

Antifungal drug development – two novel strategies

- Opportunistic fungal infections on the rise
- Efflux pumps critical to emerging fungal drug resistance
- Pump inhibitors and new class of antifungal agents identified

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The Opportunity

Opportunistic fungal infections are associated with increasing rates of mortality and morbidity, especially amongst immunocompromised patients. Existing treatment options are limited and fungal infections are now recognised for killing as many people, worldwide, as tuberculosis and malaria.

Current antifungal agents are hampered by their narrow spectrum activity, poor bioavailability, toxicity, interactions with other drugs, or by having fungistatic rather than fungicidal activity. Current treatments are ineffective against some fungal infections, such as aspergillosis, and emerging multiple drug resistance, particularly to azole class drugs, is now a serious issue in the treatment of fungal infections.

We have used two innovative strategies to address the urgent need to develop new antifungal agents:

- A novel pilot screen to identify compounds with either drug sensitising or antifungal activity has yielded a number of confirmed hits in both classes.
- Investigation of the mode of action of a potentially new class of antifungal agents, identified from an in-house drug discovery program.

The Technology

Fungal resistance to azole drugs, such as fluconazole, is often caused by increased expression of plasma membrane efflux pumps. Pump inhibition would enable drug resistant fungal infections to be effectively treated with existing azole class agents. Our team has developed a screening platform to identify new candidate compounds that block efflux pumps in fungi (Figure 1). Efflux of a fluorescent azole surrogate is used to quantify pump activity. The assay has been multiplexed such that viability is simultaneously assessed using a luminescent readout (Figure 2).

A pilot screen of 10,000 compounds, that included known drugs and diverse lead-like compounds, was conducted using yeast cells expressing a key fungal efflux pump.

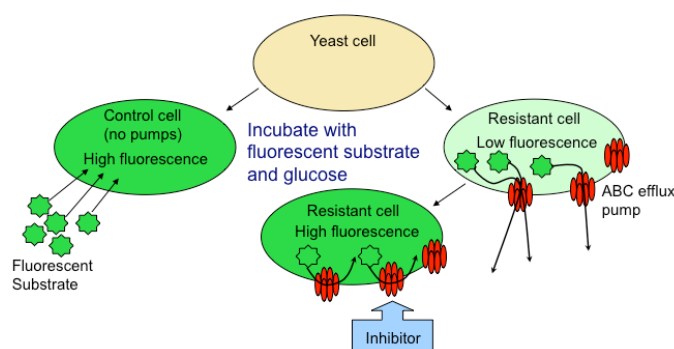


Figure 1. Efflux pump inhibitor assay design.

The screen identified novel pump inhibitors and antifungals, as well as agents with known antifungal activity.

The second arm of the antifungal program involves optimising novel drug candidates with demonstrated antifungal activity. The compounds inhibit the growth of human and animal pathogenic fungi, including *Aspergillus* and *Trichophyton*. *Trichophyton* causes fungal nail infections, which are estimated to affect 2-14% of the Western adult population. Investigation into the mechanism of action and molecular targets of these compounds is ongoing.

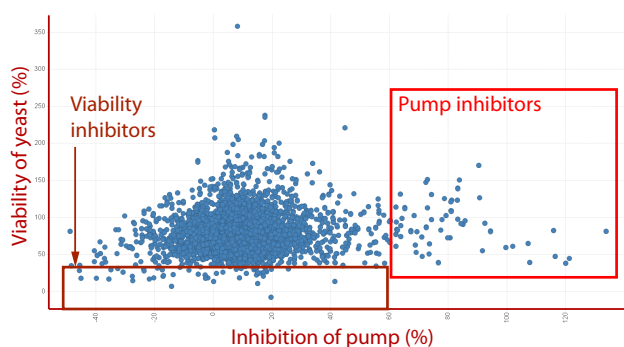


Figure 2. Results of the pilot screen for novel antifungal compounds and efflux pump inhibitors in yeast.

Applications

Our dual strategies will address key limitations of current treatment options.

- Identification and development of compounds capable of overcoming resistance to azole class drugs will result in a cost-effective means of treating drug-resistant fungal infections.
- Development of new compounds will add to the very limited existing antifungal armamentarium and facilitate early and rapid treatment of fungal infections.

The multiplexed efflux pump inhibitor and viability high-throughput screening assay is a flexible discovery tool that can easily be adapted to screen for inhibitors of other ABC transporters.

Opportunity for Partnership

WEHI and UoO are seeking a partner to co-invest in the development of compounds with antifungal activity, identified in the pilot screen, and to identify further promising compounds via a more substantive high-throughput screen of WEHI's compound libraries. WEHI has a successful track record in medicinal chemistry programs focused on hit-to-lead and lead optimisation.

Intellectual Property

The intellectual property regarding the fluorescence-based assays to measure efflux pump function is protected (WO/2003/018817, August 2002).

Compound structures have not been publically disclosed. An opportunity exists to generate novel composition of matter intellectual property.

Figure Legend

Figure 1: Efflux pump inhibitor assay design. Efflux pumps from a pathogenic fungus are over-expressed in a *Saccharomyces cerevisiae* host strain depleted of endogenous transporter proteins. Pump activity is monitored following addition of the fluorescent pump substrate R6G and compound. Pump inhibition results in intracellular retention of R6G.

Figure 2: Results of the pilot screen for novel antifungal compounds and efflux pump inhibitors in yeast. The multiplexed approach provides clear readouts for compounds affecting either pump activity or cell viability.

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