

# Virtual screening for small molecular non-covalent binders of the SARS-CoV-2 main protease

Piotr F.J. Lipiński<sup>1</sup>, Jolanta Zaborniak<sup>2</sup>, Piotr Garnuszek<sup>3</sup>, Przemysław Szurmak<sup>4</sup>

<sup>1</sup>Mossakowski Medical Research Centre Polish Academy of Sciences, Poland

<sup>2</sup>National Centre for Nuclear Research, Poland

<sup>3</sup>Radioisotope Centre POLATOM, National Centre for Nuclear Research, Poland

<sup>4</sup>MacShur Calculus, Poland

**Submitted:** 4 December 2020; **Accepted:** 7 February 2021;

**Online publication:** 19 March 2021

Arch Med Sci

DOI: <https://doi.org/10.5114/aoms/133122>

Copyright © 2021 Termedia & Banach

**Corresponding author:**

Piotr F.J. Lipiński  
Mossakowski Medical  
Research Centre Polish  
Academy of Sciences  
5 Pawińskiego St  
02-106 Warsaw, Poland  
E-mail: [plipinski@imdik.  
pan.pl](mailto:plipinski@imdik.pan.pl)

Among several molecular targets that were proposed for fighting coronaviruses in general and have been proposed again in the case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, is a chymotrypsin-like cysteine protease known as the 3C-like protease (3CL<sup>pro</sup>) or the main protease (M<sup>pro</sup>) [1, 2]. The enzyme is involved in proteolytic processing of coronavirus polyproteins indispensable for forming the replicase-transcriptase complex. Inhibition of M<sup>pro</sup> blocks the viral replication, which in turn gives an antiviral effect.

A dominant approach to blocking M<sup>pro</sup> is by using covalent inhibitors. Several potent compounds of this type have been already reported for SARS-CoV-2 M<sup>pro</sup> [3–5]. However, the covalent active compounds have their issues, e.g. a propensity to interact with off-targets causing side-effects and toxicity. Hence it is also important to develop non-covalent M<sup>pro</sup> inhibitors.

In order to aid the efforts along this track, we decided to perform a virtual screening (VS) campaign towards the identification of potential SARS-CoV-2 M<sup>pro</sup> non-covalent inhibitors. The results of this work are freely available to parties interested in COVID-19 drug discovery via the webpage: [www.cov2mpro.com](http://www.cov2mpro.com) [6]. In the current contribution we describe our protocol and its validation, and comment on some of the findings.

Virtual screening is a computational (in silico) procedure, the aim of which is to identify probable binders (active compounds) for a given molecular target:

- from a certain large database of possible compounds,
- by means of a particular in silico technique.

While it is unrealistic to expect VS to find ready-for-market drugs, the screening may provide good-quality starting points (hits) for further medicinal chemistry programmes. With the VS being only the initial step, the screened compounds should have some space for structural optimization (not too large size) and be readily acquirable. In light of this, the chosen screening pool comprised 2,658,170 million molecules of 300–400 Da molecular weight, moderate lipophilicity (logP between 1 and 3), and “in-stock” status found in the ZINC database [7]. Additionally, we removed molecules with reactive or otherwise problematic functional groups or substructures.

As to choose which computational technique to employ for our problem, we decided to perform receptor-based screening by molecular docking simulations to the 6LU7 crystal structure of SARS-CoV-2 M<sup>pro</sup> [5].

An alternative choice of QSAR/pharmacophore approaches (ligand-based) for our task would not be easily realizable because only scarce empirical data on SARS-CoV-2  $M^{pro}$  inhibitors were available. Three docking programs (freely-available to academic researchers) were considered: AutoDock 4.2.6 (AD) [8], AutoDock Vina 1.1.2 (Vina) [9], and DOCK 6.9 (DOCK) [10, 11].

In order to validate the usefulness of these programs for docking-based virtual screening towards the discovery of non-covalent binders of the SARS-CoV-2 main protease, we performed 2 retrospective tests on available data pertinent to the main proteases of other  $\beta$ -coronaviruses. As these proteins are highly similar in sequence and structure, the results of such trials might be expected to give some reasonable indication of the software performance in the case of SARS-CoV-2  $M^{pro}$ . The tests were supposed to answer the following questions:

How well do the programs reproduce binding poses of non-covalent inhibitors of  $\beta$ -coronaviruses  $M^{pro}$  reported in a few available crystal structures?

How good are they at finding true non-covalent inhibitors of SARS-CoV-1  $M^{pro}$  among structurally similar decoys?

For the first test, we considered 6  $M^{pro}$  structures from 3 different  $\beta$ -coronaviruses: SARS-CoV-1 (96% sequence identity with SARS-CoV-1  $M^{pro}$ , 2 structures [12, 13]), MERS-CoV (51% sequence identity, 1 structure [14]), and Bat-CoV HKU4 (50% sequence identity, 3 structures [15]). The crystallographic ligands were redocked to their native structures with all 3 programs. The success rate of correct pose prediction was 1/6, 3/6, and 4/6, for AD, Vina, and DOCK, respectively. Considering the closest homologue of our target, that is  $M^{pro}$  of SARS-CoV-1, for which there are 2 crystal structures, each program gave a correct pose in 1 of 2 cases while failing in another.

For the second test, 46 non-covalent inhibitors of SARS-CoV-1  $M^{pro}$  were collected from the literature [12, 13] and seeded with decoys found by DecoyFinder [16] (in the ratio 1 : 75, giving in total about 3500 compounds). The idea behind such a procedure is to check whether a given program/protocol is able to discern 'true' ligands from 'false' ligands (decoys). The set was docked to 3V3M and 4MDS structures of SARS-CoV-1  $M^{pro}$  using all 3 considered programs. The results of this test are given as enrichment factors (EF, Figure 1 A) and receiver operating characteristic (ROC) curves in Figure 1 B and Table I.

In this test, AutoDock 4 performed exceptionally well. In the cases of both structures, the program was able to pick the majority of 'true' ligands at the very beginning of the ranking list. The enrichment factors at the top 1% of database are

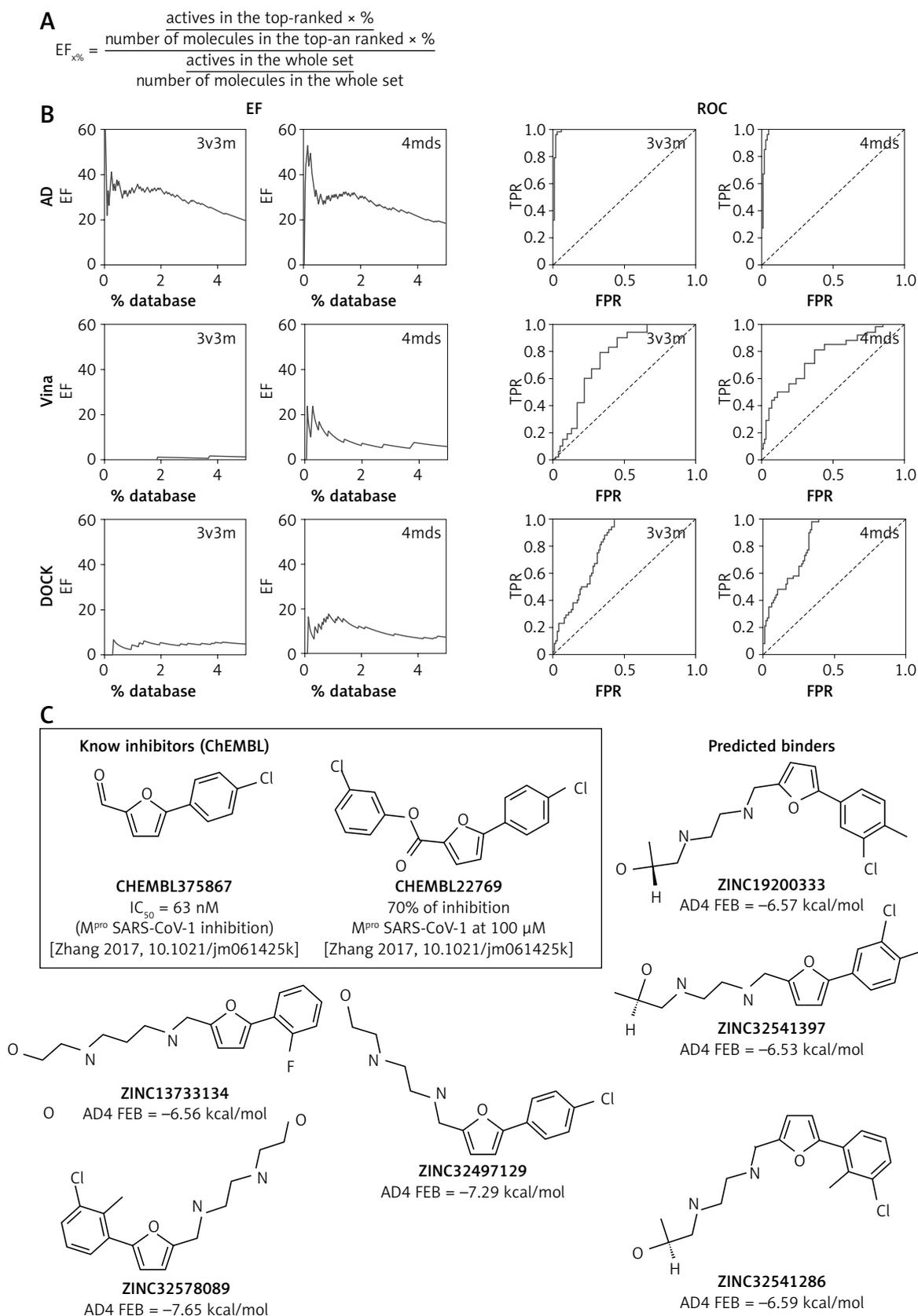
here greater than 30, meaning that the program recognizes 'true' ligands with 30-times better efficacy than random choice would.

The performance of the 2 remaining programs was much worse. Vina did not find any 'true' ligand in the first 1% of the database ( $EF_{1\%} = 0$ ) in the docking to 3V3M, and a moderate  $EF_{1\%}$  of 10.14 was found in the docking to 4MDS. DOCK performed better than that, but still with moderate success at best (3V3M  $EF_{1\%} = 4.15$ , 4MDS  $EF_{1\%} = 14.53$ ). On the other hand, Vina and DOCK were significantly faster, which is an important factor if large libraries are to be screened. In the validation set, average docking times per molecule were: 207.7 s (AD), 24.5 s (Vina), and 27.7 s (DOCK).

Weighing up both the results of the validation tests and the speed, we decided to perform the proper screening task in a 2-step procedure. First, the whole set of over 2.6 million molecules was docked to SARS-CoV-2  $M^{pro}$  (PDB: 6LU7, [5]) with DOCK. Then the best-scored 100,000 compounds were docked also with AutoDock 4 and AutoDock Vina. The ranking lists coming from these 2 stages are freely available via the webpage: [www.cov2mpro.com](http://www.cov2mpro.com). The page contains all the docked molecules in the native output format of each program, available for download in compressed archives. Furthermore, the on-site interface allows for access to the top scoring 10,000 compounds (by the AutoDock 4 scoring function). A user can filter the molecules according to several simple filters in order to download only a certain subset of the top scoring entries.

For a quantitative look-up of the interactions found in the top 10,000 entries, we performed a BINANA analysis [17] of the predicted binding modes. It revealed that the screened compounds are predicted to bind mostly in S1 and S2 subpockets of the active site, and a good number of them are able to touch S4 and S1' sites. This suggests that the potential hits can be subject to further structural optimization for more dense interactions with subsites other than S1 and S2. Reaching a wider extent of active site surface is common to covalent inhibitors, and some of them span across the whole active site [5]. Further avenue for structural optimization may be an attempt to increase the number of hydrogen bonds that should give the ligands specificity. In the AD binding poses of the best-ranked 10,000 molecules, there are on average  $2.7 \pm 1.5$  hydrogen bonds.

With regard to chemical diversity, we conducted analysis of scaffolds and rings of the best-ranked 10,000 molecules. A query in the ChEMBL database [18, 19] revealed that only 1 of the scaffolds found in our predicted binders (2-phenylfuran) is present in the previously reported SARS-CoV-1  $M^{pro}$  inhibitors. Furthermore, only a few scaffolds are substructures of inhibitors of other viral pro-



**Figure 1.** **A** – Formula for calculating the enrichment factor (EF) at a given percent of the ranking. **B** – Results of validation set docking for particular combinations of software and structure presented as EF plots and receiver operating characteristic (ROC) curve. TPR – true positive rate, FPR – false positive rate. A dashed line is the  $y = x$  line (performance of random classification). **C** – 2-phenylfuran scaffold as a core for M<sup>pro</sup> inhibitors with the examples of known inhibitors and the predictions from the presented virtual screening

**Table I.** Results of validation set docking for particular combinations of software and structure

Variable	3V3M structure			4MDS structure				
	ROC AUC <sup>1</sup>	EF <sub>1%</sub> <sup>2</sup>	EF <sub>2%</sub> <sup>2</sup>	EF <sub>5%</sub> <sup>2</sup>	ROC AUC <sup>1</sup>	EF <sub>1%</sub> <sup>2</sup>	EF <sub>2%</sub> <sup>2</sup>	EF <sub>5%</sub> <sup>2</sup>
AutoDock 4	0.990	32.92	32.92	19.58	0.987	30.86	29.83	18.33
AutoDock Vina	0.745	0.00	1.04	1.25	0.763	10.14	7.20	5.81
DOCK	0.792	4.15	5.19	4.57	0.837	14.53	11.42	7.06

<sup>1</sup>ROC AUC – area under the curve of receiver operating characteristic. <sup>2</sup>Enrichment factors (EF) at a particular percentage of the database.

teases. This means that our predicted binders, if confirmed to be hits, would provide chemical novelty to the group of M<sup>pro</sup> inhibitors.

On the other hand, the fact that our VS procedure accidentally ‘rediscovered’ the mentioned 2-phenylfuran scaffold as a core for M<sup>pro</sup> inhibitors is gratifying (Figure 1 C). This scaffold (present in 6/10,000 top entries, rather in the lower middle part of the ranking, average AD estimate of binding free energy, FEB:  $-6.87 \pm 0.48$  kcal/mol) is found in 2 relatively potent covalent inhibitors (Figure 1 C) reported previously [20]. This finding might be some remote indication of the correctness of the VS predictions.

In conclusion, the data obtained by our retrospectively validated VS protocol represent a large set of probable inhibitors of SARS-CoV-2 M<sup>pro</sup>. The molecules are chemically diverse and novel compared to the known inhibitors. Nonetheless, they are readily acquirable from commercial suppliers. Moreover, there is a reasonable space for structural optimization both in terms of the properties of the molecules themselves, and the possibility of creating additional intramolecular contacts. Thus, the data form a good basis for pursuing an experimental medicinal chemistry programme aimed at the discovery of anti-SARS-CoV-2 drugs. Still, because of the *in silico* character of the screening results, it should be underscored that the predictions require experimental validation to see if the VS hits are true SARS-CoV-2 M<sup>pro</sup> binders. If confirmed, the molecules might need to undergo structural optimization to achieve high activity and favourable physicochemical properties. Finally, pharmacological characterization will be needed to see if the putative confirmed hits are selective and safe.

Our VS results are provided freely via the webpage: [www.cov2mpro.com](http://www.cov2mpro.com). Hopefully, along with other recent *in silico* predictions [21–23], they will be useful to accelerate anti-SARS-CoV-2 drug discovery. The interested researchers are welcomed to use the data in their work. We are also open to co-operation proposals, in particular with respect to experimental testing of the VS predictions.

### Acknowledgments

The contribution of Jolanta Zaborniak was realized within the program of interdisciplinary

doctoral studies: Radiopharmaceuticals for molecularly targeted diagnostics and therapy – RadFarm; Project No. POWR.03.02.00-00-1009/17-00 (Operational Project Knowledge Education Development 2014-2020 co-financed by European Social Fund). The calculations were performed at Świerk Computing Centre, National Centre for Nuclear Research, Świerk, Poland. The authors are most grateful for the computational grant at this facility.

### Conflict of interest

The authors declare no conflict of interest.

### References

- Pillaiyar T, Meenakshisundaram S, Manickam M. Recent discovery and development of inhibitors targeting coronaviruses. *Drug Discov Today* 2020; 25: 668-88.
- Yang H, Bartlam M, Rao Z. Drug design targeting the main protease, the Achilles heel of coronaviruses. *Curr Pharm Des* 2006; 12: 4573-90.
- Dai W, Zhang B, Su H, et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science* 2020; eabb4489. DOI: 10.1126/science.abb4489.
- Zhang L, Lin D, Sun X, et al. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved  $\alpha$ -ketoamide inhibitors. *Science* 2020; 368: 409-12.
- Jin Z, Du X, Xu Y, et al. Structure of M<sup>pro</sup> from SARS-CoV-2 and discovery of its inhibitors. *Nature* 2020; 582: 289-93.
- [www.cov2mpro.com](http://www.cov2mpro.com).
- Sterling T, Irwin JJ. ZINC 15 – ligand discovery for everyone. *J Chem Inf Model* 2015; 55: 2324-37.
- Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem* 2009; 30: 2785-91.
- Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010; 31: 455-61.
- Allen WJ, Fochtman BC, Balias TE, Rizzo RC. Customizable de novo design strategies for DOCK: application to HIVgp41 and other therapeutic targets. *J Comput Chem* 2017; 38: 2641-63.
- Allen WJ, Balias TE, Mukherjee S, et al. DOCK 6: impact of new features and current docking performance. *J Comput Chem* 2015; 36: 1132-56.
- Jacobs J, Grum-Tokars V, Zhou Y, et al. Discovery, synthesis, and structure-based optimization of a series of N-(tert-butyl)-2-(N-arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent noncovalent small molecule inhibi-

- tors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3. *J Med Chem* 2013; 56: 534-46.
13. Turlington M, Chun A, Tomar S, et al. Discovery of N-(benzo[1,2,3]triazol-1-yl)-N-(benzyl)acetamido phenyl) carboxamides as severe acute respiratory syndrome coronavirus (SARS-CoV) 3CLpro inhibitors: identification of ML300 and noncovalent nanomolar inhibitors with an induced-fit binding. *Bioorg Med Chem Lett* 2013; 23: 6172-7.
  14. Tomar S, Johnston ML, St. John SE, et al. Ligand-induced dimerization of Middle East Respiratory Syndrome (MERS) coronavirus nsp5 protease (3CL pro). *J Biol Chem* 2015; 290: 19403-22.
  15. St. John SE, Tomar S, Stauffer SR, Mesecar AD. Targeting zoonotic viruses: structure-based inhibition of the 3C-like protease from bat coronavirus HKU4 – the likely reservoir host to the human coronavirus that causes Middle East Respiratory Syndrome (MERS). *Bioorg Med Chem* 2015; 23: 6036-48.
  16. Cereto-Massagué A, Guasch L, Valls C, Mulero M, Pujadas G, Garcia-Vallvé S. DecoyFinder: an easy-to-use python GUI application for building target-specific decoy sets. *Bioinformatics* 2012; 28: 1661-2.
  17. Durrant JD, McCammon JA. BINANA: a novel algorithm for ligand-binding characterization. *J Mol Graph Model* 2011; 29: 888-93.
  18. Davies M, Nowotka M, Papadatos G, et al. ChEMBL web services: streamlining access to drug discovery data and utilities. *Nucleic Acids Res* 2015; 43: W612-20.
  19. Mendez D, Gaulton A, Bento AP, et al. ChEMBL: towards direct deposition of bioassay data. *Nucleic Acids Res* 2019; 47: D930-40.
  20. Zhang J, Pettersson HI, Huitema C, et al. Design, synthesis, and evaluation of inhibitors for severe acute respiratory syndrome 3C-like protease based on phthalhydrazide ketones or heteroaromatic esters. *J Med Chem* 2007; 50: 1850-64.
  21. Reiner Ž, Hatamipour M, Banach M, et al. Statins and the COVID-19 main protease: in silico evidence on direct interaction. *Arch Med Sci* 2020; 16: 490-6.
  22. Azeez S, Ghalib Alhashim Z, Al Otaibi WM, et al. State-of-the-art tools to identify druggable protein ligand of SARS-CoV-2. *Arch Med Sci* 2020; 16: 497-507.
  23. Borgio JF, Alsuwat HS, Al Otaibi WM, et al. State-of-the-art tools unveil potent drug targets amongst clinically approved drugs to inhibit helicase in SARS-CoV-2. *Arch Med Sci* 2020; 16: 508-18.