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Synthesis and Biological Activity of Novel Macrocyclic Antifungals: Acylated Conjugates of the Ornithine Moiety of the Lipopeptidolactone FR901469

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Abstract—A series of acylated analogues of the novel macrocyclic lipopeptidolactone FR901469 has been prepared and evaluated for antifungal and hemolytic activity. Several analogues displayed markedly reduced hemolytic potential and comparable protective effects to the natural product in a mouse model of candidiasis.

Introduction

Recently the medical profession has seen an increase in the incidence of severe, life-threatening invasive fungal diseases.[1] This has been attributed primarily to an increase in the population of immunosuppressed individuals resulting from transplantation surgery and subsequent immunosuppression therapy, aggressive anticancer regimens, widespread use of powerful antibacterials and the HIV epidemic.[2] During this period, amphotericin B (AmpB) has remained the primary agent for treatment of these infections, even though it is known to produce a variety of dose-limiting toxic side-effects that result in discontinuation of treatment and clinical failure.[3,4] Lipid formulations of AmpB can reduce the toxicity associated with this agent,[5] however, the need exists for a novel antifungal agent with a comparable spectrum of activity but a superior safety profile to AmpB.

In the course of screening for novel antifungal antibiotics, we previously described the isolation of FR901379, the first naturally occurring water-soluble echinocandin,[6,7] and FR901469(**1**), a water-soluble 40-membered cyclic lipopeptidolactone.[8,9] Both natural products possess potent antifungal activity and strong inhibitory activity against 1,3-β-D-glucan synthase, a fungal-specific enzyme involved in cell wall synthesis. Side-chain deacylation of FR901379 followed by semi-synthetic modification led to the discovery of FK463,[10] with significantly improved antifungal efficacy, and without the hemolytic liability associated with FR901379. The antifungal activity of FR901469 is also excellent, however hemolytic activity is relatively high and comparable to echinocandin B,[9] indicating the potential for drug–erythrocyte interactions and subsequent lysis under in vivo conditions, especially in the multiple dose regimens of typical drug treatment protocols. Early work on echinocandin B indicated that the major toxic liabilities resulted from its high hemolytic potential, a feature not present in subsequent analogues that showed no tendency to lyse red blood cells.[11,12] Additionally, the presence of an ornithine amino group adjacent to the lactone moiety in FR901469 leads to a pH-dependent ring opening process, a feature that whilst not affecting in vivo antifungal potency significantly, due to amino group protonation at physiological pH, is potentially a source of chemical instability in the drug isolation/development process.

In this paper, we describe the synthesis and evaluation of a series of ornithine-modified analogues of FR901469, and the discovery of several derivatives with markedly reduced hemolytic activity and comparable efficacy in a murine model of disseminated candidiasis (Fig. 1).

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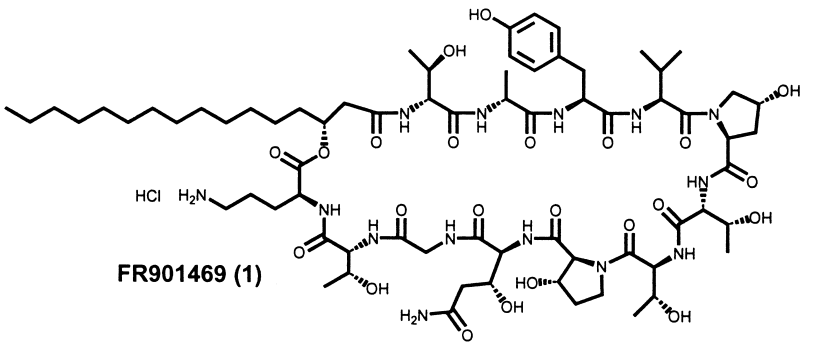
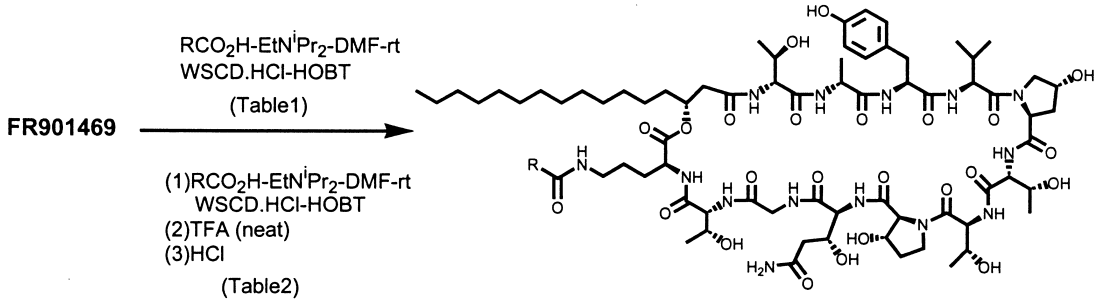


Figure 1.



Scheme 1. Synthesis of ornithine-modified FR901469 analogues.

Synthesis

The compounds prepared in this work were obtained as shown in Scheme 1. Acylation of the ornithine amino group of FR901469 with various carboxylic acids in DMF in the presence of diisopropylethylamine, 1-hydroxybenzotriazole (HOBT) and 1-ethyl-3-dimethylaminopropyl carbodiimide hydrochloride (WSCD·HCl) at room temperature afforded the amide derivatives in 66–100% yields after reverse phase ODS column chromatography and freeze-drying. In the case of BOC-protected amino acid derivatives, the BOC group was then removed by stirring at room temperature in neat TFA, followed by reverse phase ODS chromatography and finally by ion-exchange chromatography on Amberlyst A-26 and freeze-drying to afford the amorphous hydrochloride salts (Tables 1 and 2). All reactions and purifications were monitored by reverse phase HPLC. All final compounds and intermediates were characterized by ^{1}H NMR, FAB-MS, IR and elemental analysis. Purity was assessed by HPLC.

Biological Methods

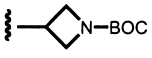
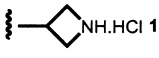
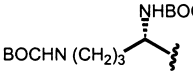
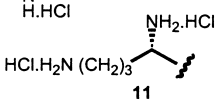
In vitro antifungal activity

Minimum inhibitory concentration (MIC) values were determined by the agar dilution method using Sabouraud Dextrose agar. Tenfold dilutions of overnight cultures in Sabouraud Dextrose broth containing about 10^4 colony forming units (CFU) were inoculated onto agar plates containing serial twofold dilutions of each compound, prior to incubation at 30 °C for 24 h by the streak method. The MIC was read as the lowest concentration required to inhibit visible growth of the organism.

Table 1. Acylation of FR901469

Compound	R	Yield (%)
2	CH_3	94
3	CH_2OH	100
6	$CH_2N(CH_3)_2$·HCl	72

Table 2. Acylation and deprotection reactions of FR901469

R	Acylation yield (%)	R	Deprotection yield (%)
$(CH_2)_n$NHBOC		$(CH_2)_n$NH$_2$·HCl	
$n=1$	84	$n=1$ (**4**)	99
$n=2$	92	$n=2$ (**7**)	96
$n=3$	66	$n=3$ (**8**)	98
$n=4$	91	$n=4$ (**9**)	95
$n=5$	90	$n=5$ (**10**)	89
CH_2NMeBOC	78	CH_2NHME.HCl(**5**)	88
	82	 **12**	80
	85	 **13**	89
	89	 **11**	80

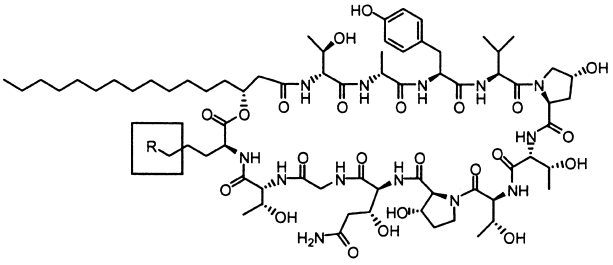
In vivo antifungal activity

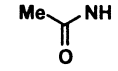
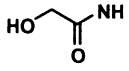
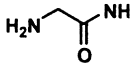
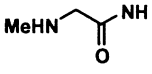
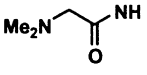
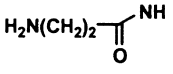
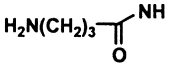
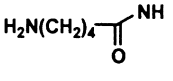
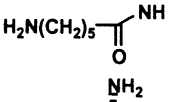
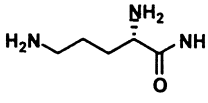
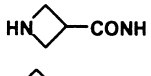
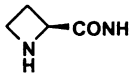
Disseminated candidiasis was induced in ICR mice by iv inoculation of 0.2 mL of a *Candida albicans* FP633 cell suspension into the lateral tail vein. A single dose of each compound was administered sc 60 min after challenge. ED_{50} was estimated on the basis of survival at day 14 after challenge.

Hemolytic activity

A microtiter red blood cell (RBC) hemolysis assay was used to determine the potential of compound to hemolyze ICR mouse RBCs. A suspension of freshly drawn heparinized ICR mouse whole blood (2 mL) was added to 50 mL of sterile saline. Test solutions were dispensed into microtiter wells and the RBC suspension was added

Table 3. In vitro antifungal and hemolytic activity of FR901469 derivatives



Compound(R)[a]		MIC (μg/mL)						
		C.a.[b]		*C.g.*	*C.t.*		*C.p.*	
		FP633	FP579	FP587	8001	8002	15001	Hemolytic activity (%)
FR901469 (**1**)	NH_2	0.39	0.39	0.39	0.39	0.39	0.39	100
2		0.39	0.78	0.78	1.56	1.56	1.56	5
3		0.39	0.78	0.39	1.56	0.78	0.78	9
4		0.39	0.78	0.78	0.78	0.78	0.78	67
5		0.2	0.39	0.39	0.78	0.78	0.39	54
6		0.39	0.78	0.2	0.78	0.78	0.39	10
7		0.39	0.39	0.39	0.78	0.78	0.39	44
8		0.78	1.56	0.78	1.56	0.78	0.78	30
9		0.39	0.78	0.39	0.78	0.78	0.39	20
10		0.39	0.78	0.78	0.78	0.78	0.39	14
11		0.39	0.78	0.39	1.56	1.56	0.39	20
12		0.1	0.39	0.39	0.39	0.39	0.39	57
13		0.39	0.78	0.78	0.39	0.39	0.78	27

[a]Except for **2** and **3**, all compounds are hydrochloride salts.
[b]*C.a.* = *Candida albicans*; *C.g.* = *Candida guilliermondi*; *C.t.* = *Candida tropicalis*; *C.p.* = *Candida parapsilosis*.

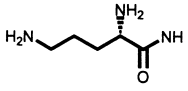
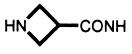
to each well. Final test concentration was 1 mg/mL. After incubation for 30 min, the blood samples were centrifuged at 1200 rpm for 10 min. Absorbance of the supernatant was measured at 450 nm. Hemolytic activity was calculated as: Hemolytic activity (%) = (A_{450nm}sample − A_{450nm}saline)/($A_{450nm}H_2O$ − A_{450nm}saline) × 100.

Results and Discussion

We designed a series of analogues of FR901469 in order to probe: (1) the requirement for and optimum location of the ornithine amino group; and (2) the influence of the ornithine moiety on hemolytic activity. Since the in vivo activity of FR901469 is strong,[8,9] we aimed to achieve a superior profile (improved therapeutic index) by reducing the hemolytic potential of the natural product, without reducing in vivo efficacy. We also aimed to remove the propensity for intramolecular attack by the amino group on the lactone carbonyl, by either blocking the amino group or placing it at a location whereby intramolecular cyclization is a less thermodynamically favorable process.

Tables 3, 4 and 5 summarize the biological activity of the derivatives prepared in this work. Whilst the simple acyl derivatives **2** and **3** had comparable in vitro anticandida activity to the natural product, the in vivo activity of **2** in a murine model of candidiasis was significantly reduced relative to **1**, indicating the requirement for an amino group to elicit a strong in vivo effect. The amino group is also an important determinant for hemolytic activity, since **2** is only very weakly hemolytic (5% at 1000 μg/mL). Comparison of **1** with the amino acid analogues **4** and **6–10** indicates that the MIC is relatively unaffected by the location of the amino group. Thus, when the length of the spacer moiety between the carbonyl group and the amino group is increased from one to five methylenes, in vitro potency is unchanged. Interestingly, the hemolytic activity was gradually reduced with an increasing number of methylenes. Furthermore, whilst the in vivo effects of **2** and **6** were reduced compared to **1**, the L-ornithine derivative **11** and the azetidine derivative **12** displayed marginally better in vivo efficacy. In the case of **11**, significantly reduced hemolytic potential as shown by only 20% hemolysis at a concentration of 1000 μg/mL was also noted. This contrasts with 100% at 1000 μg/mL for **1**. The anti-*Aspergillus fumigatus* activity of **11** was determined by the broth microdilution method[13] and shown to be comparable to **1** (Table 5), indicating that modification with an extra ornithine moiety had not modified the antifungal spectrum.

Table 4. In vivo activity: disseminated murine candidiasis

Compound	R	ED_{50} (mg/kg)
FR1469 (**1**)	NH_2	0.44–0.88[a]
2	$NHCOCH_3$	4 (6.2)[b]
6	$NHCOCH_2NMe_2$	9.1 (10.4)
11		0.54 (0.8)
12		0.54 (0.8)
Amphotericin B		0.132
Fluconazole		>20

[a]Range of values of ED_{50} for FR901469 over a number of experiments.
[b]Figures in parentheses: ratio of ED_{50}(drug)/ED_{50}(FR901469) for the same experiment.

Table 5. In vitro anti-*Aspergillus fumigatus* activity

Compound	*A. fumigatus* TIMM0063 FP1305 MIC (μg/mL)[a]
FR901469 (**1**)	0.5
2	1
4	0.5
6	0.5
11	0.5

[a]Determined by the broth microdilution method, according to NCCLS M27-A guidlines.[13]

In summary, a series of acylated analogues of the unique macrocyclic lactone FR901469 have been prepared, and several derivatives with comparable in vivo antifungal efficacy and reduced hemolytic potential identified. The in vivo efficacy displayed is strong and compares favorably with amphotericin B and fluconazole, the main drugs used for candidiasis. Further SAR studies will be reported in subsequent publications.

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