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Synthesis and Evaluation of Novel Antifungal Agents Targeted to the Plasma Membrane H+-ATPase

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SUMMARY

A library of dienones were synthesized as candidate antifungal agents. These compounds were screened against *Saccharomyces cerevisiae* and *Candida albicans* using macro-broth susceptibility assay. The dienones exhibited a broad range of inhibition against *S. cerevisiae* (0.2-99%) and *C. albicans* (0-99%). 3,5-bis(*p*-trifluoromethylbenzylidene)-1-methyl-piperidine-4-one was identified as the most potent inhibitor of *S. cerevisiae*. Whereas, the most promising inhibitor of *C. albicans* was 2,6-bis(pyridine-3ylmethylene)cyclohexan-1-one.

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INTRODUCTION

Fungal infections now contribute more significantly to microbe-related morbidity and mortality due to limited number of available antifungals. (Shreaz, *et al* 2013). In recent years only echinocandins have been introduced as a new class of antifungals. Drug resistance, adverse side effects and limited modes of drug delivery has led to an increased interest in identifying novel drug targets within the mycota. The fungal plasma membrane (PM) H^+-ATPase is a vital enzyme for the growth of fungi and it maintains an electrochemical proton gradient required for the uptake of nutrients (Manavathu, *et al* 1999). The PM H^+-ATPase has been shown to be inhibited by sulphinimides (eg. omeprazole) (Monk, *et al* 1995), ebselen and *a,β*-unsaturated carbonyls such as curcumin (Neelofar, *et al* 2011). These findings prompted us to synthesize a dienone library similar to curcumin and to investigate their antifungal activities.

MATERIALS AND METHODS

Using the Claisen-Schmidt condensation a dienone library was synthesized between N-methyl-piperidin-4-one/cycloalkanones with various substituted aldehydes. These compounds were characterized by LCMS, IR, ^1H and ^{13}C NMR and screened for their antifungal activity against *S. cerevisiae* and *C. albicans* using a macro-broth dilution susceptibility assay. Test compounds and controls were incubated at 32°C, 100 rpm. Optical density was measured at 600nm after 24 hours of incubation.

RESULTS AND DISCUSSION

The inhibitory activity of the dienones were analysed from the dose response curves. EC50, area under the curve (AUC), symmetry of the curve & slope values were obtained from the dose response curves fitted to 4 and 5 parameter equations. Representative dose-

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response curves of the most potent compounds with *S. cerevisiae* as a target cell (Fig. 1).

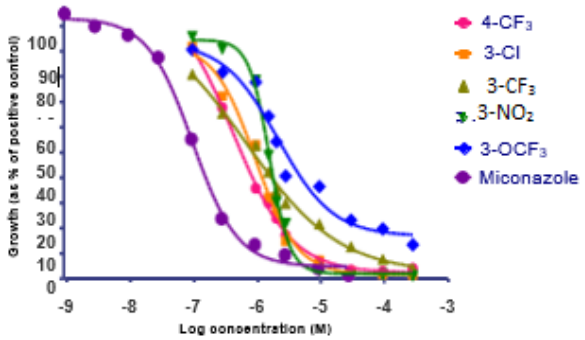

Fig. 1. *Representative dose response curves of the most potent compounds against S. cerevisiae.*

The pyridyl derivatives of N-methyl-piperidone, cyclopentanone and cyclohexanone showed good inhibitory activity against *S. cerevisiae* with EC_{50} ranged from 5.5 – 20.3 µM. In addition, the benzylidene derivatives of N-methyl-piperidones depicted a wide range of inhibition against *S. cerevisiae* with EC_{50} ranged from 0.6 – 337 µM, depending on the positioning of electron withdrawing or electron donating groups present on the benzene ring. The pyridyl derivatives of cyclopentanone and cyclohexanone were effective inhibitors of *C. albicans*. However, the thienylidene derivatives were not effective inhibitors of either *S. cerevisiae* or *C. albicans*.

The table 1 below reveals the inhibitory activity of the most potent compounds against *S. cerevisiae* and *C. albicans*. Miconazole was employed as positive control in all tests.

Table 1. *In vitro antifungal testing results of the dienones against S. cerevisiae and C. albicans.*

Most potent compounds	***S. Cerevisiae* EC_{50} (µM) Mean ± SD, n=6**	***C. albicans* EC_{50} (µM) Mean ± SD, n=4**
3,5-bis((E)-3-trifluoromethylbenzylidene)-1-methylpiperidin-4-one	1.53 ± 0.73	525.00 ± 33.94
3,5-bis((E)-4-trifluoromethylbenzylidene)-1-methylpiperidin-4-one	0.62 ± 0.26[a]	473.50 ± 7.78
3,5-bis((E)-3-trifluoromethyoxybenzylidene)-1-methylpiperidin-4-one	2.83 ± 0.96	548.50 ± 115.26
3,5-bis((E)-3-chlorobenzylidene)-1-methylpiperidin-4-one	1.21 ± 1.29	95.82 ± 15.82
3,5-bis((E)-3-nitrobenzylidene)-1-methylpiperidin-4-one	1.87 ± 0.60	666.00 ± 70.71
2,6-bis(pyridin-3-ylmethylene) cyclohexan-1-one	5.57 ± 2.47	57.57 ± 5.78
2,6-bis(pyridin-4-ylmethylene) cyclohexan-1-one	6.25 ± 1.49	50.66 ± 3.50[b]
2,5-bis(pyridin-4-ylmethylene) cyclopentan-1-one	8.46 ± 0.68	68.48 ± 3.27
Miconazole	0.12 ± 0.01	0.04 ± 0.02

[a] most potent against *S. cerevisiae*, [b] most potent against *C. albicans*.

CONCLUSIONS

3,5-bis((E)-(4-trifluoromethyl-benzylidene)-1-methyl-piperidin-4-one was the most potent inhibitor of *S. cerevisiae* (EC_{50} = 0.62 µM), whereas 2,6-bis-[(E)-(4-pyridyl)methylidene] cyclohexanone showed the highest inhibitory activity against *C. albicans* (EC_{50} = 50 µM). Although not as effective as the control azole antifungal. These results offer an encouraging framework that could lead to the discovery of novel antifungal agents.

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