A New Triazole, Voriconazole (UK-109,496), Blocks Sterol Biosynthesis in *Candida albicans* and *Candida krusei*

HOMAYOON SANATI,[1] PAUL BELANGER,[1] RUTILIO FRATTI,[1]
AND MAHMOUD GHANNOUM[2,3]*

*Division of Infectious Diseases, Harbor-UCLA Medical Center, Torrance, California 90509,[1] and
Mycology Reference Laboratory, Center for Medical Mycology, University Hospitals
of Cleveland,[2] and Department of Dermatology, Case Western
Reserve University,[3] Cleveland, Ohio 44106-5028*

Voriconazole (UK-109,496) is a novel triazole derivative with potent broad-spectrum activity against various fungi, including some that are inherently resistant to fluconazole, such as *Candida krusei*. In this study we compared the effect of subinhibitory concentrations of voriconazole and fluconazole on sterol biosynthesis of fluconazole-resistant and -susceptible *Candida albicans* strains, as well as *C. krusei*, in an effort to delineate the precise mode of action of voriconazole. Voriconazole MICs ranged from 0.003 to 4 μg/ml, while fluconazole MICs ranged from 0.25 to >64 μg/ml. To investigate the effects of voriconazole and fluconazole on candidal sterols, yeast cells were grown in the absence and presence of antifungals. In untreated *C. albicans* controls, ergosterol was the major sterol (accounting for $53.6\% \pm 2.2\%$ to $71.7\% \pm 7.8\%$ of the total) in *C. albicans* and *C. krusei* strains. There was no significant difference between the sterol compositions of the fluconazole-susceptible and -resistant *C. albicans* isolates. Voriconazole treatment led to a decrease in the total sterol content of both *C. albicans* strains tested. In contrast, exposure to fluconazole did not result in a significant reduction in the total sterol content of the three candidal strains tested ($P > 0.5$). Gas-liquid chromatographic analysis revealed profound changes in the sterol profiles of both *C. albicans* strains and of *C. krusei* in response to voriconazole. This antifungal agent exerted a similar effect on the sterol compositions of both fluconazole-susceptible and -resistant *C. albicans* strains. Interestingly, a complete inhibition of ergosterol synthesis and accumulation of its biosynthetic precursors were observed in both strains treated with voriconazole. In contrast, fluconazole partially inhibited ergosterol synthesis. Analysis of sterols obtained from a fluconazole-resistant *C. albicans* strain grown in the presence of different concentrations of voriconazole showed that this agent inhibits ergosterol synthesis in a dose-dependent manner. In *C. krusei*, voriconazole significantly inhibited ergosterol synthesis (over 75% inhibition). *C. krusei* cells treated with voriconazole accumulated the following biosynthetic intermediates: squalene, 4,14-dimethylzymosterol, and 24-methylenedihydrolanosterol. Accumulation of these methylated sterols is consistent with the premise that this agent functions by inhibiting fungal P-450-dependent 14α-demethylase. As expected, treating *C. krusei* with fluconazole minimally inhibited ergosterol synthesis. Importantly, our data indicate that voriconazole is more effective than fluconazole in blocking candidal sterol biosynthesis, consistent with the different antifungal potencies of these compounds.

The incidence of fungal infections has increased significantly in the past two decades (1, 5, 21). This increase could be attributed, in part, to the growing number of immunocompromised patients and the frequent use of cytotoxic and/or antibacterial drugs (9). Until recently, amphotericin B was the standard therapy for many fungal infections, but a high frequency of renal toxicity has limited its use (20). Azoles and triazoles, such as fluconazole, have increased our ability to treat many fungal infections (1, 2, 22). However, mortality due to these infections, even with antifungal therapy, is still unacceptably high (27). There is, therefore, a great demand for new antifungals. The development of new antifungal agents targeting specific fungal structures or functions is being actively pursued (8).

Voriconazole (UK-109,496) is a new triazole derivative of fluconazole with potent broad-spectrum activity against fungi, including filamentous fungi as well as fluconazole-resistant *Candida albicans* and other fluconazole-resistant *Candida* spp.

(4, 12, 14). Studies have shown that voriconazole has both fungistatic and fungicidal activities against *Aspergillus* spp. (7). Although all azole antifungals belong to the class of 14α-demethylase inhibitors, with their antifungal activity originating from binding to this enzyme (13), there is a heterogeneity of action among them (3, 13, 23, 24). The earlier imidazole derivatives (such as miconazole, econazole, and ketoconazole) have a complex mode of action. For example, these azoles inhibit several membrane-bound enzymes and membrane lipid biosynthesis (for a review, see reference 13), with some of them affecting membranes directly by binding to lipids (6). In contrast, the more recent triazole derivatives, such as fluconazole and itraconazole, owe their antifungal activity exclusively to the inhibition of cytochrome P-450-dependent 14α-sterol demethylase. For example, fluconazole-treated fungi are depleted of ergosterol and they accumulate 14α-methylated sterols, such as lanosterol, which is thought to disrupt membrane structure and function, thereby causing a cessation of fungal growth and morphogenesis (13). In this study, to elucidate the precise mechanism of action of voriconazole, we compared the sterol patterns of fluconazole-susceptible and -resistant *C. albicans* strains as well as the sterol pattern of *Candida krusei*, an innately resistant species, and examined the effects of subin-

---

TABLE 1. MIC_{80} of fluconazole and voriconazole
against candidal isolates

Strain	MIC_{80} (µg/ml) of:	
	Fluconazole	Voriconazole
C. albicans OY-2-76 (fluconazole susceptible)	0.25	0.003
C. albicans OY-12-99 (fluconazole resistant)	>64	4.0
C. krusei	32	0.5

hibitory concentrations of voriconazole and fluconazole on the sterol biosynthesis of these candidal isolates. Our data showed that ergosterol is the major sterol in fluconazole-susceptible and -resistant *C. albicans* strains as well as *C. krusei*. Other components detected in untreated yeast included obtusifoliol, lanosterol, calciferol, and squalene. There was no significant difference between the sterol compositions of the fluconazole-susceptible and -resistant *C. albicans* isolates. Treatment of candidal cells with voriconazole resulted in the accumulation of methylated sterol intermediates, consistent with the premise that this agent functions by inhibiting fungal P-450-dependent 14α-demethylase.

MATERIALS AND METHODS

Microorganisms. The two isolates of *C. albicans* (OY-2-76 and OY-12-99) used in this study have been described previously (19). These isolates are part of a series of candidal isolates collected over 2 years from a single patient with AIDS who developed azole-resistant candidiasis. Fluconazole-susceptible *C. albicans* OY-2-76 (MIC at which 80% of the isolates are inhibited [MIC_{80}] of fluconazole, 0.25 µg/ml) was isolated after initial candidal infection, and fluconazole-resistant *C. albicans* OY-12-99 (MIC_{80} of fluconazole, >64 µg/ml) was isolated after the 12th episode of candidiasis. Karyotyping showed that both isolates had the same DNA profile, suggesting that they are the same strain (19). A clinical strain of *C. krusei* was obtained from Michael Rinaldi (San Antonio, Tex.). These isolates were stored on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) slants at 4°C and recultured onto fresh medium every 30 days.

Antifungals. Fluconazole and voriconazole were provided as powder by Pfizer Central Research, Sandwich, England. Fresh stock solutions (1.0 and 0.32 mg/ml for fluconazole and voriconazole, respectively) of these two agents were prepared in distilled water on the day of experimentation.

Susceptibility testing. MICs of fluconazole and voriconazole for the candidal isolates were determined by using the National Committee for Clinical Laboratory Standards microbroth dilution method, M27T (16).

Growth. Yeast cells were grown as a shake culture (200 rpm) at 37°C in RPMI 1640 (American Bioorganics, Inc., Niagara Falls, N.Y.) with MOPS (morpholinepropanesulfonic acid) buffer (165 mM, pH 7.0) with an initial inoculum concentration of 10^4 CFU/ml. Cells were allowed to grow in the presence of either fluconazole or voriconazole (one-half the MIC) and in the absence of these two agents (control) up to early stationary phase, according to their growth rate (20 to 24 h). Yeast cells were harvested by centrifugation at 3,000 × *g* and washed three times with sterile saline (0.85% [wt/vol]).

Since earlier experiments demonstrated complete ergosterol inhibition using one-half MIC of voriconazole, fluconazole-resistant *C. albicans* cells were grown in the presence of different concentrations of voriconazole (1/16, 1/8, and 1/2 the MIC) to determine whether ergosterol inhibition is dose dependent.

Sterol extraction. Sterols were extracted by the method of Ghannoum et al. (10). Briefly, 1.5 g of KOH in 2 ml of distilled water and 8 ml of ethanol was added to 0.2 g (wet weight) of yeast cells. The mixture was refluxed for 3 h under nitrogen. The refluxed mixture was filtered, diluted with an equal amount of water, and extracted four times with heptane. The extract was dried with anhydrous Na_2SO_4, and the solvent was removed by using a rotovaporizer. The purified sterols were weighed and dissolved in chloroform (10 mg/ml) and stored under nitrogen at −20°C until being analyzed.

Sterol analysis. Sterols were analyzed by gas-liquid chromatography (GLC). Silylated sterols were prepared for GLC studies by a modification of the method of Vandenheuvel and Court (26). Briefly, 2 mg of extracted sterols was reacted with 0.1 ml of hexamethyldisilazane (Sigma Chemical Co., St. Louis, Mo.) and 0.1 ml of 10% (vol/vol) trimethylchlorosilane (Sigma) in chloroform. The reaction mixture was gently mixed, covered with aluminum foil, and stored at room temperature for 4 h. Next, the excess solvent was evaporated under nitrogen and the samples were redissolved in 50 µl of hexane. One-microliter samples were

analyzed by GLC with an OV-1 column (3% on 100/120 gaschrome Q) in a Vista 6000 (Varian, Sugerland, Tex.) gas chromatograph. The samples were eluted with helium as the carrier gas (30 ml/min). The column temperature was 230°C, while the injection temperature was set at 250°C and the flame ionization temperature was set at 300°C. Sterols were identified by a comparison of their retention times with authentic standards or relative to the retention time of ergosterol. GLC peaks were quantified by using a Varian Vista 402 integrator.

Unless otherwise specified, all chemicals used were of the highest purity and were purchased from Fisher Scientific (Pittsburgh, Pa.).

Data analysis. Differences in sterol content between treated and untreated candidal cells were expressed as percent variability.

RESULTS

MICs. Table 1 summarizes the MICs for the candidal isolates tested against voriconazole and fluconazole. Voriconazole was more effective than fluconazole in inhibiting the growth of both *C. albicans* and the *C. krusei* strains. Voriconazole MICs ranged from 0.003 to 4 µg/ml, and fluconazole MICs ranged from 0.25 to >64 µg/ml. Voriconazole was 16- and 64-fold more potent than fluconazole in inhibiting the growth of fluconazole-resistant *C. albicans* and *C. krusei*, respectively.

Sterol content. The total sterol contents of candidal isolates grown in the presence and absence of fluconazole or voriconazole are summarized in Table 2. Variation in total sterol content among the three isolates grown in drug-free medium was minimal. Data obtained from two separate experiments revealed that voriconazole-treated cells showed a trend towards lower total sterol content, with percent variation ranging between 12.4 and 41.7% reduction in sterol content (Table 2). In contrast, fluconazole was not as effective as voriconazole in reducing the sterol content of treated cells (Table 2).

Sterol pattern. The compositions of different sterols present in the *C. albicans* and *C. krusei* strains tested are shown in Table 3. In the untreated control, ergosterol was the major sterol, representing over 50% of the total amount of sterols in all *Candida* strains tested. In addition, *C. albicans* accumulated obtusifoliol and lanosterol (each fraction was >5% of the total amount of sterols [Table 3]), while *C. krusei* accumulated more than 10% each of lanosterol and calciferol. The sterol precursor squalene was also detected in all strains analyzed (between 8.5 and 11.5% of the total amount of saponified fraction [Table 3]).

GLC analysis revealed profound changes in the sterol patterns of both *C. albicans* and *C. krusei* in response to voriconazole. A complete inhibition of ergosterol and obtusifoliol synthesis was observed in both *C. albicans* strains. Accumulation of the following ergosterol biosynthetic precursors was observed in the two *C. albicans* isolates grown in the presence of voriconazole: squalene, zymosterol, 4,14-dimethylzymosterol, and 24-methylenedihydrolanosterol. In contrast, fluconazole partially inhibited ergosterol synthesis, at about 44% for both *C. albicans* strains. To a lesser extent, the same biosynthetic

TABLE 2. Total sterol content of candidal cells

Category	Total sterols[a]		
	C. albicans OY-2-76	C. albicans OY-12-99	C. krusei
Control	3.33 ± 0.42	3.05 ± 1.47	2.59 ± 1.50
Fluconazole (1/2 MIC)	3.82 ± 1.23	2.30 ± 0.07	1.32 ± 0.39
Voriconazole (1/2 MIC)	1.94 ± 0.09	1.87 ± 0.02	2.27 ± 1.48

[a] Mean total sterols as a percentage of the wet weight of *Candida* cells grown in the absence of drugs (control) and in the presence of 1/2 the MIC of either fluconazole or voriconazole. The values are the means ± standard deviations of two separate experiments.

TABLE 3. GLC analysis of sterols of *Candida* isolates

Sterol	C. albicans OY-2-76			C. albicans OY-12-99			C. krusei		
	Control	FLU	VOR	Control	FLU	VOR	Control	FLU	VOR
Squalene	8.5 ± 0.5	11.1 ± 0.7	19.7 ± 7.4	10.8 ± 3.7	12.1 ± 6.6	25.9 ± 2.2	11.5 ± 1.2	5.8 ± 1.8	28.6 ± 5.7
Calciferol	4.2 ± 1.5	10.9 ± 2.7	5.8 ± 1.2	3.4 ± 0.3	5.0 ± 2.6	2.8 ± 3.3	10.3 ± 1.7	5.0 ± 2.2	7.8 ± 1.1
Zymosterol	ND	2.0 ± 0.8	4.1 ± 2.6	ND	2.8 ± 2.3	5.0 ± 4.6	2.8 ± 0.5	2.9 ± 0.6	3.8 ± 0.0
Ergosterol	71.7 ± 7.8	40.0 ± 6.2	ND	58.6 ± 10.5	33.1 ± 2.2	ND	53.6 ± 2.2	47.4 ± 0.3	12.6 ± 6.1
4,14-Dimethylzymosterol	ND	6.3 ± 1.2	15.2 ± 1.0	ND	6.1 ± 1.8	10.0 ± 4.8	ND	ND	11.0 ± 2.4
Obtusifoliol	8.3 ± 3.5	ND	ND	7.8 ± 0.4	ND	ND	3.0 ± 0.8	ND	ND
Lanosterol	7.2 ± 2.3	19.8 ± 3.0	23.8 ± 10.6	19.3 ± 7.5	19.3 ± 1.1	12.8 ± 0.5	18.8 ± 2.4	14.7 ± 0.2	16.1 ± 0.8
24-Methylenedihydrolanosterol	ND	9.9 ± 0.7	31.4 ± 0.8	ND	21.6 ± 1.0	43.4 ± 15.4	ND	24.3 ± 0.8	20.1 ± 1.9

[a] Amounts are shown in the absence of antifungals (control) and in the presence of 1/2 the MIC of either fluconazole (FLU) or voriconazole (VOR). Values are percents (wt/wt) of total amounts of sterols (means ± standard deviations) from two separate experiments. ND, not detected.

FIG. 1. Sterols of a fluconazole-resistant *C. albicans* strain grown in different concentrations of voriconazole.

intermediates were accumulated in cells grown in the presence of fluconazole. The two antifungals completely blocked obtusifoliol synthesis in both *C. albicans* strains tested.

Since voriconazole at one-half the MIC completely inhibited ergosterol synthesis, the effects of different concentrations of this agent were tested in the fluconazole-resistant *C. albicans* strain. Figure 1 shows the effects of voriconazole at various concentrations (1/16, 1/8, and 1/2 the MIC) on the sterol pattern of this isolate. Voriconazole inhibited ergosterol synthesis in a dose-dependent manner. Reductions in ergosterol synthesis of 46% at 1/16 the MIC, 89% at 1/8 the MIC, and 100% at 1/2 the MIC were observed. Voriconazole, even at a very low concentration (1/16 the MIC), was able to completely block obtusifoliol synthesis.

In *C. krusei*, voriconazole significantly inhibited ergosterol synthesis (over 75% inhibition), but unlike in *C. albicans*, some ergosterol synthesis was evident (Table 3). Additionally, *C. krusei* cells treated with voriconazole accumulated the following intermediates: squalene, 4,14-dimethylzymosterol, and 24-methylenedihydrolanosterol. In contrast, treating *C. krusei* with fluconazole inhibited ergosterol synthesis by less than 12%, with a correspondingly small increase in 24-methylenedihydrolanosterol. Similarly to the findings with *C. albicans*, both voriconazole and fluconazole completely inhibited obtusifoliol synthesis in the *C. krusei* isolate.

DISCUSSION

Analysis of sterols obtained from fluconazole-susceptible and -resistant *C. albicans* strains showed no major differences in either the sterol contents or the sterol patterns of the untreated controls of the two isolates. Additionally, ergosterol was the predominant sterol in these strains. Lanosterol, obtusifoliol, and calciferol were also detected in the *C. albicans* strains tested. Data for *C. krusei* were similar. Our finding that

calciferol is a sterol component of candidal cells confirms earlier reports showing that calciferol constitutes about 12.0% ± 4.0% of the total sterol of the plasma membrane from the yeast form of *C. albicans* (15).

Growth of *C. albicans* in the presence of subinhibitory concentrations of voriconazole altered the sterol patterns of the fluconazole-resistant and -susceptible strains in a similar manner. Voriconazole completely blocked ergosterol synthesis and caused a significant increase in the levels of squalene, 4,14-dimethylzymosterol, 24-methylenedihydrolanosterol, and zymosterol (Table 3). Accumulation of 24-methylenedihydrolanosterol was observed in both strains of *C. albicans* and in *C. krusei*. Accumulation of the methylated sterols (4,14-dimethylzymosterol and 24-methylenedihydrolanosterol) is consistent with the premise that voriconazole inhibits fungal growth by interfering with cytochrome P-450-dependent 14α-demethylase, a known target enzyme for azoles (13). Additionally, our inability to detect ergosterol following voriconazole treatment of the two *C. albicans* strains suggests that voriconazole is an efficient inhibitor of the demethylation process of ergosterol precursors. Studying the effect of voriconazole at various subinhibitory concentrations on the sterols of the fluconazole-resistant *C. albicans* strain showed that voriconazole acts in a dose-dependent fashion to decrease ergosterol content (Fig. 1). Interestingly, voriconazole was able to completely inhibit obtusifoliol synthesis, even at a low concentration (1/16 the MIC). This indicates that the methylation route through obtusifoliol is most sensitive to triazoles. The inhibition of ergosterol synthesis and the accumulation of methylated sterol intermediates (4,14-dimethylzymosterol and 24-methylenedihydrolanosterol) following voriconazole treatment of *C. albicans* and *C. krusei* suggest that this antifungal utilizes the same mechanism of action for the inhibition of different *Candida* spp., namely, inhibition of cytochrome P-450-dependent 14α-demethylase.

In addition to an increase in the level of methylated sterol intermediates, accumulation of zymosterol and squalene was observed. These intermediates represent a small percentage of the overall sterol fractions (between 8 and 14% of the total sterol contents of the cells). We were unable to determine whether the accumulation of these intermediates is due to voriconazole's ability to disrupt sterol biosynthesis by interacting with various enzymes involved in ergosterol synthesis, apart from 14α-demethylase, or whether it is a secondary effect of the inhibition of 14α-demethylase. The decrease in the amount of obtusifoliol in the presence of both fluconazole and voriconazole lends credence to the possibility that these agents may inhibit other enzymes involved in ergosterol biosynthesis, including 3-ketosteroid reductase, in addition to 14α-demethylase. For example, Vanden Bossche et al. (25) demonstrated that in addition to inhibiting the 14α-demethylase in *Cryptococcus neoformans*, itraconazole affects the reduction of obtusifolione to obtusifoliol. Our group reported similar findings with fluconazole and *C. neoformans* (11). Therefore, it is likely that voriconazole may have a similar mode of action. Furthermore, inhibition of one branch of the sterol biosynthetic pathway may greatly influence the other branches.

Growth of fluconazole-susceptible *C. albicans* in the presence of fluconazole resulted in changes in the sterol pattern similar to those observed when cells were treated with voriconazole. However, voriconazole was more active than fluconazole in blocking ergosterol synthesis; only partial inhibition of ergosterol synthesis was observed following fluconazole treatment. Additionally, although fluconazole caused a significant accumulation of methylated sterols, the levels were not as high as those observed following voriconazole treatment. Thus, our data indicate that both fluconazole and voriconazole have similar modes of action in that they inhibit cytochrome P-450-dependent 14α-demethylase. *C. krusei* cells grown in the presence of voriconazole had a significantly lower ergosterol content than that of the untreated control (76.5% inhibition). In contrast, growth of this yeast in the presence of fluconazole led to minimal inhibition of ergosterol synthesis (12% inhibition) compared to that in control cells. These findings may explain the potency and broad-spectrum antifungal activity of voriconazole against opportunistic pathogens, including some organisms that are inherently resistant to fluconazole (4, 12).

In another study, using scanning and transmission electron microscopy, we showed that voriconazole treatment leads to the formation of protuberances on the yeast cell surface, cell wall thinning with detachment, and cell membrane degradation (4). These structural manifestations are likely to be secondary effects due to depletion of ergosterol and/or accumulation of 14α-methylsterols brought about by voriconazole treatment (17). It is known that depletion and/or accumulation of sterol precursors causes generalized activation of chitin synthesis (18). Chitin is known to be an important determining factor in fungal morphogenesis (13), and regulation of its synthesis is critical for the formation of normal cell wall structure. Moreover, indiscriminate chitin synthesis may lead to deleterious effects on the fungal cell wall (17).

In conclusion, our data show that voriconazole, like fluconazole, exerts its antifungal activity primarily by inhibiting the cytochrome P-450-dependent 14α-demethylase and that this new triazole possesses higher activity than fluconazole. The conclusion that voriconazole has higher antifungal activity than fluconazole is based on the differences in drug concentrations needed to inhibit yeast growth (Table 1) as well as drug concentrations needed to inhibit the P450-dependent 14α-demethylase. Hitchcock et al. (12) showed that voriconazole is 1.6- and 160-fold more active than fluconazole in inhibiting ergosterol P450-dependent 14α-demethylase in *C. albicans* and *Aspergillus fumigatus* lysates, respectively.

REFERENCES

1. **Anaissie, E. J.** 1992. Opportunistic mycoses in the immunocompromised hosts: experience in a cancer center and review. Clin. Infect. Dis. **14**(Suppl. 1):43–53.
2. **Arndt, C. A. S., T. J. Walsh, C. L. McCully, F. M. Balis, P. A. Pizzo, and D. G. Poplack.** 1988. Fluconazole penetration into cerebrospinal fluid: implications for treating fungal infections of the central nervous system. J. Infect. Dis. **157**:178–180.
3. **Beggs, W. H.** 1983. Comparison of miconazole- and ketoconazole-induced release of K+ from *Candida* species. J. Antimicrob. Chemother. **11**:381–383.
4. **Belanger, P., H. Sanati, R. Fratti, A. Ibrahim, and M. Ghannoum.** 1996. Effect of UK-109,496 on growth and ultrastructure of fluconazole-sensitive (CA^S) and fluconazole-resistant (CA^R) *Candida albicans* strains, abstr. F.75. *In* Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. American Society for Microbiology, Washington, D.C.
5. **Bodey, G. P.** 1988. Fungal infections in cancer patients. Ann. N.Y. Acad. Sci. **544**:431–442.
6. **Cope, J. E.** 1980. Mode of action of miconazole on *Candida albicans*: effects on growth, viability and K+ release. J. Gen. Microbiol. **119**:245–251.
7. **Denning, D., A. del Favero, E. Gluckman, D. Norfolk, M. Ruhnke, S. Youren, P. Troke, and N. Sarantis.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: clinical efficacy in acute invasive aspergillosis, abstr. F80. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
8. **Georgopapadakou, N. H., and T. J. Walsh.** 1996. Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob. Agents Chemother. **40**:279–291.

9. **Ghannoum, M. A.** 1988. Mechanisms potentiating *Candida* infections: a review. Mycoses **31:**543–557.

10. **Ghannoum, M. A., N. A. Moussa, P. Whittaker, I. Swairjo, and K. H. Abu-Elteen.** 1992. Subinhibitory concentration of octenidine and pirtenidine: influence on the lipid and sterol contents of *Candida albicans*. Chemotherapy (Basel) **38:**46–56.

11. **Ghannoum, M. A., B. Spellberg, A. S. Ibrahim, J. A. Ritchie, B. Currie, E. D. Spitzer, J. E. Edwards, and A. Casadevall.** 1994. Sterol composition of *Cryptococcus neoformans* in the presence and absence of fluconazole. Antimicrob. Agents Chemother. **38:**2029–2033.

12. **Hitchcock, C. A., G. W. Pye, G. P. Oliver, and P. F. Troke.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: antifungal activity and selectivity *in vitro*, abstr. F72. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

13. **Hitchcock, C. A., and P. J. Whittle.** 1993. Chemistry and mode of action of fluconazole, p. 183–197. *In* J. W. Rippon and R. A. Fromtling (ed.), Cutaneous antifungal agents: selected compounds in clinical practice and development. Marcel Dekker, Inc. New York, N.Y.

14. **Jezequel, S. G., M. Clark, S. Cole, K. E. Evans, and P. Wastall.** 1995. UK-109,496, a novel, wide-spectrum triazole derivative for the treatment of fungal infections: pre-clinical pharmacokinetics, abstr. F76. *In* Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

15. **Marriott, M. S.** 1975. Isolation and characterization of plasma membranes from the yeast and mycelial forms of *Candida albicans*. J. Gen. Microbiol. **86:**115–132.

16. **National Committee for Clinical Laboratory Standards.** 1995. Reference method for broth dilution antifungal susceptibility testing of yeast; tentative standard M27T. National Committee for Clinical Laboratory Standards, Wayne, Pa.

17. **Pancaldi, S., G. Dall'Olio, F. Poli, and M. P. Fasulo.** 1994. Stimulation of autophagic activity in blastospores of *Candida albicans* exposed in vitro to fluconazole. Microbios **80:**55–61.

18. **Pesti, M., J. M. Campbell, and J. F. Peberdy.** 1981. Alterations of ergosterol content and chitin synthase activity in *Candida albicans*. Curr. Microbiol. **5:**187–190.

19. **Redding, S., J. Smith, G. Farinacci, M. Rinaldi, A. Fothergill, M. Rhine-Chalberg, and M. Pfaller.** 1994. Resistance of *Candida albicans* to fluconazole during treatment of oropharyngeal candidiasis in patients with AIDS: documentation of in vitro susceptibility testing and DNA subtype analysis. Clin. Infect. Dis. **18:**240–242.

20. **Sabra, R., and R. A. Branch.** 1990. Amphotericin B nephrotoxicity. Drug Saf. **5:**94–108.

21. **Selik, R. M., E. T. Starcher, and J. W. Curran.** 1987. Opportunistic diseases reported in AIDS patients: frequencies, associations, and trends. AIDS **1:**175–182.

22. **Stern, J. J., B. J. Hartman, P. Sharkey, V. Rowland, K. Squires, H. Murray, and R. Graybill.** 1988. Oral fluconazole therapy for patients with acquired immunodeficiency syndrome and cryptococcosis: experience with 22 patients. Am. J. Med. **85:**477–480.

23. **Sud, I. J., and D. S. Feingold.** 1981. Mechanisms of action of the antimycotic imidazoles. J. Investig. Dermatol. **76:**438–441.

24. **Sud, I. J., and D. S. Feingold.** 1981. Heterogeneity of action mechanisms among antimycotic imidazoles. Antimicrob. Agents Chemother. **20:**71–74.

25. **Vanden Bossche, H., P. Marichal, L. le Jeune, M.-C. Coene, J. Gorrens, and W. Cools.** 1993. Effects of itraconazole on cytochrome P-450-dependent sterol 14α-demethylation and reduction of 3-ketosteroids in *Cryptococcus neoformans*. Antimicrob. Agents Chemother. **37:**2101–2105.

26. **Vandenheuvel, F. A., and A. S. Court.** 1968. Reference high-efficiency nonpolar packed column for the gas-liquid chromatography of nanogram amounts of sterols. Part I. Retention time data. J. Chromatogr. **38:**439–459.

27. **Wey, S., M. Mori, M. A. Pfaller, R. F. Woolson, and R. P. Wenzel.** 1988. Hospital acquired candidemia. The attributable mortality and excess length of stay. Arch. Intern. Med. **148:**2642–2645.