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Comparison of In Vitro Activities of the New Triazole SCH56592 and the Echinocandins MK-0991 (L-743,872) and LY303366 against Opportunistic Filamentous and Dimorphic Fungi and Yeasts

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The in vitro antifungal activities of SCH56592, MK-0991, and LY303366 against 83 isolates of *Acremonium strictum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Bipolaris* spp., *Blastomyces dermatitidis*, *Cladophialophora bantiana*, *Fusarium oxysporum*, *Fusarium solani*, *Histoplasma capsulatum*, *Phialophora* spp., *Pseudallescheria boydii*, *Rhizopus arrhizus*, *Scedosporium prolificans*, and *Sporothrix schenckii* were compared. The in vitro activities of these agents against 104 isolates of yeast pathogens of *Candida* spp., *Cryptococcus neoformans*, and *Trichosporon beigelii* were also compared. MICs were determined by following a procedure under evaluation by the National Committee for Clinical Laboratory Standards (NCCLS) for broth microdilution testing of the filamentous fungi (visual MICs) and the NCCLS M27-A broth microdilution method for yeasts (both visual and turbidimetric MICs). The in vitro fungicidal activity of SCH56592 was superior (minimum fungicidal concentrations [MFCs], 0.25 to 4 μg/ml for 7 of 18 species tested) to those of MK-0991 and LY303366 (MFCs, 8 to >16 μg/ml for all species tested) for the molds tested, but the echinocandins had a broader spectrum of fungicidal activity (MFCs at which 90% of strains are inhibited [MFC_{90}s], 0.5 to 4 μg/ml for 6 of 9 species tested) than SCH56592 (MFC_{90}s, 0.25 to 8 μg/ml for 4 of 9 species tested) against most of the yeasts tested. Neither echinocandin had in vitro activity (MICs, >16 μg/ml) against *C. neoformans* and *T. beigelii*, while the SCH56592 MICs ranged from 0.12 to 1.0 μg/ml for these two species. The MICs of the three agents for the other species ranged from <0.03 to 4 μg/ml. These results suggest that these new agents have broad-spectrum activities in vitro; their effectiveness in the treatment of human mycoses is to be determined.

Patients who are immunocompromised due to cancer chemotherapy (1), organ or bone marrow transplantation (12, 20), or human immunodeficiency virus infection (14, 31) are predisposed to severe fungal infections. Although *Candida albicans* is the organism most often associated with serious fungal infections (4, 8, 20), other species of *Candida* as well as filamentous fungi such as *Aspergillus* and *Fusarium* species, *Pseudallescheria boydii*, and a variety of phaeoid (dematiaceous) fungi have emerged as important pathogens in immunocompromised hosts (13, 22, 25). The numbers of infections caused by the dimorphic fungi *Blastomyces dermatitidis*, *Histoplasma capsulatum*, and *Sporothrix schenckii* (4) have also increased in these patient populations. However, the number of effective antifungal agents available for their treatment has not increased. Amphotericin B and azole derivatives, most notably, fluconazole and itraconazole, are the primary drugs used for the treatment of serious fungal infections (13a). Limitations in the efficacy and/or tolerability of established agents, however, have prompted a search for new drugs that may be effective in the management of severe and refractory fungal infections in these patients. Most of the investigational agents are either azole derivatives or glucan inhibitor echinocandins.

SCH56592 is a new triazole derivative that has in vitro activity against *Candida* spp. (9, 17) and *Aspergillus* spp. (23) and in vivo activity against *Cryptococcus neoformans* (26), *Coccidioides immitis* (18), and *Aspergillus fumigatus* (24). The echinocandins MK-0991 and LY303366 also have been shown to have in vitro and in vivo activities against molds (2, 3, 32) and yeasts (2, 10, 11, 15, 27, 29, 30, 32). These previous studies have compared the in vitro activity of each agent against those of established agents. Although a standard method for the testing of the wide variety of filamentous fungi is not available, the National Committee for Clinical Laboratory Standards (NCCLS) Subcommittee for Antifungal Susceptibility Tests has evaluated the clinical relevance of the microdilution procedure (6, 7) that was used for the susceptibility testing of the molds in this study. The present study was undertaken to determine the in vitro activities of SCH56592, MK-0991, and LY303366 against a wide spectrum of opportunistic filamentous and dimorphic fungi as well as against pathogenic yeasts by following the M27-A microdilution method for the yeasts (21) and the NCCLS testing conditions for the molds (6, 7). Both the MICs and the minimum fungicidal concentrations (MFCs) of each drug for each organism were determined. Since the determination of MICs by the M27-A method is performed by the subjective visual evaluation of growth inhibition, MICs for the yeasts were also determined by a spectrophotometric procedure (28).

MATERIALS AND METHODS

Antifungal agents. SCH56592 (Schering-Plough Research Institute, Bloomfield, N.J.), MK-0991 (Merck Research Laboratories, Rahway, N.J.), and LY303366 (Eli Lilly & Co., Indianapolis, Ind.) were obtained from the manufacturers as standard powders. Drug stock solutions (1,600 μg/ml) were prepared by dissolving the antifungal powders in either polyethylene glycol (SCH56592), 100% dimethyl sulfoxide (LY303366), or sterile distilled water (MK-0991). Additive twofold dilutions of SCH56592 and LY303366 were prepared at 100 times the final concentrations in the 100% corresponding solvents followed by further dilutions (1:50) in the NCCLS standard RPMI 1640 medium to yield two times

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TABLE 1. Susceptibilities of 83 opportunistic filamentous and dimorphic fungi to SCH56592, MK-0991, and LY303366[a]

Fungus (no. tested)	Antifungal agent	MIC range (μg/ml)	Geometric mean MIC (μg/ml)	MFC range (μg/ml)
Opportunistic filamentous fungi				
Acremonium strictum (1)	SCH56592	0.06	ND[b]	>16
	MK-0991	0.5	ND	>16
	LY303366	>16	ND	ND[c]
Aspergillus flavus (11)	SCH56592	0.03–0.12	0.10	0.25–2
	MK-0991	0.5	0.5	ND
	LY303366	<0.03–0.12	0.08	ND
Aspergillus fumigatus (13)[d]	SCH56592	<0.03–1.0	0.13	0.25–>16
	MK-0991	0.5–>16	2.15	ND
	LY303366	0.06	0.06	ND
Aspergillus terreus (2)	SCH59592	<0.03–0.5	ND	1.0
	MK-0991	0.5	ND	ND
	LY303366	<0.03	ND	ND
Bipolaris spp.[e] (6)	SCH56592	0.06–0.25	0.14	0.5–4
	MK-0991	1.0–2	1.7	ND
	LY303366	1.0–4	2.7	ND
Cladophialophora bantiana (5)	SCH56592	<0.03–0.06	0.05	0.06–0.5
	MK-0991	2–8	3.6	8–>16
	LY303366	1.0–4	2	8–>16
Fusarium oxysporum (6)	SCH56592	1–>16	4.16	2–>16
	MK-0991	>16	>16	ND
	LY303366	16–>16	>16	ND
Fusarium solani (6)	SCH56592	>16	>16	ND
	MK-0991	16–>16	>16	ND
	LY303366	>16	>16	ND
Phialophora spp.[f] (5)	SCH56592	0.06–1.0	0.4	2–>16
	MK-0991	1.0–16	2.8	>16
	LY303366	1.0–>16	9	>16
Pseudallescheria boydii (6)	SCH56592	0.5–2	1.0	>16
	MK-0991	0.5–4	1.3	>16
	LY303366	2–4	2.5	>16
Rhizopus arrhizus (5)	SCH56592	2	2	2–16
	MK-0991	>16	>16	ND
	LY303366	>16	>16	ND
Scedosporium prolificans (2)	SCH56592	>16	ND	ND
	MK-0991	4–8	ND	>16
	LY303366	4	ND	ND
Dimorphic fungi				
Blastomyces dermatitidis (5)	SCH56592	<0.03–0.06	0.05	2–16
	MK-0991	0.5–8	2	ND
	LY303366	2–8	4	ND
Histoplasma capsulatum (5)	SCH56592	<0.03–0.06	0.04	0.5–2
	MK-0991	0.5–4	1.3	ND
	LY303366	2–4	3.6	ND
Sporothrix schenckii (5)	SCH56592	0.12–1.0	0.7	1–4
	MK-0991	1.0–>16	5.4	ND
	LY303366	0.25–>16	3.9	ND
Total (83)				

[a] Visual MICs-2 correspond to prominent growth inhibition (approximately ≤50% of that of the growth control).
[b] For the geometric mean MIC column, ND indicates not obtained.
[c] For the MFC range column, ND indicates not done.
[d] The set included two isolates resistant to itraconazole (MICs, >8 μg/ml).
[e] *Bipolaris hawaiiensis* and *Bipolaris spicifera*.
[f] *Phialophora parasitica*, *Phialophora repens*, and *Phialophora verrucosa*.

TABLE 2. Susceptibilities of 104 selected pathogenic yeasts to SCH56592, MK-0991, and LY303366 as determined by a spectrophotometric procedure

Fungus (no. tested)	Antifungal agent	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MFC range (μg/ml)	MFC_{90} (μg/ml)
C. albicans (10)[a]	SCH56592	0.06–1.0 (>16)[b]	0.5	1.0	ND[c]	ND[c]
	MK-0991	0.25–2	0.5	1.0	0.5–>16	2
	LY303366	<0.03–0.25	0.06	0.25	0.25–4	1.0
C. albicans (10)[d]	SCH56592	<0.03–0.06 (>16)	<0.03	<0.03	ND	ND
	MK-0991	0.25–2	0.5	1.0	0.25–1.0	1.0
	LY303366	<0.03–0.25 (<0.03)	0.06	0.06	0.25–1.0	0.5
C. glabrata (12)	SCH56592	<0.03–4	1.0	4	>16	≥16
	MK-0991	0.5–2	0.5	1.0	1–4	2
	LY303366	0.06–0.25	0.12	0.25	0.12–1.0	0.5
C. guilliermondii (8)	SCH56592	<0.03–0.25 (0.06–4)	0.25	ND[e]	4–>16	ND
	MK-0991	>16 (0.5–8)	2	ND	ND	ND
	LY303366	0.25–4	2	ND	4–8	ND
C. krusei (13)	SCH56592	0.5–1.0	1.0	1.0	1.0–2	1.0
	MK-0991	0.5–4	1.0	2	1.0–2	2
	LY303366	0.12–1.0 (<0.03–0.5)	0.5	1.0	0.25–1.0	1.0
C. lusitaniae (12)	SCH56592	<0.03–0.25 (<0.03–1.0)	0.06	0.06	0.06–4	0.25
	MK-0991	1.0–4	1.0	2	1.0–4	1.0
	LY303366	0.25–2 (0.06–2)	1.0	2	1.0–2	2
C. parapsilosis (12)	SCH59592	0.06–0.5 (0.06–>16)	0.25	0.5	1.0–>16	8
	MK-0991	0.5–2 (2–4)	1.0	2	1.0–>16	2
	LY303366	0.5–2	2	2	1.0–4	4
C. tropicalis (12)	SCH56592	0.06–8 (>16)	0.25	0.25	ND	ND
	MK-0991	0.5–2 (<0.03–2)	1.0	1.0	0.5–1.0	1.0
	LY303366	0.12–0.5 (<0.03–0.2)	0.25	0.5	0.12–1.0	1.0
C. neoformans (10)	SCH56592	0.25–0.5	0.25	0.25	0.25–0.5	0.5
	MK-0991	16–>16	>16	>16	ND	ND
	LY303366	>16	>16	>6	ND	ND
T. beigelii (5)	SCH56592	0.12–1.0	1.0	ND	0.5–>16	ND
	MK-0991	16–>16	>16	ND	>16	ND
	LY303366	>16	>16	ND	ND	ND
Total (104)						

[a] Fluconazole MIC, >16 μg/ml; itraconazole MIC, 0.06 to >8 μg/ml.
[b] Values in parentheses are MIC-0 (SCH56592) and MIC-2 (other agents) endpoints.
[c] For MFC columns, ND indicates not done.
[d] Fluconazole MIC, ≤1.0 μg/ml; itraconazole MIC, <0.03 to 0.06 μg/ml.
[e] For the MIC_{90} column, ND indicates not obtained.

the final strength required for the test. Dilutions of MK-0991 were diluted directly in RPMI 1640 medium instead of solvent. The drugs at their final concentrations (0.03 to 16 μg/ml) were frozen at −70°C until they were needed.

Filamentous fungal isolates. One to 13 isolates each of the opportunistic filamentous fungi *Acremonium strictum*, *Aspergillus flavus*, *A. fumigatus* (including two of the three available itraconazole-resistant isolates [MICs, >8 μg/ml]), *Aspergillus terreus*, *Bipolaris* spp., *Cladophialophora bantiana*, *Fusarium oxysporum*, *Fusarium solani*, *Phialophora* spp., *P. boydii*, *Rhizopus arrhizus*, and *Scedosporium prolificans* and the dimorphic fungi *B. dermatitidis*, *H. capsulatum* var. *capsulatum*, and *S. schenckii*, were evaluated. These isolates were recovered from clinical specimens from 83 individual patients with severe fungal infections. These cultures were received at the Medical College of Virginia, Virginia Commonwealth University, from different medical centers in the United States during the last 3 years. Identification of each strain was performed by using routine mycological techniques. Twenty of the opportunistic mold isolates were evaluated in two previous collaborative studies conducted by the NCCLS Subcommittee for Antifungal Susceptibility Tests (6, 7) to identify the optimal testing conditions for this group of fungi. The mold isolates were maintained in sterile water as described previously (19) and were subcultured on antimicrobial agent-free potato dextrose agar to ensure viability and purity.

Yeast isolates. The 104 pathogenic yeast and yeast-like isolates from the Medical College of Virginia, Virginia Commonwealth University, culture collection included 5 to 20 isolates each of *C. albicans*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida tropicalis*, *C. neoformans*, and *Trichosporon beigelii*. The isolates were recovered during the last 3 years from either oral cavities, urine samples, or blood and other sterile body fluids. Each strain represented a unique isolate from a patient managed in one of several medical centers in the United States or Europe. In order to evaluate isolates with different susceptibility patterns, the set included 13 *Candida* sp. strains from AIDS patients with recurrent thrush. The amphotericin B MICs for the strains were high (MICs, ≥2 μg/ml) or the strains were fluconazole and itraconazole resistant or susceptible-dose dependent (MICs, 16 to ≥64 μg/ml and 0.25 to ≥16 μg/ml, respectively). Yeast isolates were also maintained in sterile water (19) and were subcultured on antimicrobial agent-free medium to ensure viability and purity.

Inoculum preparation. (i) Molds. Stock inoculum suspensions of the molds were prepared as described previously (6, 7) from 7-day-old cultures grown on potato dextrose agar. Cultures of *B. dermatitidis* and *H. capsulatum* were incubated for 10 days at 35°C, and cultures of *Fusarium* spp. were grown at 35°C for 48 to 72 h and then at 25 to 28°C until day 7. The stock suspensions were adjusted spectrophotometrically to optical densities (ODs) that ranged from 0.01 to 0.2 and contained conidia or sporangiospores and hyphal fragments. The diluted (two times) inoculum sizes ranged from 0.9×10^4 to 4.7×10^4 CFU/ml, as demonstrated by quantitative colony counts on Sabouraud dextrose agar (SDA).

(ii) Yeasts. Stock inoculum suspensions of the yeasts were obtained from 24-h-old cultures (48-h-old cultures for *C. neoformans*) on SDA at 35°C. The

turbidity of the yeast suspensions was adjusted by the spectrophotometric method and the diluted (two times) yeast inoculum concentrations ranged from 0.8×10^3 to 4.2×10^3 CFU/ml, as demonstrated by quantitative colony counts on SDA.

Microdilution tests. On the day of the test, each microdilution well containing 100 μl of the diluted (two times) drug concentrations was inoculated with 100 μl of the diluted (two times) inoculum suspension (final volume in each well, 200 μl). Growth and sterility control wells were included for each isolate tested, and the growth control well contained medium plus 2% of the corresponding solvent. The final concentration of solvent in each well used for MIC determinations and in the growth control well was 1%. The NCCLS M27-A quality control (QC) isolates *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were tested as described above each time that a set of isolates was evaluated. In addition, when performing antifungal susceptibility testing with the filamentous fungi, MICs were determined for the reference isolate *Paecilomyces variotii* ATCC 22319, which has served as a control for drug activity in previous collaborative studies of the NCCLS subcommittee (6, 7). Microdilution trays were incubated at 35°C and were examined at 24 or 48 h or until growth was sufficient (heavy growth) for MIC determination (24 to 72 h for yeasts and opportunistic molds and up to 5 to 7 days for the dimorphic and phaeoid fungi).

(i) Visual MIC determinations for the yeasts. MICs for the yeasts were determined after 24 h (*Candida*) and 48 h (*C. neoformans*) of incubation by visual examination of growth inhibition and were determined again after 48 h (*Candida*) and 72 h (*C. neoformans*) of incubation. The growth in the control well (drug-free medium) was compared with that in each MIC well with the aid of a reading mirror. Both conventional criteria of MIC determination were used: (i) the lowest concentration showing prominent growth inhibition (MIC-2, approximately ≥50% inhibition) and (ii) the lowest concentration showing complete (100%) growth inhibition (MIC-0). This resulted in two visually determined MICs for each drug-organism combination.

(ii) Spectrophotometric MIC determinations for the yeasts. Upon completion of the second visual MIC determination, the microdilution plates were agitated for 5 min at 60 rpm, and MICs were determined spectrophotometrically at 490 nm with a kinetic microplate reader. For spectrophotometric MIC determinations, the concentration of drug in the first well in which the OD was 90% lower (MK-0991 and LY303366) and 50% or lower (SCH56592) than the OD of the growth control well was considered the MIC.

(iii) MIC determinations for the molds. The MICs for the molds were determined by the visual inspection of growth inhibition as described above for the yeasts. Both prominent and complete inhibition MICs also were obtained for each mold-drug combination.

(iv) MFCs. The in vitro fungicidal activity of each agent was determined by streaking 10 μl from each well that showed complete inhibition (100% inhibition or an optically clear well), from the last positive well (growth similar to that for the growth control well), and from the growth control well onto SDA plates. The plates were incubated at between 28 and 30°C until growth was seen in the growth control subculture. The MFC was the lowest drug concentration at which there was either no growth or fewer than three colonies.

Data analysis. MIC and MFC ranges were obtained by use of both criteria and by the two procedures of MIC determination for each yeast species-drug combination tested. MICs and MFCs for 50% (MIC_{50}s and MFC_{50}s, respectively) and 90% (MIC_{90}s and MFC_{90}s, respectively) of the isolates tested were determined for yeast species for which ≥10 isolates were available. Since the MIC ranges for the molds were generally narrow, geometric mean MICs were determined to facilitate comparisons of the activities of the drugs.

For comparisons of MIC pairs (e.g., first and second days of incubation, MICs-0 and MICs-2, and visual and spectrophotometric MICs), values were considered to be in agreement when the differences were within 3 dilutions, as previously evaluated in other studies (6, 7).

RESULTS

MICs for *B. dermatitidis*, *C. bantiana*, and *Phialophora verrucosa* were determined on day 5, and those for *H. capsulatum* were determined on day 7. All other isolates produced sufficient growth to determine the MICs at between 24 and 72 h of incubation.

Effect of testing variables on MIC data. When the MIC-0 endpoints of MK-0991 and LY303366 were determined for the molds, they were more than 3 dilutions (three wells) higher than the corresponding MICs-2, while most MICs-0 and MICs-2 of SCH56592 were within 2 dilutions. MICs for the yeasts were determined by the NCCLS microdilution method (M27-A document) (21), which involved the subjective visual examination of growth inhibition (visual MICs-0 and MICs-2). In addition, the in vitro activities of the three agents were evaluated by a spectrophotometric procedure. As for the other azoles, the agreement between visual and spectrophotometric MICs was higher for SCH56592 MICs-2 than for SCH56592 MICs-0. For the two echinocandins, spectrophotometric MICs were similar either to both of the visual MICs (MICs-0 and MICs-2) or to MICs-0. Differences between MICs-0 and MICs-2 were more frequently found for *Candida* spp. The MICs-0 of SCH56592 were >16 μg/ml for all *C. albicans* and *C. tropicalis* isolates, and those of MK-0991 were >16 μg/ml for all *C. guilliermondii* isolates, while the corresponding MICs-2 (see Table 2) were much lower. Other differences (mostly 3 dilutions higher) were also seen between the MICs-0 and the MICs-2 of the three agents for one to three isolates of other species (see Table 2). When MICs obtained after the two incubation times were compared, they were either the same or no more than 2 dilutions higher after the second day of incubation with the three agents. The exceptions were some SCH56592 MICs-0 for *C. lusitaniae* (MICs at 24 h, <0.03 μg/ml; MICs at 48 h, 1.0 μg/ml) and MK-0991 MICs for *C. guilliermondii* (MICs at 24 h, 0.25 μg/ml; and MICs at 48 h, 2 μg/ml).

TABLE 3. MICs of SCH56592, MK-0991, and LY303366 for QC and reference isolates[a]

Isolate	Antifungal agent	MIC range (μg/ml)
C. parapsilosis ATCC 22019	SCH56592	0.12–0.25
	MK-0991	0.5–2.0
	LY303366	0.5–1.0
C. krusei ATCC 6258	SCH56592	0.25–0.5
	MK-0991	0.5–2
	LY303366	0.06–0.25
P. variotii ATCC 22319	SCH56592	0.06–0.12
	MK-0991	0.06
	LY303366	<0.03

[a] Expected MICs correspond to prominent growth inhibition (approximately 50% growth inhibition or visual MICs-2). Each isolate was tested three to six times.

Susceptibilities of the molds. Because trailing (MICs-0, >16 μg/ml) was demonstrated when testing SCH56592, MK-0991, and LY303366 against *Aspergillus* spp., *Bipolaris* spp., *Fusarium* spp., *P. boydii*, *R. arrhizus*, and *S. schenckii*, only the MICs-2 of the three agents are listed in Table 1 to facilitate comparisons of their in vitro activities. Fungicidal activity (MFCs, 0.06 to 4 μg/ml) was demonstrated for SCH56592 for 63% of the molds tested (MICs-0, below 4 μg/ml; data not shown in Table 1). SCH56592 did not show fungicidal activity against *A. strictum*, *F. solani*, *P. boydii*, and *S. prolificans* isolates (SCH56592 MICs-0, 1.0 to >16 μg/ml; data not shown in Table 1). The highest level of fungicidal activity of SCH56592 was determined to be against *A. flavus*, *C. bantiana*, and *H. capsulatum* (geometric mean MFCs, 0.18 to 0.76 μg/ml) and the lowest fungicidal activity of SCH56592 was determined to be against *B. dermatitidis* and *Phialophora* spp. (geometric mean MFCs, 9.2 to 10 μg/ml). Although the other two agents were found to have fungicidal activities against 17% of the isolates tested (MICs-0, 2 to 8 μg/ml; data not shown in Table 1), they demonstrated very little activity. Overall, the in vitro activity of SCH56592 was superior to those of the two other agents by use of both criteria of MIC determination for most of the mold species, as demonstrated by the MICs and MFCs.

Susceptibilities of the yeasts. Table 2 lists only the spectrophotometric MICs of each agent, which were determined after the second day of incubation, for the nine species of yeast or yeast-like organisms. Low SCH56592 MICs (MIC_{90}s, <2.0 μg/ml) were obtained for eight of the nine yeast species tested

TABLE 4. Summaries of published MIC data for four antifungal agents and for the three investigational agents from this study[a]

Species	Antifungal agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Geometric mean MIC (μg/ml)
Yeasts				
Candida spp.	Amphotericin B		1–2	
	Fluconazole		2–64	
	Itraconazole		0.25–2	
	Voriconazole		0.06–4	
	SCH56592		<0.03–4	
	MK-0991		1.0–2	
	LY303366		0.06–2	
Cryptococcus neoformans	Amphotericin B		1.0	
	Fluconazole		16	
	Itraconazole		1.0	
	Voriconazole		0.12	
	SCH56592		0.25	
	MK-0991		>16	
	LY303366		>16	
Trichosporon beigelii	Amphotericin B	2		
	Fluconazole	2		
	Itraconazole	0.25		
	Voriconazole	<0.03		
	SCH56592	1.0		
	MK-0991	>16		
	LY303366	>16		
Opportunistic filamentous fungi				
Aspergillus spp.	Amphotericin B			1.0–1.07
	Itraconazole			0.1–0.24
	Voriconazole			0.29–0.57
	SCH 56592			0.1–0.13
	MK-0991			0.5–2.15
	LY303366			0.06–0.08
Bipolaris spp.	Amphotericin B			0.65
	Itraconazole			0.06
	Voriconazole			0.33
	SCH56592			0.14
	MK-0991			1.7
	LY303366			2.7
Fusarium spp.	Amphotericin B			1.31–2
	Itraconazole			8
	Voriconazole			4–10.5
	SCH56592			4.16–>16
	MK-0991			>16
	LY303366			>16
Pseudallescheria boydii	Amphotericin B			2.6
	Itraconazole			0.76
	Voriconazole			0.33
	SCH56592			1.0
	MK-0991			1.3
	LY303366			2.5
Rhizopus arrhizus	Amphotericin B			0.57
	Itraconazole			0.43
	Voriconazole			18.37
	SCH56592			2
	MK-0991			>16
	LY303366			>16
Dimorphic fungi				
Blastomyces dermatitidis	Amphotericin B			0.14
	Itraconazole			0.06
	Voriconazole			0.1
	SCH56592			0.05
	MK-0991			2
	LY303366			4
Histoplasma capsulatum	Amphotericin B			0.42
	Itraconazole			0.06
	Voriconazole			0.06
	SCH56592			0.04
	MK-0991			1.3
	LY303366			3.6
Sporothrix schenckii	Amphotericin B			1.5
	Itraconazole			0.5
	Voriconazole			16
	SCH56592			0.7
	MK-0991			5.4
	LY303366			3.9

[a] Data for amphotericin B, fluconazole, itraconazole, and another new triazole, voriconazole, have been published previously (5).

(MIC_{90}, 4 μg/ml for *C. glabrata*), and low echinocandins MICs (MICs, 16 to >16 μg/ml for *C. neoformans* and *T. beigelii*) were obtained for seven of the nine species (Table 2). The MIC_{90} endpoints of the three agents were within 2 dilutions for the 10 isolates of *C. albicans* with low levels of susceptibility to fluconazole and itraconazole and for *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. SCH56592 MICs were 4 dilutions lower for the 10 *C. albicans* isolates susceptible to fluconazole and itraconazole than for the 10 isolates that were more resistant to those two drugs. For the other species, the MICs of the two echinocandins were within 2 dilutions of each other, whereas SCH56592 MICs were mostly lower than those of the echinocandins (Table 2). Fungicidal activity, on the other hand, was demonstrated for more *Candida* spp. with the echinocandins than with SCH56592. The exceptions were *C. guilliermondii* (MK-0991 MICs-0, >16 μg/ml) and some isolates of *C. parapsilosis* (MK-0991 MFC range, 1.0 to >16 μg/ml).

QC and reference isolates. The MICs of the three agents for the two NCCLS QC isolates *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 and the control isolate *P. variotii* ATCC 22319 are presented in Table 3. These are the expected values in my laboratory. The reference MIC ranges of the investigational agents are not yet available.

DISCUSSION

Although several evaluations of the in vitro activities of the three investigational agents described here have been conducted, none of these have included head-to-head comparisons. These studies have focused on *Aspergillus* spp. (2, 3, 23, 32), *B. dermatitidis* with LY303366 (32), and a group of mold species (one to eight isolates each) with MK-0991 (3). While only 1 to 3 isolates were available in the present study for 8 of the 18 mold species tested, 5 to 13 isolates of the other 10 species were included. The association of some of these molds with human disease is rare. However, the in vitro antifungal activities of these investigational agents should be assessed against representative isolates of these species and other emerging mold and yeast pathogens.

A reference method is not available for the antifungal susceptibility testing of molds. For this study, the MIC data for the three agents were obtained by the standard testing guidelines for molds that have been proposed by the NCCLS Subcommittee for Antifungal Susceptibility Tests; this broth microdilution assay also has been used in my laboratory for an in vitro evaluation of voriconazole (5). Other investigators have used

different testing conditions and MIC determination criteria for their evaluation of the antifungal activities of these investigational agents. However, despite the discrepant testing conditions, comparable in vitro data for these three agents for *Aspergillus* spp. (MICs, ≤1.0 μg/ml) have been obtained in this and other studies (2, 3, 24, 32). Furthermore, the high SCH56592 MICs (1.0 μg/ml), which were determined for two of the three available itraconazole-resistant (MICs, >8 μg/ml) isolates of *A. fumigatus*, are similar to those reported previously (23, 24). The SCH56592 MFCs (>16 μg/ml) for these two isolates also matched the data obtained during a comparison of the efficacy of SCH56592 to that of itraconazole in the treatment of experimental invasive aspergillosis in neutropenic mice (24). Those investigators found a correlation between their high (0.5 μg/ml) and low (0.01 μg/ml) SCH56592 MICs and the quantitative organ culture results, which suggested a certain degree of cross-resistance between itraconazole and SCH56592. SCH56592 was found to be superior to itraconazole for the treatment of this experimental infection in animals infected with both types of isolates (high and low itraconazole MICs). The reliability of the MIC data for *Aspergillus* spp. and their preliminary correlation with the in vivo response (24) suggest the potential clinical utility of MIC data, but this relevance should be determined with humans during clinical trials.

In this study, MK-0991 MIC data for *Fusarium* spp., *P. boydii*, *R. arrhizus*, and *S. prolificans* are similar to those reported previously (geometric mean MICs, >16, 0.38, >16, and 8.8 μg/ml, respectively) (3). In vitro data obtained by other investigators for the other mold species are available only for *B. dermatitidis* with LY303366; the MIC_{90} for 29 isolates of *B. dermatitidis* was higher (16 μg/ml) (32) than the MICs obtained in this study (2 to 8 μg/ml) for five isolates. The incubation temperature in the previous study was 30°C, which may have enhanced the fungal growth and provided higher MICs; standard testing conditions are needed for the susceptibility testing of molds. The MFCs for the molds (Table 1) suggest that the in vitro activity of SCH56592 is superior to those of the other two compounds. However, it has been reported that the measurement of morphologic changes may provide a more relevant assessment of the activities of the echinocandin compounds against the molds, because those indicators correspond to the in vivo response better than conventional indicators of in vitro activity (16). Again, the clinical relevance of the in vitro activities of these compounds should be validated in clinical trials.

The isolates of *C. albicans* resistant to fluconazole and itraconazole were less susceptible to SCH56592 than the fluconazole- and itraconazole-susceptible isolates (Table 2). This suggested cross-resistance has been reported for another group of fluconazole-resistant *C. albicans* strains (17). On the other hand, the MIC_{90}s and MFC_{90}s of the two echinocandins for the two groups of *C. albicans* isolates were similar, as has been previously reported for MK-0991 (30). Such comparisons have not been described for LY303366. Overall, SCH56592 MICs for the other species of *Candida* and *C. neoformans* (Table 2) are also similar to those described by other investigators (9, 17, 26). However, these other studies did not provide fungicidal data for any of these species and did not evaluate *T. beigelii* isolates.

The MICs and MFCs of MK-0991 have been described in several studies of yeasts (2, 15, 30), but MFC data are not available for LY303366. Although MK-0991 MICs in this and other studies (2, 15, 30) are comparable, the MFCs for *C. albicans*, *C. guilliermondii*, and *C. parapsilosis* reported here (1 to >16 μg/ml) are higher than those reported previously (0.25 and 6.2 μg/ml) (2, 30). LY303366 MICs are more variable in the different studies: LY303366 MIC_{90}s of >8 μg/ml for *C. glabrata*, *C. lusitaniae*, and *C. parapsilosis* were described in one study (15), while the MICs for these isolates ranged from 0.25 to 5.12 μg/ml in this and other studies (27, 29, 32). This variability may be due to the different testing conditions used or to the different yeast populations that were studied. Collaborative studies, such as those that have been conducted with the established agents (21), are needed to investigate the nature of these variable results.

The data in this study also indicate that the in vitro activities of the three agents tested are species dependent. The lack of activity of the echinocandins against *C. neoformans* has been attributed to the postulated different cell wall compositions of these cells (2): they appear to lack the target molecules (1,3-β-D-glucans) of these related agents. In contrast to the established triazoles, SCH56592 appears to have fungicidal activity against some yeasts and molds, including the fluconazole and itraconazole-resistant yeasts. The echinocandins appeared to have in vitro fungicidal activity superior to that of SCH56592 against some *Candida* spp.; however, the activities of these agents against the yeasts should also be validated in vivo.

In summary, these three agents appear to have similar or better in vitro activities (Table 4) than those of the established agents and voriconazole (5). Preliminary in vivo data from animal models have corroborated these results for some of these drug-organism combinations (10, 11, 18, 24, 26). However, the potential use of these agents as therapeutic drugs must be determined by clinical trials.

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