REPURPOSED SINGLE INHIBITOR FOR SERINE PROTEASE AND SPIKE GLYCOPROTEINS OF SARS-CoV-2

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ABSTRACT

In this research, structure of SARS-CoV-2 spike glycoprotein S1 and S2 along with TMPRSS2, TMPRSS4, TMPRSS11A, TMPRSS11D and TMPRSS11E serine protease (which activates S1 and S2) are used for docking with repurposed inhibitor drug molecules. We searched for a universally active drug molecule which binds with glycoproteins and serine protease with binding energy above a pre-set threshold value, thus single handedly inhibits the virus glycoprotein interaction with ACE-II receptor on human cell preventing the virus RNA transfer to human cell. Through data analysis performed on binding energies of the selected repurposed inhibitors, we found out five molecules to have high binding energies on both spike glycoproteins and serine protease, while showing less variance in their binding energies. Among these five, Edoxaban is an FDA approved commercially available drug molecule. Hence, high binding molecular inhibitors for spike glycoprotein and serine protease for treatment of SARS-CoV-2 were identified.

Keywords: SARS-CoV-2; Docking; Spike glycoprotein; Serine protease, Protease inhibitors.

INTRODUCTION

SARS-CoV-2 is a single positive strand RNA virus [1] with enveloped structure. The known strategies to inhibit the virus by drugs have so far included identification of drug molecules for interaction with reception sites related to SARS-CoV-2 main protease (Mpro), RNA polymerase (RdRp), receptor-binding domain (RBD) and angiotensin-converting enzyme II (ACE-II). Identified RdRp molecular inhibitors have showed less specificity and side effects [2]. Mpro protease has proven to be potential drug target for anti-viral drug molecules for SARS-CoV in past [3][4][5]. It provides an opportunity for structure-based protease inhibitor drug molecular identification and design [6][7]. In

this work we are focusing on serine protease TMPRSS2, 4, 11E, 11D, 11E along with S1 and S1 subunits of spike glycoproteins of SARS COV-2. Previous studies focused on inhibiting the entry of SARS-COV-2 into the host cell by inhibit interaction of spike glycoprotein with ACE-II and TMPRSS (**Figure**-1) [9].

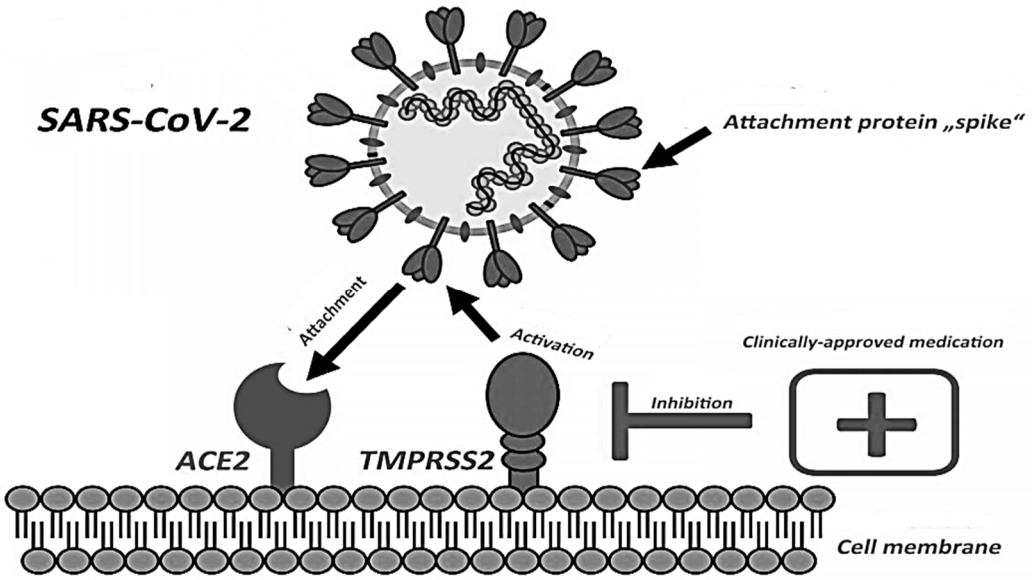


Figure-1. Mechanism of SARS-CoV-2 binding with ACE-II.

Serine Protease

The entry of SARS-COV2 into the human cell is initiated by transmembrane serine protease family members such as TMPRSS2, 4, 11E, 11D, 11E. These serine proteases cleave and activate the spike protein. TMPRSS2 is present along with angiotensin converting enzyme 2 (ACE-II) on surface of the cell and acts as receptor for SARS and SARS-CoV-2 [8]. The inhibition of TMPRSS2 can reduce the interaction of SARS-CoV, SARS-CoV-2 [9] MERS-CoV [10] glycoproteins with ACE-II. In this work, we checked already known commercially available drug molecules for binding with TMPRSS2, 4, 11E, 11D, 11E serine protease inhibitors. The advantage of screening for approved drugs is that they can be readily deployed for treatment [8].

Spike S1 and S2

The spike glycoprotein is located on the surface of SARS COV-2. It mediates the virus interactions with ACE-II receptor of the human cell. Trimeric S monomer protein contains two subunits proteins. The S1 subunit of S protein has receptor binding domain (RBD) interacts with the ACE-II receptor on the cell after which the S2 subunit of S protein is responsible for virus and cell membrane fusion. This facilitates the entry of viral genetic material into the human cell. Inhibition of S protein can thus avert the process of virus binding with ACE-II receptor [11]. Hence. spike glycoprotein is a

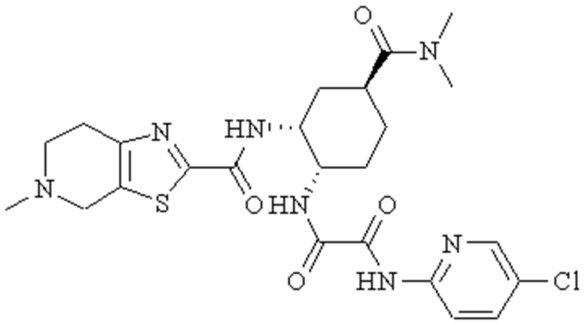
main drug target as SARS-CoV-2 uses it to bind to its receptor ACE-II on human cell, mediate membrane fusion and virus entry. S1 itself consists of N-terminal domain (NTD) and C-terminal domains (C-domain). Depending on type of the virus, either NTD or C-domain can serve as the receptor-binding domain (RBD) [8].

MATERIAL AND METHODS

Docking simulations were performed using molecular operating environment (MOE version 2015.10) software. Structures of all drug molecules (**1-27**) were downloaded from PubChem website in smiles format. For preparation of drug target receptor sites, the structure of the spike glycoprotein S1 (6LZg) [12] and S2 (6LXT) [13] along with TMPRSS2 (O15393), TMPRSS4 (Q9NRS4), TMPRSS11A (Q6ZMR5), TMPRSS11D (O60235), TMPRSS11E (Q9UL52) were obtained from the Protein Data [14] and UniProt in .pdb formats [15]. Docking simulations were performed for at pH 7.

RESULTS AND DISCUSSION

Binding of coronaviruses to the receptor site on the surface of the cell and entry to the cell mainly depend on the spike proteins (S protein) on the virus that are attached to the envelope structure. Its extracellular domain contains two subunits, S1 receptor binding subunit and S2 which is membrane fusion subunit. S1 unit contains receptor binding unit which play a major role in the in the interaction of virus spikes to ACE-II receptor [16]. It has been reported that the spike protein RBD can interact strongly with ACE-II receptor of the host cell enhancing the pathogenicity [17]. During fusion of virus to the host cell membrane, the spikes are cleaved at S1 and S2 boundary by proteases at the host cell, depending upon the type of coronavirus [18]. The proteases responsible for the cleavage of SARS CoV-2 spike glycoproteins subunit are TMPRSS2, 4, 11E, 11D, 11E transmembrane serine protease family members. Previously attempts have been made at inhibition of TMPRSS2, spike proteins and ACE-II interfaces. Our study is aimed to inhibit all these sites that enable SARS-CoV-2 entry into the host cell by a single repurposed drug molecule. Docking analysis was done on the basis of hydrogen bonding, Van der Waals and π-stacking interactions. Known serine protease, S-protein and S-protein-ACE-II receptor interface inhibitor molecules were used for docking (**1-27**) (**Table**-1). We used repurposed ligands or drug molecules for serine protease and spike glycoprotein inhibition. By setting up a pre-set threshold value of –6 Kcal/mol, we found out that (**11**), (**12**), (**15**), (**16**) and (**24**) have more than –6 Kcal/mol binding energy on all spike proteins and serine protease substrates (**Table**-2). These molecules also showed less variance in range of 0.3 to 0.4 indicating that these molecules have similar binding with both spike glycoproteins and also with serine protease. In this way a single molecule can inhibit the virus spike interaction with ACE-II receptor site on human cell. Repurposing a known drug molecule provides the benefit that molecule is already medicinally known. The above mentioned repurposed drugs for inhibiting entry of SARS-CoV-2 into the host cell are used as anticoagulants and in trials for treating coronary artery disease. Among (**11**), (**12**), (**15**), (**16**) and (**24**), Edoxaban (**16**) is a FDA approved anticoagulant drug and a direct factor Xa inhibitor (**Figure**-2).



Edoxaban
(16)

Figure-2. Molecular structure of Edoxaban (**16**).

Table 2. Binding energies and variance of (**1-27**)

S. No.	Ligand	Binding energy = - Kcal/mol							Variance
		TMPRSS4	TMPRSS11A	ZMPRSS11D	TMPRSS11E	TMPRSS2	S1	S2	
Code		Q9NRS4	Q6ZMR5	O60235	Q9UL52	O15393	6LZG	6LXT	
1	Phenformin HCl	5.34	4.75	5.39	5.03	5.091	4.84	4.58	0.09022
2	Demethyl-Coclaurine	5.558	6.15	5.2509	6.006	5.241	5.04	4.86	0.235007
3	AEBSF	4.61	4.99	4.68	5.3	4.55	4.61	3.71	0.239062
4	Nafamostat	5.71	6.73	6.15	6.66	5.73	5.75	5.19	0.310948
5	Tazobactu	5.84	5.93	5.25	5.44	6.03	5.33	4.77	0.201357
6	Benserazid	5.33	6.05	5.215	6.398	5.47	4.821	5.087	0.308194
7	Eriodictyol	5.507	6.16	5.59	6.499	5.44	4.94	5.11	0.305258
8	Argatroban	7	7.42	6.73	7.64	6.63	5.94	6.57	0.323457
9	Vildaglipti	5.624	5.43	5.795	6.666	5.224	5.168	4.936	0.325758
10	Quercetol	5.34	6.19	5.398	6.475	5.42	4.93	4.817	0.376636
11	Letaxaban	6.74	7.65	6.94	7.43	6.66	6.15	6.18	0.327648
12	Otamixaba	6.9	6.71	6.68	7.89	6.13	6.88	6.29	0.322324
13	Nitrofurantoi	4.61	6.19	4.958	5.933	5.44	5.07	4.65	0.378666
14	Protirelin	6.689	7.09	6.122	6.927	5.843	5.407	6.178	0.369657
15	MAFP	6.39	6.47	6.55	7.82	6.01	6.59	6.34	0.327929
16	Edoxaban	6.55	7.51	7.45	8.14	6.28	6.6	7.49	0.455762
17	PMSF	4.28	4.78	4.23	5.19	4.54	5.63	6.21	0.544267
18	Sapropterin	5.208	6.81	5.24	5.77	5.47	5.096	3.63	0.895047
19	Pemirolast	4.47	6.03	5.2	5.93	5.67	4.93	3.91	0.617157

20	Pyruvic acid calcium isoniazid	4.52	5.54	4.95	5.81	4.84	4.6	3.498	0.566973
21	Carbazochrome	4.87	5.61	5.05	6.007	5.263	5.03	6.196	0.266323
22	Camostat	6.51	6.28	6.52	7.95	6.27	6.42	5.61	0.500381
23	Vidarabine	5.319	5.85	5.37	6.413	5.739	4.96	3.836	0.662792
24	Darexaban	6.81	7.33	7.45	8.1	7.34	6.71	6.41	0.319395
25	Benserazide	-5.45	5.49	5.33	6.14	5.31	5.17	4.87	16.92122
26	Luteolin-monoarabinoside	5.45	6.01	5.12	6.3996	-5.38	5.13	5.33	17.36413
27	BIA 10-2474	5.94	6.32	-5.89	6.91	6.16	5.16	5.4	20.471

Table 2. Binding energies of (**1-27**) above threshold value of -6kcal/mol are highlighted.

		Binding energy > -6 Kcal/mol						
S. No.	**Name of ligand**	**TMPRSS4**	**TMPRSS11A**	**ZMPRSS11D**	**TMPRSS11E**	**TMPRSS2**	**S1**	**S2**
Code		**Q9NRS4**	**Q6ZMR5**	**O60235**	**Q9UL52**	**O15393**	**6LZG**	**6LXT**
1	Phenformin hcl	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
2	Demethyl-Coclaurine	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE
3	AEBSF	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
4	Nafamostat	FALSE	TRUE	TRUE	TRUE	FALSE	FALSE	FALSE
5	Tazobactu	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE
6	Benserazid	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE
7	Eriodictyol	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE
8	Argatroban	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE
9	Vildaglipti	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE
10	Quercetol	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE
11	Letaxaban	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
12	Otamixaba	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
13	Nitrofurantoi	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE
14	Protirelin	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE
15	MAFP	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
16	Edoxaban	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
17	PMSF	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE
18	Sapropterin	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE
19	Pemirolast	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE
20	Pyruvic acid calcium isoniazid	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
21	Carbazochrome	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE
22	Camostat	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	FALSE
23	Vidarabine	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE
24	Darexaban	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
25	Benserazide	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE
26	Luteolin-monoarabinoside	FALSE	TRUE	FALSE	TRUE	FALSE	FALSE	FALSE
27	BIA 10-2474	FALSE	TRUE	FALSE	TRUE	TRUE	FALSE	FALSE

CONCLUSION

We used SARS-Cov-2 spike glycoprotein S1 receptor-binding domains (RDB), S2 along with serine protease of human cell i.e. TMPRSS2, 4, 11E, 11D, 11E as a drug target sites for binding with repurposed and known protease inhibitors **(1-27).** We carried out the binding energy data analysis by filtering out the molecules with binding energies above the pre-set threshold value of –6 Kcal/mol across all substrates. Among the selected molecules, **(11), (12), (15), (16)** and **(24)** were found to have the higher than –6 Kcal/mol binding energies of interactions on all the reception sites. Through variance analysis we also found out that all of the above mentioned molecules have consistent binding energies across the glycoproteins and serine protease. Among these (**16**) Edoxaban is an FDA approved drug molecule. Hence, by data manipulation, we found out the most potent inhibitors that can bind with spike glycoprotein subunits and serine protease substrates at the same time. Previous studies focused on inhibiting spike glycoproteins and serine protease individually. Thus, a single repurposed molecule can inhibit the entry of the SARS-CoV-2 into human cell by binding to all the reception sites that enable the entry of virus RNA into the host cell.

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