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Fragment screening as a tool for the search of human dihydroorotate dehydrogenase inhibitors

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Our work aims at contributing to the development of an innovative therapy against COVID-19 based on the selective inhibition of the human enzyme dihydroorotate dehydrogenase (HsDHODH). HsDHODH takes part of the *de novo* pyrimidine biosynthetic pathway [1]. The fact that the virus relies on the host cell biochemical machinery to provide nucleosides for its viral replication cycle DHODH a potential target for the development of broad spectrum antivirals. Our objectives envolve fragment synthesis, evaluation and characterization of DHODH fragment-based inhibitors as a strategy for the development of innovative treatment for viral diseases such as COVID-19. Inhibiton assays against HsDHODH were carried out using 2,6-dichloroindophenol (DCIP) as the final electron acceptor. Co-crystallization assays were performed using vapour diffusion methods. Inhibitors were evaluated regarding their citotoxicity and antiviral properties in Calu-3 cells. At 10 uM, 8 non-citotoxic nanomolar inhibitors were found to inhibit 100% of viral replication.

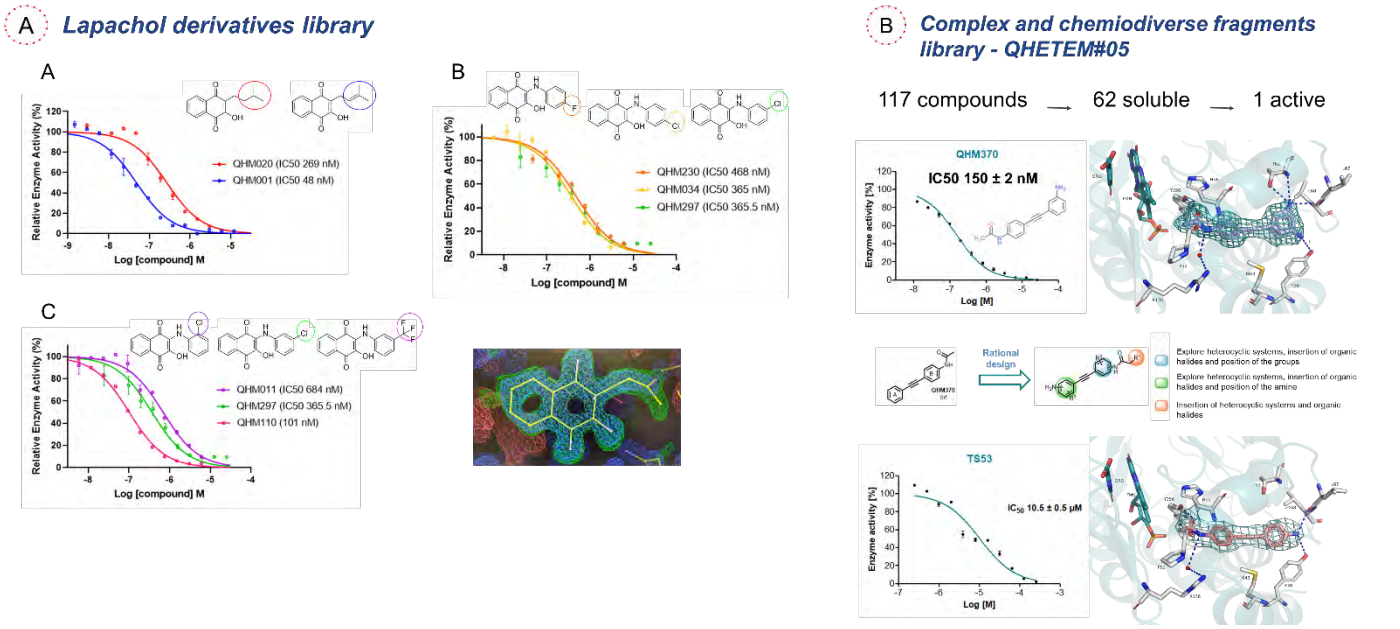

Figure 1. Screening and characterization of human DHODH inhibitors. Different libraries have been tested and potent inhibitors have been identified and characterized by inhibition assays and X-ray crystallography.

The search for new inhibitors for the HsDHODH enzyme is an important strategy in the fight against SARS-CoV-2, responsible for the COVID-19 pandemic with devastating consequences. In this scenario, the fragment screening (FS) is a great tool that can be exploited. In our work, we approched FS by screening different libraries and mapping the molecular basis of ligand interaction by X-ray crystallography. Our results show promising hits tools can be used for the discovery of new inhibitors for the HsDHODH.

[1] Reis RAG, Calil FA, Feliciano PR, Pinheiro MP, Nonato MC. (2017) Arch Biochem Biophys. **632**:175-191.

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