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Azasordarins: Susceptibility of Fluconazole-Susceptible and Fluconazole-Resistant Clinical Isolates of *Candida* spp. to GW 471558

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The in vitro activity of the azasordarin GW 471558 was compared with those of amphotericin B, flucytosine, itraconazole, and ketoconazole against 177 clinical isolates of *Candida* spp. GW 471558 showed potent activity against *Candida albicans*, *Candida glabrata*, and *Candida tropicalis*, even against isolates with decreased susceptibility to azoles. *Candida krusei, Candida parapsilosis, Candida lusitaniae,* and *Candida guilliermondii* are resistant to GW 471558 in vitro (MICs, >128 μg/ml).

The sordarins are a new class of antifungal drugs with a novel and unusual mode of action in antifungal therapies. These compounds interfere with protein synthesis through inhibition of protein elongation factor 2 (2, 3). The sordarins have shown in vitro activity against *Candida* species, *Pneumocystis carinii*, and some filamentous fungi (5). They have demonstrated synergy or additive effects against yeasts, *Aspergillus* spp., and *Scedosporium apiospermum* when combined with other systemic antifungal agents (M. E. Alvarez, E. Herreros, A. Sanchez-Sousa, D. Gargallo-Viola, and F. Baquero, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-12, p. 454). In vivo, these compounds have shown efficacy in murine models of candidosis, and a recent report has pointed out activities of sordarins in murine histoplasmosis (1, 4). In addition, sordarins display good bioavailability and low toxicity in murine models (1).

The azasordarins are a new family of sordarins characterized by the presence of a 6-methylmorpholin-2-yl group with different N-4 substituents instead of the sugar moiety and show an improved profile of biological properties. GW 471558 is an azasordarin that shows in vitro activity against clinical isolates of yeasts and filamentous fungi (E. Herreros, M. J. Almela, S. Lozano., and D. Gargallo-Viola, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-202, p. 353; E. Herreros, M. J. Almela, S. Lozano, C. M. Martinez, and D. Gargallo-Viola, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-201, p. 353; M. Lozano-Chiu, V. L. Paetznich, J. R. Rodriguez, and J. Rex, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-292, p. 351). However, the number of clinical isolates included in previous studies has been limited. This work evaluated the in vitro activity of GW 471558 against 177 isolates of *Candida* spp. (123 fluconazole [FLZ] susceptible and 54 with decreased susceptibility to FLZ) and compared the results obtained with those obtained with amphotericin B (AMB), itraconazole (ITZ), ketoconazole (KTZ), and flucytosine (5FC).

Organisms. A collection of 177 clinical isolates were tested. Isolates were recovered during 1998 and 1999 from 62 Spanish hospitals. Tables 1 and 2 display the species distribution. Sixty-four strains were recovered from oropharyngeal exudates, 58 were from blood cultures, 15 were from vaginal samples, and 40 were from other specimens. Each strain represented a unique isolate from a patient and was sent to our laboratory for identification or antifungal susceptibility testing. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as quality control strains in each set of experiments.

Antifungal susceptibility testing. A broth microdilution test was performed by using the National Committee for Clinical Laboratory Standards (NCCLS) reference method (6), with minor modifications (RPMI–2% glucose) (7). GW 471558 (Glaxo-Wellcome S.A., Madrid, Spain), AMB (Squibb, Madrid, Spain), FLZ (Pfizer, Madrid, Spain), ITZ (Janssen Farmaceútica, Madrid, Spain), KTZ (Janssen Farmaceútica), and 5FC (Sigma Aldrich Química, Madrid, Spain) were obtained as standard powders. Stock solutions were prepared in 100% dimethyl sulfoxide (Sigma Aldrich Química), except for 5FC, which was dissolved in sterile distilled water. Sterile flat-bottom microtitration trays were prepared with the antifungal agents and were inoculated with 100 μl into each well (final inocula, 0.5×10^5 to 2.5×10^5 CFU/ml). Spectrophotometric readings were performed with a Labsystems IEMS Reader MF (Labsystems, Madrid, Spain) at 540 nm. The MIC of GW 471558 was defined as the lowest concentration resulting in 95% inhibition of growth compared to that of a drug-free control (Lozano-Chiu et al., 40th ICAAC). The MIC of AMB was defined as 80% inhibition, and the MICs of 5FC, FLZ, ITZ, and KTZ were defined as 50% inhibition.

Statistical analysis. Data are reported as the MIC ranges and the MICs of each antifungal agent necessary to inhibit 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates tested. The significance of the differences in the distribution of MICs of GW 471558 and the other antifungal agents between isolates

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TABLE 1. Drug susceptibilities of 123 FLZ-S[a] isolates of *Candida* spp.

Organism (no. of isolates) and antifungal agent	MIC_{50}[b]	MIC_{90}[b]	MIC range[b]
C. albicans (58)			
AMB	0.50	1.00	0.12–1.00
5FC	0.12	0.50	0.03–1.00
ITZ	0.06	0.25	0.015–0.50
KTZ	≤0.0002	0.001	≤0.0002–0.06
GW 471558	≤0.0002	0.001	≤0.0002–0.06
C. parapsilosis (25)			
AMB	1.00	2.00	0.12–2.00
5FC	0.12	0.50	0.06–0.50
ITZ	0.03	0.25	0.015–0.25
KTZ	0.001	0.007	≤0.0002–0.015
GW 471558	128.0	>128.0	128.0–>128.0
C. tropicalis (24)			
AMB	0.50	1.00	0.03–1.00
5FC	0.06	0.25	0.015–16.0
ITZ	0.06	0.25	0.15–0.50
KTZ	0.001	0.007	≤0.0002–0.15
GW 471558	0.06	1.00	≤0.0002–1.00
C. glabrata (8)			
AMB	0.50	ND[c]	0.50–1.00
5FC	0.12	ND	0.03–0.25
ITZ	0.50	ND	0.50–1.00
KTZ	0.12	ND	0.06–0.50
GW 471558	0.50	ND	0.06–2.00
C. guilliermondii (4)			
AMB	1.00	ND	0.50–1.00
5FC	0.50	ND	0.50–1.00
ITZ	0.50	ND	0.25–0.50
KTZ	0.06	ND	0.06
GW 471558	>128.0	ND	>128.0
C. lusitaniae (4)			
AMB	0.50	ND	0.50–1.00
5FC	0.25	ND	0.25–0.50
ITZ	0.06	ND	0.03–0.12
KTZ	≤0.0002	ND	≤0.0002
GW 471558	>128.0	ND	>128.0

[a] MIC, <16 μg/ml.
[b] All of the values shown are in micrograms per milliliter.
[c] ND, not determined when the number of isolates was <10.

TABLE 2. Drug susceptibilities of 54 FL2-R[a] isolates of *Candida* spp.

Organism (no. of isolates) and antifungal agent	MIC_{50}[b]	MIC_{90}[b]	Range[b]
C. albicans (19)			
AMB	0.50	1.00	0.12–1.00
5FC	0.25	0.50	0.03–0.50
ITZ	0.50	1.00	0.12–1.00
KTZ	0.015	0.06	≤0.0002–0.25
GW 471558	0.007	0.03	≤0.0002–0.06
C. krusei (16)			
AMB	1.00	2.00	0.12–2.00
5FC	2.00	4.00	0.06–0.50
ITZ	0.50	1.00	0.015–0.25
KTZ	1.00	2.00	0.03–2.00
GW 471558	128.0	>128.0	128.0–>128.0
C. glabrata (11)			
AMB	1.00	1.00	0.50–1.00
5FC	0.12	0.50	0.06–0.50
ITZ	1.00	16.0	0.50–16.0
KTZ	1.00	4.00	0.06–8.00
GW 471558	0.50	1.00	0.06–1.00
C. tropicalis (5)			
AMB	0.50	ND[c]	0.50–1.00
5FC	0.06	ND	0.06–0.12
ITZ	0.25	ND	0.12–0.25
KTZ	0.06	ND	0.015–0.06
GW 471558	0.50	ND	0.50–1.00
C. guilliermondii (3)			
AMB	0.50	ND	0.50–1.00
5FC	0.12	ND	0.12–0.25
ITZ	0.50	ND	0.50
KTZ	0.06	ND	0.06–0.12
GW 471558	>128.0	ND	>128.0

[a] MIC, ≥16 μg/ml.
[b] All of the values shown are in micrograms per milliliter.
[c] ND, not determined when the number of isolates was <10.

was determined by the unpaired Student *t* test; $P < 0.01$ was considered to show a statistically significant difference. The correlation among the MICs of the antifungal agents was determined by Pearson's *r* coefficient that was expressed over a maximum value of 1. MICs were transformed on $\log_2$ data. Control limits for quality control strains were defined as ranges which included 1 doubling concentration on either side of the mode. All statistical analyses were done with the Statistical Package for the Social Sciences, version 10.0 (SPSS S.L., Madrid, Spain).

FLZ-susceptible isolates (FLZ-S) were isolates for which the FLZ MIC was <16 mg/liter. FLZ-resistant isolates (FLZ-R) were strains for which the FLZ MIC was increased (>16 mg/liter) and included the susceptible dose-dependent (S-DD) and resistant categories of the NCCLS (6). An analysis of the S-DD and resistant strains done separately showed no differences in azasordarin MICs.

The MICs obtained for the control organisms varied by no more than 3 twofold dilutions and were similar in range to the reference values of AMB, FLZ, ITZ, KTZ, and 5FC (6). The MICs of GW 471558 were 128 to >128 μg/ml for *C. parapsilosis* ATCC 22019 and >128 μg/ml for *C. krusei* ATCC 6258.

Table 1 displays the distribution of the MICs of AMB, 5FC, ITZ, KTZ, and GW 471558 for 123 FLZ-S isolates of *Candida* spp. Table 2 summarizes the MICs for the 54 FLZ-R strains tested. GW 471558 had higher in vitro activity than AMB, 5FC, and ITZ and had activity similar to that of KTZ against both FLZ-S and FLZ-R *Candida albicans* isolates. Tables 1 and 2 show that for FLZ-resistant isolates, the GW 471558 MICs were proportionally higher than those for FLZ-S isolates. This trend not was statistically significant, however ($P = 0.241$ by the Student *t* test). The new azasordarin showed good in vitro activity against FLZ-S and FLZ-R isolates of *Candida glabrata* and *Candida tropicalis*, and no significant differences were observed between FLZ-S and FLZ-R strains ($P = 0.780$ and 0.719, respectively). This activity was similar to those of the other drugs tested. Finally, *C. parapsilosis, C. krusei, Candida lusitaniae*, and *Candida guilliermondii* are resistant to GW 471558 in vitro (MIC, >128 μg/ml). The MICs of GW 471558 did not correlate with those of AMB, 5FC, FLZ, ITZ, and

KTZ, and the Pearson coefficients obtained were not statistically significant ($P > 0.01$). Likewise, no significant differences were encountered when an analysis by species was performed.

The sordarins are a new class of compounds causing selective inhibition of the fungal protein synthesis system (2). In 1997, the agent GR 135402 was isolated from *Graphium putredinis* and characterized (3, 5). It was the first sordarin with potent and selective activity against protein synthesis by *C. albicans*. Azasordarins are a new family of sordarins characterized by an increased profile of antifungal properties. GW 471558 has been identified as a lead compound of this family. In addition, more agents belonging the azasordarin group and presenting antifungal activity have been identified (GW 471552, GW 506540, GW 531920, and GW 560849) (Herreros et al., 40th ICAAC, abstr. J-210; Lozano-Chiu et al., 40th ICAAC). This family constitutes a promising group of antifungal agents that merits more comprehensive studies.

This work shows the in vitro susceptibility to GW 471558, AMB, 5FC, ITZ, and KTZ of 123 FLZ-S and 54 FLZ-R clinical isolates of *Candida* spp. The azasordarin showed potent in vitro activity against *C. albicans* and *C. tropicalis*. No evidence of cross-resistance was found among FLZ-R isolates belonging to these species. In addition, GW 471558 showed in vitro activity against *C. glabrata*, a species usually considered refractory to azoles. On the other hand, *C. parapsilosis, C. krusei, C. guilliermondii,* and *C. lusitaniae* were intrinsically resistant to GW 471558.

In summary, it can be concluded from the in vitro data presented here that GW 471558 has extremely potent activity against both FLZ-S and FLZ-R isolates of *C. albicans*. This sordarin derivative shows in vitro activity similar to that of other antifungal agents against *C. tropicalis* and *C. glabrata*, even against strains with decreased susceptibility to azoles. The other *Candida* spp. tested are resistant to GW 471558 in vitro. The excellent in vitro activity against azole-resistant *C. albicans* strains may have important implications for the treatment of infections due to this yeast. GW 471558 is a promising new antifungal agent that merits more comprehensive clinical studies to determine the correlation between these data and in vivo outcome.

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