

MeetEU Project - Team Heidelberg - Team 1 –  
Identification and Enhancement of novel Sars-CoV-2 NSP13 helicase  
inhibitors

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February 2024

## 1 Abstract

Even though the development of vaccines against Sars-CoV-2 was successful during the recent pandemic, the amount of FDA approved drugs for the therapy of Covid-19 is still limited to Paxlovid and Veklury, Olumiant and Actemra FDA 2023. One possibility to accelerate the development of new therapies for Covid19 is to screen already approved drugs for effects against the viral reproduction. In this years MeetEU project, we investigated the NSP13 helicase of Sars-CoV-2 and tried to find compounds that could be repurposed for this therapy, as well as novel compounds that could lead to an effective treatment of Covid19. Using our *in-silico* pipeline enables us to evaluate possible drug candidates, suggest novel structures based on already approved drugs and investigate their toxicity, while being cheaper and less labor intensive than projects limited to wet-lab work. HIER KOMMT EINE ABBILDUNG HIN

## 2 Introduction

### 2.1 Identification of consensus binding pocket

In drug discovery, the initial step is to investigate the protein structure in order to analyse potential binding sites. These are cavities on the surface or interior of the protein with suitable properties to bind a ligand. The functionality of a binding pocket is determined by its shape and location, but also by the amino acid residues which define its physicochemical characteristics Stank et al. 2016. There are both different experimental and theoretical procedures existent to analyse the druggability of such binding pockets. In this work, we combined three different in-silico tools, each following a different algorithm. Fpocket Le Guilloux et al. 2009 utilises a geometry-based algorithm based on Voronoi tessellation and sequential clustering to determine potential binding sites. We also implemented P2Rank Krivák and Hoksza 2018; Jendele et al. 2019; Jakubec et al. 2022 which is based on a machine-learning algorithm. It assigns structural, physico-chemical, and evolutionary features to points on the solvent accessible surface of a protein. From this information, the machine-learning model is built and used to predict and rank potential ligand binding sites. Lastly, FTMAP Brenke et al. 2009 was used to validate the binding pocket found with the other two approaches. This tool uses docking results of sixteen small molecules differing in polarity, shape and size to identify binding hot spots with a fast Fourier transform correlation. The most favorable docked conformations are determined by energy minimization and clustering. Finally, the results of the three approaches were combined to identify a consensus binding pocket of the NSP13 helicase. The resulting coordinates of the consensus binding pocket were then used for molecular docking simulations.

### 2.2 Molecular Docking

Molecular Docking programs are used in order to calculate binding affinities between a potential drug candidate and the target protein. Most crucial to this task is the prediction of the ligand position, orientation and conformation. Search-based methods approach this task by continuously modifying the ligand pose, while estimating its quality/likelihood (score) and stochastically trying to presume their scoring-functions global optimum. Among the most widely known are also AutoDock Vina and Glide, which mainly differ in their scoring function **Trott.2010; Halgren.2004**. However, such search based methods are computationally highly expensive. Therefore, in order to be able to screen large datasets, search-based methods are generally restricted to a previously defined binding pocket Corso et al. 2022. Consequently, potential other binding sites of a ligand are not assessed. Machine learning based blind docking approaches try to address that problem by stochastically predicting

binding pocket and ligand pose based on learned characteristics and aligning them. Most promising results are thereby archived by Diffdock, a generative model which applies a reverse diffusion process to the docking paradigm. Thereby, Diffdock iteratively transforms an uninformed noisy distribution over ligand poses defined by the degrees of freedom involved in docking (position, turns around its centre of mass and twists of torsion angles) into a learned model distribution Corso et al. 2022. Corso et al. thereby describes this process as progressive refinement of random ligand poses via updates of their translations, rotations and torsion angles.

### 2.3 Lead Drug Enhancement

In order to enhance the binding affinity of our drug candidates and thus their performance, we used AutoGrow4 (Version 4.0.3) **packageAutogrow4** to generate novel compounds. Starting with the best binding compounds of our initial docking simulation with AutoDock Vina as generation zero, multiple new structures are generated by combining sub-structures of the first generation or by passing them through a set of possible chemical reactions after converting them into their respective SMILES codes. All of the generated compounds are ranked by their binding affinity. After passing several filters the best performing compounds are used as the seed for the next generation. Using this algorithm, compounds are found, which show higher binding affinities than the first generation. As AutoGrow4 labels all new structures by the path by which they were obtained, we can also evaluate the synthesizability.

### 2.4 Estimation of Toxicity and Synthetic Accessibility

After identifying the lead compounds that exhibit optimal binding affinity within the consensus pocket, an evaluation of the general toxicity and synthetic accessibility of these compounds was performed using *eToxPred* (Pu et al. 2019). This additional step helps estimate the suitability of the compounds as real-life pharmaceuticals against COVID-19. The Tox-score allows for a general assessment of the predicted risk vs. benefit ratio. Moreover, *eToxPred* allows for an insight into the ease of synthesis, reflected by the synthetic accessibility score (SAscore), of the compounds in a laboratory setting.

### 2.5 Molecular Dynamics Simulation

As the last step of our pipeline, a MD simulation is conducted using the best scoring compounds as a ligand in the binding pocket of the NSP13 protein. Using GROMACS (Version 2023.3) **packageGROMACS**, this enables us to interpret the stability of the protein-ligand interaction, as well as to identify important residues for the interaction. Using a given force-field, a set of equations describing different forces between the atoms and residues in the protein and ligand, the movement of all atoms in the system can be simulated and analysed. However, this is only possible in a very limited timeframe with a small time step size. As this process is rather resource heavy, it has to be conducted on a cluster with access to a GPU.

## 3 Material and Methods

### 3.1 Toxicity and Synthetic Accessibility Prediction using *eToxPred*

Following lead optimisation, the toxicity and synthetic accessibility of the given compounds was estimated using the machine-learning tool *eToxPred* (Pu et al. 2019) **on molecular fingerprints**. The toxicity predictor was pre-trained on the FDA-approved and the KEGG-drug datasets whose compounds were considered non-toxic as well as the TOXNET and the T3DB datasets whose compounds

were considered toxic using a deep-belief-network based model. This predictor yields a Tox-score between 0 and 1 and in accordance to the paper, all compounds with a Tox-score below 0.58 are deemed non-toxic. The synthetic accessibility was reflected in a synthetic accessibility score (SAscore) which was obtained by training an extra-trees-based classifier on NuBBE, UNPD, FDA-approved, and DUD-E-active datasets. As input for the pre-trained model served the SMILES files of the Top100 compounds from AutoDock Vina **CITATION**.

## 4 Results

## 5 Discussion and Outlook

## 6 Supplementary Material

## References

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