Activity of Isavuconazole and Other Azoles against *Candida* Clinical Isolates and Yeast Model Systems with Known Azole Resistance Mechanisms

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Isavuconazole is a novel, broad-spectrum, antifungal azole. In order to evaluate its interactions with known azole resistance mechanisms, isavuconazole susceptibility among different yeast models and clinical isolates expressing characterized azole resistance mechanisms was tested and compared to those of fluconazole, itraconazole, posaconazole, and voriconazole. *Saccharomyces cerevisiae* expressing the *Candida albicans* and *C. glabrata* ATP binding cassette (ABC) transporters (CDR1, CDR2, and CgCDR1), major facilitator (MDR1), and lanosterol 14-α-sterol-demethylase (ERG11) alleles with mutations were used. In addition, pairs of *C. albicans* and *C. glabrata* strains from matched clinical isolates with known azole resistance mechanisms were investigated. The expression of ABC transporters increased all azole MICs, suggesting that all azoles tested were substrates of ABC transporters. The expression of MDR1 did not increase posaconazole, itraconazole, and isavuconazole MICs. Relative increases of azole MICs (from 4- to 32-fold) were observed for fluconazole, voriconazole, and isavuconazole when at least two mutants were present in the same ERG11 allele. Upon MIC testing of azoles with clinical *C. albicans* and *C. glabrata* isolates with known resistance mechanisms, the MIC	extsubscript{50} of *C. albicans* for fluconazole, voriconazole, itraconazole, posaconazole, and isavuconazole were 128, 2, 1, 0.5, and 2 μg/mL, respectively, while in *C. glabrata* they were 128, 2, 4, 4, and 16 μg/mL, respectively. In conclusion, the effects of azole resistance mechanisms on isavuconazole did not differ significantly from those of other azoles. Resistance mechanisms in yeasts involving ABC transporters and *ERG11* decreased the activity of isavuconazole, while MDR1 had limited effect.
that of once-daily fluconazole in the primary treatment of un-
complicated esophageal candidiasis (19). In vitro, isavucona-
zole is highly active against bloodstream isolates of Candida
spp. and has demonstrated good efficacy in animal models of can-
didiasis (20–22). However, it is important to understand how isavucona-
zole interacts with known mechanisms of azole resistance and
what patterns of cross-resistance it shares with other azoles. In
this study, we assessed the possible role of isavuconazole as a sub-
strate for yeast multidrug efflux transporters. We also evaluated
the susceptibility profile of isavuconazole when cytochrome P450
proteins encoded by different ERG11 alleles are expressed in a
heterologous yeast host. In addition, isavuconazole susceptibility
of clinical strains of Candida spp. with different azole susceptibil-
ity profiles and known azole resistance mechanisms were evalu-
ated and compared to other azoles (fluconazole, itraconazole,
voriconazole, and posaconazole).

MATERIALS AND METHODS

Strains. Saccharomyces cerevisiae isolates expressing Candida albicans
ATP binding cassette (ABC) transporter genes CDR1 and CDR2, the C. albicans
major facilitator BENT+ (MDR1), FLU1, and ERG11 alleles, and C. glabrata CDR1
and CDR2 (CgCDR1 and CgCDR2) were used in this study; these resistance-conferring alleles have been described elsewhere (23–25).

The S. cerevisiae strain YKKB13 (MATα ura3-53 lys2-801 ade2-101trp1-Δhis3-Δ200 leu2-Δ1 pdr5Δ:TRP1) was used for all trans-
porter and ERG11 allele expression experiments with Yep24- and Yep51-
derived plasmids, respectively (23, 26, 27).

Antifungal drugs. Azole antifungal drugs were obtained as pure sub-
stances from pharmaceutical companies (fluconazole and voriconazole,
Basilea Pharmaceutica International Ltd., Basel, Switzerland), and Laboratory Standards Institute (CLSI) (28). This protocol uses selective
broth microdilutions based on protocol M27-A3 of the Clinical
Susceptibility Testing of Candida Albicans Clinical Isolates
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RESULTS

Susceptibility testing of S. cerevisiae containing drug resistance genes. In order to test whether isavuconazole is a potential sub-
strate for ABC and MFS transporters, drug susceptibility testing
was carried out with S. cerevisiae containing specific transporter

genes associated with drug resistance. Changes to the MICs of
isavuconazole upon expression of a given transporter indicated
whether this azole was a substrate for the expressed transporter.

The expression of ABC transporters, including CDR1, CDR2,
CgCDR1, and CgCDR2, elevated the MICs of isavuconazole from
2- to 32-fold compared with the MICs of the recipient S. cerevisiae
strain (Table 2). These values depended on the expressed ABC
transporters. MDR1 expression had the greatest impact on MICs
(32-fold increase), while CgCDR2 expression had the least impact
(2-fold increase). Fluconazole and voriconazole showed the larg-
est increases in MICs, ranging from 16- to 128-fold and 8- to
56-fold, respectively.

Expression of MFS transporters MDR1 and FLU1 did not in-
crease the MICs of isavuconazole, which was a characteristic
shared with itraconazole and posaconazole (Table 2). In contrast,
MDR1 and FLU1 expression increased the MICs of fluconazole by
128-fold and 16-fold, respectively, and those of voriconazole by
32-fold and 8-fold, respectively. These results indicated that, in
contrast to isavuconazole, fluconazole and voriconazole were sub-
strates for these MFS transporters.

Ten S. cerevisiae strains, each expressing an ERG11 allele from
different C. albicans clinical isolates, were tested for their suscep-
tibility to isavuconazole and other azoles and were compared to a
strain carrying a wild-type (WT) allele. The ERG11 alleles used in
this study carried nine distinct single or combined mutations that
are known to be involved inazole resistance (23). Isavuconazole
MICs ranged from 0.125 to 1 μg/ml. The highest increase for
isavuconazole was between 4- and 8-fold and was associated with
the substitution Y132H alone or combined with the substitution
S405F or G464S.

When C. albicans ERG11 mutant alleles were expressed in S.
cerevisiae, increases inazole MICs relative to those of the control
strain were observed for fluconazole (4- to 32-fold) and voricona-
olecule (1- to 16-fold) when at least two mutations were present in
the same ERG11 allele (Table 3). Correlation coefficient analyses
between the different azoles demonstrated that isavuconazole was
more closely related to voriconazole and itraconazole than to flu-
conazole (see Fig. SA1 in the supplemental material). Interest-
ingly, fluconazole MICs in S. cerevisiae showed the highest varia-
tions, followed by voriconazole MICs. No changes in posaconazole
MICs were observed with any of the ERG11 mutant alleles ex-
pressed in S. cerevisiae.

Susceptibility testing of Candida species isolates with known
azole resistance mechanisms. Our strain collection included a
number of clinical isolates of C. albicans and C. glabrata with
known and diverse resistance mechanisms. Paired isolates exhib-
ted closely related genotypic patterns deduced from multilocus
sequence typing (31, 40) (see Table SA1 in the supplemental ma-
terial). Therefore, it was possible to compare azole MICs from the most susceptible isolates to those of the most resistant isolates. All quality controls were observed to have azole MICs within the accepted range (20, 41) (Table 4).

Susceptibility testing of *C. albicans* clinical isolates. The MIC range for isavuconazole (0.004 to 8 μg/ml) was similar to that for voriconazole (0.004 to 4 μg/ml) (Table 5). The MICs for fluconazole, itraconazole, and posaconazole ranged from 0.125 to 128, 0.016 to 2, and 0.016 to 4 μg/ml, respectively. The relative increase in MICs for each azole compared to that of a susceptible control ranged from 32- to 512-fold for fluconazole, voriconazole, and isavuconazole but from only 4- to 32-fold for posaconazole.

### Table 1 Clinical isolates used in the study

<table>
<thead>
<tr>
<th>DSY no.</th>
<th>Alternative no.</th>
<th>Species</th>
<th>Parent strain</th>
<th>Resistance mechanism/genotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSY281</td>
<td>C23</td>
<td><em>C. albicans</em></td>
<td>Related to DSY284</td>
<td>WT</td>
<td>32</td>
</tr>
<tr>
<td>DSY284</td>
<td>C39</td>
<td><em>C. albicans</em></td>
<td>WT</td>
<td>ERG11 (S405F), TAC1 (G980E)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32</td>
</tr>
<tr>
<td>DSY347</td>
<td>C33</td>
<td><em>C. albicans</em></td>
<td>Related to DSY348 and DSY289</td>
<td>WT</td>
<td>25</td>
</tr>
<tr>
<td>DSY288</td>
<td>C34</td>
<td><em>C. albicans</em></td>
<td>WT</td>
<td>ERG11 (S405F)</td>
<td>25</td>
</tr>
<tr>
<td>DSY289</td>
<td>C26</td>
<td><em>C. albicans</em></td>
<td>WT</td>
<td>ERG11 (S405F), Y132H, TAC1 (A736V)</td>
<td>25, 29</td>
</tr>
<tr>
<td>DSY348</td>
<td>C82</td>
<td><em>C. albicans</em></td>
<td>WT</td>
<td>ERG11 (S405F), TAC1 (A736V)</td>
<td>25, 29</td>
</tr>
<tr>
<td>DSY290</td>
<td>C27</td>
<td><em>C. albicans</em></td>
<td>Related to DSY291, DSY292</td>
<td>WT</td>
<td>25</td>
</tr>
<tr>
<td>DSY291</td>
<td>C37</td>
<td><em>C. albicans</em></td>
<td>WT</td>
<td>ERG11 (G464S) (R467K)</td>
<td>15, 25</td>
</tr>
<tr>
<td>DSY292</td>
<td>C40</td>
<td><em>C. albicans</em></td>
<td>WT</td>
<td>ERG11 (G464S) (R467K, Y132H),&lt;sup&gt;b&lt;/sup&gt; MRR1 (P683H)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15, 25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mutations are indicated in parentheses. Amino acid changes at given codon numbers of the corresponding gene are given. i (5L), isochromosome 5L.

<sup>b</sup> Heterozygous state.

<sup>c</sup> Deletion of methionine at position 677 in TAC1.
fold for itraconazole and 2- to 128-fold for posaconazole. The MIC$_{90}$s for the matched clinical C. albicans isolates for fluconazole, voriconazole, itraconazole, posaconazole, and isavuconazole were 128, 2, 1, 0.5, and 2 μg/ml, respectively. This suggests that isavuconazole had activity similar to those of itraconazole, posaconazole, and voriconazole.

Islaconazole MICs for each isolate were compared to those of other azoles, separating non-WT isolates from WT isolates, i.e., those with and without known azole resistance mechanisms (Fig. 1A). The upper isavuconazole MIC value for WT isolates was 0.031 μg/ml. This value is in agreement with another study which reported that 90% of C. albicans isolates exhibit isavuconazole MICs of ≤0.03 μg/ml (42). Correlation curves between isavuconazole MICs of WT and non-WT isolates and other azoles were established (Fig. 1). Isavuconazole MICs correlated with other azoles MICs in the following order of correlation strength: itraconazole < voriconazole < fluconazole < posaconazole. However, the analysis of relative MIC increases of matched isolates for each azole correlation coefficient demonstrated that the MIC profile of isavuconazole was related significantly to those of posaconazole and itraconazole, while profiles of fluconazole and voriconazole were significantly more similar to each other (see Fig. SA2 in the supplemental material).

Susceptibility testing of C. glabrata isolates. The MIC range for isavuconazole (0.25 to 16 μg/ml) was similar to the range for posaconazole (0.125 to 16 μg/ml) (Table 6). The MIC ranges for fluconazole, itraconazole, and voriconazole were 1 to 16, and 0.0156 to 2 μg/ml, respectively. MIC$_{90}$s of clinical isolates for fluconazole, voriconazole, itraconazole, posaconazole, and isavuconazole were 128, 2, 4, 4, and 16 μg/ml, respectively. These values were higher than those observed for the C. albicans isolates DSY565 and DSY2325 and were associated with the highest relative increase in MICs for isavuconazole (32-fold). Comparison of isavuconazole MICs from WT isolates and non-WT isolates revealed that the upper isavuconazole MIC value of WT isolates was 1.0 μg/ml (Fig. 1B). This value was equivalent to that observed in another study (42). Correlation curves between isavuconazole MICs of WT and non-WT isolates and other azoles highlighted that isavuconazole MICs were correlated with MICs of other azoles in the following order: voriconazole < fluconazole < itraconazole < posaconazole. When we analyzed correlation coefficients between relative MIC increases of matched isolates for each azole in C. glabrata, we observed that the profile of isavuconazole was related significantly only to that of fluconazole, in contrast to the case for C. albicans (see Fig. SA3 in the supplemental material).

Susceptibility testing of C. albicans multidrug transporter mutants. MICs for isavuconazole and other azoles were determined for C. albicans mutants lacking the major multidrug transporters involved in azole resistance in order to test their impact on drug resistance. The absence of CDR1 had the largest impact on azole MICs (Table 7). For isavuconazole, the MIC decreased from 0.0078 μg/ml in WT CAF2-1 to <0.001 μg/ml in the cdr1ΔΔ mutant. The fluconazole MIC decreased from 0.25 to <0.0625 μg/ml.

### Table 2 Activity of azoles in S. cerevisiae containing several Candida multidrug transporters

<table>
<thead>
<tr>
<th>S. cerevisiae strain</th>
<th>Resistance mechanism/genotype</th>
<th>MIC (μg/ml [fold difference$^a$])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fluconazole</td>
</tr>
<tr>
<td>DSY595$^b$</td>
<td>YKKB13 and C. albicans ERG11</td>
<td>4 (1)</td>
</tr>
<tr>
<td>DSY1109</td>
<td>YKKB13 and C. albicans ERG11 (G129A)</td>
<td>4 (1)</td>
</tr>
<tr>
<td>DSY949</td>
<td>YKKB13 and C. albicans ERG11 (S405F)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>DSY952</td>
<td>YKKB13 and C. albicans ERG11 (Y132H)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>DSY950</td>
<td>YKKB13 and C. albicans ERG11 (G464S)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>DSY951</td>
<td>YKKB13 and C. albicans ERG11 (G464S/G129A)</td>
<td>8 (2)</td>
</tr>
<tr>
<td>DSY963</td>
<td>YKKB13 and C. albicans ERG11 (R467K)</td>
<td>128 (32)</td>
</tr>
<tr>
<td>DSY965</td>
<td>YKKB13 and C. albicans ERG11 (S405F/Y132H)</td>
<td>32 (8)</td>
</tr>
<tr>
<td>DSY1107</td>
<td>YKKB13 and C. albicans ERG11 (G464S/R467K)</td>
<td>32 (8)</td>
</tr>
<tr>
<td>DSY1108</td>
<td>YKKB13 and C. albicans ERG11 (G464S/G129A)</td>
<td>15 (4)</td>
</tr>
<tr>
<td>DSY612</td>
<td>YKKB13 and YEp$^c$</td>
<td>1 (0.25)</td>
</tr>
</tbody>
</table>

$^a$ Fold difference is relative to the level for DSY595 for each azole.  
$^b$ Baseline strain.  
$^c$ Yeast replicating vector (parent vector) into which ERG11 alleles were cloned.
µg/ml in the absence of CDR1. MIC decreases in itraconazole and posaconazole were less pronounced, 0.0625 to 0.0156 µg/ml, when multiple transporters were deleted.

Susceptibility testing of *C. albicans* mutants with inactivation of known azole resistance mechanisms. The DSY3706 derivative, which lacks TAC1 (the transcriptional activator of CDR1 and CDR2) and contains WT ERG11, had MICs for all azoles tested that were similar to those of DSY294, which is the susceptible parent strain (Table 8). Reintroduction of the TAC1 mutation in DSY3606-1 increased the MIC of fluconazole by approximately 32-fold. The largest increases in MICs for isavuconazole were observed in the DSY3606-1 (64-fold) and DSY296 (128-fold) strains. These strains contain TAC1 GOF mutations, suggesting that increases in CDR1 and CDR2 are associated with reduced susceptibility to isavuconazole. The G446S mutation in DSY296 resulted in the largest increases in MICs (128-fold) for fluconazole, voriconazole, and isavuconazole. The reintroduction of ERG11 WT alleles into the TAC1 deletion mutant DSY3083 re-

### Table 4 Azole MIC quality controls

<table>
<thead>
<tr>
<th>Reference strain</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Posaconazole</th>
<th>Voriconazole</th>
<th>Isavuconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> ATCC 928</td>
<td>0.125</td>
<td>0.0625</td>
<td>0.0078</td>
<td>&lt;0.0039</td>
<td>0.0078</td>
</tr>
<tr>
<td><em>C. krusei</em> ATCC 6258</td>
<td>32</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td><em>C. tropicalis</em> ATCC 750</td>
<td>0.5</td>
<td>0.0078</td>
<td>0.0156</td>
<td>0.0078</td>
<td>0.0312</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> ATCC 22019</td>
<td>2</td>
<td>0.0625</td>
<td>0.0625</td>
<td>0.0156</td>
<td>0.125</td>
</tr>
<tr>
<td><em>C. glabrata</em> ATCC 930</td>
<td>4</td>
<td>0.125</td>
<td>0.125</td>
<td>0.0312</td>
<td>0.125</td>
</tr>
</tbody>
</table>

### Table 5 Activity of azoles in *C. albicans* clinical isolates with known resistance mechanisms

<table>
<thead>
<tr>
<th><em>C. albicans</em> clinical strain</th>
<th>Resistance mechanism/genotype</th>
<th>MIC (µg/ml) [fold differencea)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSY281 WT</td>
<td></td>
<td>0.250 (1) 0.125 (1) 0.031 (1) 0.004 (1) 0.016 (1)</td>
</tr>
<tr>
<td>DSY284 ERG11 (S405F), TAC1 (G980E)</td>
<td></td>
<td>8 (32) 0.25 (2) 0.25 (8) 0.063 (16) 0.25 (16)</td>
</tr>
<tr>
<td>DSY347 WT</td>
<td></td>
<td>0.250 (1) 0.125 (1) 0.125 (1) 0.004 (1) 0.031 (1)</td>
</tr>
<tr>
<td>DSY288 ERG11 (S405F)</td>
<td></td>
<td>0.5 (2) 0.125 (1) 0.125 (1) 0.031 (8) 0.063 (2)</td>
</tr>
<tr>
<td>DSY289 ERG11 (S405F), Y132H, TAC1 (A736V)</td>
<td></td>
<td>&gt;128 (512) 2 (16) 0.5 (4) 4 (1026) 8 (256)</td>
</tr>
<tr>
<td>DSY348 ERG11 (S405F), TAC1 (A736V)</td>
<td></td>
<td>8 (32) 1 (8) 0.5 (4) 0.063 (16) 1 (32)</td>
</tr>
<tr>
<td>DSY290 WT</td>
<td></td>
<td>0.500 (1) 0.063 (1) 0.063 (1) 0.004 (1) 0.008 (1)</td>
</tr>
<tr>
<td>DSY291 ERG11 (G464S, R467K)</td>
<td></td>
<td>2 (1) 0.25 (4) 0.063 (1) 0.031 (8) 0.031 (4)</td>
</tr>
<tr>
<td>DSY292 ERG11 (G464S, R467K, Y132H), a MRR1 (P683H)</td>
<td></td>
<td>128 (256) 1 (16) 0.25 (4) 0.5 (128) 2 (256)</td>
</tr>
<tr>
<td>DSY294 WT</td>
<td></td>
<td>0.5 (1) 0.016 (1) 0.063 (1) 0.004 (1) 0.016 (1)</td>
</tr>
<tr>
<td>DSY296 ERG11 (G464S), TAC1 (N977D)</td>
<td></td>
<td>64 (128) 0.125 (8) 0.25 (4) 0.5 (128) 2 (128)</td>
</tr>
<tr>
<td>DSY2321 ERG11 (S405F)</td>
<td></td>
<td>&lt;0.125 (1) 0.031 (1) 0.063 (1) 0.004 (1) &lt;0.0039 (1)</td>
</tr>
<tr>
<td>DSY2322 ERG11 (S405F), TAC1 (G980E)</td>
<td></td>
<td>16 (128) 1 (32) 0.5 (8) 0.125 (32) 2 (513)</td>
</tr>
<tr>
<td>DSY2323 ERG11 (S405F), TAC1 (G980E)</td>
<td></td>
<td>32 (256) 0.5 (16) 0.5 (8) 0.125 (32) 2 (513)</td>
</tr>
<tr>
<td>DSY731 WT</td>
<td></td>
<td>0.250 (1) 0.063 (1) 0.063 (1) 0.004 (1) 0.008 (1)</td>
</tr>
<tr>
<td>DSY775 ERG11 (G464S), TAC1 (G980W)</td>
<td></td>
<td>64 (512) 1 (16) 0.5 (8) 0.5 (128) 2 (513)</td>
</tr>
<tr>
<td>DSY2309 WT</td>
<td></td>
<td>2 (1) 0.125 (1) 0.125 (1) 0.004 (1) &lt;0.0039 (1)</td>
</tr>
<tr>
<td>DSY750 MRR1 (N378D)</td>
<td></td>
<td>16 (8) 0.25 (2) 0.125 (1) 0.063 (16) 0.063 (16)</td>
</tr>
<tr>
<td>DSY751 ERG11 (S405F), MRR1 (N378D)</td>
<td></td>
<td>128 (64) 0.5 (4) 0.25 (2) 0.125 (32) 1 (256)</td>
</tr>
<tr>
<td>DSY2243 ERG11 (S442F, G465K)</td>
<td></td>
<td>0.25 (1) 0.125 (1) 0.063 (1) &lt;0.0039 (1) &lt;0.0039 (1)</td>
</tr>
<tr>
<td>DSY2242 ERG11 (S442F, R467K), TAC1 (G980E)</td>
<td></td>
<td>8 (32) 0.5 (4) 0.5 (8) 1 (16) 1 (256)</td>
</tr>
<tr>
<td>DSY2284 WT</td>
<td></td>
<td>0.125 (1) 0.063 (1) 0.063 (1) 0.004 (1) 0.004 (1)</td>
</tr>
<tr>
<td>DSY2285 MRR1 (T986D), i (5L)</td>
<td></td>
<td>64 (512) 0.25 (4) 0.25 (4) 0.125 (32) 0.25 (64)</td>
</tr>
<tr>
<td>DSY550 WT</td>
<td></td>
<td>0.125 (1) 0.016 (1) 0.016 (1) 0.004 (1) 0.008 (1)</td>
</tr>
<tr>
<td>DSY551 ERG11 (G464S, Y132H)</td>
<td></td>
<td>64 (512) 0.5 (32) 0.5 (32) 2 (513) 2 (256)</td>
</tr>
<tr>
<td>DSY520 TAC1 (N972D)</td>
<td></td>
<td>16 (1) 0.063 (1) 0.5 (1) 1 (1) 0.250 (1)</td>
</tr>
<tr>
<td>DSY522 ERG11 (G464S), TAC1 (N972D)</td>
<td></td>
<td>128 (8) 2 (32) 1 (2) 2 (2) 2 (8)</td>
</tr>
<tr>
<td>DSY2250 ERG11 (S442F, G465S)</td>
<td></td>
<td>1 (1) 0.125 (1) 0.031 (1) 0.004 (1) 0.008 (1)</td>
</tr>
<tr>
<td>DSY2251 ERG11 (S442F, G465S), TAC1 (N972D)</td>
<td></td>
<td>32 (32) 1 (8) 4 (128) 0.25 (64) 4 (513)</td>
</tr>
<tr>
<td>DSY741 WT</td>
<td></td>
<td>0.125 (1) 0.063 (1) 0.125 (1) 0.008 (1) 0.008 (1)</td>
</tr>
<tr>
<td>DSY742 MRR1 (T360I)</td>
<td></td>
<td>16 (128) 0.250 (4) 0.125 (1) 0.03 (4) 0.125 (16)</td>
</tr>
<tr>
<td>DSY757 WT</td>
<td></td>
<td>0.5 (1) 0.031 (1) 0.063 (1) &lt;0.0039 (1) &lt;0.0039 (1)</td>
</tr>
<tr>
<td>DSY758 ERG11 (G464S, F145L), a TAC1 (A736V)</td>
<td></td>
<td>32 (64) 0.5 (16) 0.25 (4) 0.125 (32) 1 (256)</td>
</tr>
</tbody>
</table>

Note: [Heterogenous state.](#) [Fold difference is relative to the WT or to the most susceptible isolate for each related isolate group.](#)
specific azole resistance genes or various *C. albicans* and *C. glabrata*. This study assessed the mechanisms of isavuconazole resistance in *C. albicans* isolates with known resistance mechanisms to determine the profile of fluconazole was distinct from those of isavuconazole and voriconazole. Interestingly, isavuconazole MICs exhibited no changes with any of the mutant alleles expressed in *S. cerevisiae*. The largest effect on the MIC for isavuconazole was with *S. cerevisiae* strains expressing ERG11 alleles with the Y132H mutation either alone or combined with mutation S405F or G464S. These results are consistent with data in the literature and are in line with the expression of specific azoles.

**FIG 1** Relationship of isavuconazole MICs to those of other azoles for *C. albicans* isolates (MICs are from Tables 5, 7, and 8) (A) and *C. glabrata* isolates (MICs are from Table 6) (B). Correlations were calculated with nonlinear regression fits and log-log lines as an option using GraphPad Prism 6. The R² values are indicated within figure captions. Wild-type (WT) isolates and non-WT isolates with resistance mechanisms are shown with empty and filled symbols, respectively.

resulted in MICs that were decreased by 16-fold (fluconazole) and 4-fold (voriconazole) in strain DSY3706.

**DISCUSSION**

This study tested the mechanisms of isavuconazole resistance in *Candida* species using either *S. cerevisiae* as a vector for expressing specific azole resistance genes or various *C. albicans* and *C. glabrata* isolates with known resistance mechanisms to determine azole susceptibility. Isavuconazole had mechanisms of resistance similar to those of other azoles. Its range of activity was comparable to that of voriconazole in the *Candida* strains tested in this study.

The presence or absence of ABC transporters had the greatest effect on the MICs of isavuconazole as well as those of voriconazole, posaconazole, and itraconazole. The expression of *CDR1*, *CDR2*, *CgCDR1*, and *CgCDR2* in *S. cerevisiae* resulted in elevated isavuconazole MICs compared to those of the controls, indicating that isavuconazole is a substrate for these transporters. This is particularly the case with the *CDR1* and *CgCDR1* transporters of *C. albicans* and *C. glabrata*, respectively. The expression of these transporters resulted in the largest increases in MICs for isavuconazole. Changes in MICs due to the expression of *CDR* genes were similar to those for isavuconazole, voriconazole, and itraconazole, although isavuconazole exhibited average MIC increases compared to those of other drugs.

*MDR1* and *FLU1* expression did not result in increases in MIC levels for isavuconazole, demonstrating that isavuconazole is not a substrate for MFS transporters. The opposite was observed for fluconazole and voriconazole, for which increases in MICs were linked to the expression of these transporters. This is supported in a study by Cheng et al. (43) in which *MDR1* overexpression in a *C. albicans* petite mutant was associated with increased resistance to fluconazole and voriconazole, but the strain remained susceptible to itraconazole and ketoconazole.

The MIC increases for isavuconazole were moderate when associated with *ERG11* mutations in the *S. cerevisiae* model. The MICs for fluconazole demonstrated the greatest variations, followed by those for voriconazole. A closer look at all azole susceptibility profiles indicated that the profile of fluconazole was distinct from those of isavuconazole and itraconazole. Interestingly, posaconazole MICs exhibited no changes with any of the mutant alleles expressed in *S. cerevisiae*. The largest effect on the MIC for isavuconazole was with *S. cerevisiae* strains expressing *ERG11* alleles with the Y132H mutation either alone or combined with mutation S405F or G464S. These results are consistent with recent Erg1p crystallographic data published by Monk et al. (44), who posited that individualazole molecules interact with specific Erg1p residues depending on their structure. Therefore, we expect that *ERG11* mutations do not have the same effect on the binding efficiency of specific azoles.

When testing *C. albicans* and *C. glabrata* isolates, MIC changes for isavuconazole were similar to those observed for fluconazole, voriconazole, itraconazole, and posaconazole. In general, isavuconazole was more active in *C. albicans* than in *C. glabrata* (MIC ranges of 0.004 to 8 μg/ml and 0.25 to 16 μg/ml, respectively). This difference also has been observed in other studies (20, 45). *C. albicans* clinical isolate DSY289 had the highest isavuconazole MIC (8 μg/ml). This strain originally was isolated from an HIV-positive patient who had oropharyngeal candidiasis treated with fluconazole (25); this strain is associated with the *ERG11* mutation S405F/Y132H and with the A736V mutation in *TAC1*. The *TAC1* mutation is associated with increased *CDR1* and *CDR2* levels (29). This suggests that combined resistance mechanisms result in high resistance levels against isavuconazole and other azoles. This is consistent with data in the present study, in which the MICs of non-WT isolates were shifted to higher values than those of WT isolates. However, comparison of relative increases in MICs for fluconazole (512-fold) and voriconazole (1,026-fold) to that of isavuconazole (256-fold) indicates that the effect of a combined azole resistance mechanism was reduced on isavuconazole.

Increases in MICs for isavuconazole in *C. glabrata* clinical isolates were observed when the *CgPDR1* GOF mutation was present in non-WT isolates. These mutations cause the upregulation of *CgCDR1* and *CgCDR2* (46, 47). Relative increases for isavuconazole ranged from 8- to 32-fold, similar to values reached by other azoles. However, the analysis of correlation coefficients between relative MIC increases by each azole in specific isolates highlighted...
that the susceptibility profile of isavuconazole was closely related to those of posaconazole and itraconazole in *C. albicans* but distinct from that of fluconazole. In *C. glabrata* this tendency was not verified, since the profile of isavuconazole was more closely related to that of fluconazole. The present study also reflects these features, as the MIC profile for isavuconazole was similar to the MIC profile for fluconazole in *C. albicans* and for the fluconazole profile in *C. glabrata*. Azole resistance mechanisms in *C. albicans* and *C. glabrata* are not equally distributed between the two species and therefore may have contributed to the observed susceptibility profile differences. On the other hand, isavuconazole exhibits a chemical structure that is different from that of the two structurally related groups of fluconazole/voriconazole and posaconazole/itraconazole (48). Therefore, isavuconazole might adopt susceptibility profiles that cannot be predicted simply from structural resemblance to the two known azole groups.

The absence of *CDR1* from *C. albicans* mutants had the greatest effect on the MICs of azoles. The lowest MICs for both fluconazole and isavuconazole were associated with *CDR1* knockout either on its own or in association with the deletion of other transporters. Isavuconazole and fluconazole had similar MIC profiles in these mutants, while decreases in MICs were less pronounced for itraconazole and posaconazole. The inactivation of known resistance mechanisms demonstrated that TAC1 had the greatest effect on isavuconazole, with its deletion resulting in decreases in MICs and its reintroduction increasing them.

This study suggests that isavuconazole follows the resistance patterns observed in other azoles, with slight variations depending on the investigated fungal species. Currently, there is a lack of data on isavuconazole susceptibility patterns among clinical *Candida* isolates. In an Egyptian epidemiology study, all strains of *Candida* isolated from 187 patients were susceptible to isavuconazole (range, <0.016 to 1 µg/ml), while overall resistance to voriconazole was 2.5% in all strains (49). Another study demonstrated isavuconazole to be highly active against 296 *Candida* bloodstream isolates; the activity of isavuconazole was more potent than that of fluconazole against all organisms tested, and often it was more potent than that of itraconazole or voriconazole (20). It also was noted that only two isolates, both *C. glabrata*, had a MIC for isavuconazole of >0.5 µg/ml. In a study investigating approximately 1,400 *Candida* species, isa-
iuconazole exhibited high activity. A few isolates with high isavuconazole MICs in this study (1 to 8 μg/ml) could not be analyzed for their cross-resistance to other azoles (42). C. albicans and C. glabrata isolates were directly compared by using isavuconazole MICs. Fluconazole- and voriconazole-resistant isolates corresponded to high isavuconazole MICs in C. albicans (0.12 to 1 μg/ml) and C. glabrata (1 to 8 μg/ml), which overlap values obtained in the study reported here (42).

Isavuconazole activity has been investigated in other fungal species with respect to a possible correlation betweenazole resistance and levels of isavuconazole MICs. One study on Aspergillus fumigatus highlighted that the acquisition of resistance to voriconazole and itraconazole due to Cyp51 mutations was followed by an increase of isavuconazole MICs compared to those of susceptible isolates (50). Thus, isavuconazole does not deviate from other azoles in the characteristic decrease of activity in the presence of azole resistance mechanisms.

In conclusion, isavuconazole, as a substrate of multidrug ABC transporters, has properties in common with other azoles. However, in contrast to fluconazole and voriconazole, isavuconazole is not a substrate for the MFS transporter MDR1 or FLU1. Isavuconazole was sensitive to mutations in ERG11, but these mutations had less impact on MIC increases in isavuconazole than they did on MIC increases for fluconazole and voriconazole. These mutations had minimal effect on MICs of posaconazole. Isavuconazole had an activity range similar to that of voriconazole in the Candida strains tested in this study.

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REFERENCES


TABLE 8 Activity of azoles in C. albicans mutants reconstituting the azole-susceptible parent

<table>
<thead>
<tr>
<th>C. albicans ERG11/TAC1 mutant</th>
<th>Resistance mechanism/genotype</th>
<th>MIC (μg/ml) (fold difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSY294</td>
<td>WT</td>
<td>Fluconazole: 0.5 (1) 0.0156 (1) 0.0156 (1) 0.0039 (1) 0.0156 (1)</td>
</tr>
<tr>
<td>DSY296</td>
<td>ERG11 (G464S), TAC1 (N977D)</td>
<td>64 (128) 0.125 (8) 0.25 (16) 0.5 (128) 2 (128)</td>
</tr>
<tr>
<td>DSY3082</td>
<td>ERG11, tac1ΔΔ</td>
<td>0.5 (1) 0.0156 (1) 0.0156 (1) 0.0078 (2) 0.0312 (2)</td>
</tr>
<tr>
<td>DSY3083</td>
<td>ERG11 (G464S), tac1ΔΔ</td>
<td>4 (8) 0.0078 (0.5) 0.0312 (2) 0.0156 (4) 0.0312 (2)</td>
</tr>
<tr>
<td>DSY3706</td>
<td>tac1ΔΔ, ERG11</td>
<td>0.25 (0.5) 0.0156 (1) 0.0156 (1) 0.0039 (1) 0.0156 (1)</td>
</tr>
<tr>
<td>DSY3606-1</td>
<td>tac1ΔΔ, ERG11, TAC1-5 (N977D)</td>
<td>8 (32) 0.125 (4) 0.0625 (2) 0.0625 (16) 0.5 (64)</td>
</tr>
<tr>
<td>DSY3608-2</td>
<td>tac1ΔΔ, ERG11, TAC1-1</td>
<td>0.125 (0.5) 0.0312 (1) 0.0156 (1) 0.0039 (1) 0.0156 (2)</td>
</tr>
<tr>
<td>DSY3604</td>
<td>tac1ΔΔ, ERG11 (G464S)</td>
<td>0.5 (1) 0.0312 (2) 0.0312 (2) 0.0078 (2) 0.0078 (0.5)</td>
</tr>
</tbody>
</table>

a Fold difference relative to the level of the WT for each azole.

b Heterozygous state.
Mechanisms of Azole Resistance to Isavuconazole


