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🌐 Screening of compounds for inhibition of *Sporothrix* spp. growth

PLOS Oneluanaborba¹¹Universidade Federal do Rio de Janeiro

L. P. Borba-Santos

luanaborba

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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 medium¹ 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 μ M using sterile microtubes;
2. Aliquots of 100 μ l 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 μ l containing 2×10^5 CFU/ml was added in microplates containing compounds;
4. The final concentration of compounds was 1 μ M, 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 μ M itraconazole¹, (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 °C, in a 5% CO₂ 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: $I = 100 - (A \times 100/C)$, 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.

¹Sigma Chemical Co., USA.