Secondary Metabolites from *Streptomyces* sp CSDX076 for the First Time

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**ABSTRACT**

**Objective:** To investigate the secondary metabolites from the cultures of *Streptomyces* sp CSDX076.

**Methods:** The compounds were isolated using column chromatography and RP-18 medium-pressure liquid chromatography. Their structures were elucidated by one-dimensional and two-dimensional nuclear magnetic resonance spectroscopic methods in combination with mass spectrometry experiments.

**Results:** Four compounds were isolated from the cultures of *Streptomyces* sp CSDX076 and identified as aurantiamide benzoate, deoxytryptoquivaline, 2-acetyl-3,5-dihydroxyl-benzene acetic acid, and 2-acetyl-3,5-dihydroxyl-benzene ester.

**Conclusion:** It was the first time that the four isolated compounds were obtained from the *Streptomyces* genus.

**Keywords:** Isolation and identification, secondary metabolites, *Streptomyces* sp CSDX076

Metabolitos secundarios de *Streptomyces* SP CSDX076 por primera vez

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**RESUMEN**

**Objetivo:** Investigar los metabolitos secundarios de los cultivos de *Streptomyces* sp CSDX076.

**Métodos:** Los compuestos fueron aislados usando la cromatografía de columna y cromatografía líquida RP-18 de presión media. Sus estructuras fueron dilucidadas mediante métodos espectroscópicos de resonancia magnética nuclear unidimensional y bidimensional, combinados con experimentos de espectrometría de masa.

**Resultados:** Cuatro compuestos de culturas de *Streptomyces* sp CSDX076 fueron aislados e identificados como benzoato de aurantiamida, deoxitriptoquivalina, ácido acético 2-acetil-3,5-dihidroxil-benzeno, y éster 2-acetil-3,5-dihidroxil-benzeno.

**Conclusión:** Fue la primera vez que los cuatro compuestos aislados se obtuvieron del género *Streptomyces*.

**Palabras clave:** Aislamiento e identificación, metabolitos secundarios, *Streptomyces* sp CSDX076
INTRODUCTION
The secondary metabolites (1–6) of Streptomyces played an important role in natural products which have attracted great attention of researchers due to their complicated structures and potential biological activities. Previously reported chemical investigations of the genus Streptomyces include the antifungal hygrolidin (7), cytotoxic elmonin (8), antibacterial angumycinone C (9) and antitumour streptonoate (10). Additionally, it had been implied that the genus Streptomyces was a good source of antibiotic (11–13). In our previous research, we conducted the isolation, and activity screening of the strains from the soil at different altitudes in Chishuidanxia city and Fanjinshan mountain, Guizhou Province, People’s Republic of China (PRC). Nine activity strains of the Streptomyces genus were established. Phytochemistry of Streptomyces sp FJS31-2 resulted in a new antibacterial compound, Zunyimycin A (14). In an ongoing research for bioactive secondary metabolites, secondary metabolites of Streptomyces CSXD076 were investigated. Four compounds were isolated and identified as auranthiamide benzoate (1), deoxytryptoquivaline (2), 2-acetyl-3,5-dihydroxyl-benzene acetic acid (3) and 2-acetyl-3,5-dihydroxyl-benzene ester (4). These compounds were reported from this genus for the first time. This paper reports the isolation, and structural elucidation of these compounds.

MATERIALS AND METHODS
General experimental procedures
High-resolution electrospray ionisation mass spectrometry (HRESIMS) data were obtained on a Waters Xevo G2 QTOF mass spectrometer with an ACQUITY UPLC BEH C-18 column (2.1 mm × 50 mm, 1.7 μm). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV 500 NMR spectrometer with tetramethylsilane as an internal standard. The concentration was performed on an EYELA N-1100S-W rotary evaporator. Silica gel (200–300 mesh) for column chromatography and GF254 for thin layer chromatography (TLC) were obtained from Qingdao Marine Chemical Factory, Qingdao, PRC, and sprayed with Dragendorff’s reagent. Rp-C18 silica gel (20–45 μm) was bought from Fuji Chemical Ltd. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co, Ltd, Qingdao, PRC), and spots were visualized by heating after spraying with 5% H2SO4 in ethanol. Medium-pressure liquid chromatography (MPLC) was carried out on a Büchi pump system coupled with C18 silica gel-packed glass columns (15 × 230 mm and 26 × 460 mm).

Materials
The strain was isolated from the soil of Chishuidanxia city, PRC, and identified as a Streptomyces sp CSXD076 based on a 16S ribosomal ribonucleic acid gene analysis. The strain was preserved at China Center for Type Culture Collection (CCTCC no. M2014241), Wuhan University, PRC.

Fermentation, extraction and isolation
A stock culture of Streptomyces sp CSXD076 strain was maintained at 4°C on GYM agar slant consisting of glucose 4 g, malt extract 4 g, yeast extract 10 g, calcium carbonate 2 g and microelement mixed liquid 0.5 ml in 1 L distilled water (pH 8.0). The stock culture was inoculated into a 500 ml Erlenmeyer flask containing 200 ml of seed medium which was the same as above without agar. The flask culture was incubated on a rotary shake (160 rpm) for 72 hours. Two hundred milliliters of the flask culture was transferred to a 5000 ml Erlenmeyer flask containing 1000 ml of the producing medium, which contained the same ingredients as the seed medium. The formation was carried out at 28°C for 196 hours on a rotary shaker (160 rpm).

The whole fermented cultures (70 L) were extracted with ethyl acetate two times at room temperature. The solvent was evaporated in vacuo to give a crude extract (17.8 g). The extract was subjected to a silica gel column eluting with CHCl3-acetone in gradient (30:1 to 1:2) to obtain eight fractions (Fractions 1–8). Fraction 3 (2.82 g) was chromatographed by MPLC eluted with methanol-water (30–80%) to give six fractions (Fractions 3-1 to 3-6). Fraction 3-2 (200 mg) was chromatographed on a silica gel eluted with petroleum ether-acetone (4:1 to 2:1) to give 1 (40 mg). Fraction 3-1 (1.0 g) was chromatographed on a RP-18 silica gel eluted with methanol-water (20–50%) to give 2 (348 mg) and 3 (46 mg). Fraction 3-6 was chromatographed on a silica gel eluted chloroform-acetone (10:1 to 4:1) to give 4 (20 mg).

RESULTS
Compound 1 (Figure) was obtained as a white amorphous powder. Its molecular formula was established as C32H26N2O4 by HRESIMS data (m/z 529.2106 [M + Na]+), indicating 19 degrees of unsaturation. In the 1H NMR spectrum, the aromatic proton signals at 7.70 (2H, dd, J = 8.5, 1.5 Hz), 7.66 (2H, dd, J = 8.5, 1.5 Hz), 7.50
(1H, t, J = 7.4 Hz) and 7.21–7.52 (15H, m, Ar-H) suggested the presence of four benzene rings. Two amide NH proton was also observed at 6.68 (1H, d, J = 8.5 Hz, H-3) and 6.59 (1H, d, J = 6.5 Hz, H-6). The $^{13}$C NMR and distortionless enhancement by polarization transfer (DEPT) spectra of compound 1 displayed 32 carbon resonances ascribable to three methylenes, 22 methines, and five quaternary carbons. Compared to the literature (15), the $^1$H and $^{13}$C NMR data were similar to those of aurantiamide benzoate. As a result, compound 1 was determined to be aurantiamide benzoate.

Compound 2 was obtained as a white powder. Its $^1$H NMR spectrum displayed five methyl groups [δ$_H$ 2.13 (3H, s, H-14), 1.46 (3H, s, H-34), 1.43 (3H, s, H-35), 1.21 (3H, d, J = 6.6 Hz, H-33) and 1.01 (3H, d, J = 6.8 Hz, H-31)], eight aromatic proton signals [δ$_H$ 8.21 (1H, dd, J = 8.0, 1.0 Hz, H-6), 7.89 (1H, ddd, J = 8.5, 7.2, 1.5 Hz, H-4), 7.84 (1H, d, J = 7.6 Hz, H-5), 7.75 (1H, d, J = 7.9 Hz, H-3), 7.60 (1H, m, H-11), 7.55 (1H, d, J = 7.5 Hz, H-19), 7.50 (1H, d, J = 7.7, 1.0 Hz, H-20) and 7.30 (1H, td, J = 7.6, 1.1 Hz, H-22)]. The $^{13}$CNMR data and DEPT spectrum indicated that compound 2 possessed 29 carbons, including five methyls, one methylene, 12 methines and 11 quaternary carbons. The signal at δ$_C$ 172.2 (s, C-13) and δ$_H$ 2.13 (3H, s, H-14) revealed the existence of an ester group. The signals at δ$_H$ 1.21 (3H, d, J = 6.6 Hz, H-33), 1.01 (3H, d, J = 6.8 Hz, H-31) and δ$_C$ 33.7 (d, C-32) illustrated the presence of one isopropyl group. According to the literature (16), compound 2 was the same as deoxytryptoquivaline. Thus, compound 2 was confirmed as deoxytryptoquivaline.

**DISCUSSION**

The strain of *Streptomyces* sp CSDX076 was isolated from the soil of Chishuidanxia city, PRC. A chemical study was performed and resulted in the isolation of four compounds, aurantiamide benzoate (1), deoxytryptoquivaline (2), 2-acetyl-3,5-dihydroxyl-benzene acetic acid (3) and 2-acetyl-3,5-dihydroxyl-benzene ester (4), by separation methods and their structural elucidation on the basis of NMR and mass spectrometry. These compounds were obtained for the first time from *Streptomyces* genus by separation methods and their structural elucidation on the basis of NMR and mass spectrometry. This research provided a basis for further development of the constituents of the *Streptomyces* genus.

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REFERENCES


