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Preprocessing of the Candidate Antiviral Drugs against COVID-19 in Models of SARS cov2 Targets

Abdul Kadhim AH[1]*, Hadi NR[1], Abdulhussein M[2], Zamil ST[2] and Zamil ST[2]

[1]Department of Pharmacology and Therapeutics, College of Medicine, University of Kufa, Iraq
[2]Department of Pharmacy, College of Pharmacy, University of Kufa, Iraq

Abstract

Although many viral infections are self-limiting, other are real health challenges like COVID-19 since many viruses possess just few drug gable targets to be treated with small drug molecules. Corona virus genome encodes for up to 17 main proteins. Orf1ab encodes for polyprotein. COVID-19 structural proteins are the spike S, membrane M, envelope E and the nucleocapsid N protein while other are non-structural proteins designated as NSP1-13 for non-structural proteins. Among NSP the most important corona virus targets for developing antiviral drugs are the papain-like protease, PDB ID: 6m03 and RNA polymerase NSP12, PDB ID: 6nur. NCBI, NIH Genbank, Uniprot, PDB, DrugBank, ChemSpider databases and bioinformatics editor software's like ICM Mol soft pro and Swiss Dock were used in addition to the in vitro lab model of viral protease were integrated to retrieve and analyze corona virus targets and to select the candidate ligands in an attempt to evaluate the inhibitory efficacy of different experimental and approved drugs which were further optimized and searched for the highly similar approved drug.

This step aims to adopt drug repurposing to speed the development of antiviral drugs and recommend rational in vivo and clinical studies. After COVID-19 targets had been analyzed the drugs that shared > 70% similarity to the binding sites of those targets were reversin, pentagastrin, remdesivir, norfloxacin and nitazoxanide against COVID-19 papain-like protease whereas benzyl glutathione, lopinavir and hydroxymethylglutathione against RNA polymerase. The anti-resistance reversin showed the highest inhibitory efficacy against COVID-19 papain-like protease as indicated by the ligand-protease binding energy with Mol soft pro analysis. The calculated inhibitory binding was -137.30 kJ/mol z > 1.9 as compared with the tetrazapentadecanoate -129.57 kJ/mol z = 4.0, whereas remdesivir, pentagastrin, nitazoxanide and norfloxacin had a moderate antiprotease activity (>- 100 kJ/mol). Norfloxacin shoresults showed a slight consistency between in vitro and in silico models. Although benzyl glutathione is an experimental compound, however it had the highest RNA polymerase inhibiting efficacy with -129 kJ/mol binding energy which is even higher than lopinavir and Favinavir.

From the overall results, reversin, oligopeptides, quinolones and antiviral drugs may widen the treatment options for COVID-19 if further evaluated in clinical studies.

Keywords: COVID-19; NSP; RDRP; Papain-Like Protease; Replicas; Antiviral Drugs

Correspondence to: Hussein Abdul Kadhim A, Department of Pharmacology and Therapeutics, College of Medicine, University of Kufa, Iraq; E-mail: Husseina.ahussein@uokufa.edu.iq

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Introduction

Severe acute respiratory syndrome corona virus 2 (SARS cov2 or COVID-19) is a highly spreading viral infection caused by a novel type of Coronaviridae family called SARS nCov [1]. Similar to other viral infections, Covid-19 has unique virulence characteristics in that viruses possess just a few drug gable target proteins [2] like hemagglutinin, neuraminidase, polymerase, proteases, envelope and membrane proteins in addition to few polyproteins associated with their genome [3]. Viruses are semi-dormant units that are interactive with drugs only in their cycles of proliferation [4]. These factors diminish the ability to treat viral infections. However in concern to the COVID-19, the viral proteome is composed of 17 main proteins: four are structural proteins, envelope E, membrane glycoprotein M, surface glycoprotein spikes S, and nucleocapsid protein N for +RNA binding whereas hemagglutinin and other non-structural proteins NSP1-13 are encoded with different orf genes (PDB, Genbank, NCBI ID: 6lu7, 6lvn, 6lxt, 5r7y, 5r7z, 5r80, 5r81, 5r82, 5r83, 5r84) [5-7]. The following set of COVID-19 proteome contents is a reasonable pharmacological target for developing new or repurposed antiviral drugs. The orf1ab encoded polyprotein: COVID-19 replicas are a polyprotein that has multiple activities like -RNA transcription, RNA template, mRNAs and virion RNA. This polyprotein has also multiple proteinases activity to cleave this polyprotein into functional products. The non-structural protein 1 NSP1 (host translation inhibitor) blocks 40S of human ribosome so that it arrests host transcription and mediate mRNA lysis and spare COVID-19 mRNAs. Non-structural protein 2: through cell PHB and PHB2, NSP2 maintains cells mitochondria viable. Papain-like proteinases PLP: lyses the N-terminus of the replicas polyprotein and activates Lys-48 and Lys-63 linked polyubiquitin chains in cells. PLP mediates viral membrane assembly. In addition, PLP inhibits INF production and NFkB. Non-structural protein 4

NSP4: mediates viral membrane assembly. Proteinases 3CL-PRO: activates lysis C-terminus of replicas polyprotein. Non-structural protein 6 NSP6: Mediates endocytosis and prevents lysosomal fusion. Non-structural protein 7 NSP7: with NSP8 it acts as a primase. Non-structural protein 8 NSP8: synthesizes longer products than Oligonucleotide primers. Non-structural protein 9 NSP9: by ssRNA-binding protein it mediates viral replication. Non-structural protein 10 NSP10: mediates COVID-19 transcription by stimulating both nsp14 (exoribonuclease) and nsp16 (O-methyltransferase) for methylating viral mRNAs cap. RNA-directed RNA polymerase NSP12 RDRP: for replication and transcription of the viral RNA genome. Viral Helicase: a Mg-dependent and Zn-binding protein that unwinds RNA and DNA. Guanine-N7 methyltransferase: Have exoribonuclease activity (on both ssRNA and dsRNA) and a N7-guanine methyltransferase activity. Uridylate-specific endoribonuclease: Mn-dependent, uridyl enzyme. 2'-O-methyltransferase: for viral mRNA cap methylation at 2'-O-ribose site [8,9] in addition to the non-protein target against the viral membrane like terpenoids [10]. Another host factors that were investigated as reasonable targets to treat COVID-19 include angiotensin converting enzymes 2 ACE2 [11-13] furins and passive and active immunostimulants like INFs and ILs [14,15]. Many studies analyze viral protein targets virtually for docking sites [16,17] then the predicted compound with high energy of affinity to target site is optimized for predicting the 3D conformational isomers and analogues [18]. Such lead experimental or drug compounds are then rearranged in a rank according to their target binding energy in kj/mol with classifying types of ionic, vW, and hydrogen bonds by which they interact with target residues [19,20]. Many compounds and approved drugs like antiviral drugs are potential resources to be repurposed against different health challenges like corona virus infections. Of these potential drugs are the quinolones, small peptide molecules like reversin and pentagastrin, benzyl glutathione and antiviral drugs that inhibit viral replication like lopinavir, Favinavir in addition to the protease inhibitors like ritanovir, remdesivir and ribavirin while others are ant parasitic nitro-azole like nitazoxanide. In vitro viral model is a critical technical step for testing antiviral compounds [21] since that direct access of the researchers into the active viruses are forbidden according to the international regulation policy and the strict requisites for level 4 laboratories owning to their health risks [22] so that the artificial viral models including killed virion or its partial components are critical in conducting such researches [23]. In vitro testing is followed to determine pharmacokinetics and target binding dynamics. Although viruses have just limited number of proteins, however each protein like COVID-19 main protease and NSP12 has in average 3 docking sites for surveying many test compounds [24]. This means that developing a rapid medical treatment against the highly transmittable viral epidemics like COVID-19 is a possible approach. This study aimed to analyze all COVID-19 proteins and evaluate the highly predictive ligands for further optimization and to study ligand similar to the approved drugs to be re-evaluated and arranged for further clinical studies.

Models, Materials and Methods

This study had been accomplished in serial stages and originally designed to include both computer-based and laboratory assessment of candidate list of drugs against COVID-19.

I. The database retrieval and review study for COVID-19, SARS cov and Human corona virus Hcov OC43, NL63 whole genome, proteome, envelope and membrane analysis in addition to reviewing relevant host factors like furin and ACE2. NCBI, NIH, Uniprot, GenBank, Proteinpedia, PDB, NeXtProt, Genomix, Gene Card and Viral genome were the used database to specify the target genes, proteins in addition to analyze and optimize the control targets with BLAST analysis to determine synonymous and diverse segments. The whole genome FASTA format had been downloaded using different bioinformatics software NCBI (appendix IV). Ugene software was used to graph and conduct blast analysis between each corresponding proteins related to the SARS cov, SARS cov2 and Hcov, S, M, E, N, Orf1ab, orf3, orf6, orf7, orf8, orf9, orf10, HA and other NSP proteins that were deposited on softwares were assessed by multiple BLAST with NCBI to determine the exact synonym and structural variations and to be further confirmed by pdb superimposition with ICM. Viral protein complexes like S-ACE2 and N-RNA were also identified for their interacting sites. Host relevant proteins furin and ACE2 had been downloaded and assessed for their role in the pathogenesis of steps of viral infection. Those steps were conducted using pdb and ICM mol soft.

II. COVID-19 protein analysis was done using PDB, Uniprot, ICM mol soft pro, NCBI, NIH, Swiss Dock, BioXLab and Ugene software for determining viral protein 2D, 3D structures, similarity, biological roles , pathological effects, ligands, binding residues, surface map, protein health, strain points and target sites.

III. Ligand prediction: The target proteins were preprocessed for determination of the full fit ligand with highest binding energy, in addition to determining of the ligand trajectory and types of force field energy at the binding site.

IV. Ligand optimization: Using ICM, mol soft pro the ligand is optimized to best fit binding site after selecting a highly effective pocket among the determined table of protein binding sites and the final structure of the inhibitor is then edited and stored in several molecule formats like sdf, mol, mol2 for the next steps.

V. RCSB pdb, PDBe and PDBj were the main database softwares used to obtain pdb format of the viral and host proteins according to the following IDs: 6lu7, 6m3m, 6m03, 6lvn, 6lxt, 5r7y, 5r7z, 5r80, 5r81, 5r82, 5r83, 5gwy, 5r84.

Preprocessing of COVID-19 Papain-like Protease

With Mol soft pro, the target protein is loaded and converted to PDB format with water deletion. The protein is analyzed for health, surface electro potential and reviewing all domains and cofactors and then a project of table drug surveying is conducted by creating the project directory. Receptor mapping and surface map. The test drugs table is loaded too for later trajectory. Adjusting the cut-off distances and selecting the candidate drugs table (Figure 1).

Preparing the table of candidate drugs for surveying for PL Protease

The ligand based drug predictions were formulated by analyzing the ligand skeleton formula or functional group similarity in addition to the isomer and tautomer search were adopted, further optimization of the candidate to maximally fit the binding site in the target protein then retrograde surveying of the present drug to be tested for their binding energies to the candidate target COVID-19 proteins. These steps are analyzed with different editing softwares like Molsoft pro (Figure 2).

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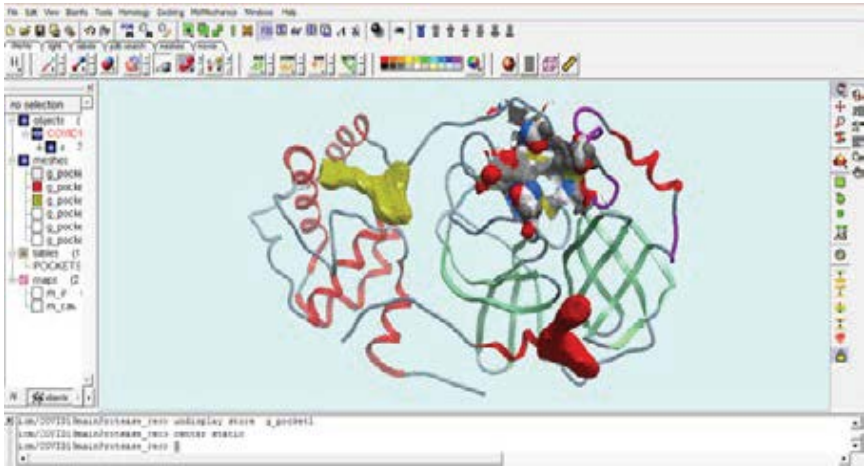

Figure 1: The main predicted binding scaffolds of PL protein at cut-off grid = 4.5.

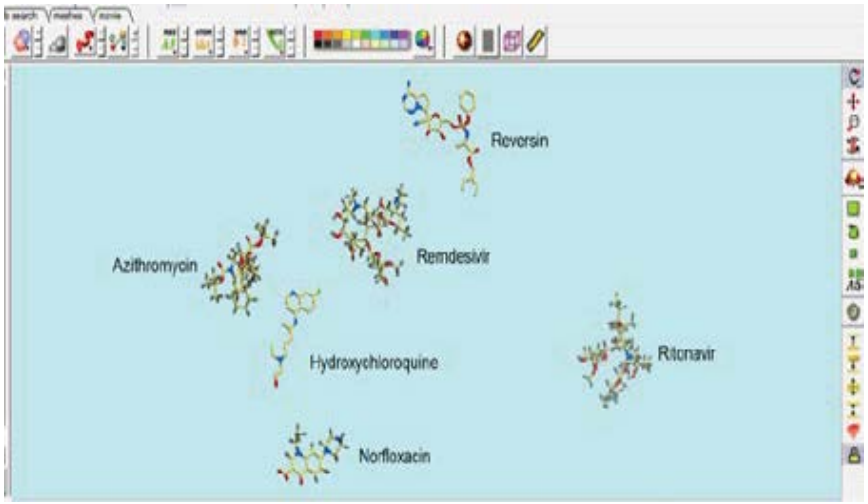

Figure 2: The drugs which were re-evaluated for their inhibitory efficacy against PL protease in Mol soft pro to analyze the trajectory, pockets, force field energy types and binding energy as indicators for the drug selectivity and affinity toward the binding site.

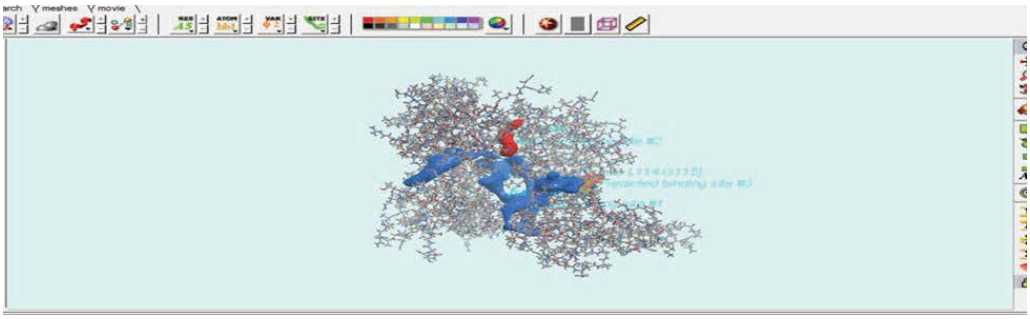

Figure 3: Predicting and evaluating the main target sites in COVID-19 N protein for surveying inhibitor compounds and drugs.

Preparing COVID-19 Nucleocapsid protein N for ligand prediction

The same steps were followed in preparing the previous targets (Figure 3).

Preprocessing of COVID-19 NSP12 (RDRP), PDB ID: 6nur

Similar steps were followed in preparing the previous targets (Figures 4-8).

Processing of COVID-19 NSP9 the replicase associated protein

Similar steps were followed in preparing the previous targets (Figure 9).

Processing of the viral spike S protein

Similar steps were followed in preparing the previous targets (Figures 10-12).

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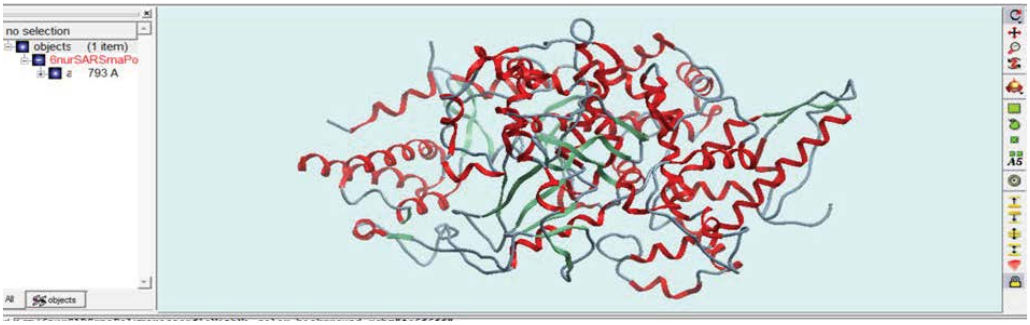

Figure 4: Ribbon representation of COVID-19 NSP12 the main viral target is the RDRP before complexing with NSP7 and NSP8.

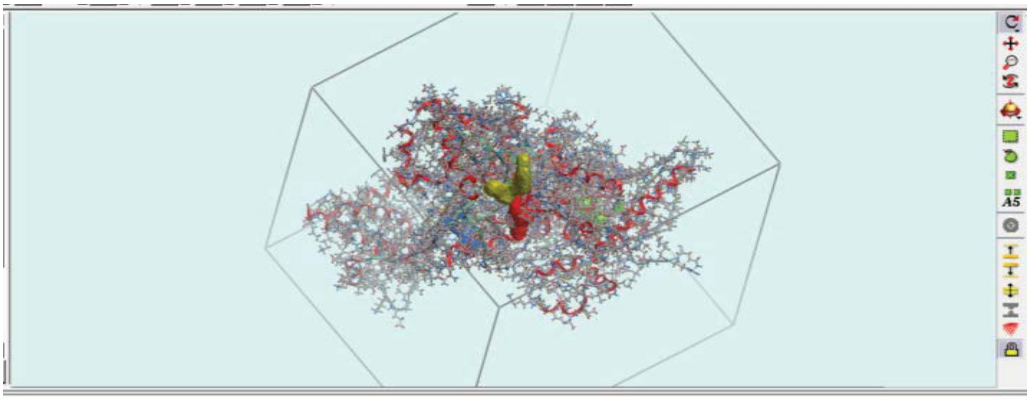

Figure 5: Predicting and evaluating the target sites of RDRP.

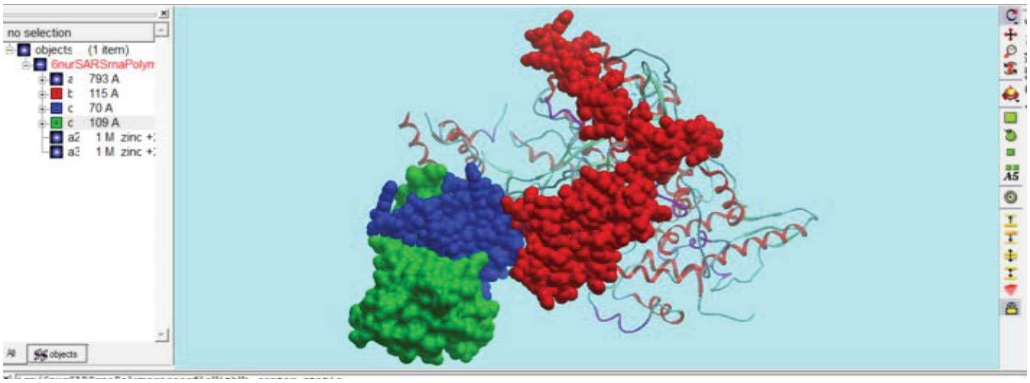

Figure 6: The complexes multi domain of COVID-19 NSP12 with NSP7, NSP8 and NSP9.

Figure 7: The FASTA format of the complexes multi domain of COVID-19 NSP12 with NSP7, NSP8 and NSP9.

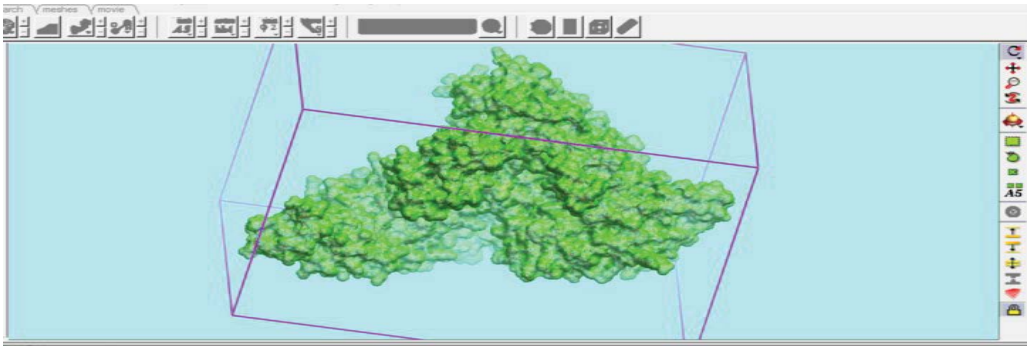

Figure 8: Processing of the surface receptor of NSP12 complex.

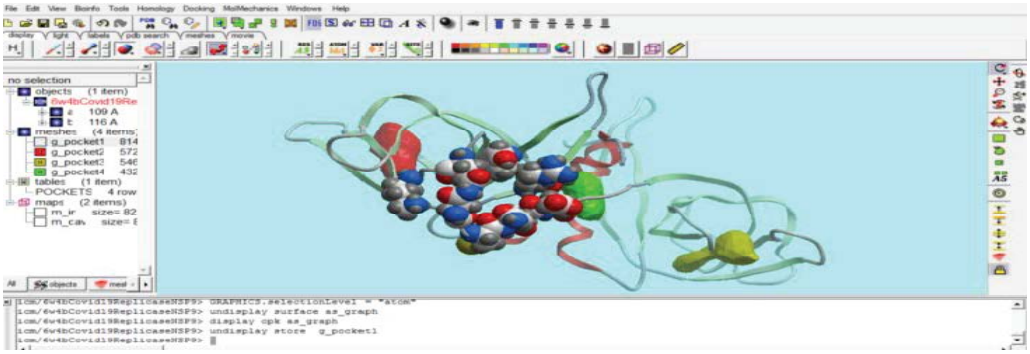

Figure 9: The 3D presentation of NSP9 (replicase associated protein) of COVID-19 with evaluating the target sites for drug design.

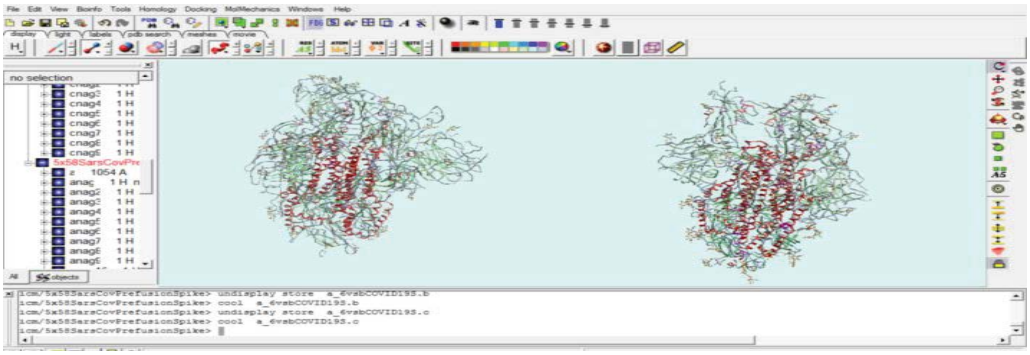

Figure 10: The 3D grid superimposition of the COVID-19 S protein (Rt) and Hcov S protein (Lt).

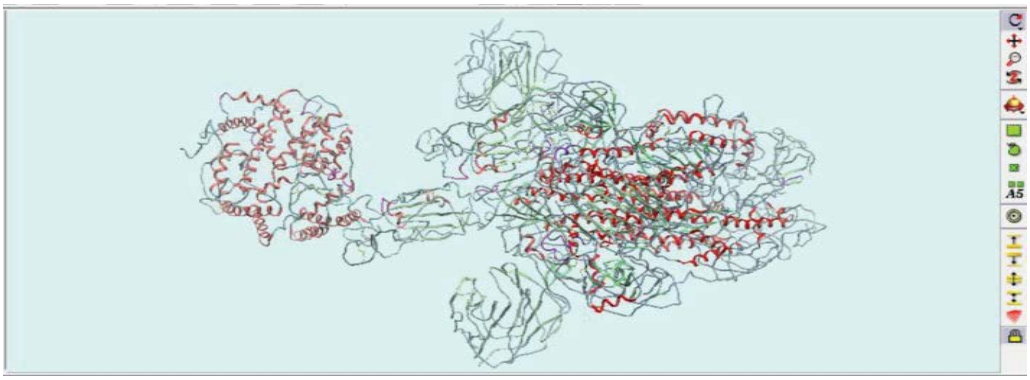

Figure 11: SARS CovS protein connected to the host angiotensin converting enzyme ACE2 to mediate cell membrane penetration.

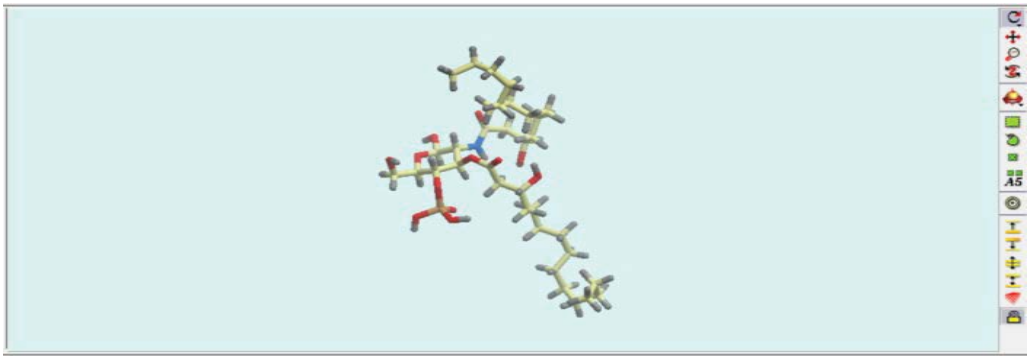

Figure 12: The ball and sticks representation of decaglucopyranose ligand of COVID-19 S protein.

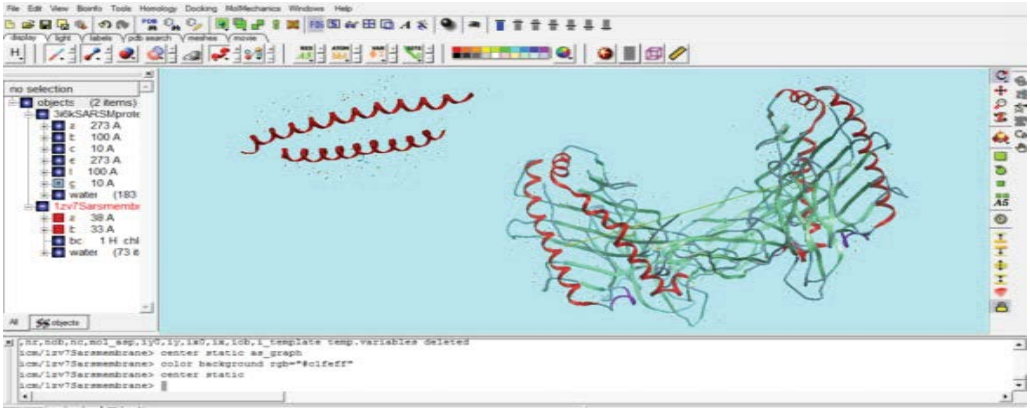

Figure 13: Represent a prior assessment and visualizing the M protein related to SARS Cov linked to HLA (Rt) and free (Lt).

The 3D representation of the structural (S, E, M, N) and non-structural viral proteins were processed and viewed with Mol soft pro and RasMol as shown below (Figure 13).

Processing and representing of the corona virus membrane M protein

Similar steps were followed in preparing the previous targets.

Laboratory assessment of the test antiviral compounds

To review drugs physicochemical, pharmacokinetics and toxicity properties in order to select the candidate of choice if it shows highly safe profile otherwise it is submitted to the next step. Steps of in silico and in vitro study were conducted in University of Kufa/College of Medicine/ the research lab of the department of Pharmacology and Therapeutics in addition to some steps conducted in private lab. Tis-phosphate buffer, alcohol 70%, norfloxacin pure powder, casein powder, papain-like protease powder, normal saline, distilled water, protease inhibitor powder were prepared. Simulink image intensity analyzer was used to measure casein opalescence in response to the test papain-like proease inhibiting drug, 1000X Olympus microscope, centrifuge; ultr-high speed mechanical stirrer, micropore filter and a mechanical shaker were used.

The artificial COVID-19 viral model

This model is aimed to test the bioavailability of the test drugs and their diffusion through the artificial viral membrane in addition to determining the inhibitory effects of those test drugs on papain-like protease by casein opalescence assay. The model is made of an artificial membrane which is obtained from human RBCs to formulate RBCs vesicles RV as it has been described in the appendix I procedure. After preparing the artificial viral membrane, papain-like protease is then incorporated into the RV to for the artificial virus AV. Casein in a pure form and was formulated as described in procedure in appendix II. Addition of 5 microg/ml of norfloxacin to the test well 1 in comparison to the control well and then the rate of casein opalescence change is measured with serial monitoring of the sample every one hour for 8 hrs.eir inhibitory effects on viral protease with microscopic imaging and spectrophotometric analysis of absorbance in relation with casein hydrolysis. Data is compared with the in silico model to assess the consistency of the antiviral property of a test drug. Data of the test and control wells are compared to assess norfloxacin inhibitory effect on viral papain-like protease. Such procedure could be repeated to assess other drugs for their protease inhibitor efficacy.

A drug repositioning step includes evaluating the level of similarity to an approved drug to be reassessed for antiviral use against that target protein.

I. Designing an in vitro model of antiviral assessment of the already preprocessed compounds and drugs. This model is formulated by incorporating the target COVID-19 papain-like protease into the lipid bilayer vesicles to evaluate the inhibitory efficacy of the experimental or approved drug on this enzyme function which causes casein opalescence.

II. Further surveying drug substructure and similarity to evaluate the optimal anti-COVID-19 drug.

III. Recommending further in vitro and in vivo study conduction and clinical study designs since the increase in antiviral treatment options is important for controlling viral infections

IV. Data analysis with the compatible statistical tests for in silico and in vitro outcomes.

Results

The outcomes of in vitro and in silico evaluation of compounds against COVID-19 were divided according the main pharmacological proteins targets related to this virus.

1. Findings of the inhibitory efficacy of test drugs repurposed against the COVID-19 main protease or the papain-like (PLP) protease (Figure 14).

The parameter was evaluated as the binding energy to PL protease in kJ/mol. Reversin, remdesivir, pentagastrin and norfloxacin showed enthalpy > 100 kJ/mol.

• The highest negative value the highest inhibitory efficacy

Reversin showed the highest inhibitory efficacy against COVID-19 papain-like protease as indicated by the ligand-PLP binding energy with Mol soft pro and BioXLab analysis. The calculated inhibitory binding was -137.30 kJ/mol z > 1.9. As compared with the tetrazapentadecanoate -129.57 kJ/mol z = 4.0, whereas remdesivir, pentagastrin, nitazoxanide and norfloxacin had a moderate anti PLP activity (> -100 kJ/mol).

2. Findings of the in vitro study design of COVID-19 papain-like protease (Figures 16 and 17).

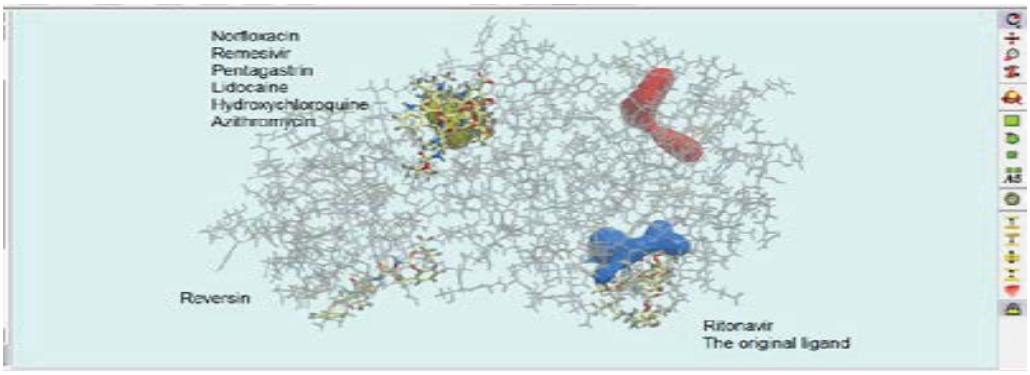

Figure 14: The binding sites of different tested drugs against COVID-19 protease PLP.

Table 1: COVID-19 protease inhibition efficacy of the test drugs as a binding energy in kJ\mol as analyzed with Mol soft pro (Figure 15).

Test drugs against COVID-19 protease	The inhibitory efficacy In kJ\mol	Statistics Z score	Drug approval
1- Reversin	-137.30	>1.9	Approved
2- Tetrazapentadecanoate	-129.57	>4.0	Experimental
3- Remdesivir	-119.50	>1.6	Approved
4- Pentagstrin	-118.82	>1,9	Approved
5- Nitazoxanide	110.29	>1.9	Approved
6- Norfloxacin	-103.70	>1.9	Approved
7- Ritonavir	-95.22	>1.6	Approved
8- Hydroxychloroquine	-86.42	>1.6	Approved
9- Azithromycin	-85.78	>1.6	Approved
10- Lidocaine	-80.69	>1.6	Approved
Red = Very Potent			

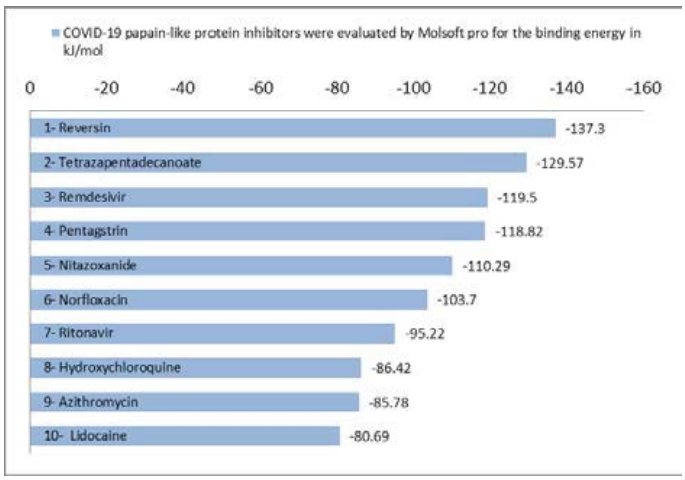

Figure 15: The comparative efficacy of drugs that were selected according to their skeletal superimposition to the predicted ligand of PL protease.

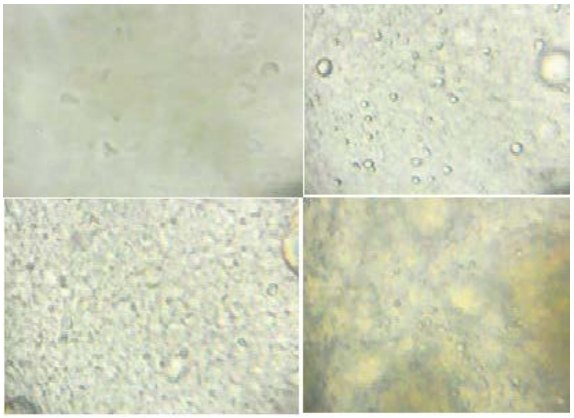

Figure 16: In vitro antiviral model under 1000X microscopic image showing the casein micelles of the blank sample (upper left) and the artificial papain-like protease containing vesicles AV (upper right). The lower left sample represents casein sample mixed with AV and the lower right sample shows the casein sample mixed with AV and norfloxacin to assess its effect on papain-like protease.

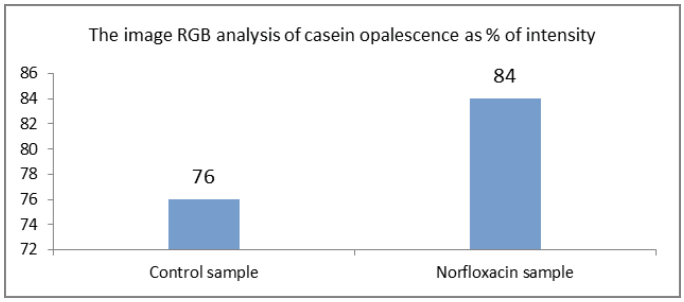

Figure 17: The relative protection with norfloxacin against casein decrease in opalescence. This decrease in opalescence is due to the effect of papain-like protease in hydrolyzing casein at cysteine residues. It was obvious that norfloxacin had a protective effect which indicates its papain-like protease inhibitor action. Casein palescence was measured with Simulink image analysis to determine the relative loss in image intensity.

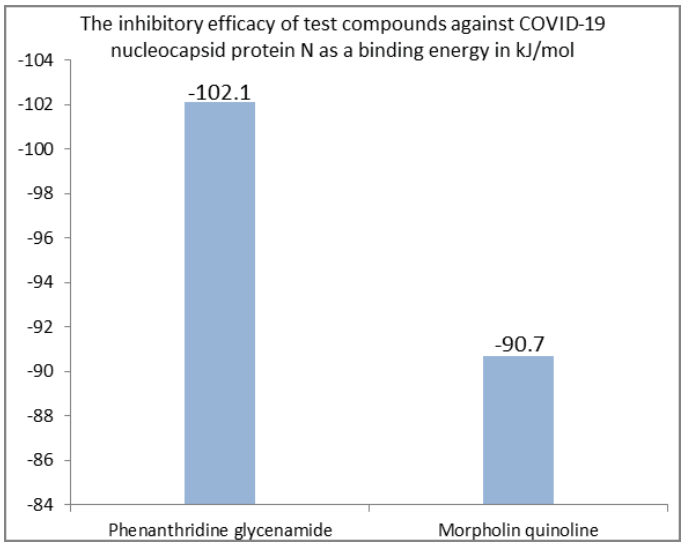

Figure 18: The main COVID-19 nucleocapsid N binding ligands and their binding energy in kJ/mol.

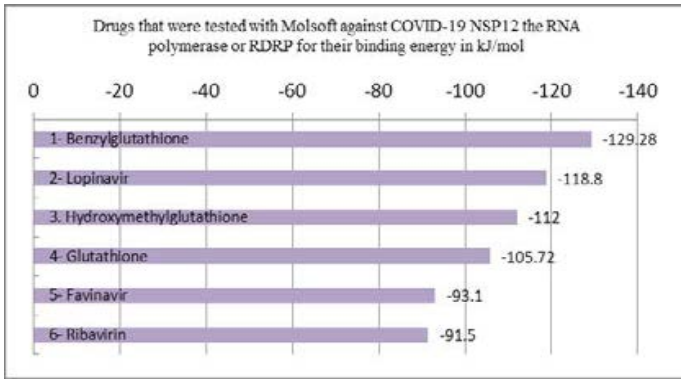

Figure 19: The comparative efficacy of test drugs against COVID-19 RDRp, the parameter was evaluated as the binding energy to RDRP in kJ/mol. Benzylglutathion, lopinavir, hydroxymethylchloroquine and glutathione had a binding enthalpy > 100 kJ/mol and z score >1.9.

3. Findings of COVID-19 nucleocapsid N inhibiting ligands.

Assessment of the drugs activity against NSP12 (RDRp) included selectedligandanaloguesbenzylglutathione,hydroxymethylglutathione, lopinavir, glutathione, Favinavir and ribavirin showed promising results against COVID-19 infection. Benzyl glutathione had the highest inhibitory efficacy against COVID-19 RDRp with a binding energy of -129.28 kJ/mol z > 1.9 and lopinavir came second in efficacy with a binding energy of -118.80 kJ/mol, z = 2.2. Glutathione had a moderate RDRp binding energy (> -105.72 kJ/mol).

Table 2: COVID-19 nucleocapsid N inhibiting ligands evaluated and represented by Mol soft pro and iGemdock (Figure 18).

COVID-19 Nucleocapsid N inhibitor drugs	Anti-N binding energy in kJ\mol	Statistics Z score	Drug approval
Phenanthridine Glycenamide	-102.10	4.2	Experimental
Morpholin Quinoline	-90.70	4.0	Experimental

Table 3: The inhibitory efficacy of a group of drugs evaluated for their NSP12 (RDRP) binding energy with Mol soft pro (Figure 19).

COVID-19 NSP12 inhibiting drugs	Anti-RNA polymerase Efficacy in kJ/mol	Statistics Z score	Change
1- Benzyl glutathione	-129.28	>1.9	Experimental
2- Lopinavir	-118.80	2.2	Approved
3. Hydroxymethylglutathione	-112.00	>1.9	Experimental
4- Glutathione	-105.72	>1.9	Approved
5- Favinavir	-93.10	2.2	Approved
6- Ribavirin	-91.50	2.2	Approved
Red = Highly Potent			

Discussion

Although viruses like COVID-19 having limited number of protein, however these proteins contain multiple pockets and binding sites to guide the rational for developing many antiviral compounds [25]. Among the COVID-19 genome set of proteins, the current study concerned with papain-like protease (PLpro) and RNA-dependent RNA polymerase (RdRp) as the main targets. The near future steps will include more presice optimization of the ligands against other COVID-19 proteins [26-28]. Ten drugs where assessed by in silico model of studying the PLP binding energy in kJ/mol. These drugs included reversin, pentagastrin, the original ligand tetrapentazadecanoate, remdesivir, nitazoxanide, norfloxacin, hydroxychloroquine, ritanovir, Azithromycin and lidocaine. The anti-resistance reversin showed the highest inhibitory efficacy against COVID-19 papain-like protease as indicated by the ligand-PLP binding energy. The calculated inhibitory binding energy was -137.30 kJ/mol z > 1.9 as compared with the tetrazapentadecanoate -129.57 kJ/mol z = 4.0, whereas remdesivir, pentagastrin, nitazoxanide and norfloxacin had a moderate PLP binding energy (>-100 kJ/mol). In vitro PLP inhibiting activity for norfloxacin was slightly consistent with the in silico outcomes. The designed in vitro model for COVID-19 was not highly reliable due to the limited facilities under the current epidemic, however adopting a more sophisticated in vitro models against risky viral infections is the key of development of new antiviral drugs decause this model is accessible for a wider number of researchers [29-31]. Other tested drugs against PLP showed just a weak binding energy (-80 to -95 kJ/mol). These drugs included hydroxychloroquine, Azithromycin, ritanovir and lidocaine. Antiviral activities of remdesivir, ritanovir, nitazoxanide, quinolones and some oligopeptides were confirmed by different studies against viral infections other than COVID-19 [32]. Assessment of the drugs activity against NSP12 (RDRp) included selected ligand analogues benzyl glutathione, hydroxymethylglutathione, lopinavir, glutathione, Favinavir and ribavirin showed promising results against COVID-19 infection. Benzyl glutathione had the highest inhibitory efficacy against COVID-19 RDRp with a binding energy of -129.28 kJ/mol z > 1.9 and lopinavir came second in efficacy with a bindind energy of -118.80 kJ/mol, z = 2.2. Glutathione had a moderate RDRp binding energy (> -105.72

kJ/mol). Glutathione, lopinavir, Favinavir and ribavirin had also antiviral effects on other viral infections [33,34]. Comparing the 3D conformational binding was also evaluated and showed that some of the evaluated drugs had different docking sites while others had the same docking sites with different binding residues and energies. Evaluation of the ligands against NSP9 COVID-19 replicase associated protein. The predicted ligands binding to NSP9 were evaluated using Mol soft pro and they showed no significant similarity in their structure or formula although the similarity cut-off was set at 0.4. Evaluation of the ligands against COVID-19 spike S protein. This protein was relatively large in size and it performs a structural unit and cell penetration mechanisms which mean it has a macromolecular surface of binding site so that direct inhibitory actions of small drugs may have a limited efficacy against S protein [35-37]. One ligand was predicted to be of applied value which was the decaglucopyranose.

The COVID-19 relevant host components Furin and ACE2

Furin is an essential housekeeping enzyme for activating many metabolic and cellular proteins it needs for a highly selective mechanism of modifying its action in order to spare the physiologic effects [38]. On the other hand, angiotensin converting enzyme ACE2 is an essential cytoprotective enzyme although it's a one mediator of corona virus's penetration into the cell [39,40].

Other host components like INF gamma and ILs and vaccines

Immunotherapy is critically important in treating and controlling viral diseases. Vaccines may comprise the versatile health care measures against future viral epidemics; however several weak points are correspondent with immunotherapy in the future of viral infections. Of these drawbacks of immunotherapy is its expiry of protection since most of pathogens have the virulent strategy to change their antigens, moreover, host immune response has its own duration of action which may extend from weeks to few years, however immune protection is uncommonly to be lifelong [41]. As it was confirmed by bioinformatics of different databases, another critical point in viral immunotherapy is that most of the viral infections will eventually exaggerate immune system. This response is at most the cytotoxic and pathogenic event that in many instances gives rise to the seriousness of even simple viral infection so that immunotherapy will remain risky and in many times it is cautious [42-44]. For all these reasons the antiviral drug therapy is highly promising to compact the epidemic infections.

Conclusion

From the overall results quinolones, antiviral drugs, glutathione and peptides like reversin and pentagastrin had a promising inhibitory efficacy against COVID-19 protein targets so that developing COVID-19 proteins blockers from the approved drugs is an accessible approach and could provide rapid and safe therapeutic option against the risky viral epidemics.

Statement of Noveltys

Quinolones, antiviral drugs, glutathione and the small peptide compounds are promising inhibitors for different COVID-19 proteins and provide the rational to be repurposed in further studies against corona virus infections.

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References

1. Rabi FA, Al Zoubi MS, Kasasbeh GA, Salameh DM, Al-Nasser AD (2020) SARS-CoV-2 and Coronavirus disease 2019: What we know so far. Pathogens 9: 231.
2. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, et al. (2020) Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B.
3. Prajapat M, Sarma P, Shekhar N, Avti P, Sinha S (2020) Drug targets for corona virus: A systematic review. Indian J Pharmacol 52: 56-65.
4. Fehr AR, Perlman S (2015) Coronaviruses: An overview of their replication and pathogenesis. Methods Mol Biol 1282: 1-23.
5. Masters PS (2006) The molecular biology of coronaviruses. Adv Virus Res 66: 193-292.
6. Schoeman D, Fielding BC (2019) Coronavirus envelope protein: current knowledge. Virol J 16: 69.
7. Yang S, Fu C, Lian X, Dong X, Zhang Z (2019) Understanding human-virus protein-protein interactions using a human protein complex-based analysis framework. mSystems 4: e00303.
8. Dyall J, Coleman CM, Hart BJ, Venkataraman T, Holbrook MR, et al. (2014) Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob Agents Chemother 58: 4885-4893.
9. Wink M (2015) Modes of Action of Herbal Medicines and Plant Secondary Metabolites, Medicines 2: 251-286.
10. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, et al. (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503: 535-538.
11. Han DP, Penn-Nicholson A, Cho MW (2006) Identification of critical determinants on ACE2 for SARS-CoV entry and development of a potent entry inhibitor. Virology 350: 15-25.
12. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, et al. (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426: 450-454.
13. Baxter D (2007) Active and passive immunity, vaccine types, excipients and licensing. Occup Med (Lond) 57: 552-556.
14. Prompetchara E, Ketloy C, Palaga T (2020) Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol 38: 1-9.
15. Afzal O, Kumar S, Kumar R, Firoz A, Jaggi M, et al. (2014) Docking based virtual screening and molecular dynamics study to identify potential monoacylglycerol lipase inhibitors. Bioorg Med Chem Lett 24: 3986-3996.
16. Karthick V, Nagasundaram N, Doss CGP, Chakraborty C, Siva R, et al. (2016) Virtual screening of the inhibitors targeting at the viral protein 40 of Ebola virus. Infect Dis Poverty 5: 12.
17. Leidner F, Kurt Yilmaz N, Schiffer CA (2019) Target-Specific prediction of ligand affinity with structure-based interaction fingerprints. J Chem Inf Model 59: 3679-3691.
18. Zou X, Sun Y, Kuntz ID (1999) Inclusion of solvation in ligand binding free energy calculations using the generalized-born model. J Am Chem Soc 121: 8033-8043.
19. Wang R, Lai L, Wang S (2002) Further development and validation of empirical scoring functions for structure-based binding affinity prediction. J Comput Aided Mol Des 16: 11-26.
20. Totura AL, Bavari S (2019) Broad-spectrum coronavirus antiviral drug discovery. Expert Opin Drug Discov 14: 397-412.
21. Janosko K, Holbrook MR, Adams R, Barr J, Bollinger L, et al. (2016) Safety

precautions and operating procedures in an (A) BSL-4 laboratory: 1. Biosafety level 4 suit laboratory suite entry and exit procedures. J Vis Exp 2016: 52317.

22. Wimmer E, Mueller S, Tumpey TM, Taubenberger JK (2009) Synthetic viruses: a new opportunity to understand and prevent viral disease. Nat Biotechnol 27: 1163-1172.

23. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S (2020) Potential inhibitor of COVID-19 main protease (Mpro) from several medicinal plant compounds by molecular docking study. Preprints.

24. Báez-Santos YM, John SE, Mesecar AD (2015) The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. Antiviral Res 115: 21-38.

25. Lu A, Zhang H, Zhang X, Wang H, Hu Q, et al. (2004) Attenuation of SARS coronavirus by a short hairpin RNA expression plasmid targeting RNA- dependent RNA polymerase. Virology 324: 84-89.

26. Wang Z, Ren L, Zhao X, Hung T, Meng A, et al. (2004) Inhibition of severe acute respiratory syndrome virus replication by small interfering RNAs in mammalian cells. J Virol 78: 7523-7527.

27. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, et al. (2020) Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS Central Science.

28. Arya R, Das A, Prashar V, Kumar M (2020) Potential inhibitors against papain-like protease of novel coronavirus (SARS-CoV-2) from FDA approved drugs. ChemRxiv.

29. Pizzorno A, Terrier O, Nicolas de Lamballerie C, Julien T, Padey B, et al. (2019) Repurposing of drugs as novel influenza inhibitors from clinical gene expression infection signatures. Front Immunol 10.

30. Smith T, Bushek J, Prosser T (2020) COVID-19 Drug Therapy-Clinical Drug Information | Clinical Solutions. Elsevier.

31. Te Velthuis AJ, Arnold JJ, Cameron CE, van den Worm SH, Snijder EJ (2011) The RNA polymerase activity of SARS-coronavirus nsp12 is primer dependent. Nucleic Acids Res 39: 9458-9458.

32. Xu X, Liu Y, Weiss S, Arnold E, Sarafianos SG, et al. (2003) Molecular model of SARS coronavirus polymerase: implications for biochemical functions and drug design. Nucleic Acids Res 31: 7117-7130.

33. Sutton G, Fry E, Carter L, Sainsbury S, Walter T, et al. (2004) The nsp9 replicase protein of SARS-coronavirus, structure and functional insights. Structure 12: 341-353.

34. Bavan S, Sherman B, Luetje CW, Abaffy T (2014) Discovery of novel ligands for mouse olfactory receptor MOR42-3 using an in silico screening approach and in vitro validation. PLoS One 9: e92064.

35. Smith RD, Engdahl AL, Dunbar Jr JB, Carlson HA (2012) Biophysical limits of protein-ligand binding. J Chem Inf Model 52: 2098-2106.

36. Thomas G (2002) Furin at the cutting edge: From protein traffic to embryogenesis and disease. Nat Rev Mol Cell Biol 3: 753-766.

37. Wong SK, Li W, Moore MJ, Choe H, Farzan M (2004) A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 279: 3197-3201.

38. Xiao X, Chakraborti S, Dimitrov AS, Gramatikoff K, Dimitrov DS (2003) The SARS-CoV S glycoprotein: expression and functional characterization. Biochem Biophys Res Commun 312: 1159-1164

39. Naran K, Nundalall T, Chetty S, Barth S (2018) Principles of immunotherapy: implications for treatment strategies in cancer and infectious diseases. Front Microbiol 9.

40. Troy NM, Bosco A (2016) Respiratory viral infections and host responses; insights from genomics. Respir Res 17: 156.

41. UniProt (2020) Human SARS coronavirus (SARS-CoV). UniProtKB-P0C6X7 (R1AB_CVHSA).

42. Canadian Institutes of Health Research (2020) Drugbank, Canada.

43. Xia Q, Zhang Y, Li Z, Hou X, Feng N (2019) Red blood cell membrane-camouflaged nanoparticles: a novel drug delivery system for anti-tumor application. Acta Pharm Sin B 9: 675-689.

44. Yoshino U (2014) Studies on the opalescence in Sodium-caseinate solution developed by the milk coagulating enzymes. J Agric Chem Soc Japan 22: 242-248.