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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 CLSI¹ with modifications.

1. Stock solutions of compounds in dimethyl sulfoxide (DMSO) at 1 mM were diluted in RPMI 1640 medium² supplemented with 2% glucose³ 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. Itraconazole² (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 2×10^5 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 1×10^5 CFU/ml⁴;
8. Samples were incubated at 35 °C for 48 h, in a 5 % CO₂ atmosphere⁵;
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: $I = 100 - (A \times 100/C)$, 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 (IC₅₀ and IC₈₀, respectively) were estimated.

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

²Sigma Chemical Co., USA.

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

⁴ The inoculum of 1×10⁵ CFU/ml was used due to the slow growth of *Sporothrix* yeasts.

⁵ Microplates were incubated in a 5% CO₂ atmosphere to maintain the culture in the yeast form.