these assays, suggesting that the sugar moiety is important for the antiproliferative activity of **1**. This is rather surprising, as most xanthone-containing pentangular polyphenols show potent activity in the absence of a sugar moiety.[25−29] Whether this is an indication that arixanthomycin A (**1**) has a distinct mode of action from other pentangular polyphenols or that the sugar is needed to improve the bioavailability of **1** in light of the new carboxylate remains to be determined.

The biosynthesis of the arixanthomycins can be rationalized on the basis of the predicted function of the genes found within BAC-AZ1076/33/378 (Figure 4). The predicted ARX gene cluster spans 33 kb and contains 35 genes predicted to be involved in the biosynthesis, regulation, and resistance of the

arixanthomycins. The structure of arixanthomycin A (**1**) can be divided into three parts, the naphthaxanthone core, the oxazolidine ring, and the quinovose sugar moiety. Key ARX enzymes that are predicted to be involved in the biosynthesis of each of these substructures are shown in Figure 4. The hexacyclic naphthaxanthone core is predicted to arise from a pradimicin-type anthraquinone intermediate by a Baeyer–Villiger oxidation catalyzed by Arx30 (62% identity to llpOVIII and 61% identity to xanO4).[11,14] Stereospecific formation of the oxazolidine (G ring) is likely to occur spontaneously upon incorporation of serine into the terminal carboxylate by the predicted amidotransferase Arx5. The addition of the trimethylated quinovose sugar moiety is predicted to occur through the action of the glycosyltransferase Arx9. A detailed biosynthetic proposal appears in the Supporting Information (Figure S3).

This work demonstrates the power of using single gene sequence tags to rapidly and economically bridge the gap between genotype and chemotype in complex microbiomes. This methodology is easy to scale using high throughput sequencing technology and archived eDNA libraries. Sequence-based eDNA mining approaches like those used here should allow for the movement away from the traditional new species-based natural product discovery paradigm to a new bioinformatics-driven, clade-based, natural products discovery paradigm. Using this general approach, it should be possible to systematically access untapped chemical diversity encoded within diverse natural microbiomes.

■ METHODS

Clone Recovery and Bioinformatics Analysis. eDNA clone AZ33 was recovered from the AZ eDNA library by serial dilution of the 5,000-member subpool from which the AZ33 amplicon was originally amplified. To do this, an overnight culture of the AZ33 subpool was inoculated into 96-well microtiter plates at a dilution of 10^{-6}. After 18 h of shaking at 37 °C, the diluted cultures were screened by whole-cell PCR using the KS_{β} screening primers and touchdown PCR protocol. PCR positive wells were plated onto solid media (LB agar) to yield distinct colonies that were screened individually in a second round of whole-cell PCR. Overlapping clones for AZ33 were subsequently identified in the AZ library using clone-specific primers designed to recognize the sequence at each end of AZ33 (AZ33F_FW: CAGGAACTCGAGTCCATCTC, AZ33F_RV: ACTTCACCAGTA-CCTCGAC, AZ33B_FW: CACATGAAGCTGATGACTC, and AZ33B_RV: GCCATTCGATCTCGATAC). The PCR protocol used was as follows: denaturation (95 °C, 5 min), 36 standard cycles (95 °C, 30 s; 55 °C, 30 s; 72 °C, 40s), and a final extension step (72 °C, 7 min). Hits were identified in subpools AZ1076 and AZ378. Overlapping eDNA cosmids (designated AZ1076 and AZ378) were recovered from these pools using the serial dilution method outlined above. All three cosmids were sequenced by 454-pyrosequencing. ORFs (open reading frames) were predicted using MetaGeneMark,[30] and predicted ORFs were annotated by Blast search and Pfam domain analysis.

TAR Assembly of Overlapping eDNA Clones. The three overlapping eDNA cosmid clones that are predicted to contain the AZ33 gene cluster (AZ1076, AZ33 and AZ378) were assembled into a single bacterial artificial chromosome (BAC) clone using transformation-associated recombination (TAR) in yeast and the pTARa BAC shuttle vector.[19,20] The pathway-specific TAR capture vector was constructed using InFusion cloning methodology (Clontech). Five hundred bases upstream and downstream homology arms were amplified from the AZ1076 and AZ378 cosmids, respectively, using the following primers:

AZ1076UPS_FW (5′-CTATCGATCTCGAGGACGACCTG-ATGAGCAGTA-3′),

AZ1076UPS_RV (5′-CGTGCCACTGTTAACTCAACTCC-ATCCTCACCT-3′),

AZ378DWS_FW (5′-**GTTAAC**AGTGGCACGTTGACGTTCTT-3′),

AZ378DWS_RV (5′-CCCTGCAGGAGCTCGTCTCTTTCTC-GTCCACTGGT-3′)

Primers AZ1076UPS_FW and AZ1076DWS_RV include 15 bp sequences (underlined) that overlap with BmtI/SphI linearized pTARa capture vector. Primer AZ1076UPS_RV was designed to contain a 15 bp overlap (underlined) with the AZ378DWS_FW primer and an *Hpa*I site (bold), which was added to facilitate the subsequent linearization of the pathway-specific pTARa capture vector. Gel-purified amplicons (Qiagen) and BmtI/SphI linearized pTARa were combined in a standard InFusion cloning reaction (Clontech) to yield the pathway-specific capture vector.

For TAR assembly, 200 ng of DraI-digested AZ1076, AZ33, and AZ378 were mixed with 100 ng of the *Hpa*I-cut pathway-specific capture vector and then transformed into 200 μL of *Saccharomyces cerevisiae* CRY1-2 spheroplasts prepared according to published protocols.[19,20] Transformed spheroplasts were mixed with synthetic complete (SC) top agar (1 M sorbitol, 1.92 g/L synthetic complete uracil dropout supplement, 6.7 g/L yeast nitrogen base, 2% glucose, and 2.5% agar) and overlaid onto SC uracil dropout plates. Plates were incubated at 30 °C for 72 h. DNA was prepared from 12 yeast colonies using a zymolyase lysis protocol (ZYMO RESEARCH) and screened by PCR with primers designed to recognize sequences from AZ1076, AZ33, and AZ378. A BAC clone that was "PCR positive" with all the primer sets was electroporated into *E. coli* EPI300. DNA isolated from the resulting *E. coli* was PGM (Ion Torrent Personal Genome Machine) sequenced to confirm the correct reassembly of the three overlapping cosmids into a 80 kb insert BAC clone (BAC-AZ1076/33/378).

Heterologous Expression. BAC-AZ1076/33/378 was transformed into *E. coli* S17.1 and conjugated into *S. albus* to generate *S. albus* BAC-AZ1076/33/378 (apramycin selection). This strain was cultured in R5A[31] liquid medium for 7 days (200 rpm at 30 °C) and then extracted with ethyl acetate (1/3 volume to the culture broth). The resulting ethyl acetate extract was compared by HPLC to the extract from a similarly grown culture of *S. albus* transformed with a pTARa empty vector control (HPLC conditions: 30 min gradient from 5% to 100% aqueous acetonitrile containing 0.1% trifluoroacetic acid, C_{18}, 4.6 mm × 150 mm, 1 mL/min).

Extraction and Isolation. Compounds **1**−**3** were isolated from cultures of *S. albus* BAC-AZ1076/33/378 grown (200 rpm at 30 °C) in 125 mL baffled flasks containing 50 mL of R5A media. Seven-day-old cultures were extracted with ethyl acetate (1/3, v/v to the culture broth), and the ethyl acetate was dried *in vacuo*. Arixanthomycins A (**1**), B (**2**), and C (**3**) were isolated from the resulting ethyl acetate extract using two rounds of reversed-phase HPLC (C_{18} column, 10 mm × 250 mm, 3.5 mL/min). The first round of HPLC (isocratic, 55% acetonitrile containing 0.1% trifluoroacetic acid) yielded three crude compounds **1** (17.9 min), **2** (9.8 min), and **3** (6.6 min), which were repurified by a second round of HPLC using 80%, 72%, and 70% aqueous methanol with 0.1% trifluoroacetic acid to yield arixanthoym-cins A (**1**, 1.6 mg L^{-1}), B (**2**, 4.6 mg L^{-1}), and C (**3**, 4.1 mg L^{-1}), respectively.

Arixanthoymcin A (1). Yellowish powder; $[\alpha]^{25}_{D}$ −197; UV (MeOH) λ_{max} (log ε) 287 (4.24), 327 (shoulder; 4.13), 346 (shoulder; 3.98), 403 (3.57) nm; IR (neat) ν_{max} 3375, 2981, 2945, 2912, 2835, 1680, 1641, 1631, 1606, 1548, 1529, 1492, 1462, 1431 cm^{-1}; ^{1}H and ^{13}C NMR, COSY, and HMBC data, see Supplementary Table 1; HRESIMS *m/z* 720.2300 [M + H]$^+$ (calcd for $C_{37}H_{38}$ NO$_{14}$, 720.2292).

Arixanthomycin B (2). Greenish powder; $[\alpha]^{25}_{D}$ −127; UV (MeOH) λ_{max} (log ε) 285 (4.42), 331 (shoulder; 4.12), 399 (3.47) nm; IR (neat) ν_{max} 3110, 2980, 2891, 2846, 1681, 1614, 1586 1488, 1467, 1437 cm^{-1}; ^{1}H and ^{13}C NMR data, see Supplementary Table 2; HRESIMS *m/z* 546.1379 [M + H]$^+$ (calcd for $C_{29}H_{24}NO_{10}$, 546.1400).

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dx.doi.org/10.1021/cb500141b | *ACS Chem. Biol.* 2014, 9, 1267−1272

Arixanthoymcin C (3). Yellowish powder; $[\alpha]^{25}_D$ −351; UV (MeOH) λ_{max} (log ε) 287 (4.74), 324 (shoulder; 4.55), 347 (shoulder; 4.40), 399 (3.90) nm; IR (neat) ν_{max} 3429, 2982, 2940, 2893, 2837, 1679, 1640, 1610, 1590, 1492, 1440 cm^{-1}; ^{1}H and ^{13}C NMR data, see Supplementary Table 2; HRESIMS *m/z* 532.1246 [M + H]$^+$ (calcd for $C_{28}H_{22}NO_{10}$, 532.1244).

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Methods for library construction, library screening, and bioassays and discussions for structure determination. NMR data table, gene annotation table and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Cragg, G. M., and Newman, D. J. (2013) Natural products: a continuing source of novel drug leads. *Biochim. Biophys. Acta 1830*, 3670−3695.

(2) Handelsman, J., Rondon, M. R., Brady, S. F., Clardy, J., and Goodman, R. M. (1998) Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chem. Biol. 5*, R245−R249.

(3) Reddy, B. V., Kallifidas, D., Kim, J. H., Charlop-Powers, Z., Feng, Z., and Brady, S. F. (2012) Natural product biosynthetic gene diversity in geographically distinct soil microbiomes. *Appl. Environ. Microbiol. 78*, 3744−3752.

(4) Harvey, A. (2000) Strategies for discovering drugs from previously unexplored natural products. *Drug Discovery Today 5*, 294−300.

(5) Feng, Z., Kallifidas, D., and Brady, S. F. (2011) Functional analysis of environmental DNA-derived type II polyketide synthases reveals structurally diverse secondary metabolites. *Proc. Natl. Acad. Sci. U.S.A. 108*, 12629−12634.

(6) Lackner, G., Schenk, A., Xu, Z., Reinhardt, K., Yunt, Z. S., Piel, J., and Hertweck, C. (2007) Biosynthesis of pentangular polyphenols: deductions from the benastatin and griseorhodin pathways. *J. Am. Chem. Soc. 129*, 9306−9312.

(7) Zhou, H., Li, Y., and Tang, Y. (2010) Cyclization of aromatic polyketides from bacteria and fungi. *Nat. Prod. Rep. 27*, 839−868.

(8) Walsh, T. J., and Giri, N. (1997) Pradimicins: a novel class of broad-spectrum antifungal compounds. *Eur. J. Clin. Microbiol. Infect. Dis. 16*, 93−97.

(9) Winter, D. K., Sloman, D. L., and Porco, J. A., Jr. (2013) Polycyclic xanthone natural products: structure, biological activity and chemical synthesis. *Nat. Prod. Rep. 30*, 382−391.

(10) Seow, K. T., Meurer, G., Gerlitz, M., Wendt-Pienkowski, E., Hutchinson, C. R., and Davies, J. (1997) A study of iterative type II polyketide synthases, using bacterial genes cloned from soil DNA: a means to access and use genes from uncultured microorganisms. *J. Bacteriol. 179*, 7360−7368.

(11) Zhang, W., Wang, L., Kong, L., Wang, T., Chu, Y., Deng, Z., and You, D. (2012) Unveiling the post-PKS redox tailoring steps in biosynthesis of the type II polyketide antitumor antibiotic xantholipin. *Chem. Biol. 19*, 422−432.

(12) Saito, H., Bruenker, P., Sterner, O., Bailey, J. E., and Wolfgang, M. (2002) Molecular analysis of the rubromycin biosynthesis gene cluster isolated from *Streptomyces collinus* DSM2012 and synthesis of novel recombinant polyketides. *Nippon Nogei Kagakkai Taikai Koen Yoshishu*, 299.

(13) Kim, B. C., Lee, J. M., Ahn, J. S., and Kim, B. S. (2007) Cloning, sequencing, and characterization of the pradimicin biosynthetic gene cluster of *Actinomadura hibisca* P157-2. *J. Microbiol. Biotechnol. 17*, 830−839.

(14) Lopez, P., Hornung, A., Welzel, K., Unsin, C., Wohlleben, W., Weber, T., and Pelzer, S. (2010) Isolation of the lysolipin gene cluster of *Streptomyces tendae* Tu 4042. *Gene 461*, 5−14.

(15) Wendt-Pienkowski, E., Huang, Y., Zhang, J., Li, B., Jiang, H., Kwon, H., Hutchinson, C. R., and Shen, B. (2005) Cloning, sequencing, analysis, and heterologous expression of the fredericamycin biosynthetic gene cluster from *Streptomyces griseus*. *J. Am. Chem. Soc. 127*, 16442−16452.

(16) Kudo, F., Yonezawa, T., Komatsubara, A., Mizoue, K., and Eguchi, T. (2011) Cloning of the biosynthetic gene cluster for naphthoxanthene antibiotic FD-594 from *Streptomyces* sp. TA-0256. *J. Antibiot. 64*, 123−132.

(17) Xu, Z., Schenk, A., and Hertweck, C. (2007) Molecular analysis of the benastatin biosynthetic pathway and genetic engineering of altered fatty acid-polyketide hybrids. *J. Am. Chem. Soc. 129*, 6022−6030.

(18) Li, A., and Piel, J. (2002) A gene cluster from a marine *Streptomyces* encoding the biosynthesis of the aromatic spiroketal polyketide griseorhodin A. *Chem. Biol. 9*, 1017−1026.

(19) Kim, J. H., Feng, Z., Bauer, J. D., Kallifidas, D., Calle, P. Y., and Brady, S. F. (2010) Cloning large natural product gene clusters from the environment: piecing environmental DNA gene clusters back together with TAR. *Biopolymers 93*, 833−844.

(20) Kallifidas, D., and Brady, S. F. (2012) Reassembly of functionally intact environmental DNA-derived biosynthetic gene clusters. *Methods Enzymol. 517*, 225−239.

(21) Omura, S., Nakagawa, A., Kushida, K., and Lukacs, G. (1986) Structure of cervinomycin, a novel antianaerobic antibiotic. *J. Am. Chem. Soc. 108*, 6088−6089.

(22) Nakagawa, A., Omura, S., Kushida, K., Shimizu, H., and Lukacs, G. (1987) Structure of cervinomycin, a novel xantone antibiotic active against anaerobe and mycoplasma. *J. Antibiot. 40*, 301−308.

(23) Kunimoto, S., Someno, T., Yamazaki, Y., Lu, J., Esumi, H., and Naganawa, H. (2003) Kigamicins, novel antitumor antibiotics. II. Structure determination. *J. Antibiot. 56*, 1012−1017.

(24) Rodriguez, J. C., Fernandez Puentes, J. L., Baz, J. P., and Canedo, L. M. (2003) IB-00208, a new cytotoxic polycyclic xanthone produced by a marine-derived *Actinomadura*. II. Isolation, physico-chemical properties and structure determination. *J. Antibiot. 56*, 318−321.

(25) Ratnayake, R., Lacey, E., Tennant, S., Gill, J. H., and Capon, R. J. (2007) Kibdelones: novel anticancer polyketides from a rare Australian actinomycete. *Chemistry 13*, 1610−1619.

(26) Ando, T., Chen, Z., Chu, Y., Kawashima, A., and Terui, Y. (2002) Xanthone compound. Patent WO2002027010 A1.

(27) Pultar, T. (1988) Lysolipin X und I—Fermentation, Isolierung, Biologische Wirkung und Interaktion mit Mg^{2+}. PhD thesis at the University of Tübingen, Germany.

(28) Carter, G. T., Nietsche, J. A., Williams, D. R., and Borders, D. B. (1990) Citreamicins, novel antibiotics from *Micromonospora citrea*: isolation, characterization, and structure determination. *J. Antibiot. 43*, 504−512.

(29) Liu, L. L., He, L. S., Xu, Y., Han, Z., Li, Y. X., Zhong, J. L., Guo, X. R., Zhang, X. X., Ko, K. M., and Qian, P. Y. (2013) Caspase-3-dependent apoptosis of citreamicin epsilon-induced HeLa cells is associated with reactive oxygen species generation. *Chem. Res. Toxicol. 26*, 1055−1063.

(30) Zhu, W., Lomsadze, A., and Borodovsky, M. (2010) Ab initio gene identification in metagenomic sequences. *Nucleic Acids Res. 38*, e132.

1271

dx.doi.org/10.1021/cb500141b | *ACS Chem. Biol.* 2014, 9, 1267−1272

(31) Fernandez, E., Weissbach, U., Sanchez Reillo, C., Brana, A. F., Mendez, C., Rohr, J., and Salas, J. A. (1998) Identification of two genes from *Streptomyces argillaceus* encoding glycosyltransferases involved in transfer of a disaccharide during biosynthesis of the antitumor drug mithramycin. *J. Bacteriol. 180*, 4929−4937.