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Screening of compounds for inhibition of Sporothix spp. growth

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Troubleshooting

Screening of compounds for inhibition of Sporothrix spp. growth

1. Stock solutions of compounds in dimethyl sulfoxide (DMSO) at 1 mM were diluted in RPMI 1640 medium[1] supplemented with 2% glucose and buffered to pH 7.2, with 0.165 M MOPS (from here on referred to as "supplemented RPMI") to obtain concentrations of 2 µM using sterile microtubes;

2. Aliquots of 100 µl of each compound were added in four wells of a flat-bottom 96-well microplate (KASVI, Brazil);

3. A standardized suspension of *Sporothrix* yeasts was adjusted using a Neubauer chamber, prepared in supplemented RPMI, and 100 µl containing 2×10^5 CFU/ml was added in microplates containing compounds;

4. The final concentration of compounds was 1 µM, while the final concentration of cells was 1×10^5 CFU/ml;

5. The following controls were included in the experiment: (i) antifungal control containing 1 µM itraconazole[1], (ii) diluent control containing 0.1% DMSO in supplemented RPMI, (iii) negative control containing only supplemented RPMI, and (iv) positive control with Sporothrix cells growing in supplemented RPMI without compounds (all in quadruplicate);

6. Samples were incubated for 48 h, at 35 ºC, in a 5% CO_2 atmosphere;

7. Fungal growth was analyzed by visual inspection in an inverted light microscope (Axiovert 100, ZEISS Company, Germany);

8. After visual inspection, samples were homogenized and the optical density was quantified by spectrophotometric readings at 492 nm, in a microtiter plate reader (EMax Plus, Molecular Devices, USA);

9. The absorbance value for each well was subtracted from the mean value for the negative control;

10. Inhibition of fungal growth (I) relative to positive controls was calculated according to the following equation: I = 100 – (A x 100/C), where A is the absorbance of treated wells, and C is the absorbance of positive controls;

11. Inhibitions of more than 80% were defined as the cutoff, corresponding to clearly visible preventions of growth when samples were analyzed by visual inspection.

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