SUPPORTING INFORMATION

The aquastatin biosynthetic gene cluster encodes a versatile polyketide synthase capable of synthesising heteromeric depsides with diverse alkyl side chains

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Experimental Procedures

1. Strains, strain maintenance, and transformation conditions.

A. gemini MST-FP2131 has been maintained in YPD agar or Malt Extract agar (MEA) plates. *Saccharomyces cerevisiae* strain BJ5464-NpgA was employed for yeast-mediated homologous recombination cloning and heterologous expression of *aquA* gene.¹ The preparation of competent cells and transformation processes have been previously described.¹ *A. nidulans* LO8030 was used for heterologous expression.² The methods for protoplast preparation, transformation, and selection have been described in previous studies.^{3,4} *A. nidulans* was cultured in Glucose Minimal Medium (GMM) for all experiments.³ GMM was supplemented with riboflavin, and pyridoxine if necessary.³ *Escherichia coli* NEB10-beta® electrocompetent cells were used to store and propagate the generated plasmids.

2. Isolation and purification of compounds from A. gemini MST-FP2131.

A. gemini MST-FP2131 was cultivated on sterilised (121 °C for 40 min) jasmine rice in 90 × 250 mL Erlenmeyer flasks each containing 50 g of rice. Agar squares from a 7 day culture on Petri plates were used as inoculum for the flasks. Flasks were incubated at 24 °C for 14 days and the grains were pooled and extracted with acetone (2 \times 4 L). The combined extracts were reduced in vacuo to produce an aqueous slurry (1 L). The slurry was partitioned against EtOAc $(2 \times 4 \text{ L})$ and the combined EtOAc layer was reduced in vacuo to give a crude extract (42 g). The extract was redissolved in 90% MeOH/H₂O (500 mL) and defatted with hexane (2×500 mL) to provide an enriched extract (38 g). The enriched extract was adsorbed onto silica gel (85 g) and dry-loaded onto a silica gel column (100 g, 300×50 mm). The column was washed once with hexane, then eluted with 50% CHCl₃/hexane, 75% CHCl₃/hexane and 100% CHCl₃, followed by a stepwise gradient of 1, 2, 4, 8, 16, 32 and 100% MeOH/CHCl₃ (500 mL each step), to yield 11 fractions (Fr 1–11). Fr 10 (11.1 g) was purified by isocratic preparative HPLC (Zorbax C18, isocratic 60% MeCN/H₂O containing 0.1% TFA, 60 mL/min) to yield 3a (t_R 19.19 min; 67 mg), 4a (t_R 22.15 min; 81 mg), 2a (t_R 28.67 min; 343 mg) and 1a (t_R 46.89 min; 2.8 g). Fr 7 (430 mg) was purified by isocratic preparative HPLC (Zorbax C18, isocratic MeCN containing 0.1% TFA, 60 mL/min) to yield 1b (t_R 17.82 min; 38 mg) and an enriched fraction that was further purified by isocratic preparative HPLC (Zorbax C18, isocratic 90% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield 2b (t_R 23.10 min; 7.9 mg). A workflow of the purification process is presented in Figure S1.

3. Chemical degradation studies.

Preparation of ariestatin B (3b). A solution of **3a** (15 mg) in 5% aqueous acetone (2 mL) was treated with 37% aqueous HCl (200 μ L) and incubated at 24 °C in a sealed vial for 48 h. The reaction mixture was diluted with H₂O (200 mL) and partitioned against EtOAc (200 mL). The EtOAc was evaporated in vacuo and purified by preparative HPLC (Zorbax C18; isocratic 85% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **3b** (t_R 31.42 min; 2.4 mg, 16%).

Preparation of ariestatin C (3c). A solution of **3a** (15 mg) in MeOH (2 mL) was heated at 80 °C in a sealed vial for 8 h. The reaction mixture was purified by preparative HPLC (Zorbax C18, isocratic 90% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **3c** (t_R 9.78 min; 3.7 mg, 25%).

Preparation of ariestatin D (3d). A solution of **3a** (15 mg) in 5% aqueous acetone (2 mL) was heated at 80 °C in a sealed vial for 8 h. The reaction mixture was purified by preparative HPLC (Zorbax C18, isocratic 70% MeCN/H2O containing 0.1% TFA, 20 mL/min) to yield **3d** (t_R 8.60 min; 1.8 mg, 12%).

Preparation of ariestatin E (3e). A solution of **3b** (10 mg) in MeOH (2 mL) was heated at 80 °C in a sealed vial for 8 h. The reaction mixture was purified by preparative HPLC (Zorbax C18, isocratic 90% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **3e** (t_R 38.24 min; 5.5 mg, 55%).

Preparation of ariestatin F (3f). A solution of **3b** (10 mg) 5% aqueous acetone (2 mL) was heated at 80 °C in a sealed vial for 6 h. The reaction mixture was purified by preparative HPLC (Zorbax C18, isocratic 95% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **3f** (t_R 9.58 min; 4.2 mg, 42%).

Preparation of capricostatin B (4b). A solution of **4a** (25 mg) in 5% aqueous acetone (2 mL) was treated with 37% aqueous HCl (200 μ L) and incubated at 24 °C in a sealed vial for 72 h. The reaction mixture was diluted with H₂O (200 mL) and partitioned against EtOAc (200 mL). The EtOAc was evaporated in vacuo and purified by preparative HPLC (Zorbax C18; isocratic 95% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **4b** ($t_{\rm R}$ 10.02 min; 20 mg, 80%).

Preparation of capricostatin C (4c). A solution of **4a** (25 mg) in MeOH (2 mL) was heated at 80 °C in a sealed vial for 24 h. The reaction mixture was purified by preparative HPLC (Zorbax C18, isocratic 87.5% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **4c** (t_R 12.74 min; 9.3 mg, 37%).

Preparation of capricostatin D (4d). A solution of **4a** (25 mg) in 5% aqueous acetone (2 mL) was heated at 80 °C in a sealed vial for 24 h. The reaction mixture was purified by preparative HPLC (Zorbax C18, isocratic 70% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **4d** (*t*_R 10.05 min; 3.9 mg, 16%).

Preparation of capricostatin E (4e). A solution of **4c** (15 mg) in MeOH (2 mL) was treated with 37% aqueous HCl (200 μ L) and incubated at 24 °C in a sealed vial for 6 h. The reaction mixture was diluted with H₂O (200 mL) and partitioned against EtOAc (200 mL). The EtOAc was evaporated in vacuo and purified by preparative HPLC (Zorbax C18, isocratic 95% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **4e** (t_R 37.87 min; 5.2 mg, 35%).

Preparation of capricostatin F (4f). A solution of **4b** (15 mg) in MeOH (2 mL) was heated at 80 °C in a sealed vial for 8 h. The reaction mixture was purified by preparative HPLC (Zorbax C18, isocratic 85% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield **4f** (t_R 19.92 min; 5.2 mg, 35%).

Ozonolysis of capricostatin A. Capricostatin A (**4a**; 30 mg) was dissolved in MeOH (10 mL) and ozone was bubbled through the solution for 1 min at a rate of 10 mg/min. The reaction mixture was purified by preparative HPLC (Zorbax C18; isocratic 40% MeCN/H₂O containing 0.1% TFA, 20 mL/min) to yield ozonolysis product (t_R 6.55 min; 4.2 mg, 14%).

4. Compound Characterisation.

Ariestatin A (3a). White powder; $[\alpha]_D^{24} - 29$ (*c* 0.25, MeCN); UV (MeCN) λ_{max} (log ε) 216 (4.59), 266 (4.25), 308 (3.96) nm; IR (ATR) ν_{max} 3776, 3714, 3336, 2923, 2849, 2376, 2321, 2234, 1658, 1609, 1456, 1291, 1244, 1199, 1180, 1139, 1064, 1032, 890, 848, 780, 708, 608, 589, 539, 512 cm⁻¹; ¹H and ¹³C NMR see Table S17. HR-ESI(–)-MS *m/z* 647.3072; calcd for C₃₄H₄₇O₁₂⁻ [M – H]⁻, 647.3073.

Ariestatin B (3b). White powder; UV (MeCN) λ_{max} (log ε) 217 (4.63), 269 (4.27), 307 (4.03) nm; IR (ATR) ν_{max} 3083, 2920, 2851, 1654, 1594, 1453, 1379, 1294, 1241, 1197, 1135, 1070, 1017, 888, 829, 797, 712, 690, 606, 530 cm⁻¹; ¹H and ¹³C NMR see Table S17. HR-ESI(–)-MS *m/z* 485.2544; calcd for C₂₈H₃₇O₇⁻ [M – H]⁻, 485.2544.

Ariestatin C (3c). White powder; $[\alpha]_D^{24} - 29$ (*c* 0.20, MeCN); UV (MeCN) λ_{max} (log ε) 217 (4.44), 259 (4.09), 303 (3.66) nm; IR (ATR) v_{max} 3665, 3522, 3422, 3326, 2972, 2918, 2850, 1932, 1644, 1620, 1572, 1455, 1434, 1388, 1324, 1261, 1221, 1176, 1067, 957, 897, 870, 837, 784, 711, 634, 597, 544 cm⁻¹; ¹H and ¹³C NMR see Table S18. HR-ESI(-)-MS *m/z* 511.2911; calcd for C₂₇H₄₃O₉⁻ [M – H]⁻, 511.2912.

Ariestatin D (3d). White powder; $[\alpha]_D^{24}$ –96 (*c* 0.10, MeCN); UV (MeCN) λ_{max} (log ε) 218 (4.28), 258 (3.84), 303 (3.48) nm; IR (ATR) ν_{max} 5729, 3365, 2922, 2848, 2667, 2358, 2297, 1609, 1463, 1358, 1243, 1210, 1175, 1141, 1085, 1030, 843, 789, 709, 680, 585, 544 cm⁻¹; ¹H and ¹³C NMR see Table S18. HR-ESI(–)-MS *m/z* 497.2754; calcd for C₂₆H₄₁O₉⁻ [M – H]⁻, 497.2756.

Capricostatin A (4a). White powder; $[\alpha]_D^{24} - 16$ (*c* 0.50, MeCN); UV (MeCN) λ_{max} (log ε) 214 (4.62), 266 (4.24), 309 (3.95) nm; IR (ATR) v_{max} 3728, 3315, 2924, 2852, 2359, 2297, 1658, 1610, 1582, 1454, 1345, 1242, 1198, 1178, 1138, 1063, 1034, 927, 886, 835, 763, 695, 612, 583, 526 cm⁻¹; ¹H and ¹³C NMR see Table S20. HR-ESI(–)-MS *m/z* 673.3227; calcd for C₃₆H₄₉O₁₂⁻ [M – H]⁻, 673.3229.

Capricostatin B (4b). White powder; UV (MeCN) λ_{max} (log ε) 215 (4.59), 269 (4.25), 307 (4.00) nm; IR (ATR) ν_{max} 3728, 3070, 2923, 2851, 2614, 2559, 2359, 2299, 1878, 1646, 1609, 1452, 1342, 1308, 1238, 1194, 1166, 1136, 1070, 1020, 994, 899, 843, 796, 688, 603, 528 cm⁻¹; ¹H and ¹³C NMR see Table S21. HR-ESI(–)-MS *m/z* 511.2697; calcd for C₃₀H₃₉O₇⁻ [M – H]⁻, 511.2701.

Capricostatin C (4c). White powder; $[\alpha]_D^{24}$ –30 (*c* 0.50, MeCN); UV (MeCN) λ_{max} (log ε) 218 (4.46), 259 (4.11), 303 (3.69) nm; IR (ATR) v_{max} 3662, 3608, 3357, 2923, 2854, 156, 1650, 1617, 1577, 1435, 1391, 1324, 1299, 1257, 1208, 1175, 1138, 1075, 956, 888, 852, 802, 711, 575, 539 cm⁻¹; ¹H and ¹³C NMR see Table S22. HR-ESI(–)-MS *m/z* 537.3068; calcd for C₂₉H₄₅O₉⁻ [M – H]⁻, 537.3069.

Capricostatin D (4d). White powder; $[\alpha]_D^{24} - 31(c \ 0.20, MeCN)$; UV (MeCN) λ_{max} (log ε) 218 (4.43), 258 (4.02), 303 (3.62) nm; IR (ATR) ν_{max} 3729, 3595, 3365, 2925, 2850, 2665, 2359, 2296, 1612, 1461, 1357, 1245, 1213, 1175, 1141, 1086, 1031, 985, 948, 845, 782, 680, 583, 542 cm⁻¹; ¹H and ¹³C NMR see Table S23. HR-ESI(–)-MS *m/z* 523.2912; calcd for C₂₈H₄₃O₉⁻ [M – H]⁻, 523.2912.

Capricostatin F (4f). White powder; UV (MeCN) λ_{max} (log ε) 218 (4.39), 260 (3.99), 303 (3.56) nm; IR (ATR) v_{max} 3798, 3706, 3387, 3006, 2921, 2851, 2537, 2359, 1894, 1619, 1461, 1356, 1242, 1176, 1113, 1022, 854, 802, 754, 717, 671, 619, 538 cm⁻¹; ¹H and ¹³C NMR see Table S25. HR-ESI(–)-MS *m/z* 361.2401; calcd for C₂₂H₃₃O₄⁻⁻ [M – H]⁻, 361.2384.

5. A. nidulans metabolite profiles analysis.

Metabolite profile analyses of the *A. nidulans* strain LO8030, harbouring the constructed plasmids, as well as the control plasmids, were performed using an Agilent 1260 liquid chromatography (LC) system, coupled to a diode array detector (DAD) and an Agilent 6130 Quadrupole MS with an ESI source. Chromatographic separation was carried out at 40 °C, employing a Kinetex C18 column (2.6 μ m, 2.1 × 100 mm; Phenomenex). For small-scale metabolic profile analyses, *A. nidulans* spores were inoculated (~10⁸/L) into 50 mL of liquid GMM medium in 250 mL Erlenmeyer flasks. The cultures were incubated at 37 °C with shaking at 180 rpm for 24 h. After this period, 3 mL/L of methyl ethyl ketone was introduced to induce the expression of the *alcA* promoter. Subsequently, the cultures were incubated at 25 °C with shaking at 180 rpm for 120 h to allow for the accumulation of secondary metabolites. Experiments were conducted employing three biological replicates. The supernatant was isolated by vacuum filtration and extracted employing an organic solvent mixture containing EtOAc, MeOH, and AcOH in a ratio of 89.5:10:0.5. While mycelia were extracted using acetone. The crude extracts were dried in vacuo and redissolved in MeOH for LC-DAD-MS analysis. Chromatographic separation was achieved with a linear gradient of 5–95% MeCN-H₂O (0.1% (v/v) formic acid) over 10 min, followed by 95% MeCN for 3 min, with a flow rate of 0.6 mL/min. The MS data were collected in the *m/z* range of 100–1000.

To compare compound production between the *A. gemini* MST-FP2131 and *A. nidulans* transformants harbouring the *aqu* BGC, both fungi were cultured for 13 days in GMM supplemented with pyridoxine. The *A. gemini* MST-FP2131 cultures were maintained at 25 °C with shaking at 180 rpm throughout the experiment. In contrast, the *A. nidulans* cultures were maintained at 37 °C and induced as previously described, followed by 12 days at 25 °C with shaking at 180 rpm. Crude extract preparation and chromatographic separation was performed as described previously.

6. Nucleic acid extraction and genome assembly and annotation.

For genomic DNA extraction, *A. gemini* MST-FP2131 was cultivated in malt extract broth at room temperature for 7 days with gentle shaking. DNA was extracted from mycelia using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA). For genome sequencing, Nextera XT libraries were prepared using Illumina kits (sample prep kit, Illumina, San Diego, CA, USA) and then subjected to quality control using a Bioanalyzer 2100 (Agilent Technologies) before whole genome sequencing on the Illumina HiSeq 2000 platform with 100 bp paired-end reads. Raw sequencing reads were processed and assembled using the AAFTF pipeline.⁵ BBDuk (BBTools software package, 2014, https://sourceforge.net/projects/bbmap/) was used to trim adapter sequences as well as filter reads for common contaminants and mitochondrial DNA. Reads were then assembled using SPAdes v.3.13.0.⁶ Assembly scaffolds containing non-ascomycete sequences identified by sourmash,⁷ low-coverage scaffolds identified by BWA⁸ and SAMtools,⁹ and duplicate scaffolds identified by Minimap2,¹⁰ were removed. Gene model prediction and functional annotation was performed using the Funannotate v1.5.2 pipeline.¹¹

7. RNA extraction and cDNA synthesis.

For cDNA synthesis, the *A. nidulans* LO8030 harbouring the *aqu* BGC was cultured and induced as described earlier, but maintained for only 24 h at 25 °C. The resulting fungal mass was ground into a powder in liquid nitrogen, and RNA extraction was performed using a phenol: chloroform protocol as previously described.¹² The extracted RNA was then treated with DNase I (Sigma-Aldrich, Burlington, MA, USA). Reverse transcription and cDNA synthesis were carried out using ProtoScript® II Reverse Transcriptase (New England Biolabs, Ipswich, MA, USA) with a mix of oligo-dT and random hexamer primers.

8. Genome mining and heterologous expression of aqu cluster in A. nidulans and S. cerevisiae.

To identify potential gene cluster of interest in the genome of *A. gemini* MST-FP2131, it was initially analysed using antiSMASH v. $7.0.^{13}$ The boundaries of BGCs were defined based on cblaster analysis (Fig. S56).¹⁴ The GenBank accession number for the *aqu* BGC is available at PQ095580.

For the heterologous expression of the *aqu* BGC in *A. nidulans* LO8030, the promoters of *aquA* and *aquC* were replaced with the *aclA* promoter, while the promoter of *aquB* was replaced with the *alcS* promoter using our previously described pYFAC fungal episomal vector system.¹⁵ *AquA* was cloned into the PacI site of the plasmid pYFAC-CH2, resulting in the creation of plasmid pYFACNS00020. *AquA* and *aquB* were cloned into the PacI and NotI sites, respectively, of the plasmid pYFAC-CH2, which led to the creation of plasmid pYFACNS00021. *AquC* was cloned into the PacI site of the plasmid pYFAC-CH3, resulting in the creation of plasmid pYFAC-CH3.

For the heterologous expression of *aquA* gene and truncated versions in *S. cerevisiae* BJ5464-NpgA, intron-free fragments amplified from the generated cDNA were cloned into the SpeI and PmII sites of the plasmid pXW55. This led to the creation of plasmid pXW55::*aquA*. The vectors, constructed strains, and primer sequences used and generated in the cloning steps are listed in Table S10–Table S12.

9. S. cerevisiae BJ5464-NpgA culturing and feeding assay.

Generated *S. cerevisiae* BJ5464-NpgA transformants expressing the *aquA* gene were screened for compound production in both liquid YPD and Drop-Out (without uracil and uridine)⁴ media. For the feeding assay, a selected *S. cerevisiae* BJ5464-NpgA transformant was initially cultured in 50 mL of liquid Drop-Out medium (without uracil and uridine) in a 250 mL Erlenmeyer flask. The culture was incubated at 30 °C with shaking at 220 rpm for 48 h. Subsequently, 2 mL of the starter culture was transferred to new flasks containing 50 mL of the same liquid Drop-Out (without uracil and uridine) medium. These cultures were then incubated at 30 °C with shaking at 220 rpm for for 24 h, after which they were fed with 200 μ L of a 2.5 M solution of linoleic acid (Sigma-Aldrich, Burlington, MA, USA) in ethanol. Control cultures were fed with an ethanol solution only. The cultures were further incubated

for 96 h under the same conditions. Cells and supernatants were separated by centrifugation (5 min at $3220 \times g$). Crude extract preparation and chromatographic separation were performed as previously described. The experiment was conducted in triplicate.

10. HR-ESI-MS and MS-based molecular networking analysis.

High resolution metabolite profile analyses of the *A. nidulans* LO8030, harbouring the *aqu* BGC, as well as the control plasmids, were performed using a Vanquish UHPLC System, coupled to a diode array detector (DAD) and an Orbitrap Exploris 120 Quadrupole MS with an ESI source. Chromatographic separation was carried out at 25 °C, employing an InfinityLab Poroshell 120 SB-C18 column (2.7 μ m, 2.1 × 100 mm; Agilent). Chromatographic separation was achieved with a linear gradient of 5–95% MeCN-H₂O (0.1% (v/v) formic acid) over 13 min, followed by 95% MeCN for 3 min, with a flow rate of 0.3 mL/min. The MS data were collected in the *m/z* range of 100–1000.

MS-based molecular networking analysis was conducted using Compound Discoverer Software (Thermo Fisher Scientific, Waltham, MA, USA) to compare a control strain (*A. nidulans* LO8030 with empty plasmids) with *A. nidulans* LO8030 expressing the *aqu* BGC. The generated network was inspected for known and unknown compounds linked to the nodes of **1a**, **2a**, **3a**, and **4a**. Subsequently, the fragmentation patterns of the identified compounds were inspected using FreeStyle Software v1.5 (Thermo Fisher Scientific, Waltham, MA, USA), with potential fragments being manually assigned.

11. Phylogenetic analysis.

Phylogenetic analyses were conducted by incorporating AquA sequences together with previously described entries.^{16,17} SAT and TE domain boundaries were predicted employing the Conserved Domain Database (CDD).¹⁸ The amino acid alignments were built using Clustal Omega, without manual curation.¹⁹ The phylogenetic reconstruction was conducted employing PhyML 3.1 (Maximum Likelihood) with aLRT SH-like (approximate likelihood ratio test Shimodaira–Hasegawa) branch support estimation, and the best-fit evolutionary model predicted by the Smart Model Selection (SMS), implemented in the PhyML environment.^{20,21}

12. Bioactivity screening.

Purified metabolites were dissolved in DMSO to create stock solutions of 10,000 μ g/mL. An aliquot from each stock solution was transferred to the first well of Rows B to G on a 96-well microtitre plate and then serially diluted two-fold with DMSO across the 12 wells of the plate to achieve a 2,048-fold concentration gradient. Bioassay medium was added to an aliquot of each diluted solution to achieve a 100-fold dilution in the final bioassay. This resulted in a testing range from 100 to 0.05 μ g/mL in 1% DMSO for most of our test organisms, and from 200 to 0.1 μ g/mL in 2% DMSO for yeasts. Row A served as a control with no test compound (indicating no inhibition), and Row H was left uninoculated (serving as a control for complete inhibition).

B. subtilis (ATCC 6633), *S. aureus* (ATCC 25923) and MRSA (ATCC 33592) were used as indicator species for antibacterial activity, employing the ProTOX bioassay platform. A bacterial suspension (50 mL in a 250 mL flask) was prepared in nutrient media by cultivating it for 24 h at 250 rpm and 28 °C. The suspension was diluted to an absorbance of 0.01 absorbance units per mL, and 10 μ L aliquots were added to the wells of a 96-well microtitre plate containing the test compounds dispersed in 190 μ L of nutrient broth (Amyl) with 10 μ L resazurin (12.5 μ g/mL). The plates were incubated at 28 °C for 48 h, during which the positive control wells changed colour from blue to light pink. Tetracycline was employed as a control for *B. subtilis*, while ampicillin was used for *S. aureus* and MRSA. MIC endpoints were determined visually. Absorbance was measured using a Multiskan Skyhigh Microplate Spectrophotometer (Thermo Fisher Scientific) at 605 nm.

Saccharomyces cerevisiae (ATCC 9763) was used as indicator species for antifungal activity, employing the EuTOX bioassay platform. A yeast suspension (50 mL in a 250 mL flask) was prepared in 1% malt extract broth by cultivating it for 24 h at 250 rpm and 24 °C. The suspension was diluted to absorbances of 0.03 absorbance units per mL and 30 μ L were added to the wells of a 96-well microtitre plate containing the test compounds dispersed in 100 μ L of malt extract agar with bromocresol green (50 μ g/mL). The plates were incubated at 28 °C for 48 h, during which the positive control wells changed colour from blue to yellow. Blasticidin S HCl was used as a control. MIC endpoints were determined visually. Absorbance was measured using a Multiskan Skyhigh Microplate Spectrophotometer (Thermo Fisher Scientific) at 605 nm.

NS-1 (ATCC TIB-18) mouse myeloma and NFF (ATCC PCS-201) human neonatal foreskin fibroblast cells were each inoculated in 96-well microtitre plates (190 μ L) at 50,000 cells/mL in DMEM (Dulbecco's Modified Eagle Medium + 10% fetal bovine serum (FBS) + 1% penicillin/streptomycin (10,000 U/mL / 10,000 μ g/mL, Life Technologies Cat. No. 15140122), together with resazurin (250 μ g/mL; 10 μ L) and incubated in 37 °C (5% CO₂) incubator. The plates were incubated for 96 h during which time the positive control wells change colour from a blue to pink colour. The absorbance of each well was measured using Multiskan Skyhigh Microplate Spectrophotometer (Thermofisher Scientific) at 605 nm. Sparsomycin was used as a control. The IC₅₀ values were determined using a sigmoidal dose-response model with variable slope in GraphPad Prism 8.

Supplementary Tables

Table S1. Presence or absence of homomeric depside aglycone molecules in extracts derived from *A. nidulans*, *S. cerevisiae* and *A. gemini*. The presence of compounds was examined using LC-MS and HR-ESI-MS analysis.



R	A. nidulans	S. cerevisiae	A. gemini
CH ₃	-	-	-
$C_{15}H_{31}$	-	-	-
$C_{17}H_{31}$	-	-	-

Table S2. Presence or absence of glycosylated homomeric depside molecules in extracts derived from *A. nidulans* and *A. gemini*. The presence of compounds was examined using LC-MS and HR-ESI-MS analysis.



R	A. nidulans	A. gemini
CH ₃	-	-
$C_{15}H_{31}$	-	-
C ₁₇ H ₃₁	-	-

Table S3. Presence or absence of potential glycosylated heteromeric depside molecules in extracts derived from *A. nidulans* expressing all genes from the *aqu* BGC. The presence of compounds was examined using LC-MS and HR-ESI-MS analysis.



R	Building block	Identifier	Present /Absent
C_2H_5	(C3:0)		-
C_3H_7	(C4:0)		-
C ₄ H ₉	(C5:0)		-
C ₅ H ₁₁	(C6:0)		-
$C_{6}H_{13}$	(C7:0)		-
C7H15	(C8:0)		-
C9H19	(C10:0)		-
$C_{11}H_{23}$	(C12:0)		-
$C_{12}H_{25}$	(C13:0)		-
$C_{13}H_{27}$	(C14:0)	ariestatin A (3a)	Present
$C_{15}H_{31}$	(C16:0)	aquastatin A (1a)	Present
$C_{15}H_{29}$	(C16:1)	capricostatin A (4a)	Present
$C_{17}H_{35}$	(C18:0)		-
$C_{17}H_{31}$	(C18:2)	geministatin A (2a)	Present
$C_{18}H_{37}$	(C19:0)		-
$C_{19}H_{39}$	(C20:0)		_
C ₁₉ H ₃₁	(C20:4)		-

Table S4. Presence or absence of potential heteromeric depside aglycone molecules in extracts derived from *A. nidulans* expressing all genes from the *aqu* BGC. The presence of compounds was examined using LC-MS and HR-ESI-MS analysis.



R	Building block	Identifier	Present /Absent
C ₂ H ₅	(C3:0)		-
C_3H_7	(C4:0)		-
C_4H_9	(C5:0)		-
$C_{5}H_{11}$	(C6:0)		-
$C_{6}H_{13}$	(C7:0)		-
$C_{7}H_{15}$	(C8:0)		-
$C_{9}H_{19}$	(C10:0)		-
$C_{11}H_{23}$	(C12:0)		-
$C_{12}H_{25}$	(C13:0)		-
$C_{13}H_{27}$	(C14:0)	ariestatin B (3b)	Present
$C_{15}H_{31}$	(C16:0)	aquastatin B (1b)	Present
$C_{15}H_{29}$	(C16:1)	capricostatin B (4b)	Present
$C_{17}H_{35}$	(C18:0)		-
$C_{17}H_{31}$	(C18:2)	geministatin B (2b)	Present
$C_{18}H_{37}$	(C19:0)		-
$C_{19}H_{39}$	(C20:0)		-
$C_{19}H_{31}$	(C20:4)		_

Table S5. Presence or absence of *O*-methylated and glycosylated alkylresorcylic acid molecules in extracts derived from *A*. *nidulans* expressing all genes from the *aqu* BGC.



R	Building block	Number	Present /Absent
C ₁₃ H ₂₇	(C14:0)	ariestatin C (3c)	Present
$C_{15}H_{31}$	(C16:0)	aquastatin C (1c)	-
$C_{15}H_{29}$	(C16:1)	capricostatin C (4c)	-
C ₁₇ H ₃₁	(C18:2)		-

Table S6. Presence or absence of glycosylated alkylresorcylic acid molecules in extracts derived from *A. nidulans* expressing all genes from the *aqu* BGC.



R	Building block	Number	Presence/Absence
$C_{13}H_{27}$	(C14:0)	ariestatin C (3d)	Present
$C_{15}H_{31}$	(C16:0)	aquastatin D (1d)	Present
$C_{15}H_{29}$	(C16:1)	capricostatin D (4d)	Present
$C_{17}H_{31}$	(C18:2)		Present

Table S7. Presence or absence of *O*-methylated alkylresorcylic acid molecules in extracts derived from *A. nidulans* expressing all genes from the *aqu* BGC.



R	Building block	Number	Present / Absent
$C_{13}H_{27}$	(C14:0)	ariestatin E (3e)	Present
$C_{15}H_{31}$	(C16:0)	aquastatin E (1e)	-
$C_{15}H_{29}$	(C16:1)	capricostatin E (4e)	Present
$C_{17}H_{31}$	(C18:2)		Present

Table S8. Presence or absence of alkylresorcylic acid molecules in extracts derived from *A. nidulans* expressing all genes from the *aqu* BGC.



R	Building block	Identifier	Presence/Absence
$C_{13}H_{27}$	(C14:0)	ariestatin F (3f)	-
$C_{15}H_{31}$	(C16:0)	corticiolic acid (1f)	Present
$C_{15}H_{29}$	(C16:1)	capricostatin F (4f)	Present
$C_{17}H_{31}$	(C18:2)	_	Present

Table S9. Presence or absence of alkylresorcylic acid molecules and orsellinic acid in extracts derived from *S. cerevisiae* expressing *aquA*.



R	Building block	Identifier	Presence/Absence
CH ₃	(C2:0)	Orsellinic acid (5)	Present
$C_{13}H_{27}$	(C14:0)	ariestatin F (3f)	-
$C_{15}H_{31}$	(C16:0)	corticiolic acid (1f)	Present
$C_{15}H_{29}$	(C16:1)	capricostatin F (4f)	Present
$C_{17}H_{31}$	(C18:2)		-

Vector name	Backbone plasmid	Fungal marker	Purpose/Genes
pYFAC-CH2		pyrG	Heterologous expression A. nidulans
pYFAC-CH3		ribo	Heterologous expression A. nidulans
pXW55		URA3	Heterologous expression S. cerevisiae
pYFACNS00020	pYFAC-CH2	pyrG	aquA
pYFACNS00021	pYFAC-CH2	pyrG	aquAB
pYFACNS00022	pYFAC-CH3	ribo	aquC
pXW55::aquA	pXW55	URA3	aquA
pXW55:: <i>aquA-∆SAT</i>	pXW55	URA3	$aquA-\Delta SAT$
pXW55:: <i>aquA-∆TE</i>	pXW55	URA3	$aquA-\Delta TE$

Table S10. Vectors used in this study.

Table S11. Strains built in this study for heterologous expression.

Strain number	Strain name
1	A. nidulans aquABC
2	A. nidulans aquAB
3	A. nidulans aquAC
4	A. nidulans aquA
5	S. cerevisiae aquA
6	S. cerevisiae aquA-∆SAT
7	S. cerevisiae $aquA-\Delta TE$

Table S12.	Oligonucleotides used in this	study.
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Oligonucleotide	Sequence (5' to 3')	Purpose
FP2131_AquA_fg1CH2_PacI_F	TTAATTAGAACTCTTCCAATCCTATCACCTCGC CTTAATGGCCTCTCCACCCCG	Heterologous expression of aquA in A. nidulans
FP2131_AquA_fg1CH2_R	GAGGTACTCTGCGCTGAAGG	Heterologous expression of aquA in A. nidulans and S. cerevisiae
FP2131_AquA_fg2CH2_F	TCGTCCTCAGCGGTAAAGTC	Heterologous expression of aquA in A. nidulans and S. cerevisiae
FP2131_AquA_fg2CH2_PacI_R	CGCGCTCCACGGGGACTCGCTTCAATTTGTTCC GCTTAATCTAGATCGTGAACTTCTCC	Heterologous expression of aquA in A. nidulans
FP2131_AquB_CH2_NotI_F	TGAGATACCAAAGCATTGAGCCCAGAAACAGCA GAAGCATGAGCGCAACGACGACTC	Heterologous expression of aquB in A. nidulans
FP2131_AquB_CH2_NotI_R	AGTCTAAAGGTCTACAATCAATTCAGGCCGTAT TCAGGGCCTAGAATGCAGCTGGCCTC	Heterologous expression of aquB in A. nidulans
FP2131_AquC_CH3_PacI_F	TTAATTAGAACTCTTCCAATCCTATCACCTCGC CTTAATGACTCTCCCGTCGATTC	Heterologous expression of <i>aquC</i> in <i>A. nidulans</i>
FP2131_AquC_CH3_PacI_R	CGCGCTCCACGGGGACTCGCTTCAATTTGTTCC GCTTAATCTAAAAAAAGCTTCGCGC	Heterologous expression of aquC in A. nidulans
FP2131_AquA_pXW55_SpeI_F	CTAGCGATTATAAGGATGATGATGATAAGACTA GTATGGCCTCTCCACCCCG	Heterologous expression in S. cerevisiae
FP2131_AquA_pXW55_PmlI_R	ATTTAAATTAGTGATGGTGATGGTGATGCACGT GGATCGTGAACTTCTCCAGCG	Heterologous expression in S. cerevisiae
FP2131_KS-AT-PT-	CTAGCGATTATAAGGATGATGATGATAAGACTA	Domain deletion in S. cerevisiae
ACP_pXW55_Spel_F	GTATCGCTGTTGTGGGGCATGGCAGGTC	
FP2131_KS-AT-PT-	ATTTAAATTAGTGATGGTGATGGTGATGCACGT	Domain deletion in S cereviside
ACP pXW55 PmlI R	GGCTCGCGGCGTTGGAGCTGCGCTTGG	

Compound	Mini	mum inhibitory co	IC ₅₀ (μM)			
Compound	B. subtilis	S. aureus	MRSA	S. cerevisiae	NS-1	NFF
aquastatin C (1c)	>100	>100	>100	>200	>100	>100
aquastatin E (1e)	>100	>100	>100	>200	>100	>100
corticiolic acid (1f)	0.8	3.1	3.1	>200	25	>100
capricostatin C (4c)	>100	>100	>100	>200	6.3	6.3
orsellinic acid (5)	>100	>100	>100	>200	>100	>100
adipostatin (6)	>100	>100	25	>200	6.3	6.3
Controls	6.3	3.1	>100	3.1	1.7	1.7

Table S13. Additional	bioassay	results	for	compounds	investigated	in	this	study.
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Controls: *B. subtilis* ATCC 6633 = tetracycline; *S. aureus* ATCC 25923 and MRSA ATCC 33592 = ampicillin; *S. cerevisiae* ATCC 9763 = blasticidin S HCl; NS-1 ATCC TIB-18 and NFF TCC PCS-201) = sparsomycin.



Table S14. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for 1a and 1b in DMSO- d_6

Dec	Aquastatin A (1a)		Aquastatin B (1b)		
POS.	δc, type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	δc, type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	
1	166.2, C		166.8, C		
2	113.0, C		109.3, C		
3	157.2, C		158.7, C		
3-OH		10.20, s		10.12, s	
4	101.4, CH	6.46, d (2.2)	100.5, CH	6.22, d (2.2)	
5	159.6, C		160.5, C		
5-OH				9.84, s	
6	108.4, CH	6.43, d (2.2)	108.4, CH	6.17, d (2.2)	
7	143.1, C		144.1, C		
8	33.5, CH ₂	2.60, m	33.8, CH ₂	2.59, m	
9	30.8, CH ₂	1.54, m	31.0, CH ₂	1.51, m	
10-19	28.7-29.9 ^a , CH ₂	1.18–1.30 ^b , m	28.7–29.9 ^a , CH ₂	1.20–1.27 ^b , m	
20	31.3, CH ₂	1.21 ^b , m	31.3, CH ₂	1.21 ^b , m	
21	22.1, CH ₂	1.24 ^b , m	22.1, CH ₂	1.24 ^b , m	
22	13.9, CH ₃	0.84, t (6.9)	13.9, CH ₂	0.83, t (7.0)	
1'	170.6, C		170.7, C		
1'-OH		13.33, br s		13.33, br s	
2'	116.6, C		116.3, C		
3'	158.9, C		159.2, C		
3'-OH		11.18, br s		11.12, br s	
4'	107.2, CH	6.58, d (2.1)	107.2, CH	6.56, d (2.2)	
5'	152.3, C		152.4, C		
6'	114.4, CH	6.52, br d (2.1)	114.5, CH	6.51, dd (2.2, 0.6)	
7'	139.6, C		139.6, C		
8'	21.0, CH ₃	2.36, s	21.1, CH ₃	2.36, br s	
1"	100.5, CH	4.81°, d (7.7)			
2"	70.2, CH	3.54°, m			
5° 4″	73.2, CH	3.41, dd (9.7, 3.2)			
4	07.9, СП 75.4. СН	3.71, br u(3.2)			
5	75.4, CH	3.55, III 3.56^{d} m			
0	00.0, 0112	3.48 m			
2″-ОН		5.15° br s			
3″-OH		$4.81^{c,e}$ br s			
4″-OH		4.49°, br s			
6″-OH		4.62°, br s			

^a assignments interchangeable, ^{b-d} overlapping resonances, ^e assignments based on those for **4c** (Table S22).



Table S15. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for 1c and 1d in DMSO- d_6

Dec	Aquastatin C (1c)	Aquastatin D (1d)
r us.	δc, type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	δc, type	δ _H , mult (J in Hz)
1	169.0, C		171.9, C	
1-OCH ₃	51.6	3.74, s		
1-OH				13.38, br s
2	113.5, C		109.3, C	
3	157.9, C		161.6, C	
3-OH		9.78, br s		11.45, br s
4	101.4, CH	6.37, d (2.2)	101.3, CH	6.39, d (2.2)
5	159.3, C		160.3, C	
6	108.0, CH	6.32, d (2.2)	109.8, CH	6.36, d (2.2)
7	143.0, C		145.8, C	
8	33.7, CH ₂	2.47, m	34.9, CH ₂	2.27, m
9	30.7, CH ₂	1.44, m	31.2, CH ₂	1.48, m
10–19	28.7–29.0ª, CH ₂	1.21–1.26 ^b , m	28.7–29.1ª, CH ₂	1.15–1.29 ^b , m
20	31.3, CH ₂	1.21 ^b , m	31.3, CH ₂	1.21 ^b , m
21	22.1, CH ₂	1.24 ^b , m	22.1, CH ₂	1.23 ^b , m
22	13.9, CH ₃	0.84, t (7.0)	13.9, CH ₂	0.84, t (6.9)
1″	100.5, CH	4.76°, d (7.5)	100.2, CH	4.83, d (7.7)
2″	70.2, CH	3.52 ^d , m	70.1, CH	3.52 ^d , m
3″	73.2, CH	3.39, dd (9.5, 3.3)	73.2, CH	3.39, dd (9.5, 3.4)
4″	67.9, CH	3.69, br d (3.3)	68.0, CH	3.69, br d (3.2)
5″	75.4, CH	3.53 ^d , m	75.5, CH	3.53 ^d , m
6″	60.0, CH ₂	3.54 ^d , m	60.2, CH ₂	3.57 ^d , m
		3.46, m		3.45, m
2″-ОН		5.15°, br s		5.12 ^e , br s
3″-ОН		4.81 ^{c,e} , br s		4.83 ^{c,e} , br s
4″-OH		4.49°, br s		4.47 ^e , br s
6"-OH		4.62°, br s		4.61°, br s

^a assignments interchangeable, ^{b-d} overlapping resonances, ^e assignments based on those for **4c** (Table S22).



Table S16. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for 1e and 1f in DMSO- d_6

Dos	Aquastatin E (1e)	quastatin E (1e)		lf)
1 05.	δC, type $ δH, mult (J in Hz)$		δc, type	δ _H , mult (<i>J</i> in Hz)
1	169.6, C		172.7, C	
1-OCH ₃	51.6	3.74, s		
1-OH				13.31, s
2	108.8, C		105.2, C	
3	159.3, C		163.5, C	
3-OH		10.20, br s		11.78, s
4	100.4, CH	6.13, d (2.2)	100.5, CH	6.14, d (2.4)
5	160.7, C		161.5, C	
5-OH		10.20, br s		9.99, s
6	108.8, CH	6.10, d (2.2)	109.9, CH	6.11, d (2.4)
7	144.4, C		147.1 C	
8	34.2, CH ₂	2.51, m	35.3, CH ₂	2.72, m
9	30.9, CH ₂	1.42, m	31.3, CH ₂	1.46, m
10–19	$28.7-29.0^{a}, CH_{2}$	1.20–1.27 ^b , m	28.7–29.1ª, CH ₂	1.21–1.27 ^b , m
20	31.3, CH ₂	1.22 ^b , m	31.2, CH ₂	1.22, m
21	22.1, CH ₂	1.24 ^b , m	22.1, CH ₂	1.24, m
22	13.9, CH ₃	0.84, t (6.9)	13.9, CH ₂	0.84, t (6.9)

^a assignments interchangeable, ^b overlapping resonances



Table S17. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for 3a and 3b in DMSO- d_6

Dec	Ariestatin A (3a)		Ariestatin B (3b)		
r us.	δ _C , type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	δ _C , type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	
1	166.2, C		166.8, C		
2	113.0, C		109.3, C		
3	157.2, C		158.7, C		
3-OH		10.20, s		10.11, s	
4	101.4, CH	6.46, d (2.2)	100.4, CH	6.22, d (2.2)	
5	159.6, C		160.5, C		
5-OH				9.84, s	
6	108.4, CH	6.43, d (2.2)	108.4, CH	6.17, d (2.2)	
7	143.2, C		144.1, C		
8	33.5, CH ₂	2.60, m	33.8, CH ₂	2.59, m	
9	30.8, CH ₂	1.54, m	31.0, CH ₂	1.51, m	
10-17	28.7–29.0ª, CH ₂	1.18–1.30 ^b , m	28.7–29.0 ^a , CH ₂	1.19–1.27 ^b , m	
18	31.3, CH ₂	1.21, m	31.2, CH ₂	1.20, m	
19	22.1, CH ₂	1.24, m	22.0, CH ₂	1.23, m	
20	13.9, CH ₃	0.83, t (6.9)	13.9, CH ₂	0.83, t (7.2)	
1'	170.6, C		170.7, C		
1'-OH		13.33, br s		13.44, br s	
2'	116.6, C		116.3, C		
3'	158.9, C		159.2, C		
3′-OH		11.27, br s		11.06, br s	
4'	107.2, CH	6.58, d (1.9)	107.2, CH	6.57, d (2.2)	
5'	152.3, C		152.4, C		
6'	114.4, CH	6.52, br d (1.9)	114.6, CH	6.52, dd (2.2, 0.6)	
7'	139.6, C		139.6, C		
8'	21.0, CH ₃	2.36, s	21.1, CH ₃	2.36, br s	
1″	100.5, CH	4.81°, d (7.7)			
2″	70.2, CH	3.54 ^d , m			
3″	73.2, CH	3.41, dd (9.7, 3.2)			
4″	67.9, CH	3.71, br d (3.2)			
5″	75.4, CH	3.55 ^d , m			
6″	60.0, CH ₂	3.56 ^d , m			
		3.48, m			
2″-ОН		5.15 ^e , br s			
3″-OH		4.81 ^{c,e} , br s			
4″-OH		4.50°, br s			
6"-OH		4.63°, br s			

^a assignments interchangeable, ^{b-d} overlapping resonances, ^e assignments based on those for **4c** (Table S22).



Table S18. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for 3c and 3d in DMSO- d_6

Doc	Ariestatin C (3c)		Ariestatin D (3d)	
ros.	δc, type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	δc, type	δ _H , mult (<i>J</i> in Hz)
1	168.8, C		172.0, C	
1-OCH ₃	51.7, CH ₃	3.75, s		
				13.43, br s
2	113.2, C		109.6, C	
3	157.4, C		160.9, C	
3-OH		10.11, s		11.47, br s
4	101.2, CH	6.39, d (2.3)	101,1, CH	6.35, br s
5	159.3, C		160.13, C	
5-OH				
6	108.5, CH	6.36, d (2.3)	109.8, CH	6.31, br s
7	143.2, C		146.0, C	
8	33.7, CH ₂	2.49, m	34.9, CH ₂	2.75, m
9	30.7, CH ₂	1.44, m	31.3, CH ₂	1.47, m
10-17	28.7–29.0 ^a , CH ₂	1.22–1.24 ^b , m	$28.7-29.2^{a}, CH_{2}$	1.20–1.29 ^b , m
18	31.3, CH ₂	1.22 ^b , m	31.3, CH ₂	1.22 ^b , m
19	22.1, CH ₂	1.24 ^b , m	22.1, CH ₂	1.24 ^b , m
20	13.9, CH ₃	0.84, t (6.8)	14.0, CH ₂	0.84, t (7.0)
1″	100.5, CH	4.77, d (7.7)	100.3, CH	4.82°, d (7.7)
2″	70.2, CH	3.51°, m	70.1, CH	3.52 ^d , m
3″	73.2, CH	3.39, ddd (9.5, 5.8, 3.3)	73.2, CH	3.39, m
4″	67.9, CH	3.69, br dd (4.7, 3.3)	68.0, CH	3.69, m
5″	75.4, CH	3.53°, m	75.4, CH	3.56 ^d , m
6″	60.1, CH ₂	3.54°, m	60.2, CH ₂	3.53 ^d , m
		3.46, m		3.45, m
2″-ОН		5.12, d (5.2)		5.11, d (5.3)
3″-ОН		4.82, d (5.8)		4.82, m
4″-OH		4.46, d (4.7)		4.47, d (4.5)
6″-OH		4.61, m		4.61, dd (5.5, 5.1)

^a assignments interchangeable, ^{b-c} overlapping resonances



Table S19. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for 3e and 3f in DMSO- d_6

Doc	Ariestatin E (3e)		Ariestatin F (3f)	
1 05.	δc, type	δ _H , mult (J in Hz)	δc, type	δ _H , mult (J in Hz)
1	169.5, C		172.7, C	
1-OCH ₃	51.6	3.74, s		
1-OH				13.31, s
2	109.1, C		105.2, C	
3	159.1, C		163.5, C	
3-OH		10.24, br s		11.78, s
4	100.4, CH	6.14, d (2.3)	100.5, CH	6.14, d (2.4)
5	160.2, C		161.5, C	
5-OH		9.79, br s		9.99, s
6	108.6, CH	6.11, d (2.3)	109.9, CH	6.11, d (2.4)
7	144.3, C		147.1 C	
8	34.1, CH ₂	2.51, m	35.3, CH ₂	2.72, m
9	30.9, CH ₂	1.42, m	31.3, CH ₂	1.46, m
10-17	28.7–29.6 ^a , CH ₂	1.20–1.26 ^b , m	28.7–29.1ª, CH ₂	1.21–1.27 ^b , m
18	31.2, CH ₂	1.22, m	31.3, CH ₂	1.22 ^b , m
19	$22.0, CH_2$	1.24, m	22.1, CH ₂	1.24 ^b , m
20	13.9, CH ₃	0.84, t (6.8)	13.9, CH ₂	0.84, t (6.9)

^a assignments interchangeable, ^b overlapping resonances



Table S20. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for capricostatin A (4a) in DMSO- d_6

Pos.	δ _C , type	δ _H , mult (<i>J</i> in Hz)	НМВС	COSY	ROESY
1	166.2, C				
2	113.0, C				
3	157.2, C				
3-OH		10.20, s	2, 3, 4		4
4	101.4, CH	6.46, d (2.2)	2, 3, 5, 6	6	3-OH, 1″
5	159.6, C				
6	108.4, CH	6.43, d (2.2)	2, 4, 6, 8	4	8,1"
7	143.1, C				
8	33.5, CH ₂	2.60, m	2, 6, 7, 9, 10		6
9	30.8, CH ₂	1.54, m	7, 8, 10, 11		
10	28.7, CH ₂	1.23–1.29°, m			
11	28.9ª, CH ₂	1.23–1.29°, m			
12	28.5 ^b , CH ₂	1.23–1.29°, m			
13	29.1ª, CH ₂	1.23–1.29°, m	15	14	15
14	26.5, CH ₂	1.94 ^d , m	12, 15, 16	13, 15	
15	129.6, CH	5.29 ^e , m	13, 14, 16, 17	14	13
16	129.6, CH	5.29 ^e , m	14, 15, 17, 18	17	18
17	26.5, CH ₂	1.94 ^d , m	15, 16, 18	16, 18	
18	29.0ª, CH ₂	1.23–1.29°, m	16	17	16
19	28.2 ^b , CH ₂	1.22°, m			
20	31.0, CH ₂	1.20°, m			
21	$22.0, CH_2$	1.23°, m		22	
22	13.9, CH ₃	0.82, t (7.0)	20, 21	21	
1'	170.6, C				
1'-OH		11.42, br s			
2'	116.7, C				
3'	159.1, C				
3'-OH		13.13, br s			
4′	107.2, CH	6.57, d (2.2)	2', 3', 5', 6'	6'	
5'	152.3, C				
6'	114.4, CH	6.51, br d (2.2)	2', 4', 5', 8'	4'	8'
7'	139.6, C				
8'	21.0, CH ₃	2.36, s	2', 6', 7'		6'
1″	100.5, CH	4.80, d (7.8)	5, 2", 3", 5"	2″	4, 6, 3", 5"
2″	70.2, CH	3.54 ¹ , m	1", 3"	1", 3"	3″
3″	73.2, CH	3.41, dd (9.5, 3.3)	2", 5"	2", 4"	1", 2", 4"
4″	67.9, CH	3.71, br d (3.3)	2", 3"	3″	3", 5"
5″	75.4, CH	3.55 ^r , m	4", 6"		1", 4"
6″	$60.0, CH_2$	3.55 ¹ , m	4", 5"		
A # 633		3.48, m	4", 5"		
2"-OH		5.15^{g} br s			
3"-OH		4.84 ^g br s			
4"-OH		4.49 ^g br s			
6"-OH		4.62 ^g br s			

^{a-b} assignments interchangeable, ^{c-f} overlapping resonances, ^g assignments based on those for **4c** (Table S22).



Table S21. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for capricostatin B (4b) in DMSO- d_6

Pos.	δc, type	δ _H , mult (<i>J</i> in Hz)	HMBC	COSY	ROESY
1	166.7, C				
2	109.3, C				
3	158.7, C				
3-OH		10.11, s	2, 3, 4		4
4	100.4, CH	6.22, d (2.2)	2, 3, 5,6	6	3-OH, 5-OH
5	160.5, C				
5-OH		9.84, s	4, 5, 6		4, 6
6	108.4, CH	6.17, d (2.2)	2, 4, 5, 8	4	8, 5- OH
7	144.1, C				
8	33.8, CH ₂	2.59, m	2, 6, 7, 9, 10	9	6, 9
9	31.0, CH ₂	1.51, m	8, 10, 11	8, 10	8
10	28.6 ^a , CH ₂	1.18–1.29°, m		9	
11	28.9 ^b , CH ₂	1.18–1.29°, m			
12	28.5ª, CH ₂	1.18–1.29°, m			
13	29.0 ^b , CH ₂	1.18–1.29°, m	15	14	
14	$26.5, CH_2$	1.94, m	12, 13, 15, 16	13, 15	15
15	129.6, CH	5.29, m	13, 14, 17	14, 16	14
16	129.6, CH	5.29, m	14, 17, 18	15, 17	17
17	$26.5, CH_2$	1.94, m	15, 16, 18, 19	16, 18	16
18	29.0 ^b , CH ₂	1.18–1.29°, m	16	17	
19	28.2, CH ₂	1.18–1.29°, m			
20	31.1, CH ₂	1.21°, m	22		
21	22.0, CH ₂	1.23°, m		22	
22	13.9, CH ₃	0.82, t (7.0)	20, 21	21	
1′	170.6, C				
1'-OH		11.05, br s			
2'	116.4, C				
3'	159.0, C				
3'-OH		13.44, br s			
4′	107.2, CH	6.57, d (2.0)	2', 3', 5', 6'	6'	
5'	152.4, C		· · · ·		
6'	114.5, CH	6.52, d (2.0)	2', 4', 5', 8'	4′	8'
7'	139.5, C				
8'	21.0, CH ₃	2.36, s	2', 6', 7'		6'

^{a-b} assignments interchangeable, ^c overlapping resonances



Pos.	δc, type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	HMBC	COSY	ROESY
1	168.8, C				
1-OCH ₃	51.7, CH ₃	3.75, s	1		
2	113.2, C				
3	157.4, C				
3-OH		10.11, s	2, 3, 4		4
4	101.2, CH	6.39, d (2.3)	2, 3, 5, 6	6	1″, 3-ОН
5	159.3, C				
6	108.5, CH	6.36, d (2.3)	2, 4, 5, 8	4	1″
7	143.2, C				
8	33.7, CH ₂	2.49, m	2, 6, 7, 9, 10	9	10
9	30.7, CH ₂	1.44, m	11	8, 10	
10	$28.6^{a}, CH_{2}$	1.22–1.29°, m		9	8
11	28.8 ^b , CH ₂	1.22–1.29°, m			
12	$28.5^{a}, CH_{2}$	1.22–1.29°, m			
13	$28.6^{a}, CH_{2}$	1.22–1.29°, m		14	
14	29.0 ^b , CH ₂	1.97, m	12, 13, 15, 16	13, 15	
15	129.6, CH	5.31, m	14, 17	14, 16	
16	129.6, CH	5.31, m	14, 17	15, 17	
17	26.6, CH ₂	1.97, m	15, 16, 18	16, 18	
18	29.0 ^b , CH ₂	1.22-1.29°, m		17	
19	28.2ª, CH ₂	1.22-1.29°, m			
20	31.1, CH ₂	1.23°, m			
21	22.0, CH ₂	1.24 ^c , m	20	22	
22	13.9, CH ₃	0.84, t (6.8)	20, 21	21	
1″	100.5, CH	4.77, d (7.7)	5, 2", 5"	2"	4, 6, 5"
2''	70.2, CH	3.51, m	2", 3"	1", 3", 2"-OH	
3″	73.2, CH	3.39, m	2"	2", 4", 3"-OH	
4''	67.9, CH	3.69, m	2", 3"	3″, 4″-ОН	
5''	75.4, CH	3.53 ^d , m	6″		1″
6″a	60.1, CH ₂	3.54 ^d , m	4", 5"	6"b, 6"-OH	
6‴b		3.46, m	4", 5"	6‴a, 6″-OH	
2′′-ОН		5.11, d (5.1)	1", 2", 3"	2"	
3′′-ОН		4.82, d (5.7)	2", 3", 4"	3″	
4''-OH		4.46, d (4.6)	4", 5"	4″	
6''-OH		4.61, m	5", 6"	6″a, 6″b	

Table S22. 1 H (600 MHz) and 13 C (150 MHz) NMR data for capricostatin C (4c) in DMSO- d_{6}

^{a-b} assignments interchangeable, ^{c-d} overlapping resonances



Pos.	δ _C , type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	НМВС	COSY	ROESY
1	171.9, C				
1-OH		11.48, br s			
2	109.3, C				
3	161.7, C				
3-OH		13.40, br s			
4	101.1, CH	6.38, d (2.2)	2, 3, 5, 6	6	1″
5	160.3, C				
6	109.8, CH	6.35, d (2.2)	4, 5, 8, 9	4	8,1"
7	145.8, C				
8	34.9, CH ₂	2.73, m	2, 6, 7, 9		6
9	31.2, CH ₂	1.48, m	7,8		
10	28.7ª, CH ₂	1.20–1.31°, m			
11	29.0 ^b , CH ₂	1.20–1.31°, m			
12	$28.6^{a}, CH_{2}$	1.20–1.31°, m			
13	29.1 ^b , CH ₂	1.20–1.31°, m	15		
14	26.6, CH ₂	1.97, m	12, 13, 15, 16	13, 15	
15	129.6, CH	5.31, m	13, 14, 17	14, 16	
16	129.6, CH	5.31, m	14, 17, 18	15, 17	
17	26.6, CH ₂	1.97, m	15, 16, 18, 19	16, 18	
18	29.1 ^b , CH ₂	1.20–1.31°, m	16		
19	28.2, CH ₂	1.20–1.31°, m			
20	31.1, CH ₂	1.23°, m	22		
21	22.0, CH ₂	1.24°, m	20, 22		
22	13.9, CH ₃	0.84, t (7.1)	20, 21		
1″	100.2, CH	4.83 ^d , d (7.7)	5, 2", 3", 5"	2"	4, 6, 3", 5"
2''	70.1, CH	3.53°, m	1″	1", 2"-OH	
3″	73.2, CH	3.39, br dd (9.8, 2.3)		3″-ОН	1″
4''	68.0, CH	3.69, br s	2", 3"	3″-ОН	5''
5''	75.5, CH	3.56 ^e , m	4", 6"		1", 4"
6‴a	$60.2, CH_2$	3.53 ^e , m	5"	6''-OH	
6‴b		3.46, m	5"	6''-OH	
2′′-ОН		5.11, br d (3.8)		2"	
3''-OH		4.83 ^{d,f} , br s		3″	
4''-OH		4.47 ^f , br m		4″	
6''-OH		4.61 ^f , br m		6″a, 6″b	

Table S23. 1 H (600 MHz) and 13 C (150 MHz) NMR data for capricostatin D (4d) in DMSO- d_{6}

^{a-b} assignments interchangeable, ^{c-e} overlapping resonances, ^f assignments based on those for **4c** (Table S22).



Pos.	δc, type	$\delta_{\rm H}$, mult (<i>J</i> in Hz)	HMBC	COSY	ROESY
1	169.5, C				
$1-OCH_3$	51.6, CH ₃	3.74, s	1		
2	108.9, C				
3	159.3, C				
3-OH		10.28, br s			
4	100.4, CH	6.13, d (2.3)	2, 3, 5,6		
5	160.6, C				
5-OH		9.83, br s			
6	108.7, CH	6.10, d (2.3)	2, 4, 5, 8		8
7	144.4, C				
8	34.2, CH ₂	2.51, m	2, 6, 7, 9, 10	9	6, 9
9	30.9, CH ₂	1.42, br m		8, 10	8
10	28.6 ^a , CH ₂	1.26–1.29°, m		9	
11	28.9 ^b , CH ₂	1.26–1.29°, m			
12	28.5ª, CH ₂	1.26–1.29°, m			
13	29.0 ^b , CH ₂	1.21–1.26°, m			
14	26.5, CH ₂	1.97, dt (7.0, 6.9)	12, 13, 15, 16	13, 15	15
15	129.6, CH	5.31, m	14, 17,	14	14
16	129.6, CH	5.31, m	14, 17	17	17
17	26.5, CH ₂	1.97, dt (7.0, 6.9)	15, 16, 18, 19	16, 18	16
18	29.0 ^b , CH ₂	1.21–1.26°, m			
19	28.2, CH ₂	1.21–1.26°, m			
20	31.1, CH ₂	1.22°, m			
21	22.0, CH ₂	1.24°, m		22	
22	13.9, CH ₃	0.84, t, (6.9)	20, 21	21	

Table S24. ¹H (600 MHz) and ¹³C (150 MHz) NMR data for capricostatin E (4e) in DMSO- d_6

^{a-b} assignments interchangeable, ^c overlapping resonances



Pos.	δc, type	δ _H , mult (<i>J</i> in Hz)	HMBC	COSY	ROESY
1	172.7, C				
1-OH		13.19, br s			
2	105.2, C				
3	161.5, C				
3-OH		12.00, br s			
4	100.5, CH	6.10, d (2.4)	1, 2, 3, 5, 6		
5	163.6, C				
5-OH		9.99, s			6
6	109.9, CH	6.13, d (2.4)	1, 2, 5, 8		8, 5- OH
7	147.1, C				
8	35.3, CH ₂	2.73, m	2, 6, 7	9	6, 10
9	31.3, CH ₂	1.46, m	7, 8, 10, 11	8	
10	28.7ª, CH ₂	1.22–1.29°, m			
11	29.0 ^b , CH ₂	1.22–1.29°, m			
12	28.5ª, CH ₂	1.22–1.29°, m			
13	29.1 ^b , CH ₂	1.22–1.29°, m			
14	26.6, CH ₂	1.97, m	12, 13, 15, 16	13, 15	
15	129.6, CH	5.31, m	13, 14, 16, 17	14	13
16	129.6, CH	5.31, m	14, 15, 17, 18	17	18
17	26.5, CH ₂	1.97, m		16, 18	
18	29.1 ^b , CH ₂	1.22–1.29 °, m			
19	28.2, CH ₂	1.22–1.29 °, m			
20	31.1, CH ₂	1.22°, m			
21	22.0, CH ₂	1.24 ^c , m		22	
22	13.9, CH ₃	0.83, t (7.0) m	20, 21	21	

Table S25. ¹ H (600 MHz)	nd ¹³ C (150 MHz) NMR	data for capricostatin	F (4f) in DMSO- d_6
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a-b assignments interchangeable, ^c overlapping resonances
Supplementary Figures



Figure S1. Fractionation scheme for the isolation of metabolites from A. gemini MST-FP2131 38



Figure S2. Comparison of the metabolic profiles of A. nidulans and A. gemini

LC-DAD (254 nm) chromatograms of *A. nidulans* LO8030 harbouring the *aqu* BGC and *A. gemini* MST-FP2131 grown under similar conditions and in the same medium. The peaks for aquastatin A (1a) and geministatin A (2a) are highlighted to emphasize the differences in the ratio between both compounds in the two species. Both samples are in the same y-axis scale.



Figure S3. MS-based molecular networking analysis.

The MS-based molecular networking analysis was conducted using Compound Discoverer Software to compare a control strain (*A. nidulans* LO8030 with empty plasmids - Blue) with *A. nidulans* LO8030 expressing the *aqu* BGC (Green). Node styles are represented as pie charts, and node size is determined by the area of the peaks. Link styles are coloured according to the coverage. Node links and cluster size are defined based on default values (10 and 100, respectively). The score threshold is set to 40, the coverage threshold to 70, and the matched fragments threshold to 40. The nodes of some compounds are indicated in the figure.

NOrbi90 #5099 RT: 8,02 AV: 1 NL: 1.28E+007 T: FTMS - c ESI d Full ms2 675.3375@hcd43.33 [70.9558-709.5583]



Figure S4. HRESI(-)MS/MS fragmentation pattern of orsellinic acid (5).



Figure S5. HRESI(–)MS/MS fragmentation pattern of aquastatin A (1a).



Figure S6. HRESI(-)MS/MS fragmentation pattern of geministatin A (2a).



Figure S7. HRESI(–)MS/MS fragmentation pattern of ariestatin A (3a).



Figure S8. HRESI(–)MS/MS fragmentation pattern of capricostatin A (4a).





Figure S9. HRESI(–)MS/MS fragmentation pattern of 7a.



Chemical Formula: C38H52O14 Exact Mass: 732.3357



Figure S10. HRESI(–)MS/MS fragmentation pattern of 8a.





Figure S11. HRESI(-)MS/MS fragmentation pattern of 9a.



Chemical Formula: C₃₈H₅₆O₁₃ Exact Mass: 720.3721



Figure S12. HRESI(–)MS/MS fragmentation pattern of 10a.



Chemical Formula: C₃₈H₅₀O₁₂ Exact Mass: 698.3302



Figure S13. HRESI(-)MS/MS fragmentation pattern of 11a.



Figure S14. ¹H NMR spectrum (600 MHz) of aquastatin A (1a) in DMSO-*d*₆



Figure S15. ¹³C NMR spectrum (150 MHz) of aquastatin A (1a) in DMSO- d_6



Figure S16. ¹H NMR spectrum (600 MHz) of aquastatin B (1b) in DMSO-d₆



Figure S17. ¹³C NMR spectrum (150 MHz) of aquastatin B (1b) in DMSO- d_6



Figure S18. ¹H NMR spectrum (600 MHz) of aquastatin C (1c) in DMSO-*d*₆



Figure S19. ¹³C NMR spectrum (150 MHz) of aquastatin C (1c) in DMSO- d_6



Figure S20. ¹H NMR spectrum (600 MHz) of aquastatin D (1d) in DMSO-*d*₆



Figure S21. ¹³C NMR spectrum (150 MHz) of aquastatin D (1d) in DMSO- d_6



Figure S22. ¹H NMR spectrum (600 MHz) of aquastatin E (1e) in DMSO- d_6



Figure S23. ¹³C NMR spectrum (150 MHz) of aquastatin E (1e) in DMSO-d₆



Figure S24. ¹H NMR spectrum (600 MHz) of corticiolic acid (**1f**) in DMSO-*d*₆



Figure S25. ¹³C NMR spectrum (150 MHz) of corticiolic acid (1f) in DMSO-*d*₆



Figure S26. ¹H NMR spectrum (600 MHz) of ariestatin A (3a) in DMSO-*d*₆



Figure S27. ¹³C NMR spectrum (150 MHz) of ariestatin A (3a) in DMSO- d_6



Figure S28. ¹H-¹³C HSQC NMR spectrum (600 MHz) of ariestatin A (3a) in DMSO-*d*₆



Figure S29. ¹H-¹³C HMBC NMR spectrum (600 MHz) of ariestatin A (3a) in DMSO-*d*₆



Figure S30. COSY NMR spectrum (600 MHz) of ariestatin A (3a) in DMSO-d₆



Figure S31. ROESY NMR spectrum (150 MHz) of ariestatin A (3a) in DMSO-d₆



Figure S32. ¹H NMR spectrum (600 MHz) of ariestatin B (3b) in DMSO-*d*₆



Figure S33. ¹³C NMR spectrum (150 MHz) of ariestatin B (3b) in DMSO-*d*₆



Figure S34. ¹H-¹³C HSQC NMR spectrum (600 MHz) of ariestatin B (**3b**) in DMSO-*d*₆



Figure S35. ¹H-¹³C HMBC NMR spectrum (600 MHz) of ariestatin B (3b) in DMSO-*d*₆


Figure S36. COSY NMR spectrum (600 MHz) of ariestatin B (3b) in DMSO-d₆



Figure S37. ROESY NMR spectrum (150 MHz) of ariestatin B (3b) in DMSO-d₆



Figure S38. ¹H NMR spectrum (600 MHz) of ariestatin C (3c) in DMSO-d₆



Figure S39. ¹³C NMR spectrum (150 MHz) of ariestatin C (3c) in DMSO-d₆



Figure S40. ¹H NMR spectrum (600 MHz) of ariestatin D (3d) in DMSO-*d*₆



Figure S41. ¹³C NMR spectrum (150 MHz) of ariestatin D (3d) in DMSO-d₆



Figure S42. ¹H NMR spectrum (600 MHz) of ariestatin E (3e) in DMSO- d_6



Figure S43. ¹³C NMR spectrum (150 MHz) of ariestatin E (3e) in DMSO- d_6



Figure S44. ¹H NMR spectrum (600 MHz) of ariestatin F (**3f**) in DMSO- d_6



Figure S45. ¹³C NMR spectrum (150 MHz) of ariestatin F (3f) in DMSO-d₆



Figure S46. ¹H NMR spectrum (600 MHz) of capricostatin A (4a) in DMSO-*d*₆



Figure S47. ¹³C NMR spectrum (150 MHz) of capricostatin A (4a) in DMSO- d_6



Figure S48. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin A (4a) in DMSO-*d*₆



Figure S49. ¹H-¹³C HMBC NMR spectrum (600 MHz) of capricostatin A (4a) in DMSO- d_6



Figure S50. COSY NMR spectrum (600 MHz) of capricostatin A (4a) in DMSO-d₆



Figure S51. ROESY NMR spectrum (150 MHz) of capricostatin A (4a) in DMSO-d₆



Figure S52. ¹H NMR spectrum (600 MHz) of capricostatin B (4b) in DMSO-d₆



Figure S53. ¹³C NMR spectrum (150 MHz) of capricostatin B (4b) in DMSO-d₆



Figure S54. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin B (**4b**) in DMSO-*d*₆



Figure S55. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin B (4b) in DMSO-*d*₆



Figure S56. COSY NMR spectrum (600 MHz) of capricostatin B (4b) in DMSO-d₆



Figure S57. ROESY NMR spectrum (600 MHz) of capricostatin B (4b) in DMSO-d₆



Figure S58. ¹H NMR spectrum (600 MHz) of capricostatin C (4c) in DMSO-*d*₆



Figure S59. ¹³C NMR spectrum (150 MHz) of capricostatin C (4c) in DMSO- d_6



Figure S60. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin C (4c) in DMSO-*d*₆



Figure S61. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin C (4c) in DMSO-*d*₆



Figure S62. COSY NMR spectrum (600 MHz) of capricostatin C (4c) in DMSO-d₆



Figure S63. ROESY NMR spectrum (600 MHz) of capricostatin C (4c) in DMSO-d₆



Figure S64. ¹H NMR spectrum (600 MHz) of capricostatin D (4d) in DMSO-*d*₆



Figure S65. ¹³C NMR spectrum (150 MHz) of capricostatin D (4d) in DMSO-*d*₆



Figure S66. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin D (4d) in DMSO-d₆



Figure S67. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin D (4d) in DMSO-*d*₆



Figure S68. COSY NMR spectrum (600 MHz) of capricostatin D (4d) in DMSO-d₆



Figure S69. ROESY NMR spectrum (600 MHz) of capricostatin D (4d) in DMSO-d₆



Figure S70. ¹H NMR spectrum (600 MHz) of capricostatin E (4e) in DMSO-*d*₆



Figure S71. ¹³C NMR spectrum (150 MHz) of capricostatin E (4e) in DMSO- d_6


Figure S72. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin E (4e) in DMSO-*d*₆



Figure S73. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin E (4e) in DMSO-*d*₆



Figure S74. COSY NMR spectrum (600 MHz) of capricostatin E (4e) in DMSO-d₆



Figure S75. ROESY NMR spectrum (600 MHz) of capricostatin E (4e) in DMSO-d₆



Figure S76. ¹H NMR spectrum (600 MHz) of capricostatin F (4f) in DMSO-*d*₆



Figure S77. ¹³C NMR spectrum (150 MHz) of capricostatin F (4f) in DMSO- d_6



Figure S78. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin F (4f) in DMSO-*d*₆



Figure S79. ¹H-¹³C HSQC NMR spectrum (600 MHz) of capricostatin F (**4f**) in DMSO-*d*₆



Figure S80. COSY NMR spectrum (600 MHz) of capricostatin F (4f) in DMSO-d₆



Figure S81. ROESY NMR spectrum (600 MHz) of capricostatin F (4f) in DMSO-d₆



Figure S82. HRESI(-) mass spectrum of aquastatin A (1a)



Figure S83. HRESI(-) mass spectrum of aquastatin B (1b)



Figure S84. HRESI(-) mass spectrum of aquastatin C (1c)



Figure S85. HRESI(-) mass spectrum of aquastatin D (1d)



Figure S86. HRESI(-) mass spectrum of aquastatin E (1e)



Figure S87. HRESI(-) mass spectrum of corticiolic acid (1f)



Figure S88. HRESI(-) mass spectrum of ariestatin A (3a)



Figure S89. HRESI(-) mass spectrum of ariestatin B (3b)



Figure S90. HRESI(-) mass spectrum of ariestatin C (3c)



Figure S91. HRESI(-) mass spectrum of ariestatin D (3d)



Figure S92. HRESI(-) mass spectrum of ariestatin F (3f)



Figure S93. HRESI(-) mass spectrum of capricostatin A (4a)



Figure S94. HRESI(-) mass spectrum of capricostatin B (4b)



Figure S95. HRESI(-) mass spectrum of capricostatin C (4c)



Figure S96. HRESI(-) mass spectrum of capricostatin D (4d)



Figure S97. HRESI(-) mass spectrum of capricostatin E (4e)



Figure S98. HRESI(-) mass spectrum of capricostatin F (4f)



Figure S99. HRESI(-) mass spectrum of capricostatin A (4a) ozonolysis product



Figure S100. UV-vis spectrum of ariestatin A (3a) in MeCN.



Figure S101. UV-vis spectrum of ariestatin B (3b) in MeCN.



Figure S102. UV-vis spectrum of ariestatin C (3c) in MeCN.



Figure S103. UV-vis spectrum of ariestatin D (3d) in MeCN.



Figure S104. UV-vis spectrum of capricostatin A (4a) in MeCN.



Figure S105. UV-vis spectrum of capricostatin B (4b) in MeCN.



Figure S106. UV-vis spectrum of capricostatin C (4c) in MeCN.



Figure S107. UV-vis spectrum of capricostatin D (4d) in MeCN.



Figure S108. UV-vis spectrum of capricostatin F (4f) in MeCN.



Figure S109. IR spectrum (ATR) of ariestatin A (3a).



Figure S110. IR spectrum (ATR) of ariestatin B (3b).



Figure S111. IR spectrum (ATR) of ariestatin C (3c).



Figure S112. IR spectrum (ATR) of ariestatin D (3d).



Figure S113. IR spectrum (ATR) of capricostatin A (4a).



Figure S114. IR spectrum (ATR) of capricostatin B (4b).



Figure S115. IR spectrum (ATR) of capricostatin C (4c).



Figure S116. IR spectrum (ATR) of capricostatin D (4d).



Figure S117. IR spectrum (ATR) of capricostatin F (4f).



Figure S118. Phylogenetic analysis of SAT domain sequences of selected fungal NR-PKSs.

Depside-forming enzymes are highlighted in yellow. AquA and enzymes with characterised SAT and TE domains are displayed in bold. Branches with aLRT SH-like support < 0.8 have been collapsed.



Figure S119. Phylogenetic analysis of TE domain sequences of selected fungal NR-PKSs.

Depside-forming enzymes are highlighted in yellow. AquA and enzymes with characterised SAT and TE domains are displayed in bold. Branches with aLRT SH-like support < 0.8 have been collapsed.

GyrPKS	140 * : ~~~~~VLLRG	160 NPRSNLPRI	.FLIS	180 *	200 AAMIHFPE	* FDAGVVTYGIDSP	220 HCPEDFF	* 2	240 DCSIEVRAIQ-	* 260	0 * QPHGPYLIG <mark>GW</mark>	: 82
AquA	: DLSSFHSNVVLLHG : ~~~~~VLV <mark>QG</mark>	DTASAQTPI	FLIS	DGAGSA	SAYNYIPRLP	-SGTPVYALESP	FLRNPFDPS	CSMEE	VATLYKDALK-	ST(OPAGPYLIG G W	: 204
AN7909	:TT			-IDSSRQHKLDAAVSF	RASYIHEKA	PKGRRIYALESP	FLEQPELPD	LSIEE	MATIFLETIR	RI(QPHGPYLIG <mark>GW</mark>	: 202
ExoA	: AAKKYSSNVVLLQG	RPSSGQTPI	FLLV	DGAGSS	STATLH PF	FPTKLPVYALESP	FUHCPIEYT	ISIHE	TAQLEVEATRE	K	-TQSKGPYMLG <mark>GW</mark>	: 118
PKS16	: SSQALRAKSVLLQG	RPEKGKPAI	FLLP	DGAGSI	FSYISHPS	PSGLPIYGDSP	HNNPSERT	ISFSI	VATIVIAMIR-	AI(QPKGPYMIGGW	: 116
PFUR17	SNOSVRSKSSIIDE	RPASNRPAL	PETTR	DGAG	CDAMEDVC	PSGVPVYGIDSP	HNSEKUTI-	COPUNIT INCOMA	ASTIKER.	ALDELLE LOCI	OPRGPY OF GGW	: 190
Dreus	·TUSDDUALTIHCG				-GHAT HTOKH	INPRHTEMI ODI G	DI DUSUDUDI	LOPEVNLINGPMAL	ARDANNORI	TIPCIPU_CSEI	DUDKEHUMUTOV	. 102
DrcA	NKPMPTAIMIHGG				-GYMTISBRA	RPHOTOMLUTNN	TUPTSLOVE	LCPEVDVLSGPTTT	CDALWWART	TLPGIAK-EOGY	VIVDVDKLVVVG	: 103
Preu6	:SPMPLALMIHGG				-GHMTI SRKA	RPTOAKYL SHG	PISIDYR	LCPEVNLIDGPIA	VRDAVWACOL	NLGTHLA-EHS	ISVDGGRVVVVGW	: 100
MollE	:EAMDIALLIHGG				-GHMTLSRKA	RPAQTAHL SQN	IL PVSIDYR:	LCPEVTLIEGPIA	VRDAYVWAQK(QLPGMVK-PMG	ITVKADNIAVV <mark>G</mark> W	: 100
Se	r1937				0	1	II 5		a		65	
	280	* 300	- *	320	* 3	10*	360	*	380	*	400	
GyrPKS	: SIGMYAYEVAR-QLIE	RGETVIGLVFIDS	ACER	RLLGLFRIT		MEVCEETGMFM	GMA-KAGK-		KAPLTT	AQKLHVT	GCVR	: 159
ThiA	SIGGMYMYETAR-OLVA	AGERVEGLULIDS	SACER	RLEGIRAIT		VETCEETGWFL	GFA-NTGR-		KQPLTV	AQKLHVT	GCVR	: 281
AQUA	A SAVIGIEVSR-QULV	EGELVQGLLLLDM FGETTOALLIDM		KNEIIAAP		-TEVVQQANWVI	RUH-GDH		AVEDELIA:	SVKQHLL	ASSL	: 160
Exol	SASSAVAVEAR-TIT	GGETVIGUTUTO	IRVPR	PMPDGLFLT		-MDTLDOVGUTA	GIKRAG			MT.BOHLT.	STVK	. 196
PKS16	SIGGIHAVETAR-OLIE	OGETISNLIMIDS	PCPG	TLPPLPAPT		LSLLEKAGHED	GI S-TSG		APITER	RTRLHFL	GCVR	: 191
PFUR17	: SIGGILAYEASR-QLIA	OGETITNLIMIDS	PCPG	TLPPLPSPT		INLLEKAGIFD	GLS-ASS		GPITER	RTRLHFL	GSVR	: 265
DepH	: STGGHLAMSLAW-TAAE	AGMDPPKGV-LAP	YAPVOFEDGD	LDA~~~~~~~~~			~~~~~~~~~	~~~~~~			~~~~ <mark>~</mark> ~~~~~~~~	: 141
Preu3	: SIGHLALTTAF-TTRV	RGFKPPSAI-LGF	YCPINYSADW	WRSPIYPELAQQS		SSETFD	GVNEHAIAG	YTPTVNNNVAP	ALLMSLDDPRW	REVLHANWRAQ	TLPMLINGLPSKS	: 216
DrcA	: SIGHLAMTIGW-ICFA	KGVKPPTAI-LSE	YAPTDFEHEY	WTRRDLGAFPAPT		MNVESTER	ALSGKVISH	YDGPKGIGASEENI	GLLRQGDPRSH	ELILNLFSGGN(GLSLLLNGFTGSL	: 219
Preu6	SIGGHLAMSLGW-SLEE	AGVPPPKAV-LSE	YAPVDFESGE	LDNQKNPALPKPR		MTLDQUTK	AUPRTPVTQ	YGASS-TDETNI	LGWLHPGDPRSH	ELLLHVFHSDI	GLPLILHGLPI	: 211
MollE	SIGHLGMTLGW-THKE	AGVSPPKAN-USP	YGPTDFESGD	LDVRRAEEYFERQ		MSMANUIA	SMPKQPITN	YATTT-IDATNM	IGWVRPGDPRSI	ELVESEFREGN	GLPLLLNGLSSPD	: 213
	5 a	6 00	F									
	* 420	* 4	40	* 460	*	480	*	500 +	520	*	540	
GyrPKS	* 420 : AVADYEPLPI	* 4	40 GKREA-	* 460	• 	480 OSKFG-EFD-	•	500 * TLSFKVKEVGD	520 -KMAEEQGLAKS	* SGISHDWLTGE	540 RKD FG PK GN DR	: 232
GyrPKS ThiA	* 420 : AVADYEPLPI : SAAEYEPAPI	* 4 EE EV	140 GKRPA- AGRPA-	* 460 H-TFVI R-AFVI	•	480 NSKFG-LFD- NSRFG-MFD-	*	500 TLSFKVKEVGD KLSLKVKEVGE	520 -KMAEEQGLAKS -RVAEERGLEKT	* SGISHDWLTGE IGVNQDWLTAE	540 RKDFGPKGWDR RTSFGPKGWDR	: 232 : 354
GyrPKS ThiA AquA	* 420 : AVADYEPLPI : SAAEYEPAPI : CVRGYEPKPX	* 4 PE PV PD	140 GKRPA- AGRPA- DRRPT-	* 460 R-TFVI R-AFVI KGTFII	•	480 NSKFG-LFD- NSRFG-MFD- NARSG-LCDT	* MRNPDEVEE	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN-	520 -KMAEEQGLAKS -RVAEERGLEKS -VMYDNDI	* SGISHDWLTGE IGVNQDWLTAE DMRTFLFSK	540 RKDFGPKGWDR RTSFGPKGWDR RYHFGINGWDK	: 232 : 354 : 241
GyrPKS ThiA AquA AN7909	* 420 : AVADYEPLPI : SAAEYEPAPI : CVRGYEPKPY : ALSRYDAPAE	* 4 PE PV PD PS	140 GKRPA- AGRPA- DRRPT- DRQPK-	* 460 R-TFVI 	*	480 WSKFG-LFD- WSRFG-MFD- WARSG-LCDT WALLG-LDNR	* MRNPDEVEE	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIGP	520 -KMAEEQGLAKS -RVAEERGLEKS -VMYDNDI (SMYEMNLDEFS	* SGISHDWLTGE IGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR	540 RKDFGPKGWDR RTSFGPKGWDR RYHFGINGWDK RQQFGTNGWED	: 232 : 354 : 241 : 359
GyrPKS ThiA AquA AN7909 ExoA	* 420 : AVADYEPLPI : SAREYEPAPI : CVRGYEPKPM : ALSRYDAPAF : QLNVYNAQPM	* 4 PE PV PD PS PE	140 GKRPA- DRRPT- DRQPK- GKRPL-	* 460 R-TFVI 	*	480 WSKFG-LFD- SRFG-MFD- WARSG-LCDT WALLG-LDNR WAKKG-MIDV	* MRNPDEVEE PDAPIA IKEQGL	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIGP EIPEWAQEIE	520 -KMAEEQGLAK -RVAEERGLEK -VMYDNDI (SMYEMNLDEF EEMDGNVMEDE(* SGISHDWLTGE IGVNQDWLTAE -DMRTFLFSK ERYFNSWFYGR GLGLVGWFYGK	540 RKDFGPKGWDR RTSFGPKGWDR RYHFGINGWDK RQQFGTNGWED RTDFGPNGWDK	: 232 : 354 : 241 : 359 : 275
GyrPKS ThiA AquA AN7909 ExoA PKS16	* 420 : AVADWEPLPT : SAEWEPAPI : CVRGWEPKP : AISRVDAPAF : QLRVYNAQPN : ALENYTVTPI	* 4 PE PD PS PE PP	140 GKRPA- DRRPT- DRQPK- GKRPL- GKSPG-	* 460 	*	480 SKFG-LFD- SRFG-MFD- ARSG-LCDT ALLG-LDNR AKKG-MIDV 	* MRNPDEVEE PDAPIA IKEQGL	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIGP EIPEWAQEIE REEQG-KEYMAATS	520 -KMAEEQGLAK -RVAEERGLEK -VMYDNDI (SMYEMNLDEF EEMDGNVMEDEC SSGDLNKD)	* SGISHDWLTGE IGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR GLGLVGWFYGK MDKAKEWLTGK	540 RKDFGPKGWDR RTSFGPKGWDR RYHFGINGWDK RQQFGINGWDK RTDFGPNGWDK RTSFGPSGWDK	: 232 : 354 : 241 : 359 : 275 : 264
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH	* 420 : AVADWEPLFI : SVRGWEPKPL : CVRGWEPKPL : ALSRVDAPAF : QLENWTVFFI : ALENWTVFFI : ALENWTVFFI	* 4 PE PD PS PE PA	140 GKRPA- DRRPT- DRQPK- GKRPL- GKSPG- DRSPG-	* 460 R-TFV 	*	480 SSKFG-LFD- SRFG-MFD- 	* MRNPDEVEE PDAPIA: IKEQGL	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIGP EIPEWAQEIF REEQG-KEYMAATS REDVGGEEWMADS	520 -KMAEEQGLAK -RVAEERGLEK -VMYDNDI (SMYEMNLDEF -EMDGNVMEDE (SSGDLNKD) GDANAD)	* SGISHDWLTGE IGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR GLGLVGWFYGK MDKAKEWLTGK MEKAKQWLTGK	540 RKDFGPKGMDR RTSFGPKGMDR RYHFGINGMDK RQCFGTNGWDZ RTSFGPSGMDK RTSFGPSGMDK RTSFGPSGMDK	: 232 : 354 : 241 : 359 : 275 : 264 : 339
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3	* 420 : AVADVEPPPT : SVRGVEPXPT : CVRGVEPXPT : QINVVNAQPT : ALENVVTVPT : ALENVVVPT : DLADSGOTUDSVINPT	* 4 PV PD PS PP PA	140 GKRPA- DRPT- DRQFK- GKRPL- GKSPG- DRSPG- DRSPG-	* 460 	*	480 	* 	500 * TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIG EIPEWAQEIF REEQC-KEYMAATS REEQC-KEYMAATS	520 -KMAEEQGLAK: -RVAEERGLEK: -VMYDNDI SMYEMNLDEFI SEMDGNVMEDE(SSGDLNKD) SGGDLNKD)	* GGISHDWLTGE DMRTFLFSK GLGLVGWFYGR GLGLVGWFYGK MDKAKEWLTGK MEKAKQWLTGK	540 RKDFGPKGNDR R-TSFGPKGNDR RYHFGINGWDK R-QCFGTNGWD RDFGPNGWDK R-TSFGPSGWDK R-TSFGPSGWDK	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : -
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA	* 420 : A VAD VEPLPI : S VRGVEPRP : C VRGVEPRP : A ISN DAPAF : Q ISN DAPAF : A LEN VNNAUPF : A LEN MTVKPI : RLARSGQT VDSVINREI : NRLOP	* 4 PZ PV PS PZ PZ PA PDAEDVASISPID PTTIOIOSISPID	140 GKRPA- DRPFT- DRQPK- GKRPL- GKSPG- DRSPG- 0Q-IVRGSYS- H-VRGSYS-	* 460 	*	480 SKFG-LFD- 	* PDAPIA: IKEQGL	500 TLSFKVKEVGD KLSLKVKEVGE SMGRPGLDIGP EIPEWAQEIE REEQG-KEYMAATS REDVGGEEWMADSS VEIIEGAE VEVIEGAE	520 -KMAEEQGLAK -RVAEERGLEK -VMYDNDI	* GGISHDWLTGE DMRTFLFSK GLGLVGWFYGR GLGLVGWFYGR MDKAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL	540 RKDFGPKG DR -TSFGPKG DR -YHFGING DK RQQFGTNS BD RTDFGPNG DK RTSFGPSG DK RTSFGPSG DK DKWDG	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : - : 308 : 303
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Preu6	* 420 : AVADWEPPPT : SVRGWEPAPT : CVRGWEPKPM : AISRYDAPAF : AISRYDAPAF : AISRYDAPAF : AISRYDAPAF : A	+ 4 PV	140 GKRPA- DRPT- 	* 460 	+ 	480 	* 	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN SMGRPGLDIG EIPEWAQEIE REEQG-KEYMAATS REDVGGEEWMADS VEIIEGAE VLVVPHRR	520 KMAEEQGLAK: RVAEERGLEK: VMYDNDI	SGISHDWLTGE IGVNQDWLTAE DNRTFLFSK ERYFNSWFYGR GLGLVGWFYGR MDKAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLT HVFDVTM	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : - : 308 : 303 : 295
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Preu6 MollE	* 420 : AVADWEPLPI : SAEWEPAPI : CVRGMEPKP : ALSWIDAPP : ALENWIVKPI : ALENWIVKPI : RLARSGQTVDSVINREI : NRLQP	* 4 PV	140 GKREA- 	* 460 	* 	480 	* MRNPDEVEE PDAPIA: IKEQGL	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN SMGRPGLDIGP EIPEWAQEIE REEQG-KEYMAATS REDVGGEEWMADS VEIIEGAE VLVVFHRR FLSLSGTG	520 KMAEEQGLAK: RVAEERGLEK: VMYDNDI	SGISHDWLTGE IGVNQDWLTAE -DNRTFLFSK ERYFNSWFYGR GLGLUGWFYGK MCKAKEWLTGK MEKAKQWLTGK -HCFDVWS HVFDUTL HVFDUTM HIHDLEA	540 KDFGPKGDP TFFGPKGDP YHFGINGDPK QOFGTNGDP TFFGPSGDK TFFGPSGDK TFFGPSGDK FFGDAC KPGAC KPGSAC KDSK-G2D 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 :
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Preu6 MollE	* 420 : A VAD VEPLPI : S VRGVEPRP : A ISRYDAPAF : Q ISRYDAPAF : A LENYTVTPI : A LENYTVTPI : RLARSGQTVDSVINREI : NRLQP	+ 4 PV	140 GKREA- 	* 460 	+ 	480 SKFG-LFD- 	* MRNPDEVEE PDAPIA: IKEQGL LARRGVNAR MKARGLDGE MKARGLDGE CREKGVECG	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN SMGRPGLDIGP EIPEWAQEIE REEQG-KEYMAADS REDVGGEEWMADS VEIIEGAE VLVVPHRR FLSLSGTG FLTVPGAR	520 KMAEEQGLAK: VVAYDNDI	SGISHDWLTGE IGVNQDWLTAE DNRTFLFSK ERYFNSWFYGR GLGLVGWFYGR MDKAKEWLTGK MEKAKQWLTGK -HCFDVWS -HLFDLTL HVFDVTM	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : - : 308 : 303 : 295 : 297
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Preu6 MollE	* 420 : AVADWEPLPI : SAEWEPAPI : CVRGWEPKP : ALSRVDAPAP : ALENMTVTPI : ALENMTVTPI : RLARSGOTVDSVINREL : RLARSGOTVDSVINREL : SGSGR	4 PD	140 GKRPA- DRPT- DROPK- GKRPC- GKSPG- DRSPG 	* 460 	*	480 	MRNPDEVEE PDAPIA IKEQGL LARRGVNAR MKARGLDGE MSEKGVKSG CREKGVECG	500 * TLSFKVKEVGD VSRDLAIDPIGKN- SMGRPGLDIG PIPEWAQEIE REEQG-KEYMAATS REDVGGEEWMADSS VEITEGAE FLSLSGTG FLIVPGAR	520 KMAEEQGLAK: VVAERGLEK: VMYDNDI SSMYEMNLDEFF EEMDGNVMEDE(SSGDLNKD) SGGDLNKD	A GGISHDWLTGE IGUNQDWLTAE DMRTFLFSK ERYFNSWFYGR BLGLVGWFYGK MDXAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL HVFDVTM HIHDLEA	540 	: 232 : 354 : 241 : 255 : 264 : 339 : - : 308 : 303 : 295 : 297
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Preu6 MollE GyrPKS	* 420 : AVADWEPPPT : SVRGWEPKPN : AURWUNAQPF : ALENYTVTPI : ALENYTVTPI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : SGSGR	* 4 PL	140 GKREA- DRET- DRET- GKRE GKSEG- DRSEG- DRSEG- DRSEG- 	* 460 	*	480 	* PDAPIA: IKEQGL LARRGVNAR' MKARGLDGE' MSEKGVKSG CREKGVECG	500 * TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIG FLEVGQEEWAQUIR REEQG-KEYMAATS VEILEGAE FLSLSGTG FLSLSGTG FLSLSGTG FLTVPGAR	* 520 KMAEEQGLAK: -VVAERGLEK: -VMYDNDI (SMYEMNLDEF) SSGDANADI 	SGISHDWLTGE GGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR MDRAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL HVFDVTM HIHDLEA 660	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : - : 308 : 308 : 295 : 297 : 297
GyrFKS ThiA AquA AN7909 ExoA PKS16 DrcH Preu3 DrcA Preu6 MollE GyrPKS ThiA	* 420 : AVADWEPPPT : SVRGWEPKPM : AURWVNAQPH : ALENWTVTPT : ALENWTVKPT : RLARSGQTWDSVINRE : NRLQP : SGSGR	* 4 PL	140 GKREA- DRRPT- DRCPK- GKRPL- GKSPG- DRSPG- 0Q-IVRGSYS- HR-VRDGSYT- HR-VRDGSYT- 580 THAPELQCMV THTGFLQQLV	* 460 	*	480 	MRNPDEVEE PDAPIA: IKEQGL	500 * TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIG FIEVGAUNAQUIE REEQG-KEYMAATS VEIIEGAE FLSLSGTG FLSLSGTG FLSLSGTG FLIVPGAR 640	<pre>520 KMAEEQGLAKS RVAEERGLEKT VMYDNDI (SMYEMNLDEFF EMDGNVMEDE() SGGDANAD)</pre>	SGISHDWLTGE IGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR GLGLVGWFYGK MEKAKQWLTGK -HCFDVWS -HCFDVWS -HVFDVTM -HIFDLTL -HIHDLEA 660	540 RKDFGPKG DR RTSFGPKG DR RYHFGING DK RQOFGING DK RISFGPSG DK RTSFGPSG DK RTSFGPSG DK RKPDSA-Q EK RKPDSK-S IED RKPDSK-S IED W \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$: 232 : 354 : 241 : 359 : 275 : 264 : 309 : : 308 : 303 : 295 : 297 : 274 : 485
GyrFKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA MollE GyrFKS ThiA AquA	* 420 : AVADWEPPPT : SVRGWEPRP : AURWEPRP : AURWUNAOPH : ALENMTVTPT : ALENMTVKPT : ALENMTVKPT : RLARSGQTVDSVINRET : NRLOP	* 4 PL	140 GKREA- DRRET- DRQEX- GKREL GKSEG- DRSEG- DRSEG- DRSEG- 	* 460 	* 	480 	* MRNPDEVEE' PDAPIA: IKEQGL LARRGVNAR' MKARGLOGE' MSEKGVKSG; CREKGVECG' * EEGCGDFMT;	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN SMGRPGLDIGP EIPEWAQEIE REEQG-KEYMAADS REDVGGEEWMADSS VEIIEGAE FLSLSGTG FLSLSGTG FLIVPGAR 640 LVYPQQRRLVIAYV	S20 KMAEEQGLAK: RVAEERGLEK: VMYDNDI (SMYEMNLDEFF EEMDGNVMEDEC SSGDINKD) SGGDANAD)	SGISHDWLTG IGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR GLGLVGWFYGR MDKAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL HVFDVTM HIHDLEA 660	540 RKDFGPKG DR -TSFGPKG DR -YHFGING DK RQQFGTNS ED RTDFGPNG DK RTSFGPSG DK RTSFGPSG DK RTSFGPSG DK RTSFGPSG DK KPGDA-QTEK KPGDA-QTEK KPGDA-QTEK KPGSK-G ED KPGSK-G ED KPGSK-G ED 	: 232 : 354 : 241 : 359 : 275 : 264 : 308 : 303 : 295 : 297 : 297 : 274 : 4745 : 281
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Freu6 MollE GyrPKS ThiA AquA AN7909	* 420 : AVADWEPLPI : SAEWEPAPI : CVRGWEPKP : ALSRVDAPAF : ALSRVDAPAF : ALENWIVKP : ALENWIVKP : RLARSGQIVDSVINREI : NRLQP	+ 4 PL	140 GKRPA- DRPT- DROPR- GKRPG- GKSPG- GKSPG- 	* 460 	+ 	480 	* MRNPDEVEE' PDAPIA: IKEQGL LARRGVNAR' MKARGLDGE' MSEKGVKSG; CREKGVECG' * EEGCGDFMT:	500 TLSFKVKEVGD VSRDLAIDPIGKN- SMGRPGLDIG PURAQEIE REEQG-KEYMAATS REDVGGEEWMADSS VEITEGAE VLVVPHRR FLSLSGTG FLTVPGAR 640 LVYPQQRRLVIAYV	* 520 KMAEEQGLAK: FVAEERGLEK: VMYDNDI SSMYEMNLDEFF EEMDGNVMEDE(SSGDLNKD) SGGDANAD SGGDANAD 	-HURDVLTGE GUNQDWLTAE DMRTFLFSK ERVFNSWFYGR BLGLVGWFYGK MDKAKEWLTGK -HOFDVWS -HUFDLTL -HUFDLTL HUFDLTL 660 LQDLPAGTPVV	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : : 308 : 297 : 297 : 274 : 485 : 299
GyrPKS Thia Aqua AN7909 ExoA PFUR17 DepH Preu3 DrcA Preu6 MollE GyrPKS Thia Aqua AN7909 ExoA	* 420 : AVADWEPPPT : SVRGWEPKP : AURWUNAQPF : ALENTVTPT : ALENTVTPT : ALENTVTPT : RLARSGQTVDSVINRET : RLARSGQTVDSVINRET : SGSGR	* 4 PL	140 GKRPA- DRPT- DRPFX- GKRPI- GKSPG- DRSPG- DRSPG- DRSPG- 	* 460 	*	480 	* MRNPDEVEEY PDAPIA: IKEQGI LARRGVNAR' MKARGLDGEY MSEKGVKSG: CREKGVECG: * EEGCGDFMT:	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIGP EIPEWAQEIE REEQG-KEYMAATS REEQG-KEYMAATS VEIIEGAE FLSLSGTG FLSLSGTG FLSLSGTG 640 LVYPQQRRLVIAYV	S20 KMAEEQGLAKS VMYDNDI (SMYEMNLDEF) SSGDLNKD SGGDLNKD SGGDANAD	GISHDWLTGE GUNQDWLTAE DMRTFLFSK ERYFNSWFYGR MDKAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL HUFDVTM HIHDLEA 660 LQDLPAGTPVV	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : 303 : 295 : 297 : 297 : 274 : 485 : 291 : 399 : 317
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu6 MollE GyrPKS ThiA AquA AN7909 ExoA PKS16	* 420 : AVADVEPPPI : SVRGVEPAPI : CVRGVEPAPI : QURVVNAQPH : ALENTVTPI : ALENTVTPI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : SGSGR	* 4 PE	140 GKRPA- DRPT- 	* 460 	*	480 	* MRNPDEVEE' PDAPIA IKEQGL LARGVNAR' MKARGLOGE' MSEKGVKSG CREKGVECG * EEGCGDFMT	500 * TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIG FLEVGQEEWAQUIR REEQG-KEYMAATS VEIIEGAE FLSLSGTG FLSLSGTG FLSLSGTG FLVPGAR 640	* 520 KMAEEQGLAKS VAYENDDI SMYEMNLDEF(SSGDANAD) SGGDANAD)	SGISHDWLTGE GGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR MDRAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL HVFDVTM HIHDLEA 660 LQDLPAGTPVVV	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : : 303 : 295 : 297 : 274 : 485 : 281 : 399 : 317 : 289
GyrFKS ThiA AquA AN7909 ExoA PKS16 DrcA Preu6 MollE GyrFKS ThiA AquA AN7909 ExoA PKS16 PFUR17	* 420 : AVADVEPPPI : SVRGVEPKP : AURVVNAOPH : ALENVVNQ : ALENVVVPI : ALENVVVPI : RLARSGQTVDSVINRE : NRLOP : SGSGR	* 4 PL	140 GKRPA- DRPT- DRCPK- GKSPG- GKSPG- DRSPG- 02-IVRGSYS- H-VRDGSYT- 580 CHTAPLLQEMV HTAPLLQEMV HTAPLLQEMV HTAPLLQUV E-LGQSIQKAL -VGDIVIETV 	* 460 	*	480 	* MRNPDEVEE' PDAPIA: IKEQGL ILARRGVNAR' MKARGLOGE' MSEKGVKSG: CREKGVECG: * EEGCGDFMT:	500 * TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIG FLIPEWAQEIE REEQG-KEYMAATS VEIIEGAE FLSLSGTG FLSLSGTG FLIVPGAR 640	* 520 KMAEEQGLAX: RVAEERGLEK: -/MYDNDI SMYEMNLDEFF EEMDGNVMEDE(SSGDANAD) 	SGISHDWLTAE DMRTFLFSK ERVFNSWFYGR GLGLVGWFYGR MDRAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL HVFDVTM HIHDLEA 660	540 RKDFGPKG DR RTSFGPKG DR RYHFGING DK RQCFGTNS IEL RTSFGPSG DK RTSFGPSG DK RTSFGPSG DK RKPGDA-Q EK RKPGDA-Q EK RKPGDK-S IEL W \$ 680 VSGVPPKHAQLLD	: 232 : 354 : 241 : 359 : 265 : 264 : 339 : : 308 : 295 : 297 : 297 : 297 : 297 : 297 : 297 : 297 : 299 : 399 : 376
GyrFKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu6 MollE GyrFKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3	* 420 : AVADWEPLPI : SAEWEPAPI : CVRGMEPKP : ALSRVDAPAF : QLSRVDAPAF : ALENVIVVP : ALENVIVVP : ALENVIVVP : RLARSGQTVDSVINRE : SLGGT : SLGGT : SLGGT : LLGHVETFALDCDHF : LLGPVECFALDCDHF : LLGD-FVECFALDCDHF : LLGDHIAVYTVNGDHF : LLGDVETH-VVEGNHF : LLGDVETH-VVEGNHF : LTGA-EVQCHVVGGNHF	+ 4 PL	140 GKRPA- 	* 460 	*KDD: HGTKDD: HGTKDV: HGTQDE) * PALSYSSVRGA	480 	*PDAPIA: IKEQGL LARRGVNAR MKARGLDGE MSEKGVKSG CREKGVECG	500 ***********************************	520 KMAEEQGLAK: VMYDNDI	* GGISHDWLTGE IGUNQDWLTAE DMRTFLFSK ERYFNSWFYGR MDKAKEWLTGK MEKAKQWLTGK MEKAKQWLTGK -HCFDVWS -HLFDLTL -HUFDLTL HIHDLEA 660	540 	: 232 : 354 : 241 : 359 : 275 : 265 : 339 : : 308 : 303 : 295 : 297 : 297 : 297 : 297 : 297 : 297 : 317 : 289 : 317 : 289 : 317 : 289 : 317 : 289 : 317 : 281 : 295 : 275 : 265 : 265 : 265 : 265 : 265 : 275 : 265 : 295 : 297 :
GyrPKS Thia Aqua AN7909 ExoA PFKS16 PFUR17 DepH MollE GyrPKS Thia Aqua An7909 ExoA PKS16 PFUR17 DepH Freu3 Drca	* 420 : AVADWEPLPI : SAEVEPAPI : CVRGWEPKP : ALSRVDAPAF : QLENMTVTPI : ALENMTVTPI : ALENMTVKP : RLARSGOTVDSVINREI : RLARSGOTVDSVINREI : RLGP	* 4 PL	140 GKRPA- DRPT- DROPK- 	* 460 	*	480 	*PDAPIA: IKEQGL IKEQGL LARRGVNAR' MKARGLDGE' MSEKGVKSG CREKGVECG' * EEGCGDFMT'	500 * TLSFKVKEVGD VSRDLAIDPIGKN- SMGRPGLDIGP EIPEWAQEIE REEQG-KEYMAATS REDVGGEEWMADSS VEITEGAE VLVVPHRR FLSLSGTG FLTVPGAR 640	520 KMAEEQGLAK: VMYDNDI	A CONTRACT OF CONT	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : 303 : 295 : 297 : 297 : 297 : 297 : 297 : 297 : 317 : 399 : 317 : 399 : 317 : 289 : 376 : 324 : 324 : 324 : 295 : 297 : 297 : 297 : 297 : 297 : 297 : 297 : 297 : 297 : 399 : 207 : 397 : 297 : 399 : 397 : 397
GyrPKS ThiA AquA AN7909 ExoA PFKS16 PFUR17 DepH Preu6 MollE GyrPKS ThiA AquA AN7909 ExoA PFUR17 DepH PFUR3 DepH Preu3 Drc4 PFue16	* 420 : A VADWEPLPI : S AREWEPAPI : C VRGWEPKPI : A LENTVNAQPF : A LENTVVFI : A LENTVVFI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : RLARSGQTVDSVINREI : LLGHVETFALDCDHF : LLG-HVETFALDCDHF : LLG-HVETFALDCDHF : LLGDLAVYTVNG-DHF : LLGDLAVYTVNG-DHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDLAVYTVNG-DHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGD-HVEFFALDCDHF : LLGDVETH-VVEGPDHF : LLGD-HVEFFALDCDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGD-HVEFFALDCDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGDVETH-VVEGPDHF : LLGD-HVEFFALDCDHF : LLGDVETH-VVEGPDHF : LLGD-HVEFFALDCDHF : LLGDVETH-VVEGPDHF : LLGD-HVEFFALDCDHF : LLGDVETH-VVEGPDHF : LLGD-HVEFFALDCDHF : LLGDVETH-VVEGPDHF : LLGDVETHF : LLGDVETHF	* 4 PL	140 GKRPA- 	* 460 	*	480 SRFG-LFD- 	*	500 TLSFKVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIGP EIPEWAQEIP REEQG-KEYMAATS REEQG-KEYMAATS VEIIEGAE FLSLSGTG FLSLSGTG FLSLSGTG 640 LVYPQQRRLVIAYV	520 KMAEEQGLAK: RVAEERGLEK: CMYDNDI SMYEMNLDEFI SSGDLNKDI SGGDLNKDI SGGDANADI COMPANY COMPAN	sgishdwlige GGISHDWlige DMRTFLFSK ERYFNSWFYGR MDKAKEWLIGK HCFDVWS HLFDLTL HVFDVTM HHDLEA 660 LQDLPAGTPVVV	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : 303 : 295 : 297 : 297 : 297 : 297 : 297 : 281 : 317 : 289 : 317 : 297 : 297 : 297 : 303 : 295 : 297 : 297 : 303 : 295 : 297 : 297 : 303 : 295 : 297 : 297 : 303 : 295 : 297 : 297 : 297 : 303 : 295 : 297 : 297 : 297 : 297 : 303 : 295 : 297 : 303 : 295 : 297 : 317 : 295 : 317 : 297 : 317 : 281 : 317 : 289 : 317 : 324 : 326 : 324 : 326 : 324 : 326 : 326
GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Preu6 MollE GyrPKS ThiA AquA AN7909 ExoA PKS16 PFUR17 DepH Preu3 DrcA Preu3 DrcA Preu3 DrcA	* 420 : AVADVEPLPI : SVRGVEPAPI : CVRGVEPAPI : AURVVNAQPA : ALENTVVPI : ALENTVVPI : RLARSGOTVDSVINREI : RLARSGOTVDSVINREI : SGSGR	* 4 PE PD PD PE PA PDAEDVASISPLA PSPSLVASISPLA PSPSLVASISPLA PDNKVAYISPLS OO SIML	140 GKRFA- 	* 460 	*	480 	*	500 * TLSFRVKEVGD KLSLKVKEVGE VSRDLAIDPIGKN- SMGRPGLDIG FIIPEWAQEIP REEQG-KEYMAATS REEQG-KEYMAATS VEIIEGAE FLSLSGTG FLSLSGTG FLSLSGTG 640 LVYPQQRRLVIAYV	× KMAEEQGLAK: -RVAEERGLEK: -VMYDNDI (SMYEMNLDEF) SSGDANADI 	SGISHDWLTGE GGVNQDWLTAE DMRTFLFSK ERYFNSWFYGR MDRAKEWLTGK MEKAKQWLTGK -HCFDVWS HLFDLTL HVFDVTM HIHDLEA 660 LQDLPAGTPVVV	540 	: 232 : 354 : 241 : 359 : 275 : 264 : 339 : 303 : 295 : 297 : 297 : 297 : 297 : 297 : 281 : 312 : 317 : 289 : 317 : 324 : 326 : 324 : 312

Figure S120. Sequence alignment of TE domain sequences of depside-forming enzymes.

Ser1937 and His2100 of ThiA are highlighted in green.



Figure S121. Heterologous expression of truncated versions of *aquA* (ΔTE and ΔSAT) in *S. cerevisiae*.

EIC(-)MS of 1b (*m/z* 511), 1f (*m/z* 361), 5 (*m/z* 167), and lecanoric acid (*m/z* 317).

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