Biosynthetic Potential-based Strain Prioritization for Natural Product Discovery – a Showcase for Diterpenoid Producing Actinomycetes

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Table S1. Ten media selected for fermentation of *Streptomyces griseus* CB00830.

| mediums          | ingredients                                                                                                                                 |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| medium A<sup>a</sup> (g/L) | Soluble starch 20, glucose 10, peptone 5, yeast extract 5, NaCl 4, K<sub>2</sub>HPO<sub>4</sub> 0.5, MgSO<sub>4</sub>•7H<sub>2</sub>O 0.5, CaCO<sub>3</sub> 2, pH 7. |
| medium B (g/L)   | Dextrin 40, tomato paste 7.5, NZ Amine A 2.5, primary yeast 5, pH 7.                                                                             |
| medium C (g/L)   | Glycerol 50, corn meal 25, ardamine 5, pH 7.                                                                                                 |
| medium D (g/L)   | Lactose 20, glucose 10, primary yeast 4, cotton seed flour 7.5, pH 7.                                                                           |
| medium E (g/L)   | Soluble starch 30, soybean flour 10, yeast extract 5, CaCO<sub>3</sub> 3, pH 7.                                                                  |
| medium F<sup>a</sup> (g/L) | Sucrose 100, glucose 10, casamino acids 0.1, yeast extract 5, MOPS 21, trace elements<sup>b</sup> 1 mL, K<sub>2</sub>SO<sub>4</sub> 0.25, MgCl<sub>2</sub>•6H<sub>2</sub>O 10, pH 7. |
| medium G (g/L)   | Mannitol 20, malt extract 10, ardamine 2.5, pH 7.                                                                                                |
| medium H (g/L)   | Glucose 15, soluble starch 10, beef extract 3, malt extract 2, yeast extract 2, dried yeast 15, corn steep liquor 3, K<sub>2</sub>HPO<sub>4</sub> 0.5, NaCl 0.5, MgSO<sub>4</sub>•7H<sub>2</sub>O 0.5, pH 7. |
| medium I (g/L)   | Glucose 20, yeast extract 5, soytone 10, CoCl<sub>2</sub>•6H<sub>2</sub>O 0.001, CaCO<sub>3</sub> 2, pH 7.                                         |
| Medium J (g/L)   | Glycerol 20, malt extract 4, yeast extract 4, NZ Amine A 2, trace elements 1mL, pH 7.                                                         |

<sup>a</sup>The two media used in constructing the natural product library at TSRI.

<sup>b</sup>Trace elements solution was prepared according to a literature protocol.<sup>23</sup>

Table S2. The 16 strains used to develop and optimize the method (see Fig. S1 for structures of the four classes of natural products).

| classes            | natural products       | producers                      | genes targeting each of the biosynthetic machineries | Ref |
|--------------------|------------------------|--------------------------------|------------------------------------------------------|-----|
|                    |                        |                                | type I PKS                                          |     |
| reduced polyketides|                        |                                | type II PKS                                         |     |
|                    |                        |                                | NRPS                                               |     |
|                    |                        |                                | DTS                                                |     |
| Erythromycin       | *Saccharopolyspora erythraea* | +                              | +                                                    | 1   |
| FK506              | *Streptomyces sp.*     |                                | +                                                    | 2   |
| Tylosin            | *Streptomyces fradiae* |                                | +                                                    | 3   |
| Geldanamycin       | *Streptomyces autolyticus* |                             | +                                                    | 4   |
| Avermectin         | *Streptomyces avermitilis* |                           | +                                                    | 5   |
| Rapamycin          | *Streptomyces hygroscopicus* |                         | +                                                    | 6   |
| aromatic           |                        |                                |                                                     |     |
| polyketides        |                        |                                |                                                     |     |
| Tetracenomycin     | *Streptomyces glaucescens* | +                              |                                                      | 7   |
| Daunorubicin       | *Streptomyces peucetius* |                                | +                                                    | 8   |
| Actinorhodin       | *Streptomyces coelicolor* |                              | +                                                    | 9   |
| Fredericamycin     | *Streptomyces griseus*  |                                |                                                      | 10  |
| nonribosomal       |                        |                                |                                                     |     |
| peptides           |                        |                                |                                                     |     |
| Vancomycin         | *Amycolatopsis orientalis* |                              | +                                                    | 11  |
| Bleomycin          | *Streptomyces verticillus* |                             | +                                                    | 12  |
| Tallysomycin       | *Streptomyces alloteichus hindustanus* |                         | +                                                    | 13  |
| Zorbamycin         | *Streptomyces flavoviridis* |                         | +                                                    | 14  |
| diterpenoids       |                        |                                |                                                     |     |
| Platensimycin      | *Streptomyces platensis* MA7327 | +                              |                                                      | 15  |
| Platencin          | *Streptomyces platensis* MA7339 | +                              |                                                      | 15  |


| position | **viguiepinol (1)** | | **oxaloterpin E (2)** | | **oxaloterpin C (3)** |
|----------|-------------------|-----------------|----------------------|----------------------|
|          | \( \delta_C, \text{ type} \) | \( \delta_H (J \text{ in Hz}) \) | \( \delta_C, \text{ type} \) | \( \delta_H (J \text{ in Hz}) \) | \( \delta_C, \text{ type} \) | \( \delta_H (J \text{ in Hz}) \) |
| 1        | 38.9, \( \text{CH}_2 \) 1.89, dt (13.5, 3.4), \( J \) | 38.5, \( \text{CH}_2 \) 1.89, dt (13.6, 3.4), \( J \) | 38.4, \( \text{CH}_2 \) 1.95, dt (13.8, 3.1), \( J \) |
|          | 1.32, m* | 1.42, m* | 1.44, m* |
| 2        | 28.2, \( \text{CH}_2 \) 1.64, m* | 24.9, \( \text{CH}_2 \) 1.70, m* | 24.5, \( \text{CH}_2 \) 1.81, m*, 1.73, m* |
| 3        | 79.2, \( \text{CH} \) 3.23, dd (10.1, 5.8) | 81.8, \( \text{CH} \) 4.38, dd (11.4, 4.6) | 85.4, \( \text{CH} \) 4.70, dd (11.8, 4.3) |
| 4        | 39.1, C | 38.2, C | 38.3, C |
| 5        | 44.8, \( \text{CH} \) 1.41, m* | 45.0, \( \text{CH} \) 1.51, m* | 45.1, \( \text{CH} \) 1.52, dd (12.4, 5.9) |
| 6        | 18.4, \( \text{CH}_2 \) 1.79, m*, 1.56, m | 18.2, \( \text{CH}_2 \) 1.79, m, 1.56, m* | 18.2, \( \text{CH}_2 \) 1.83, m*, 1.60, m* |
| 7        | 26.8, \( \text{CH}_2 \) 1.67, m*, 1.22, m | 26.7, \( \text{CH}_2 \) 1.66, m*, 1.22, m | 26.6, \( \text{CH}_2 \) 1.68, m*, 1.25, m |
| 8        | 29.4, \( \text{CH} \) 2.28, m | 29.4, \( \text{CH} \) 2.29, m | 29.4, \( \text{CH} \) 2.29, m |
| 9        | 151.1, C | 150.8, C | 150.3, C |
| 10       | 37.6, C | 37.6, C | 37.6, C |
| 11       | 115.9, \( \text{CH} \) 5.36, m | 116.1, \( \text{CH} \) 5.36, m | 116.5, \( \text{CH} \) 5.37, m |
| 12       | 37.6, \( \text{CH}_2 \) 2.02, br d (17.3), 1.76, m | 37.5, \( \text{CH}_2 \) 2.02, br d (15.6), 1.77, m* | 37.5, \( \text{CH}_2 \) 2.03, br d (17.8), 1.72, m* |
| 13       | 34.9, C | 34.9, C | 34.9, C |
| 14       | 41.6, \( \text{CH}_3 \) 1.39, m*, 1.02, m | 41.6, \( \text{CH}_3 \) 1.39, m*, 1.02, m | 41.5, \( \text{CH}_2 \) 1.40, m*, 1.04, m* |
| 15       | 150.3, \( \text{CH} \) 5.82, dd (17.5, 10.7) | 150.3, \( \text{CH} \) 5.82, dd (17.5, 10.7) | 150.2, \( \text{CH} \) 5.82, dd (17.5, 10.7) |
| 16       | 109.1, \( \text{CH}_2 \) 4.93, dd (17.5, 1.0), 4.86, dd (10.8, 1.0) | 109.1, \( \text{CH}_2 \) 4.93, dd (17.5, 1.4), 4.86, dd (10.7, 1.4) | 109.2, \( \text{CH}_2 \) 4.93, dd (17.5, 1.4), 4.87, dd (10.7, 1.4) |
| 17       | 22.5, \( \text{CH}_3 \) 0.97, s | 22.4, \( \text{CH}_3 \) 0.96, s | 22.5, \( \text{CH}_3 \) 0.96, s |
| 18       | 25.3, \( \text{CH}_3 \) 1.07, s | 25.3, \( \text{CH}_3 \) 1.09, s | 25.3, \( \text{CH}_3 \) 1.11, s |
| 19       | 27.8, \( \text{CH}_3 \) 0.99, s | 27.7, \( \text{CH}_3 \) 0.92, s* | 27.8, \( \text{CH}_3 \) 1.04, s |
| 20       | 15.3, \( \text{CH}_3 \) 0.87, s | 16.4, \( \text{CH}_3 \) 0.92, s* | 16.5, \( \text{CH}_3 \) 0.90, s |
| 1'       | 157.0, C | 4.57, br s | 158.2, C |
| 2'       |             |               | 154.2, C |
| \( \text{NH}_2 \) |             | 4.57, br s | 9.60, br s |
| N-OH     |             |               |             |

*a Assignments were based on COSY, HMBC and HSQC experiments.

* These signals overlapped with others.
Table S4. $^1$H (700 MHz) and $^{13}$C (175 MHz) NMR data for 4-chloroanthranilamide (8) in $d_6$-DMSO and methyl 4-chloroanthranilate (9) in CDCl$_3$.\textsuperscript{a}

| position | 4-chloroanthranilamide (8) | | methyl 4-chloroanthranilate (9) | |
|----------|----------------------------|----------------------------|--------------------------------|
| 1        | 112.8, C                    | 109.3, C                    |                                |
| 2        | 151.9, C                    | 151.2, C                    |                                |
| 3        | 115.5, CH 6.73, d (2.2)     | 115.9, CH 6.66, d (2.0)     |                                |
| 4        | 136.8, C                    | 140.0, C                    |                                |
| 5        | 114.5, CH 6.48, dd (8.5, 2.2) | 116.7, CH 6.60, dd (8.6, 2.0) |                                |
| 6        | 131.0, CH 7.53, d (8.5)     | 132.6, CH 7.77, d (8.6)     |                                |
| 7        | 170.9, C                    | 168.0, C                    |                                |
| 8        |                             | 51.7, CH$_3$ 3.86, s        | 5.80, br s                     |
| NH$_2$   | 6.82, br s                  |                            |                                |
| CONH$_2$ | 7.77, br s, 7.14 br s       |                            |                                |

\textsuperscript{a} Assignments were based on COSY, HMBC and HSQC experiments.
Table S5. $^1$H (700 MHz) and $^{13}$C (175 MHz) NMR data for seco-dinactin (12) in comparison with dinactin (10) and feigrisolid C (11) in CDCl$_3$.\(^{a}\)

| position | $\delta_c$, type | $\delta_H$ (J in Hz) | $\delta_c$, type | $\delta_H$ (J in Hz) | $\delta_c$, type | $\delta_H$ (J in Hz) |
|----------|------------------|----------------------|------------------|----------------------|------------------|----------------------|
| 1        | 174.3, C         | 176.8, C             | 175.9, C         |                      |                  |                      |
| 1’       | 174.3, C         |                      | 174.5, C         |                      |                  |                      |
| 2        | 45.1, CH 2.52, m* | 45.5, CH 2.55, m*    | 45.7, CH 2.55, m*|                      |                  |                      |
| 2’       | 45.1, CH 2.52, m* |                      | 45.6, CH 2.55, m*|                      |                  |                      |
| 3        | 79.7, CH 4.03, m* | 80.7, CH 4.04, m*    | 80.3, CH 4.03, m*|                      |                  |                      |
| 3’       | 79.7, CH 4.03, m* |                      | 80.2, CH 4.03, m*|                      |                  |                      |
| 4        | 27.9, CH 1.95, m* | 29.2, CH$_2$ 1.99, m*, 1.64, m*| 29.2, CH$_2$ 2.00, m*|                      |                  |                      |
| 4’       | 27.9, CH 1.97, m* |                      | 28.6, CH$_2$ 1.62, m*|                      |                  |                      |
| 5        | 31.4, CH$_2$ 1.97, m* | 31.1, CH$_2$ 2.06, m*, 1.63, m*| 31.4, CH$_2$ 2.02, m*|                      |                  |                      |
| 5’       | 31.4, CH$_2$ 1.61, m* |                      | 31.3, CH$_2$ 1.56, m*|                      |                  |                      |
| 6        | 76.1, CH 3.83, m* | 76.5, CH 4.04, m*    | 76.5, CH 3.87, m  |                      |                  |                      |
| 6’       | 76.1, CH 3.83, m* |                      | 76.5, CH 3.87, m  |                      |                  |                      |
| 7        | 42.2, CH$_2$ 1.75, m* | 42.3, CH$_2$ 1.80, m  | 42.5, CH$_3$ 1.85, m*|                      |                  |                      |
| 7’       | 42.2, CH$_2$ 1.75, m* |                      | 42.3, CH$_3$ 1.85, m*|                      |                  |                      |
| 8        | 68.9, CH 4.92, m* | 68.9, CH 5.07, m    | 69.6, CH 5.05, m  |                      |                  |                      |
| 8’       | 68.9, CH 4.92, m* |                      | 68.8, CH 5.00, m  |                      |                  |                      |
| 9        | 174.0, C         | 174.4, C             | 174.4, C         |                      |                  |                      |
| 9’       | 174.0, C         |                      | 174.2, C         |                      |                  |                      |
| 10       | 45.0, CH 2.52, m* | 45.1, CH 2.55, m*    | 45.6, CH 2.55, m*|                      |                  |                      |
| 10’      | 45.0, CH 2.52, m* |                      | 45.5, CH 2.55, m*|                      |                  |                      |
| 11       | 79.9, CH 4.03, m* | 81.1, CH 4.04, m*    | 80.9, CH 4.03, m*|                      |                  |                      |
| 11’      | 79.9, CH 4.03, m* |                      | 80.9, CH 4.03, m*|                      |                  |                      |
| 12       | 28.1, CH$_2$ 1.95, m | 28.8, CH$_2$ 1.99, m*, 1.64, m*| 28.4, CH$_3$ 1.97, m*|                      |                  |                      |
| 12’      | 28.1, CH$_2$ 1.47, m* |                      | 28.2, CH$_3$ 1.60, m*|                      |                  |                      |
| 13       | 31.2, CH$_2$ 1.97, m | 30.5, CH$_2$ 2.06, m*, 1.63, m*| 31.1, CH$_3$ 2.05, m*|                      |                  |                      |
| 13’      | 31.2, CH$_2$ 1.61, m* |                      | 30.7, CH$_3$ 1.63, m*|                      |                  |                      |
| 14       | 76.2, CH 3.83, m* | 77.1, CH 4.17, m    | 76.6, CH 4.17, m  |                      |                  |                      |
| 14’      | 76.2, CH 3.83, m* |                      | 76.6, CH 4.05, m*|                      |                  |                      |
| 15       | 39.8, CH$_2$ 1.75, m* | 40.5, CH$_2$ 1.73, m*| 40.8, CH$_3$ 1.78, m*|                      |                  |                      |
| 15’      | 39.8, CH$_2$ 1.75, m* |                      | 40.2, CH$_3$ 1.78, m*|                      |                  |                      |
| 16       | 73.1, CH 4.92, m* | 70.3, CH 3.77, m    | 73.5, CH 4.87, m  |                      |                  |                      |
| 16’      | 73.1, CH 4.92, m* |                      | 70.3, CH 3.78, m  |                      |                  |                      |
| 17       | 12.5, CH$_3$ 1.08, m* | 13.7, CH$_3$ 1.14, d (7.0) | 13.3, CH$_3$ 1.19, d (7.0) |                      |                  |                      |
| 17’      | 12.5, CH$_3$ 1.08, m* |                      | 13.1, CH$_3$ 1.12, d (6.9) |                      |                  |                      |
| 18       | 20.3, CH$_3$ 1.21, br s | 20.3, CH$_3$ 1.27, d (6.3) | 20.6, CH$_3$ 1.27, d (6.3) |                      |                  |                      |
| 18’      | 20.3, CH$_3$ 1.21, br s |                      | 20.4, CH$_3$ 1.25, d (5.8) |                      |                  |                      |
| 19       | 13.1, CH$_3$ 1.08, m* | 13.6, CH$_3$ 1.19, d (7.0) | 13.4, CH$_3$ 1.11, d (5.6) |                      |                  |                      |
| 19’      | 13.1, CH$_3$ 1.08, m* |                      | 13.4, CH$_3$ 1.10, d (5.6) |                      |                  |                      |
| 20       | 27.3, CH$_2$ 1.60, m* | 30.0, CH$_2$ 1.55, m | 30.0, CH$_2$ 1.64, m*|                      |                  |                      |
| 20’      | 27.3, CH$_2$ 1.45, m* |                      | 27.5, CH$_3$ 1.54, m*|                      |                  |                      |
| 21       | 9.2, CH$_3$ 0.86, br s | 10.1, CH$_3$ 0.95, t (7.4) | 9.3, CH$_3$ 0.90, t (7.4) |                      |                  |                      |
| 21’      | 9.2, CH$_3$ 0.86, br s |                      | 10.1, CH$_3$ 0.95, t (7.4) |                      |                  |                      |

\(^{a}\) Assignments were based on COSY, HMBC and HSQC experiments.

\(^{*}\) These signals overlapped with others.
**Table S6.** Primers used for the amplification and sequencing of the diterpene synthases and the housekeeping genes from *S. griseus* CB00830.

| Primer       | Nucleotide Sequence (5'-3')                      | Function                          |
|--------------|--------------------------------------------------|-----------------------------------|
| 830T2_F      | GAATGCAGACGACCACCGATCTC                         | Amplification/sequencing of *ent-cpps* |
| 830T2_R      | CAGATCGGAGCAGGACCTTCAG                          | Amplification/sequencing of *ent-cpps* |
| 830T2_MF     | CGGGCATACCTCGATGATCAGACCTAC                     | Sequencing of *ent-cpps*          |
| 830PMDS_F    | CGAGGACAGATCGCGAGAATCC                          | Amplification/sequencing of *pmds* |
| 830PMDS_R    | TCAGTCGTTCCGT CGA TCA GGC                       | Amplification/sequencing of *pmds* |
| 830GGDP_F    | GTGGTCACCTTGGTGGAACACACGG                       | Amplification/sequencing of *ggs*  |
| 830GGDP_R    | CGGTACACTTACTACAGGG                           | Amplification/sequencing of *ggs*  |
| 16SrRNA_for  | AGAGTTTGATCCTGGCTCAG                            | Phylogenetic analysis             |
| 16SrRNA_rev  | ACGGCTACCTTGGTGACTCTT                         | Phylogenetic analysis             |
| recA_for     | TAATACGACTCAGATAGGCCGCGCCTGGCTGC               | Phylogenetic analysis             |
| recA_rev     | CGATCAAAAGAGTCGGTCGCTGGGTGTTGTCTAAGGG          | Phylogenetic analysis             |
| rpoB-2       | CATCGACCACTTCGGCAAC                            | Phylogenetic analysis             |
| ActRpoB3303R | GAANCGCTGDCRCGCCAACATTG                        | Phylogenetic analysis             |
| trpBfor      | TAATACGACTCAGATAGGCCGCGCAGA                    | Phylogenetic analysis             |
| trpBrev      | CGATCAAAAGAGTCGGCGATGGCCGGG                   | Phylogenetic analysis             |
|              | ATGATGCC                                           |                                    |
Figure S1. Structures of the 16 natural products chosen as representatives for the four major classes of natural products (see Table S2 for each of the producers).

**Reduced polyketides**

- Erythromycin
- FK506
- Tylosin
- Geldanamycin
- Avermectin
- Rapamycin

**Aromatic polyketides**

- Tetracenomycin
- Daunorubicin
- Adriamycin
- Fredericamycin

**Nonribosomal peptides**

- Zoribactam
- Tallysonycin
- Bioxymycin
- Vancocycin

**Diterpenes**

- Patansinmycin
- Patencin
**Figure S2.** Validation of the method using 16 known producers. (A) Sequence alignments of genes encoding the selected type I PKSs, type II PKSs, NRPSs, and DTSs from the 16 strains. Asterisks (*) indicate complete conservation among the selected protein sequences; double dots (:) indicate conserved substitutions; single dots (.) indicate semi-conserved substitutions; underlined asterisks (*) indicate the protein sequences selected for degenerate primer design. The number of amino acids between two underlined sequences are shown below each of the sequence alignments. Protein sequences used in Type I PKS sequence alignment: Aves1 (NP822113), GdmAI (ADY00167), FkbC (ADX99523), RapB (CAA60459) and TylG (AAB66504); protein sequences used in Type II PKS sequence alignment: Fdm1 (KSα AAQ08916, KSβ AAQ08917), Act1 (KSα NP_629237, KSβ NP_629238), Dauno1 (KSα AAA65206, KSβ AAA65207) and Tetra1 (KSα P16538, KSβ P16539); protein sequences used in NRPS sequence alignment: ZbmX (ACG60782), BlmVI (AAG02359), TlmVI (ABL74940) and Vcm2 (YP_008010469); protein sequences used in DTS sequence alignment: ent-CPPS (BAD86797), PtmT2 (ACO31276) and PtntT2 (ADD83015). (B) Agarose gel electrophoresis showing distinct products with the predicted size of the pool of PCR products; lane 1, type I PKS; lane 2, type II PKS; lane 3, NRPS; lane 4, DTS; 5, DNA marker. (C) Dot blot of the 16 strains; (1) type I PKS; (2) type II PKS; (3) NRPS; (4) DTS. All the known producers for the four representative classes of natural products are highlighted with red boxes. (D) PCR confirmation for the dot blot results; (1) type I PKS; (2) type II PKS; (3) NRPS; (4) DTS. All the known producers for the four representative classes of natural products are highlighted with red boxes.
**A**

**Type I PKS**

- Aves1 (59) QGGLFLDAAHTDAGFGISPREALAMDPQQRLLETASE-----RLSDAVRHLGKVVLAVRGSASVQDGASNLTAAPGFAQERVQALNAG (298)
- GdmAI (61) BEGGFLTGAQFFTDAAFGKISPREALAMDPQQRLLETASE-----RSLDAARMHEVLAVRGSATVQDGASNLTAAPGFAQERVQALNAG (298)
- FlbC (59) --------GALRAGGDAAFGKISPREATAALPDQQRLLETASE-----RSLDAARMHEVLAVRGSATVQDGASNLTAAPGFAQERVQALNAG (298)
- RapB (58) VQGGFLQDAFFGKISPREALAMDPQQRLLETASE-----RSLDAARLGRQKVLAVRGSASVQDGASNLTAAPGFAQERVQALNAG (288)
- Ery1 (50) BEGGFLHADAFDPSVFKISPREALAMDPQQRLLETASE-----RSLDAARLGRQKVLAVRGSASVQDGASNLTAAPGFAQERVQALNAG (288)

**≈204 aa**

**Type II PKS**

- KSα
  - Pdm1 (291) ALDEARINPDEVDYANAHGSGTKQNDRHETAAYKR SLGEHA (332)
  - Act1 (335) ALDESRTDATDIDYINAHGSGTRQNDRHETAAYKRALGEHA (375)
  - Dauno1 (292) ALDEARRDPSDVDYVNAHGTATKQNDRHETSAFKRSLGEHA (332)
  - Tetra1 (295) ALDQARRTGDDLHYINAHGSGTRQNDRHETAAFKRSLQRA (336)

- KSβ
  - ZlnX (94) QAGPGNAAYILFTSGSTGEPKGVVVEHRNVVASLR-----ERHYTVGDVRCLSGLGDFRQDQKVIRGRFVLESIIEAVLDAHPDVHAA-- (382)
  - BlnVI (84) SVHPEQLAAVMATSGSTGTPKTIGVPRALAGYLR-----ARRYRTGDLARRLPDGTLLAGRADRQVKIRGRHRVEPGEVEQVLGGHPVRE---- (364)
  - TlmVI (88) BTHALACVMPTSSTGVPVLVGVHGMDYLR-----ARRYRTGDLARRLPDGTLLAGRADRQVKIRGRHRVEPGEVEQVLGGHPVRE---- (364)
  - Vcm2 (111) RVDPRSAAYVIYTSGSTGTPKGVVVTHT GLGNLA-----------ERHYRTGDLAYWTDQGELVSAGRADDQVKIRGFRVEPREIEFALSAYPGITQATV (391)

**≈300 aa**

**NRPS**

- ZlnX (94) QAGPGNAAYILFTSGSTGEPKGVVVEHRNVVASLR-----ERHYTVGDVRCLSGLGDFRQDQKVIRGRFVLESIIEAVLDAHPDVHAA-- (382)
  - BlnVI (84) SVHPEQLAAVMATSGSTGTPKTIGVPRALAGYLR-----ARRYRTGDLARRLPDGTLLAGRADRQVKIRGRHRVEPGEVEQVLGGHPVRE---- (364)
  - TlmVI (88) BTHALACVMPTSSTGVPVLVGVHGMDYLR-----ARRYRTGDLARRLPDGTLLAGRADRQVKIRGRHRVEPGEVEQVLGGHPVRE---- (364)
  - Vcm2 (111) RVDPRSAAYVIYTSGSTGTPKGVVVTHT GLGNLA-----------ERHYRTGDLAYWTDQGELVSAGRADDQVKIRGFRVEPREIEFALSAYPGITQATV (391)

**≈263 aa**

**DTS**

- ent-CPPS (57) AQQHEDGTWGGPAAYGLLPTLSAVDALLSVAGTQDARR RGQGDTRYDHAAERVGGWLVEQQQPDGRWMDKWHASPYYATACG (391)
- PtmT2 (61) KEQNQDGSWGAPDGYGLVPTLSAVEALLTELARTDSGA RPDDAGRYGAEIRMISDWLLDNQLPDGSWMDKWHASPYYATACG (414)
- PtnT2 (61) EEQNQDGSWGAPDGYGLVPTLSAVEALLTELARP RPDDAGRYGAEIRMISDWLLDNQLPDGSWMDKWHASPYYATACG (414)

**≈335 aa**

**B**

**C**

**D**

1 2 3 4 5

1500 bp
1000 bp
750 bp
500 bp
250 bp

(1) (2) (3) (4)

A A A A A A A B B B C C C C C C C

(1) (2) (3) (4)
Figure S3. Dot blot survey of the randomly selected 100 strains for their biosynthetic potential of four targeted classes of natural products. (1) Type I PKS; (2) type II PKS; (3) NRPS; (4) DTS. The strain selected to do large scale fermentation and isolation, *Streptomyces griseus* CB00830, is highlighted with a red box.
Figure S4. Key COSY and HMBC correlations of compounds 1-3.

1

H
H
H
H
1H-1H COSY correlation

H
C
HMBC correlation

2

H
N
O
2

3

H
N
O
3

Figure S5. Key COSY and HMBC correlations of compounds 10 and 12.

10

H
H
1H-1H COSY correlation

H
C
HMBC correlation

12
Figure S6. The biosynthetic loci of compounds 1-3 in *S. griseus* CB00830 and *S. sp. KO-3988*. The diterpene synthase genes proposed for diterpene biosynthesis in *S. griseus* CB00830 (accession ##) are aligned with the viguiepinol and mevalonate biosynthetic gene cluster from *S. sp. KO-3988* (AB183750). DNA and protein identities are listed below each corresponding gene. The three diterpene synthase genes sequenced were *ent*-copalyl diphosphate synthase (*ent-cpps*), pimaradiene synthase (*pmds*), and geranylgeranyl diphosphate synthase (*ggpps*).
Figure S7. Phylogenetic analysis assigns CB00830 (highlighted in red box) as a *Streptomyces griseus* species. The phylogenetic tree was generated from the alignment of concatenated partial sequences\(^\text{18,19}\) of the four housekeeping genes 16S rRNA, *recA*, *rpoB*, and *trpB* (2975 bp total) using the Tamura-Nei evolutionary distance method\(^\text{20}\) and the neighbor-joining algorithm. Bootstrap\(^\text{21}\) values >60% (based on 1000 resampled trials) are given at nodes. Bar, 0.02 substitutions per nucleotide position. Sequence alignment and phylogenetic tree construction were conducted with MEGA 5.05.\(^\text{22}\) *S. coelicolor* A3(2) (AL645882), *S. avermitilis* MA-4680 (BA000030), *S. rimosus* ATCC 10970 (ANSJ00000000), *S. griseus* NBRC 13350 (AP009493), *S. scabies* 87.22 (NC_013929), *S. venezuelae* ATCC 10712 (FR845719), and *S. clavuligerus* ATCC 27064 (ADWJ00000000) were obtained from the NCBI database and used for representative *Streptomyces* spp. *Mycobacterium tuberculosis* H37Rv (AL123456) was used as an outgroup.
Figure S8. $^1$H NMR (700 MHz) and $^{13}$C (175 MHz) spectra of grisechelin A (4) in $d_6$-DMSO.
Figure S9. $^1$H NMR (700 MHz) and $^{13}$C (175 MHz) spectra of grisechelin B (5) in CDCl$_3$. 
Figure S10. $^1$H NMR (700 MHz) and $^{13}$C (175 MHz) spectra of grisechelin C (6) in CDCl$_3$. 
Figure S11. $^1$H NMR (700 MHz) and $^{13}$C (175 MHz) spectra of grisellin D (7) in $d_6$-DMSO.
Figure S12. $^1$H NMR (700 MHz) and $^{13}$C (175 MHz) spectra of seco-dinactin (12) in CDCl$_3$. 
Figure S13. Circular dichroism spectrum of grisechelin A (4) (317 μM in CHCl₃).
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