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TMC-69, a New Antitumor Antibiotic with Cdc25A Inhibitory Activity, Produced by *Chrysosporium* sp. TC1068

Taxonomy, Fermentation and Biological Activities

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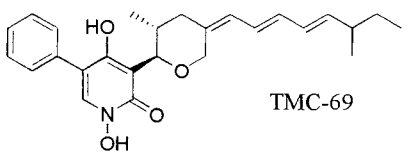
A new antibiotic designated TMC-69 has been isolated from the fermentation broth of a fungal strain *Chrysosporium* sp. TC 1068. TMC-69 exhibited moderate *in vitro* cytotoxic activity. TMC-69-6H, a derivative of TMC-69 prepared by hydrogenation, possessed more potent *in vitro* cytotoxicity than TMC-69, and exhibited *in vivo* antitumor activity against murine P388 leukemia and B16 melanoma. TMC-69-6H was found to specifically inhibit Cdc25A and B phosphatases.

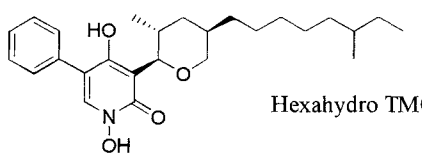
In the course of our screening program aimed at new antitumor substances from microorganisms, we isolated new cytotoxic antibiotics, TMC-1 and TMC-135 A and B from *Streptomyces* sp. strains[1,2], TMC-151 A~F, TMC-154, and TMC-171 A~C from *Gliocladium* sp. strains[3,4], and TMC-169 from *Aspergillus flavipes*[5]. Further screening has resulted in isolation of a unique active metabolite named TMC-69 (Fig. 1) from the culture broth of a fungus identified as *Chrysosporium* sp. TC 1068. Hexahydro-TMC-69 (TMC-69-6H, Fig. 1) derived from TMC-69 had an improved stability in various organic solvents as compared to TMC-69. TMC-69 and TMC-69-6H showed potent antitumor activities both *in vitro* and *in vivo*. As a result of mode of action studies, we found that TMC-69-6H inhibited Cdc25A and B phosphatase.

Cdc25 phosphatases are classified as dual-specificity protein phosphatases (DSPase) that act as key regulators of the cell cycle progression and mitogenic signaling pathways. Mammalian cells express at least three Cdc25 isoforms named A, B, and C that activate cyclin dependent kinases (Cdks) by catalyzing the dephospholylation of their phospholylated tyrosine and threonine[6~8]. Cdc25A and B are overexpressed in some types of tumors. Patients with these tumors have a tendency to result in poor prognosis[9,10]. Therefore, Cdc25s would be attractive candidates of molecular targets for development of

Fig. 1. Structures of TMC-69 and TMC-69-6H.



TMC-69



Hexahydro TMC-69

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anticancer agents. Several compounds (dysidiolide, dnacins, naphthoquinone analogs, nocardiones, steroidal derived acids) have been found to inhibit Cdc25 phosphatases[11~15].

In this paper, we report taxonomy and fermentation of the producing strain of TMC-69, and the biological activities of TMC-69 and TMC-69-6H. Isolation, the physico-chemical properties and structure elucidation of TMC-69 and TMC-69-6H were reported in elsewhere[16].

Experimental

Taxonomy

The producer strain was isolated from a soil sample that was collected at Moyoro Shell Mound near Lake Notori, Abashiri, Hokkaido, Japan. For identification of the fungus, phytone-yeast extract agar (BBL), cornmeal agar (Nissui), oatmeal agar (ISP No. 3, Difco), Czapek-yeast extract agar, malt extract agar (Difco) were used. Morphological observation was done under a microscope (Olympus BH-2). The color name used in this study was taken from Munsell color system[17].

Fermentation

Chrysosporium sp. TC 1068 was inoculated into a 500-ml Erlenmeyer flask containing 70 ml of a seed medium composed of 2% glycerol, 0.5% glucose, 2% soybean flour, 0.2% yeast extract, 0.25% NaCl, and 0.4% $CaCO_3$. The culture was incubated for 4 days at 27°C on a rotary shakar (220 rpm). One milliliter of the seed culture was transferred to a 500-ml Erlenmeyer flask containing 70 ml of the identical medium. The culture was incubated for 6 days at 27°C on a rotary shaker (220 rpm).

Hexahydrogenation of TMC-69

Hexahydro TMC-69 (TMC-69-6H) was obtained by catalyzed hydrogenation of the triene moiety of TMC-69 with palladium charcoal as described elsewhere[16].

Cell Culture

Human or murine tumor cells were cultured in the following medium: HCT-116 human colon carcinoma and SK-BR-3 human breast adenocancinoma, complete McCoy's 5A supplemented with 10% fetal bovine serum; B16 murine melanoma and HeLa S3 human epitheloid adenocarcinoma, complete D-MEM supplemented with 10% fetal bovine serum; HL-60 human promyelocytic leukemia, complete RPMI-1640 supplemented with 20% fetal bovine serum; WiDr human colon adenocarcinoma, complete RPMI-1640 supplemented with non-essential amino acid solution and 10% fetal bovine serum; P388D1 murine lymphoid neoplasm, complete RPMI-1640 supplemented with 5% fetal bovine serum.

Cell Proliferation Assays

In vitro cytotoxic activities against the various tumor cell lines were tested in 96-well microtiter plates. Ten thousand cells were seeded in 135 μl of appropriate medium per well. The test samples were dissolved in 10% DMSO. The serially diluted DMSO solution (15 μl) was added to each well of plates, and then the cells were incubated at 37°C for 72 hours in a humidified 5% CO_2 atmosphere. Five mM WST-1 (2-(4-iodophenyl)-3-(nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, Dojindo) solution (25 μl) was added to each well. After 3-hour incubation at 37°C, *in vitro* cytotoxicity was evaluated by the measurement of absorbance at 405 nm[18].

In Vivo Antitumor Activity

Growth inhibitory effects on P388 leukemia were evaluated by determination of survival days after tumor implantation. P388 leukemia was implanted at 1×10^6 cells/mouse into the peritoneal cavity of CDF1 mice weighing 22~24 g on day 0. TMC-69 and TMC-69-6H were intraperitoneally administrated to the mice once daily on the days 1 to 3.

B16 melanoma were inoculated by injecting intraperitoneally 0.5 ml of 20% homogenate into BDF1 mice weighing 22~24 g on day 0. TMC-69 and TMC-69-6H were intraperitoneally administrated to the mice once daily on days 1 to 3 and once daily on days 1 to 4, respectively.

Preparation of Cdc25A, B and VHR

The full-length human Cdc25A and Cdc25B cDNAs (a kind gift of Dr. HIROTO OKAYAMA, The University of Tokyo, Japan) was cloned into the pGEX-2T expression vector to generate pGEX-Cdc25A and pGEX-Cdc25B, respectively. The cDNA encoding the human dual-specificity phosphatase, VHR (VH1-related human protein) was obtained using the polymerase chain reaction (PCR) from a human placenta cDNA library (TOYOBO). The PCR primers used were 5′-AGCCGGATCCATGTCGGGC-TCGTT and 5′-CACGGAATTCTCGAGCAGAGGCGG for 5′ and 3′ primers, respectively. The PCR product was cut by *Eco*RI and *Bam*HI, and ligated into pUC118. The coding region of VHR was then removed by *Eco*RI and *BamH*I digestion and transferred into the plasmid pGEX-2T to generate pGEX-VHR for overexpression. The

resulting pGEX-Cdc25A, pGEX-Cdc25B, and pGEX-VHR plasmid was transformed into *E. coli* strain BL21 (DE3).

Recombinant GST-Cdc25A, Cdc25B and VHR fusion protein were purified as below. Each of the *E. coli* BL21 transformants was induced by isopropyl-β-D-thiogalacto-pyranoside (IPTG) and disrupted by sonication on ice in phosphate-buffered saline (PBS), pH 7.2, containing 1% NP-40, 1 mM EDTA, 1 mM dithiothreitol (DTT), 100 μM leupeptin, 2 μg/ml aprotinin, and 1 mM PMSF. Cell debris was removed by centrifugation for 30 minutes at 100,000×*g*. The GST fusion proteins were purified from the soluble extract by chromatography over glutathione-agarose. Active fractions were pooled and supplemented with 40% glycerol to prior to storage at −80°C.

Phsophatase Assays

The enzymatic activities of GST-Cdc25A, -Cdc25B, -VHR, YOP (protein phosphatese from *Yersinia enterocoltica*, Calbiochem) and CIAP (calf intestine alkaline phosphatase, Takara Shuzo) were determined colorimetrically by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (*p*NPP). The reaction mixture (200 μl) for Cdc25A, Cdc25B and VHR comprised 50 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM EDTA, 10 mM DTT with the appropriate *p*NPP concentration, namely 50 mM for Cdc25A and Cdc25B, and 20 mM for VHR, respectively. The reaction mixture (200 μl) for YOP assay contained 50 mM Tris-HCl pH 7.2, 150 mM NaCl, 5 mM DTT, 2.5 mM EDTA, 100 μg/ml BSA and 20 mM *p*NPP. For the CIAP assay the mixture (200 μl) contained 50 mM Tris-HCl pH 9.0, 1 mM $MgCl_2$ and 20 mM *p*NPP. The mixtures were incubated at 37°C for 60 minutes for Cdc25A and Cdc25B; at 30°C for 60 minutes for VHR; at 30°C for 15 minutes for YOP and CIAP, respectively. The reactions were stopped by addition of 0.2 M NaOH (20 μl). The released *p*-nitrophenol was measured at 405 nm. PP2A (protein phosphatase-2A) activity was determined with Ser/Thr Phosphatase Assay kit using PP2A catalytic units obtained from Upstate Biotechnology. PTP1B activity was measured with Protein Tyrosine Phosphatase 1B Assay kit from Calbiochem.

Results and Discussion

Taxonomy

On phytone-yeast extract agar, the colonies grew rapidly, were floccose covered with white mycelia and attainied a diameter of 62~65 mm after 14 days at 25°C. When sporulated, the surface of the colonies showed pale reddish yellow to pale greenish yellow color (Munsell 10Y9/2), and were slightly powdery. The reverse was dull orange to light yellowish brown (Munsell 5YR6/6). No exudates or diffusible pigments were produced.

On cornmeal agar, the colonies were restricted thin and white, attaining a diameter of 24 mm after 14 days at 25°C. On oatmeal agar, the colonies were floccose and powdery and attained 52~56 mm in diameter after 14 days at 25°C.

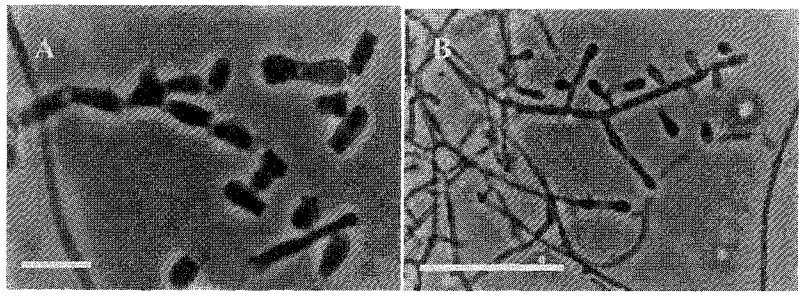
The fungus grew between 10 and 37°C on Czapek-yeast extract agar, but the optimal temperature for growth was between 27 and 32°C. The fungus did not grow at 37°C on phytone-yeast extract agar or malt extract agar. The maximum temperature for growth on these media was 32°C. The pH for growth was from 4.9 to 10.2, with an optimal between 4.9 and 5.9. No keratinophilic property was observed.

Aerial hyphae were hyaline, thin-walled, 1.5~3.5 μm in width, and branched several times. They formed terminal or lateral aleurioconidia as well as arthroconidia by fragmentation of the aerial hyphae. No distinct conidiophores were observed in either case. Aleurioconidia were formed directly on the apex or in the middle of hyphae (terminally or laterally), mostly solitary, but sometimes connected with another conidium. They were hyaline, smooth, slightly thick-walled, obovate, clavate or pear-shaped with a truncate base, one-celled; 6.0~10.0×3.5~5.0 μm (average 8.0×4.0 μm). The arthroconidia were formed intercalarily in the hyphae, solitary or in chain, hyaline, smooth, slightly thick-walled. They were dome-shaped, cylindrical or racket-shaped, 5.5~14.5×2.5~3.5 μm (average 8.0×3.0 μm). When the arthroconidia were formed, the adjacent cells became empty so that both ends of the conidia were truncate with reminiscent of cell wall (Fig. 2).

The present fungus was characterized by the following morphological properties; formation of aleurioconidia in addition to arthroconidia, fragmentation of arthroconidia by separating cells, broader width of conidium compared to fertile hyphae, hyaline conidia, lack of macro-conidia, and absence of erect conidiophores. Based on these distinct characteristics, it was placed in the genus *Chrysosporium*[19]. The present fungus was therefore identified as *Chrysosporium* sp. F 2358.

Over the latest decade, only 6 substances were isolated and described from *Chrysosporium* such as chrysosporin and pannorin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase[20,21]; RPR113228, a farnesyl-protein transferase inhibitor[22]; semicochliodinol A and B, inhibitors of HIV-1 protease and EGF-R protein tyrosine kinase[23]; dechlorogeodin and its dihydro derivatives,

Fig. 2. Microphotographs of *Chrysosporium* sp. TC 1068.



A: Arthroconidia on cornmeal agar after 7 days at 25°C (bar=10 μm).
B: Aleurioconidia laterally disposed along the hypha on oatmeal agar after 7 days at 25°C (bar=50 μm).

Table 1. *In vitro* antitumor activity of TMC-69 and TMC-69-6H (IC_{50}, μM).

	IC_{50}(μM)						
Compound	HCT-116	B16	HeLaS3	SK-BR3	WiDr	HL-60	P388D1
TMC-69	6.79	6.56	4.92	7.96	1.87	0.43	6.56
TMC-69-6H	0.77	1.87	0.23	1.17	0.47	0.10	0.94

herbicidal activity[24]; and adenopeptin, a new apoptosis inducer[25]. However, *Chrysosporium pannorum*, a producer of chrysosporin and pannorin, is classified as *Geomyces pannorum* (Link) Sigler & Carmichael at present.

Cytotoxic and Antitumor Activities

The cytotoxic activity of TMC-69 and TMC-69-6H against several tumor cell lines is shown in Table 1. TMC-69 was cytotoxic to murine tumor cells (P388D1 and B16) as well as human tumor cells such as HCT-116, HeLa S3, SK-BR3, WiDr and HL-60 cells. TMC-69 exhibited the most potent cytotoxicity against HL-60 human promyelocytic leukemia cells with IC_{50} value of 0.43 μM. It had less potent activities against the other cell lines tested. TMC-69-6H showed potent cytotoxicities with IC_{50} values of 0.1 to 1.87 μM against all of the cells tested. The cytotoxicities of TMC-69-6H against solid tumor and leukemia cells were nearly equal to each other.

The *in vivo* antitumor activity was assessed in tumor-bearing mice. As shown in Table 2 and 3, TMC-69 demonstrated moderate therapeutic activity against P388 murine leukemia by the qd×3 schedule administration with a maximum ILS value of 47.3% at a dose of 30 mg/kg. Against B16 melanoma at a dose of 30 mg/kg and the qd×3 schedule administration, TMC-69 gave a maximum ILS value of 89.8%. On the other hand, TMC-69-6H exhibited potent antitumor activity against P388 murine leukemia with maximum ILS value of 58.1% at a dose of 3 mg/kg. Moreover, TMC-69-6H showed the superior antitumor activity against B16 melanoma with ILS value of 105.9% at a dose of 1.25 mg/kg.

Phosphatase Inhibitory Activities

Since TMC-69 was unstable in various organic solvents[16], we used TMC-69-6H to study the mode of action.

TMC-69-6H caused G1 arrest when tested on HL-60 cells as evidenced by flow cytometry analysis (data not shown). Then, we examined whether or not TMC-69-6H inhibited several serine/threonine kinases and Cdc25A phosphatase which are involved in cell cycle progression (Table 4). TMC-69-6H exhibited a dose-dependent inhibition of Cdc25A activity with an IC_{50} of 3.1 μM. This was comparable to the inhibition of Cdc25A activity by

Table 2. *In vivo* activities of TMC-69 and TMC-69-6H against P388 murine leukemia.

	Dose (mg/kg)	Schedule	Mean survival days	Increase in life span (%)
control	-		9.3	-
TMC-69	3	Days 1-3	10.3	10.8
	10		13.0	39.8
	30		13.7	47.3
TMC-69-6H	1	Days 1-3	11.7	25.8
	3		14.7	58.1
	10		7.3	-21.5

P388 (1 x 10^6 cells/mouse) were incubated i.p. into CDF1 mice on the day 0, then compounds were administrated i.p. on each day following indicated schedule.

Table 3. *In vivo* activities of TMC-69 and TMC-69-6H against B16 melanoma.

		Dose (mg/kg)	Schedule	Mean survival days	Increase in life span (%)
Exp. 1	control	-		25.5	-
	TMC-69	5	Days 1-3	32.0	25.5
		10		28.2	10.6
		20		33.8	32.5
		30		48.4	89.8
Exp. 2	control	-		20.4	-
	TMC-69-6H	0.32	Days 1-4	28.4	39.2
		0.63		40.4	98.0
		1.25		42.0	105.9
		2.5		23.4	14.7
		5		8.2	-59.8

B16 melanoma (0.5ml of 20 homogenate/mouse) were incubated i.p. into BDF1 mice on the day 0, then compounds were administrated i.p. on each day following indicated schedule.

Table 4. Phosphatase inhibitory activities of TMC-69-6H.

	IC_{50} (μM)						
	Cdc25A	Cdc25B	VHR	PP2A	PTP1B	YOP	CIAP
TMC-69-6H	3.2	4.4	>30	>30	>100	>100	>30
Na_3VO_4	3.0	4.8	3.9	-	-	12	24
okadaic acid	-	-	-	0.0015	-	-	-

The concentration showing 50% inhibition (IC_{50}) was determined from a plot of percent inhibition versus the concentration.

sodium orthovanadate[26] which had an inhibitory IC_{50} activity of 3.0 μM in our assay system. TMC-69-6H also inhibited Cdc25B with an IC_{50} value of 4.4 μM. TMC-69-6H did not significantly inhibit dual-specific phosphatase, VHR, tyrosine protein phosphatases, PTP1B and YOP, serine/threonine protein phosphatase, PP2A, or alkaline

phosphatase, CIAP, up to 30 μM. These results suggested that TMC-69-6H was a specific inhibitor of Cdc25A and B.

Tumor cells proliferate abnormally due to a disturbance of regulation of the cell cycle. Cdc25A and B are key regulators of the cell cycle progression. Cdc25A and B were frequently expressed at high levels in several tumor types such as human breast cancer and gastric carcinoma[8,9]. This overexpression was associated with an abnormal increase of Cdks activities without increase of Cdks expressions[9]. Inactivation of Cdc25A by antisense oligonucleotide inhibited S-phase entry in MCF-7 breast cancer cells. This treatment blocked activation of Cdk2[9]. Therefore, TMC-69-6H, a specific inhibitor of Cdc25A, might be a lead compound useful for development of antitumor agents.

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References

1) KOHNO, J.; M. NISHIO, K. KAWANO, N. NAKANISHI, S. SUZUKI, T. UCHIDA & S. KOMATSUBARA: TMC-1 A, B, C and D, new antibiotics of the manumycin group produced by *Streptomyces* sp. Taxonomy, production, isolation, physico-chemical properties, structure elucidation and biological properties. J. Antibiotics 49: 1212~1220, 1996
2) NISHIO, M.; J. KOHNO, M. SAKURAI, S. SUZUKI, N. OKADA, K. KAWANO & S. KOMATSUBARA: TMC-135A and B, new triene-ansamycins, produced by *Streptomyces* sp. J. Antibiotics 53: 724~727, 2000
3) KOHNO, J.; M. NISHIO, M. SAKURAI, K. KAWANO, H. HIRAMATSU, N. KAMEDA, N. KISHI, T. YAMASHITA, T. OKUDA & S. KOMATSUBARA: Isolation and structure determination of TMC-151s: Novel polyketide antibiotics from *Gliocladium catenulatum* Gilman & Abbott TC 1280. Tetrahedron 55: 7771~7786, 1999
4) KOHNO, J.; Y. ASAI, M. NISHIO, M. SAKURAI, K. KAWANO, H. HIRAMATSU, N. KAMEDA, N. KISHI, T. OKUDA & S. KOMATSUBARA: TMC-171A, B, C and TMC-154, novel polyketide antibiotics produced by *Gliocladium* sp. TC 1304 and TC 1282. J. Antibiotics 52: 1114~1123, 1999
5) KOHNO, J.; N. NONAKA, M. NISHIO, T. OHNUKI, K. KAWANO, T. OKUDA & S. KOMATSUBARA: TMC-169, a new antibiotic of the aspochalasin group produced by *Aspergillus flavipes*. J. Antibiotics 52: 575~577, 1999
6) NAGATA, A.; M. IGARASHI, S. JINNO, K. SUTO & H. OKAYAMA: An additional homolog of the fission yeast *cdc25*$^+$ gene occurs in humans and is highly expressed in some cancer cells. New Biologist 3: 959~968, 1991
7) DUNPHY, W. G. & A. KUMAGAI: The cdc25 protein conteines an intrinsic phosphatase activity. Cell 67: 189~196, 1991
8) DRAETTA, G. & J. ECKSTEIN: Cdc25 protein phosphatases in cell proliferation. Biochim. Biophys. Acta 1332: M53~63, 1997
9) KUDO, Y.; W. YASUI, T. UE, S. YAMAMOTO, H. YOKOZAKI, H. NIKAI & E. TAHARA: Overexpression of cyclin-dependent kinase-activating CDC25B phosphatase in human gastric carcinomas. Jpn. J. Cancer Res. 88: 947~952, 1997
10) CANGI, M. G.; B. CUKOR, P. SOUNG, S. SIGNORETTI, G. MORERIRA Jr., M. RANASHINGE, B. CADY, M. PAGANO & M. LODA: Role of the cdc25A phosphatase in human breast cancer. J. Clin. Invest. 106: 753~761, 2000
11) GUNASEKERA, S. P.; P. J. MCCARTHY, M. KELLY-BORGES, E. LOBKOVSKY & J. CLARDY: Dysidiolide: A novel protein phosphatase inhibitor from the Caribbean sponge *Dysidea etheria* de Laubenfels. J. Am. Chem. Soc. 118: 8759~8760, 1996
12) HORIGUCHI, T.; K. NISHI, S. HAKODA, S. TANIDA, A. NAGATA & H. OKAYAMA: Dnacin A1 and dnacin B1 are antitumor antibiotics that inhibit cdc25B phosphatase activity. Biochem. Pharm. 48: 2139~2141, 1994
13) HAM, S. W.; J. PARK, S.-J. LEE, W. KIM, K. KANG & K. H. CHOI: Naphthoquinone analogs as inactivators of cdc25 phosphatase. Bioorg. Med. Chem. Lett. 8: 2507~2510, 1998
14) OTANI, T.; Y. SUGIMOTO, Y. AOYAGI, Y. IGARASHI, T. FURUMAI, N. SAITO, Y. YMADA, T. ASAO & T. OKI: New cdc25B tyrosine phosphatase inhibitors, nocardiones A and B, produced by *Nocardia* sp. TP-A0248. Taxonomy, fermentation isolation structural elucidation and biological properties. J. Antibiotics 53: 337~344, 2000
15) PENG, H.; W. XIE, D.-I. KIM, L. H. ZALKOW, G. POWIS, D. M. OTTERNESS & R. T. ABRAHAM: Steroidal derived acids as inhibitors of human cdc25A protein phosphatase. Bioorg. Med. Chem. 8: 299~306, 2000
16) KOHNO, J.; N. HIRANO, K. SUGAWARA, M. NISHIO, T. HASHIYAMA, N. NAKANISHI & S. KOMATSUBARA: Structure of TMC-69, a new antitumor antibiotic from *Chrysosporium* sp. TC 1068. Tetrahedron 57: 1731~1735, 2001
17) Japan Color Enterprise Co.: Munsell Color System, Tokyo, 1977
18) ISHIYAMA, M.; H. TOMINAGA, M. SHIGA, K. SASAMOTO, Y. OHKURA & K. UENO: A combined assay of cell viability and *in vitro* cytotoxicity with a highly water-soluble tetrazolium salt, neutral red and crystal violet. Biol. Pharm. Bull. 19: 1518~1520, 1996
19) SIGLER, L. & J. W. CARMICHAEL: Taxonomy of *Malbranchea* and some other hyphomycetes with arthroconidia. Mycotaxon 4: 349~488, 1976
20) PARK, J. K.; K. SAKAI, H. OGAWA, K. HASUMI & A. ENDO: Chrysosporin, a new inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase produced by *Chrysosporium pannorum*. J. Antibiotics 46: 1170~1172, 1993
21) OGAWA, H.; K. HASUMI, K. SAKAI, S. MURAKAWA & A. ENDO: Pannorin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor produced by *Chrysosporium pannorum*. J. Antibiotics 44: 762~767, 1991
22) VAN DER PYL, D.; P. CANS, J. J. DEBERNARD, F. HERMAN, Y. LELIEVRE, L. TAHRAOUI, M. VUILHORGNE & J. LEBOUL:

RPR113228, a novel farnesyl-protein transferase inhibitor produced by *Chrysosporium lobatum*. J. Antibiotics 48: 736~737, 1995

23) FREDENHAGEN, A.; F. PETERSEN, M. TINTELNOT-BLOMLEY, J. ROSEL, H. METT & P. HUG: Semicochliodinol A and B: Inhibitors of HIV-1 protease and EGF-R protein tyrosine kinase related to asterriquinones produced by the fungus *Chrysosporium merdarium*. J. Antibiotics 50: 395~401, 1997

24) TANAKA, Y.; K. MATSUZAKI, C.-L. ZHONG, H. YOSHIDA, T. KAWAKUBO, R. MASUMA, H. TANAKA & S. OMURA: Dechlorogeodin and its new dihydro derivatives, fungal metabolites with herbicidal activity. J. Antibiotics 49: 1056~1059, 1996

25) HAYAKAWA, Y.; H. ADACHI, J. W. KIM, K. SHIN-YA & H. SETO: Adenopeptin, a new apoptosis inducer in transformed cells from *Chrysosporium* sp. Tetrahedron 54: 15871~15878, 1998

26) GORDON, J.: Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. *In* Methods. Enzymol. *Ed.*, HUNTER, T. *et al.*, 201, 477~482, Academic Press San Diego, California, 1991