Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1474–1477
Bioorganic & Medicinal Chemistry Letters

Novel echinocandin antifungals. Part 1: Novel side-chain analogs of the natural product FR901379

Masaki Tomishima,[a,*] Hidenori Ohki,[a] Akira Yamada,[a] Katsuyuki Maki[b] and Fumiaki Ikeda[b]

[a]*Medicinal Chemistry Research Laboratories, Astellas Pharma Inc., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan*
[b]*Medicinal Biology Research Laboratories, Astellas Pharma Inc., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan*

Received 11 September 2007; revised 20 December 2007; accepted 22 December 2007
Available online 28 December 2007

Abstract—A series of novel acylated analogs of the novel water-soluble echinocandin FR901379 have been prepared and evaluated for antifungal and hemolytic activity. A relationship between antifungal activity and lipophilicity of the acyl side chain, expressed as *C*log *P* was demonstrated, and an analog (**3c**) with 5.5- to 8-fold superior in vivo activity relative to the previously disclosed 4-(*n*-octyloxy)benzoyl side chain analog, FR131535 obtained.

Fungal diseases in humans are serious in immunocompromised individuals who are susceptible to many opportunistic systemic mycoses.[1] An inability of the host immune response to fight off attack from normally benign environmental fungal pathogens leads to an increase in the incidence of deep-seated, disseminated mycoses.[2] Difficulty in diagnosis of fungal infections and delays in initiation of treatment are important factors in morbidity and mortality, however, drugs for effective treatment are also in short supply. A large effort has been dedicated to the discovery of novel antifungal agents that are safer and more efficacious than known drugs, however the difficulty in obtaining a good safety profile has meant that the number of available drugs is still actually quite small. Available drugs include the polyene natural product amphotericin B and various lipid formulations, azole compounds such as fluconazole and voriconazole, and the recently introduced candins.[3]

Our research in this area started with a search for antifungal natural products.[4] In particular, we focused on 1,3-β-glucan synthesis as an attractive target, since this polysaccharide is a primary component of the fungal cell wall, a structure with no counterpart in mammalian cells.

We have already reported the discovery and chemical modification of several unique antifungal natural products that express antifungal activity by inhibition of the synthesis of 1,3-β-glucan, including FR901379, the first natural echinocandin with outstanding intrinsic water solubility due to the presence of sulfonate moiety on the hexapeptide nucleus.[5,6] This natural product displayed good antifungal activity, but had problems with hemolysis and relatively weak activity against certain molds, e.g. *Aspergillus fumigatus*. In earlier work reported by our group, the analog FR131535 (**2**), a novel water soluble echinocandin-like lipopeptide, was obtained from FR901379 through a semi-synthetic sequence involving microbial deacylation and chemical reacylation with an octyloxybenzoyl side chain.[7] This derivative showed potent activities against *C. albicans*, *A. fumigatus*, and *P. carinii*, comparable to the natural product, but showed the major advantage of dramatically reduced hemolytic activity compared to FR901379. The activity was comparable, but not superior to fluconazole against *C. albicans* infection in a murine infection model, hence further optimization of activity was required.

These early efforts clearly established that semi-synthetic modification of FR901379 could lead to improved antifungal activity, especially against *A. fumigatus*, whilst at the same time, reducing the hemolytic potential associated with the lipophilic palmitoyl side chain and maintaining the excellent water-solubility, a feature critical for development of a parenteral formulation.

Keywords: Antifungal; Natural product; FR901379; FR13153; Echinocandin; *Candida albicans*; *Aspergillus fumigatus*.
* Corresponding author. Tel.: +81 6 6390 1285; e-mail: masaki.tomishima@jp.astellas.com

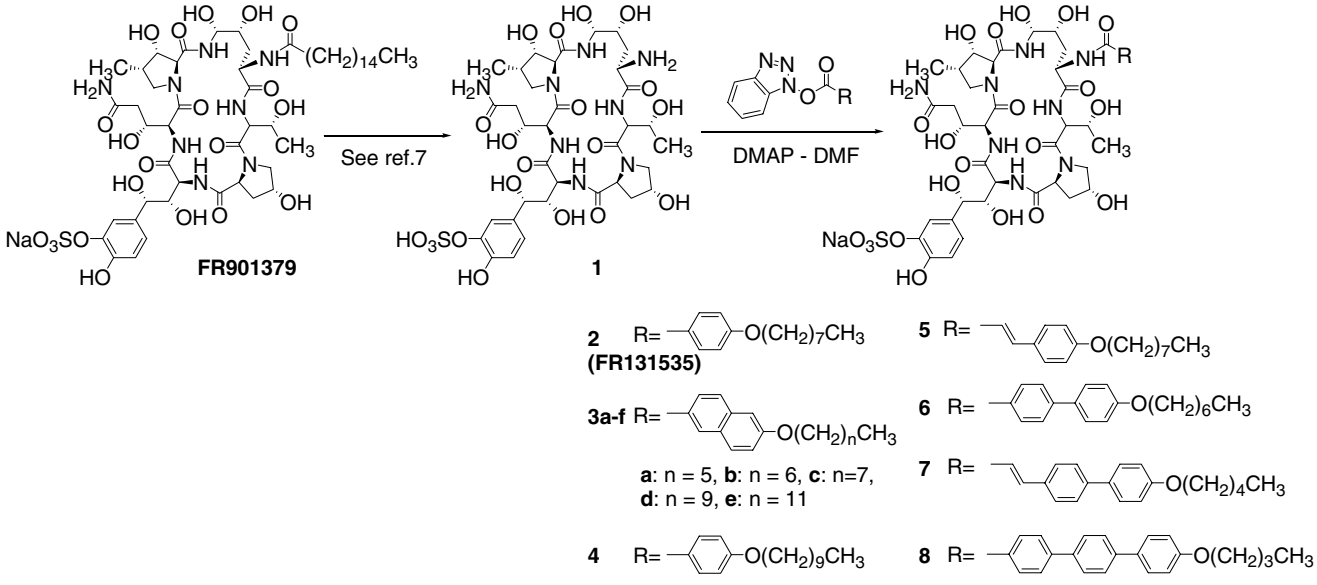
0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.12.062

Accordingly, we thus aimed to further improve antifungal activity whilst maintaining low hemolytic activity and good water solubility, by optimizing the acyl side chain. As a result, we have discovered a series of novel naphthyl side chain analogs (**3**) which displayed improved antifungal activity compared to FR131535 (**2**) whilst maintaining the low hemolytic activity and excellent water solubility. In this letter, we wish to describe the synthesis and biological activities of naphthyl side-chain derivatives (**3**) as well as related analogs **4**–**8** of FR901379 and structure–activity relationship (SAR) leading to identification of the optimal lipophilicity for expression of excellent activity.

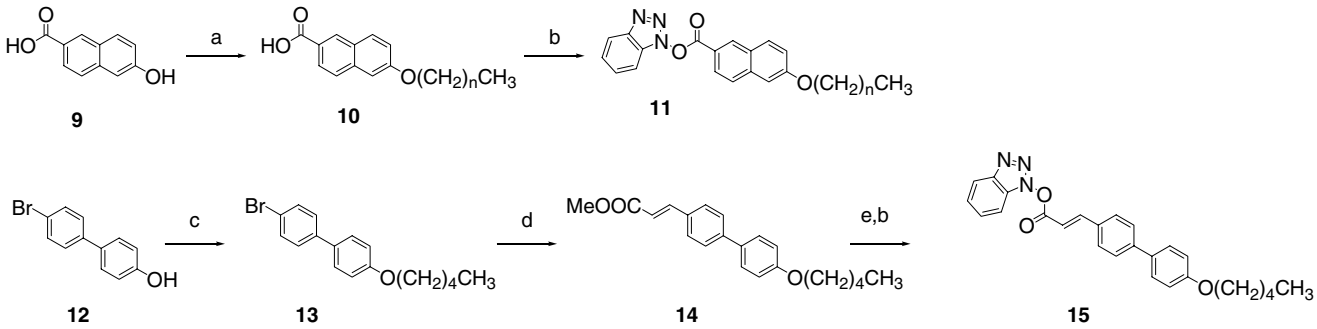
The compounds prepared in this work were synthesized as shown in Scheme 1. The deacylated hexapeptide nucleus (**1**) was prepared by enzymatic deacylation of FR901379.[8] Acylation of **1** with the respective activated ester in DMF in the presence of base (DMAP or Hunigs base) led to the crude final products after trituration of the reaction mixture with ethyl acetate. Reactions were monitored by HPLC. Conversion of the crude products to sodium salts with DEAE-Toyopearl (Cl-type) resin and ODS column chromatography led to lyophilized final products as amorphous powders.[9]

The side-chain activated esters were prepared from the corresponding carboxylic acids, which were readily prepared over several steps from commercially available materials. Scheme 2 shows the synthesis of activated esters **11** and **15** as typical examples. Alkylation of the phenol group of **9** with the corresponding alkyl bromides followed by activation as the HOBT ester gave the activated esters **11** in good yield. Compound **12** was alkylated with pentyl bromide, converted to acrylate derivative **14** by Heck reaction, and then converted to the activated ester **15** by hydrolysis and HOBT ester formation using standard conditions.

Our first efforts at optimizing the acyl side chain were aimed at establishing the optimal lipophilicity for the side chain. The series of naphthyl analogs **3a**–**e** shown in Table 1 were designed and prepared. As can be clearly seen from the data in Table 1 and Figure 1, for the anti-



Scheme 1. Synthesis of FR131535 and its analogs.



Scheme 2. Synthesis of selected activated side chain esters. Reagents and conditions: (a) $CH_3(CH_2)_nBr$, 10% NaOH aq, DMSO, 60–80%; (b) WSCD·HCl, HOBt, CH_2Cl_2, 80–100%; (c) $CH_3(CH_2)_4Br$, K_2CO_3, DMF, 72%; (d) methyl acrylate, $Pd(OAc)_2$, P(o-tol)$_3$, Et_3N, 100%; (e) 1 N NaOH, EtOH, 100%.

Table 1. *C*log *P*, antifungal and hemolytic activity of naphthalene analogs

Compound	*C*log *P*	*C. albicans* FP633 MIC[a] (μg/ml)	*A. fumigatus* FP1305 IC_{50}[b] (μg/ml)	Hemolysis LC_{30}[c] (mg/ml)
2 (FR131535)	4.77	0.78	0.015	>10
3a ($n = 5$)	4.74	0.78	0.025	>10
3b ($n = 6$)	5.27	0.39	0.004	>10
3c ($n = 7$)	5.80	0.1	0.003	>10
3d ($n = 9$)	6.86	0.2	0.015	0.75
3e ($n = 11$)	7.91	0.78	NT[d]	0.41

[a] Minimum inhibitory concentration.
[b] 50% inhibitory concentration of hyphal growth.
[c] Lytic concentration 30%.
[d] NT, not tested.

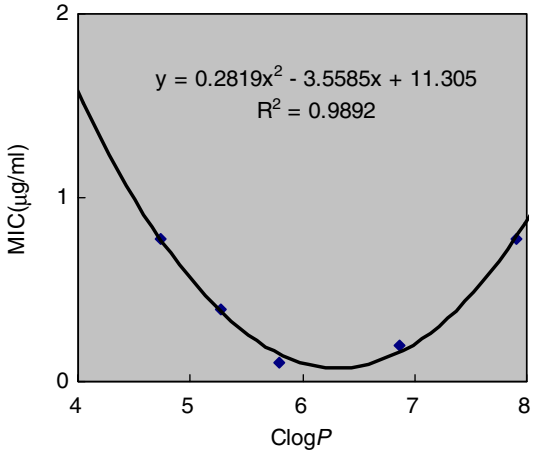


Figure 1. Correlation between *C*log *P* and MIC against *C. albicans* FP633.

fungal activity against *C. albicans*, as expressed by minimum inhibitory concentration (MIC), was at a maximum when the calculated log *P* (*C*log *P*) of the side-chain (calculated as the methylamide analog) was about 6. We also studied the correlation between other parameters and MIC, however no parameter correlated with MIC except for *C*log *P* and parameters which are correlated with *C*log *P*, such as the length of side chain. In particular, activity against *C. albicans* was optimal for **3c**, showing an MIC of 0.1 μg/ml. It is noteworthy that whilst the hemolytic potential of **2** and the naphthyl analogs up to $n = 7$ was weak, with LC_{30} values of >10 mg/ml, the $n = 9$ analog (**3d**) and the $n = 11$ analog (**3e**) showed potent hemolytic potential, as well as reduced antifungal activity. The data in Table 1 clearly indicate that antifungal activity and hemolytic activity are not linearly related and that at the optimum lipophilicity for activity, hemolysis can be weak.

Having established that a *C*log *P* of 6 was optimal for the activity of the naphthyl series of analogs, we turned attention to further enhancement of antifungal activity and introduced a series of rigid side chain analogs (**4**–**8**) as shown in Table 2. Interestingly, addition of a *trans* olefin linker between the carbonyl group and the phenyl ring of **2** led to the analog **5** with comparable activity.

Table 2. *C*log *P*, antifungal, and hemolytic activity of aromatic ring conversion analogs

Compound	*C*log *P*	*C. albicans* FP633 MIC[a] (μg/ml)	*A. fumigatus* FP1305 IC_{50}[b] (μg/ml)	Hemolysis LC_{30}[c] (mg/ml)
2 (FR131535)	4.77	0.78	0.015	>10
4	5.80	0.2	0.005	>10
5	5.38	0.78	NT[d]	>10
3c	5.80	0.1	0.003	10
6	5.90	0.1	0.002	0.91
7	5.68	0.05	0.002	3.95
8	6.14	0.0125	0.002	0.37

[a] Minimum inhibitory concentration.
[b] 50% inhibitory concentration of hyphal growth.
[c] Lytic concentration 30%.
[d] NT, not tested.

Table 3. In vivo antifungal activities of FR131535 and compound **3c**

Compound	ED_{50}[a] (mg/kg)	
	C. albicans FP633	*A. fumigatus* 8004
2 (FR131535)	3.71	4.31
3c	0.46	0.79

[a] Based on survival at 4 weeks after infection.

Whilst the naphthyl analog **3c** did not show strong hemolysis, the linear biphenyl analog **6** showed strong hemolytic potential ($LC_{30} = 0.91$ mg/mL). This result suggested a relationship between linearity of the acyl side chain and the potential for hemolysis. Introduction of an olefin linker led to analog **7**, which had reduced hemolysis and comparable antifungal activity, whereas the completely linear tertphenyl analog **8** displayed potent hemolysis.

The compound with the best balance of antifungal activity and hemolytic potential (**3c**) was compared with FR131535 (**2**) in in vivo models of *C. albicans* and *A. fumigatus* infections. As shown in Table 3, **3c** displayed approximately 5.5- to 8-fold superior activity compared to **2** in terms of ED_{50} values for both fungal species.

In summary, a series of acylated analogs of the unique echinocandin natural product FR901379 have been prepared. We established that antifungal activity correlates well with calculated log *P*, and several derivatives displayed potent in vivo antifungal efficacy and reduced hemolytic potential. We also obtained evidence which suggested that a key for reducing the hemolytic potential of these lipophilic peptides involves introduction of less-linear rigid structures to the acyl side chains. Further SAR and optimization studies will be reported in subsequent publications.

References and notes

1. Dismukes, W. E. *Clin. Infect. Dis.* **2006**, *42*, 1289.
2. (a) Patterson, T. F. *Lancet* **2005**, *366*, 1013; (b) De Pauw, B. E. *Surg. Infect.* **2006**, *7*, S93.
3. Spanakis, E. K.; Aperis, G.; Mylonakis, E. *Clin. Infect. Dis.* **2006**, *43*, 1060.

4. Barrett, D. *Biochim. Biophys. Acta* **2002**, *1587*, 224.
5. Iwamoto, T.; Fujie, A.; Sakamoto, K.; Tsurumi, Y.; Shigematsu, N.; Yamashita, M.; Hashimoto, S.; Okuhara, M.; Kohsaka, M. *J. Antibiot.* **1994**, *47*, 1084.
6. Iwamoto, T.; Fujie, A.; Nitta, K.; Hashimoto, S.; Okuhara, M.; Kohsaka, M. *J. Antibiot.* **1994**, *47*, 1092.
7. Fujie, A.; Iwamoto, T.; Sato, B.; Muramatsu, H.; Kasahara, C.; Furuta, T.; Hori, Y.; Hino, M.; Hashimoto, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 399.
8. (a) Boeck, L. D.; Fukuda, D. S.; Abbott, B. J.; Debono, M. *J. Antibiot.* **1989**, *42*, 382; (b) Debono, M.; Abbott, B. J.; Fukuda, D. S.; Barnhart, M.; Willard, . K. E.; Molloy, R. M.; Michel, K. H.; Turner, J. R.; Butler, T. F.; Hunt, A. H. *J. Antibiot.* **1989**, *42*, 389.
9. Representative synthetic procedure and spectrum data for title compound **3c**.
   To a solution of 6-hydroxy-2-naphthoic acid (1 g) in DMSO (17 ml) was added 2.5 M NaOH (4.25 ml) and stirred at 80 °C for 15 min. To this mixture *n*-octyl bromide (0.918 ml) was added and stirring continued for 2 h. The mixture was cooled to room temperature and adjusted to pH 3 with concd HCl. The precipitate was collected by filtration and dried to give 6-*n*-octyloxy-2-naphthoic acid (0.91 g). To a suspension of the acid (0.85 g) and 1-hydroxybenzotriazole (0.38 g) in ethyl acetate (26 ml) was added WSCD·HCl (0.70 g) and the mixture was stirred at ambient temperature overnight. The mixture was diluted with ethyl acetate, washed with water and brine, dried over magnesium sulfate, and evaporated under reduced pressure. The residue was triturated with isopropyl ether and the solid was collected by filtration and dried to give 1-[6-(octyloxy)-2-naphthoyl]-1H-benzotriazole 3-oxide **11** (0.74 g). A mixture of cyclic hexapeptide **1** (0.5 g), the activated ester **11** (0.27 g), and DMAP (0.083 g) was stirred at ambient temperature overnight in DMF (1.5 ml). To the mixture was added ethyl acetate and the precipitate was collected by filtration. The product was purified by reverse-phase HPLC and the product lyophilized to give compound **3c** (214 mg).
   FAB-MS: *e*/*z* = 1264(M+Na); ^{1}H NMR (DMSO-d_6 + D_2O, δ): 0.86 (3H, t, J = 6.8 Hz), 0.97 (3H, d, J = 6.8 Hz), 1.06 (3H, d, J = 6.8 Hz), 1.2–1.5 (10H, m), 1.6–2.0 (5H, m), 2.2–2.6 (4H, m), 3.18 (1H, m), 3.6–3.9 (1H, m), 4.0–4.6 (15H, m), 4.84 (1H, d, J = 3 Hz), 4.90 (1H, d, J = 3 Hz), 5.11 (1H, d, J = 3 Hz), 6.76 (1H, d, J = 8.3 Hz), 6.93 (1H, d, J = 8.3 Hz), 7.13 (1H, s), 7.25 (1H, d, J = 8.3 Hz), 7.39 (1H, s), 7.8–8.0 (3H, m), 8.44 (1H, s); IR (Nujol, cm^{-1}): 3300, 1620, 1500.