Supplementary material (ESI) for Organic & Biomolecular Chemistry

Electronic Supplementary Information

Epigenetic remodeling of the fungal secondary metabolome†

Russell B. Williams,[a] Jon C. Henrikson,[a] Ashley R. Hoover,[a] Andrlynn E. Lee,[a] Robert H. Cichewicz[*a,b]

[a] *Natural Products Discovery Group, Department of Chemistry and Biochemistry, 620 Parrington Oval, Room 208, University of Oklahoma, Norman, Oklahoma, 73019-3032, USA. Fax: (405) 325-6111; Tel: (405) 325-6969; E-mail: rhcichewicz@ou.edu*

[b] *Graduate Program in Ecology and Evolutionary Biology*

Supplementary Methods

General Chemical Methods. Initial HPLC separation of secondary metabolite mixtures was performed on a Shimadzu preparative HPLC using a SCL-10A VP system controller, SPD-10AV VP UV-VIS detector, LC-6AD pumps, DGU-14A solvent degasser, and FRC-10A programmable fraction collector. Samples were separated over a Phenomenex C_{18} Gemini column (5 μm, 110 Å, 250 × 21.2 mm) with fractions collected at a rate of one per minute. Semi-preparative HPLC was performed on a similar system using LC-10AT VP pumps and a Phenomenex C_{18} Gemini column (5 μm, 110 Å, 250 × 10 mm). Mass data from electrospray ionization were acquired on a LCT Premier (Waters Corp.) time of flight mass spectrometer. Corrections for exact mass determinations were made automatically with the lock mass feature in the MassLynx data system. A reference compound in an auxiliary sprayer was sampled every third cycle by toggling a 'shutter' between the analysis and reference needles. Samples for mass determination were dissolved in MeOH-H_2O (9:1) and introduced for ionization with a 20 μL loop on an auto injector system. NMR data were obtained on a Varian VNMR spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). All solvents were of ACS grade or better.

Fungal Test Strains. Fungal test strains for initial screening were obtained from the Agricultural Research Services (ARS) Culture Collection (formerly NRRL) and The University of Oklahoma Natural Products Discovery Group (OU NPDG) collections. The OU NPDG collection consists of >1,200 fungi obtained from soil samples and marine sediments collected from across the United States including Alaska and it costal waters, Arizona, California and it costal waters, Indiana, Maine and it costal waters, Oklahoma, Washington, and Wyoming. All strains were grown on appropriate media and cryopreserved in 15% glycerol at -80 ºC. Fungi selected for this study included the following: *Aspergillus flavus* (ARS), *Aspergillus westerdijkiae* (ARS), *Cladosporium cladosporioides* (OU NPDG), *Clonostachys* sp. (OU NPDG), *Diatrype* sp. (OU NPDG), *Penicillium chrysogenum* (ARS), *Penicillium citrinum* (ARS), *Rhizopus sp.* (OU NPDG), and *Verticillium sp*. (OU NPDG), and three additional marine-derived isolates (referred to as Isolates 1-3) that have not yet been identified.

Small Molecule Epigenetic Modifiers. Small molecule epigenetic modifiers were selected to include both DNA methyltransferase and histone deacetylase inhibitors. The DNA methyltransferase inhibitors consisted of 5-azacytidine, 5-aza-2′-deoxycytidine, hydralazine, procaine, and procainamide which were all purchase from Sigma. Histone deacetylase inhibitors

included sodium butyrate, suberohydroxamic acid, and valproic acid from Sigma and suberoylanilide hydroxamic acid from TRC Biomedical Research. Compounds were prepared in deionized H_2O and filter sterilized (0.22 μM) before being added to fungal cultures.

Screening Epigenetic Modifiers. For MIC determinations, stock cultures were prepared by lawning fungi onto Petri plates and the resulting mycelial mats were submerged in sterile water and mechanically disrupted so as to uniformly disperse cells/spores. Microplates (96-well sterile polystyrene plates, 300 μL well volume, with low-evaporation covers, Corning, Inc.) were loaded with 200 μL of media/well and 1 μL portions of filter sterilized epigenetic compounds dissolved in water or water controls. Pure compounds were tested in a series of six, ten-fold dilutions ranging in concentration from 0.1 μM to 10 mM. Aliquots of 20 μL fungal cell/spore suspension were used to inoculate each well of the plates. Plates were covered and incubated in a humidified chamber in the dark at 25 ºC. After 3-7 days (dependant upon growth characteristics of each fungal strain), the MIC values of the compounds were determined visually based on turbidity. The MIC value was assigned as the lowest compound concentration capable of inhibiting fungal growth.

Epigenetic modifying agents were tested for their capacity to induce secondary metabolite production by culturing organisms in 25 mL of media, and after 24 h, compounds were added at concentrations 10-fold less than their respective MIC values. The cultures were incubated an additional 6 days at which point 12 mL of MeOH was added to each culture before being partitioned twice against 30 mL of dichloromethane. The combined organic extracts were dried under vacuum and stored at -20 ºC prior to analysis.

Isolation and Identification of Fungi. Fungal strains (*Cladosporium cladosporioides* and *Diatrype* sp.) were isolated in a three phase process. The *Cladosporium cladosporioides* was obtained from a sediment sample collected from a tidal pool along the coastline of Casco Bay, Portland, Maine, USA. The *Diatrype* sp. was isolated from the fore gut of a fifth instar *Actias luna* (Saturniidae) larva that was cultured on an exclusive diet of *Liquidambar styraciflua* L. (Hamamelidaceae) leaves. Substrates containing fungi (~500 mg) were mixed with 15 mL sterile water, vortexed vigorously, and two, ten-fold dilutions made. Aliquots (200 μL) of the three suspensions were lawned onto primary isolation plates containing potato-dextrose agar with chloramphenicol to inhibit bacterial growth and cycloheximide to restrict expansion of rapidly growing fungi. Dilution plates were prepared in triplicate and the maintained inverted at 20 ºC.

After three days, plates were checked for colony formation and monitored every other day for three weeks. Emerging colonies exhibiting filamentous character were excised from the primary plate using a sterile probe and transferred to a secondary Petri plate containing potato dextrose agar with chloramphenicol (cycloheximide was omitted). Colonies were selected from primary plates for sub-culturing on secondary plates based on their differential morphologies and time of appearance. Rapidly spreading colonies and those having morphological features identical to isolates already sub-cultured were 'pruned' from the primary plates in order to allow the slower growing and less abundant strains to mature. Secondary plates were maintained upright at 20 ºC and the hyphae of the growing organisms allowed to radiate outward from the point of inoculation. After 1-2 weeks, sterile probes were used to remove small samples from the leading edges of the spreading fungal colonies and transferred to tertiary Petri plates containing potato-dextrose agar (chloramphenicol and cycloheximide were omitted). Plates were incubated upright at 20 ºC for an additional 1-2 weeks after which the fungal cultures were prepared for cryostorage. Sterile probes were used to remove pieces of mycelia and deposited into sterile cryostorage vials containing 15% glycerol in water. The samples were then stored at -80 ºC.

Fungi were tentatively identified based on analysis of the 300 base pair sequence of the D2 region of the large ribosomal subunit (26S rRNA gene). Analysis of samples was performed under the expert review of Michael G. Sinclair, Ph.D., Mycologist and Laboratory Director, Microcheck, Inc. Briefly, portions of the mycelia mats were scraped from the surface of an agar plate and placed in a sample tube with Prepman reagent (Applied Biosystems) to lyse the cells and liberate DNA. The mixture was boiled and briefly centrifuged to pellet cellular debris. The DNA in solution was transferred to a fresh tube and PCR reagents added (buffers, primers, and dNTPs) and the 26S rRNA gene amplified in a thermal-cycler. Next, ExoSAP-IT was added to remove unreacted primers and dNTPs. Finally sequencing reagent was added containing fluorescently-tagged ddNTPs yielding 3′ end labeled product and purified. After clean-up, the samples were loaded into a 3130*xl* Genetic Analyzer (Applied Biosystems) for sequencing. The final consensus sequence was compared to a library of 1,113 fungal standards using MicroSeq ID software. The software allowed for comparisons of sample sequences to those stored in the library by weighting mismatches (i.e. purine to purine substitutions are weighted lower than purine to pyrimidine substitutions) thereby providing a percent match score against known fungi. Most fungi that we encounter can be readily identified to the genus level (match score of >95% to known species); however, identification to species is less typical. Most genera assignments are

then confirmed by morphological/microscopic evaluation according to standard mycological methods.

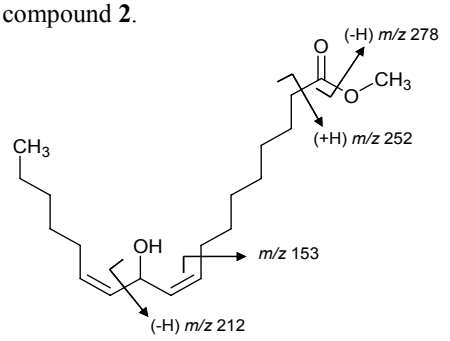
Fungal Culture Conditions and Initial Screening. Seed cultures of fungi were prepared by inoculating five 25 mL portions of media with agar plugs from Petri plates containing hypae of either *Cladosporium cladosporioides* or *Diatrype* sp. The cultures were allowed grow with shaking for seven days at 20 ºC. Scale-up fermentations consisted of sets of ten 1 L Erlenmeyer flasks containing 250 mL of sterile potato-dextrose media that were inoculated with 2 mL of their respective seed cultures. The newly prepared cultures were allowed to incubate with rotary shaking (100 rpm) for 24 h at 25 ºC. Each set of 10 cultures was treated with epigenetic modifying compounds or water blank (control) and returned to further incubate on the shaker for an additional six days. The cultures were harvested by adding 100 mL of MeOH to each flask before being partitioned twice against 300 mL of dichloromethane. The combined organic extracts from replicate flasks were dried under vacuum and stored at -20 ºC prior to analysis. All treatments and controls for each fungus were performed in tandem with test and control cultures randomly interspersed on the shaker.

Culture Comparison and Compound Isolation. For each experiment, sets composed of ten culture flasks (each with 250 mL of potato-dextrose media each inoculated 24 h prior with 1 mL of fungal seed culture) were treated with 5-azacytidine (DNA methyltransferase inhibitor), suberoylanilide hydroxamic acid (histone deacetylase inhibitor), or sterile water blank. Parallel experiments also examined the effects of ten different media conditions on secondary metabolite production (Supplementary Information Table 2). Cultures of *C. cladosporioides* were incubated with shaking for six days and the contents within each treatment group pooled. Cultures of *Diatrype sp.* were incubated with shaking for 14 days and the contents within each treatment group pooled. An additional experiment was performed on the *Diatrype* sp. by treating growing cultures of the fungus with 1 mL aliquots of autoclaved *Escherichia coli* culture instead of epigenetic modifiers. A 1 L portion of methanol was added to the media-cells mixture and then extracted three times with equal volumes of dichloromethane. The total organic extract from each treatment was injected onto C_{18} preparative HPLC (20-100% acetonitrile-water over 50 minutes) and monitored by dual wavelength UV detection at 210 nm and 254 nm. Difference chromatograms were generated for the 210 nm trace by subtracting the untreated control chromatogram from the treatment groups (Lab Solutions). Peaks phasing upward represent metabolites that were expressed only under epigenetic treatment or produced at enhanced

concentrations. Compounds from *C. cladosporioides* and *Diatrype* sp. were isolated by semi-preparative HPLC with repeated re-injection and separation across C_{18} using acetonitrile-water gradient conditions.

Compound Characterization. Secondary metabolites were characterized by NMR, HRESIMS, and CD. The HRESIMS data of compounds **1-3** from *C. cladosporioides* treated with 5-azacytidine were readily obtained providing molecular formulae of $C_{18}H_{32}O_3$ [*m/z* $[M + Na]^+$ 319.2210 (calcd for $C_{18}H_{32}NaO_3$ 319.2249)], $C_{19}H_{34}O_3$ [*m/z* $[M + Na]^+$ 333.2387 (calcd for $C_{19}H_{34}NaO_3$ 333.2406)], and $C_{21}H_{38}O_5$ [*m/z* $[M - OH]^-$ 353.2635 (calcd for $C_{21}H_{37}O_4$ 353.2692)] for **1**, **2**, and **3**, respectively. Compounds **1-3** exhibited strong aliphatic methylene signals (δ ^{1}H NMR ~1.34) and ^{13}C data suggesting that these metabolites were composed of hydrocarbon backbone. Scrutiny of the downfield regions for each compound showed that **1-3** all possessed four proton spins (H-9, H-10, H-12, and H-13) indicating the presence of two substituted double bonds with *cis* geometry. A single downfield proton (H-11) in each compound also indicated that a heteroatom substitution had occurred somewhere along these structures' carbon backbone. Examining the carbon data and HMBC correlations allowed us to confidently construct a (2*Z*,5*Z*)-hepta-2,5-dien-4-ol substructure however the placement of this moiety in the extended carbon chain remained ambiguous. Fortunately, MS/MS fragmentation generated (see Supplementary Methods Fig. 1 for example fragments generated for compound **2**) distinct masses allowing us to assign the double bond positions as 9*Z*,12*Z* as is illustrated for **1-3**. Moreover, NMR data obtained for these compounds in $CDCl_3$ and CD_3OD exhibited severe overlap; however, procurement of spectra in C_6D_6 allowed for a confident assignment of double bond positions. Based on these data, we were able to assign the structures of compounds **1-3** as (9*Z*,12*Z*)-11-hydroxyoctadeca-9,12-dienoic acid, its methylester derivative (9*Z*,12*Z*)-methyl-11-hydroxyoctadeca-9,12-dienoate, and glycerol derivative (9*Z*,12*Z*)-2,3-dihydroxypropyl 11-hydroxyoctadeca-9,12-dienoate, respectively.

Supplementary Methods Fig. 1. Key fragments generated by ESI MS/MS for compound **2**.



Compounds obtained from *C. cladosporioides* treated with suberoylanilide hydroxamic acid exhibited a rich blood-red color which combined with distinctive proton spins (H-5 and H-8 singlets; four -OCH_3 singlets) and supporting HRESIMS data (see summary data

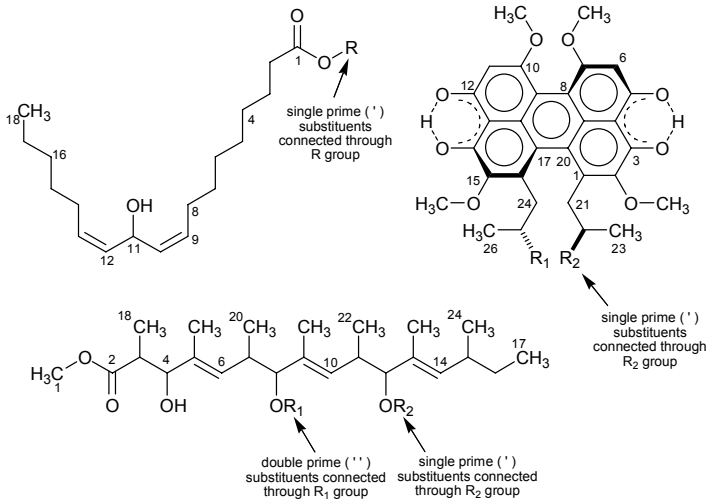
for compound **4-10** below) were characteristic for perylenequinone-type metabolites. Detailed examination of the accurate mass, ^{1}H NMR ($CDCl_3$ and CD_3OD), and HMBC data for compounds **6-10** allowed us to rapidly confirm their structures as the known cladochromes A (**6**), B (**7**), D (**8**), and E (**9**) and calphostin B (**10**). Compound **4** and **5** exhibited the same distinctive spectroscopic signatures as the other cladochromes; however, no previously reported structures shared their molecular formulas of $C_{37}H_{34}O_{12}$ (based on *m/z* $[M + Na]^+$ 693.1934, calcd for $C_{37}H_{34}NaO_{12}$ 693.1948) for compound **4** and $C_{37}H_{34}O_{13}$ (based on *m/z* $[M + H]^+$ 687.2078, calcd for $C_{37}H_{35}O_{13}$ 687.2078) for compound **5**. Therefore we embarked on deciphering the planar structures of these two new metabolites. Compound **4** showed distinct ^{1}H and ^{13}C spins arising from a single *p*-hydroxybenzoate moiety [δ ^{1}H NMR 6.30 (2H, d, H-4′ and H-6′), 6.68 (2H, d, H-3′ and H-7′); δ ^{13}C NMR 114.0 (C-4′ and C-6′), 119.4 (C-2′), 130.0 (C-3′ and C-7′), 161.4 (C-5′), 164.6 (C-1′)] whereas ^{1}H and ^{13}C NMR data for compound **5** was indicative of a *p*-hydroxycarbonateand functionality [δ ^{1}H NMR 6.02 (2H, d, H-4′ and H-6′), 6.50 (2H, d, H-3′ and H-7′); δ ^{13}C NMR 114.7 (C-3′ and C-7′), 119.4 (C-2′), 120.7 (C-4′ and C-6′), 154.6 (C-5′), 154.8 (C-1′)]. HMBC data allowed for the inverse detection and assignment of the majority of the carbon skeleton and confirmed our assignments of *p*-hydroxybenzoate and *p*-hydroxybenzoate moieties both with ester linkages at position C-22 of their respective cladochrome cores. Therefore, we were able to determine the planar structures of compounds **4** and **5** as the new cladochromes F and G, respectively. Examination of the absolute configuration of **4** and **5** was achieved by comparison of CD data [CD (EtOH) nm (*Δε*) 291 (+79.1), 360 (-19.8), 451 (+34.9), 550 (-9.0), 591 (-16.0)] to that previously reported for known cladochromes/calphostins[1, 2] and found in good agreement. Therefore, the absolute stereochemistry for **4** and **5** was established as shown.

Compound **11** and **12** were obtained as amorphous solids via HPLC fractionation of the 5-azacytidine treated *Diatrype* sp. cultures. Data from HRESIMS exhibited a peak of *m/z* 595.3494 $[M + Na]^+$ indicating the molecular formula of **11** to be $C_{30}H_{52}O_{10}$ (calc. for $C_{30}H_{52}O_{10}Na$, 595.3458). The ^{1}H and ^{13}C NMR spectra suggested the presence of nine methyls, two methylenes, and fifteen methines. In addition, four quaternary carbons were observed, one of which was a carbonyl based on its chemical shift and the other three were incorporated into double bonds. HMBC, HSQC, and COSY correlations were used to piece together a polyketide backbone, with a sugar moiety attached at C-12, with distinct anomeric proton (^{1}H δ 4.37 ppm) and carbon (^{13}C δ 97.5 ppm) chemical shifts. The sugar moiety was determined to be β-mannopyranose based on 1D-NOE correlations and susceptibility to enzymatic hydrolysis with β-

mannosidase whereas attempts to remove the sugar residue with α-mannosidase were unsuccessful. Data from HRESIMS exhibited a peak of *m/z* 757.3971 $[M + Na]^+$ indicating the molecular formula of **12** to be $C_{36}H_{62}O_{15}$ (calc. for $C_{36}H_{62}O_{15}Na$, 757.3986). Observing the ^1H NMR spectrum indicated the presence of two anomeric proton signatures (^1H δ 4.35 and 4.58 ppm). The proton spectrum, along with the difference in mass data between compounds **11** and **12**, supported that compound **12** had the same polyketide backbone, with an additional β-mannopyranose moiety being present. A combination of 2-D HMBC, HSQC, and COSY data confirmed these suspicions, with the new β-mannopyranose substituted at the at the C-8 hydroxyl. Again, the sugar moieties were determined to be β-mannopyranose based on enzymatic hydrolysis. Further support as to the identity of the β-mannopyranose was obtained by careful comparison of our ^1H and ^{13}C NMR data to that of authentic D-mannose.

Note: Number schemes for reported spectroscopic data are shown below in Supplementary Methods Fig. 2.

Supplementary Methods Fig. 2. Numbering schemes for new natural products.



Compound 1. (9*Z*,12*Z*)-11-hydroxyoctadeca-9,12-dienoic acid; ^1H-NMR(500MHz, CD_3OD): δ 0.91 (3H, t, *J*=7.5 Hz), 1.34 (14H, m), 1.60 (2H, m), 2.14 (6H, m), 5.21 (1H, dd, *J*=8 Hz, 8Hz), 5.40 (4H, m); HRESIMS *m/z* $[M + Na]^+$ 319.2210 (calcd for $C_{18}H_{32}NaO_3$ 319.2249); this compound has been previously described in the literature[3, 4]; however, proton data have not been previously reported.

Compound 2. (9*Z*,12*Z*)-methyl-11-hydroxyoctadeca-9,12-dienoate; ^{1}H-NMR(500MHz, CD_3OD): δ 0.92 (3H, t, *J*=7.5 Hz), 1.35 (14H, m), 1.61 (2H, m), 2.14 (4H, m), 2.32 (2H, t, *J*=7.5 Hz), 3.66 (3H, s), 5.21 (1H, dd, *J*=7.5 Hz, 8 Hz), 5.41 (4H, m);HRESIMS *m*/*z* $[M + Na]^+$ 333.2387 (calcd for $C_{19}H_{34}NaO_3$ 333.2406); data were in agreement with that previously reported for **2**[5] and exhibited similar fragmentation by MS[6].

Compound 3. (9*Z*,12*Z*)-2,3-dihydroxypropyl 11-hydroxyoctadeca-9,12-dienoate; ^{1}H-NMR (500MHz, CD_3OD): δ 0.92 (3H, t, *J*=7.5 Hz, H-18), 1.35 (14H, m, H-4, H-5, H-6, H-7, H-15, H-16, and H-17), 1.62 (2H, m, H-3), 2.13 (4H, m, H-8 and H-14), 2.36 (2H, t, *J*=8 Hz, H-2), 3.55 (2H, dd, *J*=2.5 Hz, 5.5 Hz, H-3′), 3.82 (1H, m, H-2′), 4.06 (1H, dd, *J*=6 Hz, 12 Hz, H-1a′), 4.15 (1H, dd, *J*=4.5 Hz, 11 Hz, H-1b′), 5.20 (1H, dd, *J*=7.5 Hz, 8 Hz, H-11), 5.41 (4H, m, H-9, H-10, H-12, and H-13); ^{13}C-NMR (125 MHz, CD_3OD) δ 14.6 (C-18), 23.8 (C-17), 26.1 (C-3), 29.0 (C-8, C-14), 30.3 (C-4), 30.4, 30.5, and 30.7 (C-5, C-6, and C-7), 30.9 (C-15), 32.9 (C-16), 35.1 (C-2), 64.2 (C-2′), 64.5 (C-11), 66.6 (C-1′), 71.3 (C-3′), 131.9 and 132.9 (C-9 and C-13), 132.0 and 132.8 (C-10 and C-12), 176.6 (C-1); HRESIMS *m*/*z* $[M - OH]^-$ 353.2635 (calcd for $C_{21}H_{37}O_4$ 353.2692).

Compound 4. Cladochrome F; ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.97 (3H, d, J=6.5 Hz, H-26), 1.30 (3H, d, J=6.0 Hz, H-23), 2.88 (1H, m, H-24a), 3.20 (1H, m, H-21a), 3.50 (1H, m, H-24b), 3.56 (1H, m, H-25), 3.66 (1H, m, H-21b), 3.99 (3H, s, 10-OCH_3), 4.00 (3H, s, 7-OCH_3), 4.22 (3H, s, 15-OCH_3), 4.29 (3H, s, 2-OCH_3), 5.10 (1H, m, H-22), 6.30 (2H, d, H-4′ and H-6′), 6.44 (1H, S, H-11), 6.57 (1H, s, H-6), 6.68 (2H, d, H-3′ and H-7′); ^{13}C-NMR (CD_3OD) δ: 19.9 (C-23), 22.6 (C-26), 39.3 (C-21), 42.2 (C-24), 55.7 (7-OCH_3 and 10-OCH_3), 60.2 (2-OCH_3 and 15-OCH_3), 68.3 (C-25), 71.4 (C-22), 100.5 (C-6), 100.6 (C-11), 105.7 (C-13), 106.0 (C-4), 114.0 (C-4′ and C-6′), 116.9 (C-8), 117.0 (C-9), 119.4 (C-2′), 128.0 (C-20), 128.2 (C-17), 130.0 (C-3′ and C-7′), 135.2 (C-1), 137.4 (C-16), 151.5 (C-15), 151.6 (C-2), 161.4 (C-5′), 164.6 (C-1′), 166.8 (C-10), 167.4 (C-7), 177.4 (C-12), 177.6 (C-5), (C-3, C-14, C-18, C-19 not observed); HRESIMS *m*/*z* $[M + Na]^+$ 693.1934 (calcd for $C_{37}H_{34}NaO_{12}$ 693.1948).

Compound 5. Cladochrome G; ^{1}H-NMR (500 MHz, CD_3OD) δ: 1.00 (3H, d, J=6.5 Hz, H-26), 1.22 (3H, d, J=6.5 Hz, H-23), 2.88 (1H, m, H-24a), 3.20 (1H, m, H-21a), 3.50 (1H, m, H-24b), 3.56 (1H, m, H-25), 3.66 (1H, m, H-21b), 3.84 (3H, s, 10-OCH_3), 4.09 (3H, s, 7-OCH_3), 4.19 (3H, s, 15-OCH_3), 4.25 (3H, s, 2-OCH_3), 4.79 (1H, m, H-22), 6.02 (2H, d, H-4′ and H-6′), 6.50

(2H, d, H-3′ and H-7′), 6.52 (1H, S, H-11), 6.73 (1H, s, H-6); ^{13}C-NMR (CD_3OD) δ: 19.9 (C-23), 22.6 (C-26), 38.8 (C-21), 42.2 (C-24), 55.7 (10-OCH_3), 56.0 (7-OCH_3), 60.2 (2-OCH_3 and 15-OCH_3), 68.3 (C-25), 76.1 (C-22), 100.2 (C-11), 100.5 (C-6), 105.6 (C-13), 105.8 (C-4), 114.7 (C-3′ and C-7′), 116.6 (C-8), 116.8 (C-9), 119.4 (C-2′), 120.7 (C-4′ and C-6′), 128.0 (C-20), 128.2 (C-17), 135.2 (C-1), 137.4 (C-16), 151.8 (C-15), 152.0 (C-2), 154.6 (C-5′), 154.8 (C-1′), 167.0 (C-10), 167.4 (C-7), 176.4 (C-12), 176.6 (C-5), (C-3, C-14, C-18, C-19 not observed); HRESIMS *m/z* $[M + H]^+$ 687.2078 (calcd for $C_{37}H_{35}O_{13}$ 687.2078).

Compound 6. Cladochrome A; HRESIMS *m/z* $[M + H]^+$ 723.2581 (calcd for $C_{38}H_{43}O_{14}$ 723.2653); ^{1}H and ^{13}C NMR were in agreement with published values[7].

Compound 7. Cladochrome B; HRESIMS *m/z* $[M + Na]^+$ 763.2356 (calcd for $C_{41}H_{40}NaO_{13}$ 763.2367); ^{1}H and ^{13}C NMR were in agreement with published values[7].

Compound 8. Cladochrome D; HRESIMS *m/z* $[M + H]^+$ 807.2271 (calcd for $C_{44}H_{39}O_{15}$ 807.2289); ^{1}H and ^{13}C NMR were in agreement with published values[8].

Compound 9. Cladochrome E; HRESIMS *m/z* $[M + H]^+$ 791.2340 (calcd for $C_{44}H_{39}O_{14}$ 791.2340); ^{1}H and ^{13}C NMR were in agreement with published values[8].

Compound 10. Calphostin B; HRESIMS *m/z* $[M + Na]^+$ 677.2001 (calcd for $C_{37}H_{34}NaO_{11}$ 677.1999); ^{1}H and ^{13}C NMR were in agreement with published values[2].

Compound 11. Lunalide A; ^{1}H-NMR (500 MHz, CD_3OD) δ 0.77 (3H, d, *J*=7.0 Hz, H-20), 0.82 (3H, d, *J*=7.5 Hz, H-22), 0.87 (3H, t, *J*=7.5 Hz, H-17), 0.94 (3H, d, *J*=7.5 Hz, H-18), 0.99 (3H, d, *J*=6.5 Hz, H-24), 1.24 (1H, m, H-16a), 1.40 (1H, m, H-16b), 1.59 (3H, d, 1.0 Hz, H-23), 1.64 (3H, d, 1.0 Hz, H-19), 1.66 (3H, d, 1.0 Hz, H-21), 2.39 (1H, m, H-15), 2.61 (1H, m, H-3), 2.64 (1H, m, H-7), 2.70 (1H, m, H-11), 3.07 (1H, m, H-5′), 3.36 (1H, m, H-3′), 3.52 (1H, dd, *J*=9.5 Hz, 9.5 Hz, H-4′), 3.70 (3H, s, H-1), 3.70 (1H, d, *J*=9.5 Hz, H-8), 3.71 (1H, m, H-6a′), 3.73 (1H, m, H-2′), 3.86 (1H, dd, *J*= 2.5 Hz, 11.5 Hz, H-6b′), 3.95 (1H, d, *J*=10 Hz, H-12), 4.06 (1H, d, *J*=10 Hz, H-4), 4.37 (1H, bs, H-1′), 5.26 (1H, d, *J*=9 Hz, H-14), 5.33 (1H, d, *J*=10 Hz, H-6), 5.38 (1H, d, *J*=9.5 Hz, H-10); ^{13}C-NMR (125 MHz, CD_3OD) δ 10.8 (C-19), 11.2 (C-21 and C-23), 12.7 (C-17), 14.9 (C-18), 17.9 (C-20 and C-22), 21.7 (C-24), 31.5 (C-16), 35.4 (C-11), 35.5 (C-15), 36.8 (C-7), 44.8 (C-3), 52.3 (C-1), 63.0 (C-6′), 68.9 (C-4′), 73.2 (C-2′), 75.8 (C-3′), 78.6 (C-

5′), 82.0 (C-4), 84.5 (C-8), 88.9 (C-12), 97.5 (C-1′), 131.8 (C-13), 134.9 (C-10), 35.5 (C-6), 136.2 (C-5 and C-9), 140.8 (C-14), 179.2 (C-2); HRESIMS *m*/*z* $[M + Na]^+$ 595.3494 (calcd for $C_{30}H_{52}NaO_{10}$ 595.3458).

Compound 12. Lunalide B; ^{1}H-NMR (500 MHz, CD_3OD) δ 0.77 (3H, d, *J*=7.0 Hz, H-20), 0.81 (3H, d, *J*=7.0 Hz, H-22), 0.87 (3H, t, *J*=7.5 Hz, H-17), 0.93 (3H, d, *J*=6.5 Hz, H-18), 1.00 (3H, d, *J*=6.5 Hz, H-24), 1.24 (1H, m, H-16a), 1.40 (1H, m, H-16b), 1.58 (3H, d, *J*=1.5 Hz, H-19), 1.61 (6H, bs, H-21, H-23), 2.40 (1H, m, H-7), 2.60 (1H, m, H-3), 2.68 (1H, m, H-15), 2.74 (1H, m, H-11), 3.03 (1H, m, H-5′), 3.33 (1H, m, H-3′), 3.41 (1H, m, H-5″), 3.49 (2H, m, H-4′ and H-4″), 3.68 (1H, m, H-3″), 3.69 (1H, m, H-6a′), 3.70 (3H, s, H-1), 3.72 (2H, m, H2′ and H2″), 3.78 (1H, dd, *J*=7 Hz, 12 Hz, H-6a″), 3.85 (1H, dd, *J*=2.5 Hz, 12 Hz, H-6b′), 3.89 (1H, dd, *J*=2.5 Hz, 12 Hz, H-6b″), 3.98 (1H, d, *J*=10 Hz, H-8), 4.02 (1H, d, *J*=10 Hz, H-12), 4.08 (1H, d, *J*=10 Hz, H-4), 4.35 (1H, bs, H-1′), 4.58 (1H, bs, H-1″), 5.27 (1H, d, *J*=9.5 Hz, H-14), 5.42 (1H, d, *J*=8 Hz, H-6), 5.45 (1H, d, *J*=8.5 Hz, H-10); ^{13}C-NMR (125 MHz, CD_3OD) δ 10.7 (C-19), 11.1 (C-21 and C-23), 12.7 (C-17), 15.0 (C-18), 17.9 (C-20), 18.0 (C-22), 21.7 (C-24), 31.5 (C-16), 35.4 (C-7), 35.5 (C-11 and C-15), 44.8 (C-3), 52.3 (C-1), 63.3 (C-6′ and C-6″), 69.3 (C-4′), 69.5 (C-4″), 73.0 (C-2′), 73.4 (C-2″), 75.0 (C-3″), 75.9 (C-3′), 77.6 (C-5″), 78.9 (C-5′), 82.0 (C-4), 88.4 (C-8), 88.5 (C-12), 97.1 (C-1″), 97.5 (C-1′), 131.8 (C-13), 132.7 (C-9), 134.9 (C-5), 136.4 (C-6), 138.6 (C-10), 141.1 (C-14), 178.3 (C-2); HRESIMS *m*/*z* $[M + Na]^+$ 757.3494 (calcd for $C_{36}H_{62}NaO_{15}$ 757.3986).

Table 1. Screening of Epigenetic Modifiers against Fungal Cultures[a].

	Organism[b]											
Compound[c]	AF	AW	CC	CS	DS	PC1	PC2	RS	VP	I1	I2	I3
A	+	+	+	-	+	+	+	-	+	+	+	+
B	+	+	nt	nt	nt	+	+	nt	nt	nt	nt	nt
C	-	-	nt	nt	nt	-	-	nt	nt	nt	nt	nt
D	+	+	+	-	nt	+	+	-	+	+	-	-
E	-	-	+	-	nt	-	-	-	+	-	-	-
F	-	-	-	-	nt	-	-	-	-	-	-	-
G	+	+	-	+	nt	+	+	-	+	-	-	+
H	+	-	-	+	nt	+	+	-	+	-	-	+
I	-	-	-	+	nt	-	-	-	-	-	-	-
J[d]	+	+	+	-	nt	-	-	-	-	+	-	-
K	-	-	-	-	nt	-	-	-	-	-	-	-
L	-	-	-	-	nt	-	-	-	-	-	-	-
M	-	-	-	-	nt	-	-	-	-	-	-	-

[a]Results described in this table should be interpreted as follows: + (active), - (not active), and nt (not tested); activity was ascribed to those compounds inducing the production of new secondary metabolites not observed under control culture conditions or enhanced levels of constitutively expressed compounds as based on analysis by HPLC, MS, ^{1}H-NMR, or TLC

[b]Fungi used in this study: AF (*Aspergillus flavus*), AW (*Aspergillus westerdijkiae*), CC (*Cladosporium cladosporioides*), CS (*Clonostachys* sp.), DS (*Diatrype* sp.), PC1 (*Penicillium chrysogenum*), PC2 (*Penicillium citrinum*), RS (*Rhizopus* sp.), VP (*Verticillium psalliotae*), I1 (Unidentified isolate 1), I2 (Unidentified isolate 2), and I3 (Unidentified isolate 3).

[c]Compounds used in this study: A (5-azacytidine), B (5-aza-2′-deoxycytidine), C (hydralazine), D (procaine), E (procainamide), F (sodium butyrate), G (suberohydroxamic acid), H (suberoylanilide hydroxamic acid), I (valproic acid), J (5-azacytidine and suberoylanilide hydroxamic acid), K (amphotericin B), L (cycloheximide), and M (5-fluorouracil).

[d]None of the 'active' compound mixture treatments produced metabolites different from those generated following the single administration of either 5-azacytidine or suberoylanilide hydroxamic acid alone. Most fungi exhibited significantly reduced growth when treated with the compound mixture.

Table 2. Media Used for Screening Fungi.

Media	Composition (g/L)
Cornmeal Broth	Yellow Cornmeal 40.0 g Distilled Water 1,000 mL
Czapek Broth	Sucrose 30 g $NaNO_3$ 3.0 g K_2HPO_4 1.0 g $MgSO_4 + 7H_2O$ 0.05g KCl 0.5g $FeSO_4 + 7H_2O$ 10.0mg Distilled Water 1,000 mL
Lactose-Peptone Broth	Lactose 1.0 g Peptone 1.0 g Distilled Water 1,000 mL
Malt Extract Broth	Malt Extract 6.0 g Mannose 1.80 g Glucose 6.0 g Yeast Extract 1.2g Distilled Water 1,000 mL
Malt Yeast Peptone Broth	Malt Extract 7g Yeast Extract 0.5g Peptone 1g Distilled Water 1,000 mL
Oatmeal II	Oatmeal 30.0 g Distilled Water 1,000 mL
Potato Dextrose Broth	Dried Potatoes 10.0 g Glucose 5.0 g Distilled Water 1,000 mL
Potato Peptone Broth	Dried Potatoes 10.0 g Glucose 10.0 g Peptone 5.0 g Distilled Water 1,000 mL
Sabouraud Peptone-Glucose Broth	Peptone 10.0 g Glucose 40.0 g Distilled Water 1,000 mL
Yeast Glucose Broth	Yeast Extract 5.0 g Glucose 10.0 g Distilled Water 1,000 mL
Yeast Peptone Dextrose Broth	Glucose 20.0 g Peptone 5.0 g Yeast Extract 10.0 g Distilled Water 1,000 mL
Yeast Peptone Sucrose Broth	Yeast Extract 5.0 g Peptone 10.0 g Sucrose 15.0 g Distilled Water 1,000 mL
Wickerhan′s Yeast Extract Malt Broth	Yeast Extract 3.0 g Malt extract 3.0 g Peptone 5.0 g Glucose 10.0 g Distilled Water 1,000 mL

Supplementary Information – References Cited

1. A. Arnone, L. Camarda, G. Nasini, and L. Merlini, *J. Chem. Soc., Perkin Trans. 1,* 1985, 1387-1392.
2. T. Iida, E. Kobayashi, M. Yoshida, and H. Sano, H. *J. Antibiot.,* 1989, **42**, 1475-1481.
3. M. Hamberg *J. Chem. Soc., Perkin Trans. 1,* 1993, 3065-3072.
4. I. D. Brodowsky, M. Hamberg, and E. H. J. Oliw, *J. Biol. Chem.*, 1992, **267**, 14738-14745.
5. A. V. Muehldorf *Tetrahedron Lett.,* 1994, **35**, 6851-6852.
6. M. Hámberg, W. Gerwick, and P. Åsen *Lipids,* 1992, **27**, 487-493.
7. A. Arnone, G. Assante, V. Di Modugno, L. Merlini, and G. Nasini, *Phytochemistry,* 1988, **27**, 1675-1678.
8. A. Arnone, G. Assante, L. Merlini, and G. Nasini, *Gazz. Chim. Ital.,* 1989, **119**, 557-559.