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Activity of Isavuconazole and Comparator Antifungals Tested against Contemporary (2012) Fungal Clinical Isolates Collected Worldwide

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AMENDED ABSTRACT

Background: Isavuconazole (ISA) is a new broad-spectrum triazole that is in late-stage clinical development for the treatment of invasive fungal infections (IFIs) caused by *Candida* spp. (CANS), *Aspergillus* spp. (ASP) and rare moulds (rMO). We report the activity of ISA and comparators tested against 1,670 fungal organisms collected in 26 countries during 2012.

Methods: 1,421 CANS, 130 ASP, 31 non-*Candida* yeasts, 50 *Cryptococcus neoformans* (CNEO), and 38 rMO clinical isolates causing IFI were consecutively collected and susceptibility (S) tested by CLSI reference broth microdilution in a central laboratory against ISA and comparators. Yeasts were identified (ID) using CHROMagar, biochemical methods and sequencing of ITS and/or 28S regions (IGS was used for a small subset). Moulds were ID by sequencing of 1 or 2 of the following genes: ITS, 28S, beta-tubulin, TEF.

Results: ISA displayed good activity against most prevalent and relevant fungal species/groups (**Table**). ISA inhibited 93.3 and 97.7% of the CANS at MICs of 0.5 and 1 µg/ml, respectively; and only 15 isolates had MIC values at >2 µg/ml (14 *C. glabrata* [1 echinocandin-non-S] and 1 *C. tropicalis*). These isolates also displayed elevated MICs for other azoles (MIC ranges, 32->128, 1->8 and 2-8 µg/ml for fluconazole, posaconazole and voriconazole, respectively). All CNEO from variants *grubii* (46 isolates) and *neoformans* (4 isolates) had ISA MIC values ≤0.25 µg/ml. These strains had fluconazole MICs ranging from 1-8 µg/ml. *A. niger* and *A. terreus* had ISA MIC values at 2-8 µg/ml, whereas *A. fumigatus* and *A. flavus* had lower MICs (1-4 µg/ml).

Conclusions: Although unusual, reports of increasing resistance among various fungal species to key antifungals and breakthrough infections highlight the challenges of IFI therapy. ISA has shown good activity against common pathogens associated to IFIs.

Organism/group	Range (µg/ml)	MIC_{50}	MIC_{90}
Candida spp.[a] (1,421)	≤0.008 - >8	0.015	0.5
C. albicans[a] (671)	≤0.008 - >8	0.015	0.03
C. glabrata[a] (291)	0.015 - 8	0.5	2
C. parapsilosis[a] (236)	≤0.008 - 0.25	0.03	0.12
C. tropicalis[a] (122)	≤0.008 - 4	0.06	0.12
C. neoformans[a] (50)	0.03 - 0.25	0.12	0.25
Aspergillus spp.[c] (130)	0.25 - 8	1	2
A. fumigatus[c] (90)	1 - 4	1	2

a. Results read at 50% inhibition after 24h incubation.
b. Results read at 50% inhibition after 72h incubation.
c. Results read at 100% inhibition after 48h incubation.

INTRODUCTION

Isavuconazole (formerly BAL4815) is a new triazole antifungal agent with broad-spectrum *in vitro* activity against clinically relevant fungi. This compound may be administered either orally or intravenously as a prodrug that is then converted by plasma esterases into the active component. Previous *in vitro* studies have demonstrated potent antifungal activity against both common and uncommon fungal pathogens including *Candida, Aspergillus,* non-*Candida* yeasts and non-*Aspergillus* moulds. Furthermore, isavuconazole significantly reduced the organism burden in kidneys of mice infected with *Candida tropicalis* and the organism burden in both kidneys and brains of neutropenic mice infected with *C. krusei*. It was as effective as voriconazole and much more effective than fluconazole in reducing the brain burden in mice infected with *C. krusei*. Presently, isavuconazole is in late-stage clinical development for the treatment of invasive candidiasis (IC), invasive aspergillosis (IA) and non-*Aspergillus* moulds.

We recently demonstrated that isavuconazole had acceptable broad-spectrum activity against a global collection of opportunistic fungi. In this study, we expand that knowledge examining the activity of isavuconazole and comparator agents using reference broth microdilution methods against 1,670 clinical fungal strains collected during 2012 from sterile body sites or respiratory tract in 70 hospitals located worldwide.

MATERIALS AND METHODS

Organisms. A total of 1,670 non-duplicate fungal strains were collected prospectively from 70 hospitals located in North America (n=29), Europe (n=24), Latin America (n=10) and the Asia-Pacific Region (n=7). These strains were recovered consecutively from patients with bloodstream (BSI; 1,094 strains), normally sterile body fluids, tissues, abscesses (162 strains), respiratory tract specimens (255 strains) and 189 were collected from non-specified sites. Identification of the organisms were confirmed at a central laboratory. Yeast isolates were subcultured on CHROMagar *Candida* (Becton Dickinson and Company, Sparks, Maryland, USA) to differentiate *C. albicans/dubliniensis, C. tropicalis* and *C. krusei.* Biochemical tests, such as growth at 45ºC (*C. albicans, C. dubliniensis*) and assimilation of trehalose *(C. glabrata*) were applied and identifications were considered definitive. Yeasts that were not identified by these methods were identified using sequencing-based methods for internal transcribed spacer (ITS) region and/or 28S ribosomal subunit or IGS1 for *Trichosporon* spp., according to protocols previously described. Moulds were cultured and identified by sequencing analysis of 28S (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. 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 documents M27-A3 and M38-A2. Frozen-form panels used RPMI 1640 broth supplemented with MOPS (morpholinepropane sulfonic acid) buffer and 0.2% glucose and inoculated with 0.5 to 2.5 X 10^3 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. Recently published CLSI clinical breakpoints were used for the five most common species of *Candida* (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* and *C. krusei*) for echinocandins, fluconazole and voriconazole. Epidemiological cutoff values (ECV) were applied when available.

Quality control (QC) was performed as recommended in M27-A3 (2008) using the following strains: *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, *Aspergillus flavus* ATCC 204304 and *A. fumigatus* MYA-3626 and results were in range as published in the CLSI guidelines.

RESULTS

- A total of 670 (99.9%) of the *C. albicans* isolates tested were inhibited by isavuconazole at ≤0.12 µg/ml (MIC_{90}, 0.03 µg/ml; **Tables 1** and **2**) and the potency of this azole was two-fold lower than the potency for voriconazole (MIC_{90}, 0.015 µg/ml; **Table 2**), and two-fold greater than those for itraconazole and posaconazole (MIC_{90}, 0.06 µg/ml for both; **Table 2**).
- Isavuconazole inhibited 69.9, 89.7 and 95.2% of the *C. glabrata* strains at ≤0.5, ≤1 and ≤2 µg/ml, respectively (**Table 1**). *C. glabrata* isolates with MIC results above the ECV ranged from 3.8% for posaconazole to 9.6% for voriconazole, and 7.9% of the isolates were considered fluconazole-resistant using species-specific breakpoints (**Table 2**).
- All *C. parapsilosis* isolates were inhibited by isavuconazole at ≤0.25 µg/ml (**Table 1**). Fluconazole and voriconazole non-wildtype (MIC>ECV) *C. parapsilosis* strains were observed among 6.8 and 2.1% of the isolates, respectively (**Table 2**).
- Isavuconazole activity tested against *C. tropicalis* was acceptable ($MIC_{50/90}$, 0.06/0.12 µg/ml) to other tested azoles (**Table 2**). A total of 4.1 and 2.5% of the *C. tropicalis* isolates were categorized as non-susceptible to fluconazole and voriconazole, respectively (**Table 2**). Three isolates had isavuconazole MIC values at >0.5 µg/ml. Two isolates displaying isavuconazole MIC values at 1 or 4 µg/ml were recovered in the same hospital from New York and both strains displayed voriconazole MIC values at 2 µg/ml. One strain from Belgium had an isavuconazole MIC value of 1 µg/ml.
- *C. krusei* isolates displayed isavuconazole MIC values ranging from 0.12 to 2 µg/ml, and 100.0% of *C. lusitaniae* and *C. dubliniensis* isolates were inhibited at ≤0.12 and ≤0.06 µg/ml of isavuconazole, respectively. Comparator agents were also active against these candidal species (**Tables 1** and **2**).
- The activity of isavuconazole tested against *C. neoformans* isolates was very good ($MIC_{50/90}$, 0.06/0.12 µg/ml; **Table 1**). All isolates were inhibited at 0.25 µg/ml of isavuconazole and the activity of this newer azole was similar to that of voriconazole ($MIC_{50/90}$, 0.06/0.12 µg/ml; **Table 2**). Other azoles were also active against *C. neoformans* with only 4.0% of the isolates having MIC values above established ECVs for this species versus posaconazole and voriconazole (**Table 2**).
- *A. fumigatus* isolates displayed isavuconazole MIC values ranging from 1 to 4 µg/ml and the activity of this newer azole ($MIC_{50/90}$, 1/2 µg/ml; **Tables 1** and **2**) was most similar to that of itraconazole and slightly lower than posaconazole and voriconazole ($MIC_{50/90}$, 0.25/0.5 µg/ml for both; **Table 2**).
- Isavuconazole MIC_{90} values were 2 µg/ml for *A. flavus* and 4 µg/ml for *A. niger* (**Tables 1** and **2**), but the activity of this newer azole was slightly lower when compared to other azoles for these species.

Table 1. MIC distributions for isavuconazole against most common fungal species collected in a worldwide surveillance program during 2012.

Organism species/groups (no. tested)	Number (cumulative %) of isolates inhibited at isavuconazole MIC (µg/ml):											
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
Candida albicans (671)	306 (45.6)	289 (88.7)	66 (98.5)	5 (99.3)	4 (99.9)	0 (99.9)	1 (100.0)					
Candida glabrata (291)	0 (0.0)	2 (0.7)	0 (0.7)	5 (2.4)	31 (13.1)	48 (29.6)	117 (69.8)	58 (89.7)	16 (95.2)	12 (99.3)	2 (100.0)	
Candida parapsilosis (236)	24 (10.2)	46 (29.7)	91 (68.2)	49 (89.0)	23 (98.7)	3 (100.0)						
Candida tropicalis (122)	2 (1.6)	18 (16.4)	40 (49.2)	33 (76.2)	22 (94.3)	2 (95.9)	2 (97.5)	2 (99.2)	0 (99.2)	1 (100.0)		
Candida krusei (32)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (12.5)	6 (31.3)	19 (90.6)	2 (96.9)	1 (100.0)			
Candida lusitaniae (24)	3 (12.5)	11 (25.0)	6 (70.8)	(95.8)	(100.0)							
Candida dubliniensis (13)	5 (38.5)	6 (84.6)	1 (92.3)	1 (100.0)								
Cryptococcus neoformans (50)	0 (0.0)	0 (0.0)	1 (2.0)	5 (12.0)	25 (62.0)	17 (96.0)	0 (96.0)	2 (100.0)				
Aspergillus fumigatus (90)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	73 (81.1)	16 (98.9)	1 (100.0)		
Aspergillus flavus SC[a] (11)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (54.5)	5 (100.0)			
Aspergillus niger SC (11)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (45.5)	5 (90.9)	1 (100.0)	

a. SC= species complex.

Table 2. Activity of isavuconazole and comparator antifungal agents when tested against the most common fungal species collected in a worldwide surveillance program during 2012.

Organism (no. tested)/ Antifungal agent	MIC_{50}	MIC_{90}	Range	CLSI[a] %S[a] / %R[a]	ECV[b] %WT[b] / %NWT[b]
C. albicans (671)					
Isavuconazole	0.015	0.03	≤0.008 – 0.5	- / -	- / -
Itraconazole	0.03	0.06	≤0.008 – 1	- / -	99.1 / 0.9
Posaconazole	0.03	0.06	≤0.008 – 0.5	- / -	94.9 / 5.1
Voriconazole	≤0.008	0.015	≤0.008 – 0.5	99.7 / 0.0	99.6 / 0.4
Fluconazole	0.25	0.25	≤0.06 – 16	99.6 / 0.3	98.7 / 1.3
Micafungin	0.015	0.03	≤0.008 – 0.06	100.0 / 0.0	99.7 / 0.3
Anidulafungin	0.015	0.06	≤0.008 – 0.12	100.0 / 0.0	100.0 / 0.0
Caspofungin	0.03	0.06	≤0.008 – 0.12	100.0 / 0.0	100.0 / 0.0
C. glabrata (291)					
Isavuconazole	0.5	2	0.015 – 8	- / -	- / -
Itraconazole	1	2	0.06 – >8	- / -	94.2 / 5.8
Posaconazole	1	2	0.03 – >8	- / -	96.2 / 3.8
Voriconazole	0.25	0.5	≤0.008 – 4	- / -	90.4 / 9.6
Fluconazole	8	32	0.25 – >128	- / 7.9	92.1 / 7.9
Micafungin	0.015	0.03	0.015 – 2	97.6 / 2.1	96.9 / 3.1
Anidulafungin	0.06	0.12	0.015 – 2	96.2 / 2.1	97.9 / 2.1
Caspofungin	0.06	0.06	0.015 – 4	97.3 / 2.4	97.3 / 2.7
C. parapsilosis (236)					
Isavuconazole	0.03	0.12	≤0.008 – 0.25	- / -	- / -
Itraconazole	0.12	0.25	0.03 – 0.5	- / -	100.0 / 0.0
Posaconazole	0.06	0.12	0.015 – 0.25	- / -	100.0 / 0.0
Voriconazole	0.015	0.06	≤0.008 – 0.5	97.9 / 0.0	97.9 / 2.1
Fluconazole	1	2	0.12 – 64	93.2 / 3.8	93.2 / 6.8
Micafungin	1	2	0.015 – 4	98.7 / 0.0	100.0 / 0.0
Anidulafungin	2	4	0.12 – 4	86.4 / 0.0	100.0 / 0.0
Caspofungin	0.5	0.5	0.06 – 2	100.0 / 0.0	99.1 / 0.9
C. tropicalis (122)					
Isavuconazole	0.06	0.12	≤0.008 – 4	- / -	- / -
Itraconazole	0.06	0.12	0.015 – 1	- / -	98.4 / 1.6
Posaconazole	0.03	0.12	0.015 – 1	- / -	96.7 / 3.3
Voriconazole	0.015	0.06	≤0.008 – 2	97.5 / 1.6	96.7 / 3.3
Fluconazole	0.5	0.5	0.12 – 64	95.9 / 2.5	95.9 / 4.1
Micafungin	0.03	0.06	≤0.008 – 0.12	100.0 / 0.0	100.0 / 0.0
Anidulafungin	0.015	0.03	≤0.008 – 0.12	100.0 / 0.0	100.0 / 0.0
Caspofungin	0.03	0.06	0.015 – 0.12	100.0 / 0.0	100.0 / 0.0
C. krusei (32)					
Isavuconazole	0.5	0.5	0.12 – 2	- / -	- / -
Itraconazole	0.25	0.5	0.25 – 4	- / -	96.9 / 3.1
Posaconazole	0.25	0.5	0.12 – 2	- / -	93.8 / 6.3
Voriconazole	0.25	0.25	0.12 – 4	96.9 / 3.1	96.9 / 3.1
Fluconazole	32	32	8 – 64	- / -	100.0 / 0.0
Micafungin	0.12	0.12	0.015 – 0.12	100.0 / 0.0	100.0 / 0.0
Anidulafungin	0.06	0.12	0.03 – 1	96.9 / 3.1	96.9 / 3.1
Caspofungin	0.12	0.25	0.06 – 1	96.9 / 3.1	96.9 / 3.1
C. lusitaneae (24)					
Isavuconazole	0.03	0.06	≤0.008 – 0.12	- / -	- / -
Itraconazole	0.12	0.25	0.06 – 0.25	- / -	100.0 / 0.0
Posaconazole	0.06	0.12	0.015 – 0.25	- / -	95.8 / 4.2
Voriconazole	≤0.008	0.015	≤0.008 – 0.03	- / -	100.0 / 0.0
Fluconazole	0.5	2	0.12 – 2	- / -	100.0 / 0.0
Micafungin	0.25	0.5	0.03 – 2	- / -	95.8 / 4.2
Anidulafungin	0.5	1	0.06 – 2	- / -	100.0 / 0.0
Caspofungin	0.25	0.5	0.03 – 2	- / -	95.8 / 4.2
C. dubliniensis (13)					
Isavuconazole	0.015	0.03	≤0.008 – 0.06	- / -	- / -
Itraconazole	0.06	0.06	0.015 – 0.12	- / -	100.0 / 0.0
Posaconazole	0.06	0.06	0.015 – 0.12	- / -	100.0 / 0.0
Voriconazole	≤0.008	≤0.008	≤0.008 – 0.015	- / -	100.0 / 0.0
Fluconazole	0.25	0.25	0.12 – 0.25	- / -	100.0 / 0.0
Micafungin	0.03	0.06	0.015 – 1	- / -	92.3 / 7.7
Anidulafungin	0.03	0.06	0.015 – 2	- / -	92.3 / 7.7
Caspofungin	0.03	0.06	0.03 – 2	- / -	92.3 / 7.7
C. neoformans (50)					
Isavuconazole	0.12	0.25	0.03 – 1	- / -	- / -
Itraconazole	0.12	0.25	0.03 – 1	- / -	100.0 / 0.0
Posaconazole	0.12	0.25	0.03 – 0.5	- / -	96.0 / 4.0
Voriconazole	0.06	0.12	0.015 – 1	- / -	96.0 / 4.0
Fluconazole	4	8	1 – 8	- / -	100.0 / 0.0
Micafungin	>16	>16	16 – >16	- / -	- / -
Anidulafungin	>16	>16	8 – >16	- / -	- / -
Caspofungin	16	16	1 – >16	- / -	- / -
A. fumigatus (90)					
Isavuconazole	1	2	1 – 4	- / -	- / -
Itraconazole	1	1	0.25 – 1	- / -	100.0 / 0.0
Posaconazole	0.25	0.5	0.12 – 1	- / -	94.4 / 5.6
Voriconazole	0.25	0.5	0.12 – 1	- / -	100.0 / 0.0
Micafungin	0.015	0.015	≤0.008 – 0.03	- / -	- / -
Anidulafungin	0.015	0.015	≤0.008 – 0.06	- / -	- / -
Caspofungin	0.03	0.06	≤0.008 – 0.12	- / -	98.9 / 1.1
A. flavus SC (11)					
Isavuconazole	1	2	1 – 2	- / -	- / -
Itraconazole	1	1	0.5 – 1	- / -	100.0 / 0.0
Posaconazole	0.5	1	0.25 – 1	- / -	81.8 / 18.2
Voriconazole	0.5	1	0.25 – 1	- / -	100.0 / 0.0
Micafungin	0.015	0.03	≤0.008 – 0.03	- / -	- / -
Anidulafungin	≤0.008	0.015	≤0.008 – 0.015	- / -	- / -
Caspofungin	0.015	0.03	≤0.008 – 0.03	- / -	100.0 / 0.0
A. niger SC (11)					
Isavuconazole	4	4	2 – 8	- / -	- / -
Itraconazole	2	2	1 – 4	- / -	90.9 / 9.1
Posaconazole	1	1	0.5 – 1	- / -	100.0 / 0.0
Voriconazole	1	1	0.5 – 2	- / -	100.0 / 0.0
Micafungin	≤0.008	0.015	≤0.008 – 0.03	- / -	- / -
Anidulafungin	≤0.008	0.015	≤0.008 – 0.015	- / -	- / -
Caspofungin	0.015	0.03	0.015 – 0.06	- / -	100.0 / 0.0

a. Clinical and Laboratory Standards Institute (CLSI), susceptible (S), resistant (R). Criteria on M27-S4.
b. Epidemiologic cutoff value (ECV), wild-type (WT), non wild-type (NWT). According to Pfaller et al., 2010, 2011 and 2012 (*Candida* spp); Espinel-Ingroff et al., 2012 and Pfaller et al., 2011 (*C. neoformans*); Espinel-Ingroff et al., 2010 and 2011 and Pfaller et al., 2010 (*Aspergillus* spp.).

CONCLUSIONS

- Isavuconazole displayed very good activity against this contemporary collection of fungal isolates, including the most common species of *Candida, C. neoformans* and *Aspergillus* species. The activity was comparable to itraconazole, posaconazole and voriconazole when read at the same test/endpoint conditions.
- Overall, fungal isolates from this contemporary global collection remain very susceptible to antifungal agents clinically used for the treatment of invasive fungal infections. However, recent reports of increasing cross-resistance among azoles and echinocandins in *C. glabrata* warrants continued surveillance for antifungal agents, including new agents (isavuconazole).

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