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Activity of Isavuconazole and Comparator Mould-Active Triazoles Tested against Contemporary Invasive Mould Isolates

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Amended Abstract

Background: The mould-active triazoles (MAT) are important agents for the treatment of invasive infections due to *Aspergillus* spp. and other selected rare moulds. We evaluated the *in vitro* activity of isavuconazole, a new broad-spectrum MAT, and comparators against 639 invasive mould isolates collected worldwide during 2014-2015 using CLSI broth microdilution methods.

Methods: 594 *Aspergillus* spp. (4 species/species complex [SC]), 22 *Fusarium solani* SC, 10 *Rhizopus microsporus* group and 13 *Scedosporium apiospermum* SC were susceptibility tested against isavuconazole, itraconazole, posaconazole, and voriconazole. Species identification was performed by MALDI-TOF and/or DNA sequencing methods. Epidemiological cutoff values (ECVs) were applied to distinguish wild-type (WT) versus non-WT strains where appropriate.

Results: The activity of isavuconazole versus *Aspergillus* spp. was similar to that of the other MATs. All *A. terreus*, 98.1% of *A. flavus,* and 98.4% of *A. fumigatus* were inhibited by ≤1 µg/ml of isavuconazole and were WT (MIC≤ECV) to the other three MATs. Similarly, 100% of *A. niger* SC were WT to isavuconazole, itraconazole, and voriconazole. The MATs had limited *in vitro* activity ($MIC_{50/90}$, ≥8 µg/ml) against *F. solani* SC. Although voriconazole was not active against *R. microsporus* group, 90% were inhibited by ≤4 µg/ml of isavuconazole and itraconazole and 100% were inhibited by ≤2 µg/ml of posaconazole. Isolates of *S. apiospermum* SC were most susceptible to voriconazole followed by posaconazole>itraconazole>isavuconazole.

Conclusions: *In vitro*, isavuconazole was as active as the other MATs against *Aspergillus* spp. and showed moderate activity against *R. microsporus* group and *S. apiospermum* SC. None of the MATs showed *in vitro* activity against *F. solani* SC. Considering its *in vitro* activity and data from recently published phase 3 studies, isavuconazole represents a new therapeutic option for the treatment of invasive infections due to a variety of clinically important filamentous fungi.

Organism (no. tested)	$MIC_{50/90}$ (µg/ml)			
	Isavuconazole	Itraconazole	Posaconazole	Voriconazole
A. fumigatus (495)	0.5/1	0.5/1	0.25/0.5	0.5/0.5
A. flavus (54)	1/1	0.5/0.5	0.25/0.5	1/1
A. niger (24)	1/2	1/2	0.5/0.5	0.5/1
A. terreus (21)	0.5/1	0.5/0.5	0.25/0.5	0.5/0.5
F. solani SC (22)	>8/>8	>8/>8	>8/>8	8/8
R. microsporus (10)	2/4	1/2	0.5/2	8/>8
S. apiospermum/boydii (13)	2/8	1/4	1/2	0.5/1

Introduction

The systemically-active antifungal armamentarium currently includes the polyenes, flucytosine, fluconazole, the extended-spectrum triazoles (itraconazole, posaconazole, and voriconazole), and the echinocandins. Despite the fact that in total these agents cover the vast majority of opportunistic fungal pathogens and are increasingly employed in either a prophylactic or preemptive treatment strategy, breakthrough invasive fungal infections continue to be reported and increasingly involve moulds that are relatively uncommon and tend to exhibit decreased susceptibility to the available antifungal agents.

Isavuconazole is new extended-spectrum triazole that has recently been approved by the FDA for the treatment of invasive aspergillosis and invasive mucormycosis. Studies to assess the clinical activity of isavuconazole against *Candida* and uncommon yeasts and moulds are ongoing. Isavuconazole may be administered orally or parenterally and exhibits broad antifungal activity against common and uncommon fungal pathogens including *Candida*, *Aspergillus*, non-*Candida* yeasts, and non-*Aspergillus* moulds.

In this study, we examined the activity of isavuconazole and comparator agents tested against 639 clinical mould isolates collected during 2014-2015 from sterile sites, respiratory tract infections (RTI) and bloodstream infections (BSI) as part as the SENTRY Antifungal Surveillance Program worldwide.

Methods

Organisms and sources. A total of 639 non-duplicate fungal strains were collected prospectively from 46 medical centers located in North America (21 sites), Europe (16), the Asia-Pacific region (7) and Latin America (2). These strains were recovered consecutively from patients with invasive fungal infections (IFI) during 2014-2015. Isolate identification was confirmed by MALDI-TOF MS or DNA sequencing-based methods using 28S for all isolates and one of the following: β-tubulin for *Aspergillus* spp., translation elongation factor (TEF) for *Fusarium* spp. or ITS for all other species of filamentous fungi when an acceptable identification was not achieved by MALDI-TOF MS. Nucleotide sequences were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin, USA) and compared to available sequences through the internet using BLAST (http://www.ncbi.nlmnih.gov/blast). TEF sequences were analyzed using the *Fusarium*-II database (http://www.isolate.fusariumdb.org/index.php) and the *Fusarium* multilocus sequence typing (MLST) database (http://www.chs.knaw.nl/fusarium/).

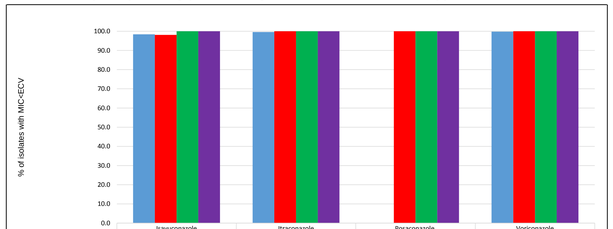
Susceptibility testing. All isolates were tested by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) methods outlined in document M38-A2. Frozen-form panels used RPMI 1640 broth supplemented with MOPS (morpholinepropane sulfonic acid) buffer and 0.2% glucose and inoculated with 0.4-5.0 X 10^4 cells/ml suspensions. MIC/MEC values were determined visually, after 24, 48, or 72 hours of incubation at 35ºC, as the lowest concentration of drug that resulted in ≥50% inhibition of growth relative to the growth control or complete (100%) inhibition. Epidemiological cutoff values (ECVs) recently published by CLSI (M59 document) were used to differentiate wild-type (WT, no acquired resistance mechanisms) from non-WT (may harbor acquired resistance mechanisms) isolates.

Antifungal agents. Antifungal agents included isavuconazole (range 8-0.008 µg/ml; 11 dilutions), itraconazole (range 8-0.008 µg/ml; 11 dilutions), posaconazole (range 8-0.008 µg/ml; 11 dilutions) and voriconazole (range 8-0.008 µg/ml; 11 dilutions). Quality control (QC) was performed as recommended in M38-A2 (2008) using the following strains: *Aspergillus flavus* ATCC 204304 and *Aspergillus fumigatus* MYA-3626.

Results

- Among the 639 fungal clinical isolates tested 495 (77.5%) were *Aspergillus fumigatus* and 45 (7.0%) were other moulds, including 22 isolates of *Fusarium solani* Species Complex (SC), 10 isolates of *Rhizopus microsporus* group, and 13 isolates of *Scedosporium apiospermum/S. boydii* (**Table 1**).
- Isavuconazole and itraconazole ($MIC_{50/90}$, 0.5/1 µg/ml for both compounds; **Table 1**) had similar activities against 495 *A. fumigatus* isolates that was slightly lower than those of posaconazole and voriconazole ($MIC_{50/90}$, 0.25/0.5 and 0.5/0.5 µg/ml, respectively).
- Isavuconazole MIC values ranged from 0.25 to >8 µg/ml for *A. fumigatus,* and 98.4% of these isolates were wild-type (WT) for this azole applying the current ECV interpretative criteria (**Figure 1**).
- Greater than 99% of isolates of *A. fumigatus* were WT for itraconazole (99.6%), posaconazole (99.4%), and voriconazole (99.8%). Cross resistance to all mould active triazoles was seen in a single isolate.
- Isavuconazole $MIC_{50/90}$ values were 1/1 µg/ml for *A. flavus* (**Table 1**). The activity of this newer azole was comparable to voriconazole ($MIC_{50/90}$, 1/1 µg/ml) and slightly lower when compared to itraconazole and posaconazole ($MIC_{90,}$ 0.5 µg/ml for both compounds).
- Isavuconazole $MIC_{50/90}$ values were 1/2 µg/ml for *Aspergillus niger* (**Table 1**). The activity was comparable to that of itraconazole (1/2 µg/ml) and two-to- eight-fold lower when compared to voriconazole (0.5/1 µg/ml) and posaconazole (0.5/0.5 µg/ml). All isolates were WT to the mould-active triazoles.
- Isavuconazole $MIC_{50/90}$ values were 0.5/1 µg/ml for *Aspergillus terreus* (**Table 1**) and the activity was slightly lower when compared to itraconazole (0.5/0.5 µg/ml), voriconazole (0.5/0.5 µg/ml), and posaconazole (0.25/0.5 µg/ml). All isolates were WT to the mould-active triazoles.
- None of the triazoles, including isavuconazole, appeared to be active *in vitro* against the 16 isolates of *Fusarium solani* SC (MIC_{90} ≥8 µg/ml; **Table 1**).
- Isavuconazole $MIC_{50/90}$ values were 2/8 µg/ml for *Scedosporium apiospermum/S. boydii* (**Table 1**) and the activity was two- to eight-fold lower when compared to itraconazole (1/4 µg/ml), voriconazole (0.5/1 µg/ml), and posaconazole (1/2 µg/ml), respectively.

Figure 1. Activity of mould-active azoles against main *Aspergillus* spp. tested. Epidemiological cutoff values (ECVs) recently published by CLSI M59 document were applied.



	Isavuconazole	Itraconazole	Posaconazole	Voriconazole
A. fumigatus (495)	98.4	99.6	—[a]	99.8
A. flavus species complex (54)	98.1	100.0	100.0	100.0
A. niger (24)	100.0	100.0	100.0	100.0
A. terreus (21)	100.0	100.0	100.0	100.0

[a] Posaconazole ECV interpretative criteria is not available in the M59 document.

Table 1. Antifungal activity of isavuconazole, itraconazole, posaconazole, and voriconazole tested against the organisms included in this study.

Organisms / Organism Groups	No. of isolates at MIC (µg/ml; cumulative %)											MIC_{50}	MIC_{90}
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8		
***Aspergillus fumigatus* (495)**													
Isavuconazole					28 (5.7)	329 (72.1)	130 (98.4)	7 (99.8)	0 (99.8)	0 (99.8)	1 (100.0)	0.5	1
Itraconazole				1 (0.2)	36 (7.5)	341 (76.4)	115 (99.6)	0 (99.6)	0 (99.6)	1 (99.8)	1 (100.0)	0.5	1
Posaconazole		3 (0.6)	3 (1.2)	55 (12.3)	334 (79.8)	97 (99.4)	3 (100.0)					0.25	0.5
Voriconazole				5 (1.0)	177 (36.8)	296 (96.6)	16 (99.8)	0 (99.8)	0 (99.8)	0 (99.8)	1 (100.0)	0.5	0.5
***Aspergillus flavus* species complex (54)**													
Isavuconazole						6 (11.1)	47 (98.1)	1 (100.0)				1	1
Itraconazole					14 (25.9)	37 (94.4)	3 (100.0)					0.5	0.5
Posaconazole				4 (7.4)	31 (64.8)	19 (100.0)						0.25	0.5
Voriconazole				4 (7.4)	1 (9.3)	14 (35.2)	35 (100.0)					1	1
***Aspergillus niger* (24)**													
Isavuconazole					2 (8.3)	1 (12.5)	10 (54.2)	11 (100.0)				1	2
Itraconazole					3 (12.5)	3 (25.0)	15 (87.5)	3 (100.0)				1	2
Posaconazole		1 (4.2)	1 (8.3)	3 (20.8)	17 (91.7)	2 (100.0)						0.5	0.5
Voriconazole				1 (4.2)	2 (12.5)	10 (54.2)	11 (100.0)					0.5	1
***Aspergillus terreus* (21)**													
Isavuconazole					5 (23.8)	12 (81.0)	4 (100.0)					0.5	1
Itraconazole				1 (4.8)	5 (28.6)	15 (100.0)						0.5	0.5
Posaconazole				3 (14.3)	15 (85.7)	3 (100.0)						0.25	0.5
Voriconazole				1 (4.8)	8 (42.9)	11 (95.2)	1 (100.0)					0.5	0.5
***Fusarium solani* species complex (22)**													
Isavuconazole											22 (100.0)	>8	>8
Itraconazole											22 (100.0)	>8	>8
Posaconazole											22 (100.0)	>8	>8
Voriconazole									8 (36.4)	13 (95.5)	1 (100.0)	8	8
***Rhizopus microsporus* (10)**													
Isavuconazole						1 (10.0)	0 (10.0)	6 (70.0)	2 (90.0)	0 (90.0)	1 (100.0)	2	4
Itraconazole						2 (20.0)	5 (70.0)	2 (90.0)	0 (90.0)	0 (90.0)	1 (100.0)	1	2
Posaconazole		1 (10.0)	0 (10.0)	1 (20.0)	3 (50.0)	3 (80.0)	2 (100.0)					0.5	2
Voriconazole						1 (10.0)	0 (10.0)	0 (10.0)	0 (10.0)	7 (80.0)	2 (100.0)	8	>8
***Scedosporium apiospermum/boydii* (13)**													
Isavuconazole					1 (7.7)	0 (7.7)	4 (38.5)	2 (53.8)	3 (76.9)	3 (100.0)		2	8
Itraconazole							7 (53.8)	2 (69.2)	4 (100.0)			1	4
Posaconazole						2 (15.4)	9 (84.6)	2 (100.0)				1	2
Voriconazole				2 (15.4)	0 (15.4)	6 (61.5)	5 (100.0)					0.5	1

Conclusions

- Isavuconazole has demonstrated activity comparable to itraconazole, posaconazole, and voriconazole when read at the same test/endpoint conditions.
- Isavuconazole also showed good coverage for most common contemporary and geographically diverse isolates of *Aspergillus* spp. tested as part of this study.

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