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SHORT REPORT

Germicidin T: A new trimethyl-substituted α-pyranone from the Mangrove-derived *Streptomyces ardesiacus* GXIMD 03502

Chun-Xiao Chen[1,2,3], Rong-Chun Qin[1,2,3], Si-Ni Liu[1,2,3], Meng Bai[1,2,3], Zhen-Zhou Tang[1,2,3], Hai-Mei Wei[1,2,3], Cheng-Hai Gao[1,2,3], Xin-Ya Xu[1,2,3*] and Xiao-Dong Jiang[1,2,3*]

[1]Institute of Marine Drugs, Guangxi University of Chinese Medicine, Nanning, 530200, P.R. China
[2]Guangxi Key Laboratory of Marine Drugs, Nanning, 530200, P.R. China
[3]University Engineering Research Center of High-efficient Utilization of Marine Traditional Chinese Medicine Resources, Guangxi, Nanning, 530200, P.R. China

Abstract: An in-depth investigation into the chemical constituents of *Streptomyces ardesiacus* GXIMD 03502, a strain known to produce 2-hydroxyphenylthiazoline derivatives, resulted in the isolation of a new α-pyrone compound, germicidin T (**1**), together with six known compounds (**2**–**7**). Their structures were elucidated using comprehensive spectroscopic analyses. The germicidin biosynthetic gene cluster was identified in the genome of this strain. Notably, ORF 17, a type III polyketide synthase (Germicidin synthase, GCS), was found to utilize trimethylmalonyl-ACP as a starter unit in the biosynthesis of compound **1**. Based on this finding, the biosynthetic pathway for compounds **1**–**7** is proposed. Bioactivity evaluation revealed that all compounds exhibited weak toxicity against *Artemia salina*, while compounds **3** and **7** showed moderate anti-inflammatory activity.

Keywords: *Streptomyces ardesiacus*, α-pyrone, germicidin, trimethylmalonyl-ACP, type III polyketide synthase, biosynthesis

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1 Microroganism Source

The strain, designated *Streptomyces ardesiacus* GXIMD 03502, was isolated from mangrove sediments in Hainan, China. It was identified as *Strepomyces ardesiacus* with 100% similarity based on its 16S rDNA gene sequence (GenBank accession number: PP249927) (Liu et al., 2025). The strain is preserved in the Beibu Gulf Marine Microbial Resource Collection, Institute of Marine Drugs, Guangxi University of Chinese Medicine.

2 Previous Studies

α-Pyrones represent an important structural group of polyketide-derived natural products, characterized by a six-membered unsaturated lactone ring (Schäberle, 2016) with varying alkyl substituents at the C-3 and C-6 positions. Among these, germicidins are a group of α-pyranone compounds primarily produced by actinomycetes. To date, 21 members of the germicidin family have been identified, including germicidins A-S and isogermicidins A-B. Despite their relatively simple core skeletons, these compounds exhibited a range of bioactivities, such as inhibition of spore germination (Aoki et al., 2011), suppression of hexokinase II (Zhang et al., 2020), neurostimulatory effects in zebrafish (Wu et al., 2022), and DPPH radical-scavenging activity (Sugiyama et al., 2010). Such bioactivity diversity arised from variations in chain length, saturation level, and oxidation state of the alkyl side chains introduced during biosynthesis. Normally, germicidin biosynthesis was catalyzed by a type III polyketide synthase, which exhibited a preference for utilizing β-ketoacyl-ACP intermediates from fatty acid metabolism as starter units and accepted malonyl-, methylmalonyl-, or ethylmalonyl-CoA as extender units. Following a single elongation cycle, followed by intramolecular cyclization and dehydration yield the germicidin scaffold. Notably, our study revealed for the first time that trimethylmalonyl-ACP could also serve as a direct starter unit in the biosynthesis of germicidin-type scaffold, a role that has not previously described.

3 Present Study

In this study, a new trimethyl-substituted germicidin-type α-pyranone, germicidin T (**1**), was isolated from the

*Corresponding Authors: Xin-Ya Xu. Email: xuxy@gxtcmu.edu.cn; Xiao-Dong Jiang. Email: jxd374487986@163.com

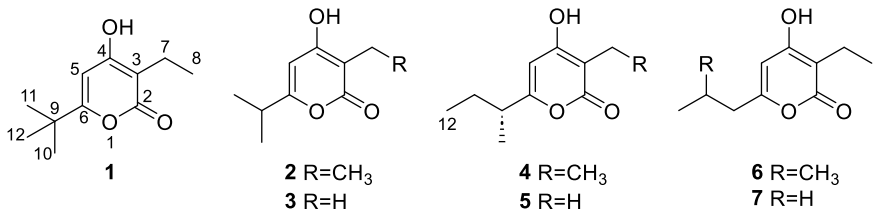

Figure 1. The chemical structures of compounds **1**–**7**

Table 1. NMR data for compound **1** (*J* in Hz, *δ* in ppm)

Position	δ_H[a]	δ_C (mult.)[b]	HMBC Correlations
2	–	168.6 (C)	
3	–	105.0 (C)	
4	–	168.1 (C)	
5	6.02, s	98.3 (CH)	C-3, 4, 6, 9
6	–	171.6 (C)	
7	2.40, q, *J* = 7.4	17.2 (CH_2)	C-2, 3, 4, 8
8	1.03, t, *J* = 7.4	12.8 (CH_3)	C-3, 7
9	–	36.7 (C)	
10, 11, 12	1.26, s	28.1 (CH_3)	C-6, 9

Note: [a]500 MHz in CD_3OD
[b]125 MHz in CD_3OD

mangrove-derived *S. ardesiacus* GXIMD 03502, along with six known compounds: germicidin B (**2**) (Zhao et al., 2022), germicidin D (**3**) (Song et al., 2006), germicidin A (**4**) (Song et al., 2006), germicidin C (**5**) (Aoki et al., 2011), isogermicidin A (**6**) (Song et al., 2006) and isogermicidin B (**7**) (Zhang et al., 2020) (Figure 1). This work presents a comprehensive analysis encompassing the detailed structural elucidation, biosynthetic analysis, and bioactivity evaluation of compounds **1**–**7**.

Detailed descriptions of the general experimental procedures, fermentation, extraction and isolation, as well as assays for cytotoxicity, *Artemia salina* lethality, *α*-glucosidase inhibition activity, antifungal activity, anti-inflammatory activity, and statistical analysis are provided in the Supplementary Information.

*Germicidin T (**1**)*: yellow solid; UV (MeOH) λ_{max} (log ε) 207 (1.90), 288 (1.28) nM. ^1H (CD_3OD, 500 MHz) and ^{13}C (CD_3OD, 125 MHz) NMR data, see Table 1; HR-ESI-MS *m/z* 197.1178 $[M + H]^+$ (calcd. for $C_{11}H_{17}O_3^+$, 197.1172).

Compound **1** was isolated as a yellow solid. Its molecular formula was established as $C_{11}H_{16}O_3$ by HR-ESI-MS (*m/z* 197.1178 $[M + H]^+$, calcd. 197.1172) and NMR data. The ^1H NMR and HSQC spectra of compound **1** revealed four methyl proton δ_H 1.26 (9H, s, CH_3-10, CH_3-11, CH_3-12) and δ_H 1.03 (3H, t, *J* = 7.4 Hz, CH_3-8), one methylene proton δ_H 2.40 (2H, q, *J* = 7.4 Hz, H-7), one alkenyl hydrogen proton δ_H 6.02 (1H, s, H-5). The ^{13}C NMR and HSQC spectra displayed eleven carbon signals, including four methyl carbons δ_C 12.8 (C-8) and δ_C 28.1 (C-10, C-11, C-12), one methylene carbon δ_C 17.2 (C-7), one vinyl methylene carbon δ_C 98.3 (C-5), one tetrasubstituted quaternary carbon δ_C 36.7 (C-9), one olefinic carbon δ_C 105.0 (C-3), two oxygen-connected olefinic carbons δ_C 168.1 (C-4) and δ_C 171.6 (C-6), and one carbonyl carbon δ_C 168.6 (C-2). The HMBC spectrum revealed the correlations from H-5 to C-3, 4, 6, 9, from H-7 to C-2, 3, 4, 8, from CH_3-8 to C-3, 7, and from CH_3-10/11/12 to C-6, 9 (Figure 2). These signals were highly consistent with the NMR data of germicidin B (**2**) reported in the literature (Zhao et al., 2022). A detailed comparison between compound **1** and germicidin B (**2**) revealed that the sole difference was that the presence of an additional methyl group at C-10 in **1**, replacing the hydrogen atom in **2** (Table S10). This modification induced a downfieldshift of the C-6 in the NMR spectrum of compound **1**, attributable to deshielding effect. The novelty of compound **1** was established through a SciFinder database search, and it was designated as germicidin T.

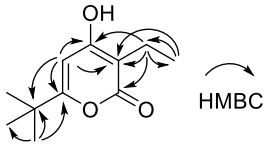

Figure 2. The key HMBC correlations of compounds **1**

Localization Analysis of Biosynthetic Gene Clusters and Proposal of Biosynthetic Pathway: Based on structural features of germicidin-type *α*-pyrones **1**–**7** isolated from *S. ardesiacus* GXIMD 03502, and previous studies (Shen, 2003; Katsuyama & Ohnishi, 2012; Wiker et al., 2019), it is suggested that compounds **1**–**7** are also biosynthesized by type III polyketide synthases (PKSs). In this study, four type III PKS gene clusters, including clusters 2, 11, 12 and 18, were identified from the genome of the strain GXIMD 03502 through analysis using the antiSMASH software. Annotation of these gene clusters revealed that ORF17, a type III polyketide synthase in the cluster 18, shared 81% similarity with GCS A from a germicidin gene cluster in *Streptomyces argillaceus* (Becerril et al., 2018). This finding suggested that cluster 18 was responsible for the biosynthesis of compounds **1**–**7**. Based on literature reports, it is proposed that ORF17 recognizes and utilizes distinct acyl-CoA as starter units. These starter units are extended and condensed with methylmalonyl-CoA or ethylmalonyl-CoA to generate linear *β*, *δ*-diketothiolate intermediates in a single catalytic cycle. These linear intermediates subsequently underwent intramolecular cyclization followed by dehydration to yield compounds **1**–**7** (Figure 3B). Notably, this study revealed for the first time that ORF17

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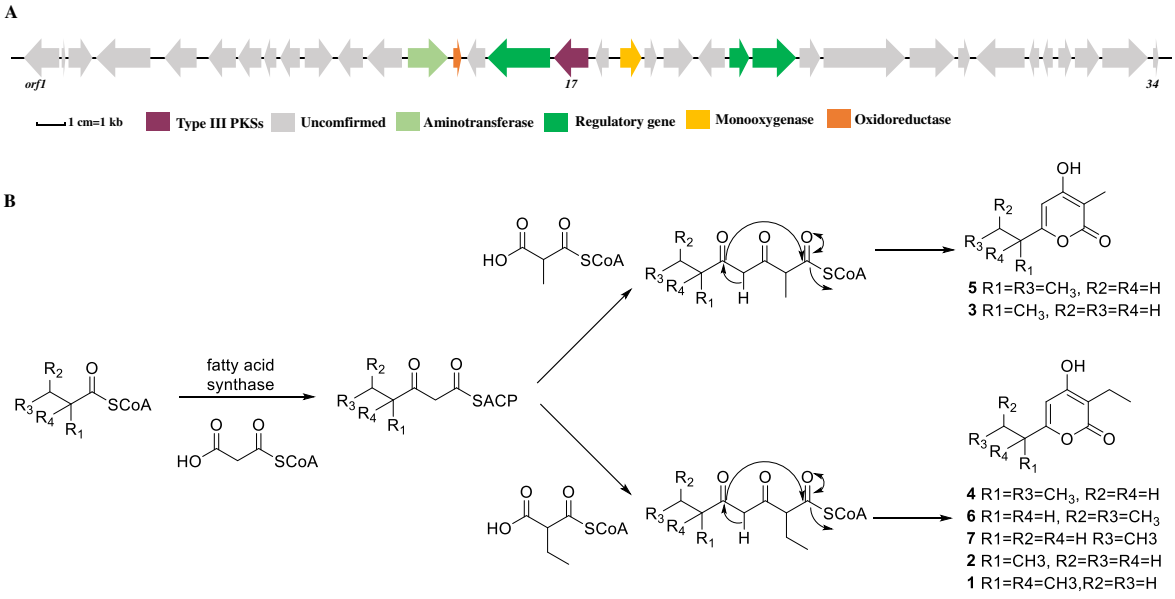

Figure 3. Biosynthetic gene clusters and proposed pathway for germicidins (**1**–**7**). (A) Biosynthetic gene clusters of germicidins (**1**–**7**). (B) Proposed biosynthetic pathway for germicidins (**1**–**7**)

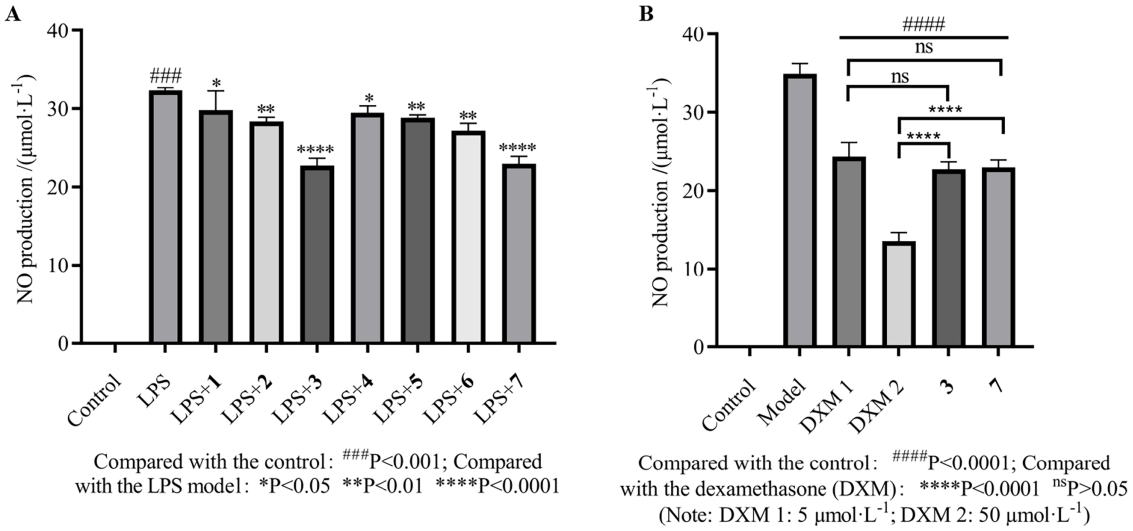

Figure 4. Effects of compounds **1**–**7** (50 μmol·L^{-1}) on nitric oxide (NO) production in LPS-stimulated RAW264.7 cells ($\bar{x}$ ± s, n = 3). A: The effects of compounds **1**–**7** and model group on NO production; B: Comparison of NO inhibitory potency of compounds **3** and **7** with dexamethasone

(Germicidin synthase, GCS), a type III polyketide synthase from *S. ardesiacus* GXIMD 03502, which is capable of recognizing and utilizing the starter unit trimethylmalonyl-ACP for the biosynthesis of the novel compound **1**. This finding demonstrated that ORF17 exhibited broad substrate flexibility.

Based on characteristics of the germicidin-type α-pyrone skeleton, compounds **1**–**7** were evaluated for various bioactivities (Lee et al., 2015; Pei et al., 2017). Their anti-inflammatory effects were assessed by measuring nitric oxide (NO) production in RAW 264.7 macrophages co-treated with lipopolysaccharide (LPS) for 24 hours (Figure 4A). Compared with the blank control, NO production was significantly increased in the model group ($P < 0.001$). At a concentration of 50 μmol·L^{-1}, compounds **3** and **7** significantly inhibited this LPS-induced NO overproduction ($P < 0.001$), while the remaining compounds showed no significant effect. Notably, the inhibitory potency of compounds **3** and **7** at 50 μmol·L^{-1} was comparable to that of 5 μmol·L^{-1} dexamethasone (Figure 4B), with no statistically significant difference observed ($P > 0.05$). These results indicated that compounds **3** and **7** exhibited moderate anti-inflammatory activity at 50 μmol·L^{-1}.

In additional bioactivity assays, compounds **1**–**7** exhibited weak toxicity against *Artemia salina* at 50 μg·mL^{-1}, with mortality rates ranging from 15% to 40%. However,

none of the compounds exhibited significant activity in the other assays. They showed neither α-glucosidase inhibition (EC_{50} > 500 μmol·L^{-1}), nor antifungal activity (IC_{50} > 50 μg·mL^{-1}), nor notable cytotoxicity (IC_{50} > 20 μmol·L^{-1}).

It has been documented that compounds **2**–**5** could inhibit spore germination in *Streptomyces coelicolor* A3(2) (Aoki et al., 2011). Compounds **2**, **5** and **6** exhibited potent hexokinase II inhibitory activity with IC_{50} values of 7.11 to 8.78 μmol·L^{-1} (Zhang et al., 2020). Compounds **2** and **7** induced neuroexcitatory effects in zebrafish (Wu et al., 2022), and compound **3** showed mild DPPH radical-scavenging activity (Song et al., 2006).

This study expanded the structural and bioactivity diversity of the germicidin family, and the ORF17 (GCS) was discovered to recognize and assemble trimethylmalonyl-ACP into the germicidin scaffold for the first time. It provided a theoretical basis and genetic resources for subsequent structural diversification and functional enhancement of germicidins via synthetic biology and metabolic engineering strategies.

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Author Contributions

Chun-Xiao Chen: Investigation, Formal analysis and Writing original manuscript; Rong-Chun Qin: Investigation and Formal analysis; Si-Ni Liu: Investigation and Methodology; Meng Bai: Formal analysis and Visualization; Zhen-Zhou Tang: Investigation; Hai-Mei Wei: Methodology; Cheng-Hai Gao: Formal analysis; Xin-Ya Xu: Writing-review & editing, Conceptualization and Methodology; Xiao-Dong Jiang: Writing-review & editing, Conceptualization, Methodology and Project administration.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

ORCID

Chun-Xiao Chen: 0009-0002-1088-4500
Rong-Chun Qin: 0009-0006-9922-3520
Si-Ni Liu: 0009-0003-9388-3569
Meng Bai: 0000-0002-1024-3458
Zhen-Zhou Tang: 0000-0002-5519-7467
Hai-Mei Wei: 0009-0001-9660-2799
Cheng-Hai Gao: 0000-0002-1088-4087
Xin-Ya Xu: 0000-0001-5968-6869
Xiao-Dong Jiang: 0000-0003-2849-6194

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