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In-silico analysis on potential anti-SARS-CoV-2 protease agents by structure-based docking and cheminformatics

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ABSTRACT

The treatment of COVID-19 patients has caused serious problems for the scientists. There are many routinely used drugs in clinical settings without definite effects, and more studies should be done so as to find a successful treatment for COVID-19. Our aim was to evaluate four suggested chemicals using virtual analysis tools based on the drug-screening approach and application of cheminformatics, pharmacotoxicology and docking.

Four repurposed drugs rizatriptan, dasabuvir, pravastatin, and empagliflozin were used in this study. The 3D structure of COVID-19 Main Protease (M Pro) was obtained from protein data bank (PDB) with PDB code: 6LU7, as the target of binding site screening. Besides, cheminformatics, pharmacotoxicology and human proteins targets for each drug was evaluated using SwissADME interface, SwissTarget Prediction web server, toxicity estimation software tool (T.E.S.T) and Toxtree-v3.1.0.1851 offline software. The docking scores (DOS) were -139.399, -125.707, -102.183 and -99.6642 for dasabuvir, rizatriptan, empagliflozin and pravastatin, respectively. In addition, the quantitative structure-activity relationship (QSAR) and pharmacotoxicologic evaluations showed that dasabuvir had more acceptable results than the others. Human protein target-exploration showed that rizatriptan interacted with G protein-coupled receptor and kinase enzymes, pravastatin targeted the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, while empagliflozin interacted with sodium/glucose cotransporters (SLC). But, dasabuvir targeted human protein with too low scores.

Virtual screening applied to four potential anti-COVID-19 drugs showed that dasabuvir could be a safer and efficient agent, regarding pharmacotoxicology and therapeutic purposes. However, virtually screened agent/s should be evaluated by experimental models for ultimate confirmation.

Keywords: 3C-like proteinase, Coronavirus, Cheminformatics, Molecular Docking Simulation, Quantitative Structure-Activity Relationship

Introduction

The binding between a protein and its ligand is based on complex interaction at defined sites. These interactions have major roles in docking mechanism [1]. Following docking of protein-ligand, there are atomic interactions between them with subsequent changes in protein activity [2]. Calculations of atomic interactions and potential energy should be evaluated as a function of geometrical atomic data with several other parameters such as covalent bond-stretching, angle-bending, torsion potentials, or non-bond

parameters including Lennard-Jones repulsion and dispersion as well as Coulomb electrostatic forces [3]. Moreover, this molecular modeling could assist the investigators in finding the binding mode of protein-ligand as a structural feature of their interaction [4]. Also, structure–activity studies were reported to be essential in finding the potential therapeutic agent [5], as well as predicting the binding model of the active site-ligand interaction, which could help in identifying new ligands [6].

Protein-ligand docking has been done for several viruses such as Ebola Virus [7], influenza [8], SARS-CoV [9], MERS [10] and SARS-CoV-2 [11]. Recently, Ton et al. reported a method for identifying 1.3 billon compounds for inhibition of SARS-CoV-2 Main Protease (also known as 3CL protease or M pro), which one thousand compounds were selected for testing as anti-SARS-CoV-2 [12]. Moreover, Ekins et al. reported repurposing approved drugs such as atazanavir and lopinavir as inhibitors for SARS-CoV-2 virus main protease, whereas rizatriptan, dasabuvir, pravastatin, and empagliflozin were documented as inhibitors to spike-ACE2 interface [13]. In this study, we intended to evaluate the docking of rizatriptan, dasabuvir, pravastatin, and empagliflozin to main protease of SARS-CoV-2. In addition, the toxicology and pharmacokinetic properties were evaluated for the addressed ligands so as to introduce the best therapeutic option with acceptable or low side effects. Further, the SwissTarget prediction search tool assisted us in this drug-screening approach.

Materials and methods

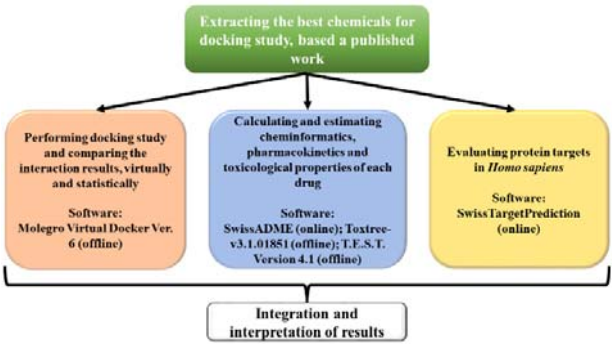
In this study three types of virtual analysis were carried out that are briefly shown in Figure 1.

Docking study, ADME (pharmacokinetic and toxicology) calculations and protein target predictive were done in three different stages.

Docking study

The docking study was done so as to evaluate the interaction of COVID-19 protease with the four selected FDA-approved drugs: rizatriptan, dasabuvir, pravastatin and empagliflozin. SDF format and the 3-dimensional structures (3D) of compounds were downloaded from ZINC Docking database [14].

Figure 1: Study steps and software tools for performing the virtual analysis and integration of data followed by interpretation of results.



The 3D of COVID-19 main protease was from the protein data bank (PDB) repository with the PDB code 6LU7 [15]. We used Molegro Virtual Docker (MVD) Ver.6 for the docking study, which was performed by this order: SDF file format and protein 3D-structures were imported into the software without water and ligand molecules, in order to explore the cavities on the protein surface. A grid space (with 0.3 Å) was selected, we set the search algorithm based on energy-minimization and optimization of hydrogen bonds, ran the software and saved ten docking top results for each ligand and subsequent analysis. The 3D structure of 6LU7 contains Leucinamide (N-[(5-METHYLISOXAZOL-3-YL) CARBONYL] ALANYL-L-VALYL-N~1~-((1R,2Z)-4-(BENZYLOXY)-4-OXO-1-[[(3R)-2-OXOPYRROLIDIN-3-YL] METHYL]BUT-2-ENYL)-L-LEUCINAMIDE), that is an inhibitor molecule. We used the binding pocket that was detected by leucinamide in a crystallography. The docking results were obtained as MolDoc scores (DOS). Ultimately, the one-way statistical analysis of variances (ANOVA) was used in comparing the ligand-protein interactions with 95% confidence interval.

Cheminformatics analysis and target forecasting

The quantitative structure-activity relationship (QSAR) is a technique for predicting the activity and reaction of the molecules based on structural analysis. Adsorption, distribution, metabolism, excretion (ADME) and the pharmacokinetic properties of the chemical compounds help to draw a rough view of toxicity characteristics of

the drug of interest. QSAR is widely used for ADME determination using cheminformatics software. In this regard, the anticipated outcome of toxicity and pharmacokinetic characteristic for the compounds and probable genetics, metabolism and hazardous complications in animal model (rat) and human beings. In the present study, we used QSAR approach to determine the ADME for each studied ligand using online and offline computational tools. We also used SwissADME interface [16-18], SwissTarget Prediction (STP) web server [19] available at: http://www.swisstargetprediction.ch/, toxicity estimation software tool (T.E.S.T) and Toxtree-v3.1.0.1851 offline software [20-22]. STP was used in forecasting protein target of human for each ligand. The major variables evaluated by the aforementioned estimation tools include: Physical properties, water solubility, pharmacokinetics, drug-likeness, oral rat LD50, bioaccumulation factor, developmental toxicity, mutagenicity, carcinogenicity, biodegradability, DNA and protein binding and toxicity class based on Cramer rules, in addition to the cytochrome P-450 metabolism predicted products. The cheminformatics were estimated, briefly, SMILES format of each ligand was introduced to each software or server followed by running the calculations and selecting the needed information. There are vast majority of data that were not in the direction of the current study objectives and were not considered in the results.

Predicting protein targets in human body

The swissTarget prediction webserver was used in predicting the potential targets for each intended compound. This online software searches a large collection of compounds (376342) that are experimentally reactive to macromolecular (approximately 3068) [23]. For this type of evaluation, SMILE format formula of each chemical structure was introduced to the online software, and the targets were explored for Homo sapiens species. Fifty targets were selected to be shown in pie-charts and 15 top scores are presented in the related tables.

Integration of results

The docking and cheminformatics results were integrated so as to evaluate either benefits or hazards of each ligand and introducing the best one as a desirable therapeutic agent.

Results

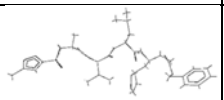
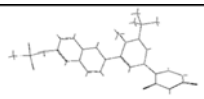
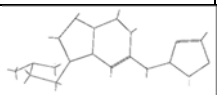
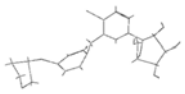
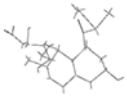
Docking study

DOS are representative of calculated ligand-receptor/protein interaction energy; therefore, more negative scores mean more favorable binding tendency. Table 1 shows detailed information of the priority of the best docking scores, hydrogen bond energy, chemical forms of each drug, related unique Zinc docking code and FDA-approval. Except leucinamide, which has equal DOS to -214.902 (Figure 2), the best drug interacting with COVID-19 main protease was dasabuvir with DOS= -139.399 (Figure 3) followed by rizatriptan with DOS= -125.707 (Figure 4), empagliflozin with DOS= -102.183 (Figure 5) and finally pravastatin, with DOS= -99.6642 (Figure 6). Figure 7, is a comparative box plot for comparison of mean±SD of MolDoc scores obtained for 10 main positions of interactions for each ligand.

QSAR and ADME results; prediction of pharmacotoxicology properties

The Cheminformatics evaluation and the pharmacological properties of dasabuvir, rizatriptan, empagliflozin and pravastatin are summarized in Table 2. Except dasabuvir that was moderately soluble, others were water-soluble. Lipophilicity, molecular weight, solubility and topological polar surface area were suitable for all of the chemicals but rizatriptan had better values than the others. Moreover, rizatriptan could penetrate to the blood-brain barrier, and could be extruded from the cell by the ATP-binding cassette proteins. While rizatriptan, empagliflozin and pravastatin could be substrate for P-glycoproteins, they could be ineffective against the virus and toxic to the cell. Four therapeutic compounds inhibit one or more types of cytochrome detoxification enzymes. Compared to the other three drugs, empagliflozin had more negative Log Kp with better skin permeation [24].

Table 1: Chemical compounds docked with the main protease of COVID-19 crystallographic structure [PDB code= 6LU7]. The parameters used in the table are based on chemical structure, hydrogen bond energy, docking score, Zinc docking database unique code and FDA approval. The drugs listed are based on descending Docking scores.

HBond	MolDoc Score	Structure and Zinc Code	FDA Approved for/as
-9.29529	-214.902	 Leucinamide; included in the PDB structure	Not applicable
-6.22906	-139.399	 Dasabuvir; ZINC95616937	treatment of hepatitis C
-3.335	-125.707	 Rizatriptan; ZINC5895	treatment of migraine headaches
-5.27574	-102.183	 Empagliflozin; ZINC36520252	Treatment of type 2 diabetes
-5.55218	-99.6642	 Pravastatin; ZINC3798763	preventing cardiovascular disease in those at high risk and treating abnormal lipids

In cheminformatics studies, other important properties that routinely are evaluated for drugs are Lipinski criteria (LC) and bioavailability score. All evaluated chemicals had desirable LC with acceptable toxicity, because their molecular weight are less than 500 g/mol, MLOGP≤ 4.15, the number of nitrogen and oxygen atoms≤ 10; and the number of NH or OH ≤ 5 [25]. Also, the bioavailability quality for all of the drugs were similar, approximately 55%.

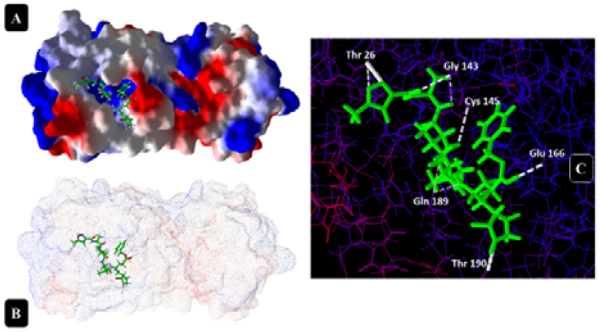


Figure 2. Leucinamide interaction with 6LU7 protein at the best position with the highest DOS (**A** and **B**); DOS= -214.902. As it is obvious, at this site, leucinamide makes 9 hydrogen bonds (**C**).

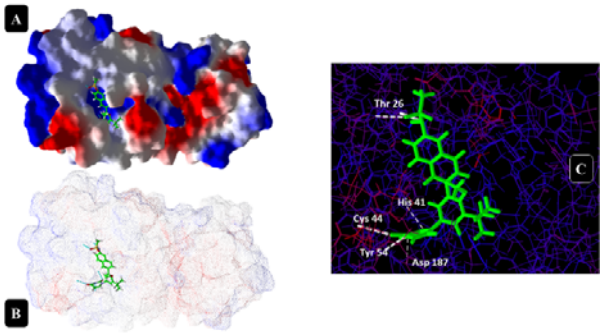


Figure 3. Dasabuvir interaction with 6LU7 protein at the best position with the highest DOS (**A** and **B**); DOS= -139.399. As it is obvious, at this site, dasabuvir makes 6 hydrogen bonds (**C**).

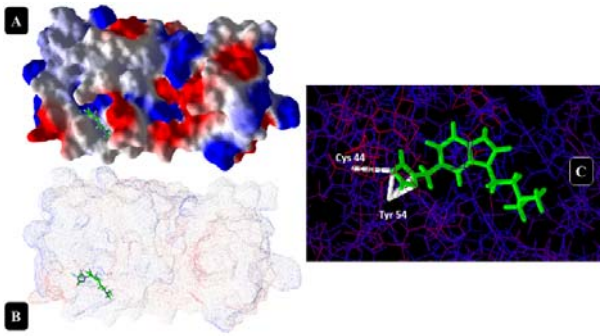


Figure 4. Rizatriptan interaction with 6LU7 protein at the best position with the highest DOS (**A** and **B**); DOS= -125.707. As it is obvious, at this site, rizatriptan makes 4 hydrogen bonds (**C**).

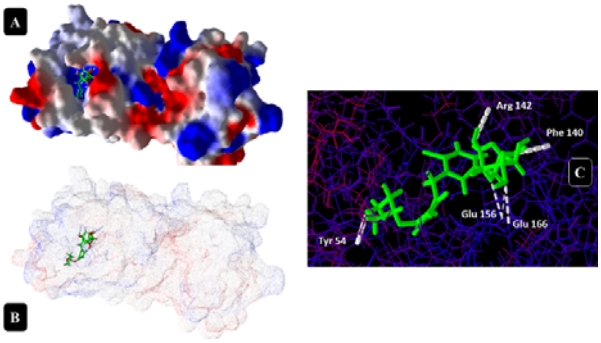


Figure 5. Empagliflozin interaction with 6LU7 protein at the best position with the highest DOS (**A** and **B**); DOS= -102.183. As it is obvious, at this site, empagliflozin makes 5 hydrogen bonds (**C**).

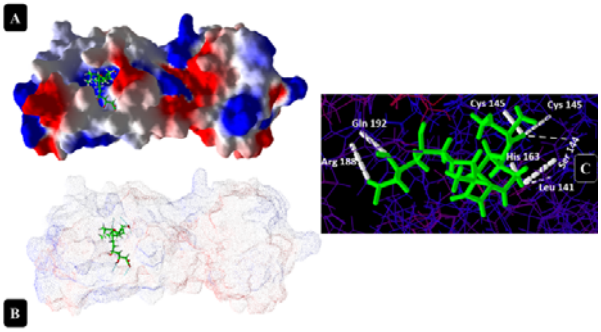


Figure 6. Pravastatin interaction with 6LU7 protein at the best position with the highest DOS (**A** and **B**); DOS= -99.6642. As it is obvious, at this site, pravastatin makes 6 hydrogen bonds (**C**).

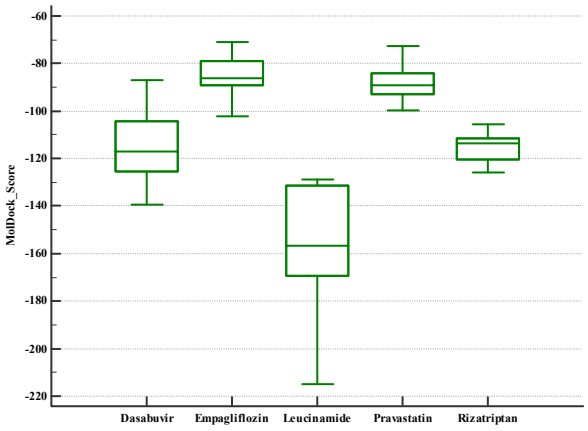


Figure 7: Comparative box plot for MolDock scores obtained from docking study; except leucinamide which was the main inhibitor and present in the 6LU7 in crystallography structure, the order of best scores were obtained for Dasabuvir, rizatriptan, empagliflozin and ultimately pravastatin, respectively. However, it should be noted that the leucinamide is not considered as a drug and only is evaluated for proper comparisons.

Based on the estimated rat LD50, the lowest to highest toxic agent was as follows: pravastatin> rizatriptan> dasabuvir> empagliflozin. While the bioaccumulation factor showed that rizatriptan had the highest (more toxic) and pravastatin had the lowest (lower toxic). Moreover, empagliflozin was estimated to be developmental toxicant with a higher score compared to the other chemicals, but only rizatriptan was determined as both developmental and mutagen toxicant.

Anti-viral agents should be evaluated for their carcinogenicity, by using Toxtree software, the genotoxic and non-genotoxic carcinogenicity of these compounds were assessed. None was genotoxic, whereas, dasabuvir and pravastatin were estimated to be non-genotoxic carcinogens. The four evaluated ligands were persistent to biodegradation (class 2). Their evaluation was based on Cramer rule, the results showed high class of toxicity (class III). They could interact with proteins and DNA molecules, except pravastatin which may not bind to DNA.

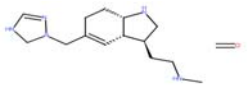
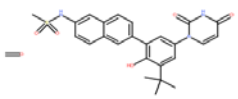
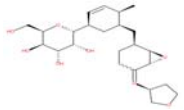
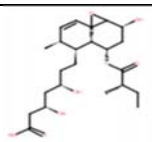
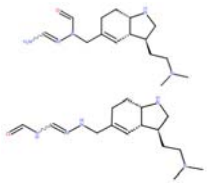
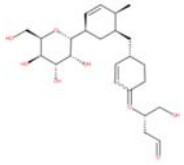
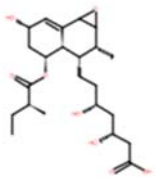
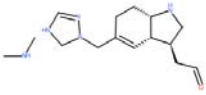
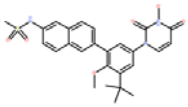
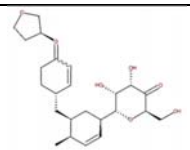
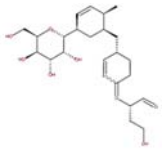
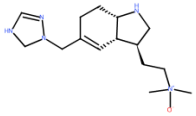
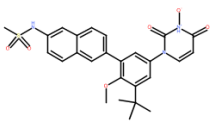
Drug metabolism could change the effective dose and lower its therapeutic impact; however, the drug metabolites could be more toxic than the main compound. Thus, predicting metabolites of a ligand with the detoxification system is interesting and valuable for toxicology and computational chemistry. We used Toxtree software in order to predict the probable reactions and metabolites of each drug after cytochrome P-450 metabolism. The most important catabolic reactions forecasted by the software include: N-dealkylation, O-dealkylation, epoxidation, aliphatic hydroxylation, N-oxidation and alcohol oxidation. Table 3, indicates the predicted reactions and metabolites of cytochrome P-450 system catabolism.

Table 2. Cheminformatics evaluations on the pharmacotoxicology properties of the investigated ligands. For detailed method of estimations and data gathering, see the material and methods section.

Toxicological criteria		Rizatriptan	Dasabuvir	Empagliflozin	Pravastatin	Reference or lower-toxic
Physicochemical and Lipophilicity	MW (g/mol)	269.34	493.57	450.91	424.53	**lower is desirable**
	TPSA (Å²)	49.74	118.64	108.61	124.29	**lower is desirable**
	Lipophilicity (Log $P_{o/w}$)	2.28	3.80	1.97	2.36	**lower is better**
Water solubility	Class	Soluble	Moderately soluble	Soluble	Soluble	**Soluble**
	Solubility (mol/l) [Log *S* (ESOL)]	1.07e-03	2.26e-06	1.57e-04	4.84e-04	**Log S scale Insoluble < -10 < Poorly< -6 < Moderately< -4 < Soluble < -2 < Very < 0 < Highly**
Pharmacokinetics	GI absorption	High	Low	High	High	**Low**
	BBB permeant	Yes	No	No	No	**Yes: neurotoxic**
	P-gp substrate	Yes	No	Yes	Yes	**No: more efficient**
	CYP1A2 inhibitor	Yes	No	No	No	**Yes: toxic and No: non-toxic for detoxification organ (exist in liver, brain,)**
	CYP2C19 inhibitor	No	Yes	No	No	
	CYP2C9 inhibitor	No	Yes	No	No	
	CYP2D6 inhibitor	Yes	No	Yes	No	
	CYP3A4 inhibitor	No	Yes	No	Yes	
	Log K_p (skin permeation) (cm/s)	-6.51	-6.29	-7.61	-7.12	**Higher: lower toxic**
Drug-likeness	Lipinski criteria (**LC**) are OK **LC:** MW ≤ 500; MLOGP ≤ 4.15; N or O ≤ 10; NH or OH ≤ 5	Yes	Yes	Yes	Yes	**Yes: desirable drug with lower toxicity**
	Bioavailability Score	0.55	0.55	0.55	0.56	**Near to 1 is better**
Oral Rat LD50 (mg/kg)		352.99	1742.48	1687.96	2311.53	**Lower value indicate more toxicity**
Bioaccumulation factor		14.96	10.39	11.19	3.81	**Higher value indicate more toxicity**
Developmental toxicity		0.67 toxicant	0.78 toxicant	0.82 toxicant	0.25 NON-toxicant	**Toxicant if > 0.5**
Mutagenicity (Consensus method)		0.66 Positive	0.43 Negative	0.05 Negative	0.21 Negative	**Mutagen if > 0.5**
Carcinogenicity	Genotoxic	No	No	No	No	**No**
	Non-genotoxic	No	Yes	No	Yes	**No**
Biodegradability		Class 2	Class 2	Class 2	Class 2	**Class 1: easily degradable Class 2: persistent Class 3: Unknown**
DNA binding		Yes	Yes	Yes	No	**No**
Binding to protein		Yes	Yes	Yes	Yes	**No**
Cramer rule of toxicity		High (Class III)	High (Class III)	High (Class III)	High (Class III)	**Low class (I) Intermediate class (II) High (III)**

Abbreviations: TPSA, total polar surface area; Log $P_{o/w}$, logarithm of octanol/water partition; MW, molecular weight; GI, gastrointestinal; BBB, blood-brain barrier; CYP, cytochrome; P-gp, P-glycoprotein; a plasma membrane protein which actively exports drugs out of the cell.

Table 3. Prediction of potential products of Rizatriptan, Dasabuvir, Empagliflozin and Pravastatin, after Cytochrome P-450 system metabolism; the ranks and the related predicted end-products are shown.

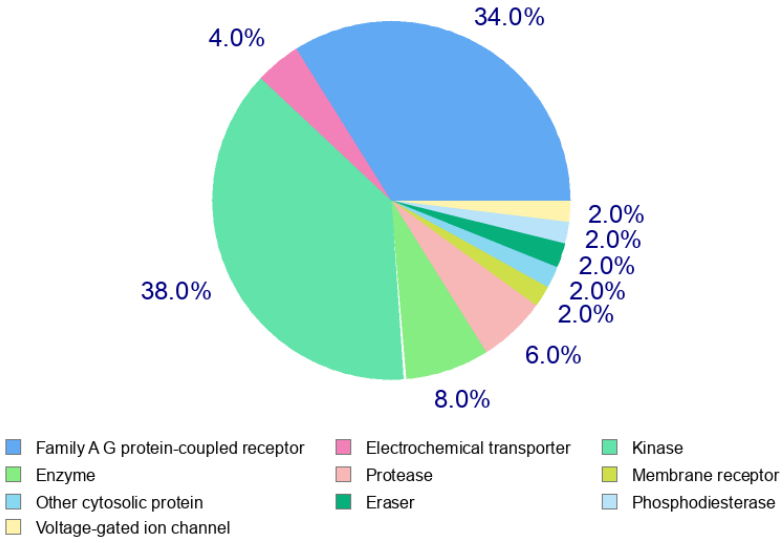
		Rizatriptan	**Dasabuvir**	**Empagliflozin**	**Pravastatin**
Metabolism by Cyt-P450	Rank I	 N-dealkylation	 O-dealkylation	 Epoxidation	 Epoxidation
	Rank II	 N-dealkylation	Aliphatic hydroxylation Structure not developed by the software	 O-dealkylation	 Epoxidation
	Rank III	 N-dealkylation	 N-oxidation	 Alcohol oxidation  O-dealkylation	Aliphatic hydroxylation Structure not developed by the software
	Rank IV	 N-oxidation	 N-oxidation	---	Aliphatic hydroxylation Structure not developed by the software

Proteins targets in human body

Protein-target prediction was done so as to find anticipated receptors based on the simiarity principle, through reverse screening by SwissTarget tool, as described in the materials and methods section. Rizatriptan interacts with the family-A of G protein-coupled receptor and kinase enzymes, with 100% probability estimated score (Figure 8). G protein-coupled receptor and kinase enzymes have a central role in signaling pathways [26].

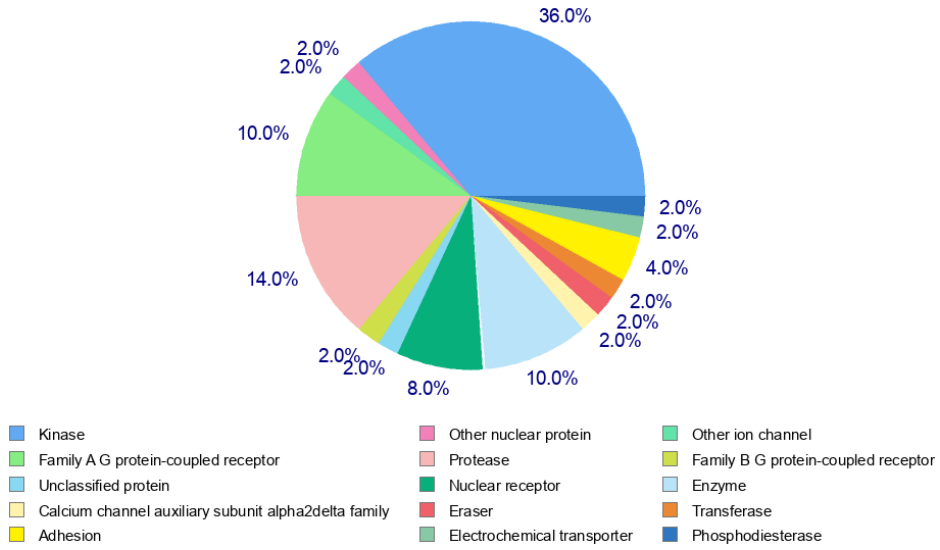
For pravastatin, the best predicted target was 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Figure 9). HMG-CoA reductase is the regulatory enzyme in the cholesterol and other isoprenoids biosynthesis pathway, mostly found in hepatocytes [27]. Aside from its lower docking score than the others, pravastatin was predicted to be associated with severe hepatotoxicity due to its tendency to the HMG-CoA reductase, therefore, pravastatin is suspected not to be an improper compound against COVID-19 infection. Empagliflozin targets the sodium/glucose cotransporters (SLC), with a probability rate around 44% to 100% (Figure 10). SLC proteins are necessary for glucose transport via enterocytes of the small intestine and nephron [28]. As empagliflozin is approved as an anti-diabetic agent, such interaction is expected. However, it can reduce the glucose serum levels

and make hypoglycemia, if administered in toxic doses which, is necessary for treating COVID-19 Dasabuvir targets no protein with good probability score (Figure 11). Thus, it is promising that the drug interacts weakly with human proteins but strongly with COVID-19 main protease, based on the docking results. Furthermore, around 14% of human proteases could be targeted by this drug, as seen in the pie chart (Figure 11); such prediction may confirm the anti-protease activity of dasabuvir. Hence, dasabuvir could be a good therapeutic option for COVID-19, without specific interaction with human proteins and with desirable binding score with 3CL COVID-19 protease.



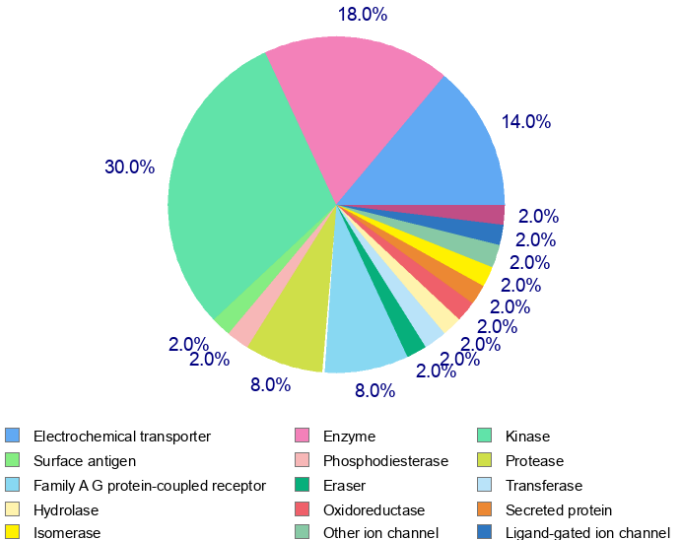
Rizatriptan			
Target	**Common name**	**Target Class**	**Probability***
Serotonin 1b (5-HT1b) receptor	HTR1B	Family A G protein-coupled receptor	1.0
Serotonin 1d (5-HT1d) receptor	HTR1D	Family A G protein-coupled receptor	1.0
Serotonin 1a (5-HT1a) receptor	HTR1A	Family A G protein-coupled receptor	1.0
Serotonin 2a (5-HT2a) receptor	HTR2A	Family A G protein-coupled receptor	1.0
Serotonin 2c (5-HT2c) receptor	HTR2C	Family A G protein-coupled receptor	1.0
Dopamine transporter	SLC6A3	Electrochemical transporter	0.100578902067
Serotonin transporter	SLC6A4	Electrochemical transporter	0.100578902067
Interleukin-1 receptor-associated kinase 4	IRAK4	Kinase	0.100578902067
Ribosomal protein S6 kinase alpha 5	RPS6KA5	Kinase	0.100578902067
Serotonin 6 (5-HT6) receptor	HTR6	Family A G protein-coupled receptor	0.100578902067
Nitric oxide synthase, inducible	NOS2	Enzyme	0.100578902067
Nitric-oxide synthase, endothelial	NOS3	Enzyme	0.100578902067
Serine/threonine-protein kinase PRKX	PRKX	Kinase	0.100578902067
Tyrosine-protein kinase SYK	SYK	Kinase	0.100578902067
Serotonin 2b (5-HT2b) receptor	HTR2B	Family A G protein-coupled receptor	0.100578902067

Figure 8. Rizatriptan's targets in human body based on SwissTarget prediction webserver; as is seen, the best predicted targets are family-A of G protein-coupled receptor and kinases enzymes which are millstone of signaling pathways of hormones and growth factors.



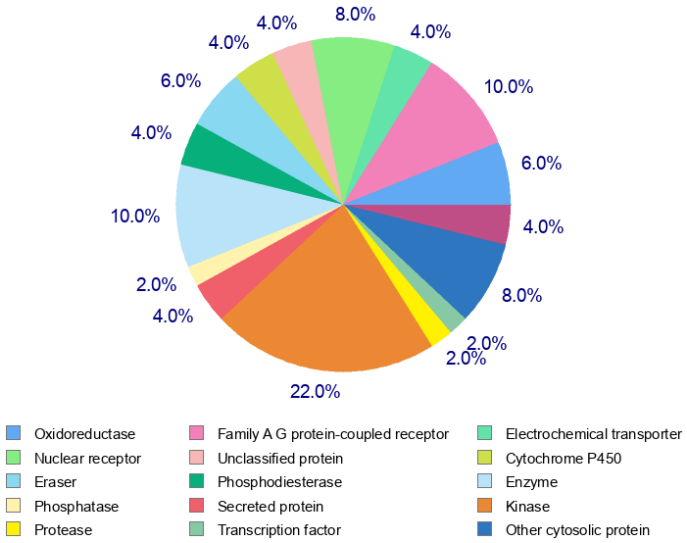
Dasabuvir			
Target	**Common name**	**Target Class**	**Probability***
Rho-associated protein kinase 1	ROCK1	Kinase	0.110612204199
p53-binding protein Mdm-2	MDM2	Other nuclear protein	0.110612204199
Cystic fibrosis transmembrane conductance regulator	CFTR	Other ion channel	0.110612204199
Vasopressin V1b receptor	AVPR1B	Family A G protein-coupled receptor	0.110612204199
Platelet activating factor receptor	PTAFR	Family A G protein-coupled receptor	0.110612204199
Cathepsin K	CTSK	Protease	0.110612204199
Cathepsin S	CTSS	Protease	0.110612204199
Cathepsin L	CTSL	Protease	0.110612204199
Calcitonin gene-related peptide type 1 receptor	CALCRL	Family B G protein-coupled receptor	0.110612204199
Protein Mdm4	MDM4	Unclassified protein	0.110612204199
Proteinase-activated receptor 1	F2R	Family A G protein-coupled receptor	0.110612204199
Bile acid receptor FXR	NR1H4	Nuclear receptor	0.110612204199
Thrombin	F2	Protease	0.110612204199
Trypsin I	PRSS1	Protease	0.110612204199
Urokinase-type plasminogen activator	PLAU	Protease	0.110612204199

Figure 9. Dasabuvir's targets in human body based on SwissTarget prediction webserver; as is seen, there are no good predicted targets for dasabuvir. Thus, it is promising that the drug interacts weakly with human proteins but strongly with COVID-19 main protease, based on docking results.



Empagliflozin			
Target	**Common name**	**Target Class**	**Probability***
Sodium/glucose cotransporter 2	SLC5A2	Electrochemical transporter	1.0
Sodium/glucose cotransporter 1	SLC5A1	Electrochemical transporter	1.0
Sodium/myo-inositol cotransporter 2	SLC5A11	Electrochemical transporter	0.440573968022
Low affinity sodium-glucose cotransporter	SLC5A4	Electrochemical transporter	0.440573968022
Equilibrative nucleoside transporter 1	SLC29A1	Electrochemical transporter	0.118883306718
Adenosine kinase	ADK	Enzyme	0.118883306718
Serine/threonine-protein kinase PIM1	PIM1	Kinase	0.118883306718
Dual specificity mitogen-activated protein kinase kinase 1	MAP2K1	Kinase	0.118883306718
Glucose transporter *(by homology)*	SLC2A1	Electrochemical transporter	0.118883306718
Coagulation factor VII/tissue factor	F3	Surface antigen	0.118883306718
Phosphodiesterase 5A	PDE5A	Phosphodiesterase	0.118883306718
Neprilysin *(by homology)*	MME	Protease	0.118883306718
Adenosine A2a receptor *(by homology)*	ADORA2A	Family A G protein-coupled receptor	0.118883306718
Beta-glucocerebrosidase	GBA	Enzyme	0.118883306718
NAD-dependent deacetylase sirtuin 2	SIRT2	Eraser	0.118883306718

Figure 10. Empagliflozin's targets in human body based on SwissTarget prediction webserver; as is seen, the best predicted targets are sodium/glucose cotransporters which are necessary for glucose metabolism and absorption, especially from gastrointestinal tract. As empagliflozin is approved as an anti-diabetic agent, such interaction is expectable. However, the other proteins in human body are not good interactive receptors for this drug and low toxicity is suggested if used as an anti-viral agent. However, it can reduce the glucose serum levels and make hypoglycemia.



Pravastatin			
Target	**Common name**	**Target Class**	**Probability***
HMG-CoA reductase	HMGCR	Oxidoreductase	1.0
Neurokinin 2 receptor	TACR2	Family A G protein-coupled receptor	0.139453235615
Norepinephrine transporter	SLC6A2	Electrochemical transporter	0.114494790121
Dopamine transporter	SLC6A3	Electrochemical transporter	0.114494790121
Vitamin D receptor	VDR	Nuclear receptor	0.106165761464
Thyroid hormone receptor alpha	THRA	Nuclear receptor	0.106165761464
Thyroid hormone receptor beta-1	THRB	Nuclear receptor	0.106165761464
Splicing factor 3B subunit 3	SF3B3	Unclassified protein	0.106165761464
Thromboxane-A synthase	TBXAS1	Cytochrome P450	0.106165761464
Histone deacetylase 6	HDAC6	Eraser	0.106165761464
Histone deacetylase 2	HDAC2	Eraser	0.106165761464
Histone deacetylase 1	HDAC1	Eraser	0.106165761464
Phosphodiesterase 5A	PDE5A	Phosphodiesterase	0.106165761464
Inosine-5'-monophosphate dehydrogenase 1	IMPDH1	Oxidoreductase	0.106165761464
Inosine-5'-monophosphate dehydrogenase 2	IMPDH2	Oxidoreductase	0.106165761464

Figure 11. Pravastatin's targets in human body based on SwissTarget prediction webserver; as is seen, the best predicted targets are HMG-CoA reductase, the regulatory enzyme in the cholesterol and isoprenoids biosynthesis which has a high activity in hepatocytes. Aside its low docking score, administration of this drug to cure COVID-19 infection, is predicted to be associated with severe hepatotoxicity for its tendency to the HMG-CoA reductase.

Discussion

The data in this investigation were based on computational analysis. Therefore, the discussion is based on the probabilities results and should be examined using proper experimental models at *in vitro* and *in vivo* levels, which require cell culture system and animal models. They are substantially hazardous and need sophisticated technologies, instrumentation and biosafety level 4 (BSL-4) or at least 3 (BSL-3) virology laboratories [29, 30]. This justifies the virtual investigations regarding these drugs, which reflects the necessity and the logic of this approach.

Cheminformatics studies help to predict toxicology and pharmacological characteristics of the drug of interest. Choosing the potential chemical is based on in-silico analysis and in this study, the selection of dasabuvir, rizatriptan, empagliflozin and pravastatin was based on the experiment of other investigators [13] who suggested that these compounds could serve as anti- COVID-19 virus by binding to SARS-CoV-2 spike-ACE2 model and SARS-CoV-2 Main Protease. The results of this investigation show interaction between each of these compounds with SARS-CoV-2 Main Protease which suggests their potential interaction in a multiple target mode.

The data of computational analysis show that dasabuvir with DOS= -139.399 has more favorable score, followed by rizatriptan, empagliflozin and pravastatin, respectively. Convincingly, based on DOS, dasabuvir is expected to be a more potent inhibitor than the other evaluated ligands. Dasabuvir is an FDA-approved medicine for treatment of hepatitis-C virus infection [31]. By considering pharmacotoxicology, in addition to docking and target prediction results, dasabuvir seems to be the drug of choice for remediation of COVID-19 compared with rizatriptan, empagliflozin and pravastatin.

As shown in the results section, structurally, rizatriptan, an antimigrane drug, has lower molecule weight than the other compounds. Although, rizatriptan generally show better results, it was reported to be hepatotoxic and has negative effect on neural tube closure [32, 33]. On the one hand, empagliflozin, an anti-diabetic agent used in treating type 2 diabetes mellitus, is a selective inhibitor of sodium glucose cotransporter 2, which at high doses could disrupt the glucose metabolism with hypoglycemia episodes [34]. Moreover, glycosuria with urinary tract infection and gastrointestinal tract distribution was reported by other investigators [35, 36]. The other proposed medicine which is considered as potential remedy for COVID-19 is pravastatin, an HMG-CoA reductase inhibitor.

Aside from the weak docking score compared with the other tested drugs, pravastatin is not the drug of choice and the results suggested that it is not efficient against COVID-19 3CL protease. Also, evaluation of these drugs based on the SwissTarget prediction tools, showed that human proteins were not interacting with dasabuvir and suggests that it could target the COVID-19 main protease, and thus dasabuvir could be a specific medicine against COVID-19 virus.

Our virtual analysis showed that dasabuvir had molecular weight < 500 g/mol, thus, it is a potential therapeutic agent for SARS-CoV-2, also, it has a moderate water solubility. This finding is consistent with previous investigations [37]. It was not excreted from urine [38], which gave it more chance to reach the therapeutic level targeting the virus [13]. The bioaccumulation factor was equal to 10.39 which is less (toxic) than rizatriptan and empagliflozin, but higher than pravastatin. Moreover, dasabuvir was predicted not to have mutagenic, genotoxic and carcinogen effects.

There are more than 13 virtual screening on the FDA-approved drugs, all published in 2020 which deals with inhibiting the Mpro molecule [39]. Due to the type of library of compounds, each virtual screening in these studies, proposed a unique result, docking tools with different algorithm for calculating the scores and previous evidences about the drugs were used for assessment. However, in the present study we have post-docking approach that consisted of toxicological and pharmacological evaluations using in-silico analysis. In this study, four compounds suggested by Ekins et al. were used. They evaluated millions of molecules on the Zika virus and related flaviviruses protein structures. They also did the docking study on COVID-19 virus with these four compounds and showed that the best DOSs was in this order: dasabuvir> pravastatin> rizatriptan>

empagliflozin [13]. Besides, the more DOS negativity order in our work was as follows: dasabuvir> rizatriptan> empagliflozin> pravastatin. Furthermore, we compared the DOS results with leucinamide, as a reference inhibitor which is the main inhibitory ingredient in crystallography evaluations, as noted in material and methods. Dasabuvir has been consistently better than the others, in addition, it was found in the current virtual study that rizatriptan, empagliflozin and pravastatin were not desirable against SARS-CO-2 due to the non-specific interactions with human proteins and probable subsequent side effects.

Rizatriptan was evaluated as potential therapy for SARS-CoV-2 virus using artificial intelligence approach combined with *in vitro* cell-based assay using feline coronavirus proliferation, the results suggested that rizatriptan was not the drug of choice [40]. Whereas empagliflozin may cause dehydration and predispose to acute kidney injury, and precipitate diabetic keto-acidosis [41]. Željko Reiner et. al. evaluated the interaction of standard ligand (Leucinamide), favipiravir, nelfinavir, lopinavir, simvastatin, rosuvastatin, pravastatin, pitavastatin, lovastatin, fluvastatin, and atorvastatin with SARS-CoV-2 main protease (Mpro) using AutoDock/Vina software. They showed that some statin drugs could be efficient in inhibiting the SARS-CoV-2 Mpro enzyme. Further, favipiravir and rosuvastatin had higher scores (not well) than Leucinamide and atorvastatin had similar score to the standard ligand. However, they didnt test Paravastatin, a statin drug that was tested in the present investigation. Paravastatin had the weakest result than other tested ligands in our work. Željko Reiner et. al. has reported that pitavastatin had higher binding energy than that of protease or polymerase inhibitors, whereas pravastatin was not selected [42]; also, myotoxicity in some patients cause acute kidney injury due to rhabdomyolysis [43]. On the other hand, renal excretion of dasabuvir and metabolites were negligible; besides, the highest dasabuvir concentrations were in the liver and the lowest in eye lens [38] and nervous tissues due to blood–brain barrier protection [44]. Dasabuvir administration should be combined with other anti-viral agents in treating HCV [45]. Collectively, our results from the dasabuvir and SARS-CoV-2 protease docking and Ekins et al. results of dasabuvir docking with SARS-CoV-2 spike-ACE2, suggest that dasabuvir has multi-target interaction with SARS-CoV-2 virus resulting in inhibition of viral entry to the cell and subsequent cell infections [46].

Conclusion

Using in-silico study on dasabuvir shows that it is a potential multi-target therapeutic agent that worth further experimental and pre-clinical evaluation for SARS-CO-2.

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Conflict of interests

The authors declare that they have no conflict of interests

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