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In Vitro Activities of Luliconazole, Lanoconazole, and Efinaconazole Compared with Those of Five Antifungal Drugs against Melanized Fungi and Relatives

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ABSTRACT The *in vitro* activities of novel azoles compared to those of five antifungal drugs against clinical ($n = 28$) and environmental ($n = 102$) isolates of black mold and melanized yeast were determined. Luliconazole and lanoconazole had the lowest geometric mean MICs, followed by efinaconazole, against tested isolates compared to the other drugs. Therefore, it appears that these new imidazole and triazole drugs are promising candidates for the treatment of infections due to melanized fungi and their relatives.

KEYWORDS melanized fungi, *in vitro*, MIC, efinaconazole, lanoconazole, luliconazole

Black mold and melanized yeast, characterized by the presence of a pale-brown to dark melanin-like pigment in the cell wall, are a diverse group of filamentous, yeasts, yeast-like fungi and relatives which are commonly found on decomposing plant debris, dead plant material, rotten wood, air, and soil. Several genera belonging to the ascomycetous orders, i.e., *Capnodiales*, *Chaetothyriales*, *Diaporthales*, *Dothideales*, *Pleosporales*, *Sordariales*, and *Venturiales*, are characterized as black mold and melanized yeast and are relatively often encountered in human and animal disorders, ranging from mild cutaneous lesions to severe encephalitis in otherwise healthy individuals (1, 2). The importance of melanized fungi has recently gained increased focus, owing to the significant increase in the number of patients with predisposing risk factors throughout the world (1). Although dematiaceous fungi as agents of invasive infections are basically susceptible to most antifungal agents *in vitro*, treatment regimens are controversial and difficult, because frequent relapses and failures are observed when antifungal resistance is involved (3). Antifungal therapy or application of drug combinations is based on the experience from case series which mostly involved amphotericin B, itraconazole, terbinafine, and flucytosine as promising treatment options (1, 4). Although triazoles might be more effective based on the observation of low *in vitro* MIC values, excessive use of azole fungicides in the environment with long-term use of drugs in the prophylactic treatment of hospitalized patients has resulted in the devel-

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TABLE 1 *In vitro* antifungal susceptibilities of 28 clinical melanized fungal isolates against eight antifungal agents

Isolate no.	Age (yr)/sex/yr of isolation[a]	Source	Species	ITS rRNA accession no.	MIC (μg/ml)							
					AMB	FLC	VRC	ITC	EFN[b]	LUL	LCZ	TRB
1	36/F/2016	Sinus discharge	*Alternaria alternata*	KY788023	8	32	0.25	1	0.25	0.0002	0.016	>0.5
2	53/M/2016	Sinus discharge	*Alternaria malorum*	KY788040	2	32	0.25	1	0.25	0.016	0.063	>0.5
3	27/M/2013	Skin lesion	*Alternaria malorum*	JQ219160	0.125	32	1	0.125	ND	0.008	0.004	>0.5
4	57/F/2015	Sinus discharge	*Neoscytalidium dimidiatum*	KY788092	0.5	32	0.125	0.5	0.063	0.0005	0.004	0.5
5	49/F/2015	Nail lesion	*Neoscytalidium dimidiatum*	KY788091	2	32	0.125	1	0.063	0.001	0.004	0.5
6	52/F/2016	Nail lesion	*Neoscytalidium novaehollandiae*	KY788097	0.5	32	0.125	4	0.063	0.0002	0.002	>0.5
7	55/M/2014	Nail lesion	*Aureobasidium pullulans*	KY788108	2	32	0.125	1	0.063	0.0002	0.002	>0.5
8	65/M/2015	Oral lesion	*Curvularia hawaiiensis*	KY788102	1	16	0.125	1	0.031	0.0002	0.002	>0.5
9	65/F/2014	Nail lesion	*Cladosporium sphaerospermum*	KY788060	8	32	0.125	1	0.25	0.016	0.016	>0.5
10	56/M/2010	Skin lesion	*Rhinocladiella aquaspersa*	GU079659	1	32	2	0.063	ND	0.002	0.004	>0.5
11	62/F/2011	Skin lesion	*Rhinocladiella aquaspersa*	GU053606	2	32	2	0.125	ND	0.004	0.004	>0.5
12	50/M/2010	Brain abscess	*Rhinocladiella mackenziei*	GQ863214	16	32	0.5	0.063	ND	0.002	0.001	>0.5
13	67/M/2017	Brain abscess	*Rhinocladiella mackenziei*	MF401515	16	64	0.5	0.125	ND	0.008	0.004	>0.5
14	39/M/2011	Brain abscess	*Thilavia subthermophila*	HM448442	0.5	8	0.016	0.016	ND	0.0002	0.002	>0.5
15	32/F/2013	Skin lesion	*Veronaea botryosa*	JX566723	4	64	1	0.25	ND	0.001	0.004	>0.5
16	4/F/2008	Toe lesion	*Cladophialophora saturnica*	EU103984	2	16	1	0.25	ND	0.0005	0.002	>0.5
17	–/M/–	Brain abscess	*Cladophialophora bantiana*	EU103989	0.5	16	0.5	0.063	ND	0.0005	0.002	>0.5
18	49/M/2006	Skin lesion	*Cladophialophora immunda*	EU137318	2	32	0.25	0.031	ND	0.0002	0.002	>0.5
19	43/M/1982	Skin lesion	*Cladophialophora samoensis*	EU137291	2	32	4	0.25	ND	0.0002	0.002	>0.5
20	31/M/2013	Skin lesion	*Exophiala jeanselmei*	KP132049	0.125	16	0.125	0.25	ND	0.0005	0.001	0.5
21	43/M/2010	Skin lesion	*Exophiala jeanselmei*	KP132048	0.25	16	0.25	0.25	ND	0.0005	0.002	0.5
22	65/M/2013	Skin lesion	*Fonsecaea pedrosoi*	JX660491	2	>64	2	0.5	ND	0.001	0.001	>0.5
23	46/M/2013	Skin lesion	*Fonsecaea monophora*	JX660490	2	64	1	0.125	ND	0.0005	0.002	>0.5
24	67/F/2010	Skin lesion	*Fonsecaea monophora*	FJ785473.1	1	32	1	0.25	ND	0.0005	0.002	>0.5
25	47/F/2010	Subcutaneous cyst	*Medicopsis romeroi*	KF015657	4	>64	4	0.5	ND	0.002	0.001	>0.5
26	55/M/2012	Skin lesion	*Exophiala spinifera*	GU980971	0.5	16	0.5	0.125	ND	0.0005	0.002	0.25
27	–/M/–	Skin lesion	*Exophiala dermatitidis*	FJ974060	0.016	64	0.25	0.125	ND	0.0005	0.002	0.5
28	20/F/2011	Sinus discharge	*Exophiala oligosperma*	KF928424	2	16	1	0.063	ND	0.0005	0.002	0.5

[a]F, female; M, male. Dashes represent unavailable data.
[b]ND, not determined.

opment of drug resistance (5). Therefore, alternative antifungal agents with better activities and fewer side effects can help improve the management of these infections. Recently, the new azole agents luliconazole, lanoconazole, and efinaconazole were approved for the treatment of dermatophytosis and onychomycosis (6–9). The frequency of application and duration of treatment with these drugs are also favorable compared with those of other regimens used for the treatment of tinea pedis (6, 7). Previous studies have also shown potent activities of these antifungal agents against *Candida albicans*, Dermatophyte species, *Malassezia* species, and azole-resistant and azole-susceptible *Aspergillus fumigatus* strains, but resistance to these drugs has not been described so far (7–12). To the best of our knowledge, only limited data are available on the *in vitro* activities of these novel azoles against dematiaceous fungi and their relatives (13). Therefore, we aimed to investigate the *in vitro* activities of luliconazole, lanoconazole, efinaconazole, and five comparators against a large collection of dematiaceous fungi and their relatives from different clinical and environmental sources.

A total of 130 well-characterized dematiaceous isolates were obtained from the reference culture collections of the Invasive Fungi Research Center (IFRC), Sari, Iran. The clinical collections ($n = 28$) were recovered from a variety of specimens (Tables 1 and 2) comprising sinus discharge ($n = 4$), skin lesions ($n = 13$), cerebral abscesses ($n = 4$), nail lesions ($n = 5$), subcutaneous cyst ($n = 1$), and an oral lesion ($n = 1$). In addition, environmental isolates ($n = 102$) were collected from air ($n = 39$), soil ($n = 33$), wood, plant, and organic debris ($n = 30$) (see Table S1 in the supplemental material). Isolates were identified to the species level by DNA sequencing of the internal transcribed spacer 1-5.8 ribosomal DNA-ITS2 (ITS1-5.8S rDNA-ITS2) rDNA region, as previously described (14). *In vitro* antifungal susceptibility testing was performed according to the

TABLE 2 MIC data for all clinical isolates

MIC data (μg/ml)	Results by antifungal drug							
	AMB	FLC	VRC	ITC	EFN	LUL	LCZ	TRB
Range	0.016 to 16	8 to >64	0.016 to 4	0.016 to 4	0.031 to 0.25	0.0002 to 0.016	0.001 to 0.063	0.25 to >0.5
Geometric mean	1.2506	28.006	0.4313	0.2383	0.0966	0.0008	0.0028	0.4528
MIC_{50}	2	32	0.5	0.25	ND	0.0005	0.002	>0.5
MIC_{90}	8	64	2	1	ND	0.008	0.008	>0.5

Clinical and Laboratory Standards Institute (CLSI) document M38-A2 (15). Concentration ranges of 0.016 to 16 μg/ml for amphotericin B (AMB; Bristol-Myers-Squib, Woerden, The Netherlands), itraconazole (ITC; Janssen Research Foundation, Beerse, Belgium), and voriconazole (VRC; Pfizer, Central Research, Sandwich, United Kingdom); 0.063 to 64 μg/ml for fluconazole (FLU; Pfizer), 0.00005 to 0.031 μg/ml for luliconazole (LUL; Nihon Nohyaku Co. Ltd., Osaka, Japan), 0.0002 to 0.125 μg/ml for lanoconazole (LCZ; Nihon Nohyaku Co. Ltd.), 0.001 to 0.5 μg/ml for efinaconazole (EFN; Nihon Nohyaku Co. Ltd.), and terbinafine (TRB; Novartis Research Institute, Vienna, Austria) were used. Conidial suspensions were prepared from up-to-week-old cultures on potato dextrose agar (PDA; Difco) by gently scraping the surfaces of the colonies with a sterile cotton swab wetted with sterile saline containing Tween 40 (0.05%). The supernatants containing mostly nongerminated conidia were adjusted spectrophotometrically at a wavelength of 530 nm to an optical density (OD) that ranged from 2×10^6 to 5×10^6 CFU/ml, the supernatants were diluted 1:50 (except for *Alternaria* species, which were diluted 1:25) in RPMI 1640 medium, and the final inoculum in assay wells was between 2×10^4 and 5×10^4 CFU/ml, as determined by the use of quantitative colony counts to determine the viable numbers of CFU per milliliter (15). Plates were incubated at 35°C for 48 h (30°C only for *Alternaria* species) (16, 17). The MIC endpoints were defined with the aid of a reading mirror as the lowest concentration of drug that prevents any recognizable growth (100% inhibition) for all tested drugs, whereas a prominent reduction in growth (>50%) compared to the growth of the drug-free control was used for fluconazole (15). *Candida parapsilosis* (ATCC 22019) and *Paecilomyces variotii* (ATCC 3630) were included as quality controls. All tests were performed in duplicate, and the differences of the mean values were evaluated statistically using Student's *t* test with the statistical SPSS package (version 7.0). *P* values of 0.05 or less were considered statistically significant.

Based on molecular characterization, Tables 1 and 3 and Table S1 in the supplemental material show the species distribution of black mold and dematiaceous yeast isolates according to their origins.

Tables 1 to 3 summarize the MIC range, geometric mean (GM) MIC, MIC mode, MIC_{50}, and when appropriate, the MIC_{90} of the tested antifungal drugs. All clinical and environmental strains had low MICs for luliconazole and lanoconazole, followed by efinaconazole, in comparison with other drugs, whereas the less active drugs were fluconazole and amphotericin B. Indeed, the widest ranges and the highest MICs were seen for fluconazole (8 to >64 μg/ml and 16 to 64 μg/ml) and amphotericin B (0.016 to 16 μg/ml and 0.5 to 8 μg/ml) against both clinical and environmental strains, respectively. The GM MICs against all clinical black fungal strains of various genera were as follows, in increasing order: luliconazole, 0.0008 μg/ml; lanoconazole, 0.0028 μg/ml; efinaconazole, 0.0966 μg/ml; itraconazole, 0.2383 μg/ml; voriconazole, 0.4313 μg/ml; terbinafine, 0.4528 μg/ml; amphotericin B, 1.25 μg/ml; and fluconazole, 28.006 μg/ml. The GM MICs of all environmental isolates were as follows: luliconazole, 0.002 μg/ml; lanoconazole, 0.009 μg/ml; efinaconazole, 0.10 μg/ml; voriconazole, 0.20 μg/ml; itraconazole, 1.18 μg/ml; amphotericin B, 2.2 μg/ml; terbinafine, >0.5 μg/ml; and fluconazole, 38.4 μg/ml. Generally, the GM MIC values of luliconazole and lanoconazole against all clinical and environmental isolates of melanized fungi were >6-$\log_2$ and >4-$\log_2$-dilution steps lower than those of efinaconazole and other azoles, respectively. However, no statistically

TABLE 3 *In vitro* susceptibilities of 102 environmental melanized fungal isolates against eight antifungal agents

Antifungal drug by strain type[a]	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)[b]	Geometric mean (μg/ml)	No. of isolates susceptible by MIC (μg/ml)[c]																				
				0.00005	0.0001	0.0002	0.0005	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64
All strains (n = 102)																								
AmB	0.5 to 8	2/8	2.2														5	7	**72**	4	14			
FLU	16 to 64	32/64	38.4																			14	**47**	41
VRC	0.063 to 1	0.25/1	0.20											6	**45**	33	3	15						
ITC	0.25 to 4	1/4	1.18													1	6	**75**	7	13				
EFN	0.016 to 0.5	0.063/0.25	0.10									2	7	**48**		38	7							
LUL	0.0001 to 0.031	0.002/0.031	0.002		2	11	**24**	13	8	14	1	18	11											
LCZ	0.0002 to 0.063	0.016/0.063	0.009			2			11	23	13	**40**		13										
TRB	0.5 to >0.5	>0.5/>0.5	>0.5														102							
Alternaria alternata (n = 14)																								
AmB	2 to 8	2/2	2.20																**13**		1			
FLU	32 to 64	32/64	39.008																				**10**	4
VRC	0.06 to 1	0.12/1	0.21											1	**7**	3		3						
ITC	1 to 1	1/1	1															**14**						
EFN	0.06 to 0.5	0.25/0.25	0.15											5		**8**	1							
LUL	0.0002 to 0.03	0.0005/0.03	0.0019			3	**4**	1		1		2	3											
LCZ	0.002 to 0.06	0.015/0.06	0.014						1	2	2	**5**		4										
TRB	0.5 to >0.5	>0.5/>0.5	>0.5														**14**							
Alternaria tenuissima (n = 10)																								
AmB	2 to 2	2/2	2																**10**					
FLU	32 to 64	32/64	45.2																				**5**	5
VRC	0.06 to 1	0.25/1	0.24											1	2	**5**		2						
ITC	1 to 4	1/1	1.14															**9**		1				
EFN	0.06 to 0.5	0.25/0.25	0.17											3		**6**	1							
LUL	0.0002 to 0.03	0.015/0.03	0.0057			1	2			1		2	**4**											
LCZ	0.008 to 0.06	0.015/0.06	0.021								1	**6**		3										
TRB	>0.5	>0.5	>0.5														**10**							
Other *Alternaria* species (n = 6)[d]																								
AmB	1 to 8	ND	2															2	**3**		1			
FLU	32 to 64	ND	40.31																				**4**	2
VRC	0.25 to 1	ND	0.39													**3**	2	1						
ITC	0.25 to 4	ND	0.79													1	**2**	**2**		1				
EFN	0.06 to 0.25	ND	0.15											2		**4**								
LUL	0.0005 to 0.015	ND	0.0021				**3**			1		2												
LCZ	0.004 to 0.06	ND	0.013							1	1	**3**		1										
TRB	>0.5	ND	>0.5														**6**							
Cladosporium cladosporioides (n = 12)																								
AmB	2 to 8	2/8	2.5																**10**		2			
FLU	16 to 64	32/64	42.7																			1	5	**6**
VRC	0.06 to 0.5	0.12/0.25	0.15											1	**7**	3	1							
ITC	0.5 to 2	1/1	0.94														2	**9**	1					
EFN	0.03 to 0.25	0.06/0.25	0.076										2	**7**		3								
LUL	0.0001 to 0.015	0.001/0.015	0.0012		**2**	1	2	2	2		1	2												
LCZ	0.0002 to 0.015	0.004/0.015	0.0034			1		**4**	3		2	2												
TRB	>0.5	>0.5	>0.5														**12**							

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TABLE 3 (Continued)

Antifungal drug by strain type[a]	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)[b]	Geometric mean (μg/ml)	No. of isolates susceptible by MIC (μg/ml)[c]																				
				0.00005	0.0001	0.0002	0.0005	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64
Cladosporium sphaerospermum (n = 10)																								
AmB	2 to 8	2/8	3.2																**6**	1	3			
FLU	32 to 64	32/64	39.39																				**7**	3
VRC	0.12 to 1	0.12/0.25	0.18												**6**	3		1						
ITC	1 to 4	1/2	1.41															**6**	**3**	1				
EFN	0.06 to 0.25	0.25/0.25	0.18											2		**8**								
LUL	0.0005 to 0.015	0.002/0.015	0.0024				2	2	1	**3**		2												
LCZ	0.0002 to 0.015	0.004/0.015	0.0053			1				**4**	1	4												
TRB	>0.5	>0.5	>0.5														**10**							
Ulocladium tuberculatum (n = 19)																								
AmB	1 to 8	2/8	2.6															1	**13**	1	4			
FLU	16 to 64	64/64	57.3																			1	1	**17**
VRC	0.125 to 1	0.25/1	0.38												2	**10**		7						
ITC	1 to 4	1/4	1.8															**10**	1	8				
EFN	0.06 to 0.5	0.25/0.5	0.17											**7**		7	5							
LUL	0.0002 to 0.031	0.004/0.031	0.003			2	3	3	1	2		**4**	4											
LCZ	0.002 to 0.063	0.016/0.063	0.01						1	1	4	**9**		4										
TRB	>0.5	>0.5	>0.5														**19**							
Bipolaris species (n = 8)[e]																								
AmB	0.5 to 2	ND	1														2	**4**	2					
FLU	16 to 32	ND	22.6																			**4**	4	
VRC	0.063 to 0.25	ND	0.11											2	**5**	1								
ITC	0.5 to 1	ND	0.9														1	**7**						
EFN	0.016 to 0.25	ND	0.06									1	2	**3**		2								
LUL	0.0002 to 0.016	ND	0.003			1	1		1	1		**4**												
LCZ	0.002 to 0.063	ND	0.011						1	1		**5**		1										
TRB	>0.5	ND	>0.5														**8**							
Neoscytalidium dimidiatum (n = 6)																								
AmB	0.5 to 2	ND	1														**3**		3					
FLU	32 to 64	ND	35.9																				**5**	1
VRC	0.125 to 0.25	ND	0.13												**5**	1								
ITC	0.5 to 4	ND	1.1														1	**4**		1				
EFN	0.063	ND	0.06											**6**										
LUL	0.0002 to 0.004	ND	0.0007			1	**2**	2		1														
LCZ	0.002 to 0.016	ND	0.004						1	**4**		1												
TRB	0.5 to >0.5	ND	>0.5														**6**							
Phoma glomerata (n = 5)																								
AmB	2 to 8	ND	3.03																**3**	1	1			
FLU	16 to 64	ND	27.8																			**2**	2	1
VRC	0.063 to 0.25	ND	0.12											1	**3**	1								
ITC	1 to 2	ND	1.3															**3**	2					
EFN	0.033 to 0.25	ND	0.06										1	**3**		1								
LUL	0.001 to 0.004	ND	0.002					1	**3**	1														
LCZ	0.002 to 0.016	ND	0.005						1	1	**2**	1												
TRB	0.5	ND	0.5														**5**							

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TABLE 3 (Continued)

Antifungal drug by strain type[a]	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)[b]	Geometric mean (μg/ml)	No. of isolates susceptible by MIC (μg/ml)[c]																				
				0.00005	0.0001	0.0002	0.0005	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64
Drechslera dematioidea ($n = 4$)																								
AmB	2 to 4	ND	2.3																**3**	1				
FLU	16 to 32	ND	19.2																			**3**	1	
VRC	0.125 to 0.25	ND	0.14												**3**	1								
ITC	1	ND	1															**4**						
EFN	0.063 to 0.25	ND	0.12											**2**		2								
LUL	0.0005 to 0.004	ND	0.001				**2**	1		1														
LCZ	0.004 to 0.016	ND	0.007							**2**		2												
TRB	>0.5	ND	>0.5														**4**							
Aureobasidium pullulans ($n = 3$)																								
AmB	2	ND	2																**3**					
FLU	32 to 64	ND	40.3																				**2**	1
VRC	0.125 to 0.25	ND	0.15												**2**	1								
ITC	1	ND	1															**3**						
EFN	0.063	ND	0.06											**3**										
LUL	0.0002 to 0.001	ND	0.0004			**1**	1	1																
LCZ	0.002 to 0.016	ND	0.004						**1**	1		1												
TRB	>0.5	ND	>0.5														**3**							
Ochroconis constricta ($n = 2$)																								
AmB	2 to 8	ND	4																**1**		1			
FLU	32 to 64	ND	45.2																				**1**	1
VRC	0.125 to 1	ND	0.34												**1**			1						
ITC	1 to 4	ND	2															**1**		1				
EFN	0.031 to 0.063	ND	0.04										**1**	1										
LUL	0.0005 to 0.002	ND	0.001				**1**		1															
LCZ	0.004	ND	0.004							**2**														
TRB	0.5 to >0.5	ND	>0.5														**2**							
Didymella rabiei ($n = 1$)																								
AmB	8	ND	ND																		1			
FLU	16	ND	ND																			1		
VRC	0.12	ND	ND												1									
ITC	1	ND	ND															1						
EFN	0.016	ND	ND									1												
LUL	0.0002	ND	ND			1																		
LCZ	0.002	ND	ND						1															
TRB	>0.5	ND	ND														1							
Exophiala phaeomuriformis ($n = 1$)																								
AmB	2	ND	ND																1					
FLU	16	ND	ND																			1		
VRC	0.125	ND	ND												1									
ITC	1	ND	ND															1						
EFN	0.063	ND	ND											1										
LUL	0.0005	ND	ND				1																	
LCZ	0.004	ND	ND							1														
TRB	0.5	ND	ND														1							

(Continued on next page)

TABLE 3 (Continued)

Antifungal drug by strain type[a]	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)[b]	Geometric mean (μg/ml)	No. of isolates susceptible by MIC (μg/ml)[c]																				
				0.00005	0.0001	0.0002	0.0005	0.001	0.002	0.004	0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64
Embellisia astragali ($n = 1$)																								
AmB	2	ND	ND																1					
FLU	16	ND	ND																			1		
VRC	0.25	ND	ND													1								
ITC	1	ND	ND															1						
EFN	0.06	ND	ND											1										
LUL	0.002	ND	ND						1															
LCZ	0.016	ND	ND									1												
TRB	>0.5	ND	ND														1							

[a]AmB, amphotericin B; FLU, fluconazole; VRC, voriconazole; ITC, itraconazole; EFN, efinaconazole; LUL, luliconazole; LCZ, lanoconazole; TRB, terbinafine.

[b]MIC_{50}, concentration at which 50% of the isolates were inhibited; MIC_{90}, concentration at which 90% of the isolates were inhibited; ND, not determined.

[c]Numbers in bold are modal values.

[d]Includes *A. malorum*, $n = 2$; *A. chlamydospora*, $n = 2$; *A rosae*, $n = 1$; and *A. japonica*, $n = 1$.

[e]Includes *B. spicifera*, $n = 4$; and *B. hawaiiensis*, $n = 4$.

significant ($P > 0.05$) differences in the lanoconazole, luliconazole, and efinaconazole susceptibility patterns were detected between strains. While species-based analysis of the MIC values is required, we provided overall MIC_{50}, MIC_{90}, and MIC ranges for the clinical isolates, since the number of strains per each genus/species is low. These data may still appear to be useful for clinical guidance due to difficulties and frequent absence of availability of species identification of dematiaceous fungi in routine practice.

The term "phaeohyphomycoses" is used to describe a heterogeneous group of cutaneous, subcutaneous, cyst, and disseminated fungal infections in which black mold and dematiaceous yeast are noted in samples through histopathology (3); however, chromoblastomycosis is a chronic progressive disorder histologically characterized by a granulomatous lesion with muriform cells caused by black yeast-like fungi and their relatives (1). Due to the increasing number of antifungal agents, there has been a great interest to evaluate the activities of these new imidazole and triazole drugs against different fungal pathogens and compare them to the reference antifungal agents (6–12). Despite the increasing number of studies on the efficacy of the newer agents for several pathogenic fungi, the *in vitro* antifungal susceptibility profiles against black fungi remain to be investigated. In addition, no comprehensive data on *in vitro* antifungal susceptibility profiles of black fungi have yet been published (13). Lanoconazole, luliconazole, and efinaconazole were found to interfere with ergosterol biosynthesis by inhibiting sterol 14-alpha demethylase and blocking fungal membrane ergosterol biosynthesis (6, 7). Based on the results in the present study, luliconazole, lanoconazole, and efinaconazole were the most active drugs against all tested isolates. Previous studies have shown that these novel imidazoles had low MICs against azole-resistant *Aspergillus fumigatus* and dermatophyte species (10, 12). Furthermore, the great potency of luliconazole against *Trichophyton* species has been shown by Koga et al. (18, 19). Uchida et al. also showed that the GM MICs of luliconazole for *Malassezia furfur*, *Malassezia sympodialis*, and *Malassezia slooffiae* were approximately 1.4 μg/ml, 0.1 μg/ml, and 1 μg/ml, respectively (12). In addition, the current investigation showed potent activities of luliconazole, lanoconazole, and efinaconazole against clinical and environmental black mold and dematiaceous yeast isolates. Although data on the *in vitro* activity of efinaconazole against black fungi are limited, in previous studies by Tatsumi et al., the MICs of efinaconazole against *Trichophyton rubrum* and *Trichophyton mentagrophytes*, the causative agents of dermatophytosis, were in the ranges of 0.015 to 0.5 μg/ml and 0.06 to 0.5 μg/ml, respectively (20, 21). The MIC range of efinaconazole against *T. rubrum* was similar to that detected in the present study on black fungi. Moreover, efinaconazole was active against a variety of pathogenic fungi associated with onychomycosis (9). Of all the antifungal agents tested, fluconazole was the drug with the highest MIC values against black fungi, which is in line with the results from previous studies (16, 17, 22, 23). In conclusion, given the fact that fungal infection due to dematiaceous fungi has increased and treatment is still challenging, the selection of proper antifungal agents is therefore critical for appropriate therapy and in order to improve the management of infected patients. Therefore, it appears that these two new imidazoles and new triazoles are promising candidates for the treatment of phaeohyphomycosis and chromoblastomycosis caused by black mold and dematiaceous yeast.

Accession number(s). The nucleotide sequences for the determined environmental isolates have been deposited in GenBank under the accession numbers KY788018 to KY788122 and MF422635 to MF422636 (see Table S1 in the supplemental material).

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We declare no potential conflicts of interest.

We alone are responsible for the content and writing of the paper.

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