Short Communication

Drug Repurposing Against Angiotensin-Converting Enzyme-Related Carboxypeptidase (ACE2) Through Computational Approach

Golnaz Vaseghi, Ali Golestaneh[1], Leila Jafari[2], Fahimeh Ghasemi[3]

Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, [1]Applied Physiology Research Center, Cardiovascular Research Institute, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, [2]Department of Bioinformatics and Systems Biology, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Medical Image and Signal Processing Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Ongoing novel coronavirus (COVID-19) with high mortality is an infectious disease in the world which epidemic in 2019 with human-human transmission. According to the literature, S-protein is one of the main proteins of COVID-19 that bind to the human cell receptor angiotensin-converting enzyme 2 (ACE2). In this study, it was attempted to identify the main effective drugs approved that may be repurposed to the binding site of ACE2. High throughput virtual screening based on the docking study was performed to know which one of the small-molecules had a potential interaction with ACE2 structure. Forasmuch as investigating and identifying the best ACE2 inhibitors among more than 3,500 small-molecules is time-consuming, supercomputer was utilized to apply docking-based virtual screening. Outputs of the proposed computational model revealed that vincristine, vinbelastin and bisoctrizole can significantly bind to ACE2 and may interface with its normal activity.

Keywords: *Angiotensin-converting enzyme 2, computer simulation, coronavirus disease 19, drug repurposing, high-throughput virtual screening*

Submitted: 07-Sep-2020 **Revised:** 29-Oct-2021 **Accepted:** 03-Feb-2022 **Published:** 10-Nov-2022

Introduction

In December 2019, an unknown respiratory disease emerged in Wuhan, Hubei province, China, which is now known as coronavirus disease 19 (COVID-19). A novel coronavirus is closely related to severe acute respiratory syndrome coronavirus (SARS-CoV). SARS-CoV-2 binds to human angiotensin-converting enzyme 2 (ACE2) which may lead to severe pneumonia and lung fibrosis in patients.[1] ACE2 is a cell membrane enzyme which expresses in the outer surface of cells, mainly in the lungs,[2] and converts angiotensin II into angiotensin 1–7.[3] It has been shown that exogenous Ang-(1–7) and upregulation of the ACE2 may protect against lung fibrosis by blocking the MAPK/NF-κB pathway.[4] On the other hand, SARS-CoV-2 spike protein entrance site is different from the active site of ACE enzyme.[5] ACE2 is a receptor to help SARS-S to entry to human cell, using the cellular serine protease TMPRSS2 for S protein priming.[6]

Few numbers of articles have been published about ACE2 using computational approach. For example, identification of exact amino acid residues in the place of interaction of S-protein with ACE2 was investigated to develop antiviral inhibitor by Zhang *et al.* in 2005.[6] Pharmacophore model and virtual screening was the other model that was published by Rella *et al.* in 2005.[7] Simulation S-protein in complex with ACE2 and their interaction with four host species-specific receptors was another computational manuscript that was published by Zhang *et al.* in 2007,[8] and changing conformational active site and reducing level of ligand binding was the other model considered by Lokeshwari *et al.* in 2015.[9]

Besides, numerous computational methods have been proposed to find the best lead compound as a *de novo* drug candidate for other homo-sapience targets since 1980. As evidenced by the recent publications in drug discovery, the search for finding drug-like compounds with desired biological activities in the large libraries of chemical compounds such as ZINC, called

How to cite this article: Vaseghi G, Golestaneh A, Jafari L, Ghasemi F. Drug repurposing against angiotensin-converting enzyme-related carboxypeptidase (ACE2) through computational approach. J Med Sign Sens 2022;12:333-8.

Address for correspondence:
Dr. Fahimeh Ghasemi, Department of Bioinformatics and Systems Biology, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: f_ghasemi@amt.mui.ac.ir

Access this article online

Website: www.jmssjournal.net

DOI: 10.4103/jmss.JMSS_66_20

Quick Response Code:

high-throughput virtual screening (HTVS), is a really risky, time-consuming procedure as well as low success percentages. Drug repurposing or repositioning is one of the proposed virtual screening approaches which refers to rediscover a narrow the list of drug candidates which already passed safety tests in clinical trials and a great chance of taking the desired activity via computational approaches.[10] Various computational approaches have been used in drug repositioning which can be categorized into three main groups: (i) ligand-based models,[11] i.e., machine learning-based models, (ii) target-based screening such as docking-based method,[12] and (iii) network-based methods.[13]

Since March 2020, various manuscripts were published based on mentioned approaches to find out the best appropriate drugs among existed ones. Some of the main of them are reviewed in the following text. Basu *et al*. focused on some of the natural products and their effects on the ACE2. In another study, it was tried to find compounds which can bind to spike, ACE2, and the ACE2:spike complex with good affinity.[14] Joshi *et al*. focused on the main effective inhibitors of ACE2 among natural compounds.[15] In another study, the current efforts of exploiting ACE2 as a therapeutic target were reviewed via Jia *et al*.[16]

Machine learning-based models depend on the known drug-target interaction.[17] Shallow learning methods such as k-nearest neighbor and deep learning model, for instance, convolutional neural network, have been the main techniques which exploited to achieve drug repurposing prediction.[11,18,19] Target-based virtual screening, most of the time, is based on the docking studies with supercomputer, and the best top-ranked drugs can be tested via molecular dynamic simulation or experimental test.[20] Moreover, some of the scientists, efforts to find out drug-disease associations and drug-target interactions simultaneously with network-based methods.[13]

In this study, it was attempted to figure out the best approved drugs to inhibit ACE2 binding site based on the drug re-purposing methodology. Hence, more than 3500 existed drugs in DrugBank website were downloaded and drug-target interaction for each drug was simulated via high-throughput docking virtual screening. Finally, the best identified effective drugs were extracted and grouped according to their categories [Figure 1]. We hypothesized that binding proposed drug to the ACE2 may exacerbate pulmonary fibrosis.

Materials and Methods

Receptor and ligand preparation

ACE2 receptor structure was downloaded from Protein Data Bank (PDB) website (PDB ID: 1r4l, resolution 3 Å) and its three-dimensional conformation was refined with AutoDock4 software, such as add hydrogen and remove nonpolar hydrogen and add total Kollman charge. ACE2 binding site was chosen around ZN (x = 37, y = 5, z = 25, with search box 60 × 60 × 60) set its binding site[21]

Besides, as the aim of this work was identifying the best drugs to inhibit COVID-19, a library of approved drugs, found in Supplementary Table S1, were downloaded from DrugBank website in xml format. The version of database is 5.1.5 released on January 3, 2020, and contained more than 3000 approved drugs. After that, xml format was changed to the sdf and then all molecules were extracted as a pdb formatted file. After that, because of the lack of hydrogen in the converted structures, all molecular hydrogens were added with Open Babel software, automatically. Besides, one of the main steps in computational drug design is optimizing 3D structures of ligands. There are several tools available to generate 2D/3D structure/conformers, but, because of existing huge number of molecules as well as time-consuming optimization procedure, Open Babel was utilized to optimize 3D structure, automatically, with the aim of finding low-energy conformations via conformer searching. In this software, accessing several algorithms for conformer searching is performed by Gen3D library with steepest descent geometry optimization and the MMFF94 force field. Finally, to confirm the optimization procedure, some of the structures were optimized with HyperChem

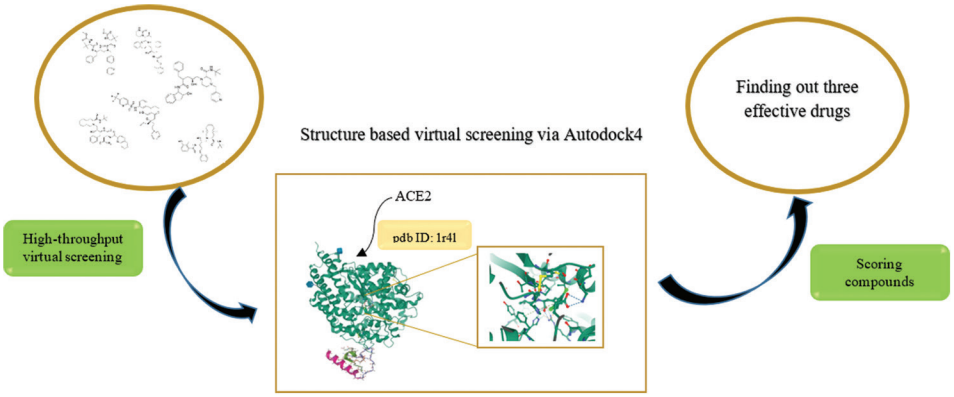

Figure 1: Schematic of the proposed model

software and the results were compared with Open Babel outputs. Finally, all optimized structures were converted to pdbqt format via Open Babel software for docking studies.

High-throughput docking-based virtual screening

HTVS methods have been one of the main computational drug design approaches to rapidly investigate hundreds number of chemical compounds which could be appropriate for finding the best drug-like molecules.[22] One of the common computational methods in HTVS is molecular docking.[23] The main goal of molecular docking is given a ligand-target interaction in two main steps: (i) minimizing ligand conformation in receptor binding site and (ii) scoring these conformations.[24] Vina AutoDock is one of the docking methods useful for HTVS by using multithreading on multicore machines and widely utilized for drug repurposing. The main drawback of Vina docking is that did not contain flexible binding sites residues.[25]

Structure-based virtual screening

Structure-based virtual screening (SBVS) has been one of the main computational drug design approaches based on the simulation ligand-target interaction for identification of hit molecules. Molecular docking is one of the SBVS methods with the aim of optimized matching prediction of ligand orientation according to the 3D structures of receptors. The main goal of molecular docking is investigating affinity and binding energy of DTI. Finally, all suggested conformers are clustered according to the computed free energies and grouped together by scoring function.

Results and Discussion

As mentioned, this study was founded on two different parts, i.e., HTVS and molecular docking, which were discussed in the following by details.

High-throughput virtual screening

To perform the docking study, two main input datasets must be prepared, containing approved drugs and protein crystallography structure. For the first one, all FDA-approved drugs (more than 3500 molecules) were downloaded from DrugBank website and their structures were optimized (discussed in material section). 3D structure of ACE2 was the second essential information which was downloaded from PDB, i.e., ID 1r4l, with resolution After that, protein PDB structure was rectified with AutoDock4 software; then, molecular docking simulation was performed between drugs and receptor via AutoDock Vina software. According to the root mean square error (RMSE), the best conformers of each ligand were extracted, and finally, the ligands were sorted based on their affinities. More than 200 drugs had an affinity with score better than 11 Kcal/Mol. Hence, ligands with pose scores of more than 15 Kcal/Mol and weight <1000 (g/mol) were selected (corresponding to 30 molecules, i.e., about the top 10% compounds) [Table 1]. Among these drugs, some of them are used widely, especially in patients with underlying disease such as antiviral. As shown, it was figured out seven different potent drugs – with ΔG <−15 (μM) and weight <700 (g/mol) – that could be effective to inhibit ACE2 enzyme activity.

Before to consider molecular interaction precisely, we were interested to investigate proposed drug performance in the body, i.e., their side effects and mechanism of actions which were extracted from DrugBank website [Table 2].

Molecular docking

At the second step, seven suggested molecules were docked to the receptor via AutoDock4. There are two main steps for docking procedure, i.e., setting the search space and optimization and docking procedure. In the first step, choosing appropriate grid box to search in 3D space of protein is a critical point which conducted researcher to have reliable decision. Three different parameters are vital which must be adjusted that are selecting suitable box center, number of points in each dimension, and spacing between the points. To achieve the best selection for box center, existed ligand in protein crystallography was extracted via Schrodinger software and docked again via blind docking method. The result was matched with experimental approach. Its binging energy was 10.7 (μM). Hence, the center of grid box was defined on the center of ligands (x = 40.12, y = 1.32, z = 23.68) in crystallography of proteins with size 60 × 60 × 60 and 0.375°A. After that, according to the defined atoms, probes were serially located at each grid point and internal energy between the probs and protein were computed for each atom type, individually. Computed energies for each point were utilized as a lookup table during the docking simulation.

The second step was docking procedure with the aim of finding out binding energy of each ligand as well as its interaction with the target. Thus, Lamarckian genetic algorithm (LGA) was utilized to extract the population of ligand conformations, randomly. In LGA method, two optimization methods, genetic algorithm and local search, are combined to enhance docking performance. Van der Waals potentials and a dihedral angle term are two main critical parameters to calculate internal energy. The results of interaction-binding energies and interactions are summarized in Table 3. As shown, indinavir, retapamulin, and saquinavir have the lowest binding energies (<−12.5 [μM]).

Besides, the interactions between suggested drugs, indinavir, retapamulin, and saquinavir, and protein are illustrated in Figure 2a-c. As demonstrated, various amino acids were contributed on the interaction, which are helpful to increase binding affinities.

Table 1: Fifteen percent of top-ranked extracted drug after docking simulation based on virtual screening

ID	Name	Pharmacologic category	Affinity	Weight	logC
1	**Indinavir**	Highly active antiretroviral therapy to treat HIV/AIDS	−18.5	627.64	3.68
2	Teicoplanin	Antibiotic, miscellaneous	−26.4	1198.93	2.43
3	**Prednisolone**	Endocrine, rheumatic, and hematologic disorders; collagen, dermatologic, ophthalmic, respiratory, and gastrointestinal diseases; allergic and edematous states; and other conditions like tuberculous meningitis	−16.3	486	4.08
4	**Retapamulin**	Antibiotic	−15.5	517	5.21
5	Bisoctrizole	Sunscreen agent in cosmetic products	−19.5	658	6.11
6	Vindesine	Antineoplastic agent, antineoplastic agent, vinca alkaloid antimicrotubular	−23.8	753.94	3.42
7	Vancomycin	Glycopeptide antibiotics	−23.7	1449.27	−1.14
8	Vinblastine	Antineoplastic agent, antineoplastic agent, vinca alkaloid antimicrotubular	−23.6	810.99	5.23
9	Histrelin	Antineoplastic agent, gonadotropin-releasing hormone agonist; gonadotropin	−23.6	1323.53	−1.9
10	Vincristine	Antineoplastic agent, antimicrotubular; antineoplastic agent, vinca alkaloid	−23.5	824.97	4.04
11	Glycyrrhizic	Anti-inflammatory agents, liver therapy, lipotropics, triterpenes	−23.4	822.94	3.03
12	Levocabastine	An ophthalmic for the temporary relief of the signs and symptoms of seasonal allergic conjunctivitis. Also used as a nasal spray for allergic rhinitis	−15.5	420.53	1.86
13	Anhydrovinblastine	Not available	−23.1	792.97	6.11
14	Zotarolimus	Immunosuppressant	−22.9	966.23	7.45
15	Betadex	Biopolymers	−22.7	1134.99	−17.46
16	Vinorelbine	Antineoplastic agent, antimicrotubular; antineoplastic agent, vinca alkaloid	−22.5	778.95	5.94
17	Octreotide	Antidiarrheal, antidote, somatostatin analog	−22.3	1019.25	2.51
18	Nafarelin	Gonadotropin releasing hormone agonist	−22	1322.50	−1.22
19	Sirolimus	Immunosuppressant agent; mTOR kinase inhibitor	−22	914.19	7.04
20	Adapalene	Indicated for the topical treatment of acne vulgaris in patients aged 12 and over			
21	Saquinavir	In combination with ritonavir and other antiretroviral agents, for the treatment of HIV-1 infection in patients 16 years of age and older	−16.5	670.86	4.73
22	Aclarubicin	Antineoplastic agent: Cytotoxic antibacterial from the group of anthracyclines	−21.7	811.88	3.43
23	Docetaxel	Antineoplastic agent, antimicrotubular, taxane derivative	−21.6	807.89	4.08
24	Flumetasone	For the treatment of contact dermatitis, atopic dermatitis, eczema, psoriasis, diaper rash, and other skin conditions	−17.5	494	3.6
25	Carperitide	Treatment of heart failure, atrial natriuretic peptide	−20.8	3080.48	0
26	Everolimus	Antineoplastic agent, mTOR kinase inhibitor; immunosuppressant agent	−20.7	958.24	7.1
27	Buserelin	Gonadotropin releasing hormone agonist	−20.4	1239.45	−1.45
28	Dalbavancin	Glycopeptide antibacterials	−20.3	1816.71	5.64
29	Goserelin	Antineoplastic agents, hormonal	−20.3	1269.43	−2.86
30	Acetoxolone	Drugs for peptic ulcer and GORD	−20	512.73	7.42

* Bold names were related to the selected drugs among others. **GORD – Gastroesophageal reflux disease

Conclusion

High-blood pressure (hypertension) has been one of the main regular situations reported in most patients with severe illness in COVID-19. The main worry in the medical treatment of these conditions, such as using RAAS inhibitors, is occurring adverse outcomes which emerge as the robust estimator of COVID-19-related death. Hence, hypertension condition has been key determined prognostic. In the recent studies, it was released that spike protein of coronaviruses binds to the human receptor in the cell surface, i.e., ACE2 which its expression is increased in the patients with type 1 or type 2 diabetes. Hence, it can be concluded that ACE2 expression and activity have a significant rule in SARS-CoV-2 patients. Therefore, the drugs with high affinity for ACE2 enzymatic site should be prescribed with caution in these patients

In this study, it was attempted to find the main ACE2 inhibitors among 3981 approved drugs downloaded from DrugBank website. The receptor structure was downloaded from PDB website (PDB ID: 1r4l, resolution 3 Å) and its three-dimensional conformation was refined with AutoDock4 software, such as add hydrogen and remove nonpolar hydrogen and add total Kollman charge. To evaluate docking results, firstly, the ligands in the 1r4l crystallography were docked again. The results revealed that selected centers of grid box were appropriate. In HTVS step, approved drugs were docked to the receptor via AutoDock Vina. The best conformers of each ligand were selected based on the RMSE, and finally, the ligands were sorted based on their affinities. Ligands with pose scores <20 (Kcal/Mol) were extracted and are shown in Table 1. After that, to find the best inhibitors, drugs with weights <700 (g/mol) were investigated via AutoDock4.

Table 2: Side effects and action mechanisms of proposed drugs (https://go.drugbank.com/drugs)

ID	Name	Toxicity
1	Indinavir	Symptoms of overdose include myocardial infarction and angina pectoris
2	Prednisolone	Patients experiencing an overdose of prednisolone may present with gastrointestinal disturbances, insomnia, and restlessness. Overdose of oral prednisolone may be treated by gastric lavage or inducing vomiting if the overdose was recent, as well as supportive and symptomatic therapy. Chronic overdosage may be treated by dose reduction or treating patients on alternate days. An overdose by the ophthalmic route is not expected to cause problems
3	Retapamulin	Not available
4	Levocabastine	Visual disturbances, dry mouth, cough, nausea, eyelid edema, and lacrimation
5	Adapalene	Toxicity information regarding adapalene is not readily available. Patients experiencing an overdose are at an increased risk of severe adverse effects such as redness, scaling, and skin discomfort. Symptomatic and supportive measures are recommended
6	Flumetasone	Can lead to signs of irritation such as burning sensation, itching, or skin rash at the site of application; hypersensitivity reactions
7	Saquinavir	Data regarding overdose with saquinavir are limited. No acute toxicities or sequelae were noted in a patient ingesting 8 g of saquinavir as a single dose, and a second subject ingesting 2.4 g as a single dose experienced throat pain that lasted for 6 h and subsequently resolved. Treatment of overdose should consist of symptomatic and supportive measures. Dialysis is unlikely to be of benefit given saquinavir's extensive protein binding

Table 3: The binding energy of molecular docking computed with AutoDock4

Molecule ID	Drug commercial name	Lowest binding energy	Mean binding energy	Number of conformers in selected cluster (%)	Interactions		
					Hydrogen bound	Pi-Pi	Pi-cation
1	Indinavir	−13.58	−13.16	10	HIS378, TYR515, PRO346	HIS378	ARG514
2	Prednisolone	−10.66	−10.07	49	GLU406	-	-
3	Retapamulin	−12.91	−12.19	44	HIS345, ARG273	-	-
4	Levocabastine	−11.49	−11.29	46	HIS378	Phe274, HIS345	-
5	Adapalene	−10.94	−10.85	70	HIS378	HIS378	-
6	Flumethasone	−8.05	−7.85	86	HIS345, 2×ARG518	-	-
7	Saquinavir	−15.12	−13.07	23	ARG273	-	-

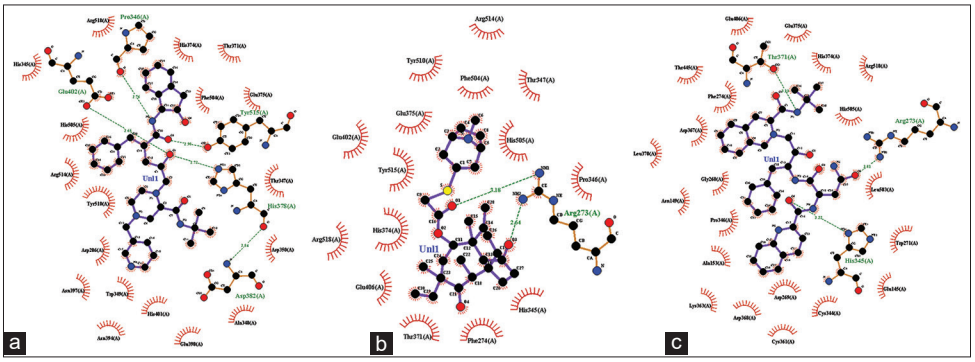

Figure 2: ACE2 in complex with (a) Indinavir, (b) Retapamulin, (c) Saquinavir

Outputs of the proposed computational model revealed that indinavir, retapamulin, and saquinavir can significantly bind to ACE2 and may interface with its normal activity.

Acknowledgment

This project was supported by the vice chancellery of research at Isfahan University of Medical Sciences (Contract grant number: 198220).

Financial support and sponsorship

None.

Conflicts of interest

There are no conflicts of interest.

References

1. Xu YH, Dong JH, An WM, Lv XY, Yin XP, Zhang JZ, *et al.* Clinical and computed tomographic imaging features of novel coronavirus pneumonia caused by SARS-CoV-2. J Infect 2020;80:394-400.
2. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004;203:631-7.

3. Keidar S, Kaplan M, Gamliel-Lazarovich A. ACE2 of the heart: From angiotensin I to angiotensin (1-7). Cardiovasc Res 2007;73:463-9.
4. Meng Y, Yu CH, Li W, Li T, Luo W, Huang S, *et al.* Angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas axis protects against lung fibrosis by inhibiting the MAPK/NF-κB pathway. Am J Respir Cell Mol Biol 2014;50:723-36.
5. Towler P, Staker B, Prasad SG, Menon S, Tang J, Parsons T, *et al.* ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis. J Biol Chem 2004;279:17996-8007.
6. Zhang Y, Zheng N, Hao P, Cao Y, Zhong Y. A molecular docking model of SARS-CoV S1 protein in complex with its receptor, human ACE2. Comput Biol Chem 2005;29:254-7.
7. Rella M, Rushworth CA, Guy JL, Turner AJ, Langer T, Jackson RM. Structure-based pharmacophore design and virtual screening for novel angiotensin converting enzyme 2 inhibitors. J Chem Inf Model 2006;46:708-16.
8. Zhang Y, Zheng N, Nan P, Cao Y, Hasegawa M, Zhong Y. Computational simulation of interactions between SARS coronavirus spike mutants and host species-specific receptors. Comput Biol Chem 2007;31:134-7.
9. Lokeshwari D, Krishna Kumar NK, Manjunatha H. Multiple mutations on the second acetylcholinesterase gene associated with dimethoate resistance in the melon aphid, *Aphis gossypii* (*Hemiptera*: *Aphididae*). J Econ Entomol 2016;109:887-97.
10. Valera-Vera EA, Sayé M, Reigada C, Miranda MR, Pereira CA. *In silico* repositioning of etidronate as a potential inhibitor of the *Trypanosoma cruzi* enolase. J Mol Graph Model 2020;95:107506.
11. Zhu Z, Wang X, Yang Y, Zhang X, Mu K, Shi Y, *et al*. D3Similarity: A Ligand-Based Approach for Predicting Drug Targets and for Virtual Screening of Active Compounds Against COVID-19. ChemRxiv. Cambridge: Cambridge Open Engage; 2020; This content is a preprint and has not been peer-reviewed.
12. Talluri S. Molecular Docking and Virtual Screening based prediction of drugs for COVID-19, Combinatorial Chemistry & High Throughput Screening 2020;23:1. DOI: 10.2174/1386207323666200814132149.
13. Zhou Y, Hou Y, Shen J, Huang Y, Martin W, Cheng F. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-CoV-2. Cell Discov 2020;6:14.
14. Basu A, Sarkar A, Maulik U. Molecular docking study of potential phytochemicals and their effects on the complex of SARS-CoV2 spike protein and human ACE2. Sci Rep 2020;10:17699.
15. Joshi T, Joshi T, Sharma P, Mathpal S, Pundir H, Bhatt V, *et al. In silico* screening of natural compounds against COVID-19 by targeting Mpro and ACE2 using molecular docking. Eur Rev Med Pharmacol Sci 2020;24:4529-36.
16. Jia H, Neptune E, Cui H. Targeting ACE2 for COVID-19 therapy: Opportunities and challenges. Am J Respir Cell Mol Biol 2021;64:416-25.
17. Ghasemi F, Mehridehnavi A, Pérez-Garrido A, Pérez-Sánchez H. Neural network and deep-learning algorithms used in QSAR studies: Merits and drawbacks. Drug Discov Today 2018;23:1784-90.
18. Zhang W, Xu H, Li X, Gao Q, Wang L. DRIMC: An improved drug repositioning approach using Bayesian inductive matrix completion. Bioinformatics 2020;36:2839-47.
19. Gao K, Nguyen DD, Wang R, Wei GW. Machine intelligence design of 2019-nCoV drugs. bioRxiv 2020;1-16, doi: 10.1101/2020.01.30.927889.
20. Smith M, Smith JC. Repurposing Therapeutics for COVID-19: Supercomputer-Based Docking to the SARS-CoV-2 Viral Spike Protein and Viral Spike Protein-Human ACE2 Interface. ChemRxiv. Cambridge: Cambridge Open Engage; 2020; This content is a preprint and has not been peer-reviewed.
21. Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ. Angiotensin-converting enzyme-2 (ACE2): Comparative modeling of the active site, specificity requirements, and chloride dependence. Biochemistry 2003;42:13185-92.
22. Dhasmana A, Raza S, Jahan R, Lohani M, Arif JM. High-throughput virtual screening (HTVS) of natural compounds and exploration of their biomolecular mechanisms: An in silico approach. In: Ahmad Khan MS, Ahmad I, Chattopadhyay D, editors. New Look to Phytomedicine. Ch. 19. Advancements in Herbal Products as Novel Drug Leads: Academic Press; 2019. p. 523-48.
23. Zhang DH, Wu KL, Zhang X, Deng SQ, Peng B. *In silico* screening of Chinese herbal medicines with the potential to directly inhibit 2019 novel coronavirus. J Integr Med 2020;18:152-8.
24. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 2011;7:146-57.
25. 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.