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PAPER

Genome mining of cryptic tetronate natural products from a PKS-NRPS encoding gene cluster in *Trichoderma harzianum* t-22

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Trichoderma harzianum is a widely used biocontrol agent in agriculture. Obtaining a full inventory of the small molecules that can be biosynthesized from the encoded biosynthetic gene clusters (BGCs) is therefore useful for understanding associated plant-microbe and microbe-microbe interactions. Here we heterologously reconstituted a polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) encoding gene cluster from *T. harzianum* t-22 in *Aspergillus nidulan*s A1145. Six new tetronate natural products trihazone A-F (**1**-**6**) were isolated and elucidated by HRESIMS, 1D and 2D NMR data. Three of the products contain an exocyclic olefin, which is derived from the oxidative decarboxylation of an α-ketoglutarate-dependent dioxygenase ThnC as shown by biochemical assays.

Introduction

Fungal polyketide synthase and nonribosomal peptide synthetases (PKS-NRPSs) are multidomain megasynthetases that synthesize structurally complex natural products with diverse biological activities.[1, 2] Typically, these PKS-NRPS hybrids consist of a PKS module fused to a C-terminal NRPS module, and produce polyketide chains amidated by a single amino acid residue that is activated by the adenylation (A) domain of the NRPS.[3] This product bound to the thiolation domain of the NRPS module can be released either by a reductive domain followed by Knoevenagel condensation to yield a pyrrolinone product; or undergo a Dieckmann cyclization to afford a tetramic acid product. Further downstream modifications can elaborate the structures into a wide array of scaffolds, including pyridones, decalins, isoindolones, etc.[4] Genome sequencing of filamentous fungi has revealed each strain can contain ~ 2-6 PKS-NPRS encoding biosynthetic gene clusters (BGCs), many of them have remained cryptic due to the suppressed transcription under laboratory conditions. Phylogenic classification of PKS-NRPS sequences has suggested signficant potential to find new structures,[5] making such BGCs attractive targets for genome-driven natural product discovery.

Trichoderma harzianum t-22 is a biofertilizer fungus that is widely applied to plants from gardening to agriculture.[6] Secondary metabolites, including natural products, produced by the strain are considered to play important roles in mediating plant-microbe and microbe-microbe interactions in the rhizosphere.[7, 8] Bioinformatic analysis of the *T. harzianum* t-22 genome sequence by the Joint Genome Institute (JGI)[9] revealed the fungus encodes a large number of BGCs based on the presence of core enzymes, including 25 PKSs,[10], 14 NRPS and 4 PKS-NRPS encoding clusters. In contrast, only a small number (<10) of natural products have been isolated from *T. harzianum* t-22, including harzianic acid,[11] abscisic acid, etc. We recently mined one dual-PKS encoding gene cluster and showed it produces the novel redox-active metabolite tricholignan A, which can reduce Fe^{3+} and aid the plant in assimilation of Fe^{2+} under iron-deficient conditions.[10] Hence, new compounds discovered from *T. harzianum* t-22 may have agricultural applications. In this work, a dormant PKS-NRPS encoding gene cluster from *T. harzianum* t-22 was activated through heterologous expression in *Aspergillus nidulans* A1145, leading to six new tetronate natural products triharone A-F (**1**-**6**). An α-ketoglutarate dependent enzyme in the pathway was biochemically verified to catalyze the exocyclic double bond formation.

Results and discussion

Among the 4 PKS-NRPS encoding BGCs (Fig S1†), We focused on the cluster containing genes 577708 - 577712, hereby renamed the *thn* BGC (Fig. 1A). The putative boundaries of *thn* BGC were assigned based on the likelihood of the biosynthetic enzymes encoded and BLAST analysis (Table S1† and Fig. S2†). One reason for choosing this cluster was it is relatively compact, containing only five genes with one of them encoding a putative transporter protein (*thnB*). The *thn* BGC is anchored by *thnA,* which encoded a PKS-NRPS with the domain organization of *N*-terminus: ketosynthase (KS), malonyl-CoA:ACP acyltransferase (MAT), dehydratase (DH), C-methyltransferase (cMT), nonfunctional enoylreductases (ER^0),

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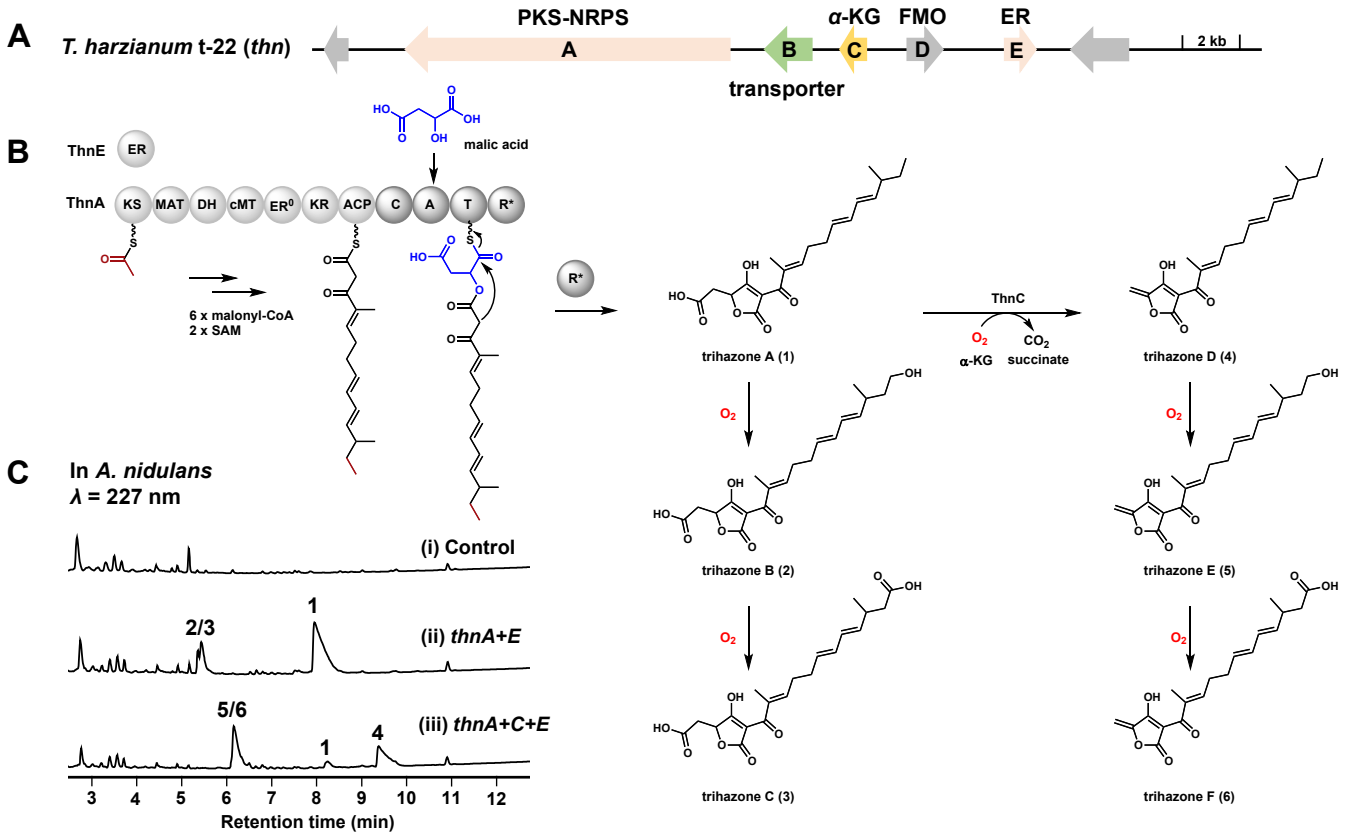

Fig. 1 Biosynthesis of trihazones. (A) The thn cluster identified from T. harzianum t-22. ThnA, a PKS-NRPS (KS-MAT-DH-cMT-ER0-KR-ACP-C-A-T-R*); KS, ketosynthase; MAT, malonyl-CoA:ACP acyl transferase; DH, dehydratase; cMT, C-methyltransferase; ER0, nonfunctional enoylreductases; KR, ketoreductase; ACP, acyl carrier protein; C, condensation; A, adenylation; T, thiolation; R*, Dieckmann cyclization); ThnB, a transporter, ThnC, a non-heme, iron and α-ketoglutarate-dependent oxygenase, ThnD, a flavin-dependent monooxygenase, ThnE, a trans-acting ER. (B) Proposed biosynthetic pathway of trihazones. (C) Product profiles from heterologous expression of different combinations of thn cluster in A. nidulans A1145. Control in trace i is A. nidulans A1145 transformed with vectors not carrying any biosynthetic genes.

ketoreductase (KR), acyl-carrier protein (ACP), condensation (C), adenylation (A), thiolation (T), and Dieckmann cyclization (R*). The remaining genes in the BGC encode a *trans*-acting ER (*thnE*), a non-heme, iron and α-ketoglutarate-dependent oxygenase (α-KG, *thnC*) and a flavin-dependent monooxygenase (FMO, *thnD*). Further sequence analysis of the A domain of ThnA revealed that the conserved aspartic acid residue that electrostatically interacts with the α-amino group of amino acid substrate is replaced with a neutral asparagine residue (Fig. S3†). This is similar to the A domain of CaaA[12] and TraA[13], both of which use L-malic acid instead of an α-amino acid. In these PKS-NRPS pathways, the α-OH of malic acid serves as the nucleophile to form an ester linkage with the polyketide chain, and the product is released as a tetronate instead of a tetramate. Therefore, we anticipate the product of *thn* cluster to be a tetronate-containing compound, which has not been isolated from *Trichoderma* sp.

To investigate the metabolites that can be produced by the *thn* cluster, combinations of the biosynthetic genes were heterologously expressed in *Aspergillus nidulans* A1145 ΔSTΔEM.[14] Expressing the PKS-NRPS alone did not lead to any products, since the putative partnering trans-acting ER ThnE was not present (Fig S4†). Upon coexpression of ThnA and ThnE under the control of glaA and gpdA promoter respectively, we observed the emergence of three new compounds **1**-**3** following three days of growth (Fig. 1C, trace ii), when compared to the control strain transformed with the empty vector only (Fig. 1C, trace i). Further coexpression of the α-KG ThnC led to formation of a new set of products **4**-**6**, with corresponding decreases in levels of **1**-**3** (Fig 1C, trace iii). Unexpectedly, further expression of the FMO ThnD led to complete abolishment of all metabolites, with no new compound formed. Coexpressing ThnD with ThnA and ThnE, however, did not abolish the production of 1-3 (Fig S4†). Finally, introduction of thnB that encodes a putative transporter along with the four other thn genes, did not lead to restoration of products (Fig S4†). Transcription analysis of thnD confirmed this gene is expressed, hence its role in the biosynthetic pathway remains cryptic. Given that compounds **1**-**6** were formed by the functions of ThnA, C and E, we renamed these compounds trihazone A-F and established the structures by extensive analysis of HRESIMS, 1D and 2D NMR data.

Trihazone A (**1**) was isolated as a yellow powder. Its molecular formula $C_{20}H_{26}O_6$ was determined by HRESIMS at *m/z* 361.1633 $[M+H]^+$ (calcd for 361.1657), corresponding to an index of hydrogen deficiency of 8. Analysis of 1D NMR and HSQC spectrum of **1** (Table S2† and Fig. S5†) recorded in CD_3OD showed three methyls at δ_H 1.88 (s), 0.98 (d, J = 6.8 Hz) and 0.86 (t, J = 7.4 Hz), one oxygenated methine at δ_H 4.97 (m), five unsaturated methines at δ_H 6.84 (t, J =

6.8 Hz), 6.06 (dd, *J* = 14.9, 10.3 Hz), 5.97 (dd, *J* = 15.0, 10.3 Hz), 5.62 (m) and 5.44 (dd, *J* = 15.0, 7.9 Hz). The ^{13}C NMR and DEPT (Table S2† and Fig. S5†) revealed the presence of 20 carbon resonances, including three methyls, four methylenes, seven methines (five olefinic, one oxygenated), six nonprotonated carbons (three olefinic including one enolic, one carboxyl, one ester carbonyl, and one conjugated carbonyl). All these proton resonances were unambiguously assigned to their respective carbons by HSQC spectrum. Taking the molecular formula and the above NMR data into consideration, four double bonds and three carbonyl groups consumed seven of the eight degrees of unsaturation. Therefore, the remaining one unsaturation unit required that compound **1** possessed a monocyclic ring system.. The ^{1}H-^{1}H COSY correlations of H-4/H_2-5 and the HMBC correlations from H_2-5 to C-3, C-4 and C-6 suggested the presence of acetic acid group, which is located at C-4 of the five-membered lactone (Fig. 2). Detailed analysis of above NMR data revealed that these signals in compound **1** are partially similar to those of carlosic acid core fragment.[12] Furthermore, the ^{1}H-^{1}H COSY correlations of H-3'/H_2-4'/H_2-5'/H-6'/H-7'/H-8'/H-9'/H-10'/(H_3-14')/H_2-11'/H_3-12' and HMBC correlations from H_3-13' to C-2' and C-3' confirmed the existence of a 10-methyldodeca-2,6,8-triene fragment, in which the double bonds $\Delta^{2',3'}$, $\Delta^{6',7'}$ and $\Delta^{8',9'}$ were determined as 2'*Z*, 6'*E* and 8'*E* configuration, respectively, supported by the NOESY correlation between H_3-13' and H_2-4', the coupling constants 14.9 Hz and 15.0 Hz between H-6'/7' and H-8'/9' (Fig. 2 and Table S2†). The Key HMBC correlations from H-3'/H_3-13' to C-1' verified that the above 10-methyldodeca-2,6,8-triene and carlosic acid core fragments were directly connected by the carbon bond C-1'–C-2' (Fig. 2). Thus, compound **1** was determined to be a new carlosic acid derivative in which the tetronate has a 4-carboxylmethyl group.

The molecular formula of trihazone B (**2**) and C (**3**) were determined as $C_{20}H_{26}O_7$ and $C_{20}H_{24}O_8$ by HRESIMS (Table S2† and Fig. S6-S7†). The 1D NMR data of **2** and **3** were similar to those of trihazone A (**1**). The main difference between **2** and **1** was the presence of one hydroxymethyl unit at the end of side chain, while the difference between **3** and **1** was the replacement of one methyl in trihazone A (**1**) by one carboxyl group in trihazone C (**3**). These deductions were further supported by the HMBC correlations of H-10' (δ_H 2.27) with C-12' (δ_C 61.1) in **2**, and H-10' (δ_H 2.64) with C-12' (δ_C 176.5) in **3**, respectively (Figure 2).

Trihazone D (**4**), E (**5**), and F (**6**) were also obtained as yellow powders. Their molecular compositions were determined to be $C_{19}H_{24}O_4$, $C_{19}H_{24}O_5$, and $C_{19}H_{22}O_6$ by HRESIMS, respectively. Their ^{1}H and ^{13}C NMR data (Table S3† and Fig. S8-S10†) showed considerable resemblance to those of trihazone A (**1**), B (**2**), and C (**3**), respectively. The main difference between them is the loss of the 4-carboxylmethyl group and the appearance of the exocyclic double bond, which was located at C-4 on the basis of HMBC correlations from H_2-5 to C-3 and C-4. The olefinic acyl-tetronate compounds are structurally related to dehydroterrestric acid and agglomerin previously isolated.

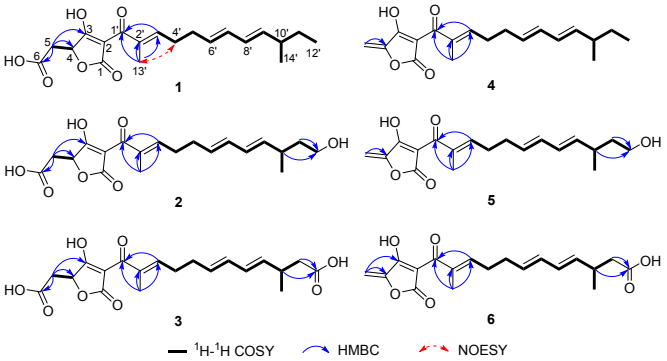

Fig. 2. Selected 2D NMR correlations for **1**-**6**.

Based on the heterologous reconstitution studies and previous work by Li et al on the terrestric acid biosynthetic pathway,[13] a putative pathway for trihazones isolated in this work is outlined in Fig. 1B. The carboxylmethyl containing **1** is proposed to be the products of ThnA and ThnE. The PKS portion of ThnA synthesizes β-keto-triene chain from one acetyl-CoA and six equivalents of malonyl-CoA, in collaboration with ThnE, which selectively reduces the enoyl intermediate during the first and fourth iteration of the PKS. The NRPS domain selects and activates malate, of which the α-hydroxyl group attacks the completed polyketide acyl-S-ACP chain to form the ester product. Intramolecular Dieckmann cyclization catalyzed by the terminal R* domain releases the product as **1** from the PKS-NPRS. Formation of the hydroxylated **2** and carboxylated **3** was unexpected, since no additional oxidative enzymes were introduced into the heterologous host and no such domain exists in ThnA. We attribute the formation of **2** and **3** to the activities of endogenous P450 enzymes in *A. nidulans* under aerobic culturing conditions. Similar endogenous enzyme-catalyzed hydroxylation has been reported in the biosynthesis of isocoumarins and other natural products.[15] The α-KG ThnC catalyzes the oxidative decarboxylation of **1** to **4**, performing a similar role as TraH[13] and CaaC[12] in the biosynthesis of terrestric acid and agglomerin, respectively. Compound **4** represents the most advanced natural product encoded in this BGC, as formation of **5** and **6** are also derived from the oxidation by endogenous P450 enzymes.

ThnC is a 320-aa protein that belongs to the family of α-KG dependent dioxygenase and shares a sequence identity of 38% with TropC.[16] Sequence alignments showed that ThnC contains the conserved His^1-X-Asp/Glu-X_n-His^2 iron-binding motif (Fig. S11†).[17] To directly confirm that ThnC catalyzes the decarboxylation reaction that transforms **1** into **4**, the uninterrupted open reading frame of *thnC* was cloned using RT-PCR from *A. nidulans* ZYG003 (Table S4†) total RNA into pET28b for expression in *Escherichia coli* BL21(DE3). The C-$(His)_6$-tagged ThnC protein was purified to homogeneity (Fig. S12†). In the presence of α-ketoglutarate, L-ascorbic acid and Fe^{II}, ThnC indeed catalyzes the conversion of **1** to **4** in a time-dependent fashion (Fig. 3A). Without ThnC or α-ketoglutarate, the production of **4** cannot be observed, while the lack of additional Fe^{II} or L-ascorbic acid led to severe reduction of product turnover (Fig. S13†). ThnC is expected perform decarboxylation of **1** with the same mechanism as that of TraH proposed by Li and coworkers (Fig. 3B).[13] The C4 hydrogen is first abstracted by the iron-oxo species generated in ThnC to give a tertiary radical at C4. This is followed by decarboxylation and removal of the second electron by the Fe^{III}-OH

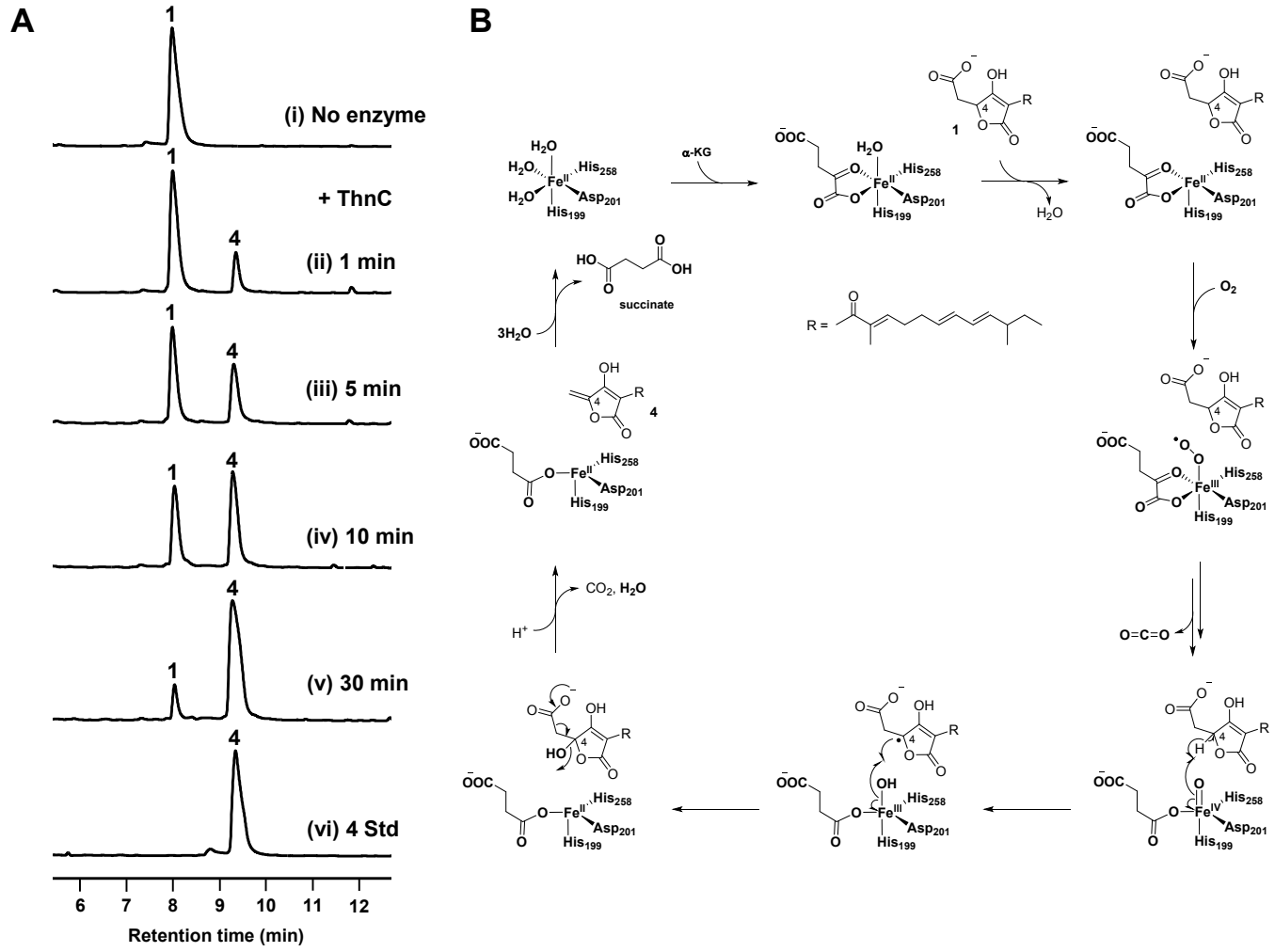

Fig. 3 ThnC catalyzes oxidative decarboxylation of **1** to **4.** (A) time course of ThnC assay with purified enzyme: (i) control assay minus ThnC; A time course of a ThnC assay for 1 min (ii), 5 min (iii), 10 min (iv), 30 min (v); (vi) standard **4**. A general ThnC assay was performed in Tris-HCl buffer (50 mM, pH 8.0) containing 200 μM **1**, 2 μM ThnC, 5 mM L-ascorbic acid, 5 mM α-ketoglutarate, 1 mM $FeSO_4 \cdot 7H_2O$ and incubated at 28 °C. (B) Proposed mechanism of ThnC.

center to give **4.** The fungal strategy to generate tetronate is therefore in contrast to the bacterial one. In the biosynthesis of agglomerin, the precursor 4-hydroxymethyl tetronate is first acetylated by the acetyltransferase Agg4 followed by elimination of acetate by the elimination enzyme Agg5 to yield the exo-olefinic tetronate that is the dienophile for spirotetronate biosynthesis.[18, 19]

Conclusions

In summary, we have reconstituted the *thn* gene cluster from *T. harzianum* t-22 in the heterologous *A. nidulans* host and identified the tetronate-containing trihazones as the products of the pathway. Trihazone D (**4**) is the most advanced product of the pathway and can be synthesized through the actions of PKS-NRPS, ER and α-KG dependent dioxygenase. The discovery of trihazones adds to the inventory of secondary metabolites that can be biosynthesized by *T. harzianum* t-22. Linear tetronates have been reported to display a range of bioactivities. In particular, two didehydro-acaterin analogs isolated from *Pseudomonas jessenii* EC-S101, structurally similar to trihazones, were shown to have inhibitory activity on the phytopathogens *Aphanomyces cochlioides* and *Pythium aphanidermatum*, indicating that trihazones may also have antimicrobial activities.[20]

Experimental

Bacterial strains and reagents and culture conditions

Bacterial strains and plasmids used and constructed in this study are listed in Table S4. *T. harzianum* t-22 was cultivated in potato dextrose broth (PDB, Sigma-Aldrich) at 28 °C for 5-6 days.[10] *A. nidulans* A1145 ΔSTΔEM was grown at 28°C in CD media for sporulation and in CDST media for heterologous expression of biosynthetic gene cluster, compound production and mRNA extraction.[21] *Escherichia coli* BL21(DE3) was used for protein expression and *E. coli* TOP10 was used for cloning. *Saccharomyces cerevisiae* BJ5464-NpgA was used as the yeast host for *in vivo* yeast homologous recombination to

construct *A. nidulans* plasmids and was cultured in uracil drop-out media at 28°C.

Construction of the heterologous expression vector

The plasmids for heterologous expression in *A. nidulans* A1145 ΔSTΔEM were built using pYTU, pYTP, and pYTR[10] (Table S4) with auxotrophic markers for uracil (*pyrG*), pyridoxine (*pyroA*), and riboflavin (*riboB*), respectively, as backbones to insert genes. The genes in *thn* BGC were amplified from genomic DNA of *T. harzianum* t-22. The *thnC* gene was amplified by PCR with primers TH22-22C-1 and TH22-22C-2 (Table S5†). The DNA fragment and *Pac*I/*Swa*I digested pYTU expression vector were co-transformed into *S. cerevisiae* BJ5464-NpgA and selected on uracil drop-out media to generate plasmid pZYG002 (Table S4†). Similarly, *thnA and thnE* were amplified and ligated into vector pYTP, yielding plasmid pZYG001, *thnD* was inserted into vector pYTR, yielding plasmid pZYG003, *thnB and thnD* were cloned into vector pYTR, yielding plasmid pZYG004 and *thnA* was cloned into vector pYTP, yielding plasmid pZYG005 (Table S4†). Plasmids were extracted from yeast using Zymoprep I™ Yeast Plasmid Miniprep I Kit (Zymo Inc. USA), and transformed into *E. coli* TOP10 by electroporation for storage.

Fermentation and isolation of compounds from heterologous expression strains

For small scale *A. nidulans* transformant product analysis, the transformants were grown in CDST agar for 3-4 days at 28°C and extracted with acetone. The organic phase was dried under reduced pressure and dissolved in methanol for LC-MS analysis. LC-MS analyses were performed on a Shimadzu 2020 EV LC-MS with a reverse phase column (Phenomenex Kinetex, C18, 1.7 µm, 100 Å, 2.1 × 100 mm) using positive-and negative-mode electrospray ionization with a linear gradient of 5-95% acetonitrile and water with 0.1% formic acid in 18 min followed by 95% acetonitrile for 3 min with a flow rate of 0.3 mL/min.

For large scale compounds purification, *A. nidulans* transformants were grown for 4-5 days in 3 L CDST agar and then was extracted with 3 L acetone. The extracts were dried under reduced pressure. The extracts were subjected to reversed phase CombiFlash system, using a linear gradient of 5-100% H_2O-acetonitrile in 60 min, then eluted by 100% acetonitrile for 10 min. Fractions containing target compounds were combined and used for further purification by HPLC with a Phenomenex semi-preparative reverse phase column. Compounds **1** and **4** with an isocratic wash of 70% acetonitrile in water supplemented with 0.1% formic acid, Compound **2, 3, 5,** and **6** with an isocratic wash of 50% acetonitrile in water supplemented with 0.1% formic acid. Compound **1**-**6** were obtained as a yellow wish powder.

Overexpression and purification of ThnC

The *thnC* gene was PCR amplified from the cDNA of *A. nidulans* ZYG003 using primers ThnC-EF and ThnC-ER (Table S5). PCR products were inserted into pET28a by MultiS One Step Cloning Kit (Vazyme Biotech Co., Ltd) to afford plasmid pZYG105 (Table S4) after sequence confirmation. The plasmid pZYG105 was introduced to *E. coli* BL21(DE3) to produce *C*-$(His)_6$-tagged ThnC fusion proteins. The overnight cultures (5 mL) of *E. coli* BL21(DE3)/pZYG105 were inoculated into 200 mL LB media in 1 L flask (5 flasks in total). The cultures were grown at 37 °C to OD_{600} of about 0.6. After adding IPTG at a final concentration of 0.1 mM, the cultures were cultivated at 16 °C for additional 12 hours. The cells were then collected by centrifugation, and washed with 50 mM Tris-Cl (pH 8.0) twice. The cell pellets were resuspended in the binding buffer (20 mM Tris-Cl, 500 mM NaCl, and 5 mM imidazole, pH 8.0) and sonicated. After sonication, the mixtures were centrifuged and the supernatants were filtered using 0.45 µm membrane. The filtrates were loaded onto a Ni-NTA affinity column (Novagen, Germany) with the binding buffer, and washed twice by the washing buffer (20 mM Tris-Cl, pH 8.0, 500 mM NaCl, 40 mM imidazole), and then eluted by the eluting buffer (20 mM Tris-Cl, pH 8.0, 500 mM NaCl, 200 mM imidazole). The fractions containing ThnC were desalted by PD-10 column (GE Healthcare, USA), and finally stored in the storage buffer (10% glycerol, 1 mM DTT, 50 mM Tris-Cl, pH 8.0) at -80 °C for further experiments. The expression yield of ThnC is approximately 9 mg/L.

Enzyme assays of ThnC

For *in vitro* assay of ThnC with **1**, a typical reaction was conducted in 100 µL reaction mixture consisting of 200 µM **1**, 2 µM ThnC, 5 mM L-ascorbic acid, 5 mM α-ketoglutarate, 1 mM $FeSO4{\cdot}7H_2O$ in Tris-Cl buffer (50 mM, pH 8.0). The reaction mixtures were incubated at 28 °C and were stopped by adding 100 µL ice cold CH_3OH.The assays were monitored by HPLC analysis. HPLC purification was carried out using a Sync Polar RP column, 4 µm, 80A, 150 × 4.6 mm (ES Industries, Inc.) with UV detection at 227 nm under the following program: solvent system (solvent A, 0.1% trifluoroacetic acid (TFA) in water; solvent B, 100% acetonitrile); 5% B to 100% B (0 - 20 min), 100% B (20 - 25 min), 100%B to 5% B (25 - 26 min), 5% B (26 - 30 min); flow rate at 1 mL/min.

Author contributions

Y. Zhu conducted biological experiments; J. Wang, P. Mou performed chemical experiments; Y. Zhu, Y. Yan, M. Chen, Y. Tang conceived and designed the project; Y. Zhu, J. Wang, Y. Tang wrote the manuscript with the assistance of all authors. All authors participated in data analyses and discussions.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1. D. Boettger and C. Hertweck, *Chembiochem*, 2013, **14**, 28-42.
2. K. M. Fisch, *Rsc Adv.,* 2013, **3**, 18228-18247.
3. H. Li, T. J. Booth and Y.-H. Chooi, in *Comprehensive Natural Products III*, eds. H.-W. Liu and T. P. Begley, Elsevier, Oxford, 2020, 415-444.
4. M. C. Tang, Y. Zou, D. Yee and Y. Tang, *AIChE. J.*, 2018, **64**, 4182-4186.
5. A. Minami, T. Ugai, T. Ozaki and H. Oikawa, *Sci. Rep.*, 2020, **10**, 13556.
6. M. Lorito, S. L. Woo, G. E. Harman and E. Monte, *Annu. Rev. Phytopathol.*, 2010, **48**, 395-417.
7. H. A. Contreras-Cornejo, L. Macias-Rodriguez, E. del-Val and J. Larsen, *FEMS. Microbiol. Ecol.*, 2016, **92**, fiw036.
8. G. E. Harman, *Plant. Dis.*, 2000, **84**, 377-393.
9. H. Nordberg, M. Cantor, S. Dusheyko, S. Hua, A. Poliakov, I. Shabalov, T. Smirnova, I. V. Grigoriev and I. Dubchak, *Nucleic. Acids. Res.*, 2014, **42**, D26-31.
10. M. Chen, Q. Liu, S. S. Gao, A. E. Young, S. E. Jacobsen and Y. Tang, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 5499-5504.
11. F. Vinale, G. Flematti, K. Sivasithamparam, M. Lorito, R. Marra, B. W. Skelton and E. L. Ghisalberti, *J. Nat. Prod*, 2009, **72**, 2032-2035.
12. X. L. Yang, T. Awakawa, T. Wakimoto and I. Abe, *Chembiochem*, 2014, **15**, 1578-1583.
13. J. Fan, G. Liao, L. Ludwig-Radtke, W. B. Yin and S. M. Li, *Org. Lett.*, 2020, **22**, 88-92.
14. M. Sato, F. Yagishita, T. Mino, N. Uchiyama, A. Patel, Y. H. Chooi, Y. Goda, W. Xu, H. Noguchi, T. Yamamoto, K. Hotta, K. N. Houk, Y. Tang and K. Watanabe, *Chembiochem,* 2015, **16**, 2294-2298.
15. P. Xiang, L. Ludwig-Radtke, W. B. Yin and S. M. Li, *Org. Biomol. Chem.*, 2020, **18**, 4946-4948.
16. J. Davison, A. al Fahad, M. Cai, Z. Song, S. Y. Yehia, C. M. Lazarus, A. M. Bailey, T. J. Simpson and R. J. Cox, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 7642-7647.
17. R. P. Hausinger, *Crit. Rev. Biochem. Mol. Biol.*, 2004, **39**, 21-68.
18. W. X. Tao, M. H. Zhu, Z. X. Deng and Y. H. Sun, *Sci. China. Chem.*, 2013, **56**, 1364-1371.
19. C. Kanchanabanca, W. Tao, H. Hong, Y. Liu, F. Hahn, M. Samborskyy, Z. Deng, Y. Sun and P. F. Leadlay, *Angew. Chem., Int. Ed.*, 2013, **52**, 5785-5788.
20. A. Deora, E. Hatano, S. Tahara and Y. Hashidoko, *Plant. Pathol*., 2010, **59**, 84-99.
21. M. Liu, M. Ohashi and Y. Tang, *Org. Lett*., 2020, **22**, 6637-6641.