Design, synthesis and evaluation of potential inhibitors of main protease (Mpro) of SARS-CoV-2

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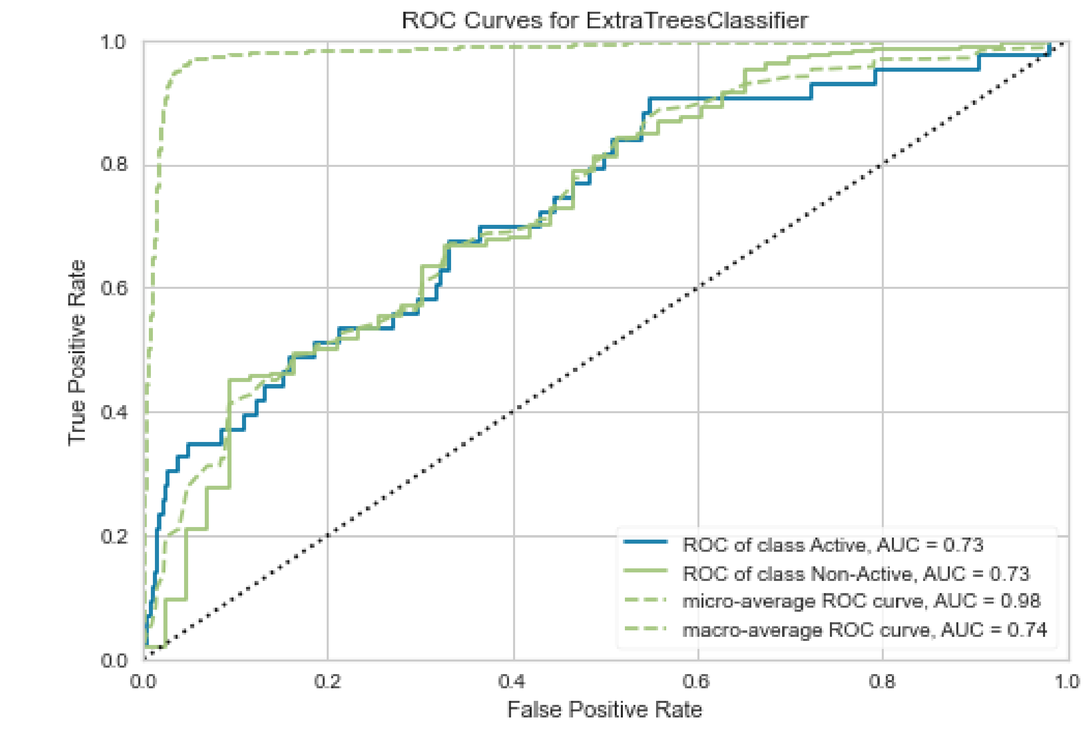
Introduction

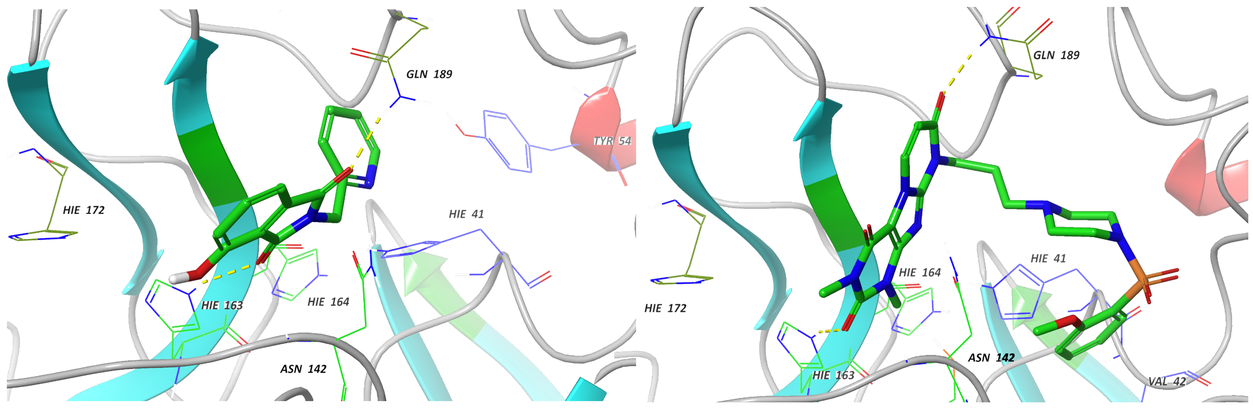
The World Health Organization (WHO) on March 12, 2020, declared a pandemic state of the global epidemic caused by a new coronavirus SARS-CoV-2. The identification of Main protease (Mpro) of SARS-CoV-2 as a drug target led to a speed-up drug design and discovery process. However, to indicate the activity against (Mpro) during virtual screening, an effective algorithm is needed. In our studies, we proposed a virtual screening protocol combining molecular modeling and machine learning techniques that will allow us to discover novel inhibitors of SARS-CoV-2 Mpro.

Virtual Screening

For our research, we used the PYCARET software to integrate molecular descriptors (Padel) and molecular docking (Induce Fit Docking; Maestro–Schrödinger) results into a classification model.
Models were built on a group of 8702 marketed drugs from a screening campaign against SARS-CoV-2 Mpro (10.6019/CHEMBL4495564). Compounds that inhibit the enzyme over 50% at 20 µM concentration were categorized as active. The final model based on the extra trees algorithm (AUC = 0.73) significantly outperformed molecular docking alone (AUC = 0.61)



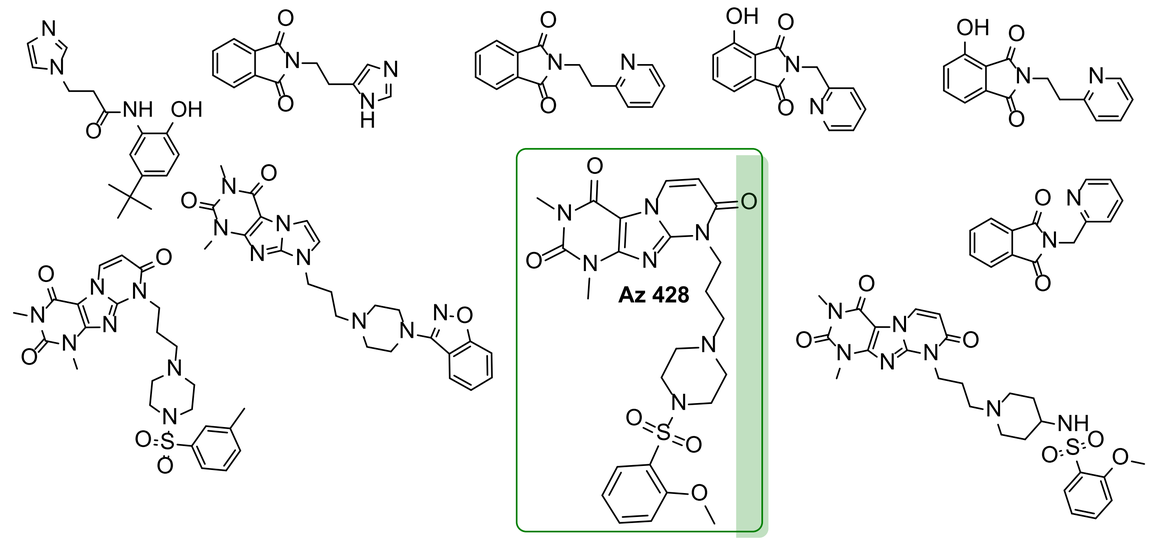
The binding mode of compounds classified as active was very similar to that of the reference compound crystallized in the 7B2J Mpro complex (compound 5, IC_{50} = 20 µM). Each of the molecules formed a hydrogen bond with His163 and was additionally stabilized by a hydrogen bond with Gln183 or Glu166. As in the case of the reference compound, the key to enzyme inhibition seems to be the ability to restrict access to S2 pocket in the His41 region by ligand.



NAWA4 **Az 428**

Chemistry

Based on results from virtual screening, derivatives of mono-, di- and tri-heterocyclic azole systems with various substituents (amine, amide, or ester) were synthesized in average to good yields (30-75%). Compounds were prepared in a multistep synthetic sequence of alkylation reaction, hydrolysis, and/or cyclocondensation. The structures of newly synthesized compounds were confirmed by ^{1}H NMR and ^{13}C-NMR spectra, LC/MS, and elemental analysis. The investigated compounds were in vitro tested as free bases.



Cell cytotoxicity

Compounds were tested on Vero E6 cells and viability was determined by MTT assay, in order to select the doses to be tested on SARS-CoV-2 infected cells. IC_{50} on cells (CC_{50}) and CC_{10} (concentration which inhibit 10% of cell growth) (Table 1).
Based on the results of CC_{10}, compound doses were selected as follows: Az428 (CC_{10}>15 µM) was tested at 20 µM, Az416 and NAWA8 (CC_{10} <2 µM) were tested at 1 µM, the other compounds (2 µM <CC_{10}<15 µM) were tested at 10 µM.

Anti-SARS-CoV-2 activity

Viral titer was determined by measuring the viral RNA load in the cell supernatant of control cells and cells treated with different concentrations of compounds. Results summarized in Table 1 are expressed as mean ΔCt (mean of at least three experiments), and as % inhibition of SARS-CoV-2 replication. The most active compound (Az 428) showed 52.6% inhibition of SARS-CoV-2 replication at the dose 20 µM.

Table 1. Cell cytotoxicity and anti-SARS-CoV-2 activity of selected compounds

	Citotoxicity against uninfected VeroE6 cells		Activity against SARS-CoV-2 infected cells		
	CC50 (µM)	CC10 (µM)	Tested concentrations	Mean Delta Ct	% inhibition of SARS-CoV-2 replication
Az 541	34.2	5.4	10 µM	-0.24 ± 0.01	13.1 ± 0.9
Az 523	39.5	5.8	10 µM	-0.41 ± 0.49	28.2 ± 14.9
Az 1051	53.7	5.5	10 µM	0.50 ± 0.15	Not Active
Az 428	>100	17.8	20 µM	-1.80 ± 0.54	52.6 ± 27.1
	-	-	2 µM	-0.10 ± 0.52	19.0 ± 9.4
Az 416	0.2	0.1	1 µM	-0.15 ± 0.47	Not Active
NAWA 4	63.7	5.1	10 µM	-0.79 ± 0.41	31.1 ± 8.5
NAWA 5	>100	12.1	10 µM	0.07 ± 0.07	10.8 ± 19.5
NAWA 7	89.3	7.2	10 µM	-0.72 ± 0.13	27.5 ± 2.5
NAWA 8	32.2	1.0	1 µM	0.49 ± 0.28	Not Active
NAWA 9	89.3	4.3	10 µM	0.19 ± 0.03	Not Active

Conclusion

The application of the proposed virtual screening protocol allowed for the selection of new compounds that can be used as a starting point for the development of SARS-Cov-2 Mpro inhibitors.

Acknowledgements

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