Targeting virus-host interaction: An *in silico* approach to develop promising inhibitors against COVID-19

Jitendra Subhash Rane[a], Aroni Chatterjee[b#], Rajni Khan [c#], Abhijeet Kumar[d*] and Shashikant Ray[e *]

[a]Department of Biosciences & Bioengineering, Indian Institute of Technology Bombay, Mumbai -400076, India

[b]Indian Council of Medical Research (ICMR)—Virus Research Laboratory, NICED, Kolkata, India

[c]Motihari College of Engineering, Motihari-845401, India

[d]Department of Chemistry, Mahatma Gandhi Central University Motihari-845401, India

[e]Department of Biotechnology, Mahatma Gandhi Central University Motihari-845401, India

[#] **Both authors contributed equally to this work.**

***Correspondence may be addressed to these authors**

[d*]. Abhijeet Kumar, Department of Chemistry, Mahatma Gandhi Central University Motihari-845401, India, E-mail: abhijeetkumar@mgcub.ac.in

[e*]. Shashikant Ray, Assistant Professor, Department of Biotechnology, Mahatma Gandhi Central University Motihari-845401, India, E-mail: shashikantray@mgcub.ac.in

Key words: hACE2, Receptor, Coronavirus, Pyrimidine derivatives, Binding site

Running Head: AP-NP and AP-4-Me-Ph may perturb SARS-CoV-2S-hACE2 receptor complex interaction: an *in silico* study for drug development against COVID-19

Abstract

The entire human population all over the globe is currently facing appalling conditions due to the spread of infection from COVID-19 (corona virus disease-2019). In the last few months an enormous amount of studies have been continuously trying to target several potential drug sites to identify a novel therapeutic target. Spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is also being targeted by several scientific groups as a novel drug target. The spike glycoprotein protein is present on the surface of the virion and binds to the human angiotensin-converting enzyme-2 (hACE2) membrane receptor thereby promoting its fusion to the host cell membrane. The binding and internalization of the virus is a crucial step in the process of infection and hence any molecule that can inhibit this, certainly holds a significant therapeutic value. We have identified AP-NP (2-(2-amino-5-(naphthalen-2-yl)pyrimidin-4-yl)phenol) and AP-4-Me-Ph (2-(2-amino-5-(p-tolyl)pyrimidin-4-yl)phenol) from a group of diaryl pyrimidine derivatives which appear to bind at the interface of hACE2-SARS-CoV-2S complex (human angiotensin converting enzyme 2 and spike glycoprotein complex) with a low binding energy (<-8 Kcal/mol). In this *in-silico* study we also found that AP-NP interacts with the S1 domain of C-terminal of SARS-CoV-2S however AP-4-Me-Ph was found to interact with S2 domain of SARS-CoV-2S. The result suggested that these compounds have the potential to inhibit the interaction between spike protein and hACE2 receptor also AP-4-Me-Ph might be prevent internalization of the virion within the host. Further *in vitro* and *in vivo* study will strengthen these drug candidates against the COVID-19.

Introduction

The world is currently going through a debilitating phase of acute health disaster attributed to the global pandemic brought about by the novel COVID-19 (Kirchdoerfer et al. 2016). Sequencing and simultaneous phylogenetic identification of the virus responsible for COVID-19 confirmed that it was a novel β-coronavirus that shared 88% sequence identity with two bat-derived SARS-like CoV (Lu et al. 2020; Wang, Chen & Qin 2020). Additionally, it was shown that this coronavirus (CoV), which was termed 2019-nCoV (Gorbalenya et al. 2020) shared 79.5% sequence identity with SARS-CoV (Lu et al. 2020; Wang et al. 2020; Xia et al. 2020). Hence it was called SARS-CoV-2. The virus gains entry into the host cells via the transmembrane spike (S) glycoprotein that forms a homotrimeric structure extending outwards from the viral surface (White, Delos, Brecher & Schornberg 2008; Belouzard, Millet, Licitra & Whittaker 2012). The monomeric S protein comprises of two distinct functional domains S1 and S2. The S1 domain is responsible for binding to the host cell receptor while S2 domain helps in the fusion of the viral and cellular membranes (Kirchdoerfer et al. 2016; Jitendra Subhash, Aroni, Abhijeet & Shashikant 2020). The S protein is cleaved at the boundary between the S1 and S2 subunits, which remains bound via a non-covalent attachment in the prefusion conformation (Walls et al. 2020) . The S1 protein subunit contains a unique receptor-binding domain (RBD) in its C-terminal region and contributes to stabilization of the prefusion state of the membrane-anchored S2 subunit that contains the fusion machinery (Shang et al. 2020; Walls et al. 2020). Transmembrane protease serine protease-2 (TMPRSS-2) further cleaves the S protein at a specific site located immediately upstream of the fusion peptide (Shen, Mao, Wu, Tanaka & Zhang 2017). This cleavage brings about huge irreversible conformational changes that essentially activate the protein for membrane fusion (Shen et al. 2017). After binding of the RBD present in the S1 subunit of S protein to the hACE2 receptor on target cells, the heptad repeat 1 (HR1) and 2 (HR2) domains in its S2 subunit of S protein interact with each other to form a six-helix bundle (6-HB) fusion core, bringing viral and cellular membranes into proximity for fusion and infection (Du et al. 2009; Zhu, Liu, Du, Lu & Jiang 2013; Li et al. 2019; Xia et al. 2020). Scientists throughout the world are looking for effective therapeutic strategies to prevent the spread of SARS-CoV-2. Till date no suitable vaccines or anti-viral agents have been put forward.

The several types of pyrimidine bases like thymine, cytosine, and uracil are found as basic skeleton or building blocks of genetic materials i.e DNA and RNA (Sharma, Chitranshi &

Agarwal 2014). This prime importance makes pyrimidine moiety applicable for broad therapeutic applications (Sharma et al. 2014). Pyrimidine moieties has gained considerable attention in the chemotherapy of various diseases like cancer (Xie et al. 2011), HIV, diabetes, cardiovascular, bacterial diseases, fungal diseases (Sharma et al. 2014) and several viral diseases (Balzarini & McGuigan 2002). It also used as anti-inflammatory drugs (Amir, Javed & Kumar 2007), analgesics (Vega, Alonso, Diaz-Martin & Junquera 1990), antipyretic drugs, anti-leishmanial drugs, herbicidal agents and anti-oxidants (Abu-Hashem, Youssef & Hussein 2011). Since pyrimidine base is a constituent of both DNA and RNA so it is useful in the chemotherapy of both DNA and RNA viruses (Sharma et al. 2014). Compounds with *N*-heterocyclic scaffolds are highly effective against a diverse range of diseases and it has already been studied for their potential pharmacological activities. In particular, pyrimidine derivatives have demonstrated outstanding biological activities. For example, Dayvigo (Lemborexant), Inrebic (Fedratinib) are few examples of drugs containing pyrimidine scaffolds which received FDA-approval in 2019. Because of the enormous therapeutic importance, we selected some well characterized pyrimidine substituted phenols (Kumar & Rao 2018) (Table S1) to investigate their inhibitory action against SARS-CoV-2 virus through molecular docking.

In this *in silico* study we have used 6 analogues of diaryl pyrimidine derivatives to find out the potent compound which may bind at the interface of the hACE2-SARS-CoV-2S complex with high affinity. Interestingly, we have found AP-NP and AP-4-Me-Ph which had the potential to bind at the interface of the hACE2-SARS-CoV-2S complex with high affinity. Moreover, the FDA approved antimalarial drug chloroquine (CQ) was also found to interact with the interface of the hACE2-SARS-CoV-2S complex (Vincent et al. 2005) with lesser affinity as compared to AP-NP and AP-4-Me-Ph. Similar to AP-4-Me-Ph, CQ was also found to binds with S2 domain of spike protein but has less affinity to the S2 domain. Besides these derivatives also possess drug likeliness property. The result together in this study suggested that AP-NP and AP-4-Me-Ph are promising compounds that bind at the interface of hACE2-SARS-CoV-2S complex and may have the ability to perturb SARS-CoV-2 binding and fusion with host cells. Further experimental studies with these lead molecules might generate fascinating results suitable for anti-COVID drug development.

Material and method:

Preparation of Ligands and Receptor:

The 3-dimensional structure of SARS-CoV-2S protein (spike glycoprotein) (PDB-ID: 6VYB) (Walls et al. 2020) and hACE2-SARS-CoV-2S complex (human angiotensin converting enzyme 2 and spike glycoprotein complex) (PDB ID: 6VW1) (Shang et al. 2020) were downloaded from the RCSB protein data bank. The structures of all diaryl pyrimidine derivatives were prepared in Discovery studio 2020. The conformation of all ligands was refined using "Clean geometry" command and the structure with minimum clean energy was saved in pdb format.

Molecular docking of AP-NP with SARS-CoV-2S (spike protein) and with hACE2-SARS-CoV-2S complex:

The molecular docking analysis of all diaryl pyrimidine deviates were performed in similar fashion. Here we are explaining the docking methodology of AP-NP with SARS-CoV-2S in detail. AP-NP compound were docked on the cryo-electron microscopic structure of SARS-CoV-2S protein (PDB-ID: 6VYB). This protein is trimer made up of chain A, B and C. All docking analysis was performed on the chain A of SARS-CoV-2S protein. First, polar hydrogen and gasteiger charges were added on the spike glycoprotein and AP-NP compound using MGL tool (Morris et al. 2009) and saved in pdbqt format. The molecular docking tool auto dock vina (Trott & Olson 2010) was used for the docking of AP-NP on the SARS-CoV-2S protein. To know the probable putative binding site, the blind docking was performed. For, this SARS-CoV-2S protein kept as rigid while AP-NP kept as flexible. Both molecules were covered by grid box with dimension of 98 Å × 104 Å × 160 Å with grid spacing 1 Å. Five sets of docking were performed with exhaustiveness 100. The auto dock vina produced 9 docked conformations with the binding energy in kcal/mol. Among them the five conformations with lowest binding energy were bind at S1 domain of spike glycoprotein. Therefore, S1 domain was choose for local docking. For this, S1 domain covered by the grid box with the dimension of 48 Å × 120 Å × 86 Å with grid spacing 1 Å and docking was done with exhaustiveness 100 (Table 1). The five sets of local docking performed and the conformation with the least binding energy was chosen for analysis. The analyses of auto dock vina results were done by MGL tools 1.5.6 (Morris et al. 2009) and inter molecular interactions were determined using PyMoL (DeLano 2002).

Similar to Spike protein, AP-NP was docked on the hACE2-SARS-CoV-2S complex. The blind docking and local docking was done. The docking parameters were shown in Table 2.

Further, the docking of all other molecules was done on spike protein and its complex with hACE2 as mentioned above and parameters are given in Table 1 and Table 2.

ADME analysis for drug likeliness: The drug likeliness properties of all diaryl pyrimidine derivatives were monitored as described earlier (Lipinski 2004; Jayaram et al. 2012). The pdb structure of all diaryl pyrimidine derivate compounds were constructed and ADME properties which are explained in Lipinski's rule of five i.e molecular weight, solubility, H-bond donor, H-bond acceptor and molar refractivity were calculated by online software tool (Table 3).

Results and discussion:

Designing the targeted drug molecules by using cheminformatics tools is the initial step in the drug discovery (Xu & Hagler 2002). In this study our focus was to investigate the efficacy of diaryl pyrimidine derivative compounds in obstructing the interaction between spike glycoprotein of COVID-19 and hACE2 receptor which is considered to be an important step towards the progress of infection. The hACE2 present at the outer surface of the human lungs cells arteries cells, kidney cells and intestine cells (Donoghue et al. 2000; Hamming et al. 2004). In the lungs cell and acts as a receptor for spike glycoprotein of COVID-19 (Li, Li, Farzan & Harrison 2005; Ou et al. 2020; Walls et al. 2020). The interaction of spike protein and hACE2 facilitates the attachment and internalization of the virus in the host cell (Belouzard, Millet, Licitra & Whittaker 2012). The COVID-19 virus attaches itself to the host cell using S1 domain spike glycoprotein whereas the S2 domain of spike protein facilitates the internalization of the virus into the host membrane to begin infection (Du et al. 2009; Belouzard et al. 2012; Belouzard et al. 2012). So both domains play a crucial role in the initialization of the infection in the host cell. The study presented here, using molecular docking tool revealed significant binding interaction of diaryl pyrimidines with the interface of hACE2-SARS-CoV-2S receptor complex (hACE2-spike protein complex: PDB ID 6VW1) (Table 4). Further, we also predicated the putative binding site and binding energy of all diaryl pyrimidine derivatives compounds on individual SARS-CoV-2S protein (Table 5).

In this study, we have analyzed the binding energies of all diaryl pyrimidine derivatives on the hACE2-SARS-CoV-2S complex. Here we found that all diaryl pyrimidine derivatives bind at the interface of the hACE2-SARS-CoV-2S complex. Interestingly, these molecules share the same binding site which lies near the binding junction of hACE2 and C-terminal S1 domain of spike glycoprotein. However, these molecules only show interaction

with the hACE2 residues of the hACE2-SARS-CoV-2S receptor complex. Among these diaryl pyrimidine derivatives, AP-NP, AP-4-Me-Ph and AP-3-OMe-Ph have displayed high binding affinity with the hACE2 receptor with the binding energies of, -8.95, -8.1 and -8.1 Kcal/mol, respectively (Table 4). These pyrimidine containing phenols binds with the active site through hydrogen bonding using -OH of phenol part and and an $-NH_2$ group of the amino pyrimidine ring. In addition to that the non-covalent interactions such as π-π interaction are also involved in the stabilization of ligand with the active site. In particular, the significant binding energy observed in case of AP-NP could be attributed to the such non-covalent hydrophobic interaction of phenyl as well as napthyl ring with non-polar amino acid residues such as LEU 391, PHE 390, LEU 73, TRP 69 and PHE 40 along with the interaction through hydrogen bonding with polar amino acid residues such as ASP 350, ASN 394 and ARG 393 (Figure 1). Slightly lower binding energy observed in case of AP-4-Me-Ph could probably be due to slight reduction in non-covalent as planar naphthyl group gets replaced with tolyl ring containing tetrahedral methyl group. In case of AP-4-Me-Ph, hydrophobic ALA 348, TRP 349, SER 47, SER 44, PHE 40 of hACE2 forms hydrophobic interaction with the toluene ring (Figure 2). However, the phenol-pyrimidine base forms hydrogen bonding with ASP 350, TYR 385, HIS 401 and ASP 382. The similarity in the binding energy of AP-3-OMe-Ph (Figure S1) with AP-4-Me-Ph could also be explained due to the presence of tetrahedral –OMe group instead of the planar aromatic group such as naphthyl. It also has the same hydrophobic interaction as AP-4-Me-Ph. This might be due to their structural similarity. Compared to the AP-4-Me-Ph, additional e hydrogen bonding interactions were observed with ARG 393 residue due to the involvement of oxygen atom of –OMe group.

CQ, a well-known drug which is recommended to use in the COVID-19 therapy and was reported to interfere with the terminal glycosylation of spike protein of SARS-CoV-2S and therefore known to reduce initial infection (Vincent et al. 2005; Hu, Frieman & Wolfram 2020). So we used CQ as a positive control and performed molecular docking experiment. Similar to all diaryl pyrimidine derivatives we also found that CQ binds at the interface of the hACE2-SARS-CoV-2S complex (Figure 3) binding energies of -5.7 Kcal/mol **.** We observed that CQ has lesser binding affinity with hACE2-SARS-CoV-2S complex or SARS-CoV-2S it may be may be due to its high affinity with its primary target i.e endosome (Al-Bari 2017).

Further, we also performed the docking analysis to find out the strength of interaction of this pyrimidine derivate compounds with SARS-CoV-2S protein (Table 5). Here we found that all diaryl pyrimidine derivatives bind strongly with the SARS-CoV-2S than CQ. Among them the AP-NP and AP-4-Me-Ph have high affinity to SARS-CoV-2S with the binding energies; -7.9 and -8.1 Kcal/mol, respectively. AP-NP binds to the C terminal of S1 domain of spike glycoprotein (Figure 4). The bulky and planar naphthalene ring of AP-NP forms strong hydrophobic interaction with PHE 464, PRO 426 and PRO 463. However, the phenyl ring forms hydrophobic interaction with GLU 516, LEU 517. The most hydrogen interactions were shown by pyrimidine ring with PHE 515, SER 514, THR 733 and ASP 428. The AP-4-Me-Ph binds to the S2 domain of spike glycoprotein. Compared to AP-NP, replacement of naphthalene ring with the toluene in the AP-4-Me-Ph changes its binding domain (Table 5) .The change in the binding domain could be attributed due to slight loss in planarity while moving from naphthyl ring to tolyl one as the later contain tetrahedral methyl group instead of fused phenyl ring The toluene ring of AP-4-Me-Ph forms the hydrophobic interaction with ALA 1056, PRO 1057 and HIS 1058 while pyrimidine and phenyl ring forms hydrophobic interactions with LYS 733, PRO 863, THR 778 and ILE 870. Further, SER 730, MET 731 forms hydrogen bond nitrogen of pyrimidine ring of AP-4-Me-Ph (Figure 5). However, CQ binds to the S2 domain and possess less affinity (-6.1 Kcal/mol) to SARS-CoV-2S (Figure 6). Similar to AP-4-Me-Ph the others diaryl pyrimidine derivatives AP-3-OMe-Ph (Figure S2), AP-Ph-4-OMe, AP-Ph-4-Br binds to the S2 domain of spike glycoprotein with the binding affinity -7.7, -7.5 and -7.5 Kcal/mol respectively. However, AP-Ph-4-I was found to interacts with the S2 domain of spike protein with binding affinity of -7.7 Kcal/mol.

Therefore the molecular docking study performed here reveals the potential effectiveness of AP-NP and AP-4-Me-Ph in targeting actives sites present on host cells as well as viral cells which are involved in the initialization of viral infection. Among all diaryl pyrimidine derivatives, these molecules show high affinity with both hACE2 and SARS-CoV-2S spike glycoprotein. The binding of AP-NP on the S1 C-terminal domain SARS-CoC-2S and the interface of hACE2-SRAS-CoV-2S shows that it might perturb the interaction of spike protein and hACE2 receptor**.** The S2 domain of spike glycoprotein helps to internalize the virus in the host cell. Therefore, the binding of AP-4-Me-Ph to S2 domain of spike protein might hamper the internalization of the viral particle inside the host cell. Besides it also binds to the hACE2 receptor, near the binding junction of hACE2 and SARS-CoV-2S protein. It shows that AP-4-Me-Ph might be able to hamper the interaction of spike

protein with its receptor, i.e. hACE2. However, further *in vitro* and in vivo study will give the detailed insight about the effect of these drug candidates on the binding of spike protein and hACE2.

To determine whether any compound with a particular biological activity has the potential to serve as a pharmacological agent/drug the Lipinski rule of five is generally used. This rule acts as a filter in *in-silico* analyses and helps to screen potential drugs during the very beginning of drug designing programme thus minimizing the cost of exercises, time and labour in case of clinical drug development (Gombar, Silver & Zhao 2003; Hughes, Rees, Kalindjian & Philpott 2011). The rule judges some of the basic molecular properties a compound possesses like absorption, distribution, metabolism and excretion (ADME) for a selected range (Hughes et al. 2011). If a compound possesses at least two of the properties it's good to go (Lipinski 2004; Jayaram et al. 2012). All the diaryl pyrimidine derivatives used in this study were found to pass all the five criteria's mentioned in the Lipinski's rule (Table 3). Thus we suggest that AP-NP and AP-4-Me-Ph have the potential ability to work effectively as novel drugs against COVID-19.

Conclusion: *In-silico* studies provide the best alternatives to screen potential drug candidates. In this study we have investigated the ability of some diaryl pyrimidine derivative compounds in binding to the SARS-COV-2S protein and obstructing its receptor binding ability. We found that all the chosen compounds were capable of binding to the interface of human cell receptor ACE2 and spike protein and with high affinity and fulfills all the criterias of Lipinski's rule of five. We believe that all of these compounds can be effective anti-COVID drugs and therefore should be experimentally verified by researchers working on this scheme.

Acknowledgments

We thank the Supercomputing Facility for Bioinformatics and Computational Biology, Indian Institute of Technology Delhi, for online facilities. AK and SR thanks Mahatma Gandhi Central University Motihari, Bihar. We are also thankful to Prof. Ashutosh Kumar (IIT Bombay) and Dr. Gautam Das (Co-founder and Chief Scientist at Mibiome Therapeutics) for thoughtful discussion.

Conflict of interest

The authors report no conflicts of interest.

Abbreviations	
COVID-19	Coronavirus Disease 2019
SARS-CoV-2S	Severe Acute Respiratory Syndrome Coronavirus 2 Spike Protein
hACE2	Human Angiotensin Converting Enzyme-2
SARS-CoV-2S-hACE2	Severe Acute Respiratory Syndrome Coronavirus 2 Spike protein and Human Angiotensin Converting Enzyme-2 receptor complex
AP-NP	2-(2-amino-5-(naphthalen-2-yl)pyrimidin-4-yl) phenol
AP-4-Me-Ph	2-(2-amino-5-(p-tolyl)pyrimidin-4-yl) phenol
AP-3-OMe-Ph	2-(2-amino-5-(3-methoxyphenyl)pyrimidin-4-yl) phenol
CQ	Chloroquine
RBD	Receptor-Binding Domain
TMPRSS-2	Transmembrane Protease Serine Protease-2
HR1	Heptad Repeat 1
HR2	Heptad Repeat 2
6-HB	Six-Helix Bundle,
PDB	Protein Data Bank
ADME	Absorption, Distribution, Metabolism and Excretion

References

Abu-Hashem, A. A., Youssef, M. M. & Hussein, H. A. R. (2011). Synthesis, Antioxidant, Antituomer Activities of Some New Thiazolopyrimidines, Pyrrolothiazolopyrimidines and Triazolopyrrolothiazolopyrimidines Derivatives. *Journal of the Chinese Chemical Society 58*: 41-48.

Al-Bari, M. A. A. (2017). Targeting endosomal acidification by chloroquine analogs as a promising strategy for the treatment of emerging viral diseases. *Pharmacol Res Perspect 5*: e00293.

Amir, M., Javed, S. & Kumar, H. (2007). Pyrimidine as antiinflammatory agent: A review. *Indian Journal of Pharmaceutical Sciences 68*.

Balzarini, J. & McGuigan, C. (2002). Bicyclic pyrimidine nucleoside analogues (BCNAs) as highly selective and potent inhibitors of varicella-zoster virus replication. *Journal of Antimicrobial Chemotherapy 50*: 5-9.

Belouzard, S., Millet, J., Licitra, B. & Whittaker, G. (2012). Mechanisms of Coronavirus Cell Entry Mediated by the Viral Spike Protein. *Viruses 4*: 1011-1033.

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*: 1011-1033.

DeLano, W. L. (2002). PyMOL. DeLano Scientific, San Carlos, CA, 700.

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., Donovan, M., Woolf, B., Robison, K., Jeyaseelan, R., Breitbart, R. E. & Acton, S. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res 87*: E1-9.

Du, L., He, Y., Zhou, Y., Liu, S., Zheng, B. J. & Jiang, S. (2009). The spike protein of SARS-CoV--a target for vaccine and therapeutic development. *Nat Rev Microbiol 7*: 226-236.

Gombar, V. K., Silver, I. S. & Zhao, Z. (2003). Role of ADME characteristics in drug discovery and their in silico evaluation: in silico screening of chemicals for their metabolic stability. *Curr Top Med Chem 3*: 1205-1225.

Gorbalenya, A. E., Baker, S. C., Baric, R. S., de Groot, R. J., Drosten, C., Gulyaeva, A. A., Haagmans, B. L., Lauber, C., Leontovich, A. M., Neuman, B. W., Penzar, D., Perlman, S., Poon, L. L. M., Samborskiy, D. V., Sidorov, I. A., Sola, I., Ziebuhr, J. & Coronaviridae Study Group of the International Committee on Taxonomy of, V. (2020). The species Severe acute respiratory syndrome-related coronavirus:

classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology 5*: 536-544.

Hamming, I., Timens, W., Bulthuis, M. L., Lely, A. T., Navis, G. & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol 203*: 631-637.

Hu, T. Y., Frieman, M. & Wolfram, J. (2020). Insights from nanomedicine into chloroquine efficacy against COVID-19. *Nat Nanotechnol 15*: 247-249.

Hughes, J. P., Rees, S., Kalindjian, S. B. & Philpott, K. L. (2011). Principles of early drug discovery. *Br J Pharmacol 162*: 1239-1249.

Jayaram, B., Singh, T., Mukherjee, G., Mathur, A., Shekhar, S. & Shekhar, V. (2012). Sanjeevini: a freely accessible web-server for target directed lead molecule discovery. *BMC Bioinformatics 13 Suppl 17*: S7.

Jitendra Subhash, R., Aroni, C., Abhijeet, K. & Shashikant, R. (2020). *Targeting SARS-CoV-2 Spike Protein of COVID-19 with Naturally Occurring Phytochemicals: An in Silco Study for Drug Development*.

Kirchdoerfer, R., Cottrell, C., Nianshuang, W., Pallesen, J., Yassine, H., Turner, H., Corbett, K., Graham, B., McLellan, J. & Ward, A. (2016). Pre-fusion structure of a human coronavirus spike protein. *Nature 531*: 118-121.

Kumar, A. & Rao, M. L. N. (2018). Pot-economic synthesis of diarylpyrazoles and pyrimidines involving Pd-catalyzed cross-coupling of 3-trifloxychromone and triarylbismuth. *Journal of Chemical Sciences 130*: 165.

Li, F., Li, W., Farzan, M. & Harrison, S. (2005). Structure of SARS Coronavirus Spike Receptor-Binding Domain Complexed with Receptor. *Science (New York, N.Y.) 309*: 1864-1868.

Li, Z., Tomlinson, A. C., Wong, A. H., Zhou, D., Desforges, M., Talbot, P. J., Benlekbir, S., Rubinstein, J. L. & Rini, J. M. (2019). The human coronavirus HCoV-229E S-protein structure and receptor binding. *Elife 8*.

Lipinski, C. A. (2004). Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov Today Technol 1*: 337-341.

Lu, R., Zhao, X., Li, J., Niu, P., Yang, B., Wu, H., Wang, W., Song, H., Huang, B., Zhu, N., Bi, Y., Ma, X., Zhan, F., Wang, L., Hu, T., Zhou, H., Hu, Z., Zhou, W., Zhao, L., Chen, J., Meng, Y., Wang, J., Lin, Y., Yuan, J., Xie, Z., Ma, J., Liu, W. J., Wang, D., Xu, W., Holmes, E. C., Gao, G. F., Wu, G., Chen, W., Shi, W. & Tan, W. (2020).

Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet 395*: 565-574.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem 30*: 2785-2791.

Ou, X., Liu, Y., Lei, X., Li, P., Mi, D., Ren, L., Guo, L., Guo, R., Chen, T., Hu, J., Xiang, Z., Mu, Z., Chen, X., Chen, J., Hu, K., Jin, Q., Wang, J. & Qian, Z. (2020). Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun 11*: 1620.

Shang, J., Wan, Y., Liu, C., Yount, B., Gully, K., Yang, Y., Auerbach, A., Peng, G., Baric, R. & Li, F. (2020). Structure of mouse coronavirus spike protein complexed with receptor reveals mechanism for viral entry. *PLoS Pathog 16*: e1008392.

Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A. & Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature*.

Sharma, V., Chitranshi, N. & Agarwal, A. K. (2014). Significance and biological importance of pyrimidine in the microbial world. *Int J Med Chem 2014*: 202784.

Shen, L. W., Mao, H. J., Wu, Y. L., Tanaka, Y. & Zhang, W. (2017). TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections. *Biochimie 142*: 1-10.

Trott, O. & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem 31*: 455-461.

Vega, S., Alonso, J., Diaz-Martin, J. & Junquera, F. (1990). Synthesis of 3-substituted-4-phenyl-2-thioxo-1,2,3,4,5,6, 7,8-octahydrobenzo[4,5]thieno[2,3-d]pyrimidines [1]. *Journal of Heterocyclic Chemistry 27*: 269-273.

Vincent, M. J., Bergeron, E., Benjannet, S., Erickson, B. R., Rollin, P. E., Ksiazek, T. G., Seidah, N. G. & Nichol, S. T. (2005). Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J 2*: 69.

Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T. & Veesler, D. (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell 181*: 281-292 e286.

Wang, Y., Chen, Y. & Qin, Q. (2020). Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. *J Med Virol*.

White, J. M., Delos, S. E., Brecher, M. & Schornberg, K. (2008). Structures and mechanisms of viral membrane fusion proteins: multiple variations on a common theme. *Crit Rev Biochem Mol Biol 43*: 189-219.

Xia, S., Liu, M., Wang, C., Xu, W., Lan, Q., Feng, S., Qi, F., Bao, L., Du, L., Liu, S., Qin, C., Sun, F., Shi, Z., Zhu, Y., Jiang, S. & Lu, L. (2020). Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Research 30*: 343-355.

Xie, F., Zhao, H., Li, D., Chen, H., Quan, H., Shi, X., Lou, L. & Hu, Y. (2011). Synthesis and Biological Evaluation of 2,4,5-Substituted Pyrimidines as a New Class of Tubulin Polymerization Inhibitors. *Journal of Medicinal Chemistry 54*: 3200-3205.

Xu, J. & Hagler, A. (2002). Chemoinformatics and Drug Discovery. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry 7*: 566-600.

Zhu, X., Liu, Q., Du, L., Lu, L. & Jiang, S. (2013). Receptor-binding domain as a target for developing SARS vaccines. *J Thorac Dis 5 Suppl 2*: S142-148.

Table 1

S. No.	Compound	Docking Parameters	
		Blind Docking	**Local Docking**
1	AP-NP	98 Å × 104 Å × 160 Å	48 Å × 120 Å × 86 Å
2	AP-Ph-4-I	96 Å × 106 Å × 160 Å	60 Å × 92 Å × 66 Å
3	AP-3-OMe-Ph	96 Å × 106 Å × 160 Å	40 Å × 52 Å × 118 Å
4	AP-4-Me-Ph	96 Å × 106 Å × 160 Å	70 Å × 120 Å × 86 Å
5	AP-Ph-4-OMe	96 Å × 106 Å × 160 Å	40 Å × 38 Å × 118 Å
6	AP-Ph-4-Br	96 Å × 106 Å × 160 Å	52 Å × 66 Å × 126 Å
7	Chloroquine	96 Å × 100 Å × 165 Å	40 Å × 52 Å × 118 Å

Table 1. Docking Parameters used for diaryl pyrimidine derivatives and SARS-CoV-2S protein

Table 2

S. No.	Compound	Docking Parameters	
		Blind Docking	**Local Docking**
1	AP-NP	116 Å × 82 Å × 86 Å	58 Å × 82 Å × 120 Å
2	AP-Ph-4-I		
3	AP-3-OMe-Ph		
4	AP-4-Me-Ph		
5	AP-Ph-4-OMe		
6	AP-Ph-4-Br		
7	Chloroquine		

Table 2. Docking Parameters used for diaryl pyrimidine derivatives and hACE2-SARS-CoV-2S complex

Table 3

S.No.	Compound/Ligand	ADME Properties (Lipinki's Rule of Five		Drug Likeliness
		Properties	**Values**	
1.	**AP-NP**	Molecular weight (<500 Da)	313	Yes
		LogP (<5)	4.3	
		H-bond donar (5)	3	
		H-bond acceptor (<10)	4	
		Molar Refractivity (40-130)	96.5	
		Violations	**NO**	
2.	**AP-Ph-4-I**	Molecular weight (<500 Da)	390	Yes
		LogP (<5)	3.7	
		H-bond donar (5)	3	
		H-bond acceptor (<10)	4	
		Molar Refractivity (40-130)	91.7	
		Violations	**NO**	
3.	**AP-3-OMe-Ph**	Molecular weight (<500 Da)	293	Yes
		LogP (<5)	3.1	
		H-bond donar (5)	3	
		H-bond acceptor (<10)	5	
		Molar Refractivity (40-130)	85.5	
		Violations	**NO**	
4.	**AP-4-Me-Ph**	Molecular weight (<500 Da)	277	Yes
		LogP (<5)	3.4	
		H-bond donar (5)	3	
		H-bond acceptor (<10)	4	
		Molar Refractivity (40-130)	83.7	
		Violations	**NO**	
5.	**AP-Ph-4-OMe**	Molecular weight (<500 Da)	293	Yes
		LogP (<5)	3.1	
		H-bond donar (5)	3	
		H-bond acceptor (<10)	5	
		Molar Refractivity (40-130)	85.6	
		Violations	**NO**	
		Molecular weight (<500 Da)	341	

6.	**AP-Ph-4-Br**			Yes
		LogP (<5)	3.9	
		H-bond donar (5)	3	
		H-bond acceptor (<10)	4	
		Molar Refractivity (40-130)	86.7	
		Violations	NO	
7.	**Chloroquine**	Molecular weight (<500 Da)	319.5	Yes
		LogP (<5)	4	
		H-bond donar (5)	1	
		H-bond acceptor (<10)	3	
		Molar Refractivity (40-130)	94	
		Violations	**NO**	

Table 3: ADME value of diaryl pyrimidine derivatives used in molecular docking experiment

Table 4

Compound	**Binding Affinity (Kcal/mol)**	**Interacting amino acid Residue of hACE2**
AP-NP	-8.95	LEU 391, PHE 390, LEU 73, TRP 69, PHE 40, ASP 350, ASN 394, ARG 393
AP-Ph-4-I	-7.9	PHE 40, ASP 350, TRP 349, ALA 348, ASP 382, TYR 385, PHE 390
AP-3-OMe-Ph	-8.1	PHE 40, TRP 349, ALA 348, ASP 382, TYR 385, ARG 393, PHE 390, ASP 350, GLY 352
AP-4-Me-Ph	-8.1	ALA 348, TRP 349, SER 47, SER 44, PHE 40, ASP 350, TYR 385, HIS 401, ASP 382
AP-Ph-4-OMe	-7.8	TYR 385, ASP 382, HIS 401, ALA 348, TRP 349, PHE 40, PHE 390, GLY 352, ARG 393
AP-Ph-4-Br	-7.9	PHE 40, TRP 349, ALA 348, ASP 350, ASP 382, TYR 385, PHE 390
Chloroquine	-5.7	TRP 69, PHE 40, ASN 394, ARG 393, PHE 390, LEU 391, ALA 99, LEU 100, LEU 73

Table 4: Data of molecular docking experiments to find out the putative binding sites of diaryl pyrimidine derivatives on hACE-2

Table 5

Compound	Binding Affinity (Kcal/mol)	Interacting Domain of SARS-CoV-2S	Interacting amino acid Residue of SARS-CoV-2S
AP-NP	-7.9	C-Terminal of S1 Domain	PHE 464, PRO 426, PRO 463, ASP 428, GLU 516, LEU 517, PHE 515, SER 514, THR 733
AP-Ph-4-I	-7.7	C-Terminal of S1 Domain	ALA 520, LEU 517, CYS 391, GLY 545, ASN 544, LEU 546, PHE 543, PHE 565, GLN 564, ALA 522
AP-3-OMe-Ph	-7.7	S2 Domain	LYS 733, PRO 863, THR 866, LEU 865, ASP 867, ILE 870, ALA 1056, THR 778, HIS 1058, MET 731, GLN 774, THR 732
AP-4-Me-Ph	-8.1	S2 Domain	MET 731, LYS 733, PRO 863, THR 778, ILE 870, ALA 1056, PRO 1057, HIS 1058, SER 730
AP-Ph-4-OMe	-7.5	S2 Domain	HIS 1058, ALA 1056, ILE 870, ASP 867, THR 866, LEU 865, PRO 863, LYS 733, GLN 744, THR 778
AP-Ph-4-Br	-7.5	S2 Domain	PRO 863, ASP 867, ILE 870, THR 778, ALA 1056, PRO 1057, HIS 1058, GLN 774, LYS 733
Chloroquine	-6.1	S2 Domain	THR 778, ILE 870, HIS 1058, PRO 1057, PHE 823, VAL 826, THR 827, LYS 733, PRO 863, GLN 774, ASP 775, LEU 865

Table 5: Data of molecular docking experiments to find out the putative binding sites of diaryl pyrimidine derivatives on **SARS-CoV-2S (spike protein)**