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Identification of a Broad-Spectrum Azasordarin with Improved Pharmacokinetic Properties

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Abstract—The synthesis and antifungal activity of 5′- and 5′-6′-substituted azasordarin derivatives are described. Modification of the 5′-position led to the discovery of the spirocyclopentyl analogue **7g**, which is the first azasordarin to register single-digit MIC values versus *Aspergillus spp*. Further investigation identified the 5′-*i*-Pr derivative **7b**, which displays superior pharmacokinetic properties compared to other azasordarins.

Invasive fungi have in recent decades emerged as important pathogens, in particular among the growing and increasingly diverse population of immunocompromised patients. Specifically, infections due to *Candida spp*. constitute the fourth leading cause of nosocomial bloodstream infections, while the incidence and mortality rates associated with the fungal pathogens *Aspergillus spp*. and *Cryptoccocus neoformans* are rapidly on the rise.[1] Despite the growing medical need, however, few existing therapies with suitable safety and broad spectrum profiles are available for the treatment of systemic fungal infections. Moreover, the emergence of fungal pathogens resistant to current chemotherapies further increases the demand for the discovery and development of novel antifungal agents.

The natural product sordarin (**1**, Fig. 1) was discovered in 1971 as a metabolite of *Sordaria araneosa* and identified as a potent antifungal compound.[2] 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.[3,4] Despite the high sequence homology between fungal and human

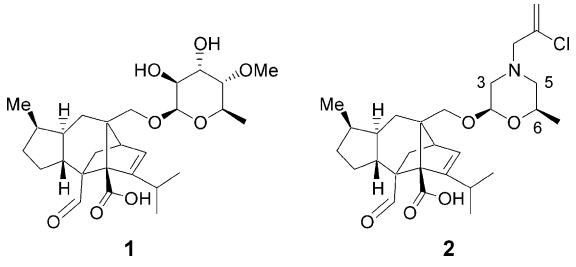

Figure 1. Sordarin (**1**) and azasordarin (**2**).

EF-2 (85%), sordarin is able to stabilize the post-translational complex through high-affinity binding to the fungal ribosome/EF-2 complex and block translocation.[5] The selective inhibition of fungal protein synthesis thus became an attractive target for the development of new antifungal agents that were mechanistically distinct from existing therapies.

Researchers at Glaxo have recently disclosed azasordarin derivatives such as **2** in which the 6′-deoxyglycoside found in the natural product has been replaced by a morpholine ring.[6,7] While compound **2** extends the antifungal spectrum of the sordarin class to include *Candida glabrata* and *C. parapsilosis*, no inhibitory activity was observed against *C. neoformans* or the filamentous fungi *Aspergillus spp*. Moreover,

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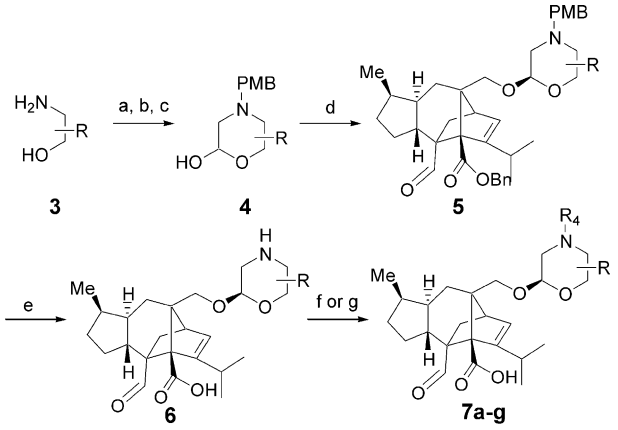
pharmacokinetic analysis demonstrated that **2** displayed a short plasma half-life in mice, and that b.i.d. (or higher) dosing frequency was required to demonstrate efficacy.[8] Having identified these limitations in spectrum and bioavailability, our goal at the outset of our discovery efforts was to identify novel azasordarins with improved antifungal and metabolic properties. Our strategy was to explore diverse substitution patterns on the morpholine ring in order to expand the SAR of the azasordarins and identify potential interactions within the active sites of less-susceptible fungal pathogens.

In order to prepare azasordarin derivatives with novel ring substitution patterns, we anticipated the synthesis of morpholino lactols and subsequent coupling with the sordarin aglycone, known as sordaricin. A wide range of enantiopure amino alcohols[9] were thus condensed with *p*-anisaldehyde, and then subjected to a second reductive amination event with dimethoxyacetaldehyde (Scheme 1). Acidic hydrolysis of the dimethyl ketal intermediate then afforded the ring-closed morpholino lactols **4** in 20–40% overall yield from amino alcohol.

To construct the anomeric bond, we treated the morpholino lactols **4** with NaH in THF, and then alkylated with a suitably protected sordaricin triflate derivative as has been previously reported.[10] The diastereoselectivity of this lactol alkylation appeared to depend on the resident chirality in the morpholino ring, as the lactol derived from (*R*)-valinol afforded the β-anomeric configuration found in the natural product as the major adduct, whereas the (*S*)-derived diastereomer gave rise to the unnatural α-configuration. While we do not fully understand the origins of this stereoselectivity, some insight may be gleaned by examining the low-energy chair conformations of the glycosidated products (Fig. 2). The observed major products allow the diequatorial disposition of both the 5-*i*-Pr and the glycosidic substituents, and presumably this is lower in energy than a transition-state assembly that situates either of these groups axial.[11] Following lactol alkylation, the amine



Scheme 1. Synthesis of azasordarin derivatives. (a) *p*-anisaldehyde, polymer-supported BH_4^-, MeOH; (b) dimethoxyacetaldehyde, $ZnCl_2$, $NaBH_3CN$, MeOH; (c) 6 N HCl, 80 °C; (d) Bn sordaricin triflate, NaH, THF; (e) H_2, $Pd(OH)_2$ on C, MeOH; (f) R_4-X, $NaHCO_3$, NaI, EtOH, 80 °C; (g) RCHO, $NaBH_3CN$, CH_3CN.

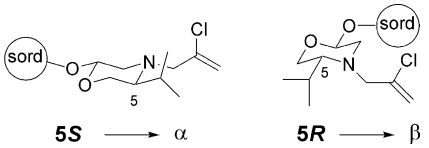


Figure 2. Impact of C_5 stereochemistry on glycosidic linkage.

and ester protecting groups were removed to reveal the morpholino acid **6**, which was *N*-alkylated under standard conditions (R-X, $NaHCO_3$, EtOH, 80 °C) to afford the final azasordarin targets **7a–g**.[12]

The in vitro activity of the novel 5-substituted morpholin-2-yl azasordarins was determined against a diverse panel of fungal pathogens (Table 1). A measurable improvement in spectrum was observed in transposing the methyl substituent from the 6′- to 5′-position of the morpholino ring. Namely, the 6′-Me analogue **2** was devoid of activity versus the pathogens *C. neoformans* and *Aspergillus fumigatus*, whereas the 5′-Me analogue **7a** registered MIC values of <0.06 and 32 μg/mL, respectively. Polar functionality was not tolerated at the 5′-position (**7c**, $R = CH_2OH$), which is consistent with structure–activity relationships identified in other heterocyclic series.[7b]

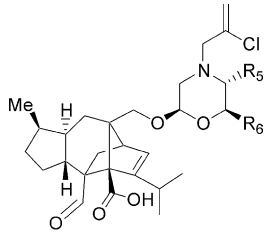
The 5,6-disubstituted morpholine derivatives **7e** ($R_5 = R_6 = Me$) and **7f** [R_5, $R_6 = -(CH_2)_3-$] confirmed our observation that substitution of the 5′-position broadened the antifungal spectrum of the azasordarins, with single digit values observed for *C. parapsilosis* and *Aspergillus flavus* and values of <0.25 μg/mL versus *C. albicans*, *C. glabrata*, and *Cryptococcus neoformans*.[13] Excellent potency was also observed with the *i*-Pr-substituted derivative **7b**, with MIC values less than 0.06 μg/mL observed versus *C. albicans, C. glabrata*, and *C. neoformans*. The geminally-substituted *spiro*-cyclopentyl morpholin-2-yl azasordarin **7g** exhibited the best MIC profile of all the derivatives listed in Table 1, and is also the only compound to register a single digit (8 μg/mL) MIC versus *A. fumigatus*.[14] Collectively, the high degree of lipophilicity and steric bulk tolerated at the 5′-position of the morpholine ring suggest a possible binding mode for the azasordarins within a hydrophobic pocket of the fungal protein synthesis machinery.

We next embarked on identifying the optimal nitrogen substitution pattern on the morpholine ring. Synthetic considerations prompted us to investigate this position in the 5′-Me series, as analogues with bulkier 5′-substituents (i.e., *i*-Pr) were either unreactive or afforded mixtures of amine and carboxylate alkylation products under our reaction conditions. An extremely stringent SAR emerged in this 5′-Me azasordarin series (Table 2). Namely, *N*-allyl substitution appeared to be required in order to observe antifungal activity that included *C. parapsilosis*, *C. neoformans*, and *Asp. spp.* Within the *N*-allyl derivatives, halogenation at the 2-position was preferred over 3-halo substitution, while surprisingly the 2-cyano derivative **8e** was less active than electronically-similar halogenated analogues. The importance of the sidechain olefin is underscored by the disparate MICs

observed with the 2-methallyl derivative **8d** and the corresponding saturated analogue **8n**, which completely lacks measurable activity versus *C. parapsilosis* and *Asp. spp.*, and diminished potency versus *C. neoformans*. However, *N*-benzyl (i.e., **8o** and **8p**) or heteroaromatic derivatives (**8q**) are not suitable replacements for the allyl moiety.

A known liability of previously disclosed azasordarin analogues is their short plasma half-lives in mice,

Table 1. Antifungal activity of sordarin morpholino derivatives **2** and **7a–g**

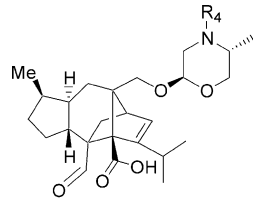


7a-g

Compd	R_5	R_6	MIC[a] (μg/mL)					
			CP	CA	CN	CG	AF[a]	AL[b]
2	—	Me	8	<0.06	>128	0.125	>128	4
7a	Me	—	1	<0.06	<0.06	0.125	32	0.5
7b	*i*-Pr	—	2	<0.06	<0.06	<0.06	32	2
7c	CH_2OH	—	128	0.5	2	1	>128	>128
7d	CF_3	—	>128	<0.06	>128	1	>128	>128
7e	Me	Me	8	<0.06	0.5	0.25	64	1
7f	Cyclopentyl		4	<0.06	0.25	0.125	64	4
7g	Spirocyclopentyl	—	0.5	<0.008	<0.008	0.015	8	0.125

[a]MIC value defined as the lowest drug concentration required to inhibit 90–100% visible growth over 48 h relative to controls. CP = *C. parapsilosis* CA = *C. albicans* CN = *Cryptococcus neoformans* CG = *C. glabrata* AF = *Aspergillus fumigatus* AL = *Aspergillus flavus*.
[b]MIC determined after 24 h.

Table 2. Variation of *N*-substitution pattern



7a, 8a-s

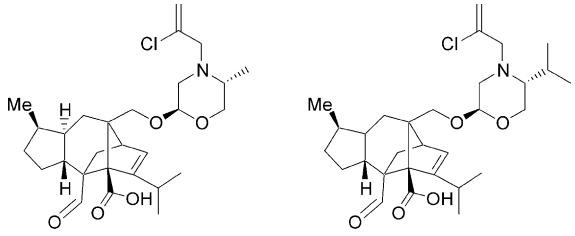
Compd	R_4	MIC (μg/mL)					
		CP	CA	CN	CG	AF	AL
8a	H	>128	8	>128	64	>128	>128
8b	Allyl	64	0.125	128	0.25	>128	>128
7a	2-Chloroallyl	1	<0.06	<0.06	0.125	32	0.5
8c	2-Bromoallyl	4	<0.06	<0.06	0.125	128	4
8d	2-Methallyl	8	<0.06	0.125	0.125	>128	8
8e	2-Cyanoallyl	>128	0.25	4	1	>128	128
8f	2-Chloroallyloxy	>128	0.125	1	2	>128	>128
8g	*cis*-3-Chloroallyl	>128	0.125	128	1	>128	>128
8h	*cis*-3-Bromoallyl	>128	<0.06	>128	1	>128	>128
8i	3,3-Dichloroallyl	32	<0.06	16	<0.06	>128	>128
8j	3,3-Difluoroallyl	64	0.125	64	1	>128	>128
8k	3,3-Dimethylallyl	>128	0.25	>128	8	>128	>128
8l	Cyclohex-2-enyl	>128	2	>128	8	>128	>128
8m	Cyclopropylmethyl	>128	2	>128	8	>128	>128
8n	*i*-Bu	>128	<0.06	32	1	>128	>128
8o	4-MeO benzyl	4	<0.06	0.5	<0.06	>128	>128
8p	2-Cl benzyl	>128	<0.06	4	1	>128	>128
8q	2-(5-Chlorothiophenyl)	>128	<0.06	2	0.125	>128	>128
8r	Acroyl	>128	8	128	64	>128	>128
8s	Cyclopropylsulfonyl	>128	32	>128	128	>128	>128

necessitating dose frequencies of b.i.d. or higher to demonstrate efficacy.[8] Indeed, pharmacokinetic profiling of the 5′-Me substituted analogue **7a** revealed rapid clearance from mice plasma following iv administration (total body clearance rate = 95 mL/min/kg, Table 3).[15] In vitro biotransformation studies identified *N*-dealkylation of azasordarin analogues as a significant liability that limited in vivo exposure. While the stringent SAR requirements outlined above prohibited drastic structural variation on the *N*-substituent, we discovered that increasing steric bulk at the 5′-position of the morpholine slowed metabolic *N*-dealkylation while maintaining broad spectrum antifungal activity. For example, the rate of disappearance of *i*-Pr-substituted **7b** following incubation with human (rate = 0.092 nmol/min/mg) and mouse (0.082 nmol/min/mg) liver microsomes was considerably lower than for Me-substituted analogue **7a** (human: 0.214 nmol/min/mg; mouse: 0.154 nmol/min/mg).

The improved metabolic stability of **7b** versus **7a** was validated in vivo by measuring serum concentrations of each compound in mice following iv and oral dosing (Table 3). The total body clearance of **7b** was significantly reduced to 20 mL/min/kg, which correlates well with the slower rate of metabolism observed for this analogue. With respect to **2**, compound **7b** displayed a longer iv half-life ($T_{1/2}$ = 1.6 h), which may be attributed to the greater volume of distribution for **7b**. Compound **7b** appears therefore to have favorable pharmacokinetic properties compared to other azasordarins with respect to metabolic stability and plasma half-life, suggesting that further structural modifications to enhance the bioavailability of other azasordarin derivatives should be possible.

In conclusion, we have identified a series of 5′-substituted morpholin-2-yl azasordarin derivatives with superior antifungal and pharmacokinetic properties. The 5′-position of the morpholino sidechain proved to be quite versatile towards improving potency against less-susceptible pathogens (i.e., *A. flavus*) as well as reducing hepatic clearance, and led to the discovery of the most potent sordarin derivative (**7g**) disclosed to date. The in vivo efficacy of **7g** and other analogues such as **7b** in systemic fungal infection models (i.e., *Cryptococcus*) thought to be resistant to the sordarins will be reported in due course.

Table 3. Mouse pharmacokinetic parameters of azasordarin analogues **2**, **7a**, and **7b** following iv (5 mg/kg) and oral (20 mg/kg) administration (n = 3)



7a **7b**

	2	**7a**	**7b**
C_{max} (ng/mL)	N/A	5946	3882
Total CL (mL/min/kg)	13[a]	95	20
Vss (L/kg)	0.49[a]	7.1	1.6
iv $T_{1/2}$ (h)	0.44[a]	1.8	1.6
Oral $T_{1/2}$ (h)	N/A	2.1	3.1
F(%)	N/A	100	83

[a]Data obtained from ref 8.

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12. The purity and identity of all new compounds were established by ^{1}H NMR and HRMS. For instance, analytical

data for **7b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.80 (s, 1H), 6.06 (m, 1H), 5.42 (s, 1H), 5.34 (s, 1H), 4.43 (dd, J = 7.0, 2.7 Hz, 1H), 4.23 (d, J = 9.5 Hz, 1H), 4.12 (app q, J = 7.0 Hz, 2H), 3.88 (dd, J = 11.9, 3.4 Hz, 1H), 3.59 (d, J = 15.0 Hz, 1H), 3.44 (dd, J = 11.6, 7.3 Hz, 1H), 3.40 (d, J = 9.5 Hz, 1H), 3.02 (d, J = 14.6 Hz, 1H), 3.00 (dd, J = 11.9, 4.0 Hz, 1H), 2.55 (m, 1H), 2.33 (m, 1H), 1.75-2.24 (m, 8H), 1.26 (m, 3H) 1.03 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H), 0.81 (d, J = 7.0 Hz, 3H). HRMS (ES) exact mass calcd for $C_{30}H_{44}ClNO_5$ ($M + H^+$): 534.2986; found 534.2987.

13. (a) The exceptional MIC observed with several analogues versus *C. neoformans* and *Asp. fumigatus* is interesting in light of recent studies which suggest that EF-2 in these fungi should not be susceptible to sordarin derivatives. See: Shastry, M.; Nielsen, J.; Ku, T.; Hsu, M.; Liberator, P.; Anderson, J.; Schmatz, D.; Justice, M. C. *Microbiology* **2001**, *147*, 383. (b) Santos, C.; Ballesta, J. P. G. *Molec. Microb.* **2002**, *43*, 227.

14. The anomeric configuration of **7g** was established by X-ray crystallography of the corresponding morpholine precursor **6**. The α-anomer of compound **7g** was considerably less active against all pathogens tested (*C. albicans* MIC = 0.5 μg/mL; *C. glabrata* MIC = 4 μg/mL; all others > 16 μg/mL).

15. Although **7a** exhibited high total body clearance after iv administration, the oral bioavailability was 100%, which may be attributed to the rapid absorption of **7a** and the resulting saturation of first-pass liver metabolism.