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Novel echinocandin antifungals. Part 2: Optimization of the side chain of the natural product FR901379. Discovery of micafungin

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Abstract—Further optimization of the potent antifungal activity of side chain analogs of the natural product FR901379 led to the discovery of compound **8** with an excellent, well-balanced profile. Potent compounds with reduced hemolytic potential were designed based upon a disruption of the linearity of the terphenyl lipophilic side chain. The optimized compound (**8**, FK463, micafungin) displayed the best balance and was selected as the clinical candidate.

In earlier publications from these laboratories, we have described our research on the chemical modification of natural products with potent antifungal activity.[1–3] Serious fungal infections in immunocompromised individuals are contributing to an increase in the incidence of deep-seated, disseminated mycoses, and new agents are needed.[4,5] Our efforts have been directed at the discovery of novel antifungal agents that are safer and more efficacious than known drugs.

In previous reports,[2,3] we have described the chemical modification of the side chain of the echinocandin-type 1,3-β-glucan synthase inhibitor FR901379, the only natural echinocandin with high intrinsic water solubility.[6,7] These efforts led to the discovery of a number of analogs with potent antifungal activity, and in particular, we established a relationship between the calculated log *P* (C log *P*) of the side chain moiety (calculated as the methylamine amide) and antifungal activity, as well as data which suggested that the key to reducing the strong hemolytic activity associated with the natural product and a number of the earlier derivatives was to introduce less-linear, but rigid structures into the side chain moiety.

In the work described herein, we have further optimized the antifungal activity and reduced the hemolytic activity of the derivatives by the introduction of a series of five- and six-membered heterocyclic moieties in place of the central phenyl ring of the terphenyl analog **3**, which was the most potent analog reported in the previous paper.[3] The basic design concept employed is outlined in Figure 1. We speculated that the reason for the reduced hemolysis of the compounds reported in the previous paper[3] was due to the lipophilic side chain having a less-linear, but a rigid structure, leading to reduced interaction with the membrane structure of red blood cells. It is known that the membrane of mammalian cells and fungal cells contains different amounts of unsaturated phospholipids,[8] thus suggesting that less-linear side chains may only interact weakly with the mammalian cell membrane, leading to reduced potential for hemolysis of red blood cells. Accordingly, we designed a series of analogs containing saturated central rings and heteroatoms, as well as five-membered ring heteroaromatic derivatives.

The compounds prepared in this work were synthesized as shown in Scheme 1, and as indicated in the earlier publications. Deacylated hexapeptide nucleus (**1**) was

Keywords: Antifungal; Natural product; FR901379; FR13153; FK463; Micafungin; Echinocandin; *Candida albicans*; *Aspergillus fumigatus*; Isoxazole.
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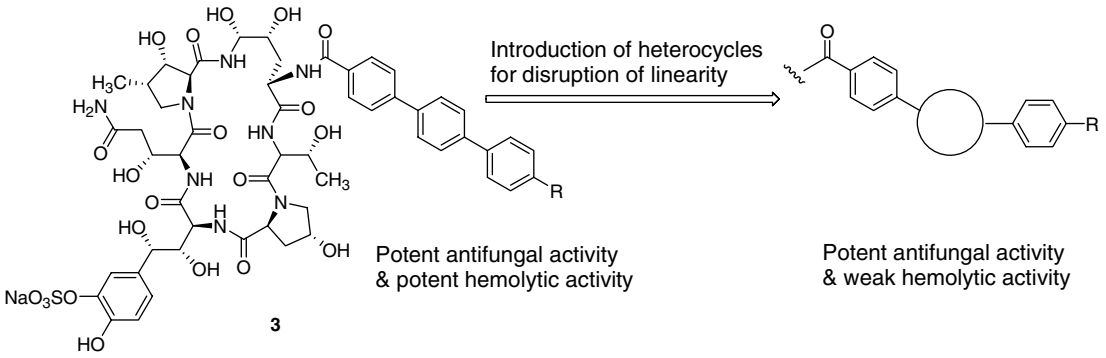
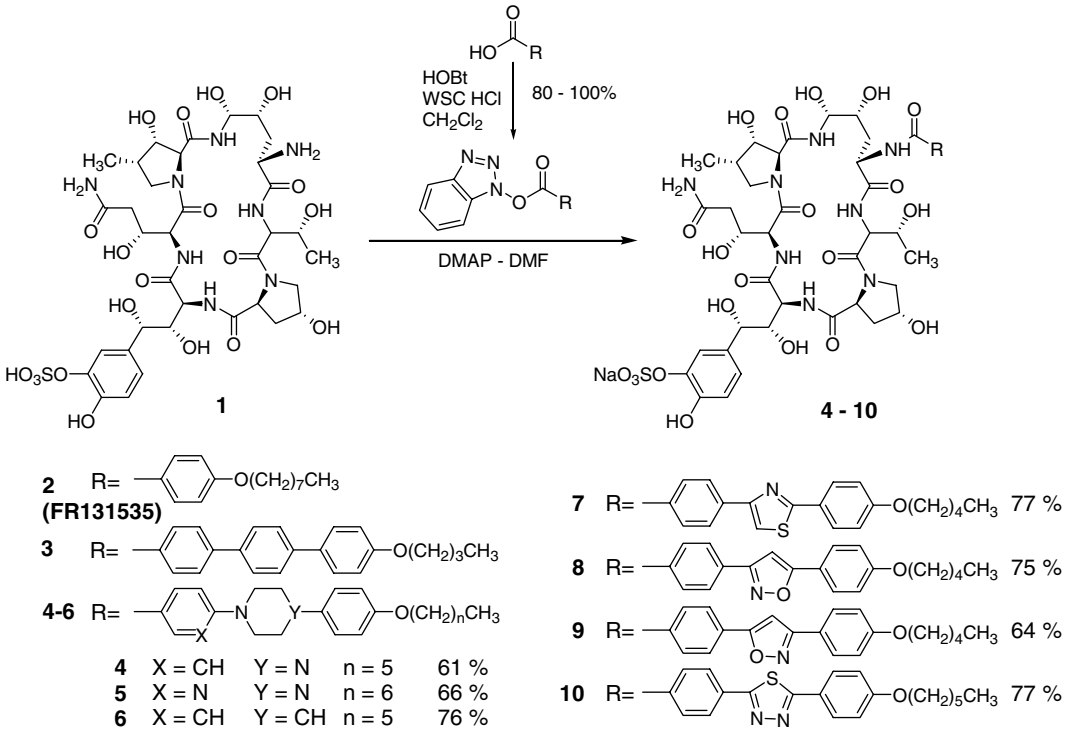


Figure 1. Side chain design for potent antifungal activity and weak hemolytic activity.

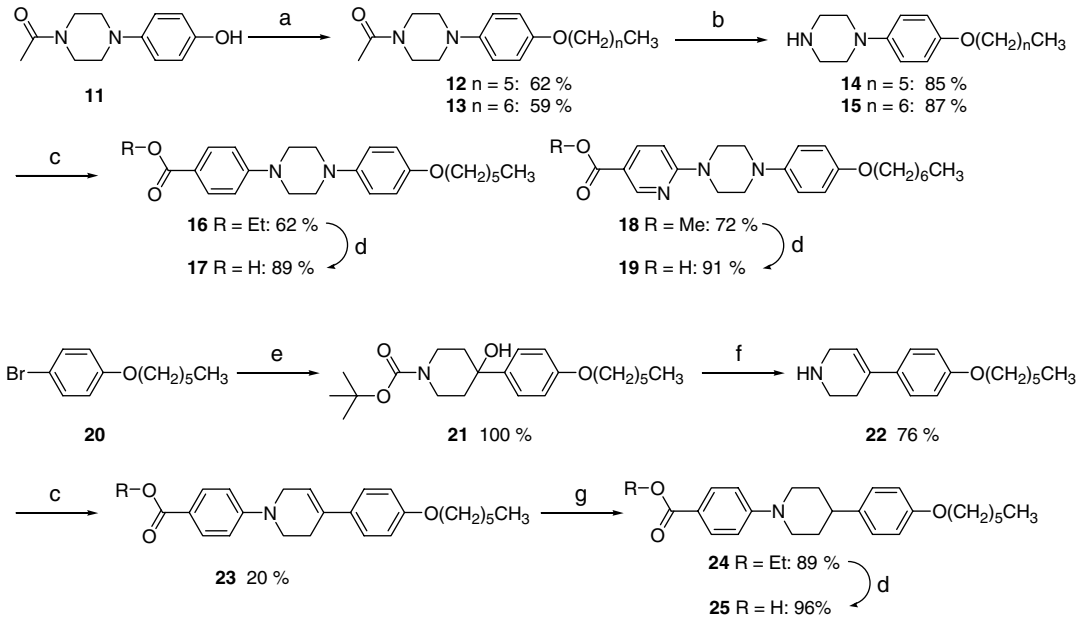


Scheme 1. Synthesis of FR131535 and novel tri-cyclic analogs.

prepared by enzymatic deacylation of FR901379.[9] Reacylation of compound **1** with the respective HOBT activated esters, prepared conveniently from the corresponding carboxylic acids, led to the crude final products as DMAP salts after trituration of the reaction mixture with ethyl acetate. Conversion to the sodium salts and ODS column chromatography eluting with acetonitrile-water mixtures and lyophilization afforded the final products as amorphous powders.

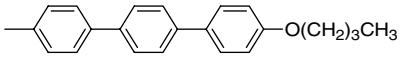
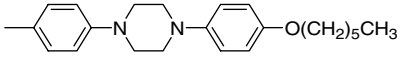
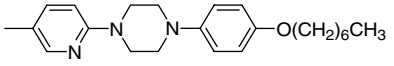
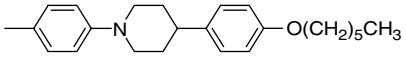
The synthesis of the novel six-membered heterocyclic ring-containing side-chain carboxylic acids is as outlined in Scheme 2. Acids **17** and **19** were obtained by hydrolysis of the corresponding ethyl or methyl ester with sodium hydroxide in THF–EtOH or THF–MeOH. The esters were obtained by coupling of the respective piperazine derivatives with ethyl 4-fluorobenzoate or methyl 4-chloronicotinate. The piperazines were prepared by alkylation and amide hydrolysis of 1-acetyl-4-(4-hydroxyphenyl)piperazine under standard conditions. Acid **25** was prepared by the reaction of the Grignard reagent prepared from 4-bromo-*n*-hexyloxybenzene with *tert*-butyl 4-oxo-1-piperidinecarboxylate, elimination of water and deprotection of the *t*-boc group with TFA, followed by coupling of the resulting piperidine derivative with ethyl 4-fluorobenzoate and alkaline hydrolysis.

Biological evaluation of the derivatives containing the novel six-membered heterocyclic ring was performed, and the results are summarized in Table 1. As shown in Table 1, the replacement of the central phenyl ring in the terphenyl side chain of compound **3** by saturated six-membered heterocyclic ring led to compounds with significantly reduced hemolysis, as indicated by the percentage of hemolysis at a drug concentration of 1 mg/ml. The introduction of nitrogen atoms to the central ring, as well as the disruption of linearity by including saturated, rather than aromatic rings, meant that the



Scheme 2. Reagents and condition: (a) alkyl bromide, K_2CO_3, DMF; (b) concd HCl; (c) ethyl 4-fluorobenzoate or methyl nicotinate, K_2CO_3, DMSO; (d) 1 N NaOH, corresponding alcohol, THF; (e) Mg, ether, then 1-*t*-boc-4-piperidone; (f) TFA, CH_2Cl_2; (g) H_2, Pd/C, THF.

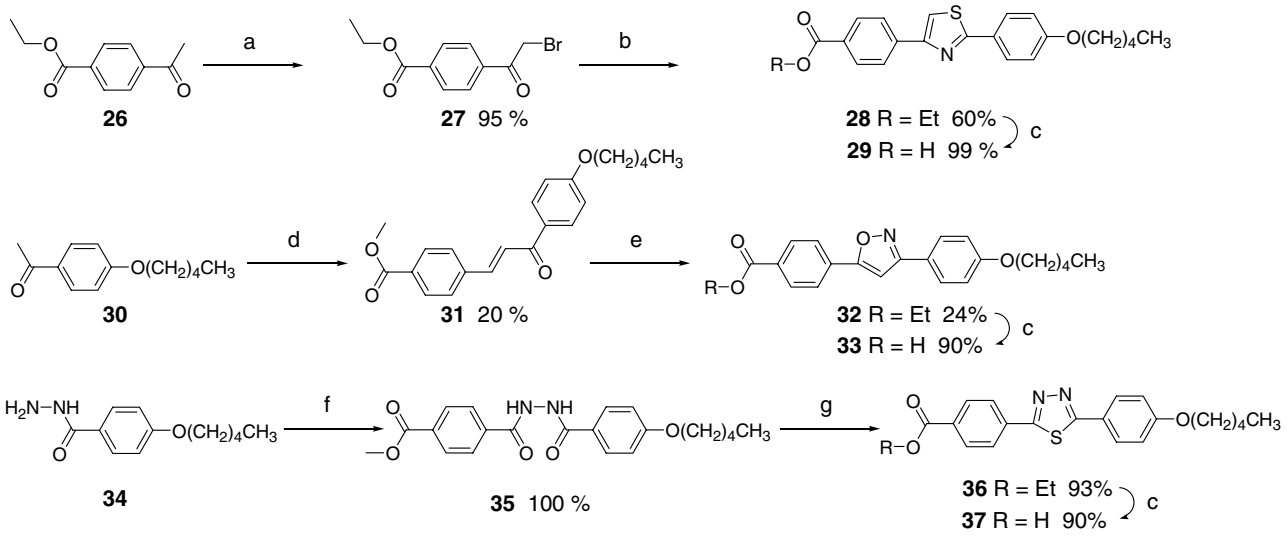
Table 1. Antifungal and hemolytic activities of compounds containing six-membered heterocyclic ring

Compound	R	C log *P*	MIC[a] (μg/ml)				Hemolysis (%, 1 mg/ml)
			C. albicans FP633		*A. fumigatus* FP1305		
			RPMI[b]	Serum[c]	RPMI[b,d]	Serum[c]	
3		6.14	0.025	1.56	0.002	1.56	79
4		5.17	0.1	3.13	0.002	1.56	<20
5		5.30	0.2	3.13	0.005	6.25	<20
6		5.87	0.05	1.56	0.003	1.56	33

[a] Minimum inhibitory concentration.
[b] In RPMI media.
[c] In mouse serum.
[d] IC_{50} values, 50% inhibitory concentration of hyphal growth.

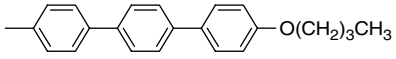
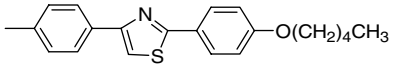
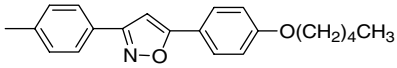
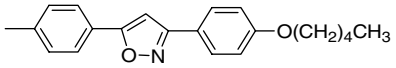
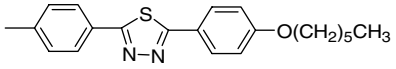
C log *P*[10] was lower, hence the C log *P* was adjusted between 5 and 6 by extending the length of the alkyl chain at the terminus of the side chain. Compounds **4**–**6** displayed comparable in vitro antifungal activity against *Candida albicans* and *Aspergillus fumigatus* to the terphenyl analog **3**. In particular, compound **4** displayed equivalent, potent activity to **3**, but very low hemolytic potential, and was selected for further evaluation. Encouraged by the results outlined in Table 1, we next turned our attention to five-membered heterocyclic rings, as their intrinsic nature means that the structures are somewhat bent away from linearity.

The novel, five-membered ring-containing side-chain carboxylic acids were prepared as shown in Scheme 3. Acid **29** was prepared from the α-bromoketone derived from ethyl 4-acetylbenzoate by reaction with pyridinium tribromide in HBr–acetic acid, followed by thiazole ring formation with the corresponding thioamide, and basic hydrolysis. The isoxazole acid **33** was prepared from 4-*n*-pentyloxyacetophenone by coupling with methyl 4-formylbenzoate using titanium tetrachloride and triethylamine, followed by the reaction of the resulting enone with hydroxylamine and oxidation with MnO_2 and hydrolysis of the ester group. Thiadiazole acid **37** was prepared by hydrolysis of the ester derived by cyclization of a diacyl hydrazide in the presence of P_2S_5 in THF. The side chain acid for compound **8** was prepared as outlined in our preliminary report, involving a 1,3-dipolar cycloaddition reaction between an acetylene



Scheme 3. Reagents: (a) pyridium tribromide, HBr–AcOH; (b) 4-pentyloxybenzthioamide, THF; (c) NaOH aq, corresponding alcohol, THF; (d) methyl 4-formylbenzoate, $TiCl_4$, Et_3N, CH_2Cl_2; (e) hydroxyamine hydrochloride, EtOH then MnO_2, dichloroethane; (f) 4-methoxycarbonylbenzoyl chloride, pyridine, THF; (g) P_2S_5, THF.

Table 2. Antifungal and hemolytic activities of compounds containing aromatic five-membered ring

Compound	R	Clog *P*	MIC[a] (μg/ml)				Hemolysis (%, 1 mg/ml)
			C. albicans FP633		*A. fumigatus* FP1305		
			RPMI[b]	Serum[c]	RPMI[b,d]	Serum[c]	
3		6.14	0.025	1.56	0.002	1.56	79
7		5.68	0.1	6.25	NT	3.13	<20
8		5.32	0.05	1.56	0.002	1.56	<20
9		5.32	0.05	1.56	0.001	0.78	38
10		5.74	0.05	0.78	0.002	0.78	42

[a] Minimum inhibitory concentration.
[b] In RPMI media.
[c] In mouse serum.
[d] IC_{50} values, 50% inhibitory concentration of hyphal growth.

Table 3. MIC in mouse serum, in vivo antifungal effect and PK profiles of selected compounds

Compound	*C. albicans* FP633		*A. fumigatus* FP1305		PK (mice, 5 mg/kg, iv)		
	Serum MIC[a] (μg/ml)	ED_{50}[b] (mg/kg)	Serum MIC[a] (μg/ml)	ED_{50}[b] (mg/kg)	C_{30min} (μg/ml)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (μg ml/ml)
2	25	3.71	12.5	4.31	30.3	2.0	109
3	1.56	0.447	1.56	NT[c]	NT[c]	NT[c]	NT[c]
4	3.13	0.395	1.56	0.531	18.8	3.5	105
8	1.56	0.325	1.56	0.228	10.1	5.1	78

[a] Minimum inhibitory concentration in mouse serum.
[b] Based on survival at 2 weeks after infection.
[c] Not tested.

and a nitrile oxide.[11] Biological evaluation of the derivatives containing the novel five-membered heterocyclic ring was performed, and results are summarized in Table 2. As indicated in Table 2, thiazole **7**, isoxazole **8**, isoxazole isomer **9**, and thiadiazole **10** all displayed reduced hemolytic potential compared to the terphenyl compound **3**. In particular, compound **8** displayed potent in vitro antifungal activity and low hemolytic potential and was selected for further evaluation.

Table 3 indicates the in vivo antifungal effects of the selected compounds (**4**, **8**) against the principal fungal species causing life-threatening infections in humans, *C. albicans* and *A. fumigatus*, along with pharmacokinetic (PK) data in mice. It can be clearly seen from the data in this table that a more potent serum MIC (minimum inhibitory concentration) gives superior in vivo efficacy for *C. albicans* infections. As indicated in the earlier paper[2] FR131535 displays comparable in vivo efficacy to fluconazole against *C. albicans*. Compounds **4** and **8** showed about 10 times superior in vivo efficacy compared to FR131535. Against *A. fumigatus* systemic infection, compound **8**, in particular showed excellent efficacy, displaying an ED_{50} value of 0.228 mg/kg. The data in Table 3 suggest that the potent efficacy in the *Candida* model results from the combination of good serum MIC and a high AUC in the PK study, whereas for good efficacy in the *Aspergillus* model, it is necessary to have a good balance of MIC, elimination half-life. Compound **8** was selected for further evaluation as a clinical candidate.

In summary, further optimization of the acyl side chain of a series of analogs of the water-soluble 1,3-β-glucan synthase inhibitor FR901379 led to the discovery of an analog (**8**) with potent in vivo activity against both *C. albicans* and *A. fumigatus*. This analog (FK463, micafungin) was selected as a clinical candidate and detailed in vivo and clinical trial results have been disclosed. The key to success was the introduction of various heterocycles in place of the central phenyl ring of the terphenyl analog **3** as such modifications resulted in potent antifungal activity and significantly reduced hemolysis.

Acknowledgment

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Supplementary data

Representative synthetic procedure and spectrum data for micafungin (**8**) and determination method for MICs in mouse serum are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.093.

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