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In Vitro Antifungal Activities of the New Triazole UR-9825 against Clinically Important Filamentous Fungi

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We used a modified reference microdilution method (the M-38P method) to evaluate the in vitro activities of the new triazole UR-9825 in comparison with those of amphotericin B against 77 strains of opportunistic filamentous fungi. UR-9825 was clearly more active than amphotericin B against all fungi except *Fusarium solani* and *Scytalidium* spp. Notably, UR-9825 had low MICs for *Aspergillus fumigatus* and *Paecilomyces lilacinus* (MICs at which 90% of isolates are inhibited, 0.125 μg/ml for both species).

The incidence of opportunistic infections caused by molds is continuously increasing, and such infections can be severe and difficult to treat, especially in immunocompromised patients. Immunosuppressive and cytotoxic therapies and hematological malignancies, among others, are important risk factors for such infections (6, 12, 18). Nowadays, the options for treatment of opportunistic infections are still led by amphotericin B and, to a lesser extent, a few azole derivatives, which frequently fail. For this reason the development of new potent and broad-spectrum antifungal agents is an important challenge for modern medicine. UR-9825 is a new triazole with a potent, broad spectrum of antifungal activity, good pharmacokinetics, and excellent bioavailability (3). It has been demonstrated to have good in vitro activities against pathogenic yeasts (17; J. Bartroli, E. Turmo, M. Algueró, E. Boncompte, L. Vericat, L. Conte, J. Ramis, J. García-Rafanell, and J. Forn, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E67, p. 125, 1997) and some filamentous fungi (10; B. Fernández-Torres, A. Carrillo, E. Martín, A. del Palacio, M. K. Moore, A. Valverde, M. Serrano, and J. Guarro, 6th Congr. Eur. Confed. Med. Mycol. Soc., abstr. P9–014, p. S161, 2000; J. Guarro, J. Cano, J. Gené, M. Solé, and A. J. Carrillo-Muñoz, Abstr. 14th Congr. Int. Soc. Hum. Anim. Mycol., p. 84, 2000). It has also been shown to have activity for the treatment of systemic aspergillosis and candidiasis in experimental animal models (M. L. Vericat, M. Algueró, M. Merlos, L. Perez, A. Araño, and J. Forn, Final Programme Abstr. Book, Trends in Invasive Fungal Infections 4, abstr. P-94, p. 142, 1997).

In the study described here we have compared the in vitro activities of this compound and those of amphotericin B against 77 clinically important filamentous fungi from our collection (Facultat de Medicina de Reus). They included 10 isolates of *Aspergillus fumigatus*, 11 isolates of *Aspergillus flavus*, 11 isolates of *Aspergillus niger*, 10 isolates of *Fusarium solani*, 10 isolates of *Paecilomyces variotii*, 10 isolates of *Paecilomyces lilacinus*, 10 isolates of *Chaetomium globosum*, 2 isolates of *Scytalidium lignicola*, and 3 isolates of *Scytalidium dimidiatum*. Isolates were retrieved from storage in water or slant cultures covered with sterile parafin oil and were subcultured on potato dextrose agar plates at 35°C for 7 to 10 days and, in the case of *C. globosum*, on oatmeal agar plates at 25°C for 15 days (9). *P. variotii* ATCC 36257 was included as the quality control strain, and it was tested in each series. UR-9825 and amphotericin B were provided as pure powders by J. Uriach & Co. (Barcelona, Spain) and the U.S. Pharmacopeia (Rockville, Md.), respectively. Both drugs were dissolved in dimethyl sulfoxide (Panreac Química S.A., Barcelona, Spain) and diluted in RPMI 1640 buffered with morpholinepropanesulfonic acid (MOPS) to a final concentration range of 16 to 0.03 μg/ml. The inocula were prepared by scraping the sporulated fungi from the agar plates with a loop and suspending them in sterile saline solution. The fungal suspensions were filtered once through sterile gauze to remove the hyphae. For the preparation of ascospores and conidial suspensions of *C. globosum* and *Aspergillus* spp., respectively, we used 0.05% Tween 20 (Panreac Química S.A.) in sterile saline solution. The resulting suspensions were vigorously vortexed and adjusted spectrophotometrically (λ = 530 nm) to 80 to 82% transmittance for *Aspergillus* spp., 68 to 70% transmittance for *Fusarium* spp. (14), and 74 to 76% transmittance for *Paecilomyces* spp. Inocula of *Scytalidium* spp. and *C. globosum* were prepared with a hemocytometer. The final inocula in the microtiter plates were 0.34×10^4 to 6.5×10^4 spores/ml.

MICs were determined by a broth microdilution method mainly by following National Committee for Clinical Laboratory Standards guidelines for molds (14). MICs were determined in 96-well round-bottom microtiter plates, with a final volume of 200 μl per well. A 100-μl fungal inoculum was added to each well of the microdilution trays, which were incubated without agitation at 35°C with the exception of trays containing *C. globosum*, which were incubated at 25°C (9). Readings were taken when growth in the drug-free well was evident. The MICs of UR-9825 were determined visually with a reading mirror and were the lowest drug concentrations that inhibited 50% fungal growth compared with the growth in the drug-free well. The MICs of amphotericin B were the lowest drug concentrations that inhibited 100% of the fungal growth.

Most of the fungal isolates tested produced adequate growth in microtiter plates within 48 h. MICs for *Scytalidium* spp. were interpreted at 96 h, and those for *C. globosum* isolates were interpreted at 120 h. The in vitro activities of UR-9825 and

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TABLE 1. In vitro activities of UR-9825 and amphotericin B against pathogenic mold isolates

Species (no. of isolates) and antifungal agent	MIC (μg/ml)		
	Range	50%	90%
Aspergillus fumigatus (10)			
UR-9825	0.06–0.125	0.06	0.125
Amphotericin B	1–2	1	2
Aspergillus flavus (11)			
UR-9825	0.06–0.25	0.125	0.25
Amphotericin B	0.25–4	1	2
Aspergillus niger (11)			
UR-9825	0.06–0.5	0.25	0.5
Amphotericin B	0.06–1	0.125	1
Fusarium solani (10)			
UR-9825	4–>16	16	>16
Amphotericin B	1–2	2	2
Paecilomyces variotii (10)			
UR-9825	0.03–>16	0.06	0.125
Amphotericin B	0.125–1	0.25	0.5
Paecilomyces lilacinus (10)			
UR-9825	0.06–0.5	0.125	0.125
Amphotericin B	>16	>16	>16
Chaetomium globosum (10)			
UR-9825	1–2	2	2
Amphotericin B	1–16	4	16
Scytalidium lignicola (2)			
UR-9825	>16		
Amphotericin B	2		
Scytalidium dimidiatum (3)			
UR-9825	2–>16		
Amphotericin B	2–4		

amphotericin B against the 77 strains are summarized in Table 1. The data are presented as MIC ranges and as the lowest drug concentration required to inhibit 50 and 90% of the isolates of each species (MIC_{50} and MIC_{90}, respectively). With the exception of the MICs for *F. solani* and *Scytalidium* spp., UR-9825 had lower MICs than amphotericin B. The high MICs of amphotericin B and UR-9825 for *F. solani* (MIC_{90}s, 2 and >16 μg/ml, respectively) were not a surprise because of the poor activities of all the available antifungals against this fungus that have previously been reported by different investigators (5, 8, 15, 16). UR-9825 and amphotericin B were also poorly active against the two species of *Scytalidium* tested (MIC_{90} of UR-9825, >16 μg/ml for both species; MIC_{90}s of amphotericin B for *S. lignicola* and *S. dimidiatum*, 2 and 4 μg/ml, respectively). *Scytalidium* spp. are common agents of onychomycosis. Amphotericin B and ketoconazole are the drugs recommended for the treatment of these infections, although in many cases they are ineffective. Recently, we tested the in vitro activities of six antifungal agents including amphotericin B, miconazole, itraconazole, ketoconazole, fluconazole, and flucytosine against 17 strains of *Scytalidium* spp. (7). In that study, amphotericin B (MIC_{90}, 1 μg/ml) and miconazole (MIC_{90}, 4 μg/ml) showed the best results. As miconazole is not well tolerated when it is administered intravenously, amphotericin B seems to be the only drug available for the treatment of these systemic infections.

One of the most noteworthy aspects of the present study is the good activity of UR-9825 against the three species of *Aspergillus* tested. The MIC ranges and MIC_{90}s were 0.06 to 0.125 and 0.125 μg/ml for *A. fumigatus*, respectively; 0.06 to 0.25 and 0.25 μg/ml for *A. flavus*, respectively; and 0.06 to 0.5 and 0.5 μg/ml for *A. niger*, respectively. These values were lower than those for amphotericin B. The latter is still the antifungal most frequently used to treat aspergillosis. Recently, Verweij et al. (P. E. Verweij, A. J. M. M. Rijs, J. P. Donnelly, and J. F. G. M. Meis, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J33, p. 460, 1998) also tested UR-9825 against *A. fumigatus* and obtained similar results (MIC range, 0.06 to 0.5 μg/ml). The new triazole also demonstrated excellent activity against the 20 strains of *P. variotii* and *P. lilacinus* tested. The MIC_{90} was 0.125 μg/ml for both species. This is very interesting, especially in the case of *P. lilacinus*, an emerging opportunistic fungus which in recent years has caused larger numbers of infections in humans ranging in severity from nail infections to fatal endocarditis (4, 6, 10, 19). Until now this fungus has always been resistant to the available antifungals. In the present study amphotericin B was ineffective (MIC, >16 μg/ml) against all isolates tested. In a previous study by Aguilar et al. (1), numerous strains of *Paecilomyces* were tested against six antifungals, and none of them was shown to be effective against *P. lilacinus*. Few data on the susceptibilities of *Chaetomium* spp. exist. This genus has been involved in several cases of infections in humans (2, 6, 9, 11, 13). In our study we have included 10 strains of *C. globosum* as a representative species of the genus. The MICs of both antifungals were relatively high for this species; however, those of UR-9825 (MIC_{90}s, 2 μg/ml) were clearly lower than those of amphotericin B (MIC_{90}s, 16 μg/ml). Guarro et al. (9) previously tested the activities of six antifungal agents against numerous clinical and environmental strains of *Chaetomium* spp. and showed that the MICs of the six agents were similar to the MIC of amphotericin B (MIC_{90}, 9.23 μg/ml at 72 h of incubation), despite the use of a different methodology (the macrodilution method).

In conclusion, the MICs of UR-9825 were, in general, lower than those of amphotericin B for several strains of opportunistic fungi, including some species refractory to treatment such as *P. lilacinus*. This potent triazole constitutes a promising therapeutic agent for the treatment of those fungal infections for which there is no effective therapy; however, further in vivo studies with experimental animal models are needed to confirm this activity.

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