SUPPLEMENTARY MATERIAL

Discovery and evaluation on the antibacterial and cytotoxic activities of a novel antifungalmycin N2 produced from *Streptomyces* sp. strain N2

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ABSTRACT

Antifungalmycin N2 (3-methyl-3,5-amino-4-vinyl-2-pyrone, $C_6H_7O_2N$) was a novel metabolite produced from *Streptomyces* sp. strain N2, and the present study aimed to evaluate its antibacterial and cytotoxic properties. By using Oxford cup method, the obtained results revealed that antifungalmycin N2 exhibited a significant antibacterial activity against the pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Micrococcus kristinae*, especially the Gram-positive *S. aureus*. Meanwhile, the MTT assay showed that antifungalmycin N2 could exert a marked inhibitory action on tumor cell lines, such as the cell lines of BEL-7402 (human hepatocellular carcinoma), Hela (human cervical carcinoma), HCT116 (human colon cancer), and SW620 (human colon cancer). And the IC_{50} values antifungalmycin N2 against the above cell lines ranged from 11.23 to 15.37 μg/mL. In conclusion, the antibacterial and cytotoxic activities suggested that the novel antifungalmycin N2 was

a promising active structure to be developed as new drug for treating infectious diseases and cancers.

KEYWORDS: Antifungalmycin N2; Antibacterial activity; Cytotoxic activity; *Streptomyces* sp. strain N2

Experimental

Preparation of antifungalmycin N2

The antifungalmycin N2-producing *Streptomyces* sp. strain N2 was carried out in a 250-mL Erlenmeyer flask containing 40 mL of fermentation medium (sucrose, 40 g; corn starch, 20 g; corn steep liquor, 20 g; $(NH)_2SO_4$, 2 g; KH_2PO_4, 1 g; $MgSO_4$, 1 g; $MnSO_4 \cdot H_2O$, 0.01 g; $ZnSO_4 \cdot 7H_2O$, 0.01 g; distilled water, 1000 mL; pH 7.2~7.4), and cultured at 28 °C on a rotary shaker at 200 rpm. After 6 days of cultivation, the fermentation broth was centrifuged at 5,000 rpm for 20 min, and then the mycelium was used for the preparation of antifungalmycin N2. The separation and purification processes were according to the method described in our previous report (Xu et al. 2015).

In vitro determination of antibacterial activity of antifungalmycin N2

The antibacterial activities of antifungalmycin N2 on the pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Micrococcus kristinae*, were measured by using Oxford cup method (Wang et al. 2015). Bacterial suspensions (1 mL, about 10^8 CFU/mL) of *S. aureus*, *E. coli*, and *M. kristinae* were mixed with 100 mL of LB agar medium (cooling to about 45 °C after sterilization at 121 °C for 20 min) to make four bacterial plates (90 mm), respectively. Then four stainless Oxford

cups were placed evenly on the surface of each bacterial plate. An aliquot of 200 μL of antifungalmycin N2 aqueous solution (2.88, 5.77 and 11.53 μg/mL, respectively) was added into one of the four Oxford cups of each bacterial plate, and repeated in triplicate. The control group was using 200 μL distilled water instead of antifungalmycin N2 aqueous solution. After incubation at 30 °C for 24 h in an incubator, each bacterial plate was taken out to measure the diameter of the inhibition zones, and the results were given as Mean±SD (standard deviation).

In vitro assay of** cytotoxic **activity of antifungalmycin N2

The cytotoxic evaluation of antifungalmycin N2 was performed on the cell lines of BEL-7402 (human hepatocellular carcinoma, purchased from the Typical Culture Preservation Committee of the Chinese Academy of Sciences, Shanghai, China), Hela (human cervical carcinoma, ATCC), HCT116 (human colon cancer, ATCC), and SW620 (human colon cancer, ATCC) by using MTT assay (van Meerloo et al. 2011; Al-Jameel and Youssef 2018). The cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), and 1% antibiotics (penicillin-G and streptomycin) at 37°C in an incubator with 5% CO_2. Then the cells were harvested at exponential phase cultures by trypsinization, counted and plated as cell monolayer with 10% PBS in 96-multiwell plate (10^4 cells/well, and 200 μL/well). The plates were incubated at 37 °C in 5 % CO_2 environment for 12 h, and nine different concentrations of antifungalmycin N2 (0.75, 1.5, 3, 6, 12, 24, 48, 96, and 192 μg/mL) were added to the wells in six repetitions, respectively. The control was using double distilled water

instead of antifungalmycin N2. After further 24 h of incubation at 37 °C for 24 h in 5 % CO_2, 20 μL of MTT (5 mg/mL) was added to each well, and the plate was again incubated at 37 °C for 4 h in 5 % CO_2 environment. The media were carefully removed from the wells, and 150 μL of DMSO was added to each well and gently shaken for 10 min, so as to completely solubilize the formazan crystals produced by metabolically active cells. The optical density (OD) of the resulting aliquots was recorded at 492 nm using a microplate spectrophotometer system. The viability rate for each cell line at given antifungalmycin N2 concentration was calculated as the percentage of absorbance in wells with the antifungalmycin N2 treated cells to that of vehicle control cells (100%), and given as Mean±SD. The IC_{50} (half maximal inhibitory concentration) values were calculated through the corresponding dose dependent curve.

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Figure S1. The diameter of inhibition zone of *Staphylococcus aureus*, *Escherichia coli*, and *Micrococcus kristinae* under the treatments with different concentrations of antifungalmycin N2

Figure S2. The viability rates of BEL-7402 (human hepatocellular carcinoma), Hela (human cervical carcinoma), HCT116 (human colon cancer), and SW620 (human colon cancer) cell lines under different treatment concentrations of antifungalmycin N2

Figure S3. The IC_{50} values of antifungalmycin N2 against the cell lines of BEL-7402 (human hepatocellular carcinoma), Hela (human cervical carcinoma), HCT116 (human colon cancer), and SW620 (human colon cancer)

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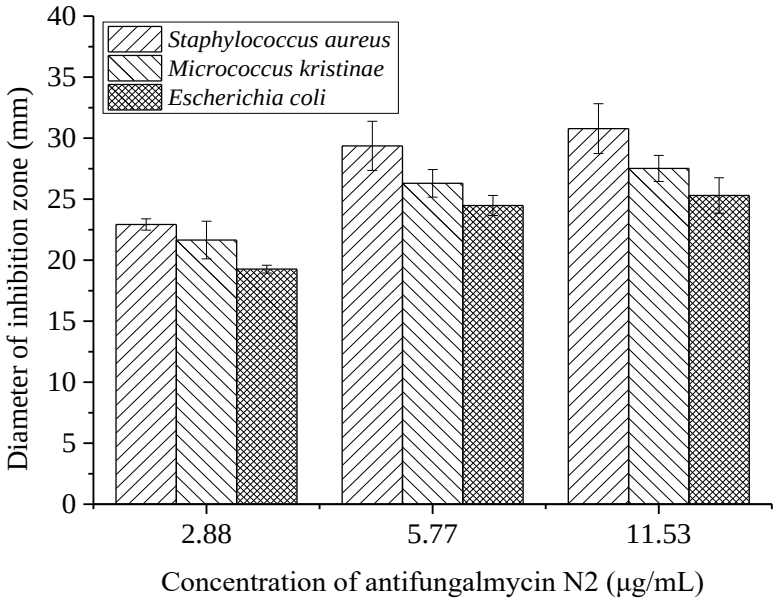

Figure S2. The viability rates of BEL-7402 (human hepatocellular carcinoma), Hela (human cervical carcinoma), HCT116 (human colon cancer), and SW620 (human

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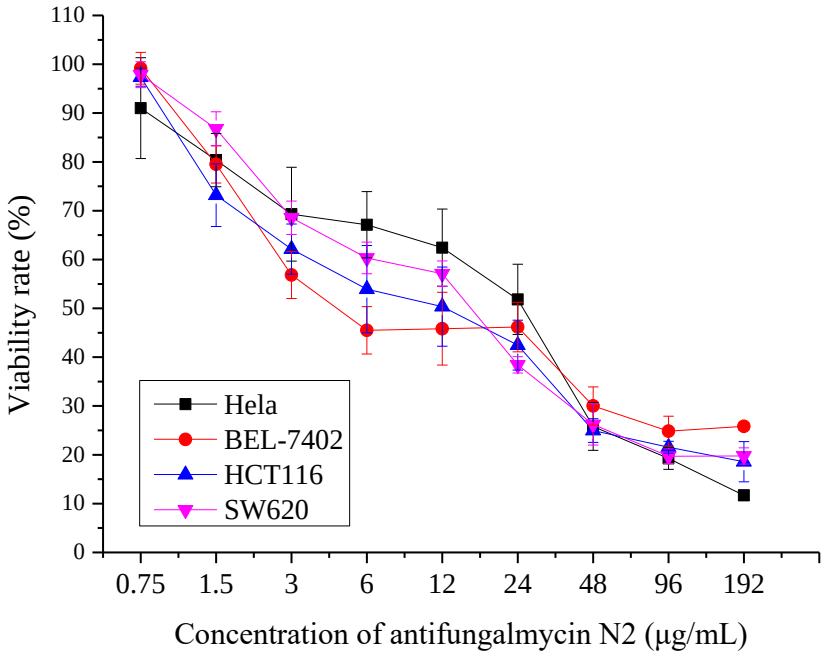

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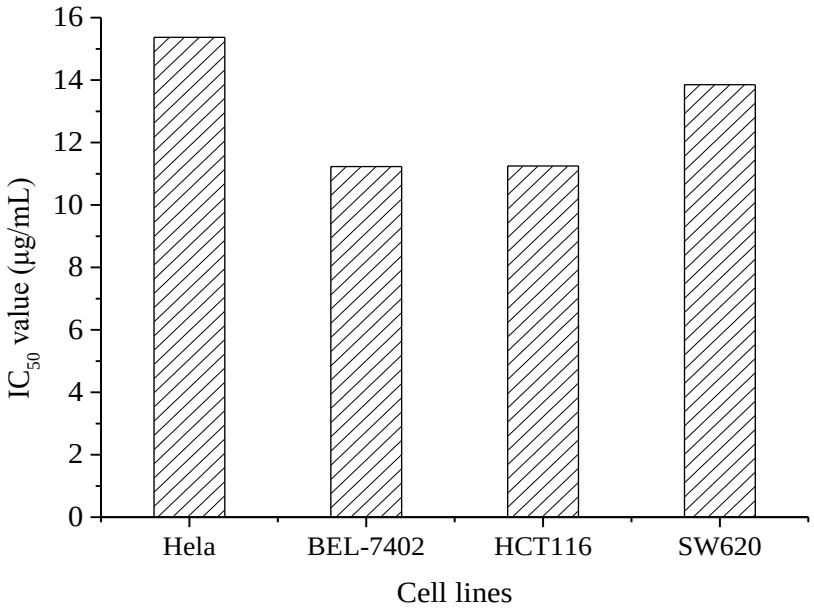

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