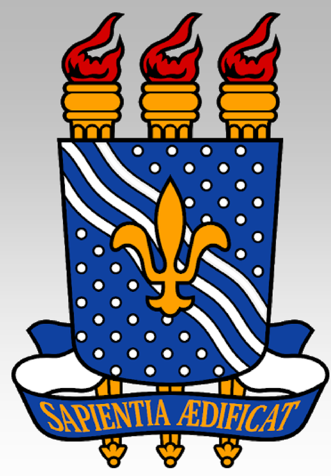

Virtual Screening Suggests Affinity Between Essential *C. Ulcerans* Proteins and Inedited Synthetic Derivatives of Tetraisoquinoline Alkaloids

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INTRODUCTION

Corynebacterium ulcerans is an aerobic, Gram-positive bacteria and one of the three species of the genus *Corynebacterium* that causes diphtheria. However, in contrast with the other causative agents, *C.ulcerans* is able to infect non-human hosts, and therefore is able to transmit diphtheria by zoonotic means. As such, *C.ulcerans* has a larger reservoir than the other causative agents, which consists of both humans and animals. In the last few years, isolated cases of diphtheria due to *C. ulcerans* have increased even in immunized nations, which has prompted the infection to be considered reemergent (KÖNIG et al, 2014). This highlights the importance to seek new drugs and treatments that are more specific and effective in order to better treat this infection. In a previous work, using the interolog mapping method, we generated the interactome and identified the conserved hub proteins for 10 strains of *C. ulcerans*, whose validation in the Database of Essential Genes (DEG), COG classification and analysis in GO, confirmed the essentiality of 457 proteins, which are potential pharmacological targets. In this work, we selected the hub proteins that had low levels of similarity with the host proteins, used homology modelling to develop viable 3D structures, identified potential molecular pockets and used molecular docking to predict the potential biding affinity between the identified hub proteins and a private library of 42 inedited tetrahydroisoquinoline alkaloid derivatives, in order to select the best candidates for synthesis and gain insight in the potential structural mechanisms that might occur between molecule and protein.

MATERIALS & METHODS

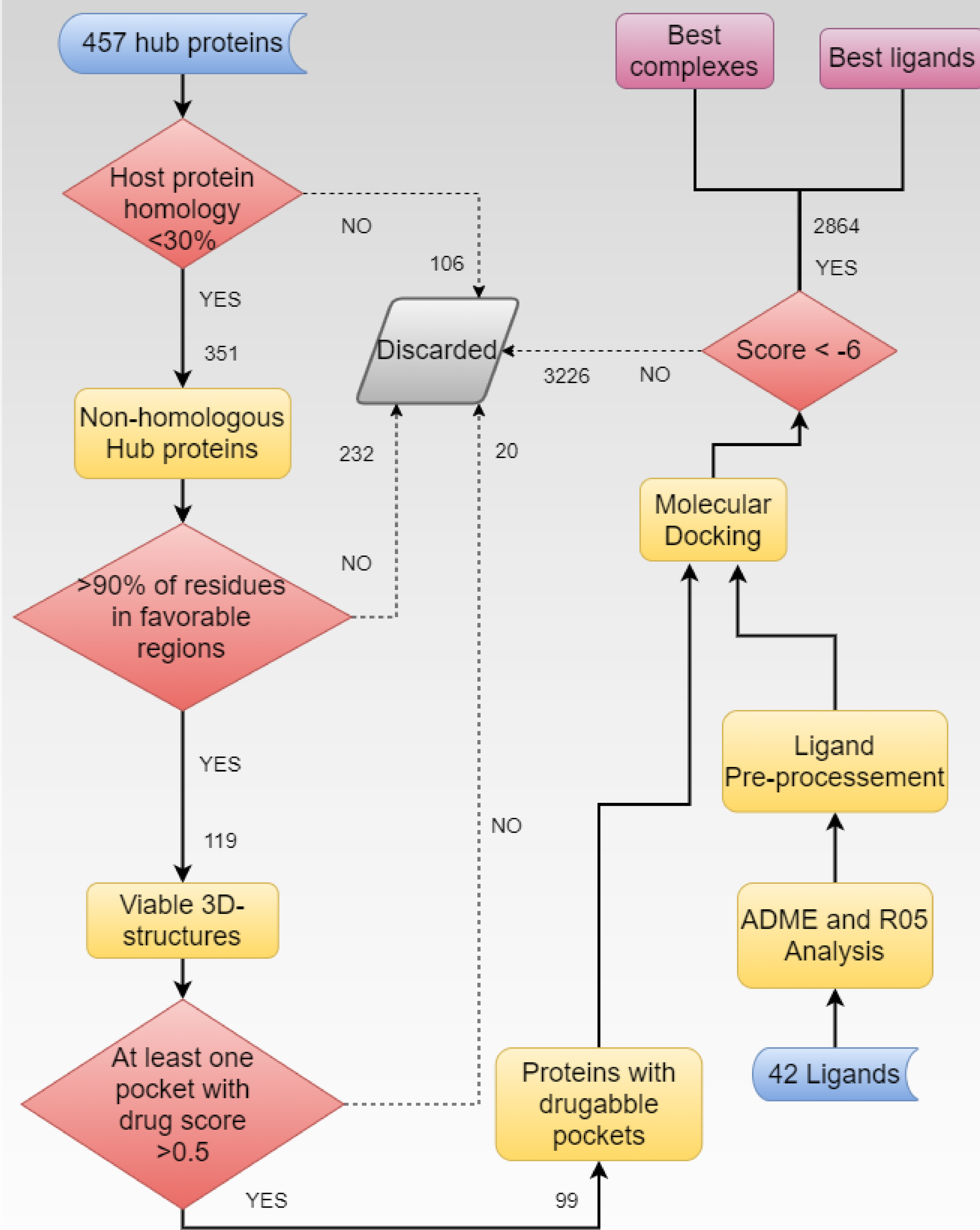

Fig. 1. Flowchart of the methodology

In order to consider only proteins that are non-homologous with the host, the hub proteins were aligned against *H.sapiens* proteome using the BLASTp program (ALTSCHUL et al., 1990), with an e-value set to 1e-10. The hub proteins with an identity*coverage greater than or equal to 30% were discarded. The 3D structure prediction of the target proteins was performed by the Phyre2 tool (KELLEY et al., 2015) with standard configurations and validated by the Ramachandran graph, excluding the models that had less than 90% of their amino acids in the favorable regions. Pockets were identified with the fpocket software (LE GUILLOUX; SCHIMIDTKE; TUFFERY, 2009), removing those with a druggability score smaller than 0.5. Proteins were then prepared for virtual screening by the script "prepare_receptor4.py" from the MGLTools version 4.1 package (MORRIS et al., 2009), converting the files in PDB format to PDBQT. Subsequently, the 42 inedited synthetic derivatives of tetrahydroisoquinoline alkaloids were then prepared using the program "prepare_ligand4.py" from the MGLTools version 4.1 (MORRIS et al., 2009). Molecular docking was performed in triplicates using the Autodock Vina software (TROTT; OLSON, 2010), with parameters "num_modes" set to 9 and "exhaustiveness" set to 50. Scripts written in-house were used to automatically generate configuration files and execute the dockings in a way that all ligands were docked into all pockets.

RESULTS & DISCUSSION

Of the 457 hub proteins considered, 351 did not present relevant homology with the host and were therefore considered promising pharmacological targets. After structure prediction and validation, 119 proteins had more than 90% of their residues in favorable regions, with 99 of these proteins having at least one pocket with a druggability score higher than 0.5, totaling 145 pockets. Even though a good amount of hub proteins were discarded due to not having good structures or druggable pockets, they continue to be potentially essential to *C. ulcerans*. In total, 6,090 dockings were performed and sorted in ascending order of the score (value that represents the free energy of binding), so the more negative the score, the better the affinity of the ligand with the protein. Thus, 2,864 dockings with a score equal to or less than -6 were considered to be relevant.

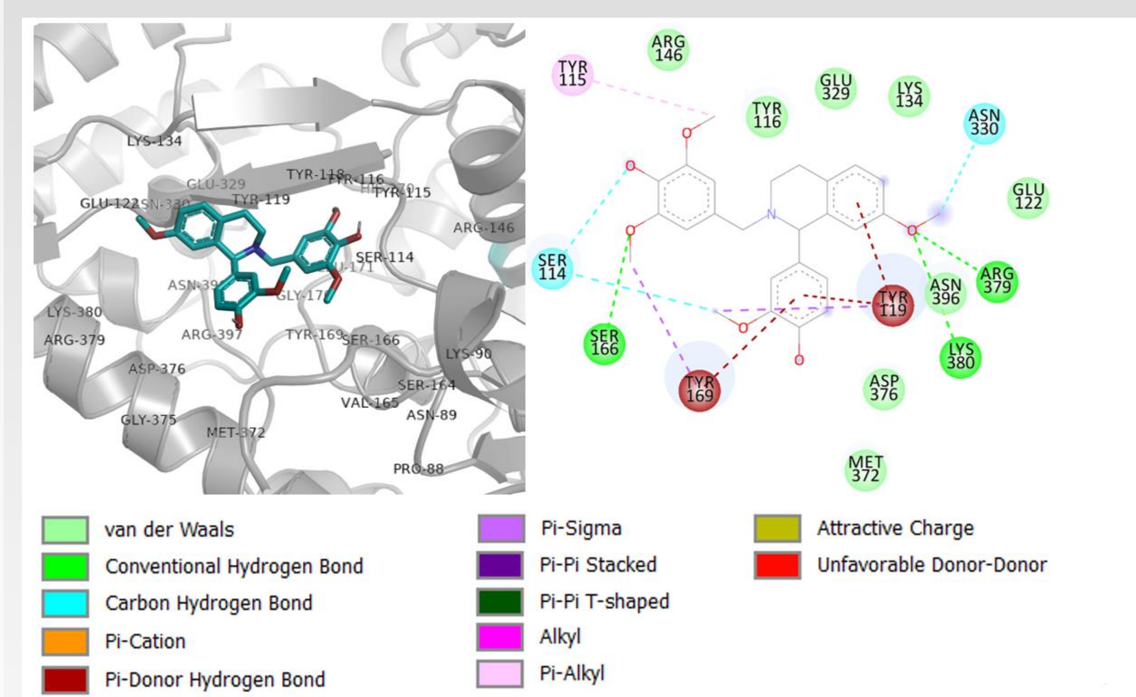

Fig. 2. Interactions between molecule 23 and UvrABC system protein B

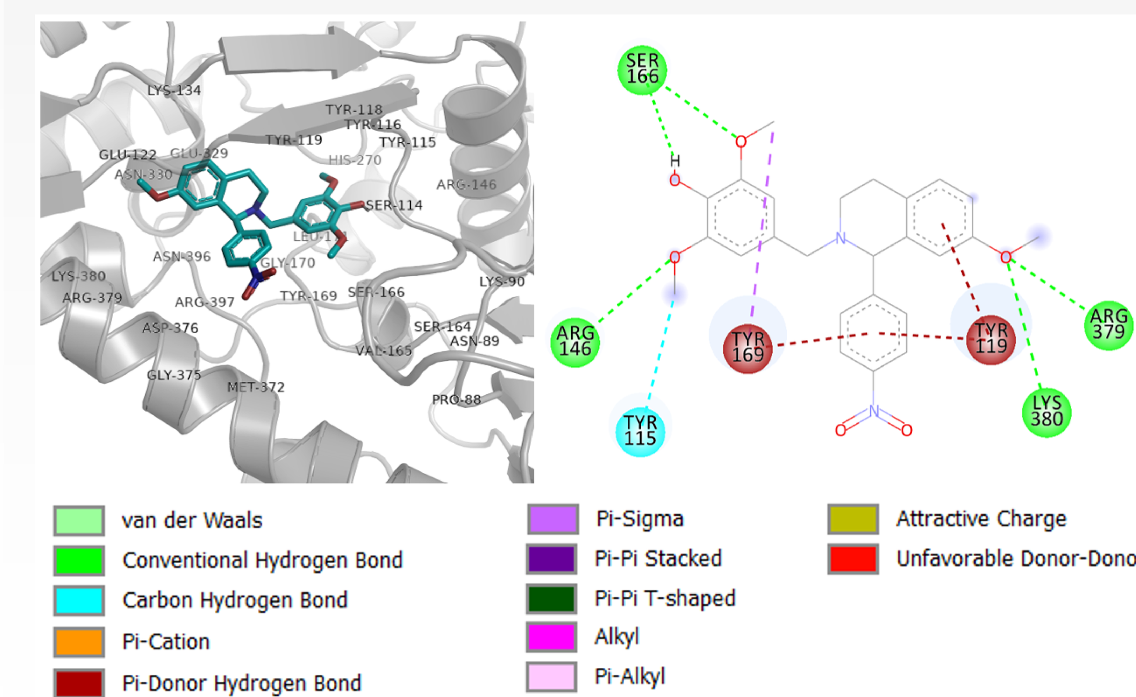

Fig 3. interactions between molecule 27 and UvrABC system protein B

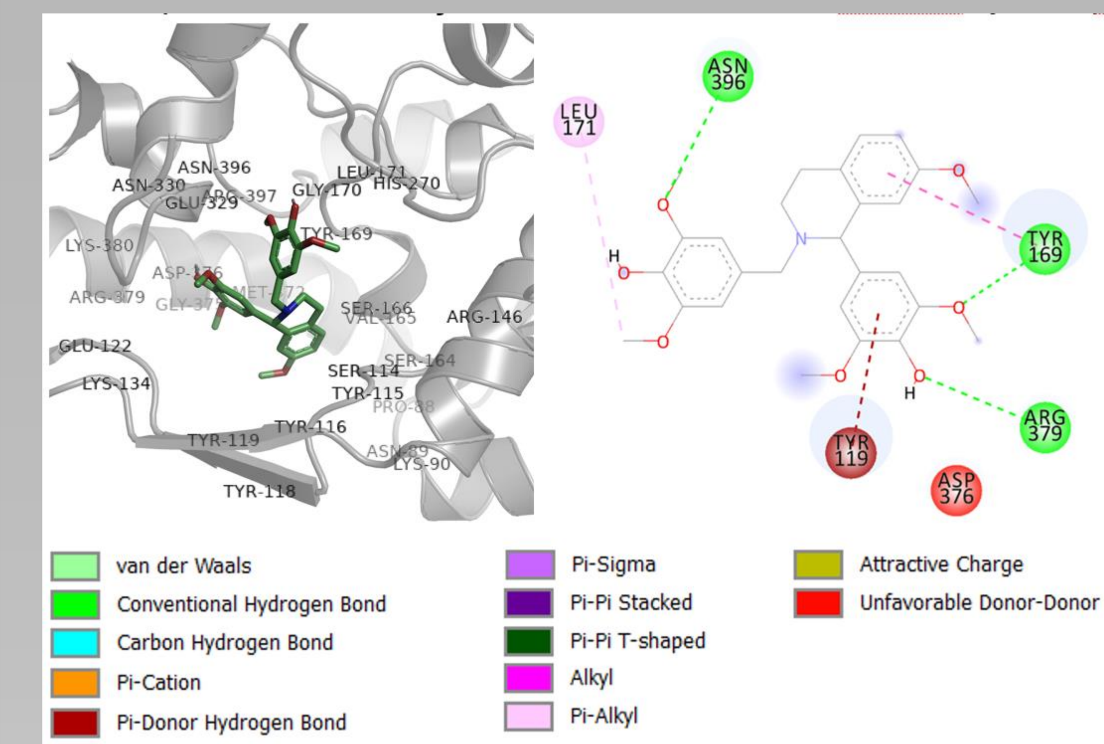

Fig. 4. Interactions between molecule 24 and UvrABC system protein B

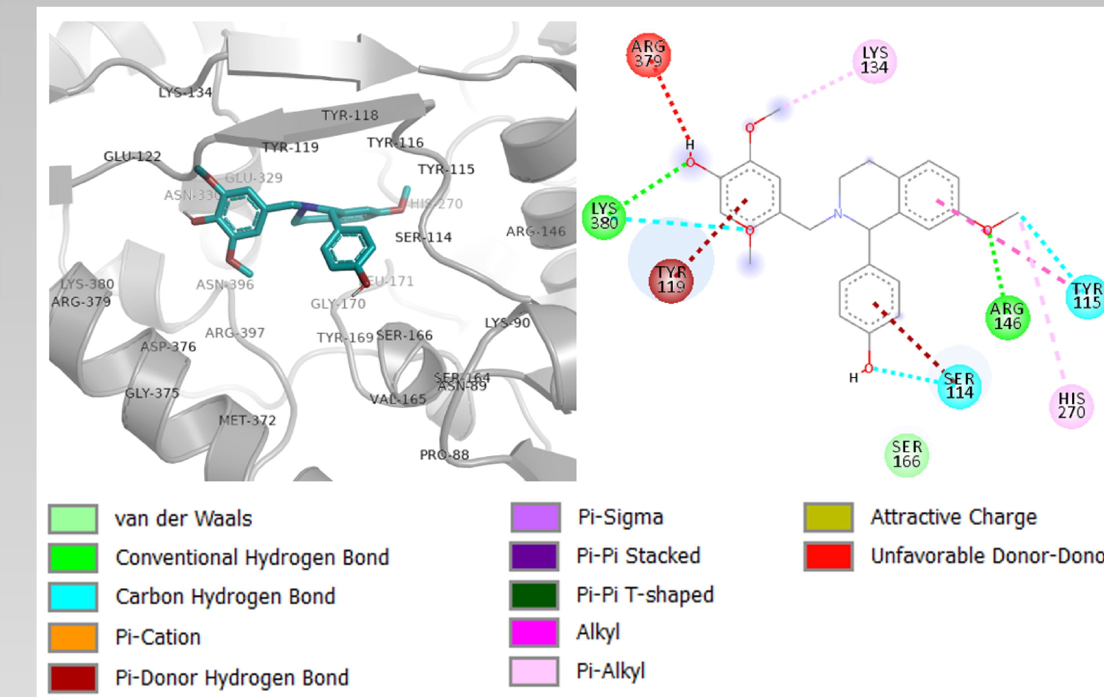

Fig. 5. Interactions between molecule 26 and UvrABC system protein B

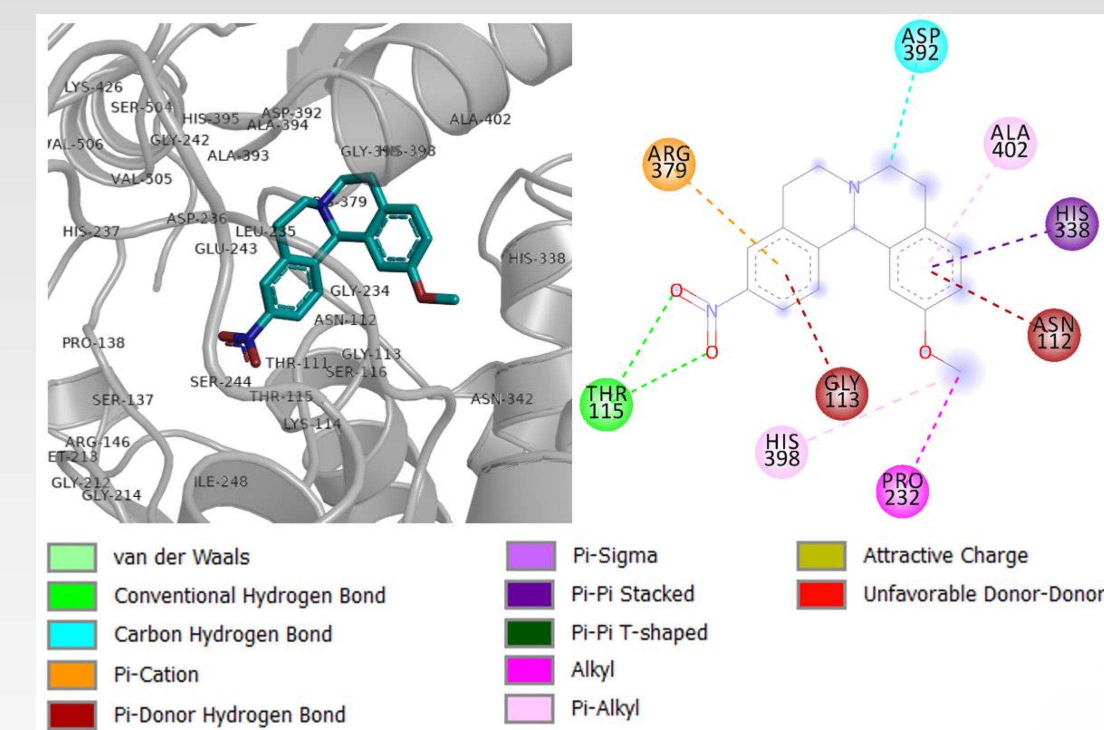

Fig. 6. Interactions between molecule 34 and Bifunctional protein

The top 5 best complexes occurred between the UvrABC system protein B with molecules 23, 27, 24, 26 and Bifunctional protein with molecule 34, with binding energies of -9.9, -9.9, -9.3, -9.0 and -9.6, respectively. The UvrABC system protein B, in conjunction with the UvrABC system protein A, is responsible for the repair of damaged bacterial DNA, therefore being an essential protein for bacterial replication. All four molecules docked within the actual DNA biding site, forming favorable interactions precisely with the protein residues that bind to DNA, such as: hydrogen bonds (ARG379, LYS380 and SER166), Van der Waals interactions (ARG146, ASP376, ASP396, GLU122, GLU32, LYS134, MET372 and TYR116), pi-electron interactions (TYR119, TYR119 and TYR169), among others. Thus, this molecules may act as competitive inhibitors by blocking the DNA interactions with the binding site residues (Fig.7). The Bifunctional protein is an vital protein in the tetrahydrofolate biosynthesis pathway (Vitamin B9) and therefore, is essential for amino acid synthesis in bacteria. Molecule 34 docked within the ADP binding site and may compete with ADP for the binding site, disrupting the protein activity (Fig.8).

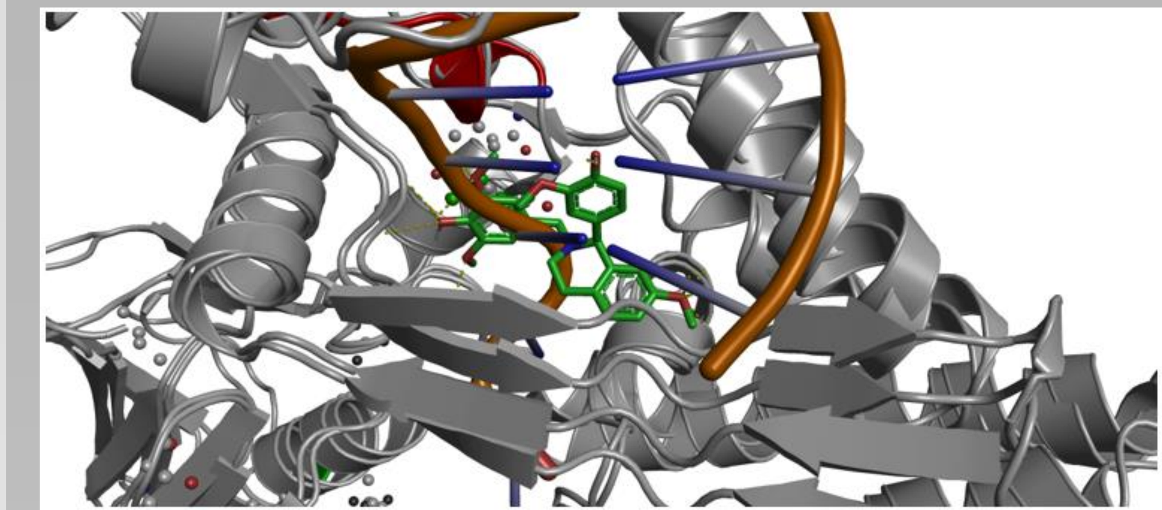

Fig. 7. UvrABC system protein B binding site with DNA and molecule 23

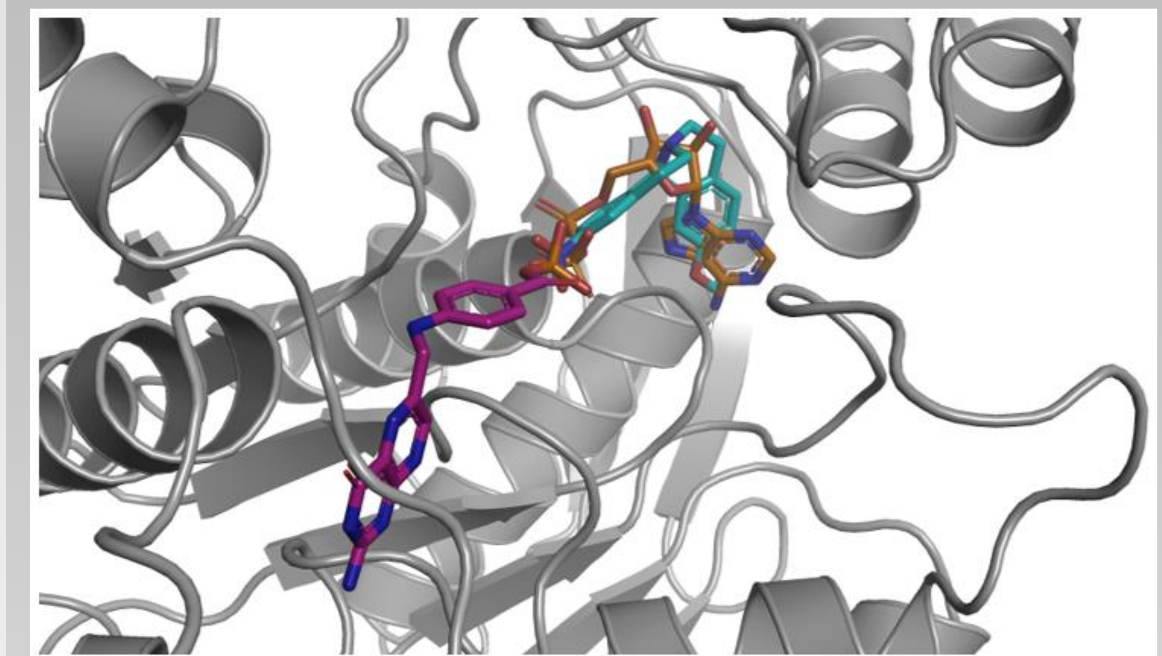

Fig. 8. Bifunctional protein ADP binding site with ADP and molecule 34

CONCLUSIONS

Due to is zoonotic capabilities, *C.Ulcerans* is considered a reemergent organism and as of now, there isn't any treatments designed to specifically treat these bacterial infections. Hence, we used 457 hub proteins indentified in a previous work as targets for the development of new treatments. Through homology modeling it was possible to obtain three-dimensional structures that have not yet been elucidated and with the usage of the Ramachandran plot it was possible to verify the viability of these structures. By predicting the pockets, it was possible to identify binding sites even for proteins without native ligands, turning them into viable targets for docking. By using molecular docking, we were capable of evaluating the potential activity of the 42 inedited derivatives of tetrahydroisoquinoline alkaloids without having to synthesize them, avoiding costs related to synthesis. Also, it was possible to gain insight into the potential molecular mechanisms that modulate the molecules activity, enabling the organic chemists to optimize and even design new molecules based on the interactions observed. Even though we only analysed the potential activity of the derivatives, with the usage of the hub proteins of *C.ulcerans*, any other molecule can be tested *in silico* or *in vitro* with these proteins as targets. This work was unprecedented when using *C.ulcerans* hub proteins to test the affinity of the derivatives with molecular docking and selecting the best results.

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