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SHORT COMMUNICATION

Two new streptovaricin derivatives from mutants of *Streptomyces spectabilis* CCTCC M2017417

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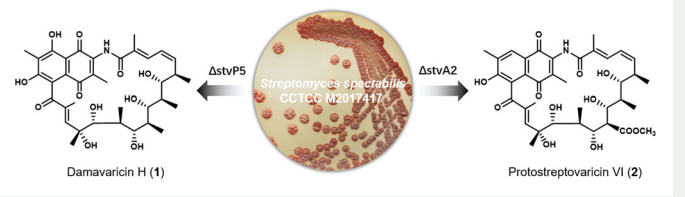
ABSTRACT

Two new ansamycin derivatives, damavaricin H (**1**) and protostreptovaricin VI (**2**) were isolated from the *Streptomyces spectabilis* CCTCC M2017417 derived mutants of ΔstvP5 and ΔstvA2, respectively. The structures of **1** and **2** were established by analysis of the HRESIMS as well as 1D and 2D NMR datasets. The minimum inhibitory concentration (MIC) results showed that compounds **1** and **2** possessed the corresponding anti-MRSA bioactivities of 4 ~ 8 μg/ml and 8 ~ 16 μg/ml, which confirmed the structure-activity relationships of streptovaricins reported previously and also revealed that addition of the hydroxyl group at C-8 increased the anti-MRSA activity.



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1. Introduction

Ansamycins are a group of nature compounds structurally characterised by a macrocycle composed of a benzene ring or naphthalene chromophore, which is further bridged by an aliphatic ansa chain and terminated with an amide bond of the chromophore. They are usually synthesised by type I modular polyketide synthases (PKSs) and started with loading of a 3-amino-5-hydroxybenzoic acid (AHBA) into the chain initiation domain, and finally ended by intramolecular amidation (Kakinuma et al. 1976; Zhang et al. 2017). Ansamycins have broad bioactivities and many on market drugs, such as antituberculosis rifamycins (Floss and Yu 2005), anti-tumour geldanamycins (Fukuyo et al. 2010). Streptovaricins are members of the ansamycin family

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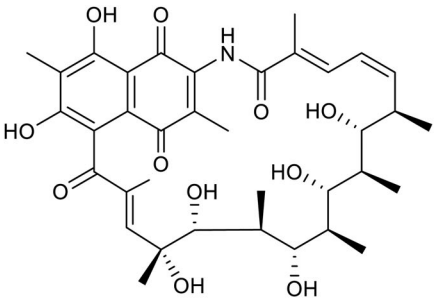
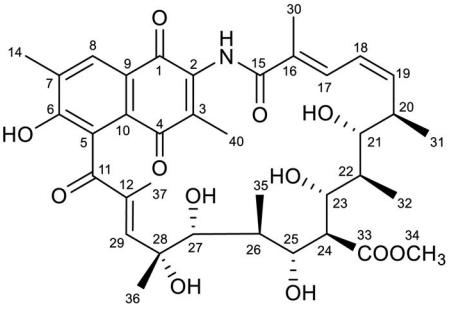


Damavaricin H (**1**)



Protostreptovaricin VI (**2**)

Figure 1. Chemical structures of the damavaricin H (**1**) and protostreptovaricin VI (**2**) from mutants of *Streptomyces spectabilis* CCTCC M2017417.

with a naphthalene chromophore, and their structure are so similar to rifamycins (Floss and Yu 2005). This should be the reason of that they also showed similar prominent antibacterial activities as rifamycins, especially against *Mycobacterium tuberculosis*.

In our previous searching for anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) drug leads from *Streptomyces*, we obtained a *Streptomyces* from the soil of the campus of Wuhan University, which was identified as *Streptomyces spectabilis* CCTCC M2017417, and its crude extracts exhibited significant bioactivities against MRSA (Liu et al. 2017). Bioassay guided isolation and identification showed that streptovaricin C is responsible for the anti-MRSA activity of extract crude (Liu et al. 2017). Subsequent biosynthetic studies of streptomycin C found that cytochrome P450 enzymes stvP1, stvP4 and stvP5 are responsible for the hydroxylation of C-20, Me-24 and C-28, respectively, while stvP2 is involved in the formation of methylenedioxy bridges in the biosynthesis of streptomycin C, and also found that stvA2 is responsible for the acetylation at C-4 (Liu et al. 2017; Sun et al. 2020). Due to the anti-MRSA activities of streptomycin C, we hope to achieve more streptomycin derivatives from the mutants of *Streptomyces spectabilis* CCTCC M2017417. During our previous study of the biosynthesis of streptomycin C, we found that the addition of AB-8 macroporous adsorption resin in the fermentation medium can significantly increase the production of streptovaricins (Sun et al. 2020). Based on this, we here obtained and elucidated two new streptovaricin derivatives damavaricin H (**1**) and protostreptovaricin VI (**2**) (Figure 1) from mutants ΔstvP5 and ΔstvA2, respectively, and reported their anti-MRSA bioactivities.

2. Results and discussion

Compound **1** was obtained as red power from mutant ΔstvP5. It has a molecular formula of $C_{36}H_{47}NO_{11}$ deduced from the protonated ion at *m/z* 670.3226 ($[M+H]^+$) observed in its the High-Resolution Electrospray Ionization Mass Spectroscopy (HRESIMS) (calcd. for $C_{36}H_{48}NO_{11}$, 670.3222), indicating 14 unsaturation. The UV/Vis

spectrum of **1** was similar to that of streptovaricins, suggesting it is a streptovaricin derivative. Careful analysis of the ^{13}C-NMR spectrum unveiled signals for 36 carbons, including 4 carbonyls, 8 aromatic quaternary carbons, 6 unsaturated double bond carbons, 5 oxygenated carbons and 13 aliphatic carbons. The ^{1}H-NMR, ^{13}C-NMR and HSQC spectrum of **1** further assigned some signals as nine methyl carbons, four oxygenated methine carbons, one oxygenated quaternary carbon, four aliphatic methine carbons and fifteen quaternary carbons. The naphthoquinone structure chromophore was assigned by the signals of four carbonyls, eight aromatic quaternary carbons together with the HMBC correlations of H-40 (δ_H 1.72 (s, 3H)) to δ_C 134.0 (C-2), 140.4 (C-3), 183.7 (C-4), H-14 (δ_H 2.09 (s, 3H)) to δ_C 161.0 (C-6), 116.7 (C-7), 159.4 (C-8), and NH (δ_H 9.49 (s, 1H)) to δ_C 183.5 (C-1), 134.0 (C-2), 140.4 (C-3). The aliphatic ansa chain was determined by spin system of C-16 (C-30)/C-17/C-18/C-19/C-20(C-31)/C-21/C-22(C-32)/C-23/C-24(C-33)/C-25/C-26(C-35)/C-27/C-28(C-36)/C29/C-12(C-37) obtained by the COSY and HMBC correlations of **1**. Two fragments were linked by the HMBC correlations of NH to C-1, C-2, C-3, C-15; H-30 to C-15, C-16, C-17; H-37 to C-11, C-12, C-29 to form **1** (Figure 1 and Figure S1, Supplementary material). Comparing the NMR data with that of damavaricin C (Rinehart et al. 1976; Onodera et al. 2014), we found that **1** only loss a methyl ester moiety relative to damavaricin C, which is consistent with the HMBC correlations of H_3-33 to C-23, C-24 and C-25. Thus it was named as damavaricin H.

For compound **2** from ΔstvA2, its molecular formula $C_{37}H_{47}NO_{12}$ was determined on the basis of HRESIMS and it also has the characterised UV/Vis spectrum of streptovaricins. The ^{1}H and ^{13}C-NMR spectroscopic data of **2** was highly similar to that of **1.** Detailed comparison unveiled that the C-33 methyl group in **1** was replaced by methyl ester in **2** and the C-8 hydroxyl in **1** was lost in **2**. These were corresponding with the signals of C-33 (δ_H 0.66, δ_C 9.6) in **1** were replaced with the signals of (δ_H 3.66, δ_C 52.2, δ_C 174.4) in **2** and the signals of C-8 (δ_C 159.4) in **1** were replaced with the signals of (δ_H 7.11, δ_C 126.3) in **2**. This is further confirmed by the signals of C-24 (δ_H 1.68, δ_C 37.2) in **1** is up shift to C-24 (δ_H 2.68, δ_C 51.3) in **2**, and the HMBC correlations of δ_H 7.11 (H-8)/δ_C 161.3 (C-6), 131.1 (C-10), 183.6 (C-1) and δ_H 2.68 (H-24)/δ_C 72.1 (C-25), 75.2 (C-23), 174.4 (C-34) (Figure 1 and Figure S1). Thus **2** was finally assigned as protostreptovaricin VI.

To measure the antibacterial activity of compounds **1** and **2**, ATCC 43300, USA300 LAC and USA400 MW2 were used as MRSA indicator strains with the method reported previously (Liu et al. 2017; Luo et al. 2020). The results showed that compounds **1** and **2** have a little weaker anti-MRSA bioactivities than that of streptovaricin C with a MIC value of $4\sim8\,\mu g/ml$ and $8\sim16\,\mu g/ml$ (Table S2, Supplementary material). Consistent with our previous discovery, this result further confirmed the structure-activity relationships of streptovaricins. For example, since the presence of the methoxycarbonyl side chain at C-24 in protostreptovaricin VI (**2**), it showed better anti-MRSA activity compared with counterpart protostreptovaricin III (Liu et al. 2017). Significantly, the hydroxyl group at C-8 plays a crucial role to the antibacterial activity. It led to an obvious increase of the activity of damavaricin H (**1**) when compared with protostreptovaricin III, an ideal reference without a hydroxyl group at the corresponding position.

3. Experimental

3.1. General experimental procedures

Optical rotations were carried out with an MCP 500 polarimeter (Anton Paar). UV spectra were recorded with a U-2600 spectrometer (Shimadzu). NMR spectra were obtained with a DD2 spectrometer (Agilent) at 600 MHz for ^{1}H nuclei and 150 MHz for ^{13}C nuclei. Chemical shifts (δ) are given in ppm with reference to TMS. HRESIMS spectra were performed with a Maxis quadruple-time-of-flight mass spectrometer (Bruker). Column chromatography (CC) was conducted with silica gel (200–300 mesh, Yantai Jiangyou Silica Gel Development Co., Ltd.). Medium-pressure liquid chromatography (MPLC) was carried on a CHEETAH 100 (Bonna-Agela) flash chromatography system. Preparative HPLC was carried out using a QuikSep-50 system (H&E Co., Ltd.) with an C18 column (250 × 21.2 mm, 5 μm, YMC).

3.2. Strain material

The mutants of ΔstvA2 and ΔstvP5 were constructed and confirmed previously (Liu et al. 2017; Sun et al. 2020).

3.3. Fermentation

The mutants of ΔstvA2 and ΔstvP5 were inoculated into 60 mL of TSBY seed medium (3% tryptone soy broth, 0.5% yeast extract, 10.5% sucrose) in 250 mL Erlenmeyer flasks and cultured for 2 days on a rotary shaker (200 rpm and 28 °C), respectively. Then 10 mL of each seed cultures was transferred into a 2 L Erlenmeyer flask containing 700 mL modified modified SFM medium (2% mannitol, 2% soya flour, 5% AB-8 macroporous adsorption resin) and cultured at 28 °C and 200 rpm for 7 days.

3.4. Extraction and isolation

For mutant ΔstvP5, the AB-8 macroporous adsorption resin along with the mycelium from 10 L culture was separated by filtration through a metal sieve (40 meshes) after fermentation for 7 days, then the resin and mycelium were extracted three times with 1.5 L of ethyl acetate. At the same time, the 10 L filter liquor was extracted three times with 10 L ethyl acetate to afford another residue following solvent evaporation. The obtained two residues were combined and subjected to silica gel CC to obtain eight fractions (Fr.A1 − Fr.A7) using a gradient elution of CH_2Cl_2/MeOH (100:0, 98:2, 96:4, 92:8, 90:10, 80:20, 70:30, v/v). Fr.A3-A4 were combined and purified by MPLC to afford Fr.B1 − Fr.B15. Fr.B4 were combined and purified with preparative HPLC equipment with an C18 column (250 × 10 mm, 5 μm), eluting with a mixture of CH_3CN/H_2O (0–20 min, 70:30–90:10) at a flow rate of 3 mL min^{-1} to yield **1** (15.0 mg). Compound **2** was purified by a similar way from ΔstvA2, and got a yield of 12.5 mg.

Damavaricin H (**1**): Red powder; [α]25 D + 48 (0.05, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.24), 258 (4.66), 315 (1.56) nm; ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR

(150 MHz, DMSO-d_6) data (Table S1, Supplementary material); HRESIMS *m/z* 670.3226 $[M+H]^+$ (calcd. for $C_{36}H_{48}NO_{11}$, 670.3222).

Protostreptovaricin VI (**2**): Red powder; [α]25 D + 62 (0.06, MeOH); UV (MeOH) λ_{max} (log ε) 219 (4.64), 243 (4.46), 264 (4.72), 332 (1.05) nm; ^{1}H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (150 MHz, DMSO-d_6) data (Table S1); HRESIMS *m/z* 698.3178 $[M+H]^+$ (calcd. for $C_{37}H_{48}NO_{12}$, 698.3171).

3.5. Antimicrobial assays

Briefly, the tested compounds **1** and **2** dissolved in DMSO were serially diluted to designed concentrations, then each of them were added with the tested strain. The experiment was repeated three times independently. The MRSA stains of ATCC 43300, USA300 LAC and USA400 MW2 were used in this study (Murphy et al. 2006; Wardenburg and Schneewind 2008). Streptovaricin C and protostreptovaricin III were used as positive controls, while DMSO was used as blank control.

4. Conclusions

In summary, two new streptovaricins were isolated from the mutant strains of ΔstvP5 and ΔstvA2 derived from *Streptomyces spectabilis* CCTCC M2017417, respectively. Their structure was elucidated extensively on the basis of NMR and HRESIMS data by comparison with the known reported analogs of the literature. Their antimicrobial activities against to MRSA were measured as 4 ∼ 8 μg/ml and 8 ∼ 16 μg/ml of compounds **1** and **2**, respectively (Table S2). This result is consistent with the structure-activity relationships of streptovaricins reported previously (Liu et al. 2017). The hydroxyl group substitution at C-8 makes a significant contribution to the anti-MRSA activities of the corresponding streptovaricins.

Disclosure statement

The authors declare no competing financial interest.

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