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In Vitro Activities of the New Antifungal Triazole SCH 56592 against Common and Emerging Yeast Pathogens

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A broth microdilution method performed in accordance with the National Committee for Clinical Laboratory Standards guidelines was used to compare the in vitro activity of the new antifungal triazole SCH 56592 (SCH) to that of fluconazole (FLC), itraconazole (ITC), and ketoconazole (KETO) against 257 clinical yeast isolates. They included 220 isolates belonging to 12 different species of *Candida*, 15 isolates each of *Cryptococcus neoformans* and *Saccharomyces cerevisiae*, and seven isolates of *Rhodotorula rubra*. The MICs of SCH at which 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were inhibited were 0.06 and 2.0 μg/ml, respectively. In general, SCH was considerably more active than FLC (MIC_{50} and MIC_{90} of 1.0 and 64 μg/ml, respectively) and slightly more active than either ITC (MIC_{50} and MIC_{90} of 0.25 and 2.0 μg/ml, respectively) and KETO (MIC_{50} and MIC_{90} of 0.125 and 4.0 μg/ml, respectively). Our in vitro data suggest that SCH has significant potential for clinical development.

The risk of opportunistic infections is greatly increased in patients who are severely immunocompromised due to cancer chemotherapy, organ or bone marrow transplantation, or human immunodeficiency virus infection (1, 8). Although *Candida albicans* is the organism most often associated with serious fungal infections, other *Candida* species have emerged as clinically important pathogens associated with opportunistic infections (1, 8). Recently, many yeasts belonging to genera other than *Candida* have been reported as causative agents of systemic fungal infections (8).

This rising incidence of fungal infections has made the pursuit of safe and effective therapies an area of much activity over the past two decades. Although amphotericin B remains the "gold standard" therapy for life-threatening fungal infections, its use reveals important clinical limitations, including toxic side effects and inconvenience of intravenous dosing. Azole drugs represent a therapeutic advance (2).

SCH 56592 (SCH) is a new broad-spectrum antifungal triazole currently under development. SCH has been shown to have potent in vitro and in vivo activities against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., *Blastomyces dermatitidis*, and *Coccidioides immitis* (3–6, 9–13).

The aim of the present study was to compare the in vitro activity of SCH with those of three currently available azole drugs used in systemic fungal infections: fluconazole (FLC), itraconazole (ITC), and ketoconazole (KETO). The drugs were tested by a broth microdilution method, performed as recommended by the National Committee for Clinical Laboratory Standards (NCCLS), against common and emerging yeast pathogens.

Yeast isolates. A total of 257 clinical yeast isolates were used in this study. They included 220 isolates of *Candida* spp. (84 strains of *C. albicans*; 20 strains of *C. tropicalis*; 15 strains each of *C. glabrata* and *C. parapsilosis*; 14 strains each of *C. kefyr* and *C. krusei*; 10 strains each of *C. famata*, *C. guilliermondii*, *C. inconspicua*, *C. lusitaniae*, and *C. pelliculosa*; and 8 strains of *C. lipolytica*), 15 isolates of *Cryptococcus neoformans*, 15 isolates of *Saccharomyces cerevisiae*, and 7 isolates of *Rhodotorula rubra*. The isolates were recovered from gastrointestinal, respiratory, urinary tract, blood, cerebrospinal fluid, or other sterile body fluid specimens. With the exception of nine strains of *C. albicans* isolated from four patients with AIDS suffering from recurrent oropharyngeal candidiasis, each of the other strains represented a unique isolate from a patient. Yeast isolates were identified at the species level by conventional morphological and biochemical methods and stored at −70°C in 10% glycerol (14). Before the initiation of the study, yeast isolates were subcultured on antimicrobial agent-free medium to ensure viability and purity. *C. krusei* ATCC 6258 was used as the quality control and tested in each run of the experiments (7).

Antifungal agents. SCH was obtained as a standard powder from Schering-Plough Research Institute (Kenilworth, N.J.). FLC, ITC, and KETO were obtained from their respective manufacturers. Stock solutions were prepared in polyethylene glycol (SCH, ITC, and KETO) or water (FLC). Serial twofold dilutions were prepared as recommended in NCCLS approved standard M27-A (7). Final dilutions were made in RPMI 1640 medium (Sigma, Milan, Italy) 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.

Antifungal susceptibility testing. Antifungal susceptibility testing was performed by a broth microdilution method in accordance with the NCCLS recommendations (7). The final concentrations of the antifungal agents were 0.0078 to 4.0 μg/ml for SCH and ITC, 0.03 to 16 μg/ml for KETO, and 0.125 to 64 μg/ml for FLC. Plates were incubated at 35°C for 48 h (72 h for *C. neoformans* and *R. rubra*). MICs of all drugs were defined as the lowest concentrations resulting in 80% inhibition of growth compared to that of the growth control. MIC ranges were obtained for each species-drug combination

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TABLE 1. In vitro susceptibilities of 257 clinical yeast isolates to four azole drugs[a]

Organism (no. of isolates) and antifungal agent	MIC (μg/ml)			Organism (no. of isolates) and antifungal agent	MIC (μg/ml)		
	Range	50%	90%		Range	50%	90%
C. albicans (84)				*C. inconspicua* (10)			
SCH	≤0.0078–>4.0	0.015	0.25	SCH	0.125–4.0	0.5	0.5
FLC	≤0.125–>64	0.25	8.0	FLC	4.0–>64	16	64
ITC	0.03–>4.0	0.125	1.0	ITC	0.25–>4.0	1.0	2.0
KETO	≤0.03–16	0.06	1.0	KETO	0.5–>16	2.0	8.0
C. tropicalis (20)				*C. lusitaniae* (10)			
SCH	≤0.0078–0.125	0.03	0.06	SCH	≤0.0078–0.06	0.03	0.06
FLC	0.125–2.0	0.25	2.0	FLC	≤0.125–1.0	0.25	1.0
ITC	0.06–0.5	0.25	0.25	ITC	0.06–0.25	0.125	0.25
KETO	0.06–0.25	0.06	0.125	KETO	≤0.03–0.125	0.06	0.125
C. glabrata (15)				*C. pelliculosa* (10)			
SCH	0.015–1.0	0.25	1.0	SCH	2.0	2.0	2.0
FLC	0.25–8.0	4.0	8.0	FLC	4.0–32	4.0	32
ITC	0.06–2.0	0.5	2.0	ITC	0.5–2.0	1.0	2.0
KETO	0.125–2.0	1.0	2.0	KETO	1.0–2.0	1.0	
C. parapsilosis (15)				*C. lipolytica* (8)			
SCH	0.015–0.5	0.06	0.25	SCH	0.06–2.0	1.0	
FLC	0.25–4.0	1.0	2.0	FLC	2.0–>64	4.0	
ITC	0.25–2.0	0.25	0.5	ITC	0.06–2.0	1.0	
KETO	0.06–2.0	0.25	1.0	KETO	0.5–8.0	2.0	
C. kefyr (14)				*S. cerevisiae* (15)			
SCH	0.015–4.0	0.125	0.5	SCH	0.015–>4.0	0.125	4.0
FLC	≤0.125–16	0.25	16	FLC	0.25–>64	4.0	64
ITC	0.125–1.0	0.25	1.0	ITC	0.015–>4.0	0.5	>4.0
KETO	0.06–1.0	0.06	0.5	KETO	0.06–>16	0.5	4.0
C. krusei (14)				*C. neoformans* (15)			
SCH	0.25–>4.0	0.5	2.0	SCH	0.015–0.25	0.125	0.25
FLC	16–>64	32	>64	FLC	0.5–8.0	4.0	8.0
ITC	1.0–>4.0	2.0	4.0	ITC	0.03–0.5	0.25	0.5
KETO	0.125–8.0	4.0	4.0	KETO	0.125–1.0	0.5	1.0
C. famata (10)				*R. rubra* (7)			
SCH	0.06–4.0	0.5	4.0	SCH	2.0–>4.0	2.0	
FLC	1.0–64	8.0	64	FLC	64–>64	>64	
ITC	0.125–4.0	1.0	4.0	ITC	2.0–>4.0	>4.0	
KETO	0.25–16	0.5	16	KETO	0.5–2.0	1.0	
C. guilliermondii (10)				All organisms (257)			
SCH	0.03–0.25	0.125	0.25	SCH	≤0.0078–>4.0	0.06	2.0
FLC	1.0–8.0	4.0	8.0	FLC	≤0.125–>64	1.0	64
ITC	0.25–1.0	0.5	0.5	ITC	0.015–>4.0	0.25	2.0
KETO	0.125–0.5	0.25	0.25	KETO	≤0.03–>16	0.125	4.0

[a] MICs were determined after 48 h of incubation for all yeasts with the exception of isolates of *C. neoformans* and *R. rubra*, for which MICs were determined after 72 h of incubation under the NCCLS conditions (7).

tested. MICs for 50 and 90% of the isolates of each species tested (MIC_{50} and MIC_{90}, respectively) were determined for yeast species with ≥10 isolates.

Determination of CFU per milliliter. The antifungal activities of SCH and FLC were compared by determination of CFU per milliliter of two selected clinical isolates, *C. albicans* isolate 3474 and *C. neoformans* isolate 486, incubated at multiples of the respective MICs. Briefly, three to five colonies of the isolate from a 72-h growth plate were suspended in 10 ml of sterile distilled water and the turbidity was adjusted according to spectrophotometric methods to a McFarland turbidity standard (approximately 1×10^6 to 5×10^6 CFU/ml). One milliliter of the adjusted fungal suspension was added to 9 ml of either RPMI 1640 medium buffered with MOPS or a solution of growth medium plus an appropriate amount of antifungal stock solution. This resulted in a 1:10 dilution of the fungal suspension and yielded a starting inoculum of approximately 1×10^5 to 5×10^5 CFU/ml. Drug concentrations in test solution were equal to 0.25, 0.5, 1, 2, 4, 8, and 16 times the MICs of both drugs. At predetermined time points (0, 6, 12, 24, 48, and 72 h) following the introduction of the isolate into the system, a 100-μl aliquot was removed from either the control tube (drug-free) or each test solution. Tenfold serial dilutions were performed on samples, and a 50-μl aliquot from each dilution was streaked onto Sabouraud dextrose agar plates for colony count determination. Following incubation at 35°C for 48 h, the number of CFU on each plate was determined. Each experiment was conducted in triplicate. The results were ex-

TABLE 2. Antifungal activities of SCH and FLC against *C. albicans* 3474 and *C. neoformans* 486 at multiples of MICs[a]

Drug	h	Log variation of CFU/ml for the indicated isolate at the indicated multiple of MIC[b]													
		C. albicans							*Cryptococcus neoformans*						
		0.25	0.5	1	2	4	8	16	0.25	0.5	1	2	4	8	16
FLC	6	−0.07	−0.17	−0.11	−0.26	−0.33	−0.74	−0.82	−0.05	−0.05	−0.15	−0.15	−0.15	−0.15	−0.15
SCH 56592		−0.16	−0.16	−0.12	−0.48	−0.40	−0.49	−0.54	−0.10	−0.10	−0.09	−0.09	−0.09	−0.10	−0.08
FLC	12	0.00	+0.01	0.00	+0.01	−0.98	−1.35	−1.41	−0.09	−0.31	−0.55	−0.73	−0.83	−0.84	−0.89
SCH 56592		−0.14	−0.47	−0.46	−0.92	−0.99	−0.98	−1.06	−0.19	−0.59	−0.64	−0.75	−0.77	−0.77	−0.80
FLC	24	+0.02	−0.03	−0.02	+0.02	−0.93	−0.88	−0.82	0.00	−0.12	−0.71	−1.66	−1.85	−1.87	−1.91
SCH 56592		−0.13	−0.55	−0.54	−0.67	−0.71	−0.74	−0.68	−0.30	−1.47	−2.10	−2.31	−2.31	−2.37	−2.44
FLC	48	0.00	−0.01	−0.01	−0.04	−0.07	−0.61	−0.61	0.00	−0.07	−0.13	−2.12	−2.36	−2.44	−2.44
SCH 56592		−0.08	−0.30	−0.61	−0.66	−0.63	−0.63	−0.69	0.00	−0.67	−2.08	−2.42	−2.62	−2.77	−2.77
FLC	72	ND[c]	ND	ND	ND	ND	ND	ND	−0.04	−0.08	−0.09	−1.22	−2.10	−2.14	−2.26
SCH 56592		ND	ND	ND	ND	ND	ND	ND	0.00	−0.39	−1.65	−2.34	−3.20	−3.21	−3.25

[a] The antifungal activity is expressed as the logarithmic variation of the ratio of CFU of treated cells per milliliter to CFU of untreated cells per milliliter for each time point considered.
[b] FLC MICs were 0.25 and 4.0 μg/ml and SCH 56592 MICs were 0.06 and 0.25 μg/ml for *C. albicans* and *C. neoformans*, respectively.
[c] ND, not determined.

pressed as the logarithmic variation of the ratio of CFU of treated cells per milliliter to CFU of untreated cells per milliliter for each time point considered.

The median MICs of SCH, FLC, ITC, and KETO for *C. krusei* ATCC 6258 were 0.25, 32, 0.25, and 0.5 μg/ml, respectively. Table 1 summarizes the in vitro susceptibilities of 257 clinical yeast isolates to four azole drugs. In general, a broad range of MICs was observed with each antifungal agent. SCH was from 4- to 128-fold more active than FLC. The smallest difference between drugs was observed for strains of *C. lipolytica*, for which SCH and FLC MIC_{50}s were 1.0 and 4.0 μg/ml, respectively. The widest difference was observed for strains of *C. inconspicua*, for which SCH and FLC MIC_{90}s were 0.5 and 64 μg/ml, respectively. SCH was from two- to fourfold more active than ITC against all the yeast isolates tested with the exception of strains of *C. famata*, *C. pelliculosa*, and *C. lipolytica*, for which no differences in MIC_{90}s (or MIC_{50} in the case of *C. lipolytica*) were observed between drugs. SCH was from 2- to 16-fold more active than KETO against all the yeast isolates tested, with the exception of strains of *C. kefyr*, *C. guilliermondii*, *C. pelliculosa*, *S. cerevisiae*, and *R. rubra*. For the first two *Candida* spp. and *S. cerevisiae*, no differences in MIC_{90}s were observed between drugs. On the other hand, KETO was twofold more active than SCH against strains of *C. pelliculosa* and *R. rubra* (Table 1).

To compare the antifungal activity of the new triazole with that of FLC, we determined the CFU per milliliter of two selected isolates incubated with the drugs at multiples of the MICs: *C. albicans* 3474 (FLC MIC, 0.25 μg/ml; SCH MIC, 0.06 μg/ml) and *C. neoformans* 486 (FLC MIC, 4.0 μg/ml; SCH MIC, 0.25 μg/ml). The results are shown in Table 2. Overall, the antifungal activities of both drugs were higher for *C. neoformans* than for *C. albicans*. For the latter isolate, a reduction in CFU per milliliter of >1 log, compared with that of the growth control, was reached at 12 h of incubation with FLC at concentrations ≥8 times the MIC and with SCH at a concentration equal to 16 times the MIC. The anticryptococcal activity of SCH was superior to that of FLC. At concentrations equal to their respective MICs, SCH produced a reduction of >1 log of CFU/ml with respect to FLC from 24 to 72 h of incubation. The same phenomenon was seen at concentrations equal to 0.5 times the MICs at 24 h and of ≥2 times the MICs at 72 h of incubation (Table 2).

Overall, our data confirmed the potent in vitro activity of SCH against the most common yeast pathogens and showed its potent in vitro activity against a wide spectrum of less commonly isolated yeasts. Similar to previous reports of other investigators (3, 5, 9, 12), SCH was found to be more active than both ITC and FLC against isolates of *C. albicans*. SCH was slightly more active than both ITC and KETO against strains of *C. glabrata* and *C. krusei*, two species of *Candida* often reported, in vitro as well in vivo, to be resistant to FLC (2). Our SCH MICs for the strains of *C. glabrata* were similar to those reported previously by Pfaller et al., while SCH MICs for strains of *C. krusei* were slightly higher than those reported by the same authors (12). The other three species of *Candida* which have been reported as relatively resistant to FLC were included in this study: *C. famata*, *C. inconspicua*, and *C. pelliculosa* (1, 8). In general, SCH was as active as ITC against strains of *C. famata* and *C. pelliculosa*, and it was slightly more active than ITC against strains of *C. inconspicua*. On the other hand, KETO showed slightly more activity than both SCH and ITC against strains of *C. pelliculosa*. Overall, with the exception of one isolate each of *C. famata* and *C. inconspicua* which were inhibited by SCH at a concentration of 4.0 μg/ml, all the other strains belonging to these three species of *Candida* showed an SCH MIC of ≤2.0 μg/ml. Interestingly, preliminary data on SCH pharmacokinetics showed that serum levels over 2.0 mg/liter may be achieved in several animal models (12).

Among the four azoles tested, SCH was the most active drug against isolates of *C. neoformans*. Our in vitro data on this yeast paralleled those previously reported by Galgiani and Lewis (3), having found SCH MICs ranging from 0.015 to 0.25 μg/ml. Interestingly, the anticryptococcal activity of SCH, measured by determination of CFU per milliliter of cells incubated at multiples of the MIC, was higher than that observed with FLC. Recently, Perfect et al. showed that SCH was as effective as FLC in a rabbit model of cryptococcal meningitis (11). Taken together, these data suggest the potential beneficial effect of this new triazole as an alternative to FLC for the treatment of cryptococcal infections.

In conclusion, our study underlines the potent and broad-

spectrum activity of the new antifungal triazole SCH 56592. Clearly, other in vitro and in vivo studies to further elucidate its potential for clinical development are warranted.

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