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ALKYL SIDE-CHAIN DERIVATIVES OF SORDARICIN AS POTENT ANTIFUNGAL AGENTS AGAINST YEAST

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Abstract. Sordarin (**1**) was converted to **5** and **6**, which showed potent antifungal activity against yeast. A series of C1-C9 alkyl side-chain derivatives was prepared, from which it was found that the optimal activity occurred with C5. A comparison of side chains with different unsaturation showed that the *cis*-alkene was the most active. This result suggested that the folding of the side chains might be crucial for the optimal activity.

Sordarin (**1**), produced by species of the fungal genus *Sordaria*, was described as an antifungal agent in 1970.[1,2] Sordarin itself shows only weak activity against the yeast *Saccharomyces cerevisiae* and its aglycone, sordaricin (**2**), shows no activity at all. Interestingly, when aliphatic side chains were attached to sordaricin, potent activity was observed. The *iso*-butyl ether derivative **5** (R = *i*-Bu) (L-493,422) was reported earlier. With the use of its tritiated form, we have discovered that the mode of action of sordarin analogs involves selective inhibition of elongation factor 2 (EF-2) in the elongation phase of translation in fungi, making sordarin analogs an attractive target for the development of new antifungal agents.[3] A series of similar aliphatic side-chain derivatives has now been prepared. In this communication, we report that the activity of these analogs correlated with the lipophilicity of the side chains.

The sugar unit of sordarin was effectively hydrolyzed with concentrated HCl in acetone.[1] After protection of the carboxylic acid as a *para*-methoxybenzyl (PMB) ester, compound **3** was obtained (67% over 2 steps). For derivatives containing C1 to C6 side chains, alkylation was carried out with the corresponding iodo compound and NaH in DMF. The iodo compounds for C7 and above failed to alkylate satisfactorily, however. The alkylation of such compounds was performed with the use of the benzenesulfonates of the corresponding propargyl alcohols. Following alkylation, hydrogenolysis yielded the saturated side-chain analogs **5**. We were concerned about the stability of the aldehyde groups in these analogs and more stable substitutes for the aldehyde were sought. Most chemical modifications of the aldehyde group, however, abolished the activity of the analogs. Fortunately, it was found that when the aldehyde was replaced by a nitrile group, the analogs generated had comparable activity to the aldehyde analogs. These nitrile analogs are expected to have higher chemical stability, especially toward oxidation. To prepare the nitrile compounds, the aldehydes were first converted to the oximes. Dehydration of the oximes was efficiently performed with Burgess' reagent[4] (91% yield over 2 steps). Hydrogenolysis yielded the nitrile analogs. The activities of all the analogs prepared are summarized in Table 1.[5,6]

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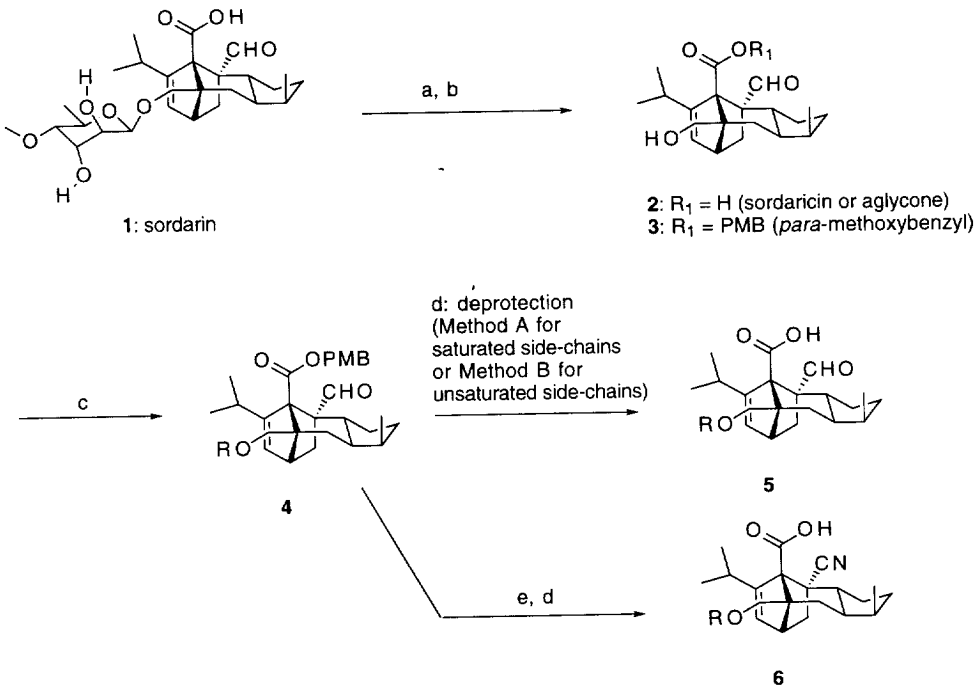
Scheme 1



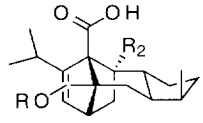
Reagents and reaction conditions: a. concentrated HCl, acetone, rt. b. *p*-methoxybenzyl chloride, $NaHCO_3$, DMF, rt. c. RX, NaH, DMF, rt. (RX = R-I for C1-C6; RX = $CH_3(CH_2)_nC{\equiv}CCH_2OSO_2Ph$ for C7 and above)
d. Method A: H_2, $Pd(OH)_2$ on C, MeOH. Method B: HCOOH.
e. 1. $NH_2OH{\cdot}HCl$, pyridine-EtOH, 70 °C. 2. Burgess' reagent, toluene, 70 °C.

From the results shown in Table 1, it was evident that the activity against *S. cerevisiae* was much enhanced when the sugar unit of sordarin was replaced by an aliphatic chain. Among the C1 to C9 derivatives for both the aldehyde series and the nitrile series, the activity increased from C1 to C5 and decreased for longer chains. This clearly demonstrated that a critical degree of lipophilicity of side chains was important for the optimal activity of sordarin analogs.

Since the optimal activity occurred with C5, it would be interesting to investigate the effect of unsaturation of the C5 side chains on the activity. Thus, *cis*-pent-2-enyl (IC_{50} 0.000001 μg/mL), *trans*-pent-2-enyl (IC_{50} 0.01 μg/mL) and pent-2-ynyl (IC_{50} 0.025 μg/mL) side-chain derivatives of the aldehydes were also prepared with the same alkylation method as above using the corresponding iodo compounds as alkylating agents. With the unsaturation, however, HCOOH was used to remove the PMB ester instead of hydrogenation. The *cis*-analog was found more active than the saturated analog (IC_{50} 0.00001 μg/mL), which was in turn significantly more active than both the *trans*- and the alkynyl analogs. From the geometrical constraints, it was expected that the *cis*-alkene promoted folding of the aliphatic chain, whereas both the *trans*- and the alkynyl side-chains hindered the

folding. Thus, it is speculated that the active forms of these aliphatic side-chain analogs of sordarin involved folding of the side chains.

Our earlier report on the specificity of sordarin analogs for elongation factor EF2 in fungal protein synthesis established the potential of these analogs as new antifungal agents. The finding in this paper unravels the importance of lipophilicity of the side chains.



5: R_2 = CHO
6: R_2 = CN

Compound	**5**	**6**
Sordarin (**1**)	10	2
Sordaricin (**2**)	> 250	20
R = Me	0.1	1
R = Et	0.06	0.5
R = *n*-Pr	0.06	0.09
R = *n*-Bu	0.0001	0.03
R = *iso*-Bu	0.0001	0.03
R = *n*-Pentyl	0.00001	0.00001
R = *iso*-Pentyl	0.00001	0.00001
R = *n*-Hexyl	0.015	0.0003
R = *n*-Heptyl	0.3	0.1
R = *n*-Octyl	0.4	0.2
R = *n*-Nonyl	50	1

Table 1. Antifungal Activity of Sordarin and Derivatives. The activities of compounds **5** and **6** against *Saccharomyces cerevisiae* (strain YPH98) are reported. The data are reported as IC_{50} (μg/mL), which was the concentration required to inhibit 50% of growth in comparison to untreated control.

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References and Notes

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5. Experimental procedure:

Sordaricin PMB ester 3. To a solution of sordarin **1** (1.0 g, 2.03 mmol) in 40 mL of acetone was added 4 mL of conc. HCl. The mixture was stirred at rt for 1 day. After aqueous workup (CH_2Cl_2, then EtOAc), the mixture was concentrated *in vacuo*, and was dissolved in 25 mL of DMF. *p*-Methoxybenzyl chloride (1.5 mL, 11.0 mmol) and $NaHCO_3$ (1.7 g, 20.2 mmol) were added. The mixture was stirred at rt overnight. After aqueous workup (ether) and chromatography, 614.8 mg (67% over 2 steps) of **3** was obtained.

Aldehyde 5. Only the methyl ether is described. To a solution of **3** (97.6 mg, 0.22 mmol) in 3 mL of DMF were added MeI (0.1 mL, 1.6 mmol) and NaH (25 mg of a 60% oil dispersion, 0.63 mmol). The mixture was stirred at rt overnight. After aqueous workup (ether) and purification by PTLC, 90.6 mg (90% yield) of **4** (R = Me) was obtained. To a solution of **4** (8.0 mg, 0.017 mmol) in 1.5 mL of MeOH was added Pearlman's catalyst (15 mg). The mixture was stirred under H_2 (balloon pressure) for 15 min. After filtration and concentration *in vacuo*, 5.7 mg (96% yield) of **5** (R = Me) was obtained. ^{1}H NMR ($CDCl_3$): δ 0.82 (3H, d, J = 6.9), 0.96 (3H, d, J = 6.6), 1.03 (3H, d, J = 6.9), 1.20-2.20 (11H, m), 2.33 (1H, m), 2.36 (1H, t, J = 3.7), 3.26 (1H, d, J = 9.1), 3.38 (3H, s), 3.93 (1H, d, J = 9.1), 6.03 (1H, d, J = 2.8), 9.88 (1H, s). HRMS (EI) calcd for $C_{21}H_{30}O_4$ 346.2144, found 346.2140.

Nitrile 6. Only the methyl ether is described. To a solution of **4** (R = Me) (45.0 mg, 0.097 mmol) in 1.5 mL of EtOH and 1.5 mL of pyridine was added NH_2OH·HCl (33.6 mg, 0.48 mmol). The mixture was stirred at 70 °C for 2 hours. After concentration *in vacuo*, aqueous workup (CH_2Cl_2) and purification by PTLC, 44.2 mg (95% yield) of the desired oxime was obtained. To a solution of this oxime (44.2 mg, 0.092 mmol) in 3 mL of toluene was added Burgess' reagent (110.0 mg, 0.46 mmol). The mixture was stirred at 70 °C for 2 hours. After concentration *in vacuo* and purification by PTLC, 40.0 mg (94% yield) of the desired nitrile was obtained. Using the same hydrogenation method as in the aldehyde, 7.4 mg of **6** (R = Me) was obtained from 10.3 mg of the PMB ester (97% yield). ^{1}H NMR ($CDCl_3$): δ 0.78 (3H, d, J = 7.1), 1.03 (3H, d, J = 6.9), 1.14 (3H, d, J = 6.6), 1.20-2.20 (10H, m), 2.35 (1H, dd, J = 4.1, 12.6), 2.48 (1H, m), 2.74 (1H, m), 3.35 (3H, s), 3.36 (1H, d, J = 8.5), 3.75 (1H, d, J = 8.5), 6.14 (1H, d, J = 2.8). HRMS (EI) calcd for $C_{21}H_{29}N_1O_3$ 343.2147, found 343.2149.

6. Biological assays: IC_{50} values were determined by growth inhibition in rich medium (YPAD containing in g/L: Bacto Yeast Extract 10, Bacto Peptone 20, glucose 20, adenine 60mg) in which *Saccharomyces cerevisiae* strain YPH98 (Mata ade2 leu2 lys2 trp1 ura3)[3] previously grown to early stationary phase in the same medium was inoculated at $2x10^5$ cells/mL in a flat-bottomed microtiter dish. We did not find significant variation in sensitivity in yeast depending on wild type strain background. Drug was added to the last column of wells from stocks at 5 mg/mL in DMSO to a drug concentration of 80 μg/mL and serial dilutions (2- to 5-fold depending on drug potency) were performed. Static incubation in a humidified chamber at 29 °C for 16 h resulted in a growth plateau at an A600 of about 0.8 in a 100 μL well. Cells were resuspended by 2 min rapid reciprocal shaking prior to determination of A600 and drug concentration for half maximal growth.