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Computational identification of disulfiram and neratinib as putative SARS-CoV-2 main protease inhibitors

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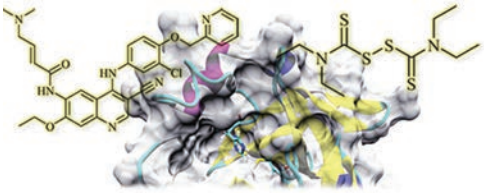
Identification of disulfiram and neratinib as putative covalent inhibitors of SARS-CoV-2 virus main protease M^{pro} by a combination of 'on-top docking' procedure, expert evaluation of potential hits and molecular dynamics is reported herein. This finding shows the importance of further development of virtual screening add-ons.



Keywords: SARS-CoV-2, molecular dynamics, main protease M^{pro}, molecular docking, neratinib, disulfiram.

Since the beginning of year 2020, the whole world has been watching the spread of novel coronavirus, which caused an epidemic in Wuhan, China in December 2019 and then disseminated to other countries. With the epidemic spreading around the world, the World Health Organization declared outbreak of the new coronavirus a pandemic on March 11th, 2020. Graveness of the situation calls seeking for a solution in all possible ways, including sharing all available information around the scientific community as widely as possible.[1] Due to the lack of time to create a new drug entity, cure should be sought among the already known drugs, and the fastest method to do so is a computer aided simulation of off-target activity.

On January 26th, 2020, the experimentally determined structure of SARS-CoV-2 main protease M^{pro} (PDB ID 6LU7) was published, enabling virtual screening for inhibitors of this protein.[2]

It is easy to notice that in virtual screening for M^{pro} blockers drugs for HIV and hepatitis C that inhibit proteases of these viruses are often 'winners'. This seems obvious as proteases can be similar in different viruses, and the use of lopinavir and ritonavir combination against coronavirus suggests that a protease inhibitor can be found without molecular modeling.[3] Other broad-spectrum antiviral drugs are also common guests among the results of virtual screening against M^{pro}.[4]

In this paper we report on the identification of new potential M^{pro} inhibitors in registered medicinal products. The search was conducted among a database of FDA approved drugs and active metabolites[5] using recently described 'on-top docking' methodology.[†,7]

During result analysis it was found that sulfur-containing drugs show unusually high ligand efficiency at the active center of SARS-CoV-2 main protease M^{pro} (Figure 1 and Table 1). Despite a number of publications already reported the potential applicability of this structural class of drugs,[8,9] an additional

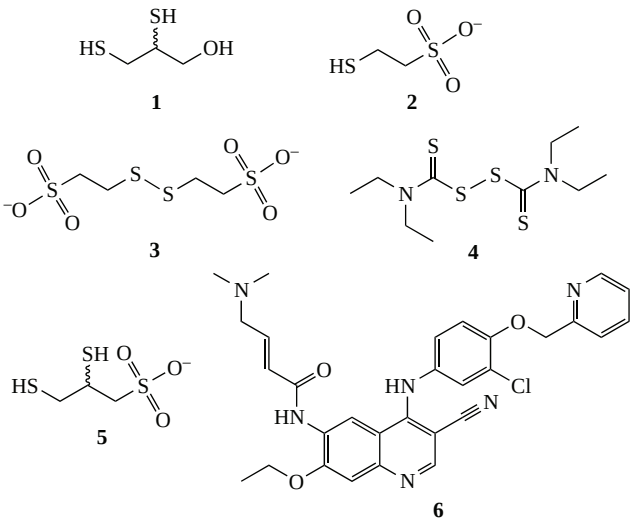


Figure 1 Potential SARS-CoV-2 main protease M^{pro} inhibitors identified in primary virtual screening.

binding study for primary hits using molecular dynamics[‡] showed that compounds **1**, **2**, **3** and **5** dissociate very quickly from M^{pro} active site, while only disulfiram **4** retains stable interactions [Figure 2(*a*)]. It should be noted that disulfiram was reported as a putative inhibitor in a recent computational study.[12] This drug can affect coronavirus infection in two ways at once. First, disulfiram has previously been shown to be a covalent inhibitor of MERS and SARS coronavirus proteases *in vitro* with IC_{50} of 14 and 24 μM, respectively.[13] And second, published data suggests that coronavirus infection in humans is accompanied by a significant decrease in reduced glutathione, and an approach

[†] Full-atom spatial model of M^{pro} (PDB ID 6LU7) was used for virtual screening performed with LeadFinder.[6]

[‡] Best ligand conformation obtained after docking was used as a starting geometry for 300 ns MD simulations in explicit TIP3P solvent. MD simulations were performed using the GROMACS 2018.1[10] package with the OPLS/AA force field. Ligand topologies for putative inhibitors were created using LigParGen service.[11]

Table 1 Docking and MD analysis of ligand binding in SARS-CoV-2 main protease M^{pro}.

Compound		Docking score[a]/ kcal mol^{-1}	Calculated dG[a]/ kcal mol^{-1}	Ligand efficiency[a]/ kcal mol^{-1} atom^{-1}	Calculated inhibition constant K_i/μM	MD[b]
1	*R*-BAL	–6.76	–8.3	–1.38	0.82	–
1	*S*-BAL	–7.09	–8.62	–1.43	0.48	–
2	MESNA	–6.37	–6.86	–0.98	9.43	–
3	DIMESNA	–8.91	–8.56	–0.68	0.54	–
4	Disulfiram	–8.05	–8.95	–0.56	0.28	+
5	*R*-DMPS	–7.39	–8.06	–1.02	0.51	–
5	*S*-DMPS	–8.25	–8.59	–1.07	1.25	–
6	Neratinib	–12.62	–10.99	–0.28	0.01	+

[a] Average over 6 independent runs; [b] '+' denotes stable position in the active site, '–' denotes dissociation.

aimed at eliminating this symptom has already been proven effective.[14,15]

We are also reporting here for the first time on the identification of another drug as a potential M^{pro} inhibitor – a tyrosine kinase inhibitor neratinib **6** [Figure 2(*b*)] used in HER2-positive breast cancer as an adjuvant therapy. This candidate was identified during 'on-top docking' and successfully passed a molecular dynamic simulation without dissociation from M^{pro} active site.

Interestingly, both potential M^{pro} inhibitors reported here are presumed to be covalent. According to published data, disulfiram can block M^{pro} enzymatic activity by thiol–disulfide exchange reaction with the main active site residue Cys145,[13] while neratinib binding suggests the possibility of covalent interaction with the same residue by Michael addition to its nitrile group (similarly to already described covalent peptide inhibitors).[16] Since molecular mechanics based methods can only suggest (but not prove) covalent interactions, this issue warrants further experimental investigation.

We have recently shown that the use of 'on-top docking' is a very effective approach to improve virtual screening accuracy in case of proteins with large and open active site.[7] We also know that the role of human expertise in the success of a computational experiment is very large and underestimated.[17] One of the main goals of the reported study was to stack both fast and accurate computational methods of drug discovery. The accuracy would undoubtedly benefit from additional quantum-mechanical simulations, however this class of methods still cannot be considered fast.[18]

In conclusion, using a combination of 'on-top docking', expert evaluation of potential hits and molecular dynamics, we identified two new potential covalent inhibitors of the SARS-CoV-2 virus main protease M^{pro}. This finding shows the importance of further development of virtual screening add-ons. Developments on improvement of virtual screening selectivity and efficacy are underway in our laboratory.

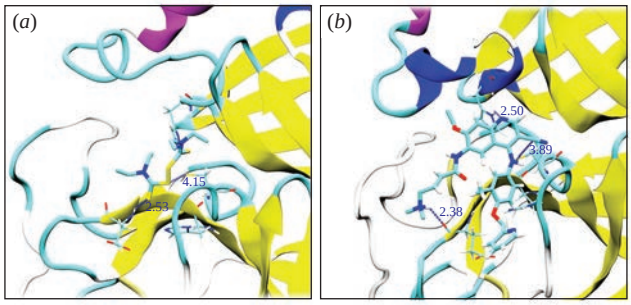


Figure 2 Binding modes of (*a*) disulfiram and (*b*) neratinib in the active site of SARS-CoV-2 main protease M^{pro}. Key active site residues Cys145, His41, His163 and Glu166 are shown in licorice. Hydrogen bond interactions are shown with blue dashed lines.

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