Cyclic dimeric guanosine monophosphate (c-di-GMP) is a common bacterial second messenger that regulates cellular processes in bacteria. High concentrations of c-di-GMP usually imply in biofilms formation. Bacterial communities are highly resistant to treatment with antibiotics and represent the predominant phenotype in most chronic infections. The c-di-GMP is synthesized from two GTP molecules by enzymes diguanilate cyclases (DGC) belonging to GGDEF family, which are potential targets for developing new therapies that interfere with the process of biofilm formation. Thus, the objective of this study relies on in silico and in vitro screening of DGC enzymes inhibitors. Hence, it was performed in the Drug Bank database a virtual screening campaign based on substrate and enzyme structures. Openeye® package was used and ligand (LBVS) and target (TBVS) based virtual screening methods were employed. The substrate GTP was used as template for similarity search and PleD enzyme (PDB code 2V0N) for molecular docking. A consensual analysis allowed the selection of 30 potential ligand for in vitro assays. The DGC YdeH from Escherichia coli has been employed in this study, the YdeH gene was amplified from genomic DNA and cloned into pET-SUMO vector. High levels of expression were obtained using E. coli Rosetta strain and the YdeH enzyme was successfully purified using IMAC and size exclusion chromatographies. Kinetic studies using the EnzCheck kit® were performed in order to establish a full characterization the YdeH enzyme. It is expected that analysis of interactions between selected compounds and DGCs allow understanding the molecular mechanisms involved in signaling by c-di-GMP.

Word Keys: YdeH, virtual screening, in vitro assay
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