VT1129 Binds Potently and Selectively to Recombinant Cryptococcal CYP51 Consistent with Its In Vitro Anti-Cryptococcal Activity

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Background

Cryptococcal meningitis (CM) is the second leading cause of death in HIV-positive individuals in sub-Saharan Africa. The most accessible therapy, high-dose fluconazole monotherapy, has mortality rates of 40-60%. Even in the U.S., mortality rates using combination therapy range from 10-20%. VT-1129 is a novel fungal CYP51 inhibitor with potent in vitro and in vivo activity against *Cryptococcus*. We disclose its chemical structure (Figure 1) and highlight the use of 1-tetrazole to bind the active site heme iron, resulting in exquisite selectivity versus human CYPs while maintaining high potency of inhibition of fungal CYP51.

Materials & Methods

CYP51 Biochemical Studies – CYP51 enzymes from *Cryptococcus neoformans* var. *neoformans* (Cneo) *Cryptococcus neoformans* var. *grubii* (Cgru) *Cryptococcus gattii* (Cgat) and human (Hsap) were expressed in *Escherichia coli*, purified and characterized. Binding constants (K_d) were determined using difference spectra assays. Inhibition potencies (IC_{50}) were measured in enzyme activity assays.

Human CYP IC50 Studies – IC_{50} values for inhibition of human CYP enzymes were determined using either human microsomes or microsomes from yeast expressing the human recombinant enzyme, at 1 mg/ml microsomal protein and substrate concentration at its K_m value.

In Vitro Antifungal Studies – Minimum inhibitory concentration (MIC) studies were performed under the methods outlined in CLSI M27-A3, and MIC was the lowest concentration that resulted in a 50% reduction of growth of *Cryptococcus neoformans* compared to control at 72 hour at 37 C. The optimal concentration range for VT-1129 was empirically determined to be 0.0005 to 0.25 μg/ml; a range of 0.12 to 64 μg/ml was used for fluconazole.

Fig 1: Chemical Structure of VT-1129

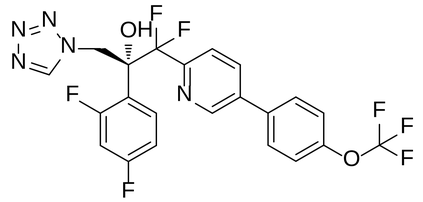

Fig 2: VT-1129 Potently Binds *Crypto* CYP51

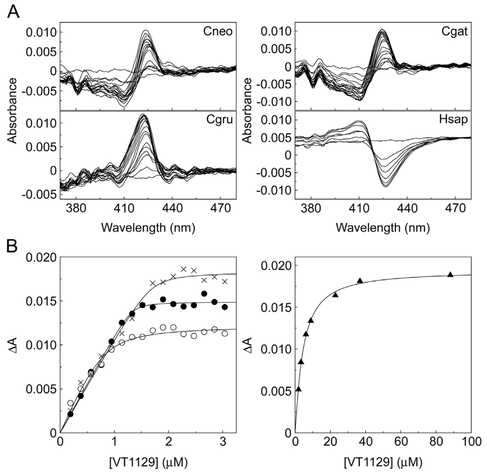

Figure 2. (A) Difference spectrum titrations of VT-1129 against the various CYP51s. (B) For each Crypto CYP51 (left graph), determination of potency was limited by concentration of CYP51 in assay; K_d values: ≤11 nM (Cneo), ≤25 nM (Cgru), and ≤24 nM (Cgat). Titration with human CYP51 lead to a K_d value of 4500 nM (right graph).

Fig 3: VT-1129 Selective Inhibition of Crypto CYP51

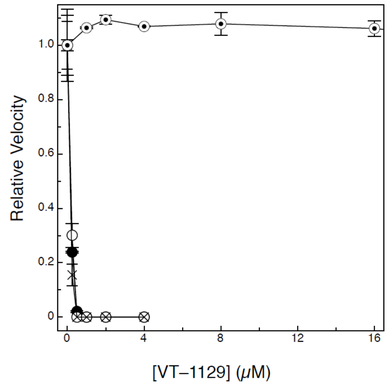

Figure 3. Inhibition of CYP51 activity by VT-1129. For each Crypto CYP51, determination of potency was limited by concentration of CYP51 in assay; IC_{50} values: ≤0.16 μM (Cneo, solid circles), ≤0.18 μM (Cgru, open circles), ≤0.15 μM (Cgat, X). VT-1129 showed little inhibition of human CYP51 (dotted circles); IC50 ~600 μM.

Table 1: VT-1129 is a Weak Inhibitor of Human CYPs

IC_{50} (μM) against Human CYPs							
Agent	2C9	2C19	3A4 (midazolam)	3A4 (testoster.)	17 OHlase	17 lyase	19
VT-1129	87	108	79	178	>200	>200	>200
Fluconazole	34	13	32	6.4	>200	>200	17
Voriconazole	10	10	13	3.8	>200	3.1	30
Posaconazole	25	7.2	0.05	0.12	0.71	0.04	6.0

Table 2: VT-1129 is a Potent Inhibitor of Cryptococcal Growth

Cryptococcus neoformans (50 clinical isolates)				
Agent	MIC Range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	MIC Geo Mean (μg/mL)
VT-1129	0.0005 - >0.25	0.008	0.06	0.013
Fluconazole	≤0.12 - 64	2	8	2.5

Conclusions

- Through interaction of 1-tetrazole with the active site heme iron, VT-1129 potently and selectively binds to and inhibits cryptococcal CYP51.
- Its antifungal potency against *Cryptococcus neoformans* is consistent with a low nanomolar potency against the target CYP51.
- VT-1129 showed very weak if any inhibition against key human CYP enzymes, suggesting a low risk of related off-target effects such as drug-drug interactions or endocrine toxicities.
- A Phase 1 study of VT-1129 in healthy volunteers is scheduled to begin by the end of this year, with Phase 2 studies in CM patients targeted by the end of 2016.