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Synthesis and antifungal activity of ASP9726, a novel echinocandin with potent *Aspergillus* hyphal growth inhibition

Hiroshi Morikawa [a,*], Masaki Tomishima [a], Natsuko Kayakiri [a], Takanobu Araki [a], David Barrett [a], Souichirou Akamatsu [b], Satoru Matsumoto [b], Satoko Uchida [b], Toru Nakai [b], Shinobu Takeda [b], Katsuyuki Maki [b]

[a] *Chemistry Research Laboratories, Astellas Pharma. Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan*
[b] *Pharmacology Research Laboratories, Astellas Pharma. Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan*

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ABSTRACT

The synthesis and antifungal activity of ASP9726, a novel echinocandin with potent *Aspergillus* hyphal growth inhibition and significantly improved MIC against *Candida parapsilosis* and echinocandin resistant-*Candida* is described.

In recent years, with the growing number of immunosuppressed patients (transplants, cancer, AIDS, etc.), invasive mycoses have become an increasingly serious problem.[1] Especially, deep seated mycosis caused by *Aspergillus spp.* and *Candida spp.* is highly lethal and immediate treatment with antifungal agents is required.[2] Only a limited number of antifungal substances are currently available at the market as azoles, polyenes, and candins. However, the treatment of invasive fungal diseases still remains unsatisfied as mortality rate, even under treatment, is still unacceptable high.[3] For example, azoles and polyenes often have side effects and/or drug–drug interactions, and some organisms are resistant to these antifungals. Furthermore, effective treatment of aspergillosis is still a major challenge.[4] In addition, mycoses due to non-albicans *Candida* and development of fungi resistant to newer drugs are also of concern.[5]

Our research in this area started with a search for antifungal natural products.[6,7] In particular, we focused on 1,3-β-glucan synthesis as an attractive target, since 1,3-β-glucan is a primary component of the fungal cell wall with no counterpart in mammalian cells. In earlier publications from our laboratories, we described the chemical modification of the side chain of a natural echinocandin-type 1,3-β-glucan synthase inhibitor FR901379,[7,8] and we identified a number of echinocandin derivatives with improved MIC/MEC against *Aspergillus spp.* and *Candida spp.*, efforts that culminated in the discovery of micafungin (**2**) (Fig. 1).[9] Subsequently, our efforts were directed towards the identification of next generation echinocandins with superior antifungal properties. As part of these efforts, we found that modification of the lipophilic side chain, the primary amide group of the glutamate side chain, and the homotyrosine sulfate ester group, lead to enhancement of the antifungal potency. Additionally, to our surprise, we also found that the maximum inhibitory effect on *Aspergillus spp.* hyphae (E_{max}) differs between echinocandin structural types, and that agents with a strong E_{max} potentially have significantly improved in vivo antifungal activity against *Aspergillus spp*. as compared with existing echinocandins. E_{max} was assessed by a scoring system (1–6; 6 is the strongest effect) based on microcolony size and growth of hyphal tip in human serum at 4-fold MEC by microscopy.[10]

Extensive synthetic modification and screening by E_{max} led to the discovery of ASP9726 (**1**), as a novel echinocandin with potent *Aspergillus* hyphal growth inhibition and significantly improved MIC against *Candida parapsilosis* (*C. parapsilosis*) and echinocandin-resistant-*Candida glabrata* (echinocandin-resistant-*C. glabrata*), as compared with caspofungin. In this communication, we wish to report the synthesis and antifungal activity of this new agent.

ASP9726 (**1**) was synthesized as shown in Figure 2. The de-acylated hexapeptide nucleus **3** was prepared by enzymatic

* Corresponding author.
E-mail address: hiroshi.morikawa@astellas.com (H. Morikawa).

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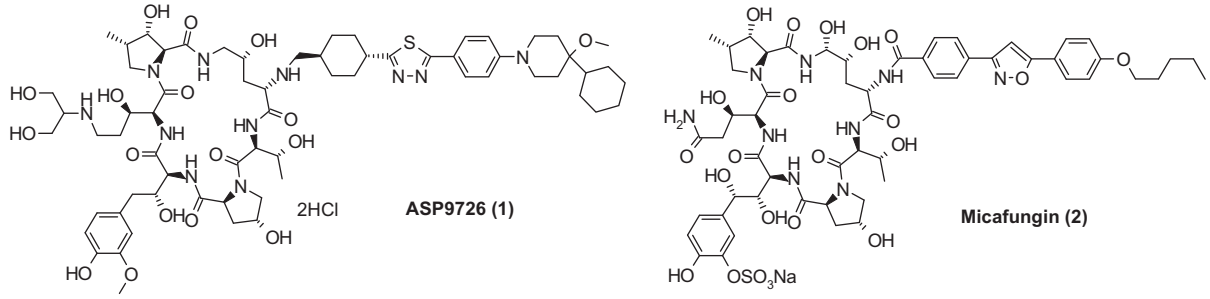

Figure 1. Structure of ASP9276 (**1**) and Micafungin (**2**).

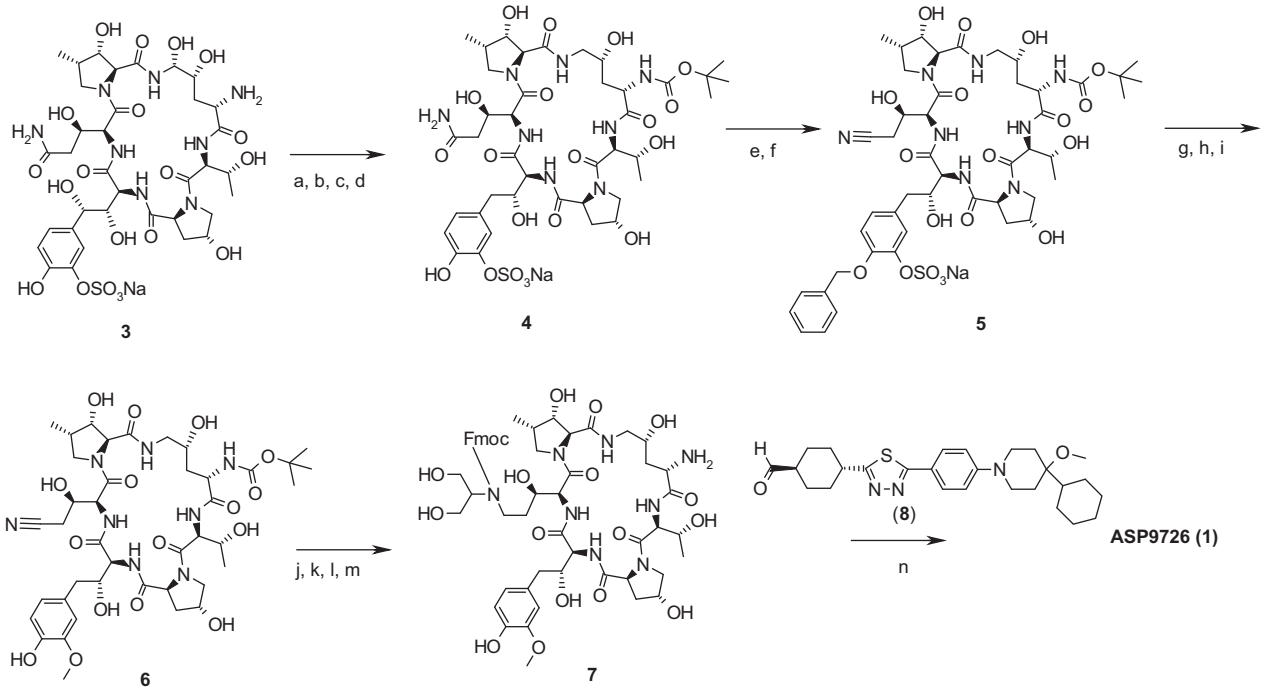

Figure 2. Synthesis of the modified hexapeptide core nucleus **7** and APS9726 (**1**). Reagents and conditions:[13] (a) benzyl chloroformate, THF, pH6.86 standard buffer solution, 68%; (b) Et_3SiH, TFA, CH_2Cl_2, 45%; (c) H_2, Pd/C, H_2O, 73%; (d) $(Boc)_2O$, NaOH, H_2O, 1,4-dioxane, 88%; (e) BnBr, LiOH–H_2O, DMF, 85%; (f) MsCl, $NaHCO_3$, *i*-Pr_2NEt, zeolite, DMF, 41%; (g) 10% HCl–MeOH, MeOH; 86%; (h) MeI, LiOH·H_2O, DMF, 76%; (i) H_2, Pd/C, MeOH, quant.; (j) $NaBH_4$, $CoCl_2$–$6H_2O$, MeOH, H_2O, 85%; (k) $NaBH_3CN$, dihydroxyacetone, AcOH, MeOH, 73%; (l) Fmoc-Cl, *i*-Pr_2NEt, DMF 46%; (m) TFA, Et_3SiH, CH_2Cl_2, 83%; (n) **8**, $NaBH_3CN$, AcOH, MeOH, DMF, $CHCl_3$, then piperidine; 67%.

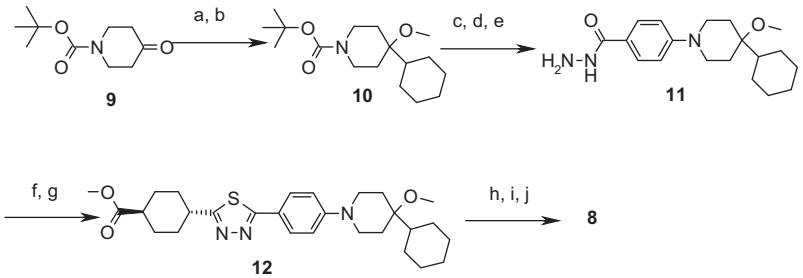

Figure 3. Synthesis of the echinocandin side chain 8. Reagents and conditions: (a) chloro(cyclohexyl)magnesium, $CeCl_3$, THF, 95%; (b) MeI, NaH, DMF, 89%; (c) TFA, anisole, CH_2Cl_2; (d) ethyl 4-fluorobenzoate, K_2CO_3, DMSO, 76% (2 steps); (e) hydrazine monohydrate, EtOH, THF, 97%; (f) *trans*-4-(methoxycarbonyl)cyclohexanecarboxylic acid, EDC, HOBt, Et_3N, DMF, quant.; (g) P_2S_5, THF, 81%; (h) KOH, THF, EtOH, 86%; (i) *N,O*-dimethylhydroxylamine hydrochloride, HBTU, *i*-Pr_2NEt, DMF, 88%; (j) $LiAlH_4$, THF, 96%.

deacylation of the natural product FR901379.[11] The primary amino group of **3** was protected with a carboxybenzyloxy moiety, the aminal hydroxy group and homotyrosine benzylic hydroxyl group were reduced with TFA-Et_3SiH, followed by hydrogenolysis to remove the carbobenzoxy group (cbz), followed by reprotection as a *tert*-butoxycarbonyl group (*t*-Boc) moiety, to afford compound **4** in 68%, 45%, 73%, and 88% yields, respectively. Protection of the homotyrosine phenol group as a benzyl ether, followed by dehydration of the primary amide group of the glutamate side chain with MsCl led to the nitrile **5** in 85%, and 41% yields, respectively.

Table 1

Compound	E_{max} (score) *A. fumigatus* #20024	MIC/MEC [μg/ml] (in human serum)			
		A. fumigatus #20024	*C. albicans* #20015	*C. parapsilosis* #20009	Echinocandin-resistant-*C. glabrata* FP2307
ASP9726 (**1**)	5.5	0.25	0.25	4	2
Caspofungin	1	0.5	0.25	64	>64

Table 2
Survival efficacy of ASP9726 (**1**) against mouse invasive pulmonary aspergillosis (IPA) model infected with *A. fumigatus* #20030, day 11[17]

Compound	Survival rate, day 11	
	1.5 mg/kg	3 mg/kg
ASP9726 (**1**)	40%*	70%*
Caspofungin	10%	20%

* Significant survival advantage was shown with log-rank test.

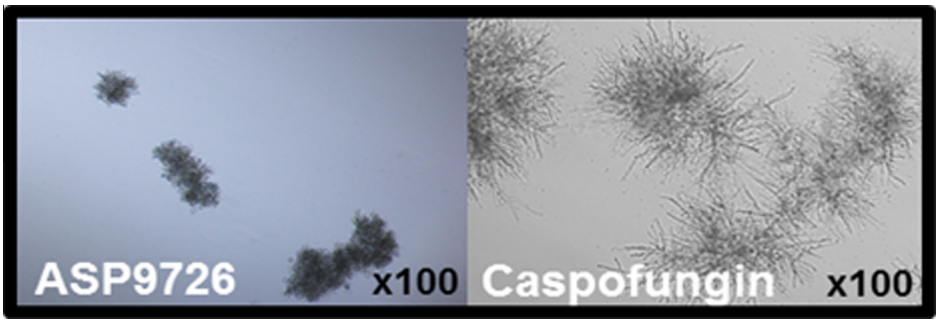

Figure 4. Microphotograph of *A. fumigatus* #20024 microcolonies treated with ASP9726 (**1**) and caspofungin in human serum.[18]

Subsequently, the sulfate ester group of **5** was removed, even in the presence of the *t*-Boc amine protecting group, by treatment with hydrogen chloride gas in MeOH and the resulting phenol group was methylated and the benzyl ether group was removed by hydrogenation, to afford nitrile **6** in 86%, 76%, and quantitative yields, respectively. The nitrile group of **6** was reduced with $NaBH_4$ and $CoCl_2$–$6H_2O$ to the primary amine, followed by reductive alkylation with dihydroxyacetone using $NaBH_3CN$, protection of the secondary amino group as a 9-fluorenylmethyloxycarbonyl group (Fmoc) and removal of the *t*-Boc group, to afford key skeleton **7** in 85%, 73%, 46%, and 83% yields, respectively. Reductive amination of **7** with aldehydye **8** using $NaBH_3CN$, followed by removal of the Fmoc protecting group with piperidine afforded ASP9726 dihydrochloride as an amorphous powder after lyophylization in 67% yield.[12]

Key aldehyde **8**, the side chain of ASP9726 (**1**), was synthesized as shown in Figure 3. Commercially available piperidinone **9** was treated with the organocerium reagent derived from cyclohexylmagesium chloride and $CeCl_3$ in THF, followed by methylation of the hydroxy group with MeI to yield **10** in 95%, and 89% yields, respectively. Removal of the *t*-Boc moiety of **10** with TFA, followed by reaction of the resulting amine with ethyl 4-fluorobenzoate and conversion of the ester moiety to acyl hydrazide with hydrazine, led to **11** in 76% in two steps, and 97% yields, respectively. Coupling of **11** with *trans*-4-(methoxycarbonyl)cyclohexanecarboxylic acid, formation of the thiadiazole ring system by reaction with P_2S_5, provided methyl ester **12** in quantitative yield, and 81% yields, respectively. Hydrolytic cleavage of **12**, conversion to the Weinreb amide, and reduction with $LiAlH_4$ led to key aldehyde **8** in 86%, 88%, and 96% yields, respectively.

In vitro antifungal activity of ASP9726 (**1**) and caspofungin against *Aspergillus fumigatus* (*A. fumigatus*), *Candida albicans* (*C. albicans*), *C. parapsilosis* and echinocandin-resistant-*C. glabrata* are shown in Table 1.[14] ASP9726 (**1**) displayed greatly superior E_{max} against *A. fumigatus* as compared with caspofungin, independent of MEC. In vivo efficacy of ASP9726 (**1**) and caspofungin in an aspergillosis animal model are shown in Table 2. This model is very severe, and as is clearly shown in Table 2, survival with caspofungin is very low (20% at 3 mpk), whereas for ASP9726 it was 70%. ASP9726 (**1**) displayed superior in vivo efficacy by inhibition of hyphal growth as compared with caspofungin.[15] ASP9726 (**1**) exhibited potent MIC/MEC in human serum against *A. fumigatus* and *C. albicans*, and was also effective against *C. parapsilosis* and echinocandin-resistant-*C. glabrata*.[16]

The potent inhibitory effort of ASP9726 (**1**) on hyphal elongation of *A. fumigatus* in comparison to caspofungin is shown in Figure 4. Microcolonies produced by exposure to ASP9726 (**1**) had severely stunted hypha, and were small, rounded and compact, whereas those under caspofungin exposure were large and had hyphal elongation in all directions.

In this communication, we have reported the discovery of ASP9726 (**1**), a novel potent echinocandin, discovered by extensive synthetic modification of a natural product FR901379, using a novel screening efficacy endpoint, E_{max}. Potent *Aspergillus* hyphal growth inhibition and significantly improved MIC against *C. parapsilosis* and echinocandin-resistant-*C. glabrata*[19] make this compound a suitable candidate for further development as a novel echinocandin. Future publications will report the detailed structure activity relationships of this novel class of echinocandin antifungal agents, as well as detailed investigations of the in vivo antifungal efficacy of ASP9726 (**1**).

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12. Characterization data for compound **1**. NMR (DMSO-d_6 + D_2O, δ): 0.89–1.27 (14H, m), 1.30–2.01 (20H, m), 2.07–2.18 (3H, m), 2.20–2.94 (1H, m), 2.32–2.49 (3H, m), 2.54–2.63 (1H, m), 2.66–2.18 (1H, m), 0.89–1.27 (14H, m), 2.90–3.07 (5H, m), 3.09 (3H, s), 3.10–3.15 (1H, m), 3.22 (1H, t, J = 8.0 Hz), 3.52–3.71 (8H, m), 3.74 (3H, s), 3.82–4.05 (5H, m), 4.16–4.22 (3H, m), 4.36–4.44 (3H, m), 4.78–4.82 (2H, m), 6.55–6.59 (1H, m), 6.67 (2H, d, J = 8Hz), 7.02 (2H, d, J = 8.9Hz), 7.73 (2H, d, J = 8.8Hz), HRMS (ESI) calcd for $C_{66}H_{99}N_{11}O_{17}S$ $(M+H)^+$ 1350.7019, found 1350.7029.
13. Step (a)–(h), (j)–(n) were purified by ODS column chromatography.
14. Susceptibility testing was conducted based on CLSI M27-A3 or M38-A2 standard by human serum as growth medium. Inoculum concentration was 5×10^3 cells/ml. Fungi were treated with ASP9726 (**1**) and caspofungin and incubated at 37 °C under 5% CO_2 for 48 h. E_{max} were scored (1–6) according to the degree of hyphal growth inhibition by microscopy after *A. fumigatus* #20024 was treated with ASP9726 (**1**) and caspofungin at 4-fold MEC and incubated at 37 °C under 5% CO_2 for 48 h.
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17. A mouse IPA model was established by intratracheal inoculation of A. fumigatus #20030 conidia into 4-week-old ICR mice (n = 10) immunosuppressed by cyclophosphamide + hydrocortisone. Intravenous treatment of ASP9726 (**1**), caspofungin was initiated 1 day after infection and continued for 10 days QD, and survivals was monitored for 11 days. Statistical significance in survival at day 11 was calculated using log-rank test.
18. Original magnification of the microphotograph is 100-fold. *A. fumigatus* #20024 was treated with ASP9726 (**1**) and caspofungin at 4-fold MEC and incubated at 37 °C under 5% CO_2 for 48 h.
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