TITLE: ANTI-PERSISTENCE ACTIVITY OF SYNTHETIC *N,O*-ACETALS DERIVED FROM 2-AMINO-1,4-NAPHTHOQUINONES AGAINST VANCOMYCIN-REFRACTORY CELLS OF METHICILLIN-RESISTANT *Staphylococcus aureus*.

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ABSTRACT:

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important agent of recalcitrant infections that are attributed to biofilm and consequent emergence of cells that resist to antimicrobials. Persisters are a type of refractory cells that do not present genetic differences in relation to their susceptible relatives but show a slow-growing, latent metabolic state and are able to tolerate high concentrations of bactericidal antibiotics. After the killing of normal-growing cells, persisters, which survive antibiotic treatment, can regrow upon treatment cessation, resulting in infection relapse. Since conventional antibiotics are poorly effective against infections caused by persisters, the development of novel drugs with anti-bacterial and anti-persistence activity is pressingly needed. In previous studies, we showed that synthetic *N,O*-acetals derived from 2-amino-1,4-naphthoquinones have promising antimicrobial and anti-biofilm activity against MRSA. The aim of this study was to evaluate the action of three *N,O*-acetal naphthoquinone derivatives (7a, 7b, 7c) on killing MRSA cells refractory to vancomycin. Refractory cells were induced by culturing MRSA cells using high concentration of bacterial cells (~109 CFU/ml) on the TSA surface containing 8 µg/mL vancomycin (24 h at 37˚C). Essays using vancomycin alone was performed to control the induction of refractory cells. For testing the effect of the derivatives, we used vancomycin (8 µg/mL) with different concentrations of 7a (256 µg/mL, 128 µg/mL, 64 µg/mL and 32 µg/mL), 7b (128 µg/mL, 64 µg/mL, 32 µg/mL and 16 µg/mL) and 7c (64 µg/mL, 32 µg/mL, 16 µg/mL, 8 µg/mL). Bacterial growth was determined by removing refractory cells from the plates and determining UFC/ml. All naphthoquinone derivatives were very effective to inhibit MRSA refractory cells. Complete eradication was verified using 256 µg/mL of 7a (p = 0.002) and 128 µg/mL of 7b (p = 0.002), while 64 µg/ml of 7C showed 90% growth reduction (p = 0.01). Taken all together, our results showed that the naphthoquinones derivatives showed a promising anti-persistence effect against *in vitro* generated refractory cells, which might be of potential interest for the design of new drugs for the treatment of recalcitrant infections caused by MRSA.

Keywords: Refractory cells; MRSA; naftoquinone derivatives; vancomycin.

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