Novel chemotypes of angiotensin-converting enzyme 2 binders *via* successive *in silico* screening and *in vitro* evaluation approaches

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Background and aim of the investigation

• ACE2 is a protein being expressed in the intestines, kidney, testis, gallbladder, lungs and heart and is an integral part of the renin-angiotensin-aldosterone system maintaining normal blood pressure;
• pathogenesis of SARS-CoV-2 infection is tightly bound to virus induced ACE2 receptor downregulation, and, hence, figuring out this relationship will fold the puzzle of how the virus impact a human's organism functioning.

• investigation of ACE2 is currently complicated due to the very limited data on small organic molecules selectively binding to ACE2.
• there are very few ACE2-binder discovery projects in comparison with its homologue ACE.

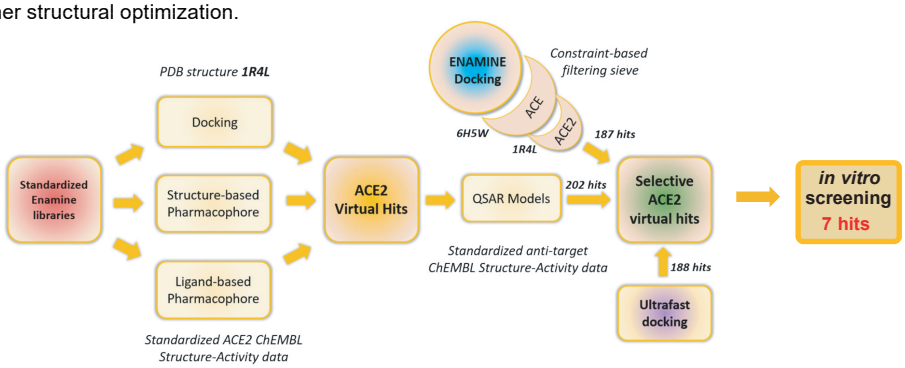
THE WORK AIMS to find the most promising molecule-candidates that fit the best chemical probe criteria for ACE2-receptor, applying *in silico* virtual screening and molecular docking/molecular dynamics approaches:
• world screening collection, Enamine – 3.2 million compounds;
• "on-demand" database, Enamine – 14.1

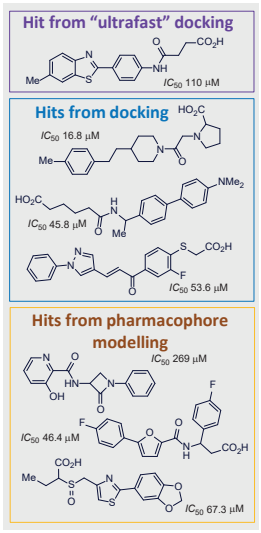
The general workflow of *in silico* search for selective ACE2 inhibitors

• **we devised chemical diversity applied workflows** that enabled us to deal with huge compound collections and demonstrated high cost-effectiveness while carrying out the docking of billions of compounds;

• **the chemo-type based approach** allowed fishing out small sets of molecular candidates with good potential for further *in vitro* optimization;

• **the strong point is novelty:** ligands present two novel ACE2-binding chemotypes and could be used for further structural optimization.





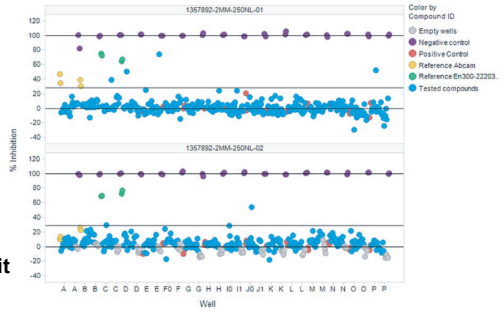
In vitro screening part of the research

Despite the commercial availability of ACE2 Inhibitor Screening Kits the optimal HTS protocols are still not published and their validation is still needed.

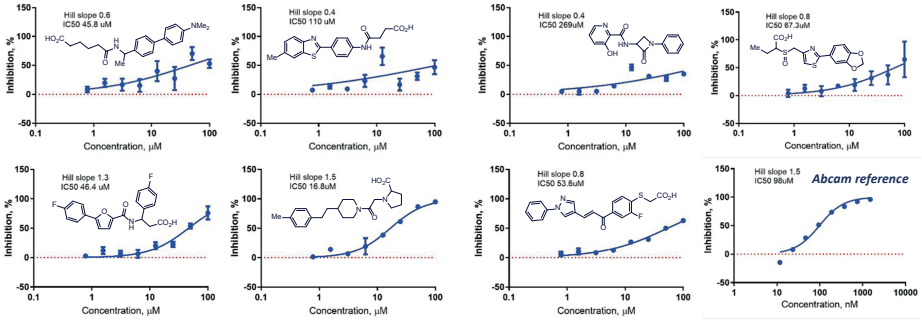
The screening milestones:

1) optimization of the test compounds solutions used volume;
2) optimization of reaction volume and concentrations of the reagents;
3) determination of IC_{50} for known and reference inhibitors in new conditions;
4) primary screening of 577 virtual hits;
5) hit confirmation;
6) dose-response curve of confirmed hits.

The seven compounds out of 577 screened were selected according to the **hit criteria Inh% > 3 SD + Avg.**



DOSE-RESPONSE CURVES OF THE CONFIRMED HITS



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References

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