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Synthesis of Sordaricin Analogues as Potent Antifungal Agents against *Candida albicans*

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Abstract—Sordaricin derivatives possessing a cyclohexane ring appendage attached via an ether, thioether, amine, oxime, ester or amide linkage were synthesized and their antifungal activity was evaluated in vitro. Compounds containing a thioether bond or an oxime bond as a linkage exhibited potent MICs ($\leq$0.125 μg/mL) against four *Candida albicans* strains including azole-low-susceptible strains. They were also active (MIC $\leq$0.125 μg/mL) against *Candida glabrata*. Their in vivo efficacy was confirmed in a murine intravenous infection model with *Candida albicans*. © 2002 Elsevier Science Ltd. All rights reserved.

Zofimarin (**1**) is an antifungal natural product isolated from *Zopfielle marina* SANK21274.[1] Sordarin (**2**) contains a 6-desoxyaltrose moiety that does not have any acyl chains, and it is also a natural glycoside isolated from *Sordaria araneosa*.[2] Sordaricin (**3**) is a common tetracyclic diterpene aglycon present in both **1** and **2**. While **1** exhibits moderate inhibitory activity in the growth of pathogenic fungi, **2** and **3** show almost no inhibitory activity. Recently, some potent antifungal derivatives among the sordarin family have been reported.[3,4] When a hydrophobic aliphatic side chain was attached to **3** instead of the sugar moiety, its activity increased enough to inhibit the growth of fungi in an in vitro assay. In particular, the isopentyl ether derivative **4** exhibited good potency as an antifungal agent.[5] The sordarin family inhibits fungal protein synthesis by selectively interrupting the functions of fungal elongation factor 2, which plays important roles with ribosome in the translation process.[6–8] Since the disclosure of the mode of action, chemical modification of **3** has been considered as one of new attractive targets for the development of novel antifungal agents.[9]

In the course of our modification, it was found that a cyclohexyl group was one of the most effective aliphatic components as a side chain. Therefore, our attention was focused on the biological activity of derivatives with various linkages between the aglycon and a cyclohexyl group. Herein, we describe the synthesis of the compounds having a cyclohexyl ring linked by various bonds and their in vitro antifungal activity against *Candida* spp (Fig. 1).

Synthesis of Sordaricin Analogues

The synthetic routes of various derivatives are shown in Scheme 1. Sordaricin benzyl ester **5a** and sordaricin benzhydryl ester **5b** were prepared according to the reported methods.[5] For cyclohexyl ether derivative **7**,

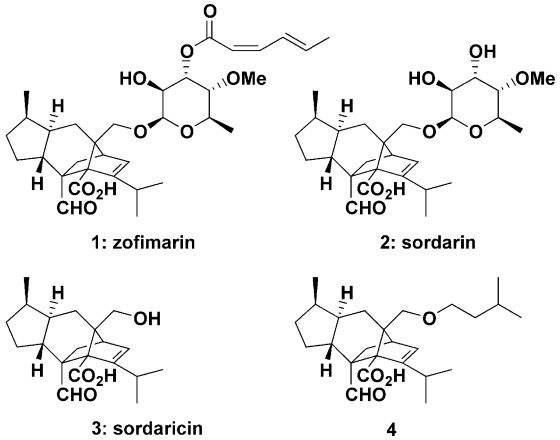

Figure 1. Chemical structures of zofimarin **1**, sordarin **2**, sordaricin **3** and isopentyl ether derivative **4**.

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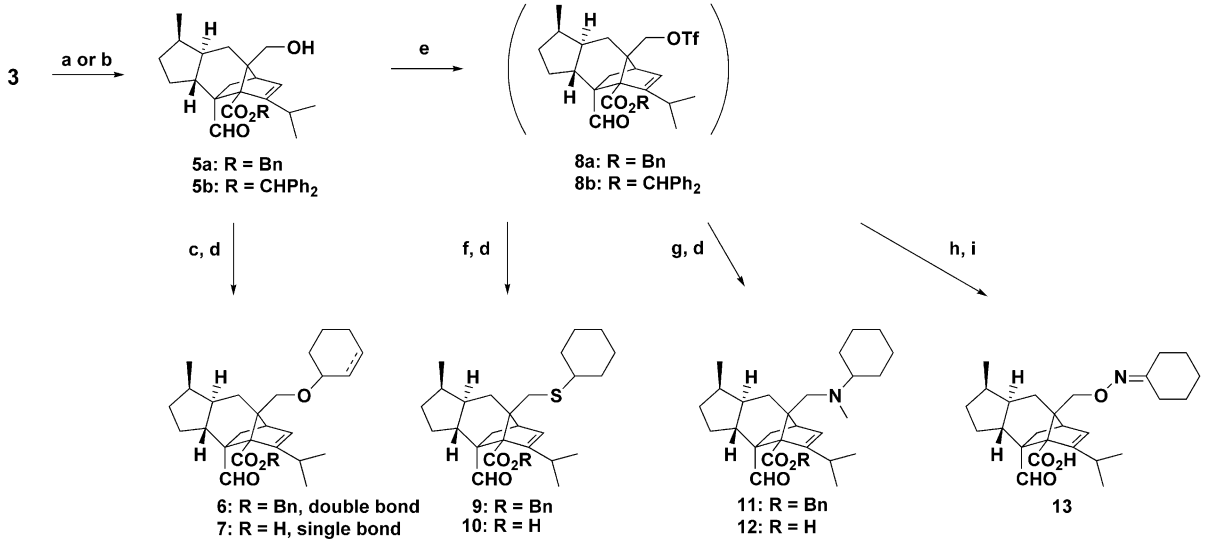
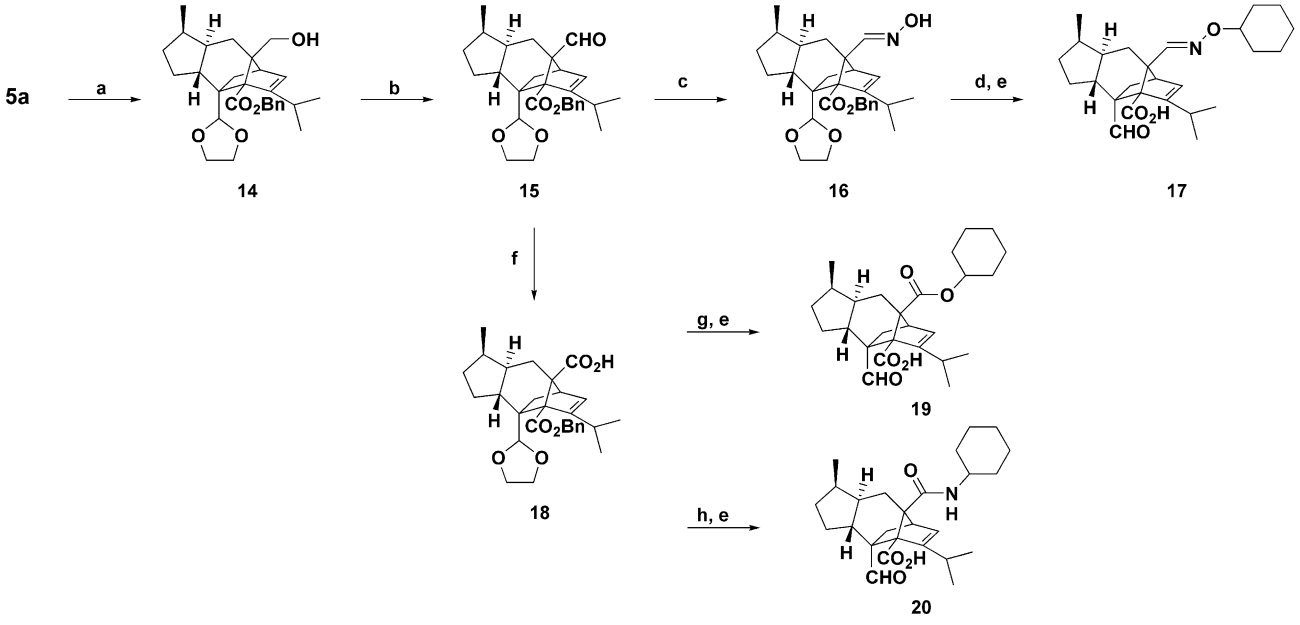
alkylation of the hydroxyl group of **5a** was carried out with 2-cyclohexenyl bromide and silver oxide at room temperature in dichloromethane, to afford **6** (36%). Subsequent hydrogenolysis brought about both saturation of the cyclohexene ring and removal of the benzyl ester, to yield **7** (70%). For cyclohexyl thioether derivative **10**, compound **5a** was first converted to unstable triflate **8a** (95%). Thioether formation was performed by treatment with mercaptocyclohexane and sodium hydride at 0 °C in *N,N*-dimethylformamide to give **9** (59%). Then, hydrogenolysis of **9** yielded **10** (56%). For *N*-methyl cyclohexylamine derivative **12**, substitution of **8a** was carried out with lithium *N*-methyl cyclohexylamide at −50 °C in tetrahydrofuran, to give **11** (21%). Subsequent hydrogenolysis yielded **12** (61%). For cyclohexanone oxime derivative **13**, compound **5b** was first converted to triflate **8b**. A displacement reaction of **8b** was carried out with potassium hydride and cyclohexanone oxime at 0 °C in *N,N*-dimethylformamide. Finally, deprotection was performed with trifluoroacetic acid at 0 °C in dichloromethane to give **13** (57% in two steps).

Next, we set out for the oxidation of the hydroxylmethyl group of **5a** as shown in Scheme 2. The aldehyde of **5a** was initially protected as an ethylene acetal. Treatment of **5a** with ethylene glycol catalyzed by toluenesulfonic acid in methanol afforded **14** (78%). Swern oxidation of **14** gave aldehyde **15** (87%). For *O*-cyclohexyl oxime derivative **17**, formation of oxime **16**[10] was performed with hydroxylamine hydrochloride in a



Scheme 1. (a) BnBr, $NaHCO_3$/DMF; (b) Ph_2CN_2/MeOH; (c) 2-cyclohexenyl bromide, Ag_2O, MS^4A/CH_2Cl_2; (d) H_2, $Pd(OH)_2$-C/MeOH; (e) Tf_2O, Py/CH_2Cl_2; (f) mercaptocyclohexane, NaH/DMF; (g) *N*-methyl cyclohexylamine, BuLi/THF; (h) cyclohexanone oxime, KH/DMF; (i) TFA/CH_2Cl_2.



Scheme 2. (a) $CH(OMe)_3$, $(CH_2OH)_2$, TsOH/MeOH; (b) $(COCl)_2$, Me_2SO/CH_2Cl_2; Et_3N; (c) $NH_2OH{\cdot}HCl$/EtOH-Py; (d) cyclohexyl mesylate, NaH/DMF; (e) (i) 1N-HCl/MeOH; (ii) H_2, $Pd(OH)_2$-C/MeOH; (f) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene/^{t}BuOH-H_2O; (g) cyclohexanol, Ph_3P, DEAD/DMF; (h) 2,4,6-triisopropylbenzenesulfonyl chloride, Et_3N, DMAP/CH_2Cl_2; cyclohexylamine.

Table 1. In vitro antifungal activity of novel sordaricin derivatives

Organism	MIC (μg/mL)									
	FCZ[a]	ITCZ[b]	**1**	**7**	**10**	**12**	**13**	**17**	**19**	**20**
Candida albicans ATCC24433	0.5	0.125	0.5	0.125	0.031	8	0.031	0.031	≤0.125	>4
Candida albicans SANK51486	0.25	0.031	0.25	≤0.063	≤0.016	8	0.031	0.031	≤0.125	>4
Candida albicans TIMM3164	>4	0.25	0.5	0.25	0.063	16	0.063	0.063	0.25	>4
Candida albicans ATCC64550	>4	1	0.5	0.25	0.125	16	0.063	0.063	0.5	>4
Candida glabrata ATCC90030	>4	1	>4	≤0.063	0.031	8	0.063	0.125	0.5	0.5
Candida tropicalis ATCC750	0.5	0.5	0.5	2	2	>16	1	1	4	>4

[a]Fluconazole.
[b]Itraconazole.

mixture of pyridine and ethanol (1:1) (99%). *O*-alkylation of **16** was carried out with cyclohexyl mesylate and sodium hydride at 0 °C in *N,N*-dimethylformamide (60%). Finally, cleavage of ethylene acetal and benzyl ester gave **17**[10] (92%).

Carboxylic acid **18** was obtained by $NaClO_2$ oxidation of **15** (70%). For cyclohexyl ester derivative **19**, esterification of **18** was achieved by Mitsunobu reaction. Treatment of **18** with cyclohexanol, triphenylphosphine and diethylazodicarboxylate in *N,N*-dimethylformamide gave the cyclohexyl ester (42%). Subsequent hydrolysis and hydrogenolysis yielded **19** (75%). For cyclohexyl amide derivative **20**, amidation of **18** was carried out with 2,4,6-triisopropylbenzenesulfonyl chloride, triethylamine and cyclohexylamine to afford the desired amide. Hydrolysis and hydrogenolysis of this amide produced **20** (39% in three steps).[11]

Biological Activity

Minimum inhibitory concentrations (MICs) of the analogues are summarized in Table 1.[12]

Compounds **10**, **13**, and **17** showed excellent activity against *Candida albicans*. In particular, the MIC value of thioether **10** was ≤ 0.016 μg/mL against *C. albicans* SANK51486. Moreover, these compounds were active against azole-low-susceptible strains as well as azole susceptible ones. In particular, the inhibitory potency of **13** and **17** was 16-fold stronger than that of itraconzole against *C. albicans* ATCC64550. In addition, these compounds showed potent activity against *C. glabrata* with MICs of 0.125 μg/mL or lower. However, compounds **7** and **19** only showed moderate activity and compounds **12** and **20** had extremely weak activity against most tested strains. From these results, it is speculated that lipophilicity of the linkage is very important to exhibit the biological activity, and that the linkage directly affects the binding interaction with EF-2. The two representative compounds (**10** and **17**) were evaluated for their in vivo activity using a murine model of systemic candidasis.[13] The results are illustrated in Figure 2. Remarkably, both compounds showed good efficacy against systemic candidasis after both oral and subcutaneous administration.

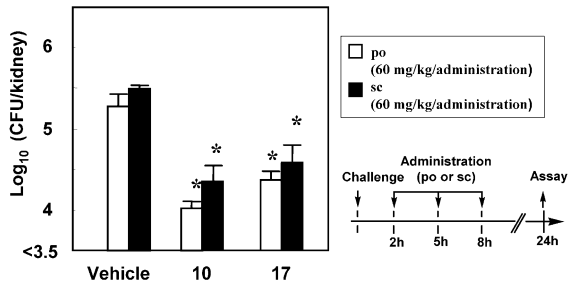


Figure 2. In vivo efficacy of sordaricin derivatives against murine intravenous infection with *Candida albicans* SANK51486. *$P<0.05$.

In conclusion, several novel sordaricin derivatives were synthesized. It was found that thioether **10** and oxime **13** and **17** had excellent activity as antifungal agents. These compounds will serve to investigate the mode of action of this class of compounds. They are considered to be promising candidates for the therapeutic treatment of fungal infection.

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10. The geometry of oximes **16** and **17** was determined by ^{1}H NMR chemical shifts. *Syn*-aldoximes show their –CH=N– signals at a magnetic field lower than approximately $\bar{\delta}$ 7.0 ppm, whereas *anti*-isomers show their signals at a higher field. The corresponding signals of **16** and **17** appeared at δ 7.72 and δ 7.35, respectively. In addition, α-branched aldehydes tend to predominantly afford thermodynamically more stable *syn*-isomers. See: (a) Karabatsos, G. J.; Hsi, N. *Tetrahedron* **1967**, *23*, 1079. (b) Karabatsos, G. J.; Taller, R. A. *Tetrahedron* **1968**, *24*, 3347.

11. All new compounds gave satisfactory spectroscopic and analytical data. The representative data are shown as follows. **10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.66 (1H, s), 6.11 (1H, dd, J = 3.4, 1.2 Hz), 3.07 (1H, d, J = 12.0 Hz), 2.80 (1H, t, J = 3.8), 2.65 (1H, d, J = 12.0 Hz), 2.60–1.10 (m), 1.06 (3H, d, J = 6.8 Hz), 0.99 (3H, d, J = 6.8 Hz), 0.79 (3H, d, J = 6.8 Hz); FABMS (*m*/*z*): 431 $[M+H]^+$. **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.82 (1H, s), 6.07 (1H, d, J = 2.8 Hz), 4.20 (1H, d, J = 9.6 Hz), 4.13 (1H, d, J = 9.6 Hz), 2.70 (1H, t, J = 3.6 Hz), 2.46 (2H, m), 2.36–2.24 (3H, m), 2.18–1.52 (m), 1.32 (1H, d, J = 12.4 Hz), 1.25 (2H, m), 1.00 (3H, d, J = 6.4 Hz), 0.96 (3H, d, J = 7.6 Hz), 0.81 (3H, d, J = 6.6 Hz); FABMS (*m*/*z*): 428 $[M+H]^+$. **17**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (1H, s), 7.35 (1H, s), 6.03 (1H, d, J = 3.7 Hz), 4.02 (1H, m), 2.78 (1H, t, J = 4.4 Hz), 2.33–2.25 (2H, m), 2.11–1.28 (m), 1.00 (3H, d, J = 6.6 Hz), 0.98 (3H, d, J = 6.6 Hz), 0.84 (3H, d, J = 6.6 Hz); FABMS (*m*/*z*): 428 $[M+H]^+$.

12. In vitro antifungal activity was determined at 35 °C in RPMI1640 at pH 7.0. Minimum inhibitory concentration (MIC) was defined as the minimum concentration of the test compound that inhibited the growth of the fungi by 80%.

13. In vivo activity was determined in mice (each group consisted of 8–10 male mice, ddY strain, 5 weeks old) infected systemically by an intravenous administration of 6×10^5 CFU of *C. albicans* SANK51486. Test compounds (60 mg/kg/administration) were administered orally (po) or subcutaneously (sc) 2, 5 and 8 h post infection. An aqueous solution of carboxymethyl cellulose sodium salt (0.5%) containing dimethyl sulfoxide (10%) was used as a vehicle for administration. Viable cell numbers in the kidneys were determined 24 h post infection.