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Fungal CYP51 Inhibitors VT-1161 and VT-1129 Exhibit Strong *In Vitro* Activity against *Candida glabrata* and *C. krusei* Isolates Clinically Resistant to Azole and Echinocandin Antifungal Compounds

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ABSTRACT The *in vitro* activities of fungal CYP51 inhibitors VT-1161 and VT-1129 were determined for *Candida glabrata* ($n = 34$) and *C. krusei* ($n = 50$). *C. glabrata* isolates were screened for FKS gene mutations. All isolates were resistant clinically and/or *in vitro* to at least one standard antifungal compound. VT-1161 and VT-1129 MICs for all isolates were at least 5-fold below achievable human plasma levels for VT-1161. VT-1161 and VT-1129 are promising for the treatment of resistant *C. glabrata* and *C. krusei* infections.

KEYWORDS *Candida glabrata*, *Candida krusei*, antifungal susceptibility testing, resistance

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The occurrence of invasive candidiasis is increasing, particularly among populations of patients receiving critical care support and in patients with severe immune suppression, such as those undergoing myeloablative chemotherapies and hematopoietic stem cell or solid organ transplantation (1, 2). Based on population-based surveillance of candidemia in the United States, *Candida glabrata* is the second most common and *C. krusei* is the fifth most common *Candida* species recovered from clinical specimens in the U.S. and worldwide (3). Resistance to currently available antifungal compounds is increasing for these two pathogens and appears to be coemerging within isolates of *C. glabrata* (4, 5). *C. krusei* is intrinsically resistant to fluconazole, and in both of these species, the emergence of voriconazole resistance following exposure to fluconazole is a significant concern.

VT-1161 and VT-1129 represent a new generation of fungal CYP51 inhibitors. Through the rationale of using a tetrazole moiety to bind the active site heme iron, these inhibitors are designed for an enhanced affinity and selectivity for fungal CYP51 versus human cytochrome P450 proteins (CYPs) (6, 7). These compounds showed potent *in vitro* activity against *C. albicans* in initial tests (6, 7). Subsequently, a study of VT-1161 against 10 isolates of fluconazole-resistant *C. albicans* from acute and recurrent vulvovaginal candidiasis showed MICs ranging from ≤0.015 to 2 μg/ml, with an MIC_{90} of ≤0.015 μg/ml (8). Whereas VT-1161 shows potency against yeasts (7, 8) and dermatophytes (9), VT-1129 distinguishes itself with potent activity against *Cryptococcus* spp. (10). Both compounds are now in clinical development, with VT-1161 nearing completion of separate phase 2b studies in recurrent vulvovaginal candidiasis (NCT02267382) and onychomycosis (NCT02267356) and VT-1129 in phase 1 studies.

In this study, we determined the *in vitro* activities of VT-1161 and VT-1129, as well as those of fluconazole, voriconazole, anidulafungin, and micafungin, for clinical iso-

TABLE 1 MIC results for 84 drug-resistant *Candida* isolates

Microorganism (no. of isolates)	Antimicrobial compound[a]	MIC (μg/ml)			
		Range	Geometric mean	50%	90%
C. krusei (50)	VT-1129	<0.015–2	0.34	0.5	1
	VT-1161	<0.015–1	0.16	0.25	0.5
	FLC	16–128	34.3	32	64
	VRC	<0.015–4	0.23	0.25	0.5
	ANF	<0.015–0.25	0.03	0.03	0.03
	MCF	<0.015–0.25	0.06	0.06	0.12
C. glabrata (34)[b]	VT-1129	0.03–2	0.22	0.12	1
	VT-1161	<0.015–1	0.16	0.12	1
	FLC	1–128	5.19	2	64
	VRC	<0.015–4	0.25	0.12	2
	ANF	0.06–8	0.92	1	8
	MCF	<0.015–16	0.44	0.5	8

[a]ANF, anidulafungin; FLC, fluconazole; MCF, micafungin; VRC, voriconazole. All drugs were tested according to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method within concentrations ranging from 0.015μg/ml to 16μg/ml, except for fluconazole which ranged in concentration from 0.12μg/ml to 128μg/ml.

[b]Two isolates required 48 h of incubation.

lates of *C. glabrata* ($n = 34$) and *C. krusei* ($n = 50$) using the Clinical and Laboratory Standards Institute M27-A3/S4 broth microdilution method (11, 12). Each isolate of *C. krusei* represents a unique episode of bloodstream infection from 2003 to 2013, and each isolate of *C. glabrata* represents a unique episode of bloodstream infection from 2001 to 2010. *C. glabrata* isolates were screened for FKS gene mutations as previously described (13). VT-1161-M and VT-1129-G (powders >99% pure) were provided by Viamet Pharmaceuticals, Inc. (Durham, NC), and stock solutions were prepared at a concentration of 1,600 μg/ml in pure dimethyl sulfoxide (DMSO). Anidulafungin, micafungin, and voriconazole were purchased in the form of frozen custom-made microtiter plates without a colorimetric indicator (Trek Diagnostics, Inc., Independence, OH). Fluconazole was purchased as a powder (99% pure; Alfa Aesar, Inc., Ward Hill, MA). Drugs were tested in concentrations ranging from 0.015 μg/ml to 16 μg/ml, except for fluconazole, which ranged in concentration from 0.12 μg/ml to 128 μg/ml. Inoculum concentrations were verified by quantitative culture. VT-1161 and VT-1129 endpoints were determined visually at 24 and 48 h and defined as 50% growth inhibition compared to that of drug-free controls. *C. parapsilosis* ATCC 22019 was used as the quality control strain.

All *C. glabrata* and *C. krusei* isolates were inhibited by VT-1129 and VT-1161 at concentrations of ≤2 μg/ml after 24 h of incubation (Table 1). For VT-1129, the mean MICs for *C. glabrata* and *C. krusei* were 0.22 μg/ml and 0.34 μg/ml, respectively, and the MIC_{90} values for both species were 1 μg/ml. For VT-1161, the mean MIC for both species was 0.16 μg/ml and the MIC_{90} values were 1 μg/ml and 0.5 μg/ml for *C. glabrata* and *C. krusei*, respectively. Per Table 2, VT-1129 and VT-1161 exhibited low

TABLE 2 Distribution of antifungal MICs and FKS mutations for 34 clinically resistant *Candida glabrata* isolates

Antifungal compound[a]	No. of isolates (no. having FKS mutation) at MIC shown (μg/ml)													
	<0.01	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128
VT-1129	—[b]	—	—	3 (1)	8[c] (6)	10 (7)	8 (7)	5 (5)	—	—	—	—	—	—
VT-1161	—	—	1	5[c] (1)	13 (12)	5 (4)	8 (7)	2 (2)	—	—	—	—	—	—
FLC	—	—	—	—	—	1	2 (1)	6[c] (2)	9 (8)	4 (4)	2 (1)	4 (4)	2 (1)	4 (4)
VRC	1	—	5 (2)	12[c] (9)	4 (4)	3 (2)	4 (4)	3 (3)	2 (2)	—	—	—	—	—
ANF	—	—	3 (2)	3 (2)	7 (6)	1 (1)	3	7 (7)	5 (4)	5[c] (4)	—	—	—	—
MCF	1 (1)	2 (2)	6 (5)	4 (2)	2 (2)	7 (5)	2 (2)	1	5 (4)	3[c] (2)	1 (1)	—	—	—

[a]ANF, anidulafungin; FLC, fluconazole; MCF, micafungin; VRC, voriconazole.

[b]—, no isolates tested had an MIC at this value.

[c]One of the 34 strains was not tested for FKS mutations.

MICs for C. *glabrata* isolates demonstrating FKS gene mutations, including those with FKS2-S663P mutations, which were uniformly associated with echinocandin-resistant MICs.

Ninety percent of the 84 isolates were inhibited by $\leq$1 μg/ml of VT-1129 and VT-1161. Such MIC values are substantially lower than the well-tolerated and achievable plasma concentrations of VT-1161 measured during phase I and phase II clinical trials (Tavakkol, Degenhardt, Brand, Jet, Viamet Pharmaceuticals, unpublished data). Furthermore, preclinical and phase 1 data for VT-1129 indicate similar safety and PK profiles. Thus, although the clinical breakpoints for these compounds are not yet known, the MIC values reported here likely represent clinically relevant antifungal potencies.

These data suggest VT-1161 and VT-1129 have uniformly potent activities against *C. glabrata* and *C. krusei*, two *Candida* species in which the resistance to standard antifungal compounds can be intrinsic or acquired and for which resistance to standard antifungal compounds is a growing public health concern. VT-1161 and VT-1129 show strong potential for the treatment of fluconazole-resistant *C. krusei* and fluconazole- and echinocandin-resistant *C. glabrata* infections. Further investigation is warranted.

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