MPC1001, a New Antitumor Antibiotic Produced by *Cladorrhinum* sp.

Noriko Tsumagari, Ryuichiro Nakai,[†]
Hideyuki Onodera, Atsuhiro Hasegawa,
Endang S. Rahayu,[††] Katsuhiko Ando and
Yoshinori Yamashita[†]

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.,
3-6-6 Asahimachi, Machida-shi, Tokyo 194-8533, Japan
[†] Pharmaceutical Research Institute,
Kyowa Hakko Kogyo Co., Ltd.,
1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka
411-8731, Japan
[††] Faculty of Agricultural Technology, Gadjah Mada University,
Bulaksumur Yogyakarta 55281, Indonesia

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In the course of our screening for antitumor antibiotics, MPC1001 was isolated from a microorganism, *Cladorrhinum* sp. KY4922. MPC1001 exhibited antimicrobial activity, and antiproliferative activity against human tumor cell lines. In this paper, we describe the taxonomy of the producing organism, fermentation, isolation, physico-chemical properties and biological activities of MPC1001.

The cultural and taxonomical characteristics of the producing fungal strain KY4922 were as follows. KY4922 was isolated from a soil sample collected in Indonesia. The size of colonies on the malt extract agar medium varies from 60 to 68 mm in diameter after culturing at 25°C for a week. The surface of the colony is white at the center and gray at the marginal area. The color of reverse side is pale beige at the center and bright gray at the marginal area. The size of colonies on potato - glucose agar medium are 57 to 67 mm in diameter after culturing at 25°C for a week. The surface of the colony is white at the center and bright gray at the marginal area. The color of reverse side is pale beige at the center and bright gray at the marginal area. The temperature range for growth is from 10.5 to 36.5°C and optimum growth temperature is about 27°C. The pH range for growth is from 5.3 to 10.0 and the optimal pH for growth is around 7.3. On malt extract agar medium at 25°C for two weeks, hyphae that composed of septate and branched well are smooth, colorless to pale brown, and 2 to 3 μm wide. The conidial ontogeny is enteroblastic. Conidia are produced at a phialidic flaring collarette that formed from fertile hypae or cylindrical intercalary phialide with lateral openings. The phialidic conidia adhering in slimy heads are single-celled, hyaline, smooth, globose to subglobose or dacryoid, and 1.5 to 3.0 μm in diameter. No teleomorph was observed in this strain.

From the characteristics mentioned above, the fungal strain KY4922 was identified as *Cladorrhinum* sp.[1)]. The fungus has been deposited at International Patent Organism Depositary, the National Institute of Advanced Industrial Science and Technology, Japan, as *Cladorrhinum* sp. FERM BP-7266.

A loopful of the cells from a mature slant of a strain KY4922 was inoculated into each of glass long test tubes containing 10 ml of the seed medium composed of glucose 10%, dried mashed potato (Snow Bland Milk Co. Ltd.) 3%, yeast extract (Wako Co. Ltd.) 0.5% in deionized water (pH adjusted to 6.5 with NaOH before sterilization). The inoculated tubes were incubated on a shaker (320 rpm) at 28°C for 5 days. The seed culture (2.5 ml) was added to two 300 ml Erlenmeyer flasks containing 50 ml of the same medium. After incubation on a rotary shaker at 25°C for 2 days (260 rpm), 2.5 ml of seed culture was transferred into each of twenty two 300 ml Erlenmeyer flasks containing 50 ml of the fermentation medium composed of glucose 2%, dried mashed potato 2%, peptone (Kyokuto Co. Ltd.) 0.5%, KH_2PO_4 0.5%, $Mg_3(PO_4)_2 \cdot 8H_2O$ 0.5% in deionaized water (pH adjusted to 6.0 with NaOH before sterilization). The fermentation was carried out at 25°C for 5 days on a rotary shaker (260 rpm).

After culture broth (1.1 liters) was separated by filtration using aspirator, methanol (800 ml) was added to the mycelial cake and stired sufficiently. The extract was filtrated and added with 1.6 liters of water. The diluted extract (2.4 liters) was applied to a column of Diaion HP-20 (60 ml, Mitsubishi Chemical Industries). The column was washed with 90% methanol and then the active substances were eluted with 100% methanol (200 ml) and 100% acetone (200 ml). The two active fractions were concentrated *in vacuo* to yield a yellow oil. The oil was applied to a column of silica gel (70 ml, LiChroprep Si60, Merck). The column was developed with hexane - AcOEt (1 : 1) and active substance was eluted with AcOEt. The eluate was concentrated *in vacuo*, and then purified through HPLC using a packed ODS column (HG-5, Nomura

* Corresponding author: noriko.tsumagari@kyowa.co.jp

Chemical Co.) under the conditions, in which the fraction was developed with developers of 67% to 100% CH_3CN. The active fraction was concentrated and afforded MPC1001 19.4 mg as a white powder.

The physico-chemical properties of MPC1001 are summarized in Table 1. The molecular formula of MPC1001 was determined to be $C_{28}H_{24}N_2O_{10}S_2$ (F.W.612) by HR-FAB MS and ^{13}C-NMR spectra. Interpretations of 1D and 2D NMR spectra along with physico-chemical data, MPC1001 was revealed to be a new 2″-*O*-methyl derivative of emestrin, a 15-membered antifungal macrolide possessing a unique epidithiodioxopiperazine skeleton (Fig. 1). The relative configuration of MPC1001, elucidated by proton coupling constants and NOE experiments, and the absolute structure *via* CD spectrum were identical to those of emestrin.[2] In the HPLC analysis, MPC1001 was definitely detected in the fungal broth and mycelium extracted with other solvent than methanol such as acetone and acetonitrile, while any trace of emestrin was not appeared. We, therefore, concluded that MPC1001 is not the methylated artifact of emestrin that generated during extraction and purification processes using methanol. The details in the structure determination of MPC1001 will be

Fig. 1. Structures of MPC1001 and emestrin.

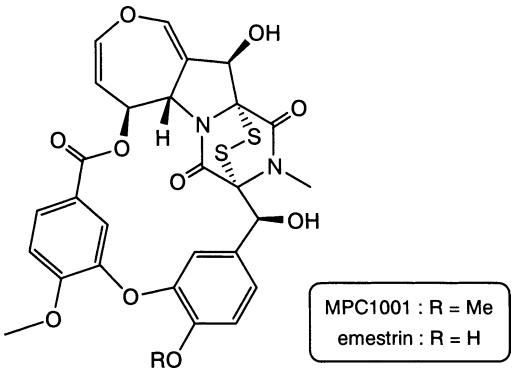

Table 1. Physico-chemical properties of MPC1001.

Appearance	white powder
Melting point	181.0～184.0℃
Molecular formula	$C_{28}H_{24}N_2O_{10}S_2$
FAB MS (Pos.) m/z	613 $[M+H]^+$, 635 $[M+Na]^+$, 548$[M-S_2]^+$, 531 $[M-S_2-H_2O+H]^+$
FAB MS (Neg.)	611 $[M-H]^-$
HR FAB MS (Neg.)	611.0802 (Δ+0.8mmu calcd. for $C_{28}H_{23}N_2O_{10}S_2$)
IR ν (KBr) cm^{-1}	3442, 1716, 1684, 1668, 1614, 1515, 1346, 1271, 1200 1174, 1136, 1122, 1051, 1024, 891, 822, 762
UV λ_{max} (MeOH) nm (e)	205 (38,000), 263 (14,000), 283 (8,000)
TLC (Rf value[a])	0.45
Solubility	
Soluble	MeOH, Acetone, AcOEt, $CHCl_3$, DMSO
Insoluble	Hexane, H_2O

[a] Silica gel TLC (Kieselgel 60 F_{254}, Merck), solvent: hexane-acetone (1:1)

Table 2. Antimicrobial activities of MPC1001.

Test microorganisms	MIC (μg / ml)
Staphylococcus aureus subsp. *aureus* ATCC 6538P	10
Bacillus subtilis No.10707	2.6
Enterococcus hirae ATCC 10541	21
Proteus vulgaris ATCC 6897	>83
Klebsiella pneumoniae subsp. *pneumoniae* ATCC 10031	>83
Escherichia coli ATCC 26	>83
Pseudomonas aeruginosa BMH No.1	>83
Shigella sonnei ATCC 9290	>83
Candida albicans ATCC 10231	>83

Table 3. Antiproliferative activity of MPC1001.

	IC_{50} (nmol / L)
MPC1001	9.3
Adriamycin	20
Mitomycin C	25
Etoposide	400

reported elsewhere.

Antimicrobial activities of MPC1001 are shown in Table 2. MPC1001 exhibited antimicrobial activity against Gram-positive bacteria, such as *Staphylococcus aureus* and *Bacillus subtilis*, whereas weak activities were detected against Gram-negative bacteria.

Table 3 shows the antiproliferative activity of MPC1001 and known antitumor agents, obtained from the experiment carried out under the same assay condition. MPC1001 exhibited an antiproliferative activity against a human prostate cancer cell line (DU145) with an IC_{50} value of 9.3 nM. The activity of MPC1001 was shown to be more potent than those of known antitumor agents such as adriamycin, mitomycin C and etoposide. This antiproliferative activity of MPC1001 against human cancer cell is consistent with previous studies in which emestrin induces apoptotic nuclear changes at 167.2 nM and shows the cytotoxicity at 83.6 nM against a human promyelotic leukemia cells (HL-60)[3]. The investigation on the mechanism of action and antitumor activity of MPC1001 is in progress.

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Reference

1) VON ARX, J. A.: The genera of fungi sporulating in pure culture, 2nd ed., Cramer, 1974
2) SEYA, H.; K. NOZAWA, S. NAKAJIMA, K. KAWAI & S. UDAGAWA: Studies on fungal products. Part 8. Isolation and structure of emestrin, a novel antifungal macrocyclic epidithiodioxiopiperazine from *Emericella striata*. X-Ray molecular structure of emestrin. J. Chem. Perkin Trans. I, 1986: 109～115
3) UENO, Y.; K. UMEMORI, E. NIIMI, S. TANUMA, S. NAGATA, M. SUGAMATA, T. IHARA, M. SEKIJIMA, K. KAWAI, I. UENO, *et al.*: Induction of apoptosis by T-2 toxin and other natural toxins in HL-60 human promyelotic leukemia cells. Natural Toxins. 3(3): 129～137, 1995