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REVIEW ARTICLE

Micafungin: a sulfated echinocandin

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Micafungin is the second approved antifungal agent in the echinocandin series and is now used worldwide in chemotherapy for life-threatening fungal infections. It is water-soluble and is semi-synthesized from the acylated cyclic hexapeptide FR901379, a natural product from the fungus *Coleophoma empetri* F-11899, through enzymatic deacylation of FR901379, followed by chemical reacylation with the optimized *N*-acyl side chain. The water solubility of micafungin is ascribed to a sulfate moiety in the molecule. This feature differentiates micafungin from other echinocandin members. Micafungin is a potent inhibitor of 1,3-β-glucan synthase, an enzyme necessary for cell-wall synthesis of several fungal pathogens.

INTRODUCTION

Fungal infections cause not only superficial diseases such as athlete's foot and onychomycoses, but also life-threatening diseases. Serious deep-seated fungal infections caused by *Candida* spp., *Aspergillus* spp. and *Cryptococcus neoformans* are a threat to human health. Incidences of these systemic fungal infections have increased significantly over the past few years. The major reasons for this dramatic increase are the extensive use of broad-spectrum antibiotics and the growing number of immunocompromised patients with acquired immunodeficiency syndrome (AIDS), cancer and transplants.[1,2]

In the mid 1900s, few compounds, such as polyenes (for example, nystatin and amphotericin B) and flucytosine, were available for antifungal chemotherapy. Although the development of azole drugs started in the early 1970s, only a limited number of antifungal agents were available for treatment of life-threatening fungal infections. Moreover, the existing agents had disadvantages, such as the significant nephrotoxicity of amphotericin B[3] and the emergence of resistance to the azoles.[4] To overcome these defects, lipid formulations of polyenes were developed to reduce toxicity, and new triazoles (for example, voriconazole, ravuconazole and posaconazole) were developed to improve the antifungal spectra or susceptibility to azole-resistant isolates.[5] Despite a number of therapeutic advancements, there was a need to develop a new class of antifungal agents with novel mechanisms of action.

The echinocandins were a new class of antifungal drugs developed for the first time since azoles. The first launched echinocandin was caspofungin acetate (Merck & Co. Inc. (Merck), Readington, NJ, USA), followed by micafungin (Fujisawa Pharmaceutical Co., Ltd, now Astellas Pharma Co., Ltd, Fujisawa, Japan) and anidulafungin (Vicuron Pharmaceuticals Inc., now Pfizer Inc., New York, NY, USA), which was originally developed by Eli Lilly and Company (Indianapolis, IN, USA)

(Lilly) as LY 303366 and subsequently licensed to Vicuron (formerly Versicor) as VER-002. The approved echinocandins are synthetically modified lipopeptides that originate from natural compounds produced by filamentous fungi. The original anidulafungin, caspofungin and micafungin compounds were echinocandin B from *Aspergillus nidulans var. echinulatus*,[6] pneumocandin B0 from *Glarea lozoyensis*[7] and FR901379 from *Coleophoma empetri*,[8] respectively.

Although natural echinocandins have potent antifungal activity *in vitro*, their structures were chemically altered to improve their absorption, distribution, metabolism and excretion characteristics. Such operations were initiated by Lilly on echinocandin B to yield cilofungin.[9] This compound was subjected to Phase II clinical trials, but was abandoned due to toxicity. Further modification of the structure by converting the phenolic hydroxyl to a sodium phosphate ester produced the more soluble prodrug LY307853, which resulted in the active form, LY303366.[10] Merck has produced MK-0991 using pneumocandin B0 as the starting material.[11] MK-0991 likewise possesses increased water solubility. Other reviews on echinocandins or individual antifungal agents have reported their usefulness in clinical practice.[12–16] This review describes the discovery and development of micafungin, focusing on the chemical diversity of echinocandins.

DISCOVERY OF FR901379

The seed compounds of micafungin, FR901379 and two related compounds (FR901381 and FR901382), were discovered at Fujisawa Pharmaceutical Co., Ltd in 1989 from the screening of approximately 6000 microbial broth samples (Figure 1). These new compounds were categorised as members of the echinocandin class of lipopeptides. Echinocandin B, pneumocandin B0 and other echinocandin lipopeptides are structurally characterized by a cyclic hexapeptide acylated with a long side chain, and have an excellent anti-*Candida* activity
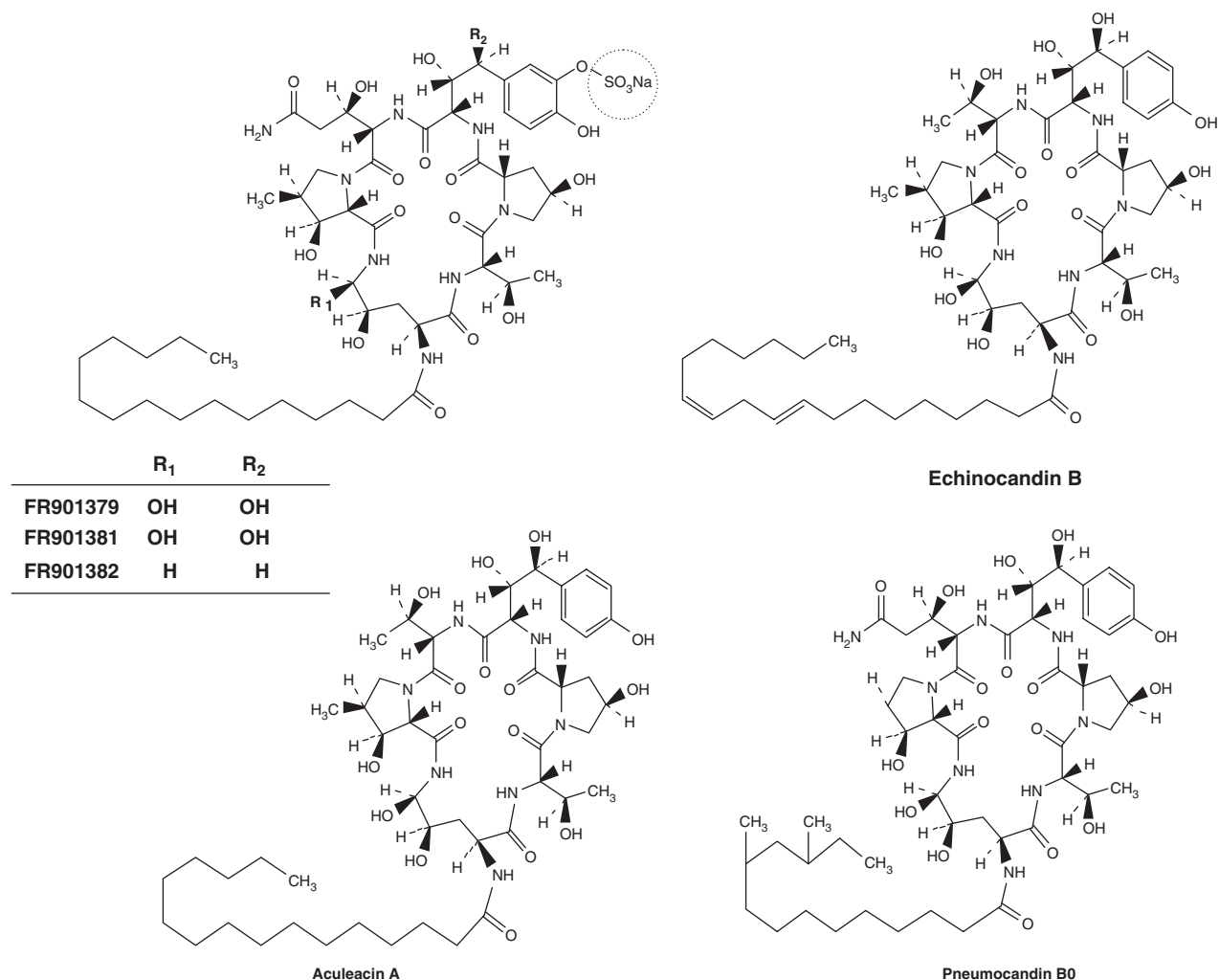
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Figure 1 Structures of echinocandins.

attributed to selective inhibition of 1,3-β-glucan synthesis, although their intrinsic water insolubility is a major barrier for drug development.[17–19] However, FR901379 and related compounds showed both high water solubility and a strong antifungal effect on *Candida* spp.[20] The structural difference between FR901379 and the other echinocandins is that FR901379 has a sulfate moiety in its molecule (Figure 1, circled). This residue was speculated to be the basis for the high water solubility of FR901379 (soluble in water even at a concentration of $50 \, \text{mg ml}^{-1}$, a concentration at which other compounds have low solubility). To probe this hypothesis, FR901379 was digested with aryl sulfatase from *Aerobacter aerogenes* (Table 1), after which the water solubility of the desulfated molecule (FR133302) was decreased to $1 \, \text{mg ml}^{-1}$, even though the inhibitory activity on 1,3-β-glucan synthase did not decrease markedly.[21] This result indicated that the excellent water solubility of FR901379 was attributed to the sulfate moiety in its structure.

The IC_{50} value of FR901379 on 1,3-β-glucan synthase is $0.7 \, \mu\text{g ml}^{-1}$, which is superior to that of echinocandin B (Table 1). The *in vitro* antifungal activity of FR901379 and related compounds against both *Candida albicans* and *A. fumigatus* indicates a higher potency than that of aculeacin A (Table 2); however, it is only weakly active against *A. fumigatus*. None of these compounds show antifungal activity against *C. neoformans*. Table 3 shows the therapeutic effect of

FR901379 in a murine *C. albicans* infection model in which drugs were administered s.c. for four consecutive days. FR901379 and related compounds significantly prolonged the survival of infected mice. FR901379 was the most potent compound, with an ED_{50} value of $2.7 \, \text{mg kg}^{-1}$ 14 days after the infection. This value was almost comparable to that of fluconazole. In spite of its potent antifungal activity and its good water solubility, FR901379 could not be developed further because of class-specific reticulocyte lysis at low concentrations (Table 4), although the lytic activity of FR901379 was weaker than that of amphotericin B.

The producer strain of FR901379, identified as *C. empetri* F-11899, was originally isolated from a soil sample collected at Iwaki City, Fukushima Prefecture, Japan. Its morphological characteristics were determined on the basis of cultures on sterilized azalea leaf affixed to a Miura's LCA plate, because the strain produced conidial structures on the leaf segment alone.

GENERATION OF THE LEAD COMPOUND, FR131535
When FR901379 was discovered by Fujisawa, both Merck and Lilly had already investigated this area of echinocandins. However, FR901379 had two advantages over other analogs. One was its excellent intrinsic water solubility, and the other was that the hemolytic activity of echinocandins was reduced by substituting the acyl side chain. As

Table 1 Water solubility and inhibitory effect of echinocandins on 1,3-β-glucan synthase

Compound	Solubility in water (mg ml^{-1})	Inhibition of 1,3-β-glucan synthase IC$_{50}$ (μg ml^{-1})
FR901379	> 50	0.7
FR133302	1	1.3
Echinocandin B	0.008	2.6
Cilofungin	0.1	nt

Abbreviation: nt, not tested.

Arylsulfatase from *Aerobacter aerogenes*

FR901379   **FR133302**
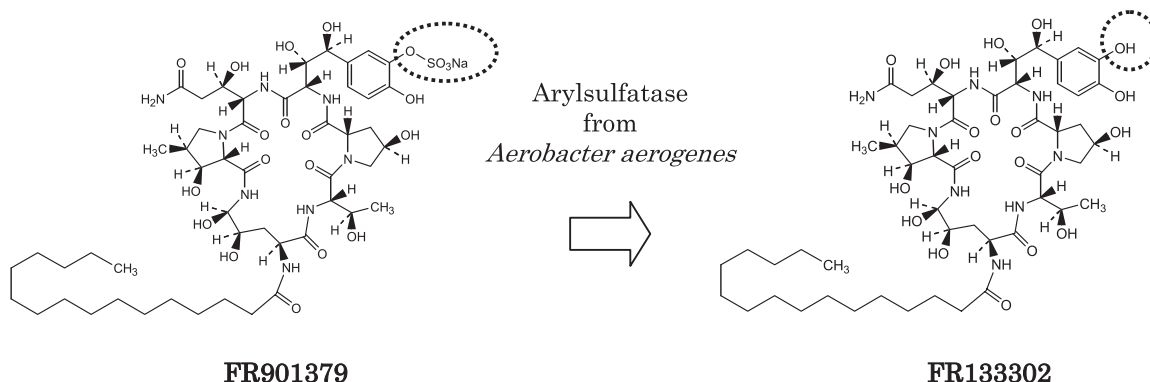
Table 2 *In vitro* antifungal activity of FR901379 and related compounds

Test organism	IC$_{50}$ (μg ml^{-1})			
	FR901379	FR901381	FR901382	Aculeacin A
Candida albicans FR578	0.008	0.008	0.008	0.008
C. albicans FP582	0.025	0.015	0.03	0.06
C. albicans FP629	0.008	0.004	0.008	0.015
C. albicans FP633	0.025	0.025	0.03	0.06
C. tropicalis YC118	0.025	0.05	0.015	0.31
C. krusei YC109	0.16	0.16	0.16	0.62
C. utilis YC123	0.03	0.003	0.003	0.06
Aspergillus fumigatus FD050	1.9	1.6	0.62	2.5
A. niger ATCC9642	0.03	0.03	0.03	2.5
Cryptococcus neoformans YC203	> 2.5	> 2.5	> 2.5	> 2.5

Table 3 *In vivo* efficacy in a neutropenic mouse model of disseminated candidiasis

Compound	ED$_{50}$ (mg kg^{-1})
FR901379	2.7
Aculeacin A	6.4
Fluconazole	4.5

Infection: *Candida albicans* FP633.

Table 4 Hemolytic activity of FR901379

Compound	MLC[a] (μg ml^{-1})
FR901379	62
Aculeacin A	31
Echinocandin B	125
Amphotericin B	8

[a]Minimum lytic concentration.

chain was prepared starting from 1-bromooctane and 4-hydroxybenzoic acid. Thus, 2,4,5-trichlorophenyl 4-(*n*-octyloxy) benzoate was obtained from 4-(*n*-octyloxy) benzoic acid and 2,4,5-trichlorophenol using *N*, *N*′-dicyclohexylcarbodiimide (DCC) in ether. The reacylation of FR179642 by the 2,4,5-trichlorophenyl active ester method yielded FR131535 (Figure 2).[23] The water solubility of FR131535 was as high as that of FR901379, even after replacement of the acyl side chain. FR131535 inhibited 1,3-β-glucan synthase prepared from *C. albicans* 6406 with an IC$_{50}$ value of 2.8 μg ml^{-1} in a non-competitive manner (Ki 4.0 mM) and exhibited potent activity against a variety of fungal species by the micro broth dilution method. The protective efficacy of FR131535 administered s.c. against a murine systemic infection with *C. albicans* was reported to be superior to those of echinocandin B and cilofungin, and almost as potent as fluconazole. Furthermore, FR131535 showed potent *in vivo* activity against *A. fumigatus*: the ED$_{50}$ value of FR131535 was improved dramatically from 70 to 4.3 mg kg^{-1} (Table 5). Thus, FR131535 was the first echinocandin to show an effective anti-*Aspergillus* activity. The hemolytic activity of FR131535 had also been greatly reduced compared with that of FR901379 (Table 5). These findings led to extensive research on the optimization of the acyl side chain.

LEAD COMPOUND OPTIMIZATION TO FK463[24]
As the acyl side chain substitution led to the expansion of the antifungal spectrum to include *Aspergillus* spp., the relationship between the side-chain lipophilicity and antifungal activity was

other compounds were not as soluble, Fujisawa's researchers focused on side-chain replacement, while keeping the sulfate group intact. Considering that the hemolytic activity may be related to the long alkyl side chain, they attempted to reduce the hemolytic activity of FR901379 by substituting the side chain, as Lilly's researchers had.[22]

FR901379 was treated with acylase from *Actinoplanes utahensis* to remove the palmitoyl group to yield FR179642, and a new acyl side
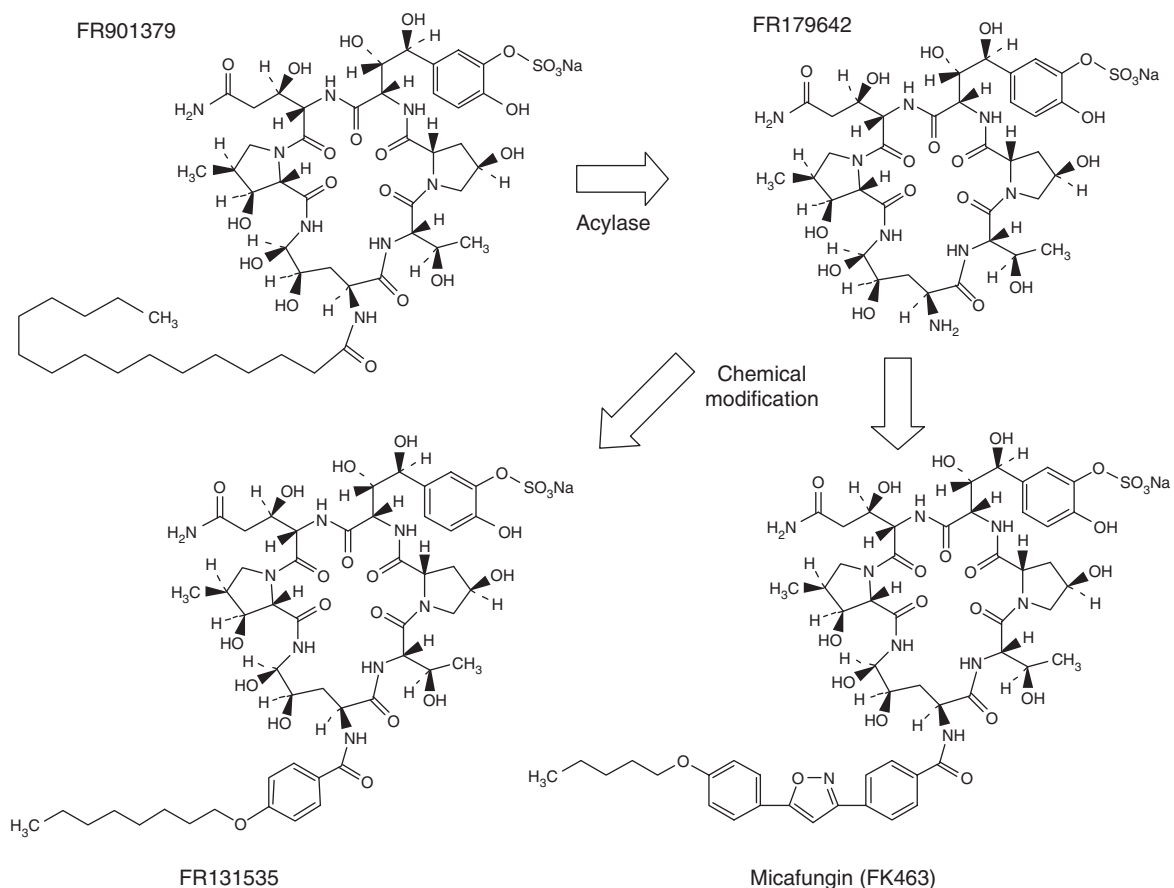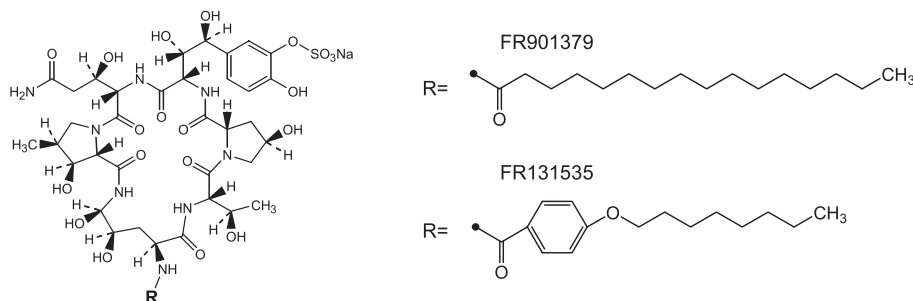
Figure 2 Semi-synthesis of FR131535 and micafungin (FK463).

Table 5 Influence of acyl side chain on MIC, ED$_{50}$ and hemolysis



| Compound | Candida albicans FP633 | | Aspergillus fumigatus FP1305 | Hemolysis |
	MIC ($\mu g\,ml^{-1}$)	ED$_{50}$ ($mg\,kg^{-1}$)	ED$_{50}$ ($mg\,kg^{-1}$)	LC$_{30}$[a] ($mg\,ml^{-1}$)
FR901379	0.2	1.8	70	0.456
FR131535	0.78	3.7	4.3	>8

[a]Lytic concentration 30%.

examined.[25,26] Naphthalene side chains, which are compact and amenable to modulation of lipophilicity, were chosen as the initial acyl side chains to be substituted. Meanwhile, the relationship between antifungal activities and hemolysis was examined by changing the lipophilicity by varying the length of the alkyl chains. The lipophilicity increase resulted in improved anti-*Candida* activity, which was most potent with an octyloxy group ($n=7$; Figure 3a). Hemolytic activity was enhanced in proportion to the length of the alkyl chains. To make the analog design more quantitative, the ClogP value [octanol–water partition coefficient (calculated value)] of the side chain was used to evaluate the correlation with anti-*Candida* activity. The strongest *in vivo* effect was observed in compounds with ClogP values of
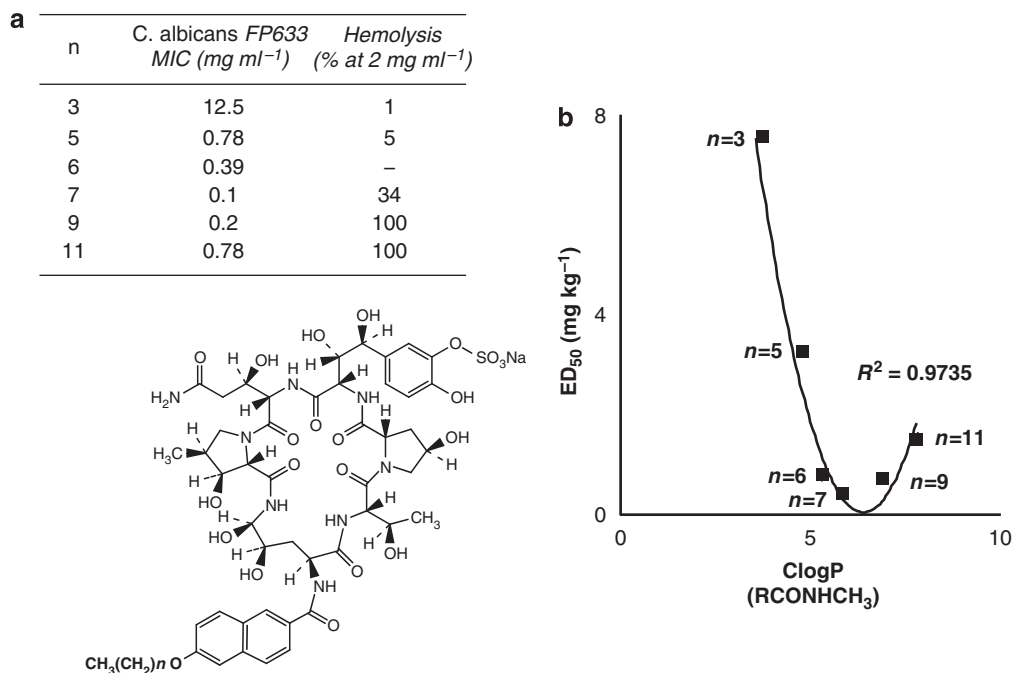
Figure 3 Effect of lipophilicity on MIC, hemolysis and ED_{50}: (a) length of alkyl chain; (b) ClogP of the side chain determined for $CH_3(CH_2)_nCONHCH_3$.

Table 6 Key compounds in optimization for side chain

Compound	Side chain	ClogP	Candida albicans FP633		Aspergillus fumigatus FP1305		Hemolysis	
			MIC ($\mu g\,ml^{-1}$)	Serum MIC ($\mu g\,ml^{-1}$)	ED_{50}[a] ($mg\,kg^{-1}$)	ED_{50} ($mg\,kg^{-1}$)	LC_{30}[a] ($mg\,ml^{-1}$)	At $1\,mg\,ml^{-1}$ (%)
FR131535	$O(CH_2)_7CH_3$	4.77	0.78	25	1.5–4.3	4.31	>10	<20
1	$O(CH_2)_7CH_3$	5.80	0.1	6.25	0.742	0.788	10	
2	$O(CH_2)_5CH_3$	5.37	0.2	6.25	0.658		1.74	
3	$O(CH_2)_3CH_3$	6.14	0.025	1.56	0.447		0.37	79
Micafungin	$O(CH_2)_4CH_3$	5.32	0.05	1.56	0.658	0.228		<20

[a]Lytic concentration 30%.

approximately 6; therefore, the side chains were thereafter prepared by adjusting the ClogP value to approximately 6 (Figure 3b).

Conversion of a single benzene ring of FR131535 into a naphthalene ring improved anti-*Candida* activity, and further introduction of aromatic rings into the side chain was also effective (Table 6). Anti-*Candida* activity was improved as the number of benzene rings increased, and compound **3** showed the strongest MIC. Furthermore, compound **1**, which had a naphthalene ring, showed a large improvement in anti-*Aspergillus* activity when compared with FR131535. However, even though compound **3** showed the lowest MIC value, its *in vivo* efficacy (ED_{50} 0.447 mg kg^{-1}) was only slightly better than that of compound **2** (ED_{50} 0.658 mg kg^{-1}), which had a 10-fold greater MIC. This apparent large discrepancy was reduced using serum MIC values that were measured in a medium supplemented with mouse serum. A good correlation between the *in vivo* effect (ED_{50}) and *in vitro* activity (serum MIC) was obtained.[27] The marked difference in the MIC values was deduced to result from a decreased concentration of the free form due to binding of the compound to serum protein. On the basis of the observed correlation, prediction of *in vivo* effects by measuring the serum MIC of the compound became more feasible, and structure–activity relationships were established rapidly. Consequently, compound **3**-type derivatives with three linearly linked aromatic rings revealed strong anti-*Candida* and anti-*Aspergillus* activities. However, the hemolytic activity of these analogs was still evident. This was overcome by converting the central benzene ring of compound **3** into various heterocycles. Initially, hemolytic

activity reduction was difficult because FR901379 derivatives have an amphiphilic structure and surfactant activity. The difference in the amount of unsaturated fatty acids between erythroid and eukaryotic cell membranes was noted, which led to the hypothesis that reduction of hemolytic activity might be possible by decreasing the linearity of the acyl side chains. As expected, the introduction of a heterocycle into the acyl side chain resulted in a marked lowering of hemolytic activity without a decrease in the potent antifungal activity of compound **3**. Finally, the cyclic peptide nucleus FR179642, obtained by enzymatic cleavage of the natural product FR901379, was reacylated with an optimal side chain containing an isoxazole ring to yield FK463 (later named micafungin) (Figure 2).

PRE-CLINICAL STUDY OF FK463
Micafungin is a non-competitive inhibitor of 1,3-β-glucan synthase, a key enzyme necessary for the synthesis of 1,3-β-glucan, that is a main component of fungal cell walls.[28–30] Fungal cells unable to synthesize 1,3-β-glucan lose the rigidity required to resist osmotic pressure, resulting in cell lysis. This mechanism is common to the echinocandin class of antifungal agents. Most fungal cell walls are composed of chitin, glucan and mannoprotein, although the quantity and relative content of each component depend on fungal species. The multi-enzyme complex 1,3-β-glucan synthase is composed of the catalytic subunit FKS1p/FLs2p and the regulatory subunit Rho1p GTPase.[31] The potent inhibitory effect of micafungin on the activity of 1,3-β-glucan synthase was determined in membrane preparations of both *C. albicans* and *A. fumigatus* by measuring incorporation of [^{14}C]UDP-glucose into a trichloroacetic-acid-insoluble precipitate. The short-term exposure of *C. albicans* to 0.1–0.5 mg ml^{-1} micafungin induced abnormal swelling and irregular shape of fungal cells, a reported feature resulting from inhibition of cell wall synthesis.[32]

Micafungin shows potent fungicidal activity against clinically isolated yeast species of *Candida*, including *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. glabrata* and *C. krusei*, and against *C. parapsilosis*, *C. lusitaniae* and *C. guilliermondii* with slightly higher MIC$_{90}$ values.[33–35] No cross-resistance to fluconazole-resistant clinical isolates of *Candida* has been observed.[36] Characteristically, micafungin has potent *in vitro* inhibitory activity against *Aspergillus* species at lower concentrations than amphotericin B and itraconazole, but micafungin was not fungicidal against *Aspergillus* spp.[37] There are no reports on acquired resistance to micafungin in susceptible yeast species in clinical practice, except for a case of resistance developed in *C. parapsilosis*.[38] Repeated passage of *C. albicans* in sub-inhibitory concentrations of micafungin resulted in negligible changes in MIC values, which suggests a low possibility of developing resistance in a clinical setting.

Among the compounds prepared in the optimization study, micafungin had the strongest *in vivo* effects against *Candida* spp. and *Aspergillus* spp. The efficacy of micafungin was determined in neutropenic murine models of disseminated candidiasis and aspergillosis in comparison with amphotericin B and fluconazole.[39] As shown in Table 7, the ED$_{50}$ values of micafungin against disseminated infections with *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei* ranged from 0.14–0.77 mg kg^{-1}. Although micafungin efficacy was 1.4–3.1 times inferior to that of amphotericin B (0.09–0.26 mg kg^{-1}), it was 9.6 ⩾77 times superior to that of fluconazole. The ED$_{50}$ of micafungin against disseminated *C. parapsilosis* infection was 1.0 mg kg^{-1}, which was 11 times superior to that of fluconazole (10.9 mg kg^{-1}) and 18 times inferior to that of amphotericin B (0.06 mg kg^{-1}). Micafungin was also effective against disseminated *A. fumigatus* infection, with ED$_{50}$ values in the range of 0.25–0.50 mg kg^{-1}. The efficacies of micafungin were 1.7–2.3 times inferior to those of amphotericin

Table 7 *In vivo* efficacy of micafungin in a neutropenic mouse model of disseminated candidiasis and aspergillosis

Organism	ED$_{50}$ (mg kg^{-1})		
	Micafungin	Fluconazole	Amphotericin B
C. albicans FP633	0.18	1.54	0.07
C. glabrata 16011	0.18	4.89	0.10
C. tropicalis 16004	0.35	7.2	0.21
C. krusei 15001	1.61	>20.0	0.71
C. parapsilosis 16005	3.21	4.57	0.08
C. guilliermondii 13003	0.77	6.27	0.32
A. fumigatus TIMM0063	0.33	>20.0	0.25
A. fumigatus IFM40835	0.26	>20.0	0.25
A. fumigatus IFM40836	0.45	>20.0	0.46

B (0.11–0.29 mg kg^{-1}) and more than 80 times superior to those of fluconazole. These results indicated that micafungin would be a potent parenteral therapeutic agent for disseminated candidiasis and aspergillosis.

CLINICAL STUDY OF FK463
The clinical efficacy of micafungin was evaluated in the treatment and prophylaxis of *Candida* and *Aspergillus* infections. A dose–response trial and two randomized double-blinded trials were first conducted to evaluate the safety and efficacy of micafungin in the treatment of esophageal candidiasis in patients with AIDS.[40–42] A total of 120 patients with endoscopically proven esophageal candidiasis were included in the initial study, with an i.v. micafungin dosing of 12.5, 50, 75 and 100 mg day^{-1}. Clinical improvement was endoscopically noted in all patients, especially those who received 75 or 100 mg day^{-1}. Symptoms improved or resolved within 3–5 days of treatment in the majority of patients, irrespective of the underlying CD4+ lymphocyte count. The efficacies of i.v. micafungin and i.v. fluconazole were compared in a randomized, double-blind, dose–response study. In all, 245 patients with a prior diagnosis of AIDS/human immunodeficiency virus (HIV) infection and esophageal candidiasis confirmed by endoscopy and culture were randomized to receive micafungin (50, 100 or 150 mg day^{-1}) or fluconazole (200 mg day^{-1}). Both agents were administered once daily by 1-h i.v. infusion for 14–21 days and the response was endoscopically determined. Cure rates were 68.8, 77.4 and 89.8% with 50, 100 and 150 mg of micafungin, respectively. The average cure rate with two higher doses of micafungin (83.5%) was comparable to that of 200 mg of fluconazole (86.7%). This study confirmed the efficacy of micafungin in treatment of esophageal candidiasis in HIV-positive patients.

Other treatment and prophylaxis trials against candidemia, invasive candidiasis and aspergillosis were conducted to assess the efficacy, adverse effects, pharmacokinetics, drug–drug interactions and dosing of micafungin. A convenient dosing regime, an excellent safety profile and remarkably few drug interactions were observed, indicating that micafungin is a potent weapon in antifungal therapy.

OTHER SULFATED ECHINOCANDINS
The acyl side chain of echinocandin has been readily replaced by established semi-synthesis methods; however, modification of the cyclic peptide by chemical synthesis is difficult. As an alternative, Fujisawa's researchers attempted to obtain structural diversity in the cyclic peptide by extensive screening of fungal products. A number of

Figure 4 Summary of the structure of echinocandins.
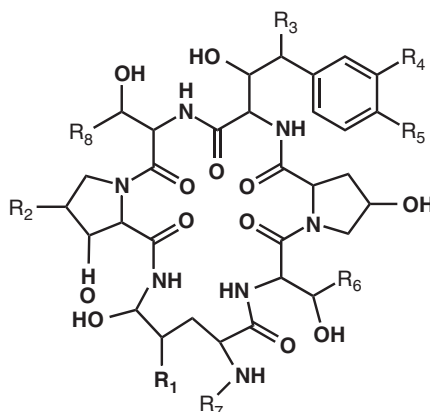
	R1	R2	R3	R4	R5	R6	R7	R8
FR901379	OH	CH_3	OH	OSO_3H	OH	CH_3	Palmitoyl	CH_2CONH_2
FR901381	OH	CH_3	H	OSO_3H	OH	CH_3	Palmitoyl	CH_2CONH_2
FR901382	H	CH_3	H	OSO_3H	OH	CH_3	Palmitoyl	CH_2CONH_2
FR220897	OH	H	H	OH	OSO_3H	CH_3	Palmitoyl	CH_2CONH_2
FR220899	OH	CH_3	H	OH	OSO_3H	CH_3	Palmitoyl	CH_2CONH_2
FR209602	OH	CH_3	H	OSO_3H	OH	H	Palmitoyl	CH_2CONH_2
FR209603	OH	H	H	OSO_3H	OH	H	Palmitoyl	CH_2CONH_2
FR209604	H	CH_3	H	OSO_3H	OH	H	Palmitoyl	CH_2CONH_2
FR190293	OH	CH_3	OH	OSO_3H	OH	CH_3	Branched acyl	CH_2CONH_2
FR227673	OH	CH_3	OH	OSO_3H	OH	CH_3	Branched acyl	CH_2CONH_2
Echinocandin B	OH	CH_3	OH	H	OH	CH_3	Linoleoyl	CH_3
Aculeacin Aγ	OH	CH_3	OH	H	OH	CH_3	Palmitoyl	CH_3
Pneumocandin B0	OH	H	OH	H	OH	CH_3	Branched acyl	CH_2CONH_2
Mulundocandin	OH	CH_3	OH	H	OH	CH_3	Branched acyl	H

sulfated echinocandins, which differ not only in the amino acid constituents of the cyclic peptide but also in the acyl chain, have been isolated (Figure 4). FR209602, FR209603, FR209604, FR220897 and FR220899 have different methylation and hydroxylation modifications of amino acids in the cyclic peptide, with the same palmitoyl acyl chain as FR901379,[43,44] whereas FR190293 and FR227673 have the same cyclic peptide as FR901379, with different branched acyl side chains.[45] Furthermore, the sulfated hydroxyl residue presents at a different position in FR209602, FR209603 and FR209604 than those in other sulfated echinocandins. The strain producing FR209602, FR209603 and FR209604, identified as *C. crateriformis* no. 738, also belongs to the same genus as that producing FR901379. The strain producing FR220897 and FR220899, identified as *C. empetri*, belonged to the same species as that producing FR901379. On the contrary, FR190293 and FR227673 were produced from non-*Coleophoma* strains; that is, *Tolypocladium parasiticum* and *Chalara* sp., respectively. Whereas structural diversity in cyclic peptides was observed among the compounds produced by the three strains belonging to the *Coleophoma* genus, chemical diversity in the acyl side chain was exhibited by non-*Coleophoma* species.

Considering other echinocandins, we have proposed a classification model for the echinocandin-producing strains, based on the chemical diversity of their product: type I as a sulfated echinocandin-producing strain and type II as a non-sulfated echinocandin-producing strain (Table 8).[46] Type I has been further classified into two sub-groups. Type IA is a coelomycetes group that produces a sulfated cyclic peptide nuclear structure with a palmitoyl chain. The strains belonging to this group form conidial structures only on leaf segments. Type IB, a hyphomycetes group, produced FR190293 and FR227673, which comprise the same peptide nuclear structure as FR901379, with a variety of branched acyl side chains. These strains form hyphal conidia in a general medium. Type II includes strains (for example, *Aspergillus* spp. and *G. lozoyensis*) that produce non-sulfated echinocandins, such as echinocandin B, aculeacin A, mulundocandin and pneumocandin B0. Type I fungi may have a specific enzyme, aryl sulfotransferase, to sulfate a phenolic hydroxyl residue in the cyclic peptide structure. This putative classification model may help in elucidating the molecular evolution of echinocandins, as well as the phylogenetics of the strains producing them.

PROSPECTS

A novel series of sulfated echinocandins has been reviewed in this paper. The compounds are highly soluble in water, even at a concentration of $50\,mg\,ml^{-1}$. Their structural and physico-chemical properties enabled the efficient development of micafungin. Thus, chemical diversity in screening sources is a key factor in successful

Table 8 A classification model for echinocandin-producing microorganisms

Class	Sub group	Compound	Producing strain
Type I sulfated		FR901379	
		FR901381	*Coleophoma empetri*
		FR901382	
	A	FR209602	
		FR209603	*C. crateriformis*
		FR209604	
		FR220897	
		FR220899	*C. empetri*
	B	FR190293	*Tolypocladium parasiticum*
		FR227673	*Chalara* sp.
Type II non-sulfated		Aculeacin Ar	*Aspergillus japonicus var. aculeatus*
		Echinocandin B	*A. nidulans var. echinulatus*
		Pneumocandin B0	*Glarea lozoyensis*
		Mulundocandin	*A. sydowi*

drug discovery and subsequent development. In the case of microbial screening, such diversity can be achieved by suitable selection of producing strains and culture conditions. Chemical diversity in the echinocandin family has been observed not only in the cyclic peptide nuclear structure but also in the acyl side chain moiety. Interestingly, various fungi produce common structures, such as an acylated cyclic hexapeptide, with some modifications. Thus far, the mechanism for the biosynthesis of echinocandins has not been elucidated, although it is likely to include a non-ribosomal peptide synthetase to produce the cyclic peptide core, with a fatty acid or polyketide synthase to produce the acyl side chain. An increase in echinocandin chemical diversity may be realized using genetic engineering of the biosynthetic genes.

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