The Fungal Cyp51 Inhibitors VT-1161 and VT-1129 Maintain *in vitro* Activity Against *Candida albicans* Isolates with Reduced Antifungal Susceptibility

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Introduction

Candida spp. are a major cause of invasive mycoses with the majority of infections caused by *C. albicans* (CA). As treatment options are limited in the setting of resistance to available agents, we compared the *in vitro* activity of the fungal Cyp51 inhibitors VT-1161 and VT-1129 to fluconazole (FLC), caspofungin (CAS), and voriconazole (VRC) against a panel of clinical CA isolates, including those with reduced antifungal susceptibility.

Reports of antifungal resistance are prevalent in the published literature. While some mechanisms of resistance have the ability to cause cross-resistance within the class, this is not always the case. Therefore, it is important to evaluate the relevance of known resistance mechanisms in relation to investigational compounds.

Material and Methods

A total of 20 CA isolates from patients with candidemia or oropharyngeal candidiasis were tested including 8 that were susceptible dose-dependent (SDD) or resistant to FLC.

Stock solutions were prepared by dissolving drug (10mg) in 1 ml of DMSO. Further dilutions were made in DMSO and RPMI for a final DMSO concentration of 1%.

Methods for testing were those outlined in the CLSI document M27-A3. All drugs were tested in a microdilution format using RPMI-1640 with L-glutamine but without bicarbonate, an inoculum of 0.5-2.5 x 10^3, incubation at 35˚C, and were read manually with an inverted mirror. Testing range for VT compounds and VRC was 0.03-16 µg/ml while the range for CAS was 0.015-8 µg/ml and for FLC was 0.125-64 µg/ml respectively. Endpoints were determined at 50% inhibition of fungal growth as compared to the drug-free growth control

Results

Fluconazole SDD / Resistant Isolates (MIC ≥16 µg/ml)

Agent	VT-1161	VT-1161	VT-1129	VT-1129	CAS	FLC	VRC
Time Pt.	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	24 hrs	48 hrs
MIC Range	≤0.03 – 0.5	≤0.03 – 1	≤0.03 – 0.5	≤0.03 – 1	0.25 – 2	32 – >64	0.06 – >16
GM MIC	0.11 p < 0.001	0.34 p < 0.001	0.12 p < 0.001	0.36 p < 0.001	0.50 p < 0.001	58.7	1.88 p < 0.01

p-value vs. FLC

Results

Antifungal Time Point (hr)		VT-1161 24	VT-1161 48	VT-1129 24	VT-1129 48	CAS 24	FLC 24	VRC 48
Control	CP	≤0.03	≤0.03	≤0.03	≤0.03	0.5	1	0.06
Control	CK	0.06	0.25	0.06	0.125	0.5	16	0.25
Fluconazole & Caspofungin Susceptible Isolates								
1649	CA	≤0.03	≤0.03	≤0.03	≤0.03	0.5	0.25	≤0.03
2307	CA	≤0.03	≤0.03	≤0.03	≤0.03	0.015	0.5	≤0.03
412	CA	≤0.03	≤0.03	≤0.03	≤0.03	0.125	≤0.125	≤0.03
1002	CA	≤0.03	≤0.03	≤0.03	≤0.03	0.25	≤0.125	≤0.03
3034	CA	≤0.03	≤0.03	≤0.03	≤0.03	0.5	1	≤0.03
4018	CA	≤0.03	>16	≤0.03	>16	0.25	2	>16
SC5314	CA	≤0.03	≤0.03	≤0.03	≤0.03	0.25	≤0.125	≤0.03
Isolates with Reduced Susceptibility to Fluconazole and Caspofungin								
6482	CA	0.25	0.25	0.5	0.5	0.25	>64	8
CLY719	CA	0.5	1	0.5	1	1	>64	16
43001	CA	≤0.03	≤0.03	≤0.03	≤0.03	2	32	2
42379	CA	≤0.03	≤0.03	≤0.03	≤0.03	2	32	0.06
53264	CA	≤0.03	≤0.03	≤0.03	≤0.03	2	≤0.125	≤0.03
4254	CA	0.06	≤0.03	≤0.03	≤0.03	1	≤0.125	0.06
2274	CA	0.125	0.25	0.125	0.25	0.25	32	0.25
6431	CA	0.5	0.5	0.5	0.5	0.25	64	8
2440	CA	0.06	0.06	0.06	0.125	0.5	8	0.125
3795	CA	≤0.03	≤0.03	≤0.03	≤0.03	0.25	8	≤0.03
2257	CA	0.25	0.5	0.5	0.5	0.25	>64	0.5
4380	CA	0.06	0.125	≤0.03	0.125	0.5	32	0.5

Isolate	Mechanisms of Resistance
1649	Wild-type
2307	*ERG11* point mutation & over-expression *CDR1*, *CDR2*, & *ERG11*
412	Wild Type – Control for 2307
1002	Wild-type – Control for 3034
3034	Over-expression of *CDR1*, *CDR2*, & *MDR1*
4018	Wild-type
SC5314	Wild-type (genome available)
6482	*FKS1* point mutation; unknown mechanism of azole resistance
CLY719	*FKS1* point mutation; unknown mechanism of azole resistance
43001	*FKS1* point mutation; unknown mechanism of azole resistance
42379	*FKS1* point mutation; unknown mechanism of azole resistance
53264	*FKS1* point mutation
4254	*FKS1* point mutation
2274	*ERG11* point mutation
6431	Unknown mechanism of azole resistance
2440	*ERG11* Point mutation & over-expression of *MDR1*
3795	*ERG11* point mutation
2257	*ERG11* point mutation
4380	*ERG11* point mutation & over-expression *CDR1*, *CDR2*, & *ERG11*

	WT
	Fluconazole SDD / Resistance & Echinocandin Resistance
	Echinocandin Resistance
	Fluconazole SDD / Resistance

Results

1. VT-1161 and VT-1129 exhibited greater potency and significantly lower GM MICs than FLC including isolates SDD and resistant isolates with point mutations in *ERG11* and those that overexpress *CDR1*, *CDR2* and *MDR1.*

2. VT-1161 and VT-1129 had similar or superior potency to CAS and VRC against all isolates tested.

3. VT-1161 and VT-1129 maintained good activity against isolates with resistance to both azoles and CAS.

Conclusions

In maintaining activity despite the presence of resistance mechanisms, the activity of VT-1161 and VT-1129 against *Candida* spp. compared favorably with both caspofungin and voriconazole and was more potent than fluconazole.

The results for these compounds are very promising and suggest that further development of these agents is warranted in an effort to improve the treatment options for patients with fungal infections.

References

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