IN VITRO ACTIVITY OF SF001, A NEXT GENERATION POLYENE ANTIFUNGAL, AGAINST YEASTS AND MOULDS

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ABSTRACT

Background: SF001, a next generation renal sparing polyene with long-acting, potent, broad spectrum fungicidal activity, is currently undergoing development. It is important to understand SF001 activity against pathogenic yeasts and moulds.

Methods: The minimum inhibitory concentration (MIC) of SF001 and comparators, including liposomal amphotericin B (L-AMB), was determined against 171 *Candida* spp. and 84 *Aspergillus* spp. isolates by broth microdilution (CLSI M27/M38). The minimum fungicidal concentration (MFC) of SF001 and L-AMB was evaluated against 30 *Candida* spp. and 9 *Aspergillus* spp. isolates. Time-kill for both SF001 and L-AMB was determined against 13 *Candida* spp. isolates at 4X/8X/16X the L-AMB MIC.

Results: SF001 $MIC_{50/90}$ values (MIC_{50s}=0.12-0.25 and MIC_{90s}=0.12-0.5 µg/mL) were lower than amphotericin B (AmB) and L-AMB (MIC_{50s}=0.25-2 and MIC_{90s}=0.5-4 µg/mL) against evaluated *Candida* spp. and were comparable to L-AMB and AmB when tested against *Aspergillus* spp., excluding *A. niger* where L-AMB showed slightly lower MIC values than SF001 and AmB.

SF001 displayed MFC:MIC ratios of ≤4 (indicative of fungicidal activity) against 90% of the evaluated isolates, while L-AMB showed fungicidal activity against 77%. When evaluating SF001 at the same concentration as L-AMB by timekill kinetics, all 13 *Candida* isolates had a ≥3-log decrease in CFU in the presence of SF001, indicative of fungicidal activity.

Conclusion: SF001 showed potent in vitro activity against both yeasts and moulds, with activity exceeding L-AMB and AmB against yeasts. Both MFC and time-kill kinetics indicated that SF001 exhibits potent fungicidal activity.

BACKGROUND

- Significant mortality and morbidity results from fungal infections with around 1 billion infections globally per year and 1.7 million deaths annually.[1, 2]
- Resistance and associated toxicity for currently available antifungals highlight the need for novel and safer antifungal therapies.
- Efforts to develop less toxic alternatives to AmB that maintain its potent activity and low propensity for resistance development have led to the discovery of AmB urea derivatives.[3]
- This led to the development of SF001, an AmB urea derivative formulated in micelles that is less toxic based on selective binding of ergosterol without binding cholesterol, the latter of which is believed to result in human toxicity.[3]
- In this study, the activity of SF001 against pathogenic yeast and mould was evaluated including assessment of fungicidal activity by MFC and time-kill kinetic analysis.

METHODS

- Susceptibility testing of yeast and mould was conducted by broth microdilution in accordance with CLSI guidelines M27 and M38. MIC interpretations were made based on interpretive criteria in CLSI M60 and M61.
- For MIC testing, a total of 171 non-consecutive non-duplicate clinical *Candida* spp. and 84 *Aspergillus* spp. isolates were evaluated.
- Agents tested included SF001 (micelles), L-AMB, AmB, caspofungin (CAS), and fluconazole (FLC).
- A subset of 30 *Candida* spp. and 9 *Aspergillus* spp. were evaluated for MFC with SF001 and L-AMB using an increased inoculum density for yeast to enable detection of 3-log killing.[4] MFC:MIC ratios ≤4 indicate fungicidal activity.
- Killing of yeast was further evaluated for SF001 and L-AMB against 13 *Candida* spp. isolates by time-kill[4]; SF001 and L-AMB were tested at 4X, 8X, and 16X the L-AMB MIC and viable bacteria were assessed at 2, 4, 6, 24, and 48 hr by serial dilution and plating.

RESULTS

Table 1. Activity of SF001 and comparators against yeast

Organism	Test Agent	MIC (µg/mL)				%S	%I/SDD	%R
		Range	Mode	MIC_{50}	MIC_{90}			
C. albicans (n=30)	SF001	0.06-0.25	0.12	0.12	0.12	-	-	-
	L-AMB	0.25-1	0.5	0.5	0.5	-	-	-
	AmB	0.12-0.5	0.25	0.25	0.5	-	-	-
	CAS	≤0.008-4	0.03	0.03	0.06	96.6	0.0	3.4
	FLC	0.25->64	0.5	0.5	1	89.9	3.4	6.7
C. tropicalis (n=30)	SF001	0.06-0.25	0.12	0.12	0.12	-	-	-
	L-AMB	0.25-4	1	1	1	-	-	-
	AmB	0.25-1	0.5	0.5	1	-	-	-
	CAS	≤0.008-1	0.06	0.06	0.12	96.6	0.0	3.4
	FLC	0.12->64	0.5	0.5	16	83.3	0.0	16.7
C. glabrata (n=31)	SF001	0.12-0.25	0.12	0.12	0.25	-	-	-
	L-AMB	0.25-2	0.5	0.5	1	-	-	-
	AmB	0.25-1	0.5	0.5	0.5	-	-	-
	CAS	0.06-8	0.12	0.12	1	83.9	0.0	16.1
	FLC	1->64	4	4	>64	-	74.2	25.8
C. parapsilosis (n=30)	SF001	0.06-0.12	0.12	0.12	0.12	-	-	-
	L-AMB	0.25-1	0.25	0.25	0.5	-	-	-
	AmB	0.25-1	0.5	0.5	0.5	-	-	-
	CAS	0.12-0.5	0.25	0.25	0.25	100	0.0	0.0
	FLC	0.25-32	0.5	0.5	16	86.7	0.0	13.3
C. krusei (n=31)	SF001	0.12-0.5	0.25	0.25	0.25	-	-	-
	L-AMB	0.5-4	2	2	4	-	-	-
	AmB	0.5-2	1	1	1	-	-	-
	CAS	0.06-0.12	0.12	0.12	0.12	100	0.0	0.0
	FLC	8-64	16,32	32	64	-	-	-
C. auris (n=19)	SF001	0.06-0.5	0.12	0.12	0.5	-	-	-
	L-AMB	1-4	2	2	4	-	-	-
	AmB	0.25-2	0.5	1	2	-	-	-
	CAS	0.03-1	0.06	0.06	0.12	-	-	-
	FLC	2->64	>64	>64	>64	-	-	-

S: susceptible, I: intermediate, R: resistant, SDD: susceptible dose-dependent

Table 2. Activity of SF001 and comparators against mould

Organism	Test Agent	MIC (µg/mL)			
		Range	Mode	MIC_{50}	MIC_{90}
A. fumigatus (n=30)	SF001	0.12->16	0.25	0.25	0.5
	L-AMB	0.12->16	0.5	0.5	1
	AmB	0.25-2	1	1	1
	CAS	0.03-0.12	0.06	0.06	0.06
	FLC	8->64	>64	>64	>64
A. niger (n=29)	SF001	0.06-0.25	0.12	0.12	0.12
	L-AMB	≤0.016-0.5	≤0.016	≤0.016	0.06
	AmB	0.06-1	0.12	0.12	0.25
	CAS	≤0.008-0.06	0.016	0.016	0.03
	FLC	32->64	64	64	>64
A. flavus (n=14)	SF001	0.06-0.5	0.25	0.25	0.5
	L-AMB	≤0.016-0.5	1	1	2
	AmB	0.03-1	0.5	0.5	1
	CAS	0.015-0.06	0.03	0.03	0.03
	FLC	32->64	64	64	>64
A. terreus (n=10)	SF001	0.25-0.5	0.25	0.25	0.5
	L-AMB	0.12->16	0.5	0.5	2
	AmB	0.25-2	0.5	0.5	1
	CAS	0.016-0.03	0.03	0.03	0.03
	FLC	32-64	64	64	64

Figure 1. MIC distribution of SF001, L-AMB, and AmB against yeast (A; N=171) and mold (B; N=83)

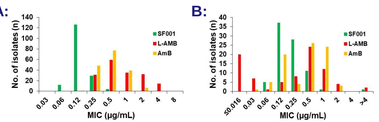


Table 3. Fungicidal Activity of SF001 and L-AMB against yeast and mould based on MFC:MIC ratio

Organism	N	Test Agent	MFC:MIC ratio (n (%))					
			1	2	4	8	>8	≤4	≥8
Candida spp.	30	SF001	4 (13.3)	14 (46.7)	9 (30.0)	1 (3.3)	2 (6.7)	27 (90.0)	3 (10.0)
		L-AMB	3 (10.0)	18 (60.0)	3 (10.0)	5 (16.7)	1 (3.3)	24 (80.0)	6 (20.0)
C. albicans	10	SF001	1 (10.0)	6 (60.0)	3 (30.0)			10 (100)	
		L-AMB	2 (20.0)	6 (60.0)	1 (10.0)	1 (10.0)		9 (90.0)	1 (10.0)
C. glabrata	5	SF001		2 (40.0)	3 (60.0)			5 (100)	
		L-AMB		3 (60.0)	1 (20.0)	1 (20.0)		4 (80.0)	1 (20.0)
C. tropicalis	5	SF001	2 (40.0)	3 (60.0)				5 (100)	
		L-AMB		3 (60.0)	1 (20.0)	1 (20.0)		4 (80.0)	1 (20.0)
C. parapsilosis	5	SF001			2 (40.0)	1 (20.0)	2 (40.0)	2 (40.0)	3 (60.0)
		L-AMB	1 (20.0)	1 (20.0)		2 (40.0)	1 (20.0)	2 (40.0)	3 (60.0)
C. krusei	5	SF001	1 (20.0)	3 (60.0)	1 (20.0)			5 (100)	
		L-AMB		5 (100)				5 (100)	
Aspergillus spp.	9	SF001	5 (55.5)	3 (33.3)		1 (11.1)		8 (88.9)	1 (11.1)
		L-AMB	5 (55.5)	1 (11.1)		2 (22.2)	1 (11.1)	6 (66.7)	3 (33.3)
A. fumigatus	3	SF001		2 (66.7)		1 (33.3)		2 (66.7)	1 (33.3)
		L-AMB	1 (33.3)	1 (33.3)		1 (33.3)		2 (66.7)	1 (33.3)
A. niger	3	SF001	3 (100)					3 (100)	
		L-AMB	3 (100)					3 (100)	
A. flavus	1	SF001	1 (100)					1 (100)	
		L-AMB				1 (100)			1 (100)
A. terreus	2	SF001	1 (50.0)	1 (50.0)				2 (100)	
		L-AMB	1 (50.0)				1 (50.0)	1 (50.0)	1 (50.0)

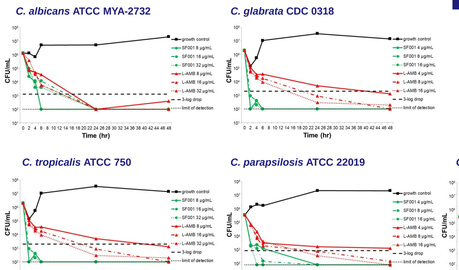
RESULTS

Table 4. Summary of log-killing observed by time-kill for SF001 and L-AMB against yeast

Organism	Test Agent	MIC (µg/mL)	Log-kill relative to growth control at 0 hr													
			2 hr	4 hr	6 hr	24 hr	48 hr	2 hr	4 hr	6 hr	24 hr	48 hr	2 hr	4 hr	6 hr	24 hr	48 hr
Test concentration:			**4 µg/mL**					**8 µg/mL**					**16 µg/mL**				
C. albicans ATCC 90028	SF001	0.12	+	++	+++	+++	+++	+	++	+++	+++	+	++	++	+++	+++	
	L-AMB	1		+	++	+++	+++		+	+	+++	+++		+	+++	+++	
C. albicans MMX 7063	SF001	0.12		+	++	+++	+++		+	+	+++	+++		+	+	+++	+++
	L-AMB	1		++	+++	+++	+++		+	+	++	+		+	++	+++	+++
C. glabrata ATCC 90030	SF001	0.12	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	L-AMB	1			++	++	+++			+	++	+++			++	++	+++
C. glabrata CDC 0318	SF001	0.12	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	L-AMB	1				+	+				+++	++				++	+++
C. parapsilosis ATCC 22019	SF001	0.06	++	++	++	+++	+++	++	+++	+++	+++	++	+++	+++	+++	+++	+++
	L-AMB	1		+	++	++	++		++	++	+++	+++		+	++	+++	+++
C. parapsilosis ATCC 90018	SF001	0.06	+	+	++	+++	+++	++	+++	+++	+++	+++	++	++	++	+++	+++
	L-AMB	1			+	++	+++				+	+++			+	++	+++
C. tropicalis ATCC 66029	SF001	0.06	++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	L-AMB	1	++	+++	+++	+++	+++	++	+++	+++	+++	+++	+	++	+++	+++	+++
Test concentration:			**8 µg/mL**					**16 µg/mL**					**32 µg/mL**				
C. albicans ATCC 90029	SF001	0.12		+	+	+++	+++		+	+	+++	+++		+	+	+++	+++
	L-AMB	2			+	+++	+++			+	+++	+++		++	+++	+++	+++
C. albicans ATCC MYA-2732	SF001	0.12	+	++	+++	+++	+++	+	++	++	+++	+++	+	++	++	+++	+++
	L-AMB	2	+	+	+	+++	+++	+	+	++	+++	+++		+	++	+++	+++
C. albicans ATCC 96901	SF001	0.12		+	+	+++	+++		+	+	+++	+++		+	+	+++	+++
	L-AMB	2			+	++	+++			+	++	+++			++	++	+++
C. krusei ATCC 6258	SF001	0.25	+	++	+++	++		++	+++	+++	+++	++	+++	+++	+++	+++	+++
	L-AMB	2			+	++	++			+	++	++			+	+++	+++
C. krusei ATCC 201748	SF001	0.25	+	++	++	++		+	++	+++	+++	+++	+	++	+++	+++	+++
	L-AMB	2				+++	++			+	++	++			+	+++	+++
C. tropicalis ATCC 750	SF001	0.06	++	++	++	+++	+++	++	++	+++	+++	+++	++	+++	+++	+++	+++
	L-AMB	2		+	++	++	++		+	++	+++	+++		+	++	+++	+++

+ = 1-2 log-kill, ++ = 2-3 log kill, +++ = >3-log kill

Figure 2. Representative kill-curves for SF001 and L-AMB against yeast



- SF001 had potent activity against yeast (**Table** 1) with an $MIC_{50/90}$ of 0.12/0.12 µg/mL against *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, 0.12/0.25 µg/mL against *C. glabrata,* 0.25/0.25 µg/mL against *C. krusei* and 0.12/0.5 µg/mL against *C. auris*, values lower than L-AMB and AmB.
- The increased potency of SF001 against yeast relative to L-AMB and AmB was also apparent by MIC distribution (**Figure 1A).**
- Against mould (**Table 2),** SF001 had an $MIC_{50/90}$ of 0.25/0.5 µg/mL against *A. fumigatus*, *A. flavus*, and *A. terreus* which was lower than that of AmB and L-AMB; against *A. niger* the SF001 $MIC_{50/90}$ of 0.12/0.12 µg/mL matched that of AmB while L-AMB had an an $MIC_{50/90}$ of ≤0.016/0.12 µg/mL against this species.
- SF001 had lower MIC values against mould than L-AMB and AmB based on MIC distribution excluding the subset of *A. niger* with L-AMB MIC values of ≤0.015 g/mL (**Figure 1B**).
- As shown in **Table 3**, SF001 MFC:MIC ratios were ≤ 4 for 89.7% (35/39) indicating fungicidal activity.
- Time-kill kinetic analysis demonstrated rapid fungicidal activity for SF001 (**Table 4**, **Figure 2**); at equivalent concentrations, SF001 killed more rapidly than L-AMB against *C. glabrata*, *C. tropicalis*, and *C. krusei* and had similar kill kinetics to L-AMB for *C. albicans* and *C parapsilosis*.

CONCLUSIONS

- SF001 had potent activity against yeast and moulds with potency that typically exceeded that of L-AMB and AmB
- SF001 demonstrated fungicidal activity against both yeast and mould by MFC:MIC ratio, and rapid killing was observed against yeast by time-kill kinetic analysis.

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