Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Spermine phosphate Inhibits the SARS-CoV-2 Spike–ACE2 Protein-Protein Interaction–as an *in silico* approach contribute to its antiviral activity against COVID-19

Estari Mamidala[1*], Rakesh Davella[2], Pujala Shivakrishna[3]
[1,2,3]Department of Zoology, Kakatiya University, Warangal, Telangana State, India

ABSTRACT

Coronavirus 2019 (COVID-19), triggered by SARS-CoV-2, is reportedly under attack worldwide. The purpose of this survey was to re-subject drugs with possible antiviral activity from the selected drugs to human ACE-2 receptor protein. Molecular docking analysis of the ACE-2 receptor protein against seven drugs identified using the AutoDock 4.2 platform has been conducted to screen with improved possible antiviral activity and other computational methods have been further analysed for potential drugs or COVID-19 drugs. For the purpose of understanding the complex behaviour, the binding-mode of ACE-2 inhibitors was performed by Molecular Dynamic Simulation studies by using GROMACS version 5.1.4. The ability of anti-viral and COVID-19 therapeutic effectiveness is predicted by Spermine phosphate, Bromhexicin hydrochloride, Amentoflavone, Tiotropium, Ipratropium, fenugreekine and theophylline against ACE-2 receptor protein. The maximum binding affinity and reactivity to ACE-2 with −8.61kcal/mol was observed in Spermine phosphate. Spermine phosphate is the anti-viral medicine dependent on in silico results as compared to Chloroquine phosphate, which was found during the current study. Furthermore, the advancement of quick treatment for COVID-19 needs to be facilitated through laboratory and clinical investigations.

Keywords
Spermine phosphate, SARS-CoV-2 Spike, ACE2, Chloroquine phosphate, Molecular Docking

Introduction

SARS-CoV-2, which ranges from 27 to 32 kb, refers to one of the main RNA virus genomes. The SARS-CoV-2 viral genome comprises non-structural protein genes, which are encoded by the spick genes, envelopes, membrane (M) and nuclear capesid (N)1a (ORF) and ORF1b (1a), and four main structural proteins (Lu et al, 2020). CoV spicing (S) glycoprotein has also been identified as the main target for the development of antibodies, vaccines and inhibitors of infection and has been stated to play the most important role in viral adhesion and in fusion as well as the entry. Significant criteria for the evaluation of the effects of the natural products, which could attach to the surface between SARS-CoV-2 spike protein and their ACE-2 receptor. The binding energy is a physiochemical parameter, also regarded as a steady dissociation (Kd). Binding affinity prediction between SARS-CoV-2 spike protein and ACE2 receptors was carried out using the PRODIGY Web Server (Figure-1).

The genome-specific vaccinations of the SARS-CoV-2 are currently being studied along with their therapeutical antibodies. Alternatively, for clinical medicinal trials of COVID-19, methods for medication repurposition of known therapeutic agents have been implemented. Medicines for the management of COVID-19 were repeated based upon their past clinical history of possible therapeutics for other viral infections and diseases, such as Remedesivir, Favipiravir, Hydroxychloroquine, Ivermectine and Lopinavir/Ritonavir (Ahmed et al, 2020; EstariMamidalaet al, 2021). It is essential to consider how a coronavirus infects the human body, its attachment to the cells and its success in the host cells in order to create a new medicine or repurposite. It is understood that SARS-CoV-2, the angiotensin converting enzyme (ACE-2), uses its Spike

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

protein to attach to the cells of the host cells via the protein-based receptor on the cells' surface. In order to check that the ACE-2 receptor is a significant mediator in SARS CoV entering, the complex structure between the SARS-CoV-2 Spike protein receptor-binding domain (RBD) and the ACE-2 receptor has been determined (Wu et al, 2020).

ACE-2, which plays an important role in the regulation of the renin-angiotensin (RAS), has been reported to minimise the ACE-2 expression of SARS-CoV infection and to mitigate acute pathway renin-angiotensin insufficiency (Zhang et al, 2020). A compound that may prevent or interrupt the development of this complex was found in the RBD-ACE2 complex as an appropriate approach for the development of a legitimate drug discovery for COVID-19 (Wang et al, 2020). While it takes many years to formulate and discover a new medication, the release into the market of a new drug greatly decreases the prices and the time spent. It was also proposed that a medicine be recycled with the aforementioned technique to address the new pandemic epidemic quickly. While a number of attempts are under way and has not been published as a potential antiviral agent against SARS-CoV-2. Therefore, to explore their antiviral properties, we will need to study current medicines. This strategy attempts to recast COVID-19 drugs that could suppress computational interactions with possible antiviral activity for SARS-CoV-2 Spike-ACE2 Protein.

Material & Methods

Ligand preparation:

In-silico docking of ACE2 receptors has been selected and downloaded to individual PDB files using the OpenBabel 2.4.1 (https://pubchem.nlm.nlm.gov, in PubChem (Kim et al. 2019) database in 3D structures from seven FDA drugs, Spermines, Bromhexins, Amentoflavone, Tiotropium, Ipratropium, Fenugretkin and Theophyllines, and in SDF format (Table-1). Converting these substances with the AutoDockMGLTools 1.5.6 (http://mgltools.scripps.edu/downloads) was also processed. The control agent in this research, classified as the ACE-2 inhibitor, consists of the usage of chloroquine phosphate (commercial drug PubChem CID-64927).

Protein preparation:

A Research Collaboratory for Structural Bioinformatics (RCSB) data base (https://www.rcsb.org /structure/6LZG) has been used for the critical structure 6LZG (Resolution: 2.50 A as) of the spike protein-binding domain SARS-CoV-2 receptor complex with the human ACE2 receptor (6LZG). In the RSCB database, there are two ligands (Zn2+ and NAG) available in 3D spike protein structures (PDB ID: 6LZG). Based on the structure consistency and completeness of the ACE-2 receptor, the structure was chosen. To prepare the docking protein, removed the RBD domain with a spike protein, water molecules and two ligands with using BIOVIA Discovery Studio 4.5 Visualizer (BIOVIA, San Diego, CA, USA). Both chains were therefore omitted with the exception of Chain-A where only a ligand site chain is shown. Using Auto Dock Tools, the ACE-2 receptor has been reduced and transformed into pdbqt format.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Docking parameters:

A molecular docking process aims to anticipate and interpret the prevailing binding pose of a ligand and a protein. The seven FDA medicinal products Spermine phosphate, Bromhexine Hydrochloride, Amentoflavone, Tiotropium, Ipratropium, Fenugreekine and Theophylline were examined by*in silico*computational method. The whole protein surface has been scanned to screen for the ligands that are linked directly to the association site ACE2-S-protein. The parameters of docking have been maintained by convention. In this context, molecular docking study was performed by usingAutoDock version 4.2 (http://autodock.scripps.edu/). The best resulting docked complexes were defined and processed for further computational study based on the binding energy values, ligand efficiency, intermolecular hydrogen (H) bonding and other hydrophobic and electrostatic interactions.

Post docking analysis:

Based on its binding free energy and binding location, the ligands were evaluated. For further evaluation, ligands were selected which attach on the S-protein-ACE2 interface. Using UCSF Chimera (http://www.cgl.ucsf.edu/chimera) we chosen the best hits based on the interactions between ligands and the macromolecule.

Molecular dynamic simulations

For additional experiments utilising atomic molecular dynamic (MD) simulations to evaluate the versatility and stability of protein ligand interactions, the docked position for effective ligand spermine phosphate complex with the ACE2 of the S-protein has been prepared. To this end, all simulation experiments with the GROMACS 5.4.1 suite on a LINUX-based workstation utilising the GROMOS96-43a1 strength area were completed. The PRODRG server created ligand parameters and topology files. In addition, to neutralise the whole cubic structure, solvation, ions and water molecules have been introduced. The most pronounced process of decay accompanied the balancing of constant particle numbers, volume and temperature (NVT), constant particle count, pressure and temperature (NVT) (NPT). NVT equilibrium was achieved by using the Berendsen temperature combinations technique, using 300K electrostatic short range cut-offs of 1.2 nm and temperature control. In addition, the next NPT balance step was conducted and co-ordinates were produced every 1 ps. Finally, an integration timeframe of 2fs took 30 ns MD output, and every 10 ps trajectory was produced. For the calculation of RMSD values of protein-ligand complexes, the conformation produced during the production phase was used.

Results and Discussion

Identification of ACE2 Receptor Binding Molecules

The S-RBD trimericcoronaviral protein links to the host cell surface receptor ACE-2 through S-RBD from S-protein to mediate within the host cell (Shang et al, 2020). ACE-2 is a glycoprotein membrane with an outer surface claw such as the N-terminal peptidase dominion composed of α-helical lobes which associate with bowl-shaped, S-RBD-shaped cavity ((Wu et al, 2009). S-RBDs communicating specifically with the ACE-2 receptor in the SARS-CoV-2 series are identical to SARS-CoV, significantly showing that ACE-2 plays a central role in the entry of

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

SARS-CoV-2 in host cells (Shang et al, 2020). The Lys31 and Lys353 virus reception sites on ACE-2 are confirmed to be two major hotspot SARS-CoVbinding sites(Wan et al, 2020). Recent results show that hotspot 31 is made up of a bridge from Lys31 to Glu35, whereas hotspot 353 is a bridge from Lys353 to Asp38, all in hydrophobic conditions (Yan et al, 2020).

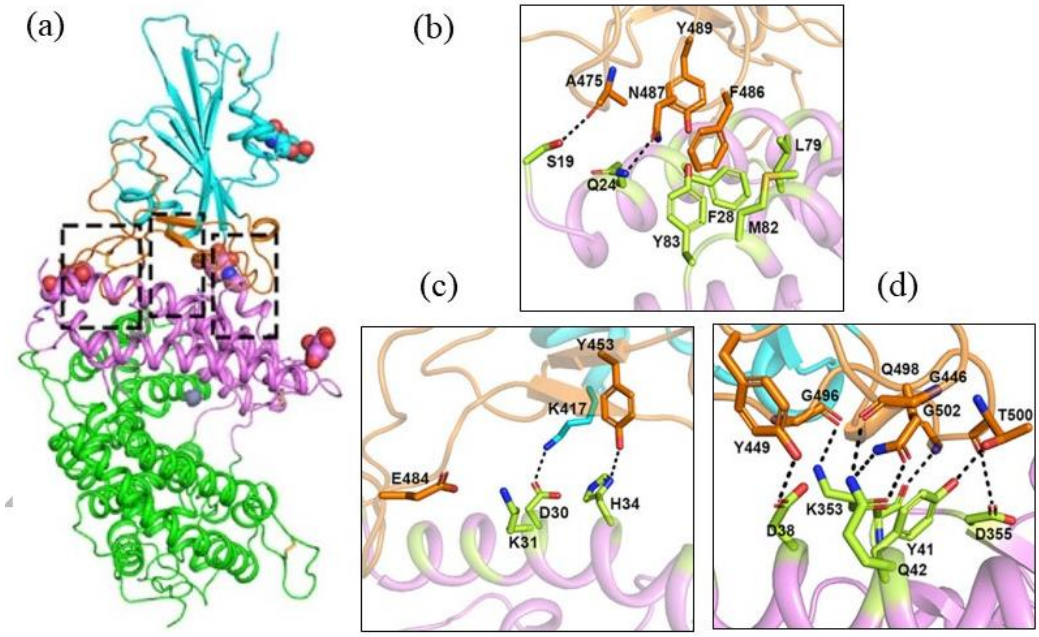


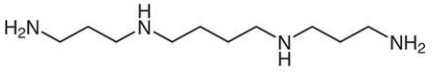
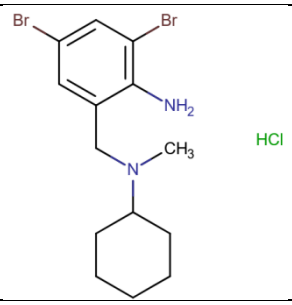
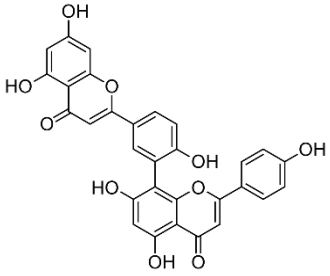
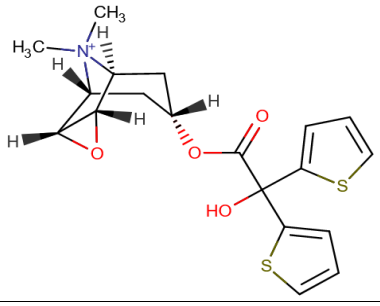
Figure-1. The structure of SARS-CoV-2 spike (S) protein bound to human ACE2 (hACE2). (A) A cartoon representation of the complex structure. The contacting sites are further delineated in (B)–(D) for the amino acid interaction details.

The binding site was split into the electrostatic surface area at the S-protein ACE2 interface, respectively location 1, location 2 and location 3. (Figure-1A). The 'Site 1' hydraulic area consists of the ACE2 receptor's residues Asp38, Tyr41, Gln42, Lys353 which Asp355 and communicate with the S-Protein residues Gly446, Tyr449, Gly496,Gln498, Thr500 and Asn501 (Figure-1B). Residue Asp30 and Glu35 on the ACE2 receptor interacting with the residents Lys417 and Gln493 from the S-Protein are present in the mild hydrophillic area 'Site 2' (Figure-1C). The field 'Site 3' also comprises of the Ser19, Glu24, and Tyr83 residues of ACE2, and interacts with residual S-protein Ala475 and Asn487 (Figure-1D).

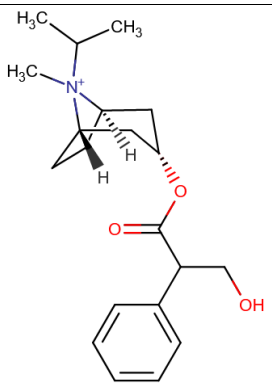
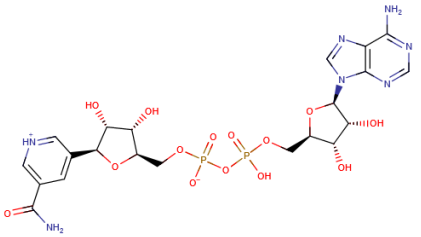
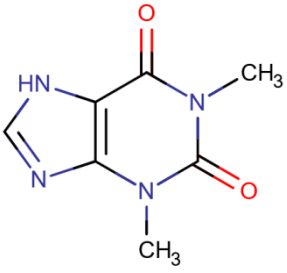
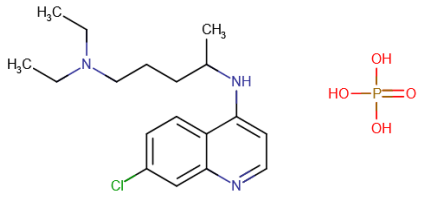
A thorough study showed a significant hydrophilic area at the top of the 'Site 1' region, according to the topology of the binding site. Consequently, the ACE2 receptor virus binding hotspots were designed to recognise FDA authorised library molecules, which are supposed to block the ACE2 receptor and its interactions with the virus. AutoDock was achieved with high-performance computer-based scanning. Any of the selected molecules was docked to ACE2 using AutoDock to get additional information about the interactions that exist at the ligand-ACE2 interface. Topping scores for hydrogen bonds and for other relationships between these ligands are defined in Table-1 on the basis of their binding affinities and visual analyses of docked complexes.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Table-1. Molecular docking scores of selected drugs participating in hydrogen bonding with human ACE-2 receptor protein

Pub Chem ID	Compound Name	Structure	Docking Score (Binding Free Energy) (Kcal/mol)	No. of Hydrogen bonds with 6LZG	H bond form with Binding residues
517358	Spermine phosphate		-8.61	5	Thr371, Glu406, Glu375, Glu402, Pro346
5702220	Bromhexine hydrochloride		-7.45	1	Asp350
5281600	Amentoflavone		-7.13	6	Trp69, Tyr385, Ser44, Ala348, Asp382, Tyr385
5487427	Tiotropium		-6.21	1	Asp394

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

657309	Ipratropium		-6.04	2	Leu95, Lys562
444170	Fenugreekine		-5.88	7	Asn53, Arg357, Gln340, Thr334, Trp48, Asn330 , Ser331
2153	Theophylline		-4.66	1	Asn290
64927	Chloroquine phosphate		-6.3	2	Glu208, Asp206

Molecular Docking Analysis

A total of 7 compounds were screened with binding energy ranging from – 8.61 to -4.66 kcal/mol against ACE-2 receptor protein and three compounds out of these seven screened leads showed low binding energy (-8.61, -7.45, and -7.13 kcal/mol). The binding force of the chloroquine phosphate reference molecule was −6,3 kcal/mol.The docking studies thus show that the screened compounds will operate as the reference molecule.The 3D interaction pictures of Spermine phosphate, Bromhexine hydrochloride and amentoflavonecompounds with ACE-2 showed inFigures 2-4with binding energy – 8.61, -7.45 and - 7.13 kcal/mol.It displays lower docking rates (higher binding energy) compared to those of the reference molecule, but certain ligands often have stronger interactions with the objective protein that can be regarded as a successful ACE-2 inhibitor.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Interaction of Spermine phosphate with human ACE-2 receptor protein

The relationship between Spermine phosphate and human ACE-2 receptor protein is shown in **Figure 2.**Spermine phosphate is a polyamine. It is an organic molecule required for the growth of eukaryotic cells. Spermine phosphate acts as a free radical scavenger directly and forms a range of adductives that keep DNA from being oxidative. The compound with an energy value of -8.61 kcal/mol has shown the most powerful residue of the ACE-2 receptor protein in the production of hydrogen bond with Thr371, Glu406, Glu375, Glu402 and Pro346 residues. Simulation dynamics have further confirmed the conclusion gained from molecular docking.

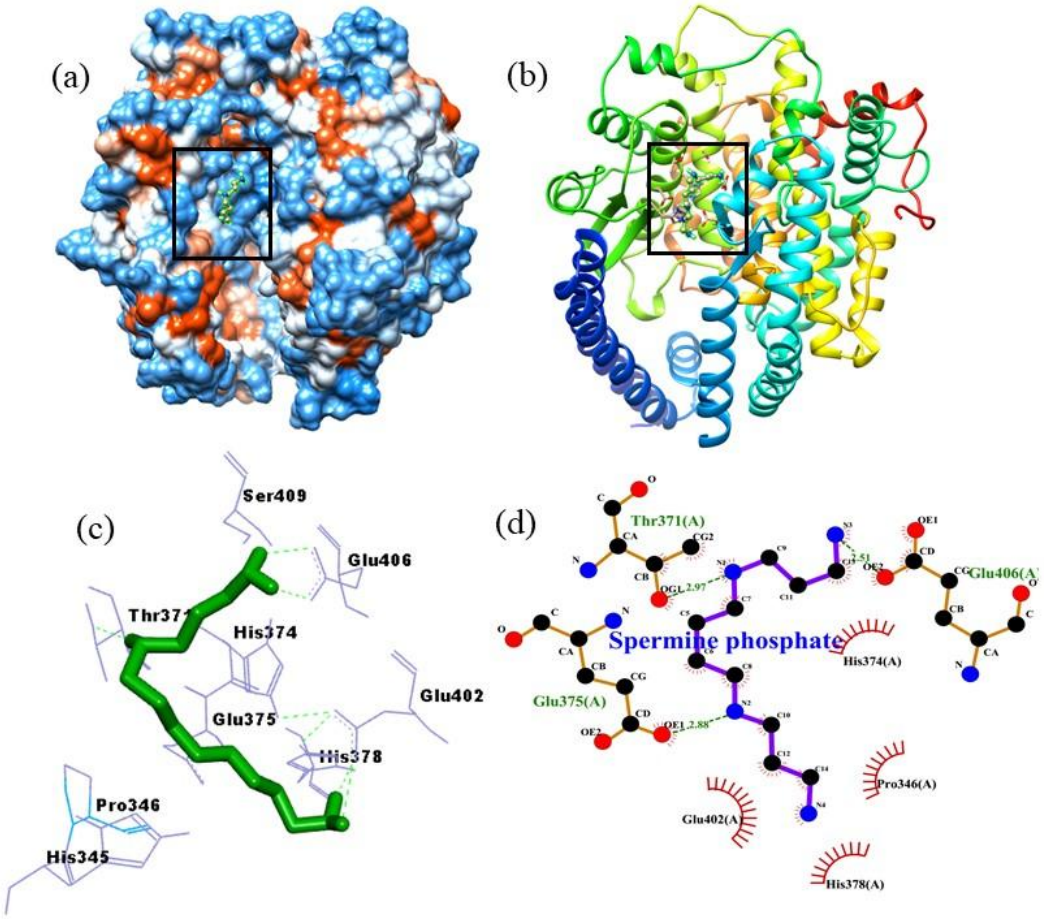


Figure-2. Molecular docking interaction of Spermine phosphate with human ACE-2 receptor protein (A chain of 6VYZ).

2a: Molecular surface map built in UCSF chimera; 2b: Docked Complex map built in UCSF chimera; 2c: The 3D contact map built in Discovery Studio; 2d: The 2D contact map built in ligplot+

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Interaction of Bromhexine hydrochloride with human ACE-2 receptor protein

The molecular agent Bromhexine is used to treat a number of respiratory disorders combined with enhanced secretion of mouth. It comes from the herb *Adhatodavasica* and helps to clear excess mucus, to better breathe and to reduce toxin. It was launched in 1963 and is currently available in several countries as an overall medication (Zanasi et al, 2017). **Figure-3** illustrates Bromhexine hydrochloride's molecular relationship with human ACE-2 receptor protein. Bromhexine hydrochloride binds to ACE-2 receptor with binding energy of –7.45 kcal/mole. This docked configuration is retained with hydrogen bond in between Asp350 of ACE-2 withBromhexine hydrochloride, with a bond duration of 2.53 Å. The human ACE-2 receptor protein interactions was further investigated by studies in the docking complexes and Bromhexinhydrohloride is centered primarily by hydrophobic interactions with ACE-2 residues Phe40, Phe390, Tyr385, Arg383, Ala348 and His401.

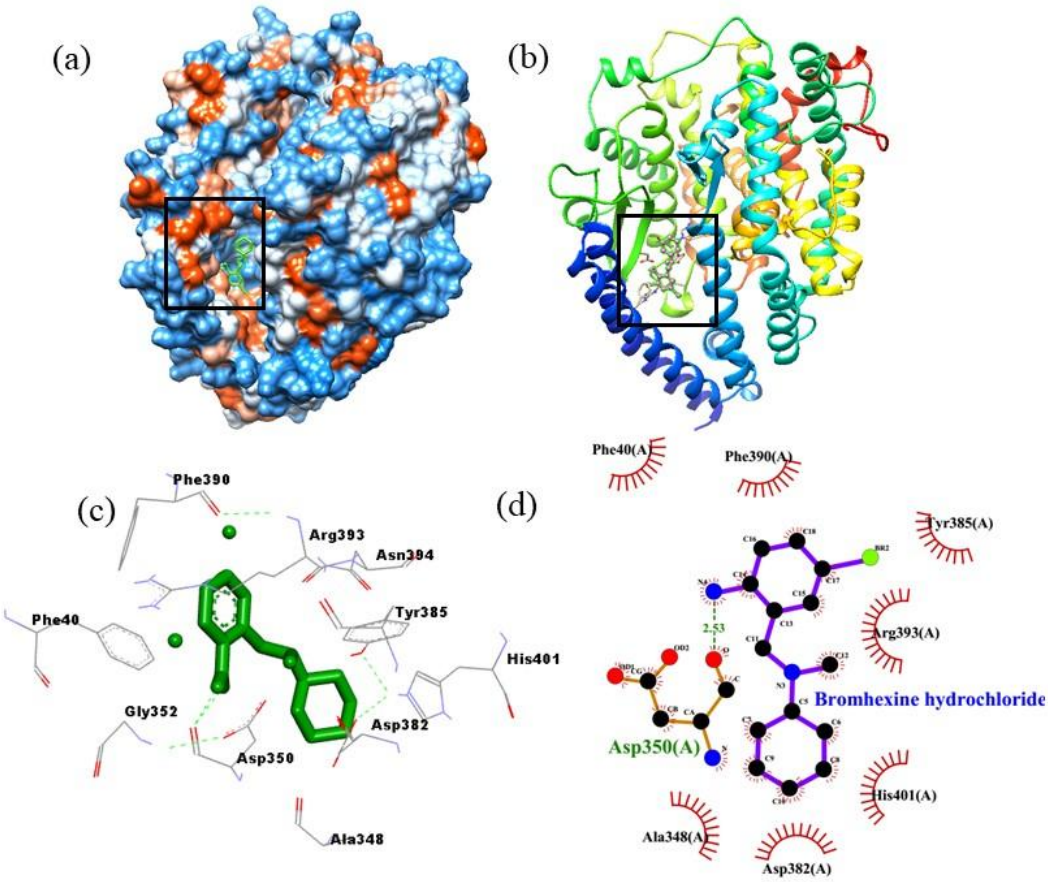


Figure-3. Molecular docking interaction of Bromhexine hydrochloridewith human ACE-2 receptor protein (A chain of 6VYZ).

3a: Molecular surface map built in UCSF chimera; 3b: Docked Complex map built in UCSF chimera; 3c: The 3D contact map built in Discovery Studio; 3d: The 2D contact map built in ligplot+

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Interaction of Amentoflavone with human ACE-2 receptor protein

Amentoflavone is a biflavonoid present in *Ginkgo biloba* and *Hypericumperforatum* and a natural product. It plays a function in cathepsin B, an antiviral, angiogenesis, P450 and plant metabolites. It also acts as a cathepsin B inhibitor. **Figure-4** shows Amentoflavone docking associations with the ACE-2 receptor protein. Binding energy of Amentoflavone is -7.13 kcal/mole with human ACE-2 receptor protein. There are some Pi-Pi bond reactions, connections between Pi-Alkyl and 6 hydrogen bonds withdistance of < 2.48 Å. Two Pi-Pi stack interactions with Phe40 and His401, and one Pi-Alkyl with Asp382 residue are found. The protein forms six hydrogen bonds withTrp 69, Tyr385, Ser44, Ala348, Asp382 and Tyr385 amino acid residues. The hydrogen bond with Ser44 with distance 1.85 Å apart, which ensures greater affinity for binding.

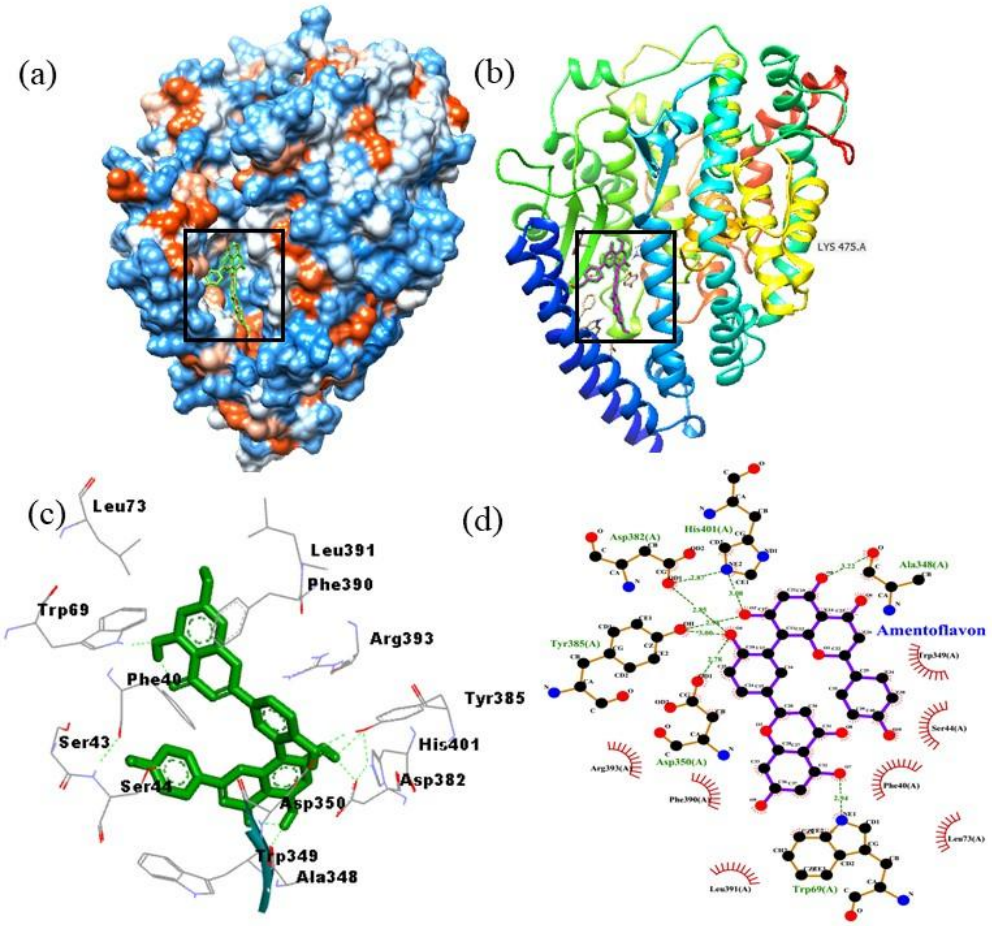


Figure-4. Molecular docking interaction of Amentoflavone with human ACE-2 receptor protein (A chain of 6VYZ).

4a: Molecular surface map built in UCSF chimera; 4b: Docked Complex map built in UCSF chimera; 4c: The 3D contact map built in Discovery Studio; 4d: The 2D contact map built in ligplot+

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Interaction of Chloroquine phosphate with human ACE-2 receptor protein

Chloroquine phosphate is a derivative of aminoquinolones, originally used to combat malaria in the 1940s (Li et al, 2017). Chloroquin and hydroxychloroquine are repurposed after then in order to control a range of other diseases such as HIV, chronic erythematosis of lupus and rheumatoid arthritis (Plantone et al, 2018). The FDA's permit for emergency usage of COVID-19 hydroxychloroquine and chloroquine was withdrawn on 15 June 2020 (FDA, 2020). In this analysis which is regarded as an ACE-2 inhibitor, chloroquine phosphate thus is used as a reference medicine. Chloroquine phosphate associations with ACE-2 receptor protein are seen in **Figure-5.** The docking score for chloroquine phosphate is -6.3 kcal/mol which is above the three compounds, and this shows the their effectiveness and Chloroquine phosphate forms one hydrogen bonds with ACE-2 receptor proteinresidue of Glu208.

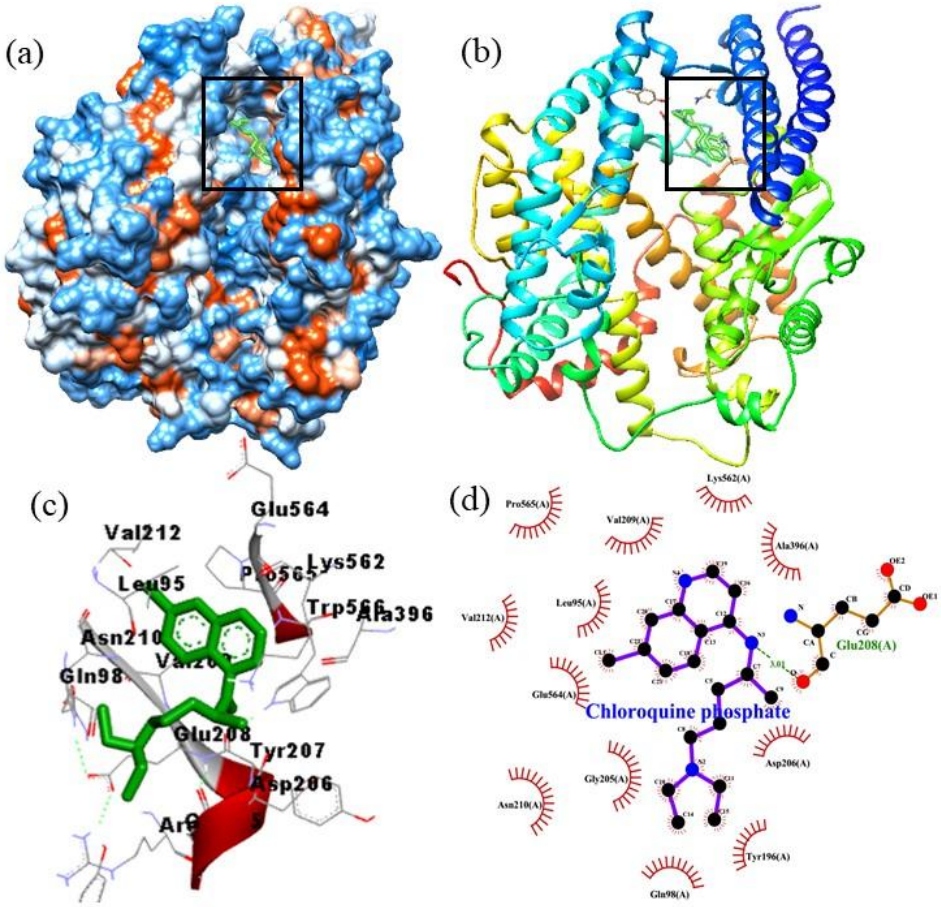


Figure-5. Molecular docking interaction of Chloroquine phosphate **with human ACE-2 receptor protein (A chain of 6VYZ).**

5a: Molecular surface map built in UCSF chimera; 5b: Docked Complex map built in UCSF chimera; 5c: The 3D contact map built in Discovery Studio; 5d: The 2D contact map built in ligplot+

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

Analysis of MD Simulation

In addition, MD analyses of the Spermine phosphate molecule was conducted to assess the stability of the protein-ligand complexes. A cubic water box with minimised volume and set-up for a simulation period of 30% solved the chosen protein-ligand complexes. The basic parameters were inspected and the ligands were interacting in depth to clarify the right ligand.

Root mean square deviation (RMSD)analysis of ACE-2 protein backbone and ligand atom Spermine Phosphate has been computed in **Figure-6.** These complexes revealed that the ligand atom stabilising at 1.25 and 0.09 nm respectively over the 30ns simulation period and protein backbone were not significantly fluctuated. The ligand RMSD for the protein was measured and analysed to verify the stability of the compound at the binding site expected by docking. Initially, RMSD rises to 2.5 ns. The plateau after 2.5 ns shows that there is no fluctuation in the complex. After 2.5 ns simulation the complex has robust RMSD (figure 6). The versatility of the capping loop and terminal residues will result in a sudden leap in RMSD at around 26 ns. However, the inhibitor does not distinguish from the receptor protein ACE-2 and the interface of the complex is stable. For complementary concentration inhibitors to be more closely related to their target. For essential amino acids, an enzyme must be selectively bound and have low RMSD. Such peptides may easily be synthesised chemically, or recombinant overexpression can be generated in large amounts. Though our results have been checked by different methods of *in silico*, more test checks are warranted. The RMSD finding has been further confirmed by fluctuation of residues by RMSF.

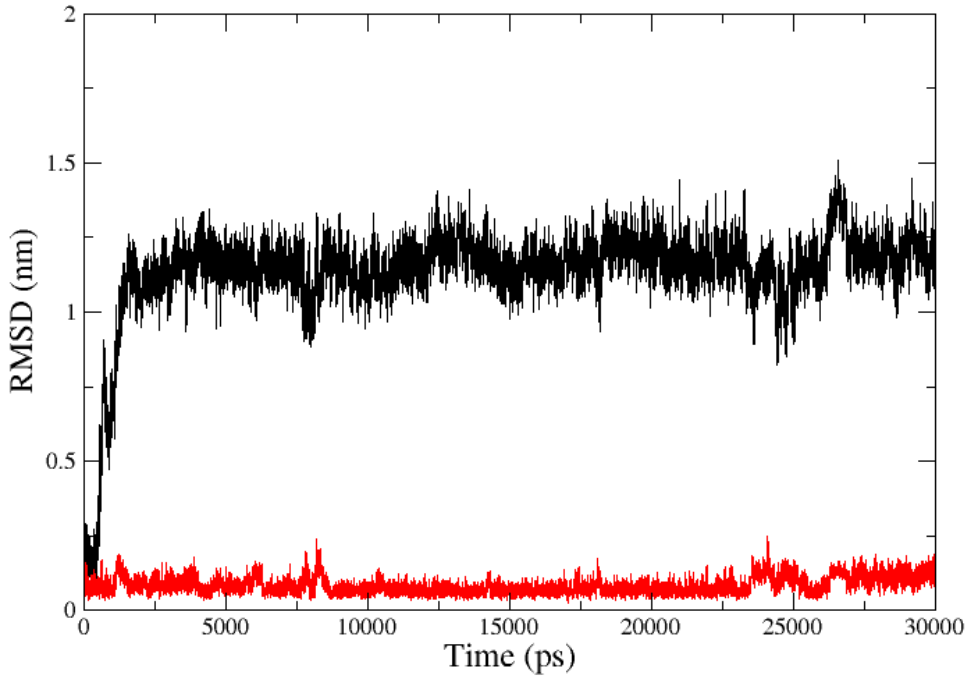


Figure-6. Root mean square deviation (RMSD) analysis of the protein backbone (black colour) and Spermine phosphate ligand (Red colour) for the ACE-2-Spermine phosphate complex throughout 30 ns time period of MD simulation.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

During the simulation in a structure, the Root mean square deviation**(**RMSF) has average residual mobility. In ACE-2 receptor protein, mobility of many residues was measured by RMSF (Fig. 7). In the complex of the ACE-2 receptor, there were more variations in their Cα atoms than in other regions between 1100-1200 and 4500-4600. This plot denotes the reduction of residue mobility in the ACE-2 receptor protein by the binding of Spermine phosphate.

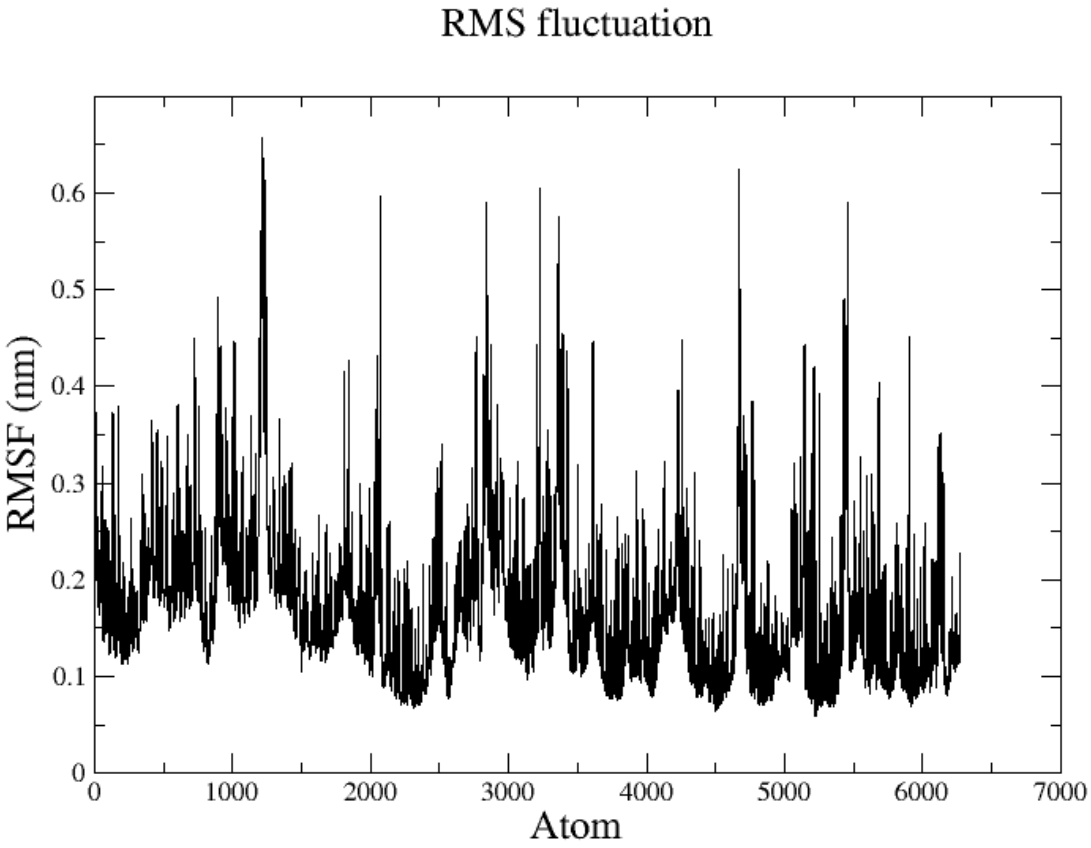


Figure-7. Root mean square deviation (RMSD) analysis of the Spermine phosphate for the ACE-2 complexes.

Figure-8 reveals, ACE-2 receptor protein gyration (Rg) profile with Spermine phosphate. The total compactness for complex was determined by Rg. Radius gyration vs time graphs to verify compaction were drawn. The ligand Rg of ACE-2 ranged from ~.54 nm to ~2.62 nm, with a higher Rg. The energy plot shows the decline in residue mobility in ACE-2, which was verified in RMSF (Figure-8).

In an*in silico* investigation, the results may be supportive proof that the effectiveness and antiviral drug power of Spermine phosphate against human receptor protein ACE-2 must be confirmed by *in vivo* and *in vitro* research.

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 5, 2021, Pages. 4814 - 4827
Received 25 April 2021; Accepted 08 May 2021.

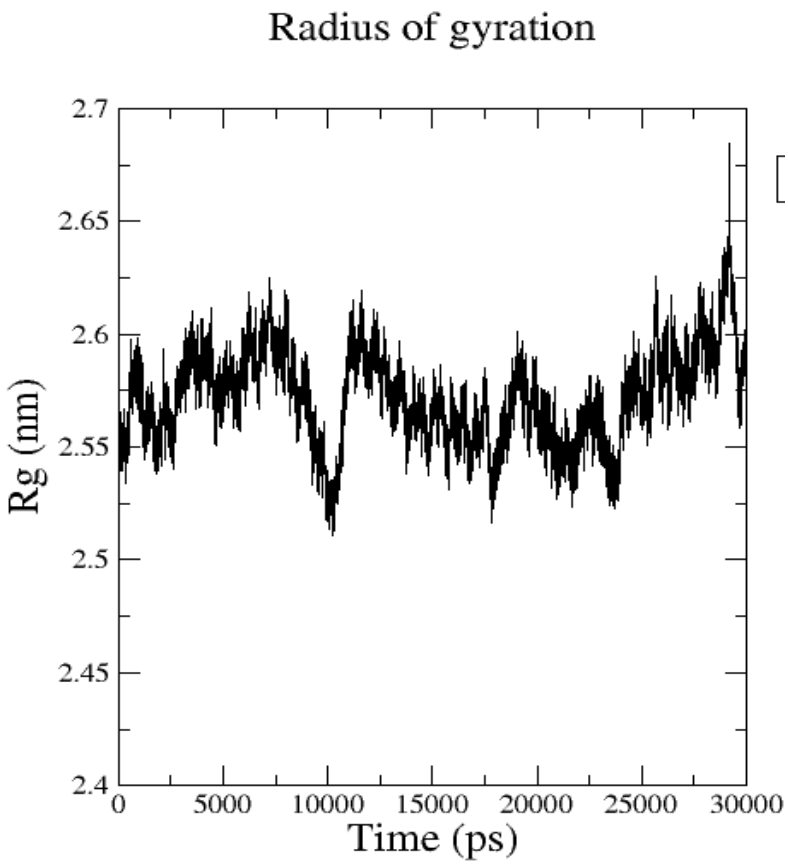


Figure-8. Radius of gyration (Rg) analysis of the Spermine phosphate for the ACE-2 complex.

Conclusion

The findings of this *in silico* analysis showed possible compounds that could prevent the infection of the virus. These Docking and MD simulation experiments show the possible blocker of SARS-CoV-2 infection in Spermine phosphate. The inhibitor's predicted binding affinity is higher than the control drugChloroquine, thereby competitively binding the ACE-2 receptor with the SARS-CoV-2 Spike safety. As a result, the suggested inhibitor could be an ACE-2 receptor protein potential blocker and thus impede viral entry to cells. The results in this study could proved that the efficacy and anti-viral drug strength of Spermine phosphate with human receptor protein ACE-2 for inhibiting entry into the host SARS-CoV-2 was to be confirmed in *in vivo* and *in vitro* tests.

Acknowledgment:

Authors thank to Head, Department of Zoology, Kakatiya University, Warangal, Telangana State, India, for his support.

Financial support & sponsorship: None.

Conflicts of Interest: None

References

[1] Ahmed, S. F., Quadeer, A. A. & McKay, M. R. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. *Viruses***12**, 254 (2020)
[2] EstariMamidala, RakeshDavella , SwapnaGurrapu and PujalaShivakrishna , In Silico Identification of Clinically Approved Medicines Against the main Protease of Sars-Cov-2 – A Causative Agent of Covid-19.(2021).*Int. J. Life Sci. Pharma Res*.11(1), L107- 122
[3] FDA: Emergency use Authorization for Hydroxychloroquine and Chloroquine Revoked, 2020.
[4] Li C, Zhu X, Ji X, Quanquin N, Deng YQ, Tian M, Aliyari R, Zuo X, Yuan L, Afridi SK, Li XF, Jung JU, Nielsen-Saines K, Qin FX, Qin CF, Xu Z, Cheng G: Chloroquine, a FDA-approved Drug, Prevents Zika Virus Infection and its Associated Congenital Microcephaly in Mice. *EBioMedicine.* 2017 Oct;24:189-194.
[5] Lu R., Zhao X., Li J., Niu P., Yang B., Wu H., Wang W., Song H., Huang B., Zhu N. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395:565–574
[6] Mamidala, E., Davella, R., Gurrapu, S. and Shivakrishna, P., 2020. In silico identification of clinically approved medicines against the main protease of SARS-CoV-2, causative agent of covid-19. arXiv preprint arXiv:2004.12055.
[7] Plantone D, Koudriavtseva T: Current and Future Use of Chloroquine and Hydroxychloroquine in Infectious, Immune, Neoplastic, and Neurological Diseases: A Mini-Review. *Clin Drug Investig*. 2018 Aug;38(8):653-671.
[8] Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. J Virol. (2020) 94:e00127-20.
[9] Wang L, Xiang Y. Spike Glycoprotein-Mediated Entry of SARS Coronaviruses. *Viruses*. 2020;12(11):1289. Published 2020 Nov 11.
[10] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG. A new coronavirus associated with human respiratory disease in China. Nature 2020; 579 : 265-9
[11] Wu K, Li W, Peng G, Li F. Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. ProcNatlAcadSci USA. (2009) 106:19970–4.
[12] Yan R, Zhang Y, Li Y, Xia L, Zhou Q. Structure of dimeric full-length human ACE2 in complex with B 0 AT1. bioRxiv [Preprint]. (2020)
[13] Zanasi A, Mazzolini M, Kantar A: A reappraisal of the mucoactive activity and clinical efficacy of bromhexine. MultidiscipRespir Med. 2017 Mar 20;12:7. doi: 10.1186/s40248-017-0088-1.
[14] Zhang, H., Penninger, J.M., Li, Y. *et al.* Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med***46,** 586–590 (2020).