FABAD J. Pharm. Sci., 46, 2, 147-158, 2021

RESEARCH ARTICLE

Hydroxychloroquine: Similarity Search and Structure-Based Virtual Screening for Identification of Potential Hits for Chemoprophylaxis Against SARS-CoV-2

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SUMMARY

The current eruption of the novel severe acute respiratory syndrom causing coronavirus 2 (SARS-CoV-2) is an atrocious health tragedy. In this virulent disease, the computational approach appears to be the most hopeful choice to make out an efficient remedial medicinal agent for the treatment of an infected population. This current exploration inclined to analyze the similar druggable compounds as hydroxychloroquine to combat unworn coronavirus (COVID-19), using a pharmacoinformatics study. Docking-based virtual screening was carried-out using Glide, followed by Absorption Distribution Metabolism Excretion (ADME) anticipation. Hydroxychloroquine is being used as the criterion for comparison as it showed potential effect for symptomatic relief. Target-based virtual screening study divulged 28 top-ranked compounds based on their binding energy and dock score from 10695 PubChem compounds. In the additional weed-out process, 07 compounds were selected based on their similar interactions as hydroxychloroquine, comparable binding energy, and shape complementarity of the binding pocket of 6LU7. The three-dimensional binding pose of screened 07 hits and their chemo-essential features were successfully matched with reference compound. These candidates showed potential interactions with the amino acid residues of the active site of SARS-CoV-2 (PDB ID 6LU7). Therefore, they may have the capability as lead compound(s) against COVID-19.

Key Words: *Binding energy, COVID-19, Docking, Hydroxychloroquine, SARS-CoV-2, Virtual Screening*

Hidroksiklorokin: SARS-CoV-2'ye Karşı Kemoprofilaksi İçin Potansiyel Aday Bileşiklerin Tanımlanması Yönünde Benzerlik Araştırması ve Yapı Tabanlı Sanal Tarama

ÖZ

Yeni şiddetli akut solunum yolu sendromu koronavirüsü 2'nin (SARS-CoV-2) mevcut pandemisi korkunç bir sağlık trajedisidir. Bu virülan hastalıkta, hesaplamalı yaklaşım, enfekte popülasyonun tedavisi için etkili bir iyileştirici tıbbi ajan oluşturmak için en umut verici seçenek olarak görünmektedir. Mevcut araştırma, yeni koronavirüsle (COVID-19) mücadelede farmakoinformatik çalışması kullanarak, hidroksiklorokine benzer ilaç olma özelliği gösteren bileşikleri analiz etme eğilimindedir. Moleküler yerleştirme (docking) esaslı sanal tarama Glide kullanılarak gerçekleştirilmiş ve ardından Absorbsiyon Dağılım Metabolizma Eliminasyon (ADME) tahmini yapılmıştır. Hidroksiklorokin, semptom hafifletici etki potansiyeli gösterdiği için karşılaştırma kriteri olarak kullanılmıştır. Hedefe dayalı sanal tarama çalışması, 10695 pubChem bileşiğinden bağlanma enerjilerine ve docking skorlarına göre en üst sırada yer alan 28 bileşiği ortaya çıkarmıştır. İlave ayıklama işleminde, 7 bileşik, hidroksiklorokine benzer etkileşimleri, karşılaştırılabilir bağlanma enerjisi ve 6LU7 bağlanma cebinin şekil tamamlayıcılığı temelinde seçilmiştir. Üç boyutlu bağlanma pozu ve taranan 7 aday bileşiğin kimyasal olarak gerekli özellikleri, referans bileşik ile başarılı bir şekilde eşleştirilmiştir. Bu adaylar, SARS-CoV-2'nin (PDB ID 6LU7) aktif bölgesinin amino asit kalıntıları ile potansiyel etkileşimler göstermiştir. Bu nedenle, COVID-19'a karşı öncü bileşik(ler) olma kapasitesine sahip olabilirler.

Anahtar Kelimeler: *Bağlanma enerjisi, COVID-19, Moleküler yerleştirme (Docking), Hidroksiklorokin, SARS-CoV-2, Sanal Tarama*

Received: 21.08.2020
Revised: 15.10.2020
Accepted: 09.02.2021

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INTRODUCTION

The 2019-novel coronavirus (nCoV) is a significant source of disaster in the 21st century. The global pandemic of novel coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in the Wuhan city of China in December 2019 and has since spread worldwide to almost every country.

Coronaviruses (CoVs) have a single-stranded RNA genome (range between 26.2-31.7 kb, positive sense), concealed by an encapsulated structure. The shape is either pleomorphic or globular, and it is characterized by bears club-shaped bulge of glycoproteins on its surface (diameter 80–120 nm) (Yang, 2006). Among all the RNA viruses, the RNA genome of SARS-CoV-2 is one of the largest (Belouzard, 2012.). The number of open reading frames (ORFs) in the CoV genome ranges from six to ten. The genetic material of CoV is susceptible to frequent recombination processes, which can give rise to new strains with an alteration in virulence (Hilgenfeld, 2014).

There are seven strains of human CoVs including 229E, NL63, OC43, HKU1, Middle East respiratory syndrome (MERS)-CoV, SARS-CoV-2 and nCoV which are responsible for the infection of both of lower and upper respiratory tract and lead to common cold, pneumonia, bronchiolitis, rhinitis, pharyngitis, sinusitis and other symptoms such as watery diarrhea (Chang, 2020). (SARS)-CoV, and (nCoV), responsible for the infection with particular reference to the involvement of the respiratory tract (both lower and upper respiratory tract), e.g., common cold, pneumonia, bronchiolitis, rhinitis, pharyngitis, sinusitis, and other symptoms such as occasional watery diarrhea (Chang, 2016; Paules, 2020). Among the seven strains, three strains proved highly pathogenic (SARS-CoV, MERS-CoV, and 2019-nCoV), which caused endemic of severe CoV disease (Abramo, 2012; Paules, 2020). SARS-CoV reservoir is unknown, but bats and subsequent spread to Himalayan palm civets are being hypothesized (Saif, 2004). The MERS-CoV also has a zoonotic origin in the Middle East region, and the transmission is through the camels (Al-Osail Al-Wazzah, 2017). Among these, the SARS-CoV outbreak started in 2003 in Guangdong province of China and the second outbreak of the MERS-CoV outbreak in 2012 in Saudi Arabia (Saif, 2004; Chang, 2016; Yang, 2006). The most important structural proteins of CoV are spike (S) protein (trimeric), membrane (M) protein, envelop (E) protein, and the nucleocapsid (N) protein. Some viruses, such as beta-CoVs, also have hemagglutinin esterase (HE) glycoprotein (Hilgenfeld, 2014). The RNA genome of CoV has seven genes which are conserved in the order: ORF1a, ORF1b, S, OEF3, E, M, N in 5' to 3' direction. The two-third part of the RNA genome is covered by the ORF1a/b, which produces the two viral replicase proteins which are polyproteins (PP1a and PP1ab). The remaining genome part of the virus encodes the mRNA, which has the structural proteins, i.e., spike, envelope, membrane, nucleocapsid, and other accessory proteins (McBride, van Zyl, Fielding, 2014). Another essential envelope-associated protein that is expressed by only some strains of CoV is the HE protein. The RNA genome of CoV is packed in the nucleocapsid protein and further covered with an envelope (Guo, Korteweg, McNutt, Gu, 2008).

In silico based screening has proven to be a very advantageous tool to meet the challenges of antiviral drug discovery. PubChem has diverse nature of compounds with an enormous extent of chemical scaffolds that are available for digging out the potential compounds using the computational approach (Kristensen, 2013; Kim, 2016; Patidar, 2016). An antimalarial agent, Chloroquine acts by expanding the pH of intracellular vacuoles and adjusting protein debasement pathways through acidic hydrolases. These pathways mainly occur in lysosomes, macromolecule union in the endosomes, and post-translational protein change in the Golgi contraption (Plantone Koudriavtseva, 2018; Nicola Principi, 2020). Hydroxychloroquine, a less harmful aminoquinoline with N - hydroxyethyl side chain instead of the N - diethyl gathering of Chloroquine (Savarino, 2003). This alteration makes ease in the dissolving capability of Hydroxychloroquine than Chloroquine (Gautret, 2020). Therefore, Chloroquine, Hydroxychloroquine build the pH and present antiviral impacts (Sahraei,

Shabani, Shokouhi, Saffaei, 2020). The present study focused on the ≥80% similar compounds to the Hydroxychloroquine were delivered to hierarchical target-based virtual screening to identify sound hits that could bind at the N3 site of SARS-CoV-2 (PDB ID 6LU7) and thus, could play a vital role in hindering extension of COVID-19 bug (Colson, 2020; Gbinigie Frie, 2020; Jin, 2020; Kapoor KM; Kapoor A, 2020). We concluded by using this virtual screening and extracted out most successful hit candidates according to their rank, binding energy, and protein-ligand interactions from Hydroxychloroquine like 10695 PubChem compounds. Further, Hydroxychloroquine and these seven hits comprise similar protein-ligand interaction along with the 3D-orientation in the binding pocket of SARS-CoV-2. Therefore, it may serve as hit candidates for lead finding studies as precised in Figure 1.

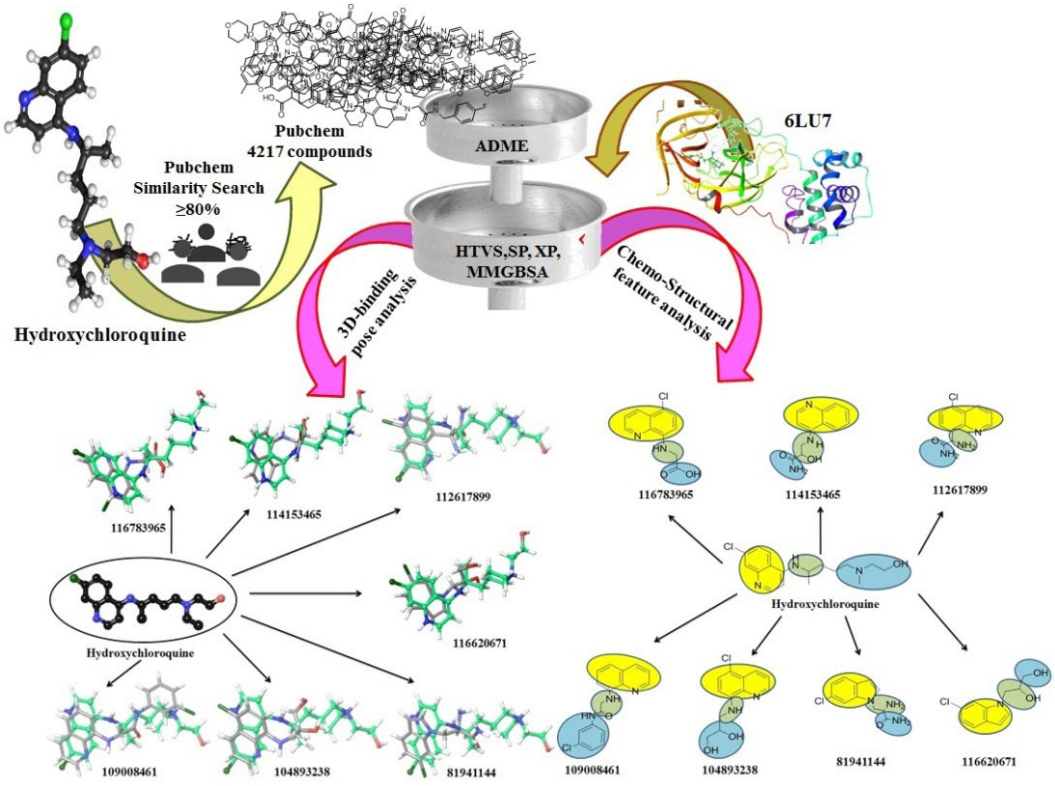

Figure 1. Pictorial representation of the application of Structure-Based Approach

MATERIALS AND METHODS

The crystal structure of SARS-CoV-2, which causes COVID-19, was obtained from protein databank (PDB ID 6LU7) for docking-based virtual screening, and it has virtuous resolution i.e., 2.16 Å. This is the only validated synthetic crystal structure of SARS-CoV-2 that was found as per the experimental records, and its sequence similarity was 95% to bat-SL-CoVZC45 and 88% to SARS-CoV-ZSc with complex with N3 inhibitor (Jin, 2020; Prajapat, 2020). The protein was prepared using protein preparation in which the addition of the hydrogens, disulfide bonds establishment, and assignment of bond orders with the exclusion of water molecules far from 5Å. Removal of steric hindrances in the amino acid residues of protein was done by improving and minimizing hydrogen bonds. The missing side chains and loops were filled by using the Prime module (Genheden Ryde, 2015). Docking studies were performed on a primed structure of SARS-CoV-2. The 3D grid was generated at the N3 site, which is an inhibitor having CC50 >133 μM; substrate-binding site is situated between

domains I and II as reported in previous literature. N3 can precisely inhibit Mpro from numerous coronaviruses, including SARS-CoV and MERS-CoV, and has exhibited potential as an antiviral agent against contagious bronchitis virus in an animal model. A docking pose revealed that N3 could suitably privilege for the substrate-binding pocket in the homology model of SARS-CoV-2 Mpro. The kinetic studies were also proved a progressive plot disclosed that it is a time-dependent irreversible inhibitor of this enzyme in the reported literature (Jin, 2020; Prajapat, 2020).

Similarity Search and Structure-Based Virtual Screening

As per the reported literature, Hydroxychloroquine has the potential to fight with SARS-CoV-2. Therefore; all 2D structures of PubChem compounds having ≥80% similarity with Hydroxychloroquine were downloaded in .sdf format (Kristensen, 2013; Kim, 2016; Patidar, 2016). The hydroxychloroquine and these 4,217 similar structures were processed using the Ligprep module of Schrodinger 2019-1 package for desalting, cleaning, and addition of hydrogen atoms using OPLS3e force filed. Various stereoisomers, tautomers, and ionization states at pH 7 ± 2 were also generated (Sirin, 2014). Target based *in silico* screening was used to recognize the best hit(s), which may act against SARS-CoV-2 as hydroxychloroquine. The Docking studies of these similar compounds along with the hydroxychloroquine were done on active site of 6LU7 by hierarchal computer-generated screening from GLIDE module v8.2 (ADME, Lipinski filter, HTVS, SP, and XP) for searching of hit candidate compounds based on protein-ligand interactions and predicted binding energy of ligands in Kcal/mol.

Binding Energy Calculation

Retrieved PubChem compounds as hits against 6LU7 were in use for relative binding energy computation from pose viewer file of protein-ligand complexes using Prime module of Schrodinger 2019-1 software. The process, which is physics-centered drives the force field in an implicit solvent system of the bound and unbound candidates contributing in the binding course of accomplishment is well known as MM-GBSA (Molecular Mechanics, the Generalized Born model, and Solvent Accessibility). The MM-GBSA calculate the binding energy in Kcal/mol. and comprises of requisites such as protein-ligand Vander Waals connections, electrostatic ionic exchanges, desolvation of ligand, and energies in internal strain. The binding energies by the VSGB2.0 implicit solvent model have been established in Prime (Genheden Ryde, 2015). The hits candidate compounds succeeded from hierarchal virtual screening were further calculated to the MM-GBSA consideration. In the progression of these explorations, the active site of protein was established to modify itself up to a 5 Å for docked ligands accordingly. This practice introduced the XP docked file, which was "out.maegz" of protein-ligand complex, which resulted in the compounds rank founded on computational binding energies, and the full methodology was figured out in Figure 2 (Adem, 2020; Khaerunnisa, 2020).

ADME Predictions

Numerous druggable compounds were rejected due to their poor pharmacokinetic features such as absorption, distribution, metabolism, and excretion (ADME). Therefore, these pharmacokinetic features of best 07 candidates were screened using ADME parameters from QikProp module v5.9 of Schrodinger 2019-1 by conforming to the rule of five and three to evaluate the blood-brain barrier permeability, polar surface area, human oral absorption percentage, and molecular weight (Dzierba, 2007; Chatterjeeet al., 2014;)

RESULTS AND DISCUSSION

Target Based Screening of Ligands

The structure-based *in silico* screening was performed in the hierarchal model for elimination technique, i.e., ADME, Lipinski filter, high throughput virtual screening (HTVS) pursued by simple precision (SP) and extra precision (XP). The compounds were being passed from ADME and Lipinski filter were further delivered to gradual screening to weed out lower ranked compounds, then top-ranked 50 compounds were selected for next level screening consisting of binding energy prediction and protein-ligand interactions.

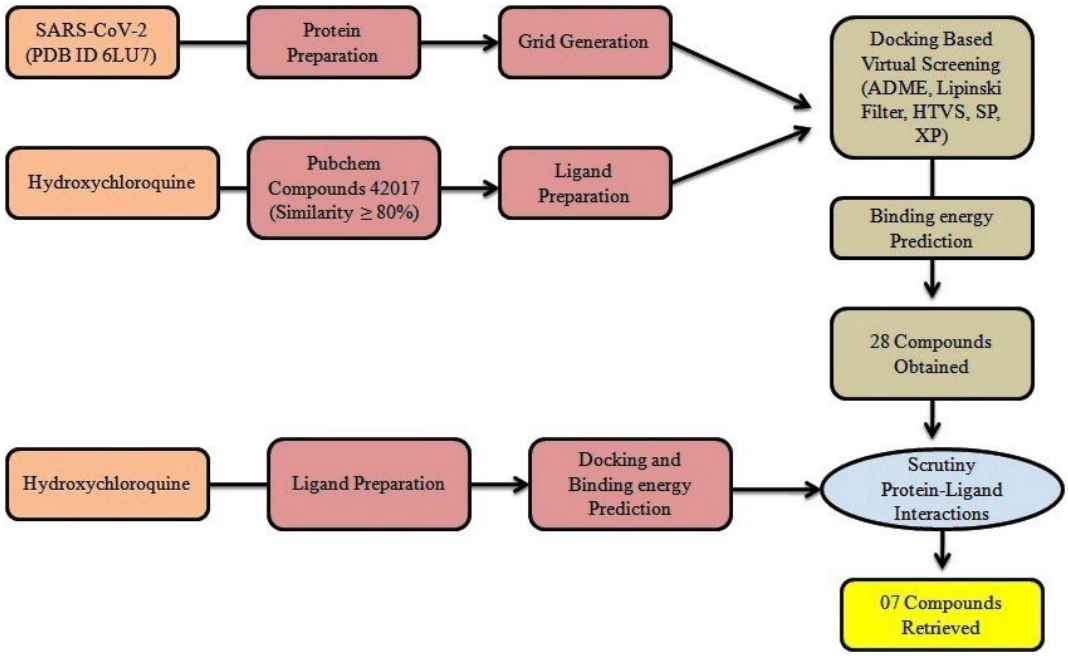

Figure 2. The methodology of Structure-based virtual screening

The PDB ID 6LU7 was selected for targeting COVID-19 in structure-based virtual screening, and it indicated that the similar compounds to the hydroxychloroquine were able to form bonds with diverse amino acids in the active site of 6LU7 for the computational model of screening. Hydroxychloroquine (anti-malarial) was successfully acted against COVID-19; therefore XP docked and prediction of binding energy was done for the finding of essential interacting residues and formed different types of contacts with amino acids of the binding pocket of 6LU7. Structure-based virtual screening and binding energy prediction gave 28 hydroxychloroquine like compounds.

Assessment of Target Based Screening

Assessment of screened 28 hits was done based on protein-ligand interaction study with predicted binding energy and 07 compounds were selected. These 07 hit compounds have a similar type of interactions to the hydroxychloroquine in the binding pocket and may cure symptoms of COVID-19.

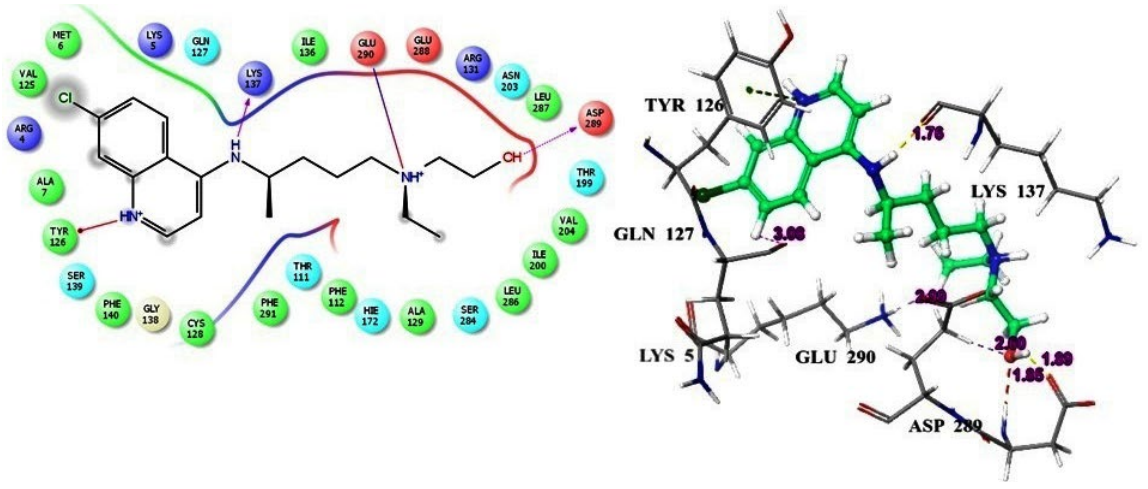

Figure 3. 2D and 3D interaction of hydroxychloroquine with 6LU7

The hydroxychloroquine establishes hydrogen bonding with Asp289, Lys137, Gln127, and having 1.5Å to 3Å length, pi-cation bond formation with Tyr126, Glu290 as shown in Figure 3. More than 80% of similar PubChem compounds to hydroxychloroquine were screened and found 07 hits based on the interaction, scores, and acceptable ADME properties. These hits were found to form pi-pi bonding of heterocyclic ring with Tyr126 of 6LU7 and Lys5, Lys137, Glu127, Glu290 coordinated by the formation of hydrogen bonds and illustrated through 2D 3D contacts from Figure 4-10. All these best hit candidates have better dock scores (≥ -4.5 Kcal/mol) and comparable binding energy than reference drug i.e., hydroxychloroquine (-3.612 Kcal/mol), along with their predicted ADME properties were tabulated in Table 1 and Table 2, respectively. Insight of 07 retrieved hits, three hits i.e., compound 2, compound 5, and compound 7 have similar interaction as hydroxychloroquine in the binding domain of 6LU7, and these three compounds interacted with Glu290, Lys137, Tyr126. Whereas, other four compounds have 50% similar interactions as hydroxychloroquine, besides the interactions, obtained hits have exhibit shape complementarity with the reference compound in the catalytic pocket of 6LU7. Among all of the obtained hits, compound 4 [PubChem ID 109008461, (*N*-(3-chlorophenyl)-2-(quinolin-8-ylamino)acetamide)] has more structural similarity with the reference drug.

Site selectivity has been analyzed by overlapping of all candidates in the binding pocket where all hits have similar nature of the spatial arrangement and protein-ligand interactions as hydroxychloroquine as shown in Figure 11. These hits have a similar kind of topology and functional group features as the reference drug. Plainer structure and 3D- contiguity of all hit compounds along with the commonness in patterns of protein-ligand interactions were matched with hydroxychloroquine as explained in Figure 12. Where, the yellow-colored region is prerequisites of heterocyclic moieties (quinoline, isoquinoline, indole, phenyl with furan or thiophene) for pi-pi or pi-cation bond formation with Tyr126 of 6LU7. Blue denoted that the region which is essential and specific for hydrogen bond formation such as acidic, hydroxyl moiety, and amides (carboxamide, sulphonamides), hydroxylamines, etc. While the necessity of smaller green section for link-up the head and tail moieties of compounds and it may have a longer or shorter chain of carbon with hetero atom group which is also played a vital role in hydrogen bond formation.

These retrieved hydroxychloroquine similar candidates from PubChem have proven affinity potential, therefore they may serve as a lead against the SARS-CoV-2 virus i.e., which causes the outbreak of COVID-19 in the hit to lead finding process and control the pandemic condition.

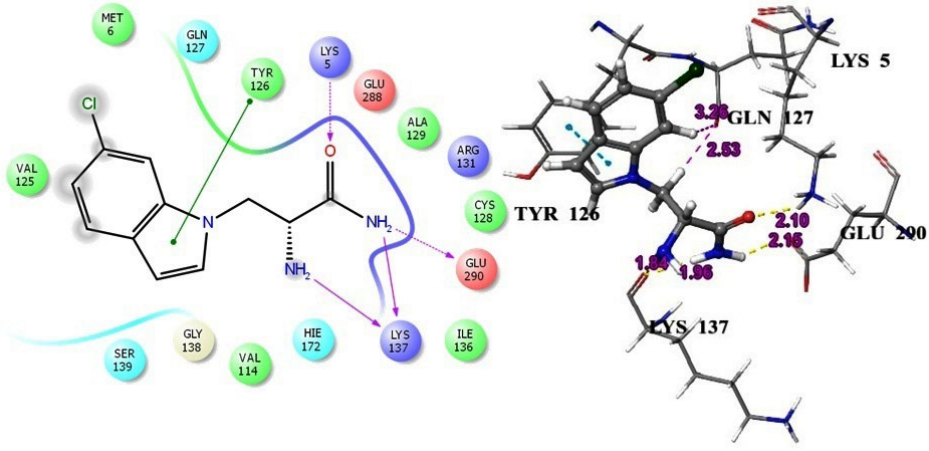

Figure 4. 2D and 3D interaction of 81941144 with 6LU7

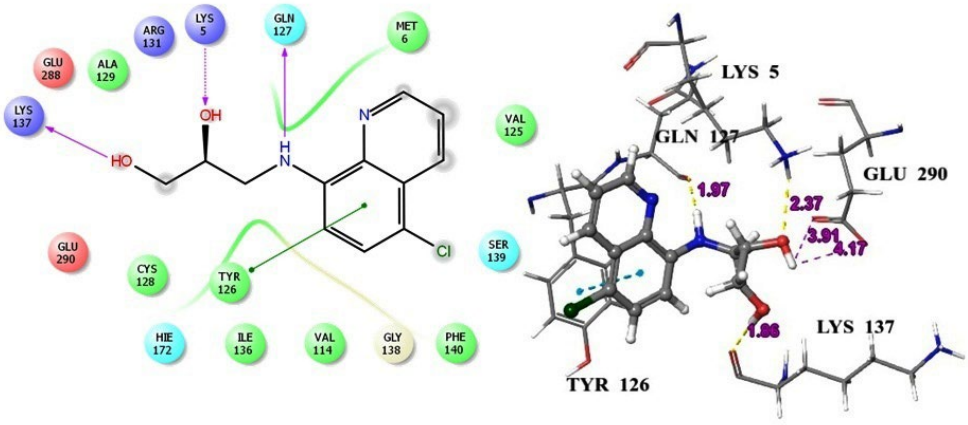

Figure 5. 2D and 3D interaction of 104893238 with 6LU7

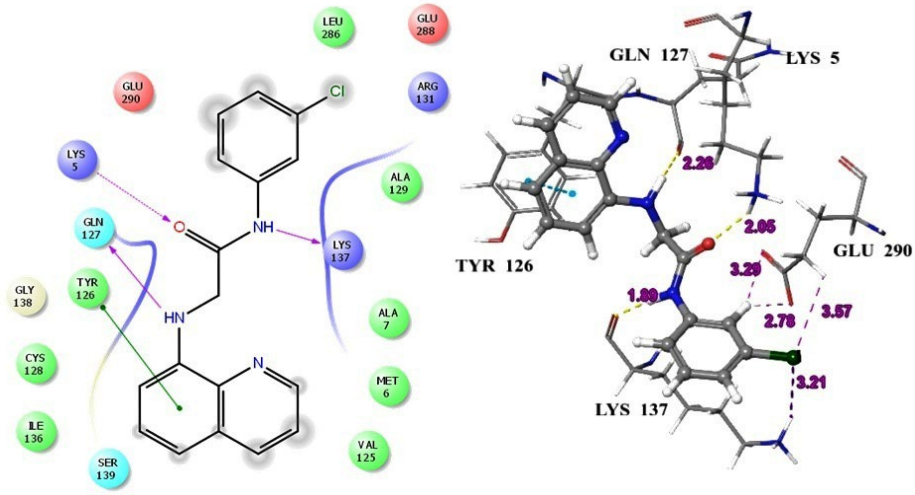

Figure 6. 2D and 3D interaction of 109008461 with 6LU7

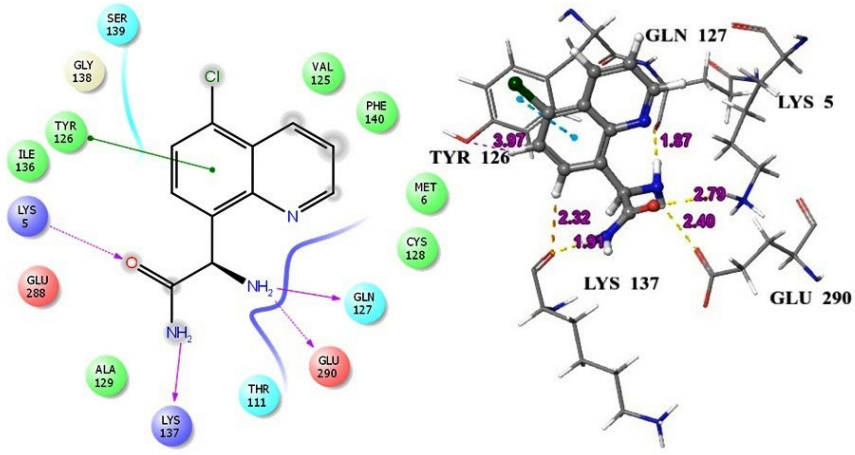

Figure 7. 2D and 3D interaction of 112617899 with 6LU7

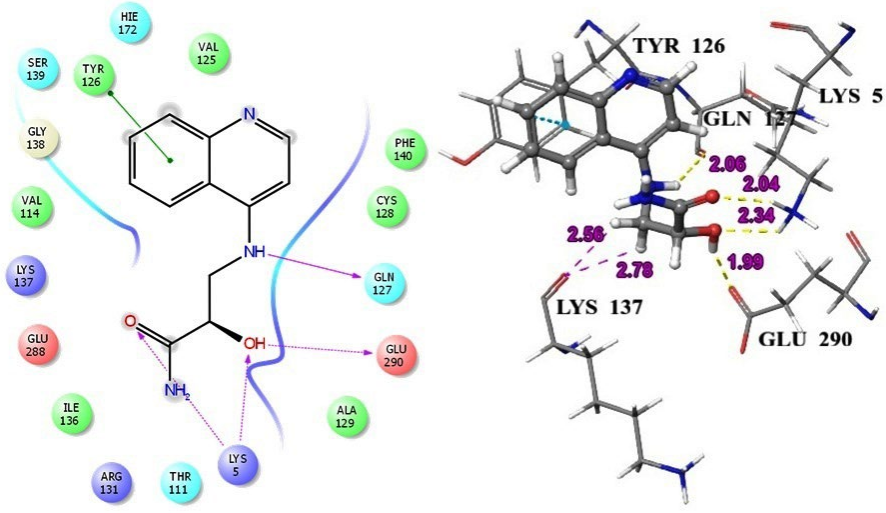

Figure 8. 2D and 3D interaction of 114153465 with 6LU7

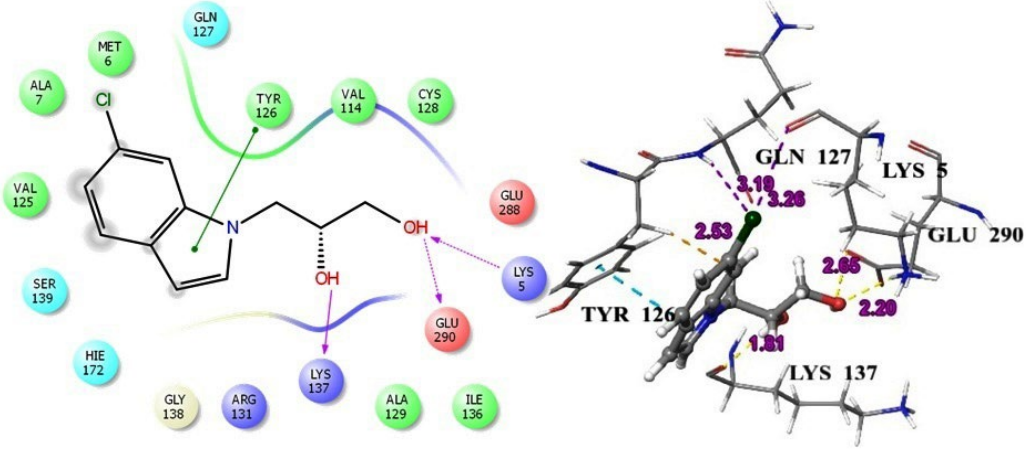

Figure 9. 2D and 3D interaction of 116620671 with 6LU7

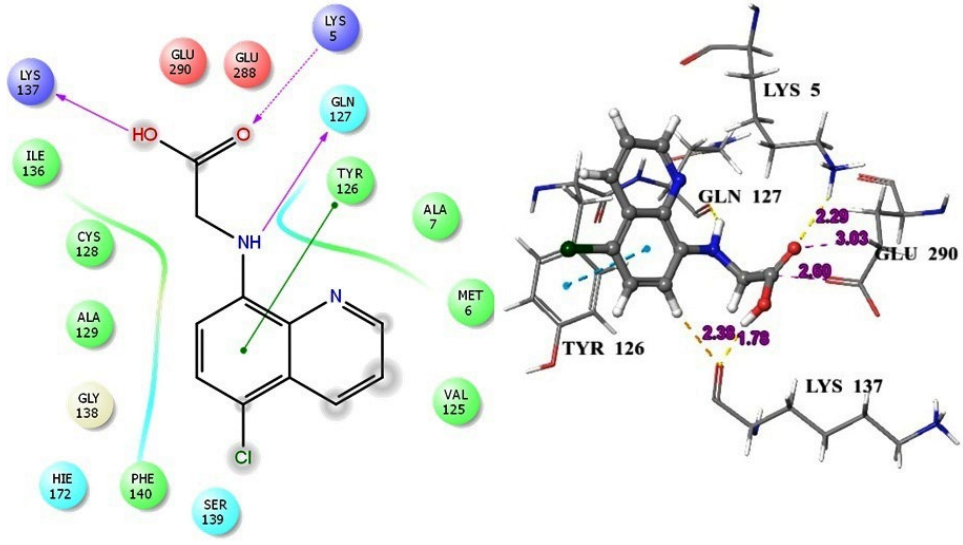

Figure 10. 2D and 3D interaction of 116783965 with 6LU7

Table 1. Scores of hits with 6LU7 and their protein-ligand interactions

S. No.	PubChem Compound ID	IUPAC Name	Dock Score (Kcal/mol)	Binding energy (Kcal/mol)	Protein-Ligand Interactions
1	Hydroxychloroquine		-3.612	-50.052	Glu290, Asp289, Lys137, Tyr126, Gln127
2	81941144	2-amino-3-(6-chloroindol-1-yl) propanamide	-4.429	-42.324	Glu290, Lys5, Lys137, Tyr126
3	104893238	(2*S*)-3-[(5-chloroquinolin-8-yl) amino]propane-1,2-diol	-4.959	-47.600	Lys5, Lys137, Tyr126, Glu127
4	109008461	*N*-(3-chlorophenyl)-2-(quinolin-8-ylamino)acetamide	-4.920	-46.328	Lys5, Lys137, Tyr126, Glu127
5	112617899	2-amino-2-(5-chloroquinolin-8-yl)acetamide	-4.467	-33.769	Lys5, Lys137, Tyr126, Glu127, Glu290
6	114153465	2-hydroxy-3-(quinolin-4-ylamino) propanamide	-5.106	-32.323	Lys5, Tyr126, Glu127, Glu290
7	116620671	3-(6-chloroindol-1-yl)propane-1,2-diol	-4.447	-47.414	Lys5, Lys137, Tyr126, Glu290
8	116783965	2-[(5-chloroquinolin-8-yl)amino] acetic acid	-5.503	-49.206	Lys5, Lys137, Tyr126, Glu127

Table 2. ADME prediction results with their PubChem compound IDs

S. No.	PubChem Compound ID	logPo/w (-2.0-6.5)	logS (-6.5-0.5)	logHERG (below-5)	QPPCaco (<25poor, >500great)	logBB (-3.0-1.2)	Madin-Darby Canine Kidney cells permeability (<25 poor, >500 great)	Human oral absorption(%)
1	81941144	0.277	-0.409	-3.531	71.236	-0.296	124.807	61.726
2	104893238	1.424	-2.419	-4.582	672.51	-0.718	712.191	85.892
3	109008461	3.737	-5.079	-6.596	2013.312	-0.244	2600.15	100
4	112617899	-0.254	0.211	-3.433	46.93	-0.301	91.896	55.334
5	114153465	0.148	-1.099	-2.919	165.074	-1.116	116.706	67.506
6	116620671	2.146	-2.435	-4.077	1674.379	-0.231	2107.65	100
7	116783965	2.102	-2.851	-2.769	121.911	-0.671	143.012	76.589

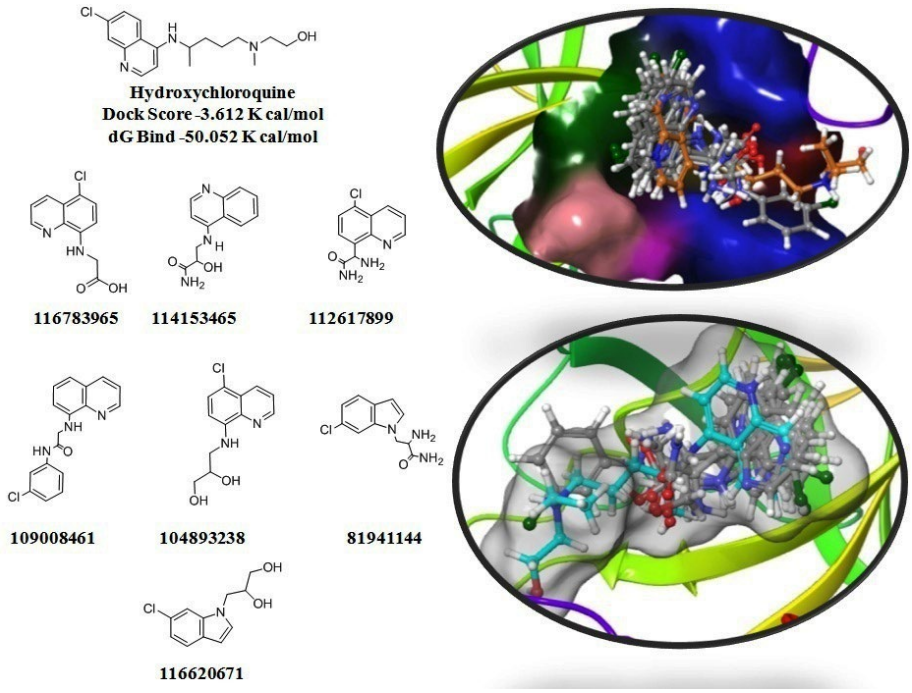

Figure 11. The proximity of Hydroxychloroquine with obtained hits in Protein surface and Ligand surface view

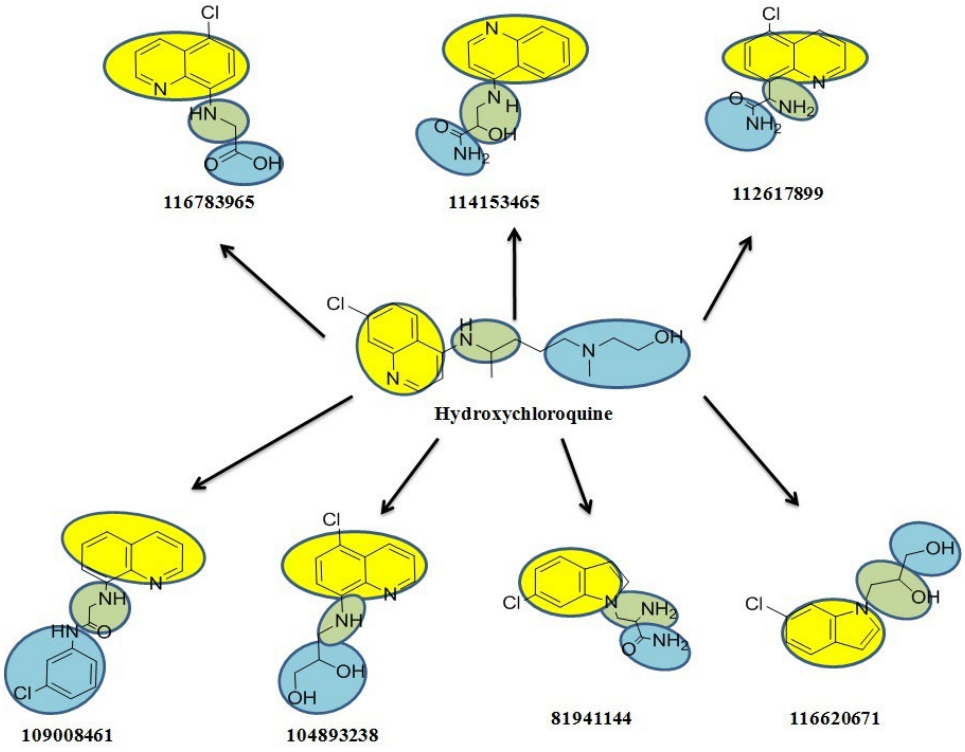

Figure 12. Functional group assessment of Hydroxychloroquine matched with each hit compound

CONCLUSION

The overall COVID-19 wellbeing crisis has developed as a potential danger to worldwide health. Additionally, the serious issue lies in the un-accessibility of any affirmed medicate against the SARS-CoV-2 bug. The targeted ligand virtual screening in drug discovery could prove as a fast and the most appropriate option to find a potential hit identification of hits against coronavirus (COVID-19). The present study gave screened 07 promising hits and having chemo- structural similarity with hydroxychloroquine. This work also indicates that these identified 07 compounds effect in limiting SARS-CoV-2's infection as they were superimposed well with the 3D binding pose of hydroxychloroquine in the binding pocket of 6LU7. However, various steps in clinical trial investigations are essential to authenticate these potential hits intended for lead development. We expect that this study may confirm precious for investigating newer remedial representatives as anti-SARS-CoV-2 in the future.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTION STATEMENT

Developing hypothesis and experimenting (Paswan S.K., Nath V.), preparing the study text and literature Research (Verma P.), reviewing the text (Sikarwar A.P.), Analysis and interpretation of the data (Verma S.K., Nath V.).

REFERENCES

Abramo, J. M., Reynolds, A., Crisp, G. T., Weurlander, M., Söderberg, M., Scheja, M., Rugg, G. (2012). Individuality in music performance. *Assessment Evaluation in Higher Education*, *37*(October), 435. https://doi.org/10.1007/82

Adem, S., Eyupoglu, V., Sarfraz, I., Rasul, A., Ali, M. (2020). Identification of potent COVID-19 main protease (Mpro) inhibitors from natural polyphenols: An in silico strategy unveils a hope against CORONA. *Preprints*, (March), 2020030333. https://doi.org/10.20944/PREPRINTS202003.0333.V1

Al-Osail, A. M., & Al-Wazzah, M. J. (2017). The history and epidemiology of Middle East respiratory syndrome coronavirus. *Multidisciplinary Respiratory Medicine*, *12*(1), 1–6. https://doi.org/10.1186/s40248-017-0101-8

Belouzard, S., Millet, J. K., Licitra, B. N., Whittaker, G. R. (2012). Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses*, *4*(6), 1011–1033. https://doi.org/10.3390/v4061011

Chang, C. K., Lo, S. C., Wang, Y. S., Hou, M. H. (2016). Recent insights into the development of therapeutics against coronavirus diseases by targeting N protein. *Drug Discovery Today*, *21*(4), 562–572. https://doi.org/10.1016/j.drudis.2015.11.015

Chatterjee, A., Cutler, S. J., Doerksen, R. J., Khan, I. A., Williamson, J. S. (2014). Discovery of thienoquinolone derivatives as selective and ATP non-competitive CDK5/p25 inhibitors by structure-based virtual screening. *Bioorganic and Medicinal Chemistry*, *22*(22), 6409–6421. https://doi.org/10.1016/j.bmc.2014.09.043

Colson, P., Rolain, J. M., Lagier, J. C., Brouqui, P., Raoult, D. (2020). Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. *International Journal of Antimicrobial Agents*, 105932. https://doi.org/10.1016/j.ijantimicag.2020.105932

Dzierba, C. D., Tebben, A. J., Wilde, R. G., Takvorian, A. G., Rafalski, M., Kasireddy-Polam, P., Gilligan, P. J. (2007). Dihydropyridopyrazinones and dihydropteridinones as corticotropin-releasing factor-1 receptor antagonists: Structure-activity relationships and computational modeling. *Journal of Medicinal Chemistry*, *50*(9), 2269–2272. https://doi.org/10.1021/jm0611410

Gautret, P., Lagier, J. C., Parola, P., Hoang, V. T., Meddeb, L., Mailhe, M., Raoult, D. (2020). Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. *International Journal of Antimicrobial Agents*, *56*(1), 105949. https://doi.org/10.1016/j.ijantimicag.2020.105949

Gbinigie, K., & Frie, K. (2020). Should chloroquine and hydroxychloroquine be used to treat COVID-19? A rapid review. *BJGP Open*, *4*(2), 1-7. https://doi.org/10.3399/bjgpopen20x101069

Genheden, S., & Ryde, U. (2015). The MM / PBSA and MM / GBSA methods to estimate ligand-binding affinities. *Expert Opinion on Drug Discovery*, *10*(5), 449–461.

Guo, Y., Korteweg, C., McNutt, M. A., Gu, J. (2008). Pathogenetic mechanisms of the severe acute respiratory syndrome. *Virus Research*, *133*(1), 4–12. https://doi.org/10.1016/j.virusres.2007.01.022

Hilgenfeld, R. (2014). From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. *The FEBS Journal*, *281*(18), 4085–4096. https://doi.org/10.1111/febs.12936

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., ... Yang, H. (2020). Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. *Nature*, *582*(7811), 289–293. https://doi.org/10.1038/s41586-020-2223-y

Kapoor KM & Kapoor A. (2020). Role of chloroquine and hydroxychloroquine in the treatment of COVID-19 infection- A systematic literature review. *MedRxiv*, 2020.03.24.20042366. https://doi.org/10.1101/2020.03.24.20042366

Khaerunnisa, S., Kurniawan, H., Awaluddin, R., Suhartati, S. (2020). Potential inhibitor of COVID-19 main Protease (Mpro) from several medicinal plant compounds by molecular docking study. *Preprints*, (March), 1–14. https://doi.org/10.20944/preprints202003.0226.v1

Kim, S. (2016). Getting the most out of PubChem for virtual screening. *Expert Opinion on Drug Discovery*, *11*(9), 843–855. https://doi.org/10.1080/17460441.2016.1216967

Kristensen, T. G., Nielsen, J., Pedersen, C. N. S. (2013). Methods for similarity-based virtual screening. *Computational and Structural Biotechnology Journal*, *5*(6), e201302009. https://doi.org/10.5936/csbj.201302009

McBride, R., van Zyl, M., Fielding, B. C. (2014). The coronavirus nucleocapsid is a multifunctional protein. *Viruses*, *6*(8), 2991–3018. https://doi.org/10.3390/v6082991

Patidar, K., Deshmukh, A., Bandaru, S., Lakkaraju, C., Girdhar, A., Gutlapalli, V. R., Singh, S. K. (2016). Virtual screening approaches in identification of bioactive compounds akin to delphinidin as potential HER2 inhibitors for the treatment of breast cancer. *Asian Pacific Journal of Cancer Prevention*, *17*(4), 2291–2295. https://doi.org/10.7314/APJCP.2016.17.4.2291

Paules, C. I., Marston, H. D., Fauci, A. S. (2020). Coronavirus infections—More than just the common cold.. *JAMA*, *323*(8), 707–708. https://doi.org/10.1001/jama.2020.0757

Principi, N., & Esposito, S. (2020). Chloroquine or hydroxychloroquine for prophylaxis of COVID-19. The Lancet Infectious Diseases, 20(10), 1118. doi:10.1016/s1473-3099(20)30296-6

Plantone, D., & Koudriavtseva, T. (2018). Current and future use of chloroquine and hydroxychloroquine in infectious, immune, neoplastic, and neurological diseases: A mini-review. *Clinical Drug Investigation*, *38*(8), 653–671. https://doi.org/10.1007/s40261-018-0656-y

Prajapat, M., Sarma, P., Shekhar, N., Avti, P., Sinha, S., Kaur, H., Medhi, B. (2020). Drug targets for coronavirus: A systematic review. *Indian Journal of Pharmacology*, *52*(1), 56–65. https://doi.org/10.4103/ijp.IJP_115_20

Sahraei, Z., Shabani, M., Shokouhi, S., Saffaei, A. (2020). Aminoquinolines against coronavirus disease 2019 (COVID-19): Chloroquine or hydroxychloroquine. *International Journal of Antimicrobial Agents*, *55*(4), 105945. https://doi.org/10.1016/j.ijantimicag.2020.105945

Saif, L. J. (2004). Animal coronaviruses: What can they teach us about the severe acute respiratory syndrome? *OIE Revue Scientifique et Technique*, *23*(2), 643–660. https://doi.org/10.20506/rst.23.2.1513

Savarino, A., Boelaert, J. R., Cassone, A., Majori, G., Cauda, R. (2003). Effects of chloroquine on viral infections: An old drug against today's diseases? *Lancet Infectious Diseases*, *3*(11), 722–727. https://doi.org/10.1016/S1473-3099(03)00806-5

Sirin, S., Kumar, R., Martinez, C., Karmilowicz, M. J., Ghosh, P., Abramov, Y. A., Sherman, W. (2014). A computational approach to enzyme design: Predicting ω - aminotransferase catalytic activity using docking and MM-GBSA scoring. *Journal of Chemical Information and Modeling*, *54*, 2334–2346.

Yang, H., Bartlam, M., Rao, Z. (2006). Drug design targeting the main protease, the Achilles heel of coronaviruses. *Current Pharmaceutical Design*, *12*(35), 4573–4590. https://doi.org/10.2174/138161206779010369