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Review

# From natural products to clinically useful antifungals

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I would like to dedicate this paper to the memory of Dr. Kazuo Sakane, Director of these laboratories, who passed away on October 1st 2001. His caring, considerate nature, inspirational leadership and keen sense of responsibility contributed critically to the success of this project and he will be sorely missed.

**Abstract**

In our search for natural products with a broad spectrum of antifungal activity as lead compounds for novel treatments for mycoses, we have isolated echinocandin-type lipopeptide FR901379 and lipopeptidolactone FR901469, as novel water-soluble antifungal agents that inhibit the synthesis of 1,3-β-glucan, a key component of the fungal cell wall. Since the cell wall is a feature unique to fungi and is not present in nonfungal eukaryotic cells, inhibitors of the synthesis of fungal cell wall components such as 1,3-β-glucan have potential for selective toxicity to fungi and not to the host. In this short review, we describe efforts directed at synthetic modification of FR901469 and FR901379 with the ultimate goal of identifying new entities with suitable profiles as development candidate compounds. The main thrust of our work to date has been replacement of the highly flexible lipophilic side chains of the natural products with a view to reducing the hemolytic potential associated with these compounds, and to enhance chemical stability and/or in vivo antifungal efficacy. As a result of these efforts, we recently discovered a novel analog, FK463 (micafungin). Micafungin is currently in phase III clinical trials worldwide as a parenteral agent for various mycoses, and a new drug application (NDA) was recently filed in Japan. 

*Keywords:* Antifungals; Natural product; Micafungin; Drug discovery

## 1. Introduction

Fungal diseases in humans can be classified into (a) allergic reactions to fungal proteins, (b) toxic reactions to toxins present in certain fungi and (c) infections (mycoses). By far the most serious and difficult to diagnose and treat, mycoses come in many forms. Otherwise healthy individuals are susceptible to a host of superficial, cutaneous, subcutaneous, and in certain instances, systemic infections that cause a variety of conditions ranging from Athletes foot and nail infections to severe life-threatening disseminated disease (e.g. histoplasmosis). On the other hand, immunocompromised individuals are susceptible to a large number of opportunistic systemic mycoses, resulting from the inability of host immune response to fight off attack from normally benign environmental fungal pathogens. Indeed, the last 20 years has witnessed a remarkable increase in the incidence of deep-seated, disseminated mycoses [1]. The reasons are manifold; however, the advent of aggressive cancer chemotherapy, highly effective immunosuppressants for organ transplantation, widespread use of powerful broad spectrum antibacterial agents and the explosion in the number of cases of human immunodeficiency virus (HIV) infection have all contributed to the increase in life-threatening fungal disease [2]. Furthermore, ongoing demographic trends would tend to strongly suggest that the number of fungal infections will continue to increase due to the aging of the population in developed countries [3].

Whilst difficulty in diagnosis of fungal infections and delays in initiation of treatment are important factors, drugs

*Abbreviations:* MIC, minimum inhibitory concentration; $IC_{50}$, 50% inhibitory concentration; $ED_{50}$, 50% effective dose; NDA, new drug application; HIV, human immunodeficiency virus; MLC, minimum lytic concentration; BOC, *tert*-butoxycarbonyl; SCID Mouse, severe combined immunodeficient mouse; PCP, *Pneumocystis carinii*-associated pneumonia; Amp-B, amphotericin B; ITCZ, itraconazole; CFU, colony forming units; FLCZ, fluconazole; AcOH, acetic acid; MeOH, methanol; THF, tetrahydrofuran; EtOAc, ethyl acetate; DMF, *N*,*N*-dimethylformamide; TFA, trifluoroacetic Acid; HOBT, 1-hydroxybenzotriazole; WSCD.HCl, 1-ethyl-3-dimethylaminopropylcarbodiimide hydrochloride; $EtN^iPr_2$, diisopropylethylamine; LiOH, lithium hydroxide; Z, benzyloxycarbonyl; MTPA, α-methoxy-α-(trifluoromethyl)phenylacetic acid

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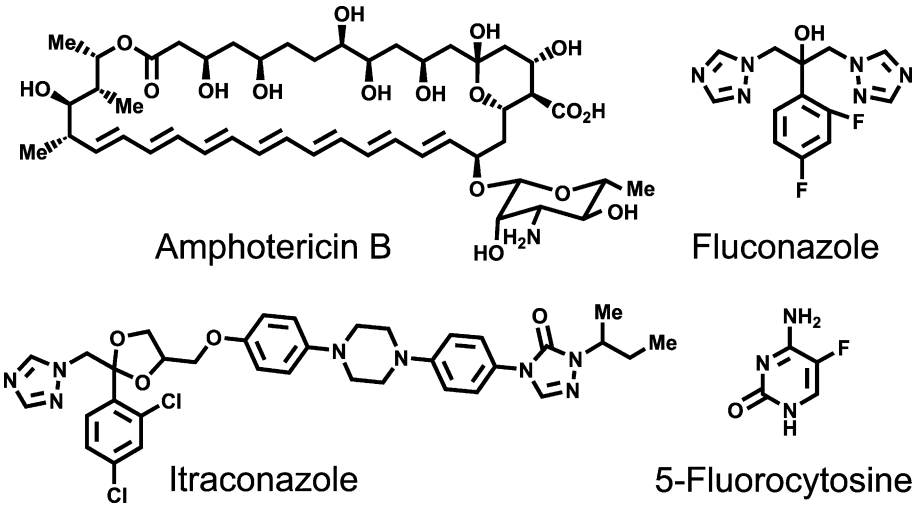



Fig. 1. Major available drugs for systemic mycoses.

for effective treatment of these emerging infections are in short supply, thereby contributing to a high mortality rate. Available drugs are essentially limited to the polyene natural product amphotericin B [4,5] and various newer lipid formulations [6], the azole compounds such as fluconazole and itraconazole, and flucytosine (5-fluorocytosine) (Fig. 1). These established agents suffer from a number of limitations that can render their use difficult; for example, dose-limiting nephrotoxicity associated with amphotericin B, rapid development of resistance with flucytosine, drug–drug interactions, fungistatic mode of action and resistance development with the azoles. There is thus an urgent need for new antifungals with a broad, fungicidal spectrum of action, and with fewer dose-limiting side effects [7,8]. Whilst a number of lipid-based formulations of amphotericin B have been shown to be effective in ameliorating the toxic liabilities associated with this agent, and a number of new azoles are in the final stages of clinical development, the fundamental problems described above remain. Accordingly, it is particularly imperative that we bring agents to the clinic that have new mechanisms of action and have good, broad-spectrum, cidal antifungal activity.

## 2. Natural product screening: novel antifungal agents

As a part of our efforts to discover new compounds with potential as lead compounds for optimization studies leading to novel antifungal candidates, we first must answer a simple key question: how do we identify and select a lead

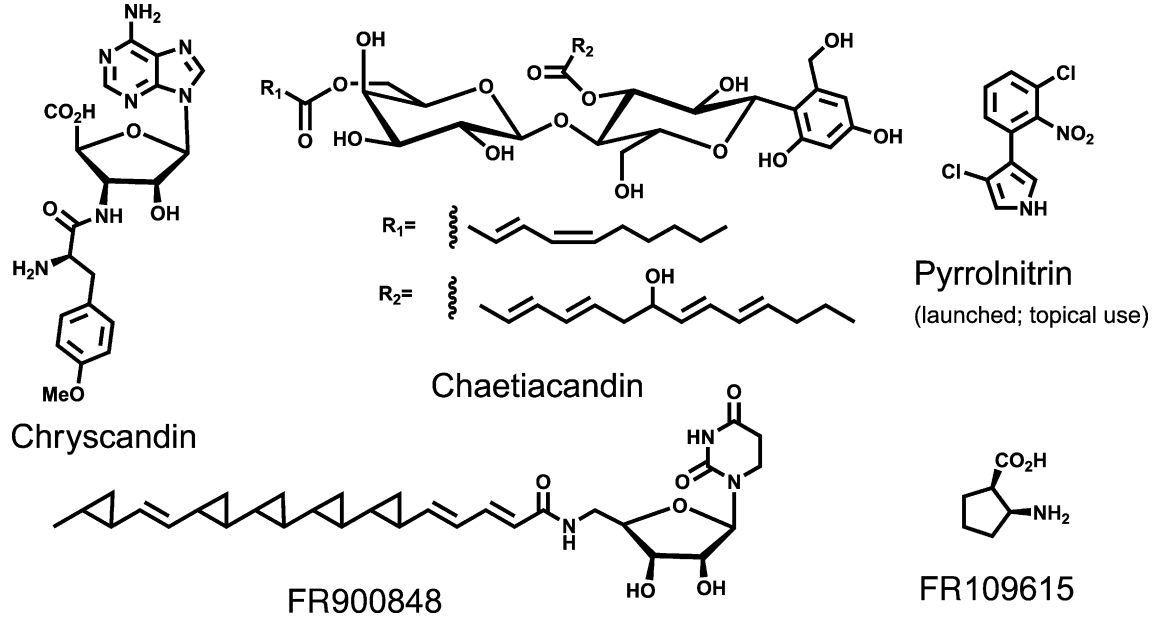



Fig. 2. Antifungal natural products discovered at Fujisawa.

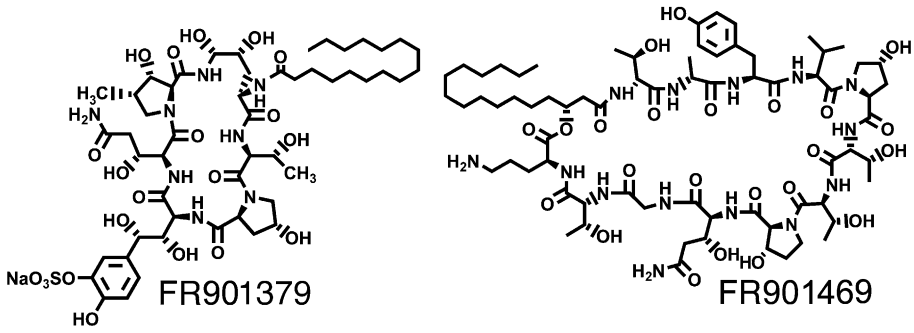



Fig. 3. Water-soluble antifungal natural products discovered at Fujisawa.

compound for further study? This is an important question as it is clearly critical that any potential compound must meet a number of criteria, otherwise the chances of ultimate success will not be high. The questions that we ask when selecting compounds for further investigation are as follows: (1) Is the compound structurally novel? (2) Is the mechanism of action novel and/or potentially useful? (3) Is intrinsic biological activity good? (4) Is clinical proof of concept possible? (5) Is chemical modification/optimization of the structure possible? It has become clear over a number of years that all of these questions are critical and impact greatly upon the chances of final success.

Since natural products have been proven to be an excellent source of novel chemical entities, we have employed screening of microbial extracts in our search for compounds that match the criteria discussed above, and have disclosed a number of structurally unique biologically active materials. In the course of screening for novel antifungal antibiotics, Fujisawa scientists have previously described the isolation of antifungals such as chryscandin [9], chaetiacandin [10], pyrrolnitrin [11], FR900848 [12] and FR109615 [13,14] (Fig. 2). Whilst pyrrolnitrin is marketed in Japan for topical indications, the other natural products had various limitations in terms of spectrum of in vitro activity, in vivo activity, water-solubility, lack of success in the chemical modification studies, mechanism of action limitations and potential for resistance development.

More recently, we have been engaged in the search for water-soluble inhibitors of fungal 1,3-β-D-glucan synthase [15], an enzyme critical to the synthesis of 1,3-β-D-glucan, a major component of the cell wall of a number of key pathogenic fungii [16]. The fungal cell wall is an attractive target for the discovery of new antifungal agents [17], primarily because it is essential for the viability of fungal cells, and also because the fungal cell wall has no counterpart in mammalian cells. Accordingly, compounds that selectively target the biosynthesis of key components of the fungal cell wall have potential to be nontoxic to mammalian hosts.

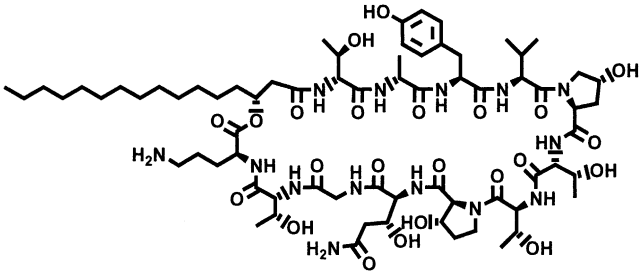
As a result of our continuous screening of microbial extracts, we have discovered two unique, water-soluble antifungals that express their activity by inhibition of the synthesis of 1,3-β-D-glucan (Fig. 3). FR901379 is the first example of a naturally water-soluble echinocandin derivative [18,19]. This natural product displays excellent water-solubility by virtue of the presence of a sulfate moiety on the homotyrosine group. FR901469 is the first example of a new type of 1,3-β-glucan synthase inhibitor based upon a 40-membered cyclic lipopeptidolactone structure [20–22]. FR901469 also displays excellent water-solubility.

In this short review, we briefly describe some of our efforts directed at synthetic modification of FR901469 and FR901379. The main thrust of our work has been replacement of the highly flexible lipophilic side chains and/or chemical derivatization with a view to reducing the hemolytic potential associated with the natural products, and to enhance chemical stability and/or in vivo antifungal efficacy. As a result of these efforts, we have discovered a novel analog, FK463 (micafungin), which is currently in worldwide phase III clinical trials as a parenteral agent for various mycoses.

## 3. Discovery and biological activity of FR901469 and related derivatives

FR901469 was isolated as a hydrochloride salt from an unidentified fungus no. 11243 [20]. This compound displayed water solubility of > 50 mg/ml, which compares with

Table 1
FR901469, a water-soluble lipopeptidolactone



β-1,3-glucan synthase inhibition (from *C. albicans* 6406)

| Compound | $IC_{50}$ (μg/ml) |
|---|---|
| FR901469 | 0.05 |
| FR901379 | 0.7 |
| Echinocandin B | 2.6 |
| Cilofungin | 2.9 |
| Papulacandin B | 2.5 |

Table 2
Biological properties of FR901469

| Compound | Hemolysis | *C. albicans* FP633 | | *A. fumigatus* FP1305 |
|---|---|---|---|---|
| | MLC (μg/ml) | MIC (μg/ml) | $ED_{50}$ (mg/kg/day) | $ED_{50}$ (mg/kg/day) |
| FR901469 | 250 | 0.1 | 0.4 | 0.42 |
| Echinocandin B | 125 | 2.5 | >32 | >100 |
| Fluconazole | NT | >100 | >20 | >40 |
| Amphotericin B | <50 | 0.39 | 0.13 | 0.13 |

0.008 mg/ml for echinocandin B, the prototype 1,3-β-glucan synthase inhibitor. Furthermore, inhibition of the enzyme from *Candida albicans* 6406 showed an $IC_{50}$ of 0.05 μg/ml, significantly more potent than echinocandin B and papulacandin B (Table 1). The antifungal activity of FR901469 is also excellent (Table 2), however, hemolytic activity is high and comparable to echinocandin B, indicating the potential for drug–erythrocyte interactions and hemolysis under in vivo conditions, especially in the multiple dose regimens of usual drug treatment protocols. Early work on echinocandin B indicated that major toxic liabilities resulted from its high hemolytic potential [23,24]. Minimum lytic concentration (MLC) for FR901469 was 250 μg/ml, compared to 125 μg/ml for echinocandin B. Reduction of hemolysis was considered critical in order to improve the overall profile of this natural product, since echinocandin B was unsuitable as a development candidate due to its hemolysis and toxicity. An additional notable structural feature of FR901469 is the juxtaposition of an ornithine amino group adjacent to a lactone moiety. This feature leads to a pH-dependent ring opening process, which whilst not effecting in vivo antifungal potency significantly, presumably due to amino group protonation at physiological pH, is potentially a source of chemical instability in the drug isolation/development process (Fig. 4).

We have already reported some of our efforts on the site-specific chemical modification of FR901469. In particular, we have described selective direct modification of the ornithine residue by synthesis of acylated and amidine conjugates of the ornithine amino group [25,26], selective functionalization of the tyrosine moiety [27] and complete replacement of the ornithine by a novel substituted glutamic acid residue [28]. In the course of these studies, it became apparent that the hemolysis associated with the natural product could be reduced significantly by chemical modification whilst maintaining the potent in vitro and, especially, in vivo antifungal activity. After completion of this work, scientists at Nippon Roche described the discovery of aerothricins, a series of lipopeptides closely related to FR901469 [29]. A number of studies aimed at exploring modifications at the ornithine moiety with a view to improving the therapeutic index and antifungal potency have also appeared recently [30,31].

As a part of continuing studies on FR901469, in order to remove the propensity for ring opening leading to a biologically inactive linear peptide, we designed the amide analog of the natural product (Fig. 4). This design is a rational one since an amide bond is significantly more stable to hydrolysis compared to an ester, however, it was unclear how such a modification would effect biological activity. On the assumption of retention of activity, we were particularly interested in the possibility of development of new methodology that would be applicable to the synthesis of a variety of novel lipophilic side chain-modified analogs, since work in the echinocandin B area established the influence of lipophilicity of the acyl side chain on antifungal potency and hemolysis of lipopeptides [24].

We based our approach to the synthesis of the amide analog of FR901469 on deacylation of the lipophilic side chain of the natural product, introduction of a pre-formed amide-substituted side chain moiety, macrolactamization and ornithine amino group deprotection. The key linear peptide intermediate was produced in 35% yield by direct incubation of FR901469 with *Actinoplanes utahensis* IFO-13244 in 0.2 M phosphate at pH 7.8 and 60 °C (Fig. 5). This organism has been employed for deacylation of various lipopeptide compounds, including echinocandin B, and cleaves only the amide bond connecting the lipophilic side chain to the peptide skeleton [32]. In this work, we have demonstrated another feature of this organism, whereby simultaneous removal of a β-hydroxyacid side chain and an ornithine moiety is also possible [33].

For this synthesis, the appropriate side-chain fragment was prepared as shown (Fig. 6). Myristylaldehyde was converted over six synthetic steps to an activated ester

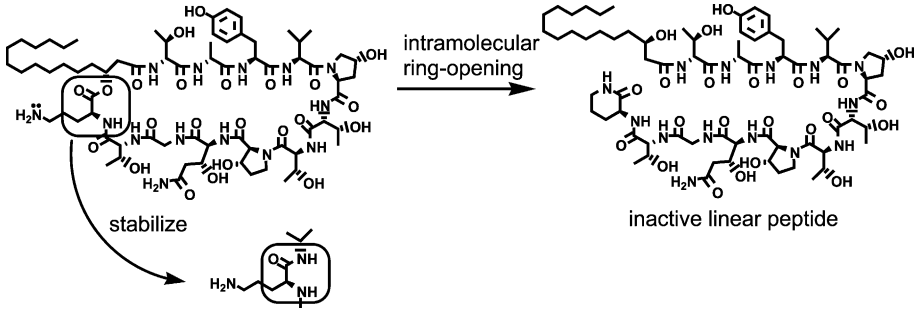



Fig. 4. Intramolecular ring-opening of FR901469.

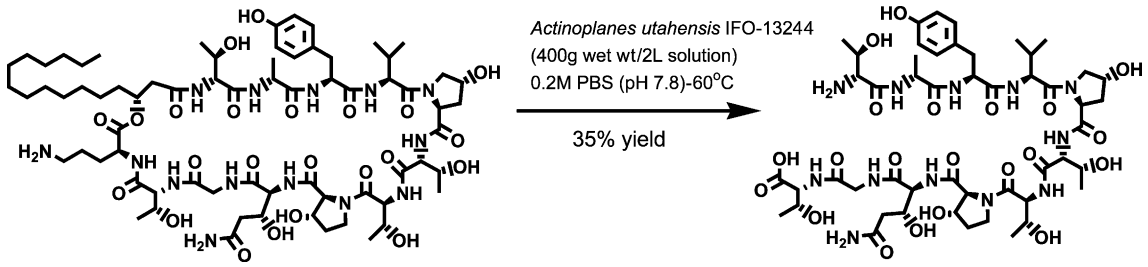



Fig. 5. Microbial deacylation of FR901469.

building block containing all required functionality. The β-amino acid portion of the fragment was prepared by adaptation of Davies methodology. The final steps in the synthesis of the amide analog of FR901469 are shown in Fig. 7. Acylation of the linear peptide produced by microbial biotransformation with the activated synthetic dipeptide, followed by protecting group manipulation, macrocyclization under high dilution conditions and removal of the *tert*-butoxycarbonyl (BOC) protecting group afforded the amide analog (FR207010) as the hydrochloride salt after ion-exchange chromatography and freeze-drying. In support of our original expectations, this compound was significantly more stable compared to FR901469 under high pH conditions, and the antifungal potency was comparable to the lactone natural product (Table 3).

Whilst FR207010 displayed comparable antifungal activity to FR901469, the presence of a linear unbranched lipophilic side chain meant that hemolysis was still high. This methodology is readily applicable to the synthesis of a variety of novel lipophilic side chain-modified analogs, and indeed, synthesis and biological activity of such analogs has revealed that nonhemolytic compounds with natural product-like levels of antifungal activity can be identified in this series (data not shown).

## 4. Discovery and biological activity of FR901379 and FK463, novel water-soluble echinocandin derivatives

The first example of a naturally occurring, water-soluble echinocandin-like lipopeptide, FR901379 (Table 4), was isolated from the culture broth of *Coleophoma empetri* F-11899 [18,19]. This compound was originally designated WF11899A and is one member of a family of three related cyclic hexapeptides (WF11899A, B, C) that differ only in the number of hydroxyl groups on the hexapeptide skeleton. FR901379 is a cyclic hexapeptide bearing a fatty acid acyl group on the N-terminal moiety and has excellent water-solubility by virtue of the presence of a sulfonate moiety on the homotyrosine residue. The water-solubility is greater than 50 mg/ml, which is much higher than the water-solubility of echinocandin B (0.008 mg/ml). FR901379

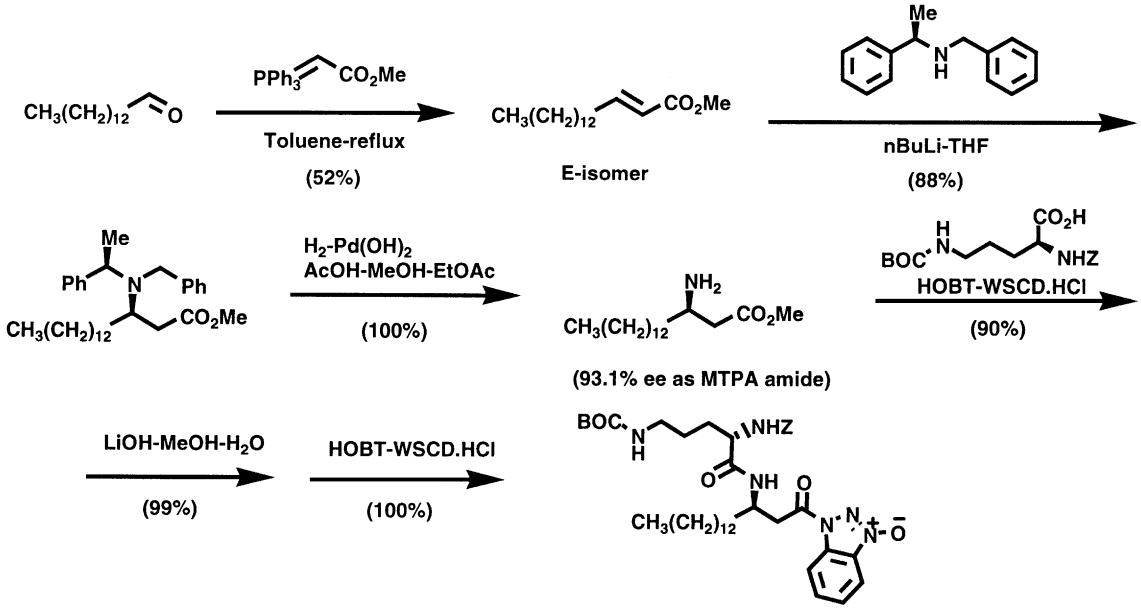



Fig. 6. FR901469 amide analog: activated ester fragment.

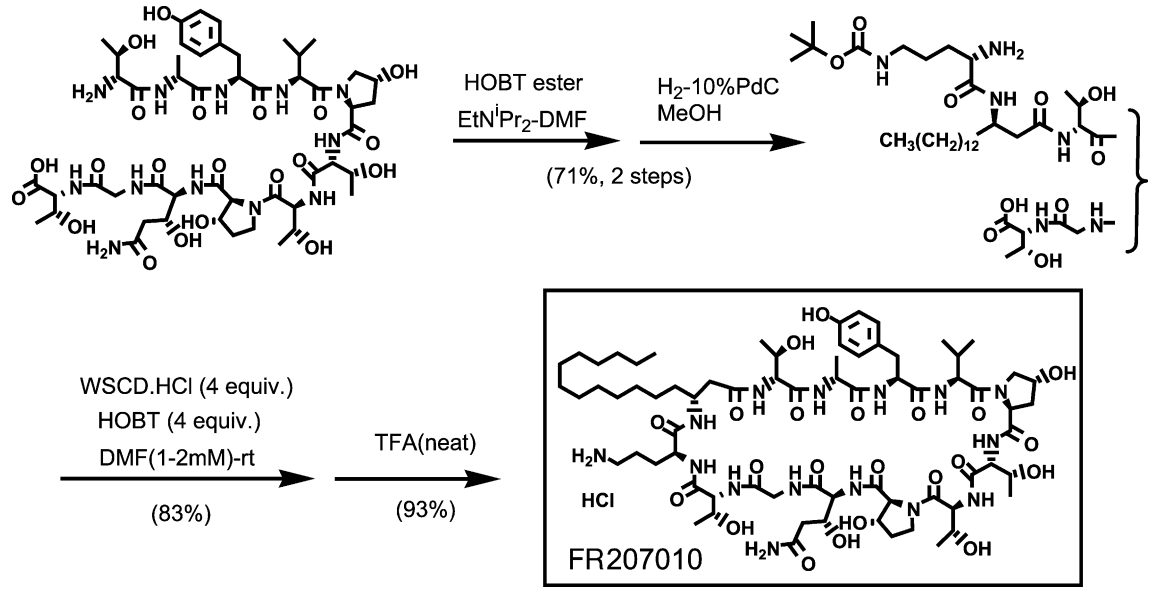



Fig. 7. FR901469 amide analog: completion of synthesis.

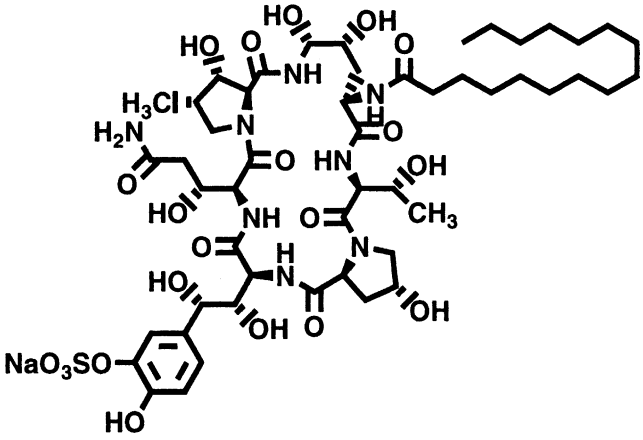
displayed more potent inhibition of β-1,3-glucan synthase from *C. albicans* 6406 ($IC_{50}$ = 0.7 μg/ml) than echinocandin B ($IC_{50}$ = 2.6 μg/ml), and in a *C. albicans* murine infection model, displayed a superior protective effect relative to echinocandin B and fluconazole, but was weaker than amphotericin B (Table 5). However, this compound was not strongly effective in prolonging survival in a model of disseminated aspergillosis, and it had an MLC of 62 μg/ml, indicating that chemical modification would be required to improve antifungal potency and to reduce hemolysis.

Recently, scientists at Lilly reported that the in vivo, but not in vitro, activity of cilofungin, an echinocandin B derivative with a modified fatty acid side chain, against *Aspergillus fumigatus* correlated with inhibition of *A. fumigatus* glucan synthase [34], and that the hemolytic potential of echinocandin B could be reduced by incorporation of benzoyl-type side chains. In our studies, enyzmatic deacylation of FR901379 was readily achieved and reacylation of the resulting key skeleton, FR179642, afforded novel side chain-modified antifungal agents. FR131535 [35,36] was the first key compound prepared and displayed excellent antifungal activity, comparable to FR901379, but without the hemolysis associated with the natural product. Subsequent optimization studies were then initiated, leading ultimately to the discovery of a novel analog, FK463, with an excellent profile. The synthesis of FK463 is outlined in Fig. 8, and involves a straightforward reacylation of the hexapeptide skeleton FR179642 with a pre-formed novel isoxazole-containing benzoyl-like side-chain [37]. In com-

Table 3
Antifungal activity of FR207010, the amide analog of FR901469

| Fungal Species | MIC (μg/ml) | | | |
|---|---|---|---|---|
| | FR207010 | FR901469 | Amp-B | Fluconazole |
| *C. albicans* ATCC90028 | 1 | 0.5 | 0.5 | 0.5 |
| *C. tropicalis* TIMM0313 | 2 | 1 | 0.5 | 4 |
| *C. krusei* ATCC6258 | 1 | 0.5 | 1 | 32 |
| *C. guilliermondii* ATCC9390 | 1 | 0.5 | 0.5 | 4 |
| *C. parapsilosis* ATCC22019 | 4 | 2 | 0.5 | 2 |
| *A. fumigatus* TIMM0063 | 1 | 0.5 | 0.5 | >64 |
| *A. niger* ATCC6275 | 0.5 | 0.25 | 0.25 | >64 |

Table 4
FR901379: a water-soluble echinocandin lipopeptide




β-1,3-glucan synthase inhibition (from *C. albicans* 6406)

| Compound | $IC_{50}$ (μg/ml) |
|---|---|
| FR901379 | 0.7 |
| FR901469 | 0.05 |
| Echinocandin B | 2.6 |
| Cilofungin | 2.9 |
| Papulacandin B | 2.5 |

Table 5
Biological properties of FR901379

| Compound | Hemolysis | *C. albicans* FP633 | | *A. fumigatus* FP1305 |
|---|---|---|---|---|
| | MLC (μg/ml) | MIC (μg/ml) | $ED_{50}$ (mg/kg/day) | $ED_{50}$ (mg/kg/day) |
| FR901379 | 62 | 0.2 | 1.8 | 70 |
| Echinocandin B | 125 | 2.5 | >32 | >100 |
| Fluconazole | NT | >100 | >20 | >40 |
| Amphotericin B | <50 | 0.39 | 0.13 | 0.13 |

mon with FR901379 and FR131535, FK463 displays excellent water-solubility.

Biological evaluation of FK463 has revealed that it possesses potent in vitro antifungal activity [38–41]. In comparison to the established agents, amphotericin B, itraconazole and fluconazole, FK463 displayed excellent activity against *Candida* species (Table 6) and *Aspergillus* species (Table 7). In common with other 1,3-β-D-glucan synthase inhibitors, FK463 was not active against *Cryptococcus neoformans* strains. [37]. FK463 was shown to be fungicidal to *C. albicans* FP633 by examination of the relationship between change in viable cell counts and drug concentration after exposure for 24 h in comparison to other drugs. A 99% or greater reduction in viability was observed after 24 h of exposure to >0.0156 μg/ml of FK463 (Fig. 9). As can be seen from the data in Fig. 9, FK463 exhibited fungicidal activity at concentrations lower than those at which amphotericin B was fungicidal, and was clearly superior to itraconazole and fluconazole, which displayed fungistatic effects.

Table 6
Antifungal spectrum of FK463 (1)

| Organism | MIC (μg/ml) | | | |
|---|---|---|---|---|
| | FK463 | Amp-B | ITCZ | FLCZ |
| *Candida albicans* ATCC90028 | 0.0156 | 0.5 | 0.0313 | 0.5 |
| *Candida tropicalis* TIMM0313 | 0.0313 | 0.5 | 0.125 | 4 |
| *Candida glabrata* ATCC90030 | 0.0156 | 0.5 | 1 | 16 |
| *Candida kefyr* ATCC28838 | 0.125 | 0.5 | 0.0625 | 0.5 |
| *Candida krusei* ATCC6258 | 0.125 | 1 | 0.25 | 32 |
| *Candida guilliermondii* ATCC9390 | 0.125 | 0.5 | 0.25 | 4 |
| *Candida parapsilosis* ATCC22019 | 2 | 0.5 | 0.25 | 2 |
| *Candida stellatoidea* IFM5491 | 0.0313 | 0.0625 | 0.0078 | 0.125 |
| *Saccharomyces cerevisiae* ATCC9763 | 0.125 | 0.5 | 0.25 | 2 |

The in vivo antifungal efficacy of FK463 is also good when drug is administered as an intravenous injection (Table 8). In murine models of disseminated candidiasis and aspergillosis, FK463 displayed comparable efficacy to amphotericin B, and superior efficacy compared to fluconazole [42]. In particular, in the candidiasis model, FK463 significantly prolonged the survival of intravenously infected mice at doses of 0.125 mg/kg of body weight or higher. In the disseminated aspergillosis model, FK463 given at doses of 0.5 mg/kg or higher significantly prolonged the survival of mice infected intravenously with *A. fumigatus* conidia. This result indicates that the efficacy of FK463 was about two times inferior to that of amphotericin B. Furthermore, FK463 has demonstrated good in vivo activity in mouse models of pulmonary aspergillosis [43]. Pulmonary aspergillosis in mice was induced by intranasal inoculation and administration was by the intravenous route.

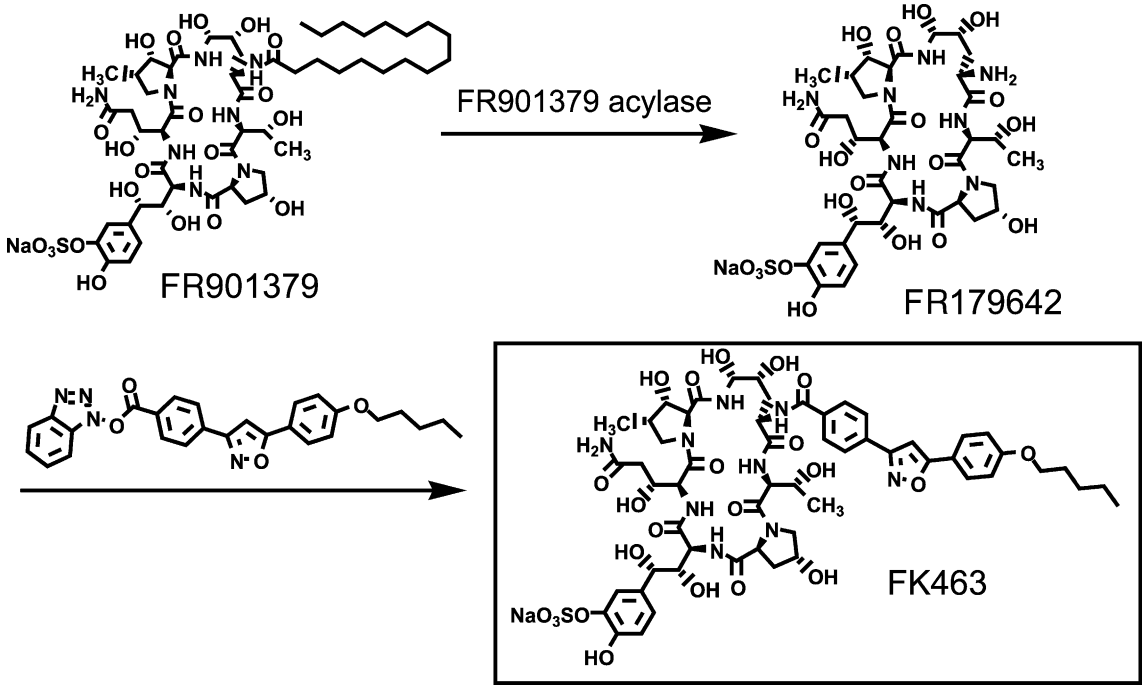



Fig. 8. Deacylation of FR901379 and synthesis of FK463.

Table 7
Antifungal spectrum of FK463 (2)

| Organism | MIC (μg/ml) | | | |
|---|---|---|---|---|
| | FK463 | Amp-B | ITCZ | FLCZ |
| *Cryptococcus neoformans* TIMM0354 | >64 | 0.25 | 0.0313 | 0.5 |
| *Trichosporon cutaneum* IFM4-1-4 | >64 | 2 | 0.5 | 8 |
| *Trichosporon asahii* TIMM3144 | >64 | 0.25 | 0.25 | 2 |
| *Aspergillus fumigatus* TIMM0063 | 0.0078 | 0.5 | 0.5 | >64 |
| *Aspergillus niger* ATCC6275 | 0.0078 | 0.25 | 0.5 | >64 |
| *Aspergillus nidulans* IFM5369 | 0.0078 | 1 | 0.0625 | 32 |
| *Aspergillus flavus* ATCC9643 | 0.0156 | 1 | 0.25 | 64 |
| *Aspergillus terreus* IFM5369 | 0.0156 | 1 | 0.125 | >64 |
| *Aspergillus versicolor* IFM41406 | 0.0156 | 0.5 | 0.0625 | 32 |
| *Fusarium solani* IFM41532 | >64 | 0.25 | >8 | >64 |

Table 8
In vivo efficacy of FK463: disseminated candidiasis and aspergillosis

| Organism | $ED_{50}$ (mg/kg/day) | | |
|---|---|---|---|
| | FK463 | FLCZ | Amp-B |
| *C. albicans* FP633 | 0.14 | 2.15 | 0.08 |
| *C. glabrata* 13002 | 0.30 | 6.27 | 0.11 |
| *C. tropicalis* 16009 | 0.28 | 3.71 | 0.09 |
| *C. krusei* FP1866 | 0.77 | 9.52 | 0.26 |
| *C. parapsilosis* FP1946 | 1.00 | 10.9 | 0.06 |
| *A. fumigatus* IFM41209 | 0.50 | >20 | 0.29 |

Once-daily treatment for 4 days starting 1 h after infection by intravenous administration to mice.

The 50% effective dose was in the range 0.26–0.51 mg/kg of body weight, which was comparable to amphotericin B. The minimum effective plasma FK463 concentration in murine pulmonary aspergillosis was determined to be 0.55–0.80 μg/ml by a viable-cell reduction assay in the target organs [43]. Good protection against infection due to azole-resistant *C. albicans* has also been described [44]. The prophylactic effect of FK463 against *Pneumocystis carinii* infection in the severe combined immunodeficient (SCID) mouse model has also been described [45]. *P. carinii* is an opportunistic pathogen that causes *P. carinii*-associated pneumonia (PCP), a cause of morbidity and mortality in immunocompromised patients. A recent summary article describes all available biological and clinical evaluation data, including pharmacokinetic data, for FK463 (micafungin) [46]. FK463 is currently in late-stage phase III clinical development.

## 5. Conclusions

The recent FDA approval of the Merck compound caspofungin as a therapy for refractory invasive aspergillosis has opened up the first chapter in a new era for antifungal therapy [47]. The confirmation that 1,3-β-D-glucan synthase inhibition can provide compounds with significant clinical utility bodes well for the future of treatment of severe systemic fungal infections. Our efforts in this area have culminated in the identification of FK463 (micafungin). This compound is in phase III clinical trials worldwide, and a new drug application (NDA) has been filed recently in

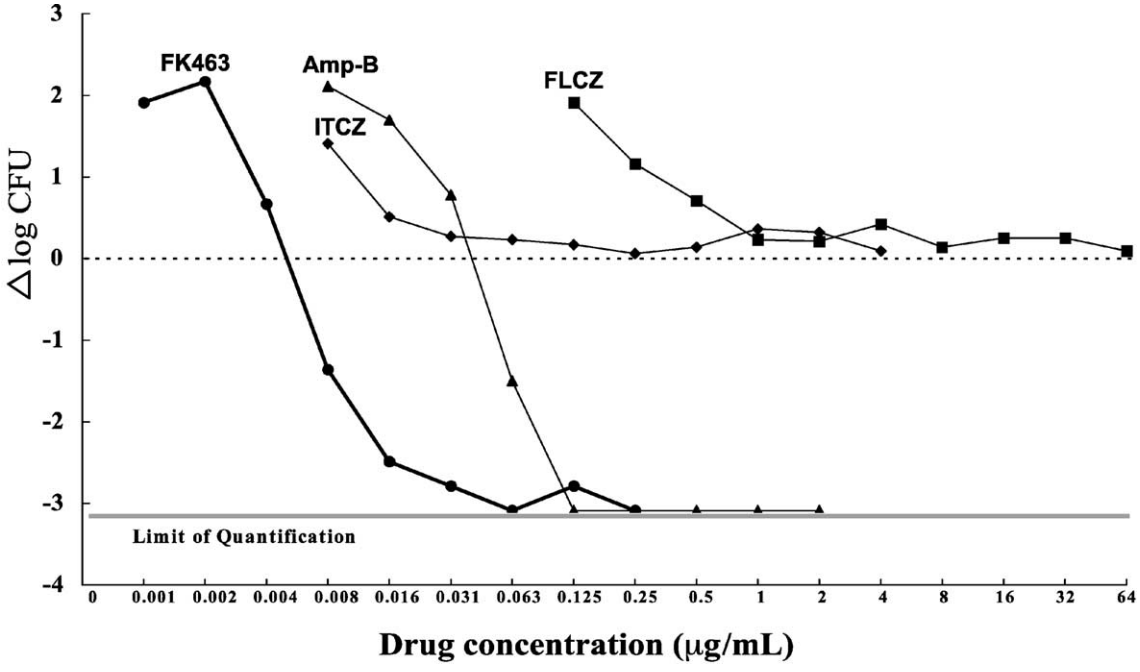



Fig. 9. Fungicidal activity against *C. albicans* FP633 after 24-h exposure.

Japan. These developments clearly show that a rational approach to antifungal drug discovery, focussing on compounds that interfere selectively with elements essential to the survival of fungal cells, can lead to new agents with the potential to become key components in the arsenal of weapons directed at serious systemic fungal disease.

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