# Rationally-Designed Metalloenzyme Inhibitors Have Potent, Broad-Spectrum Antifungal Activity

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## Abstract

While the orally-active azoles such as voriconazole, posaconazole, and itraconazole are effective antifungal agents, they potently inhibit a broad range of off-target human cytochrome P450 enzymes (CYPs) leading to various safety issues (e.g. drug-drug interactions, liver toxicity). Herein, we describe rationally-designed, broad spectrum antifungal agents that are more selective for the target fungal enzyme, CYP51, than related human CYP enzymes such as CYP3A4, CYP2C9, CYP17, etc. Using Viamet's proprietary technology platform, the triazole metal-binding group (metallophile, MTP) found in current agents has been replaced with novel metal-binding groups in concert with the design of novel small molecule scaffolds. Several series of unique inhibitors, including an oral antifungal (VT-1161) currently in Phase I, have been identified. These series exhibit potent activity against a broad spectrum of yeasts, dermatophytes, and molds. The MIC values of series representatives are in general superior to approved agents. The data exemplified in the tables illustrate the potential utility of this technology to provide new treatments for a wide range of fungal infections.

## Materials and Methods

**Synthetic.** Voriconazole analogs were prepared in 5 steps from 2,4-difluoro-benzene and 4-Cl-5-F-6-ethyl-pyrimidine as published (WO 2007/085385). VT and VMT compounds were prepared in 5-7 steps from 2,5-dibromo-pyridine and ethyl bromodifluoroacetate as published (WO 2011/133875). All compounds were prepared at SAI Advantium, Inc. (R. Akula).

***In Silico* Enthalpies.** Using Spartan 2006 program package, Me-metallophile (Me-MTP) ligands were minimized using the MMFF-94 force field and optimized with the semi-empirical PM3 method. The CYP-51 Fe-porphyrin construct (Podust, *PNAS*, **2001**, *98*, 3068) was minimized (MMFF94) and then optimized using the PM3 semi-empirical method to obtain unligated structure. Me-MTPs were introduced and the energy was determined by a single point calculation. The Fe-porphyrin and Me-MTP were complexed with only the Me-MTP ligand free to move during optimization. Next, Me-MTPs were submitted for geometry optimization and enthalpies measured (K. Page, Research Triangle Institute).

***In Silico* CYP51 Modeling.** Compound dockings in *C. albicans* (*Ca*) or *A. fumigatus* (*Af*) proprietary homology models (Dr. S. Shaver, SR Consulting) were generated using Molecular Operating Environment (MOE; 2010.10; Chemical Computing Group Inc; www.chemcomp.com). Energy-minimized small molecules were docked using a heme iron-MTP pharmacophore-directed approach followed by relaxation and induced optimal fit in the binding pocket. Binding energies were then calculated and used to rank-order molecular targets for synthesis.

***In Vitro* MIC.** Minimum inhibitory concentrations (MIC) were determined under the CLSI guidelines M27-A3 and M38-A2 and were read at both 50% and 100% inhibition of fungal growth.

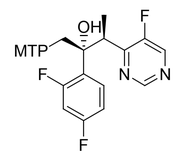
***In Vitro* Selectivity.** IC50 values for inhibition of human CYP enzymes were determined using either human microsomes or microsomes from yeast expressing the human recombinant enzyme. Reactions were analyzed for product using HPLC/MS/MS methods (K. Lewis, OpAns, LLC).

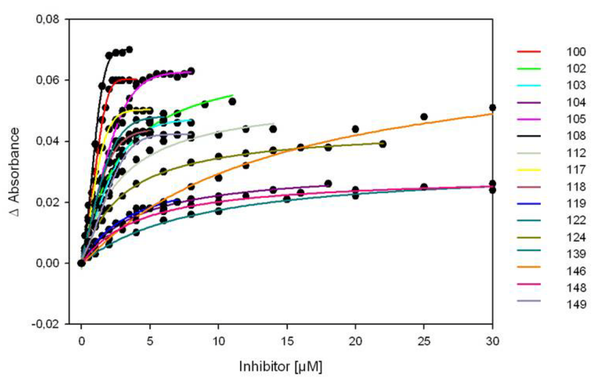
***In Vivo* Mouse Efficacy.** Female CD-1 mice (7 weeks,18-25 g) were made neutropenic with IP injections of cyclophosphamide (150 mg/kg) at 4 and 1 days before inoculation with *Ca* R303 or *Af* ATCC 204305 (tail vein). Compounds were administered orally 2h after infection on day 1 (*Ca* study), and once-daily on days 1–4 (*Af* study). Kidneys were collected from treated mice (*Ca*, day 2; *Af*, day 5), PBS added, and homogenized. CFU/kidney was determined from colony counts on SDA plates from serial dilutions of the homogenates (A. O'Leary, Ricerca Biosciences).

## Results

### Figure 1. Spectral Shift Binding Affinity of Voriconazole Analogs

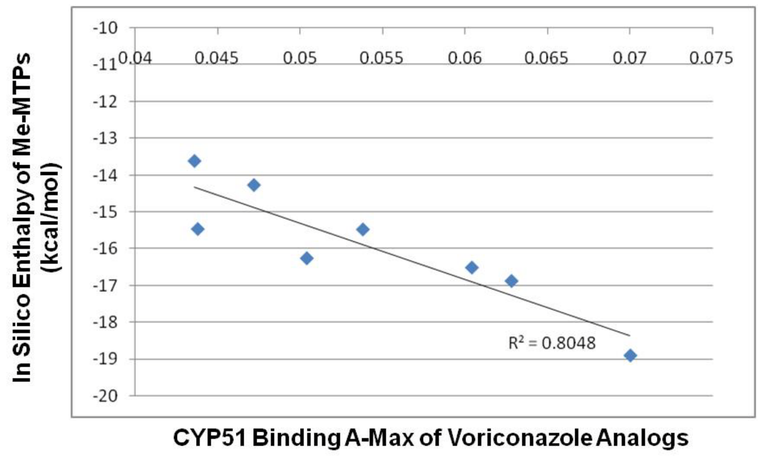
*C. albicans* CYP51 Type-II spectral shift binding curves for voriconazole analogs. Several alternative metallophiles (MTPs; the metal binding moiety) to 1-(1,2,4-triazole) bind heme-iron (compound **100** is voriconazole; for method, see Podust, *Structure*, **2004**, *12*, 1937).




### Figure 2. Voriconazole Analog Binding Affinities v. MTP Enthalpies

In silico enthalpies of Me-MTPs correlate with binding affinities of voriconazole analogs (see Fig. 1). Thus, an ample selection of low affinity MTPs, relative to 1-(1,2,4-triazole), were identified for design of new selective antifungals.




### Figure 3. Homology Model Docking & Synthesis Furnish Series 1

A preferred, low-affinity MTP was selected for further scaffold exploration (Fig. 2). To improve *C. albicans* (*Ca*) MIC potency, serial homology model dockings of select, novel scaffolds in a *Ca* construct and synthesis eventually provided potent compounds shown in Table 1 after several iterations (voriconazole docking standard., **1**; data not shown). Similarly, *A. fumigatus* (*Af*) homology model dockings and subsequent synthesis furnished compounds shown in Table 2 (posaconazole standard).

### Table 1. Yeast and Dermatophyte Activity Similar to Itra

| Agent | *C. albicans* MIC | *T. rubrum* MIC | CYP2C9 IC50 | CYP2C19 IC50 | CYP3A4 IC50 | CYP17 lyase IC50 | CYP17 hydroxylase IC50 |
|---|---|---|---|---|---|---|---|
| VT-1129 | ≤0.0001 | ≤0.0001 | 87 | 108 | 79 | >200 | >200 |
| VT-1161 | ≤0.0001 | ≤0.0001 | 99 | 72 | 65 | >200 | >100 |
| Itraconazole | 0.016 | 0.062 | >100 | 60 | 0.07 | 8.8 | >200 |

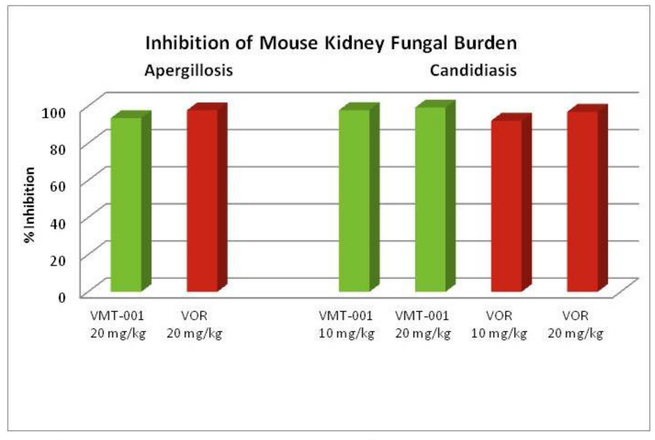
MIC values determined according to CLSI protocol. 50% inhibition for *Ca* and *Tr*. MIC units are μg/ml. IC50 units are μM.

### Table 2. Mold Activity Similar to Vori

| Agent | *A. fumigatus* MIC | *C. albicans* MIC | CYP2C9 IC50 | CYP2C19 IC50 | CYP3A4 IC50 | CYP17 lyase IC50 | CYP17 hydroxylase IC50 |
|---|---|---|---|---|---|---|---|
| VT-1148 | 1 | 0.0004 | 19 | 14 | 16 | >10 | >10 |
| VT-1546 | 6 | 0.008 | 70 | 92 | >200 | 31 | >100 |
| VMT-001 | 0.5 | 0.004 | 172 | 54 | >200 | 38 | >100 |
| Voriconazole | 0.5 | 0.016 | 10 | 10 | 13 | 79 | >200 |

MIC values determined according to CLSI protocol. 100% inhibition for *Af;* 50% inhibition for Ca. MIC units are μg/ml. IC50 units are μM.

### Figure 4. In Vivo Yeast and Mold Inhibitory Activity of VMT-001




Percent reduction in mouse kidney fungal CFUs compared to vehicle following inoculation with either *Af* lab strain ATCC 204305 or *Ca* clinical strain R303.

## Conclusions

- VT-1129 and VT-1161, designed using a *C. albicans* CYP51 homology model, are very potent inhibitors of yeast and dermatophyte growth *in vitro,* and are significantly more selective than marketed antifungal agents (e.g. itraconazole).
- VMT-001, designed using an *A. fumigatus* CYP51 homology model, is a potent inhibitor of *Aspergillus* growth *in vitro* and *in vivo*, and is significantly more selective than marketed antifungal agents (e.g. voriconazole).