

Activity of a Series of Investigational Compounds Tested Against Invasive Fungal Isolates

Paul R. Rhomberg¹, Shawn A. Messer¹, Richard W. Scott², Simon D.P. Baugh², Michael A. Pfaller¹, Mariana Castanheira¹, Cecilia G. Carvalhaes¹

¹JMI Laboratories, North Liberty, Iowa, USA; ²Fox Chase Chemical Diversity Center, Doylestown, Pennsylvania, USA

Introduction

- The development of host defense proteins (HDP) that have broad-spectrum antimicrobial and immunomodulatory properties is a new endeavor to address antimicrobial resistance.
- Small molecule non-peptide analogs of HDP (smHDP) may exhibit potent antimicrobial activity and circumvent the challenge of protease digestion faced by HDP.
- The smHDPs have shown better pharmacokinetic and tissue distribution properties due to their size and improved stability.
- We evaluated the antifungal activity of 6 novel non-peptide analogs of HDP using reference broth microdilution methods against a total of 150 invasive fungal isolates collected worldwide.

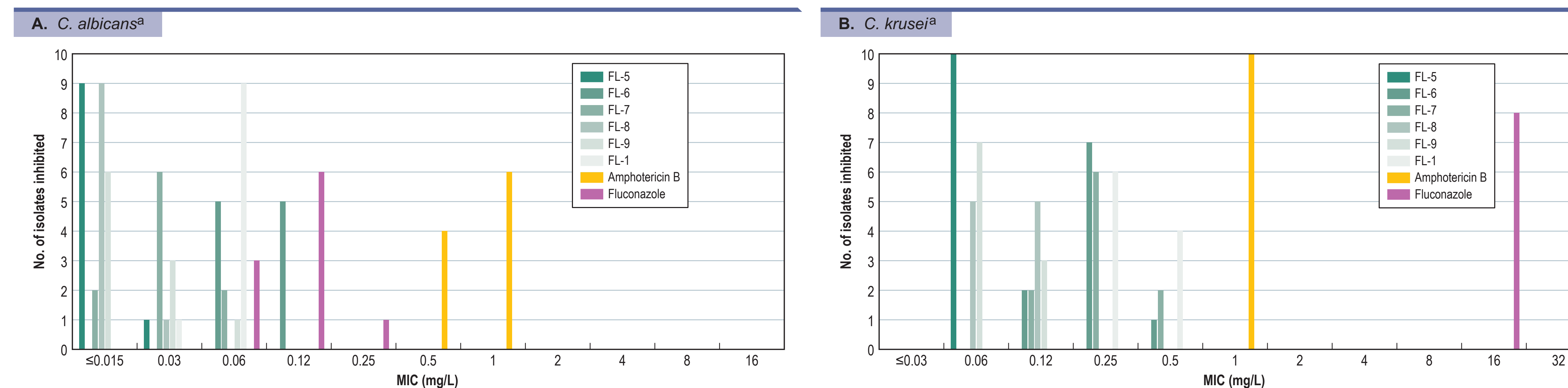
Materials and Methods

- A total of 150 non-duplicate fungal isolates (80 yeasts and 70 moulds) selected from worldwide medical centers as part of the SENTRY Antimicrobial Surveillance Program between 2017 and 2019 were included (Table 1).
- All isolates were identified using MALDI-TOF or DNA sequencing analysis when an acceptable identification was not achieved by MALDI-TOF.
- Susceptibility testing was performed for yeast and mould isolates according to CLSI M27 (2017) and M38 (2017) documents, respectively, using frozen form panels with RPMI broth supplemented with MOPS buffer and 0.2% glucose.
- Antifungal compounds FL-1, FL-5, FL-6, FL-7, FL-8, and FL-9 were tested over the range of 0.015 – 8 mg/L.
- Comparators were amphotericin B (range 0.12 – 4 mg/L) for all isolates, and fluconazole (range 0.03 – 64 mg/L) for yeasts and itraconazole (range 0.008 – 8 mg/L) for moulds.
- MIC values were read at 24 hours for *Candida* spp. and 72 hours for *Cryptococcus* spp. at the lowest concentration that resulted in $\geq 50\%$ inhibition of growth for the investigational compounds.
- Mould MIC values were read at incubation periods of either 24-, 48-, or 72 hours as designated in CLSI M38-A3 per species. MIC values were read at the lowest concentration that resulted in $\geq 50\%$ inhibition of growth and 100% inhibition of growth for the investigational compounds.
- Quality control (QC) was performed as recommended by CLSI using the following strains: *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Aspergillus flavus* ATCC 204304, and *Aspergillus fumigatus* ATCC MYA-3626.

Results

- All investigational compounds displayed MIC₅₀ results at ≤ 0.015 mg/L to 0.06 mg/L and MIC₉₀ results at ≤ 0.015 to 0.12 mg/L against *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* (Table 2; Figure 1A).
- All *C. krusei* isolates were inhibited by investigational compounds at MIC of 0.5 mg/L, and MIC₅₀ and MIC₉₀ results were 0.06-0.25 mg/L and 0.06-0.5 mg/L, respectively (Figure 1B; Table 2).
- FL-1 (MIC_{50/90}, 0.12/0.5 mg/L) and FL-6 (MIC_{50/90}, 0.25/0.5 mg/L) compounds were 4- to 8-fold more active than amphotericin B (MIC_{50/90}, 1/2 mg/L), and 256-fold more active than fluconazole (MIC_{50/90}, 64/>64 mg/L) against *C. auris* isolates (Table 2).
- All investigational compounds (MIC_{50/90} range, ≤ 0.015 -0.03/ ≤ 0.015 -0.03 mg/L) were at least 16- and 64-fold more active than amphotericin B and fluconazole, respectively, against *Cryptococcus* spp. isolates (Table 2).
- The compounds FL-5, FL-8, and FL-9 had MIC₅₀ and MIC₉₀ results at ≤ 0.015 mg/L against *A. fumigatus*, *A. flavus* species complex, and *A. section Terrei* (Table 3; Figure 2A). All *Aspergillus* spp. isolates, including *A. section Nigri*, were inhibited by investigational compounds at MIC of 0.25 mg/L.
- Fusarium* spp. isolates were inhibited by investigational compounds at MIC of 0.12 mg/L. FL-5 and FL-9 (both with a MIC_{50/90}, ≤ 0.015 / ≤ 0.015 mg/L) were the most active compounds against *Fusarium* spp. (Table 3).
- The Mucorales isolate set showed the widest range of MIC results for investigational compounds. FL-5 exhibited the greatest potency with a MIC_{50/90} at 0.5/2 mg/L (Table 3).
- The investigational compounds showed potent activity against *Scedosporium* spp. isolates, with MIC_{50/90} results of 0.03-0.25/0.03-0.25 mg/L (Figure 2B). The investigational compounds were at least 16-fold more active than amphotericin B and itraconazole against these highly resistant moulds (Table 3).
- Itraconazole was active against all *Aspergillus* spp. (MIC_{50/90}, 0.5-1/0.5-2 mg/L), but showed poor activity against *Fusarium* spp. (MIC_{50/90}, >8/>8 mg/L; Table 3).
- Amphotericin B showed a narrow range of MIC results (0.5 to 2 mg/L) for all isolates, except 1 *Aspergillus* section *Terrei* (MIC, 4 mg/L) and most (8/10) *Scedosporium* spp. isolates.

Figure 1. MIC distribution of investigational compounds and comparators against *C. albicans* and *C. krusei* isolates



* Read times were 24 hours for *Candida* isolates at $\geq 50\%$ reduction in growth.

Table 1. List of fungal clinical isolates included in this study

Organism group	# of organisms included
<i>Candida</i> spp.	10 <i>Candida albicans</i> 10 <i>Candida auris</i> 10 <i>Candida dubliniensis</i> 10 <i>Candida glabrata</i> 10 <i>Candida krusei</i> 10 <i>Candida parapsilosis</i> 10 <i>Candida tropicalis</i>
<i>Cryptococcus</i> spp.	7 <i>Cryptococcus neoformans</i> var. <i>grubii</i> 2 <i>Cryptococcus neoformans</i> var. <i>neoformans</i> 1 <i>Cryptococcus gattii</i>
<i>Aspergillus flavus</i> species complex	10 <i>Aspergillus flavus</i> species complex
<i>Aspergillus fumigatus</i>	10 <i>Aspergillus fumigatus</i>
<i>Aspergillus</i> section <i>Terrei</i>	6 <i>Aspergillus terreus</i> 4 <i>Aspergillus terreus</i> species complex
<i>Aspergillus</i> section <i>Nigri</i>	7 <i>Aspergillus niger</i> 3 <i>Aspergillus niger</i> species complex
<i>Fusarium</i> spp.	6 <i>Fusarium solani</i> species complex 2 <i>Fusarium incarnatum-equiseti</i> species complex 2 <i>Fusarium oxysporum</i> species complex
Mucorales	4 <i>Rhizopus oryzae</i> 2 <i>Mucor circinelloides</i> / <i>Mucor ramosissimus</i> 2 <i>Rhizopus microsporus</i> group 1 <i>Mucor circinelloides</i> 1 <i>Rhizomucor pusillus</i>
<i>Scedosporium</i> spp.	7 <i>Scedosporium apiospermum</i> / <i>Scedosporium boydii</i> 2 <i>Scedosporium aurantiacum</i> 1 <i>Scedosporium boydii</i>

Table 2. Summary of MIC₅₀ and MIC₉₀ results for investigational compounds and comparators tested against yeast isolates

Compound	Yeast group MIC _{50/90} (mg/L)*								
	<i>C. albicans</i>	<i>C. auris</i>	<i>C. dubliniensis</i>	<i>C. glabrata</i>	<i>C. krusei</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>Cryptococcus</i> spp.	
FL-1	0.06/0.06	0.12/0.5	0.03/0.03	≤ 0.015 /0.06	0.25/0.5	0.06/0.06	0.03/0.03	0.03/0.03	
FL-5	≤ 0.015 / ≤ 0.015	1/2	≤ 0.015 / ≤ 0.015	≤ 0.015 /0.03	0.06/0.06	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	
FL-6	0.06/0.12	0.25/0.5	0.03/0.06	≤ 0.015 /0.12	0.25/0.25	0.03/0.06	0.03/0.03	0.03/0.03	
FL-7	0.03/0.06	8/>8	≤ 0.015 / ≤ 0.015	≤ 0.015 /0.06	0.25/0.5	≤ 0.015 /0.03	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	
FL-8	≤ 0.015 / ≤ 0.015	2/4	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.06/0.12	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	
FL-9	≤ 0.015 /0.03	2/4	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.06/0.12	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	
Amphotericin B	1/1	1/2	0.5/1	1/1	1/1	1/1	1/1	0.5/1	
Fluconazole	0.12/0.12	64/>64	0.12/0.25	2/4	16/32	0.25/8	0.25/0.25	2/4	

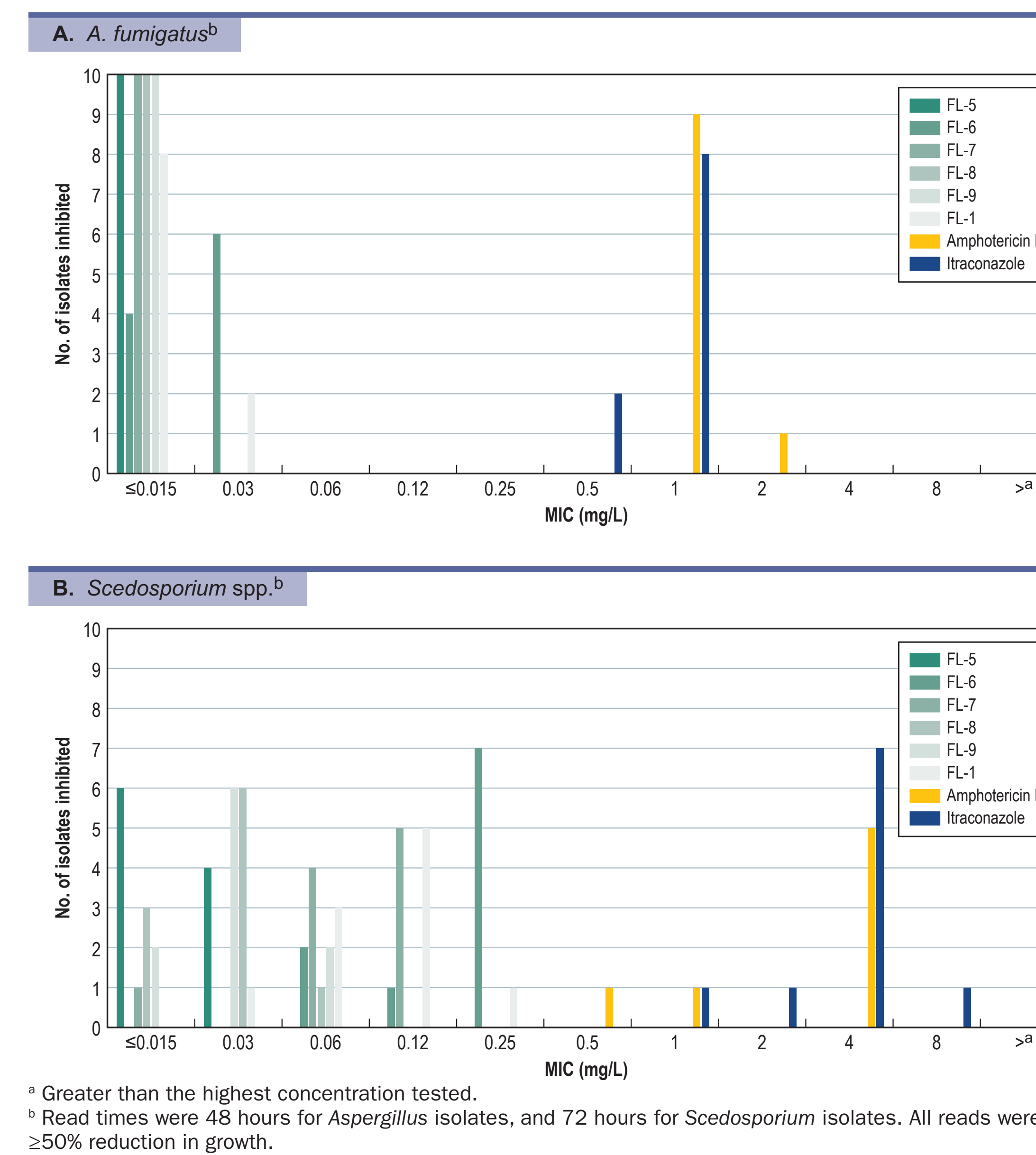
* Read times were 24 hours for *Candida* isolates and 72 hours for *Cryptococcus* isolates.

Table 3. Summary of MIC results for investigational compounds and comparators read at $\geq 50\%$ reduction in growth for mould isolates

Compound	Mould group MIC _{50/90} (mg/L)*						
	<i>A. fumigatus</i>	<i>A. flavus</i> SC	<i>A. section Nigri</i>	<i>A. section Terrei</i>	<i>Fusarium</i> spp.	Mucorales	<i>Scedosporium</i> spp.
FL-1	≤ 0.015 /0.03	0.03/0.03	0.12/0.25	0.03/0.06	0.03/0.06	1/>8	0.12/0.25
FL-5	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.06/0.12	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.5/2	0.03/0.03
FL-6	0.03/0.03	0.06/0.12	0.12/0.25	0.06/0.06	0.06/0.06	2/8	0.25/0.25
FL-7	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.06/0.12	0.03/0.03	≤ 0.015 /0.03	>8/>8	0.12/0.12
FL-8	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.06/0.12	≤ 0.015 / ≤ 0.015	≤ 0.015 /0.03	2/>8	0.03/0.03
FL-9	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.06/0.12	≤ 0.015 / ≤ 0.015	≤ 0.015 / ≤ 0.015	0.25/>8	0.03/0.06
Amphotericin B	1/1	2/2	0.5/1	2/2	2/2	1/1	4/>4
Itraconazole	1/1	0.5/1	1/2	0.5/0.5	>8/>8	2/8	4/4

* Read times were 48 hours for *Aspergillus* and *Fusarium* isolates, 24 hours for Mucorales isolates, and 72 hours for *Scedosporium* isolates. Abbreviation: SC, species complex

Figure 2. MIC distribution of investigational compounds and comparators against *A. fumigatus* and *Scedosporium* spp.



* Greater than the highest concentration tested.

* Read times were 48 hours for *Aspergillus* isolates, and 72 hours for *Scedosporium* isolates. All reads were at $\geq 50\%$ reduction in growth.

Conclusions

- The novel investigational non-peptide analogs of HDP exhibited equal or greater activity than the comparators against *Candida* spp. and *Cryptococcus* spp., including resistant organisms such as *C. auris* and *C. krusei*.
- These investigational compounds also displayed activity against *Aspergillus* spp., *Fusarium* spp., and *Scedosporium* spp. clinical isolates.
- Among the investigational compounds, FL-5, FL-8, and FL-9 showed the greatest activity against all tested fungal isolates.
- These *in vitro* results support the continued development of this series of compounds.

Acknowledgements

This study was supported by Fox Chase Chemical Diversity Center. Fox Chase Chemical was involved in the design and decision to present these results, and JMI Laboratories received compensation for preparing the poster. Fox Chase Chemical did not contribute to decisions in the collection, analysis, or interpretation of the data.

References

- Scott RW, Tew GN. Mimics of Host Defense Proteins; Strategies for Translation to Therapeutic Applications. *Curr Top Med Chem.* 2017;17(5):576-589
- Chowdhury MH, Ryan LK, Cherabuddi K, et al. Antifungal Potential of Host Defense Peptide Mimetics in a Mouse Model of Disseminated Candidiasis. *J Fungi (Basel).* 2018;4(1):30
- Clinical and Laboratory Standards Institute (2017). *M27Ed4E. Reference method for broth dilution antifungal susceptibility testing of yeasts.* Wayne, PA: CLSI
- Clinical and Laboratory Standards Institute (2017). *M60Ed1E. Performance standards for antifungal susceptibility testing of yeasts.* Wayne, PA: CLSI
- Clinical and Laboratory Standards Institute (2017). *M61Ed1E. Performance standards for antifungal susceptibility testing of filamentous fungi, first edition.* Wayne, PA: CLSI
- Clinical and Laboratory Standards Institute (2018). *M38Ed3. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, third edition.* Wayne, PA: CLSI
- Clinical and Laboratory Standards Institute (2018). *M59Ed2. Epidemiological cutoff values for antifungal susceptibility testing, second edition.* Wayne, PA: CLSI

Contact

Cecilia Carvalhaes, MD, PhD
JMI Laboratories
345 Beaver Creek Centre, Suite A
North Liberty, IA 52317
Phone: (319) 665-3370
Fax: (319) 665-3371
Email: cecilia-carvalhaes@jmilabs.com