Activity of MK-3118, a New Oral Glucan Synthase Inhibitor, Tested Against *Candida* and *Aspergillus* spp. using Two Reference Broth Microdilution Methods

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AMENDED ABSTRACT

Background: MK-3118 is a potent inhibitor of fungal glucan synthase that is derived from enfumafungin. We compared the activity of MK-3118 against *Aspergillus* spp. (ASP) and *Candida* spp. (CSP) using CLSI and EUCAST broth microdilution (BMD) methods.

Methods: 71 ASP (23 *A. flavus* [AFL], 21 *A. fumigatus* [AFU], 9 *A. niger* [ANG], and 18 *A. terreus* [ATR]) and 113 CSP (29 *C. albicans* [CA], 29 *C. glabrata* [CGLA], 15 *C. parapsilosis* [CPRP], 21 *C. tropicalis* [CTRO] and 18 *C. krusei* [CKRU]) were tested against MK-3118, amphotericin B (AMB; ASP) and fluconazole (FLC; CSP) and caspofungin (CAS) by CLSI and EUCAST methods. MK-3118 was read at 24-h, 48-h, 50% and 100% inhibition. ASP included 8 itraconazole (ITR)-resistant (R; MIC, ≥4 µg/mL) and CSP included 31 CAS-R *fks* hot spot (HS) mutants (15 also FLC-R) and 19 FLC-R.

Results: Activities of MK-3118, CAS, FLC and AMB are displayed in the **Table**. MK-3118 demonstrated good activity against ASP and CSP. MK-3118 showed excellent activity against ITR-R strains of AFU (MEC range, 0.03 to 0.5 µg/mL), ANG and ATR (MEC, 0.06 and 0.12 µg/mL, respectively). MK-3118 was very active against FLC-R CA (MIC range, 0.06-2 µg/mL), CGLA (0.5-2 µg/mL), CTRO (0.25-1 µg/mL), and CPRP (0.25-0.5 µg/mL) and was most potent against CAS-R CA (MIC range, 0.12-2 µg/mL), CGLA (0.5-2 µg/mL), CTRO (0.25-2 µg/mL) and CKRU (0.5-2 µg/mL). Overall essential agreement (±2 $\log_2$ dilution steps) was 94.3% for ASP and 97.1% for CSP.

	MIC/MEC$_{50/90}$ (µg/mL)					
	MK-3118		Caspofungin		Fluconazole/ Amphotericin B[c]	
Species	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST
C. albicans (29)[a]	0.12/2	0.06/1	0.12/2	0.12/2	0.25/≥128	0.25/≥128
C. glabrata (29)[a]	0.5/2	0.25/1	0.12/16	0.25/4	32/≥128	8/64
C. parapsilosis (15)[a]	0.25/0.5	0.25/0.5	0.5/0.5	1/1	1/64	1/32
C. tropicalis (21)[a]	0.25/1	0.25/1	0.06/1	0.25/2	0.5/≥128	1/≥128
C. krusei (18)[a]	0.5/2	0.5/1	0.12/1	0.5/1	32/≥128	32/≥128
A. flavus (23)[b]	0.06/0.12	0.06/0.12	0.015/0.03	0.06/0.12	2/2	1/1
A. fumigatus (21)[b]	0.12/0.25	0.06/0.06	0.03/0.06	0.06/0.12	1/2	0.5/0.5
A. niger (9)[b]	0.06/-[d]	0.03/-[d]	0.03/-[d]	0.06/-[d]	1/-[d]	0.25/-[d]
A. terreus (18)[b]	0.06/0.12	0.06/0.06	0.015/0.06	0.06/0.12	2/2	1/4

a. 24-h readings, 50% inhibition.
b. 48-h readings, 50% inhibition.
c. Fluconazole was tested for *Candida* spp. and Amphotericin B for *Aspergillus* spp.
d. Not calculated due to number of strains, <10.

Conclusions: MK-3118 was shown to be a potent and broad-spectrum antifungal agent regardless of the BMD method applied, with excellent activity against challenging R strains of ASP and CSP.

INTRODUCTION

The echinocandins, anidulafungin, caspofungin and micafungin, are now well-established as first-line agents for the treatment of invasive candidiasis (IC) and serve as the prime example for the efficacy of inhibitors of glucan synthase as antifungal agents. Despite this clinical success, one important limitation of the echinocandins is the requirement for intravenous administration. An orally bioavailable glucan synthase inhibitor with comparable antifungal activity to the parenteral echinocandins would provide a valuable benefit for the treatment and prophylaxis of IC and other fungal infections. Additionally, although mould-active azoles (itraconazole, posaconazole and voriconazole) are the primary class of antifungal agents used for the treatment and prevention of invasive aspergillosis (IA), an echinocandin with an oral formulation could play a role as an alternative to the azoles.

MK-3118 is an orally active, semi-synthetic derivative of the natural product enfumafungin with in vitro and in vivo activity against *Candida* and *Aspergillus* species. MK-3118 and other derivatives of enfumafungin are potent inhibitors of fungal glucan synthase, yet these compounds are structurally distinct from the echinocandins. Mutations in *fks* that are associated with resistance to the echinocandins are distinctly different from those causing decreased susceptibility to enfumafungin derivatives; likewise, echinocandin-resistant isolates remain susceptible to these agents.

In the present study, we determine the activity and potency of MK-3118, caspofungin and fluconazole against panels of *Candida* spp. and *Aspergillus* spp. isolates, selected to represent phenotypically and genotypically antifungal resistant strains; and we first examine the effect of incubation time and MIC endpoint criteria on the results of the Clinical and Laboratory Standards Institute (CLSI) method and subsequently determine the essential agreement (EA; MIC ±2 $\log_2$ dilutions) between CLSI and EUCAST broth microdilution (BMD) results using the optimized CLSI conditions.

MATERIALS AND METHODS

Organisms. A total of 113 *Candida* spp. and 71 *Aspergillus* spp. obtained from centers participating in the 2008-2010 ARTEMIS and SENTRY Antimicrobial Surveillance Programs were evaluated. The collection included: *A. flavus* species complex (SC; 23), *A. fumigatus* SC (21), *A. terreus* SC (18), *A. niger* SC (9), *C. albicans* (29), *C. glabrata* (29), *C. tropicalis* (21), *C. parapsilosis* (15) and *C. krusei* (19). The collection contained 34 fluconazole-resistant *Candida* spp. strains, 31 caspofungin-resistant *Candida* spp. strains and eight itraconazole-resistant (MIC, ≥4 µg/mL) *Aspergillus* spp. Before testing, each isolate was subcultured at least twice on potato dextrose agar (Remel, Lenexa, Kansas, USA) to ensure viability and purity.

Susceptibility testing. All isolates were tested for in vitro susceptibility to MK-3118 and comparator agents using the CLSI and EUCAST BMD methods. Reference powder of MK-3118 was obtained from the manufacturer. Stock solutions were prepared in DMSO and the final range of MK-3118 concentrations tested was 0.008-16 µg/mL. CLSI BMD testing was performed exactly as outlined in document M27-A3 by using RPMI 1640 medium with 0.2% glucose, inocula of $0.5x10^3$ to $2.5x10^3$ cells/mL and incubation at 35°C. MIC values were determined visually after both 24- and 48-h of incubation. MIC/MEC endpoint criteria at both reading times include the lowest concentration of drug that caused a significant diminution (≥50% inhibition) as well as complete (100%) inhibition of growth relative to that of the growth control.

EUCAST BMD testing was performed as outlined in document EDef. 7.1 by using RPMI 1640 with 2.0% glucose, inocula of $0.5x10^5$ to $2.5x10^5$ cells/mL and incubation at 35°C. MIC/MEC values were determined spectrophotometrically (at 530 nm) for yeasts and visually for moulds, after 24-h of incubation as the lowest concentration of drug that resulted in both ≥50% inhibition and 100% inhibition of growth relative to that of the growth control.

Quality control (QC) was ensured by testing the following strains recommended by CLSI and EUCAST; *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019.

Analysis. The MIC results for MK-3118 obtained with the CLSI method using both partial (≥50%) and complete (100%) inhibition were compared at each reading time in order to determine the EA between MIC values obtained at 24- and 48-h with each endpoint criterion and subsequently to determine the EA at 24-h between MIC values determined using partial versus complete endpoint criteria. The MIC results for MK-3118 obtained with the EUCAST method were compared with those of the CLSI method at 24-h of incubation using both complete and partial inhibition criteria for both methods. High off-scale MIC results were converted to the next highest concentration and low off-scale MIC results were left unchanged. Discrepancies of <±2 $\log_2$ dilutions among MIC results were used to calculate the EA.

RESULTS

- MK-3118 was active against *C. albicans* (MIC$_{90}$, 1 µg/mL for CLSI method read at 50% inhibition and 24-h incubation), *C. glabrata* (1 µg/mL), *C. parapsilosis* (0.5 µg/mL), *C. tropicalis* (1 µg/mL), and *C. krusei*, (2 µg/mL; **Table 1**). High levels of resistance to fluconazole (19.1 to 44.8%) and caspofungin (0.0 to 34.5%; **Table 1**) were noted among these strains selected to include challenging resistant strains.
- MK-3118 displayed similar activity to caspofungin against *C. albicans*, *C. parapsilosis*, *C, tropicalis* and *C. krusei* (**Table 1**), but was eight-fold more potent than caspofungin against *C. glabrata* strains (MIC$_{90}$, 2 and 16 µg/mL, respectively).
- All *Aspergillus* spp. were inhibited by ≤0.25 µg/mL of MK-3118 as determined by EUCAST and 69/71 (97.2%) were inhibited at this MEC value as determined by CLSI BMD methods.
- MK-3118 was active against 31 *Candida* spp. strains that were *fks1* or *fks2* mutants (**Table 2**), inhibiting all these strains at ≤2 µg/mL and 22 (71.0%) strains at ≤1 µg/mL. Nine isolates showed MK-3118 MIC values of 2 µg/mL (*C. albicans* [3], *C. glabrata* [3], *C. krusei* [2] and *C. tropicalis* [1]). Seven (78.0%) of these strains had mutations in position 641 (or 625 for *C. glabrata* numbering scheme) of the *fks1* HS1.
- Among the 34 fluconazole-resistant strains tested, the MIC for MK-3118 was ≤1 µg/mL for 32 (94.1%; data not shown) strains. Co-resistance to both fluconazole and caspofungin was evident among isolates of *C. albicans* (61.5%) and *C. glabrata* (58.3%), but was also noted among five *C. parapsilosis* and four *C. tropicalis* strains.
- MK-3118 and caspofungin were both quite active against the itraconazole-resistant (MIC, ≥4 µg/mL) *Aspergillus* spp. isolates in the collection (**Table 3**). The MEC results for caspofungin ranged from 0.015 to 0.06 µg/mL and those for MK-3118 ranged from 0.03 to 0.5 µg/mL.
- Comparison of CLSI and EUCAST methods read at 24-h for *Candida* spp. and 48-h for *Aspergillus* spp. of incubation and partial inhibition revealed an EA ranging from 95.2 to 100.0% for *Candida* spp. and from 85.7 to 100.0% and for *Aspergillus* spp. from 85.7 to 100.0% (**Table 4**).

Table 1. In vitro activity of an oral glucan synthase inhibitor, MK-3118, and comparator antifungal agents tested against *Candida* and *Aspergillus* spp. as determined by CLSI broth microdilution methods.

		MIC/MEC[a] (µg/mL)[b]:		
Species (no. tested)	Antifungal agent	Range	50%	90%
C. albicans (29)	MK-3118	0.06 - 2	0.12	1
	Caspofungin	0.015 - 8	0.12	2
	Fluconazole	0.06 - ≥128	0.25	≥128
C. glabrata (29)	MK-3118	0.5 - 2	0.5	2
	Caspofungin	0.03 - 16	0.12	16
	Fluconazole	2 - ≥128	32	≥128
C. parapsilosis (15)	MK-3118	0.25 - 1	0.25	0.5
	Caspofungin	0.25 - 0.5	0.5	0.5
	Fluconazole	0.25 - 64	1	64
C. tropicalis (21)	MK-3118	0.06 - 2	0.25	1
	Caspofungin	0.03 - 4	0.06	1
	Fluconazole	0.12 - ≥128	0.5	≥128
C. krusei (19)	MK-3118	0.5 - 2	0.5	2
	Caspofungin	0.12 - 8	0.12	1
	Fluconazole	16 - ≥128	32	≥128
A. flavus SC[c] (23)	MK-3118	0.06 - 0.12	0.06	0.12
	Caspofungin	≤0.008 - 0.03	0.015	0.03
	Amphotericin B	1 - 2	2	2
A. fumigatus SC (21)	MK-3118	0.03 - 1	0.12	0.25
	Caspofungin	0.015 - 0.25	0.03	0.06
	Amphotericin B	1 - 2	1	2
A. terreus SC (18)	MK-3118	0.03 - 0.25	0.06	0.12
	Caspofungin	≤0.008 - 0.06	0.015	0.06
	Amphotericin B	1 - 4	2	2
A. niger SC (9)	MK-3118	0.03 - 0.25	0.06	ND[d]
	Caspofungin	≤0.008 - 0.06	0.03	ND
	Amphotericin B	1	1	ND

a. MIC, minimum inhibitory concentration; MEC, minimum effective concentration.
b. 50% and 90%, MEC/MIC that encompasses 50% and 90% of isolates tested, respectively.
c. SC, species complex.
d. ND = not determined due to number of isolates <10.

Table 3. In vitro activities of MK-3118 and comparators against itraconazole-resistant (MIC, ≥4 µg/mL) *Aspergillus* spp. as determined by CLSI broth microdilution methods.

	MIC/MEC[a] (µg/mL) for:		
Species	Amphotericin B	Caspofungin	MK-3118
A. fumigatus SC	1	0.015	0.03
A. fumigatus SC	1	0.03	0.06
A. fumigatus SC	1	0.06	0.5
A. fumigatus SC	1	0.06	0.25
A. fumigatus SC	1	0.03	0.25
A. fumigatus SC	1	0.06	0.12
A. niger SC	1	0.06	0.06
A. terreus SC	1	0.03	0.12

a. MIC, minimum inhibitory concentration; MEC, minimum effective concentration.

Table 2. MK-3118 results compared to caspofungin against selected strains displaying *fks* mutations.

		FKS Alterations				MK-3118 MIC (µg/mL)[a]		Caspofungin MIC (µg/mL)[a]	
Organism	Year	FKS1 HS1	FKS1 HS2	FKS2 HS1	FKS2 HS2	CLSI	EUCAST	CLSI	EUCAST
C. albicans	2009	F641I	WT[b]	NT[b]	NT	2	1	0.5	1
C. albicans	2009	F641Y	WT	NT	NT	0.5	2	1	1
C. albicans	2010	S645P	WT	NT	NT	0.5	0.5	2	4
C. albicans	2010	F641S	WT	NT	NT	2	1	1	2
C. albicans	2010	S645Y	WT	NT	NT	0.25	0.12	2	2
C. albicans	2010	S645F	WT	NT	NT	0.12	0.12	2	2
C. albicans	2010	D648Y	WT	NT	NT	0.25	0.06	0.5	0.5
C. albicans	2010	P649H	WT	NT	NT	0.25	0.12	0.5	0.5
C. albicans	2010	S645F	R1361H	NT	NT	0.12	0.12	2	2
C. albicans	2010	S645P	WT	NT	NT	1	0.25	8	4
C. albicans	2010	F641S	WT	NT	NT	2	2	1	2
C. albicans	2010	F641S	WT	NT	NT	1	1	0.5	1
C. glabrata	2008	S629P	WT	NT	NT	1	0.5	16	8
C. glabrata	2008	D632Y	WT	NT	NT	0.5	0.25	0.25	0.25
C. glabrata	2009	L630I	WT	NT	NT	0.5	0.5	0.12	0.25
C. glabrata	2008	WT	WT	S663P	WT	1	0.5	16	16
C. glabrata	2008	WT	WT	S663F	WT	0.5	0.25	0.5	0.5
C. glabrata	2010	F625S	WT	WT	WT	2	1	1	2
C. glabrata	2010	S629P	WT	WT	WT	1	0.5	8	16
C. glabrata	2010	D632G	WT	WT	WT	1	1	2	2
C. glabrata	2010	WT	WT	S663P	WT	2	0.5	16	4
C. glabrata	2006	WT	WT	F659V	WT	2	2	1	4
C. glabrata	2010	WT	WT	D648E	WT	0.5	1	0.12	0.5
C. glabrata	2010	F625Y	WT	WT	WT	0.5	0.25	0.12	0.25
C. krusei	2010	WT	R1361H	NT	NT	0.5	0.5	8	4
C. krusei	2010	F655C	WT	NT	NT	2	1	1	1
C. tropicalis	2009	F641S	WT	NT	NT	2	2	1	2
C. tropicalis	2009	F641S	WT	NT	NT	2	1	1	1
C. tropicalis	2010	S645P	WT	NT	NT	1	2	1	2
C. tropicalis	2010	S645P	WT	NT	NT	0.5	0.5	1	2
C. tropicalis	2010	F641S	WT	NT	NT	0.25	0.25	0.25	0.5

a. All results displayed were read at 24h 50% inhibition.
b. WT = Wildtype; NT = Not tested.

Table 4. Comparison of CLSI and EUCAST broth microdilution methods[a] when testing the oral glucan synthase inhibitor, MK-3118, against *Candida* spp. and *Aspergillus* spp.

		No. of isolates at indicated MIC/MEC (cumulative %; µg/mL)[b]										
Species (no. tested)	Test Method[a]	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	≥4	% EA[c]
C. albicans (29)	CLSI				5	10	6	3	2	3		100.0
	EUCAST			3	12	7	1	2	3	1		
C. glabrata (29)	CLSI							18	8	3		100.0
	EUCAST						15	10	3	1		
C. tropicalis (21)	CLSI				1	2	10	3	3	2		95.2
	EUCAST			1		3	10	3	2	2		
C. parapsilosis (15)	CLSI						8	6	1			100.0
	EUCAST					1	11	3				
C. krusei (19)	CLSI							12	5	2		100.0
	EUCAST						3	12	4			
A. flavus SC[c] (23)	CLSI				14	9						100.0
	EUCAST			5	8	9	1					
A. fumigatus SC (21)	CLSI			2	7	5	5	1	1			85.7
	EUCAST		1	5	14	0	1					
A. terreus SC (18)	CLSI			2	11	4	1					100.0
	EUCAST			7	11							
A. niger SC (9)	CLSI			3	5	0	1					88.9
	EUCAST		2	6	1							

a. CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee for Antimicrobial Susceptibility Testing.
b. MIC, minimum inhibitory concentration; MEC, minimum effective concentration.
c. EA, essential agreement (MIC ±2 $\log_2$ dilutions).
d. SC, species complex.

CONCLUSIONS

- MK-3118 is a potent, novel antifungal agent with impressive activity against both WT and antifungal-resistant strains of *Aspergillus* and *Candida* species. The oral bioavailability of this compound coupled with mechanistic studies showing different GS target from the echinocandins suggest that it may provide a valuable benefit for the treatment and prophylaxis of invasive fungal infections.
- Optimal testing conditions using CLSI and EUCAST methods include incubation of 24-h for *Candida* and 48-h for *Aspergillus* and 50% inhibition MIC/MEC endpoint criterion. Further development of this new antifungal agent appears warranted.

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