VT-1598 is a Highly Potent Inhibitor of *Cryptococcus* spp. In Vitro and In Vivo

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Introduction

Cryptococcal meningitis causes more than half a million deaths annually, including many cases associated with HIV infection in developing countries. Even with gold standard chemotherapy [amphotericin (AMB) and 5-flurocytosine induction, followed by fluconazole (FLU) maintenance], cryptococcal meningitis mortality rates reach 20%, with much higher rates (>50%) in resource-limited countries. In addition, these regimens include significant toxicities and the potential for serious drug-drug interactions. Furthermore, amphotericin B, if available, must be delivered intravenously using a slow infusion. New treatments are urgently needed that deliver improved efficacy, a better safety and tolerability profile, as well as the potential for more convenient dosing regimens.

Methods

Minimum inhibitory concentration (MIC) was determined at 72 h (unless indicated otherwise) at 50% inhibition of growth relative to no-drug control in RPMI media (CLSI M27-A3), using FLU and AMB as comparator controls. *In vitro* selectivity was assessed by measuring IC_{50} values for key human CYP enzymes in biochemical assays, using approved fungal CYP51 inhibitors as comparator controls. *In vivo* activity was determined in a tail-vein model of cryptococcal meningitis in immunocompetent male ICR mice inoculated with *C. neoformans var. grubii* H99 (ATCC 208821) (N=10 mice/dose group). A 14-day treatment period started 1-day post-inoculation, followed by a 6-day wash-out period, after which brain fungal burden and plasma and brain drug levels were measured. Treatments included: 5, 15, and 50 mg/kg oral VT-1598 once daily, 25 mg/kg oral fluconazole twice daily, and 10 mg/kg IV AmBisome (a lipid formulation of AMB) once daily. Vehicle control was the same formulation used for VT-1598 (20% cremophor EL).

Results

Table1: MICs against *Cryptococcus* spp.

MIC(μg/ml)			
Agent	**Range**	**MIC_{50}**	**MIC_{90}**
	C. neoformans (N=36 isolates)		
VT-1598	0.004 - 0.25	0.015	0.03
Fluconazole	0.25 - 8	2	4
Amphotericin B	0.25 - 1	0.5	1
	C. gattii (N=16 isolates)		
VT-1598	≤0.015 - 0.25	0.03	0.12
Fluconazole	1 – 8	2	8
Amphotericin B	0.25 – 1	1	1
	C. neoformans H99 MIC (μg/ml)		
	24 h	48 h	72 h
VT-1598	0.03	0.03	0.03
Fluconazole	1	2	2
Amphotericin B	0.06	0.12	0.12

Table 2: Biochemical selectivity versus key human CYP enzymes.

Human CYP Selectivity (IC50, μM)								
CYP:	**2C9**	**2C19**	**3A4**	**11B1**	**11B2**	**17 lyase**	**17 OHase**	**19**
VT-1598	>200	138	>200	>100	>100	38	>100	>100
FLU	34	13	32	30	13	>200	>200	22
VOR	10	10	13	20	7.5	3.1	>200	6.4
ITR	80	78	0.085	9.3	0.37	1.6	>200	52
POS	109	7.2	0.075	0.31	0.021	0.042	0.71	8.4
ISA	12	14	8.9	9.8	2.8	2.7	37	6.6

Figure 1: Survival benefit in a murine model of cryptococcal meningitis. Single deaths in red and blue groups were due to gavage errors.

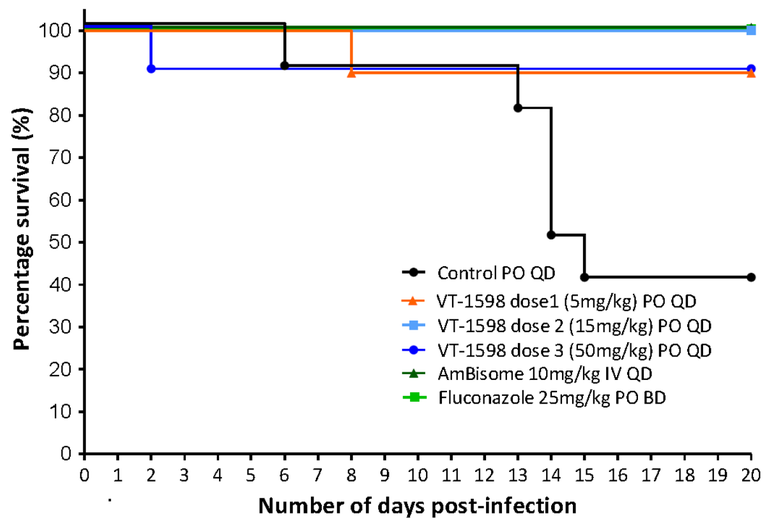

Figure 2. Brain fungal burden from end of a survival study in a murine model of cryptococcal meningitis.

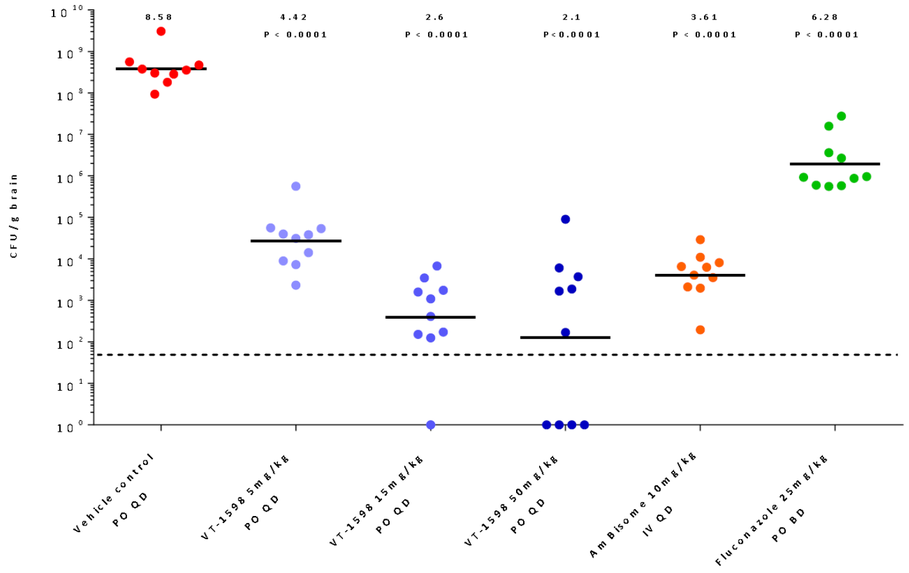

Table 3: Brain fungal burden and drug levels from plasma and brain tissue 6 days after last treatment in a murine survival model of cryptococcal meningitis.

Brain Fungal Burden vs. "Washed-Out" Drug Levels			
Treatment	**Brain Fungal Burden (CFU/g)**	**Drug Plasma Level (μg/ml)**	**Drug Brain Level (μg/ml)**
Vehicle	377,581,237	-	-
5 mg/kg VT-1598	26,564	0.027	<0.016
15 mg/kg VT-1598	398	0.038	<0.016
50 mg/kg VT-1598	127	0.10	<0.016
10 mg/kg AmBisome	4,084	0.22	<0.036
50 mg/kg/d FLU	1,910,889	0.017	<0.006

VT-1598 Conclusions

- Potent inhibition of *C. neoformans* and *C. gattii* growth *in vitro*
- Highly selective fungal CYP51 inhibitor versus critical human CYPs
- Doses as low as 5 mg/kg led to complete protection against mortality
- Mid and high doses resulted in profound 6 log_{10} brain fungal burden reductions
  - 4/10 samples in the high dose group had undetectable viable yeast
- *In vivo* antifungal activity sustained for 6 days after the last treatment
  - With low plasma levels and no detectable drug level in brain tissue
- VT-1598 is a promising drug candidate for the treatment of cryptococcal meningitis

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