Novel, multi-substituted β-lactone shows broad-spectrum antimicrobial activity

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Abstract

Pathogenic fungi continue to develop resistance to fungicidal/fungistatic compounds and pose a significant risk to immune compromised patients. Traditional fungicides are based on azole functional groups or complex polyenes and echinocandins all of which have encountered some resistance resulting in a shortage of new, effective treatments. While occurring in some known fungicidal agents, the β-lactone group has not been prominent.

Synthesized easily from a ketene heterodimer, a novel, small molecule β-lactone, (3S,4Z)-4-[1-(4-chlorophenyl)ethylidene]-3-methyloxetan-2-one) shows antifungal activity against *S. cerevisiae* and *C. albicans* and has previously shown antibacterial activity against Gram-positive *M. luteus* and Gram-negative *E. coli* K-12 in disk diffusion tests.

The broad-spectrum antimicrobial properties of this compound suggest that the β-lactone functional group is worth further consideration as a focal point in the development of new therapeutics.

Introduction

Fungal infections pose a considerable threat to those already weakened by illness or immune-compromised and effective treatment options are dwindling due to increasing resistance[1].

The compound studied here features the β-lactone group which is uncommon in known antimicrobials and unlike the prevalent β-lactam based compounds[2,3], no structure- specific evasion mechanisms to β-lactones have been identified.

A novel, multi-substituted β-lactone previously showed bactericidal properties against Gram-positive *Micrococcus luteus* and Gram-negative *Escherichia coli* K-12 and was evaluated against fungi in to evaluate any broad-spectrum behavior.

Saccharomyces cerevisiae (more commonly, "baker's yeast") and the clinically prevalent pathogenic *Candida albicans* were evaluated by disk diffusion.

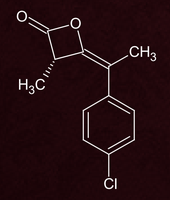

Figure 1. Compound structure of ((3S, 4Z)-4-[1-(4-chlorophenyl)ethylidene-3-methyloxetan-2-one)

Methods

Screening was performed by a disk diffusion assay. A fungal lawn was applied to YPD agar at ~ 1 x 10^6 CFU and sterile 6 mm paper disks treated with the compound at 512 μg were applied against a positive control of 100 U of nystatin. Samples were incubated for 24 hours; *S. cerevisiae* at 25 °C and *C. albicans* at 37 °C. Mechanism of action in *S. cerevisiae* was assessed by sorbitol assay in 1.00 ml of broth vs. 512 and 1,024 μg of compound.

Results

In addition to previously seen antibacterial behavior, the novel β-lactone showed efficacy in the inhibition of growth of *S. cerevisiae* as well as *C. albicans* (Figure 2), showing *ca*. 2.5 times the area of inhibition in the former against a similar number of CFU (Table 1).

Fungus	+ control area (mm^2)	β-lactone area (mm^2)	% of control
C. albicans	491	177	36
S. cerevisiae	140	126	90

Table 1. Area comparison of zones of inhibition

With a proposed minimum inhibitory concentration (MIC) of 512 μg/ml in *S. cerevisiae*, a sorbitol assay was performed to determine if the mechanism of action was targeting the cell wall. Sorbitol at a concentration of 0.8 M acts to stabilize the cell wall by osmotic pressure. When treated with MICx2, MIC, and control, growth was arrested at MIC suggesting the β-lactone is not targeting the cell wall and may be a cell membrane agent.

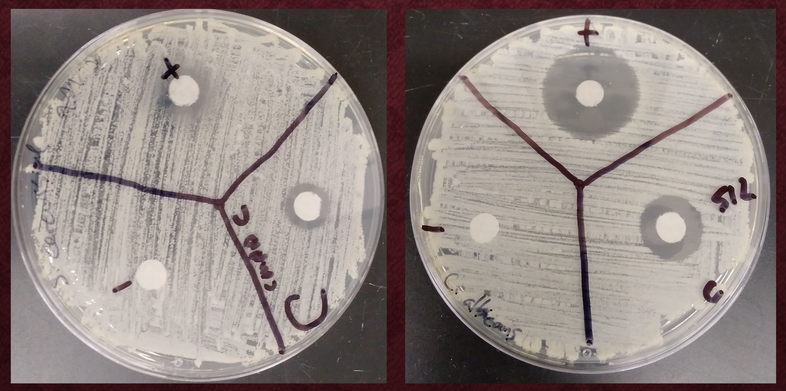

Figure 2. Disk diffusion assay with β-lactone, *S* enantiomer. Left) *S. cerevisiae*, Right) *C. albicans*. Clockwise from top: positive control, β-lactone, negative control.

The β-lactone features a single chiral center at the α carbon of the lactone ring and the samples were supplied as pure enantiomers. While the *S* enantiomer has been studied almost exclusively, the *R* enantiomer shows similar physical properties and bioactivity against all bacteria and fungi studied.

Discussion

The novel β-lactone has shown to be effective in minimizing the growth of both bacteria and fungi, including the more pathogenic *C. albicans* which is a leading cause of fatal fungal infections and has shown resistance to the major classes of current antifungals[5,6]. While a highly divergent strain genetically, it is hoped that such efficacy would also found against the latest drug-resistant fungal threat, *C. auris*[7].

Although the mechanism of action and/or target in both families of microbes remains unknown, experiments suggest that the compound is not acting against the cell wall in fungi. To further assess this hypothesis, a binding study of the compound with ergosterol, a major fungal cell wall component, is planned.

Whatever the site of action may be, both the *R* and *S* enantiomers act as growth inhibitors across the microbes studied and demonstrate that the chirality of the compound is not a significant factor.

These studies form the foundation of future work to determine a mechanism of action, toxicological data and compound stability. The data obtained here suggests that a compound featuring the β-lactone group may hold some promise in defeating microbial resistance .

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