Novel Amidine Conjugates of the Ornithine Moiety of the Macrocyclic Antifungal Lipopeptidolactone FR901469

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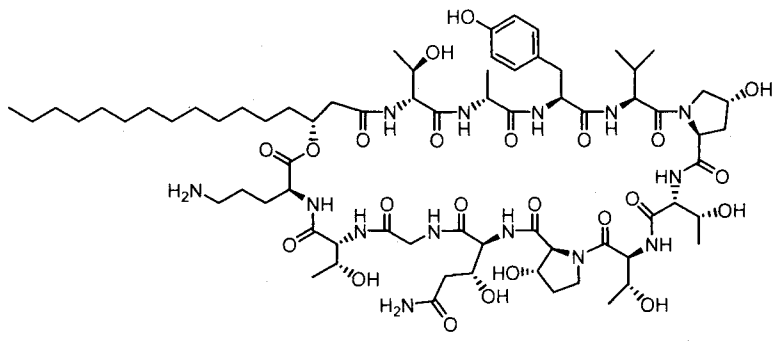
As a part of our efforts to identify new agents for the treatment of serious fungal infections, we have focussed on the discovery of novel water-soluble agents that are amenable to preparation of injectable formulations. We previously described isolation of the first naturally occurring water-soluble echinocandins,[1,2] and FR901469(**1**), a water-soluble 40-membered cyclic lipopeptidolactone.[3,4] These natural products possess potent antifungal activity due to strong inhibitory activity against the fungal-specific enzyme 1,3-β-D-glucan synthase, an enzyme involved in the synthesis of 1,3-glucan, a key structural component of fungal cell walls. FK463 is a semi-synthetic analog of FR901379 with significantly improved antifungal efficacy and is currently in phase III clinical trials.[5]

Whilst the antifungal activity of FR901469 is high, hemolytic activity, as expressed by red blood cell lysis at 1 mg/ml, is also relatively high.[4] Echinocandin B, the prototype member of the echinocandin class of 1,3-β-glucan synthase inhibitors, displayed toxic effects resulting from its high hemolytic potential.[6,7] With the goal of reducing the potential for hemolysis and maintaining the strong antifungal activity of the natural product, we recently described a series of acylated analogs of the ornithine moiety of **1**.[8] We have also reported novel methodology for the synthesis of the amide analog of **1**[9] and non-ornithine-containing macrocyclic lactones.[10] Herein, we describe the synthesis and evaluation of a new series of amidine analogs of the ornithine moiety of FR901469, and the discovery of pyrazolium derivative **8** with reduced hemolytic activity and comparable efficacy to **1** in a murine model of disseminated candidiasis.

Amidine derivatives were prepared as shown in Scheme 1. Coupling of FR901469 with imidate esters (obtained from the corresponding nitriles with HCl-EtOH) occurred smoothly using $EtNPr^i_2$ as base in DMF. Alternatively, aqueous conditions could be employed using sodium carbonate to maintain pH 7.5. The azetidine derivative **9** was obtained by hydrogenolysis of intermediate **11** produced by coupling of FR901469 with the azetidine imidate ester **10**. Guanidine derivative **4** was prepared from **1** and 3,5-dimethylpyrazole-1-carboxamidine nitrate (**12**) in 68% yield. All reactions and purifications were monitored by reverse phase HPLC. Purifications were performed by reverse phase ODS column chromatography and products obtained by pooling of the appropriate fractions, adjusting to pH 3 with 1 N-HCl, passage through a short column of

Fig. 1.



FR901469 (1)

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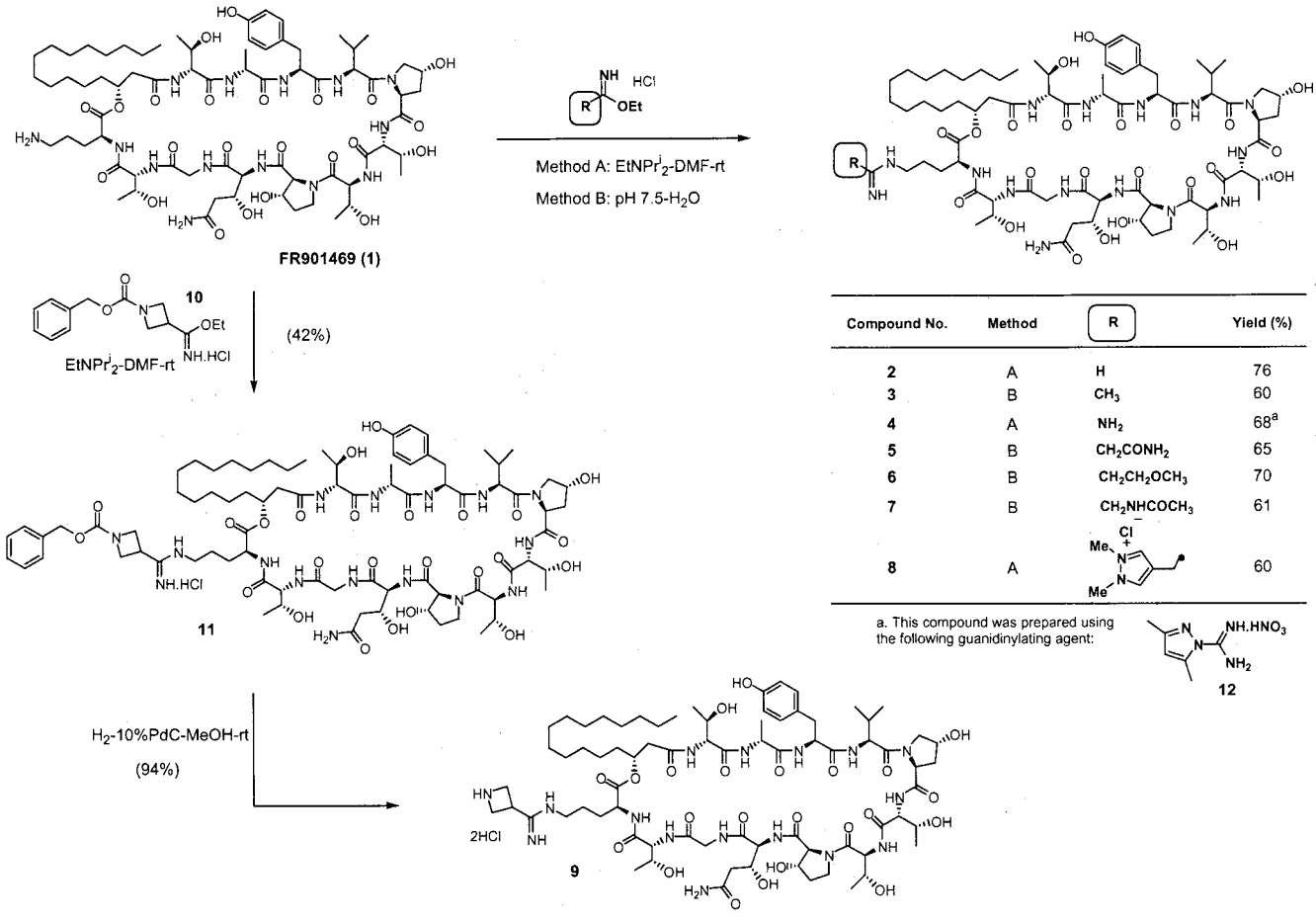
Scheme 1. Synthesis of FR901469 Amidine-type derivatives.



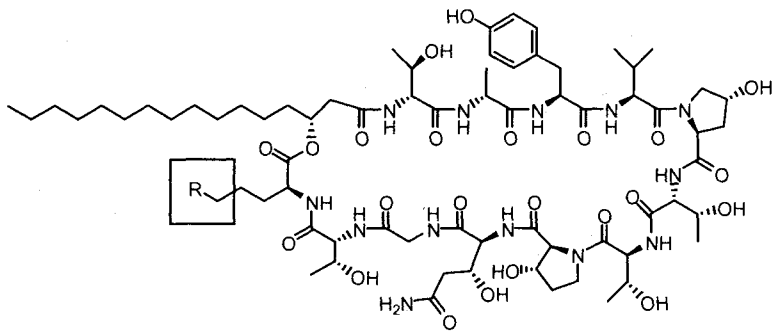
Compound No.	Method	R	Yield (%)
2	A	H	76
3	B	CH_3	60
4	A	NH_2	68[a]
5	B	CH_2CONH_2	65
6	B	$CH_2CH_2OCH_3$	70
7	B	$CH_2NHCOCH_3$	61
8	A		60

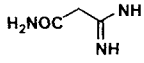
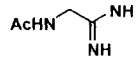
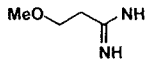
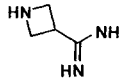
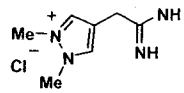
Amberlyst A-26 (chloride form) and freeze-drying. Final compounds and intermediates were characterized by ^{1}H NMR, FAB-MS, IR and elemental analysis. Purity was assessed by HPLC.

In our earlier work on acylated conjugates of the ornithine moiety of **1**, we designed analogs of FR901469 to probe the requirement for the presence of the ornithine amino group and its influence on antifungal and hemolytic activity.[8] As a result of these studies, it became apparent that hemolysis could be reduced by acylation with amino acid derivatives of varying spacer length, and a relationship was found between hemolysis at 1 mg/ml and the number of methylene groups in the spacer. In order to achieve good levels of *in vivo* efficacy, it was necessary to increase the number of amino groups close to the original ornithine amino, such that an added ornithine moiety was a particularly effective conjugate group for the natural product.[8] From these early results, we next decided to examine other types of modification of the ornithine moiety of **1**, and selected amidine-type analogs as being particularly attractive, due to retention of a basic group close to the position of the ornithine amino group. Since the *in vivo* activity of FR901469 is strong, a superior therapeutic profile should result from a reduction in hemolytic potential whilst maintaining *in vivo* efficacy.

Table 1 shows the *in vitro* antifungal activity against selected *Candida* species and the hemolytic activity of the amidine derivatives prepared in this work. Table 2 shows activity against a strain of *Aspergillus*. Formamidine derivative **2** and the acetamidine derivative **3** both displayed highly potent *in vitro* antifungal activity. In particular, compound **2** displayed superior activity towards *C. albicans* FP633, the strain used in our model of *in vivo* candidiasis.[8] However, the hemolytic activity for both **2** and **3** was essentially unchanged relative to **1**, indicating that further modification was required. The guanidine analog **4** retained

Table 1. *In vitro* antifungal and hemolytic activity of FR901469 amidine derivatives.



Compound(R)[a]	MIC(μg/ml)						Hemolytic Activity(%)
	C. a.[b]		*C. g.*	*C. t.*		*C. p.*	
	FP633	FP579	FP587	8001	8002	15001	
FR901469 NH_2	0.39	0.39	0.39	0.39	0.39	0.39	100
2 	0.1	0.2	0.2	0.2	0.39	0.39	98
3 	0.2	0.39	0.39	0.39	0.2	0.39	92
4 	0.39	0.39	0.39	0.39	0.39	0.78	100
5 	0.2	0.39	0.2	0.39	0.2	0.39	100
7 	0.39	0.39	0.39	0.78	0.78	0.39	75
6 	0.39	0.78	0.39	0.39	0.39	0.39	87
9 	0.78	0.78	0.78	1.56	1.56	0.39	47
8 	0.39	0.78	0.39	0.78	0.39	0.78	17

a. All compounds are hydrochloride salts

b. *C. a.=Candida albicans; C.g.=Candida guilliermondi; C.t.=Candida tropicalis; C.p.=Candida parapsilosis*

strong *in vitro* activity and was also equipotent to **1** in the *in vivo* model, indicating no advantage over the natural product, since hemolysis was unchanged. Addition of polar functionality to the amidine alkyl moiety resulted in a reduction in hemolysis at 1 mg/ml. For example, acetamidomethyl derivative **7** and methoxyethyl analog **6** displayed reduced hemolysis. However, introduction of an amino group or a quarternary ammonium salt produced analogs with the lowest hemolysis at 1 mg/ml. Azetidine analog **9** and pyrazolium analog **8** were particularly attractive because these analogs displayed good *in vitro* activity and were also highly efficacious in the *in vivo* candidiasis model, showing comparable efficacy to FR901469 (Table 2).

In summary, a series of amidine analogs of the unique macrocyclic lactone FR901469 have been prepared, and

Table 2. *In vivo* activity: disseminated murine candidiasis.

Compound	ED_{50}(mg/kg)
FR901469	0.44-0.88[a]
4	0.88(1)[b]
9	0.4(0.6)
8	0.54(0.6)
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 3. *In vitro* anti-*Aspergillus fumigatus* activity.

Compound	*A. fumigatus* TIMM0063 FP1305 MIC(μg/ml)[a]
FR901469	0.5
2	0.25
5	0.5
9	1

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

derivatives with good *in vivo* antifungal efficacy and reduced hemolytic potential identified. In particular, the *in vivo* efficacy displayed by the pyrazolium-substituted amidine **8** compares favorably with amphotericin B and fluconazole, the drugs most often used clinically for candidiasis.

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