

Susceptibility testing of VT-1129, a novel fungal CYP51 inhibitor, against *Cryptococcus neoformans* and *Cryptococcus gattii*

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Background-

VT-1129 is a potent and highly selective novel inhibitor of fungal CYP51 (lanosterol demethylase). As an oral induction treatment, VT-1129 is superior to fluconazole in the murine model of CM caused by *C. neoformans* (see adjoining abstract), and has a significantly longer half-life than fluconazole which may decrease the dosing schedule and improve patient compliance.

The aim of this study was to test VT-1129 against a global collection of *C. neoformans* and molecularly characterized *C. gattii* isolates.

Methods-

- *Cryptococcus* isolates were identified to the species level and isolates of *C. gattii* were further characterized to molecular type.
- Testing was performed as outlined in CLSI document M27-A3. MIC testing was performed using RPMI broth in 96-well microdilution plates with final dilution concentrations of VT-1129 ranging from 0.015 µg/ml to 8 µg/ml.
- All MIC values were determined visually as the lowest drug concentration at which there was a 50% and a 100% decrease in growth after 72 hours of incubation at 35°C.

Table 1. Distribution of *Cryptococcus* spp. isolates used in this study by country of origin and molecular type.

Origin	No. of isolates	<i>Cryptococcus neoformans</i>	<i>Cryptococcus gattii</i> molecular type						
			VGI	VGII	VGIIa	VGIIb	VGIIc	VGIII	VGIV
Australia	7		4	3					
Botswana	48							1	47
South Africa	143	100	17	1					25
British Columbia	7				5	2			
United States	195		24	3	99	18	32	19	
Total	400	100	45	7	104	20	32	20	72

Table 2. Previously determined fluconazole MIC range, mode, MIC₅₀ and MIC₉₀ for the isolates used in this analysis.

	MIC (µg/ml) at 50% inhibition			
	Range	Mode	MIC ₅₀	MIC ₉₀
<i>C. neoformans</i>	0.5-16	4	2	8
<i>C. gattii</i>	0.5-32	8	8	16
<i>C. gattii</i> VGI	0.5-8	4	2	4
<i>C. gattii</i> VGII	1-4	2	2	4
<i>C. gattii</i> VGIIa	2-16	8	8	8
<i>C. gattii</i> VGIIb	16	16	16	16
<i>C. gattii</i> VGIIc	4-32	16	16	32
<i>C. gattii</i> VGIII	1-16	4	4	8
<i>C. gattii</i> VGIV	0.5-32	8	4	8

Results- *C. neoformans* MIC values against VT-1129 were very low with a 50% inhibition range of ≤0.015 to 0.125 µg/ml and a 100% inhibition range of ≤0.015 to 2 µg/ml.

The VT-1129 MIC₅₀ and MIC₉₀ ranges were much lower (seven Log₂ dilutions) than the fluconazole MIC₅₀ and MIC₉₀ ranges against the same set of isolates at 50% inhibition.

The *C. gattii* MIC values against VT-1129 were low with a 50% inhibition range of ≤0.015 to 1 µg/ml but the MIC₅₀ and MIC₉₀ values were two and three log₂ dilutions higher, respectively, than those of *C. neoformans*.

At 50% inhibition, the MIC₅₀ and MIC₉₀ values for VGIV isolates were lowest (0.015 and 0.06 µg/ml) followed by VGI isolates (0.03 and 0.125 µg/ml). VGII isolates and VGIII isolates had the highest MIC₅₀ and MIC₉₀ values.

Conclusions- MIC values for VT-1129 against *C. neoformans* and *C. gattii* are low. VT-1129 has excellent activity against *Cryptococcus* isolates from Africa where the burden of *Cryptococcus* remains very high, and against isolates of *C. gattii* with high MIC values to fluconazole.

VT-1129 is currently undergoing IND-enabling studies that will support the clinical investigation of its potential to reduce the high mortality rate of this devastating disease.

Table 3. VT-1129 MIC data for 100 *C. neoformans* isolates and 300 *C. gattii* isolates.

Organism	Minimum Inhibitory Concentration (µg/ml) of VT-1129			
	<i>C. neoformans</i>		<i>C. gattii</i>	
Inhibition	50%	100%	50%	100%
Range	≤0.015-0.125	≤0.015-2	≤0.015-1	0.125->8
Mode	≤0.015	0.5	0.125	1
MIC ₅₀	0.015	0.25	0.125	1
MIC ₉₀	0.06	0.5	0.25	2

Table 4. VT-1129 MIC values for 300 *C. gattii* isolates grouped by molecular type.

Compound	MIC (µg/ml) VT-1129	
	50%	100%
VGI (n=45)		
Range	≤0.015-0.5	0.125-8
Mode	0.015	0.25,0.5
MIC ₅₀	0.03	0.5
MIC ₉₀	0.125	1
VGII (n=7)		
Range	0.06-0.25	0.5-2
Mode	0.25	0.5-1
MIC ₅₀	0.25	1
MIC ₉₀	0.25	1
VGIIa (n=104)		
Range	0.06-0.5	0.5-2
Mode	0.125	1
MIC ₅₀	0.125	1
MIC ₉₀	0.25-2	2
VGIIb (n=20)		
Range	0.06-0.5	0.5-2
Mode	0.125-0.25	2
MIC ₅₀	0.125	2
MIC ₉₀	0.25	2
VGIIc (n=32)		
Range	0.06-1	0.5-4
Mode	0.25	2
MIC ₅₀	0.25	2
MIC ₉₀	0.25	2
VGIII (n=20)		
Range	≤0.015-0.25	0.25-2
Mode	0.125	1
MIC ₅₀	0.125	1
MIC ₉₀	0.25	1
VGIV (n=72)		
Range	≤0.015-0.25	0.125->8
Mode	0.015	0.5
MIC ₅₀	0.015	0.5
MIC ₉₀	0.06	2

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