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Activity of SCH 56592 Compared with Those of Fluconazole and Itraconazole against *Candida* spp.

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The in vitro activity of Schering 56592, a new azole drug, was compared with those of fluconazole and itraconazole against 103 isolates of *Candida* comprising 10 different species. Schering 56592 was more active than itraconazole and fluconazole, and it was active against many fluconazole-resistant isolates.

The rising incidence of resistance to currently available antifungal drugs has led to the development of newer drugs with improved pharmacokinetics and different spectra of activity. In particular, resistance to the triazole fluconazole (FLU) has been widely documented (1, 6, 7). Treatment of infections caused by fluconazole-resistant organisms is problematic, because resistance to the other azoles may occur (5, 8). New drugs that possess activity against such isolates are required (2).

Schering 56592 (SCH) is a new triazole antifungal drug, and preliminary data indicate that SCH has good activity against a wide spectrum of fungal species in vivo and in vitro (4, 10, 11). In this study we compared the antifungal activity of SCH with those of FLU and itraconazole (ITZ) against yeasts with different degrees of resistance to the existing azole drugs.

One hundred three *Candida* yeasts belonging to 10 different species were used; 87 were clinical isolates, 7 were isolates from the American Type Culture Collection, and the remainder were isolates from our culture collection. Isolates with different patterns of susceptibility to fluconazole were selected.

The three drugs were provided by the manufacturers as standard powders. SCH was provided by Schering Plough Research Institute (Bloomfield, N.J.), FLU was provided by Pfizer Ltd. (Sandwich, England), and ITZ was provided by Janssen Pharmaceutica (Beerse, Belgium). Stock solutions of each drug were prepared by dissolving the drug in dimethyl sulfoxide to give concentrations of 1,280 μg/ml for SCH and ITZ and 5,120 μg/ml for FLU. Stock solutions were stored at −20°C in the dark before use.

MICs were determined by using a microtiter modification of the method of the National Committee for Clinical Laboratory Standards (3, 9) with RPMI 1640 (Sigma, Poole, England) supplemented with glucose to a concentration of 2% (12), buffered with 0.165 M morpholinopropanesulfonic acid, and adjusted to pH 7.0. The final inoculum was 10^3 CFU/ml, and the volume per well was 200 μl. Final drug concentration ranges were 16 to 0.015 μg/ml for SCH and ITZ and 64 to 0.06 μg/ml for FLU. Plates were incubated in a moist chamber at 37°C for 48 h. After incubation the plates were shaken for 5 min to obtain a uniform suspension before reading. The growth in each well was measured by determining the optical density at 490 nm (OD_{490}) with a spectrophotometer. The MIC was taken as the lowest drug concentration that reduced the OD_{490} by 80% compared with that for a drug-free control.

The minimum fungicidal concentrations (MFCs) of SCH for 82 yeasts, including representatives of each of the 10 species tested, were determined. For each isolate tested, a 50-μl aliquot was withdrawn from the well containing the drug at the MIC and all wells containing the drug at concentrations above the MIC, and the aliquots were inoculated onto half of a blood agar plate. The aliquot was allowed to dry, and then the plate was streaked with a sterile loop and incubated at 37°C for 48 h. The MFC was defined as the lowest concentration of drug at which two or fewer colonies grew (e.g., 98% killing). Because of the lower inoculum used, compared with bacterial MIC tests, it is not possible to calculate 99.9% killing. To check the reproducibility of the MIC method, the susceptibility test method was repeated with 25 isolates and all three drugs.

The MIC range, the geometric mean (GM) MIC, and the MIC at which 90% of isolates are inhibited (MIC_{90}) for each *Candida* species are presented in Table 1. SCH was the most active drug tested against all 103 isolates, being slightly more active than ITZ (GM MICs, 0.21 and 0.34 μg/ml, respectively) and considerably more active than FLU (GM MIC, 4.99 μg/ml). For 57 isolates FLU MICs were increased (MICs, ≥8 μg/ml); for 41 of these 57 isolates SCH and ITZ MICs were ≤1 μg/ml, indicating that both drugs had good activity against FLU-resistant isolates. SCH was particularly active against FLU-resistant *C. norvegensis*, *C. guilliermondii*, *C. krusei*, and *C. inconspicua* and the majority of FLU-resistant *C. albicans* isolates. However, its activity against *C. glabrata* was poorer; for only 3 of 14 *C. glabrata* isolates were SCH MICs below 2 μg/ml, the breakpoint suggested by Pfaller et al. (11).

A comparison of SCH and ITZ MICs indicated that they were very similar. For 35 of 103 organisms tested, the MICs were identical, and for 40 of the isolates tested, the MICs were only 1 dilution different. Major differences were observed with three *C. glabrata* isolates, for which ITZ MICs were ≥32 μg/ml and SCH MICs were 4 μg/ml, and for a single isolate of *C. tropicalis*, for which the ITZ MIC was 0.5 μg/ml but the SCH MIC was >16 μg/ml. In addition to these isolates for which SCH and ITZ MICs were different, for eight isolates (three *C. albicans*, three *C. glabrata*, and two *C. tropicalis* isolates) for which FLU MICs were high, ITZ and SCH MICs were also high (MICs, >16 μg/ml). A common mechanism of resistance may exist in these isolates, and this mechanism of resistance may be distinct from that mediating FLU resistance in other

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TABLE 1. GM MICs, MIC ranges, and MIC_{90}s of SCH, ITZ, and FLU for different yeast species

Species	No. of isolates tested	MIC (μg/ml)								
		SCH			ITZ			FLU		
		GM	Range	90%	GM	Range	90%	GM	Range	90%
C. albicans	30	0.11	<0.015–>16	1	0.22	<0.015–>16	1	2.64	0.06–>64	>64
C. albicans (FLU MIC, <8 μg/ml)	17	0.015	<0.015–0.25	0.06	0.05	<0.01–0.25	0.12	0.27	0.06–4	0.5
C. albicans (FLU MIC, >8 μg/ml)	13	1.45	0.25–>16	>16	1.62	0.5–>16	>16	51.7	16–>64	>64
C. glabrata	14	3.62	0.5–>16	>16	4.88	0.5–>16	>16	55.2	16–>64	>64
C. krusei + *C. inconspicua*	15	0.25	0.12–1	0.5	0.27	0.06–1	0.5	42.2	16–>64	64
C. guilliermondii	8	0.23	0.12–0.5	0.25	0.32	0.25–0.5	0.5	4.36	2–16	8
C. lusitaniae	7	0.01	<0.015–0.015	0.015	0.045	0.03–0.12	0.06	0.37	0.03–2	1
C. norvegensis	7	0.11	0.015–0.25	0.25	0.17	0.03–0.25	0.25	26.25	8–64	32
C. parapsilosis	8	0.028	0.015–0.12	0.06	0.066	0.03–0.12	0.12	0.76	0.12–4	4
C. tropicalis	13	0.42	0.03–>16	>16	0.38	0.03–>16	>16	8.9	0.5–>64	>64
C. kefyr	1	0.015			0.03			0.25		
All isolates	103	0.21	<0.015–>16	4	0.34	<0.015–>16	>16	4.99	0.06–>64	>64

isolates for which SCH and ITZ MICs are comparatively lower. These findings agree with those obtained by Pfaller et al. (11); they found the activities of SCH and ITZ to be similar and also detected high-level resistance to SCH and ITZ.

MFCs were determined for 82 isolates. SCH did not appear to have appreciable fungicidal activity, because MFCs were ≥16 μg/ml for 75 of 82 isolates. Of the seven isolates for which MFCs were lower, for three isolates (one *C. parapsilosis*, one *C. kefyr*, and one *C. lusitaniae* isolates) MFCs were at least fivefold higher than the MICs. For four isolates (two *C. krusei*, one *C. inconspicua*, and one *C. norvegensis* isolates), MFCs were only 1 to 3 dilutions higher than the MICs. However, for other isolates of these species MFCs were ≥16 μg/ml, indicating that low MFCs are not a species-dependent phenomenon.

Tests of reproducibility were good for all three drugs. For FLU, 18 isolates showed identical results, with MICs for 7 isolates differing by only 1 dilution. SCH MICs for 16 isolates were identical, and the MICs for 6 isolates differed by 1 dilution, and MICs for 3 isolates differed by 2 dilutions on repeat testing. ITZ MICs were identical for 11 isolates, and ITZ MICs differed by 1 and 2 dilutions for 9 and 5 isolates, respectively.

In conclusion, SCH, a new triazole drug, has good fungistatic activity in vitro against many species of yeasts including FLU-resistant isolates. Overall, its activity in vitro was slightly superior to that of ITZ and was markedly superior to that of FLU. SCH may therefore be a useful drug for the treatment of candidiasis, including infections caused by FLU-resistant isolates. Its activity in vivo must be assessed in animal models and clinical trials, and further in vitro susceptibility testing is required. Correlation of the results of in vivo and in vitro studies will help establish breakpoints for susceptibility and resistance.

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