**Objectives**:

*Cryptococcus neoformans* is an opportunistic fungal pathogen that predominantly affects immunocompromised individuals, causing as many as 200,000 cases annually, with a staggering mortality rate of 75.8%. Current therapy of antifungal drugs has been successful for many years, however several issues arose over the recent years. These include the development of antifungal drug resistance in as many as 30% of *C. neoformans* clinical isolates; current therapies having considerable side effects on patients such as nausea and nephrotoxicity; and finally, drug supply chain issues with some of the producers ceasing their operations. Targeting fungal mitochondria holds promise due to their pivotal role in C*. neoformans*physiology, including virulence, defence against immune cells, and energy production. Here we describe the results of tests to evaluate our newly developed fungal specific inhibitor of *C. neoformans* respiration, Inz-Bob.

**Materials & Methods:**

The effects of Inz-Bob were assessed by growth rate, minimum inhibitory concentration (MIC), and viability. Titan cells production in the presence of Inz-Bob was assessed by microscopy and image analysis and mitochondrial respiration was assessed using high resolution respirometry. The induction of an alternative oxidase to support respiration upon Inz-Bob treatment was conducted using an anti-Aox1 antibody developed in our lab. Macrophage *C. neoformans* engulfment assays utilised murine macrophage cell lines and effects on virulence were assessed in the *Galleria mellonella* infection model. Inz-Bob cytotoxicity was assessed in macrophage, red blood cell and *Galleria mellonella* systems.

**Results**:

We have designed a new drug, Inz-bob, that targets the bc1 subunit of complex III of C. neoformans. Here we show that Inz-bob successfully inhibits *C. neoformans* growth and respiration at low concentrations. Inz-bob treatment also reduces viability and prevents the key virulence trait of Titan cell formation. Notably, it does not decrease the respiration of murine macrophages, does not exhibit haemolytic activity and is non-toxic in a *Galleria mellonella*model organism, confirming its fungal specificity. Crucially Inz-bob does not lead to upregulation of the alternative oxidase, Aox1, which has previously been shown to be essential in *C. neoformans*response to the electron transport chain inhibition and to the development of resistance to bc1 class inhibitors.

**Conclusions**:

Overall, the mitochondria of *C. neoformans*shows great promise as a target for antifungal therapy. Our data suggests that Inz-bob is a highly effective and non-toxic drug *in-vitro*. Our data warrants further research into the development and delivery of fungal specific inhibitors of respiration in *C. neoformans*.