NOTE

Viridicatumtoxin B, a new anti-MRSA agent from *Penicillium* sp. FR11

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Abstract A new tetracycline-type antibiotic named viridicatumtoxin B along with the known compound viridicatumtoxin has been isolated from the mycelium of liquid fermentation cultures of *Penicillium* sp. FR11. The structure of viridicatumtoxin B was determined on the basis of MS and NMR data. Viridicatumtoxin B inhibited the growth of *Staphylococcus aureus* including methicillin-resistant *S. aureus* and quinolone-resistant *S. aureus* with MIC (μg/ml) of 0.5, which is similar with that of vancomycin, but 8~64 times higher activity than that of tetracycline.

Keywords viridicatumtoxin B, tetracycline, *Penicillium*, antibacterial, MRSA

Gram-positive eubacteria are representative of pathogenic microorganisms. Especially, *Staphylococcus aureus* is the most clinically important of these pathogens because of its exceptional virulence, stress tolerance, and capacity to accumulate antimicrobial resistances [1]. Methicillin-resistant *S. aureus* (MRSA) is known as a major nosocomial pathogen which has also developed resistance to many other antibiotics. Overall, in the United States and the United Kingdom, 40~60% of nosocomial *S. aureus* strains are methicillin-resistant. More deaths are associated with MRSA than with methicillin-sensitive strains. Moreover, MRSA has been reported to acquire resistance to

the last-resort antibiotic, vancomycin [2]. Therefore, it is increasingly important and necessary to find new classes of antimicrobials.

In the course of our screening for new anti-MRSA agents from microbial resources, a new tetracycline-type antibiotic named viridicatumtoxin B (**1**) along with the known compound viridicatumtoxin (**2**) were isolated from liquid fermentation cultures of *Penicillium* sp. FR11 (Fig. 1). Compound **1** is a new epoxide derivative of viridicatumtoxin. Viridicatumtoxin is a tetracycline-type but rare metabolite, which has been once isolated from *Penicillium viridicatum* [3]. The antibacterial activity of viridicatumtoxin has not been reported. In this paper, we report the fermentation, isolation, structure determination, and antibacterial activity of **1** and **2**.

Fermentation and Isolation

The fungal strain FR11 was isolated from a soil sample which was collected around Odae mountain, Kangwon-do, Korea, and identified as *Penicillium* sp. Fermentation was carried out in a liquid culture medium containing YPS medium (2.0% glucose, 0.2% yeast extract, 0.5% peptone, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.1% KH_2PO_4, pH 5.7 before sterilization). A piece of the strain FR11 from a mature plate culture was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of the above sterile seed liquid medium and cultured on a rotary shaker (150 rpm) at 28°C for 3 days. For the production of active compounds, 15 ml of the seed culture were transferred into 1000-ml Erlenmeyer

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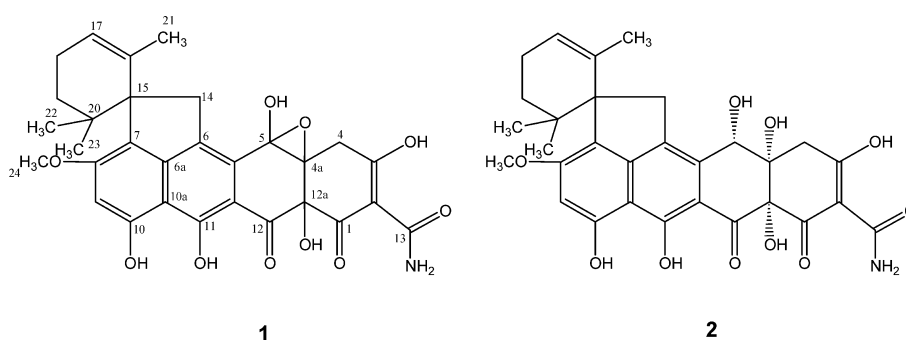
Fig. 1 Structures of viridicatumtoxin B (**1**) and viridicatumtoxin (**2**).

flasks containing 300 ml of the YPS medium, and cultivated on a rotary shaker (150 rpm) for 7 days at 28°C. After incubation, the fermented liquid cultures (20 liters) were extracted with 50% Me_2CO. The Me_2CO extracts was concentrated *in vacuo* to an aqueous solution, which was then extracted with an equal volume of EtOAc three times. The EtOAc extract (5.0 g) was subjected to ODS (YMC s-150 μm) column chromatography, followed by stepwise elution with $MeOH:H_2O$ (50:50, 70:30, 80:20, 90:10, 100:0). The active fractions eluted with both $MeOH:H_2O$ (80:20) and $MeOH:H_2O$ (90:10) were pooled and concentrated *in vacuo*. The residue dissolved in MeOH was further purified by HPLC RP 18 reverse phase HPLC column (20×250 mm, YMC C_{18}) chromatography with a photodiode array detector. The column was eluted with $MeOH:H_2O$ (85:15) containing 0.01% trifluoroacetic acid at a flow rate of 5.0 ml/minute to afford **1** (2.0 mg) and **2** (142.5 mg) at retention times of 32.1 and 38.2 minutes, respectively, as yellow powders.

Structure Elucidation

The ^1H- and ^{13}C-NMR spectroscopic data of **2** suggested that this compound is a member of the tetracycline class. Independent interpretation of the ^1H- and ^{13}C-NMR data together with the ^1H-^1H COSY, DEPT, HMQC, HMBC NMR data led to the identification of **2** as viridicatumtoxin [4]. We found that the ^{13}C-NMR chemical shifts at C-14 and C-19 of viridicatumtoxin have been wrong assigned in the literature. Their ^{13}C chemical shifts should be revised.

The molecular formula of **1** was determined as $C_{30}H_{29}NO_{10}$ on the basis of the HRESI-MS $[(M-H)^-$, 562.1711 m/z (-0.2 mmu error)] in combination with the ^1H- and ^{13}C-NMR data. Compound **1** gave characteristic UV maxima at 427 and 450 nm, which were different from that of **2** (433 nm) as shown in Table 1. IR absorptions of **1** at 1625 and 3417 cm^{-1} suggested the presence of carbonyl and hydroxyl moieties, respectively. The ^1H- and ^{13}C-NMR data (Table 2) supported by the ^1H-^1H COSY, DEPT, and

Table 1 Physico-chemical properties of viridicatumtoxin B (**1**)

Appearance	Yellow powder
$[\alpha]_D^{25}$	+18.3 (*c* 0.2, EtOH)
ESI-MS (*m/z*)	562 $[M-H]^-$
HRESI-MS (*m/z*)	
found.	562.1711 $[M-H]^-$
calcd.	562.1713 for $C_{30}H_{28}NO_{10}$
Molecular formula	$C_{30}H_{29}NO_{10}$
UV λ_{max} nm (log ε) (MeOH)	252 (4.29), 289 (4.32), 427 (3.92), 450 (3.92)
IR (KBr) γ cm^{-1}	3417, 2923, 2854, 1625, 1588, 1449, 1399, 1190

HMQC NMR data suggested the presence of an aromatic methine (δ 6.79, s, δ 102.5), two isolated methylenes (δ_H 3.12, d, $J=20.4$; δ_H 2.84, d, $J=20.4$; δ_C 42.1 and δ_H 4.02, d, $J=19.8$; δ_H 3.01, d, $J=19.8$; δ_C 44.5), a methoxy, $-CH(=)-CH_2-CH_2-$, two isolated methyls, an allylic methyl, five quaternary sp^3 carbons (δ 116.4, 80.7, 77.8, 60.6, and 38.4), twelve quaternary sp^2 carbons (δ 192.9, 165.3, 161.2, 158.4, 146.0, 144.8, 135.7, 127.2, 116.9, 106.8, 106.6, and 99.9), two ketone carbonyls (δ 195.1 and 188.6), and an amide carbonyl (δ 172.9). These spectral data of **1** were similar to those of compound **2**. The major difference between **1** and **2** in ^1H- and ^{13}C-NMR data is that one quaternary sp^3 carbon (δ 116.4) was newly observed in **1** instead of the isolated hydroxy methine (C-5) in **2**. In addition, the quaternary sp^2 carbons of C-4a and C-5a were downfield-shifted from δ 71.7 and 137.3 to δ 77.8 and 144.8, respectively, while the quaternary sp^2 carbon of C-6 was upfield-shifted from δ 124.0 and 116.9. These spectral data together with the molecular formula suggested that an epoxide could be formed between C-4a and C-5. The presence of the epoxide moiety was determined by the HMBC spectrum (Fig. 2). One methylene proton at 2.84

Table 2 ¹H- and ¹³C-NMR spectral data for **1** and viridicatumtoxin (**2**)

Position	1		2	
	δ_H (mult., J_{HH})	δ_C	δ_H (mult., J_{HH})	δ_C
1		188.6 C		190.4 C
2		99.9 C		99.8 C
3		192.9 C		193.0 C
4	Ha 2.84 (1H, d, 20.4)	42.1 CH$_2$	Ha 2.76 (1H, d, 19.0)	40.5 CH$_2$
	Hb 3.12 (1H, d, 20.4)		Hb 2.83 (1H, d, 19.0)	
4a		77.8 C		71.7 C
5		116.4 C	4.51 (1H, s)	71.9 CH
5a		144.8 C		137.3 C
6		116.9 C		124.0 C
6a		146.0 C		147.3 C
7		106.8 C		105.9 C
8		161.2 C		161.0 C
9	6.79 (1H, s)	102.5 CH	6.66 (1H, s)	100.0 CH
10		158.4 C		158.2 C
10a		127.2 C		122.9 C
11		165.3 C		166.2 C
11a		106.6 C		105.2 C
12		195.1 C		195.5 C
12a		80.7 C		80.4 C
13		172.9 C		172.6 C
14	Ha 3.01 (1H, d, 19.8)	44.5 CH$_2$	Ha 2.94 (1H, d, 17.4)	41.4 CH$_2$
	Hb 4.02 (1H, d, 19.8)		Hb 3.48 (1H, d, 17.4)	
15		60.6 C		60.1 C
16		135.7 C		136.7 C
17	5.52 (1H, m)	121.9 CH	5.51 (1H, m)	121.6 CH
18	Ha 2.08 (1H, m)	22.8 CH$_2$	Ha 2.21 (1H, m)	22.6 CH$_2$
	Hb 2.25 (1H, m)		Hb 2.24 (1H, m)	
19	Ha 1.41 (1H, m)	34.1 CH$_2$	Ha 1.35 (1H, m)	34.1 CH$_2$
	Hb 1.88 (1H, m)		Hb 1.85 (1H, m)	
20		38.4 C		38.8 C
21	1.46 (3H, s)	21.0 CH$_3$	1.53 (3H, s)	21.4 CH$_3$
22	0.92 (3H, s)	24.3 CH$_3$	0.92 (3H, s)	24.2 CH$_3$
23	0.46 (3H, s)	25.5 CH$_3$	0.48 (3H, s)	25.7 CH$_3$
24	3.89 (3H, s)	55.7 CH$_3$	3.87 (3H, s)	55.7 CH$_3$

¹H- and ¹³C-NMR spectral data were measured at 500 and 225 MHz, respectively, in CDCl$_3$. The assignments were aided by ¹H-¹H COSY, DEPT, HMQC, and HMBC.

(Ha-4) was long-ranged coupled to the quaternary sp^2 carbons at δ 192.9 (C-3) and 99.9 (C-2), and the quaternary sp^3 carbons at δ 77.8 (C-4a) and 80.7 (C-12a). The other methylene proton at 3.12 (Hb-4) showed the HMBC correlations with the quaternary sp^2 carbon at C-3 and the quaternary sp^3 carbons at δ 116.4 (C-5), indicating that the epoxide is present between C-4a and C-5. The remaining structure was also confirmed by the HMBC spectroscopic data (Fig. 2) and the ¹³C-NMR data measured in 225 MHz

(Table 2). Thus, the structure of **1** was assigned as an epoxide derivative of viridicatumtoxin as shown in Fig. 1.

Compound **1** exhibited potent antibacterial activity against *S. aureus* (*S. aureus* RN4220 and *S. aureus* 503), methicillin-resistant *S. aureus* (*S. aureus* CCARM 3167 and *S. aureus* CCARM 3506), and quinolone-resistant *S. aureus* (*S. aureus* CCARM 3505 and *S. aureus* CCARM 3519) with MIC (μg/ml) of 0.5 as shown in Table 3. The antibacterial activity of **1** against *S. aureus*, MRSA, and

quinolone-resistant *S. aureus* (QRSA) is similar with that of vancomycin, but 8~64 times higher than that of tetracycline. Compound **1** also showed strong antibacterial activity against other pathogenic bacteria including *Acinetobacter calcoaceticus*, *Enterococcus faecalis*, and *Streptococcus pneumoniae* with MIC (μg/ml) of 1~2. However, **1** didn't have antibacterial activity against some gram negative bacteria including *E. coli*, *Pseudomonas*

aeruginosa, and *Klebsiella aerogenes* like vancomycin, while tetracycline still showed potent activity on *E. coli* and *Klebsiella aerogenes*. The antibacterial potency and spectrum of **2** was similar with **1**.

Compound **1** is a new epoxide derivative of viridicatumtoxin. Viridicatumtoxin is a tetracycline-type metabolite which has been isolated from *Penicillium viridicatum* [3]. The absolute configuration of viridicatumtoxin has been determined by X-ray crystallographic methods [5]. The absolute configuration of compound **1** was not determined in this study because of a small amount. Viridicatumtoxin has been known to exhibit antitumor activity [4]. The antibacterial activity of viridicatumtoxin is reported for the first time in this study.

Compounds **1** and **2** showed potent antibacterial activity against several strains of *S. aureus* including MRSA and QRSA. They exhibited the similar activity with vancomycin, but 8~64 times higher activity than tetracycline. Thus, these compounds may be useful for development of new anti-MRSA agents.
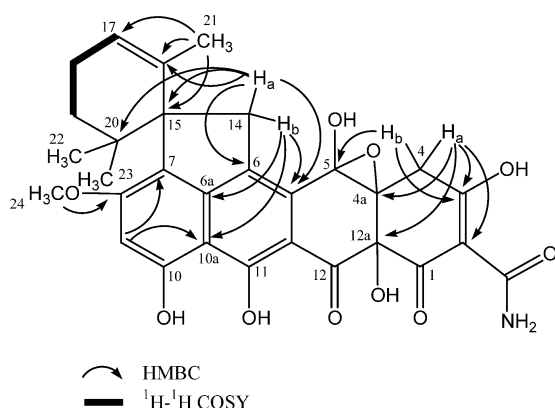

Fig. 2 Key HMBC and ^{1}H-^{1}H COSY correlations of **1**.

Table 3 Antibacterial activity for **1** and **2** (MIC, μg/ml)

Test organisms	1	2	Tetracycline	Vancomycin
Staphylococcus aureus 503	0.5	0.5	8	0.25
Staphylococcus aureus 209	0.5	0.25	4	0.25
Staphylococcus aureus RN4220	0.5	0.5	8	1
MRSA CCARM 3167	0.5	0.25	8	1
MRSA CCARM 3506	0.5	0.25	8	0.25
QRSA CCARM 3505	0.5	0.5	32	1
QRSA CCARM 3519	0.5	0.25	32	0.5
Bacillus subtilis KCTC 1021	0.06	0.06	0.03	0.12
Bacillus cerues KCTC 1661	8	16	0.25	>64
Micrococcus luteus KCTC 1056	0.12	0.12	0.06	0.06
Streptococcus pneumoniae KCTC 3932	1	2	0.5	0.25
Streptococcus pneumoiae KCTC 5412	2	2	0.25	0.25
Entercoccus faecium KCTC 3122	0.5	1	0.25	1
Entercoccus faecalis KCTC 5191	2	4	0.06	1
Entercoccus faecalis KCTC 3511	2	4	8	2
Staphycococcus epidermidis KCTC 3958	0.25	0.25	0.5	1
Salmonella typhinurium KCTC 1926	8	8	0.25	32
Acinetobacter calcoaceticus KCTC 2357	1	2	0.06	1
Escherichia coli CCARM 1356	>64	>64	0.5	64
Escherichia coli KCTC1682	>64	>64	0.25	>64
Pseudomonas aeruginosa KCTC 2004	>64	>64	8	>64
Pseudomonas aeruginosa KCTC 2742	>64	>64	16	>64
Klebsiella aerogenes KCTC 2619	>64	>64	0.5	>64
Candida albicans KCTC 7535	>64	>64	2	32

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