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Determination of minimum inhibitory concentration values (MICs) against Sporothrix brasiliensis and Sporothrix schenckii V.2

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Determination of minimum inhibitory concentration values (MICs) against *Sporothrix brasiliensis* and *Sporothrix schenckii*

The minimum inhibitory concentration (MIC) of compounds was determined using the broth microdilution technique described by the CLSI[1] with modifications.

1. Stock solutions of compounds in dimethyl sulfoxide (DMSO) at 1 mM were diluted in RPMI 1640 medium[2] supplemented with 2% glucose[3] and buffered to pH 7.2, with 0.165 M MOPS (from here on referred to as “supplemented RPMI”) to obtain solutions 4-fold concentrated using sterile microtubes;

2. Itraconazole[2] (reference antifungal) was also included in the experiment;

3. Aliquots of 100 µl of compounds were added in two wells of the first column of a flat-bottom 96-well microplate (KASVI, Brazil);

4. Aliquots of 100 µl of supplemented RPMI were added in all microplate wells (including wells containing compounds) using a multichannel pipette and a serial 2-fold dilution was performed to the tenth column;

5. The eleventh column corresponded to the positive controls (containing 100 µl supplemented RPMI without compounds) and the

twelfth to the negative controls (containing 200 µl supplemented RPMI);

6. A standardized suspension of *Sporothrix* yeasts was adjusted using a Neubauer chamber, prepared in supplemented RPMI, and 100 µl containing 2 x 10^5 CFU/ml was added in microplates containing compounds (except in the twelfth column);

7. Ten different concentrations of compounds were tested and their final concentration ranged from 0.002 to 1 µM, while the final concentration of yeasts were 1 x 10^5 CFU/ml[4];

8. Samples were incubated at 35 °C for 48 h, in a 5 % CO_2 atmosphere[5];

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

10. 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);

11. The absorbance value for each well was subtracted from the mean value for the negative controls;

12. 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;

13. Concentrations that inhibit 50% and 80% of fungal growth (IC_{50} and IC_{80}, respectively) were estimated.

[1]CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 4th ed. CLSI standard M27.Wayne, PA: Clinical and Laboratory Standards Institute; 2017.

[2]Sigma Chemical Co., USA.

[3] The RPMI medium was supplemented with 2% glucose to improve the growth of *Sporothrix* yeasts.

[4] The inoculum of 1x10^5 CFU/ml was used due to the slow growth of *Sporothrix* yeasts.

[5] Microplates were incubated in a 5% CO_2 atmosphere to maintain the culture in the yeast form.

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