ORGANIC LETTERS

2011
Vol. 13, No. 3
410−413

Histone Deacetylase Inhibitors as a Tool to Up-Regulate New Fungal Biosynthetic Products: Isolation of EGM-556, a Cyclodepsipeptide, from *Microascus* sp.

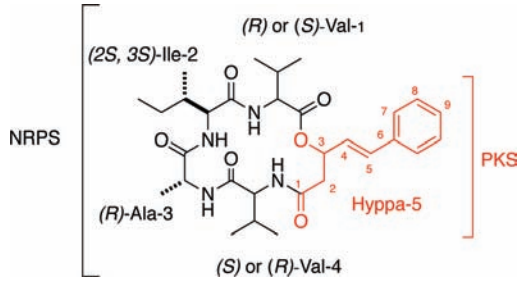
Hélène C. Vervoort, Marija Drašković, and Phillip Crews*

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064, United States

phil@chemistry.ucsc.edu

Received November 9, 2010

ABSTRACT



The histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA) was used to turn on the biosynthesis of EGM-556, a new cyclodepsipeptide of hybrid biosynthetic origin, isolated from the Floridian marine sediment-derived fungus *Microascus* sp. The absolute configurations of three chiral centers were determined by Marfey's derivatization. EGM-556 represents one of the few examples in which silent biosynthetic genes, encoding a new secondary metabolite, were activated by means of epigenetic manipulation of the fungal metabolome.

Marine-derived fungi[1] continue to be a source of novel bioactive metabolites, even in genera as well studied as *Aspergillus*[2] and *Penicillium*.[3] Continuing attention on these taxa is, in part, being motivated by advances in genome sequencing,[4] especially through genome mining.[5] A core intent of these studies usually involves identifying biosynthetic gene clusters producing novel structures[6,7] to test the hypothesis that such approaches will allow expression of 'silent' metabolite pathways.[8,6a] Another related strategy involves unraveling the regulatory mechanisms controlling the biosynthetic genes[9] using insights gained from chemical genetics[10] and epigenetics[11] experiments.

The use of small molecule HDAC (Histone Deacetylase) inhibitors[12] to perturb the fungal secondary biosynthetic machinery[10c] has been rewarding and represents a proof-of-principle of ideas summarized above. Commercially available compounds such as 5-azacytidine (Aza)[13] and suberoylanilide hydroxamic acid (SAHA),[12a] explored by several laboratories, have provided valuable outcomes. For

(1) (a) Saleem, M.; Ali, M. S.; Hussain, S.; Jabbar, A.; Ashraf, M.; Lee, Y. S. *Nat. Prod. Rep.* **2007**, *24*, 1142–1152. (b) Ebel, R. In *Comprehensive Natural Products II: Chemistry and Biology*; Moore, B. S., Crews, P., Eds.; Elsevier: 2010; Vol. 2, Ch. 2, pp 223−262.

(2) (a) Tsukamoto, S.; Umaoka, H.; Yoshikawa, K.; Ikeda, T.; Hirota, H. *J. Nat. Prod.* **2010**, *73*, 1438–1440. (b) Zhao, W. Y.; Zhu, T. J.; Fan, G. T.; Liu, H. B.; Fang, Y. C.; Gu, Q. Q.; Zhu, W. M. *Nat. Prod. Res.* **2010**, *24*, 953–957. (c) Wu, Q.-X.; Crews, M. S.; Drašković, M.; Sohn, J.; Johnson, T. A.; Tenney, K.; Valeriote, F. A.; Yao, X.-J.; Bjeldanes, L. F.; Crews, P. *Org. Lett.* **2010**, *12*, 4458–4461.

(3) (a) Drawz, S. M.; Bonomo, R. A. *Clin. Microbiol. Rev.* **2010**, *23*, 160–201. (b) Barrios-González, J.; Miranda, R. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 869–883. (c) de Silva, E. D.; Geiermann, A.-S.; Mitova, M. I.; Kuegler, P.; Blunt, J. W.; Cole, A. L. J.; Munro, M. H. G. *J. Nat. Prod.* **2009**, *72*, 477–479.

(4) Ansorge, W. J. *New Biotechnol.* **2009**, *25*, 195–203.

(5) Challis, G. L. *J. Med. Chem.* **2008**, *51*, 2618–2628.

10.1021/ol1027199
Published on Web 12/21/2010

example, the culturing of terrestrial *Aspergillus niger* in the presence of SAHA yielded the novel secondary metabolite nygerone, carrying a highly unusual 1-phenylpyridin-4(1*H*)-one core.[11c] New oxylipins plus the unique pigments, the cladochromes, were produced by *C. cladosporioides* cultured in the presence of either Aza or SAHA. As another parallel result, the lunalides, octadecadienoic acids, were produced by cultures of *Diatrype* sp. grown in the presence of Aza.[11d]

We used a test bed of 12 marine sediment-derived fungi (strain numbers and properties shown in Table S1, Supporting Information) to assess changes in their biosynthetic products induced by adding 10^{-4} M SAHA to the saltwater cultures. All fungi were isolated from shallow water sediments collected in Florida and cultured for 18 days to reach the stationary growth phase. The group of 24 ethyl acetate crude extracts (from broths + or − SAHA) were profiled by analytical RP C18 LC-ELSD-MS, and significant changes were observed in the scans between the matched pairs. One member of this set, *Microascus* sp. (strain number 098059A, identified by morphological and molecular methods[14]), showed noteworthy changes and was chosen for further study. Shown for this sample in Figure 1 are the primary data consisting of LC-ELSD-MS traces for control (**C**) and SAHA-spiked (**SP**) cultures. Significantly, the **SP** sample contained a new peak at retention time 21.5 min with an *m*/*z* of 557 ($[M+H]^+$) that was not present in the control culture. In addition, the ethyl acetate crude extract yields were very different between the two samples: **SP** = 114.6 mg vs **C** = 30.6 mg. Of further note was our literature search

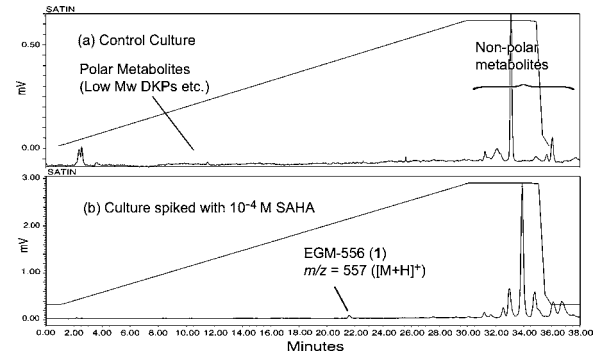


Figure 1. RP LC-ELSD-MS traces showing the absence (a) and presence (b) of EGM-556 (**1**) in cultures of *Microascus* sp.

that revealed that only ~10 secondary metabolites have been reported for *Microascus* and its asexual anamorph *Scopulariopsis* species, described as human pathogens in immunocompromised patients.[15]

Isolation of the RT = 21.5 min substance using RP HPLC (see General Experimental Procedures, Supporting Information) was straightforward and yielded 3.5 mg of a white solid. This compound, named EGM-556 (**1**) with $[\alpha]^{23}_{D} = +2.60$ (*c* 0.60, MeOH), possessed a molecular formula of $C_{30}H_{44}N_4O_6$, (11 unsaturation units) assigned by HR ESI-TOFMS data for the $[M+H]^+$ ion at *m*/*z* 557.33064 (calcd 557.33336, $\Delta = -4.8$ ppm, see General Experimental Procedures, Supporting Information). The DEPT NMR spectrum (Table 1, and Figures S2, S3, Table S2, Supporting Information) showed a partial molecular formula of $C_{30}H_{40}$, and the remaining $H_4N_4O_6$ atoms were indirectly accounted for by four amides (NH protons at δ_H 7.60, 7.74, 8.10, 8.56, see Figure S1, Supporting Information) and one ester (C═O)−Z (see five peaks δ_C 168.7−173.0). These functionalities comprised five unsaturation units with five more from an monosubstituted benzene ring (δ_H 7.44 (2H, d), 7.36 (2H, dd), δ 7.28 (1H, t)) and an *E*-disubstituted conjugated double bond (δ_H 6.28 (dd, J = 15.9, 7.1 Hz, 1H), δ_H 6.68 (d, J = 15.9 Hz, 1H)). Collectively, these observations meant that an additional ring must also be present as a macrocyclic depsipeptide. Using all of the preceding structural constraints and formulas as input, extensive dereplication searches were carried out but yielded no structural matches.

The 2D COSY NMR results clearly showed five separate proton spin systems consistent with the substructures **I**−**V** drawn in Scheme 1. The presence of seven methyl groups plus the $^1H-^1H$ COSY NMR data accounted for two valines (Val-1, Val-4), alanine (Ala-3), and isoleucine (Ile-2). The

(6) (a) Chiang, Y.-M.; Szewczyk, E.; Davidson, A. D.; Keller, N.; Oakley, B. R.; Wang, C. C. C. *J. Am. Chem. Soc.* **2009**, *131*, 2965–2970. (b) Ding, Y.; de Wet, J. R.; Cavalcoli, J.; Li, S.; Greshock, T. J.; Miller, K. A.; Finefield, J. M.; Sunderhaus, J. D.; McAfoos, T. J.; Tsukamoto, S.; Williams, R. M.; Sherman, D. H. *J. Am. Chem. Soc.* **2010**, *132*, 12733–12740. (c) Cuomo, C. A.; Birren, B. W. In *Methods in Enzymology*; Jonathan, W., Christine, G., Gerald, R. F., Eds.; Academic Press: 2010; Vol. 470, pp 833−855. (d) Yin, W.-B.; Grundmann, A.; Cheng, J.; Li, S.-M. *J. Biol. Chem.* **2009**, *284*, 100–109.

(7) (a) Ricke, D. O.; Wang, S.; Cai, R.; Cohen, D. *Curr. Opin. Chem. Biol.* **2006**, *10*, 303–308. (b) Van Lanen, S. G.; Shen, B. *Curr. Opin. Microbiol.* **2006**, *9*, 252–260. (c) Bergmann, S.; Schumann, J.; Scherlach, K.; Lange, C.; Brakhage, A. A.; Hertweck, C. *Nat. Chem. Biol.* **2007**, *3*, 213–217.

(8) (a) Chiang, Y.-M.; Lee, K.-H.; Sanchez, J. F.; Keller, N. P.; Wang, C. C. *Nat. Prod. Commun.* **2009**, *4*, 1505–1510. (b) Christian, O. E.; Compton, J.; Christian, K. R.; Mooberry, S. L.; Valeriote, F. A.; Crews, P. *J. Nat. Prod.* **2005**, *68*, 1592–1597.

(9) (a) Keats Shwab, E.; Keller, N. P. *Mycol. Res.* **2008**, *112*, 225–230. (b) Bok, J. W.; Keller, N. P. *Eukaryot. Cell* **2004**, *3*, 527–535. (c) Stadler, M.; Keller, N. P. *Mycol. Res.* **2008**, *112*, 127–130.

(10) (a) Hertweck, C. *Nat. Chem. Biol.* **2009**, *5*, 450–452. (b) Scherlach, K.; Hertweck, C. *Org. Biomol. Chem.* **2009**, *7*, 1753–1760. (c) Keats Shwab, E.; Bok, J. W.; Tribus, M.; Galehr, J.; Graessle, S.; Keller, N. P. *Eukaryot. Cell* **2007**, *6*, 1656–1664. (d) Bok, J. W.; Chiang, Y.-M.; Szewczyk, E.; Reyes-Dominguez, Y.; Davidson, A. D.; Sanchez, J. F.; Lo, H.-C.; Watanabe, K.; Strauss, J.; Oakley, B. R.; Wang, C. C. C.; Keller, N. P. *Nat. Chem. Biol.* **2009**, *5*, 462–464.

(11) (a) Cichewicz, R. H. *Nat. Prod. Rep.* **2010**, *27*, 11–22. (b) Fisch, K.; Gillaspy, A.; Gipson, M.; Henrikson, J.; Hoover, A.; Jackson, L.; Najar, F.; Wägele, H.; Cichewicz, R. *J. Ind. Microbiol. Biotechnol.* **2009**, *36*, 1199–1213. (c) Henrikson, J. C.; Hoover, A. R.; Joyner, P. M.; Cichewicz, R. H. *Org. Biomol. Chem.* **2009**, *7*, 435–438. (d) Williams, R. B.; Henrikson, J. C.; Hoover, A. R.; Lee, A. E.; Cichewicz, R. H. *Org. Biomol. Chem.* **2008**, *6*, 1895–1897.

(12) (a) Marks, P. A. *Oncogene* **2007**, *26*, 1351–1356. (b) Wiech, N. L.; Fisher, J. F.; Helquist, P.; Wiest, O. *Curr. Top. Med. Chem.* **2009**, *9*, 257–271.

(13) Raj, K.; Mufti, G. J. *Ther. Clin. Risk Manag.* **2006**, *2*, 377–388.

(14) Dr. E. H. Thompson and Dr. B. L.Wickes at the Department of Pathology UT Health Science Center at San Antonio kindly provided taxonomic identification of fungal strain 098059A placing it within the Microascus/Scopulariopsis genera (details on the identification of the strain described in the Identification of Fungal strain 098059A and Table S2, Supporting Information).

(15) (a) Mohammedi, I.; Piens, M. A.; Audigier-Valette, C.; Gantier, J. C.; Argaud, L.; Martin, O.; Robert, D. *Eur. J. Clin. Microbiol. Infect. Dis.* **2004**, *23*, 215–217. (b) Baddley, J. W.; Moser, S. A.; Sutton, D. A.; Pappas, P. G. *J. Clin. Microbiol.* **2000**, *38*, 395–397.

Table 1. NMR Assignments for EGM-556 (**1**) in DMSO-d_6

structuralunit	position	$^{13}C^a$ δ, type^b	^1H δ (m, J(Hz), int)^c
Val-1	CO	168.7, qC	
	α	58.2, CH	4.24 (dd, 9.6, 6.0, 1H)
	β	28.5, CH	2.25 (ds, 6.0, 6.6, 1H)
	β-CH_3	18.9, CH_3	0.89 (d, 6.6, 3H)
	β-CH_3	19.3, CH_3	0.89 (d, 6.6, 3H)
	*N*H		7.60 (d, 9.6, 1H)
Ile-2	CO	170.4, qC	
	α	57.2, CH	4.26 (d, 9.6, 1H)
	β	35.6, CH	2.05 (m, 1H)
	γ	23.6, CH_2	1.26 (m, 2H)
	β-CH_3	15.5, CH_3	0.81 (d, 6.6, 3H)
	γ-CH_3	11.9 CH_3	0.81 (t, 7.5, 3H)
	*N*H		8.10 (d, 9.6, 1H)
Ala-3	CO	173.0, qC	
	α	48.8, CH	4.32 (dq, 6.3, 6.4, 1H)
	α-CH_3	16.3, CH_3	1.18 (d, 7.2, 3H)
	*N*H		8.56 (d, 6.3, 1H)
Val-4	CO	172.3, qC	
	α	57.5, CH	4.14 (dd, 8.7, 7.2, 1H)
	β	29.8, CH	1.96 (ds, 6.9, 6.8, 1H)
	β-CH_3	19.6, CH_3	0.89 (d, 6.6, 3H)
	β-CH_3	18.2, CH_3	0.89 (d, 6.6, 3H)
	*N*H		7.74 (d, 8.7, 1H)
Hyppa-5	1	168.7, qC	
	2	40.1, CH_2^d	2.42 (dd. 14.4, 1.8, 1H)
			2.88 (dd, 14.4, 10.5, 1H)
	3	72.9, CH	5.50 (ddd, 10.5, 7.1, 1.8, 1H)
	4	126.7, CH	6.28 (dd, 15.9, 7.1, 1H)
	5	132.4, CH	6.68 (d, 15.9, 1H)
	6	135.7, qC	
	7	126.5, CH	7.44 (d, 7.2, 2H)
	8	128.7, CH	7.36 (dd, 7.5, 7.2, 2H)
	9	128.1, CH	7.28 (t, 7.5, 1H)

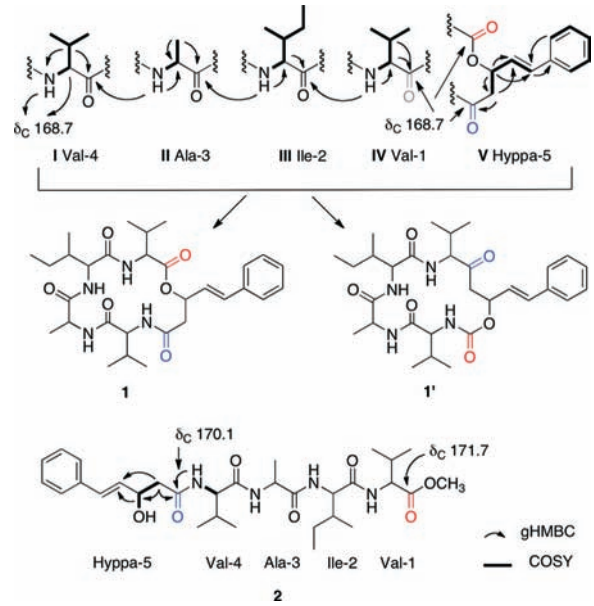
[a] Measured at 150 MHz. [b] Carbon type established from ^{13}C DEPT and/or gHMQC experiments. [c] Measured at 600 MHz. [d] Overlapping with DMSO-d_6.

remaining substructure **V**, 3-hydroxy-5-phenyl-4(*E*)-pentenoic acid, Hyppa, was visualized from the 2D NMR correlations shown in Scheme 1. The intersubstructural gHMBC correlations, also shown in Scheme 1, unambiguously indicated the sequence Val-Ile-Ala-Val. The insertion of the Hyppa (**V**) substructure was complicated by the accidental isochrony of two carbonyl groups at δ_C 168.7 (due to Val and Hyppa arrays, indicated in red and blue in Scheme 1). The gHMBC correlations between protons at δ_H 4.24, 2.25 (Val-1), 4.14, 7.74 (Val-4), 5.50, 2.88, and 2.42 (Hyppa-5) and these carbonyls were consistent with two possible ways to dock **V** into the tetrapeptide as shown by structural candidates **1** and **1′** (Scheme 1). The gray carbonyl group in substructure **IV** is common to a carbonyl group in **V**, either in red, resulting in alternative structure **1**, or in blue resulting in alternative structure **1′**. Fortunately, the latter structure was quickly ruled out because it contains a carbamate and a ketone whose diagnostic ^{13}C shifts of δ_C ~157 and δ_C ~200, respectively, were not observed.

The pathway to further confirm the structure proposed of **1** along with efforts to resolve the configurations at the six chiral centers was approached in the following way. Hydrolysis of **1** (1.5 mg, 1N NaOH) followed by esterification with diazomethane[16] afforded the acyclic methyl ester **2**,

(16) Glastrup, J. *J. Chromatogr., A* **1998**, *827*, 133–136.

Scheme 1. Substructures, Alternative Final Structures, And Key 2D NMR Correlations[a]



[a] Accidentally isochronous carbonyl groups occurred in **1** at δ_C 168.7, indicated in red, gray and blue.

$[\alpha]^{23}_D = -1.36$ (*c* 1.30, MeOH) shown in Scheme 1. Its molecular formula of $C_{31}H_{48}N_4O_7$ was confirmed by HR ESITOFMS, based on the $[M+Na]^+$ ion *m*/*z* 611.34268 (calcd 611.34152, $\Delta = 1.9$ ppm, see General Experimental Procedures, Supporting Information) and as expected was one unsaturation unit less than that of **1**. The NMR data for **2** (see Table S4, Supporting Information) were consistent with the expected sequence and all five carbonyl resonances were anisochronous, which made it possible to unequivocally confirm the juxtaposition of Hyppa-5 and Val-4 through an amide bond.

Acid mediated hydrolysis of **1** (1 mg, 6 M HCl) followed by derivatization with Marfey's reagent[17] and subsequent C18 LC-ELSD-MS analysis of the adducts was the approach taken to deduce the absolute configurations of **I**−**IV**. The overall results indicated the constituent amino acids were as follows: (*R*)-Val, (*S*)-Val, (2*S*,3*S*)-Ile, and (*R*)-Ala. Retention times of the standard amino acids under our analysis conditions were as follows: (*S*)-Ala 59.7 min, (*R*)-Ala 64.4 min, (*S*)-Val 68.9 min, (*R*)-Val 75.4 min, (2*S*,3*S*)-Ile 74.7 min, (2*R*,*3R*)-Ile 81.3 min (see also Figure S13, Supporting Information). Since both (*R*)-Val and (*S*)-Val were observed, EGM-556 (**1**) is one of four possible structures: cyclic ((*R*)-Hyppa-5)-((*R*)-Val-4)-((*R*)-Ala-3)-((2*S*,3*S*)-Ile-2)-((*S*)-Val-1), cyclic ((*R*)-Hyppa-5)-((*S*)-Val-4)-((*R*)-Ala-3)-((2*S*,3*S*)-Ile-2)-((*R*)-Val-1), cyclic ((*S*)-Hyppa-5)-((*R*)-Val-4)-((*R*)-Ala-3)-((2*S*,3*S*)-Ile-2)-((*S*)-Val-1), or cyclic ((*S*)-Hyppa-5)-((*S*)-Val-4)-((*R*)-Ala-3)-((2*S*,3*S*)-Ile-2)-((*R*)-Val-1).

Reversed phase chromatography did not differentiate between (2*S*,3*S*)-isoleucine and (2*S*,3*R*)-isoleucine, and these

(17) (a) Bhushan, R.; Brückner, H. *Amino Acids* **2004**, *27*, 231–247. (b) Marfey, P.; Ottesen, M. *Carlsberg Res. Commun.* **1984**, *49*, 585–590.

amino acids were separated using a chiral column (Chirex 3010, Phenomenex, 4.1 mm × 150 mm).[18] Solvent A was 0.1 M aqueous ammonium acetate, pH = 3 with TFA and solvent B acetonitrile. The gradient used was 20−75% solvent B in A over 50 min, 1 mL/min. Retention times of amino acids under these conditions were as follows: (2*S*,3*S*)-Ile 23.0 min, (2*R*,3*R*)-Ile 24.3 min, (2*S*,3*R*)-Ile 22.3 min, (2*R*,3*S*)-Ile 23.5 min (see also Figure S14, Supporting Information). Our results showed that a (2*S*,3*S*)-Ile is present.

Another approach considered, to assign the unresolved configuration of **1** at C3 of Hyppa-5, involved attempts to make a Mosher ester derivative[19] of the alcohol moiety of **2**. However, all of our experiments were unsuccessful. The configuration is therefore left unassigned since there is precedent for both (*R*) and (*S*) in similar compounds in the literature, as discussed below.

As an important additional note, we utilized MS^n data to also address the subunit sequence for both **1** and **2**. The complex array of fragmentations observed for **1** was consistent with principles outlined in the literature for cyclic depsipeptides.[20] By contrast, the pattern observed for **2** was quite distinct. These contrasting outcomes will be fully described elsewhere.

The EGM-556 (**1**) substructural units are likely derived from an HPN (Hybrid PKS/NRPS) biosynthetic pathway. Moreover, the 16 atom peptolide core of EGM-556 (**1**) is rare; the only other examples are the antimicrobial unnarmicins (**3**) from a marine-derived *Photobacterium* sp. MBIC06485[21] and the histone deacetylase inhibitory/antitumor active FK228 (FR901228, **4**) from *Chromobacterium violaceum* No. 968.[22] Side-by-side comparison of **1**, **3**, and **4** (see Figure 2) reveals some additional similarities and contrasts. The ketide portions of all three contain β-hydroxy amides and, analogous to the situation of **1**, FK228 (**4**) possess (*R*)-Val and (*S*)-Val units in close proximity to the PKS side chain. The monosubstituted benzene-ring-containing side chain of **1** is analogous to similar functionality present in the cryptophycins, especially −26, isolated from *Nostoc* strains of cyanobacteria.[23] Rigorous biosynthetic analysis[24] has shown the phenyl-octenoic polyketide unit of cryptophycin-26 is likely derived from a phenylalanine (via trans-cinnamic acid), which is clearly present in **1**. Finally, the 3(*R*)-hydroxy-5-phenyl-4(*E*)-pentenoic acid (**5**) coded as Hyppa in **1**, as shown in Figure 3,

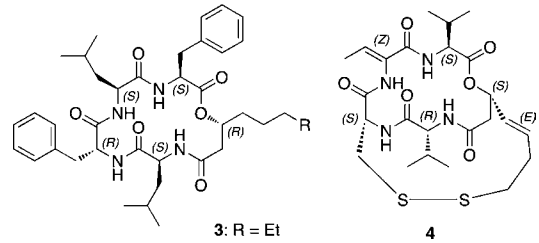


Figure 2. Unnarmicin C (**3**) from marine *Photobacterium* sp.[21] and FK228 (**4**) from *C. violaceum*.[22]

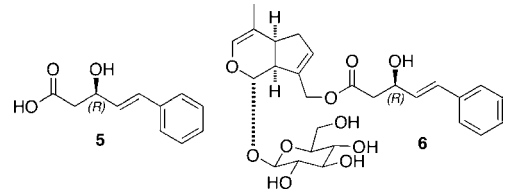


Figure 3. 3(*R*)-hydroxy-5-phenyl-4(*E*)-pentenoic acid (Hyppa) (**5**) and marinoid E (**6**) isolated from *A. marina*.[25]

has one precedent. The free acid was first isolated from the Chinese plant *Avicennia marina* together with five new iridoid glucosides, and one marinoid E (**6**) also contained the (3*R*)-Hyppa unit.[25]

Our successful initial experiments with the 12 marine-derived fungi explored here extend previous parallel results where spiking cultures with the potent actin inhibitor Jasplakinolide[8b] upregulated different biosynthetic products. The discovery of EGM-556 (**1**) represents an exciting outcome of expressing hitherto silent HPN biosynthetic genes. In the future, we will report on parallel outcomes based on additional HDAC spiking experiments to extend the biosynthetic products from well-studied fungi including *Aspergillus* and *Penicillium* but isolated from marine sources.

Acknowledgment. Support came from NIH CA 052955 to P.C. and an ARRA supplement to H.V. We thank Prof. Fenical, S.I.O., UCSD, for sediments from which *Microascus* sp. was isolated.

Note Added after ASAP Publication. The Abstract graphic and Supporting Information contained errors in the stereochemistry of (*S*)-Ala and (*R*)-Ala in the version published ASAP December 21, 2010. The abstract graphic, SI, and affected text were replaced in the version reposted January 13, 2011.

Supporting Information Available: Figures (14), tables (4), experimental procedures, and NMR data of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL1027199

(18) Capon, R. J.; Skene, C.; Stewart, M.; Ford, J.; O'Hair, R. A. J.; Williams, L.; Lacey, E.; Gill, J. H.; Heiland, K.; Friedel, T. *Org. Biomol. Chem.* **2003**, *1*, 1856–1862.
(19) Latypov, S. K.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Am. Chem. Soc.* **1998**, *120*, 877–882.
(20) Paizs, B.; Suhai, S. *Mass Spectrom. Rev.* **2005**, *24*, 508–548.
(21) Oku, N.; Kawabata, K.; Adachi, K.; Katsuta, A.; Shizuri, Y. *J. Antibiot.* **2008**, *61*, 11–17.
(22) Sasakawa, Y.; Naoe, Y.; Inoue, T.; Sasakawa, T.; Matsuo, M.; Manda, T.; Mutoh, S. *Biochem. Pharmacol.* **2002**, *64*, 1079–1090.
(23) Golakoti, T.; Ogino, J.; Heltzel, C. E.; Le Husebo, T.; Jensen, C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Mooberry, S. L. *J. Am. Chem. Soc.* **1995**, *117*, 12030–12049.
(24) Magarvey, N. A.; Beck, Z. Q.; Golakoti, T.; Ding, Y.; Huber, U.; Hemscheidt, T. K.; Abelson, D.; Moore, R. E.; Sherman, D. H. *ACS Chem. Biol.* **2006**, *1*, 766–779.
(25) Sun, Y.; Ouyang, J.; Deng, Z.; Li, Q.; Lin, W. *Magn. Reson. Chem.* **2008**, *46*, 638–642.