NOTE

Sannanine, a new cytotoxic alkaloid from *Streptomyces sannanensis*

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As reported recently, quinoline-5,8-diones have generated a lot of interest as anticancer agents.^{1,2} Most of them were obtained by synthesis, and only a few of them, such as streptonigrin, streptonigrone and lavendamycin, were obtained from microbial sources.^{2,3} Over the past few decades, major research has focused on the synthesis and biological activities of variously 6,7-functionalized quinoline-5,8-diones.^{4–9} In the course of screening for new antitumor bioactive compounds from microbial sources, a new cytotoxic quinoline-5,8-dione derivative, sannanine (**1**), was isolated from the fermentation broth of *Streptomyces sannanensis* (Figure 1). In this paper, we report the fermentation, isolation, structural elucidation and biological activities of **1**.

The producing organism was isolated from a soil sample collected from Dali, Yunnan province, China. The strain was identified as *S. sannanensis* according to phylogenetic analysis. The genomic DNA of the strain was extracted and used as a template for PCR-mediated amplification of 16S rDNA. The amplicons were used for sequencing and the resulting 16S rDNA sequence was compared with those of the type of strains of validly published species in the genus *Streptomyces*. Phylogenetic analysis showed that the strain shared a higher 16S rRNA gene sequence similar to the closely related strains *S. sannanensis* NBRC 14239^T (accession number AB184579). Meanwhile, the strain was examined for a number of key phenotypic properties known to be of value in streptomycete systematics, and the presence of LL-diaminopimelic acid in peptidoglycan, together with its colonial characteristics, supported its assignment to the genus *Streptomyces*.

A slant culture of the strain was inoculated into 500 ml Erlenmeyer flasks containing 100 ml of seed medium composed of yeast extract (0.4%), glucose (0.4%), malt extract (0.5%), multiple vitamins solution (5.0 ml) and trace element solution (1.0 ml), pH was 7.2 with no adjustment, and cultured for 2 days at 28 °C on a rotary shaker at

220 r.p.m. This seed culture was used to inoculate the fermentation medium with 5% volume. Fermentation was carried out in a 500-ml Erlenmeyer flask containing 100 ml of fermentation medium that contained starch (2.4%), beef extract (0.3%), glucose (0.1%), yeast extract (0.5%), peptone (0.3%), CaCO₃ (0.4%), pH was 7.0 with no adjustment, and cultured for 6 days at 28 °C on a rotary shaker at 220 r.p.m. for upscale fermentation.

The completed fermentation broth (80 l) was separated into filtrate and mycelium by centrifugation. The culture filtrate was absorbed onto polymeric resin Amberlite XAD-16 (Rohm & Hass, Paris, France). Salt and high molecular materials were washed out with water, and other absorbed organic materials were eluted with MeOH to yield 55 g of dried extract after removing the solvent in vacuum. The dried extract was extracted with petroleum, CHCl₃ and EtOAc; the CHCl₃ extract fraction (2 g) was applied to gel chromatography on Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden) (MeOH) to produce three fractions. Fraction 1 was then purified by silica gel column chromatography (CHCl₃-MeOH 80:1) and yielded 180 mg of a semipure material. Additional preparative HPLC (Waters SunFire C18 10 µm (Milford, MA, USA) 19×250 mm, flow rate: 10 ml min⁻¹), eluted with water-methanol of gradient 6:4, yielded 50 mg of compound **1**. **1** was obtained as an orange amorphous powder. UV (MeOH) λ_{max}=237, 274, 304 nm. IR(KBr) ν_{max}=3376, 3319, 1687, 1608, 1585, 1556, 1506, 1334, 1228 cm⁻¹. ESI-MS *m/z*=217.3 [M+H]⁺, 239.3 [M+Na]⁺, 433.4 [2M+H]⁺, 455.4 [2M+Na]⁺, 215.3 [M-H]⁻. For ¹H and ¹³C NMR data see Table 1.

The molecular formula of **1** was established as C₁₂H₁₂N₂O₂ by HRESI-MS at *m/z* 217.0975 [M+H]⁺ (calcd 217.0977). The IR spectrum of **1** indicated the presence of an amino group (3376 cm⁻¹) and a carbonyl group (1687 cm⁻¹). The ¹H NMR

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Figure 1 Structure of compound **1**.

Table 1 ^1H (300 MHz, CDCl_3) and ^{13}C NMR (75 MHz, CDCl_3) data of compound **1**

No.	δ_{C}	δ_{H}	HMBC
2	164.4	—	H-4, H-9, H-10
3	135.3	—	H-9, H-10
4	134.2	8.01 (1H, s)	H-10
4a	125.3	—	—
5	181.78 (181.75) ^a	—	H-4
6	148.3	—	H-11
7	101.1	5.83 (1H, s)	H-11
8	181.75 (181.78) ^a	—	H-7
8a	146.7	—	H-4, H-7
9	23.8	2.69 (3H, s)	H-4
10	19.3	2.40 (3H, s)	H-4
11	29.1	2.93 (3H, d, $J=5.4$ Hz)	—
NH		5.91 (1H, brs)	—

^aChemical shift were not distinguished.

(Table 1) spectrum of **1** showed two aromatic proton signals at $\delta=8.01$ (1H, s) and 5.83 (1H, s), as well as three methyl signals at $\delta=2.93$ (3H, d, $J=5.4$ Hz), 2.69 (3H, s) and 2.40 (3H, s). In addition, an active proton was presented at $\delta=5.91$ (1H, brs). ^{13}C NMR and DEPT spectra of **1** (Table 1) indicated the corresponding carbon atoms with ^1H NMR data, two aromatic methines ($\delta=134.2$, 101.1) and three methyls ($\delta=29.1$, 23.8 , 19.3). Furthermore, seven quaternary carbons were presented, including two ketone carbonyls with nearly the same chemical shifts ($\delta=181.78$, 181.75) and five aromatic quaternary carbons ($\delta=164.4$, 148.3 , 146.7 , 135.3 and 125.3). ^1H and ^{13}C NMR data suggested that **1** possesses quinoline-5, 8-dione skeleton,^{1,2} which is the basic structural moiety for a kind of significant compound with wide biological activities.^{5–9} By comparing the ^1H and ^{13}C data with the known compound 6-amino-2-methyl-quinoline-5,8-dione,¹ two more methyl groups were presented in the molecule of **1**. HMBC correlations were observed between CH_3 ($\delta=2.40$) and C-2 ($\delta=164.2$), C-3 ($\delta=135.3$), C-4 ($\delta=134.2$), CH_3 ($\delta=2.69$) and C-2 ($\delta=164.2$), C-3 ($\delta=135.3$), CH_3 ($\delta=2.93$) and C-6 ($\delta=148.3$), suggesting that two singlet methyls were located at C-2 and C-3, respectively, and the doublet methyl was linked with the amino group. Complete assignments of the ^1H and ^{13}C NMR spectra of **1** were achieved with 1D and 2D experiments (Table 1). Therefore, compound **1** was elucidated as 2,3-dimethyl-6-(methylamino)quinoline-5,8-dione, named sannanine. Almost all of the quinoline-5, 8-diones from natural and synthetic sources have no substituent on

C-3 and C-4. Sannanine was isolated as a quinoline-5,8-dione derivative with methyl substituted on C-3 first.

To evaluate the cytotoxic activity of **1**, we measured its cytotoxic effects on four human tumor cell lines, including human gastric carcinoma cell line (BGC-823), human pancreatic carcinoma cell line (PANC-1), human hepatocellular liver carcinoma cell line (HepG2) and human large-cell lung carcinoma cell line (H460) grown in RPMI-1640 medium plus 10% heat-inactivated fetal bovine serum with an MTT assay procedure.¹⁰ Assays were performed in 96-well microtiter plates. Compound **1** was dissolved in dimethylsulfoxide and diluted to six different concentrations (10, 3.3, 1.0, 0.33, 0.10 and 0.033 mM), and each solution was 10-fold diluted to six different concentrations using culture medium (1.0, 0.33, 0.10, 0.033, 0.010 and 0.0033 mM); thereafter, 10 μl of each solution was added to 90 μl (about 5000 cells) of culture medium wells. After incubation at 37°C for 72 h, 10 μl of MTT (5 mg ml^{-1}) was added to each well and incubated for 4 h; thereafter the liquid in the wells was removed. Dimethylsulfoxide (150 μl) was added to each well. Absorbance was recorded on a microplate reader at a wavelength of 590 nm, and IC_{50} was defined as a 50% reduction of absorbance in the control assay. Compound **1** showed cytotoxicity against four human tumor cell lines BGC823, PANC1, HepG2 and H460, with IC_{50} values of 6.6, 5.8, 3.1 and 1.8 μM , respectively.

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