The Clinical Candidate VT-1161 is a Highly Potent Inhibitor of *Candida albicans*, but not Human, CYP51

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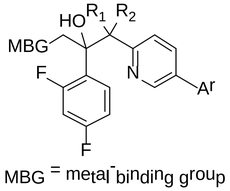
Abstract

Background: Approved antifungal therapies that block CYP51 (lanosterol demethylase) lack selectivity versus human cytochrome P450 enzymes, and better agents that avoid the safety problems inherent to the current drugs are needed. VT-1161 and the related analogs studied here have less avid metal-binding groups with the purpose of achieving greater CYP51 selectivity than the azole drug class. **Methods:** Effects on fungal sterol levels were determined after an overnight incubation of 2 strains of *C. albicans* with 0.004 μg/ml antifungal. The binding affinity (K_d) of each antifungal for recombinant *C. albicans* or human CYP51 was determined from absorbance difference spectra and enzyme inhibition potency (IC_{50}) was determined in a CYP51 reconstitution assay. **Results:** VT-1161 (MIC ≤ 0.004 μg/ml) had the same, but more pronounced, fungal sterol disruption profile (increased methylated sterols and decreased ergosterol), compared with the known CYP51 inhibitor voriconazole (MIC = 0.004 μg/ml). The spectral studies using purified recombinant fungal CYP51 demonstrated that VT-1161 and related analogs produced type II binding spectra, characteristic of heme iron coordination. The binding affinity of VT-1161 for the fungal CYP51 was high (K_d ≤ 39 nM); an accurate calculation of potency was limited by the high enzyme concentration (5 μM) needed for the spectral study. In stark contrast, VT-1161 at concentrations up to 86 μM did not perturb the spectrum of recombinant human CYP51. In reconstitution assays, VT-1161 inhibited fungal CYP51 activity in a tight-binding fashion and again failed to inhibit the human enzyme at the highest concentration tested (50 μM). **Conclusion:** VT-1161 potently inhibited isolated *C. albicans* CYP51 and in cells but did not bind to human CYP51, demonstrating selectivity of >2200. This and previous studies demonstrate the potential utility of VT-1161 in the treatment of systemic and invasive *Candida* infections.

Background

All approved azole antifungal drugs contain either an imidazole or triazole moiety that coordinates with the active site heme iron in the fungal target enzyme, CYP51. These heterocycles are potent heme iron binders and significantly contribute to the overall potency of this class of antifungals. However, they also contribute significantly to the *non-selectivity* found to varying degrees in all approved azoles. To cite one example, most azoles have profound drug-drug interactions because of low or sub-micromolar potencies against key metabolizing CYPs found in the liver. Unlike all CYP51 inhibitors that have been approved in the past 20 years and that contain a 1,2,4-triazole, VT-1161 has a novel metal-binding group that binds the active site heme iron. We demonstrate here that VT-1161 retains very high potency in binding to and inhibiting fungal CYP51, but equally important, does so with a selectivity of >2200-fold over human CYP51.



MBG = metal-binding group

Figure 1 – VT-1161 Chemical Series Structure

Materials & Methods

Difference Spectroscopy. Recombinant fungal and human CYP51 was purified essentially as described (Arase et al, 2006). Binding of CYP51 inhibitors was quantified by type II difference spectra as described (Lamb et al., 1999), with CYP51 protein concentration at 5 μM. Kd values were calculated for molecules that showed tight-binding behavior using Morrison's equation (Lutz et al, 2009), which dictates that dissociation constants can be accurately measured to 1/100th of the concentration of protein used in the study (i.e., 0.05 μM).

IC50 Determination. Inhibition of CYP51 lanosterol demethylase activity was measured in a reconstitution assay (Venkateswarlu et al., 1998), using 2.5 μM enzyme. Product analysis was measured by GC/MS.

Effect of Antifungals on *C. albicans* sterols. Antifungals at 0.004 μg/ml were incubated overnight with *C. albicans* isolates SC5314 or CA14, and sterols were extracted by standard methodology (Martel et al., 2010) and then analyzed by GC/MS. This concentration of antifungal was used because VT-1161, VT-1129, and VT-1148 had a MIC value of ≤0.004 μg/ml.

Results

Binding data with purified fungal and human CYP51 protein are shown in Figure 2, with calculated Kd values in Table 1. IC50 titrations in CYP51 reconstitution activity assays are presented in Figure 3. Effects on fungal sterol pathways after antifungal incubation are shown in Figure 4.

Figure 2. Type II difference spectra of *C. albicans* (Ca) and human (Hu) CYP51, titrating VT-1161 (left) and voriconazole (right).

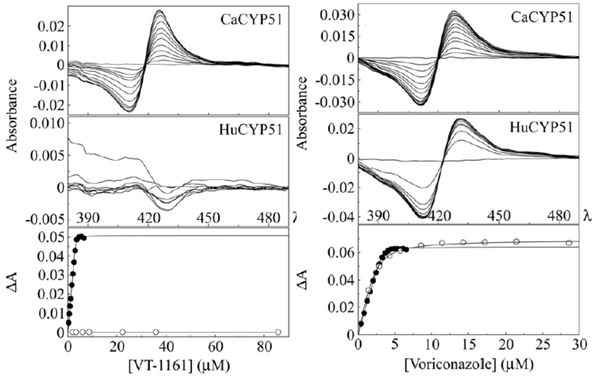
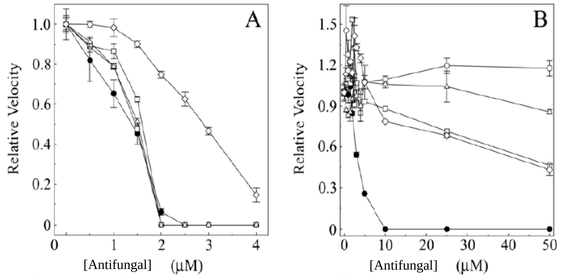


Table 1. Antifungal Dissociation Constants for fungal and human CYP51

Antifungal Agent	K_d (nM), *C. albicans* CYP51	K_d (nM), Human CYP51	Selectivity Ratio (Hu/Ca)
Itraconazole	≤27 (4)	54 (3)	≥2
Voriconazole	≤25 (4)	2290 (120)	≥92
Fluconazole	56 (4)	30300 (4100)	540
VT-1161	≤39 (2)	>86000	>2200
VT-1129	≤28 (3)	4500 (150)	≥160
VT-1148	≤32 (5)	879 (8)	≥27
VT-1163	356 (39)	3900 (160)	11

K_d values are means of three replicates with associated standard errors in parentheses. K_d values are accurate to ~1/100th of the concentration of protein used (5 μM) in study, or ~50 nM.

Figure 3 – Inhibition of *C. albicans* (A) and Human (B) CYP51



(A): fluconazole (filled circles). (B): clotrimazole (filled circles). Both: VT-1129 (empty circles), VT-1148 (empty squares), **VT-1161 (empty triangles)**, and VT-1163 (empty diamonds).

References

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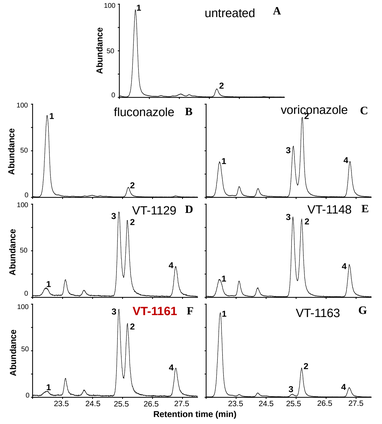
Figure 4 – Chromatograms of sterols isolated from (A) untreated *C. albicans*, and (B-G) *C. albicans* treated with 0.004 μg/ml antifungal.



Sterol chromatograms for untreated (A) and treated (B-G) SC5314 *C. albicans*, with the following sterols highlighted. 1 = Ergosterol, 2 = Lanosterol, 3 = 14α-methyl ergosta 8,24(28)-dien 3β,6α-diol, 4 = Eburicol.

Summary & Conclusions

- VT-1161 potently bound *C. albicans* CYP51 (K_d ≤ 39 nM) with a type II difference spectrum indicative of heme interaction.
- VT-1161 blocked fungal CYP51 demethylase activity in a manner consistent with tight-binding inhibition.
- In cellular studies, at a concentration of 0.004 μg/ml (7.6 nM), VT-1161 nearly completely inhibited the sterol pathway.
- In stark contrast, VT-1161 did not bind to human CYP51 at a concentration of 86 μM, nor inhibit human CYP51 activity at a concentration of 50 μM. Thus, VT-1161 bound to its fungal target >2200-fold tighter than to the human counterpart.
- **The degree of potency and selectivity of VT-1161 supports a strong potential for this novel antifungal clinical candidate.**