

TITLE: Screening of Pandemic Box library reveals the high activity of olorofim against *Sporothrix* pathogenic species

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ABSTRACT:

The increase in the prevalence and severity of fungal infections and the resistance to available antifungals highlight the imperative need for novel antifungal drugs and the exploration of new targets. High-content screening of libraries containing hundreds of drug-like compounds is a powerful strategy to search for new drug candidates. In this study, we screened the Pandemic Box library (Medicines for Malaria Venture [MMV], Switzerland) of 400 diverse drug-like molecules using as a model of pathogenic fungi the *Sporothrix* pathogenic species. The *in vitro* activity of 400 compounds was evaluated according to inhibition of *Sporothrix brasiliensis* ATCC MYA 4823 yeasts at concentrations of 1 μ M. The initial screen identified 24 compounds that inhibited *S. brasiliensis* growth by more than 80%. Most of these promising molecules were known antifungals, including olorofim (MMV 1782354). The effect of olorofim against *Sporothrix* spp. was not previously described. For this reason, we selected olorofim for further studies exploring their potential antifungal activity and targets. We evaluated the inhibitory and fungicidal activity of olorofim against *Sporothrix* yeasts, as well as biofilm inhibition and morphophysiological alterations by fluorimetry and electron microscopy analyses. Olorofim was able to inhibit and kill yeasts of *S. brasiliensis*, *Sporothrix schenckii* ATCC 32286, and *Sporothrix globosa* CBS 130104 at concentrations lower than itraconazole. Olorofim was also able to inhibit *Sporothrix* biofilms. No biofilm inhibition was found using itraconazole. Fluorimetry analyses revealed that olorofim induced DNA accumulation and changes in the cell wall composition, results that are consistent with pyrimidine synthesis block by dihydroorotate dehydrogenase inhibition. Scanning and transmission electron microscopy analyses also showed changes in cell wall structure after treatment with olorofim. In summary, our results indicate that olorofim is a promising new antifungal agent.

Keywords: antifungal development; dimorphic fungi; antifungal target.

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