Potential COVID-19 protease inhibitors: Repurposing FDA-approved drugs

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Short Title: COVID-19 protease inhibitors: Repurposing FDA drugs.

ABSTRACT

Using as a template the crystal structure of COVID-19 protease, we developed a pharmacophore model of functional centers of the protease inhibitor-binding pocket. With this model, we conducted data mining of the conformational database of FDA-approved drugs. This search brought 64 compounds that can be potential inhibitors of COVID-19 protease. The conformations of these compounds undergone 3D fingerprint similarity clusterization. Then we conducted docking of possible conformers of these drugs to the binding pocket of protease. We also conducted the same docking of random compounds. Free energies of the docking interaction for the selected compounds were clearly lower than random compounds. Three of the selected compounds were carfilzomib, cyclosporine A, and azithromycin—the drugs that already are tested for COVID-19 treatment. Among the selected compounds are two HIV protease inhibitors and two hepatitis C protease inhibitors. We recommend testing of the selected compounds for treatment of COVID-19.

One Sentence Summary: Using pharmacophore-based data mining and computational docking, we selected 64 potential COVID-19 protease inhibitors.

INTRODUCTION

Unfolding COVID-19 pandemics shows a necessity of rapid finding of drug-candidates that could be used immediately in numerous world sites of virus activity. One of the important targets of antiviral treatment is COVID-19 protease. Scientists already developed drugs that target other viral proteases: telaprevir acting against hepatitis C virus (HCV) protease, indinavir acting against HIV protease, glecaprevir acting against HCV protease. Therefore, this approach sounds promising. Recently published structure of COVID-19 protease (PDB ID 6Y2F) [1] created an opportunity for the similar approach to develop its inhibitor. The potential inhibitors of this protease were alpha-ketoamides with modifications added based on the protein–compound interactions [1].

METHODS

We used the potential binding site of the ligand in COVID-19 protease (PDB ID 6Y2F) [1] for construction of the protein-based pharmacophore of the potential fictional centers that would bind to the residues in the pocket. Using Molecular Operating Environment (MOE, CCG, Montreal, Canada), we constructed the pharmacophore model including nine features: two donors, three acceptors, one donor/acceptor, and three hydrophobic features.

Based on developed pharmacophore, we provided pharmacophore search with nine of nine, eight of nine, and seven of nine features on our conformational database (DB) of FDA-approved drugs, containing around 2500 drugs and 600 000 conformations.

Then we clustered selected 64 compounds, using Fingerprint Clusters of MOE Database Viewer with a fingerprint GpiDAPH3 and similarity–overlap parameter *SO* = 45%.

For a docking of selected compounds, we used the crystal structure of the SARS-CoV-2 (Protein Data Bank entry, 6Y2F) imported into the MOE program. Then a pocket was defined, selecting all atoms within 4.5 Å of the α-ketoamide inhibitor. Then, the compounds were docked into the pocket using the Triangle Matcher method and London dG scoring for placement; and the Rigid Receptor method and GBVI/WSA dG scoring for refinement.

The random control compounds were selected by a 100-compound, simple-random subset of the ZINC DB of drug-like compounds.

RESULTS

We used the potential binding site of the ligand in COVID-19 protease (PDB ID 6Y2F) [1] including majority of possible interactions of the protein with a potential ligand for creation of the protein-based pharmacophore model with the potential fictional centers that would bind to the residues in the pocket (Figure 1A). Using Molecular Operating Environment (MOE, CCG, Montreal, Canada), we constructed the pharmacophore model including nine features: two donors, three acceptors, one donor/acceptor, and three hydrophobic features (Figure 1B). Based on developed pharmacophore to select potential drug-candidates, we provided pharmacophore search with 9 of 9, 8 of 9, and 7 of 9 features on our conformational database (DB) of FDA-approved drugs, containing around 2500 drugs and 600 000 conformations. Then we clustered selected 64 compounds, using Fingerprint Clusters of MOE Database Viewer with a fingerprint GpiDAPH3 and similarity–overlap parameter *SO* = 45% that elucidated five clusters containing five and more compounds, along with several clusters containing one compound (Table 1). Flexible alignment of each cluster was used to illustrate compounds' common features (Figure 2).

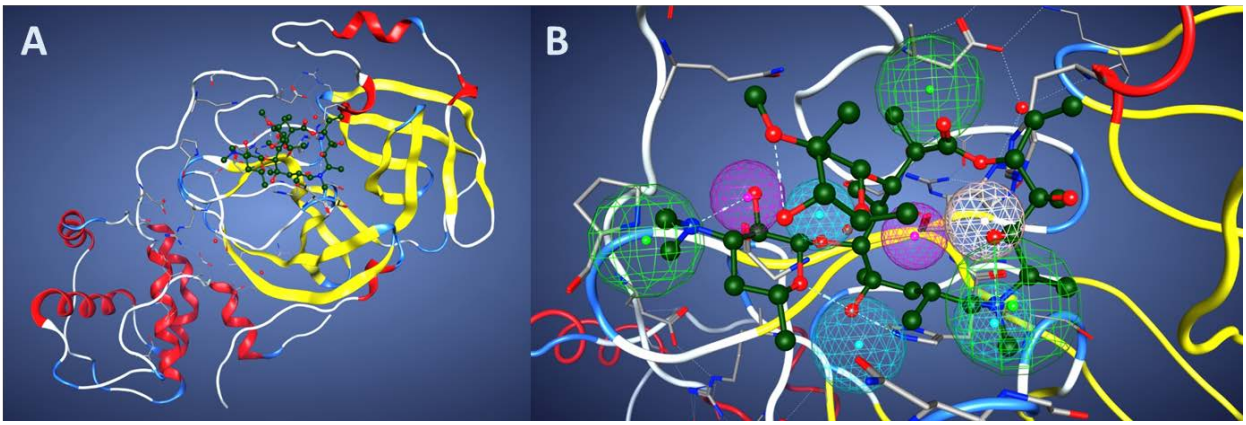

Figure 1. Azithromycin binding position in COVID-19 protease. (A) A position of azithromycin in the pocket of COVID-19 protease. (B) Azithromycin in the Pharmacophore model of a potential protease inhibitor. The model contains nine functional centers: two donors, two acceptors, one donor/acceptor center, and three hydrophobic centers.

Interesting to note that this selection contained azithromycin already tested and showed promising results in treatment of COVID-19 infection and three known inhibitors of viral proteases: telaprevir (HCV), glecaprevir (HCV), and indinavir (HIV).

Table 1. Drug-candidates clusterized by fingerprint similarity alignment. Section 'S' containing compounds that are selected as single-compound cluster

Cluster	A	B	C	D	E	S
	Bleomycin	Azithromycin	Doxycycline	Iopamidol	Cangrelor	Amprenavir
	Carbetocin	Dibekacin	Lymecycline	Ioversol	Citicoline	Bemotrizinol
	Viomycin	Calcium glubionate	Chlortetracycline	Iodixanol	Flavin Adenine Dinucleotide	Betrixaban
	Atosiban	Amikacin	Demeclocycline	Iomeprol	Hesperidin	Cefpiramide
	Boceprevir	Framycetin	Eravacycline	Iopromide	Ticagrelor	Elagolix
	Carfilzomib	Gentamicin	Methacycline			Eluxadoline
	Caspofungin	Micronomicin	Minocycline			Indinavir
	Etelcalcetide	Plazomicin	Omadacycline			Ioxaglic Acid
	Glecaprevir	Ribostamycin	Rolitetracycline			Macitentan
	Goserelin	Roxithromycin	Sarecycline			Mangafodipir
	Icatibant	Steviolbioside	Tetracycline			Mithramycin
	Octreotide	Streptomycin	Tigecycline			Neratinib
	Pentagastrin	Tobramycin				Pantethine
	Sincalide					Rutin
	Telaprevir					Valrubicin
	Polymixin B					1,2-icosapentoyl-sn-glycero-3-phosphoserine*
	Cyclosporine A					

*Approved, Experimental Drug: 1,2-icosapentoyl-sn-glycero-3-phosphoserine

Figure 2A–D shows the flexible alignments of clusters B–E. Cluster A containing 15 compounds does not have specific features that can be visually elucidated. Nevertheless, these compounds have similarities by 3D maps of their functional centers.

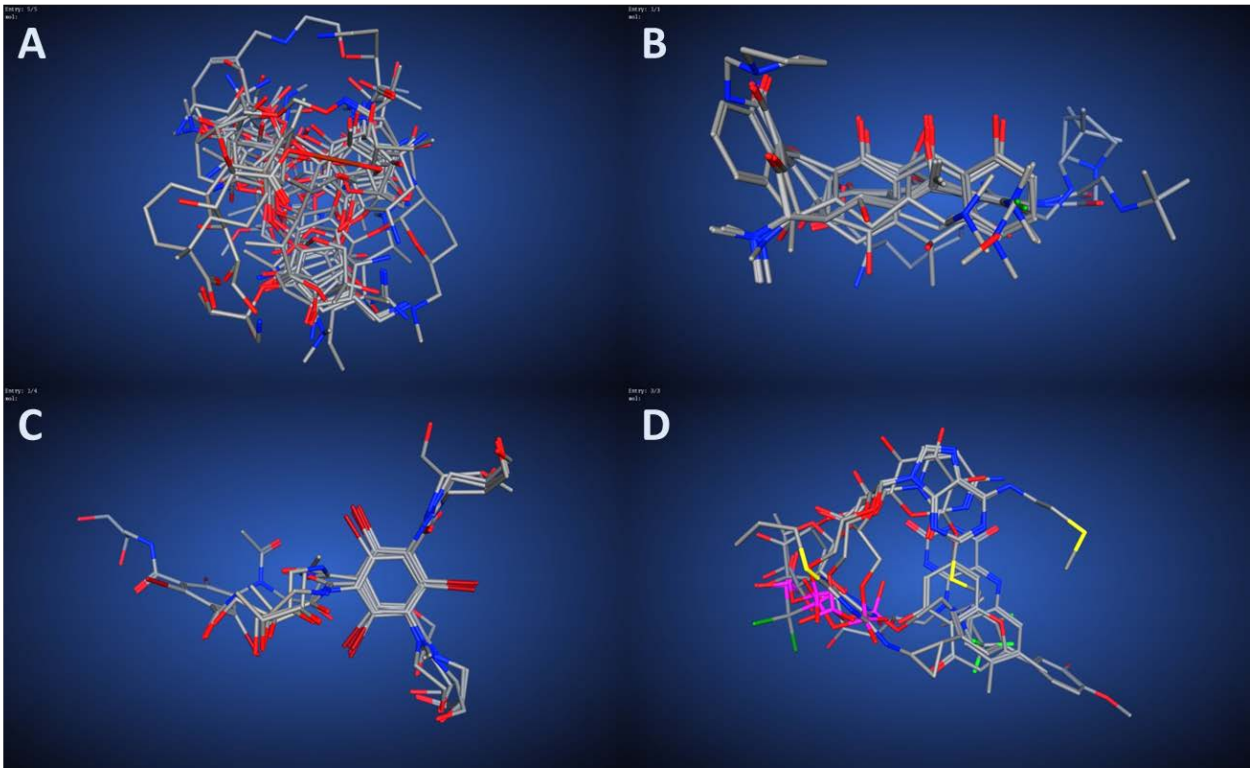

Figure 2. Flexible alignments of compounds in clusters of the selected by the pharmacophore-based search of possible drug-candidates in the conformational database of FDA-approved drugs. **(A)** Cluster B (13 compounds), **(B)** cluster C (11 compounds), **(C)** cluster D (5 compounds), **(D)** Cluster E (5 compounds).

Cluster A contains 15 drugs. Note that there are two hepatitis C viral proteases inhibitors: Boceprevir and Telaprevir. Other drugs have various targets, but they are linked together in the cluster by similarity of the 3D coordinates of their functional centers.

Cluster B contains mostly aminoglycoside antibiotics that inhibit 30S and/or 50S subunits of bacterial ribosome, this way inhibiting translation of mRNA and consequently synthesis of bacterial proteins.

Cluster C contains mostly tetracycline group of antibiotics. Note that these drugs also bind to ribosomal subunit S30 and prevent bacterial proteins synthesis.

Cluster D contains iodine-containing contrasting agents. Their use as the antiviral drugs may be questionable, but still can be a hint for development of other compounds.

Cluster E contains a group of phosphorus-containing compounds of various current medical use.

Separate group of compounds contains drugs that were included in a “cluster of one molecule “. Note two HIV protease inhibitors indinavir and amprenavir are in this group.

To further range, we ran flexible docking to the binding site of COVID-19 protease of the selected drugs and random compounds. For a docking of selected compounds, we used the crystal structure of the SARS-CoV-2 (Protein Data Bank entry, 6Y2F) imported into the MOE program. Then a pocket was defined, selecting all atoms within 4.5 Å of the α-ketoamide inhibitor. Then, the compounds were docked into the pocket using the Triangle Matcher method and London dG scoring for placement; and the Rigid Receptor method and GBVI/WSA dG scoring for refinement. The random control compounds were selected by a 100-compound, simple-random subset of the ZINC DB of drug-like compounds.

Figure 3 shows the values of docking free energies of the selected and random compounds. The energies of interaction with protease are shown in Table 2. One can see that cluster A drugs are on the top of the table. We want to note that the values of energies in the table do not have to be overestimated. Specialists know that binding positions of ligands in the pockets of proteins in many cases do not have minimal energies.

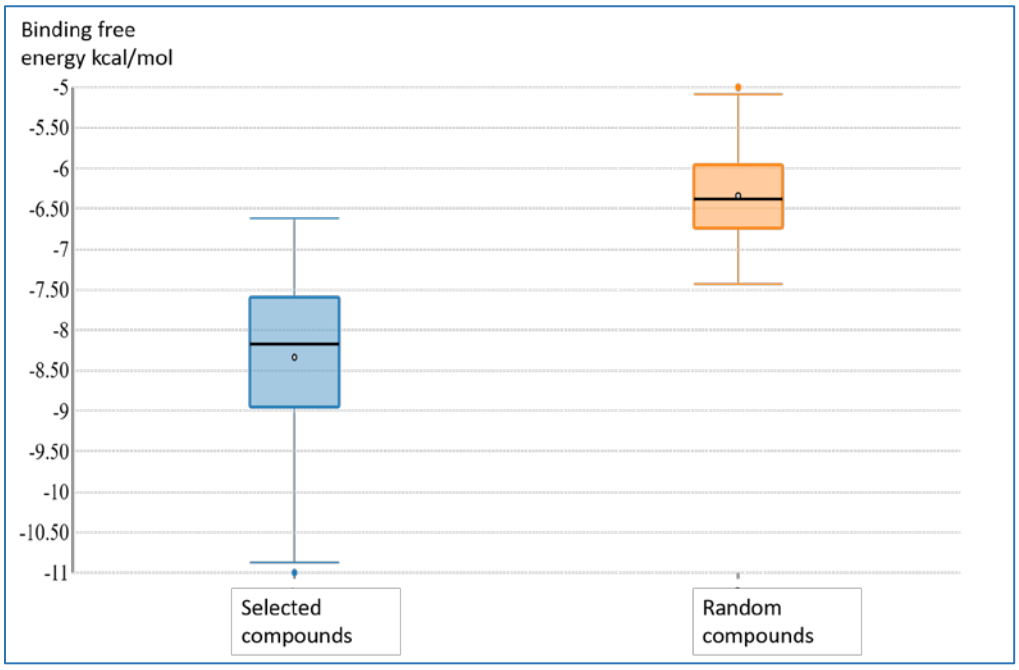

Figure 3. Free energies of docking interactions of selected and random compounds with COVID-19 protease. Medians of the selected and random compounds are −8.17 and −6.37 correspondingly.

Table 2. List of docked compounds sorted by their energies of interaction with COVID-19 protease in the flexible docked positions

Name	DFE* kcal/mol	Cluster	Name	DFE* kcal/mol	Cluster
Bleomycin	-11.58	A	Steviolbioside	-8.20	B
Mithramycin	-10.87	S	Rutin	8.13	S
Goserelin	-10.51	A	Neratinib	-8.07	S
1,2-icosapentoyl-sn-glycero-3-phosphoserine**	-10.48	S	Bemotrizinol	-8.04	S
Cyclosporin A	-10.19	A	Ioversol	-7.96	D
Polymixin B	-10.15	A	Iomeprol	-7.95	D
Carbetocin	-9.81	A	Citicoline	-7.90	E
Glecaprevir	-9.76	A	Tigecycline	-7.84	C
Caspofungin	-9.74	A	Eluxadoline	-7.84	S
Carfilzomib	-9.61	A	Gentamicin	-7.82	B
Atosiban	-9.43	A	Amikacin	-7.78	B
Iodixanol	-9.31	D	Amprenavir	-7.73	S
Icatibant	-9.28	A	Lymecycline	-7.60	C
Roxithromycin	-9.23	B	Tobramycin	-7.59	B
Framycetin	-9.14	B	Ribostamycin	-7.59	B
Pentagastrin	-9.03	A	Indinavir	-7.59	S
Micronomicin	-9.03	B	Etelcalcetide	-7.58	A
Azithromycin	-8.96	B	Betrixaban	-7.58	S
Octreotide	-8.93	A	Streptomycin	-7.50	B
Telaprevir	-8.89	A	Sarecycline	-7.45	C
Hesperidin	-8.89	E	Iopromide	-7.34	D
Flavin Adenine Dinucleotide	-8.82	E	Omadacycline	-7.32	C
Ioxaglic Acid	-8.60	S	Demeclocycline	-7.11	C
Elagolix	-8.56	S	Macitentan	-7.09	S
Valrubicin	-8.47	S	Tetracycline	-7.09	C
Cefpiramide	-8.39	S	Eravacycline	-7.06	C
Pantethine	-8.37	S	Rolitetracycline	-6.98	C
Cangrelor	-8.33	E	Calcium glubionate	-6.93	B
Viomycin	-8.33	A	Iopamidol	-6.92	D
Ticagrelor	-8.32	E	Doxycycline	-6.82	C
Boceprevir	-8.30	A	Chlortetracycline	-6.74	C
Plazomicin	-8.26	B	Methacycline	-6.62	C

*Docking free energy

**Approved, Experimental Drug

CONCLUSION

Based on a crystal structure of COVID-19 protease with the inhibitor we developed a pharmacophore model of the binding pocket of this protein. Using this model, we browsed our conformational database of FDA-approved drugs and obtained 64 hits that were clusterized and then used for flexible docking to the protease pocket. The obtained drug list includes HIV and Hepatitis C proteases inhibitors, set of antibiotics targeting 30S and 50S bacterial ribosomes, and azithromycin already demonstrated some activity in COVID-19 therapy [2]. We also note that the recent publication of the COVID-19 therapies include two inhibitors of HIV proteases, along with two drugs that were elucidated in our study—carfilzomib and cyclosporine A [3]. Currently we work on the mining of the extended DB having around 9000 FDA-approved drugs and natural compounds, but thinking about emergency situation in the world, we want to publish the obtained data ASAP.

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Author contributions: IFT and VLK introduced initial idea of the project, VLK conducted pharmacophore development and databases searches, IFT and VLK conducted interpretation of the results, DH conducted computational docking, IFT and VLK wrote the article.

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