

Bioactive Alkaloids from the Beibu Gulf Coral-associated Fungus

Acremonium sclerotigenum GXIMD 02501

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(Received April 24, 2022; Revised May 05, 2022; Accepted May 15, 2022)

Abstract: The Beibu Gulf represents an underexplored reservoir of marine micro-/organisms and secondary metabolites. Three uncommon 4-hydroxy-2-pyridone alkaloids and one phenazine alkaloid were obtained from the Beibu Gulf coral-associated fungus *Acremonium sclerotigenum* GXIMD 02501 via OSMAC approach. They were identified as campyridones D (**1**) and A (**2**), ilicicolin H (**3**), and phenazine-1-carboxylic acid (**4**), respectively, by spectroscopic analysis and comparison with literature values. All of them were evaluated for cytotoxic, anti-*Vibrio*, and NF- κ B luciferase inhibitory activities. Compounds **2** and **3** showed cytotoxicity against two prostate cancer cell lines, with IC₅₀ values of 17.6 ± 1.3 and 5.5 ± 1.2 μ M for PC-3, while 25.4 ± 1.7 and 11.9 ± 1.3 μ M for 22Rv1, respectively. Besides, compound **4** showed promising anti-*Vibrio* activity with MIC values of 0.047–0.067 mg/mL and also displayed inhibition of LPS-induced NF- κ B activation at 10 μ M.

Keywords: *Acremonium sclerotigenum*; coral-associated fungi; alkaloids; bioactivity; cytotoxicity. © 2022 ACG Publications. All rights reserved.

1. Fungal Source

The strain GXIMD 02501 was isolated from a scleractinian coral *Pocillopora damicornis* endemic to the Weizhou Islands coral reef in Guangxi Zhuang autonomous region. It was identified as *Acremonium sclerotigenum* GXIMD 02501 by sequence analysis of the internal spacer region as previously described [1]. Its voucher specimen has been deposited in Guangdong Microbial Culture Collection Center (GDMCC No. 60670).

2. Previous Studies

The coral-derived microorganisms (particularly fungi) have been found as promising sources of structurally and biologically intriguing secondary metabolites (SM) [2-4]. The *Acremonium* species, widely distributed in soil, plants, and marine organisms, are evidenced as rich sources of novel and bioactive SMs, including steroids, terpenoids, polyketides, alkaloids, peptides, and miscellaneous types [5]. The Beibu Gulf in the north of the South China Sea, harbors a tremendous diversity of marine microbial species, potential bioactive SMs of which have been little investigated. As part of

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our progressive program to explore novel biologically active SMs from the Beibu Gulf-derived marine fungi [1, 6, 7], a coral-associated fungus *Acremonium sclerotigenum* GXIMD 02501 attracted our attention owing to its interesting HPLC-UV profiles of the solid fermented products.

Further efforts of this fungus led to the isolation of structurally diverse and novel ascochlorin derivatives. Notably, acremochlorin A was identified as a novel potent hDHODH inhibitor, which efficiently suppressed tumor growth in patient-derived triple-negative breast cancer (TNBC) xenograft models without obvious toxicity, suggesting a novel potential anti-TNBC lead compound. Besides, illicicolin A displayed a significant antitumor effect in castration-resistant prostate cancer by suppressing the EZH2 signaling pathway [8]. Moreover, 3-bromoascochlorin, a brominated ascochlorin derivative, indicated anti-small cell lung cancer effects via inhibiting the mitogen-activated protein kinase pathway [9]. The above studies revealed that the fungus *A. sclerotigenum* GXIMD 02501 could predominantly produce biologically significant ascochlorins.

Naturally occurring 4-hydroxy-2-pyridone alkaloids with diverse structures and different modifications in the pyridone core showed versatile biological effects, including antifungal, antibacterial, insecticidal, and cytotoxic activities [10]. Meanwhile, the uncommon subclass featured with a decalin unit linked via a carboxide bridge was mainly obtained from marine fungi of *Arthrinium* species, which was found with anti-cancer [11, 12] and antibacterial [13] activities. Notably, 2-pyridone natural products were identified as potential inhibitors of SARS-CoV-2 main protease via the *in silico* methodology [14].

3. Present Study

Given that the strategy of one strain many compounds (OSMAC) is known as a simple and powerful tool to mine the structural diversity of microbial SMs via activating silent biogenetic gene clusters in microorganisms, the strain GXIMD 02501 was further cultured in the Czapek liquid medium. The fermented products were repeatedly extracted with EtOAc thrice and the crude extract was then purified by continuously different column chromatography involving silica gel and octadecylsilane (ODS). Interestingly, three 4-hydroxy-2-pyridone alkaloids and one phenazine alkaloid were characterized by spectroscopic analysis (Supporting Information) as well as comparison with literature data, which were identified as campyridone D (**1**) [12], campyridone A (**2**) [12], illicicolin H (**3**) [12], and phenazine-1-carboxylic acid (**4**) [15] (Figure 1). To our knowledge, the three 4-hydroxy-2-pyridone alkaloids (**1–3**) with a decalin framework were characterized from the *Acremonium* genus for the first time.

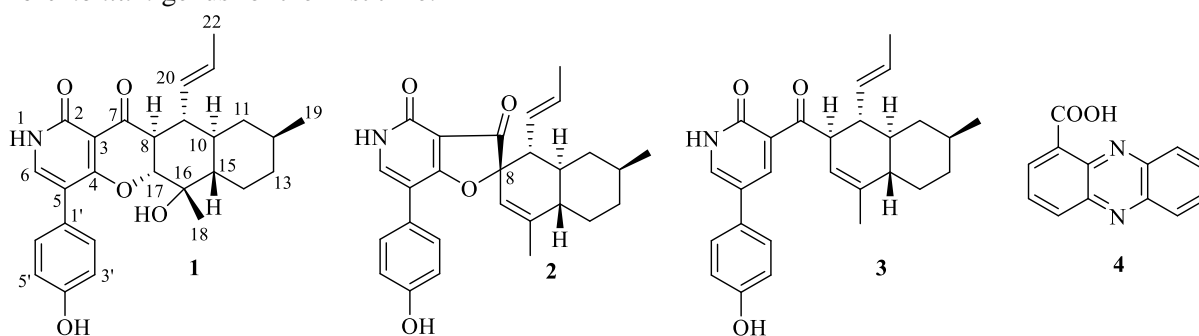


Figure 1. Chemical structures of **1–4**.

Compounds **1–4** were tested for cytotoxic activities against two human prostatic cancer cell lines, PC-3 and 22Rv1. Compounds **2** and **3** showed inhibitory effects against the above two cell lines, with IC_{50} values of 17.6 ± 1.3 and $5.50 \pm 1.2 \mu\text{M}$ for PC-3, while 25.4 ± 1.7 and $11.9 \pm 1.3 \mu\text{M}$ for 22Rv1, respectively. By comparison of the structural characteristics among compounds **1–3**, the oxidation of the Δ^{16} double bond and further cyclization in **1** would probably reduce the cytotoxicity. Besides, compounds **1–4** were also tested for inhibitory activity against a series of pathogenic *Vibrio* spp., including *V. parahemolyticus*, *V. alginolyticus*, *V. owensii*, and *V. coralliilyticus*, while **4** showed

promising anti-*Vibrio* activity with MIC values of 0.053, 0.067, 0.053, and 0.047 mg/mL, respectively. Moreover, compounds **1–4** were then evaluated for their inhibitory effects against lipopolysaccharide (LPS)-induced NF- κ B activation in RAW 264.7 macrophages using the luciferase reporter gene [6]. Compound **4** exhibited inhibition of LPS-induced NF- κ B activation at 10 μ M ($p < 0.001$) (Figure 2). The molecular docking study was further carried out to investigate the binding modes of **4** with NF- κ B p65, which showed that the carboxyl group in **4** interacted tightly with the surrounding hydrophilic arginine residue (ARG-33) through hydrogen bond, with the glide gscore of -3.2 kcal/mol (Figure 2).

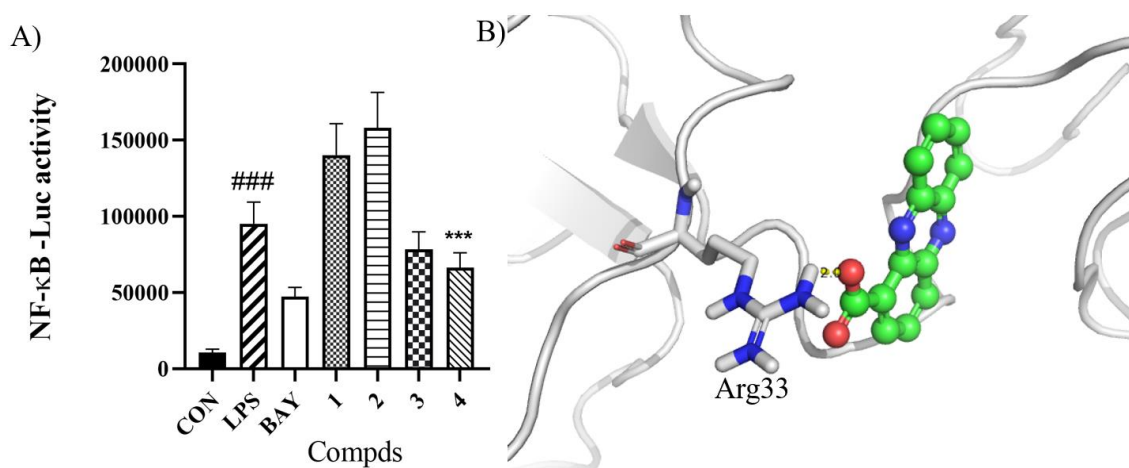


Figure 2. NF- κ B luciferase inhibitory activity. (A) The inhibitory effects of compounds **1–4** on LPS-induced NF- κ B activation in RAW264.7 cells at 10 μ M. $n = 3$. ### $p < 0.001$ vs. control group (CON, untreated); *** $p < 0.001$ vs. LPS-induced group. BAY (BAY11-7082 treated, positive control). (B) The predicted binding modes of compounds **4** with NF- κ B p65 (PDB code: 3GUT, chain A) by molecular docking. The protein receptor is shown by a cartoon and the highlighted interacting residues are shown by thick sticks. The yellow dashed lines represent hydrogen bonds.

Compounds **1** and **2** were recently reported with uncommon γ -pyrone or spiro-furanone moieties, respectively, meanwhile both of them and **3** were found with cytotoxicity against HeLa, HL-60, A549, and/or HCT116 cell lines, with the IC_{50} values of 8.8–20.4 μ M [12]. Illicicolin H (**3**) was known as a potent inhibitor of the mitochondrial cytochrome bc1 reductase with broad antifungal activities [16]. To our knowledge, the cytotoxicity against two prostate cancer cell lines PC-3 and 22Rv1 of compounds **2** and **3** is described herein for the first time. Notably, in our recent endeavor to discover novel osteoclast differentiation inhibitors from marine fungi [6], compound **4** showed inhibitory effects by suppressing LPS-induced NF- κ B activation, which is also reported herein for the first time.

In conclusion, three uncommon 4-hydroxy-2-pyridone alkaloids (**1–3**) and one phenazine alkaloid (**4**) were obtained from the Beibu Gulf coral-associated fungus *A. sclerotigenum* GXIMD 02501 based on OSMAC approach. Their structures were determined by spectroscopic analysis and comparison with reported data. Compounds **2** and **3** showed cytotoxicity against two prostate cancer cell lines PC-3 and 22Rv1, with IC_{50} values of 5.50–25.4 μ M. Besides, compound **4** showed promising anti-*Vibrio* activity with MIC values of 0.047–0.067 mg/mL and also displayed inhibition of LPS-induced NF- κ B activation at 10 μ M. This is the first report of NF- κ B luciferase inhibitory activity for **4**. This study would enrich the chemical context of the genus *Acremonium*, as well as the biological diversity of 4-hydroxy-2-pyridone or phenazine alkaloids.

Acknowledgments

This work was financially supported by the Specific Research Project of Guangxi for Research Bases and Talents (AD20297003, AD19110013), the Natural Science Foundation of Guangxi (2020GXNSFGA297002, 2021GXNSFDA075010), the Special Fund for Bagui Scholars of Guangxi (Yonghong Liu), the National Natural Science Foundation of China (22007019, U20A20101), the Scientific Research Foundation of Guangxi University of Chinese Medicine (2018006, 2018ZD005, 2019BS021), Innovation Project of Guangxi Graduate Education of GXUCM (YCXJ2021118).

Supporting Information

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

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