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

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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

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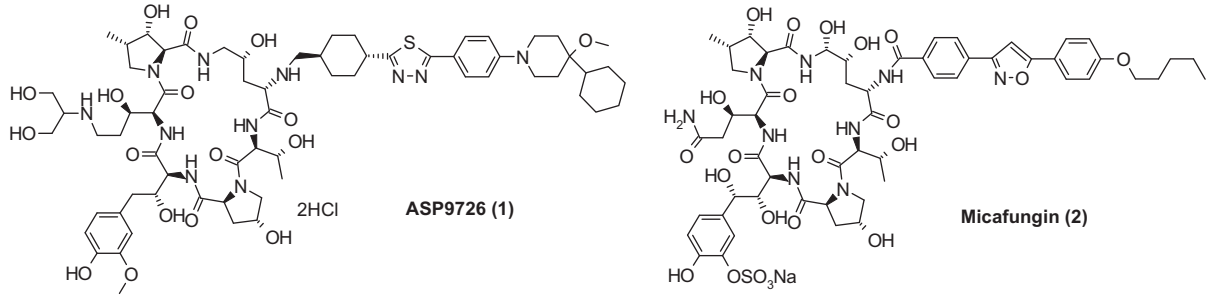

Figure 1. Structure of ASP9276 (**1**) and Micafungin (**2**).

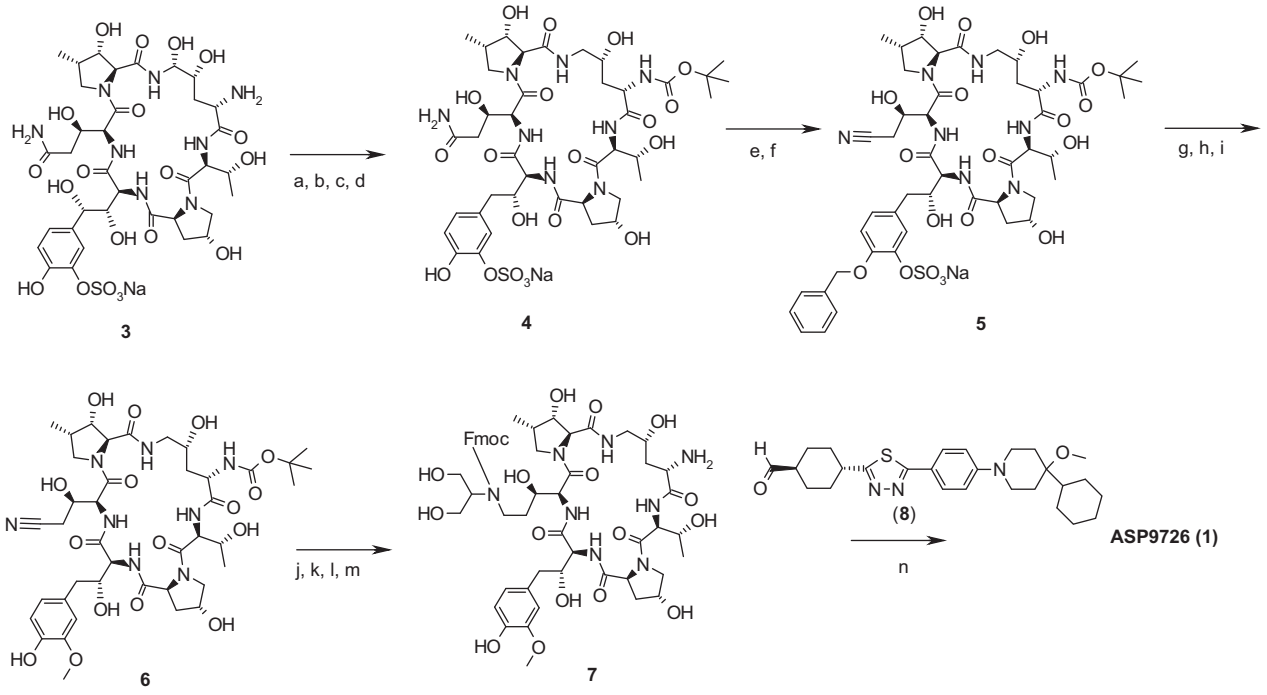

Figure 2. Synthesis of the modified hexapeptide core nucleus **7** and APS9726 (**1**). Reagents and conditions:[13] (a) benzyl chloroformate, THF, pH6.86 standard buffer solution, 68%; (b) Et_3SiH, TFA, CH_2Cl_2, 45%; (c) H_2, Pd/C, H_2O, 73%; (d) $(Boc)_2O$, NaOH, H_2O, 1,4-dioxane, 88%; (e) BnBr, 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%.

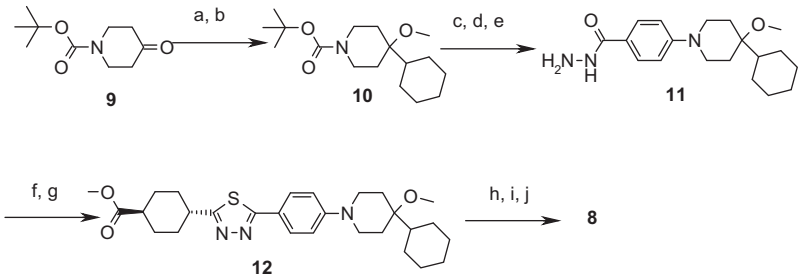

Figure 3. Synthesis of the echinocandin side chain 8. Reagents and conditions: (a) chloro(cyclohexyl)magnesium, $CeCl_3$, THF, 95%; (b) MeI, NaH, DMF, 89%; (c) TFA, anisole, CH_2Cl_2; (d) ethyl 4-fluorobenzoate, K_2CO_3, DMSO, 76% (2 steps); (e) hydrazine monohydrate, EtOH, THF, 97%; (f) *trans*-4-(methoxycarbonyl)cyclohexanecarboxylic 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.