Xylarianins A-D from the endophytic fungus Xylaria sp. SYPF 8246 as natural inhibitors of human carboxylesterase 2

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\begin{abstract}
Eighteen secondary metabolites were isolated from the fermentation broth of the endophytic fungus Xylaria sp. SYPF 8246, including four new compounds, xylarianins A-D (1–4), three new natural products, 6-methoxycarbonyl-2′-methyl-3,5,4′,6′-tetramethoxy-diphenyl ether (5), 2-chlor-6-methoxycarbonyl-2′-methyl-3,5,4′,6′-tetramethoxy-diphenyl ether (6), and 2-chlor-4′-hydroxy-6-methoxycarbonyl-2′-methyl-3,5,6′-trimethoxy-diphenyl ether (7), and eleven known compounds (8–18). Their structural elucidations were conducted by using 1D and 2D NMR, HRESIMS, and Rh\textsubscript{2}(OOCF\textsubscript{3})\textsubscript{4}-induced electronic circular dichroism (ECD) spectra analyses. The integrated \textsuperscript{1}H and \textsuperscript{13}C NMR data of three new natural products \textsuperscript{1}, \textsuperscript{5} and \textsuperscript{18} displayed significant inhibitory activities against human carboxylesterase 2 (hCE 2). Compounds 1, 5–9, and 18 displayed significant inhibitory activities against hCE 2 with IC\textsubscript{50} values of 10.43 ± 0.51, 6.69 ± 0.85, 12.36 ± 1.27, 18.25 ± 1.78, 29.78 ± 0.48, 18.86 ± 1.87, and 20.72 ± 1.51 µM, respectively. The interactions between compounds 1 and 5 with hCE 2 were analyzed by molecular docking.
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1. Introduction

Endophytic fungi are regarded as key resources for discovering bioactive metabolites [1,2], such as cytochalasins, diphenyl ethers, xanthones, isocoumarines, indole diterpenoids, and depsipeptides, that can be used in medicinal and agricultural fields [1]. Some of bioactive secondary metabolites from fungi have been used in clinic [3–5], including penicillin, cyclosporine A, FK-506, and vancomycin, therefore, endophytic fungi have received more attentions from biosynthetic chemists and pharmacists. Recently, some bioactive indole diterpenoids that displayed antimicrobial activity and agonistic effect against human pregnane X receptor have been isolated from the endophytic fungus Drechmeria sp. in our laboratory [6,7]. As part of our continuous research for discovering bioactive secondary metabolites from plants and fungi [8–12], the investigation of the fermentation broth of the endophytic fungus Xylaria sp. SYPF 8246 led to the isolation of seventeen metabolites (Fig. 1), including four new compounds, xylarianins A-D (1–4), and three new natural products, 6-methoxycarbonyl-2′-methyl-3,5,4′,6′-tetramethoxy-diphenyl ether (5), 2-chlor-6-methoxycarbonyl-2′-methyl-3,5,4′,6′-tetramethoxy-diphenyl ether (6), and 2-chlor-4′-hydroxy-6-methoxycarbonyl-2′-methyl-3,5,6′-trimethoxy-diphenyl ether (7), as well as eleven known compounds (8–18). Their structural elucidations were conducted by using HRESIMS, 1D and 2D NMR, and Rh\textsubscript{2}(OOCF\textsubscript{3})\textsubscript{4}-induced electronic circular dichroism (ECD) spectra analyses. All the isolated compounds were assayed for their inhibitory activities against human carboxylesterase 2 (hCE 2).

2. Results and discussion

Compound 1 was obtained as an amorphous powder, and had the molecular formula of C\textsubscript{18}H\textsubscript{20}O\textsubscript{7} on the basis of the quasi-molecular ion peak at m/z 371.1107 [M+Na]\textsuperscript{+} (calcd. for C\textsubscript{18}H\textsubscript{20}NaO\textsubscript{7}, 371.1101) in the HRESIMS spectrum. The \textsuperscript{1}H NMR data (Table 1) of 1 displayed signals of four aromatic protons at δ\textsubscript{H} 6.37 (1H, d, J = 2.6 Hz, H-5′), 6.28 (1H, d, J = 2.0 Hz, H-3′), 6.21 (1H, d, J = 2.6 Hz, H-3′), 6.19 (1H, d, J = 2.6 Hz, H-3′), and 5.59 (1H, d, J = 2.0 Hz, H-2′), signals of four methoxy groups at δ\textsubscript{H} 3.84 (3H, -OCH\textsubscript{3})

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Compound 2 was assigned the molecular formula C_{12}H_{10}O_{5} according to positive HRESIMS m/z 235.0602 [M + H]^+ (calcd. for C_{12}H_{10}O_{5}, 235.0606), indicating 8 degrees of unsaturation. The 1H NMR data (Table 1) of 1 displayed the presence of two aromatic protons at δ_{H} 6.76 (1H, d, J = 2.0 Hz, H-8), and 6.71 (1H, d, J = 2.0 Hz, H-6), an olefinic proton at δ_{H} 6.84 (1H, s, H-3), a methoxy at δ_{H} 3.98 (3H, s, OCH_{3}-10), and a methyl at δ_{H} 2.73 (3H, s, CH_{3}-9). The 13C NMR data (Table 1) showed 12 carbon resonances, including a carbonyl carbon at δ_{C} 181.5, an ester carbon at δ_{C} 162.4, six aromatic carbons at δ_{C} 164.3, 161.2, 144.3, 119.1, 117.0, and 102.2, one methoxy carbon at δ_{C} 54.0, and a methyl carbon at 23.2. The 1H and 13C NMR data of Compound 2 were closely resembling to those of 2,5-dimethyl-7-hydroxychromone (15) [16], except for that the chemical shift value of C-4 was deshielded from δ_{C} 31.5 in 18 to δ_{C} 63.7 in 3, signals of C-5′ (δ_{C} 22.4) and C-6′ (δ_{C} 13.9) in 18 were absent, and signals of an acetoxy moiety [δ_{H} 2.06; δ_{C} 171.3 and 21.1] were present in 3, which suggested 3 had an acetoxy moiety at C-4′ rather than an ethyl group at C-4′. In the HMBC spectrum of 3, correlations of H-4′ with OAc-4′ confirmed this deduction (Fig. 2). The configuration of C-3 in 3 was established as R according to optical rotation −32.8 similar to that of 18 [17]. Thus, the structure of 3 was showed in Fig. 1, and it was named as xylarianin C.

Compound 4 had a molecular formula of C_{12}H_{16}O_{6} established by HRESIMS (m/z 267.1204 [M + Na]^+), calcd. for C_{12}H_{16}NaO_{6}, 267.1203) and 13C NMR spectra. The 1H and 13C NMR data of 4 were similar to those of 2-hexylidene-3-methyl succinic anhydride ester (18) [17], except for that the chemical shift value of C-3′ was deshielded from δ_{C} 28.1 in 18 to δ_{C} 70.4 in 4, which indicating the location of a hydroxy group at C-3′. This deduction was confirmed by HMBC correlations of H-1′ with C-3′, H-2′a/H-2′b with C-3′, and H-3′ with C-5′. The absolute configuration of 4 was established as 3β,3′R according to a negative Cotton effect at 341 (Δε = −1.01) nm in the Rb_{2}(OCOCF_{3})_{2}-induced ECD spectrum [18], the similar NMR data of C-3 in 3 and 18, and the biosynthesis background of these analogues [17]. Accordingly, the structure of 4 was showed in Fig. 1, and it was named as xylarianin D.

In addition, the fermentation broth of the endophytic fungus Xylaria sp. SYPF 8246 was investigated, and led to the isolation of three new natural products, 6-methoxycarbonyl-2′-methyl-3,5,4′-trimethoxymethyl ether (5) [15], 2-chloro-6-methoxycarbonyl-2′-methyl-3,5,4′,6′-tetramethoxymethyl diphenyl ether [6], same as methyl 4,6-dimethoxy-2-(2,4-dimethoxy-6-methyl-phenyl)-benzoxide) [19], and 2-chloro-4′-hydroxy-6-methoxycarbonyl-2′-methyl-3,5,6′-trimethoxy-diphenyl ether (7) [19], together with eleven known compounds, grise phenone A [8] [20], 5,9,11-trimethoxy-3,13-dihydroxy benzophenone [9] [20], (R)-5-hydroxymellein [10] [21], (R)-5-carbonyl mellein [11] [22], (R)-5-methoxycarbonyl mellein [12] [23], xylarelin [13] [24], (3R)-mellein methyl ether [14] [25], 2,5-dimethyl-7-hydroxychromone [15] [16], (R)-4,6,8-trihydroxy-3,4-dihydro-1(2H)-napthalenone [16] [26], methyl orsellinate [17] [27], and 2-hexylidene-3-methyl succinic acid 4-methyl ester (18) [17]. The integrated 1H and 13C NMR data (Table 2) of 5–7 were reported for the first time.

Human carboxylesterases (hCE 1 and hCE 2) are the important enzymes that hydrolyze chemicals with functional groups, such as a carboxylic acid ester and amide, and they are known to play vital roles in drug metabolism and insecticide detoxication [28]. hCE 1 is abundantly expressed in the liver, whereas hCE 2 is predominately expressed in the gastrointestinal tract. hCE 2 is a major mediator in the gastrointestinal tract to reduce drug toxicity and enhance drug bioavailability in drug metabolism [28], and it has attracted more attentions. Therefore, all the isolated compounds were assayed for their inhibitory activities against s, OCH_{3}-7), 3.81 (3H, s, OCH_{3}-5), 3.69 (3H, s, OCH_{3}-6), and 3.63 (3H, s, OCH_{3}-3), and the signal of a methyl group at δ_{H} 2.04 (1H, s, CH_{3}-7). The 13C NMR data (Table 1) of 1 showed 18 carbon signals, including an ester carbonyl carbon at δ_{C} 169.2, twelve aromatic carbons at δ_{C} 164.2, 160.4, 159.2, 156.7, 154.5, 135.2, 134.1, 109.8, 106.9, 99.6, 92.9, and 92.3, four methoxy carbons at δ_{C} 56.6, 56.4, 55.9, and 52.8, and a methyl carbon at 16.2, which indicated that 1 was a derivative of the diphenyl ethers [13,14]. Comparison of NMR data of 1 and 6-methoxycarbonyl-2′-methyl-3,5,4′,6′-tetramethoxymethyl ether (5) [15] indicated that 1 lacked a methoxy moiety than 5. The locations of four methoxy moieties in 1 were established through an HMBC spectrum showing correlations of OCH_{3}-3 with C-3, OCH_{3}-5 with C-5, OCH_{3}-6′ with C-6′ (Fig. 2) in conjunction with NOESY correlations of OCH_{3}-5 with H-4, OCH_{3}-3 with H-2/H-4, and OCH_{3}-6′ with H-5′ (Fig. 3). Therefore, the structure of 1 was showed in Fig. 1, and it was named as xylarianin A.

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Fig. 1. Secondary metabolites isolated from Xylaria sp. SYPF 8246.

1 R₁ = R₂ = H  
5 R₁ = CH₃; R₂ = H  
6 R₁ = CH₃; R₂ = Cl  
7 R₁ = H; R₂ = Cl

8 R = Cl  
9 R = H  
10 R₁ = R₃ = H; R₂ = OH  
11 R₁ = R₂ = H; R₃ = COOH  
12 R₁ = R₃ = H; R₂ = COOCH₃  
13 R₁ = OH; R₂ = COOCH₃; R₃ = H  
14 R₁ = R₂ = H; R₃ = CH₃