Effects of Imidazole- and Triazole-derivative Antifungal Compounds on the Growth and Morphological Development of *Candida albicans* Hyphae

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Six azole-derivative antifungal compounds affected several aspects of *Candida albicans* hyphal development with only a relatively small degree of inhibition of growth rate, measured in terms of ATP concentration, whereas amphotericin B and 5-fluorocytosine affected morphology only when they also substantially inhibited fungal growth rate. At 10^{-8} M, all the azoles tested inhibited branch formation by *C. albicans* hyphae. At 10^{-7} M and higher concentrations, clotrimazole and miconazole strongly suppressed emergence of new hyphal outgrowths from parent yeast cells, whereas ICI 153066 and itraconazole had little effect on this phenomenon and ketoconazole and tioconazole had intermediate effects. At the highest concentrations tested (10^{-5} M) hyphal development was ultimately arrested by the azole compounds and the fungus grew predominantly in the form of budding yeast cells; however, none of the azole antifungals prevented initial emergence of an apparently normal germ tube. The antifungals only exerted their morphological effects when they were present in the culture medium: removal of the compounds after exposure of *C. albicans* to them led to reversion to normal growth.

INTRODUCTION

Various imidazole-derivative antifungal agents have been claimed to inhibit the morphological transformation of *Candida albicans* from budding yeast cells to hyphae (Borgers *et al.*, 1979; Borgers, 1980; Davies & Marriott, 1981). However, this inhibitory effect is not unequivocal. Johnson *et al.* (1982) were unable to detect any inhibition of germ tube outgrowth by ketoconazole in serum, and Davies & Marriott (1981) saw inhibition of hyphal extension by low concentrations of tioconazole or ketoconazole only when the drugs were added with the yeast inoculum: there was no inhibition of extension of preformed germ tubes even when drugs were added at 100 times the concentration that inhibited germ tube initiation.

Since substances with definable and specific effects on morphological changes in *C. albicans* would be of obvious value in elucidating the mechanisms determining such changes, there is a case for further study of the effects of imidazole antifungals and the newer triazole compounds on *C. albicans* morphogenesis. Most investigators of morphological change in this fungus have studied only the early stages of the process – emergence of hyphal germ tubes from stationary phase yeast cells – although later events in hyphal development, such as formation of hyphal branches or lateral buds from sites adjacent to hyphal septa, also merit attention as morphogenetic processes (Odds, 1985). The present study was therefore undertaken to establish whether one or more of several antifungal azole compounds might be sufficiently selective in its effects on morphogenetic processes to be a useful probe for further study of the processes. The study was confined to relatively low concentrations of antifungals since the aim was to demonstrate possible morphological effects that were detectable in cells whose overall growth rate was unaltered by the antifungals.

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Abbreviation: EMEM, Eagle's minimal essential medium.

METHODS

Fungi and growth conditions. *C. albicans* isolate 73/055 (originally obtained from a vaginal swab) was used in the experiments. The fungus was maintained on Sabouraud's dextrose agar (Oxoid) and inocula were grown overnight in this medium and prepared by suspension of the yeast cells in sterile water. Concentrations of yeast cells were determined by haemocytometer counts.

C. albicans was grown in its hyphal form in Eagle's minimal essential medium (EMEM) containing glutamine (Gibco) buffered at pH 7.3 with 0.02 M-HEPES and NaHCO₃ and incubated at 37 °C under 5% CO₂ in air. Serum was not included in the medium since the object of the experiments was to allow inhibitory compounds to express their effects on morphology maximally; protein binding of the compounds might be expected to complicate the experiments. The medium was prewarmed to 37 °C, inoculated to a yeast cell concentration of 10⁶ ml⁻¹, dispensed in 1 ml lots in the wells of Repli-dishes (Sterilin) and incubated statically. Unless otherwise stated, antifungal stock solutions or their solvents were added to the medium just before inoculation. Samples of cultures were taken at intervals by resuspension of the contents of a Repli-dish well with a magnetic stirrer and a small wire follower (Odds, 1982). Samples (0.5 ml) were treated immediately for extraction of ATP (see below). To the remaining 0.5 ml was added 50 µl of preservative [sodium dodecyl sulphate, 100 g l⁻¹ in 10% (v/v) formalin] and the samples were stored at room temperature for subsequent microscopic examination.

In experiments where antifungal drugs were removed from cultures at intervals after inoculation, the procedure was as follows. Cultures (10 ml) were set up in 25 ml glass universal bottles. At the time of drug removal the cultures were centrifuged at 2500 *g* for 3 min, the supernatants were discarded and the fungi were washed in antifungal-free EMEM then resuspended in 10 ml antifungal-free EMEM. Parallel cultures were washed and resuspended in fresh medium containing the same antifungal concentration as before to act as controls for the centrifugation/resuspension procedures. Results from the latter showed there was no measurable effect of the procedures on the growth rates or morphology of *C. albicans*.

Antifungal compounds. Six azole antifungals and two non-azole compounds were tested for their effects on *C. albicans* growth and morphology. Clotrimazole (Bayer), ICI 153066 (ICI Pharmaceuticals), itraconazole, ketoconazole and miconazole (Janssen Pharmaceutical), and tioconazole (Pfizer) were kindly supplied by their manufacturers. Amphoteracin B and 5-fluorocytosine were purchased from Sigma. The antifungals were prepared as stock solutions at 100 times their final test concentrations in DMSO and stored at -20 °C when not in use.

Measurement of biomass and morphology of C. albicans. Preliminary experiments showed that the effects of azole antifungals were reflected predominantly in altered dimensions of the *C. albicans* hyphae, numbers of branches per mycelium, numbers of yeast cells per mycelium, numbers of hyphal outgrowths per inoculum yeast cell, and hyphal growth rate (in terms of increase in ATP content). These parameters were therefore assessed for all samples.

Growth rates were determined by a least-squares regression analysis of plots of log fungal ATP concentration versus time. ATP was extracted from the fungal cells and ATP hydrolases inactivated by addition of 0.5 ml samples to 5 ml lots of Tris/EDTA buffer heated in a boiling water bath (Odds, 1982). ATP concentrations were determined by bioluminescence photometry (Odds, 1982). ATP concentrations in *C. albicans* have been shown to correlate well with alternative estimations of viable biomass (Ansehn & Nilsson, 1984; F. C. Odds, unpublished data).

Hyphal lengths and widths were determined by phase-contrast microscopic examination of 25 mycelia from each sample with the aid of a calibrated eyepiece graticule. Only the lengths of primary hyphae were measured, i.e. those that appeared to be longest and attached to a large, phase-bright yeast cell, presumed to be the original yeast parent cell from the inoculum. In some drug-treated cultures differentiation between primary hyphal elements and other, pleomorphic types of growth was inevitably subjective (see e.g. Figs 1*b* and 1*c*).

Numbers of hyphal branches were estimated for the same 25 mycelia that were used for determination of hyphal length, and the number of branches per hypha was calculated. Numbers of hyphal outgrowths per parent yeast cell were determined separately by counting a minimum of 30 mycelia, and the number of yeast cells (other than parent yeast cells) per mycelium was assessed on the basis of haemocytometer counts. Reductions in growth rate, mean hyphal length, hyphal outgrowths per parent yeast cell and branches per mycelium were calculated for each culture as percentages relative to the data for drug-free controls.

RESULTS

Microscopic appearance of drug-treated cultures

In antifungal-free cultures in EMEM the pattern of growth of *C. albicans* was typical of the hyphal growth form of the fungus. After 1 h germ tubes had emerged from most of the inoculum yeast cells. The germ tubes elongated into hyphae, and the earliest hyphal branches were formed after 3–4 h. The hyphae were predominantly phase-dark, but the parent yeast cells remained phase-bright throughout the observation period. By 5 h secondary hyphal shoots had formed from approximately 80% of the parent yeast cells.

Fig. 1. Phase-contrast micrographs of *C. albicans* grown in EMEM under 5% CO₂ in air in the presence and absence of azole antifungals as follows. (a) No antifungal added; hyphae are phase-dark, branched and more than one hyphal outgrowth has emerged from the parent yeast cell. Bar, 10 µm. (b) With 10⁻⁵ M-ketoconazole, (c) with 10⁻⁶ M-clotrimazole, (d) with 10⁻⁷ M-itraconazole, (e) with 10⁻⁷ M-tioconazole and (f) with 10⁻⁷ M-miconazole.

The gross effects of azole antifungals on *C. albicans* hyphal morphology could be seen by qualitative microscopic observation of the cultures. For all the azole compounds tested there was a tendency for cells grown in the presence even of drug concentrations as low as 10⁻⁸ M to appear phase-bright, unlike the control hyphae (Figs 1a to 1f). A second effect common to the azole antifungals was a substantial inhibition of hyphal branching: hyphae grown in the presence of azoles consistently emerged as phase-dark germ tubes in the same manner as control hyphae during the first 2–3 h of growth, but in their subsequent development they turned pleomorphic and generated lateral buds instead of hyphal branches (Figs 1c and 1e). At the highest azole concentrations hyphal development was sometimes arrested substantially after initial germ tube formation, and growth continued in the form of budding yeast cells that commonly remained in clumps.

Another effect of the azole antifungals was seen with some but not all of the compounds, and was most evident at the highest drug concentrations tested. This effect was an apparent failure of the original parent yeast cells to initiate new hyphal outgrowths – predominantly budding cells were formed from the parent after the initial (first-generation) germ tube emerged (Fig. 1b). In general, at the two highest drug concentrations tested (10⁻⁵ and 10⁻⁶ M) hyphal development appeared to be virtually abolished after an initial germ tube outgrowth, whereas at 10⁻⁷ and 10⁻⁸ M hyphal extension appeared to be retarded, and the hyphal shape was less regular than in controls, but hyphal development was not arrested completely.

Fig. 2. Effects of azole antifungals on morphological development of *C. albicans* 73/055. (a) Effects of itraconazole on the hyphal growth curve, measured in terms of cellular ATP concentration; the effects of the other azole compounds tested were essentially the same. (b) Effects of tioconazole on hyphal branching; similar curves were obtained for all the azole antifungals tested. (c, d) Effects of itraconazole and tioconazole respectively on the lengths of hyphae. The effects were similar over the concentration range 10^{-9} to 10^{-6} M, but tioconazole had a greater inhibitory effect on hyphal length at 10^{-5} M, whereas the effects of itraconazole appeared to be similar at this concentration. Cultures were treated with various concentrations of antifungals as follows: ○, control (no antifungal); ●, 10^{-5} M; □, 10^{-6} M; ■, 10^{-7} M; △, 10^{-8} M; ▲, 10^{-9} M. Bars indicate standard deviations for experiments done with three or more replicates.

Effects of antifungals on rates of hyphal ATP increase

Azole antifungals at concentrations from 10^{-9} to 10^{-5} M appeared to have little or no effect on the growth curves of *C. albicans* hyphae, where ATP concentration was used as a measure of fungal biomass (Fig. 2a), although regression analysis of the growth curves revealed there was in fact some inhibition of growth rate at all test concentrations above 10^{-9} M (Table 1). By contrast, amphotericin B was entirely non-inhibitory at 10^{-7} M and entirely inhibitory at higher concentrations (Table 1). Only clotrimazole and ketoconazole appeared to cause any inhibition of biomass growth rates at 10^{-9} M, and, except for ICI 153066, the extent to which growth rates were reduced by azole concentrations from 10^{-8} to 10^{-6} M was essentially similar and virtually independent of dose-related effects. A generally greater degree of growth rate inhibition was evident at drug concentrations of 10^{-5} M. ICI 153066 was rather more inhibitory than the other azole compounds at concentrations from 10^{-8} to 10^{-5} M. 5-Fluorocytosine inhibited growth rate to an extent that was proportional to the drug concentration from 10^{-7} to 10^{-5} M.

Table 1. *Effects of antifungal drugs on rate of increase of ATP concentration (biomass growth rate) in C. albicans hyphae*

The values are mean percentage decreases from the rate of ATP increase for drug-free control cultures, calculated by regression analysis of growth curve data for two or more replicate experiments. At least three replicate experiments were done with drug concentrations from 10^{-7} M to 10^{-5} M. Standard deviations ranged from 4% to 14%; NT, not tested.

Antifungal	Concn (M)	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Amphotericin B		NT	NT	0	91	100
5-Fluorocytosine		NT	NT	5	49	66
Clotrimazole		18	28	23	22	36
ICI 153066		0	26	30	41	45
Itraconazole		6	13	14	26	27
Ketoconazole		16	23	22	18	19
Miconazole		0	16	19	23	35
Tioconazole		0	18	23	26	39

Table 2. *Effects of antifungal drugs on length of C. albicans hyphae after 7.5 h growth in EMEM*

The values are percentage reductions relative to drug-free control hyphae. For details of replication data see Table 1. Standard deviations ranged from 0 to 9%; NT, not tested.

Antifungal	Concn (M)	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Amphotericin B		NT	NT	0	88	100
5-Fluorocytosine		NT	NT	0	37	66
Clotrimazole		0	33	40	34	57
ICI 153066		0	27	39	41	47
Itraconazole		0	19	28	36	37
Ketoconazole		24	29	32	30	47
Miconazole		0	35	38	45	67
Tioconazole		11	39	35	39	67

Effects of antifungals on hyphal length

The inhibitory effects of the antifungals on hyphal length correlated broadly with their effects on rates of ATP increase (Table 2). Detailed results are shown for two of the test antifungals in Figs 2(c) and 2(d). Itraconazole and tioconazole caused no reduction in hyphal length at 10^{-9} M, then essentially similar reductions in hyphal length from 10^{-8} to 10^{-6} M (Figs 2c and 2d). At 10^{-5} M, tioconazole caused a greater reduction in hyphal length than at 10^{-6} M and lower concentrations, while the inhibitory effects of 10^{-5} M-itraconazole were little different from those at the lower concentrations. The other four azole antifungals inhibited hyphal lengths in patterns similar to those of either tioconazole or itraconazole (Table 2). Thus there were negligible effects at 10^{-9} M, a roughly equal inhibitory effect from 10^{-8} to 10^{-6} M, and differences only at 10^{-5} M, where clotrimazole, ketoconazole, miconazole and tioconazole reduced hyphal length by at least 50% more than at lower concentrations, whereas ICI 153066 and itraconazole were only 3 and 15%, respectively, more inhibitory at 10^{-5} M than at lower concentrations (Table 2).

The data concerning percentage inhibition of hyphal length for amphotericin B and 5-fluorocytosine at 10^{-7} M to 10^{-5} M were of the same order as the growth rate inhibition data for these compounds (Tables 1 and 2), whereas the data for the azole antifungals suggested they often inhibited hyphal length more than growth rate. This difference was accounted for by an increased tendency for the mycelia to initiate yeast cell growth by lateral budding in the presence of azole compounds (Table 3), and by an increase in average hyphal diameter of 12 to 32%. The numbers of secondary yeast cells and the increase in hyphal diameter were usually greatest at the highest antifungal concentrations tested. Only ICI 153066 and itraconazole at 10^{-5} M caused a relatively small increase in numbers of non-primary yeast cells. This observation correlated with the data on hyphal length inhibition by these two compounds (Tables 2 and 3).

Table 3. *Effects of antifungal drugs on numbers of non-primary yeast cells per C. albicans mycelium after 7.5 h growth in EMEM*

Cultures that were not treated with drugs contained a mean of 0.15 ± 0.03 yeast cells per mycelium. Standard deviations for experiments with three or more replicates ranged from 0.00 to 0.07; NT, not tested; —, growth was totally inhibited, no mycelium formed.

Antifungal	Concn (M) . . .	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Amphotericin B		NT	NT	0.2	—	—
5-Fluorocytosine		NT	NT	0.1	0.2	0.7
Clotrimazole		0.3	1.8	2.2	2.8	3.5
ICI 153066		0.2	0.7	0.7	0.6	0.6
Itraconazole		0.2	0.5	0.6	0.6	1.7
Ketoconazole		0.2	1.5	2.2	3.6	4.0
Miconazole		0.1	0.8	2.1	4.8	4.4
Tioconazole		0.2	1.7	1.6	1.6	5.3

Table 4. *Effects of antifungal drugs on hyphal branching of C. albicans*

The values are percentage reductions in numbers of branches per mycelium measured after 7.5 h growth in EMEM. Data are the means of at least duplicate experiments. Standard deviations for experiments with three or more replicates ranged from 2% to 9%; NT, not tested.

Antifungal	Concn (M) . . .	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Amphotericin B		NT	NT	35	100	100
5-Fluorocytosine		NT	NT	23	88	100
Clotrimazole		0	92	97	99	99
ICI 153066		4	86	97	97	99
Itraconazole		25	95	88	94	97
Ketoconazole		74	98	94	96	98
Miconazole		23	91	97	95	100
Tioconazole		30	100	98	98	99

Effects of antifungals on hyphal branching of C. albicans

At least a 23% reduction in the number of branches formed per mycelium was observed at all azole concentrations tested except for clotrimazole and ICI 153066, which appeared not to affect branching at 10^{-9} M. For all azole compounds, hyphal branching was inhibited by 86 to 100% at concentrations from 10^{-8} M to 10^{-5} M (Table 4). Inhibition of branch formation of this order was seen with amphotericin B and 5-fluorocytosine only at concentrations where growth rates were inhibited by 50% or more (Tables 1 and 4). The data in Table 4 are amplified by the detailed time courses of branch formation shown for one of the test compounds in Fig. 2(b). The time at which branching commenced was the same in the presence and in the absence of the antifungals, but the extent to which branches were formed was markedly reduced in the presence of the azole compounds.

Effects of antifungals on secondary hyphal outgrowths from parent yeast cells

Of all the inhibitory effects of azole antifungals on *C. albicans* hyphae, prevention of outgrowths of secondary and later hyphae from the original parent yeast cells was the property that revealed variation in the effects of the individual compounds (Table 5). Clotrimazole and miconazole reduced the number of hyphal outgrowths by more than 70% at concentrations of 10^{-7} M and higher. Ketoconazole and tioconazole were somewhat less inhibitory than clotrimazole and miconazole at 10^{-7} M, but they were similarly inhibitory at 10^{-8} M. ICI 153066 and itraconazole had relatively little effect on secondary hyphal outgrowths at concentrations below 10^{-5} M. Amphotericin B and 5-fluorocytosine had this particular inhibitory effect only at concentrations that also caused substantial inhibition of biomass increase (Table 1).

Table 5. Effects of antifungal drugs on secondary hyphal outgrowths from parent yeast cells of *C. albicans*

The values are percentage reductions in numbers of secondary hyphal outgrowths per mycelium. Standard deviations for experiments with three or more replicates ranged from 1% to 11%; NT, not tested.

Antifungal	Concn (M)	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Amphotericin B		NT	NT	42	100	100
5-Fluorocytosine		NT	NT	4	22	83
Clotrimazole		3	75	75	80	100
ICI 153066		1	0	26	33	42
Itraconazole		0	12	4	26	64
Ketoconazole		35	57	55	75	77
Miconazole		25	45	71	75	94
Tioconazole		9	52	55	54	93

Table 6. Effects on biomass growth rates (measured in terms of ATP concentration) and hyphal branch formation of time of addition or removal of 10^{-7} M-ketoconazole in cultures of *C. albicans* grown in EMEM

For details of replication data see Table 4; NT, not tested.

Time of addition or removal of ketoconazole (h)	Ketoconazole added		Ketoconazole removed	
	Percentage inhibition of ATP growth rate	Branches per mycelium	Percentage inhibition of ATP growth rate	Branches per mycelium
0	18	100	NT	NT
1	NT	NT	14	43
2	7	69	10	50
4	7	31	14	64
6	4	19	NT	NT

Significance of time of additions or removal of antifungal on development of *C. albicans* hyphae

Ketoconazole at 10^{-7} M was selected as representative of the effects of azole antifungals on *C. albicans* hyphal development to investigate the effects of addition or removal of the drug at various times before and after the 'zero time' additions used in the previously described experiments. Inhibition of hyphal branching and growth rate were measured as the most sensitive indicators of the range of effects of ketoconazole. The results (Table 6) suggested that ketoconazole was an effective inhibitor only when it was present in the culture. Its addition at times later than 0 h caused progressively less inhibition of growth rate and branch formation. When the antifungal was removed from the culture at intervals its inhibitory effects on biomass growth rate and branching were generally greater the longer the period of exposure of fungus to antifungal. It was, however, notable that hyphal branching was reduced by 43% even in cultures that had been exposed to ketoconazole for only the first hour of growth (Table 6): the time at which branches first appeared was at least 2 h later than this.

DISCUSSION

The results of this study confirm previous reports that imidazole antifungals can affect the morphological development of *C. albicans* hyphae (Borgers *et al.*, 1979; Borgers, 1980; Davies & Marriott, 1981). They show that triazole antifungals exert similar effects and they define more precisely than before the nature of the steps in morphogenesis that are affected by azole antifungal compounds. At very low concentrations, all the azole antifungals tested substantially inhibited hyphal branching, but none of them prevented emergence of a normal germ tube in the

earliest stages of growth. Some of the compounds appeared to prevent additional hyphal outgrowths from parent yeast cells, thus favouring emergence of budding yeast cells. At the highest concentration tested, 10^{-5} M, most of the azoles tested caused substantial inhibition of hyphal length with concomitant enforcement of growth in the shape of budding yeast cells. The yeast cells commonly appeared not to separate, suggesting that the non-separation mechanism characteristic of hyphae might still be operating, but this possibility was not specifically investigated. Although none of these morphological effects were fully expressed in the absence of some inhibition of hyphal growth rate, the extent of inhibition of growth rate was substantially less than that of the morphological changes. The two control non-azole antifungals amphotericin B and 5-fluorocytosine had similar apparent morphological effects only when hyphal growth rate was also substantially reduced, and these effects are therefore considered non-specific to morphogenesis.

Two primary modes of action have been proposed for azole antifungals against *C. albicans*. The first is an effect on cytochrome P-450 (Van den Bossche & Willemsens, 1982), which is probably the cause of an inhibition of ergosterol biosynthesis that results in accumulation of 14- α -methyl sterols in the plasma membrane. The second effect is a direct interaction of the drugs with membrane lipids, leading to membrane damage (Van den Bossche *et al.*, 1983), which is probably the result of changes in the lipid transition temperature (Van den Bossche *et al.*, 1982). Interaction with cytochrome P-450 and inhibition of ergosterol synthesis in yeast cells have been demonstrated for several azoles at concentrations of the order of 5×10^{-8} M to 5×10^{-7} M (Marriott, 1980; Van den Bossche & Willemsens, 1982; Van den Bossche *et al.*, 1978, 1980, 1982) whereas direct membrane damage requires higher azole concentrations – at least 10^{-6} M and usually 10^{-5} M or more (Sreedhara Swamy *et al.*, 1974; Cope, 1980; Sud & Feingold, 1981*a*, 1981*b*; Uno *et al.*, 1982; Van den Bossche *et al.*, 1983, 1984). A slow-acting inhibitory effect of miconazole and ketoconazole on membrane fatty acid synthesis in *C. albicans* has been proposed, but little of the evidence for this has been formally published (Van den Bossche *et al.*, 1983).

The lowest antifungal concentrations known to have an effect on the cytochrome P-450 target in cell-free systems are of precisely the same order as the lowest concentrations that affected branch development of *C. albicans* in the present study (Table 4). Inhibition of growth rate (Table 1) was lowest at the lowest antifungal concentrations; some inhibition of growth rate at concentrations as low as 10^{-8} M has been noted before (Van den Bossche *et al.*, 1980).

The impression gained is therefore that the effects of the azole antifungals on hyphal branching may be related mainly to the effects of the drugs on cytochrome P-450. Because inhibition of hyphal extension and secondary hyphal shoot formation are seen only at higher drug concentrations and principally with drugs known to possess the capability for direct membrane damage, these effects probably result mainly from direct interactions of the compounds with the fungal cell membranes. All the known membrane changes caused directly and indirectly in azole-treated *C. albicans* are likely to alter the fluidity of the plasma membrane (Van den Bossche *et al.*, 1983). Because several of the enzymes involved in *C. albicans* cell wall synthesis are located in the cell membrane (Braun & Calderone, 1978; Marriott, 1975; Chiew *et al.*, 1980) it is possible that changes in membrane fluidity could affect the activity of these enzymes and their regulation, and thus lead to distorted cell wall synthesis with concomitant changes in cell shape. The fact that the azole compounds at low concentrations have an effect selectively related to hyphal branching presumably depends on a different mechanism, possibly related to the known effects of azole antifungals on cytochrome P-450 and sterol synthesis.

In some fungi with an entirely hyphal mode of development, such as *Neurospora crassa*, regulation of branch formation has been shown to depend on a Ca^{2+} /cyclic AMP mechanism, which is affected by ionophores such as A23187 (Reissig & Kinney, 1983). The possibility that azole antifungals affect a similar mechanism awaits investigation. It has been postulated that hyphal branching of *C. albicans* is a process regulated by gene expression whereas overall cell shape is regulated mainly at the phenotypic level by environmental influences on cell wall synthesizing enzymes (Odds, 1985). The fact that the azole compounds tested in the present

study were shown to affect these two aspects of cell development differentially supports the notion that they are not regulated by a common process. Further experiments are needed to establish which cell wall synthesizing enzymes and intracellular proteins are affected by azole antifungals at different stages of mycelium formation in *C. albicans*.

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