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SUSCEPTIBILITY

Potent Activities of Luliconazole, Lanoconazole, and Eight Comparators against Molecularly Characterized *Fusarium* Species

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ABSTRACT A collection of clinical (n = 47) and environmental (n = 79) *Fusarium* isolates were tested against 10 antifungal drugs, including 2 novel imidazoles. Luliconazole and lanoconazole demonstrated very low geometric mean MIC values of 0.005 and 0.013 μg/ml, respectively, compared with 0.51 μg/ml for micafungin, 0.85 μg/ml for efinaconazole, 1.12 μg/ml for natamycin, 1.18 μg/ml for anidulafungin, 1.31 μg/ml for voriconazole, 1.35 μg/ml for caspofungin, 1.9 μg/ml for amphotericin B, and 4.08 μg/ml for itraconazole. Results show that these drugs are potential candidates for (topical) treatment of skin and nail infections due to *Fusarium* species.

KEYWORDS *Fusarium* species, intrinsic resistance, luliconazole, lanoconazole

Species of *Fusarium* are globally distributed fungi of considerable ecological plasticity, causing infections in plants and humans (1, 2). Over the past few years, human infections with *Fusarium* species have shown a global increase in frequency in immunocompromised patients and healthy individuals (3). These infections can be classified into three main groups, i.e., superficial infections involving skin, nails, and corneas; deep subcutaneous infections; and disseminated infections, which occur exclusively in patients with profound neutropenia or T-cell immunodeficiency (4). Management of infections caused by *Fusarium* species is challenging because of their intrinsic multiresistance to most currently available antifungal drugs (5–7).

Fusarium and other members of the order *Hypocreales*, such as *Trichoderma* and *Acremonium*, are among the most antifungal drug-resistant organisms encountered in

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TABLE 1 MIC and MEC results of antifungal testing

Source of isolates and variable	MIC (μg/ml)[a] for:							MEC (μg/ml)[b] for:		
	LULI	LANO	EFINA	VRC	ITC	AMB	NATA	CFG	MFG	AFG
All (n = 126)										
Range	0.001 to 0.125	0.001 to 1	0.004 to 2	0.125 to >16	0.125 to >16	0.016 to 16	0.125 to 8	0.008 to >8	0.001 to >8	0.25 to >8
MIC_{50}/MEC_{50}	0.008	0.016	1	2	8	2	1	4	1	1
MIC_{90}/MEC_{90}	0.032	0.125	2	4	>16	8	2	>8	>8	>8
GM	0.005	0.013	0.85	1.31	4.08	1.9	1.12	1.35	0.51	1.18
Clinical (n = 47)										
Range	0.001 to 0.125	0.001 to 1	0.004 to 2	0.125 to >16	1 to >16	0.016 to >16	0.125 to 2	0.008 to >8	0.001 to >8	0.25 to >8
MIC_{50}/MEC_{50}	0.004	0.016	1	2	>16	2	1	8	0.064	4
MIC_{90}/MEC_{90}	0.032	0.064	2	4	>16	8	2	>8	>8	>8
GM	0.005	0.014	0.88	1.88	3.48	1.7	1.02	2.69	0.09	1.64
Environmental (n = 79)										
Range	0.001 to 0.125	0.001 to 0.5	0.5 to 2	0.125 to 8	0.125 to >16	0.125 to >16	0.125 to 8	0.008 to >8	0.008 to >8	0.5 to >8
MIC_{50}/MEC_{50}	0.008	0.008	1	1	8	2	1	4	1	8
MIC_{90}/MEC_{90}	0.032	0.125	1	4	>16	8	2	>8	>8	8
GM	0.006	0.012	0.83	1.07	4.21	2.03	1.15	2.04	1.64	5.17

[a]LULI, luliconazole; LANO, lanoconazole; EFINA, efinaconazole; (VRC), voriconazole; ITC, itraconazole; AMB, amphotericin B; NATA, natamycin.
[b]Echinocandins: CFG, caspofungin; MFG, micafungin; AFG, anidulafungin.

clinical practice (8). Intrinsic resistance to azoles and high *in vitro* MIC values to polyenes and the echinocandins have been noted, although some studies reported successful clinical outcomes with these agents (8–14). Currently, European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and European Confederation of Medical Mycology (ECMM) joint guidelines and most published studies suggest that early therapy with amphotericin B and voriconazole in conjunction with surgical debridement and reversal of immunosuppression is the treatment of choice for disseminated fusariosis (13–15). However, survival rates are low in these patient populations (~30% or less), particularly among patients with constant immunosuppression (13–17). In keratitis cases, topical natamycin is used along with voriconazole as the mainstay of *Fusarium* treatment (18).

Luliconazole and lanoconazole are novel topical FDA-approved imidazoles for treatment of superficial mycoses. These drugs have proven *in vitro* activity against most clinically important molds and yeasts, e.g., *Aspergillus fumigatus* (including strains with acquired itraconazole resistance) (19), *Aspergillus terreus* species complex (20), dermatophytes (21), black fungi and relatives (22), *Malassezia* species (23), and *Candida* species (24). No *in vitro* susceptibility data of luliconazole and lanoconazole against *Fusarium* species have been published. Therefore, we used a large panel of *Fusarium* species to evaluate the *in vitro* activity of luliconazole, lanoconazole, and eight comparator drugs based on CLSI M38-A2 guidelines (25).

A total of 126 clinical and environmental *Fusarium* isolates were included in the study. Species identification was confirmed by partial sequencing of the translation elongation factor 1α (*TEF-1*α) (8). The clinical isolates originated from nails (n = 30) and corneas (n = 17) from four clinical centers in Iran from 2014 to 2017 (Table 1). The environmental isolates were recovered from samples of rice (n = 27), poultry fodder (n = 9), maize (n = 25), wheat (n = 6), and eggplant (n = 1) (Table 1). The collection comprised 11 reference environmental strains from three reference collections: Invasive Fungi Research Center (IFRC, Iran), Teikyo University Institute of Medical Mycology (TIMM, Japan), and Centraalbureau voor Schimmelcultures (CBS) housed at Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands) (Table 1).

All strains were tested for their *in vitro* susceptibility to luliconazole and lanoconazole and eight comparator agents according to CLSI M38-A2 guidelines (25). Powders of the antifungal agents were obtained from the manufacturers (efinaconazole, luliconazole, and lanoconazole, Nihon Nohyaku Co., Osaka, Japan; itraconazole, Janssen, Beerse, Belgium; anidulafungin and voriconazole, Pfizer, Sandwich, United Kingdom; amphotericin B, Bristol-Myers-Squib, Woerden, The Netherlands; caspofungin, Merck Sharp and Dohme BV, Haarlem, The Netherlands; micafungin, Astellas, Toyama, Japan; and natamycin, Sigma-Aldrich, Steinheim, Germany). Final concentrations of antifungal

agents in the wells ranged from 0.016 to 16 μg/ml for amphotericin B, voriconazole, itraconazole, efinaconazole, and natamycin; 0.001 to 1 μg/ml for luliconazole and lanoconazole; and 0.008 to 8 μg/ml for caspofungin, micafungin and anidulafungin. Stock solutions of drugs were prepared in dimethyl sulfoxide, except for caspofungin and micafungin, which were dissolved in sterile water and stored at −80°C until used. The strains were grown on potato dextrose agar (Difco) and incubated at 35°C for 5 to 7 days for adequate sporulation. To obtain final inocula of 0.4×10^4 to 5×10^4 CFU/ml, suspensions were diluted 1:50 in RPMI 1640 medium. For micafungin, caspofungin, and anidulafungin, minimum effective concentrations (MECs) were determined microscopically as the lowest concentrations of the agent that resulted in growth of rounded and compact hyphal forms compared with those in the well of the growth control. For others drugs, MICs were the lowest concentrations that showed complete inhibition of visible growth. Microdilution plates were incubated at 35°C, and MICs and MECs were read after 48 h. *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), and *Aspergillus flavus* (ATCC 2004304) were used as quality control strains. Differences of mean values were determined by Student's *t* test with the statistical SPSS package (version 7.0). *P* values of <0.05 were considered statistically significant.

The identified species in our study, based on *TEF1* partial gene analysis, were members of the *F. fujikuroi* species complex (FFSC) ($n = 94$), consisting of *F. proliferatum* ($n = 53$), *F. verticillioides* ($n = 37$), *F. thapsinum* ($n = 1$), *F. sacchari* ($n = 1$), *F. nygamai* ($n = 1$), and *F. fujikuroi* ($n = 1$). We also identified members of other species complexes: *F. oxysporum* ($n = 11$) in *F. oxysporum* species complex (FOSC), *F. lateritium* ($n = 1$) in *F. lateritium* species complex (FLSC), *F. culmorum* ($n = 1$) in *F. graminearum* species complex (FGSC) and *F. solani sensu stricto* (FSSC) ($n = 13$), and *F. petroliphilum* ($n = 1$) in *F. solani* species complex (FSSC) (Table 2).

Table 2 summarizes the *in vitro* susceptibilities of 47 clinical and 79 environmental isolates of *Fusarium* to luliconazole, lanoconazole, and eight common comparator antifungal agents. Interestingly, *Fusarium* species demonstrated extremely low MICs to luliconazole and lanoconazole, with geometric mean (GM) MICs of 0.005 and 0.013 μg/ml, respectively; followed by micafungin, with a GM MEC of 0.51 μg/ml, and efinaconazole, with a GM MIC of 0.85 μg/ml. MICs/MECs of the other drugs were >1 μg/ml (natamycin, 1.12 μg/ml; anidulafungin, 1.18 μg/ml; voriconazole, 1.37 μg/ml; caspofungin, 1.35 μg/ml; amphotericin B, 1.9 μg/ml; and itraconazole, 4.08 μg/ml) (Table 1). While the widest MEC ranges were observed for micafungin (0.001 to >8 μg/ml) and anidulafungin (0.25 to >8 μg/ml), the lowest MIC ranges were found with luliconazole (0.001 to 0.125 μg/ml) and lanoconazole (0.001 to 1 μg/ml) (Table 1). Of the three echinocandins, micafungin had the best activity, with a GM MEC that was >2-$\log_2$ dilution steps lower than those of anidulafungin and caspofungin, although the MEC_{90} of >8 μg/ml for the clinical isolates would not qualify micafungin as an agent that can be used as monotherapy for *Fusarium*.

MIC_{50} values of luliconazole and lanoconazole against *Fusarium* isolates were 8- and 7-$\log_2$ dilutions steps lower, respectively, than those of amphotericin B and voriconazole (MIC_{50}, 2 μg/ml), the drugs of choice for the treatment of invasive fusariosis (13). These results confirm previous findings of very low MIC values for luliconazole and lanoconazole compared with those of amphotericin B, voriconazole, and itraconazole against wild-type and resistant *A. fumigatus* and melanized fungi (19, 22). Abastabar et al. (19) and Vaezi et al. (20) reported that the majority of MIC values of lanoconazole and luliconazole against azole-resistant isolates of *A. fumigatus* and *A. terreus* were ≤ 0.016 μg/ml and in some isolates even ≤ 0.001 μg/ml. In addition, Shokoohi et al. (22) showed that MIC_{50}, MIC_{90}, and GM MIC values of luliconazole and lanoconazole for clinical isolates of dematiaceous fungi and relatives were 0.0005, 0.008, and 0.0008 μg/ml, respectively. In the current study, MIC_{90} values against clinical *Fusarium* strains were as follows, in increasing order: luliconazole, 0.032 μg/ml; lanoconazole, 0.064 μg/ml; efinaconazole and natamycin, 2 μg/ml; voriconazole, 4 μg/ml; amphotericin B, 8 μg/ml; and itraconazole, >16 μg/ml; and MEC_{90} values for anidulafungin, micafungin, and caspofungin were >8 μg/ml. Moreover, MIC_{90} values of luliconazole, lanoconazole,

TABLE 2 *In vitro* susceptibilities of 10 antifungal drugs against 126 *Fusarium* isolates from different species complexes

Source and antifungal agent	Susceptibility (*n*) at MIC/MEC[a] (μg/ml) of:														
	0.001	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	≥16
Fusarium, clinical (*n* = 47)															
Luliconazole	**10**	6	8	9	8	5		1							
Lanoconazole	4	6	4	4	7	8	**9**	3	1		1				
Efinaconazole			1				1	1	1	6	**22**	15			
Voriconazole								1	1	2	9	**16**	15	2	1
Itraconazole									1		1	1	1	2	**42**
Amphotericin B					1			2	1	3	8	**14**	7	6	5
Natamycin								2	1	5	**20**	19			
Caspofungin				1	1			3			1	1	2	**38**	
Micafungin	1			4	1	5	1	1	2	2	2	4		**24**	
Anidulafungin									1	1		2	3	**40**	
Fusarium, environmental (*n* = 79)															
Luliconazole	**16**	14	6	10	15	11	5	2							
Lanoconazole	5	10	12	**14**	10	13	4	6	4	1					
Efinaconazole									3	18	**54**	4			
Voriconazole								2	9	19	**21**	18	12	1	
Itraconazole								1	1		1	3	5	12	**56**
Amphotericin B								1		10	14	**22**	20	7	5
Natamycin								1	5	10	**35**	24	3	1	
Caspofungin				1	1	1		1			2	7	16	**50**	
Micafungin				1	2		1	2	2	16	12	11	14	**18**	
Anidulafungin										11	2	3		**63**	
F. fujikuroi complex (*n* = 94)															
Luliconazole	19	13	12	12	**20**	12	5	1							
Lanoconazole	7	12	14	13	12	**14**	11	6	3	1	1				
Efinaconazole							1		4	21	**53**	15			
Voriconazole								3	6	20	24	**24**	17		
Itraconazole								1		2	2	3	4	14	**68**
Amphotericin B					1			1		12	17	**25**	23	8	7
Natamycin								1	5	13	**40**	31	4		
Caspofungin				1	1	1		2			1	7	37	**44**	
Micafungin	1			3	2	3		2	3	17	12	12	12	**27**	
Anidulafungin									1	2	1	3	13	9	**65**
F. solani complex (*n* = 14)															
Luliconazole		3	1	1	3	**3**	1	2							
Lanoconazole		1		1	2	**4**	3	1	2						
Efinaconazole										1	**11**	2			
Voriconazole										2	3	3	3	**3**	
Itraconazole													1		**13**
Amphotericin B								2	1		1	4	2	3	
Natamycin								2		2	5	4		1	
Caspofungin				1								2		**11**	
Micafungin					1				1	2	1	2		**7**	
Anidulafungin										1	1			**12**	
F. oxysporum complex (*n* = 11)															
Luliconazole	**4**	2	2	3											
Lanoconazole	1	3	2		1	1		**3**							
Efinaconazole			1					1		1	**7**	1			
Voriconazole											2	4	**5**		
Itraconazole															**11**
Amphotericin B											1	**4**	1	2	3
Natamycin									1		3	**7**			
Caspofungin					1		2	1					1	**8**	
Micafungin				2					1			1	1	**4**	
Anidulafungin										2		2		**7**	
F. graminearum complex (*n* = 1)															
Luliconazole	1														
Lanoconazole				1							1				

(Continued on next page)

TABLE 2 (Continued)

Source and antifungal agent	Susceptibility (*n*) at MIC/MEC[a] (μg/ml) of:														
	0.001	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	≥16
Efinaconazole											1				
Voriconazole												1			
Itraconazole															1
Amphotericin B													1		
Natamycin												1			
Caspofungin														1	
Micafungin														1	
Anidulafungin														1	
F. lateritium complex (*n* = 1)															
Luliconazole				1											
Lanoconazole				1											
Efinaconazole											1				
Voriconazole												1			
Itraconazole												1			
Amphotericin B											1				
Natamycin												1			
Caspofungin														1	
Micafungin														1	
Anidulafungin														1	

[a]The mode in each row is in boldface. MEC only for echinocandins caspofungin, micafungin, and anidulafungin.

and other tested drugs for the environmental *Fusarium* isolates were completely similar to those of the clinical isolates, with the exception of better activities of efinaconazole (MIC_{90}, 1 μg/ml) in environmental isolates and lanoconazole (0.64 μg/ml) in clinical isolates (Table 1).

Many studies have reported data on the poor *in vitro* activity of amphotericin B, itraconazole, and echinocandins against clinical *Fusarium* strains, with MIC values similar to those in our findings (9, 10, 18, 26–28). Regarding the poor outcome with monotherapy and in view of the reported synergistic interactions of some agents, such as, liposomal amphotericin B with terbinafine (4), amphotericin B with voriconazole (29), and natamycin with voriconazole (9), combination therapy is recommended (1, 18). In the current study, all clinical *Fusarium* isolates showed low MICs of ≤0.125 μg/ml for luliconazole and ≤1 μg/ml for lanoconazole.

Forty-three clinical isolates (91.48%) had MICs of ≥1 μg/ml for voriconazole, 40 isolates (85.1%) had MICs of ≥1 μg/ml for amphotericin B, 42 isolates (89.36%) had MICs of ≥16 μg/ml for itraconazole, and 38 isolates (80.85%) demonstrated MECs of ≥8 μg/ml for caspofungin (Table 2). All of the environmental isolates with high MICs/MECs for azoles, amphotericin B, and echinocandins were inhibited by ≤0.125 μg/ml of luliconazole and ≤0.5 μg/ml of lanoconazole (Table 2). Data on the *in vitro* activity of efinaconazole, a novel triazole, against *Fusarium* species are limited (30, 31). We found that the *in vitro* antifungal activity of efinaconazole with a GM MIC of 0.85 μg/ml was superior to those of amphotericin B, natamycin, other triazoles, and echinocandins, except for micafungin, which had a GM MEC of 0.51 μg/ml (Table 1). These results agree with previously published data on efinaconazole versus itraconazole with limited species selections and fewer isolates (30, 31). We found no significant differences between *Fusarium* species complexes regarding susceptibility to luliconazole and lanoconazole, for which most isolates demonstrated very low MIC values (Table 2). These two drugs inhibited all isolates studied within the *F. solani* species complex (FSSC) at a concentration of 0.125 μg/ml for luliconazole and ≤0.25 μg/ml for lanoconazole. In addition, members of the *F. fujikuroi* species complex (FFSC) were inhibited at ≤0.125 μg/ml of luliconazole and ≤0.5 μg/ml of lanoconazole, except for a single isolate with an MIC of 1 μg/ml. In addition, all isolates of the *F. oxysporum* species complex (FOSC) were inhibited at ≤0.008 μg/ml of luliconazole and 0.0125 μg/ml of lanoconazole (Table 2). The echinocandins are not considered to be active against *Fusarium*, and the present data support this, especially with MEC_{90} values of >8 μg/ml,

against clinical *Fusarium* strains, except for a few strains within the *F. fujikuroi* species complex (FFSC), followed by *F. oxysporum* species complex (FOSC). This strain-specific phenomenon in *Fusarium* was reported before (10), although testing *Fusarium* species routinely against echinocandins was not recommended. We conclude that luliconazole and lanoconazole exhibit potent activity against clinical and environmental *Fusarium* species. These compounds are therefore promising for the treatment of fusariosis.

Accession number(s). The nucleotide sequences of all isolates were deposited in GenBank under accession numbers MG734576 to MG734653.

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The authors alone are responsible for the content and writing of the paper.

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