JOURNAL OF NATURAL PRODUCTS

Note

pubs.acs.org/jnp

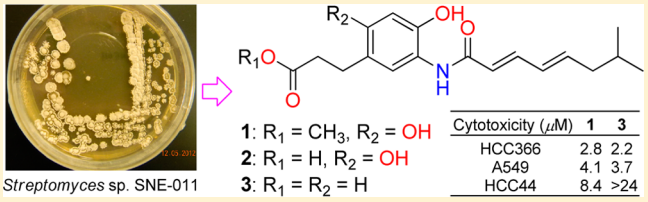
Carpatamides A–C, Cytotoxic Arylamine Derivatives from a Marine-Derived *Streptomyces* sp.

Peng Fu, Melissa Johnson, Hong Chen, Bruce A. Posner, and John B. MacMillan*

Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390, United States

Supporting Information

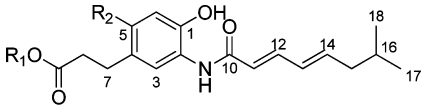
ABSTRACT: Three new acylated arylamine derivatives (**1**–**3**), carpatamides A–C, were isolated from a marine-derived *Streptomyces* sp. based on activity screening against non-small-cell lung cancer (NSCLC). The structures of **1**–**3** were established on the basis of comprehensive spectroscopic analyses and chemical methods. Compounds **1** and **3** showed moderate cytotoxicity against NSCLC cell lines HCC366, A549, and HCC44 with IC_{50} values ranging from 2.2 to 8.4 μM.



Examining the natural product profiles of underexploited organisms from the marine environment has become a research hotspot in drug discovery.[1] With more than 400 new compounds with cytotoxicity and antimicrobial activity isolated, such as abyssomycin C,[2] salinosporamide A,[3] discoipyrrole A, and marinomycin,[4] cultured marine actinomycetes have been a prolific resource of bioactive natural products.[5] This resource for natural products, combined with advancements in high-content phenotypic screening approaches, opens up tremendous possibilities for the discovery of biologically and structurally interesting compounds.[6] In order to identify molecules with selective activity against non-small-cell lung cancer (NSCLC), the most prevalent form of lung cancer, 6500 natural product fractions were screened against a panel of 17 comprehensively annotated NSCLC cell lines.[7] Analysis of the results from this screen revealed a series of natural product fractions that demonstrated selective activity against a subset of these lines at 5 μg/mL.

Herein we describe the isolation of metabolites from a cytotoxic fraction from a *Streptomyces* sp. (strain SNE-011). Analysis of the active fraction by LC-UV-MS showed it contained one main peak with the molecular weight of 347 and a UV profile suggestive of a substituted phenyl ring (Figure S1). Bioassay-guided chemical investigation resulted in the isolation of three new arylamine derivatives (**1**–**3**), which we have named carpatamides A–C. These compounds possess a novel amide structure consisting of an amino-phenylpropionic acid core and an unsaturated fatty acid chain. This aromatic substitution pattern has been previously encountered only in manumycin derivatives from *Streptomyces parvulus*,[8a,b] although there are a large number of phenylpropionic acid-containing natural products reported.[8c–f] Compounds **1** and **3** exhibited moderate cytotoxicity against non-small-cell lung cancer cell lines HCC366, A549, and HCC44, but no activity against H2122.

Marine-derived bacterium SNE-011 was isolated from a sediment sample collected from Kiawah Island, South Carolina, and isolated on a seawater-based humic acid medium. Analysis by 16S rRNA sequence revealed SNE-011 to be a *Streptomyces* sp. with closest identity (98%) to *S. carpaticus*. After identification of an active fraction in the library, a large-scale (10 L) shake fermentation was carried out to obtain sufficient material for full chemical and biological analysis of the metabolites. The excreted metabolites were collected using XAD-7-HP resin, and the resulting extract was purified by a combination of solvent/solvent extraction and reversed-phase flash chromatography to give fractions that showed cytotoxicity. Final purification by Sephadex LH-20 and gradient reversed-phase HPLC gave carpatamides A (**1**, 10.5 mg), B (**2**, 15.1 mg), and C (**3**, 2.0 mg).



1: $R_1 = CH_3$, $R_2 = OH$
2: $R_1 = H$, $R_2 = OH$
3: $R_1 = R_2 = H$

Carpatamide A (**1**) was obtained as a yellow oil. Its molecular formula was assigned as $C_{19}H_{25}NO_5$ on the basis of a molecular ion peak at m/z 348.1805 $[M + H]^+$ observed by HRESIMS. Analysis of the 1D NMR data for **1** revealed two carbonyls, four quaternary carbons, seven methines (six olefinic or aromatic), three methylenes, and three methyls (one oxygenated) (Table 1). The ^{1}H NMR spectrum showed four coupled signals at δ_H 6.16 (H-11, d, J = 14.9 Hz), 7.25 (H-12, dd, J = 14.9, 10.9 Hz), 6.26 (H-13, dd, J = 14.9, 10.6 Hz), and 6.15 (H-14, dt, J = 14.9, 7.2 Hz), due to a conjugated diene in the all *E*-configuration. The contiguous COSY correlations extending from H-11 through the H_3-17 and H_3-18 methyl groups, along with the

Received: March 4, 2014
Published: April 23, 2014

Table 1. 1D and 2D NMR Data of Compound 1[a]

no.	δ_C[b]	δ_H, mult. (J in Hz)[b]	δ_C[c]	δ_H, mult. (J in Hz)[c]	COSY	HMBC
1	149.7, C		147.9, C			
2	119.0, C		117.0, C			
3	125.4, CH	7.11, s	123.9, CH	7.20, s		1, 2, 4, 5, 7
4	119.7, C		117.8, C			
5	155.0, C		152.9, C			
6	105.1, CH	6.37, s	103.6, CH	6.37, s		1, 2, 4, 5
7	26.5, CH_2	2.79, t (7.9)	25.1, CH_2	2.65, t (7.7)	8	3, 4, 5, 8, 9
8	35.2, CH_2	2.58, t (7.9)	33.8, CH_2	2.49, t (7.7)	7	4, 7, 9
9	175.7, C		173.0, C			
10	167.3, C		164.1, C			
11	122.5, CH	6.16, d (14.9)	122.8, CH	6.27, d (15.0)	12	10, 12, 13
12	143.4, CH	7.25, dd (14.9, 10.9)	140.5, CH	7.12, dd (15.0, 10.9)	11, 13	10, 11, 13, 14
13	130.9, CH	6.26, dd (14.9, 10.6)	129.7, CH	6.22, dd (15.0, 10.9)	12, 14	11, 12, 14, 15
14	143.7, CH	6.15, dt (14.9, 7.2)	141.5, CH	6.14, dt (15.0, 7.3)	13, 15	12, 13, 15, 16
15	43.4, CH_2	2.09, t (6.9)	41.7, CH_2	2.04, t (7.0)	14, 16	13, 14, 16, 17, 18
16	29.5, CH	1.72, m	27.8, CH	1.68, m	15, 17, 18	14, 15, 17, 18
17	22.7, CH_3	0.93, d (6.7)	22.2, CH_3	0.88, d (6.7)	16	15, 16, 18
18	22.7, CH_3	0.93, d (6.7)	22.2, CH_3	0.88, d (6.7)	16	15, 16, 17
2-NH				9.50, s		1, 2, 3, 10
9-$OCHH_3$	52.0, CH_3	3.64, s	51.3, CH_3	3.57, s		9

[a]Spectra were recorded at 600 MHz for ^{1}H and 100 MHz for ^{13}C using the corresponding solvent residual signal as internal standard. [b]Measured in CD_3OD. [c]Measured in DMSO-d_6.

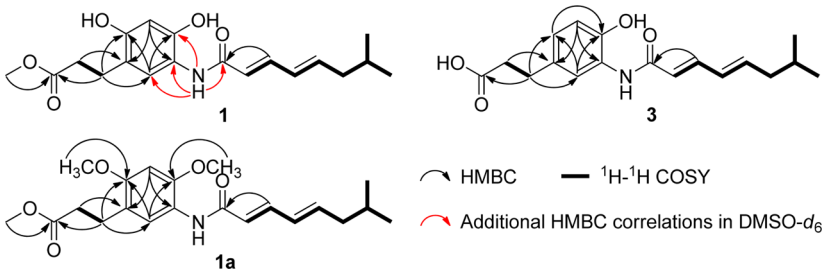
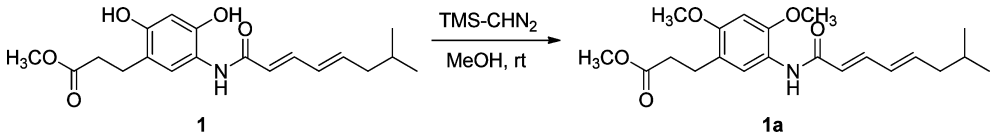


Figure 1. Key correlations for the structural assignment of **1**, **3**, and **1a**.

Scheme 1. Methylation of 1 with TMS-CHN_2



HMBC correlations from H-12 to the C-10 carbonyl (δ_C 167.3), indicated the presence of a 7-methylocta-2,4-dienoic acid residue (Figure 1). The COSY correlation of H_2-7 to H_2-8 as well as HMBC correlations from H-3 to C-1 and C-5, H-6 to C-2 and C-4, H_2-7 to C-3, C-5, and C-9, H_2-8 to C-4, and 9-OCH_3 to C-9 indicated the presence of a 1,2,4,5-tetrasubstituted benzene unit (Figure 1). COSY and HMBC correlations confirmed a methyl propionate residue was connected at C-4 (Figure 1). A combination of ^{13}C NMR, examination of exchangeable protons, and chemical derivatization allowed us to determine C-1, C-2, and C-5 were substituted by −OH, −NHR, and −OH, respectively. In particular, the downfield ^{13}C chemical shifts of C-1 (δ_C 149.7) and C-5 (δ_C 155.0) suggested oxygen substitution, while the shift of C-2 (δ_C 119.0) was indictive of −*N*-acyl substitution. In order to verify the 7-methylocta-2,4-dienoic acid residue was attached to the nitrogen at C-2, the HMBC spectrum was measured in DMSO-d_6. HMBC correlations from an exchangeable proton (δ_H 9.50) to C-1, C-2, C-3, and C-10 were observed (Figure 1), thus verifying the assignment. Finally, to confirm the C-1 and C-5 −OH substitution, methylation of **1** was carried out using TMS-CHN_2 to yield compound **1a** (Scheme 1). The LC-MS analysis showed the presence of two new methyl groups (Figure S2), while NMR analysis gave ^{1}H chemical shifts of two methoxy groups at δ_H 3.89 (1-$OCHH_3$, s) and 3.85 (5-$OCHH_3$, s) and HMBC correlations from 1-$OCHH_3$ to C-1 and from 5-$OCHH_3$ to C-5 (Figure 1).

Carpatamide B (**2**) was nearly identical to **1** by ^{1}H and ^{13}C NMR, with the molecular formula determined to be $C_{18}H_{23}NO_5$, indicating that a $-CH_3$ of **1** was replaced by a −H. Analysis of the ^{1}H and ^{13}C NMR spectra revealed that the methoxy signals at $\delta_{H/C}$ 3.64/52.0 in compound **1** disappeared. Thus, compound **2** was determined to be the carboxylic acid analogue of **1**.

dx.doi.org/10.1021/np500207p | J. Nat. Prod. 2014, 77, 1245−1248

The molecular formula of carpatamide C (**3**) was determined to be $C_{18}H_{23}NO_4$ based on the HRESIMS peak at *m/z* 318.1701 $[M + H]^+$, one oxygen atom less than **2**. The ^1H and ^{13}C NMR spectra (Table S1) of **3** were very similar to those of **2**, with the exception that an oxygenated quaternary carbon signal (δ_C 155.0) in compound **2** was replaced by a methine signal at $\delta_{C/H}$ 125.4/6.90 in compound **3**. The ^1H NMR signals at δ_H 7.49 (d, *J* = 1.9 Hz), 6.90 (dd, *J* = 8.2, 1.9 Hz), and 6.79 (d, *J* = 8.2 Hz) of compound **3** indicated a 1,2,4-trisubstituted benzene system. Furthermore, the COSY and HMBC correlations confirmed this structure (Figure 1).

Compounds **1**–**3** were evaluated for their cytotoxicity against four NSCLC cell lines (HCC366, A549, HCC44, and H2122). Compound **1** showed cytotoxicity against the cell lines HCC366 with an IC_{50} of 2.8 μM, A549 with an IC_{50} of 4.1 μM, and HCC44 with an IC_{50} of 8.4 μM. **1** showed no activity up to 24 μM against H2122. Compound **3** exhibited cytotoxicity against the HCC366 and A549 cell lines, with IC_{50} values of 2.2 and 3.7 μM, respectively. Compound **2** did not show significant cytotoxicity against any of the four cell lines tested. We believe that **2** does not show activity against cancer cell lines due to an inability to penetrate cells. It is plausible that the methyl ester of **1** acts as a prodrug and is cleaved in cells to give the active pharmacophore.

EXPERIMENTAL SECTION

General Experimental Procedures. UV spectra were recorded on a Shimadzu UV-1601 UV–vis spectrophotometer. ^1H and 2D NMR spectroscopic data were recorded at 600 MHz in CD_3OD or DMSO-d_6 solution on a Varian System spectrometer. ^{13}C NMR spectra were acquired at 100 MHz on a Varian System spectrometer. Chemical shifts were referenced to the corresponding solvent residual signal (3.31/49.00 in CD_3OD, 2.50/39.52 in DMSO-d_6). High-resolution ESI-TOF mass spectra were provided by The Scripps Research Institute, La Jolla, CA. Low-resolution LC/ESIMS data were measured using an Agilent 1200 series LC/MS system with a reversed-phase C_{18} column (Phenomenex Luna, 150 mm × 4.6 mm, 5 μm) at a flow rate of 0.7 mL/min. Preparative HPLC was performed on an Agilent 1200 series instrument with a DAD detector, using a C_{18} column (Phenomenex Luna, 250 × 10.0 mm, 5 μm). Sephadex LH-20 (GE Healthcare, Sweden) and ODS resin (50 mm, Merck) were used for column chromatography. Artificial seawater was used in microbial fermentations as described in previous references.[9]

Collection and Phylogenetic Analysis of Strain SNE-011. The actinomycete *Streptomyces* sp. SNE-011 was isolated from a marine sediment sample collected from South Carolina (32°35′10″ N, 80°07′31″ W). Bacterial spores were collected via stepwise centrifugation as follows: 2 g of sediment was dried over 24 h in an incubator at 35 °C, and the resulting sediment added to 10 mL of seawater (sH_2O) containing 0.05% Tween 20. After vigorous vortexing for 10 min, the sediment was centrifuged at 2500 rpm for 5 min (4 °C). The supernatant was removed, transferred into a new tube, and centrifuged at 18 000 rpm for 25 min (4 °C), and the resulting spore pellet was collected. The resuspended spore pellet (4 mL of sH_2O) was plated on a humic acid medium (humic acid 10 g, peptone 2 g, $MgSO_4$ 0.5 g, $FeSO_4$ 0.01 g, and agar 15 g, dissolved in 1 L of seawater), giving rise to individual colonies of SNE-011 after 2 weeks. Analysis of the 16S rRNA sequence of SNE-011 revealed 98% identity to *Streptomyces carpaticus*. The sequence is deposited in GenBank under accession no. KJ174292.

Cultivation and Extraction. Bacterium SNE-011 was cultured in 10 2.8 L Fernbach flasks each containing 1 L of a seawater-based medium (10 g starch, 4 g yeast extract, 2 g peptone, 1 g $CaCO_3$, 40 mg $Fe_2(SO_4)_3{\cdot}4H_2O$, 100 mg KBr) and shaken at 200 rpm at 27 °C. After 7 days of cultivation, sterilized XAD-7-HP resin (20 g/L) was added to adsorb the organic products, and the culture and resin were shaken at 200 rpm for 2 h. The resin was filtered through cheesecloth, washed with deionized water, and eluted with acetone. The acetone-soluble fraction was dried in vacuo to yield 7.9 g of extract.

Purification. The extract of strain SNE-011 (7.9 g) was partitioned with hexanes, CH_2Cl_2, EtOAc, and MeOH/H_2O. The hexanes and the CH_2Cl_2 extracts were combined to give an extract (750 mg). This extract was fractionated by flash column chromatography on ODS (50 μm, 30 g), eluting with a step gradient of MeOH and H_2O (10:90–100:0), and 12 fractions (Fr.1–Fr.12) were collected. Fraction 10 (25.1 mg) was purified by reversed-phase HPLC (Phenomenex Luna, C_{18}, 250 × 10.0 mm, 2.5 mL/min, 5 μm) using a gradient solvent system from 30% to 100% CH_3CN (0.1% formic acid) over 20 min to afford compounds **3** (2.0 mg, t_R = 17.4 min) and **1** (10.5 mg, t_R = 18.4 min). The EtOAc extract (480 mg) was separated by flash column chromatography on ODS (50 μm, 30 g), eluting with a step gradient of MeOH and H_2O (10:90–100:0), to give 15 fractions (Fr.1–Fr.15). Fractions 12 (45.5 mg) and 13 (11.7 mg) were combined and then separated by Sephadex LH-20, eluting with MeOH, to give 12 fractions. The subfraction 12-6 (22.9 mg) was purified by reversed-phase HPLC (Phenomenex Luna, C_{18}, 250 × 10.0 mm, 2.5 mL/min, 5 μm) using a gradient solvent system from 30% to 100% CH_3CN (0.1% formic acid) over 20 min to yield compound **2** (15.1 mg, t_R = 16.0 min).

*Carpatamide A (**1**):* yellow oil; UV (MeOH) λ_{max} (log ε) 266 (4.02), 315 (3.77) nm; ^1H and ^{13}C NMR, see Table 1; ESIMS *m/z* 346.1 $[M - H]^-$; HRESIMS *m/z* 348.1805 $[M + H]^+$ (calcd for $C_{19}H_{26}NO_5$, 348.1805).

*Carpatamide B (**2**):* yellow oil; UV (MeOH) λ_{max} (log ε) 273 (4.19), 314 (3.86) nm; ^1H and ^{13}C NMR, see Table S1; ESIMS *m/z* 332.1 $[M - H]^-$; HRESIMS *m/z* 334.1650 $[M + H]^+$ (calcd for $C_{18}H_{24}NO_5$, 334.1649).

*Carpatamide C (**3**):* yellow oil; UV (MeOH) λ_{max} (log ε) 272 (3.94), 305 (3.62) nm; ^1H and ^{13}C NMR, see Table S1; ESIMS *m/z* 316.2 $[M - H]^-$; HRESIMS *m/z* 318.1701 $[M + H]^+$ (calcd for $C_{18}H_{24}NO_4$, 318.1700).

Methylation of 1 with TMS-CH_2N_2. To a solution of **1** (1.0 mg) in MeOH (0.5 mL) was added 200 μL of TMS-CHN_2 (2.0 M in Et_2O) until a yellow color persisted upon addition. After allowing it to stir for 1 h, solvent was removed via a stream of N_2, and the reaction mixture was analyzed via LC/MS (Figure S2). The reaction product was purified by reversed-phase HPLC (Phenomenex Luna, C_{18}, 250 × 10.0 mm, 2.5 mL/min, 5 μm) using a gradient solvent system (solvents: A H_2O + 0.1% formic acid; B CH_3CN + 0.1% formic acid; gradient: 0 min, 30% B; 20 min, 100% B; 25 min, 100% B) to yield compound **1a** (0.9 mg, t_R = 22.0 min, 83% yield). Compound **1a**, yellow oil; ^1H NMR (600 MHz, CD_3OD) δ 7.71 (s, 1H, H-3), 7.21 (dd, *J* = 14.9, 10.7 Hz, 1H, H-12), 6.65 (s, 1H, H-6), 6.26 (dd, *J* = 15.1, 10.9 Hz, 1H, H-13), 6.19 (d, *J* = 15.0 Hz, 1H, H-11), 6.15 (dt, *J* = 15.1, 7.3 Hz, 1H, H-14), 3.89 (s, 3H, 1-$OCHH_3$), 3.85 (s, 3H, 5-$OCHH_3$), 3.65 (s, 3H, 9-$OCHH_3$), 2.83 (t, *J* = 7.7 Hz, 2H, H-7), 2.55 (t, *J* = 7.7 Hz, 2H, H-8), 2.09 (t, *J* = 7.0 Hz, 2H, H-15), 1.73 (m, 1H, H-16), 0.94 (d, *J* = 6.7 Hz, 6H, H-17/18); ^{13}C NMR (100 MHz, CD_3OD) δ 175.5 (C, C-9), 167.2 (C, C-10), 156.5 (C, C-5), 151.7 (C, C-1), 143.2 (CH, C-14), 142.9 (CH, C-12), 131.0 (CH, C-13), 125.6 (CH, C-3), 122.3 (CH, C-11), 121.1 (C, C-4), 120.5 (C, C-2), 96.6 (CH, C-6), 56.5 (CH_3, 1-$OCHH_3$), 56.2 (CH_3, 5-$OCHH_3$), 52.0 (CH_3, 9-$OCHH_3$), 43.4 (CH_2, C-15), 35.3 (CH_2, C-8), 29.6 (CH, C-16), 26.6 (CH_2, C-7), 22.7 (2 × CH_3, C-17/18); ESIMS *m/z* 374.2 $[M - H]^-$.

Cytotoxicity Assays. Cell lines were cultured in 10 cm dishes (Corning, Inc.) in NSCLC cell-culture medium: RPMI/L-glutamine medium (Invitrogen, Inc.), 1000 U/mL penicillin (Invitrogen, Inc.), 1 mg/mL streptomycin (Invitrogen, Inc.), and 5% fetal bovine serum (Atlanta Biologicals, Inc.). Cell lines were grown in a humidified environment in the presence of 5% CO_2 at 37 °C. For cell viability assays, HCC366, A549, HCC44, and H2122 cells (60 μL) were plated individually at a density of 1200, 750, and 500 cells/well, respectively, in 384-well microtiter assay plates (Bio-one; Greiner, Inc.). After incubating the assay plates overnight under the growth conditions described above, purified compounds were dissolved and diluted in DMSO and subsequently added to each plate with final compound

dx.doi.org/10.1021/np500207p | J. Nat. Prod. 2014, 77, 1245–1248

concentrations ranging from 50 μM to 1 nM and a final DMSO concentration of 0.5%. After an incubation of 96 h under growth conditions, Cell Titer Glo reagent (Promega, Inc.) was added to each well (10 mL of a 1:2 dilution in NSCLC culture medium) and mixed. Plates were incubated for 10 min at room temperature, and luminescence was determined for each well using an Envision multimodal plate reader (PerkinElmer, Inc.). Relative luminescence units were normalized to the untreated control wells (cells plus DMSO only). Data were analyzed using the Assay Analyzer and Condoseo modules of the Screener Software Suite (GeneData, Inc.) as described previously.[10]

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra for compounds **1**−**3** and **1a**, NMR data table for **2** and **3**, LC/MS analysis of the product of methylation of **1**, and LC/MS analysis of the active fraction of *Streptomyces* sp. SNE-011 are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1-214-648-8653. Fax: +1-214-648-8856. E-mail: john.macmillan@utsouthwestern.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the following grants for funding this project: NIH R01 CA149833, U01 CA176284, and the Welch Foundation I-1689. J.B.M. is a Chilton/Bell Foundation Endowed Scholar. We are also grateful for the support of the UT Southwestern High Throughput Screening Core Facility and the grants that support it (NCI Cancer Center Support Grant P30CA142543 and the CPRIT MIRA Grant RP110708-C2).

■ REFERENCES

(1) (a) Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discovery* **2009**, *8*, 69−85. (b) Chakraborty, C.; Hsu, C. H.; Wen, A. H.; Lin, C. S. *Curr. Top. Med. Chem.* **2009**, *9*, 1536−1545.

(2) Bister, B.; Bischoff, D.; Strobele, M.; Riedlinger, J.; Reicke, A.; Wolter, F.; Bull, A. T.; Zahner, H.; Fiedler, H. P.; Sussmuth, R. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2574−2576.

(3) Feling, R. H.; Buchanan, G. O.; Mincer, T. J.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *Angew. Chem., Int. Ed.* **2003**, *42*, 355−357.

(4) Kwon, H. C.; Kauffman, C. A.; Jensen, P. R.; Fenical, W. *J. Am. Chem. Soc.* **2006**, *128*, 1622−1632.

(5) (a) Proksch, P.; Muller, W. E. G. *Frontiers in Marine Biotechnology*; Routledge: Norfolk, 2006; pp 225−288. (b) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666−673. (c) Lam, K. S. *Curr. Opin. Microbiol.* **2006**, *9*, 245−251.

(6) (a) Ohnuki, S.; Oka, S.; Nogami, S.; Ohya, Y. *PLoS One* **2010**, *5*, e10177. (b) Yarrow, J. C.; Totsukawa, G.; Charras, G. T.; Mitchison, T. J. *Chem. Biol.* **2005**, *12*, 385−395. (c) Peach, K. C.; Bray, W. M.; Shikuma, N. J.; Gassner, N. C.; Lokey, R. S.; Yildiz, F. H.; Linington, R. G. *Mol. Biosyst.* **2011**, 1176−1184. (d) Sista, P.; Edmiston, S.; Darges, J. W.; Robinson, S.; David, J.; Burns, D. J. *Mol. Cell. Biochem.* **1994**, *141*, 129−134. (e) Cherry, J. J.; Evans, M. C.; Ni, J.; Cuny, G. D.; Glicksman, M. A.; Elliot, J.; Androphy, E. J. *J. Biomol. Screening* **2012**, *17*, 481−495.

(7) The natural product fraction library was created from bacterial extracts of 5 L fermentations of bacterial strains. Fractions were generated using reversed-phase C_{18} chromatography on an ISCO medium-pressure automatic purification system (gradient from 90:10 H_2O/CH_3CN to 0:100 H_2O/CH_3CN over 25 min) to generate 10−20 fractions.

(8) (a) Zeeck, A.; Frobel, K.; Heusel, C.; Schroder, K.; Thiericke, R. *J. Antibiot.* **1987**, *40*, 1541−1548. (b) Uosaki, Y.; Agatsuma, T.; Tanaka, T.; Saitoh, Y. *J. Antibiot.* **1996**, *49*, 1079−1084. (c) Devi, P.; Wahidullah, S.; Rodrigues, C.; Souza, L. D. *Mar. Drugs* **2010**, *8*, 1203−1212. (d) DellaGreca, M.; Previtera, L.; Purcaro, R.; Zarrelli, A. *Chem. Biodiversity* **2009**, *6*, 459−465. (e) Li, C. Y.; Tsai, W. J.; Damu, A. G.; Lee, E. J.; Wu, T. S.; Dung, N. X.; Thang, T. D.; Thanh, L. *J. Agric. Food Chem.* **2007**, *55*, 9436−9442. (f) Matsuda, H.; Ando, S.; Kato, T.; Morikawa, T.; Yoshikawa, M. *Bioorg. Med. Chem.* **2006**, *14*, 138−142.

(9) Keller, M. D.; Selvin, R. C.; Claus, W.; Guillard, R. R. L. *J. Phycol.* **1987**, *23*, 633−638.

(10) Kim, H. S.; Mendiratta, S.; Kim, J.; Pecot, C. V.; Larsen, J. E.; Zubovych, I.; Seo, B. Y.; Kim, J.; Eskiocak, B.; Chung, H.; McMillan, E.; Wu, S.; De Brabander, J. K.; Komurov, K.; Toombs, J. E.; Wei, S.; Peyton, M.; Williams, N.; Gazdar, A. F.; Posner, B. A.; Brekken, R. A.; Sood, A. K.; Deberardinis, R. J.; Roth, M. G.; Minna, J. D.; White, M. A. *Cell* **2013**, *155*, 552−566.