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FR290581, a novel sordarin derivative: Synthesis and antifungal activity

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ABSTRACT

Sordarin is a unique natural product antifungal agent that is an inhibitor of elongation factor 2. To improve biological activity, we synthesized various compounds by novel modification of the aglycone, sordaricin. As a result, we have discovered the novel sordarin derivative FR290581. This compound exhibited superior activity and a good pharmacokinetic profile, and also displayed good in vivo activity against *Candida albicans*.

In recent years, with the growing number of immunosuppressed patients (transplants, cancer, AIDS, etc.), invasive mycoses have become an increasingly serious problem. A number of antifungal agents are used commonly, for example, azoles, polyenes, and echinocandins, and although treatment of fungal diseases has been vastly improved, there is still room for improvement. For example, azoles and polyenes often have side effects and/or drug–drug interactions, and some organisms are resistant to these antifungals, whereas echinocandins are not oral agents, and are only available as a parenteral formulation. Therefore, there is a need for orally antifungal agents with a novel mode of action and good oral actvity.[1]

Sordarin (**1**) is an antifungal antibiotic that was discovered in 1971 as a metabolite of *Sordaria araneosa*.[2] A number of related natural products having the sordarin skeleton have also been reported[3–5] as antifungal agents such as zofimarin (**2**)[3] and FR231956 (**3**)[4] (Fig. 1). Semi-synthetic sordarin derivatives which have improved antifungal activity have also been reported by several groups (Fig. 2).[6] Sordarin and related compounds inhibit protein synthesis by a mechanism involving selective binding to the elongation factor 2 (EF-2) and ribosome complex in fungi.[7] Sordarin (**1**) has activity against *Candida* species, however because of the weak antifungal activity and poor pharmacokinetic profile of sordarin itself, in vivo activity is not observed, hence synthetic efforts reported to date have focused on increasing antifungal activity and improving pharmacokinetic profile. While some semi-synthetic sordarin derivatives display good antifungal activity against *Candida* species (Fig. 2), the activity in serum and the pharmacokinetic profile are not sufficiently high.

Herein, we describe the discovery of the novel sordarin derivative FR290581 (**8**). To find a more potent antifungal agent, we focused on modification of sordaricin (**7**) and synthesized various novel derivatives. We speculated that the conformation between the alcohol oxygen of sordaricin (**7**) and the amino group nitrogen of the above derivatives (**4**, **5**, and **6**)[6] was important to the antifungal activity, and that the antifungal activity in serum could be improved by optimization of the amino group. Moreover, it was thought that poor stability that originated in the acetal structure led to a poor PK profile, and we aimed to discover a non-acetal structure. As a result, we discovered the novel sordarin derivative FR290581 (**8**), bearing a unique tri-substituted tetrahydrofuran ring (Fig. 3).

The synthesis of FR290581 (**8**) is outlined in Scheme 1. Sordaricin (**7**) was prepared from sordarin according to the reported method.[8] Aldehyde (**13**) was prepared from sordaricin (**7**) by protection of the carboxyl group with diphenyl diazomethane, protection of the formyl group with ethylene glycol, and oxidation of the alcohol (**12**) to aldehyde (**13**) with catalytic TPAP. Alcohol (**14**) was obtained by nucleophilic addition reaction[9] with the lithium naphthalenide derivative derived from (**11**). Bromide (**11**) was prepared from butyl crotonate according to Uchida's general method[10] (enolate alkylation with the appropriate iodide, reduction with LAH and mesylation followed by bromination with LiBr). In this addition reaction step, when the Grignard reagent (Mg) was used instead, the yield was very low. The R selectivity of this addition reaction to the alcohol was about 6:1 (ratio determined by NMR). This selectivity is

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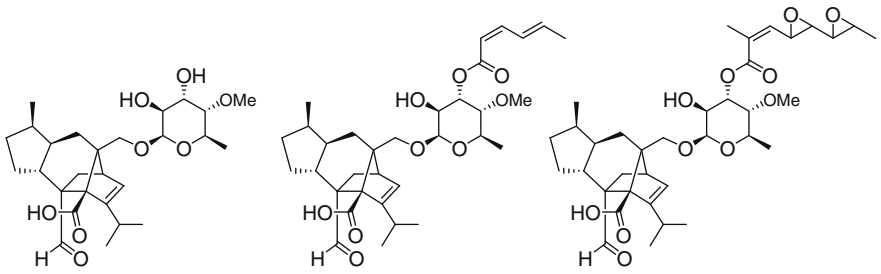


Figure 1. Natural sordarin derivatives.

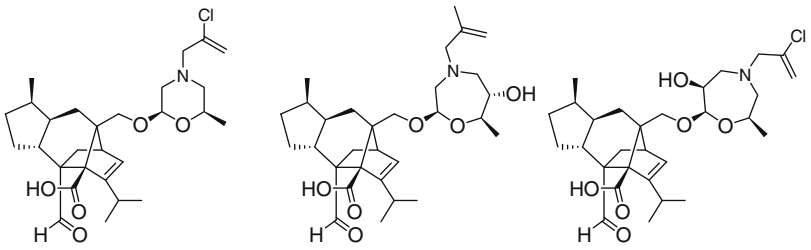


Figure 2. Semi-synthetic sordarin derivatives.

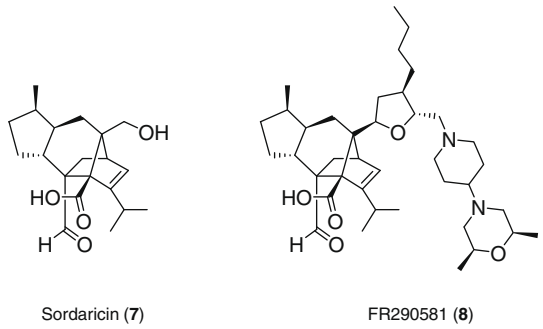


Figure 3.

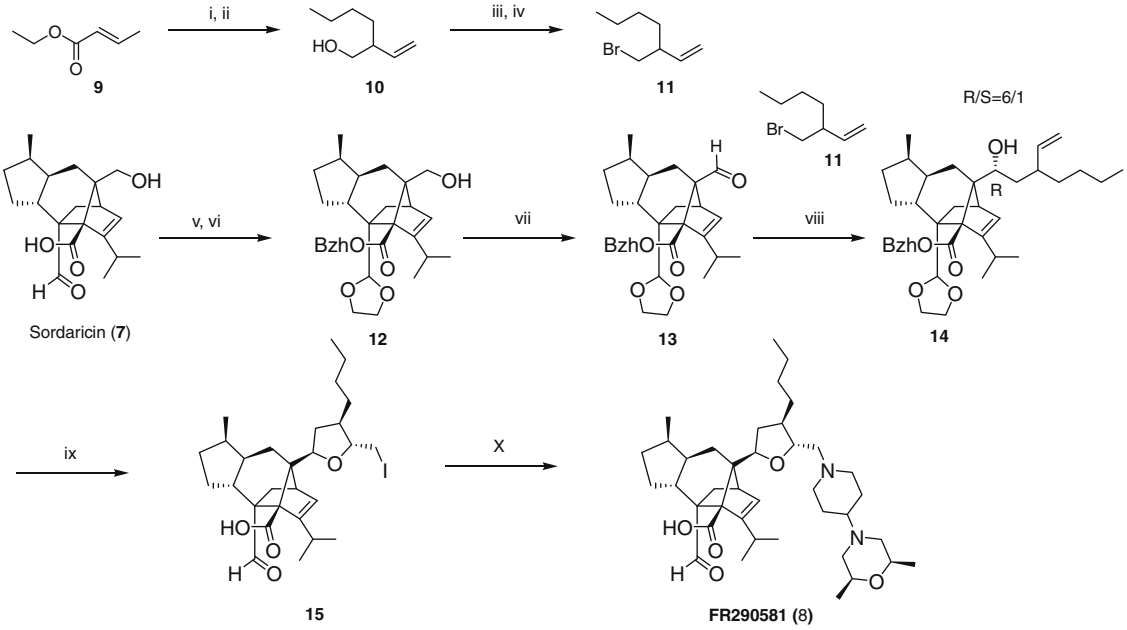
speculated to be due to the steric hindrance of the *i*-Pr group and/or benzhydryl group. The *R*/*S* configuration of the major isomer was determined after subsequent transformation to the iodide (**15**). Whilst all diastereomers were present, alcohol (**14**) was used for the following step. The key intermediate (**15**) was prepared by iodoetherification of alcohol (**14**) with deprotection. Fortunately, this reaction progressed in a *trans*-selective manner. The diastereomers of iodide were separable by crystallization with MeOH. The configuration of the major isomer of iodide (**15**) was established by X-ray crystallography. In the final step, amination of iodide (**15**) furnished the desired product FR290581 (**8**).[11]

Table 1 shows the in vitro antifungal activity of FR290581 (**8**) along with sordarin and the clinically used antifungal agent fluconazole (FLCZ). FR290581 (**8**) is clearly more potent than sordarin (**1**) against all fungi. FR290581 (**8**) exhibited good activity against *Candida* species including azole-resistant *C. albicans* as well as azole-susceptible strains. Moreover FR290581 (**8**) exhibited moderate activity against non-*Candida* albicans pathogens and *C. neoformans*, although no activity against *Aspergillus* species was displayed. This result suggested that there may be a possibility to discover a broad-spectrum oral antifungal agent by further modification of FR290581 (**8**).

Table 2 shows the in vitro antifungal activity against *C. albicans* in mouse serum, pharmacokinetics profile and in vivo activity of FR290581 (**8**) along with sordarin (**1**). FR290581 (**8**) displayed potent in vivo activity, and activity was 100-fold superior against *C. albicans* as compared with sordarin (**1**) in mouse serum. FR290581 (**8**) also showed 50-fold higher C_{max} and significantly longer half-life as compared with sordarin (**1**). The good in vivo activity reflected the good in vitro activity in serum and good pharmacokinetic profile.

Table 3 shows kidney burden in a mouse systemic candidiasis model of *C. albicans*. In this model, FR290581 (**8**) exhibited good efficacy. Although FLCZ exhibited only a fungistatic effect, FR290581 (**8**) displayed fungicidal activity against kidney fungal burden. This result suggests that FR290581 (**8**) may be useful for prophylaxis and also as a promising treatment.

In conclusion, we have discovered a novel sordarin derivative FR290581 (**8**) bearing a unique tri-substituted tetrahydrofuran ring.[12] This tetrahydrofuran ring was prepared by the key reaction of nucleophilic addition and *trans-selective* iodoetherification. FR290581 (**8**) shows good in vitro activity against *Candida* species including azole-resistant *C. albicans* and moderate activity against *C. neoformans*. FR290581 (**8**) also displayed good in vivo activity resulting from potent serum MIC and a good PK profile. Additionally, in a mouse systemic *C. albicans* infection model, FR290581 displayed fungicidal activity. These results suggest that FR290581 may be a promising orally antifungal agent. Further evaluation of FR290581 (**8**) as a candidate and structure–activity relationships (SAR) of a series of FR290581-analogs will be published elsewhere.



Scheme 1. Reagents and conditions: (i) *n*-butyl iodide, LDA, HMPA, THF, −78 °C; (ii) LAH, THF, 0 °C, 55% from **9**; (iii) MsCl, DIPEA, CH_2Cl_2, 0 °C, 82%; (iv) LiBr, acetone, 86%; (v) diphenyl diazomethane, CH_2Cl_2, 60%; (vi) TsOH, ethylene glycol, 89%; (vii) TPAP, NMO, CH_2Cl_2, 85%; (viii) Li, naphthalene, **11**, THF, −78 °C, then **13**, THF, 44%; (ix) I_2, $NaHCO_3$, CH_3CN, crystallization from MeOH, 48%; (x) (2*R*,6*S*)-2,6-dimethyl-4-(4-piperidinyl) morpholine, CH_2Cl_2, 54%.

Table 1
MIC[a] of FR290581 (**8**) against clinical isolates of fungi

Organism	FR290581	Sordarin	FLCZ
C. albicans ATCC90028	0.5	32	0.125
C. albicans 22009 (FLCZ-R)	0.5	64	128
C. glabrata ATCC90030	1	64	16
C. tropicalis 21005	0.5	64	0.25
C. parapsilosis ATCC22019	8	>128	1
C. neoformans TIMM0354	4	>128	0.25
A. fumigatus ATCC204305	128	>128	>128

[a] Minimum inhibitory concentration (μg/mL).

Table 2
Serum MIC, PK profiles and in vivo efficacy of FR290581 (**8**)

	FR290581	Sordarin
Serum MIC[a]	0.25	32
C_{max} (μg/mL)[b]	1.0	0.02
$T_{1/2}$ (h)	3.4	0.33
ED_{50} (mg/kg)[c]	2	>40

[a] Minimum inhibitory concentration in mouse serum (μg/mL).
[b] Mouse, 2 mg/kg, po.
[c] Mouse systemic candidiasis model, po.

Table 3
Kidney burden in mouse systemic *C. albicans* infection

	Δlog cfu/g kidney[a]
FR290581[b]	−1.09*
FLCZ[b]	−0.19

[a] Δlogarithm of kidney burden subtracted from inoculation after 24 h.
[b] 20 mg/kg, po, $n = 5$.
* $p < 0.01$ significant to control 0 h (Dunnett comparison).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.051.

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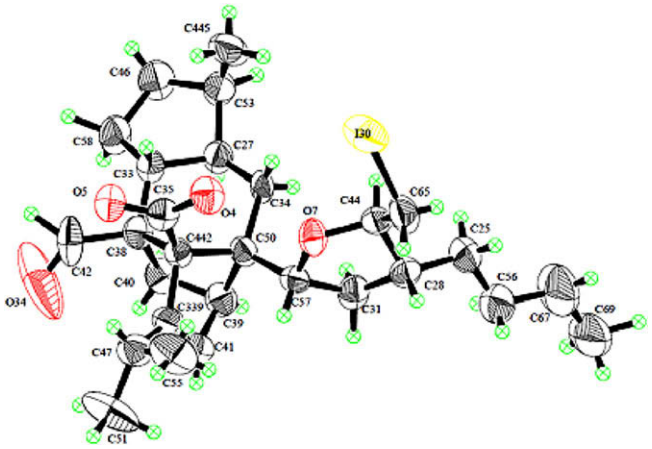
11. Representative synthetic procedure and spectral data for title compound FR290581 (**8**). Under a nitrogen atmosphere, to a solution of naphthalene (8.5 g, 67 mmol) in THF (100 mL) was added lithium turnings (0.4 g, 62 mmol) at room temperature, and the mixture was stirred at the same temperature for 1 h then cooled to −78 °C. To the cooled mixture was added dropwise **11** (10.0 g, 52 mmol) in THF (15 mL) at the same temperature, and stirring was continued for 1.5 h. To the mixture was added dropwise **13** (20.0 g, 37 mmol) in THF (15 mL) at −78 °C, and stirring was continued at the same temperature for 2 h. The reaction was quenched via addition of water at −78 °C. After being allowed to warm to room temperature, the mixture was extracted three times with AcOEt. The combined extract was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography to give compound **14** (5.3 g) as a white amorphous solid. To a suspension of iodine (2.8 g, 10.3 mmol) and sodium hydrogencarbonate (3.7 g, 44.0 mmol) in acetonitrile (20 mL) was added a solution of **14** (3.7 g, 5.7 mmol) in acetonitrile (20 mL) at room temperature. The mixture was stirred for 1 h. The resulting mixture was quenched by saturated aqueous sodium thiosulfate solution. The color turned to a white suspension. After evaporation of acetonitrile, the aqueous layer was extracted with ethyl acetate three times. The combined organic layer was washed with saturated aqueous ammonium chloride solution, and brine, dried over magnesium sulfate, then concentrated. The residue was purified by silica gel column (hexane/ethyl acetate = 100:0–85:15). The obtained white amorphous solid was purified by recrystallization from methanol to give **15** (1.9 g).To a solution of the compound of **15** (38.0 mg, 0.1 mmol) in dichloromethane (0.2 mL) was added (2*R*,6*S*)-2,6-dimethyl-4-(4-piperidinyl) morpholine (50 mg, 0.3 mmol) at room temperature. The mixture was stirred at the same temperature for 12 h and diluted with AcOEt. The solution was washed with 1.0 M aqueous sodium hydroxide, water, and brine, dried over sodium sulfate, then concentrated. The residue was purified by HPLC (C18, 20–100% acetonitrile in water (+0.2% formic acid)). The desired fraction was concentrated. The residue was diluted with saturated aqueous sodium hydrogen carbonate and extracted with AcOEt three times. The combined organic layer was washed with 1.0 M aqueous sodium hydroxide and brine, dried over sodium sulfate, and concentrated to give the compound of FR290581 (**8**) (19.1 mg) as a colorless oil. FABMS (*m*/*z*): 637.5 $[M-H]^-$; IR (ATR): 2954, 2929, 2866, 2812, 1710, 1452, 1375, 1144, 1074; ^{1}H NMR ($CDCl_3$, δ): 0.77–1.09 (14H, m), 1.11–2.58 (37H, m), 268–3.02 (4H, m), 3.58–3.87 (3H, m), 4.44–4.60 (1H, m), 5.91–5.99 (1H, m), 9.91 (1H, s).
ORTEP plot of the key intermediate iodide (**15**). Details will be provided in a subsequent publication from these laboratories.



12. A tri-substituted tetrahydrofuran ring was optimal for PK properties. Further details will be disclosed in the full paper.