Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 399–402

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

FR131535, a Novel Water-Soluble Echinocandin-Like Lipopeptide: Synthesis and Biological Properties

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Received 5 October 2000; accepted 23 November 2000

Abstract—The synthesis and biological properties of a novel water-soluble echinocandin-like lipopeptide, FR131535, are described. This compound displayed potent in vitro and in vivo antifungal activities. The hemolytic activity of FR901379 was reduced by replacing the acyl side chain. This compound showed good water-solubility, comparable to the natural product FR901379.

Introduction

Fungal infections are an increasingly important problem, particularly in immunocompromised and AIDS patients, and current therapies are limited to amphotericin B, azole compounds, and flucytosine. However, these drugs have problems regarding spectra of activity, toxicity, or resistance. Furthermore, the emergence of multi-azole-resistant strains of *Candida* is leading to many serious fungal infections. Much interest has been focused on developing safer and more effective antifungal agents, however, despite many efforts, novel and attractive parental drugs are not yet on the market.[1]

1,3-β-Glucan is one of the components primarily responsible for the structure of the fungal cell wall and is not present in mammalian cells,[2] and it is therefore an attractive target for antifungal agents. Indeed, echinocandin-like lipopeptides[3] and papulacandin B,[4] which are known 1,3-β-glucan synthase inhibitors, show potent antifungal activity against *Candida albicans* and *Aspergillus fumigatus*. Recent studies on the cell wall composition of *Pneumocystis carinii*, known as a cause of *P. carinii* pneumonia and now classified as a fungus, have revealed that 1,3-β-glucan is an important component of the cell wall in the cyst form. Thus, inhibitors of glucan synthesis are also expected to be useful as potent anti-*Pneumocystis* drugs.

In the course of our screening, we isolated FR901379 (WF11899A)[5] (**1**) and related compounds, which are novel water-soluble echinocandin-like lipopeptides from the culture broth of *Coleophoma empetri* F-11899. FR901379 is a cyclic antifungal lipopeptide that has a hexapeptide nucleus with sulfonate, and bears a fatty acid acyl group attached to the N-terminus. FR901379 showed potent in vivo antifungal activity against *C. albicans*. However, in a disseminated aspergillosis mouse model, this compound was ineffective in prolonging survival (unpublished data). Studies on aculeacin and echinocandin B have demonstrated that the different fatty acid acyl side chains of these otherwise identical cyclic peptide antibiotics were important determinants of their antifungal activity and toxicity. Recently, Lilly reported that cilofungin, an echinocandin B derivative with a 4-(*n*-octyloxy) benzoyl side acyl chain, showed a correlation between in vivo efficacy against *Aspergillus fumigatus* and the inhibitory effect of cilofungin on the *A. fumigatus* glucan synthase.[6] Concerning toxicity, it is known that fatty acids with a linear side chain of carbon atoms show hemolytic activity in vitro. The acyl side chain in the FR901379 molecule is a palmitoyl group

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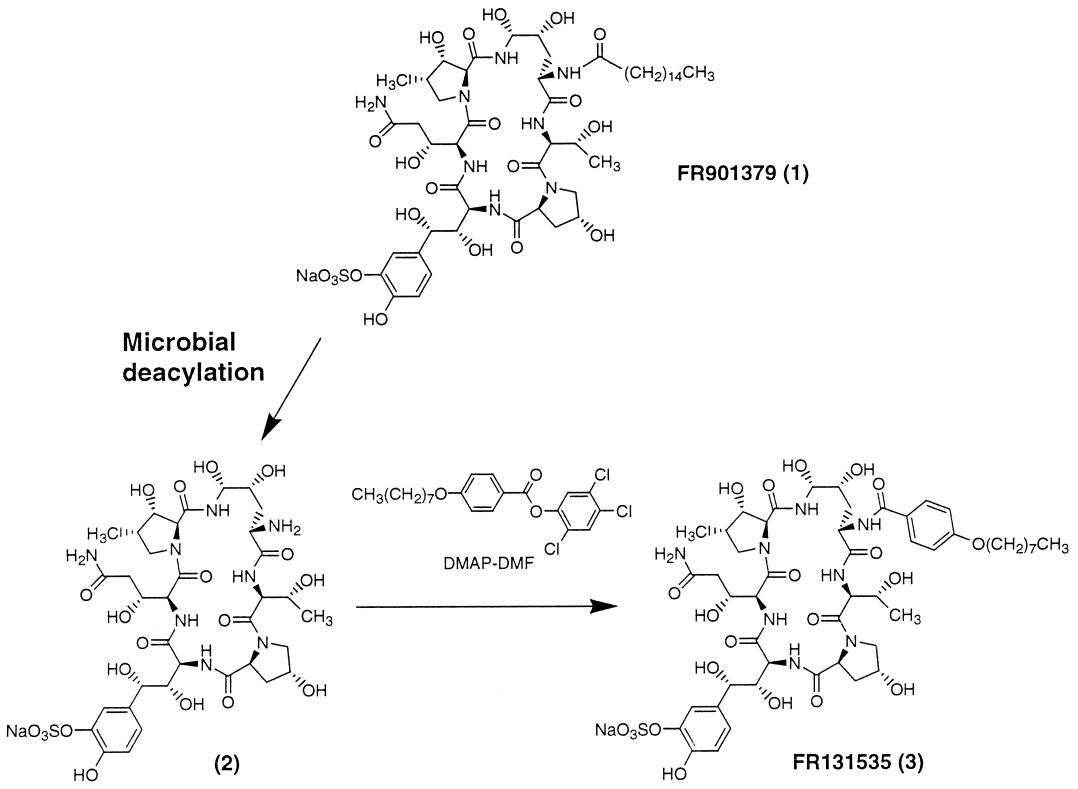
0960-894X/01/$ - see front matter
PII: S0960-894X(00)00677-6



Scheme 1. Synthesis of FR131535.

with a straight chain of 15 carbon atoms. This compound showed rather strong hemolytic activity in vitro compared to other echinocandins. We thus aimed to reduce the hemolytic activity of FR901379 by replacing the acyl side chain. As a result, we have obtained the novel echinocandin-like lipopeptide FR131535 (**3**). In this paper, we describe the synthesis and biological properties of FR131535.

Synthesis

The synthesis of this novel echinocandin-like lipopeptide is outlined in Scheme 1. The acyl side chain was prepared from 1-bromooctane and 4-hydroxybenzoic acid. 2,4,5-Trichlorophenyl 4-(*n*-octyloxy) benzoate was then obtained from 4-(*n*-octyloxy) benzoic acid and 2,4,5-trichlorophenol using DCC in ether. The cyclic peptide nucleus **2** was prepared by the method developed by Debono et al.[7] Acylation of **2** with the activated ester in DMF in the presence of DMAP, afforded crude FR131535, which was converted to the sodium salt using DEAE-Toyopearl (Cl type) resin and then purified by YMC GEL ODS-AM120-S50 column chromatography. The eluate was lyophilized to give FR131535 as a white powder. The structure of FR131535 was confirmed by elemental analysis, FAB-MS, IR and ^{1}H NMR.[8]

Biological Properties

The water-solubility of FR131535 was as high as that of FR901379 even after replacement of the acyl side chain (Table 1). Echinocandin B and cilofungin did not dissolve in water under the same conditions. FR131535 inhibited 1,3-β-glucan synthase prepared from *Candida albicans* 6406[9] with an IC_{50} value of 2.8 μg/mL (Table 2) and the inhibition was non-competitive (K_i 4.0μM). This compound displayed broad spectrum and potent activity against a variety of fungal species by the microbroth dilution method (Table 3). FR131535 was active against

Table 1. Solubility of FR131535 and other compounds[a]

Compound	Solubility in water (mg/mL)
FR131535	>50
FR901379	>50
Echinocandin B	0.008
Cilofungin	0.1

[a]Solubility of compounds were measured by HPLC (column: LiChrospher 100 RP18 (5 μm); solvent system: 1 mL/min, detection A210 nm, 50% aqueous acetonitrile containing with 0.5% $NH_4H_2PO_4$).

Table 2. IC_{50} value of FR131535 and other compounds on 1,3-β-glucan synthase from *Candida albicans* 6406

	IC_{50} (μg/mL)
FR131535	2.8
FR901379	0.7
Echinocandin B	2.6
Nikkomycin X	>100

Table 3. In vitro antifungal activities of FR131535 and other compounds by microbroth dilution method[a]

Test organism	IC_{50} (μg/mL)				
	FR131535	FR901379	Echinocandin B	Cilofungin	Amphotericin B
Candida albicans FP578	0.31	0.025	0.24	0.36	0.39
Candida albicans FP582	0.39	0.004	0.16	0.24	0.55
Candida albicans FP629	0.47	0.018	0.24	0.47	0.39
Candida albicans FP633	0.47	0.018	0.12	0.39	0.39
Candida krusei YC109	1.9	0.14	1.1	3.2	1.6
Candida tropicalis YC118	0.47	0.09	0.47	0.94	0.62
Candida utilis YC123	1.35	0.03	0.55	0.94	0.47
Aspergillus fumitgatus FD050	<0.003[b]	1.3	5.0	<0.003[b]	0.62
Aspergillus niger ATCC9642	0.16	0.004	5.0	0.08	1.9
Cryptoccocus neoformans YC203	>2.5	>2.5	>2.5	>2.5	0.39

[a]Medium: YNBD broth; incubation: *Candida* and *Aspergillus* spp. were cultured at 37 °C for 22 h, *Cryptococcus neoformans* was cultured at 30 °C for 48 h; inoculum size: 1×10^5 CFU/mL.
[b]Inhibition rate against test organism was between 30 and 50% in the range of 0.003–5.0 μg/mL.

Table 4. In vivo antifungal activities of FR131535 and other compounds against *Candida albicans* in a murine infection model[a]

Compound	ED_{50} (mg/kg)	
	Day 10	Day 14
FR131535	3.0	3.5
FR901379	1.1	2.9
Echinocandin B	>10	>10
Cilofungin	5.0	>10
Fluconazole	<1.0	2.4

[a]Mice: ICR strain, female, 4 weeks old, five mice per group; infection: 2.5×10^6 cells/mL of *Candida albicans* FP633 suspended in saline were intravenously injected; treatment: subcutaneous administration at 1 h after injection and once a day for three consecutive days.

most *Candida* species and all *Aspergillus*. It was, however, inactive against *Cryptococcus neoformans*.

The protective efficacy of FR131535 administered subcutaneously against murine systemic infection with *C. albicans* was examined. The ED_{50}s of FR131535 at days 10 and 14 after challenge were 3.0 and 3.5 mg/kg, respectively. As shown in Table 4, this compound was superior to echinocandin B and cilofungin in the above model. Furthermore, the in vivo efficacy of FR131535 was almost as potent as fluconazole. Fluconazole is fungistatic against fungal pathogens, while FR131535 is an inhibitor of cell wall biosynthesis and fungicidal against *Candida* species (data not shown). FR131535 was administered to *P. carinii*-infected nude mice to examine its efficacy on the growth of *P. carinii* cysts in the lungs. As shown in Table 5, the number of *P. carinii* cysts in the lungs of infected mice was very high at the start of drug administration. At 3 weeks after administration, a significant therapeutic effect was observed with a daily dose of 10 mg/kg.[10] Furthermore, hemolytic activity of FR131535 by both Akaishi[11] and RBC[12] methods was greatly improved in comparison with that of FR901379 and echinocandin B, as shown in Table 6. The acute toxicity of FR131535 was weak as shown by no toxic symptoms at day 7 after an iv dose of 500 mg/kg in ICR mice (female, 4 weeks old).

Table 5. *Pneumocystis carinii* in lungs after drug treatment

Time of treatment	Cysts		Number of lungs positive for trophozoites/number tested	Number of lungs positive for lesions/number tested
	Number of lungs positive/number tested	Number of cysts/lung (log_{10})		
Day 0	5/5	4.7±0.2	5/5	5/5
3 weeks				
Saline	5/5	5.4±0.3	5/5	5/5
FR131535	0/5	<1.8[a,b]	0/5	0/5
FR901379	0/5	<1.8[b]	0/5	0/5
ST[c]	0/5	<1.8[b]	0/5	0/5
8 weeks				
Saline	5/5	6.3±0.1	5/5	5/5
FR131535	4/5	2.3±0.3[b]	0/5	0/5
FR901379	2/5	2.0±0.3[b]	0/5	0/5
ST[c]	5/5	4.1±0.3[b]	0/5	0/5

[a]Below the level of detection.
[b]Significantly different from the values for the saline-treated control group ($P<0.01$) by Student *t* test.
[c]ST: sulfamethoxazole and trimethoprim.

Table 6. Hemolytic activity of FR131535 and other compounds

Compound	Akaishi method (LC_{30})[a]	RBC[b] method (MLC)[c]	
	Human	Human	Mouse
FR131535	>500	>1000	>500
FR901379	160	31	31
Echinocandin B	350	125	62
Cilofungin	>500	>1000	>500

[a]LC30: lytic concentration 30%.
[b]RBC: red blood cell.
[c]MLC (minimum lytic concentration): MLC was defined as the lowest concentration of drug that lysed red blood cells.

Conclusions

In this paper, we have reported the synthesis and biological properties of FR131535. FR131535 inhibited 1,3-β-glucan synthase in a non-competitive mode and showed potent activities against *Candida albicans*, *Aspergillus fumigatus*, and *Pneumocystis carinii* comparable to the natural product, FR901379. Furthermore, FR131535 maintained good water-solubility and reduced only the hemolytic activity compared to FR901379. FR131535 is expected to be an attractive antifungal agent, especially as an anti-*Pneumocystis* drug. Further evaluation of FR131535 and synthetic studies on more effective derivatives are currently ongoing.[13]

Acknowledgements

The authors are grateful to Dr. David Barrett, Medicinal Chemistry Research Laboratories of Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan for assistance in the preparation of this manuscript. This paper is dedicated to the memory of Dr. Takumi Yatabe, who died at the age of 37 whilst working on the synthesis of new drugs in our Exploratory Research Laboratories.

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8. Elemental analysis: calcd for $C_{50}H_{71}N_8SO_{22}Na\cdot 5H_2O$ C 46.87, H 6.37, N 8.74, Na 1.79 (%). Found: C 46.80, H 6.13, N 8.78, Na 1.81 (%). FAB-MS *m/z* 1213 (M + Na). IR (KBr) cm^{-1} 3330, 2900, 2850, 1620, 1500, 1430, 1270, 1250, 1170, 1110, 1080, 1040, 960, 940, 880, 840, 800, 750, 710 cm^{-1}. ^1H NMR (CD_3OD, 200 MHz) δ: 7.78 (2H, d, $J=8$ Hz), 7.31 (1H d, $J=2$ Hz), 7.03 (1H, dd, $J=2$ Hz and 8 Hz), 6.96 (2H, d, $J=8$ Hz), 6.87 (1H, dd, $J=8$ Hz), 5.33 (1H, d, $J=3$ Hz), 5.08 (1H, d, $J=4$ Hz), 4.99 (1H, d, $J=3$ Hz), 4.80–3.20 (17H, m), 2.83 (1H, m), 2.65–2.30 (4H, m), 2.22–1.90 (2H, m), 1.79 (2H, m), 1.56–1.25 (10H, m), 1.19 (3H, d, $J=6$ Hz), 1.06 (3H, d, $J=6.5$ Hz), 0.90 (3H, t, $J=6.5$ Hz).
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