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Sordarin Oxazepine Derivatives as Potent Antifungal Agents

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Abstract—The synthesis and biological activity of sordarin oxazepine derivatives are described. The key step features a regioselective oxidation of an unprotected triol followed by double reductive amination to afford the ring-closed products. The spectrum of antifungal activity for these novel derivatives includes coverage of *Candida albicans*, *Candida glabrata*, and *Cryptococcus neoformans*.

The natural product sordarin (**1**) was discovered in 1971 as a metabolite of *Sordaria araneosa* and identified as a potent antifungal compound.[1] Interest in this diterpene glycoside has recently been sparked by the identification of its cellular target in fungal organisms, the transcription elongation factor EF-2.[2,3] Despite the high-sequence homology between fungal and human EF-2 (85%), sordarin is able to selectively stabilize the fungal ribosome/EF-2 complex and block translocation.[4] Inhibition of fungal protein synthesis thus became an attractive target for the development of new antifungal agents that were mechanistically distinct from existing therapies.

In terms of synthetic sordarin analogues, several sugar-modified derivatives have been recently disclosed.[5,6] These analogues include morpholino derivatives such as **3**, which in addition to their structural novelty gained interest upon the demonstration of potent antifungal activity with broader spectrum than earlier analogues (Fig. 1).[7] As part of our ongoing effort to discover novel therapies for the treatment of systemic fungal infections,[8] we became interested in exploring other heterocyclic replacements for the sordarin glycoside. Herein we report our discovery of novel sordarin oxazepine derivatives and their antifungal activity against several important pathogens.

A crucial intermediate for the preparation of **3** was the 4′-hydroxy glycoside **2**, the preparation of which has been recently reported.[9] Treatment of the suitably protected triol with excess sodium periodate for prolonged reaction times affords a dialdehyde intermediate where bonds (a) and (b) are cleaved with excision of one equivalent of formaldehyde (Scheme 1). Double reductive amination of this dialdehyde then generates the ring-closed morpholino product.[10]

In light of the potent antifungal activity and broader spectrum observed with **3**, our attention focused on the design of analogues with heterocyclic sugar surrogates that could offer more synthetic opportunities to expand the SAR of this natural product. One way to achieve this from **2** hinged on the potential to exploit the *anti*- and *syn*-relationship between the respective 2′-, 3′- and 3′-,4′-diol pairs in their reactivity towards periodate oxidation. We envisioned the selective oxidation of bond (b) to generate a hydroxy dialdehyde intermediate, which could then undergo a similar reductive amination ring closure event to yield the seven-membered ring (Scheme 1). Such a strategy would not only provide a novel oxazepine ring system, but would also expose the unreacted 2′-hydroxyl for additional SAR investigation.

Our entry into heterocyclic sugar surrogates required an efficient preparation of sordarin and triol **2**. A series of medium and conduction studies resulted in an optimized fermentation yield of sordarin of greater than 2.6 mg/g in flasks.[11] Scale-up to tanks as large as 550 L facilitated comparable yields, resulting in a product that could be easily isolated via solvent extraction or resin adsorption. For the conversion of sordarin to **2**, a

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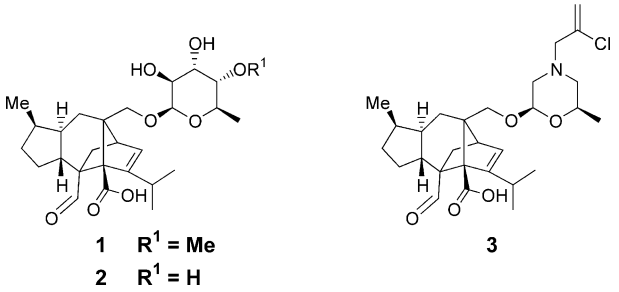
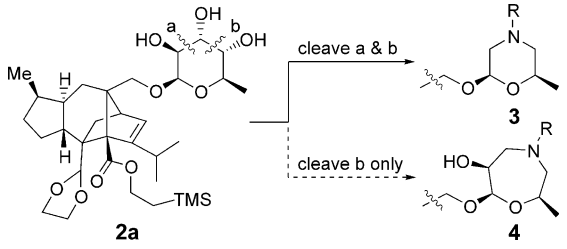


Figure 1. Sordarin derivatives.



Scheme 1. Selective oxidation to generate oxazepine heterocycles.

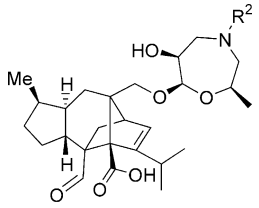
screen of micro-organisms identified a *Nocardia* spp. as the most efficient enzyme for this demethylation.[12] It is interesting to note that Hall and co-workers have recently reported the use of *Streptomyces avermitilis* (ATCC 31272) as another efficient organism for this same biotransformation.[9]

With gram quantities of **2** in hand, we were able to investigate the proposed oxidative ring expansion. Intermediate **2** was subjected to standard protection conditions to mask the aldehyde and carboxylic acid functionalities found in the sordarin core. Protected triol **2a** was then treated with a slight excess (1.25 equiv) of sodium periodate at 0 °C.[13] While attempts to characterize the proposed hydroxy dialdehyde intermediate were unsuccessful due to the existence of open-chain and cyclized ketal mixtures, the major product in the subsequent reductive amination reaction was always the expected oxazepine ring. The regiochemistry of the bond scission was confirmed by standard 2D NMR techniques (COSY, HMBC, and HMQC) on acetate derivative **6b**.

It is noteworthy that selective protection of the triol was not required to effect this ring expansion.[14] Moreover, all of the aliphatic amines examined in this unoptimized reaction afforded the ring-closed heterocycle, while anilines and other electron-poor amines (i.e., entry **4c**) generally were much less successful. This method could be easily adapted to parallel synthesis, and typically the crude reductive amination reactions were used without further purification. Deprotection of the ester and aldehyde groups (TBAF in THF, followed by 1 N HCl in MeOH) thus afforded the final targets **4a**–**n** in 13–36% overall yield for the 4 steps from **2a**.[15]

The sordarin oxazepines **4a**–**n** were tested for fungal growth inhibition in *Candida albicans*, *C. glabrata*, and *Cryptococcus neoformans* (Table 1).[16] At first glance, the seven-membered ring heterocycles appeared to be inferior in antifungal activity compared to the morpholine derivative **3** bearing a 2-chloroallyl nitrogen sidechain (*C. albicans* MIC <0.06 μg/mL). However, the *N*-

Table 1. In vitro activity of sordarin derivatives **1**–**3** and oxazepines **4a**–**n**



Compd	R^2	MIC[a] (μg/mL)		
		C. albicans	*C. glabrata*	*C. neoformans*
1	—	16	>128	8
2	—	>128	>128	>128
3	—	<0.06	0.125	16
4a	2-MeO ethyl	16	>128	>128
4b	Cyclopropyl methyl	16	>128	>128
4c	2-CF_3 ethyl	1	32	>128
4d	2-(1-Cyclohexenyl) ethyl	2	>128	>128
4e	Allyl	8	128	>128
4f	Propargyl	4	128	32
4g	2-Chloroallyl	0.5	32	4
4h	2-Methallyl	0.125	64	64
4i	2-Cl benzyl	2	>128	128
4j	4-MeO benzyl	<0.06	1	>128
4k	4-MeO phenyl	2	64	>128
4l	(2-Thiophenyl) methyl	2	128	32
4m	[2-(4-Me furanyl)] methyl	2	>128	32
4n	2-(2-Imidazoyl)-ethyl	>128	>128	>128

[a]MIC value defined as the lowest drug concentration required to inhibit 90–100% visible growth relative to controls.

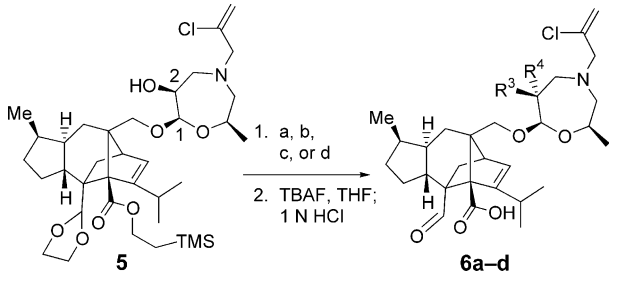
methoxybenzyl derivative **4j** inhibited fungal growth in *C. albicans* to a comparable degree, suggesting that a range of steric bulk can be accomodated at this site, and also that the oxazepine heterocycle could serve as a useful template for further SAR investigation.

Polar functionality (i.e., **4n**), was mostly detrimental to whole cell antifungal activity versus all three pathogens tested. The basicity of the oxazepine nitrogen was also important to activity, as analogue **4j** was superior in both *Candida* strains relative to *N*-methoxyphenyl analogue **4k**. Several *N*-allyl derivatives displayed good anti-*Candida* activity, with the electron-deficient 2-chloroallyl analogue **4g** offering the best balance of in vitro potency across the three pathogens. Based on this broad-spectrum profile, we selected oxazepine **4g** for additional SAR investigation.

The protected hydroxy oxazepine **5** derived from 2-chloroallyl amine served as a versatile template to explore the optimal substitution pattern on the C_2 position of the heterocycle (Scheme 2). Specifically, the hydroxyl could be converted to the methyl ether (**6a**), acetate (**6b**), the inverted fluoro (**6c**), and the desoxy derivative (**6d**) using standard procedures to afford oxazepine analogues with varying lipophilic character. Ester and aldehyde deprotection (TBAF, then 1 N HCl) afforded final targets **6a–d** for MIC determination.

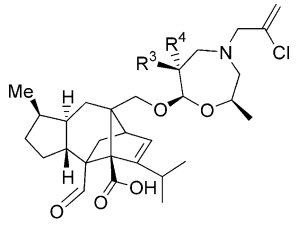
Based on our earlier SAR observations, we expected that masking the polarity of the 2′-OH group via the ether or ester would improve the antifungal properties of our lead compound. However, diminished activity was found for both **6a** and **6b**, suggesting that C_2-oxygenation of the oxazepine heterocycle was not favorable (Table 2). Loss of activity with C_2-functionalized derivatives has also been observed by GlaxoSmithKline in their naturally-derived pyranose series.[5a]

The activity of the parent compound **4g** was improved with the fluoro and desoxy derivatives **6c** and **6d**. A comparison of the inhibition of *C. albicans* growth by **6c** and **6d** suggests that a subtle inductive or H-bonding effect favors the fluoro analogue **6c** over the isosteric dihydro derivative. However, oxazepine **6d** appears to offer the best balance of antifungal potency across the



Scheme 2. Oxazepine C_2 derivatization. (a) MeI, NaH, THF (57% yield of final target **6a**, R^3 = OMe, R^4 = H); (b) Ac_2O, pyridine, DMAP (52% yield of **6b**, R^3 = OAc, R^4 = H); (c) DAST, CH_2Cl_2 (22% yield of **6c**, R^3 = H, R^4 = F); (d) (i) CS_2, NaH, THF, then MeI; (ii) Bu_3SnH, AIBN, toluene, 80 °C (6% yield of **6d**, R^3 = R^4 = H).

Table 2. In vitro activity of sordarin oxazepines **4g** and **6a–d**



6a–d

Compd	R^3	R^4	MIC (μg/mL) *C. albicans*	*C. glabrata*	*C. neoformans*
3	—	—	<0.06	0.125	16
4g	OH	H	0.5	32	4
6a	OMe	H	4	64	>128
6b	OAc	H	8	16	>128
6c	H	F	<0.06	2	16
6d	H	H	0.125	2	1

different pathogens, and in particular offers a significant advantage over morpholine derivative **3** with respect to MIC against *C. neoformans* (1 vs 16 μg/mL). It is interesting that a one-carbon homologation of the morpholine side chain in **3** affords a measurable improvement in *C. neoformans* growth inhibition while maintaining anti-*Candida* potency.

The antifungal activity of basic heterocycles such as oxazepine **6d** is in some ways unexpected given the stringent requirement for lipophilic substitution patterns identified in earlier sordaricin-based series.[8,17] Our results suggest that the moderate activity observed against certain pathogens (i.e., *C. neoformans*) that were believed to be not susceptible to sordarin-derived analogues can in part be attributed to the tertiary nitrogen in the oxazepine ring. While it is difficult to speculate whether the oxazepine nitrogen participates in an active site hydrogen bond or simply orients the chloroallyl side chain within the EF-2/ribosome assembly, it is clear that a more detailed understanding of the relevant active site interactions between drug and target in each fungal species is required.[18]

In conclusion, we have identified an oxazepine heterocycle as a competent surrogate for the sordarin sugar moiety. We arrived at this ring system via a novel *syn*-selective mono-oxidation of an unprotected triol, and we were able to elaborate the unreacted secondary alcohol to optimize for potency. This work suggests that heterocyclic replacements such as morpholines, oxazepines, and certainly others may prove useful in the exploration of other sugar-containing pharmacophores.

Note Added in Proof

A related sordarin oxazepine ring system was recently reported by Kaneko et al. in *Biorg. Med. Chem. Lett.* **2002**, *12*, 1705.

Acknowledgements

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12. This strain has been deposited with the ATCC for registration.
13. **General procedure for synthesis of 4a–n**: An aqueous solution of $NaIO_4$ (1.25 equiv) was added dropwise to a 0 °C solution of **2a** (1.0 equiv) and powdered $NaHCO_3$ (2.5 equiv) in MeOH. The mixture was stirred for 2 h at room temperature before it was filtered and concentrated in vacuo. The residue was taken up in CH_3CN and charged with the amine (2.0 equiv) followed by $NaBH_3CN$ (1.0 equiv), and the reaction was allowed to stir overnight at room temperature. Aqueous workup afforded the crude reductive amination product which was deprotected (TBAF in THF, then 1 N HCl in MeOH) according to standard procedures. The final targets **4a–n** were purified to homogeneity by silica gel chromatography.
14. For other examples of *syn*-selective oxidation of triols, see (a) Jeong, L. S.; Shinazi, R. F.; Beach, J. W.; Kim, H. O.; Nampalli, S. *J. Med. Chem*. **1993**, *36*, 181. (b) Stache, U.; Radscheit, K.; Haede, W.; Fritsch, W. *Liebigs Ann. Chem*. **1982**, *2*, 342.
15. The purity and identity of all new compounds were established by ^1H NMR, HPLC, and LRMS. For instance, analytical data for **4g**: ^1H NMR ($CDCl_3$, 400 MHz) δ 9.68 and 9.67 (2s, 1H), 6.10 (d, *J* 2.3 Hz, 1H), 5.84 (s, 2H), 5.77 (d, *J* 1.4 Hz, 1H), 4.73 (s, 1H), 4.37 (d, *J* 6.8 Hz, 1H), 4.25 (br m, 1H), 4.07 (s, 2H), 4.05–3.94 (series of m, 3H), 3.80–3.75 (m, 1H), 3.70 (d, *J* 9.6 Hz, 1H), 3.59–3.41 (series of m, 3H), 3.24–3.21 (m, 1H), 2.67–2.65 (m, 1H), 2.38–2.32 (m, 1H), 2.10–1.95 (series of m, 5H), 1.91–1.80 (m, 2H), 1.79–1.74 (m, 1H), 1.31 (d, *J* 13.0 Hz, 1H), 1.27 (d, *J* 5.6 Hz, 3H), 1.22–1.18 (m, 1H), 1.04 (d, *J* 6.8 Hz, 3H), 1.03–0.98 (m, 1H), 0.97 (d, *J* 6.7 Hz, 3H), 0.79 (d, *J* 6.7 Hz, 3H); LRMS (ESI, *m*/*z*, $M\text{-}H^-$) 534.
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