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Discovery of Oosporein as a New Inhibitor of Influenza Virus Cap-snatching Activity

Nobuhiro Koyama[1,2*], Yoshio Shibagaki[3], Mitsuru Okuwaki[3], Seisuke Hattori[3] and Hiroshi Tomoda[1,2*]

[1]*Department of Microbial Chemistry, Graduate School of Pharmaceutical Sciences, Kitasato University, Japan*

[2]*Medicinal Research Laboratories, School of Pharmacy, Kitasato University, Japan*

[3]*Division of Biochemistry, School of Pharmacy, Kitasato University, Japan*

Abstract

During our screening of microbial origins, we found that fungal strain BF-0073 produces an inhibitor of the cap-snatching activity of influenza virus. Compound (**1**) was isolated from the culture broth of fungal strain BF-0073 by solvent extraction and preparative HPLC. Based on structural analyses using MS and NMR, **1** was identified as oosporein. Compound **1** inhibited the cap-snatching activity of influenza virus A in a dose-dependent manner, with an IC_{50} value of 20.0 µg/mL.

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Our research group has focused on the discovery of new compounds from microbial sources due to their great chemical diversity and interesting biological activity [1-5]. Original assays systems were used to screen our culture collection for bioactive compounds. Over 1,000 microbial samples were screened, and fungal strain BF-0073 was selected for producing an inhibitor of the cap-snatching activity of influenza virus. Seven-day-old culture broth (200 mL) of this strain was extracted with an equal volume of ethanol, and the extract was collected by suction filtration and then evaporated *in vacuo* to obtain a water fraction. The water fraction was extracted with ethyl acetate (200 mL) and then concentrated *in vacuo* to yield a crude extract (275.6 mg). The crude extract was dissolved in a small volume of methanol and further purified by HPLC using a reverse-phase C18 column (PEGASIL ODS SP100, i.d. 20×250 mm) under the following conditions: solvent, 25% aq CH_3CN containing 0.05% TFA; flow rate, 6.0 mL/min; detection, UV at 210 nm. Under these conditions, the active compound was eluted as a peak with a retention time of 28 min. This fraction was collected, concentrated *in vacuo*, and lyophilized to dryness to yield pure compound **1** (15.3 mg) as a purple powder. The structure was elucidated by spectroscopic data, including NMR experiments. Compound **1** was identified as oosporein based on comparisons with previously reported data [6] (Figure 1). ^{1}H-NMR (400 MHz, pyridine-d_5): δ 1.70 (s, 3H). ^{13}C-NMR (100 MHz, pyridine-d_5): δ 8.1, 106.1, 107.4, 171.9, 173.4. ESI-MS: $[M-H]^-$: 305.

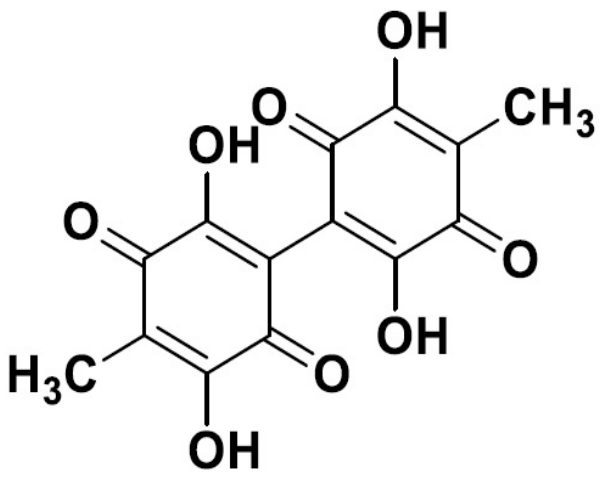

Figure 1: Structure of oosporein.

The effects of **1** on the cap-snatching activity of influenza virus were evaluated according to previously established methods [7-9]. Briefly, a reaction mixture (25 µL) containing 50 mM Tris-HCl (pH 7.9), 0.1 M ammonium acetate, 1.5 mM $MgCl_2$, 2.5 mM DTT, 0.1% Nonidet P-40, 4U of RNasin, and 30-50 fmol of [^{32}P]Cap 1-$GACU_{32}$-biot (2×10^5 cpm/pmol) was incubated with influenza virus A virions (1 µg) at 37°C for 1h. The reaction was stopped by incubation withbuffer (200 µL) containing 20 mM Tris-HCl (pH 8.0), 5 mM EDTA, 150 mM NaCl, and streptavidin beads (30 µg) at room temperature for 10 min. The streptavidin beads-bound and unbound fractions were then collected separately, and the radioactivity of each was measured to calculate the corresponding IC_{50} value. Compound **1** inhibited cap-snatching activity in a dose-dependent manner by suppressing the generation of the cleaved fragment from [^{32}P]Cap 1-$GACU_{32}$-biot. The calculated IC_{50} value was 20.0 µg/mL (Figure 2), which was comparable to that of a known inhibitor of cap-snatching activity described in the literature, 2-hydroxy-4-oxo-4-phenyl-2-butenoic acid [10].

In conclusion, we identified oosporein produced by the fungal strain BF-0073 as an inhibitor of influenza virus cap-snatching activity. Terry et al. reported that the compound inhibited DNA polymerase of herpes simplex virus type 1 with an IC_{50} value of 22.9 µg/mL [11]. Interestingly, oosporein had no observable effect on DNA polymerase of HeLa cells or *Escherichia coli*, confirming the DNA polymerase selectivity of these organisms. Further study is needed to determine whether inhibition of the cap-snatching activity of influenza virus and DNA polymerase of herpes simplex virus type I occurs via a similar mechanism.

***Corresponding Author:** Dr. Nobuhiro Koyama, Department of Microbial Chemistry, Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan; E-mail: koyaman@pharm.kitasato-u.ac.jp

***Corresponding Author:** Dr. Hiroshi Tomoda, Department of Microbial Chemistry, Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan; E-mail: tomodah@pharm.kitasato-u.ac.jp

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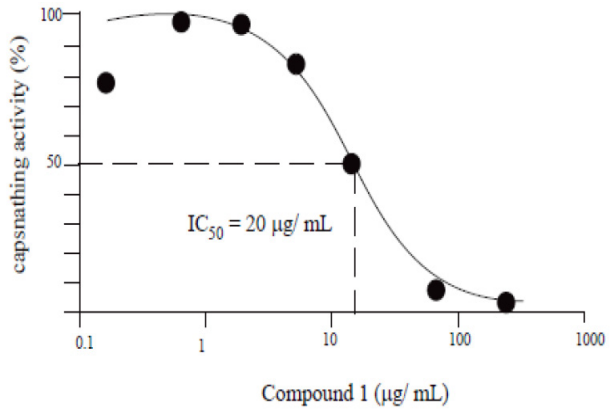

Figure 2: Assay of oosporein-mediated inhibition of cap-snatching activity. [^{32}P]Cap 1-GACU$_{32}$-biot (50 fmol) was incubated with increasing amounts of oosporein and influenza virus A virions (1 µg). Cap-snatching activity is expressed as the ratio of the amount of cleaved RNA to the amount of total capped RNA. Typical results of three independent experiments are shown.

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Competing Interests

The authors declare that they have no competing interests.

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