Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1273–1276

BIOORGANIC &
MEDICINAL
CHEMISTRY
LETTERS

Synthesis and Antifungal Activities of Novel 1,3-β-D-Glucan Synthase Inhibitors. Part 2

Kazunao Masubuchi,[a] Takehiro Okada,[a] Masami Kohchi,[a] Takeshi Murata,[a] Masao Tsukazaki,[a] Osamu Kondoh,[b] Toshikazu Yamazaki,[b] Yasuko Satoh,[b] Yoshinori Ono,[b] Toshiyuki Tsukaguchi,[b] Kazuko Kobayashi,[c] Naomi Ono,[b] Tomoaki Inoue,[c] Ikuo Horii[c] and Nobuo Shimma[a,]*

[a]*Department of Chemistry, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*
[b]*Department of Mycology, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*
[c]*Department of Preclinical Science, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan*

Received 17 January 2001; accepted 9 March 2001

Abstract—Highly potent 1,3-β-D-glucan synthase inhibitors, **7b**, **10a**, **10b** and **12**, have been identified by the chemical modification of the ornithine residue of a fungicidal macrocyclic lipopeptidolactone, RO-09-3655 (**1**), isolated from the cultured broth of *Deuteromycotinia* spp. These compounds showed stronger antifungal activity against systemic candidiasis as well as pulmonary aspergillosis in mice, and less hepatotoxicity as compared with **1**. © 2001 Elsevier Science Ltd. All rights reserved.

Introduction

The medical need for safe and effective systemic antifungal agents with novel modes of action has been intensified due to rapid growth of the immunocompromised patient population.[1] Azole antifungal agents are widely used for the treatment of systemic mycoses. However, long-term prophylactic use of azole antifungal agents generated azole resistant *Candida* spp. due to their fungistatic action. Therefore, fungicidal agents are particularly important for the treatment of severe systemic mycoces.

We isolated novel fungicidal macrocyclic lipopeptidolactones, **2** and **3**, from the cultured broth of *Deuteromycotinia* spp. as 1,3-β-D-glucan synthase (β-GS) inhibitors,[2] together with a known inhibitor FR901469[3] (**1** = RO-09-3655). In 1999, Barrett et al. reported the L-ornithine derivative (**5a**)[4] to have a reduced hemolytic effect and a slightly improved antifungal activity in a murine systemic candidiasis model as compared with **1**. We independently found the derivatives **4** and **5b** (D-ornithine derivative), which showed 4-fold antifungal activity against systemic candidiasis in mice and one-third the hepatotoxicity in vitro, respectively, as compared with **1**.[5,6]

These derivatives, however, still have insufficient efficacy against pulmonary aspergillosis, which is life threatening and one of the most difficult fungal infections to treat. In addition, further reduction of their effects on hepatocytes is required. Thus, we conducted chemical modification of **4** and **5b** to obtain safer and more effective antifungal agents.

In this paper, we describe the synthesis and biological activity of new RO-09-3655 (**1**) analogues that showed improved in vivo efficacy against pulmonary aspergillosis and reduced hepatotoxicity.

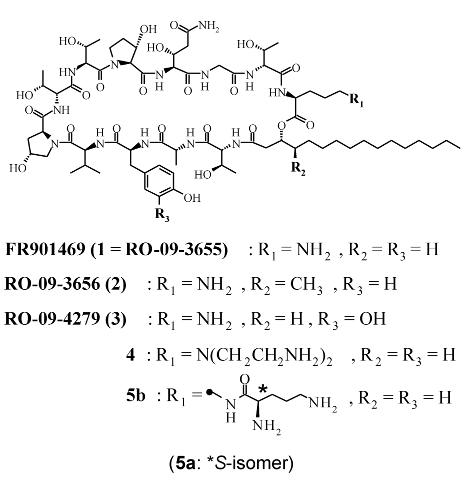
Chemistry

As reported in a previous paper,[5] we found a tendency that the introduction of additional basic amino functional groups at the ornithine residue (ornithine-1 moiety) of **1** improved 1,3-β-D-glucan synthase inhibitory activity and in vivo antifungal activities in the systemic candidiasis model and/or reduced hepatotoxicity (inclusion body formation).

---

*Corresponding author. Tel.: +81-467-47-2280; fax: +81-467-45-6824; e-mail: nobuo.shimma@roche.com

PII: S0960-894X(01)00178-0



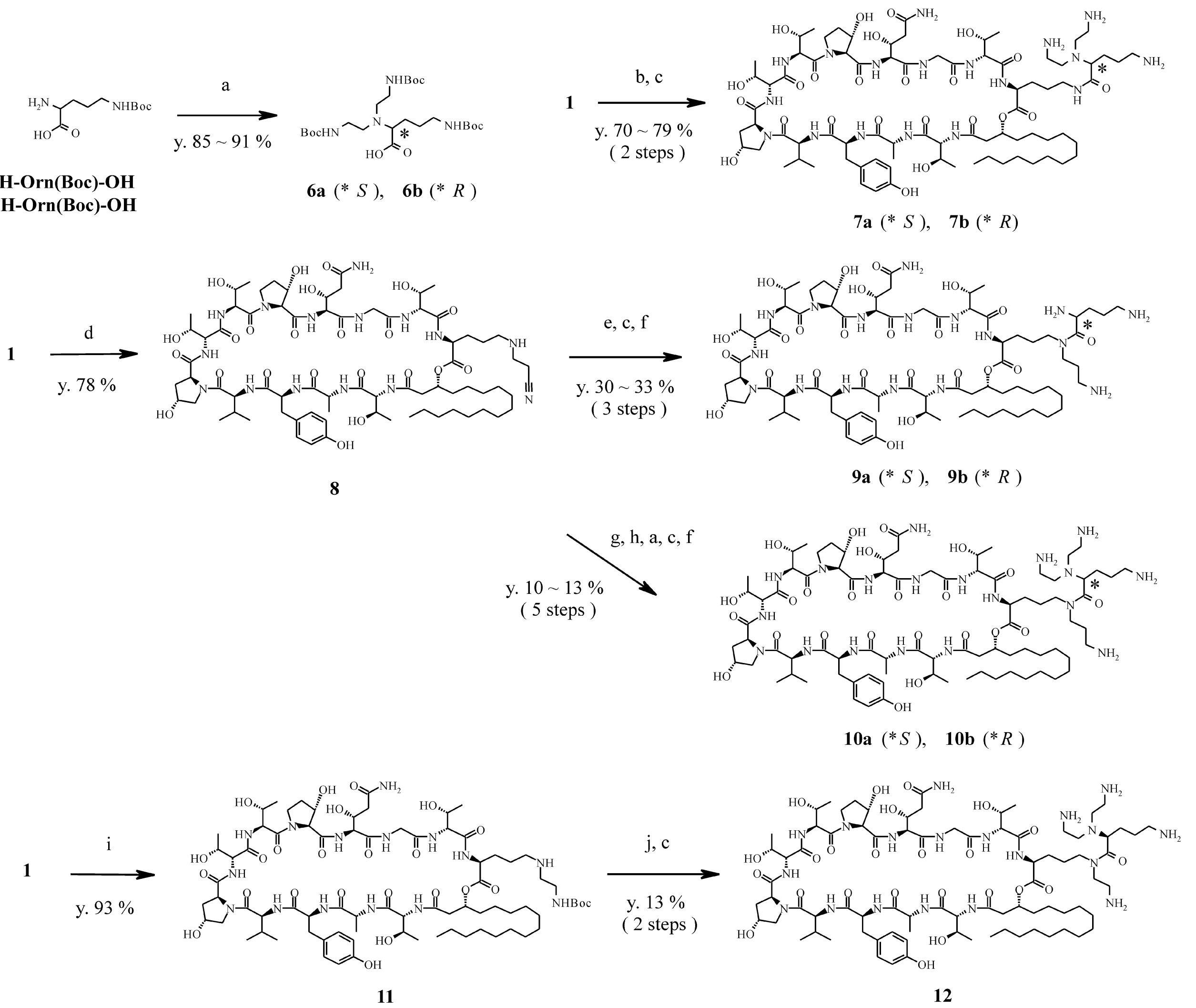
FR901469 (1 = RO-09-3655) : $R_1 = NH_2$, $R_2 = R_3 = H$
RO-09-3656 (2) : $R_1 = NH_2$, $R_2 = CH_3$, $R_3 = H$
RO-09-4279 (3) : $R_1 = NH_2$, $R_2 = H$, $R_3 = OH$
4 : $R_1 = N(CH_2CH_2NH_2)_2$, $R_2 = R_3 = H$
5b : $R_1 =$, $R_2 = R_3 = H$
(**5a**: *S-isomer)

Figure 1.

Therefore, we synthesized a series of new analogues that have hybrid structures of **4** and **5b**. The general synthetic procedures of the representative derivatives of **1** are outlined in Scheme 1.

The *N*-Boc-protected ornithine derivatives **6a** and **6b** were prepared from L- or D-H-Orn(Boc)-OH by reductive *N*-dialkylation with *N*-Boc-aminoacetaldehyde and $NaBH_3CN$.

The derivatives **7a** and **7b** were synthesized by acylation of **1** with **6a** or **6b** by BOP and HOBt in DMF, followed by removal of the Boc groups with trifluoroacetic acid. The derivatives **9a** and **9b** were obtained from **1** in four steps: (i) *N*-monoalkylation of the primary amine of **1** with acrylonitrile in the presence of *N*-ethyldiisopropylamine in EtOH; (ii) acylation of the nitrile **8** with L- or D-Boc-Orn(Boc)-OH by BOP and HOBt in DMF; (iii) removal of the Boc groups with trifluoroacetic acid; and



Scheme 1. General synthetic route of the representative derivatives of **1**. (a) *N*-Boc-aminoacetaldehyde, $NaBH_3CN$, AcOH, MeOH, rt; (b) **6**, BOP, HOBt, *N*-ethyldiisopropylamine, DMF; (c) CF_3CO_2H, 0 °C; (d) acrylonitrile, *N*-ethyldiisopropylamine, EtOH, rt; (e) Boc-Orn(Boc)-OH, BOP, HOBt, *N*-ethyldiisopropylamine, DMF; (f) H_2, 10% Pd–C, dioxane–H_2O; (g) Fmoc-Orn(Boc)-OH, BOP, HOBt, *N*-ethyldiisopropylamine, DMF; (h) 20% piperidine in DMF, rt; (i) *N*-Boc-aminoacetaldehyde (1.5 equiv), $NaBH_3CN$, AcOH, MeOH, CH_2Cl_2, rt; (j) **6a**, HATU, HOAt, *N*-ethyldiisopropylamine, DMF, rt.

(iv) reduction of the nitrile group of the resulting diamine intermediate by catalytic hydrogenation over 10% Pd–C in a 1:1 mixture of dioxane/water.

The derivatives **10a** and **10b** were obtained from **8** in five steps: (i) acylation of **8** with L- or D-Fmoc-Orn(-Boc)-OH by BOP and HOBt in DMF; (ii) removal of the Fmoc group with 20% piperidine in DMF; (iii) reductive *N*-dialkylation of the α-amino group of an additionally introduced ornithine moiety (ornithine-2 moiety) with *N*-Boc-aminoacetaldehyde and $NaBH_3CN$; (iv) removal of three Boc groups with trifluoroacetic acid; and (v) reduction of the nitrile group by catalytic hydrogenation over 10% Pd–C in a 1:1 mixture of dioxane/water.

The derivative **12** was synthesized from **1** in three steps: (i) reductive *N*-monoalkylation of **1** with 1.5 equivalents of *N*-Boc-aminoacetaldehyde and $NaBH_3CN$; (ii) acylation of **11** with **6a** by HATU and HOAt in DMF;[7] and (iii) removal of the Boc groups with trifluoroacetic acid.

Results and Discussion

The results of the biological evaluation of the new analogues are summarized in Table 1. Systemic candidiasis due to *C. albicans* CY1002 in normal mice was treated with a single iv dose, and the ED_{50} values were measured on day 7. For pulmonary aspergillosis due to *Aspergillus fumigatus* CF1083 in immunosuppressed mice with cortisone acetate, the test compound was given once daily for 4 days by iv administration, and the ED_{50} values were measured on day 14.[8] The evaluation of hepatotoxicity of the new analogues was performed by the inclusion-body formation test in hepatocytes observed at 1, 3, 10, 30 and 100 µg/mL with an optical microscope.[5]

Significant improvement in 1,3-β-D-glucan synthase inhibitory activity was achieved in **10a**, **10b** and **12** in which amino-alkyl groups were introduced at the δ-amide nitrogen of the ornithine-1 moiety and the α-amino group of the ornithine residue-2 in **5a** and **5b**. The enzyme inhibitory activity of **12** ($IC_{50} = 0.20$ nM) was 42 times higher than that of **1** ($IC_{50} = 8.5$ nM).

All new derivatives having additional amino functional groups (**7a**, **7b**, **9a**, **9b**, **10a**, **10b** and **12**) showed almost no serum effect on antifungal activities against *C. albicans* CY1002 measured in the presence of calf serum. This reduced serum effect reflected a good in vivo efficacy in the systemic candidiasis model as we expected.[5] Among these derivatives, compound **7b** having α-*N,N*-bis(aminoethyl)-D-ornithine moiety was 7 times more active than **1** in the same infection model.

In the pulmonary aspergillosis model in mice, the new derivatives (**7b**, **9a**, **9b**, **10a**, **10b** and **12**) also clearly showed better antifungal activities than **1** and MK-0991.[9,10] The efficacies of **9a** and **12** were greater than 3 times those of **1** and MK-0991. Survival curves for compound **12** and MK-0991 are illustrated in Figure 2. Compound **12** prolonged the survival of infected mice at doses of 1.7 mg/kg or higher. All of the infected mice treated with 15 mg/kg of **12** could survive at day 14 after infection.

Another important improvement in the new analogues is the reduction of hepatotoxicity in vitro. The compounds **7b**, **10a**, **10b** and **12** having the α-*N,N*-bis(aminoethyl)-ornithine moiety showed 1/10th and 1/100th the hepatotoxicity (inclusion-body formation assay) of **1** and LY303366,[11] respectively (Table 1).

The antifungal spectrum of the representative compounds **7b**, **10a**, **10b** and **12** are shown in Table 2. These compounds were active against *C. albicans* including a

Table 1. Antifungal activities and in vitro hepato-toxicity of the RO-09-3655 (**1**) derivatives

Compound	Enzyme inhibition IC_{50} (nM)	In vitro antifungal activity[a] IC_{50} (µg/mL)		In vivo efficacy ED_{50} (mg/kg)		Inclusion-body formation
	β-GS	*C. albicans* CY1002	*C. albicans* CY1002 (80% serum)	Systemic candidiasis[b] (day 7)	Pulmonary aspergillosis[c] (day 14)	Observed at (µg/mL)
7a	0.63	0.31	0.31	0.13	11.6	30
7b	0.9	0.37	0.55	0.33	8.7	100
9a	0.83	0.37	0.27	0.05	5.2	30
9b	0.89	0.47	0.27	0.07	8.7	30
10a	0.25	0.39	0.26	0.12	5.8	100
10b	0.28	0.45	0.31	0.08	8.6	100
12	0.20	0.31	0.24	0.08	5.2	100
FR901469 (1)	8.5	0.07	0.54	0.22	> 15	10
4	1.4	0.1	0.42	0.05	13.3	10
5b	3.6	0.12	0.33	0.1	14.4	30
LY303366	18	0.014	0.55	0.22	N.D.[d]	1
MK-0991	1.3	0.29	0.6	0.03	> 15	100

[a] NCCLS M27-A microdilution method using modified media: Yeast Nitrogen Base (YNB) supplemented with 1% dextrose and 0.25% K_2HPO_4. IC_{50} values were calculated as the concentration of drug yielding 50% OD (50% inhibition) of control growth.

[b] Single iv treatment in ICR mice, infection: *C. albicans* CY1002 5×10^6 cells/mouse.

[c] Once daily iv treatment for 4 days in immunosuppressed mice, infection: *A. fumigatus* CF1083 1×10^4 conidia/g.

[d] N.D., not done.

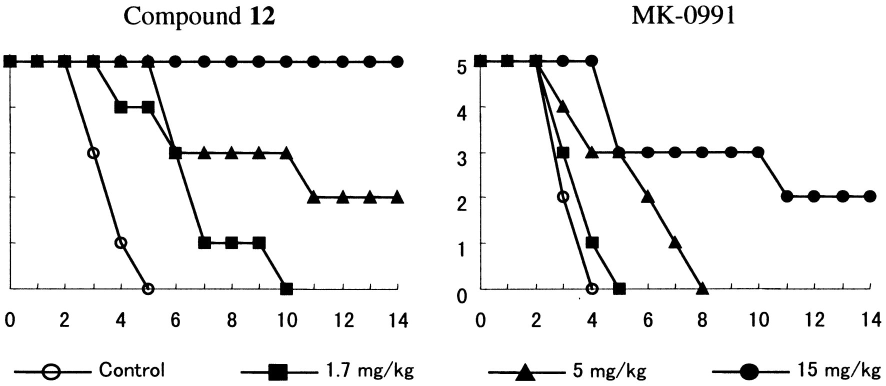


Figure 2. Efficacy of compound **12** and MK-0991 in a pulmonary *A. fumigatus* CF1083 infection (1×10^4 conidia/g) in immunosuppressed mice with cortisone acetate. Once daily iv treatment for 4 days in immunosuppressed mice (vehicle; 5% DMSO, saline).

Table 2. Antifungal spectrum of the representative RO-09-3655 (**1**) derivatives (MIC[a] = μg/mL)

		7b	**10a**	**10b**	**12**	**MK-0991**
C. albicans	ATCC48130	0.31	0.37	0.41	0.37	0.10
	KB[b]	0.68	0.31	0.35	0.35	0.088
C. glabrata	ATCC2001	1.4	1.2	1.0	1.3	0.088
C. tropicalis	ATCC13803	0.35	0.56	0.63	0.62	0.11
C. krusei	ATCC20579	0.54	0.28	0.31	0.28	0.20
A. fumigatus	MTU06002	0.67	0.58	0.37	0.34	0.09
S. prolificans	ATCC20054	N.D.[c]	0.37	0.38	2.2	8.8
S. apisopermum	IFM40731	N.D.	0.37	0.35	0.35	12

[a] NCCLS M27-A microdilution method: MIC values were calculated as the concentration of drug yeilding 20% OD (=80% inhibition) of control growth. Medium: RPMI1640 + 0.165M MOPS + 2% glucose (pH7.0) for yeast, RPMI1640 + 0.165M MOPS + 2% glucose + 0.2% LMPA (pH 7.0) for filamentous fungi.

[b] Fluconazole resistant strain.

[c] N.D., not done.

fluconazole resistant strain, non-*albicans Candida* such as *C. tropicalis* and *C. krusei*, and against filamentous fungi such as *Aspergillus fumigatus*. Interestingly, they were also active against *Scedosporium* spp. that are resistant to an echinocandin type 1,3-β-D-glucan synthase inhibitor, MK-0991.

Thus, as a whole, compounds **7b**, **10a**, **10b** and **12** exhibited a well-balanced in vivo efficacy and reduced toxicity in hepatocytes.

In summary, we identified highly potent and safe 1,3-β-D-glucan synthase inhibitors, **7b**, **10a**, **10b** and **12**, that showed improved in vivo antifungal activity against both systemic candidiasis and pulmonary aspergillosis, and reduction of the hepatotoxicity as compared with the lead compound (**1**). The in vivo antifungal activities of these compounds in a pulmonary aspergillosis model in mice were clearly better than those of MK-0991 and **1**. Thus, these compounds will be evaluated further for clinical development as systemic antifungal agents.

References and Notes

1. Willis, E. A.; Redinbo, M. R.; Perfect, J. R.; Poeta, M. D. *Emerging Therapeutic Agents* **2000**, *4*, 265.
2. The isolation and structure elucidation of compounds **1**–**3** will be reported elsewhere.
3. (a) Fujie, A.; Iwamoto, T.; Muramatsu, T.; Okudaira, T.; Nitta, K.; Nakanishi, T.; Sakamoto, K.; Hori, Y.; Hino, M.; Hashimoto, S.; Okuhara, M. *J. Antibiot.* **2000**, *53*, 912. (b) Fujie, A.; Iwamoto, T.; Muramatsu, T.; Okudaira, T.; Sato, I.; Furuta, T.; Tsurumi, Y.; Hori, Y.; Hino, M.; Hashimoto, S.; Okuhara, M. *J. Antibiot.* **2000**, *53*, 920.
4. Barrett, D.; Tanaka, A.; Harada, K.; Ohki, H.; Kawabata, K.; Takasugi, H.; Watabe, E.; Maki, K.; Ikeda, F.; Sakane, K. *Abstracts of the 19th Symposium on Medicinal Chemistry, 8th Annual Meeting of Division of Medicinal Chemistry*, Abstract No. 2P-14, Nov 11–14, 1999, Tokyo, Japan.
5. Masubuchi, K.; Okada, T.; Kohchi, M.; Sakaitani, M.; Mizuguchi, E.; Shirai, H.; Aoki, M.; Watanabe, T.; Kondoh, O.; Yamazaki, T.; Satoh, Y.; Kobayashi, K.; Inoue, T.; Horii, I.; Shimma, N. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 395.
6. A histopathological examination of **1** after a 2-week treatment revealed inclusion-body formation and single cell necrosis in hepatocytes.
7. Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561.
8. Irifune, K.; Shimoguchi, K.; Tateda, K.; Yamaguchi, K.; Hara, K. *Igaku no Ayumi* **1990**, *152*, 71.
9. For in vitro antifungal activity of MK-0991: Bartizal, K.; Gill, C. J.; Abruzzo, G. K.; Flattery, A. M.; Kong, L.; Scott, P. M.; Smith, J. G.; Leighton, C. E.; Bouffard, A.; Dropinski, J. F.; Balkovec, J. *Antimicrob. Agents Chemother.* **1997**, *41*, 2326.
10. For in vivo antifungal activity of MK-0991 in disseminated aspergillosis: Abruzzo, G. K.; Flattery, A. M.; Gill, C. J.; Kong, L.; Smith, J. G.; Pikounis, V. B.; Balkovec, J. M.; Bouffard, A. F.; Dropinski, J. F.; Rosen, H.; Kropp, H.; Bartizal, K. *Antimicrob. Agents Chemother.* **1997**, *41*, 2333.
11. Debono, M.; Turner, W. W.; LaGrandeur, L.; Burkhardt, F. J.; Nissen, J. S.; Nichols, K. K.; Rodriguez, M. J.; Zweifel, M. J.; Zeckner, D. J.; Gordee, R. S.; Tang, J.; Parr, T. R., Jr. *J. Med. Chem.* **1995**, *38*, 3271.