



# Xylanilyticolides A–C, Three New Compounds from Cultures of the Actinomycete *Promicromonospora xylanilytica* YIM 61515

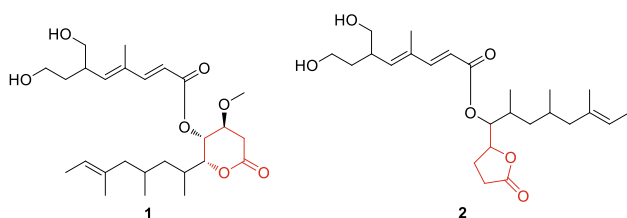
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## Abstract

Three new lactones, xylanilyticolides A–C (**1–3**), were isolated from cultures of the actinomycete *Promicromonospora xylanilytica* YIM 61515. Their structures were elucidated by 1D and 2D NMR spectroscopic data in conjunction with HRESIMS analysis. Compound **1** exhibited potent cytotoxicities against five human cancer cell lines HL-60, A-549, SMMC-7721, MCF-7 and SW480 with the IC<sub>50</sub> values of 3.9, 15.2, 11.2, 5.9, and 4.7 μM, respectively.

## Graphical Abstract



**Keywords** Actinomycete · *Promicromonospora xylanilytica* · Xylanilyticolides · Cytotoxicity

## 1 Introduction

Actinomycetes are well-known for their ability to produce natural products with structural complexity and diverse biological activities [1, 2]. Salinomycin, isolated from the culture broth of an actinomycete strain of *Streptomyces*,

shows remarkable toxicity for cancer stem cells [3, 4]. Actinomycetes, including some rare genera of actinomycetes [5], are also valuable sources in the discovery of natural antibiotics [6].

Since the genus *Promicromonospora* was proposed by Krasil'nikov et al. in 1961 [7], there has been only one report of chemical investigation on this genus with discovery of a novel macrocyclic dilactone from *Promicromonospora* sp. RL26 in 2011 [8]. Herein, the investigation of secondary metabolites from the *Promicromonospora xylanilytica* YIM 61515 led to the discovery of three new products, xylanilyticolides A–C (**1–3**) (Fig. 1) [9]. Their structures were elucidated by analyses of 1D and 2D NMR spectroscopic methods. Their cytotoxicities against five human cancer cell lines were evaluated. Report herein are the isolation, structural elucidation and cytotoxicities of them.

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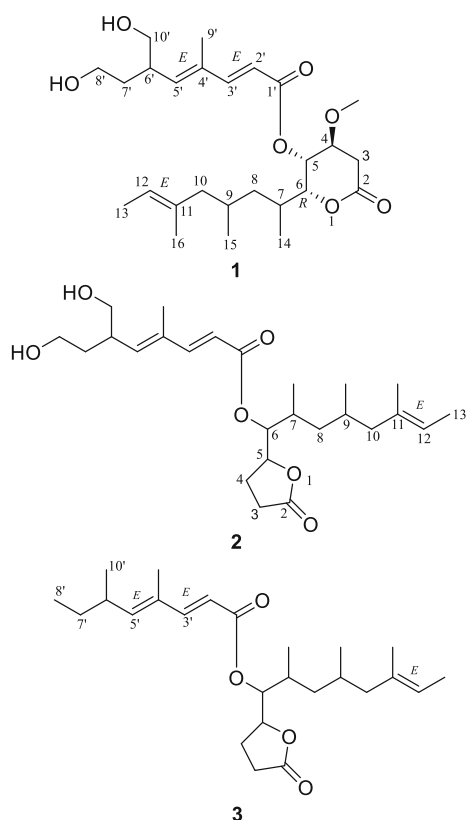


Fig. 1 Structures of compounds 1–3

## 2 Results and Discussion

### 2.1 Structural Elucidation of Xylanilytolides A–C (1–3)

Xylanilytolide A (**1**), obtained as colorless oil, had a formula of  $C_{26}H_{42}O_7$  deduced by HRESIMS sodium adduct ion peak at  $m/z$  489.2822  $[M + Na]^+$  (calcd. for  $C_{26}H_{42}O_7Na$ , 489.2823), requiring six degrees of unsaturation. The  $^1H$  NMR data displayed three methyl singlets at  $\delta_H$  1.84 (H-9'), 1.52 (H-16), 3.44 (OMe), three methyl doublets at  $\delta_H$  1.54 (H-13, d,  $J = 6.2$  Hz), 1.09 (H-14, d,  $J = 6.6$  Hz), 0.77 (H-15, d,  $J = 6.6$  Hz), and two olefinic protons at  $\delta_H$  5.81 (H-2', d,  $J = 15.7$  Hz), 7.34 (H-3', d,  $J = 15.7$  Hz). The  $^{13}C$  NMR and DEPT spectra (Table 1) exhibited signals for 26 carbon atoms, including two carbonyls ( $\delta_C$  165.9, 169.5), six olefinic carbons ( $\delta_C$  115.3, 120.2, 134.4, 134.8, 143.7, 151.1), six methyls ( $\delta_C$  12.8, 13.5, 15.7, 16.1, 20.7, 57.3). The above data coupled with a literature survey indicated that compound **1** was analogous to the reported compound rasfonin, an apoptosis inducer in *Ras*-dependent cells isolated from the fungus *Talaromyces* sp. [10]. Comparing with the reported structure, the two distinct differences in compound **1** were the reduction of olefinic bond in C-3 and the substitution of a methoxy in

C-4. The  $^1H$ - $^1H$  COSY correlations between H-3 ( $\delta_H$  2.79, 2.67)/H-4 ( $\delta_H$  3.70), H-4/H-5 ( $\delta_H$  5.29), and HMBC correlation from  $-OCH_3$  ( $\delta_H$  3.44) to C-4 ( $\delta_C$  74.1), from H-3 ( $\delta_H$  2.79, 2.67) and H-4 to C-2 ( $\delta_C$  169.5) supported these changes (Fig. 2).

$\Delta^{2'}$  was established as *E* configuration, which could be confirmed by the coupling constant (see Table 1). Verified by the ROESY correlation between H-3' ( $\delta_H$  7.34)/H-5' ( $\delta_H$  5.78),  $\Delta^{4'}$  could be identified as *E* configuration.  $\Delta^{11}$  was *E* configuration, which could be identified by the H-12 ( $\delta_H$  5.12)/H-10 ( $\delta_H$  2.00, 1.44) ROESY correlation. From the Newman projection (Fig. 3), the  $J_{H-3/H-4}$  of 3.2 Hz and 5.5 Hz,  $J_{H-4/H-5}$  of 3.2 Hz,  $J_{H-6/H-5}$  of 1.5 Hz as well as H-5/H-6 ROESY correlation were observed, which could confirm the relative configuration of the lactone ring as 4*S*\*, 5*R*\*, 6*R*\*. However, the stereoconfigurations of C-7, C-9, and C-6' can't be determined by the ROESY experiment. The calculated ECD and single crystal X-ray diffraction were also in failure. Therefore, the structure of compound **1** was deduced as xylanilytolide A.

Compound **2** was obtained as colorless oil. Its molecular formula was assigned as  $C_{25}H_{40}O_6$  by HRESIMS ion peak at  $m/z$  459.2723  $[M + Na]^+$  (calcd. for  $C_{25}H_{40}O_6Na$ , 459.2717), implying the presence of six degrees of unsaturation. The  $^1H$  NMR spectrum of **2** illustrated the presence of two methyl singlets at  $\delta_H$  1.54 (H-16) and 1.87 (H-9'); two oxygenated methylenes at  $\delta_H$  3.59/3.49 (H-8'), 3.53 (H-10'); four olefinic protons at  $\delta_H$  5.87 (H-2'), 7.34 (H-3'), 5.84 (H-5'), 5.15 (H-12); and two oxygenated methines at  $\delta_H$  4.79 (H-5), 5.06 (H-6). Combined  $^{13}C$  NMR data (Table 1) and HSQC spectrum indicated the presence of five methyls, seven methylenes, five saturated methines, four olefinic methines, two fully substituted carbons ( $\delta_C$  135.2 and 134.7), and two ketone carbons ( $\delta_C$  176.8 and 167.0). The  $^1H$  and  $^{13}C$  chemical shifts of compound **2** (Table 1) were highly similar to those of **1**, except the  $\delta$ -lactone moiety in **1** was replaced by a  $\gamma$ -lactone moiety in **2**, which could be verified by the HMBC correlation between H-5 ( $\delta_H$  4.79)/C-2 ( $\delta_C$  176.8) and  $^1H$ - $^1H$  COSY correlations of H-6 ( $\delta_H$  5.06)/H-5 ( $\delta_H$  4.79), H-5 ( $\delta_H$  4.79)/H-4 ( $\delta_H$  2.38, 1.98), H-4 ( $\delta_H$  2.38, 1.98)/H-3 ( $\delta_H$  2.53, 2.47) of compound **2** (Fig. 2). The *E/Z* configurations of the double bonds in compound **2** were deduced as the same with those of compound **1** by ROESY correlations of H-9' with H-6' and H-10'. However, the stereochemistry of C-7, C-9, and C-6' can't be established currently. The structure of compound **2** was, therefore, identified as xylanilytolide B.

The molecular of compound **3** was established by HRESIMS ion peak at  $m/z$  427.2817  $[M + Na]^+$  (calcd. for  $C_{25}H_{40}O_4Na$ , 427.2819). Detailed analyses of  $^1H$  and  $^{13}C$  NMR data of compound **3** revealed that compound **3** had the same skeleton with that of compound **2**. The main

**Table 1**  $^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR spectroscopic data of **1–3** ( $\delta$  in ppm,  $J$  in Hz)

No.	<b>1</b> ( $\text{CDCl}_3$ )		<b>2</b> (acetone- $d_6$ )		<b>3</b> (methanol- $d_4$ )	
	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$	$\delta_{\text{C}}$ , type	$\delta_{\text{H}}$
2	169.5, C		176.8, C		179.5, C	
3	32.8, $\text{CH}_2$	2.79 (17.8, 5.5) 2.67 (17.8, 3.2)	28.8, $\text{CH}_2$	2.53, ddd (17.5, 9.5, 9.5) 2.47, ddd (17.5, 9.5, 4.5)	29.3, $\text{CH}_2$	2.58, ddd (17.5, 10.0, 8.9) 2.49, overlapped
4	74.1, CH	3.70, ddd (5.5, 3.2, 3.2)	25.2, $\text{CH}_2$	2.38, m; 1.98, m	25.5, $\text{CH}_2$	2.35, m; 1.95, m
5	66.1, CH	5.29, dd (3.2, 1.5)	80.5, CH	4.79, ddd (7.4, 7.4, 6.5)	81.9, CH	4.81, ddd (7.4, 7.4, 6.4)
6	81.3, CH	4.31, dd (8.7, 1.5)	76.7, CH	5.06, dd (6.5, 4.0)	77.3, CH	5.04, dd (6.4, 4.0)
7	31.7, CH	1.99, m	31.9, CH	2.03, m	32.4, CH	2.01, overlapped
8	40, $\text{CH}_2$	1.22, br. dd (13.3, 5.0) 1.01, ddd (13.3, 9.0, 3.8)	41.7, $\text{CH}_2$	1.36, ddd (14.4, 7.6, 6.3) 0.94, m	41.9, $\text{CH}_2$	1.37, m 0.91, m
9	27.9, CH	1.67, m	28.3, CH	1.79, m	28.7, CH	1.75, m
10	46.4, $\text{CH}_2$	2.00, overlapped 1.44, dd (13.1, 9.8)	48.5, $\text{CH}_2$	1.97, overlapped 1.68, dd (13.0, 8.5)	29.6, $\text{CH}_2$	1.92, overlapped 1.72, m
11	134.4, C		135.2, C		135.5, C	
12	120.2, CH	5.12, q (6.2)	120.5, CH	5.15, q (6.6)	121.0, CH	5.15, q (6.6)
13	13.5, $\text{CH}_3$	1.54, d (6.2)	13.4, $\text{CH}_3$	1.52, d (6.6)	13.5, $\text{CH}_3$	1.53, d (6.6)
14	16.1, $\text{CH}_3$	1.09, d (6.6)	15.6, $\text{CH}_3$	0.98, d (6.8)	15.7, $\text{CH}_3$	0.99, d (6.8)
15	20.7, $\text{CH}_3$	0.77, d (6.6)	20.2, $\text{CH}_3$	0.82, d (6.5)	20.4, $\text{CH}_3$	0.84, d (6.3)
16	15.7, $\text{CH}_3$	1.52, s	15.6, $\text{CH}_3$	1.54, s	15.7, $\text{CH}_3$	1.54, s
1'	165.9, C		167.0, C		168.5, C	
2'	115.3, CH	5.81, d (15.7)	116.2, CH	5.87, d (15.7)	115.9, CH	5.85, d (15.6)
3'	151.1, CH	7.34, d (15.7)	150.9, CH	7.34, d (15.7)	150.3, CH	7.34, d (15.6)
4'	134.8, C		134.7, C		133.1, C	
5'	143.7, CH	5.78, d (10.0)	145.6, CH	5.84, d (10.0)	152.2, CH	5.73, d (10.0)
6'	39.4, CH	2.89, m	39.8, CH	2.89, m	36.2, CH	2.52, m
7'	34.9, $\text{CH}_2$	1.80, m; 1.61, m	35.7, $\text{CH}_2$	1.82, m; 1.48, m	31.1, $\text{CH}_2$	1.46, m; 1.33, m
8'	60.8, $\text{CH}_2$	3.74, m; 3.61, overlapped	60.4, $\text{CH}_2$	3.59, m; 3.49, m	12.3, $\text{CH}_3$	0.87, t (7.4)
9'	12.8, $\text{CH}_3$	1.84, s	12.8, $\text{CH}_3$	1.87, s	12.6, $\text{CH}_3$	1.83, s
10'	66.0, $\text{CH}_2$	3.63, overlapped 3.58, overlapped	66.0, $\text{CH}_2$	3.53, s, 2H	20.5, $\text{CH}_3$	1.02, d (6.6)
–OMe	57.3, $\text{CH}_3$	3.44, s				

difference was that C-8' and C-10' in compound **3** was two methyl carbons rather than two hydroxymethyl groups in **2**. Detailed analysis of 2D NMR data suggested that other parts of **3** were the same to those of **2**. Hence the structure of **3** was elucidated as xylanilyticolide C.

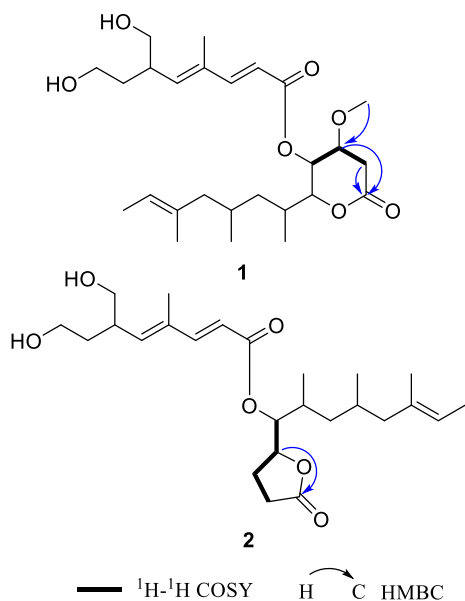
## 2.2 Cytotoxicity Assays

Compounds **1–3** were evaluated for their cytotoxicity activity utilizing an MTS cytotoxicity assay in vitro (DDP was used as a positive control). Compound **1** showed potent inhibitory activity against HL-60, A-549, SMMC-7721, MCF-7 and SW480 cells lines with the  $\text{IC}_{50}$  values of 3.9, 15.2, 11.2, 5.9, 4.7  $\mu\text{M}$ , respectively (Table 2), while compounds **2** and **3** showed no significant cytotoxicity activity under the concentration of 40  $\mu\text{M}$ .

## 3 Experimental

### 3.1 General Experimental Procedures

Optical rotations were recorded on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). UV/Vis spectra were obtained using a Shimadzu UV2401PC spectrometer (Shimadzu, Kyoto, Japan). 1D and 2D NMR spectra were measured on a Bruker Avance III 600 MHz spectrometers (Bruker Biospin GmbH, Karlsruhe, Germany). HRESIMS were recorded on an Agilent 6200 Q-TOF MS system (Agilent Technologies, Santa Clara, CA, USA). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., P. R. China) was used for chromatography column. Size exclusion chromatography was performed on Sephadex LH-20 (GE, Healthcare) columns. MPLC separation was



**Fig. 2** Key 2D NMR correlations of compounds **1** and **2**

conducted on a Biotage Isolera One using a Spherical C18 column. Semi-preparative HPLC was performed on an Agilent 1260 liquid chromatography system equipped with Zorbax SB-C18 columns (9.4 mm × 150 mm, particle size 5 μm).

### 3.2 Actinomycete Materials and Culture Condition

The strain was identified as *P. xylanilytica* YIM 61515 by Prof. Shen Qin of Yunnan University. A voucher strain (HFC: 20160920-4A) was deposited at the School of Pharmaceutical Sciences, South-Central University for Nationalities, China.

The *P. xylanilytica* YIM 61515 strain was cultured in seed medium (glucose 20 g, peptone 2 g, yeast extract 2 g, soluble starch 5 g, K<sub>2</sub>HPO<sub>4</sub> 0.5 g, MgSO<sub>4</sub> 0.5 g, NaCl 4 g, CaCO<sub>3</sub> 2 g in 1 L of deionized water, the pH was adjusted to 7.8 before autoclaving) at 25 °C with shaking at 150 rpm for 7 days. Then inoculated the seed culture into 50 Erlenmeyer flasks (500 mL), previously sterilized by autoclaving, each containing 100 g of rice and 100 mL of

**Table 2** Cytotoxicities of **1** against five human cancer cell lines (IC<sub>50</sub> μM)

Compound	HL-60	A-549	SMMC-7721	MCF-7	SW480
<b>1</b>	3.9	15.2	11.2	5.9	4.7
DDP <sup>a</sup>	4.6	27.4	28.0	29.3	30.0

<sup>a</sup>Positive control

distilled water. All flasks were incubated at 25 °C for 70 days.

### 3.3 Extraction and Isolation

Each flask added 200 mL of EtOAc, and the culture was homogenized. The suspension was extracted repeatedly with EtOAc (4 × 10 L), and the organic layer was evaporated to dryness under vacuum to yield a crude extract (49 g), which was fractionated by MPLC with a stepwise gradient of MeOH/H<sub>2</sub>O (v/v 10:90, 40:60, 70:30, 90:10, 100:0) to give nine fractions (Fr. A–J).

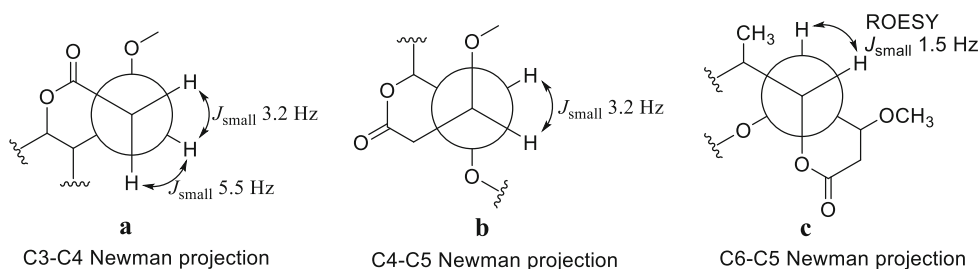
Fr. D (1.87 g) was subjected to 200–300 mesh silica gel column with stepwise elution petroleum ether–EtOAc (8:1–2:1) to obtain nine sub-fractions (D1–D9) based on TLC analyses. The third fraction Fr. D3 was purified by semi-preparative HPLC using a gradient elution (MeCN/H<sub>2</sub>O 50:50 2.5 mL/min) to give compound **1** (4.4 mg, colorless oil).

Fr. E (508 mg) was separated on Sephadex LH-20 (MeOH) to give four fractions (E1–E4), and compound **2** (3.9 mg) was obtained from Fr. E2 (66.9 mg) which purified with semi-preparative HPLC using an isocratic elution (MeCN/H<sub>2</sub>O 55:45, 3 mL/min).

Fr. F (1.9 g) was subjected to 200–300 mesh silica gel column eluting with petroleum ether–EtOAc 4:1 to give 5 fractions (F1–F5). Fr. F3 (98.4 mg) was subjected to semi-prep HPLC with isocratic elution (MeCN/H<sub>2</sub>O 90:10, 3 mL/min) to give compound **3** (0.8 mg).

*Xylanilyticolide A* (**1**) colorless oil,  $[\alpha]_D^{24}$  8.2 (c 0.11, MeOH); UV (MeOH)  $\lambda_{\max}$ (log  $\epsilon$ ) 202 (3.90), 269 (4.31) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1. HRESIMS  $m/z$

**Fig. 3** Newman projection and key ROESY correlations in compound **1**



489.2822 [M + Na]<sup>+</sup> (calcd. for C<sub>26</sub>H<sub>42</sub>O<sub>7</sub>Na, 489.2823).

*Xylanilycolide B (2)* colorless oil, [ $\alpha$ ]<sub>D</sub><sup>24</sup> 1.7 (*c* 0.13, MeOH); UV (MeOH)  $\lambda_{\max}$ (log  $\epsilon$ ) 202 (3.85), 268 (4.31) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1. HRESIMS *m/z* 459.2723 [M + Na]<sup>+</sup> (calcd. for C<sub>25</sub>H<sub>40</sub>O<sub>6</sub>Na, 459.2717).

*Xylanilycolide C (3)* colorless oil, [ $\alpha$ ]<sub>D</sub><sup>24</sup> 4.5 (*c* 0.07, MeOH); UV (MeOH)  $\lambda_{\max}$ (log  $\epsilon$ ) 202 (3.15), 262 (3.77), 544 (1.35) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1. HRESIMS *m/z* 427.2817 [M + Na]<sup>+</sup> (calcd. for C<sub>25</sub>H<sub>40</sub>O<sub>4</sub>Na, 427.2819).

### 3.4 Cytotoxicity

Five human cancer cell lines, breast cancer SK-BR-3, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, pancreatic cancer PANC-1, and lung cancer A-549 were used. Cells were cultured in RPMI-1640 or in DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO<sub>2</sub> at 37 °C. The cytotoxicity assay was performed according to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method in 96-well microplates [11]. Briefly, 100  $\mu$ L of adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1  $\times$  10<sup>5</sup> cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40  $\mu$ M in triplicates for 48 h, and all tests were done in twice with cisplatin (Sigma, Los Angeles, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC<sub>50</sub> values were calculated by Reed and Muench's method [12].

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### Compliance with Ethical Standards

**Conflict of interest** The authors declare no competing financial interest.

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