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Original article

Screening of inhibitors against SARS-CoV-2 spike protein and their capability to block the viral entry mechanism: A viroinformatics study

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ABSTRACT

SARS-CoV-2, previously named 2019 novel coronavirus (2019-nCoV), has been associated with the global pandemic of acute respiratory distress syndrome. First reported in December 2019 in the Wuhan province of China, this new RNA virus has several folds higher transmission among humans than its other family member (SARS-CoV and MERS-CoV). The SARS-CoV-2 spike receptor-binding domain (RBD) is the region mediating the binding of the virus to host cells via Angiotensin-converting enzyme 2 (ACE2), a critical step of viral. Here in this study, we have utilized *in silico* approach for the virtual screening of antiviral library extracted from the Asinex database against the Receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 spike glycoprotein. Further, the molecules were ranked based on their binding affinity against RBD, and the top 15 molecules were selected. The affinity of these selected molecules to interrupt the ACE2-Spike interaction was also studied. It was found that the chosen molecules were demonstrating excellent binding affinity against spike protein, and these molecules were also very effectively interrupting the ACE2-RBD interaction.

Furthermore, molecular dynamics (MD) simulation studies were utilized to investigate the top 3 selected molecules' stability in the ACE2-RBD complexes. To the best of our knowledge, this is the first study where molecules' inhibitory potential against the Receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 spike glycoprotein and their inhibitory potential against the ACE2-Spike has been studied. We believe that these compounds can be further tested as a potential therapeutic option against COVID-19.

1. Introduction

1.1. Background

To date we have identified three different human coronaviruses (CoVs) (Zhu et al., 2020; Decaro and Lorusso, 2020; Coleman and Frieman, 2014). First reported in 2002, the Severe acute respiratory syndrome (SARS-CoV) was the first identified member of this highly pathogenic coronavirus family (Zhu et al., 2020; Arshad Ali et al., 2020). Other members of this family include the Middle East respiratory syndrome coronavirus (MERS-CoV), which was identified in 2012 (Arshad Ali et al., 2020; Oh et al., 2018). Both SARS-CoV and MERS-CoV are capable of human-to-human transmission and were responsible for the outbreak in 2003 and 2012, respectively (Zhu et al., 2020; Oh et al., 2018; Docea et al., 2020).

Abbreviations: ACE2, Angiotensin-converting enzyme 2; COVID-19, Coronavirus Disease 2019; RBD, Receptor-binding domain; CoVs, Coronaviruses; SARS, Severe acute respiratory syndrome; MERS, Middle East respiratory syndrome coronavirus; MD, Molecular dynamics; RMSD, Root mean square deviation; RMSF, Root mean square fluctuation.

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SARS-CoV-2, previously named 2019 novel coronavirus (2019-nCoV), has been associated with the global pandemic of acute respiratory distress syndrome (Lai et al., 2020). First reported in December 2019 in the Wuhan province of China this new RNA virus has several folds higher transmission among humans than its other family members (SARS-CoV and MERS-CoV) (Lu et al., 2020; Li et al., 2020; Wang et al., 2020). Declared as a global pandemic by WHO, the disease caused by this virus (SARS-CoV-2) was termed as Coronavirus Disease 2019 (COVID-19). The person infected with SARS-CoV-2 is shown to be showing a wide range of symptoms, leading to respiratory, gastrointestinal diseases, and even death (Wang et al., 2020; Chang et al., 2020). As of 27th October 2020, a total of 43.4 million confirmed cases of COVID-19 were reported worldwide, which includes 1.16 million deaths. This major suffering of the worldwide population due to SARS-CoV-2 mediated disease has raised the alarm for the instant call for antiviral drugs for treatment/prevention against COVID-19.

Belonging to the Coronaviridae family of order Nidovirales, CoV has been classified into different genera which include α-, β-, γ-, and δ-coronaviruses (Woo et al., 2009; Woo et al., 2012). Among these different genera, α- β- and δ- CoVs infects the mammals, while the γ-coronaviruses mainly infect avian species (Mihindukulasuriya et al., 2008; Woo et al., 2009). The δ-coronaviruses are also reported to be infecting aves as well (Naqvi et al., 2020). Coronaviruses are comprised of the largest RNA viral genome (26-32 kb) (Li et al., 2020; Khan et al., 2020). The genomes of Coronaviruses share a significant similarity; for instance, the SARS-CoV-2 genome share ~82% similarity with the SARS-CoV and MERS-CoV. This high level of sequence similarity leads to these coronaviruses' common pathogenesis mechanism, and thus therapeutic targeting (Naqvi et al., 2020; Khan et al., 2020). The viral genome of SAR-CoV-2 encodes for several structural as well as non-structural proteins. There are four structural proteins in SARS-CoV-2, that include envelope (E), membrane (M), spike (S), and nucleocapsid (N) proteins, which are playing a prominent role in the viral assembly (Tai et al., 2020; Du et al., 2016) (Fig. 1). These structural proteins are found to be sharing high sequence similarity with their SARS-CoV, and MERS-CoV counterparts. Among all the structural proteins, the main role is played by the S protein, which is responsible for the cell membrane fusion, viral attachment, and viral entry. These features of S protein make it an important therapeutic target (Du et al., 2009; Du et al., 2017; Li et al., 2005). The S1 subunit of S protein (RBD region) binds to the host receptor, Angiotensin-converting enzyme 2 (ACE2), while the S2 subunit mediates the viral cell membrane fusion (Li et al., 2005; Lu et al., 2013) (Fig. 1). Studies have found that, like SARS-CoV, SARS-CoV-2 also utilizes the same host viral entry mechanism where its homotrimeric spike (S) glycoprotein binds to the host ACE2 (its functional host receptor) (Yi et al., 2020; Zhou et al., 2020; Li et al., 2003). The Receptor binding domain (RBD) of the S protein is the region involved in the most critical step of viral entry within the target host cell i.e., it is the region that mediates the binding of the virus to host cells (Li et al., 2003; Othman et al., 2020). Here in this study, we have utilized *in silico* approach for the virtual screening of Asinex antiviral compound library against the RBD of the S1 subunit of the SARS-CoV-2 spike glycoprotein.

Further, the molecules were ranked based on their binding affinity against RBD, and the top 15 molecules were selected. The affinity of these selected molecules to disrupt the ACE2-RBD interaction was also studied. Our study shows that the selected molecules demonstrate very good binding affinity against spike protein and effectively disrupt the ACE2-RBD interaction as well.

Furthermore, molecular dynamics (MD) simulation studies were utilized to study the top 3 selected compounds' stability in the ACE2-RBD complexes. This is the first study, where the inhibitory potential of molecules against the RBD of the S1 subunit of the SARS-CoV-2 spike glycoprotein and their inhibitory potential against the ACE2-RBD has been studied. We believe that these compounds may be a potential therapeutic option for the treatment of COVID-19.

2. Material and methods

The 3D structure of Spike protein (RBD) in complex with the human ACE-2 was retrieved from the RCSB protein data bank (pdb id: 6m0j) (Burley et al., 2018; Lan et al., 2020). Further both the RBD and ACE-2 were separated and the HEATM and other solvent molecules were removed. The separated structure of Spike protein (RBD) was considered for the virtual screening of antiviral compounds. The antiviral library comprising of 6827 antiviral compounds was downloaded from the Asinex database. Further, the library was screened against the SARS-CoV-2 spike protein. The virtual screening was performed using CCDC Gold (Jones et al., 1997), and the molecules were ranked based on their PLP Fitness score. The top selected molecules in complex with the SARS-CoV-2 spike protein were considered for further study.

3. Protein-protein interaction

The spike receptor-binding domain (Chain E) and ACE-2 (Chain A) were separated from the 3D complexed structure of ACE-2-RBD (pdb id: 6m0j). The separated structures of Spike protein (RBD) and human ACE-2 were redocked using Patchdock (Schneidman-Duhovny et al., 2005). The generated results were further refined for 1000 steps using firedock (Mashiach et al., 2008). The top-scoring complex was selected based on the global energy score and was compared to its crystal counterpart. The global energy of this complex was taken as a reference for further study.

Further, the protein–protein interaction study was performed using the same protocol, but this time the top-scoring molecules in complex with the Spike protein (RBD) were docked against the human ACE-2. The global score of all the complexes was compared with the reference score.

4. Molecular dynamics simulation

The structure of Spike protein (RBD) in complex with the human ACE-2 in the presence of the top selected compound was further subjected to MD simulation studies using DESMOND (Bowers et al., 2006). Here in this study, the OPLS3 force field was used to perform the MD simulation studies (Robertson et al., 2019). The first step was building the simulation systems using the OPLS3 force field for proteins, followed by their SPC solvation in the Orthorhombic box. Further, the counter ions were added for neutralizing the systems. A series of energy minimization steps were performed to relax the system. MD was performed for 10 ns using NPT ensemble. The backbone root mean square deviation (RMSD) plot for proteins, and the ligand was calculated using the initial starting structure as a reference frame. Likewise, the root mean square fluctuation (RMSF) was also calculated.

5. Result

The present study was focused on finding the potent inhibitor candidates against the RBD of the S1 subunit of the SARS-CoV-2 spike glycoprotein. We further assessed the potential of these selected inhibitor candidates to disrupt the binding of RBD to the ACE2 receptor (Table 2). The flow chart of the complete work is shown in Fig. 2. The library of 6827 molecules was screened against the S-protein (RBD) of SARS-CoV-2. The molecules were

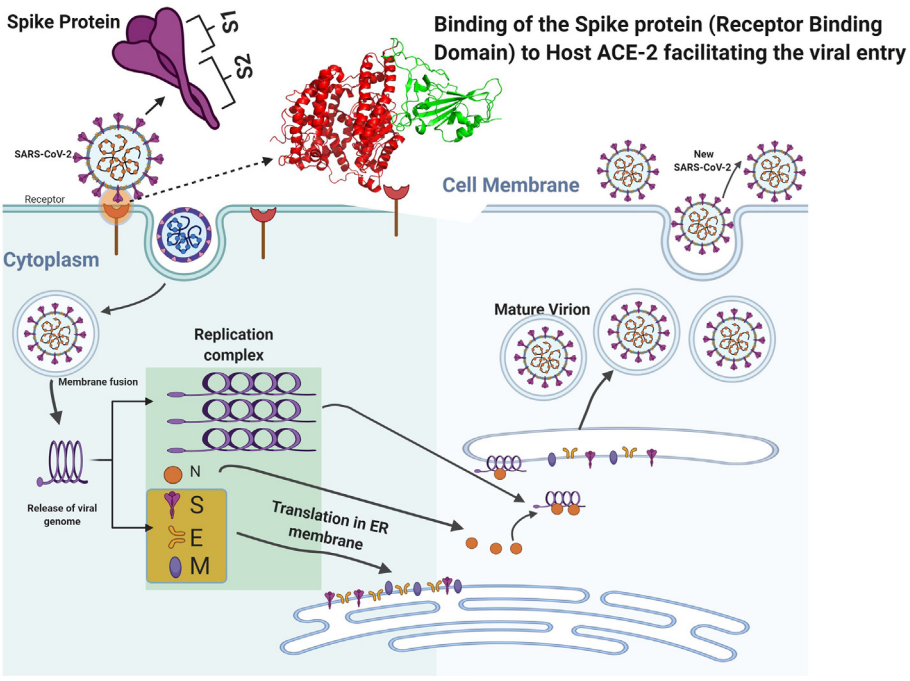

Fig. 1. The life cycle of SARS-CoV-2 in host cells.

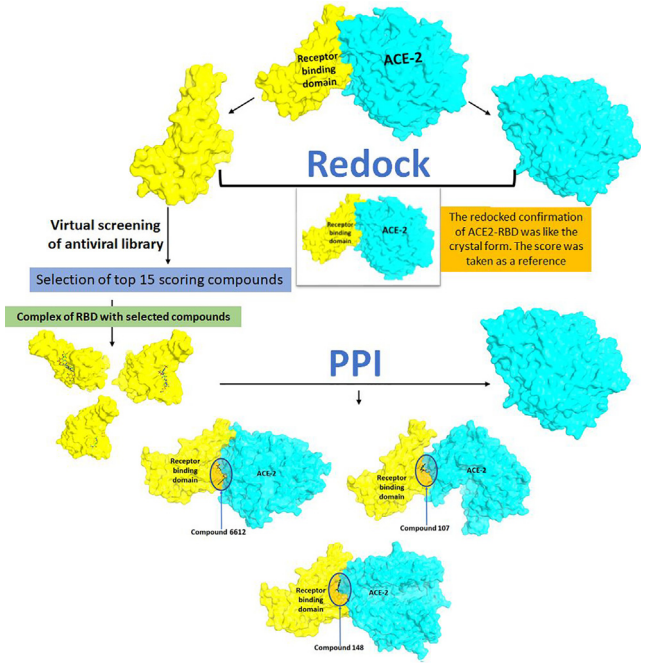

Fig. 2. The virtual screening protocol used in this study. The structure of ACE2 is shown in cyan color, while the structure of Receptor binding domain (RBD) SARS-CoV-2 spike glycoprotein is in yellow color.

Table 1
The binding score of the top 15 compounds against SARS-CoV-2spike protein (RBD).

Complex	PLP Fitness	Residues Involved	
		Hydrogen bonding	sOther interacting residues
Compound5991′	78.72	T500, N501	R403, Y495, F497, N501, G502, Y505
'Compound6604′	77.51	Y453, G496, Q498, G502	R403, Y495, N501, G502, Y505
'Compound6606′	76.34	Y453, G496, Q498, G502	R403, Y495, N501, G502, Y505
'Compound6612′	75.45	Y453, G496, Q498	R403, Y495, G496, N501, G502, V503, Y505,
'Compound96′	74.59	Y453, G502	R403, Y449, Q493, Y495, F497, N501, Y505
'Compound110′	74.17	Y453, Q493	R403, Q493, Y495, F497, N501, Y505
'Compound107′	73.13	R403, Y453	R403, K417, Y453, Y495, F497, N501, Y505
'Compound724′	72.95	Y453, N501	R403, Y449, Q493, S494, Y495, F497, N501, Y505
'Compound708′	72.77	Y453, G496, Y505	R403, Q493, S494, Y495, N501, Y505
'Compound106′	72.7	R403, Y453, G496, N501	R403, K417, Y495, F497, N501, Y505
'Compound703′	72.22	Y453, Y505	R403, Y449, Q493, S494, Y495, G496, Y505
'Compound148′	72.08	R403, Y453	R403, K417, L455, Y495, F497, N501, Y505
'Compound59′	71.84	R403, Y453, G496, N501	R403, Y495, F497, N501, Y505
'Compound692′	71.71	Y453, G496	R403, Q493, Y495, Y505
'Compound111′	71.58	Y453	R403, Y449, Q493, S494, Y495, F497, N501, Y505

evaluated based on their PLP fitness score. The top 15 effective molecules with a high binding affinity (PLP Fitness score) were selected (Table 1). The selected most effective molecules were further evaluated for their affinity to disrupt the interaction of SARS-CoV-2 spike protein (RBD) with the human host receptor (ACE-2). These 15 compounds with high binding affinity against RBD are enlisted in Table 1. Further, the complex of top-scoring 15 molecules with spike protein was prepared.

These complexes were further subjected to PPI study against ACE2. It was found that the presence of the above-mentioned compounds affects the binding of the RBD to its natural interacting partner, the ACE2 receptor. The selected top fifteen hits were ranked based on their affinity to interrupt the ACE2-RBD interaction (global energy). Compound 148, 6612, and 107 were found

to be the most potent inhibitors disrupting the ACE2-RBD interaction. These compounds expressed a high affinity against the RBD domain of the spike protein and were also capable of interrupting the binding of ACE2 with RBD. Compound 148, 6612 and, 107 have caused the reduction in the global energy of the RBD-ACE2 complex from −63.99 to −38.16, −38.34 and −38.70 respectively (Table 2 and 3, Fig. 3). The drop in the global energy score indicates that these compounds' presence restricts the binding of RBD-ACE-2. This shows that the presence of compounds 148, 6612, and 107 hinders RBD's binding to the ACE2 receptor, thereby may be a suitable therapeutic candidate blocking the viral entry mechanism (Table 2, Fig. 2).

We have also investigated the role of prominent residues involved in the binding of RBD to its receptor (ACE2) and the inhibitors. The active site residues Tyrosine (Y) 449, Tyrosine (Y) 453, Leucine (L) 455, Phenylalanine (F) 456, Phenylalanine (F) 486, Asparagine (N) 487, Tyrosine (Y) 489, Glutamine (Q) 493, Glycine (G) 496, Glutamine (Q) 498, Threonine (T) 500, Asparagine (N) 501, Glycine (G) 502 and Tyrosine (Y) 505 of the SARS-CoV-2 spike protein (RBD) were found to be involved in the binding to ACE2 receptor (Fig. 4a, Table 3). The binding of the compounds within RBD's active site was dominated by hydrogen bond interactions, involving R403, Y453, G496, Q498, G502, and Y505 residues (Table 1). Further Molecular dynamics simulation was performed to investigate the time-dependent activity of the complexes. Desmond was used to perform the MD simulation for the top 3 selected complexes (in the presence of compounds 107, 148 and 6612). Among the three identifies hits, compound 6612 exhibited greater stability while bound in the RBD-ACE-2 complex as compared to compounds 148 and 107 (Fig. 4b, c). The ligand RMSD plot revealed that Compound 148 and 6612 were very stable during simulation, while a low fluctuation was found in compound 107 (Fig. 4b). The protein RMSD and RMSF analysis were performed to investigate the proteins' dynamic stability in the presence of selected compounds. The backbone RMSD of the protein revealed greater stability for the complexes involving compound 6612, as compared to compound 148 and 107 (Fig. 4c). A large degree of fluctuation was noticed in the presence of compound 107, and the fluctuation was noticed after 6 ns (Fig. 4c). While in the presence of compound 148, fluctuation in the protein backbone RMSD was noticed after 8 ns. Further analysis of the RMSF plot revealed a large conformational change in the ACE2 protein of all three complexes (Fig. 4d, e). These conformational changes were observed in the residues lying far away from the interfacial binding region. The fluctuation was very high in the compound 107 bound complex, compared to the complexes involving other two compounds. A high degree of fluctuation was observed for the residues 365–385, 415–435, and the loop region residues (477–485) of RBD (Fig. 4d).

Table 2
The protein–protein binding score of SARS-CoV-2 spike protein (RBD) with the human host receptor (ACE-2) in absence and presence of selected compounds.

Complex	Global energy
SARS-CoV-2 SPIKE (RBD)-ACE2	−63.99
SARS-CoV-2 SPIKE (RBD)Compound5991′-ACE2	−40.58
SARS-CoV-2 SPIKE (RBD)'Compound6604′-ACE2	−41.04
SARS-CoV-2 SPIKE (RBD)'Compound6606′-ACE2	−48.44
SARS-CoV-2 SPIKE (RBD)'Compound6612′-ACE2	−38.34
SARS-CoV-2 SPIKE (RBD)'Compound96′-ACE2	−43.83
SARS-CoV-2 SPIKE (RBD)'Compound110′-ACE2	−44.29
SARS-CoV-2 SPIKE (RBD)'Compound107′-ACE2	−38.70
SARS-CoV-2 SPIKE (RBD)'Compound724′-ACE2	−41.91
SARS-CoV-2 SPIKE (RBD)'Compound708′-ACE2	−46.50
SARS-CoV-2 SPIKE (RBD)'Compound106′-ACE2	−48.23
SARS-CoV-2 SPIKE (RBD)'Compound703′-ACE2	−50.28
SARS-CoV-2 SPIKE (RBD)'Compound148′-ACE2	−38.16
SARS-CoV-2 SPIKE (RBD)'Compound59′-ACE2	−40.11
SARS-CoV-2 SPIKE (RBD)'Compound692′-ACE2	−47.63
SARS-CoV-2 SPIKE (RBD)'Compound111′-ACE2	−48.28

6. Discussion

The COVID-19 outbreak, caused by the SARS-CoV-2, has strongly affected all walks of life and has become a severe public health concern (Mattioli et al., 2020; Dong et al., 2020; Xu and Li, 2020). At present, there is no available drug or antiviral for the treatment against SARS-CoV-2, and the development of new drug molecules will take time (Choudhary et al., 2020). Several antivi-

Table 3
The active site residues involved in the protein–protein binding score of SARS-CoV-2 spike protein (RBD) with the human host receptor (ACE-2) in absence and presence of Compound6612, Compound107 and Compound148.

Complex	Global energy	Residues Involved			
		Hydrogen bonding		Other interacting residues	
		ACE2	Spike protein	ACE2	Spike protein
SARS-CoV-2 SPIKE (RBD)-ACE2	−63.99	Q24, D30, E35, Y41, Q42, Y83, K353, D355, R393	K417, N487, Y489, Q493, T500, G502, Y505	S19, Q24, T27, F28, D30, K31, H34, E35, E37, D38, Y41, Q42, L45, M82, Y83, N330, G352, K353, G354, D355, R357, R393	K417, V445, G446, G447, Y449, Y453, L455, F456, A475, P486, N487, Y489, Q493, G496, F497, Q498, T500, N501, G502, Y505
SARS-CoV-2 SPIKE				(RBD)'Compound6612′-ACE2	−38.34
N90, L91	E484	E22, Q89, N90, L91, V212, D213, T324, K353, A387	V445, G446, Y449, V483, E484, G485, F486, T500		
SARS-CoV-2 SPIKE				(RBD)'Compound107′-ACE2	−38.70
N49, T52, A330, Q340	Y449, S494, T500, N501, Q506	L45, N49, T52, N53, E329, N330, M332, P336, Q340	V445, G446, Y449, S494, Y495, G496, Q498, T500, N501, G502		
SARS-CoV-2 SPIKE				(RBD)'Compound148′-ACE2	−38.16
T27, Q81, A193	Y449, E484, T500	Q24, T27, Q81, M82, Y83, P84, A193, H195	V445, Y449, Y453, E484, G485, F490, Q493, Q498, T500		

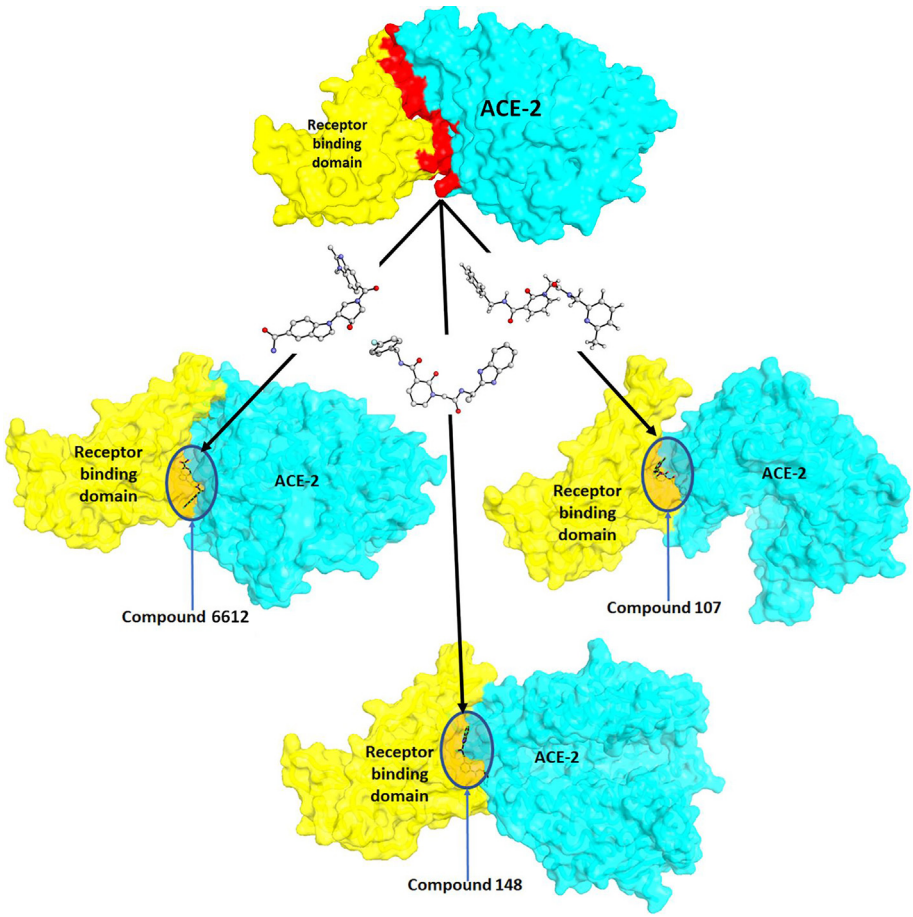

Fig. 3. The top 3 compounds selected in the study and their RBD-ACE2 interface bound structures. The structure of ACE2 is shown in cyan color, while the structure of Receptor binding domain (RBD) SARS-CoV-2 spike glycoprotein is in yellow color.

rals have been reported to inhibit the pathogenesis of chikungunya, dengue virus, or other RNA viruses by either blocking the viral entry or inhibiting their replication at different stages (Mudgal et al., 2020; Singh et al., 2018). The S protein of SARS-CoV-2 is composed of 1273 amino acids. The initial 12 residues at N-terminus form the signal peptides while the rest of the mature part is proteolytically processed into two subunits (S1 & S2) (Satarker and Nampoothiri, 2020; Chen et al., 2020). Subunit S1 comprises amino acids from 14 to 685, and residues between 686 and 1273 encodes for subunit S2 (Huang et al., 2020; Malladi et al., 2020). The S1 subunit of the spike glycoprotein is responsible for the host cell receptor binding (ACE-2), while the S2 subunit is responsible for the fusion of the virus to the host cell membrane (Huang et al., 2020; Yang et al., 2020) (Fig. 1). It has been found that the RBD (amino acids 319–541) of the S1 subunit of SARS-CoV-2 binds to the outer surface of ACE2 (claw-like structure) of the host (Li et al., 2005; Huang et al., 2020; Yang et al., 2020; Yan et al., 2020; Song et al., 2018; Kirchdoerfer et al., 2018). ACE2 is the primary host target of SARS-CoV-2 spike protein (Yan et al., 2020). It is located on the cell's outer surface and has been widely reported to be the primary host cell target, thereby playing a prominent role in the viral entry (Song et al., 2018; Ali and Vijayan, 2020; Wu et al., 2012; Gui et al., 2017) (Fig. 1). Since this interaction is an essential step facilitating the entry of SARS-CoV-2 within the host cell, the compounds targeting S-RBD–ACE2 interface and thereby blocking this protein–protein interaction could be a potential therapeutic candidate against COVID-19, inhibiting the viral entry into host cell (Adedeji et al., 2013). The crystal structure of SARS-CoV-2 spike RBD bound with ACE2 was considered for the *in silico* studies (Lan et al., 2020). We have utilized the *in silico* virtual screening approach to screen the antiviral library (Asinex) against the RBD of the SARS-CoV-2 spike glycoprotein. We determined the binding affinity and evaluated the binding conformation of molecules within the RBD's active site. The study seeks to identify small molecules carrying inhibitory potential against the spike protein (RBD), and they must be capable of disrupting the binding of RBD-ACE-2 as well. The PPI study revealed that the active site residues of SARS-CoV-2 spike protein (RBD) viz. Tyrosine (Y) 449, Tyrosine (Y) 453, Leucine (L) 455, Phenylalanine (F) 456, Phenylalanine (F) 486, Asparagine (N) 487, Tyrosine (Y) 489, Glutamine (Q) 493, Glycine (G) 496, Glutamine (Q) 498, Threonine (T) 500, Asparagine (N) 501, Glycine (G) 502 and Tyrosine (Y) 505 were found to be involved in the binding to ACE2 receptor (Fig. 4a, and Table 3). The role of these active site residues of RBD has been previously well defined (Lan et al., 2020; Ortega et al., 2020; Prajapat et al., 2020). These residues were also involved in accommodating the small molecules within the active site of spike protein (RBD) as well. Our findings reveal high flexibility in the residues remote to the RBD interface of ACE2, and the finding is in accordance with other earlier reports (Liu et al., 2020). We observed that the region at the binding interface in the ACE2-RBD complex was stable throughout the simulation compared to the other areas. The ACE2 interfacing residues exhibited minimum conformational changes. We found that the RBD-ACE2 complex was most stable in the presence of compound 6612, followed by compound 147, while it was unstable in the presence

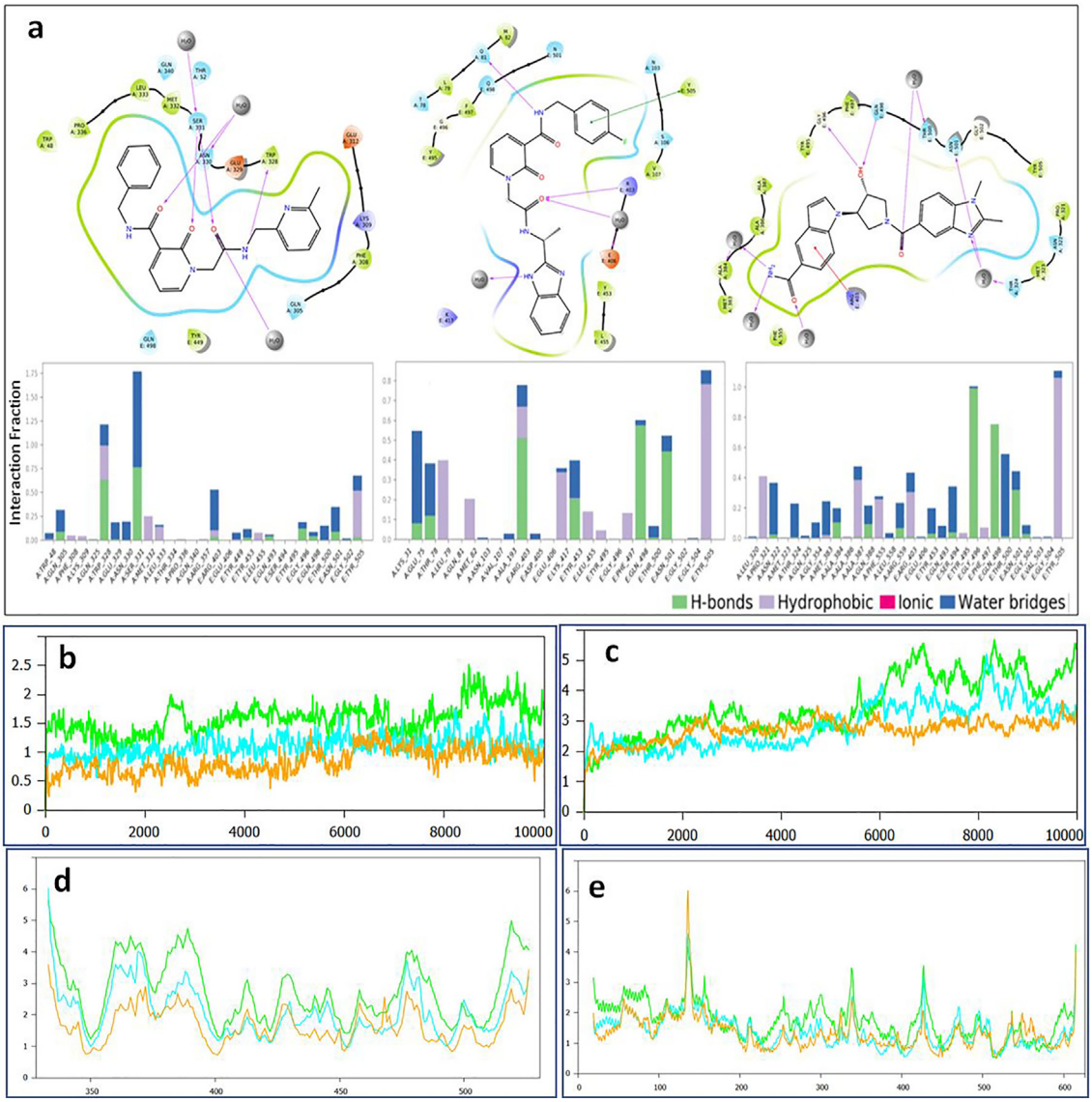

Fig. 4. The Molecular dynamics results of the SARS-CoV-2 spike protein (RBD) with the human host receptor (ACE-2) in presence of Compound 6612, Compound 107 and Compound 148. The Fig. 4**(a)** The binding of residues within the interface region of RBD-ACE-2. Fig. 4**(b)** Ligand RMSD plot of all the 3 compounds in the complex. Fig. 4**(c)** RMSD of protein in presence of the compounds. Fig. 4**(d)** The RMSF plot of ACE2 during the simulation time period. Fig. 4**(e)** The RMSF plot of Spike protein during the simulation time period.

of compound 107. A high degree of fluctuation was observed for the residues 365–385, 415–435, and the loop region residues (477–485) of RBD (Fig. 4d). We observed the prominent involvement of the residues 477–485 of the loop region at the interface binding site, and our findings are in the right perspective as has been reported earlier for the involvement of the residues of the loop region binding to the ACE2 receptor (Liu et al., 2020; Brielle et al., 2020).

7. Conclusion

The Asinex antiviral library's virtual screening against the receptor binding domain of SARS-CoV-2 spike protein provides us a list of several active compounds, with high binding affinity against the receptor binding domain. The further assessment of these compounds' potential to inhibit the ACE2-RBD interaction provides an additional valuable feature to them. Compound 107, 148, and 6612 were found to be the most active compounds, capable of binding to RBD and interrupting the ACE2-RBD interaction as well. MD simulation studies showed that in the presence of compound 6612, the ACE2-RBD complex was most stable, followed by 107 and 148. The importance of essential residues has also been explored in this study. It was noticed that the conformational changes were majorly in the residues far away (remote) from the interface region of ACE2. The information provided will be of great help in using these compounds as possible drug candidates against COVID-19, and may also help for future drug discovery.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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