Isavuconazole Activity against *Aspergillus lentulus*, *Neosartorya udagawae*, and *Cryptococcus gattii*, Emerging Fungal Pathogens with Reduced Azole Susceptibility

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Isavuconazole is an extended-spectrum triazole with *in vitro* activity against a wide variety of fungal pathogens. Clinical isolates of molds *Aspergillus lentulus* and *Neosartorya udagawae* and yeast *Cryptococcus gattii* VGII (implicated in the outbreak in the Pacific Northwest, North America) exhibit reduced susceptibilities to several azoles but higher susceptibilities to isavuconazole.

As more triazole antifungals have become available for clinical use, we have come to appreciate an increased variability in *in vitro* susceptibilities. This has been particularly notable for specific members of *Aspergillus fumigatus* section *Fumigati*, such as *Aspergillus lentulus* (1), *Neosartorya udagawae* (2), and *N. pseudofischeri* (3). These genetically distinct molds are often misidentified as *A. fumigatus* (2, 4–6). Previously unrecognized as pathogenic, these species are now implicated in invasive mycoses globally. Studies have identified *A. lentulus* among culture collections in the Netherlands (7), Australia (8), Japan (4), and Spain (5, 9). In Spain, *A. lentulus* was a cause of invasive infection in 14 of 28 samples previously identified as *A. fumigatus* (5). *Neosartorya udagawae* has been implicated in invasive aspergillosis, with a peculiar predilection for disease in patients with chronic granulomatous disease (10).

Cryptococcus gattii has recently emerged as a significant mammalian pathogen in the Pacific Northwest (PNW) of the United States and Canada (11). *C. gattii* is further distinguished into four molecular types designated VGI, VGII, VGIII, and VGIV (12–14). The molecular types are of epidemiological significance; VGII (a.k.a. AFLP6) has been implicated in the PNW outbreak, and VGIIa and VGIIb are considered, respectively, the major and minor outbreak types, along with the emergence of a novel genotype, VGIIc (14). *C. gattii* clinical and environmental isolates demonstrate various susceptibilities to several classes of antifungals (15, 16), with a high proportion of isolates from the PNW showing lower susceptibilities to fluconazole (17).

Isavuconazole, an extended-spectrum triazole antifungal, is active *in vitro* against a large number of clinically important fungal pathogens (18). It is currently in phase III clinical development for treatment of both aspergillosis and candidiasis, as well as other rare fungi. There is a relative paucity of information available regarding isavuconazole's activities against these less-common but emerging mold and yeast pathogens that exhibit various sensitivities to clinically available antifungals.

Antifungal susceptibilities were determined by the broth microdilution methods outlined in CLSI documents M38-A2 (molds) (19) and M27-A3 (yeasts) (20). For aspergilli, MIC endpoints of 100% inhibition (no discernible growth) were determined at 48 h (19). For *C. gattii* isolates, the fluconazole, voriconazole, itraconazole (20), and isavuconazole MICs (20, 21) were determined at 72 h, using an endpoint of $\geq 50\%$ reduction in growth relative to the growth of drug-free controls (20). The MICs

were derived from two independent assays; the replicate values were identical or within one dilution of each other. Using analysis functions of Microsoft Excel, the geometric mean MIC, mode MIC (the most frequent MIC value for each isolate tested) (22), MIC₅₀ (median MIC, or MIC value at which 50% of isolates tested are inhibited), and MIC₉₀ (90th percentile, or MIC value at which 90% of isolates tested are inhibited) values were computed for each antifungal; MIC₅₀/MIC₉₀ values, if intermediate, were presented as the next tested concentration on the antifungal dilution series (23). The antifungals were obtained from manufacturers as follows: isavuconazole (Basilea Pharmaceutica International, Basel, Switzerland), voriconazole (Pfizer Inc., New York, NY), and itraconazole, fluconazole, and amphotericin B (all from Sigma-Aldrich, St. Louis, MO).

We tested 15 clinical isolates of *A. lentulus* (24), 9 clinical and 1 environmental isolate of *N. udagawae* (2, 24), obtained from various U.S. centers, and one standard strain of *A. fumigatus* (Af293) (3) as a comparator. Both *A. lentulus* and *N. udagawae* exhibited relatively decreased sensitivities to itraconazole (mode MICs, 2 and 1 $\mu\text{g}/\text{ml}$, respectively) and voriconazole (mode MIC, 1 $\mu\text{g}/\text{ml}$ for both) compared to the sensitivities of *A. fumigatus* (Table 1) (25). In contrast to the MICs of these azoles, the isavuconazole MICs were lower for the *A. lentulus* and *N. udagawae* isolates (mode MICs, 0.25 and 0.125 $\mu\text{g}/\text{ml}$, respectively), as well as for *A. fumigatus*, as tested here (Table 1) and by others (26). *A. lentulus* isolates also demonstrated decreased susceptibilities to amphotericin B (range, 0.5 to 4 $\mu\text{g}/\text{ml}$; mode MIC, 2 $\mu\text{g}/\text{ml}$; 93.3% of isolates showed MICs of $\geq 2 \mu\text{g}/\text{ml}$ [data not shown separately]), corroborating observations by other investigators (2, 27).

We also tested 90 *C. gattii* VGII isolates, comprised of 58 clinical (human and veterinary) and 14 environmental isolates of *C. gattii* VGIIa (28), 7 clinical and 1 environmental isolate of VGIIb (28), and 10 clinical isolates of VGIIc (29) from the PNW (Vancouver Island, BC, Canada, and Washington and Oregon, United

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TABLE 1 Summary of drug sensitivities of all *Aspergillus* section *Fumigati* and *C. gattii* VGII isolates^a

Organism (no. of isolates)	Drug	Mode MIC (μg/ml)	MIC ₅₀ (μg/ml)	MIC ₉₀ (μg/ml)	MIC range (μg/ml)	Geometric mean MIC (μg/ml)
<i>A. lentulus</i> (n = 15)	Itraconazole	1	1	2	0.5–2	1.097
	Voriconazole	2	2	2	0.5–2	1.516
	Isavuconazole	0.25	0.25	0.25	0.063–0.5	0.188
<i>N. udagawae</i> (n = 10)	Itraconazole	1	1	1	0.25–1	0.660
	Voriconazole	1	1	1	0.25–1	0.812
	Isavuconazole	0.125	0.125	0.25	0.031–0.25	0.100
All <i>C. gattii</i> (n = 90)	Fluconazole	4	4	8	2–64	4.560
	Itraconazole	0.125	0.125	0.5	0.031–0.5	0.187
	Voriconazole	0.125	0.125	0.125	0.031–0.5	0.093
	Isavuconazole	0.031	0.063	0.125	0.031–0.5	0.057
<i>C. gattii</i> VGIIa (n = 72)	Fluconazole	4	4	8	2–16	3.849
	Itraconazole	0.125	0.125	0.5	0.031–0.5	0.177
	Voriconazole	0.125	0.125	0.125	0.031–0.25	0.088
	Isavuconazole	0.031	0.031	0.063	0.031–0.125	0.046
<i>C. gattii</i> VGIIb (n = 8)	Fluconazole	8	8	64	4–64	8.724
	Itraconazole	0.125	0.25	0.5	0.125–0.5	0.210
	Voriconazole	0.031	0.063	0.125	0.031–0.125	0.063
	Isavuconazole	0.063	0.125	0.25	0.031–0.25	0.088
<i>C. gattii</i> VGIIc (n = 10)	Fluconazole	8	8	32	4–32	9.190
	Itraconazole	0.25	0.25	0.5	0.125–0.5	0.250
	Voriconazole	0.25	0.25	0.5	0.063–0.5	0.189
	Isavuconazole	0.25	0.25	0.5	0.125–0.5	0.203

^a The MICs of a single *A. fumigatus* isolate were 0.25 μg/ml (voriconazole and itraconazole) and 0.063 μg/ml (isavuconazole). Mode MIC, most frequently occurring MIC in whole population tested; MIC₅₀, MIC at which ≥50% of all isolates are inhibited (identical to median MIC); MIC₉₀, MIC at which ≥90% of all isolates are inhibited.

States) and California (14, 28, 30, 31). The VGIIa group included type strains NIH444 (clinical) (30) and CBS7750 (environmental) (32); VGIIa isolates CBS10485 (33), CBS10866 (34), and RKI06/496 (35, 36) were recovered from European patients who traveled to Vancouver Island. *C. gattii* VGII clinical isolates exhibited a broad range (2 to 64 μg/ml) of MICs for fluconazole, similar to observations by others (22). The susceptibilities of these isolates to voriconazole and itraconazole were relatively higher; the isavuconazole MICs were similarly low. *C. gattii* subtype VGIIb and VGIIc isolates appeared less susceptible to fluconazole than subtype VGIIa isolates (Table 1). These results concur well with an earlier study of 169 *C. gattii* isolates collected globally (37). There was no difference in MICs according to the isolate source (clinical or environmental). The MICs of previously reported isolates (31) were within one dilution, except for one (B7394; data not shown).

Like other triazoles, isavuconazole inhibits fungal cytochrome P450-lanosterol 14α-demethylase (Cyp51), associated with ergosterol biosynthesis (38), thereby destabilizing membrane integrity. However, isavuconazole uniquely possesses a side arm which presumably offers a better orientation for the triazole ring to interact with the fungal Cyp51 heme moiety inside its binding pocket. The resultant tight binding likely provides isavuconazole's enhanced antifungal spectrum, including activity against fungi less sensitive to other azoles (39).

A tandem repeat (TR)/L98H point mutation in the *cyp51a* promoter of *A. fumigatus* is known to reduce its susceptibility to voriconazole (40) and isavuconazole (41). In addition, the role of the Cdr1Bp efflux transporter in non-Cyp51Ap-mediated itracona-

zole resistance in *A. fumigatus* was recently discovered (42). Efflux mechanisms in *A. lentulus* are unknown as yet; however, its Cyp51Ap homolog appears to be responsible for the decreased sensitivity to azoles (43). Prior studies evaluating *A. lentulus* echinocandin susceptibilities also suggest that nontarget characteristics, such as fungal cell wall composition, architecture, and/or hydrophobicity, may impact antifungal susceptibilities (44). The isavuconazole MICs of *A. lentulus* and *N. udagawae* isolates in this study, albeit low, varied (Table 1) and were comparable to the modal *A. fumigatus* MICs recently reported (26). Future studies are necessary to define the clinical significance of these findings.

Non-azole-target mechanisms may also influence *C. gattii* susceptibilities. *C. gattii* homologs of fungal efflux transporters (*Cryptococcus neoformans* MDR1 and AFR1 and *Candida albicans* CDR1/CDR2), expressed in *Saccharomyces cerevisiae*, confer higher fluconazole MICs and lower intracellular accumulation of [³H]fluconazole independent of *C. gattii* ERG11 (L. R. Basso, Jr., C. Gast, and B. Wong, presented at the 48th Annual Meeting of the Infectious Diseases Society of America [IDSA] Vancouver, British Columbia, Canada, 20 to 24 October 2010). High *in vitro* MIC values have been associated with clinical failure (45, 46). These findings have potential clinical implications, since fluconazole remains a recommended standard for the treatment of cryptococcosis (47); these guidelines likely need revision based on emerging evidence that VGII *C. gattii* isolates may exhibit high fluconazole MICs *in vitro*. In our study, isavuconazole, as well as voriconazole and itraconazole,

demonstrated *in vitro* antifungal activity that was superior to that of fluconazole (Table 1).

A unifying theme among these diverse fungi is the occurrence of closely related species displaying differential antifungal susceptibilities with no corresponding mechanistic explanation. Microbiology laboratories typically do not distinguish between *A. fumigatus* and *A. lentulus* or identify species of *Cryptococcus* isolates. These findings supplement an increasing literature suggesting that more-detailed species identification (including *C. gattii* genotyping) may better guide therapeutic decisions.

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