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# In Vitro and In Vivo Activities of SCH 42427, the Active Enantiomer of the Antifungal Agent SCH 39304

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**SCH 39304, a new triazole antifungal agent, is a 50:50 racemic mixture of two enantiomers, SCH 42427 and SCH 42426. The activities of these three compounds were compared in a series of in vitro and in vivo experiments. SCH 42427 was twofold more active in vitro against a variety of yeasts and dermatophytes than SCH 39304, while SCH 42426 was inactive (MICs > 64 μg/ml). In a systemic *Candida albicans* infection in mice, SCH 42427 administered orally (p.o.) (50% protective dose [$PD_{50}$], 0.17 mg/kg of body weight; 50% effective dose, [$ED_{50}$], 0.47 mg/kg) had greater efficacy than SCH 39304 ($PD_{50}$, 0.21 mg/kg; $ED_{50}$, 0.62 mg/kg) and SCH 42426 (>100 mg/kg for $PD_{50}$ and $ED_{50}$). In a pulmonary *Aspergillus flavus* infection in mice, SCH 42427 p.o. ($PD_{50}$, 13 mg/kg) was also more effective than SCH 39304 (18 mg/kg) and SCH 42426 (>250 mg/kg). In a *C. albicans* vaginal infection in hamsters, SCH 42427 p.o. ($ED_{50}$, 3.5 mg/kg) was more active than SCH 39304 (8.5 mg/kg) and SCH 42426 (320 mg/kg). Following topical administration, against a *Trichophyton mentagrophytes* infection in guinea pigs, SCH 42427 was about 2-fold more active than SCH 39304 and about 100-fold more active than SCH 42426. These and other results indicated that SCH 42427 is the active enantiomer, responsible for all the antifungal activity observed with SCH 39304.**

SCH 39304, a triazole antifungal agent, was previously shown to have broad-spectrum in vitro activity (4, 8, 9) and to be an effective oral treatment for a variety of fungal infections in experimental animals (2, 3, 5, 6, 10, 12, 14, 16, 17). SCH 39304 is a 50:50 racemic mixture of two enantiomers, SCH 42427 (RR,−) and SCH 42426 (SS,+). The activities of the enantiomers were initially described by Saji et al. (13) and recently by Tanio et al. (15). The chiral synthesis of SCH 42427 was also recently reported (6). A series of in vitro and in vivo experiments was performed to identify the microbiological characteristics of these enantiomers. Within the limits of precision of our experiments, we have determined that SCH 42427 is responsible for all the antifungal activity seen with SCH 39304. In this study, the in vitro activity and in vivo efficacy of SCH 42427 was compared with those of SCH 42426 and SCH 39304.

SCH 39304 was obtained from Sumitomo Pharmaceuticals Co., Ltd., Hyogo, Japan, and is identical to SM-8668 (16). The enantiomers (SCH 42426 and SCH 42427) of SCH 39304 were obtained from the same source, and additional quantities of all three compounds were prepared at Schering-Plough Research, Bloomfield, N.J. SCH 42427 is identical to SM-9164 (15).

**In vitro antifungal activity.** In vitro antifungal activity was determined in microtiter plate MIC tests. The media employed in these tests were Sabouraud dextrose broth (SDB) (Difco, Detroit, Mich.) and Eagle minimum essential medium with nonessential amino acids, L-glutamine, and fetal bovine serum added (EMEM), (Whittaker Bioproducts Inc., Walkersville, Md.). All three compounds were dissolved in dimethyl sulfoxide (MCB Inc., Cincinnati, Ohio) and serially diluted in medium in 96-well microtiter plates (Falcon, Lincoln Park, N.J.). SDB plates were incubated at 28°C, and plates containing EMEM were incubated at 37°C under 5% $CO_2$ for 48 h. MICs in SDB were defined as the lowest concentrations of drug to prevent growth or, in EMEM, the transformation from the yeast to mycelial phase of growth. Table 1 shows the MIC results against various fungi. SCH 42427 was about twofold more active than SCH 39304 against both yeasts and dermatophytes, while SCH 42426 was inactive at the highest concentrations tested (16 to 64 μg/ml).

***Candida albicans* infection studies in mice.** *C. albicans* infection studies used strain C43 and were as described previously (2). Antifungal agents were administered orally. All drugs were prepared in a vehicle consisting of ethanol-Emulphor EL-719P (GAF, Wayne, N.J.) (115 ml per liter of water) and lactic acid (5 ml of 20% [wt/vol] solution per liter of water), 10:90 (vol/vol). The 50% protective dose ($PD_{50}$) was defined as that dose which allowed for 50% survival of mice, and the 50% effective dose ($ED_{50}$) was defined as that dose which lowered kidney counts to $10^5$ CFU, 4 logs below those of control mice. The results are shown in Fig. 1. SCH 42427 and SCH 39304 were active at all doses above 0.125 mg/kg, while SCH 42426 was inactive, even at 100 mg/kg. From the survival data in Fig. 1A, the $PD_{50}$ for SCH 42427 was estimated to be 0.17 mg/kg, while that for SCH 39304 was 0.21 mg/kg. The $ED_{50}$, estimated from the sacrifice study (based on CFUs in kidneys) in Fig. 1B, for SCH 42427 was 0.47 mg/kg, while for SCH 39304 it was 0.62 mg/kg. All animals treated with SCH 42426 died, and no endpoints were obtained.

**Pulmonary aspergillosis in mice.** CF-1 mice (white, male, ca. 20 g, Harlan Sprague-Dawley, Inc., Indianapolis, Ind.) were compromised with cortisone acetate (100 mg/kg subcutaneously), once daily for 3 days. On day 2 of this treatment, mice were infected by exposure to spores of *Aspergillus flavus* ATCC 24133 (13-day-old culture grown at room temperature on malt extract agar) in an inhalation chamber, first described by Piggot and Emmons in 1960 (11) and modified by us. Single-dose oral treatment (5 to 250 mg/kg, prepared

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TABLE 1. In vitro activities of SCH 39304, SCH 42426, and SCH 42427 against various yeasts and dermatophytes[a]

| Species and strain | Microtiter MIC (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | SCH 39304 | | SCH 42427 | | SCH 42426 | |
| | EMEM[b] | SDB[c] | EMEM | SDB | EMEM | SDB |
| *Candida albicans* | | | | | | |
| C40 | 0.13 | >64 | 0.06 | >64 | >16 | >64 |
| C41 | 0.13 | >64 | 0.06 | >64 | >16 | >64 |
| C42 | 0.13 | >64 | 0.06 | >64 | >16 | >64 |
| C43 | 0.13 | >64 | 0.06 | >64 | >16 | >64 |
| C60 | 0.13 | >64 | 0.06 | >64 | >16 | >64 |
| C79 | 0.13 | >64 | 0.06 | >64 | >16 | >64 |
| *Candida tropicalis* | | | | | | |
| C44 | 1.0 | >64 | 0.5 | >64 | >16 | >64 |
| C90 | 1.0 | >64 | 0.5 | >64 | >16 | >64 |
| *Candida stellatoidea* | | | | | | |
| C45 | 0.13 | >64 | 0.06 | >64 | >16 | >64 |
| C184 | | >64 | | >64 | | >64 |
| *Candida parapsilosis* | | | | | | |
| C53 | | 8 | | 8 | | >64 |
| C91 | | 8 | | 4 | | >64 |
| *Trichophyton mentagrophytes* | | | | | | |
| D24 | | 16 | | 8 | | >64 |
| D30 | | 16 | | 8 | | >64 |
| *Trichophyton rubrum* | | | | | | |
| D54 | | 8 | | 4 | | >64 |
| D61 | | 32 | | 16 | | >64 |
| *Trichophyton tonsurans* | | | | | | |
| D27 | | 64 | | 64 | | >64 |
| D73 | | 64 | | 32 | | >64 |
| *Microsporum canis* | | | | | | |
| D18 | | 16 | | 8 | | >64 |
| *Aspergillus niger* | | | | | | |
| ND19 | | >64 | | >64 | | >64 |
| ND133 | | 64 | | 32 | | >64 |
| *Saccharomyces cerevisiae* | | | | | | |
| C51 | | >64 | | >64 | | >64 |

[a] The vehicle was dimethyl sulfoxide for all three drugs. Drug MICs were measured at 48 h.
[b] EMEM at pH 7.0 and 37°C. An inoculum of $3 \times 10^2$/ml was used.
[c] SDB at pH 5.7 and 28°C. An inoculum of $3 \times 10^3$/ml was used for yeasts; spores and mycelia for dermatophytes.

as described above) began 24 h postinfection. The average number of inhaled conidia was $9.8 \times 10^5$/mouse lung. Results for SCH 42427 are shown in Fig. 2. The values for percent survival on day 14 following treatment with 50, 25, 10, and 5 mg/kg of SCH 42427 were 100, 94, 44, and 0, respectively. The values for percent survival following treatment with 50, 25, and 10 mg/kg of SCH 39304 were 100, 75, and 19, respectively (not shown). The $PD_{50}$ for SCH 42427 was 13 mg/kg, and that for SCH 39304 was 18 mg/kg. No activity was seen with SCH 42426, even at 250 mg/kg (not shown).

**Vaginal *Candida* infections in hamsters.** *C. albicans* C60, grown on Sabouraud dextrose agar (SDA) slants for 48 h at 28°C, was used to intravaginally infect groups of 10 female Syrian outbred hamsters (Charles River, Kingston, N.Y.), weighing 100 to 120 g. Treatment began 4 days after completion of infection. Treatment was a single oral dose and ranged from 0.6 to 250 mg/kg. By using cotton swabs, vaginal samples were obtained after 2, 4, 7, and 9 days of treatment. Efficacy was determined on the basis of negative cultures at each of the four time periods. The results are shown in Fig. 3. The graph shows a probit analysis of the percent area under the inhibition versus time curve obtained at each dose level for all three compounds. SCH 42427 was active at all doses above 0.625 mg/kg, SCH 39304 was active at all doses above 1.25 mg/kg, while SCH 42426 was only slightly active, even at 250 mg/kg. On the basis of this analysis, $ED_{50}$s for SCH 42427, SCH 39304, and SCH 42426 were 3.5, 8.5, and 320 mg/kg, respectively.

TABLE 2. In vivo activities of SCH 39304, SCH 42427, and SCH 42426 against a topical *T. mentagrophytes* infection in guinea pigs[a]

| Compound and concn (%) | % Samples negative on day: | | | | | | Avg. lesion score |
|---|---|---|---|---|---|---|---|
| | 4 | 7 | 9 | 11 | 14 | 16 | |
| SCH 39304 | | | | | | | |
| 1.0 | 40 | 97 | 97 | 100 | 97 | 90 | 5.0 |
| 0.5 | 32 | 97 | 87 | 100 | 95 | 87 | 5.3 |
| 0.25 | 20 | 90 | 97 | 100 | 92 | 97 | 5.8 |
| 0.125 | 42 | 88 | 92 | 100 | 100 | 93 | 6.2 |
| 0.06 | 22 | 90 | 93 | 100 | 97 | 93 | 17.7 |
| 0.03 | 10 | 80 | 90 | 97 | 100 | 95 | 25.1 |
| SCH 42427 | | | | | | | |
| 0.5 | 68 | 90 | 98 | 100 | 100 | 95 | 3.3 |
| 0.25 | 58 | 80 | 100 | 92 | 95 | 90 | 3.9 |
| 0.125 | 60 | 88 | 100 | 93 | 95 | 93 | 3.1 |
| 0.06 | 48 | 80 | 100 | 93 | 95 | 93 | 6.1 |
| 0.03 | 25 | 58 | 100 | 93 | 98 | 93 | 17.9 |
| 0.015 | 35 | 37 | 88 | 87 | 83 | 100 | 25.0 |
| SCH 42426 | | | | | | | |
| 5.0 | 42 | 53 | 98 | 95 | 90 | 95 | 18.7 |
| 3.0 | 28 | 52 | 87 | 87 | 80 | 90 | 21.9 |
| 1.0 | 2 | 40 | 70 | 80 | 82 | 98 | 26.1 |
| 0.5 | 0 | 10 | 22 | 28 | 33 | 55 | 27.2 |
| None (vehicle[b]) | 0 | 0 | 13 | 10 | 12 | 55 | 29.2 |

[a] Treatment was given topically twice daily for 10 days.
[b] Glycerol-polyethylene glycol 400-ethanol (45:45:10).

**Topical dermatophyte infections in guinea pigs.** Groups of 10 male Hartley guinea pigs (Charles River) weighing 250 to 300 g were infected superficially with *Trichophyton mentagrophytes* 0176 (grown on SDA slants for 10 days at 28°C). Compounds were administered topically (0.3 ml), twice a day for 10 consecutive days. Samples were obtained from around the lesion site and implanted onto Mycosel agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.). Plates were examined after 4 and 8 days of incubation at 28°C and scored as positive or negative. Lesions were graded daily during treatment and for up to 7 days after treatment ended and were used to calculate the group lesion score averages. The results, in Table 2, show the percent samples negative at each day and the average lesion score at the end of the experiment. On the basis of culture results, both SCH 39304 and SCH 42427 were active, even at the lowest concentrations tested (0.03 and 0.015%), although SCH 42427 appeared to act more rapidly. On the basis of lesion score results, SCH 42427 was still very active

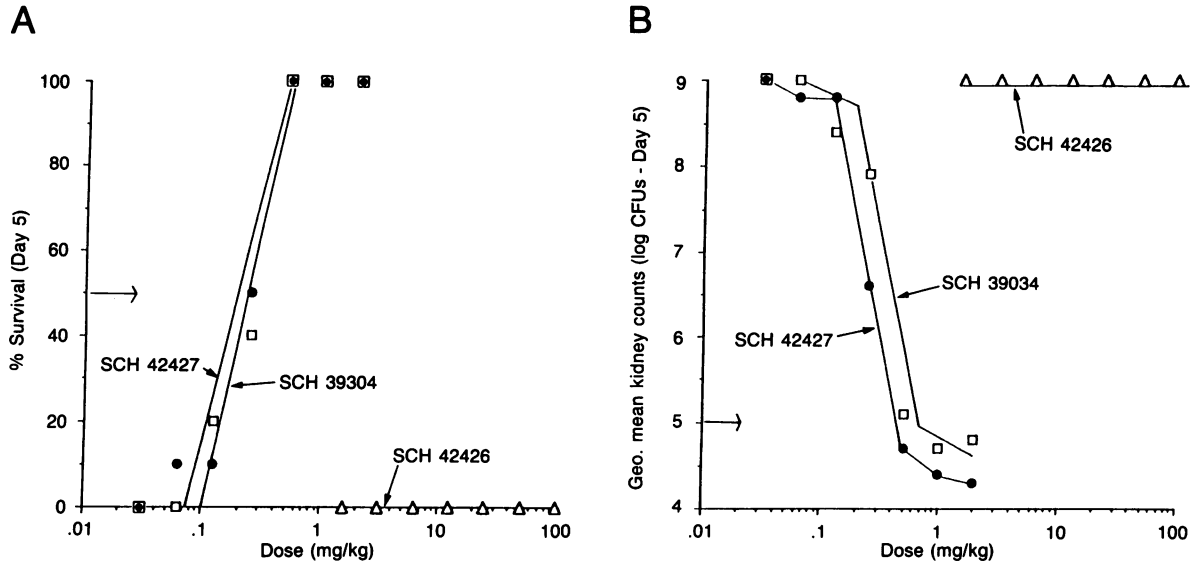


FIG. 1. Effects of SCH 39304, SCH 42427, and SCH 42426 on survival (A) and kidney counts (B) of *C. albicans*-infected mice treated orally once daily for 4 days. Arrows indicate $PD_{50}$s (A) and $ED_{50}$s (B).

at 0.06%, while SCH 39304 was not active below 0.125%. At all concentrations tested, SCH 42427 was more active than SCH 39304. SCH 42426 was inactive below 1% on the basis of culture results and was only slightly active even at 5% on the basis of lesion scores.

In summary, a series of in vivo and in vitro experiments were performed to identify some of the microbiological characteristics of SCH 42427 (RR,−) and to compare its activity with that of the other enantiomer, SCH 42426 (SS,+), and to the racemic mixture, SCH 39304. In vivo experiments were conducted in mice and hamsters (oral treatment) and in guinea pigs (topical treatment) with systemic or superficial fungal infections.

Results of these experiments indicated that SCH 42427 was the active enantiomer and was about two times more active than SCH 39304. SCH 42426 was essentially inactive. The slight activity observed with SCH 42426 in some experiments was most probably due to small amounts (≤1%) of SCH 42427 in the SCH 42426 samples tested.

In studies conducted by Allendoerfer et al. (1), SCH 42427 was more effective than SCH 39304 and SCH 42426 against meningeal *Coccidioides* and *Cryptococcus* infections in mice. Against *Coccidioides immitis*, SCH 42427 was about 5 times more active than SCH 39304 and over 50 times more active than SCH 42426. Against *Cryptococcus neoformans*, SCH 42427 was again about 5 times more active than SCH 39304 and about 100 times more active than SCH 42426.

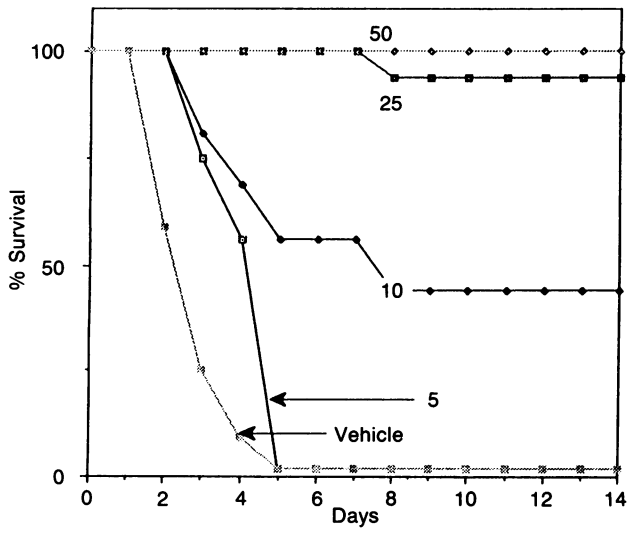


FIG. 2. Effect of various doses (in milligrams per kilogram) of SCH 42427 on survival of *A. flavus*-infected mice treated orally once daily for 4 days.

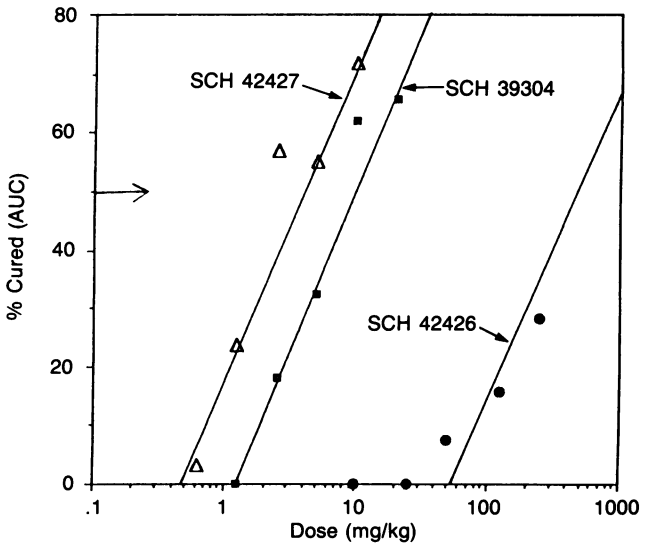


FIG. 3. Activities of SCH 39304, SCH 42427, and SCH 42426 (single dose, oral) against a *C. albicans* vaginal infection in hamsters. The arrow on the left indicates 50% cured values. AUC, area under the concentration-time curve.

In another study, SCH 42427 was also shown to be more active in vitro than SCH 39304 and to have better oral efficacy ($ED_{50}$, 0.27 mg/kg) than SCH 39304 (0.38 mg/kg) or fluconazole (1.4 mg/kg) against murine systemic candidiasis (15). These investigators also reported that SCH 42427 had better oral efficacy against murine systemic cryptococcosis and aspergillosis ($ED_{50}$, 0.71 and 4.1 mg/kg, respectively) than SCH 39304 (1.9 and 18 mg/kg, respectively) and fluconazole (29 and >125 mg/kg, respectively). SCH 42427 was also more efficacious than fluconazole in these infection models when administered intravenously. They also demonstrated that the oral bioavailability of SCH 42427 was 100%.

SCH 39304 and SCH 42427 have shown promising results in a number of in vivo infection studies with normal and immunocompromised animal models. These results, coupled with good pharmacokinetic qualities, suggested that SCH 39304 and SCH 42427 would be potentially effective antifungal drugs in humans. However, recent information from prolonged (18 to 24 months) toxicological studies indicated that hepatocellular adenomas and carcinomas were observed in rats and mice treated with SCH 39304. These effects were considered secondary to the potent liver enzyme induction potential of SCH 39304. Studies with SCH 42427 and SCH 42426 demonstrated that both enantiomers were equally potent as liver enzyme inducers. On the basis of these findings, the clinical programs for SCH 39304 and SCH 42427 have been terminated.

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