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Coronavirus (SARS-CoV-2) Deactivation via Spike Glycoprotein Shielding by Old Drugs: Molecular Docking Approach

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A B S T R A C T

Today the disease of COVID-19 comprises the most serious problem against human health worldwide with a high rate of virulence and mortality. The disease is caused by the SARS-CoV-2 virus from the beta coronavirus family. The virus makes use of its surface glycoprotein named S protein or spike to enter the human cells. The virus is attached to its receptor of angiotensin-converting enzyme 2 on the cell surface via its receptor-binding domain and fused after cleavage at S2' site that is carried out by surface protease. Vaccines or drugs interfering with S protein binding or blocking the cleavage sites of S protein could be considered as a treat to get rid of the infection. In the current work and through molecular docking and molecular dynamics experiments, 14 drugs were selected based on their molecular weights among 100 drugs to study their shielding potency toward S protein binding sites. The obtained results indicate that fidaxomicin, niclosamide, and flubendazole bind specifically to the S2' cleavage site; while ivermectin, rapamycin, heparin, azithromycin, clarithromycin, and erythromycin bind both receptor-binding domain and S2' and can prevent virus attachment to its receptor and may be useful as a prophylactic candidate for COVID-19 management after clinical approval.

Introduction

Atypical pneumonia outbreak in 2019-2020, identified first in Wuhan, China, is caused by a novel enveloped, positive-stranded RNA virus (Huang, et al., 2020; Zhou, et al., 2020). The virus is a new member of the betacoronavirus family, including Severe Acute Respiratory Syndrome (SARS-CoV) and Middle East Respiratory Syndrome (MERS-COV) coronavirus that is newly named SARS-CoV-2 (Lu, et al., 2019; Wu, et al., 2020). The disease is now called COVID-19 which is accompanied by fever, cough, and in advanced cases with severe respiratory distress (Chan, et al., 2020; Huang, et al., 2019). The virus makes use of highly glycosylated S protein (spike) to enter host cells.

The spike binds to angiotensin-converting enzyme 2 (ACE2) receptors in target cells with 10-20 fold higher affinity contrast to SARS-COV or MERS-COV coronaviruses. This underlies the high rate of the virus spreading between human cells as well as individuals that leads to a pandemic threat to worldwide safety (Lu, et al., 2014). Spike is a trimeric protein that belongs to the class I fusion proteins. Each monomer contains 1288 amino acids. There are two major subunits (scheme 1), which are called S1 and S2 subunits, formed by

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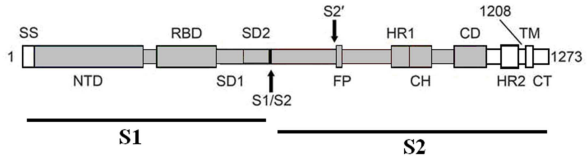
cleavage at Arg667-X668 residues (S1/S2 site). Membrane-bound furin protease is the enzyme that catalyzes this cleavage to produce a mature pre-fusion form of the virus. In humans, this protease is expressed in multiple tissues, with high concentrations in alveolar cells leading the lung to be the primary site for infection. Unlikely SARS-COV and MERS-CoV viruses do not carry this cleavage site and become less dangerous than SARS-CoV-2 (Wrapp, et al., 2020; Coutard, et al., 2020).



Scheme 1: Subunits and domains of S protein in SARS-CoV-2.

As it is indicated in scheme 1, the S1 subunit contains different domains beginning with a small signal sequence (SS), N-terminal domain (NTD), residues 14-305, receptor-binding domain (RBD), residues 319-541, and ending with subdomains of 1 and 2 (SD1/SD2), residues 542-685. Considering the overall mushroom-like shape of the spike, the S1 subunit is placed in the mushroom head with the RBD domain faced outward to bind host cell ACE2 receptor (Xia, et al., 2020; Xia, et al., 2019). The three RBD domains of the trimer adjust one of two conformations called up and down conformations. Up conformation corresponds to the receptor accessible state of the RBD domain, while down conformation is inaccessible for binding (Pallesen, et al., 2017; Walls, et al., 2019).

The next subunit, S2, contains a signal sequence (SS), a next cleavage site called S2′ that becomes accessible for furin protease upon binding to the ACE2 receptor in a pre-fusion state. Fusion peptide (FP), residues 788-806, in post-fusion complex accelerates virus fusion to host cells. Heptad repeats 1 (HR1), residues 912-984, central helix (CH), connector domain (CD), heptad repeat 2 (HR2), residues 1163-1213, transmembrane domain (TM), residues 124-1237, and cytoplasmic tail (CT), residues 1238-1273, are the rest domains of subunit S2 (Xia, et al., 2019).

Upon spike binding to ACE2 and S1/S2 cleavage, subunit S1 undergoes structural rearrangements that eventually lead to its release from the pre-fusion complex and prime the virus fusion (Coutard, et al., 2020; Xia, et al., 2020). Successful infection is accomplished by furin cleavage at S2' site that releases the fusion peptide, i.e. hydrophobic tail that is essential for post-fusion complex formation and virus fusion. During the conversion of pre-fusion to post-fusion state, HR1 and HR2 interact to form a fusion core of a six-helical bundle. This core brings viral and cellular membranes in close proximity for fusion (Song, et al., 2018).

In the current work medications with high molecular weights and therefore high binding energies among those approved for clinical use were chosen such as macrolides antibiotics, anthelmintic/antiparasitic, and anti-HIV. However, during the drug selection process, previous reports regarding the probable usefulness of these drugs in COVID-19 were considered. During this work, the drugs were undergone molecular docking analysis to study their ability in binding/masking RBD domain of spike in competition with ACE2 receptor or their preferential binding to S2' site that may prevent furin protease binding and hydrolysis.

2. Material and Methods:

2.1. Coordinate structures: Coordinate structures of SARS-CoV-2 and SARS-COVS protein with PDB IDs' 6VYB and 6CRZ as well as the coordinate structure of ACE2 receptor with PDB ID 1O8A were retrieved from protein data bank (https://www.rcsb.org/). The structures were energy-minimized in 12.85×13.13×17.12 nm, 14.68×14.28×17.72nm, and 7.21×8.30×7.75nm separate rectangular boxes respectively. The simulated boxes were filled with SPCE water with shells of 1.0 nm thickness. The energy minimization algorithm of steepest descent was used to minimize the system energy to lower than 100 kJ/mol. Neutral pH (given Asp, Glu, Arg, and Lys ionized), the temperature of 37°C, and one atmospheric pressure were used as energy minimization conditions (Macindoe, et al., 2010).

In order to study the dynamic behavior of spike proteins especially at RBD and fusion core, we performed molecular dynamics simulations using a double-precision MPI version of GROMACS 4.5.5 installed on the UBUNTU version 16.04

with GROMOS force field for 20 ns at 37degrees centigrade and 1 atmosphere (Dayer, 2016).

2.2. Sequence Alignment: Given the binding property of RBD domains for SARS-CoV-2 and SARS-CoV is determined by their amino acid sequences we compared the RBD sequence with the same sequence of SARS-CoV through sequence alignment on EMBOSS Stretcher (www.ebi.ac.uk), scheme 2, to pick up the underlying structural differences that lead to their different pathogenicity (Dayer & Dayer, 2013).

```
6VYB---001     1 RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRKRISNCVADYSVL  50
                 ||||||||||||||:|.||||||.||:||||||||||
6CRZ---001     1 -------------ITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVL  37

6VYB---001    51 YNSASF-STFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTG-  98
                 ||| :| |||||||||.|||||||:||||||||::||:||||||||||
6CRZ---001    38 YNS-TFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGV  86

6VYB---001    99 KIADYNYKLPDDFTGCVIAWNSNNLD--SKVGGNYNY-LYR-LFR--KSN  142
                 ||||||||||.|||:|||:.|:|  | .| |||| || | |  |
6CRZ---001    87 -IADYNYKLPDDFMGCVLAWNTRNIDATS-TG-NYNYK-YRYL-RHGK--  129

6VYB---001   143 LKPFERDISTEI-YQA--GSTPCN-GVEGFNCYFPLQSYGFQPTNGVGYQ  188
                 |:|||||||. : : :  |. ||.  ...|||:||..|||..|.|:|||
6CRZ---001   130 LRPFERDISN-VPF-SPDGK-PCTP--PALNCYWPLNDYGFYTTTGIGYQ  174

6VYB---001   189 PYRVVVLSFELLHAPATVCGPK-KSTNLVKNKCVNF-------------  223
                 ||||||||||:|:||||||  ||:|:||:||||
6CRZ---001   175 PYRVVVLSFELLNAPATVCGPKL-STDLIKNQCVNFNFNGLTGTGVLTPS  223
```

Scheme 2: Sequence alignment result performed on www.ebi.ac.uk for the RBD domains (residues 319-541) using FASTA files with PDB IDs' of 6VYB and 6CRZ.

***2.3. Ligands Coordinate structures*:** Coordinate structures for studied drugs including fidaxomicin, ivermectin, rapamycin, heparin, azithromycin, clarithromycin, niclosamide, erythromycin, ritonavir, flubendazole, mebendazole, bephenium, albendazole, and diethylcarbamazine were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as SDF format, converted to PDB format in Open Babel software (http://openbabel.org/) and optimized in ArgusLab software (http://www.arguslab.com/) (Abdelouahab & Abderrahmane, 2008).

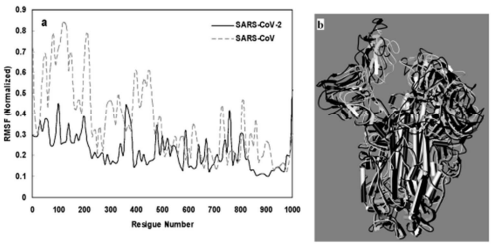
***2.4. Blind docking experiments*:** To survey the potential binding potency of the studied drugs blind docking experiments were carried out in Hex 8.0.0 (http://www.loria.fr/~ritchied/hex/) using SARS-CoV-2 spike as receptor and drugs as ligands (Ritchie, et al., 2008). The mode of Shape+Electrostatic with macro sampling was used during docking and the best 100 poses were analyzed accordingly.

***2.5. Isoelectric pH calculation*:** Isoelectric pH for RBD domains and ACE2 were calculated using www.web.expasy.org/compute_pi/. Server

***2.6. Data handling and analysis*:** All the numerical data were analyzed using excel 2012 and SPSS version 20.0 software. P-value under 0.05 was considered significant.

3. Results:

Figure 1-a, represents the root mean square fluctuations (RMSF) curve for alpha carbons during the 20ns period of simulation. The higher fluctuation for each alpha carbon represents its higher flexibility in contrast to other alpha carbons. As it is clear, the overall RMSF curve of SARS-CoV-2 shows a lower average fluctuation of 0.52nm in contrast to that's of SARS-CoV with the average RMFS of 0.65nm. This finding implies the more folded and less flexible structure for SARS-CoV-2. Figure 1-b represents the superposed structures for these two proteins, SARS-CoV-2 (white) and SARS-CoV (back), that confirm this claim.



Figurt-1: a- Root mean square fluctuation (RMSF) curves for SARS-CoV-2 and SARS-CoV spikes extracted from 20ns MD simulation. b- Superposed structures of SARS-CoV-2 (white) and SARS-CoV (black) spike proteins.

Our data in table 1 represents the statistics of S protein bindings to ACE2 receptor as percent values. As it is indicated, there are three kinds of binding patterns between ACE2 and RBD domains. The binding to the ACE2 receptor maybe take place via up or down conformation or by intervening regions of RBD domains.

Table 1: Binding pattern of S protein RBD domains to ACE2 receptor in accordance with their binding energies extracted from docking experiments performed on HEX 8.0.0.

	Up (%)	Down (%)	Inter domains region (%)	Binding Energy(kJ/mol)
SARS-CoV	53	0	1	-379.66
SARS-CoV-2	10	46	15	-450.51

As it is obvious SARS-CoV binds merely to up conformation of RBD domain while SARS-CoV-2 uses up and down conformations of RBD domains in addition to inter-domain region (table 1). Glycosylation status for SARS-CoV (right) and SARS-CoV-2 (left), is shown in figure 2. The figure graphically indicates that the spike of SARS-CoV-2 carries more quantities of N-acetyl glucosamine (NAG) in its especially RBD domains in contrast to SARS-CoV protein that may affect its more effective binding to its receptor contrast to SARS-CoV.

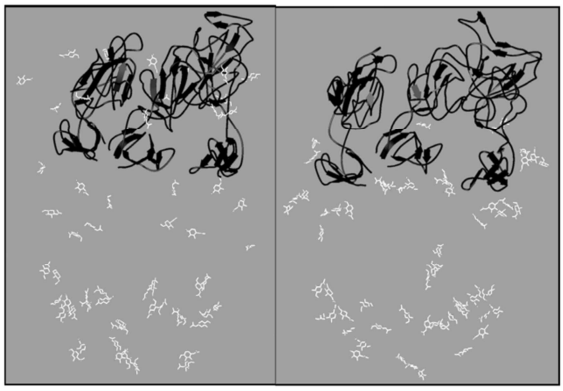


Figure 2: Schematic representations of N-acetyl glucosamine residues distribution through trimeric S protein. SARS-CoV-2 (left) and SARS-CoV (right).

Based on residue composition analysis the isoelectric pH for RBD domains and ACE2 were calculated as 5.82, 7.22, and 7.89 for ACE2, SARS-CoV, and SARS-CoV-2 proteins, respectively.

Our preliminary dockings revealed that there is a significant positive correlation between the molecular weights of drugs and their binding energies. Accordingly, we have enrolled 14 candidates from 100 approved drugs that bear relatively high molecular weights for docking (table 2) to select those specifically bind and shield RBD and/or S2' sites with considerable binding energies as candidates to interfere with spike binding.

Table-2: Total binding energies (kJ/mol) of the 14 enrolled drugs in accordance with percent occupancy of RBD and S2′ sites extracted from blind docking experiments performed on Hex 8.0.0.

	Drug	Energy±STD	S2'	RBD
1	Fidaxomicin	-652.67±19	99	1
2	Ivermectin	-539.70±19	23	23
3	Rapamycin	-520.83±18	21	8
4	Heparin	-495.04±15	41	29
5	Azithromycin	-467.35±13	41	22
6	Clarithromycin	-452.84±10	44	9
7	Niclosamide	-440.01±9	99	1
8	Erythromycin	-436.23±9	19	19
9	Ritonavir	-415.13±10	30	13
10	Flubendazole	-378.38±12	99	1
11	Mebendazole	-324.88±7	57	12
12	Bephenium	-316.43±7	28	14
13	Albendazole	-301.95±10	44	19
14	Diethylcarbamazine	-257.66±5	25	27

4. Discussion:

Studies on S proteins from SARS-CoV and SARS-CoV-2 origins indicated that despite large differences seen in the amino acid sequences, there are structural similarities seen between these two proteins, especially at their domains in S1 and S2 subunits which confirmed by low RMSD differences of only 4Å (Wrapp, et al., 2020; Coutard, et al., 2020). Accordingly, it is expected that these two proteins should behave likewise in receptor recognition and host cell infection and virulence. The mechanism for the higher rate of virulence and mortality for SARS-CoV-2 in contrast to SARS-CoV is the main question we tried to answer in this study. Our MD simulations results (Figure 1-a) show that besides structural similarities, S proteins of SARS-CoV-2 reveal different dynamic behavior. Base on our data in figure 1-b, it is evident that the S protein of SARS-CoV-2 expresses a more compacted structure than SARS-CoV, the fact that may affect its interaction with receptors. Spike to receptor docking results (table 1) indicates that SARS-CoV binds to ACE2 restrictedly using RBD in up conformation (53%), while SARS-CoV-2 binds ACE2 receptor simultaneously and more effectively via RBD domains in down conformation (46%), up conformation (10%), and

inters-domains region (5%). Our docking results also indicate that the S protein of SARS-CoV-2 can bind to ACE2 with a much higher binding energy of -450.51 kJ/mol; contrast to SARS-CoV with -379.66 kJ/mol binding energy (p-value<0.01). These findings are useful to understand the higher tendency of SARS-CoV-2 for the ACE2 receptor (Pallesen, et al., 2017; Walls, et al., 2019).

Figure 2 indicates that SARS-CoV-2 by carrying higher quantities of sugar moieties (N-acetyl glucosamine) compare to SARS-CoV expresses more affinity for the ACE2 receptor, considering that these groups enforce the hydrophobic interactions with receptor and accelerate SARS-CoV-2 entrance (Glowacka, et al., 2011; Iwata-Yoshikawa, et al., 2019). Isoelectric pH calculations show that at neutral pH of in vivo condition (~7.4), ACE2 and SARS-CoV carry negative charges, while S protein of SARS-CoV-2 carries global positive charge therefore unlike SARS-CoV an attractive electrostatic force is expected between SARS-CoC-2 and ACE2 receptor. This finding reconfirms the higher binding affinity of the SARS-CoV-2 virus ACE2 receptor, with respect to the SARS-CoV virus.

Our docking results in table 2 indicate, that among the 14 drugs, fidaxomicin (MW=1058.05g/mol) which binds merely to S2' cleavage site represents the highest average binding energy (-652.67±19 kJ/mol). Ivermectin (MW=875.106g/mol) which binds equally to the RBD domain and S2' cleavage site and with a total 23 percent of probability reveals average binding energy of -539.7±16 kJ/mol (Iwata-Yoshikawa, et al., 2019; Caly, et al., 2020). Some reports in accordance with our findings have shown that fidaxomicin and ivermectin exert beneficial effects in SARS-CoV-2 treatment by reducing COVID-19 mortality (Parvez, et al., 2020; Bryant, et al., 2021). Rapamycin or sirolimus (MW= 914.187g/mol), binds to S2' and RBD sites with 21 and 8 percent, respectively with average -520.83±18 kJ/mol energy Heparin (MW=1134.9gr/mol) is the more extensively drug masking RBD with 29 percent and average binding energy of -495.04±14.9 kJ/mol seems to be the more effective drug shielding S protein against ACE2 receptor (Mastrangelo, et al., 2012; Leone, et al., 2012). Romanelli and Mascolo, 2020, have suggested the use of sirolimus for COVID-19 treatment (Romanelli & Mascolo, 2020). Due to the negative charges present on heparin structure and the previously mentioned positive charge of S protein, we hypothesize that the electrostatic binding forces between heparin and S protein is much higher than that undertaken in our docking experiments because of software limitations. Moreover, it is very important to notice that the heparin structure used in our experiments was ultra-low molecular weight heparin with 5 sugar residues which is primarily used in acute coronary syndrome, pulmonary embolism, and deep venous thrombosis, and there are heparin variants with higher molecular weights with more binding energies (Riffo-Vasquez, et al., 2016). Gozzo et al, 2020 like many other reports indicate that heparin beyond its anticoagulation properties which is also useful in COVID-19 treatment exerts beneficial effects on these patients (Gozzo, et al., 2020). Our findings also indicate that the macrolides of azithromycin, clarithromycin, and erythromycin, masking both RBD and S2' sites, with average binding energies of -467.35±13, -452.84±10, and -436.01±9 kJ/mol, respectively. These drugs comprise good candidates for clinical trials in COVID-19, especially when considering their confirmed immunomodulatory effects, as reported before (Ohe, et al., 2020; Zimmermann, et al., 2018). Among other drugs, niclosamide binds restrictedly to S2' cleavage site with an average binding energy of -440.01±9 kJ/mol (Altenburg, et al., 2011). Ritonavir (MW=720.94gr/mol) contrast to the selected anti-HIV drugs, binds simultaneously to S2' (30%) and to RBD (13%) with an average energy of -415±10.09 kJ/mol. The rest of the anti-parasite and anthelmintic drugs including flubendazole, mebendazole, bephenium, albendazole, and diethylcarbamazine are placed next order of importance with lower binding energies.

5. Conclusion:

To this end, the disease of COVID-19 with a high rate of virulence and fast spread in the human body with multi-organ involvement and high rate of mortality comprises the greatest problem since the Second World War with more than 262 million infected cases and more than 5,210,000 deaths by December 2021 (Xu, et al., 2020; Basu-Ray, et al., 2020). Decreased lymphocytes,

increased C reactive protein (CRP), and pro-inflammatory cytokines, as well as hypercoagulability with increased d-dimer, lead to lung lesions with infiltrated immune cells (Zhang, et al., 2020; Spiezia, et al., 2020). It seems credulous to think that in a battle against COVID-19 that invades multi organs and disturbs the immune defensive system in a short period to be achieved by one or two drugs, especially at an advanced state. Based on plentiful reports in this context and considering our current and previous work (Tang, et al., 2020; Dayer, 2020) we imagine that a successful treatment regime should contain multi drugs of protease inhibitors, spike shielding drugs, and immunomodulatory drugs in the early steps of the disease. Considering the binding energy of drugs to S protein and their pattern of RBD and S2' shielding they could be considered for clinical assessment and their applications in early or in an advanced state of COVID-19.

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