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Research Article

Bioactivity-guided Isolation and Characterization of Antitumor Active Compound from Marine-derived Fungus *Penicillium chrysogenum* HGQ6

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Abstract: To isolate and characterize antitumor active compounds (bioactivity-guided fractionation) from the ethyl acetate extract of fermentation broth of marine-derived fungus *Penicillium chrysogenum* HGQ6. Following the cytotoxicity against human cancer cell line BGC823, one compound was isolated and purified by silica gel chromatography, Sephadex LH-20 chromatography and preparative high performance liquid chromatography. The isolated compound was elucidated on the basis of extensive spectroscopic data analysis as 5-hydroxymethyl-2-furancarboxaldehyde, which exhibited cytotoxicity against BGC823 cell line with an IC_{50} value of 0.19 mg/mL. Our present study concluded that, 5-Hydroxymethyl-2-furancarboxaldehyde isolated from marine-derived fungus *Penicillium chrysogenum* HGQ6 has a certain extent of antitumor potential.

Keywords: Antitumor activity, isolation and characterization, marine-derived fungus, *Penicillium chrysogenum* HGQ6

INTRODUCTION

Marine-derived fungus has been proven to be a rich source of structurally unique and biologically active secondary metabolites (Gao *et al*., 2011). Until to now, more than one thousand novel secondary metabolites with different structural type were isolated and identified from marine-derived fungus, including alkaloids, polypeptides, macrolides, polyethers, terpenes, etc., which showed significant bioactivities such as antitumor, antimicrobial and antivirus etc. (Guo *et al*., 2014). Plinabulin (NPI-2358), a synthetic analog of NPI-2350 derived from marine fungus *Aspergillus* sp. CNC-139, have been completed the clinical II trial, which was used to treat advanced non-small cell lung cancer combined with docetaxel (Millward *et al*., 2012).

As part of our ongoing efforts to discover antitumor active natural compounds from marine-derived fungus, *Penicillium chrysogenum* HGQ6, was isolated from the sea mud sample in Lianyungang, attracted our attention. This fungus showed cytotoxicity against gastric cancer cell line BGC823 and the fermentation conditions for production of antitumor active substance have been optimized (Guo *et al*., 2012). In this study, following the cytotoxicity against human cancer cell line BGC823, isolation and characterization of antitumor active compound from the ethyl acetate (EtOAc) extract of fermented broth of marine-derived fungus *Penicillium chrysogenum* HGQ6 was reported.

MATERIALS AND METHODS

Materials and instruments: *Penicillium chrysogenum* HGQ6 was separated from Lianyungang sea mud sample and conserved at the marine microbial active substances laboratory of Huaihai Institute of Technology, Jiangsu Province, China. Human gastric cancer cell line BGC823, was granted from China Pharmacetical University, Jiangsu Province, China.

UV spectra were recorded on a Waters 2487 absorbance detector. NMR data were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard and chemical shifts were recorded as δ values. ESI-MS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pack ODS-A, 10×250 mm, 5 μm, 4 mL/min] on a Waters 600 multisolvent delivery system equipped with a photodiode array detector (Waters 996). TLC and Column Chromatography (CC) were performed on plates precoated with silica gel GF254 (10-40 μm) and over silica gel (200-300 mesh, Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-Liquid Chromatography (VLC) utilized silica gel H (Qingdao Marine Chemical Factory).

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Fermentation and extraction: The fungus *P. chrysogenum* HGQ6 was cultured in 500 mL-conical flask containing 200 mL fermentation medium that was composed of peptone (10 g/L), sucrose (30 g/L), K_2HPO_4 (0.6 g/L), $FeSO_4$ (0.01 g/L), seawater, pH6.41 and was grown at agitation rate 180 r/min for 6 days at 35°C. The fermented whole broth (30 L) was filtered through cheesecloth to separate the filtrate from the mycelia. Then the filtrate was extracted three times with an equivalent volume of EtOAc to give an EtOAc solution. The mycelia were extracted three times with 80% acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution that was extracted three times with an equivalent volume of EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give EtOAc extract (10.5 g).

Bioactivity-guided fractionation and identification of active compounds: The EtOAc extract (10.5 g) was subjected to silica gel column chromatography, eluting with a stepwise gradient of petroleum ether-CH_2Cl_2 (1:1 and 0:1) and then of CH_2Cl_2-MeOH (100-0%) to achieve nine primary fractions (1-9). All the fractions 1-9 were screened for antitumor activity against cell line BGC823 by MTT method. The bioactive fraction 5 showed the antitumor activity. Fraction 5 (1.1 g) eluted with CH_2Cl_2-MeOH (25:1) was further purified by Sephadex LH-20 (MeOH) to afford three subfractions. The subfraction 5-2 (100 mg) was purified by semi-preparative HPLC eluting with 85% MeOH-H_2O to yield compound 1 (6.5 mg, t_R 7.2 min). The isolated bioactive compound was elucidated on the basis of extensive spectroscopic data analysis.

MTT method: The cytotoxicity against human cancer cell line BGC823 was determined by MTT method (Guo *et al*., 2006). 180 μL of BGC823 cells at 2.5×10^4 cells/mL were added into each well of white 96-well plate (Costar, USA). The cells were maintained in RPMI 1640 (Hyclone, Utah, USA) containing 10% fetal calf serum (Hyclone, Utah, USA) and then incubated in an atmosphere of 5% CO_2 at 37°C overnight. The media were removed the next day and 180 μL fresh media were added into each well. And then, 20 μL of different concentration of adriamycin and 5-hydroxymethyl-2-furancarboxaldehyde were added into each well and each sample has 3 repeats and the plate was incubated 44 h. After incubation, 20 μL of 3-(4, 5-Dimethyl-2-thiazodyl)-2, 5-diphenyltetrazolium Bromide (MTT, Amresco, Ohio, USA, 5 mg/mL) was added into each well respectively. The plate was oscillated for 1 min on oscillator and then it was incubated at 37°C and 5% CO_2 for 4 h continuously. After that, the supernatants were removed, while 200 μL of DMSO was added to each well and then the plate was oscillated for 10 min again. The absorbance values were measured by Synergy HT (Bio-Tek Inc., USA) at 570 nm. The inhibition ratio was defined as:

$$(1 - A_{570,\ sample}/A_{570,\ control}) \times 100\%.$$

RESULTS

Isolation of active compounds and structural determination: The fungus *P. chrysogenum* has been reported to produce polyketides, prenylated polyketides and terpenoids such as berkeleydione, deoxyartemisinin, trichodimerol and xanthohumol 4'-*O*-*β*-glucopyranoside and the sorbicillinoid alkaloids sorbicillactones A and B (Gao *et al*., 2011; Peng *et al*.,

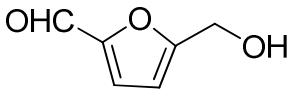

Fig. 1: Chemical structure of compound 1

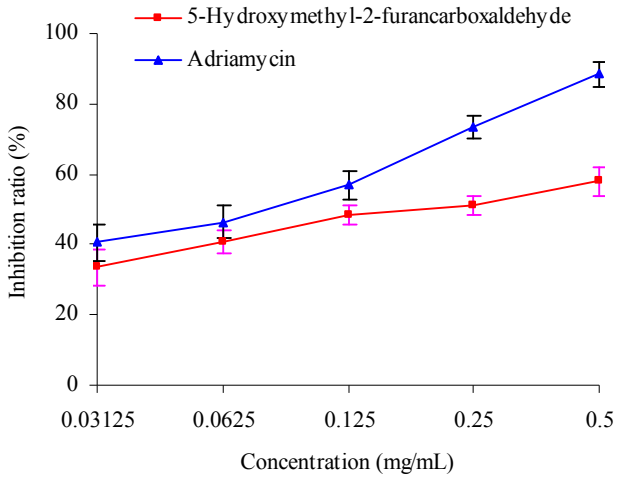

Fig. 2: Cytotoxicity test of 5-hydroxymethyl-2-furancarboxaldehyde and adriamycin

2011). In our previous study, we find the marine-derived fungus *P. chrysogenum* HGQ6 displayed obvious cytotoxicity against human gastric cancer cell line BGC823 (Guo *et al*., 2012). This fungus was therefore submitted to a large scale of fermentation for bioactive compounds isolation. One pure compound was separated from the active EtOAc extract of fermented broth of *P. chrysogenum* HGQ6 by the method mentioned above; which was identified as 5-hydroxymethyl-2-furancarboxaldehyde (Fig. 1), by comparison of its spectroscopic data with that in the literature.

5-hydroxymethyl-2-furancarboxaldehyde (1): yellow poder; ^{1}H-NMR(DMSO): δ 9.55 (1H, s, CHO), 7.51 (1H, d, *J* = 3.6 Hz, H-3), 6.60 (1H, d, *J* = 3.6 Hz, H-4), 4.51 (2H, s, H-α); ^{13}C-NMR (DMSO): δ 178.5 (CHO), 162.6 (C-5), 152.2 (C-2), 124.3 (C-3), 110.2 (C-4), 56.4 (C-α); Positive ESI-MS: *m*/*z* 127 $[M + H]^+$. Its spectroscopic data was in accordance with the report (Pyo *et al*., 2004).

Cytotoxicity of the isolated compound: The cytotoxicity of the isolated compound 1 was examined in MTT bioassay and the results indicated that 5-hydroxymethyl-2-furancarboxaldehyde (1) possessed activity against BGC823 cell with the IC_{50} value of 0.19 mg/mL, which is lower than that of adriamycin with the IC_{50} value of 0.066 mg/mL (Fig. 2).

CONCLUSION

In conclusion, one compound, 5-hydroxymethyl-2-furancarboxaldehyde, was isolated from the EtOAc extract of fermented broth of marine-derived fungus *Penicillium chrysogenum* HGQ6 under the guideline of cytotoxicity-guided fractionation. 5-hydroxymethyl-2-furancarboxaldehyde exhibited cytotoxicity against BGC823 cell line with the IC_{50} of 0.19 mg/mL and has the certain extent of antitumor potential.

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