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In Vitro Antifungal Activity of Novel Triazole Efinaconazole and Five Comparators against Dermatophyte Isolates

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ABSTRACT The objective of this study was to assess the *in vitro* activity of the novel triazole antifungal drug, efinaconazole, and five comparators (luliconazole, lanoconazole, terbinafine, itraconazole, and fluconazole) against a large collection of *Trichophyton interdigitale* and *Trichophyton rubrum* clinical isolates. The geometric mean MICs were the lowest for luliconazole (0.0005 μg/ml), followed by lanoconazole (0.002 μg/ml), efinaconazole (0.007 μg/ml), terbinafine (0.011 μg/ml), itraconazole (0.095 μg/ml), and fluconazole (12.77 μg/ml). It appears that efinaconazole, lanoconazole, and luliconazole are promising candidates for the treatment of dermatophytosis due to *T. interdigitale* and *T. rubrum*.

KEYWORDS dermatophytes, efinaconazole, *in vitro* susceptibility testing

Dermatophytes are a group of keratinophilic molds with a global distribution that based on the newly proposed taxonomy encompasses more than 50 species in the genera of *Trichophyton*, *Epidermophyton*, *Nannizzia*, *Microsporum*, *Lophophyton*, *Arthroderma*, and *Paraphyton*. They can invade and infect the nails, hairs, and stratum corneum of the skin and cause a spectrum of superficial fungal infections in human and animals, medically termed as dermatophytosis (ringworm or tinea) (1). Sources of dermatophytes can be associated with transmission via contact with infected humans (anthropophilic), animals (zoophilic), or environmental soil (geophilic) (1, 2). Although not life-threatening, dermatophytoses have been among the most common contagious diseases in the population, adversely affect the quality of life of infected patients, and have significant social, health, and economic implications (3). Although agents of dermatophytosis are generally susceptible to most antifungal drugs *in vitro* and *in vivo*, treatment is a big challenge because frequent relapses and failures are observed, especially in cases of onychomycosis (4–6). At present, oral use of itraconazole and terbinafine is the drug treatment of choice for onychomycosis, but the results of therapy for nail infection are collectively disappointing, mainly due to poor nail permeation of available antifungals (7–9). These issues signify the need for continual

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TABLE 1 Distribution[a] of the 120 clinical strains of *Trichophyton* used in the study

Clinical manifestation	Etiologic agents (no. [%])		Total no. (%)
	T. interdigitale	*T. rubrum*	
Tinea unguium	26 (21.7)	20 (16.7)	46 (38.4)
Tinea pedis	21 (17.5)	19 (15.8)	40 (33.3)
Tinea cruris	5 (4.1)	11 (9.1)	16 (13.2)
Tinea corporis	10 (8.3)	3 (2.5)	13 (10.8)
Tinea manuum	2 (1.7)	1 (0.8)	3 (2.5)
Tinea capitis	2 (1.7)	0 (0)	2 (1.7)
Tinea faciei	0 (0)	0 (0)	0 (0)
All manifestations	66	54	120

[a]In regard to the anatomical site of isolation.

clinically relevant antifungal susceptibility testing and development of new antifungals with improved safety and efficacy. A new triazole, efinaconazole, has recently been introduced specifically for the topical treatment of onychomycosis (9, 10). The drug was approved in the United States, Canada, and Japan between 2013 to 2014, and it is currently being marketed as a 10% daily topical solution under the trade names of Jublia in the United States and Canada and Clenafin in Japan (10). Aside from efinaconazole, luliconazole and lanoconazole, two agents from the imidazole class, were also recently approved for the treatment of dermatophytosis and onychomycosis (11, 12). Recent studies indicated potent activity of these azoles toward clinically important melanized fungi and their relatives, as well as azole-resistant and susceptible *Aspergillus fumigatus* strains, but resistance to these azoles has not been demonstrated thus far (13, 14). Until today, to the best of our knowledge, there have been few investigations worldwide pertaining to the *in vitro* activity of these azoles against dermatophytes (15–17). Thus, the aim of the present study was to characterize the *in vitro* activity of the novel triazole antifungal drug efinaconazole and five comparators (i.e., luliconazole, lanoconazole, terbinafine, itraconazole, and fluconazole) against a large collection of *Trichophyton interdigitale* and *Trichophyton rubrum* isolates from different clinical sources. A total of 120 clinical isolates comprised of *T. interdigitale* ($n = 66$) and *T. rubrum* ($n = 54$) isolates recovered from infected patients in Tehran, Iran, with different types of dermatophytosis, i.e., tinea unguium ($n = 46$), tinea pedis ($n = 40$), tinea cruris ($n = 16$), tinea corporis ($n = 13$), tinea manuum ($n = 3$), and tinea capitis ($n = 2$) were included (Table 1). All isolates were first screened by amplification and restriction digestion of the internal-transcribed spacer (ITS)-ribosomal DNA (rDNA) region in a PCR-restriction fragment length polymorphism (PCR-RFLP) scheme (18) and were subsequently identified to the species level by DNA sequencing of the ITS1-5.8S rDNA-ITS2 rDNA region, as previously described (19). *In vitro* antifungal susceptibility testing was adjusted in microdilution plates according to the reference method described in the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document (20). Concentration ranges of 0.002 to 0.5 μg/ml for efinaconazole (Nihon Nohyaku Co. Ltd., Tokyo, Japan), 0.0002 to 0.125 μg/ml for lanoconazole (Nihon Nohyaku Co. Ltd.), 0.00006 to 0.031 μg/ml for luliconazole (Nihon Nohyaku Co. Ltd.), 0.016 to 8 μg/ml for itraconazole (Wako Chemical Co., Ltd., Tokyo, Japan), 0.001 to 0.5 μg/ml for terbinafine (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), and 0.125 to 64 μg/ml for fluconazole (Wako Chemical Co., Ltd.). Briefly, conidial inocula were prepared from 2-week-old potato dextrose agar (PDA; Sparks, MD) cultures by gently scraping the surface of mature colonies with a sterile cotton swab moistened with sterile physiological saline containing Tween 80 (0.05%). Conidial suspensions were transferred to a sterile syringe attached to a sterile filter holder with a sterile filter (Whatman no. 40), filtered, and collected in a sterile tube to remove the majority of the hyphae. Homogeneous conidial suspensions were adjusted spectrophotometrically at a 530 nm wavelength to optical densities (ODs) that ranged from 65% to 70% transmission. The obtained inocula were diluted 1:50 in RPMI 1640 medium, corresponding to 1×10^3 to 3×10^3 CFU/ml, controlled by quantitative colony counts. Microdilution plates were incubated at 35°C

for 96 h, and MIC was visually determined by using a reading mirror and defined as the lowest concentration of each antifungal drug that resulted in 80% inhibition of growth. The strains *Trichophyton mentagrophytes* (ATCC 4439) and *T. rubrum* (ATCC 4438) served as quality control for every new series of MIC plates. All tests were performed in duplicate, and the differences of the mean values were determined by using Student's *t* test with the SPSS statistical package (version 7.0). *P* values of 0.05 or less were considered statistically significant. Based on ITS rDNA sequencing, Table 1 shows the species distribution of dermatophyte isolates according to their origins. Table 2 summarizes MIC range, geometric mean (GM) MIC, MIC mode, MIC_{50}, and (when appropriate) MIC_{90} of the tested antifungal drugs. The MICs for efinaconazole against all dermatophyte isolates ranged from 0.002 to 0.063 μg/ml, compared to 0.0005 to 0.004 μg/ml for luliconazole, 0.001 to 0.008 μg/ml for lanoconazole, 0.004 to 0.125 μg/ml for terbinafine, and 0.03 to 0.5 μg/ml for itraconazole, while the widest range (8 to 64 μg/ml) and the highest MICs were observed for fluconazole. The GM MICs against *T. rubrum* strains ($n = 54$) from various sources were as follows, in increasing order: luliconazole, 0.0004 μg/ml; lanoconazole, 0.002 μg/ml; efinaconazole, 0.005 μg/ml; terbinafine 0.09 μg/ml; itraconazole, 0.077 μg/ml; and fluconazole, 15.19 μg/ml. The GM MICs of *T. interdigitale* isolates ($n = 66$) were as follows: luliconazole, 0.0006 μg/ml; lanoconazole, 0.002 μg/ml; efinaconazole, 0.008 μg/ml; terbinafine 0.013 μg/ml; itraconazole, 0.111 μg/ml; and fluconazole, 11.07 μg/ml. Efinaconazole exhibited potent activity against *T. rubrum* and *T. interdigitale* strains, with MIC_{90}s of 0.008 μg/ml and 0.016 μg/ml, respectively. Although efinaconazole had potent activity against the dermatophyte isolates collected from clinical hosts, GM MIC values of efinaconazole against all clinical isolates of dermatophytes were 2 $\log_2$ and >6 $\log_2$ dilution steps lower than those of lanoconazole and luliconazole, respectively. However, no statistically significant (*P* value, >0.05) differences in the lanoconazole, luliconazole, and efinaconazole susceptibility patterns were detected between strains.

Although there are few reports of *in vitro* drug resistance in the genera of *Trichophyton*, *Microsporum*, and *Epidermophyton*, treatment of infections is difficult, because frequent relapses and failures are observed. Interestingly, in our investigation, efinaconazole demonstrated potent antifungal activity against *T. rubrum* and *T. interdigitale*, with a narrow range of MICs. The efinaconazole MIC ranges, MIC_{50}s, and MIC_{90}s obtained in this study for *T. interdigitale* (0.002 to 0.06 μg/ml, 0.008 μg/ml, and 0.015 μg/ml, respectively) and *T. rubrum* (0.002 to 0.06 μg/ml, 0.004 μg/ml, and 0.008 μg/ml, respectively) were approximately similar to those reported for North American (United States and Canada) and Japanese clinical isolates of *T. interdigitale* ($\leq$0.002 to 0.06 μg/ml, 0.004 μg/ml, and 0.015 μg/ml, respectively) and *T. rubrum* ($\leq$0.002 to 0.03 μg/ml, 0.002 μg/ml, and 0.008 μg/ml, respectively) (15, 16). These data demonstrate that there are no significant geographical differences between susceptibility profiles of efinaconazole for Iranian, Japanese, and North American *Trichophyton* isolates, and, therefore, that we can extrapolate the results for dermatophyte populations from other parts of the world (15, 16). Based on the MIC_{50} and MIC_{90} values, efinaconazole had activities comparable to terbinafine (1- to 2-fold) and higher than itraconazole (16- to 21-fold), two currently preferred antifungals used for treatment of dermatophytoses and onychomycosis. Similarly, based on geometric mean MIC values, efinaconazole was more effective than terbinafine and itraconazole against our *T. interdigitale* and *T. rubrum* isolates. Nonetheless, the efinaconazole MIC ranges, MIC_{50}s, and MIC_{90}s obtained in our study were significantly lower than those for *T. rubrum* and *T. interdigitale* clinical isolates (0.0156 to 0.5 μg/ml, 0.0625 μg/ml, and 0.125 μg/ml, respectively, for *T. rubrum*; 0.0625 to 0.5 μg/ml, 0.25 μg/ml, and 0.25 μg/ml, respectively, for *T. interdigitale*) reported by Tatsumi et al. (21). Such high reported MIC values can be attributed to differences in methodology. Tatsumi et al. used Sabouraud dextrose broth (pH 5.6) and a took the MIC reading at 7 days (21). In contrast, we defined the MIC according to the CLSI M38-A2 document, used RPMI 1640 medium (pH 7.0), and took an endpoint reading at 4 days (96 h). There is only one study regarding the comparison of *in vitro* activities of efinaconazole and luliconazole against *T. mentagrophytes* and *T.*

TABLE 2 *In vitro* susceptibilities of 120 clinical isolates of *T. interdigitale* and *T. rubrum*

Strain or antifungal drug	MIC range (μg/ml)	MIC_{50}/MIC_{90} (μg/ml)	Geometric mean (μg/ml)	MICs (μg/ml)[a]																
				64	32	16	8	4	0.5	0.25	0.125	0.06	0.03	0.015	0.008	0.004	0.002	0.001	0.0005	0.0002	0.0001
All strains (n = 120)																					
Efinaconazole	0.002–0.06	0.008/0.016	0.007									4	2	11	**61**	36	6				
Lanoconazole	0.001–0.008	0.002/0.004	0.002												6	21	**82**	11			
Luliconazole	0.0005–0.004	0.0005/0.001	0.0005													2	4	22	**80**	10	2
Fluconazole	8–64	16/32	12.77	5	8	52	**53**	2													
Itraconazole	0.03–0.5	0.125/0.25	0.095						3	10	**56**	43	8								
Terbinafine	0.004–0.125	0.015/0.0165	0.011								1	3	8	50	**53**	5					
T. interdigitale (n = 66)																					
Efinaconazole	0.002–0.006	0.008/0.016	0.008									3	1	9	**41**	10	2				
Lanoconazole	0.001–0.008	0.002/0.004	0.002												4	10	**48**	4			
Luliconazole	0.0002–0.004	0.0005/0.001	0.0006													2	2	16	**41**	5	
Fluconazole	4–64	8/16	11.07	2	3	21	**38**	2													
Itraconazole	0.03–0.5	0.12/0.25	0.111						3	7	**38**	15	3								
Terbinafine	0.004–0.12	0.015/0.03	0.013								1	2	6	**35**	20	2					
T. rubrum (n = 54)																					
Efinaconazole	0.002–0.06	0.004/0.008	0.005									1	1	2	20	**26**	4				
Lanoconazole	0.002–0.008	0.002/0.004	0.002												2	11	**34**	7			
Luliconazole	0.0005–0.002	0.0005/0.001	0.0004														2	6	**39**	5	2
Fluconazole	8–64	16/32	15.19	3	5	**31**	15														
Itraconazole	0.03–0.25	0.06/0.125	0.077							3	18	**28**	5								
Terbinafine	0.004–0.06	0.008/0.016	0.009									1	2	15	**33**	3					

[a]Numbers in boldface are modal values.

rubrum (16). In that study, the MIC values of luliconazole ranged from 0.0001 to 0.002 μg/ml, which was not notably different from the luliconazole MICs for our isolates (0.0005 to 0.002 μg/ml). The luliconazole MIC ranges, MIC_{50}s, and MIC_{90}s obtained for all *Trichophyton* isolates in the current investigation were 5- to 15-fold, 16-fold, and 11-fold higher, respectively, than those for efinaconazole. The MIC values for lanoconazole were 2- to 8-fold, 4-fold, and 3-fold higher, respectively, than efinaconazole. Against our isolates, two mentioned imidazoles were also significantly more potent than terbinafine, itraconazole, and fluconazole.

Overall, luliconazole and lanoconazole showed more potent *in vitro* effects than other antifungals, including terbinafine and efinaconazole, against the tested *T. interdigitale* and *T. rubrum* isolates. However, there are reports that terbinafine tends to have superior clinical efficacy compared to luliconazole and lanoconazole in the *in vivo* model of dermatophytosis, likely due to the fungicidal effect of terbinafine compared with fungistatic activities of the two imidazole agents (17, 22). Likewise, fungicidal activity was reported for efinaconazole against *T. interdigitale* and *T. rubrum* in the *in vitro* and *in vivo* models of onychomycosis (23, 24). Against terbinafine resistance, which was reported in association with *Trichophyton* clinical isolates (25, 26), efinaconazole has low potential to induce drug resistance in dermatophytes (26). On the other hand, the fungicidal activity of efinaconazole and its low affinity for binding to keratin (17, 22) compared to that of five comparator antifungals, highlights that efinaconazole may be the most promising option for the treatment of onychomycosis. In conclusion, potent topical antifungals with extensive activity may be beneficial in treatment of all dermatomycosis. This study supports efinaconazole having potent *in vitro* antifungal activity against *T. interdigitale* and *T. rubrum*, which is at least comparable to and may be more potent than that of current topical and oral medications used for treatment of dermatophytosis, especially onychomycosis.

Accession number(s). The nucleotide sequences of the ITS rDNA for the determined clinical isolates have been deposited in GenBank under the accession numbers MG980329 to MG980394.

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