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RESEARCH ARTICLE

Molecular docking studies of Indian variants of pathophysiological proteins of SARS-CoV-2 with selected drug candidates

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Abstract. SARS-CoV-2 pandemic has recently made the entire world come to a standstill. The number of cases in the world, especially India, have been increasing exponentially. The need of the hour is to assimilate as much data as possible to fast track the pipeline of bringing in new therapeutic tools against this fatal virus. In this brief communication, we aim to throw light on the various variants of the proteins involved heavily in the pathophysiology of COVID-19, namely Spike protein, ACE2, GRP78, TMPRSS2 and NSP-12. We also portray the molecular docking studies of these proteins with specific drugs that are currently being associated with the same. In our brief study, we come across a few key findings. First of all the combinations of the variants of spike protein and ACE2 binding show overall 25% unfavourable ΔΔG. Second, NSP12 is the most mutation prone among all the NSPs of the SARS-CoV-2 genome and the most common mutations are P323L and A97V. Third, we discovered the variants found in the Indian subpopulation that have greater binding with the currently investigated drugs.

Keywords. SARS-CoV-2; COVID-19; treatment; molecular docking; variant analysis; drug affinity.

Introduction

Since the SARS-CoV-2 that was first reported in Wuhan, China on the 24 December 2019, the world has been at a standstill (Zhu *et al.* 2020). A mere three months after the first reported case, the WHO declared this disease by the novel virus COVID-19, as a worldwide pandemic. As reported on 16 June, there are as many as 7.9 million people affected worldwide spanning over 215 countries with a jump of over hundred and eighteen thousand cases every day. Even more gnarly is the death count 434,796 and increasing (World Health Organization 2020). Coronavirus disease (COVID-2019) situation reports 2020 (available at https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200616-covid-19-sitrep-148-draft.pdf).

There are quite a few coronaviruses that infect human hosts but with different degrees of infectivity and lethality. Coronaviruses like the NL63, OC43 and HKU1 only cause mild respiratory infections while the Middle Eastern respiratory syndrome (MERS-CoV), severe acute respiratory syndrome (SARS-CoV) and the novel SARS-CoV-2 cause severe respiratory syndromes in the human host. The lethality of SARS-CoV-2 is markedly lower than SARS-CoV and MERS-CoV (0.9–3.3%, 11% and 34%, respectively), but the infectivity of COVID-19 outperforms SARS-CoV and MERS-CoV as the doubling rate of COVID-19 is significantly higher than these two viruses (Sun *et al.* 2020).

In the present paradigm, the pandemic has disrupted many lives around the globe and brought the world to a standstill. The emphasis in the scientific community is on the various forms of analysis of the virus to aid the therapeutic interventions and vaccine development.

COVID-19 is an enveloped virus having four different structural proteins, N (nucleocapsid), M (membrane), E (envelope) and S (spike) (Yang *et al.* 2020). The S protein mediates receptor binding and virus entry along with the ACE2 receptor on the host cell surface (Wang *et al.* 2020a,b). This process is aided by the chaperon proteins GRP78 or HSPA5 and a serine protease TMPRSS2 which primes the spike protein changing its configuration from closed to open (before binding to ACE2) (Hoffmann *et al.* 2020a, b; Ibrahim *et al.* 2020).

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Thus, here, we have focussed on the S protein and studied the recently sequenced isolates from India to identify the similarities and differences in the protein sequence with respect to Wuhan isolates. We have also analysed the key protein (GRP78, TMPRSS2 and ACE2) allelic variants present in India as assessed their binding affinity *in silico* through molecular docking studies with current SARS-CoV-2 therapeutic regimens that are employed.

Materials and methods

SARS-CoV-2 sequences are being deposited every single day from all over the world. We analysed all SARS-CoV-2 Indian sequences (197) excluding low coverage (>5% N in the 29.9 kb of each sequence) and with patient status as on 9 June 2020 from the GISAID database (Shu and McCauley 2017). To analyse the sequences, we used the CoV Surfer for all sequences against the Wuhan strain. The results were filtered for duplicates and only high coverage results were retained. The Spike protein variants were extracted from these results and the count of the mutations found were compiled.

Variant analysis

The list of variants for ACE2, GRP78 and TMPRSS2 present in the Indian population were mined from the Index Db (*J. Comput. Biol*. 2019, pp. 225–234) database along with their allelic frequency. The frequency of the Spike protein variants was calculated by the ratio of the number of patients with occurrence of a particular mutation to the total number of patient sequences obtained from GISAID. These variants were modelled using Swiss Model (Bienert *et al.* 2017) homology modelling.

The best model was obtained using the QMEAN4 score and Ramachandran plot. Further, the model quality assessment was conducted by the evaluation of the backbone conformation from the Ramachandran plot by dividing the percentage of amino acid residues of the model in the allowed and disallowed regions. The protein sequences were obtained from UniProt (The UniProt Consortium 2019). The variants were then analysed using iMUTANT (Capriotti *et al.* 2005), which is a SVM predictor that predicts the protein stability changes upon single-site mutations starting from protein sequence to get the stability of the variants ($\Delta\Delta G$) at 27°C and 37°C.

Docking analysis

Virtual screening of the list of chosen small molecule inhibitors and variants was carried out using PyRx. AutoDock 4.2 (Morris *et al.* 2009) was used to analyse the top hits obtained and their respective interactions between the ligands and macromolecules. For comparison, we also conducted the docking analysis with controls and the drugs listed above.

Proteins chosen for analysis

***Spike (S) protein and angiotensin converting enzyme 2 (ACE2)*:** The distinct morphology of the coronavirus is created by the S protein which is a homo-trimer that binds to angiotensin converting enzyme 2 (ACE2) (Kalathiya *et al.* 2020). The S protein consists of two subunits: S1 and S2, the former consisting of the receptor binding domain and the latter contributing fusion machinery (Bosch *et al.* 2003). The tight binding of SARS-CoV-2 with ACE2 is one of the likely explanations for the high transmission of the virus (Verdecchia *et al.* 2020). Needless to say, the analysis of the mutations of the S protein and identification of the conserved regions would go a long way in the creation of an effective drug or vaccine.

***Glucose regulating protein 78*:** The glucose regulating protein 78 or binding immunoglobulin protein (BiP) regulates the unfolded protein response in the endoplasmic reticulum (ER) especially when the cell is under stress (Wang *et al.* 2009). ER stress activates multiple cellular pathways that exert control at both a transcription as well as translational levels (Hebert-Schuster *et al.* 2018). The three major pathways are mediated by PERK kinase, IRE1, and ATF6. Pathogens make use of the over expression of GRP78 to gain entry into a cell (Kadowaki *et al.* 2013).

Several docking studies have been conducted to analyse the mechanism of entry of SARS-CoV-2 using GRP78 binding domain. They report that there are interactions between the substrate binding domain of GRP78 and the receptor binding domain of SARS-CoV-2, especially in regions III (C391-C525) and IV (C480-C488) (Ibrahim *et al.* 2020). This interaction can now be leveraged to design therapeutics specific to SARS-CoV-2.

***Transmembrane serine protease 2 (TMPRSS2)*:** Plays a central role in viral activation and cell entry in not just SARS-CoV-2 but almost all coronavirus and influenza viruses (Glowacka *et al.* 2011). This membrane bound molecule has serine proteases that cleave the S protein. Once cleaved, the fusion peptide is exposed for viral entry. TMPRSS2 essentially cleaves the spike protein into two subunits S1 and S2 which allows effective penetration of the virus into the cell (Belouzard *et al.* 2012). Hence, there has been extensive study on serine protease inhibitors that may be used to prevent cell entry of the virus.

***Nonstructural protein-12 (NSP12) or RNA dependent RNA polymerase*:** NSP12 is a protein encoded in the viral genome (Velthuis *et al.* 2010). After the infection by SARS-CoV-2, the coronavirus assembles an RNA-synthesis complex with the viral nonstructural proteins (NSP) that takes responsibility of replicating and transcribing the entirety of the viral

Table 1. List of Indian variants used for analysis.

ID	Variant of ACE2	Change in ΔΔG of ACE2 structure at 37°C	MAF
rs4646116	Var 1	−0.30	0.00571
rs756905974	Var 2	0.26	0.00286
rs200180615	Var 3	−1.01	0.00286
ID	Variant of GRP78	Change in ΔΔG of GRP78 structure	MAF
rs1282333876	Var 1	−1.1	< 0.01
rs1265027435	Var 2	0.56	< 0.01
rs1253850727	Var 3	−2.35	< 0.01
rs143920039	Var 4	−0.77	0.02
ID	Variant of TMPRSS2	Change in ΔΔG of TMPRSS2 structure	MAF
rs12329760	Var 1	−1.1	0.18286
rs75603675	Var 2	0.56	0.06286
rs541351488	Var 3	−2.35	0.00286

Table 2. List of spike mutations found in India.

Protein	Mutation	Count	Frequency
Spike	A930V	1	0.0051
Spike	C1250F	1	0.0051
Spike	D1153Y	3	0.0152
Spike	D574Y	3	0.0152
Spike	D614G	101	0.5127
Spike	E583D	4	0.0203
Spike	F797C	1	0.0051
Spike	I1179N	1	0.0051
Spike	K558N	1	0.0051
Spike	K77M	1	0.0051
Spike	L54F	4	0.0203
Spike	L5F	1	0.0051
Spike	N148Y	1	0.0051
Spike	P82L	1	0.0051
Spike	Q271R	2	0.0102
Spike	Q613H	3	0.0152
Spike	Q677H	2	0.0102
Spike	R408I	1	0.0051
Spike	S255F	1	0.0051
Spike	T1027I	1	0.0051
Spike	T22I	2	0.0102
Spike	T572I	6	0.0305
Spike	W258stop	1	0.0051
Spike	Y145del	1	0.0051
Spike	Y28H	1	0.0051

Table 3. List of NSP12 mutations found in India.

Protein	Mutation	Count	Frequency
NSP12	A185V	1	0.0051
NSP12	A406V	1	0.0051
NSP12	A95V	1	0.0051
NSP12	A97V	60	0.3046
NSP12	D67N	1	0.0051
NSP12	G808E	1	0.0051
NSP12	I201L	1	0.0051
NSP12	K59N	2	0.0102
NSP12	K91N	1	0.0051
NSP12	L329I	1	0.0051
NSP12	M756I	2	0.0102
NSP12	P323L	110	0.5584
NSP12	T644M	1	0.0051
NSP12	V880I	5	0.0254

genome. This NSP12 is the main structure, which is enhanced and supplemented by complexes of nsp7–nsp8, nsp10–nsp14 and nsp10–nsp16 provides high fidelity and processivity in viral replication (Kirchdoerfer *et al.* 2019).

Drugs selected for analysis

Brivudine is an antiviral drug that has been also investigated in cancer studies, as it is an effective inhibitor of HSPs. It is hence being investigated as a way to decrease the levels of GRP78 (or HSPA5) (Heinrich *et al.* 2011). Camostat mesilate (CM) had been developed in Japan as a protease inhibitor but has been used for various uses ever since. In various studies, it has been proven effective against SARS-CoV, MERS-CoV as well as in SARS-CoV-2. It is known to be a direct inhibitor of the protease TMPRSS2, an essential protein required by SARS-CoV (Uno 2020). Nafamostat mesylate (NM) is also a protease inhibitor that targets and inhibits the lucrative drug target TMPRSS2. Further, in one comparative study between CM and NM, the latter was found to be roughly 15 folds more potent than CM. The study also determined that both drugs did not interfere with cell viability and mortality (Hoffmann *et al.* 2020a, b).

Originally, perciclovir, a drug for herpesvirus has been found to be very effective as a synthetic nucleoside substitute. In its active form, perciclovir binds to the viral RNA polymerase with very high affinity and impairs viral replication. Anidulafungin is a synthetic antifungal drug. It disrupts fungal cell wall synthesis by inhibiting the enzyme complex 1,3-Δ-D-glucan synthase which is involved in the synthesis of 1,3-Δ-D-glucan, a component of the fungal cell wall. This leads to cell death and inhibition of fungal growth.

Table 4. List of combinations of docking of spike and ACE2 variants in the Indian population.

WT	AA number	Mutant	Overall effect	Torsional strain	ΔΔG
Spike 1 Ace1					
LYS	26	ARG	Destabilising	Favourable	−0.45
ASP	613	GLY	Stabilising	Favourable	2.6
Spike 1 Ace 2					
ASN	546	SER	Destabilising	Unfavourable	−0.75
ASP	613	GLY	Stabilising	Favourable	2.6
Spike 1 Ace 3					
GLU	668	LYS	None	Unfavourable	−1.1
ASP	613	GLY	Stabilising	Favourable	2.6
Spike 2 Ace 1					
LYS	26	ARG	Destabilising	Favourable	−0.45
GLU	582	ASP	Destabilising	Unfavourable	−0.94
Spike 2 Ace 2					
ASN	546	SER	Destabilising	Unfavourable	−0.75
GLU	582	ASP	Destabilising	Unfavourable	−0.94
Spike 2 Ace 3					
GLU	668	LYS	None	Unfavourable	−1.1
GLU	582	ASP	Destabilising	Unfavourable	−0.94
Spike 3 Ace 1					
LYS	26	ARG	Destabilising	Favourable	−0.45
THR	572	ILE	Stabilising	Favourable	1.52
Spike 3 Ace 2					
ASN	546	SER	Destabilising	Unfavourable	−0.75
THR	572	ILE	Stabilising	Favourable	1.52
Spike 3 Ace 3					
GLU	668	LYS	None	Unfavourable	−1.1
THR	572	ILE	Stabilising	Favourable	1.52
Spike 4 Ace 1					
LYS	26	ARG	Destabilising	Favourable	−0.45
LEU	54	PHE	Stabilising	Favourable	1
Spike 4 Ace 2					
ASN	546	SER	Destabilising	Unfavourable	−0.75
LEU	54	PHE	Stabilising	Favourable	1
Spike 4 Ace 3					
GLU	668	LYS	None	Unfavourable	−1.1
LEU	54	PHE	Stabilising	Favourable	1

ΔΔG can be used as a metric for predicting the effect of single point mutation on the protein's stability. The ΔΔG value refers to the change in Gibbs free energy (double changes intended). Intuitively, it is a measure of the change in energy between the folded and unfolded states (ΔG folding) and the change in ΔG folding when a point mutation is present. This has been found to be an excellent predictor of whether a point mutation will be favourable in terms of protein stability and subsequently, give us an understanding of potential future mutations.

Suramin is a synthetic sulphated naphthylamine with multiple uses. It was first found to be a treatment for African trypanosomiasis (African sleeping sickness). It acts by inhibiting the enzymes of energy metabolism of the parasite involved in the disease. Reportedly, it can inhibit many other enzymes and proteins like RNA polymerase, reverse transcriptase. It has also been used in the treatment of cancer as well as an antiviral agent against HIV (Dey *et al.* 2020).

Variants selected for analysis

The variants chosen for analysis are exonic and nonsynonymous variants. For ACE2 (table 1), Var 3 ('p.E668K') showed an overall maximum destabilizing effect and highest allelic frequency followed by Var 1 (p.K26R). Interestingly Var 2 ([p.N546S]) showed an overall stabilizing effect although with lower allelic frequency. In the case of GRP78 of all the variants seen, only Var 2 (p.Val432Ile.) has a mildly stabilizing effect with Var 1 (c.1276A>G, p.Ile426-Val.), Var 3 (p.Pro459Ser.) and Var 4 (p.Lys601Met, p.Lys601Thr.) having a destabilizing effect. In the case of TMPRSS2 as well all the exonic, nonsynonymous variants show significant destabilizing effect with Var 3 (p.Thr481Met, p.Thr518Met) being the highest followed by Var 1 (p.Val120Met, p.Val160Met) with the exception of Var 2 (p.Gly8Asp, p.Gly8Val) that has a mild stabilizing effect. Since the number of variants of TMPRSS2 are low, it becomes a lucrative target due to its conserved nature.

Table 5. Clustering histograms of the interactions between the chosen drugs and corresponding variants.

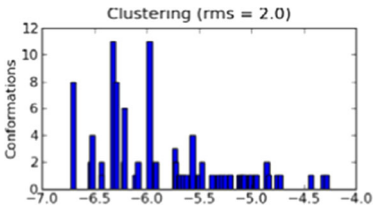
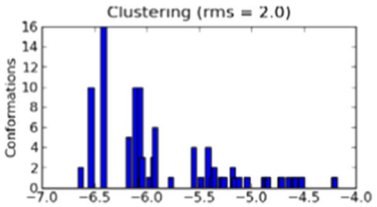
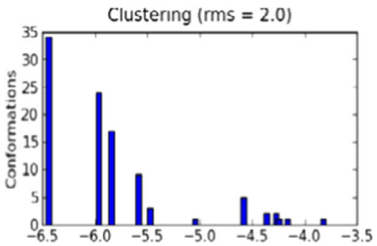
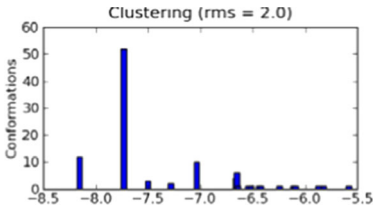
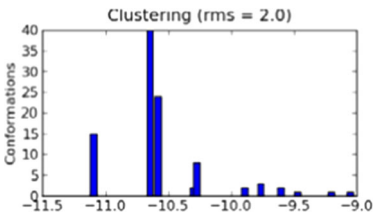
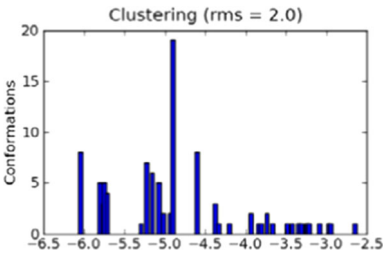
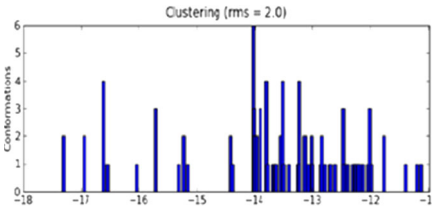
Macromolecule	Drug	Clustering histogram
GRP78 control	Brivudine	
GRP78 Var 1	Brivudine	
TMPRSS2 control	Camostat mesylate	
TMPRSS2 control	Nafamostat mesylate	
TMPRSS2 Var 1	Nafamostat mesylate	
NSP 12 control	Anidulafungin	

Table 5 (*contd*)

Macromolecule	Drug	Clustering histogram
NSP 12 Var 1	Anidulafungin	

These graphs indicate the major profiles of binding energy observed after 100 GA runs with the protein and the ligands mentioned. In tandem with the modelling results, we see that nafamostat mesylate has higher clusters of binding energy when modelled with the Indian variant of TMPRSS2 as compared to the control variant.

Results and discussion

Spike mutations found in India

When the 197 high coverage sequences with patient status were analysed, there were 24 mutations. Of these, the most significant were the D614G, E583D, L54F, Q613 and T572I mutations in the spike protein (Korber *et al.* 2020) (table 2). Previous studies have shown that the D614G mutation may confer a competitive advantage at furin binding sites (Tang *et al.* 2020). Mutations are generally seen to occur either coupled with other mutations or stand alone. The L54F mutation is seen to occur coupled with the D614G mutation. This seems to hint at a stability conferred by the coupling of the L54F and D614G mutations. The L54F mutation lies in the N terminal domain of the S protein, T572S in the SD1 domain, and E583 and Q613 in the SD2 domain (Fischer *et al.* 2020).

Three substantial mutations of NSP12 found in India

When the 197 high coverage sequences with patient status were analysed, there were 14 mutations. Of these, the most significant were the A97V, P323L and V880I mutations in NSP12 (table 3). These mutations have not been studied as extensively as the spike protein and nothing substantial could be found about these mutations. The variants themselves showed rather interesting disparities in their docking studies.

Docking analysis of spike variants and ACE2 variants show that some interactions between spike protein variants and ACE2 variants are more stable than others

The RBD domain of the spike protein is known to bind with the ACE2. Structural variation of the spike protein may cause changes in the binding of the spike protein to ACE2. Table 4 summaries the effect of binding of selected Spike and ACE2 variants. The most significant stable interaction is that of Spike variant 1 (D614G) with ACE2 variant 1 (rs4646116) this is closely followed by Spike variant 1 (D614G) with ACE2 variant 2 (rs756905974). This once again shows the exceptional stability of the D614G mutation.

Docking analysis of chosen drugs and variants of the proteins selected for the study

The selected variants of GRP78 were screened against Brivudine using PyRx. The results were used to identify the variant that showed the best binding to Brivudine. This was followed by 100 runs on Autodock to achieve the clustering histogram for statistically significant binding energy and interacting residues (table 5).

The control showed a binding affinity of around –6.5 to –6 kcal/mol. There was a difference in binding affinity with structural variation of GRP78. Of the three variants, Var 1 showed the best binding affinity with Brividuine with a binding affinity of –6.5 kcal/mol. Var 2 and Var 3 showed decreased affinity with Brivudine. The bond lengths varied from 2.1Å to 3.4Å for both control as well as Var 1. The detailed interactions are shown in (figure 1a (control), 1b (Var 1)). Var 1 showed higher binding affinity with no significant change in interactions. Both Camostat and Nafamostat mesylate are potent seriene inhibitors in the case of TMPRSS2. Nafamostat meslyate showed much better binding when compared to Camostat to the control as is seen in the clustering histograms (table 5). When screened against all the variants, Nafamostat outperformed Camostat with the best binding being shown by Var 1. The detailed interactions are shown in (figure 1c (control), 1d (Var 1)).

Anidulafungin was the most effective drug in the context of NSP12 binding energy with especially a high binding energy at –13.43 kcal/mol. This binding energy was seen primarily in the first variant of the three usually found in Indian populations with a frequency of around 0.3, which is the highest observed. Since anidulafungin is usually administered as an antifungal, it is interesting to note that it is effective in binding to NSP12. The control however has relatively poorer binding of around –4.02 kcal/mol. Further,

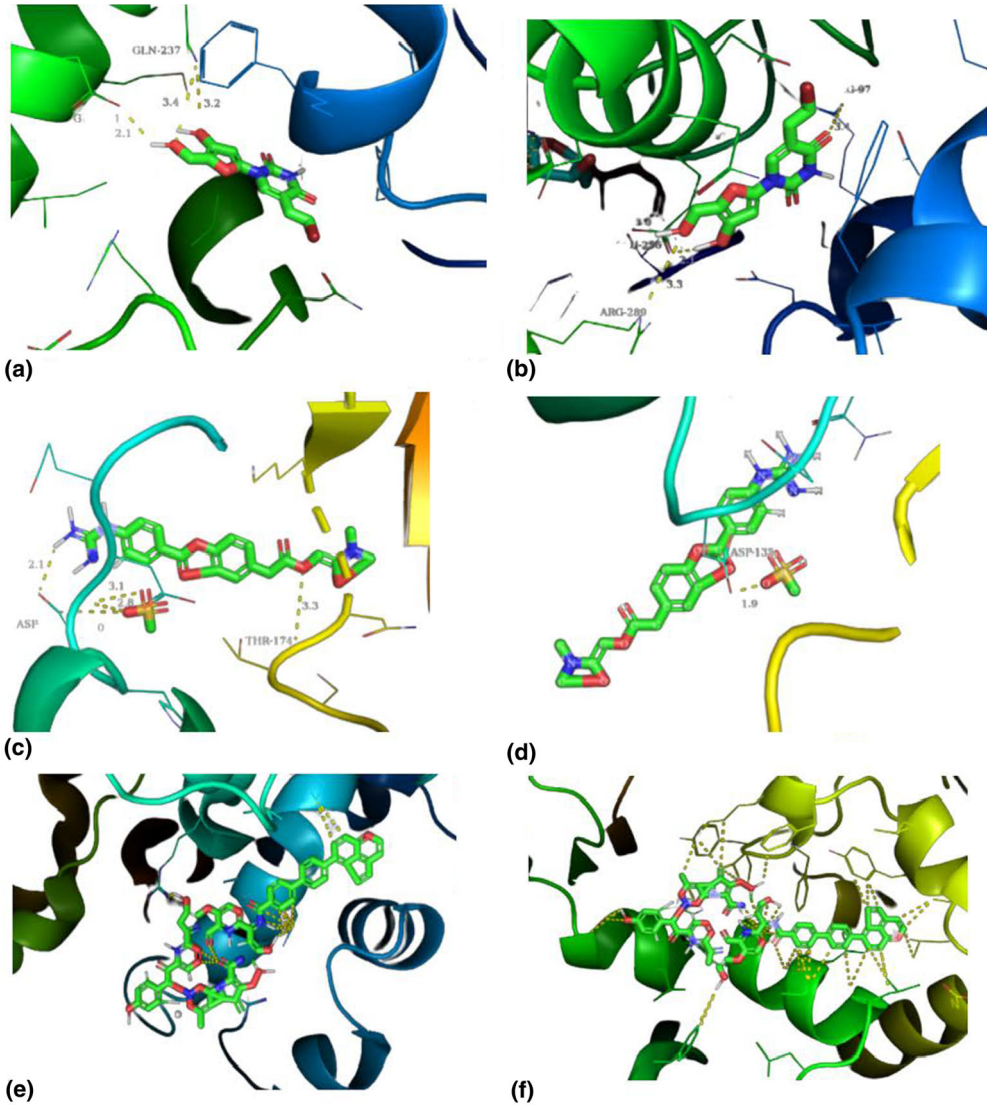


Figure 1. Interactions between the chosen drugs and variants. (a) GRP control with brivudine, (b) GRP Var 1 with Brivudine. (c) TMPRSS2 control with NM, (d) TMPRSS2 Var 1 with NM, (e) NSP 12 control with Anidulafungin, (f) NSP12 Var 1 with Anidulafungin. The interactions are shown with yellow dotted lines. More interactions correspond to greater binding.

we see lesser bonds in the docked model of the control than that of the variant 1 (figure 1e (control), 1f (Var 1)).

Conclusion

With the SARS-CoV-2 outbreak on the rise, particularly in the Indian subcontinent, it becomes more imperative that we understand the various factors that could contribute towards finding a potential treatment strategy for the pandemic. In this brief study, we focussed on the variants that are present in the Indian population and their effect in *in silico* binding to experimental SARS-CoV-2 therapeutic options. Of all the possible combinations for the variants of spike protein and ACE2 binding, 25% show overall unfavourable $\Delta\Delta G$. We see that NSP12 is the most mutation prone among all the NSPs of the SARS-CoV-2 genome and the most common mutations are P323L and A97V. With respect to the Spike protein, the most frequent mutation seen is the D614G followed by T572I. We also see that particular Indian variants (Var 1 of TMPRSS2 and Nafamostat Mesylate and Var 1 of NSP12 and Anidulafungin) show higher binding affinity to small molecule inhibitors as compared to their control counterparts. There is more research required into repurposing of small molecule inhibitors and their efficacy in the context of the Indian population. This could hopefully pave way for more robust and personalized treatment strategies.

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