INHIBITORY ACTIVITY OF A SYNTHETIC CHALCONE DERIVATIVE ON STAPHYLOCOCCUS AUREUS BIOFILM

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Staphylococcus aureus is a gram-positive, pathogenic, and opportunistic bacterium with a high capacity for biofilm formation, commonly associated with serious nosocomial infections. Part of its pathogenicity is associated with this highly organized structure, consisting of an extracellular matrix formed by polysaccharides and proteins, which provides bacterial cells with protection against the penetration of conventional antibiotics. Therefore, the search for new antimicrobial agents capable of interfering with biofilm formation has received increasing attention due to the threat to public health and infection control. This study evaluated the potential of a synthetic derivative of hydroxylated chalcone in inhibiting the formation of *Staphylococcus aureus* (ATCC 25178) biofilm. For the evaluation of biofilm inhibition, the bacterial strain cultivated overnight at 37°C was suspended in sterile saline (0.9%) until it reached a turbidity equivalent to 0.5 on the McFarland scale and subsequently diluted in Tryptic Soy Broth (TSB) (10^8 CFU/mL) supplemented with 1% (v/v) glucose. Next, 95 µL of the resulting suspension were inoculated in duplicate (n=2) into 96-well flat-bottom microtiter microplates. The synthetic compound tested was previously dissolved in 100% ethyl alcohol, generating a stock solution of 10 mg/mL. This compound was added to the previously inoculated wells at a concentration of 500 µg/mL (5 µL), and the plates were incubated at 37°C for 18-24 h. After this period, the contents of the wells were removed and washed three times with sterile saline. After drying, a safranin solution (0.2%) was added to the wells, and the plates were incubated for 40 minutes at room temperature. After incubation, the dye was removed, and the wells were washed twice with sterile saline to remove any unadhered residue. Next, the dye incorporated into the adherent cells was solubilized using 95% (v/v) ethyl alcohol. Absorbance was measured at 530 nm using a microplate reader. As a reference, the biofilm of the culture was evaluated in the presence of the compound\'s dilution solvent; the antibiotic tetracycline was used as a positive control for biofilm inhibition, and the culture medium without inoculum was evaluated as a negative control. The minimum concentration required to inhibit ≥ 80% of *S. aureus* biofilm formation (MBIC80) was determined using the broth microdilution technique. Each reaction received 100 µL as the final volume, containing the inoculum (according to the standardization) and different concentrations of the compound, ranging from 500 µg/mL to 7.81 µg/mL in a serial dilution. The results demonstrated that the compound exhibited inhibitory activity on biofilm formation, with a 95% reduction at a concentration of 500 µg/mL compared to the positive control. The minimum inhibitory concentration of biofilm (MBIC80) was 125 µg/mL, showing greater potency than the drug tetracycline. Preliminary analyses revealed that this antibiofilm capacity may be related to structural characteristics of the molecule, such as an α,β-unsaturated system in the chalcone rings and the addition of hydroxyl groups to the aromatic ring, which are crucial for pharmacological

interactions. Thus, the compound demonstrated potential as a biofilm inhibitor of *S. aureus*, highlighting its capacity as a promising new antimicrobial agent in the fight against infections.

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