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NOTES

In Vitro Antifungal Activity of Novel Azole Derivatives with a Morpholine Ring, UR-9746 and UR-9751, and Comparison with Fluconazole

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Thirty-three patient fungal isolates were studied by broth macrodilution methods for susceptibility to novel azole derivatives with a morpholine ring, UR-9746 and UR-9751, and fluconazole. MICs (μg/ml) ranged widely, but none had lower MICs for Candida albicans *or* Cryptococcus neoformans *than UR-9751. Fluconazole and UR-9751 had the most activity versus other* Candida *species. Activity was demonstrated versus endemic fungal pathogens.* Aspergillus *species were generally resistant, although modest activity was seen. UR-9746 and UR-9751 are active in vitro, with a potency comparable to that of fluconazole.* © *1997 Elsevier Science Inc.*

INTRODUCTION

The clinician has few choices for drugs for the treatment of deep fungal infections. There are currently six drugs licensed in the U.S., and they are limited by toxicity, parenteral route of administration, lack of cidal effect in vivo, deficiencies in spectrum, or combinations of these (Stevens 1994). Four of these drugs are of the azole group, and synthetic modifications in this group have given us improved agents. The search for improved drugs continues. The present study concerns novel azole derivations with a morpholine ring. The morpholine ring is also a constituent of effective topical antifungals (Polak 1992); in addition to the possible antifungal contribution of this ring, it may affect the conformation of the molecule and enable a better fit with terminal membrane enzyme targets in the ergosterol synthetic pathway. The in vitro activity of UR-9746 and UR-9751 (J. Uriach & Cia., Barcelona, Spain) (Bartroli et al. 1995) was compared to fluconazole, presently the most prescribed antifungal worldwide.

Two broth macrodilution methods were used. The methods of the NCCLS proposed standard (Fromtling et al. 1993), which uses RPMI-1640 medium, was employed for the yeasts for which the method was designed. The previously detailed method standardized in our laboratory (Hanson and Stevens 1992) for two decades, which uses an inoculum of 10^3/ml and media and procedures modified as shown in Table 1 to obtain optimal growth for each species, was used for filamentous organisms and those organisms that would not grow adequately in RPMI-1640 medium (e.g., *Blastomyces dermatitidis*). With both methods, a quality control (QC) susceptible isolate (*Candida kefyr*) was included in every run; in all instances the QC isolate gave identical results for all three drugs, or varied only one tube dilution. Minimal fungicidal concentrations (MFCs) were performed as previously described (Hanson and Stevens 1992); subculture from clear tubes to solid medium was used, and >96% killing

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TABLE 1 Details of antifungal MIC and MFC determination for various isolates

	Inoculum Preparation					MIC				MFC		
Organism	Form Tested[a]	Medium[b]	Time	Temp (°C)	Method of Enumeration	Broth Medium[b]	Temp (°C)	Time to Read	Shaking	Plate Medium[b]	Time to Read	Temp (°C)
Candida species	Y	YNB	24 h	35	$\%T_{530}$[c]	RPMI	35	48 h	No	SDA	48 h	35
C. neoformans	Y	SAAMF	24 h	35	$\%T_{530}$	RPMI	35	72 h	No	SDA	48 h	35
H. capsulatum	Y	RPMI	48 h	35	$\%T_{530}$	RPMI	35	8–13 days	Yes	BAP	3–5 days	35
B. dermatitidis	Y	BAP	72 h	35	Counted[d]	SAAMF	35	4–5 days	Yes	BAP	3–5 days	35
P. brasiliensis	Y	MVM	7 days	35	Counted	MVM	35	8 days	Yes	SDA + thiamine	6–10 days	25
Sporothrix schenckii	Y	RPMI	72 h	35	$\%T_{530}$	RPMI	35	72 h	Yes	SDA	3–5 days	35
Coccidioides immitis	M	GYE	10 days	25	Counted	SAAMF	25	7 days	No	GYE	3–5 days	25
Rhizopus species	M	SDA	48 h	35	Counted	YNB	35	24 h	Yes	SDA	24 h	35
Aspergillus species	M	SDA	48 h	35	Counted	YNB	35	48 h	Yes	SDA	24 h	35

[a] Y, yeast cells; M, mycelia.
[b] Media used: YNB, yeast nitrogen broth (Difco) with 0.5% glucose, pH 5.4; SAAMF, synthetic amino acid medium for fungi, pH 7.4, with 0.165M morpholinepropanesulfonic acid without Tris, cysteine, or cystine; MVM, modified McVeigh-Morton medium, pH 7.0; BAP, blood agar plates (Becton Dickinson); SDA, Sabouraud dextrose agar (Difco); GYE, 2% glucose—1% yeast extract (Difco) with 2% agar (BBL).
[c] $\%T_{530}$ = 87% T for NCCLS method. T, transmission. T_{530} = T at 530 A.
[d] Counted, counted in a hemacytometer chamber.

was required for the endpoint. Unselected patient isolates were used. All results are expressed in micrograms per milliliter.

As noted in Table 2, the MFC for all three drugs for all five *C. albicans* isolates were >64 μg/ml. No drug had lower MICs than UR-9751 for any individual isolate. For other *Candida* species, fluconazole was fungicidal for 3 of 10, but MFCs were ≥16 μg/ml. Neither UR-9746 nor UR-9751 was fungicidal. For four isolates, fluconazole had the lowest MIC (including both *C. parapsilosis*); for four isolates UR-9751 had the lowest MIC (including both *C. krusei*); and for two they were tied. The MIC for *C. kefyr* was the lowest of all *Candida* species for all three drugs. It could be suitable as a bioassay organism for these drugs.

For *Cryptococcus neoformans*, no drug had lower MICs than UR-9751 for any isolate. One to two isolates were killed by each drug.

Molds were generally resistant to all three drugs (MICs were ≥12.5 μg/ml for UR-9746 and UR-9751 and ≥100 μg/ml for fluconazole). That is, the MICs were above serum concentrations consistently achievable in animals (2) or likely in humans. However, the new agents showed some activity versus non-fumigatus isolates of *Aspergillus;* four of six were killed by UR-9746, six of six by UR-9751, and two of six by fluconazole. Only the new agents showed any activity versus *Mucorales.*

For the agents of the endemic mycoses, UR-9751 had the lowest MICs for all three isolates. Only one isolate was killed by any of the three drugs. All three drugs readily killed *Histoplasma capsulatum;* UR-9751 had the lowest MICs and MFCs for all three isolates. All three drugs readily killed *B. dermatitidis,* and UR-9751 had the lowest MICs and MFCs. The two new agents inhibited the *Sporothrix* isolate. All three drugs readily killed *Paracoccidioides brasiliensis;* UR-9751 had the lowest MICs and MFCs.

Thus, UR-9746 and UR-9781 are active in vitro, with potency comparable to that of fluconazole. The modest activity versus molds may suggest the need to develop related compounds of this class to treat infections due to these difficult-to-treat organisms. The activity versus the dimorphic endemic fungi is intriguing and deserves further study. We have recently shown in vivo activity of these new agents after oral administration versus coccidioidomycosis (Clemons and Stevens 1997).

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TABLE 2 Susceptibility (μg/ml)

Species and isolate	UR-9746		UR-9751		Fluconazole	
	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans						
1	16	>64	8	>64	>64	>64
2	4	>64	4	>64	>64	>64
3	2	>64	≤0.5	>64	≤0.5	>64
4	2	>64	≤0.5	>64	4	>64
5	16	>64	2	>64	6	>64
C. tropicalis						
1	>64	>64	64	>64	>64	>64
2	64	>64	16	>64	2	>64
C. parapsilosis						
1	32	>64	4	>64	2	16
2	4	>64	1	>64	≤0.5	>64
C. krusei						
1	64	>64	16	>64	64	>64
2	64	>64	8	>64	64	>64
C. kefyr	1.0	>64	1.0	>64	≤0.5	32
C. lusitaniae	8	>64	2	>64	2	32
C. glabrata						
1	>64	>64	4	>64	16	>64
2	>64	>64	16	>64	16	>64
C. neoformans						
1	4	>64	1	>64	8	>64
2	8	>64	2	>64	32	>64
3	4	>64	2	>64	4	32
4	8	>64	2	>64	16	>64
5	2	8	≤0.5	2	≤0.5	4
A. fumigatus						
1	100	>100	100	>100	>100	>100
2	100	>100	100	>100	>100	>100
3	>100	>100	>100	>100	>100	>100
4	100	>100	100	>100	>100	>100
5	100	>100	100	>100	>100	>100
A. flavus						
1	>100	>100	100	100	>100	>100
2	100	>100	100	100	100	100
3	50	50	25	25	100	100
A. niger						
1	100	100	50	100	>100	>100
2	100	100	50	50	>100	>100
A. terrus	100	100	12.5	50	>100	>100
Rhizopus species						
1	50	>100	50	>100	>100	>100
2	50	>100	50	>100	>100	>100
C. immitis						
1	25	>100	3.13	>100	6.25	>100
2	25	>100	3.13	>100	12.5	100
3	25	>100	1.56	>100	>100	>100
H. capsulatum						
1	0.5	0.5	0.25	0.25	2	2
2	0.5	0.5	0.5	0.5	4	4
3	2	2	1.0	1.0	4	4
B. dermatitidis						
1	3.13	3.13	0.20	0.39	3.13	3.13
2	0.78	0.78	0.10	0.10	6.25	6.25
S. schenckii	8	>64	8	>64	>64	>64
P. brasiliensis						
1	0.78	0.78	0.39	0.39	3.13	3.13
2	0.20	0.20	0.05	0.05	1.56	1.56

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