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Wortmannilactones I–L, new NADH-fumarate reductase inhibitors, induced by adding suberoylanilide hydroxamic acid to the culture medium of *Talaromyces wortmannii*

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ABSTRACT

With the aim of finding more potential inhibitors against NADH-fumarate reductase (specific target for treating helminthiasis and cancer) from natural resources, *Talaromyces wortmannii* was treated with the epigenome regulatory agent suberoylanilide hydroxamic acid, which resulted in the isolation of four new wortmannilactones derivatives (wortmannilactones I–L, **1**–**4**). The structures of these new compounds were elucidated based on IR, HRESIMS and NMR spectroscopic data analyses. These four new compounds showed potent inhibitory activity against NADH-fumarate reductase with the IC_{50} values ranging from 0.84 to 1.35 μM.

Fungi have remarkable capacity of producing secondary metabolites and serve as a precious database for the research and development of new drugs.[1] Numerous secondary metabolites of fungi have been widely used in the treatment of human diseases.[2–4] However, many biosynthetic gene clusters of fungi are silent under standard laboratory fermentation conditions.[5] New methods for activating silent biosynthetic genes are highly desirable to expand the natural products of the fungi.[6,7] Recent years, a significant body of work in epigenome manipulation has proved that the inhibition of histone deacetylase (HDAC) was an effective technique to turn on the silent pathways for producing interesting compounds.[8–11]

Wortmannilactones E, F, H, a new class of polyketides, possessing a dihydropyrane ring, conjugated moiety and an oxabicyclo [2.2.1] heptane moiety, were previously isolated by our group from a solid rice medium of *T. wortmannii* and showed inhibitory activities against cathepsin B.[12] These types of polyketides were first isolated from marine fungus *Penicillium rugulosum* by Gerhard Lang et al.[13] In addition, Mori et al. proved that ukulactone A, the methylated derivative of prugosene A1, showed potent and selective activities against NADH-fumarate reductase (NFRD) of *Ascaris suum*.[14] In fact, the NFRD (composed of complexes I and II) for maintaining mitochondrial energy metabolism within many anaerobic organisms (including helminths and solid tumor cells) is quite different from the electron transport system of the host or normal cell.[15,16] The NFRD has been recognized as a novel target for treating helminthiasis and cancer.[16] Thus, methods for obtaining more wortmannilactones derivatives, which will benefit our understanding of the structure–activity relationship of this new class polyketides, have drawn great interest.

In the present work, aiming to find more potential inhibitors of NFRD from natural resources, an epigenome manipulation method was selected to produce the derivatives of wortmannilactones from *Talaromyces wortmannii*. Moreover, the influences of inhibitors on the production of the wortmannilactones from the culture medium of *T. wortmannii* were also studied. Four new wortmannilactones derivatives (wortmannilactones I–L, **1**–**4**) were isolated and identified. In addition, the inhibitory activities of the wortmannilactones against NFRD were assayed and the structure-activities relationships were also discussed.

T. wortmannii was activated on potato dextrose agar medium for 3 days. Then a single colony was inoculated into a potato dextrose broth culture and treated with three types of histone deacetylase inhibitors, including SAHA (hydroxamic acids), sodium butyrate (aliphatic acid) and cyclo (l-Am7 (SAc)-Aib-L-Phe (pCl)-D-Pro)

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(cyclic peptide)[17] for 7 days in a shaking flask at 28 °C. The extracts from the PDB medium with or without different concentrations of histone deacetylase inhibitors were compared by HPLC analysis. The types and concentrations of HDAC inhibitors showed different influences on the productivity of wortmannilactones (Tables S1–4 in Supporting information), for example, adding SAHA to the medium led to the productivity of wortmannilactone F increased by 50.3% at 50 μM, but decreased by 12.4% at 100 μM, 34.6% at 150 μM, and 46.3% at 200 μM, respectively. Significant changes, in terms of the kinds of secondary metabolites, were only observed in the medium with 100 μM SAHA after seven days (Fig. 1). Peaks 1–4 possessed a typical tetraene UV spectrum, suggesting that they were derivatives of wortmannilactones. The productivities of compounds **1**–**4** were measured as 14.3%, 9.6%, 11.7%, and 8.8% of wortmannilactone F.

After scaling up the cultivation, the whole culture (6 L) was extracted with EtOAc, and then evaporated to bring dried sample (4.2 g). The extract of EtOAc was separated by silica gel to yield three fractions. The fraction 1 was separated by sephadex LH-20 and preparative HPLC (YMC-pack ODS-A) to give **4**. Fraction 3 was loaded on ODS column and then chromatographed on preparative and semi-preparative columns (YMC-pack Pro-C18 RS) successively to yield **1**. Fraction 2 from gel column was separated by preparative column to give **2** and **3**.

Wortmannilactone I (**1**)[18] was isolated as white powder; The IR spectrum indicated that **1** possessed hydroxyl and two carbonyls. The molecular formula of **1** was determined as $C_{26}H_{34}O_5$ according to the positive HRESIMS *m/z* 444.2764 $[M+NH_4]^+$ (calcd: *m/z* 444.2750) (see Supporting information). **1** had the same formula as wortmannilactones F (**6**) and H (**7**) (Fig. 2). The ^1H and ^{13}C NMR spectral data of **1** (Tables 1 and 2) were similar to wortmannilactone H, suggesting that these two compounds possessed the same groups, including seven methyls, two carbonyl groups. Correlations from COSY, HSQC and HMBC indicated that compound **1** is also composed of dihydropyrane ring, conjugated tetraene moiety and oxabicyclo [2.2.1] heptane moiety. Only 25 carbon signals were observed in the ^{13}C NMR spectrum. However, the HSQC spectrum displayed the correlations of the carbon resonance at C (16.9) with two sets of protons at δ_H 1.23 (3H, s) and 1.34 (3H, d). In addition, the HMBC correlation of H-8 with the carbon (δ_C 16.9) and the ^1H–^1H COSY correlation of the protons at δ_H 1.34 with H-20 (δ_H 4.32) assigned the resonances at δ_C 16.9 to be C-24 and C-28, respectively.

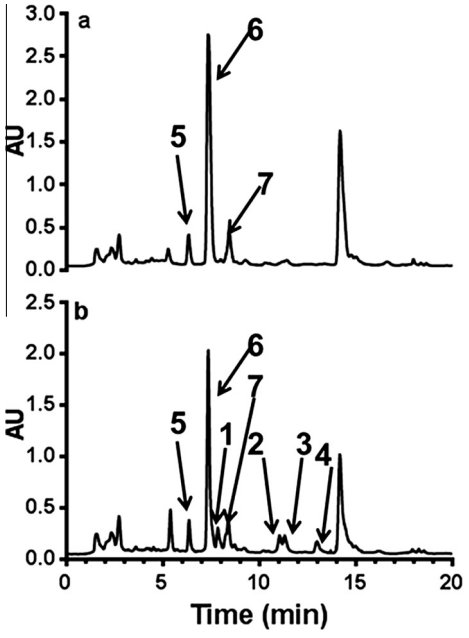

Figure 1. HPLC profile of extracts of *Talaromyces wortmannii* cultivated in PDB (a) and PDB with 100 μM SAHA (b) detected by UV absorption at 312 nm.

According to the HMBC analysis, correlations from H-16 to C-14, C-20, C-25, and C-26, H-18 to C-16, C-20, C-27 and C-26 indicated that compound **1** possessed dihydropyrane ring. Moreover, correlations from H-6 to C-2, C-3, and C-4, H-22 to C-2, C-3, C-4 and C-7 were also found. These data suggested that **1** and wortmannilactone H had the same planar structure. However, the ^{13}C NMR data demonstrated that C-17 was deshielded whereas C-16 and C-18 were shielded as compared with that of wortmannilactone H. In the meantime, distinct chemical shifts of H-26 and H-27 in the ^1H NMR spectrum were also observed. The difference between **1** and wortmannilactone H was a chemical shift of the ^1H and ^{13}C NMR spectrum in the dihydropyrane ring moiety. These data revealed that **1** was considered as a stereoisomer of wortmannilactone H. On the basis of the NOE spectrum analysis, the correlations from H-8 to H-22, H-24, H-5 to H-24, H-6 to H-9 and H-23 confirmed that the relative configuration of the two compounds were the same in the oxabicyclo [2.2.1] heptane moiety (3*S**, 5*R**, 6*S**, 7*R**) (Fig. 3). The observed correlation of H-16 to H-26 and H-28 in **1** indicated that H-16, H-26 and H-28 should be *cis* disposed. Thus, the three stereo centres of the dihydropyrane moiety of **1** were identified as 16*S**, 17*R**, and 20*S**, and the structural difference between **1** and wortmannilactone H was the relative configuration of the groups in position 17. In order to prove the relative configuration and infer the absolute configuration, the ECD spectra of **1** (3*S**, 5*R**, 6*S**, 7*R**, 16*S**, 17*R** and 20*S**) were calculated using the time-dependent density functional theory (TDDFT) method and then compared with the experimental data. The calculated CD spectrum of **1** (3*S*, 5*R*, 6*S*, 7*S*, 16*S*, 17*R*, 20*S*) agreed well with the experimental CD curve (Fig. 5).

Wortmannilactone J (**2**)[19] Wortmannilactone K (**3**)[20] were purified as white amorphous solids. IR spectrum showed that two carbonyl exist in compound **2** and **3**. A positive HRESIMS analysis showed that **2** and **3** possessed the same molecular formula of $C_{27}H_{36}O_5$, (*m/z* 463.2459 $[M+Na]^+$ for **2** and *m/z* 463.2459 $[M+Na]^+$ for **3**) (see Supporting information), and both are 14 mass units greater than those of wortmannilactone F. The ^1H and ^{13}C NMR spectra of **2** and **3** were similar to those of wortmannilactone F, except for the presence of an additioned methoxy group (δ_C 52.5, δ_H 3.31 for **2** and δ_C 50.8, δ_H 3.31 for **3**, respectively). On the basis of the COSY, HMQC, and HMBC analyses, all of the ^1H and ^{13}C NMR signals of **2** and **3** were assigned (Tables 1 and 2), and the double bonds should be at C-17 and C-18, which was consistent with those in wortmannilactone F but different from **1** and wortmannilactone H. The distinct difference was the presence of a methoxy group in **2** and **3** rather than the hydroxyl group in wortmannilactone F. The HMBC correlations of δ_H 3.31 (OCH_3) to C-19 (δ_C 71.3) in **2** and δ_H 3.31 (OCH_3) to C-19 (δ_C 75.3) in **3** demonstrated that the methoxy groups in both **2** and **3** were at C-19.

The NOESY spectrum analysis demonstrated that the relative configurations of C-3, C-4, C-5 and C-6 in **2** and **3** were the same as those in wortmannilactone F. Based on NOESY spectrum of **2**, the correlations of H-16 to H-25, H-26, H-28 and H-OCH_3 indicated that H-16, H-28, and H-OCH_3 were on the same face. The observed correlation of H-20 with H-27 was evidence of H-20 and H-27 on the other face (Fig. 3). However, in compound **3**, the correlation of H-16 and H-OCH_3 was absent whereas the correlation of H-20 and H-OCH_3 was observed in the NOESY spectrum. Therefore, the structures of **2** and **3** were assigned as having a 3*S**, 5*R**, 6*S**, 7*S**, 16*S**, 19*S**, and 20*S** and a 3*S**, 5*R**, 6*S**, 7*S**, 16*S**, 19*R**, and 20*S** relative configuration, respectively. To identify the absolute configuration of **2** and **3**, we also employed TDDFT methods to calculate the ECD spectra of relative configuration. Comparison of the