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In Vitro Activity of Syn-2869, a Novel Triazole Agent, against Emerging and Less Common Mold Pathogens

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The in vitro activity of Syn-2869 was compared with that of amphotericin B and itraconazole. MICs for 100 isolates of pathogenic molds belonging to 12 species were determined by a broth microdilution adaptation of the method recommended by the National Committee for Clinical Laboratory Standards. Syn-2869 and itraconazole showed comparable, good activity against the dematiaceous molds *Cladophialophora bantiana*, *Cladophialophora carrionii*, *Exophiala dermatitidis*, *Fonsecaea pedrosoi*, *Phialophora parasitica*, and *Ramichloridium mackenziei*. Neither of the azole agents was active against the hyaline molds *Fusarium solani*, *Scedosporium prolificans*, and *Scopulariopsis brevicaulis*, but both were more active than amphotericin B against *Scedosporium apiospermum*. The MICs of the three agents were comparable for the mucoraceous mold *Absidia corymbifera*, but Syn-2869 appeared to be the least active against the dimorphic mold *Sporothrix schenckii*. Our results suggest that Syn-2869 could be effective against a range of mold infections in humans.

The incidence of invasive mold infections is increasing, largely because of the rising number of immunocompromised patients (2, 19, 30). Although *Aspergillus* spp. are still the commonest causes of mold infection in these individuals, a growing number of other organisms, including *Fusarium* and *Scedosporium* spp., have been reported to cause lethal infection (2, 19, 30). Until recently, amphotericin B was the only effective agent against many mold infections, despite the fact that its use is seriously limited by nephrotoxicity and other side effects (11). Lipid-based preparations have reduced the toxicity but not significantly increased the efficacy of amphotericin B (16, 21, 23). In 1990 the triazole agent itraconazole became available, and it has since been used successfully to treat many patients with mold infections such as aspergillosis (4) and phaeohyphomycosis (29). However, not all mold infections respond to treatment with amphotericin B or itraconazole (3, 12), and there is a continuing need for new antifungal agents with a broad spectrum of action.

Syn-2869 (Fig. 1) is a new triazole antifungal agent (1) which has been reported to have potent in vitro and in vivo activity against isolates of *Aspergillus* spp., *Candida* spp., and *Cryptococcus neoformans* (9, 10, 13, 27, 28). To evaluate the potential usefulness of Syn-2869 in other infections, we compared its activity in vitro against 12 species of emerging and less common mold pathogens with the activities of amphotericin B and itraconazole. The in vitro testing method we employed was a microdilution adaptation of the standard broth macrodilution reference method of the National Committee for Clinical Laboratory Standards (NCCLS) (8, 20).

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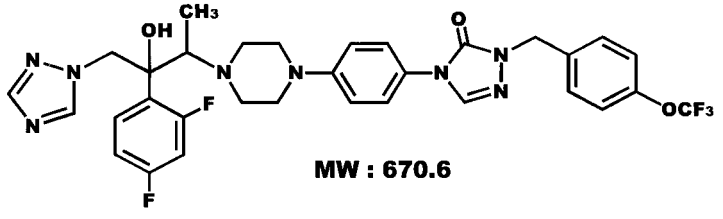
Test isolates. A total of 100 isolates were tested. These comprised 10 each of *Absidia corymbifera*, *Cladophialophora bantiana*, *Exophiala dermatitidis*, *Fonsecaea pedrosoi*, *Fusarium solani*, *Phialophora parasitica*, *Scedosporium apiospermum*, and *Sporothrix schenckii* and five each of *Cladophialophora carrionii*, *Ramichloridium mackenziei*, *Scedosporium prolificans*, and *Scopulariopsis brevicaulis*. The isolates tested came from the United Kingdom National Collection of Pathogenic Fungi (NCPF), held at the Mycology Reference Laboratory, Bristol, United Kingdom. Two reference strains, *Aspergillus fumigatus*



(2*R*, 3*R*)-2-(2,4-difluorophenyl)-3-[4-{4-[2-(4-trifluoromethoxybenzyl)-2*H*-1,2,4-triazol-3-one-4-yl]phenyl}piperazin-1-yl]-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol

FIG. 1. Chemical structure of Syn-2869.

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NCPF 7097 and *A. fumigatus* NCPF 7100, were included in each batch of tests to ensure quality control.

Isolates were retrieved from storage in liquid nitrogen or water, subcultured on plates of Oxoid Sabouraud dextrose agar (Unipath Ltd., Basingstoke, United Kingdom) supplemented with 0.5% (wt/vol) chloramphenicol, and incubated at 30°C until adequate growth was obtained. To induce spore formation, the isolates were subcultured on slopes of Oxoid potato dextrose agar and incubated at 35°C for 7 days (8). Isolates of *F. solani* were incubated at 35°C for 2 to 3 days and then at 28 to 30°C for 4 to 5 days.

Antifungal agents. Syn-2869 was obtained from SynPhar Laboratories Inc., Edmonton, Alberta, Canada, itraconazole was obtained from Janssen Research Foundation, Beerse, Belgium, and amphotericin B was obtained from Sigma Chemical Co. (St. Louis, Mo.). Stock solutions of Syn-2869 and itraconazole were prepared in polyethylene glycol 400, with the aid of heating to 70°C. Amphotericin B was dissolved in dimethyl sulfoxide. Further dilutions were made with RPMI 1640 medium (with L-glutamine, without bicarbonate) (Sigma), buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (Sigma). The recommendations stated in NCCLS document M27-A were followed for the dilution of each antifungal agent (20). The antifungal agents were tested over a final concentration range of 0.03 to 16 μg/ml.

Antifungal susceptibility testing. Broth microdilution MICs were determined in 96-well, round-bottom microtiter plates, with a final volume of 200 μl per well. Spore suspensions were prepared in RPMI 1640 medium and adjusted to a final inoculum concentration of 0.4×10^4 to 5×10^4 spores/ml (8). The plates were incubated at 35°C and read after 24 h (*A. corymbifera*) or 48 h. The growth in each well was compared with that of the controls. The MIC was defined for amphotericin B as the lowest concentration at which there was complete inhibition of growth and for Syn-2869 and itraconazole as the lowest concentration at which there was prominent or complete inhibition of growth.

Results. The in vitro activities of Syn-2869, itraconazole, and amphotericin B against the 100 mold isolates are summarized in Table 1. The data are presented as MIC ranges and, where appropriate, as the drug concentrations required to inhibit 50% and 90% of the isolates of each species (MIC_{50} and MIC_{90}). In each batch of tests, the MICs of amphotericin B and itraconazole for the control strains were within the accepted limits.

Both Syn-2869 and itraconazole were more active than amphotericin B against the dematiaceous molds *C. bantiana*, *C. carrionii*, *E. dermatitidis*, *F. pedrosoi*, and *R. mackenziei*. However, Syn-2869 was more active than the other two agents against *P. parasitica*. The MIC_{50} and the MIC_{90} of each of the three agents were comparable for the mucoraceous mold *A. corymbifera*, but Syn-2869 appeared to be the least active against the dimorphic mold *S. schenckii*. Neither of the azole agents was active against the hyaline molds *F. solani*, *S. prolificans*, and *S. brevicaulis*, but both were more active than amphotericin B against *S. apiospermum*.

TABLE 1. In vitro activities of Syn-2869, itraconazole, and amphotericin B against 100 pathogenic mold isolates

Organism (no. of isolates)	Agent[a]	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
Absidia corymbifera (10)	Syn-2869	0.25–1	0.5	0.5
	ITR	0.25–0.5	0.25	0.5
	AMB	0.06–0.25	0.25	0.25
Cladophialophora bantiana (10)	Syn-2869	≤0.03–1	0.06	0.12
	ITR	≤0.03–0.25	0.06	0.12
	AMB	0.25–0.5	0.25	0.5
Cladophialophora carrionii (5)	Syn-2869	≤0.03–0.12		
	ITR	0.06–0.25		
	AMB	0.25–4		
Exophiala dermatitidis (10)	Syn-2869	0.12–1	0.25	0.5
	ITR	0.12–0.5	0.25	0.5
	AMB	0.12–1	0.5	1
Fonsecaea pedrosoi (10)	Syn-2869	0.06–0.5	0.25	0.25
	ITR	0.12–0.25	0.12	0.25
	AMB	0.5–2	1	1
Fusarium solani (10)	Syn-2869	>16	>16	>16
	ITR	>16	>16	>16
	AMB	1–2	1	2
Phialophora parasitica (10)	Syn-2869	0.5	0.5	0.5
	ITR	1–2	1	2
	AMB	0.12–2	1	2
Ramichloridium mackenziei (5)	Syn-2869	0.12–0.5		
	ITR	0.12–0.25		
	AMB	4–>16		
Scedosporium apiospermum (10)	Syn-2869	0.5–2	1	1
	ITR	0.25–4	1	4
	AMB	1.0–>16	2	8
Scedosporium prolificans (5)	Syn-2869	>16		
	ITR	>16		
	AMB	2–>16		
Scopulariopsis brevicaulis (5)	Syn-2869	>16		
	ITR	>16		
	AMB	2–16		
Sporothrix schenckii (10)	Syn-2869	≤0.03–>16	0.5	>16
	ITR	0.06–>16	0.5	4
	AMB	0.5–4	2	4
Aspergillus fumigatus NCPF 7097[b]	Syn-2869	0.5		
	ITR	0.12–0.5		
	AMB	0.5–1		
Aspergillus fumigatus NCPF 7100[b]	Syn-2869	0.5–1		
	ITR	4–>16		
	AMB	1–2		

[a] ITR, itraconazole; AMB, amphotericin B.
[b] Quality control isolate.

Discussion. Although aspergillosis is still the commonest mold infection in immunocompromised patients, an increasing number of other environmental molds are being implicated as the cause of significant human infection (2, 19, 30). Among the more important of these emerging pathogens are *Fusarium* and *Scedosporium* spp., many isolates of which appear to be resistant to amphotericin B or itraconazole (3, 12, 17, 24). Dematiaceous molds, such as *Cladophialophora*, *Exophiala*, and *Phialophora* spp., have long been recognized as important causes of subcutaneous infection following traumatic inoculation, but they have also begun to emerge as important causes of deep fungal infection. These brown-pigmented molds are often susceptible to amphotericin B in vitro, as well as to triazole antifungal agents, such as itraconazole and voriconazole (6, 14, 18, 25). However, patients with these infections often fail to respond to currently available antifungal agents (26), and there is a need for new compounds.

Our results suggest that Syn-2869 is a broad-spectrum antifungal agent, effective in vitro against a wide range of organisms, including the mucoraceous mold *A. corymbifera* and the amphotericin B-resistant mold *S. apiospermum*. Like two other investigational triazoles, SCH 56592 and voriconazole, Syn-2869 was active against a range of dematiaceous molds but ineffective against the hyaline molds *F. solani* and *S. prolificans*

(6, 7, 14). Unlike voriconazole, Syn-2869 appears to be active against the mucoraceous mold *A. corymbifera* (14). However, some caution must be exercised in making any conclusions regarding the relative potencies of the different triazole agents. Many of the molds studied in this investigation are uncommon causes of human infection, and the number of isolates available for testing was limited. The differences in MICs between the agents might have been more or less evident had larger numbers of isolates of some molds been tested.

It remains to be seen to what extent the low MICs seen with Syn-2869 in this and other investigations (9, 13, 28) will be predictive of clinical outcome. A standardized method has been developed for determining the MICs of five antifungal agents for *Candida* spp. and *C. neoformans* (20). In addition, interpretive breakpoints for *Candida* spp. have been proposed for itraconazole and fluconazole on the basis of a comparison of the clinical outcome of treatment with the MICs of the agents for the organisms isolated (26). Although standardization of antifungal susceptibility testing of molds is at a less advanced stage, a multicenter study involving 11 laboratories and 30 isolates showed a high level of agreement among the MICs of amphotericin B and itraconazole, determined by a broth microdilution adaptation of the NCCLS M27 method (8). In addition, correlations between antifungal drug susceptibilities of some molds in vitro and treatment outcomes in animal models of infection have been reported (5, 22). However, further studies will be required before firm conclusions can be drawn.

Initial pharmacokinetic data for mice indicate that Syn-2869 is well absorbed after oral administration (15). It has a serum half-life of about 6 h in rabbits, which is shorter than that of itraconazole, but has a higher tissue-to-plasma ratio than the older compound (15).

In conclusion, our results demonstrate that Syn-2869 is effective against a range of emerging and less common mold pathogens in vitro. Based on these findings and the favorable results from animal models in the treatment of aspergillosis, candidiasis, and cryptococcosis (10, 27), this triazole compound deserves further in vitro and in vivo investigation.

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