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# In Vitro Susceptibilities of *Candida* Bloodstream Isolates to the New Triazole Antifungal Agents BMS-207147, Sch 56592, and Voriconazole

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**BMS-207147, Sch 56592, and voriconazole are three new investigational triazoles with broad-spectrum antifungal activity. The in vitro activities of these three agents were compared with those of itraconazole and fluconazole against 1,300 bloodstream isolates of *Candida* species obtained from over 50 different medical centers in the United States. The MICs of all of the antifungal drugs were determined by broth microdilution tests performed according to the National Committee for Clinical Laboratory Standards method using RPMI 1640 as a test medium. BMS-207147, Sch 56592, and voriconazole were all quite active against all *Candida* sp. isolates (MICs for 90% of the isolates tested [$MIC_{90}$s], 0.5, 1.0, and 0.5 μg/ml, respectively). *Candida albicans* was the most susceptible species ($MIC_{90}$s, 0.03, 0.06, and 0.06 μg/ml, respectively), and *C. glabrata* was the least susceptible ($MIC_{90}$s, 4.0, 4.0, and 2.0 μg/ml, respectively). BMS-207147, Sch 56592, and voriconazole were all more active than itraconazole and fluconazole against *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. There existed a clear rank order of in vitro activity of the five azoles examined in this study when they were tested versus *C. glabrata*: voriconazole > BMS-207147 = Sch 56592 = itraconazole > fluconazole ($MIC_{90}$s, 2.0, 4.0, 4.0, 4.0, and 64 μg/ml, respectively). For isolates of *Candida* spp. with decreased susceptibility to both itraconazole and fluconazole, the MICs of BMS-207147, Sch 56592, and voriconazole were also elevated. These results suggest that BMS-207147, Sch 56592, and voriconazole all possess promising antifungal activity and that further in vitro and in vivo investigations are warranted to establish the clinical value of this improved potency.**

BMS-207147, Sch 56592, and voriconazole are all investigational triazole antifungal agents that are in different stages of in vitro and clinical investigation (2, 3, 7). Previous in vitro studies of these agents indicate a spectrum of antifungal activity broader than that of fluconazole (1–4, 6, 7, 10, 11). All three investigational triazoles are lipophilic and exhibit activity similar to that of itraconazole against clinically important yeast isolates (2, 3, 7). However, earlier investigations have included limited numbers of clinical yeast isolates and have compared each of these new agents individually against fluconazole and itraconazole. None of the previously published reports have compared all three investigational triazoles simultaneously against a large (>1,000 isolates) collection of recent, clinically important *Candida* isolates. In this study, we compare the in vitro activities of BMS-207147, Sch 56592, and voriconazole with those of fluconazole and itraconazole against 1,300 clinical isolates of *Candida* spp. from bloodstream infections. The in vitro antifungal susceptibility test method employed was the broth microdilution adaptation of the macrodilution reference method described in National Committee for Clinical Laboratory Standards (NCCLS) document M27-A (5).

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## MATERIALS AND METHODS

**Organisms.** A total of 1,300 bloodstream isolates of *Candida* spp. obtained from over 50 U.S. medical centers between 1992 and 1997 were selected for testing. Approximately 550 isolates were obtained as part of a population-based survey of invasive mycoses conducted by the Centers for Disease Control and Prevention (8), and the remainder were from various medical centers throughout the United States. Included were *C. albicans* (660 isolates), *C. parapsilosis* (221 isolates), *C. glabrata* (217 isolates), *C. tropicalis* (139 isolates), *C. krusei* (33 isolates), and other *Candida* spp. (30 isolates, including 7 of *C. lusitaniae*, 7 of *C. guilliermondii*, 3 of *C. famata*, 2 of *C. rugosa*, 1 of *C. lipolytica*, 1 of *C. lambica*, and 9 of *Candida* unidentified to species level). All isolates were identified by standard methods (12) and stored as water suspensions at ambient temperature until used in the study. Prior to testing, each isolate was subcultured at least twice on potato dextrose agar plates (Remel, Lenexa, Kans.) to ensure purity and optimal growth.

**Antifungal agents.** Standard antifungal powders of BMS-207147 (Bristol-Myers Squibb), Sch 56592 (Schering-Plough), voriconazole (Pfizer), fluconazole (Pfizer), and itraconazole (Janssen) were obtained from their respective manufacturers. Stock solutions were prepared in dimethyl sulfoxide (BMS-207147 and voriconazole), polyethylene glycol (Sch 56592 and itraconazole), or water (fluconazole). Serial twofold dilutions of each antifungal agent were prepared exactly as outlined in NCCLS document M27-A (5). Final dilutions were made in RPMI 1640 medium (Sigma Chemical Co., St. Louis, Mo.) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma). The final concentration of the solvent did not exceed 1% in any well. Aliquots (0.1 ml) of each antifungal agent at a 2× final concentration were dispensed into the wells of plastic microdilution trays by using a Quick Spense II System (Dynatech Laboratories, Chantilly, Va.). The trays were sealed and frozen at −70°C until they were used.

**Antifungal susceptibilities studies.** Broth microdilution testing was performed in accordance with the guidelines in NCCLS document M27-A (5) by using the spectrophotometric method of inoculum preparation, an inoculum concentration of $(1.5 \pm 1.0) \times 10^3$ cells/ml, and RPMI 1640 medium buffered to pH 7.0 with MOPS. A 100-μl yeast inoculum was added to each well of the microdilution trays. The final concentrations of the antifungal agents were 0.008 to 8.0 μg/ml for BMS-207147, Sch 56592, voriconazole, and itraconazole and 0.12 to 128

TABLE 1. In vitro susceptibilities of 1,300 *Candida* bloodstream isolates to BMS-207147, Sch 56592, and voriconazole versus fluconazole and itraconazole

| Organism (no. of isolates) and antifungal agent | MIC (μg/ml) | | |
|---|---|---|---|
| | Range | 50% of isolates | 90% of isolates |
| *C. albicans* (660) | | | |
| BMS-207147 | 0.008–>8.0 | 0.008 | 0.03 |
| Sch 56592 | 0.008–>8.0 | 0.03 | 0.06 |
| Voriconazole | 0.008–>8.0 | 0.015 | 0.06 |
| Fluconazole | 0.12–>128 | 0.25 | 1.0 |
| Itraconazole | 0.015–>8.0 | 0.06 | 0.25 |
| *C. parapsilosis* (221) | | | |
| BMS-207147 | 0.008–4.0 | 0.03 | 0.12 |
| Sch 56592 | 0.008–>8.0 | 0.12 | 0.12 |
| Voriconazole | 0.008–2.0 | 0.03 | 0.12 |
| Fluconazole | 0.12–64 | 1.0 | 2.0 |
| Itraconazole | 0.015–2.0 | 0.12 | 0.5 |
| *C. glabrata* (217) | | | |
| BMS-207147 | 0.008–>8.0 | 0.5 | 4.0 |
| Sch 56592 | 0.015–>8.0 | 1.0 | 4.0 |
| Voriconazole | 0.03–8.0 | 0.5 | 2.0 |
| Fluconazole | 0.25–>128 | 16 | 64 |
| Itraconazole | 0.06–>8.0 | 1.0 | 4.0 |
| *C. tropicalis* (139) | | | |
| BMS-207147 | 0.008–>8.0 | 0.03 | 0.25 |
| Sch 56592 | 0.015–>8.0 | 0.06 | 0.25 |
| Voriconazole | 0.008–>8.0 | 0.06 | 0.25 |
| Fluconazole | 0.25–>128 | 0.5 | 2.0 |
| Itraconazole | 0.03–>8.0 | 0.12 | 0.5 |
| *C. krusei* (33) | | | |
| BMS-207147 | 0.06–2.0 | 0.25 | 0.5 |
| Sch 56592 | 0.12–1.0 | 0.5 | 0.5 |
| Voriconazole | 0.06–4.0 | 0.5 | 1.0 |
| Fluconazole | 8.0–128 | 64 | 64 |
| Itraconazole | 0.12–2.0 | 1.0 | 2.0 |
| *Candida* spp. (30)[a] | | | |
| BMS-207147 | 0.008–8.0 | 0.12 | 0.5 |
| Sch 56592 | 0.03–>8.0 | 0.12 | 0.5 |
| Voriconazole | 0.008–8.0 | 0.06 | 0.5 |
| Fluconazole | 0.25–128 | 2.0 | 8.0 |
| Itraconazole | 0.03–>8.0 | 0.25 | 1.0 |
| All organisms (1,300) | | | |
| BMS-207147 | 0.008–>8.0 | 0.03 | 0.5 |
| Sch 56592 | 0.008–>8.0 | 0.06 | 1.0 |
| Voriconazole | 0.008–>8.0 | 0.03 | 0.5 |
| Fluconazole | 0.12–>128 | 0.5 | 16 |
| Itraconazole | 0.015–>8.0 | 0.12 | 1.0 |

[a] Includes seven *C. lusitaniae*, seven *C. guilliermondii*, three *C. famata*, two *C. rugosa*, one *C. lipolytica*, one *C. lambica*, and nine *Candida* sp. isolates.

μg/ml for fluconazole. The trays were incubated in air at 35°C, and MIC endpoints were read after 48 h of incubation. Drug-free and yeast-free controls were included.

Following incubation, the broth microdilution wells were examined with the aid of a reading mirror; the growth in each well was compared with that of the growth control (drug-free) well. The MIC of each triazole was defined as the lowest concentration resulting in 80% inhibition of growth compared to that for untreated controls (5). The data are reported as the concentrations of each antifungal agent necessary to inhibit 50% ($MIC_{50}$) and 90% ($MIC_{90}$) of the isolates tested.

**Quality control.** Quality was controlled by testing the following strains recommended by NCCLS document M27-A (5): *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258.

## RESULTS AND DISCUSSION

Table 1 summarizes the in vitro susceptibilities of 1,300 isolates of *Candida* spp. to BMS-207147, Sch 56592, voriconazole, fluconazole, and itraconazole. A broad range of MICs was observed with each antifungal agent. Overall, BMS-207147, Sch 56592, and voriconazole were quite active ($MIC_{90}$s, 0.5, 1.0, and 0.5 μg/ml, respectively) against these isolates. *C. albicans* was the most susceptible species ($MIC_{90}$s, 0.03, 0.06, and 0.06 μg/ml, respectively), and *C. glabrata* was the least susceptible ($MIC_{90}$s, 4.0, 4.0, and 2.0 μg/ml, respectively) to the three investigational triazoles. BMS-207147, Sch 56592, and voriconazole were all more active than both itraconazole and fluconazole against *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. Voriconazole was also more active than both itraconazole and fluconazole against *C. glabrata* ($MIC_{90}$s, 2.0, 4.0, and 64 μg/ml, respectively). BMS-207147 and Sch 56592 were comparable to itraconazole ($MIC_{90}$, 4.0 μg/ml) but more active than fluconazole against *C. glabrata* ($MIC_{90}$, 4.0 versus 64 μg/ml).

One hundred forty-six strains (121 of *C. glabrata*, 14 of *C. krusei*, 6 of *C. parapsilosis*, and 4 of *C. albicans*) were inhibited by 16- to 32-μg/ml fluconazole (dose-dependently susceptibility) (9). The $MIC_{90}$s of each of the investigational triazoles ranged from 1.0 to 2.0 μg/ml for these isolates and were not significantly different from those observed for the overall isolate collection (data not shown).

Among the 1,300 isolates studied, a total of 53 strains (12 of *C. albicans*, 13 of *C. krusei*, 25 of *C. glabrata*, and 3 of *C. tropicalis*) were resistant to both fluconazole (MIC, ≥64 μg/ml) and itraconazole (MIC, ≥1 μg/ml) (9). The MICs of BMS-207147 (0.03 to >8.0 μg/ml; mode, ≥8.0 μg/ml), Sch 56592

TABLE 2. In vitro activities of BMS-207147, Sch56592, and voriconazole against isolates of *Candida* species with decreased susceptibility to fluconazole and itraconazole

| Species (no. of isolates) and antifungal agent | MIC (μg/ml) | | |
|---|---|---|---|
| | Range | 50% of isolates | 90% of isolates |
| *C. albicans* (12) | | | |
| BMS-207147 | 0.03–>8.0 | 0.12 | >8 |
| Sch 56592 | 0.03–>8.0 | 8.0 | >8.0 |
| Voriconazole | 0.25–>8.0 | >8.0 | >8.0 |
| Fluconazole | 64–>128 | >128 | >128 |
| Itraconazole | 1.0–>8.0 | >8.0 | >8.0 |
| *C. glabrata* (25) | | | |
| BMS-207147 | 0.5–>8.0 | 4.0 | 8.0 |
| Sch 56592 | 1.0–>8 | 4.0 | >8.0 |
| Voriconazole | 1.0–8.0 | 4.0 | 8.0 |
| Fluconazole | 64–>128 | 128 | >128 |
| Itraconazole | 2.0–>8.0 | >8.0 | >8.0 |
| *C. krusei* (13) | | | |
| BMS-207147 | 0.25–0.5 | 0.25 | 0.5 |
| Sch 56592 | 0.25–1.0 | 0.5 | 0.5 |
| Voriconazole | 0.25–1.0 | 0.5 | 1.0 |
| Fluconazole | 64–128 | 64 | 128 |
| Itraconazole | 1.0–2.0 | 1.0 | 2.0 |
| *C. tropicalis* (3) | | | |
| BMS-207147 | >8.0 | >8.0 | |
| Sch 56592 | 8–>8.0 | >8.0 | |
| Voriconazole | 4–>8.0 | >8.0 | |
| Fluconazole | 128–>128 | >128 | |
| Itraconazole | >8.0 | >8.0 | |

(0.03 to >8.0 μg/ml; mode, ≥8.0 μg/ml), and voriconazole (0.25 to >16 μg/ml; mode, ≥16 μg/ml) were also elevated with these isolates (Table 2).

These results support and extend findings reported previously (2, 3, 7). We found that all three investigational triazoles were more active than fluconazole against all of the *Candida* spp. tested. In almost every case, the in vitro potencies of BMS-207147, Sch 56592, and voriconazole were comparable to one another and slightly greater than that of itraconazole. In contrast to the study of Fung-Tomc et al. (2), ours found BMS-207147 to be quite active against *C. tropicalis* and to have measurable activity against *C. glabrata*. Similar to the studies of Marco et al. (3) and of Pfaller et al. (7), ours found that those isolates of *Candida* spp. with decreased susceptibility to fluconazole and itraconazole were also less susceptible to the investigational triazoles, suggesting cross-resistance that is likely to be mediated by one or more efflux mechanisms (13).

In summary, we found that BMS-207147, Sch 56592, and voriconazole all exhibit excellent in vitro activity against clinically significant isolates of *Candida* spp. These agents are slightly more active than itraconazole and offer important advantages over fluconazole in terms of spectrum and potency. As always, the translation of this in vitro activity into clinical efficacy must be established. Further investigation of these exciting new agents is encouraged.

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