Novel Metalloenzyme Inhibitors, VT-1161 and VT-1129, Exhibit Efficacy and Survival Benefit in a Murine Systemic Candidiasis Model

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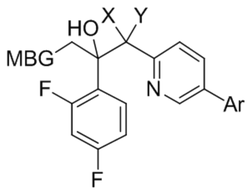
Abstract

Background: The overall mortality for invasive *Candida* infections is 30-50%, therefore new therapies are urgently needed. The efficacies of investigational lanosterol demethylase (LD) inhibitors VT-1129 and VT-1161 were compared with fluconazole (FLC) in a mouse systemic candidiasis model. **Methods:** Neutropenic female CD-1 mice were dosed orally 2h after infection (day 1) and once again on days 2 to 4 and survival (N = 10/group) monitored (day 18). CFU/kidneys was assessed in surviving mice. **Results:** VT-1129, VT-1161, and FLC significantly improved % and median survival versus vehicle and survival was greatest in the VT-1161 treated animals. Fungal burden was significantly less in the VT-1129 10 mg/kg and VT-1161 10 and 20 mg/kg groups than the FLC 10 mg/kg group. **Conclusions:** VT-1129 and VT-1161 demonstrated oral efficacy and survival benefit with greater fungal burden reductions than FLC. VT-1129 and VT-1161 are potential treatments for systemic and invasive *Candida* infections.

Background

Patients with invasive fungal infections often fail therapy with currently available antifungal agents. Mortality remains high, particularly in the immunocompromised. Fungal resistance and side-effects that result from off-target enzyme inhibition are limitations of the 'azole' class. Currently approved LD inhibitors, such as fluconazole, utilize a 1-(1,2,4)-triazole metal-binding group (MBG) group, resulting in high potency for both LD and off-target cytochrome P450s. VT-1161 and VT-1129 (Figure 1) are novel LD inhibitors with optimized metal-binding groups (MBGs) not found in current azole drugs. These novel MBGs may provide for improved potency, selectivity, and therapeutic index versus the current agents.

Figure 1 – VT-1129 & VT-1161 Chemical Structure Class



Materials & Methods

Yeast MICs – MIC values were assessed using a standardized procedure (CLSI M27-A2) at Ricerca Biosciences, LLC.

Cytochrome P-450 Inhibition - The IC_{50} values for cytochrome P450 isozymes 2C9, 2C19, and 3A4 were determined from the dose-response inhibition of conversion of diclofenac, omeprazole, and testosterone respectively, in human liver microsomes by HPLC/MS/MS (OpAns, LLC).

Mouse Candidiasis Survival/Kidney Fungal Burden - Female CD-1 mice (7 weeks, 18-25g) were made neutropenic with IP injections of cyclophosphamide (150 mg/kg) at 4 and 1 days before inoculation with *C. albicans R303* (1.22x 10^4 CFU, tail vein). Test compounds were administered orally 2 h after infection (day 0) and then once on days 1 – 3. Kidneys were collected from surviving mice, PBS added, and homogenized. CFU/kidneys was determined from colony counts on SDA plates from serial dilutions of the homogenates (detection limit, log_{10}CFU/kidneys of 1.3). In-life study performed at Ricerca Biosciences, LLC.

Results

Table 1 – MIC Values for Common Pathogens[1]

Agent	Candida albicans MIC[2] (ug/mL)	Candida glabrata MIC[3] (ug/mL)	Candida krusei MIC[4] (ug/mL)	Cryptococcus neoformans MIC[5] (ug/mL)
VT-1129	≤0.0001	≤0.0001	1.0	<0.0001
VT-1161	≤0.0001	≤0.0001	1.0	<0.0001
Fluconazole	0.5	0.125	32	0.25
Voriconazole	0.016	0.016	0.5	0.016

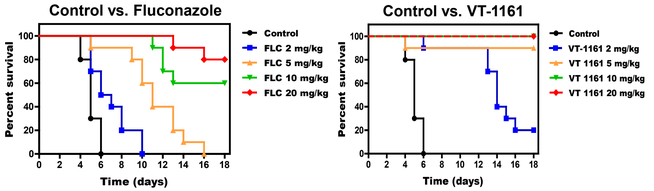
1. Testing (Ricerca Biosciences) performed using CLSI M27-A2 protocol
2. ATCC 90028
3. R362, clinical
4. ATCC 6258
5. R321, clinical

Table 2 – IC50 Values for P450 Drug Metabolizing Enzymes

Agent	CYP 2C9 IC50*	CYP 2C19 IC50*	CYP 3A4 IC50*	C. albicans Therapeutic Index**
VT-1129	110	>200	104	≥520,000
VT-1161	115	94	105	≥470,000
Fluconazole	28	7	35	4
Voriconazole	7	7	15	70

*50% inhibitory concentration values in μM. Performed at OpAns, LLC
** Lowest CYP IC50/C. albicans ATCC 90028 MIC

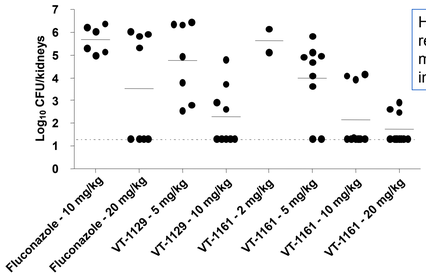
Figure 2 – Effect of LD Inhibitors on Survival in Candidiasis Model[1]



Treatment (mg/kg)	% Survival
FLC – 5	0
FLC – 10	60
FLC - 20	80
VT-1129 – 5	70
VT-1129 – 10	90
VT-1161 – 2	20
VT-1161 – 5	90
VT-1161 – 10	100
VT-1161 – 20	100

1. Agents dosed orally once daily on Day 0 (2-hours post inoculation) through Day 3.

Figure 3 – Effect of LD Inhibitors on Kidney Fungal Burden (Day 18)



Horizontal bars represent the group mean and dotted line indicates the LLQ.

Treatment (mg/kg)	CFU/kidneys (+/- SD)	p-value[1]
FLC – 10	5.68 +/- 0.60	
FLC – 20	3.54 +/- 2.4	> 0.05
VT-1129 – 5	4.75 +/- 1.71	> 0.05
VT-1129 – 10	2.28 +/- 1.31	< 0. 001
VT-1161 – 2	5.63 +/- 0.73	> 0.05
VT-1161 – 5	3.98 +/- 1.64	> 0.05
VT-1161 – 10	2.13 +/- 1.33	< 0. 001
VT-1161 – 20	1.71 +/- 0.67	< 0.001

[1]One-way ANOVA analysis, Tukey-Kramer Multiple Comparisons Test (GraphPad v 3.06), vs. FLC 10mg/Kg group.

Table 3 – Plasma Levels on Day 18

Agent	Dose (mg/kg)	n	Plasma Level (μg/ml ± SD)
VT-1129	5	7	0.32 +/- 0.062
	10	9	1.56 +/- 0.27
VT-1161	5	9	0.0090 +/- 0.0084
	10	10	0.090 +/- 0.058
	20	10	0.79 +/- 0.43

Plasma levels determined by LC/MS/MS methodology (OpAns, LLC)

Summary & Conclusion

VT-1129 and VT-1161 demonstrated potent oral efficacy in a murine systemic candidiasis survival model. VT-1129 10 mg/kg and VT-1161 10 and 20 mg/kg doses statistically reduced kidney yeast burden vs. the 10mg/kg fluconazole group. VT-1129 and VT-1161 represent potential, highly potent and selective therapies for invasive *Candida* infections.