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In silico and in vitro inhibition of quorum sensing and biofilm formation in Chromobacterium violaceum using semi synthetic compounds

Tonkin, Madison

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ABSTRACT

Introduction

Over the past decades, the number of emerging drug resistant bacteria has increased at an alarming rate. Bacteria develop drug resistance through a variety of mechanisms with the prime contributor being the misuse and overuse of antibiotics. Adding to this, the development of new antibiotics with novel drug targets has decreased. Recently, researchers have identified biofilm and quorum sensing as emerging drug targets. Both biofilm and quorum sensing are inter-related processes and provide variety of opportunities for development of novel anti-bacterial drugs with different mechanisms of action. In this study, we used five novel imidazole derivatives (IMA-1 – IMA-5) to inhibit quorum sensing and biofilm formation in *Chromobacterium violaceum* using a variety of *in silico* and *in vitro* techniques.

Materials and Methods

In silico molecular docking was first performed using Maestro software to determine the binding scores of the compounds and interactions created when the compounds bind to target protein, CviR. Following molecular docking, molecular dynamic simulations were performed using Amber18 software to determine the stability, flexibility and solubility of the complexes formed with CviR and the compounds.

In vitro anti-bacterial activity was determined by calculating MIC and MBC, following the CLSI standard guidelines. To determine qualitative anti-quorum sensing activity of the test compounds, agar plate assay was employed. To further quantify anti-quorum sensing potential of imidazole derivatives, the percentage of violacein pigment was measured spectrophotometrically. To study the effect of imidazole derivatives on biofilm formation, standard crystal violet staining assay was performed on treated and untreated *C. violaceum*. Furthermore, the ability of the test compounds to inhibit quorum sensing genes was determined by performing RT-qPCR to calculate relative fold gene expression.

Results

Results from *in silico* molecular docking indicated that all the imidazole derivatives interacted with important amino acids in the binding pocket. The interactions formed between compounds and CviR included pi-pi stacking, salt bridge formation and hydrogen bond generation. Despite all the derivatives showed high binding energies (ΔG_{bind}), IMA-1 had the highest ΔG_{bind} at -

34.44 kcal/mol with a binding score of -9.23 kcal/mol and IMA-5 had the lowest ΔG_{bind} at -30.74 kcal/mol with a binding score of -6.97 kcal/mol. Molecular dynamic simulation results showed that all compounds formed stable complexes with the target protein, CviR. Results also indicated that all complexes do not disrupt the natural manner in which the natural CviR-HS-10 complex behaves in hydrophobic or hydrophilic environments and this indicates that the compounds do not change the natural state of the CviR once in complex.

In vitro susceptibility results indicated that all the compounds had anti-bacterial activity against *C. violaceum* with IMA-1 as the most effective derivative with an MIC and MBC values of 0.0488 and 0.0977 µg/ml, respectively. Anti-quorum sensing results indicated that IMA-1 was the most effective and IMA-5 showed the least promising anti-quorum sensing. The derivatives were tested to determine the ability to inhibit biofilm formation and it was found that IMA-1 is effective at disrupting mature biofilm. Results from gene studies revealed that treatment with all the derivatives were able to significantly reduce the expression of *CviI* gene, indicating that all the derivatives were able to disrupt quorum sensing at a gene level. Genes from the *VioA, VioB, VioC, VioD* and *VioE* operon were also tested, and it was found that treatment with all the compounds significantly reduce the expression of *VioB* and *VioD*. The compounds also disrupt the expression of *VioA* and *VioE* but not to the same extent.

Conclusion

This study targeted quorum sensing and biofilm formation using five newly synthesised imidazole compounds. Various *in silico* and *in vitro* studies revealed that the compounds caused inhibition of growth at higher concentrations and disrupted quorum sensing at lower concentrations. The results from the *in vitro* studies agree with the results of *in silico* studies in that the same trend was found where IMA-1 was seen as the most effective compound and IMA-5 as the least effective. This study provides data to support the novel ideas as to how the bacterial drug resistance could be evaded by developing molecules that could target quorum sensing and biofilm formation.