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Potent Activities of Novel Imidazoles Lanoconazole and Luliconazole against a Collection of Azole-Resistant and -Susceptible *Aspergillus fumigatus* Strains

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A collection of azole-susceptible ($n = 141$) and azole-resistant ($n = 27$) *Aspergillus fumigatus* isolates was tested against seven antifungal drugs, including the new imidazoles lanoconazole and luliconazole. The luliconazole and lanoconazole MIC_{90} values for the azole-susceptible strains were 0.001 μg/ml and 0.008 μg/ml, and those for the azole-resistant strains were 0.016 μg/ml and 0.032 μg/ml.

Invasive aspergillosis caused by *Aspergillus fumigatus* is a difficult-to-diagnose, life-threatening opportunistic fungal infection associated with significant morbidity and mortality (1). Survival rates improved significantly after the introduction of triazole antifungal agents such as voriconazole (2–4). However, triazole-resistant *Aspergillus fumigatus* strains are emerging worldwide due to long-term triazole therapy or, more commonly, are selected in the environment through exposure to azole fungicides (4–7). Recently, a new antifungal agent, isavuconazole, was shown to be noninferior to voriconazole for treatment of infections caused by *Aspergillus* species (8). However, cross-resistance exists for azole-resistant *Aspergillus*. Luliconazole, a topically related compound of lanoconazole, has been approved by the FDA for topical treatment of tinea cruris, tinea corporis, and tinea pedis. Luliconazole had neither clastogenic or mutagenic effects in genotoxicity tests, and no effect on fertility or reproductive function was noted. Luliconazole affects ergosterol biosynthesis by inhibiting the azole target protein lanosterol 14α-demethylase (*cyp51A*), which is the key enzyme that catalyzes the oxidative removal of the 14α-methyl group of lanosterol to give 14-15-desaturated intermediates in ergosterol biosynthesis (9). Recently, *in vitro* antifungal susceptibility testing of lanoconazole and luliconazole demonstrated potent efficacy against *Trichophyton rubrum* and *Epidermophyton floccosum* (9–14). In addition, animal studies and small human series suggested that luliconazole and lanoconazole are effective in treating dermatophytosis and onychomycosis (15, 16). Only limited data on the *in vitro* activity of lanoconazole and luliconazole against *Aspergillus* species are available. Therefore, the aim of the present study was to investigate the *in vitro* activity of these two new imidazoles and five comparators against a large collection of azole-susceptible and -resistant *A. fumigatus* strains with various point mutations from clinical and environmental sources. A total of 168 well-characterized *A. fumigatus* strains from the culture collection of the Invasive Fungi Research Center (IFRC) were included. Azole-susceptible ($n = 141$) and -resistant ($n = 27$) strains originated from nail, sputum, bronchoalveolar lavage, sinus discharge, and skin biopsy samples. Environmental samples came from soil and air samples. Most of the azole-resistant *A. fumigatus* strains ($n = 10$) harbored a leucine-to-histidine substitution at codon 98, along with a 34-bp tandem repeat in the *cyp51A* promoter region, but TR46/Y121F/T289 ($n = 2$) and other point mutations ($n = 8$) such as G54, M220, G138C, and G432C were also included. Resistant isolates without mutations in *cyp51A* were also included ($n = 7$). MICs were determined based on CLSI M38-A2 (17). Concentration ranges of 0.001 to 1 μg/ml for luliconazole (Nihon Nohyaku Co, Osaka, Japan) and lanoconazole (Nihon Nohyaku Co.), 0.016 to 16 μg/ml for itraconazole (Janssen, Beerse, Belgium), voriconazole (Pfizer, Sandwich, United Kingdom), and amphotericin B (Bristol-Myers-Squib, Woerden, The Netherlands), and 0.008 to 8 μg/ml for posaconazole (Merck Sharp & Dohme BV, Haarlem, The Netherlands) and caspofungin (Merck Sharp & Dohme BV) were used. Stock solutions were prepared in dimethyl sulfoxide. Conidial suspensions were prepared by scraping the surface of fungal colonies with a sterile cotton swab moistened with physiological saline solution containing 0.05% Tween 40 and were adjusted to optical densities ranging from 0.09 to 0.11 (0.5×10^6 to 3.1×10^6 CFU/ml) measured at 530 nm. Inoculum suspensions, including mostly nongerminated conidia, were diluted 1:50 in RPMI 1640 medium, and the final inoculum in assay wells was between 0.4×10^4 and 5×10^4 CFU/

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TABLE 1 *In vitro* susceptibility of 168 *Aspergillus fumigatus* isolates to seven antifungal agents[a]

A. fumigatus strain category (no. of strains)	Parameter	MIC or MEC result(s) (μg/ml)						
		LANO	LULI	ITC	VRC	POS	AMB	CAS
All strains ($n = 168$)	Range	≤0.001 to 0.5	≤0.001 to 0.016	0.064 to >16	0.064 to >16	≤0.008 to 8	0.016 to 4	≤0.008 to 2
	MIC_{50}	0.002	0.001	0.5	0.125	0.064	0.5	0.032
	MIC_{90}	0.016	0.002	1	1	1	2	0.5
	GM	0.0024	0.0012	0.4243	0.2555	0.0968	0.6036	0.0535
	Mode	0.001	0.001	0.5	0.125	0.064	0.5	0.125
Susceptible strains ($n = 141$)	Range	≤0.001 to 0.016	≤0.001 to 0.016	0.064 to 2	0.064 to 1	≤0.008 to 0.25	0.1 to 4	≤0.008 to 0.5
	MIC_{50}	0.001	0.001	0.5	0.125	0.064	0.5	0.032
	MIC_{90}	0.008	0.001	1	0.5	0.25	2	0.125
	GM	0.0018	0.001	0.339	0.1776	0.0460	0.63	0.0435
	Mode	0.001	0.001	0.5	0.125	0.064	0.5	0.125
Triazole-resistant strains ($n = 27$)	Range	≤0.001 to 0.5	≤0.001 to 0.016	4 to >16	0.125 to >16	0.016 to 16	0.125 to 2	≤0.008 to 0.25
	MIC_{50}	0.016	0.002	16	4	0.5	0.5	0.032
	MIC_{90}	0.032	0.016	16	16	2	2	0.25
	GM	0.0117	0.0031	12.491	2.058	0.5728	0.6417	0.0466
	Mode	0.016	0.002	16	8	2	0.5	0.032

[a] MIC and MEC range, geometric mean MIC, MIC_{50}, and MIC_{90} values are expressed in micrograms per milliliter. Abbreviations: LANO, lanoconazole; LULI, luliconazole; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; AMB, amphotericin B; CAS, caspofungin; GM, geometric mean.

ml. Microdilution trays were incubated at 35°C for 48 h. MICs were determined visually as the lowest concentration which provided complete inhibition of growth, while minimum effective concentrations (MECs [caspofungin only]) were determined microscopically as the lowest concentration of drug promoting the growth of small, round, compact hyphae relative to the appearance of the filamentous forms seen in the control wells. *Candida krusei* (ATCC 6258) and *Paecilomyces variotii* (ATCC 3630) were included as quality controls (17). All tests were performed in duplicate, and differences of the mean values were determined by using Student's *t* test with the statistical SPSS package (version 7.0). *P* values of <0.05 were considered statistically significant. Table 1 summarizes the *in vitro* susceptibility of 168 susceptible and resistant *A. fumigatus* isolates. The novel imidazoles luliconazole and lanoconazole demonstrated potent activity against all *A. fumigatus* isolates, in comparison to voriconazole, itraconazole, and posaconazole. MICs of lanoconazole and luliconazole against all *A. fumigatus* isolates ranged from <0.001 to 0.5 μg/ml and from <0.001 to 0.016 μg/ml, respectively, compared to 0.064 to >16 μg/ml for itraconazole, 0.064 to >16 μg/ml for voriconazole, and 0.008 to 8 μg/ml for posaconazole. The lanoconazole and luliconazole geometric mean (GM) MICs against all isolates were 0.0024 μg/ml and 0.0012 μg/ml, respectively, while those of the other agents were as follows: itraconazole, 0.4243 μg/ml; voriconazole, 0.2555 μg/ml; posaconazole, 0.0968 μg/ml; caspofungin, 0.0535 μg/ml. Basically, the GM MIC value of luliconazole against all *A. fumigatus* isolates was 2 log2 dilutions lower than that of lanoconazole. However, no statistically significant ($P > 0.05$) differences in the lanoconazole and luliconazole susceptibility patterns were detected between strains. MICs of luliconazole and lanoconazole for the resistant isolates with various point mutations in the *cyp51A* gene were approximately similar to those of the susceptible isolates, but strains with TR46/Y121F/T289 mutations showed less susceptibility, with a 4-log2-dilution step compared to the other resistant strains harboring TR34/L98H, G54, M220, G138C, and G432C (Table 2).

In previous studies, we demonstrated that the prevalence of azole-resistant *A. fumigatus* with predominant TR34/L98H mutations in the *cyp51A* gene in Iran has increased remarkably from 3.3% to 6.6% (6, 7). Treatment regimens of *Aspergillus* infections with triazole agents are associated with a poor outcome when azole resistance is involved (18). In this study, molecularly identified strains of *A. fumigatus* strains from both clinical and environmental sources were subjected to antifungal susceptibility testing with newer imidazoles.

Recently, several studies have shown high *in vitro* and *in vivo* efficacy of luliconazole against a limited number of dermatophytes and other agents causative of onychomycosis (11–14, 19, 20). In addition, in the present study, lanoconazole and luliconazole showed potent activity against the wild-type strain as well as against azole-resistant mutants of *A. fumigatus*, but high MIC values for lanoconazole against two isolates with TR46/Y121F/T289 were observed. While the most common TR34/L98H mutation confers resistance to all azoles, TR46/Y121F/T289A confers resistance to voriconazole and isavuconazole but shows a variable influence on the MICs of itraconazole and posaconazole. The point mutations G54 and M220 in *cyp51A* induce resistance mainly to itraconazole and posaconazole (21). Only limited data are available on the effect and related toxicity of systemic use of luliconazole and lanoconazole. Oral luliconazole therapy in a murine model of invasive aspergillosis was superior to that of itraconazole, and intravenous luliconazole appeared to be highly effective in comparison with intravenous amphotericin B (22). In this study, 90% of the animals stayed alive when 2.5 mg/kg of body weight/day of luliconazole was used whereas only 30% of animals survived when amphotericin B (5 mg/kg/day) was used (22).

The current study demonstrated that the *in vitro* antifungal activities of luliconazole and lanoconazole against susceptible and resistant *A. fumigatus* isolates are apparently superior to those of polyenes, other azoles, and echinocandins. Clinical effectiveness in the treatment of *Aspergillus* infection and pharmacodynamic assessment and development of epidemiologic cutoff values (ECVs)/breakpoints remain to be established. In conclusion, we suggest that these two new imidazoles are promising candidates

TABLE 2 *In vitro* activity of seven antifungal drugs against triazole-resistant *Aspergillus fumigatus* isolates with different point mutations[a]

Collection no.	MIC or MEC (μg/ml)							Point mutation(s) (no.)
	LANO	LULI	ITC	VRC	POS	AMB	CAS	
IFRC 441	≤0.001	≤0.001	>16	4	4	1	0.25	TR34/L98H (n = 10)
IFRC 442	≤0.001	≤0.001	>16	4	2	1	0.125	
IFRC 836	0.016	0.008	>16	0.5	0.25	0.5	0.125	
IFRC 387	≤0.001	≤0.001	>16	8	2	0.25	≤0.008	
IFRC 795	0.008	≤0.001	>16	>16	2	0.5	0.032	
IFRC 1032	0.016	≤0.001	>16	8	0.016	0.5	0.032	
IFRC 435	0.008	0.004	16	8	2	0.5	0.016	
IFRC1000	0.008	0.004	>16	1	0.25	0.5	0.125	
IFRC 500	0.032	0.016	16	2	0.5	0.5	0.032	
IFRC 1077	0.016	≤0.001	>16	16	1	0.5	0.032	
IFRC 1503	0.5	0.002	>16	>16	1	0.125	0.016	TR46/Y121F/T289 (n = 2)
IFRC 1505	0.25	0.002	>16	>16	0.5	0.5	0.016	
IFRC 504	0.016	0.008	16	16	0.25	2	0. 25	F46Y & G89G (n = 2)
IFRC 505	0.016	0.008	16	2	2	2	0.125	
IFRC 1500	0.016	0.002	>16	0.25	1	0.25	0.032	G54 (n = 2)
IFRC 1501	0.016	0.002	>16	0.125	1	0.25	0.064	
IFRC 1502	0.016	0.002	>16	0.25	0.5	2	0.032	M220 (n = 3)
IFRC 1504	0.016	0.002	>16	0.25	0.5	0.25	0.032	
IFRC 507	0.016	0.016	16	4	16	1	0.064	
IFRC 501	0.002	0.002	16	1	0.25	1	0.064	A284T (n = 1)
IFRC 549	0.016	0.008	4	0.5	0.016	0.25	0.032	No mutation (n = 7)
IFRC 443	0.008	0.004	4	0.25	0.016	1	0.25	
IFRC 502	0.002	0.002	16	4	0.25	0.25	0.064	
IFRC 503	0.016	0.008	16	1	2	2	0.016	
IFRC 508	0.016	0.016	16	8	0.5	0.5	0.064	
IFRC 509	0.016	0.004	16	8	0.5	0.5	0.032	
IFRC 506	0.016	0.004	16	16	2	2	0.064	

[a] MIC and MEC values are expressed in micrograms per milliliter. Abbreviations: IFRC, Invasive Fungi Research Center; LANO, lanoconazole; LULI, luliconazole; ITC, itraconazole; VRC, voriconazole; POS, posaconazole; AMB, amphotericin B; CAS, caspofungin.

for treatment of invasive aspergillosis caused by either azole-susceptible or -resistant isolates.

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