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Violaceimides A–E, sulfur-containing metabolites from a sponge-associated fungus *Aspergillus violaceus*

Jiaoding Yin [a], Chanjuan Zhang [a], Jiguo Huang [a], Jianping Zhang [a], Dong Liu [a], Jian Huang [a], Peter Proksch [b], Wenhan Lin [a,*]

[a] State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, PR China
[b] Institute für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany

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ABSTRACT

Five new methylsuccinimide-based sulfur-bearing compounds, namely violaceimides A–E (**1**–**5**), were isolated from the sponge-associated fungal strain *Aspergillus violaceus* WZXY-m64-17. Their structures were determined by analyses of the HRESIMS, 1D and 2D NMR data, along with the Mosher's and Snatzke's methods, experimental and calculated ECD data, as well as chemical conversion for the configurational assignment. The structures of **1**–**5** featured the fusion of methylsuccinimide with a modified cysteine to incorporate sulfur element, which was found from nature for the first time. The biogenetic relationship of the sulfur-containing compounds was postulated. Violaceimides A and B exerted selective inhibition against the growth of human leukemia U937 and human colorectal cancer cell HCT-8 with low cytotoxicity toward Vero cells, whereas violaceimide E showed moderate activity to inhibit U937 cells. The preliminary structure-activity relationship was discussed.

Sulfur-containing natural products are ubiquitously distributed in nature with an array of diverse scaffolds. These typical natural compounds showed different biological activities and served important functions in applications in the pharmaceutical usage [1]. For instance, penicillins with a unique "penam" core from *Penicillium* fungi are a group of antibiotics among the first medications against many bacterial infections [2]; thienamycin is one of the most potent naturally produced antibiotics [3], griseoviridin exerted the inhibition of a broad spectrum of bacteria [4], while marine-derived thiocoraline possesses potent anti-cancer effects that will be tested in clinic trial [5]. With the exception of thiodiketopiperazines frequently isolated from marine-derived fungi [6–8], sulfur-incorporating natural products are uncommonly found in marine-derived microorganisms. As a part of our ongoing efforts to discover bioactive natural products from marine-derived fungi, the EtOAc extract of a sponge-associated fungal strain *Aspergillus violaceus* WZXY-m64-17 showed inhibitory effect against leukemia U937 cells. Chromatographic separation and purification of the bioactive extract resulted in the isolation of seven methylsuccinimide based alkaloids, of which five new analogues with unique sulfur-containing were named as violaceimides A–E (**1**–**5**) (Fig. 1).

Violaceimide A (**1**) has a molecular formula of $C_{12}H_{17}NO_7S$, which was provided by the HRESIMS and NMR data, requiring five degrees of unsaturation. The IR absorptions at 3374, 1743, and 1708 cm^{-1} suggested the presence of hydroxy and carbonyl groups. The ^1H NMR spectrum exhibited a methyl doublet (δ_H 1.20, d, J = 6.8 Hz, H_3-5), a methoxy singlet (δ_H 3.64, s), and a number of alkyl protons, while the ^{13}C NMR and DEPT spectra showed 12 carbons resonances including four carbonyl carbons. The COSY couplings of the methine H-2 (δ_H 2.98, ddq) with H_3-5 and methylene H_2-3 (δ_H 2.45, 3.03) in association with the HMBC correlations between H_3-5 and the carbonyl carbon C-1 (δ_C 180.4) and from H-2 to C-1 and the second carbonyl carbon C-4 (δ_C 176.3), as observed in the coexisted versimide [9], clarified the presence of a methylsuccinic anhydride moiety. Inspecting the 2D NMR data of remaining resonances revealed two additional moieties. One was attributed to a methyl ester of cysteine unit according to the COSY relationship between a methine H-7 (δ_H 4.92, dd, J = 5.0, 10.8 Hz) and the methylene protons H_2-8 (δ_H 3.00, 3.39) in association with the HMBC correlations from a carbonyl carbon C-6 (δ_C 168.7) to the methoxy protons, H-7 and H_2-8. The other unit was determined as 2-hydroxy-3-mercaptopropanoic acid based on the COSY coupling of H-10 (δ_H 4.09, brdd, J = 4.9, 6.8 Hz) with H_2-11 (δ_H 2.69, 2.76) and a D_2O exchangeable proton at δ_H 5.50 (br) along with the HMBC correlations from H_2-11 to C-10 (δ_C 71.3) and a carboxylic carbon C-9 (δ_C 174.4). The linkage of the methyl ester of cysteine

* Corresponding author.
E-mail address: whlin@bjmu.edu.cn (W. Lin).

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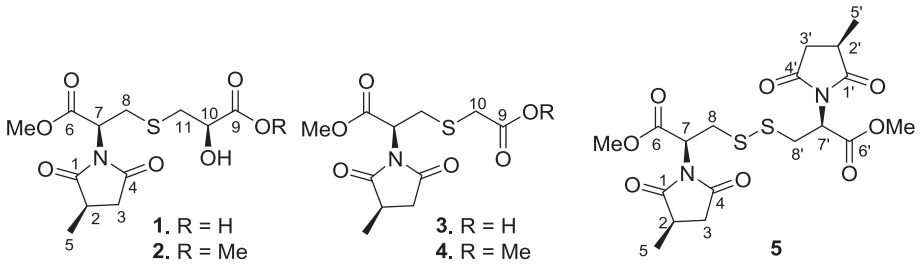

Fig. 1. Structures of violaceimides A–E (**1**–**5**).

with the latter moiety across a sulfur atom was evidenced by the HMBC correlations between H_2-8/C-11 (δ_C 35.2) and H_2-11/C-8 (δ_C 30.4). Subsequently, the connection of methylsuccinic moiety with the modified cysteine to form a methylsuccinimide by positioning C-7 (δ_C 51.1) to the nitrogen atom was deduced by the correlation of H-7 to both C-1 and C-4 in the HMBC spectrum. Based on Snatzke's method [10,11], the induced CD (ICD) data of the $[Mo_2(OAc)_4]$-**1** complex in DMSO exhibited positive Cottone effects for IV band (310 nm) and II band (430 nm). These data were in agreement with positive chirality of *g*+ favored conformation of the O—C—C—O dihedral angle (Fig. 2), reflecting 10*R* configuration. Hydrolysis of **1** under acidic condition afforded methylsuccinic acid (Fig. 3), which showed positive specific rotation ($[\alpha]_D^{23}$ +32°, H_2O), and was in agreement with that of authentic (+)-(*R*)-methylsuccinic acid [12]. Finally, the stereogenic center of cysteine unit was determined on the basis of the ECD data. The experimental ECD data of **1** with negative Cotton effect at 220 nm was in agreement with those calculated for (*S*)-cysteine-bearing **1** at the B3LYP-SCRF/6-311+G(d) level in the gas phase using the B3LYP/6-31G(d) optimized geometries after conformational searches via the MMFF94S force field (Fig. 4) [13,14]. In contrast, the ECD data of (*R*)-cysteine-bearing **1** showed positive Cotton effect at 210 nm. Thus, a D-cysteine in **1** was assumed.

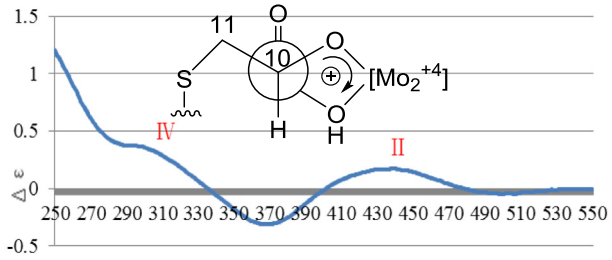

Fig. 2. ICD spectrum of $[Mo_2(AcO)_4]$-**1** in DMSO.

Violaceimide B (**2**) was determined to be a methyl ester of **1**, based on the similar NMR data with the exception of the resonances for an additional methoxy group (δ_C 53.1, δ_H 3.64). The HMBC correlation of the methoxy protons with the carbonyl carbon C-9 (δ_C 173.3) clarified the formation of a methyl ester at C-9. Methylation of **1** generated a product, which was identical to **2** by the comparison with the NMR, ESIMS data, and the value of specific rotation (Fig. 5), supporting the structural assignment. Based on the modified Mosher method [15,16], the (*R*)- and

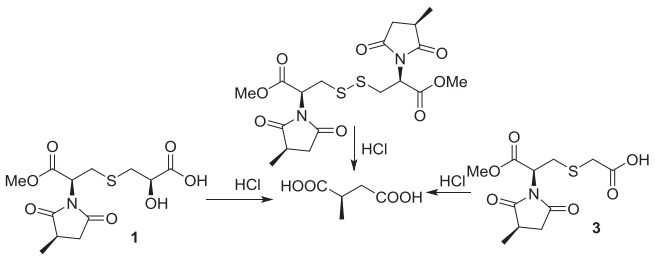

Fig. 3. Acidic hydrolysis of **1**, **3**, and **5**.

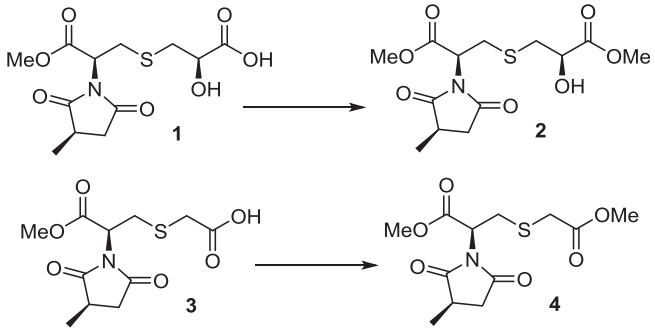

Fig. 5. Conversion of **1** and **3** to **2** and **4** by methylation.

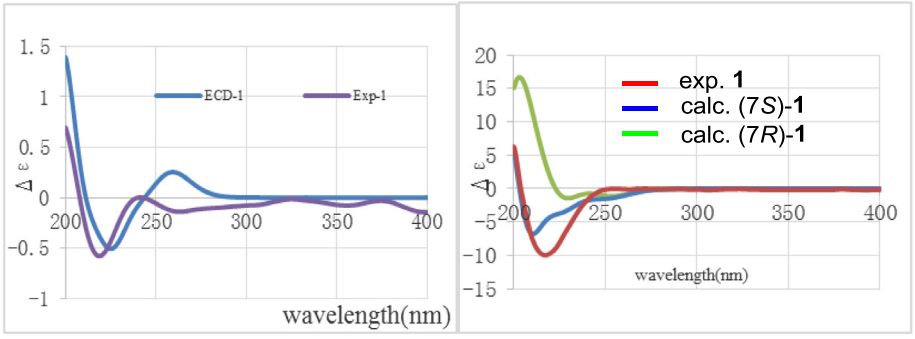

Fig. 4. Experimental and calculated ECD spectra of **1** and **2**.