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In Vitro Studies with R 51,211 (Itraconazole)

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The in vitro activity of R 51,211 (itraconazole, accepted generic name; Janssen Pharmaceutica, Beerse, Belgium), a new orally active triazole, was compared with those of two existing orally active azoles, ketoconazole and BAY n 7133, and a topical agent, Ro 14-4767/002. An agar dilution procedure (Kimmig agar) was performed with 148 isolates of pathogenic fungi. Incubation was at 30°C from 48 h to 7 days. R 51,211 was dissolved in 0.2 N HCl in absolute ethanol, ketoconazole was dissolved in 0.2 N HCl alone, BAY n 7133 was dissolved in absolute ethanol, and Ro 14-4767/002 was dissolved in dimethyl sulfoxide. R 51,211 and Ro 14-4767/002 were the most active drugs against isolates of *Histoplasma capsulatum*, and R 51,211 showed the greatest activity in vitro against isolates of *Blastomyces dermatitidis* and *Cryptococcus neoformans*. Ro 14-4767/002 was the most active drug against 30 isolates of dermatophytes, followed by R 51,211, ketoconazole, and BAY n 7133. R 51,211 showed the best activity in vitro against 19 isolates of *Aspergillus fumigatus* and *Aspergillus flavus*, as well as 19 isolates of dematiaceous fungi. All four drugs had 90% MICs of ≥16 μg/ml when tested with isolates of zygomycetous fungi.

A number of new synthetic antifungal agents, including, in particular, the imidazoles and related azoles, have been developed in the last few years for use as oral or topical antifungal agents or both (1, 3, 7, 8, 10, 11). There are three distinct groups of antifungal azoles: fused-ring imidazole compounds, the *N*-substituted (mono) imidazoles (4, 6), and the *N*-substituted triazoles (11). R 51,211 {*cis*-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methyl-propyl)-3*H*-1,2,4-triazol-3-one; accepted generic name, itraconazole; Fig. 1} is a new triazole developed by Janssen Pharmaceutica, Beerse, Belgium. It has a molecular weight of 705.64 and a melting point of 167°C (R 51,211, Basic Medical Information Brochure, Janssen Pharmaceutica, May 1983).

Limited data now are available regarding the antifungal properties of R 51,211 (R 51,211, Basic Medical Information Brochure, Janssen Pharmaceutica, May 1983). In vitro, it is active against yeasts, dermatophytes, *Aspergillus fumigatus*, *Sporothrix schenckii*, and *Phialophora verrucosa*. Its half-life in humans is 11 to 15 h, with peak serum levels of 200 to 300 ng/ml obtained 2 to 3 h after a single 200-mg dose and with serum levels of 10 to 100 ng/ml persisting through 8 h. Clinically, it has been shown to be efficacious in humans in the treatment of pityriasis versicolor and vaginal candidiasis at dosages of 100 and 200 mg given once daily.

This study reports on the comparison in vitro of R 51,211 with the topical agent Ro 14-4767/002 (Hoffmann-LaRoche Inc., Basel, Switzerland) (8, 11) and two orally active azoles: the triazole BAY n 7133 (Bayer AG, Wuppertal, Federal Republic of Germany) (3, 11) and ketoconazole (Janssen Pharmaceutica, Piscataway, N.J.).

MATERIALS AND METHODS

Drugs. Four drugs were studied: ketoconazole (R 41,400; Janssen Pharmaceutica), BAY n 7133 (micronized, batch 744288; Bayer AG), Ro 14-4767/002 (Hoffmann-LaRoche Inc.), and R 51,211 (itraconazole; Janssen Pharmaceutica).

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Because of varying solubilities, each compound was dissolved in a different solvent. Ketoconazole was dissolved in 0.2 N HCl alone, BAY n 7133 was dissolved in absolute ethanol, Ro 14-4767/002 was dissolved in 100% dimethyl sulfoxide and R 51,211 was solubilized in a mixture of 0.2 N HCl in absolute alcohol (0.2 ml of 1 N HCl in 0.8 ml of absolute ethanol). The initial concentration for each drug in its respective solvent was 2,560 μg/ml. The maximum concentration of any of the solvents when finally diluted was 10%. Previous studies have shown such concentrations of dimethyl sulfoxide to be noninhibitory for the fungi used in this study (2).

Cultures. A total of 148 isolates of pathogenic and opportunistic fungi were tested, including 38 pathogenic yeasts, 30 dimorphic fungal pathogens, 30 dermatophytic fungi, 31 nonpigmented hypomycetes, and 19 dematiaceous pathogens. Isolates were grown on Sabouraud slants. Mature cultures were harvested with sterile normal saline, and the resulting suspensions were adjusted by turbidity to approximately 10^5 CFU/ml. Approximately half of the adjusted suspensions were tested by dilution colony plate counts to determine actual counts of CFU per milliliter. The Carshalton strain of *Candida pseudotropicalis* (ATCC 28838) was used in each set of experiments as a control of drug activity and intertest reproducibility.

MIC procedure. Kimmig agar (E. R. Merck, Federal Republic of Germany) supplemented with 0.5% glycerol was used for the agar dilution method as previously described (1, 10, 11). Serial dilutions of each drug (1,280 to 0.63 μg/ml) were prepared in 2-ml volumes of sterile saline. Molten Kimmig agar (18 ml) was added to each of the 2-ml drug concentrations. These mixtures were mixed and poured into sterile, square petri dishes (100 by 15 mm) and allowed to harden. Two drug-free plates of Kimmig agar (18 ml of agar plus 2 ml of saline) were added to each set of drug concentrations. In this way, each drug set contained 14 plates. Each set of plates for each drug was inoculated with the Steer replicator. One of the drug-free plates was inoculated at the beginning and the other at the end of the set. Plates were incubated at 30°C for 2 to 7 days according to the growth

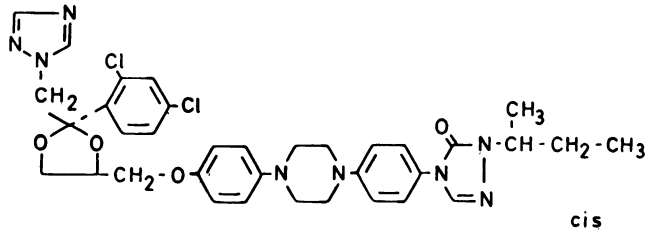

FIG. 1. Chemical structure of R 51,211

characteristics of the isolates being tested. Results with yeasts and most nonpigmented hypomycetes were determined after 48 h of incubation; the dimorphic and dematiaceous fungi and the dermatophytes required up to 7 days of incubation for production of mature colonies. Each plate was examined, and the resulting MICs were determined as the lowest concentrations of drug which permitted no visible growth.

RESULTS

Summaries of MIC statistics (range, geometric mean MIC [G-MIC], 50% MIC [MIC_{50}], and 90% MIC [MIC_{90}]) for R 51,211, ketoconazole, BAY n 7133, and Ro 14-4767/002 are presented in Table 1. Colony counts of the randomized inocula which were tested by dilution plate count generally were in the range of 10^4 to 10^5 CFU/ml. Five separate sets of experiments were performed; MIC responses for *C. pseudotropicalis* ATCC 28838 never exceeded 0.13 μg/ml with any of the four drugs tested (range, ≦0.063 to 0.13 μg/ml).

With the 38 pathogenic yeasts tested, R 51,211 was the least active drug against 24 isolates of *Candida* species with a G-MIC of 6.30 and an MIC_{90} of >128 μg/ml. Similarly, little in vitro activity was observed with the four isolates of *Torulopsis glabrata*. When data for individual species of *Candida* were analyzed, R 51,211 possessed good activity against isolates of *Candida parapsilosis* (G-MIC, 0.78 μg/ml) but was the least active against isolates of *Candida albicans* (G-MIC, 5.31 μg/ml) and *Candida tropicalis* (G-MIC, 47.8 μg/ml). Bimodal responses were observed in tests with all three azoles and isolates of *Candida* species. This was true for 6 of 11 isolates of *C. albicans* (MICs, 4 to 128 μg/ml), 2 of 6 isolates of *C. parapsilosis* (MICs, 32 to >128 μg/ml), and 6 of 7 isolates of *C. tropicalis* (MICs, 8 to >128 μg/ml). In contrast, R 51,211 was the most active drug against all 10 isolates of *Cryptococcus neoformans*, with a G-MIC of 0.07 μg/ml and an MIC_{50} and MIC_{90} of 0.063 μg/ml.

Thirty dimorphic fungi were tested. R 51,211 and Ro 14-4767/002 were the most active compounds against isolates of *Histoplasma capsulatum*, and R 51,211 was the most active drug against isolates of *Blastomyces dermatitidis*. The topical compound Ro 14-4767/002 was the most active drug against 10 isolates of *Sporothrix schenckii*, followed by ketoconazole, R 51,211, and BAY n 7133 in that order.

Thirty dermatophytic isolates were tested. In vitro activities of R 51,211, ketoconazole, and Ro 14-4767/002 were generally of the same magnitude against isolates of *Epidermophyton floccosum*. R 51,211 and Ro 14-4767/002 were the two most active compounds against isolates of *Microsporum* species; however, with the *Trichophyton* species, Ro 14-4767/002 showed the greatest in vitro activity, with a G-MIC of 0.07 μg/ml and an MIC_{90} of 0.13 μg/ml.

Thirty-one nonpigmented hypomycetes were tested. R 51,211 was, by far, the most active drug against two species of *Aspergillus*, whereas Ro 14-4767/002 was the least active. All four compounds showed little activity against eight isolates of zygomycetous fungi. Ro 14-4767/002 was the most active drug in tests with isolates of *Pseudallescheria boydii*, whereas R 51,211 was the least active.

Nineteen isolates of dematiaceous pathogens were tested. All four drugs showed an MIC_{90} of >128 μg/ml with the chromoblastomycosis group (*Fonsecaea pedrosoi* and *Phialophora verrucosa*); this was due primarily to the fact that two isolates of *Fonsecaea pedrosoi* were totally resistant to all four drugs. R 51,211, ketoconazole, and Ro 14-4767/002 showed similar activity against the other dematiaceous pathogens tested, and BAY n 7133 was the least active of the four compounds.

DISCUSSION

In vitro susceptibility data presented here show that, generally, R 51,211 has equal or greater activity against the majority of filamentous pathogenic and opportunistic fungi tested than either ketoconazole or BAY n 7133 (oral azoles) or Ro 14-4767/002. When data for individual species of *Candida* were compared in terms of G-MIC values, R 51,211, ketoconazole, and Ro 14-4767/002 demonstrated better activity against isolates of *C. parapsilosis*, whereas R 51,211 was the least active of the four drugs against isolates of *C. tropicalis* and *C. albicans*. Ketoconazole and BAY n 7133 revealed bimodal responses in tests with *C. albicans*, *C. parapsilosis*, and *C. tropicalis*; this also was observed in tests with R 51,211.

The clinical significance of in vitro bimodal patterns of susceptibility or resistance on the part of isolates of species of *Candida* to ketoconazole and other imidazole derivatives is not known. However, a recent report by Ryley et al. attributes apparent resistance in selected isolates of *C. albicans* to changes in cellular membrane properties (9). This report also raises the question of the predictive value of agar dilution procedures, such as those employed in the study reported here, in studies with imidazoles in general.

Results of in vitro studies with imidazoles and pathogenic yeasts often are difficult to evaluate in terms of both their predictive value and reproducibility. As noted above, Ryley et al. have questioned predictability (9). Others have questioned reproducibility (5). Although the data summarized here do not directly address the issue of predictability, they can be examined in terms of reproducibility. For example, 37 isolates of pathogenic yeasts were tested both in the studies reported here and in another recently reported study (11). In both studies ketoconazole and BAY n 7133 were compared in vitro by agar dilution with two new, orally active triazoles; ICI 153,066 was included in the earlier study performed in 1982, whereas R 51,211 was included in the study reported here, which was performed in 1983. Individual data for both ketoconazole and BAY n 7133 from both studies can be examined in terms of reproducibility. Such a comparison is not permitted by examination of the summarized MIC statistics alone.

Ten isolates of *C. albicans* were tested in both studies. Major discrepancies between results from the two studies were noted with two isolates and data for ketoconazole and with a third isolate and data for BAY n 7133. In all three instances, the isolates appeared to be only intermediately susceptibile to either imidazole in 1982 (MIC, ≧8 μg/ml) but were uniquely susceptible in 1983 (MIC, ≦0.5 μg/ml). In contrast, one of four isolates of *T. glabrata* was uniquely susceptible to both ketoconazole and BAY n 7133 in 1982 (MIC for both, 0.25 μg/ml) but was only partially susceptible in

TABLE 1. In vitro studies with R 51,211, ketoconazole, BAY n 7133, and Ro 14-4767/002[a]

Types of organisms (no. tested), organism (no. tested), and drugs	MIC statistics (μg/ml)			
	Range	G-MIC	MIC_{50}	MIC_{90}
I. Pathogenic yeasts (38)				
C. albicans (11)				
R 51,211[b]	0.063–128	5.31	16	128
Ketoconazole	0.063–64	2.00	4	16
BAY n 7133	0.063–64	1.77	4	32
Ro 14-4767/002	0.063–16	3.76	8	8
C. parapsilosis (6)				
R 51,211	0.063–>128	0.78	0.063	<128
Ketoconazole	0.063–4	0.25	0.13	4
BAY n 7133	0.5–64	5.66	4	64
Ro 14-4767/002	0.063–4	0.71	1	4
C. tropicalis (7)				
R 51,211	0.13–>128	47.8	>128	>128
Ketoconazole	0.063–64	5.39	8	64
BAY n 7133	0.13–128	32.0	64	128
Ro 14-4767/002	0.063–64	4.42	8	64
All *Candida* species (24)				
R 51,211	0.063–128	6.30	128	>128
Ketoconazole	0.063–64	1.59	4	16
BAY n 7133	0.063–128	5.50	16	128
Ro 14-4767/002	0.063–64	2.60	4	16
T. glabrata (4)				
R 51,211	2–128	45.3	128	128
Ketoconazole	0.13–4	1.43	2	4
BAY n 7133	0.5–4	2.38	4	4
Ro 14-4767/002	0.063–8	1.19	2	8
Cryptococcus neoformans (10)				
R 51,211	0.063–0.13	0.07	0.063	0.063
Ketoconazole	0.063–0.5	0.18	0.13	0.25
BAY n 7133	0.13–4	1.42	2	4
Ro 14-4767/002	0.063–2	0.22	0.063	2
II. Dimorphic fungi (30)[c]				
B. dermatitidis (10)				
R 51,211	0.063–0.13	0.08	0.063	0.13
Ketoconazole	0.5–2	0.71	0.5	1
BAY n 7133	0.25–4	1.62	2	2
Ro 14-4767/002	0.13–0.5	0.23	0.25	0.5
H. capsulatum (10)				
R 51,211	0.063	0.06	0.063	0.063
Ketoconazole	0.063–1	0.18	0.13	0.25
BAY n 7133	0.063–2	0.22	0.25	1
Ro 14-4767/002	0.063	0.06	0.063	0.063
S. schenckii (10)				
R 51,211	1–4	2.46	2	4
Ketoconazole	0.5–2	1.15	1	2
BAY n 7133	64	64	64	64
Ro 14-4767/002	0.063–0.13	0.08	0.063	0.13
III. Dermatophytic fungi (30)				
E. floccosum (5)				
R 51,211	0.063	0.06	0.063	0.063
Ketoconazole	0.063–0.25	0.08	0.063	0.25
BAY n 7133	0.25–0.5	0.33	0.25	0.5
Ro 14-4767/002	0.063	0.06	0.063	0.063
Microsporum species (10)				
R 51,211	0.063–0.25	0.12	0.13	0.25

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TABLE 1—*Continued*

Types of organisms (no. tested), organism (no. tested), and drugs	MIC statistics (μg/ml)			
	Range	G-MIC	MIC_{50}	MIC_{90}
Ketoconazole	0.5–2	1.07	1	2
BAY n 7133	0.5–4	1.15	1	2
Ro 14-4767/002	0.063–0.5	0.08	0.063	0.063
Trichophyton species (15)				
R 51,211	0.063–64	0.23	0.13	4
Ketoconazole	0.063–4	0.72	1	2
BAY n 7133	0.13–16	1.74	2	8
Ro 14-4767/002	0.063–0.13	0.07	0.063	0.13
All dermatophytes (30)				
R 51,211	0.063–64	0.15	0.13	0.25
Ketoconazole	0.063–4	0.58	0.5	2
BAY n 7133	0.13–16	1.15	1	8
Ro 14-4767/002	0.063–0.5	0.07	0.063	0.063
IV. Nonpigmented hypomycetes (31)				
Aspergillus flavus (9)				
R 51,211	0.063–0.13	0.07	0.063	0.13
Ketoconazole	0.25–1	0.59	0.5	1
BAY n 7133	8–32	13.72	16	32
Ro 14-4767/002	>128	<128	>128	>128
A. fumigatus (10)				
R 51,211	0.063–2	0.12	0.063	0.13
Ketoconazole	4–16	6.06	4	8
BAY n 7133	0.5–8	2.30	2	4
Ro 14-4767/002	16–>128	90.51	>128	>128
P. boydii (4)				
R 51,211	16–32	26.9	32	32
Ketoconazole	2	2.0	2	2
BAY n 7133	2–8	4.76	4	8
Ro 14-4767/002	0.063	0.06	0.063	0.063
Zygomycetes (8)[d]				
R 51,211	0.063–>128	16.02	64	>128
Ketoconazole	1–>128	14.67	4	>128
BAY n 7133	16–128	76.11	64	128
Ro 14-4767/002	4–16	13.45	16	16
V. *Dematiaceae* (19)				
Phaeohypomycosis complex (13)[e]				
R 51,211	0.063–0.25	0.08	0.063	0.13
Ketoconazole	0.063–1	0.18	0.13	0.5
BAY n 7133	1–8	3.60	4	8
Ro 14-4767/002	0.063–0.25	0.10	0.25	0.25
Chromoblastomycosis complex (6)[f]				
R 51,211	0.063–>128	1.12	0.13	>128
Ketoconazole	0.13–>128	2.0	0.25	>128
BAY n 7133	2–>128	16.0	8	>128
Ro 14-4767/002	0.13–>128	1.59	0.25	>128
All *Dematiaceae* (19)				
R 51,211	0.063–>128	0.18	0.063	0.13
Ketoconazole	0.063–>128	0.39	0.13	1
BAY n 7133	1–>128	5.76	4	16
Ro 14-4767/002	0.063–>128	0.23	0.13	0.25

[a] As determined on Kimmig agar; incubation at 30°C.
[b] Data for one isolate are excluded.
[c] Tested in the mycelial phase only.
[d] Five isolates of *Rhizopus* spp., three isolates of *Mucor* spp.
[e] *Exophiala jeanselmei* (4); *Wangiella dermatitidis* (5); *Cladosporium bantianum* (4).
[f] *Fonsecaea pedrosoi* (3); *Phialophora verrucosa* (3).

1983 (MIC for both, 4 μg/ml). The MIC for one isolate of *C. parapsilosis* and BAY n 7133 was 64 μg/ml in 1982 but only 4 μg/ml in 1983. No such discrepancies existed in paired MIC data for both drugs in studies with *C. tropicalis* and *Cryptococcus neoformans*. In summary, when individual data from these two studies are compared, MICs for 31 of 37 MIC pairs (84%) are in agreement in terms of reproducibility.

Preliminary in vivo experiments with oral dosages of 10 to 80 mg/kg of R 51,211 have demonstrated protection against experimental infections due to *C. neoformans*, *S. schenckii*, and *H. capsulatum* (R 51,211, Basic Medical Information Brochure, Janssen Pharmaceutica, May 1983). Also, it has been shown that R 51,211 has curative effects at dosages of 2.5 to 10 mg/kg in experimentally disseminated trichophytosis and systemic candidiasis (12). Thus, based on in vitro data presented here and the available preliminary in vivo data, R 51,211 appears to be a promising oral antifungal agent.

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