Biosynthesis of thiocarboxylic acid-containing natural products

Liao-Bin Dong, Jeffrey D. Rudolf, Dingding Kang, Nan Wang, Cyndi Qixin He, Youchao Deng, Yong Huang, K. N. Houk, Yanwen Duan & Ben Shen

Thiocarboxylic acid-containing natural products are rare and their biosynthesis and biological significance remain unknown. Thioplatensimycin (thioPTM) and thioplatencin (thioPTN), thiocarboxylic acid congeners of the antibacterial natural products platensimycin (PTM) and platencin (PTN), were recently discovered. Here we report the biosynthetic origin of the thiocarboxylic acid moiety in thioPTM and thioPTN. We identify a thioacid cassette encoding two proteins, PtmA3 and PtmU4, responsible for carboxylate activation by coenzyme A and sulfur transfer, respectively. ThioPTM and thioPTN bind tightly to β-ketoacyl-ACP synthase II (FabF) and retain strong antibacterial activities. Density functional theory calculations of binding and solvation free energies suggest thioPTM and thioPTN bind to FabF more favorably than PTM and PTN. Additionally, thioacid cassettes are prevalent in the genomes of bacteria, implicating that thiocarboxylic acid-containing natural products are underappreciated. These results suggest that thiocarboxylic acid, as an alternative pharmacophore, and thiocarboxylic acid-containing natural products may be considered for future drug discovery.
Thiocarboxylic acids are an underappreciated pharmacophore in drug discovery and development. In contrast to carboxylic acids, which are one of the most important and biologically active pharmacophores in modern therapeutics, the significance of thiocarboxylic acids is often overlooked due to their intrinsic instability, difficult preparation, and rare occurrence in nature. In addition, the scarcity of thiocarboxylic acid-containing natural products, of which there are only two, thioplatensimycin (PTM, 1) and thioplatencin (PTN, 2) are newly discovered thiocarboxylic acid-containing congeners of the antibacterial natural products platensimycin (PTM, 3) and platencin (PTN, 4) (Fig. 1a–c). PTM and PTN were originally isolated from Streptomyces platensis MA7327 and MA7339, respectively, representing a class of promising natural product antibiotics that target bacterial and mammalian fatty acid biosynthesis.

![Diagram](image)

**Fig. 1** In vivo characterization of thiocarboxylic acid biosynthesis. a Genetic organization of the *ptm* gene clusters from the dual PTM–PTN producers *S. platensis* MA7327, *S. platensis* CB00739, and *S. platensis* CB00765. b Genetic organization of the *ptn* gene cluster from the PTN-exclusive producer *S. platensis* MA7339. The thioacid cassette investigated in this study, *ptmU4* and *ptmA3*, are present and highlighted (red rectangle) in both the *ptm* and *ptn* gene clusters. c Structures of thioplatensimycin (1, thioPTM), thioplatencin (2, thioPTN), platensimycin (3, PTM), and platencin (4, PTN). The aliphatic ketolide and 3-amino-2,4-dihydroxybenzoic acid (5, ADHBA) moieties are highlighted in blue and red, respectively. d Structures of ADHBSH (5, ADHBA) and ADHBA (5, OH, 5). e UV at 280 nm from LC-MS analysis of metabolites from SB12039 (*ΔptmA3*), SB12040 (*ΔptmU4*), SB12041 (*ΔptmS1*), SB12042 (*ΔptmS2*), and SB12043 (*ΔptmS4*) using the PTM–PTN dual overproducer, SB12029, as a positive control.

f Extracted ion chromatograms (EIC, m/z at both 170 and 186) from LC-MS analysis of metabolites from heterologous reconstitution of 5-SH in model Streptomyces hosts. SB12306 was individually scanned using m/z 170 and 186. std standard.
Syntheses9-11. Structurally, they are comprised of two distinct moieties, a diterpene-derived lipophilic ketolide and a 3-amino-2,4-dihydroxybenzoic acid (ADHBA, 5), linked by an amide bond (Fig. 1c, d)12,13. We recently developed a dual PTM–PTN overproducing strain, SB12029, through the inactivation of the negative transcriptional regulator ptmR1 in the ptm gene cluster of Streptomyces platensis CB00739 (Fig. 1a, b), which is now used as a model strain for the study of PTM and PTN biosynthesis14,15. When following previously reported procedures for the production of 3 and 4 in SB1209, we discovered that 1 and 2 were also produced in high titers8. Although 1 and 2 were not isolated, we unambiguously established that 1 and 2 possessed thiocarboxylic acid moieties by a combination of high-resolution electrospray ionisation mass spectrometry and chemical transformation16. A sulfur-containing PTM pseudodimer, PTM D1, was also isolated supporting the presence of 1 and 2 in SB12099. The discovery of 1 and 2, and natural congeners thereof (Supplementary Fig. 1b)17,18, questioned whether 3 and 4 were final biosynthetic products of the ptm and ptn biosynthetic gene clusters and set the stage to study thiocarboxylic acid biosynthesis and explore the chemistry and biology of thiocarboxylic acid-containing natural products.

Here we report the (i) production of 1 and 2 by both wild-type strains and engineered overproducers known to produce both 3 and 4 or only 4, supporting the legitimacy of the thioacids congeners as natural products; (ii) identification and in vivo and in vitro characterization of a thioacid cassette, which, in combination with the sulfur-carrier protein machinery, is responsible for thiocarboxylic acid biosynthesis; (iii) discovery that the thioacid cassette is broadly distributed in nature; (iv) biological implications of thiocarboxylic acid biosynthesis; (iii) discovery that the thioacid cassette, which encodes PtmA3 and PtmU4, is a potential as a pair of biocatalysts for thiocarboxylic acid synthesis. This work provides clear biochemical evidence supporting that the thioacid cassette, which encodes PtmA3 and PtmU4, is responsible for carboxylate activation by coenzyme A (CoA) and sulfur transfer, respectively, and works together with the sulfur-carrier protein trafficking system for thiopTPTM and thioPTN biosynthesis. Furthermore, given the subtleties in properties between carboxylic and thiocarboxylic acids and the proven activities of thiopTPTM and thioPTN, thiocarboxylic acid and thiocarboxylic acid-containing natural products in general should now be considered as an alternative pharmacophore in future drug discovery efforts.

Results

ptm and ptn gene clusters producing thiopTPTM and thioPTN. As 1 and 2 were initially discovered from SB1209, we were curious if the thiocarboxylic acid congeners were simply a result of the overproducing nature of the strain. We selected eight strains, four wild-type strains and four engineered overproducers known to produce both 3 and 4 or only 4. The wild-type strains S. platensis MA73279 and MA73309, S. platensis CB00739, and S. platensis CB00765 (an alternative PTM–PTN dual producer)15, and the overproducing strains SB12001 (MA7327 ΔptmR)16, SB12600 (MA7339 ΔptmR)17, SB12026 (CB00739 ΔptmR)15, and SB12027 (CB00765 ΔptmR)15 were fermented under conditions known for 1 and 2 production (Supplementary Methods). Liquid chromatography-mass spectrometry (LC-MS) analysis of all eight strains revealed the production of both 1 and 2 (along with 3 and 4) in the PTM–PTN dual producers or 2 (along with 4) in PTN-exclusive producers (Supplementary Fig. 2a, b). A time-course of SB12029 fermentation revealed that only 1 and 2 were detected on day 1, with increasing amounts of 3 and 4 as the fermentation continued (Supplementary Fig. 2c, d). These findings support that 1 and 2, rather than 3 and 4, might be the bona fide natural products of the PTM and PTN biosynthetic machineries. Comparison of the ptm and ptn gene clusters (Fig. 1a, b)15,19 with gene clusters known for biosynthesis of the thiocarboxylic acid- or thiocontaining natural products TQB (qbs)9,10, PDTC (pdb)11, and yataykemycin (YT, ytk)19 revealed two conserved genes encoding an acyl-CoA synthetase [PTmA3, ∼30% protein sequence identity with QbsL (32%), OrfI (28%, from pdb), and YtkG (32%)] and a type III CoA-transferase [PTmU4, ∼36% identity with QbsK (37%), OrfI (36%, from pdb), and YtkF (35%)]. We individually inactivated ptmA3 and ptmU4 in the PTM–PTN dual overproducer SB12029 (Supplementary Tables 1 and 2, Supplementary Data 1, and Supplementary Figs. 3–6). The resultant mutants SB12039 (ΔptmA3) and SB12040 (ΔptmU4) lost their ability to produce 1 and 2 but still produced 3 and 4 (Fig. 1e). The production of 3 and 4 in SB12039 and SB12040 implies that (i) 3 and 4 are directly converted into 1 and 2, respectively, or (ii) 5 is converted into 5-SH prior to its coupling with the ketolides (Fig. 2a, b)19. To verify the timing of thiocarboxylic acid formation in the biosynthesis of 3 and 4, we introduced ptmB1, ptmB2, and ptmB3, which encodes the biosynthesis of 5 (Fig. 2a)21, together with ptmA3 and ptmU4 into four model Streptomyces hosts to afford S. albus SB12303, S. lividans SB12304, S. coelicolor SB12305, and S. avermitilis SB12306 (Supplementary Fig. 7). LC-MS analysis revealed that, while SB12303–SB12305 did not produce either 5 or 5-SH, SB12306 produced both 5 and 5-SH (Fig. 1f). Production of 5-SH in the heterologous host S. avermitilis confirms that PtmA3 and PtmU4 are required for thiocarboxylic acid biosynthesis, while the sulfur donor for thiocarboxylic acid formation is conserved between S. platensis and S. avermitilis (Supplementary Table 3), and likely among all Streptomyces species.

Utilizing sulfur-carrier proteins as the sulfur donor. Thiocarboxylic acids at the C-termini of small sulfur-carrier proteins are utilized as direct sulfur donors for the biosynthesis of essential cofactors including thiamine and molybdobenazine cofactor in nearly all bacteria species22,23. The biosynthesis of this protein thio-carboxylate species is well-established and occurs via nucleophilic attack of a protein-tethered persulfide on an adenylated carboxylate of a C-terminal glycine (G)22,23. Given that 5-SH was readily produced in S. avermitilis, and the fact that the qbs and pdb gene clusters possess their own cognate members of the sulfur-carrier protein system5-7, we suspected that the native sulfur donor for 1 and 2 is from similar primary metabolism machinery. Since no homologs are present in the ptm gene cluster, we searched the genome of CB00739 for potential candidates. Four sulfur-carrier proteins (PtmS2, PtmS3, SpSCP1, and SpSCP2), one MoA homolog (PtmS4), and one JAMM family metallopeptase (PtmS1) were encoded within the genome (Supplementary Fig. 8). Both PtmS2 and PtmS3 have capped C-termini with either two residues (MV) or a single residue (C) following the GG motif, respectively (Supplementary Fig. 9); SpSCP1 and SpSCP2 have exposed C-terminal GG sequences. Typically, sulfur-carrier proteins with capped C-termini require a protease, as exemplified by QbsD in TQB biosynthesis and whose biochemical function was previously reported, to expose the C-terminal GG sequence for subsequent activation and sulfur transfer24. Since the protease-encoding ptmS1 was found clustered with the capped sulfur-carrier protein-encoding ptmS2 in CB00739 (Supplementary Fig. 8), we hypothesized that PtmS2 may be utilized for the biosynthesis of 1 and 2. Similar two-gene cassettes were found in the qbs and pdb gene clusters5-7, as well as in the genome of S. avermitilis (Supplementary Table 3).
Fig. 2 Proposed biosynthetic pathway and in vitro characterization of thiocarboxylic acid biosynthesis. a 5-SH is the enzymatic product of the transformation of 5 by the thioacid cassette. PtmA3 and PtmU4, capturing a sulfur atom from the sulfur-carrier protein machinery. The C-terminal GG motif and capped residues (MV in PtmS2) are highlighted in red and blue, respectively. SCP sulfur-carrier protein (circled), ASA aspartate semialdehyde, DHAP dihydroxyacetone phosphate, AHBA (6) 3-amino-4-hydroxybenzoic acid, ADHBCoA (5-CoA) 5-(3-amino-2,4-dihydroxybenzoate) coenzyme A. b ThioPTM (1) and thioPTN (2) result from the coupling of 5-SH with platensiclyl- and platencinyl-CoA, respectively. c UV at 260 nm from HPLC analysis of in vitro PtmA3 reactions with 5 and 6, std standard. d UV at 260 nm from HPLC analysis of in vitro PtmU4 reactions with 5-CoA using the native sulfur donor. Sodium thiosulfate (Na2S2O3) was used as the inorganic sulfur source.

We individually inactivated ptmS1, ptmS2, and ptmS4 in SB12029, yielding mutants SB12041 (∆ptmS1), SB12042 (∆ptmS2), and SB12043 (∆ptmS4), respectively (Supplementary Figs. 10–15). Similar to SB12039 and SB12040, both SB12041 and SB12043 lost their ability to produce 1 and 2 but still produced 3 and 4 (Fig. 1e). In contrast, SB12042 retained its ability to produce 1 and 2 (Fig. 1e), indicating that PtmS2 may be functionally redundant (e.g., complemented by PtmS3). The inability of SB12041 and SB12043 to produce 1 and 2 corroborates the use of capped sulfur-carrier proteins (i.e., PtmS2 and PtmS3), as opposed to proteins with pre-exposed GG motifs (i.e., SpSCP1 and SpSCP2), by the PTM and PTN biosynthetic machineries.

Reconstituting thiocarboxylic acid biosynthesis in vitro. Upon incubation of 5, ATP, and CoA in the presence of PtmA3 (Supplementary Fig. 16a, c), high-performance liquid chromatography (HPLC) analysis of the reaction mixture revealed the disappearance of 5 and concomitant production of S-(3-amino-2,4-dihydroxybenzoate) coenzyme A (ADHBCoA, 5-CoA, Fig. 2a, c and Supplementary Figs. 17 and 18). Substitution of 5 with 3 or 4 under the same assay conditions failed to afford any new products, confirming that 3 and 4 are not the precursors of 1 and 2, respectively (Supplementary Fig. 19). Steady-state kinetics of PtmA3, using 5 or 3-amino-4-hydroxybenzoic acid (AHBA, 6, Fig. 2a, c, Supplementary Figs. 20a, b, 21, and 22), revealed 12-fold higher catalytic efficiency (kcat/Km) for 5 over 6 (Table 1), supporting 5 as the native substrate of PtmA3. When 5-CoA, ATP, and either PtmS2GG or PtmS4GG [the C-terminal caps were removed during cloning24 as the native sulfur donor, supplemented with sodium thiosulfate (Na2S2O3)23,25, were incubated with PtmS4 and PtmU4 (Supplementary Fig. 16a–c), LC-MS analysis of the assay mixture revealed the appearance of free CoA and 5-SH (Fig. 2d). Replacement of the native sulfur donor with potassium hydroxysulfide (KSH) as a sulfur donor surrogate26 led to increased 5-SH formation (Supplementary Fig. 23a), isolation of
which allowed unambiguous structural characterization by $^1$H and $^{13}$C NMR (Supplementary Figs. 24 and 25).

**PtmU4 catalyzing sulfur transfer.** PtmU4 was bioinformatically predicted to possess two type III CoA-transferase domains. CoA-transferases, which typically perform reversible CoA reactions from CoA-thioester donors to various organic acid CoA acceptors are classified into three subgroups (types I–III) based on differences in their sequences and reaction mechanisms (Supplementary Fig. 26)\(^27\). Canonical type III CoA-transferases are proposed to utilize a conserved Asp residue to form mixed anhydride and covalent thioester intermediates with the CoA donor and CoA moiety, respectively; the liberated donor free acid is not released prior to binding the CoA acceptor (Fig. 3a and Supplementary Figs. 26a and 27)\(^{27,28}\). Type III CoA-transferases can be further separated into one-domain and two-domain proteins. Members of the two-domain family have tandem CoA-transferase domains, which have highest homology at the N-termini of the individual domains, separated by a poorly conserved linker\(^29\). DddD, the only characterized two-domain type III CoA-transferase, dually functions as a CoA-transferase and a lyase mediating the first two steps of dimethyl sulfide release from dimethylsulfoniopropionate (Supplementary Fig. 26c)\(^30\). Phylogenetic analysis of selected one- and two-domain type III CoA-transferases from bacteria revealed that PtmU4/PtmU4 clades with other two-domain type III CoA-transferases and separated from all one-domain type III CoA-transferases and DddD (Fig. 3b)\(^30\). DddD forms an outgroup from both the one-domain and two-domain type III CoA-transferases.

Sequence alignment of PtmU4 and its homologs revealed that they indeed possess a conserved Asp at the C-terminal CoA transferase domain (Fig. 3a and Supplementary Fig. 27). To determine if PtmU4 requires this conserved Asp for thioarboxylic acid formation, Asp430 was mutated to Ala, Glu, or Asn by site-directed mutagenesis (Supplementary Fig. 16d). Circular dichroism indicated that the structures of these mutants were not significantly perturbed relative to native PtmU4 (Supplementary Fig. 16e and Supplementary Methods). The activities of each mutant were dramatically reduced (125–400-fold), supporting Asp430 as a key residue in PtmU4 catalysis (Supplementary Fig. 23b). Although trace amounts of 5-SH were identified in the negative control reaction without a native sulfur donor (Fig. 2d), the ∼3-fold rate enhancement of sulfur transfer by PtmU4 using either PtmS2\(^{42}\) or PtmS3\(^{42}\) (Fig. 2d), along with the near loss of activity in the Asp430 mutants, confirms genuine enzyme catalysis.

**Thiocarboxylic acids as a potential pharmacophore.** If 1 and 2 are the bona fide final metabolites of the PTM and PTN biosynthetic machineries, it is fascinating that 3 and 4, non-enzymatic hydrolyzed metabolites of the PTM and PTN pathway (Fig. 2a, b), possess such exquisite biological activity. We isolated and spectroscopically characterized 1 and 2 (Supplementary Tables 4 and 5 and Supplementary Figs. 28–37), and tested their antibacterial activities against Staphylococcus aureus ATCC 25923 and Kocuria rhizophila (previously Micrococcus luteus) ATCC 9431 (Supplementary Methods)\(^31\). Both 1 and 2 retained strong antibacterial activities with minimum inhibitory concentrations (MICs) ranging from 1 to 4 μg mL\(^{-1}\) (2–8-fold higher than those of 3 and 4, Table 2 and Supplementary Fig. 38a). As the ADHBA moiety of 3 and 4 makes key interactions with the target proteins FabF and FabH\(^9,10\), the ADHBSH moieties of 1 and 2 likely bind in a similar manner. Binding assays, using the Escherichia coli FabF C163Q mutant, which mimics the acyl-enzyme intermediate\(^6\), revealed that the dissociation constants ($K_d$) for 1 and 2 were ~2-fold tighter than those of 3 and 4 (Table 2 and Supplementary Figs. 38b and 39). Density functional theory (DFT) calculations were performed on the ADHBA moieties of 3 and 4 and the ADHBSH moieties of 1 and 2 with two interacting residues, H303 and H340, in the E. coli FabF C163Q mutant (PDB 2GFX, Supplementary Data 3)\(^9\). The carboxylate of 5 forms stronger hydrogen bonds ($\Delta G = −12.0$ kcal mol\(^{-1}\)) than the imidazoles of the His residues than the thiocarboxylate of 5-SH (two conformations, each $\Delta G = −9.6$ kcal mol\(^{-1}\), Fig. 4a). In a three-residue model (H303, H340, Q163), the geometry of the 5-SH−BH conformation is favored over 5-SH−a by 3.5 kcal mol\(^{-1}\); consistent with the two His model, ADHBA binding is favored over ADHBSH (5-SH−b) by 2.5 kcal mol\(^{-1}\) (Fig. 4b), Calculation of solvation free energies of 5 (−58.2 kcal mol\(^{-1}\)) and 5−SH (−55.1 kcal mol\(^{-1}\)) revealed that 5-SH is less well-solvated in water (Fig. 4c). Therefore, although 5 forms a more stable complex, 5−SH exhibits a higher activity (favored binding) due to a net energy difference\(^32\) of 0.6 kcal mol\(^{-1}\).

**Biocatalysts for thioarboxylic acid synthesis.** We first examined the promiscuity of PtmA3 and PtmU4 using a series of aryl acids with KSH as the sulfur donor. Both PtmA3 and PtmU4 showed broad substrate promiscuity by efficiently catalyzing the CoA activation and thiolation, respectively, to afford the corresponding aryl thiocarboxylic acids (Tables 1 and 3, Fig. 5a, and Supplementary Figs. 20a–f and 40–48). When catalysis is coupled, PtmA3 and PtmU4 convert carboxylic acids into thiocarboxylic acids in the presence of a catalytic amount of CoA (Fig. 5b, c). This biocatalytic platform provides a practical solution for thioarboxylic acid synthesis of small molecules with future opportunities to expand the substrate scope through enzyme engineering.

**Discussion**

Since the discovery of PTM and PTN as promising antibiotics over a decade ago, these natural products have garnered considerable attention. Significant progress in understanding the biosynthetic pathways of 3 and 4 has been made through the use of microbial genomics\(^11\). Given the amount of time and resources spent on studying 3 and 4, we were initially surprised to identify sulfur-containing congeners of PTM and PTN\(^8,17\). The discovery of the thioarboxylic acid-containing 1 and 2 raised a provocative question: which natural products are the true biosynthetic end products of the *ptm* and *ptn* biosynthetic gene clusters? The current study clearly demonstrates that (i) 1 and 2 are produced by several wild-type and engineered strains of *S. platensis*; the four wild-type strains were isolated from three different continents (Africa, Europe, and Asia)\(^9,10,15\), (ii) 1 and 2 is produced prior to the detection of 3 and 4, and in certain conditions, 3 and 4 are non-enzymatic hydrolysis products of 1 and 2, (iii) the biosynthesis of the thioarboxylic acid moieties in 1 and 2 is active.

**Table 1 Summary of the steady-state kinetics of PtmA3**

| Substrate | $K_m$ (μM) | $V_{max}$ (μM) | $V_{max}$ (min$^{-1}$) | rel $K_m$ kcat$^{-1}$ |
|-----------|------------|----------------|------------------------|-----------------------|
| 5         | 22.9 ± 3.0 | 184 ± 24       | 14.1 ± 0.9              | 1                     |
| 6         | 470 ± 60   | 638 ± 89       | 23.8 ± 2.2              | 0.082                 |
| 7         | (1.24 ± 0.11)×10³ | n.a.   | 6.89 ± 0.3              | 0.009                 |
| 8         | 70.9 ± 5.0 | 1.03 ± 0.02    | 0.24                    |                       |
| 9         | 290 ± 21   | 1.39 ± 0.03    | 0.008                   |                       |
| 10        | 322 ± 15   | 15.8 ± 0.3     | 0.079                   |                       |

All experiments were performed in triplicate and the data are listed with standard deviations. The relative rates are compared to the native substrate. n.a. not applicable.
In a sequence similarity network of most of which are from Actinobacteria and Proteobacteria
PtmU4 homologs (i.e., two-domain type III CoA-transferases), the conserved catalytic residue, aspartic acid (D430), is shown with blue asterisks. A full sequence alignment was included in Supplementary Fig. 27.

The isolation of PtmU4 homologs, a thioacid group, including PtmU4 from various PTM and PTN producers and the homologs involved in the biosynthesis of PDTC, TQB, and YTK, was found at an e-value threshold of $10^{-140}$ with median 58% sequence identity over 500 residues. All PtmU4 homologs in the thioacid group are from three classes of bacteria: Actinobacteria, Betaproteobacteria, and Gammaproteobacteria. Each node represents protein sequences sharing 100% sequence identity. Colors represent different classes in bacteria. Shapes in c represent the thiocarboxylic acid biosynthesis related type III CoA-transferases discussed in the paper. A complete SSN including all 2401 two-domain type III CoA-transferases in bacteria is shown in Supplementary Fig. 49.

### Table 2 Antibacterial activities and FabF binding experiments with compounds 1–4

| MIC (µg mL$^{-1}$) | Binding assay with E. coli FabF C163Q |
|---------------------|---------------------------------------|
|                     | S. aureus | K. rhizophila | $k_c$ (M$^{-1}$ s$^{-1}$) | $k_d$ (s$^{-1}$) | $K_0$ (M) |
| thioPTM (1)         | 4         | 1             | $(3.0 ± 0.1) \times 10^5$ | $(1.3 ± 0.1) \times 10^{-3}$ | $(4.4 ± 0.3) \times 10^{-9}$ |
| thioPTN (2)         | 1         | 0.5           | $(2.3 ± 1.2) \times 10^5$ | $(6.9 ± 1.7) \times 10^{-3}$ | $(3.3 ± 0.9) \times 10^{-8}$ |
| PTM (3)             | 0.5       | 1             | $(5.8 ± 0.9) \times 10^5$ | $(5.1 ± 0.9) \times 10^{-3}$ | $(8.8 ± 1.4) \times 10^{-9}$ |
| PtnU4 (4)           | 0.25      | 0.25          | $(6.2 ± 2.2) \times 10^5$ | $(4.5 ± 2.6) \times 10^{-2}$ | $(7.0 ± 1.7) \times 10^{-8}$ |

Minimum inhibitory concentrations (MICs) were determined in triplicate using the broth dilution method. Binding assays are reported as means with standard deviations of at least two replicates.

The identification and characterization of the thioacid cassette and its functional integration in the biosynthesis of 1 and 2, along with its presence in the biosynthetic gene clusters of the thio-carboxylic acid- or ester-containing natural products PDTC, TQB, and YTK, led us to examine the prevalence of the thioacid cassette in bacterial genomes. A database search (as of September 5, 2017) of putative thioacid cassettes in bacteria produced 2,401 PtmU4 homologs (i.e., two-domain type III CoA-transferases), most of which are from Actinobacteria and Proteobacteria (Supplementary Fig. 49). In a sequence similarity network of PtmU4 homologs, a thioacid group, including PtmU4 from various PTM and PTN producers and the homologs involved in the biosynthesis of PDTC, TQB, and YTK, was found at an e-value threshold of $10^{-80}$ (Fig. 3c). In contrast to the relatively conserved PtmU4 homologs from Proteobacteria, the homologs from Actinobacteria have more sequence diversity, forming 28 different clusters (Fig. 3c). Among the 175 sequences in the thioacid group, 160 (> 90%) were encoded in genetic proximity to homologs of PtmA3 (Supplementary Data 2 and Supplementary Fig. 50), confirming the broad distribution of the thioacid cassette in nature. Although it is possible that these cassettes are nonfunctional genetic remnants of evolution, the observable functional integration of the thioacid cassettes in thioPTM, thioPTN, PDTC, TQB, and YTK biosynthesis lead us to speculate that thio-carboxylic acid-containing natural products, or their derivatives thereof, are vastly underrepresented among known natural products.

The isolation of 1 and 2 gave us an opportunity to assess the biological implications of thio-carboxylic acid-containing natural products. The isolation of 1 and 2 gave us an opportunity to assess the biological implications of thio-carboxylic acid-containing natural products.

**Fig. 3** Bioinformatics analysis of type III CoA-transferases from bacteria. a Sequence alignment of selected PtmU4 homologs from bacteria. Aligned residues are colored based on the level of conservation (red box with white character and red character show strict identity and similarity, respectively).

b Phylogenetic analysis of the selected type III CoA-transferase from bacteria. The sequences used in the phylogenetic tree include selected one-domain and two-domain type III CoA-transferases (Supplementary Methods).

c A sequence similarity network (SSN) of thioacid group. The BLAST e-value threshold is $10^{-140}$ with median 58% sequence identity over 500 residues. All PtmU4 homologs in the thioacid group are from three classes of bacteria: Actinobacteria, Betaproteobacteria, and Gammaproteobacteria. Each node represents protein sequences sharing 100% sequence identity. Colors represent different classes in bacteria. Shapes in c represent the thio-carboxylic acid biosynthesis related type III CoA-transferases discussed in the paper. A complete SSN including all 2401 two-domain type III CoA-transferases in bacteria is shown in Supplementary Fig. 49.

Minimum inhibitory concentrations (MICs) were determined in triplicate using the broth dilution method. Binding assays are reported as means with standard deviations of at least two replicates.
calculations were congruent with 1 and were found to bind slightly tighter to FabF. Our DFT calculations (shown as dotted lines) and Gibbs energies are Å and kcal mol⁻¹, respectively. a Optimized geometries and energies of 5 and 5-SH (modeled as truncated versions of 1-4) in the presence of two imidazoles (the side chains of H303 and H340) in the gas phase. The units of bond length (shown as dotted lines) and Gibbs energies are Å and kcal mol⁻¹, respectively. b Optimized geometries and energies of 5 and 5-SH bound to the sides chains of three fixed amino acids (H303, H340, Q163) in the gas phase. c Free energies of solvation of 5 and 5-SH.

**Table 3 Summary of the relative activities of PtmU4**

| Substrate | Rate (s⁻¹) | Relative rate (%) |
|-----------|------------|------------------|
| 5-CoA | (2.43 ± 0.07)×10⁻³ | 100 ± 3 |
| 6-CoA | (2.46 ± 0.02)×10⁻³ | 101 ± 1 |
| 7-CoA | (5.82 ± 0.08)×10⁻³ | 240 ± 3 |
| 8-CoA | (1.50 ± 0.2)×10⁻⁴ | 6 ± 1 |
| 9-CoA | (5.23 ± 0.2)×10⁻⁴ | 22 ± 1 |
| 10-CoA | (2.44 ± 0.1)×10⁻³ | 100 ± 6 |

All experiments were performed in triplicate and the data are listed with standard deviations. The rate constants are compared to the native substrate S-CoA: S-(3-amino-4-chlorobenzoate) coenzyme A (7-CoA); S-(2-amino-3-hydroxy benzoate) coenzyme A (8-CoA); S-(2-amino-4-fluorobenzoate) coenzyme A (9-CoA); S-(6-hydroxy-2-naphthalene carboxylate) coenzyme A (10-CoA).

FabF than 3 and 4 while exhibiting minimal differences in their observed MICs. Similarly, TQB and PDTC, which are bacterial siderophores, have improved activities compared to their carboxylic acid congeners⁶,⁷. It is still unclear if and why *S. platensis* produces 1 and 2 in nature, but the biosynthetic role of the thioacid cassette and its prevalence in bacterial genomes would suggest that thioacid-containing natural products might have important and unsolved biological roles in nature. In conclusion, thioacid-containing acids, which have been an underappreciated pharmacophore in drug discovery and development, should now be considered in future studies.

**Methods**

**Bacterial strains, plasmids, and chemicals.** Strains, plasmids, and PCR primers used in this study are listed in Supplementary Information. PCR primers were obtained from Sigma-Aldrich, Q5 high-fidelity DNA polymerase, restriction endonucleases, and T4 DNA ligase were purchased from NEB and used by following the protocols provided by the manufacturer. DNA gel extraction and plasmid preparation kits were purchased from Omega Bio-Tek. DNA sequencing was conducted by Eton Bioscience. The REDIRECT Technology kit for PCR-targeting homologous recombination was provided by The John Innes Center (Norwich, UK).²⁶,²⁷. pOJ260 was used as a shuttle vector for gene homologous recombination²⁸. E. coli ET12567/pUZ8002 was used as the host for intergeneric conjugations²⁹. pUWL201PWT, which is a derivative of pUWL201PWT containing an oriT sequence that was cloned into its PstI site, was used as the shuttle vector for gene complementations, biotransformation, and heterologous production of PtmU4 in Streptomyces. Cosmid libraries were-screened by PCR using OneTaq 2× Master Mix with GC buffer (NEB). For Southern analysis, digoxigenin labeling of DNA probes, hybridization, and detection were performed according to the protocols provided by the manufacturer (Roche Diagnostics Corp.). *S. platensis* CB007391T, CB007651T, MA73279, and MA733910, and their pathway-specific products. PTM and PTN inhibit the decarboxylating condensing enzymes FabF and FabH in bacterial type II fatty acid synthesis (FASII)⁹,¹⁰. The carboxylic acid moiety of 3 and 4 mimics the malonyl-acyl carrier protein substrate and ionically interacts with two His residues in the Cys-His-His catalytic triad, resulting in competitive inhibition⁹–¹¹. Structure–activity relationship studies of 3 and 4 have concluded that modification of their ADHBA moieties results in loss of antibiotic activity¹¹,¹²,¹³. The thioacidic acid congeners 1 and 2, whose structures only differ from 3 and 4 at one atom, retained strong antibacterial activities and were found to bind slightly tighter to FabF. Our DFT calculations were congruent with 1 and 2 being better binders of...
Fig. 5 | Biocatalysis of thiocarboxylic acids using PtmA3 and PtmU4. a Chemical structures of the aryl acids tested. b UV at 260 nm of time-course analysis of the one-pot reaction to synthesize 5-SH from 5 using a combination of PtmA3 and PtmU4 using recycled coenzyme A (CoA). In this reaction, 1 equivalent of 5 was supplied with 0.5 equivalent of CoA. After 120 min, 5 was completely consumed and transformed into 5-SH, indicating CoA was recycled during the reaction. c Chemical scheme for this one-pot reaction transforming carboxylic acid to thiocarboxylic acid. The functional groups involved in this transformation are highlighted in red. 3-Amino-4-chlorobenzoic acid (7); 2-amino-3-hydroxybenzoic acid (8); 2-amino-4-fluorobenzoic acid (9); 6-hydroxy-2-naphthaleneacetic acid (10).

negative regulator ptmR1 inactivation mutants, SB1202615, SB1202914, SB1202715, SB1200116, and SB1260016 were reported previously. S. albus J107419, S. lividans K4-11418, S. avermitilis SUK22121, and S. coelicolor M11462 were used as model Streptomyces hosts for small-molecule biotransformation and protein production. Other common chemicals, biochemical, and media components were purchased from standard commercial sources.

In-frame deletion of ptmA3 in SB12029 to afford SB12039. To construct the plasmid for in-frame deletion of ptmA3, two 3-kb fragments of the genes upstream and downstream of ptmA3 were amplified from cosmids SB12037, a cosmids containing a partial ptm gene cluster15, with the primers 739A3up_F, 739A3up_R, 739A3down_F, and 739A3down_R. Both fragments were cloned into the HindIII and EcoRI sites of pOJ260 to obtain pBS12075. pBS12075 was transformed into E. coli ET12567/pUZ8002 and introduced into S. platensis SB12029 by intergeneric conjugation15. After several rounds of passaging the exconjugants, double crossovers via homologous recombination were selected by the apramycin-sensitive phenotype. The genotype of the in-frame deletion mutant SB12039 was verified by PCR analysis and Southern analysis.

Inactivation of ptmU4 in SB12029 to afford SB12040. The ptmU4 gene was replaced with the aac(3)IV-oriT resistance cassette from pJJ773 using RED-mediated PCR-targeting mutagenesis10 in E. coli BW25113/pIJ790 harboring pBS12037, a cosmids containing a partial ptm gene cluster15. The genotype of the resultant ΔptmU4 mutant cosmids, pBS12074, was confirmed by PCR analysis using primers 739U4ID_F and 739U4ID_R. pBS12074 was transformed into the non-methylating E. coli ET12567/pUZ8002 and introduced into S. platensis SB12029 by intergeneric conjugation. Single crossovers of ΔptmR1ΔptmU4 were selected by screening for apramycin resistance on ISP4 medium. After another round of passaging the single-crossover exconjugants in solid ISP4 medium, the ΔptmR1/ΔptmU4 mutant SB12040, a result of double-crossover homologous recombination, was selected for by screening for an apramycin-resistant and kanamycin-sensitive phenotype. The genotype of SB12040 was confirmed by PCR and Southern analysis.

Inactivation of ptmS1, ptmS2, and ptmS4 and disruption of ptmS3 was performed using the protocol described above for the ΔptmR1ΔptmU4 mutant SB12040. Each genotype was verified by PCR analysis and Southern analysis.

Heterologous production of 5-SH in model Streptomyces hosts. pUWL201PWT was used as an E. coli– Streptomyces expression shuttle vector to construct a 5-SH production system in model Streptomyces hosts. The candidate thiocassette genes, ptmA3 and ptmU4, were amplified by PCR using the primers 739U4pUpF_F and 739U4pUpF_R, and 739A3pUpF_F and 739A3pUpF_R from pBS12037 and individually cloned into pET-44b(+). ptmU4 was cloned into the Ndel and PstI sites and ptmA3, placed downstream of ptmU4, was cloned into the PstI and HindIII sites to yield pBS12084. The constructed fragment of ptmU4-ptmA3 was cut from pBS12084 at the Ndel and HindIII sites and cloned into pUWL201PWT at the same sites to construct pBS12085.

The three ADHBA biosynthetic genes, ptmB1, ptmB2, and ptmB3, were amplified as a single fragment by PCR using the primers 739B13pUpF_F and 739B13pUpF_R from pBS12037, which was subsequently cloned into the HindIII and EcoRI sites of pBS12085. The resulting construct, pBS12086, possessed ptmU4–ptmA3–ptmB1–ptmB2–ptmB3 (Supplementary Figure 7). pBS12086 was transformed into E. coli ET12567/pUZ8002 and introduced into four Streptomyces model strains (S. albus J1074, S. lividans K4-114, S. avermitilis SUK22, and S. coelicolor M1146) by intergeneric conjugation. Clones containing pBS12086 were selected with thiostrepton.

PTM fermentation medium, supplemented with thiostrepton, was used for the production of 5-SH in the model Streptomyces hosts. After fermentation for 2 days at 28°C, the fermentation broth was directly used for LC-MS analysis.

Gene cloning. The ptmA3, ptmU4, ptmS2ΔG12, ptmS3ΔG12, and ptmS4 genes from S. platensis CB00739 were amplified by PCR from genomic DNA with Q5 DNA polymerase (NEB). The PCR product was purified, treated with T4 polymerase, and cloned into pBS808015 according to ligation-independent procedures to afford pBS12087 (harboring ptmA3), pBS12088 (harboring ptmU4), pBS12089 (harboring ptmS4), pBS12090 (harboring ptmS2ΔG12), amino acid residues 1–90), and pBS12091 (harboring ptmS3ΔG12, amino acid residues 1–91). The E. coli fabF gene containing a site-directed mutation resulting in FabF C163Q was cloned into pBS8080 as described above, resulting in pBS12096. pUWL201PWT was used as an E. coli–Streptomyces expression shuttle vector and protein expression of PtmU4 in Streptomyces. The full-length ptmU4 gene together with an N-terminal His6-tag sequence was amplified by PCR from pBS12088 using the 739U4pUpR_F and 739SrU4_R primers. Thus, ptmU4 was cloned into the Ndel and HindIII sites of pUWL201PWT affording pBS12092. For site-directed mutagenesis of ptmU4, the ptmU4 gene from pBS12092 was amplified in two steps by primer extension14 using the 739U4pUpF_F and 739SrU4_R primers with internal primers containing the desired mutation. The mutant ptmU4 genes were then cloned into pUWL201PWT as described above yielding pBS12093–pBS12095.
Gene expression and protein production and purification. PtmA3, PtmS2GG, PtmS3GG, and PtmS4 were produced in E. coli. For enzyme activity assays, the plasmid harboring the gene of interest was transformed into E. coli BL21 (DE3) (Novagen Technologies) and grown in 1 L of lysogeny broth (LB) at 37 °C with shaking at 250 rpm until an OD_600 of 0.6 was reached. The culture was cooled to 4 °C, gene expression was induced with the addition of 0.25 mM isopropyl β-D-1-thiogalactopyranoside, and the cells were grown around 18 h at 18 °C with shaking. After harvesting the cells by centrifugation at 4000 g for 15 min at 4 °C, the pellet was resuspended in lysis buffer (50 mM Tris, pH 8.0, containing 300 mM NaCl and 10 mM imidazole), lyzed by sonication, and centrifuged at 15,000 g for 30 min at 4 °C. The supernatant was purified by nickel-affinity chromatography using an AMICON Ultra-15 column (Millipore) in 50 mM Tris, pH 7.8, containing 100 mM NaCl, 50 mM KCl, and 5% glycerol. Protein concentrations were determined from the absorbance at 280 nm using a molar absorptivity constant of each protein. Individual aliquots of each protein were stored at −80 °C until use.

2.7 μm using a 6 min solvent gradient (0.8 μL min −1) of 2.5–20% CH3CN in 10 mM ammonium acetate. Substrate and product were detected by monitoring 260 nm (for the photodiode array detector). When potassium hydrosulfide (KSH) was used to replace the native sulfur donor (sulfur-carrier protein), the reaction was performed in 50 mM phosphate, pH 7.4, containing 5 mM KSH, 200 μM S-CA, and 5 μM PtmU4 in a total volume of 50 μL. After incubation at 30 °C for 10 min, the reaction was quenched by boiling for 1 min. The reaction mixture was then centrifuged at 10 μL of the supernatant was injected and analyzed by HPLC as described above. The relative activities of all PtmU4 mutants were determined using a 5-CAo concentration of 500 μM. Due to slower turnovers of all mutants, enzyme concentration and incubation time were increased to 10 μM and 1 h, respectively, to facilitate product detection. The reaction mixture was then centrifuged and 10 μL of the supernatant were injected and analyzed by HPLC as described above. Substrate promiscuity assays of PtmU4 were determined using 500 μM of different CoA substrates and 0.5 μM PtmU4. The aryloxadil peaks were collected and analyzed by LC-MS using either positive or negative mode (Supplementary Fig. 4).

The one-pot reaction to synthesize 5-SCoA using a combination of PtmA3 and PtmU4 was performed using 5 (100 μM), CoA (50 μM), ATP (1 mM), Mg^2+ (4 mM), KSH (2 mM), PtmA3 (10 μM), and PtmU4 (5 μM) in phosphate buffer (50 mM, pH 7.4) at 30 °C.

Antibiotic binding using surface plasmon resonance. Experiments were performed on a Biacore X100 (GE Healthcare) instrument at 25 °C and the data were analyzed with Biacore X100 evaluation software. HBS-P + buffer (0.1 M HEPES, 1.5 M NaCl, 0.5% v/v surfactant P20, pH 7.4) containing 0.1% dimethyl sulfoxide (DMSO) was used as the running buffer. Cells 1 and 2 were used as the reference and experimental surface, respectively. Fab’ C163Q was diluted to 0.15 μM in HBS-P + buffer containing 0.1% DMSO and injected over both surfaces at a flow rate of 30 μL min −1. A 2-s association phase was followed by a 30-s dissociation phase. Signals from the reference surface and buffer blank injections were subtracted and the corrected results were globally fit to a 1:1 binding model. The association rate constant (k_{a}) and dissociation rate constant (k_{d}) were used to determine the equilibrium dissociation constant (K_{d}) in units of M.

Computational details. The crystal structure of the E. coli Fab’ C163Q–PTM complex (PDB 2FGX) was used to extract the ADHBA moiety of PTM and the side chains of H303 and H340, or H303, H340, and H163. For calculations with ADHBSH, one oxygen in the carboxylic acid group of ADHBA was replaced with a sulfor atom. Quantum mechanical DFT calculations were performed using Gaussian 09. Each of the geometry optimizations were performed at the M06-2X/6-311+G(dp) level of theory with the SMD implicit solvent model to account for the solvent effects of water and the interior of protein (ε = 4).

Data availability. Proteins from S. platensis CB00739 have been deposited to protein database of the National Center for Biotechnology Information (NCBI), under accession code AJIW55578 for PtmA3, AJIW55579 for PtmU4, AVR47602 for PtmS1, AVR47603 for PtmS2, AVR47604 for PtmS3, AVR47605 for PtmS4, AVR47606 for SpSSCP1, and AVR47607 for SpSSCP2. All other relevant data that support the findings of this study are available in the manuscript and the Supplementary information.

Received: 29 January 2018 Accepted: 21 May 2018
Published online: 18 June 2018

References
1. Zambresky, P. et al. Nature 479, 270–274 (2011).
2. deFigueiredo, R. M., Suppo, J.-S. & Campagne, J.-M. Nonclassical routes for drug design. Chem. Rev. 116, 8395–8403 (2016).
3. deFigueiredo, R. M., Suppo, J.-S. & Campagne, J.-M. Nonclassical routes for amide bond formation. Chem. Rev. 116, 12122–12122 (2016).
4. Durrbar, K. L., Scharf, D. H., Litomska, A. & Hertweck, C. Enzymatic carbon-sulfur bond formation in natural product biosynthesis. Chem. Rev. 117, 5521–5577 (2017).
5. Matthijs, S. et al. The Pseudomonas siderophore quinolobactin is synthesized from xanthuric acid, an intermediate of the kynurenic pathway. Mol. Microbiol. 52, 371–384 (2004).
6. Matthijs, S. & Thiolquinobactin, a Pseudomonas siderophore with antifungal and anti-Pythium activity. Environ. Microbiol. 9, 425–434 (2007).
7. Lewis, T. A. et al. A Pseudomonas stutzeri gene cluster encoding the biosynthesis of the CCL4-dechlorination agent pyridine-2,6-bis(thiocarboxylic acid). Environ. Microbiol. 2, 407–416 (2000).

NATURE COMMUNICATIONS | DOI: 10.1038/s41467-018-04747-y
ARTICLE
10. Rudolf, J. D., Dong, L.-B., Shen, B. Platensimycin and platencin: inspirations from chemistry, biology, enzymology, and medicine. Biochim. Pharmacol. 133, 139–151 (2017).

11. Singh, S. B. et al. Isolation, structure, and absolute stereochemistry of platensimycin and platencin congeners from Streptomyces platensis SB12029. Bioorg. Med. Chem. 24, 6348–6353 (2016).

12. Wang, J. et al. Platensimycin is a selective FabF inhibitor with potent antibiotic properties. Nature 441, 358–361 (2006).

13. Wang, J. et al. Discovery of platencin, a dual FabF and FabI inhibitor with in vivo antibiotic properties. Proc. Natl. Acad. Sci. USA 104, 7612–7616 (2007).

14. Rudolf, J. D., Dong, L.-B. & Shen, B. Platensimycin and platencin: inspirations from chemistry, biology, enzymology, and medicine. Biochim. Pharmacol. 133, 139–151 (2017).

15. Rudolf, J. D., Dong, L.-B., Huang, T. & Shen, B. A genetically amenable platensimycin- and platencin-overproducer as a platform for biosynthetic explorations: a showcase of PimO4, a long-chain acyl-CoA dehydrogenase. Mol. Biol. Syst. 11, 2717–2726 (2015).

16. Jurgenson, C. T., Begley, T. P. & Ealick, S. E. The structural and biochemical reinvestigation of the catalytic mechanism of formyl-CoA transferase, a class I CoA-transferase/lyase producing dimethyl sulfoxide in platensimycin. FEBS Lett. 591, 207–215 (2011).

17. Todd, J. D. et al. Structural and regulatory genes required to make the gas cording soil odor geosmin. FEBS Lett. 591, 207–215 (2011).

18. Todd, J. D. et al. Structural and regulatory genes required to make the gas cording soil odor geosmin. FEBS Lett. 591, 207–215 (2011).

19. Todd, J. D. et al. Structural and regulatory genes required to make the gas cording soil odor geosmin. FEBS Lett. 591, 207–215 (2011).

20. Todd, J. D. et al. Structural and regulatory genes required to make the gas cording soil odor geosmin. FEBS Lett. 591, 207–215 (2011).

21. Todd, J. D. et al. Structural and regulatory genes required to make the gas cording soil odor geosmin. FEBS Lett. 591, 207–215 (2011).

22. Jurgenson, C. T., Begley, T. P. & Ealick, S. E. The structural and biochemical reinvestigation of the catalytic mechanism of formyl-CoA transferase, a class I CoA-transferase/lyase producing dimethyl sulfoxide in platensimycin. FEBS Lett. 591, 207–215 (2011).

23. Mueller, E. G. Traficchi in pers��sulin: delivering sulfur in biosynthetic pathways. Nat. Chem. Biol. 2, 185–194 (2006).

24. Godert, A. M., Jin, M., McAlfferty, F. W. & Begley, T. P. Biosynthesis of the thiouinolobactin siderophore: an interesting variation on sulfur transfer. J. Bacteriol. 189, 2941–2944 (2007).

25. Sasaki, E. et al. Co-opting sulphur-carrier proteins from primary metabolic pathways for 2-thioguanosine biosynthesis. Nature 510, 431–434 (2014).

26. Park, J.-H. et al. Biosynthesis of the thiazole moiety of thiamin pyrophosphate (vitamin B1). Biochemistry 42, 12430–12438 (2003).

27. Heider, J. A new family of CoA-transferases. FEBS Lett. 509, 345–349 (2001).

28. Berthold, C. L., Toyota, C. G., Richards, N. G. J. & Lindqvist, Y. Reinvestigation of the catalytic mechanism of formyl-CoA transferase, a class III CoA-transferase. J. Biol. Chem. 283, 6519–6529 (2008).

29. Todd, J. D. et al. Structural and regulatory genes required to make the gas dimethyl sulfide in bacteria. Science 315, 666–669 (2007).

30. Alcolombri, U., Laurino, P., Lara-Astiaso, P., Vardi, A. & Tawfik, D. S. DdD is a CoA-transferase/lyase producing dimethyl sulﬁde in the marine environment. Biochemistry 53, 5473–5475 (2014).

31. Wiegand, I., Hilpert, K. & Hancock, R. E. W. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 3, 163–175 (2008).

32. Kitamura, K. et al. Binding free-energy calculation is a powerful tool for drug optimization: calculation and measurement of binding free energy for 7-azaindoles derivatives to glycogen synthase kinase-3β. J. Chem. Inf. Model. 54, 1653–1660 (2014).

33. Atkinson, H. J., Morris, J. H., Ferrin, T. E. & Babbitt, P. C. Using sequence similarity networks for visualization of relationships across diverse protein superfamilies. PLoS ONE 4, e4345 (2009).

34. Nicolau, K. C. et al. Design, synthesis, and biological evaluation of platensimycin analogs with varying degrees of molecular complexity. J. Am. Chem. Soc. 130, 13110–13119 (2008).

35. Gust, B., Challis, G. L., Fowler, K., Kieser, T. & Chater, K. F. PCR-targeted Streptomyces gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene odour geosmin. Proc. Natl. Acad. Sci. USA 100, 1541–1546 (2003).

36. Bierman, M. et al. Plasmid cloning vectors for the conjugal transfer of DNA from Escherichia coli to Streptomyces spp. Gene 116, 43–49 (1992).

37. MacNeil, D. J. et al. Analysis of Streptomyces avermitilis genes required for avermectin biosynthesis utilizing a novel integration vector. Gene 111, 61–68 (1992).

38. Dunmith, M. et al. Analysis of genes involved in 6-deoxyxylose biosynthesis and transfer in Saccharopolyspora erythraea. Mol. Gen. Genet. 264, 477–485 (2000).

39. Chater, K. F. & Wilde, L. C. Streptomyces albus G mutants defective in the suGRI restriction-modification system. J. Gen. Microbiol. 116, 323–334 (1980).

40. Ziemann, R. & Betlach, M. C. Recombinant polyketide synthesis in Streptomyces: engineering of improved host strains. Biotechniques 26, 106–119 (1999).

41. Komatsu, M. et al. Engineered Streptomyces avermitilis host for heterologous expression of biosynthetic gene cluster for secondary metabolites. ACS Synth. Biol. 2, 384–396 (2013).

42. Gomez-Escribano, J. P. & Bibb, M. J. Engineering Streptomyces coelorchis for heterologous expression of secondary metabolite gene clusters. Microb. Biotechnol. 4, 207–215 (2011).

43. Lohman, J. R., Bingman, C. A., Phillips, G. N. & Shen, B. Structure of the bifunctional acyl-CoA transferase/decarboxylase LmnK from the leinamycin biosynthetic pathway revealing novel activity for a double-histidine domain. Biochemistry 52, 902–911 (2013).

44. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K. & Pease, L. R. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene 77, 51–59 (1990).

45. Frisch, M. J. et al. Gaussian 09 Rev D.01 (Gaussian Inc., 2013).

Acknowledgements
This work is supported in part by the Chinese Ministry of Education 111 Project B0803420 (to Y. Duan.) and National Institutes of Health Grants GM114353 (to B.S.). We thank Drs. Christoph Rader and Jinsai Shang, The Scripps Research Institute, for use of and expertise in using the SPR instrument, respectively, Dr. S.B. Singh, Merck Research Laboratories, Rahway, NJ, for providing S. platensis MA7339 and MA7339 wild-type strains, and the John Innes Center, Norwich, UK, for providing the REDIRECT technology kit. J.D.R. is supported in part by an Arnold O. Beckman Postdoctoral Fellowship. N.W. is supported in part by the Institute of Applied Ecology, Chinese Academy of Sciences, and a scholarship from the Chinese Scholarship Council (201504910034). This is manuscript #29626 from The Scripps Research Institute.

Author contributions
B.S. conceived the project; L.-B.D., J.D.R., and B.S. designed the experiments; L.-B.D., J.D.R., D.K., N.W., and Y. Deng performed the experiments; L.-B.D., J.D.R., D.K., N.W., Y. Deng, Y.H., Y. Duan, and B.S. analyzed the results; C.Q.H. and K.N.H. performed the computational calculations; and L.-B.D., J.D.R., and B.S. wrote the manuscript with inputs from all co-authors.

Additional information
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-018-04747-y.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2018