The Triphenylethylenes, a Novel Class of Antifungals

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ABSTRACT **New antifungals are needed, particularly in the developing world, to treat life-threatening fungal infections, such as cryptococcosis. Drug repurposing is one strategy to identify new drug-like compounds, but it is often difficult to identify a mechanism of action. Here we discuss the outstanding effort by Butts et al. to identify calmodulin as an antifungal target of repurposed estrogen receptor antagonists [A. Butts, K. Koselny, Y. Chabrier-Roselló, C. P. Semighini, Y. C. S. Brown, et al., mBio 5(1): e00765-13, 2014, doi:10.1128/mBio.00765-13]. The authors show that these compounds bind to and directly inhibit fungal calmodulin and also reduce fungal burden in an animal disease model. These studies thus establish both the key preclinical efficacy and the antifungal mechanism of action, which will allow these compounds to progress toward development of novel antifungal therapies.**

We stood at the bedside of a tiny infant in the neonatal intensive care unit. Another member of our infectious disease team had just gotten off the phone with the microbiology lab: a yeast had grown from the blood culture.

Our little patient was sick but still holding his own. When I told the residents, "He has a one in four chance of dying from this infection," they did not believe me. Despite antifungal therapy, he passed away before rounds the next day.

Our patient highlights the urgent need for new, potent antifungal therapies. Here in the United States, our outstanding care of vulnerable populations, such as very low birth weight infants and bone marrow transplant recipients, means that these individuals no longer succumb to their primary diseases but live on with increased susceptibility to opportunistic pathogens, particularly fungi.

In resource-limited settings, the need for safe, inexpensive, oral antifungal therapies is even more pressing. According to the World Health Organization, there are now 35 million individuals living with HIV; of these, over 70% live in Sub-Saharan Africa, and only half receive antiretroviral therapy (1). As a result, opportunistic pathogens that are thankfully rare in the developed world are still both common and severe there. Critical among these is the central nervous system pathogen *Cryptococcus neoformans*, a ubiquitous environmental yeast, which causes 1 million cases of meningitis in immunocompromised hosts each year. Because the expensive intravenous therapies that comprise the standard of care are unavailable, mortality rates are high, and over half of these patients die.

New antifungal development has been slowed by several challenges, many of which are shared by efforts directed against other eukaryotic pathogens of the developing world, such as the malaria parasite. These obstacles are in large part scientific; it is inherently more difficult to identify compounds that selectively harm eukaryotic microbes, since so many essential cellular processes are indeed well conserved from yeasts to humans. In addition, new agents to treat cryptococcal meningitis must cross the blood-brain barrier, a challenge met by only a small minority of existing antimicrobials. However, the economic challenges to new antifungal development for the developing world are also substantial. There is little drive to invest significant research and development funds into new pharmaceuticals for resource-limited countries when the expected financial return on investment is negligible.

One strategy to reduce the considerable expense of anti-infective development is the concept of drug repurposing. Investigators screen for new activities present in compounds that are known to be pharmacologically active and, in many cases, are already approved for clinical use. Hits from these screens are, by definition, drug-like molecules that are typically excellent scaffolds for medicinal chemistry optimization, even if they are not immediately suitable for their new purpose. In addition, pharmaceutical companies often have panels of similar compounds from their own development efforts already synthesized and waiting on the shelf, and academic investigators may be able to "piggyback" on those previous research programs.

Drug repurposing can be an outstanding starting point for product development and may also illuminate novel biology. For example, a recent screen for compounds with *in vitro* activities against the diarrhea pathogen *Cryptosporidium parvum* identified antiparasitic activity in the widely used statin class of cholesterol-lowering drugs, thus revealing an unexpected dependence in this protozoan on host isoprenoid biosynthesis (2). However, drug repositioning screens are often far more difficult to interpret biologically, since the molecular mechanisms of action of many agents are obscure. Such was the case for the repurposing screen that identified unexpected antifungal activity in the estrogen receptor antagonist tamoxifen. While tamoxifen has chemical features that are ideal for a new antifungal treatment—it is orally bioavailable and accumulates in the brain and within lysosomes—the molecular mechanism of its antifungal activity was not clearly defined.

In an extensive and multidisciplinary effort, Butts et al. define the mechanisms of action of the estrogen receptor antagonist tamoxifen and related compounds (the triphenylethylenes) as new therapies against *Cryptococcus neoformans* (3). In this study, the authors report the key early preclinical efficacy studies, which demonstrate the promise of this new class of antifungals. A major

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limitation of the currently available oral anticryptococcal agent fluconazole is that this compound arrests the growth of *Cryptococcus* but does not kill the yeast directly. Importantly, the authors demonstrate that the triphenylethylenes not only are fungicidal in combination with fluconazole but also inhibit the intraphagocytic growth of the yeast and already show promise in a mouse model of central nervous system cryptococcal infection.

Previous studies on the triphenylethylenes had suggested that tamoxifen and related compounds might interfere with calcium homeostasis (4–6). This was a promising hypothesis, as the calcium-dependent serine-threonine phosphatase calcineurin is a well-validated target for antifungal development (reviewed in reference 7). Calcineurin inhibitors, such as the popular immunosuppressants cyclosporine (CsA) and tacrolimus (FK506), have potent activities against *C. neoformans*, which requires calcineurin for growth at elevated temperatures and therefore mammalian pathogenesis (8).

Using multiple lines of investigation, Butts et al. establish that the antifungal effects of tamoxifen and its analogs are mediated, at least in part, through direct inhibition of *C. neoformans* calmodulin (CnCAM1), a calcineurin activator (Fig. 1). CnCAM1 protein interacts directly with the triphenylethylenes, which inhibit CnCAM1-mediated calcineurin activation *in vitro*. Cellular overexpression of CnCAM1 decreased sensitivity to this group of compounds, also supporting the finding that CnCAM1 is a direct target *in vivo*. Consistently with these findings, the triphenylethylenes interfere with cellular functions downstream of calcineurin, which is known to be required for *C. neoformans* virulence. Genetic screening also revealed an apparent second, related target, calmodulin-like protein (CML1, CNAG_05655), whose deletion led to compound resistance.

The current study provides a strong foundation for future medicinal chemistry efforts to optimize the triphenylethylene scaffold in developing novel antifungal agents. Preliminary structure-activity relationships among the tamoxifen analogs indicate that potency against the CnCAM1 target is well correlated with antifungal activity. By starting with a compound class that is orally bioavailable, with intracellular activity and an optimal tissue distribution pattern, there is real hope that we may cut the time and cost of drug development and bring forward a new anticryptococcal agent for the developing world.

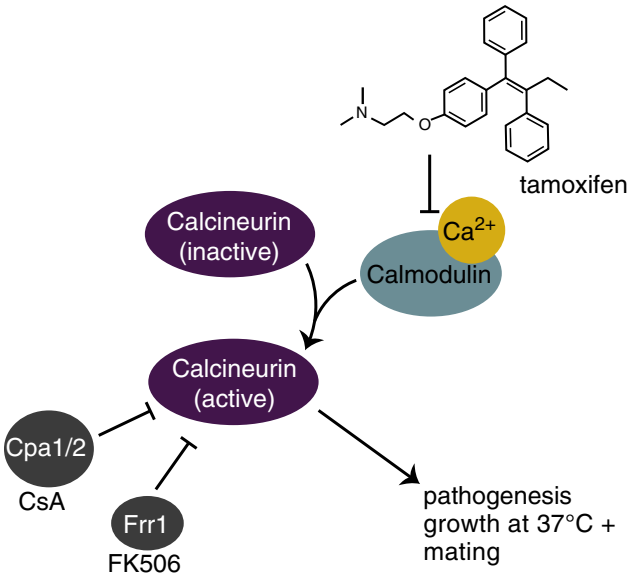

FIG 1 Calmodulin is an antifungal target of tamoxifen and other estrogen receptor antagonists. Calmodulin is an activator of the serine-threonine phosphatase calcineurin, which is required for virulence in the pathogenic fungus *Cryptococcus neoformans*. Calcineurin is the target of the commonly used immunosuppressants cyclosporine A (CsA) and tacrolimus (FK506), which also inhibit mammalian calcineurin in T cells. Tamoxifen interacts directly with calmodulin to interrupt calcineurin activation (3).

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