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SHORT COMMUNICATION

Antifungal Activity of a Novel Triazole, Efinaconazole and Nine Comparators against 354 Molecularly Identified *Aspergillus* Isolates

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Abstract Management of superficial aspergillosis is a major challenge owing to the frequent relapses and treatment failure, which may pose a potential risk, thereby gradually developing resistant species. Therefore, necessitating the development of new antifungals with higher potency should be considered as alternative strategies for efficient management of infections. We aimed to investigate the susceptibility of *Aspergillus* isolates toward a novel triazole, efinaconazole, in comparison with various classes of antifungal drugs. Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute M38-A2 guidelines. Efinaconazole exhibited poor activity against mutant *A. fumigatus* strains, *A. niger* sensu stricto, and *A. tubingensis* with GM MIC values of 3.62, 1.62, and 2 μg/ml, respectively; however, surprisingly, it efficiently inhibited the growth of *A. terreus* sensu stricto,

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followed by wild-type *A. fumigatus* and *A. flavus* with GM MIC values of 0.29, 0.42, and 0.52 µg/ml, respectively. Presumably, efinaconazole is inefficient in aspergillosis treatment due to the low susceptibility of *A. niger* sensu stricto*, A. tubingensis,* and mutant *A. fumigatus*; however, it may be effective in treating superficial aspergillosis caused by wild-type *A. fumigatus, A. terreus* sensu stricto, and *A. flavus*. Further studies are needed to determine how these findings may translate into in vivo efficacy.

Keywords *Aspergillus* species · Susceptibility profiles · Efinaconazole

Introduction

Aspergillus is a saprophytic mold commonly found in soil, water, food, and air, and particularly in decaying vegetables [1]. The spectrum of clinical manifestations associated with aspergillosis is diverse, ranging from mild allergic reactions, colonization, and cutaneous and superficial infection, to severe invasive aspergillosis [2–4]. Onychomycosis is predominantly caused by dermatophytes, but superficial infections, otitis, keratitis, and dermatomycosis are common disorders caused by *Aspergillus* species and generally result from traumatic inoculation in otherwise healthy individuals [5]. Although hyalohyphomycetes (e.g., *Fusarium* spp., *Scopulariopsis* spp., and *Acremonium* spp.) and dematiaceous molds (e.g., *Alternaria* spp., *Curvularia* spp.) cause onychomycoses, *Aspergillus* species has been increasingly reported as the primary causative agent of onychomycosis [5]. Remarkably, the global burden of onychomycosis due to *Aspergillus* species is approximately 10 million cases with a prevalence of 34.4% in Guatemala, 69.3% in Iran, and more than 71% in Sri Lanka [5]. In addition, otomycosis is an external auditory canal mycotic infection, which is prevalent in the tropical and subtropical regions, and is characterized by itching, tinnitus, inflammation, discharge, pruritus, scaling, and severe discomfort [6]. The majority of causal pathogens belong to *Aspergillus* species, predominantly *A. niger* complex, *A. fumigatus,* and *A. flavus;* however, cryptic species with low susceptibility to antifungal drugs has been reported [7]. A recent review in Iran revealed that 78.59% of otomycosis was caused by *Aspergillus* species, mainly due to *A. niger* complex (65.1%; mostly, *A. niger senso stricto, A. tubingensis, A. uvarum*), followed by *A. flavus* (21.7%) and *A. fumigatus* (9.3%) [7]. In addition, Hagiwara et al. reported that *A. niger* sensu lato is the most common species, followed by *A. terreus* sensu lato, in Japan [8]. Antifungal therapy with itraconazole and terbinafine has been used against primary superficial aspergillosis and is effective against onychomycosis and otomycosis caused by *Aspergillus*; however, complete elimination of these organisms is challenging owing to the frequent relapses and treatment failure, which can act as a potential risk factor leading to the gradual development of resistant species [9–13]. Moreover, the aforementioned drugs need to be administered twice daily for more than 6 months, and numerous side effects are frequently observed [9–13]. Furthermore, the use of other topical drugs for treating onychomycosis is not recommended as they are inferior to the systemic azoles due to their poor permeation [9–13]. Consequently, alternative antifungal strategies with higher potency should be considered to effectively manage *Aspergillus* infections. Recently, luliconazole (Luzu) and lanoconazole (Astat) have been developed and approved for the treatment of superficial, cutaneous, and nail mycotic infections. In addition, efinaconazole is currently being marketed as a 10% daily topical solution (Jublia in Canada and Clenafin in Japan) and was approved for the treatment of dermatophytosis and onychomycosis [8, 14–18] by inhibiting sterol 14-alpha demethylase and blocking fungal membrane ergosterol biosynthesis. The pharmacokinetic and pharmacodynamic properties of these drugs are more favorable than those of the other agents used for treating dermatophytosis and onychomycosis, as they can efficiently penetrate into human nails and exhibit a potent antifungal activity in the nail plate due to their lower keratin affinity [14–18]. Furthermore, previous studies reported potent activity of lanoconazole and luliconazole against medically important fungi, i.e., dematiaceous and relatives, *Candida* spp., *Malassezia* spp., dermatophytes and *Aspergillus* spp. [19–23, 25]. In contrast, only limited data are available regarding the efficacy of efinaconazole against *Aspergillus* isolates. Thus, the present study aimed to comprehensively evaluate the in vitro activity of efinaconazole in comparison with nine antifungal drugs against a huge consortium of *Aspergillus* isolates obtained from different clinical and environmental sources.

Materials and Methods

Three hundred fifty-four well-characterized *Aspergillus* isolates from different species were obtained from the reference culture collections of the Invasive Fungi Research Center (IFRC), Sari, Iran. The collection comprised clinical isolates (n = 218) from a variety of specimens mostly from nail lesions, otitis, cutaneous lesions, bronchoalveolar lavage (BAL), and sinus discharge, in addition to the environmental isolates (n = 136). All isolates were initially screened by macro- and microscopic features and were subsequently identified to the species level by DNA sequencing of the ß-tubulin gene using primers Bt2a and Bt2b, as previously described [24, 25]. Antifungal susceptibility testing was performed using 96-well microtiter plates, according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 guidelines [26]. The antifungal agents were prepared at final concentrations ranging from 0.016 to 16 µg/ml for amphotericin B (Bristol-Myers Squibb, Woerden, The Netherlands), itraconazole (Janssen Research Foundation, Beerse, Belgium), voriconazole (Pfizer, Sandwich, UK), posaconazole (Schering-Plough, Kenilworth, USA), and efinaconazole (Nihon Nohyaku Co., Osaka, Japan); from 0.008 to 8 µg/ml for caspofungin (Merck Sharp and Dohme BV, Haarlem, The Netherlands), anidulafungin (Pfizer), and micafungin (Astellas, Toyama, Japan); and from 0.001 to 1 µg/ml for luliconazole and lanoconazole (Nihon Nohyaku Co., Osaka, Japan). Minimum inhibitory concentrations (MICs) were evaluated visually as the lowest concentrations that completely inhibited the growth, and minimum effective concentrations (MECs) of echinocandins were assessed microscopically as the lowest concentration of drug presenting the growth of compact hyphae compared to the filamentous hyphae observed in the growth control wells after 48 h of incubation at 35 °C in dark. Nevertheless, the microdilution plates were incubated at 30 °C for black aspergilli (*A. niger* complex), as previously described [22]. *Candida parapsilosis* (ATCC 22019), *Pichia kudriavzevii* (*C. krusei*) (ATCC 6258), and *Aspergillus flavus (*ATCC 204304) were used as quality controls and tested with every new batch of MIC plates [26]. All tests were performed in duplicate. Data were recorded using Microsoft Excel 2007 (Microsoft Corp) and analyzed using SPSS software. P value < 0.05 was considered as statistically significant.

Results

Based on the conventional and molecular characterization, 354 *Aspergillus* isolates were identified and characterized as azole-susceptible *A. fumigatus* (74 clinical and 46 environmental), azole-resistant *A. fumigatus* (2 clinical and 19 environmental), *A. flavus* (54 clinical and 12 environmental), *A. terreus* sensu stricto (51 clinical and 52 environmental), *A. niger* sensu stricto (15 clinical and 8 environmental), and *A. tubingensis* (16 clinical and 5 environmental). As per published epidemiological cutoff values established using the CLSI M38-A2 broth microdilution method, 136 *A. fumigatus* (74 clinical and 46 environmental) were defined as wild type (azole susceptible) and 21 *A. fumigatus* with various single-nucleoid polymorphisms were characterized as non-wild type (azole resistant). The majority of the azole-resistant *A. fumigatus* strains (n = 10) harbored TR34/L98H, whereas three isolates harbored TR46/Y121F/T289 and eight strains had other point mutations (e.g., G138C, G432C, F46Y, G89G, G54, and M220) in the *cyp51A* gene. Tables 1, 2 summarizes the MIC range, MIC mode, geometric mean (GM) MIC, MIC_{50}, and MIC_{90} of 354 clinical and environmental isolates of *Aspergillus* to efinaconazole and nine common comparator antifungal agents. Interestingly, efinaconazole exhibited poor activity against azole-resistant *A. fumigatus* strains carrying point mutations, *A. niger* sensu stricto, and *A. tubingensis,* with a GM MIC of 3.62, 1.62, and 2 µg/ml, respectively; however, it showed potent activity against *A. terreus* sensu stricto, azole-susceptible *A. fumigatus*, and *A. flavus* with a GM MIC of 0.29, 0.42, and 0.52 µg/ml, respectively. Notably, the widest MIC ranges were observed for efinaconazole against azole-resistant *A. fumigatus, A. niger* sensu stricto, and *A. tubingensis* (0.25–16, 0.5–4, and 0.5–16 µg/ml, respectively). Remarkably, however, efinaconazole showing much greater potency than itraconazole shows that the in vitro effect is similar to voriconazole against azole-resistant *A. fumigatus* (Tables 1, 2). The results indicate that, in terms of MIC_{90}, the activity of efinaconazole against black aspergilli (*A. niger* sensu stricto and *A. tubingensis*) and mutant *A. fumigatus* isolates was > 8 $\log_2$

Table 1 In vitro antifungal susceptibilities of 354 *Aspergillus* isolates to 10 antifungal agents

Aspergillus species (*n*)	Antifungal agents	MIC parameter (μg/ml)				
		Range	MIC_{50}	MIC_{90}	GM	Mode
Azole-susceptible *A. fumigatus* (*n* = 120)	Amphotericin B	0.125–4	0.5	1	0.533	0.5
	Itraconazole	0.016–4	0.5	1	0.325	0.5
	Voriconazole	0.063–2	0.125	0.5	0.169	0.125
	Posaconazole	0.004–0.125	0.016	0.063	0.022	0.008
	Efinaconazole	0.25–4	0.5	0.5	0.425	0.5
	Lanoconazole	0.001–0.016	0.001	0.004	0.001	0.001
	Luliconazole	0.001–0.004	0.001	0.001	0.001	0.001
	Caspofungin	0.008–0.5	0.031	0.125	0.046	0.031
	Anidulafungin	0.008–0.25	0.016	0.063	0.022	0.008
	Micafungin	0.008–0.25	0.031	0.125	0.028	0.031
Azole-resistant *A. fumigatus* (*n* = 21)	Amphotericin B	0.125–2	0.5	2	0.57	0.5
	Itraconazole	8–> 16	16	16	15	16
	Voriconazole	0.125–> 16	4	16	3.17	16
	Posaconazole	0.016–8	2	8	1.88	8
	Efinaconazole	0.25–16	8	16	3.62	16
	Lanoconazole	0.001–0.5	0.016	0.063	0.01	0.016
	Luliconazole	0.001–0.016	0.002	0.008	0.00	0.002
	Caspofungin	0.008–0.25	0.031	0.25	0.05	0.031
	Anidulafungin	0.008–0.125	0.016	0.125	0.02	0.016
	Micafungin	0.008–0.125	0.031	0.125	0.04	0.031
A. flavus (*n* = 66)	Amphotericin B	0.125–8	1	1	0.872	1
	Itraconazole	0.125–2	0.5	0.5	0.405	0.5
	Voriconazole	0.063–1	0.25	0.5	0.325	0.25
	Posaconazole	0.016–0.25	0.125	0.25	0.125	0.125
	Efinaconazole	0.125–2	0.5	1	0.521	0.5
	Lanoconazole	0.001–0.008	0.001	0.001	0.001	0.001
	Luliconazole	0.001–0.031	0.002	0.031	0.004	0.002
	Caspofungin	0.008–0.031	0.016	0.016	0.012	0.016
	Anidulafungin	0.008–0.25	0.016	0.004	0.018	0.016
	Micafungin	0.008–0.016	0.008	0.008	0.008	0.008
A. terreus sensu stricto (*n* = 103)	Amphotericin B	0.063–4	1	2	1.02	2
	Itraconazole	0.016–2	0.125	0.25	0.138	0.125
	Voriconazole	0.063–4	0.5	1	0.39	0.5
	Posaconazole	0.016–0.125	0.016	0.031	0.019	0.016
	Efinaconazole	0.031–1	0.25	0.5	0.296	0.5
	Lanoconazole	0.001–0.031	0.001	0.008	0.002	0.001
	Luliconazole	0.001–0.031	0.001	0.016	0.003	0.001
	Caspofungin	0.004–0.031	0.008	0.008	0.008	0.008
	Anidulafungin	0.008	0.008	0.008	0.008	0.008
	Micafungin	0.008	0.008	0.008	0.008	0.008
A. niger (*n* = 23)	Amphotericin B	0.125–2	1	2	0.97	1
	Itraconazole	0.25–16	0.5	1	0.599	0.5
	Voriconazole	0.125–0.5	0.25	0.5	0.258	0.25

Table 1 continued

Aspergillus species (*n*)	Antifungal agents	MIC parameter (μg/ml)				
		Range	MIC_{50}	MIC_{90}	GM	Mode
	Posaconazole	0.016–0.25	0.063	0.125	0.082	0.125
	Efinaconazole	0.5–4	2	2	1.62	2
	Lanoconazole	0.008–0.063	0.008	0.031	0.013	0.008
	Luliconazole	0.001–0.008	0.001	0.001	0.001	0.001
	Caspofungin	0.001–0.031	0.001	0.016	0.002	0.001
	Anidulafungin	0.008–0.016	0.016	0.016	0.015	0.016
	Micafungin	0.004–0.031	0.008	0.008	0.008	0.008
A. tubingensis (*n* = 21)	Amphotericin B	0.25–2	1	2	0.768	1
	Itraconazole	0.25–16	0.5	1	0.63	0.5
	Voriconazole	0.063–1	0.5	1	0.424	0.5
	Posaconazole	0.016–0.25	0.063	0.125	0.069	0.063
	Efinaconazole	0.5–16	2	4	2	2
	Lanoconazole	0.001	0.001	0.001	0.001	0.001
	Luliconazole	0.001–0.031	0.001	0.016	0.002	0.001
	Caspofungin	0.008–0.031	0.008	0.016	0.009	0.008
	Anidulafungin	0.016–0.031	0.016	0.016	0.017	0.016
	Micafungin	0.008–0.016	0.008	0.016	0.009	0.008

MIC_{50}: concentration at which 50% of the isolates were inhibited, MIC_{90}: concentration at which 90% of the isolates were inhibited, MEC: minimum effective concentrations

GM Geometric mean

dilution step higher than that of imidazole. Noteworthy, lanoconazole and luliconazole revealed potent activity against all tested *Aspergillus* isolates with MIC_{90} values of 0.004 and 0.001 μg/ml for azole-susceptible *A. fumigatus*, 0.063 and 0.008 μg/ml for azole-resistant *A. fumigatus*, 0.001 and 0.031 μg/ml for *A. flavus*, 0.008 and 0.016 μg/ml for *A. terreus* sensu stricto, 0.031 and 0.001 μg/ml for *A. niger* sensu stricto, and 0.001 and 0.016 μg/ml for *A. tubingensis*, respectively. The results suggest that these drugs were more efficient than other azoles. Nevertheless, the MIC_{90} of efinaconazole was higher than that of lanoconazole for all tested isolates. No significant difference was observed regarding the activity of clinical versus environmental isolates ($P > 0.05$).

Discussion

In the present study, we investigated the in vitro susceptibility of 354 molecularly well-characterized *Aspergillus* isolates that originated from different sources to efinaconazole, a novel triazole in comparison with other antifungal drugs, and it was found that efinaconazole was a potent inhibitor of wild-type *A. fumigatus, A. terreus* sensu stricto, and *A. flavus* isolates; however, less activity was observed against itraconazole-resistant *A. fumigatus*, *A. niger* sensu stricto, and *A. tubingensis*. Our data showed that the MICs of efinaconazole for wild-type *A. fumigatus*, *A. flavus,* and *A. terreus* sensu stricto were approximately similar to those of itraconazole and voriconazole. Recently, triazole-resistant fungal species have emerged worldwide, which adversely impact the *Aspergillus* infection treatment [1]. Although itraconazole and terbinafine are the drugs of choice for treating superficial onychomycosis, the results are not promising and frequent relapses and treatment failure are a huge concern, mainly due to poor permeation of the drug or drug resistance [9–13]. Thus, novel therapeutic strategies are necessary for increasing the efficacy and reducing the side effect of antifungal drugs. In the last decade, the novel antifungal agent efinaconazole was introduced in the market for treating superficial infections [18, 20]. The drug displays a broad spectrum of in vitro activity against

Table 2 In vitro antifungal susceptibilities of 354 *Aspergillus* isolates to 10 antifungal agents

Aspergillus species (*n*)	Antifungal agents	MIC/MEC (μg/ml)														
		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
Azole-susceptible *A. fumigatus* (*n* = 120)	Amphotericin B								9	18	**57**	27	7	2		
	Itraconazole					1		11	17	21	**56**	11	2	1		
	Voriconazole							30	**43**	19	22	5	1			
	Posaconazole			1	**51**	14	4	43	7							
	Efinaconazole									41	**69**	8	1	1		
	Lanoconazole	**81**	18	12	8	1										
	Luliconazole	**118**	1	1												
	Caspofungin				15	9	**49**	4	33	5	5					
	Anidulafungin				**40**	35	3	34	7	1						
	Micafungin				14	23	**67**	1	14	1						
Azole-resistant *A. fumigatus* (*n* = 21)	Amphotericin B								1	2	**13**	2	3			
	Itraconazole														2	**19**
	Voriconazole								2	2		2	1	4	4	**6**
	Posaconazole					2			1			4	4		**10**	
	Efinaconazole									3	4			1	5	**8**
	Lanoconazole	2	1	1	3	**11**	1			1	1					
	Luliconazole	6	**7**	4	2	2										
	Caspofungin				1		**14**		2	4						
	Anidulafungin				6	**12**		1	2							
	Micafungin				2	2	**11**	2	4							
A. flavus (*n* = 66)	Amphotericin B								1	5	11	**44**	1	2	2	
	Itraconazole								3	25	**32**	1	5			
	Voriconazole							1	6	**29**	27	3				
	Posaconazole					2		1	**56**	7						
	Efinaconazole								4	15	**24**	19	4			
	Lanoconazole	**63**		1	2											
	Luliconazole	14	**26**	4	10	4	8									
	Caspofungin				26	**38**	2									
	Anidulafungin				5	**54**	3	2	1	1						
	Micafungin				64	2										
A. terreus sensu stricto (*n* = 103)	Amphotericin B							1	2	11	16	25	**45**	3		
	Itraconazole					4	5	12	**47**	26	6	1	2			
	Voriconazole							1	8	40	**40**	8	4	2		
	Posaconazole					**87**	7	8	1							
	Efinaconazole						1	6	13	34	**45**	4				
	Lanoconazole	**77**	5	8	11	1	1									
	Luliconazole	**56**		2	16	19	10									
	Caspofungin			2	**95**	4	2									
	Anidulafungin				**103**											
	Micafungin				**103**											
A. niger (*n* = 23)	Amphotericin B								1		6	**8**	8			
	Itraconazole									8	**9**	4				2
	Voriconazole								5	**12**	6					
	Posaconazole					1	1	10	**10**	1						
	Efinaconazole										1	7	**13**	2		

Table 2 continued

Aspergillus species (*n*)	Antifungal agents	MIC/MEC (μg/ml)														
		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
	Lanoconazole				**13**	6	3	1								
	Luliconazole	**21**		1	1											
	Caspofungin	**16**			4	2	1									
	Anidulafungin				2	**21**										
	Micafungin			1	**20**	1	1									
A. tubingensis (n = 21)	Amphotericin B									3	6	**8**	4			
	Itraconazole									5	**8**	7				1
	Voriconazole							1	1	5	**9**	5				
	Posaconazole					1	1	**10**	5	2						
	Efinaconazole										1	4	**12**	2		1
	Lanoconazole	**21**														
	Luliconazole	**18**				2	1									
	Caspofungin				**17**	3	1									
	Anidulafungin					**20**	1									
	Micafungin				**17**	4										

MIC_{50}: concentration at which 50% of the isolates were inhibited, MIC_{90}: concentration at which 90% of the isolates were inhibited, MEC: minimum effective concentrations

GM Geometric mean, mode in boldface

dermatophytes, non-dermatophyte molds, and yeasts, thus presenting a more potent activity than the presently marketed antifungal agents [27–29]. Previously, several studies have demonstrated potent in vitro activity of luliconazole, lanoconazole, and efinaconazole against filamentous fungi and dermatophytes compared to the other drugs, whereas in vivo studies revealed that terbinafine has a potent and superior activity compared to luliconazole and lanoconazole against dermatophytosis and onychomycosis due to its fungicidal and fungistatic activities, respectively [29–32]. Noteworthily, information regarding the in vitro activity of efinaconazole, a novel triazole, against *Aspergillus* species is still limited. Azole-based drugs such as efinaconazole, lanoconazole, and luliconazole presented low MICs against the *Aspergillus* species causing otomycosis [8]. Efinaconazole exhibited a low MIC against almost all strains of dermatophytes and *C. albicans,* thus demonstrating high efficacy in treating superficial fungal infections [29]. Moreover, our previous investigation revealed that the GM MICs were the lowest for luliconazole, followed by lanoconazole and efinaconazole against a comprehensive collection of dermatophytic clinical isolates [30]. Additionally, the in vitro activity of luliconazole and lanoconazole against *Fusarium* clinical isolates demonstrated geometric mean MIC values of 0.005 and 0.013 μg/ml, respectively, compared with 0.85 μg/ml for efinaconazole [30]. Furthermore, luliconazole and lanoconazole presented the lowest geometric mean MICs, followed by efinaconazole, against the melanized fungi and their relatives compared to other drugs [23]. Presumably, efinaconazole is not effective in treating aspergillosis owing to the low susceptibility of *A. niger* sensu stricto*, A. tubingensis,* and non-wild-type *A. fumigatus;* however, it may serve as the drug of choice for other *Aspergillus* species. Therefore, further studies are warranted to determine the clinical implications of these findings.

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Compliance with Ethical Standards

Conflict of interest No potential conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Ethical Approval Ethical permission for this study was approved by the Ethical Committee of Mazandaran University of Medical Sciences, Sari, Iran (nr. IR.MAZUMS.REC.1397.3211).

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