Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Two new threonine-containing metabolites from fungus *Curvularia inaequalis* strain HS-FG-257

Shao-Yong Zhang, Jian-Song Li, Hui Zhang, Huan Qi, Rui-Jun Wang, Chou-Fei Wu, Li-Qin Zhang, An-Liang Chen, Ji-Dong Wang & Zhi-Kui Hao

To cite this article: Shao-Yong Zhang, Jian-Song Li, Hui Zhang, Huan Qi, Rui-Jun Wang, Chou-Fei Wu, Li-Qin Zhang, An-Liang Chen, Ji-Dong Wang & Zhi-Kui Hao (2020): Two new threonine-containing metabolites from fungus *Curvularia inaequalis* strain HS-FG-257, Natural Product Research, DOI: 10.1080/14786419.2020.1784168

To link to this article: https://doi.org/10.1080/14786419.2020.1784168

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1784168

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

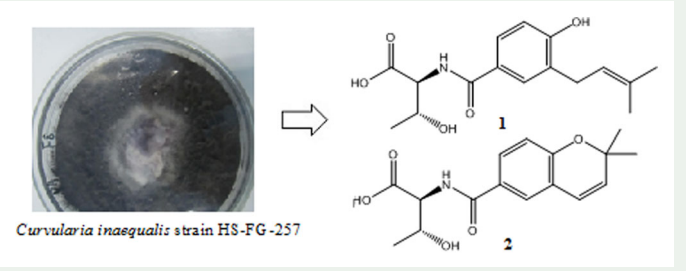
Two new threonine-containing metabolites from fungus *Curvularia inaequalis* strain HS-FG-257

Shao-Yong Zhang[a], Jian-Song Li[b], Hui Zhang[c], Huan Qi[a], Rui-Jun Wang[a], Chou-Fei Wu[a], Li-Qin Zhang[a], An-Liang Chen[d], Ji-Dong Wang[a] and Zhi-Kui Hao[b]

[a]Key Laboratory of Vector Biology and Pathogen Control of Zhejiang Province, College of Life Science, Huzhou University, Huzhou, China; [b]Institute of Applied Biotechnology, School of Medicine and Pharmaceutical Engineering, Taizhou Vocational and Technical College, Taizhou, China; [c]Zhejiang Key Laboratory of Antifungal Drugs, Zhejiang Hisun Pharmaceutical Co., Ltd, Taizhou, China; [d]Provincial Joint Engineering Laboratory of Biopesticide Preparation, School of Forestry & Biotechnology, Zhejiang Agricultural and Forestry University, China

ABSTRACT

Two new threonine-containing metabolites, *N*-[4-hydroxy-3-prenyl-benzoyl]-L-threonine (**1**) and *N*-[2,2-dimethyl-2*H*-chromene-6-carbonyl]-L-threonine (**2**), were isolated from the fermentation broth of the soil fungus *Curvularia inaequalis* strain HS-FG-257. Their structures were elucidated through the interpretation of HR-ESIMS and extensive NMR spectroscopic data. Both compounds exhibited no cytotoxic activity against the test cell lines A549 and HCT-116.



ARTICLE HISTORY
Received 12 January 2020
Accepted 7 June 2020

KEYWORDS
Curvularia inaequalis; threonine-containing metabolites; structure elucidation

1. Introduction

Fungi are a rich source of secondary metabolites and some of these secondary metabolites are important pharmaceuticals, such as penicillin, cyclosporin and statins (Keller et al. 2005). Although many fungi-derived secondary metabolites have been described, the sequencing of fungal genomes has discovered that the potential of fungi for the production of bioactive molecules is still largely untapped (Greco et al. 2019). During the course of the screening program for new fungal metabolites, three new

CONTACT J.-D. Wang 02752@zjhu.edu.cn; Z.-K. Hao haozhikui123@126.com
Supplemental data for this article can be accessed online at https://doi.org/10.1080/14786419.2020.1784168.

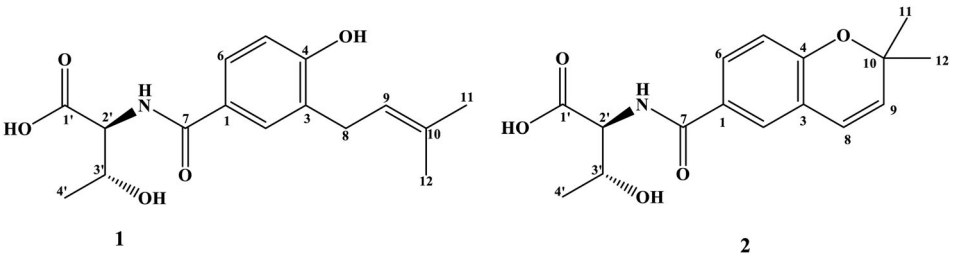


Figure 1. The structures of compounds **1** and **2.**

compounds, curvularone A, 4-hydroxyradianthin, and 4-hydroxy-3-(3-methyoxy-3-methylbutyl)-benzoic acid, have been obtained from fermentation broth of the soil fungus *Curvularia inaequalis* strain HS-FG-257 (Pang et al. 2013; Pang et al. 2012). In further studies concerning the chemical diversity of the constituents of *Curvularia inaequalis* strain HS-FG-257, two new threonine-containing metabolites, *N*-[4-hydroxy-3-prenyl-benzoyl]-L-threonine (**1**) and *N*-[2,2-dimethyl-2*H*-chromene-6-carbonyl]-L-threonine (**2**), were isolated from the *n*-BuOH soluble part of its fermentation broth. Here, we describe the isolation and chemical characterization of the two new compounds.

2. Results and discussion

2.1. Structure determination

Compound **1** was obtained as colorless oil with a specific rotation of $[\alpha]_D^{25}$ +25 (*c* 0.08, EtOH) and UV (EtOH) λ_{max} (log ε): 257 (3.72) nm. Its HR-ESIMS showed a molecular ion peak at *m/z* 308.1518 $[M+H]^+$ (calcd for $C_{16}H_{22}NO_5$, 308.1492), indicating a molecular formula of $C_{16}H_{21}NO_5$. IR absorption bands at 3360, 1728 and 1637 cm^{-1} suggested the presence of hydroxyl, carbonyl and olefinic functionalities. The ^{1}H NMR data of **1** (Table S1, Supplementary material) displayed signals for the protons of a 1,2,4-trisubstituted benzene ring [δ_H7.74 (1H, d, J = 1.6 Hz, H-2), 7.66 (1H, dd, J = 8.2, 1.6 Hz, H-6) and 6.91 (1H, d, J = 8.2 Hz, H-5)], an olefinic methine proton [δ_H 5.34 (1H, t, J = 7.2 Hz, H-9)], two oxygen or nitrogen-bearing methine protons [δ_H 4.66 (1H, dd, J = 8.3, 2.8 Hz, H-2′) and 4.44 (1H, m, H-3′)], a methylene [δ_H 3.36 (2H, d, J = 7.2 Hz, H_2-8)], two methyls connected with double bond [δ_H 1.72 (6H, s, H_3-11, H_3-12)] and a doublet for aliphatic methyl [δ_H 1.25 (3H, d, J = 6.3 Hz, H_3-4′)]. The ^{13}C NMR spectrum (Table S1, Supplementary material), complemented by DEPT and HSQC experiments, showed 16 signals including two carbonyl carbons at δ_C 172.5, 167.6, four sp^2 methines at δ_C 130.0, 127.1, 123.2, 115.2, four sp^2 quaternary carbons at δ_C 158.7, 132.7, 128.7, 126.5, two sp^3 methines (one of which contained oxygen) at δ_C 68.0, 58.6, one sp^3 methylene at δ_C 28.9, and three methyls at δ_C 25.7, 20.6, 17.7. The observed cross peak of H_2-8 and H-9 in the ^{1}H-^{1}H COSY spectrum (Figure S1, Supplementary material) together with the HMBC correlations (Figure S1, Supplementary material) from H_3-11, H_3-12 to δ_C 123.2 (C-9) and δ_C 132.7 (C-10) revealed the presence of an isoprenyl group. The HMBC correlations from H_2-8 to δ_C 128.7 (C-3), 158.7 (C-4) and from H-2 to δ_C 28.9 (C-8) indicated the isoprenyl group was substituted at C-3. An ester or amide carbonyl was situated at C-1, deduced from the long-range couplings from H-2 and H-6 to δ_C

167.6 (C-7) in the HMBC spectrum. The correlated signals of H-2′/H-3′/H_3-4′ in the ^{1}H-^{1}H COSY spectrum and the HMBC correlation between H-2′ and δ_C 172.5 (C-1′) in combination with the ^{1}H and ^{13}C NMR data of C-1′-C-4′ suggested the presence of a threonine residue (Qi et al. 2019). The ^{13}C NMR chemical shift at δ_C 58.6 (C-2′) and the ^{1}H-^{1}H COSY correlation of H-2′ with the active hydrogen (δ_H 7.21) indicated the active hydrogen was a NH group. The long-range coupling from H-2′ to C-7 established the linkage of C-2′ and C-7 via the NH group. Considered the downfield ^{13}C NMR data of C-4 (δ_C158.7) and the molecular formula $C_{16}H_{21}NO_5$ of **1**, a phenolic hydroxyl was situated at C-4. The presence of a phenolic hydroxyl was supported by the broad singlet at δ_H 8.83 (1H, br s) in the ^{1}H NMR spectrum. Accordingly, the planar structure of **1** was elucidated as shown in Figure 1.

The absolute configuration of the threonine residue in **1** was defined by the advanced Marfey's method applied to the acidic hydrolysate of **1** (Peter 1984; Soumini et al. 2016) and the configuration of the threonine moiety was determined as L.

Compound **2** was isolated as colorless oil with a positive rotation value ($[\alpha]_D^{25}$+47, EtOH) and UV (EtOH) λ_{max} (log ε): 237 (3.95) nm. Its molecular formula was established as $C_{16}H_{19}NO_5$ by HR-ESIMS at *m/z* 306.1325 $[M+H]^+$. The IR spectrum revealed carbonyl absorption at 1747 cm^{-1} and hydroxyl group absorption at 3179 cm^{-1}. Analysis of ^{1}H NMR spectrum (Table S1, Supplementary material) and the ^{1}H-^{1}H COSY correlations (Figure S1, Supplementary material) of **2** indicated a 1,2,4-trisubstituted benzene ring [δ_H 7.74 (1H, d, J = 8.3 Hz, H-6), 7.67 (1H, s, H-2), 6.80 (1H, d, J = 8.3 Hz, H-5)], one *cis*-double bond [δ_H 6.48 (1H, d, J = 9.8 Hz, H-8) and 5.80 (1H, d, J = 9.8 Hz, H-9)], two oxygen or nitrogen-bearing methine protons [δ_H 4.68 (1H, m, H-2′) and 4.45 (1H, m, H-3′)], two aliphatic methyl singlets [δ_H 1.43 (6H, s, H_3-11, H_3-12)] and one doublet for aliphatic methyl [δ_H 1.25 (3H, d, J = 6.3 Hz, H_3-4′)]. The ^{13}C and DEPT NMR data (Table S1, Supplementary material) exhibited two carbonyl carbons, three sp^2 quaternary carbons, five sp^2 methines, one oxygen-bearing sp^3 quaternary carbon, one oxygen-bearing sp^3 methine, one nitrogen-bearing sp^3 methine and three methyls. The cross peaks of H-2′/H-3′/H-4′ in the ^{1}H-^{1}H COSY spectrum and the HMBC correlation (Figure S1, Supplementary material) from H-2′ to δ_C 172.4 (C-1′) suggested the presence of a threonine residue in **2**. HMBC correlations of H_3-11/δ_C 28.2 (C-12) and H_3-12/δ_C 28.2 (C-11) showed the presence of *gem*-dimethyls. The *gem*-dimethyls and the *cis*-double bond were connected through an oxygenated aliphatic quaternary carbon (δ_C 77.1) deduced from the long-range couplings from H_3-11 and H_3-12 to δ_C 132.1 (C-9) and δ_C 77.6 (C-10). Except for the threonine moiety, the remaining ^{1}H and ^{13}C NMR data showed close similarities to those of 2,2-dimethyl-2*H*-chromene-6-carboxylic acid (Ayer and Trifonov 1994; Baldoqui et al. 1999), indicated the presence of the 2,2-dimethyl-2*H*-chromene-6-carbonyl substructure. A NH group was deduced from the chemical shift at δ_C 58.6 (C-2′) and the ^{1}H-^{1}H COSY cross signal between H-2′ and the active hydrogen (δ_H 7.29). The threonine residue and the 2,2-dimethyl-2*H*-chromene-6-carbonyl moiety were connected via the NH group evidenced by the HMBC correlation of H-2′/δ_C 167.1 (C-7).

The configuration of the threonine moiety of **2** was determined as L using the advanced Marfey's method after acidic hydrolysis.

N-[4-hydroxy-3-prenyl-benzoyl]-L-threonine (**1**). Colorless oil; $[\alpha]_D^{25}$+25 (*c* 0.08, EtOH); UV (EtOH) λ_{max} nm (log ε): 257 (3.72) nm; IR (KBr) ν_{max}: 3360, 2977, 1728, 1637, 1538, 1490, 1278, 1153, 1116 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table S1, Supplementary material; HR-ESIMS: *m/z* 308.1518 $[M+H]^+$ (calcd for $C_{16}H_{22}NO_5$, 308.1492).

N-[2,2-dimethyl-2*H*-chromene-6-carbonyl]-L-threonine (**2**). Colorless oil; $[\alpha]_D^{25}$+47 (*c* 0.17, EtOH); UV (EtOH) λ_{max} nm (log ε): 237 (3.95) nm; IR (KBr) ν_{max}: 3179, 2976, 1747, 1630, 1533, 1485, 1363, 1276, 1199, 1124 cm^{-1}; ^1H and ^{13}C NMR spectral data see Table S1, Supplementary material; HR-ESIMS: *m/z* 306.1325 $[M+H]^+$ (calcd for $C_{16}H_{20}NO_5$, 306.1336).

2.2. Cytotoxic activities

The cytotoxic activities of compounds **1** and **2** against human lung adenocarcinoma cell line A549 and human colon cancer cell line HCT-116 were evaluated. But, none of them exhibited cytotoxic activity against the test cell lines.

3. Experimental

3.1. Fermentation,extraction and isolation

The fermentation of the soil fungus *Curvularia inaequalis* (Dematiaceae) strain HS-FG-257 was the same as those described previously (Pang et al. 2013) and 30 L fermentation broth was obtained.

The fermentation broth (30 L) was filtered to obtain the mycelial cake and the supernate. The mycelial cake was extracted with MeOH (3 L) and the supernate was subjected to a Diaion HP-20 resin column eluting with 95% EtOH (5 L). The MeOH extract and the 95% EtOH eluates were combined and concentrated under reduced pressure to about 1 L at 50 °C. The residual aqueous extract was successively extracted with EtOAc followed by *n*-BuOH extraction. The *n*-BuOH phase was concentrated under reduced pressure to yield a mixture (18 g). The mixture was applied on a silica gel (Qingdao Haiyang Chemical Group, Qingdao, Shandong, China; 100–200 mesh) column and successively eluted with a stepwise gradient of $CHCl_3$/MeOH (49:1–1:1, v/v) to offer three fractions (Fr.1 to Fr.3) based on the TLC profiles. The Fr.2 (1.3 g) was separated by preparative HPLC (Shimadzu LC-8A, Shimadzu-C18, 5 μm, 250 × 20 mm inner diameter; 20 mL min^{-1}; 220/254 nm; Shimadzu, Kyoto, Japan) eluting with MeOH/H_2O (3:7, v/v) to give Fr.2-1 (t_R16.9 min, 105 mg). Fr.2-1 was further purified by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm inner diameter; 1.5 mL min^{-1}; 220 nm; Agilent, Palo Alto, CA, USA) eluting with a solvent system of 35% aqueous CH_3CN containing 0.1% acetic acid to yield compounds **1** (t_R 14.2 min, 23 mg) and **2** (t_R 17.0 min, 14 mg).

3.2. Acid hydrolysis and marfey's method

Compound **1** (1 mg) in 2 M HCl (7.4%, 1 mL) was heated at 100 °C in a sealed vial for 2 ∼ 3 h, after which the hydrolysate was concentrated to dryness under a stream of

dry N_2. The hydrolysate was then treated with 1 M $NaHCO_3$ (1 mL) and $_D$-FDAA (10% solution in acetone, 2 mL) at 40 °C for 1 h, after which the reaction was neutralized with 1 M HCl (3.7%), filtered (0.45 μm PTFE) prior to HPLC analysis. Likewise, the derivatives of compound **2** (1 mg) and the standard L-threonine (5 mg) were prepared according to themethod described above. An aliquot (5 μL) of the analytes were injected into an Agilent Eclipse XDB-C18 column (5 μm, 250 × 9.4 mm, 30 °C) with a 1 mL/min, 30 min linear gradient elution from 30% to 70% CH_3CN/H_2O with 0.1% trifluoroacetic acid in H_2O. The threonine content of analytes was assessed by UV (338 nm). Under these conditions, the derivative of standard L-threonine gave a peak at t_R(min) = 14.47(Figure S20, Supplementary material), while the derivatives of the liberated threonine residues of **1** and **2** showed peaks at 14.46 and 14.37 min (Figure S20, Supplementary material), respectively. The threonine moieties of the two new compounds **1** and **2** were identified as L-threonine.

3.3. Cytotoxic bioassay

The cytotoxicity of compounds **1** and **2** against the human tumor cell lines A549, and HCT-116 were assayed using the CCK-8 colorimetric method as described in our previous paper (Wang et al. 2009).

4. Conclusion

Two new threonine-containing metabolites, *N*-[4-hydroxy-3-prenyl-benzoyl]-L-threonine(**1**) and *N*-[2,2-dimethyl-2*H*-chromene-6-carbonyl]-L-threonine (**2**), were obtained from the *n*-BuOH soluble fraction of the fermentation broth of the soil fungus *Curvularia inaequalis* strain HS-FG-257. It revealed the structural diversity of the secondary metabolites derived from this fungus. Furthermore, the structures of **1** and **2** also suggested that the fungus *Curvularia inaequalis* strain HS-FG-257 has the potential to produce the hybrids of threonine and aromatic carboxylic acid compounds, which is worth further study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Key Research and Development Programs of Zhejiang Province (Grant No:2020C02028), (Grant No:2019C02030), (Grant No:2019C02024) and the Zhejiang Provincial Natural Science Foundation of China (HX2019069, HX2019078).

References

Ayer WA, Trifonov LS. 1994. Aromatic compounds from liquid cultures of *Lactarius deliciosus*. J Nat Prod. 57(6):839–841.

Baldoqui DC, Kato MJ, Cavalheiro AJ, Bolzani VS, Young MC, Furlan M. 1999. A chromene and prenylated benzoic acid from *Piper aduncum*. Phytochemistry. 51(7):899–902.

Greco C, Keller NP, Rokas A. 2019. Unearthing fungal chemodiversity and prospects for drug discovery. Curr Opin Microbiol. 51:22–29.

Keller NP, Turner G, Bennett JW. 2005. Fungal secondary metabolism - from biochemistry to genomics . Nat Rev Microbiol. 3(12):937–947.

Pang YW, Wang JD, Xiang WS, Liu QF, Wang XJ. 2012. A new benzoic acid derivative from a soil fungus *Curvularia inaequalis* strain HS-FG-257. Nat Prod Res Dev. 24:1331–1334.

Pang YW, Zhang LJ, Fang JS, Liu QF, Zhang H, Xiang WS, Wang JD, Wang XJ. 2013. Two new antitumor constituents from a soil fungus *Curvularia inaequalis* (strain HS-FG-257). J Antibiot. 66(5):287–289.

Peter M. 1984. Determination of D-amino acids.II.use of a bifunctional reagent, 1,5-difluoro-2,4-dinitrobenzene. Carlsberg Res Commun. 49:591–596.

Qi H, Ma Z, Xue ZL, Yu Z, Xu QY, Zhang H, Yu XP, Wang JD. 2019. A new *O*-cinnamoyl threonine derivative from gene *adpA* overexpression strain *Streptomyces* sp. HS-NF-1222A. Nat Prod Res. DOI:10.1080/14786419.2019.1573234.

Soumini V, Pritesh P, Leith JF, Ranjala R, Angela AS, Zeinab K, Robert JC. 2016. C3 and 2D C3 marfey's methods for amino acid analysis in natural products. J Nat Prod. 79:421–427.

Wang JD, Zhang H, Ying LP, Wang CX, Jiang N, Zhou Y, Wang HB, Bai H. 2009. Five new epothilone metabolites from *Sorangium cellulosum* strain So0157-2. J Antibiot. 62(9):483–487.