Rational Design of the Potent and Selective Fungal CYP51 Inhibitor VT-1129

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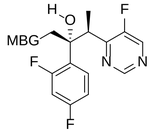
Introduction and Objective

- Cryptococcal meningitis (CM) is a fungal infection of the CNS predominantly affecting individuals with compromised immune systems including organ transplant, HIV, and cancer patients
- High mortality rates (up to 70%) among CM patients highlights the need for new therapeutic regimens to treat CM.
- Current fungal CYP51 inhibitors (e.g., fluconazole) are useful for CM consolidation and maintenance treatment following standard induction therapy with amphotericin B and 5-flucytosine; however, induction therapy with fluconazole monotherapy is associated with poor clinical outcomes.
- Furthermore, current CYP51 inhibitors potently inhibit a broad range of off-target human cytochrome P450 enzymes (CYPs) leading to safety liabilities (e.g., drug-drug interactions, liver and reproductive toxicities).
- We report rationally-designed, broad-spectrum antifungal agents that are highly selective for the target enzyme, fungal CYP51 (lanosterol demethylase), relative to human CYP enzymes (e.g., CYP3A4).
- Replacement of the triazole metal-binding group (MBG) with novel, less avid MBGs in concert with potency enhancing scaffold modifications generated a series of novel antifungal agents, including the oral agent VT-1129, with robust anti-Cryptococcal potency as well as a high degree of specificity for the fungal target relative to human CYP enzymes.

Results

- MBGs in 1st (1-imidazole) and 2nd (1-(1,2,4-triazole) generation fungal CYP51 inhibitors utilize high affinity MBG-heme-iron interaction to derive affinity.
- High affinity MBGs often carry undesired off-target inhibition of related human heme-iron containing proteins (e.g. CYP3A4).
- Exploration of a series of lower affinity MBGs (Table 1) on the voriconazole scaffold led to the identification of 1-tetrazole derivative **2**, which afforded the desired therapeutic index vs. CYP3A4.

- **Table 1**: MBG screen on voriconazole scaffold

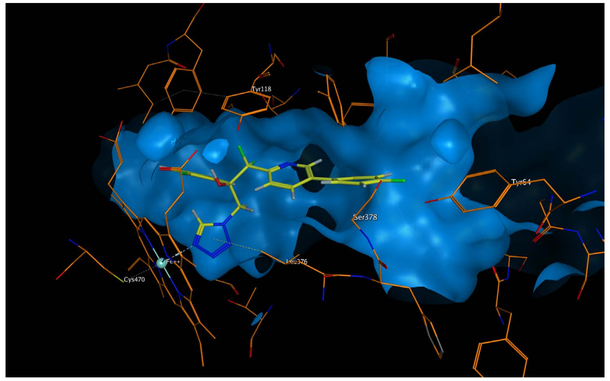


Compound	MBG	*C. albicans* MIC[a]	CYP3A4 IC_{50} [b]
1	1-(1,2,3-triazole)	> 16	8.0
2	1-tetrazole	1	32
3	4-(1,2,4)-triazole	> 16	51
4	1-imidazole	0.5	0.8
5	2-tetrazole	8	46
6 (Voriconazole)	1-(1,2,4-triazole)	0.06	13
Itraconazole	----	0.016	0.07
Posaconazole	----		0.05

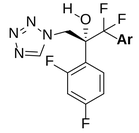
a. Minimum concentration that achieved 50% inhibition of fungal growth; MIC units in μg/mL. b. Inhibition of CYP3A4 measured in microsomes obtained from pooled human hepatocytes; IC_{50} units in μM.

- Homology modeling utilizing a *C. albicans*-CYP51 construct suggested electron-deficient 2,5-disubstituted pyridines would π-stack with Tyr-118 (Figure 1).

- **Figure 1**: Structural model of compound **11** binding to fungal-CYP51

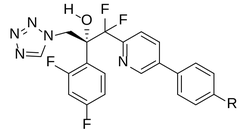


- **Table 2**: Scaffold modification with the 1-tetrazole MBG



Cmpd	Ar	*C. albicans* MIC	CYP3A4 IC_{50}
2	5-fluoropyrimidin-4-yl	1	32
7	pyridin-2-yl	0.25	136
8	5-chloropyridin-2-yl	0.25	74
9	5-(2,2,2-trifluoroethoxy)pyridine-2-yl	0.06	72
10	6-chloroquinolin-2-yl	≤ 0.001	18
11	5-(4-fluorophenyl)pyridine-2-yl	0.016	53
12	5-(4-cyanophenyl)pyridine-2-yl	≤ 0.016	16
13	5-(4-(trifluoromethyl)phenyl)pyridine-2-yl	≤ 0.016	> 60

- **Table 3**: Identification of VT-1129



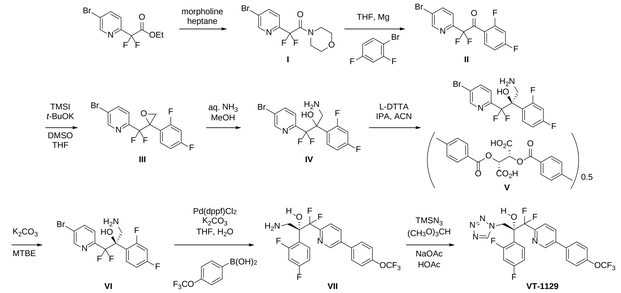
Cmpd	R	*C. albicans* MIC	CYP3A4 IC_{50}	Selectivity Index[a]
14	Cl	≤ 0.001	36	> 36,000
15	CF_3	≤ 0.001	54	> 54,000
VT-1129	OCF_3	≤ 0.001	79	>79,000
Itraconazole	----	0.016	0.07	4

a. *In vitro* selectivity calculated as CYP3A4 IC_{50}/*C. albicans* MIC.

- **Table 4**: VT-1129 is a weak inhibitor of human CYPs

IC_{50} (μM) against Human CYPs							
Agent	2C9	2C19	3A4 (midazolam)	3A4 (testoster)	17 OHlase	17 lyase	19
VT-1129	87	108	79	178	> 200	> 200	> 200
Fluconazole	34	13	32	6.4	> 200	> 200	17
Posaconazole	25	7.2	0.05	0.12	0.71	0.04	6.0

- **Scheme 1**: Synthesis of VT-1129



- **Table 5**: VT-1129 is a very potent inhibitor of Cryptococcal growth

MIC (μg/ml) at 50% inhibition of Growth		
Cryptococcus Species/Isolate	VT-1129	Fluconazole
C. neoformans/ATCC 24067	≤0.0019	0.25
C. neoformans/NCPF 8569	0.0156	2
C. neoformans/NCPF 8471	0.0078	2
C. neoformans/NCPF 8224	0.0156	1
C. gattii/NCPF 8578	0.0625	4
C. gattii/NCPF 8470	0.0156	2
C. gattii/NCPF 8204	0.0625	8

Conclusions

- Selection of a less avid MBG to provide superior selectivity followed by potency enhancing scaffold modifications led to the identification of the novel antifungal VT-1129.
- Detailed antifungal characterization of VT-1129 demonstrated robust activity against *Cryptococcus* spp.
- Clinically relevant oral doses of VT-1129 eradicated viable fungus in brain samples in a murine model of CM (Najvar et al., 2014 International Conference on Cryptococcus and Cryptococcosis).
- VT-1129 is currently in Phase 1 clinical trials in preparation for clinical antifungal studies in CM patients.