Curdlan Sulfate as a Novel Inhibitor for SARS-CoV-2 (COVID – 19): A Molecular Docking Study using Computational Tools

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COVID-19 caused by SARS-CoV-2 affected almost 2.7 million populations over worldwide. Hence there is an immediate need for a potential antiviral agent against COVID-19. Previous studies reported that curdlan sulfate has an extensive array of biological properties and has been recommended for the treatment of diseases like malaria, HIV, dengue, etc. Bioinformatics tools have positively impacted coronavirus research and have been fruitfully employed for unraveling the viral genomics and genome wide association studies, detection of SARS-CoV-2 infection, outlining of the COVID-19 pandemic, evolution of coronavirus, discovery of vaccine candidates, exploration of prospective drug targets, and development of therapeutic stratagems. Thus, the current study focuses on investigating the inhibiting property of curdlan sulfate against COVID-19 using a computational approach. AutoDock Vina was employed for molecular docking of receptor binding domains of SARS-CoV-2. Binding affinity was confirmed based on the lowest binding free energy. Docking results showed that curdlan sulfate possesses a high binding affinity with the receptorbinding domain of SARS-CoV-2. The molecular interactions between these drug candidates and receptor binding domain were studied. Subsequent experiments will reveal the stability of the targets and potential drug candidates. Hence this study will help in exploiting curdlan sulfate as an efficient antiviral therapeutic mediator for SARS-CoV-2.

Keywords: COVID-19, Inhibitors, Drug Discovery, Bioinformatics, Curdlan Sulfate.

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1 Introduction

Drug designing facilitates in the finding of potential drugs against the biological targets which is exceptionally challenging, costly, and time consuming [1]. *Insilico* screening using computational tools or computeraided drug design has been implied as a successful approach in the documentation of potential drug candidates for various infectious diseases leading to the discovery and development of novel drugs [2]. The computeraided drug design is advantageous due to costeffectiveness, speeding up the time to bring therapeutic molecules for society, and acquaintance of drugreceptor interactions [3]. With the help of a computational approach, structurebased drug designing is possible which can lead to effective drug discovery. It can be employed as a preliminary step to recognize the effective drugs against infectious diseases. COVID -19 or Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a recent pandemic, originated from Wuhan City, China [4] that lead to the death of several people across the world. SARS-CoV-2 is a single-stranded RNA enveloped virus belonging to the Coronaviridae family [5]. Coronavirus consist of four main structural target proteins and the receptorbinding domains are linked with them. The target proteins are spike, nucleocapsid, membrane, envelope, and the receptorbinding domains are spike receptorbinding domain [6], nucleocapsid protein N-terminal RNA binding domain [7], non-structural protein -1 [8], non-structural protein -5 [9]. Bioinformatics tools and methods have created a major impact in coronavirus research and have been employed fruitfully for unraveling the viral genomics and genomewide association studies, diagnosis of SARS-CoV-2 infection, mapping out of the COVID-19 pandemic and coronavirus evolution, the discovery of vaccine candidates, exploration of prospective drug targets and expansion of therapeutic stratagems [10; 11]. COVID-19 postures a severe risk to human health and hence a potential drug candidate is to be discovered by the researchers. There is a need for effective compounds for COVID-19 treatment. Currently, there are few medications such as remdesivir, dexamethasone, chloroquine, hydroxychloroquine, lopinavir, ritonavir, resveratrol, umifenovir, ivermectin, tocilizumab corticosteroids approved for clinical trials in COVID-19 treatment [12]. In current times, interest has been exposed towards polysaccharides for the treatment of COVID-19. Heparin sulfate is reported to exhibit binding affinity to the spike protein (S protein) of SARS CoV-2 [13]. 2-deoxy-D- glucose is reported to be used in adjunctive therapy and can be administered along with primary treatment for COVID -19 caused by SARS-CoV2 [14]. Sulfated polysaccharides extracted from seaweeds have been reported to exhibit antiviral properties and have a significant impact on the treatment of viral disease [15]. Curdlan is an exopolysaccharide produced by certain bacterial species such as *Agrobacterium* sp., *Alcaligenes* sp., *Cellulomonas* sp., *and Bacillus* sp. [16]. Curdlan and its sulfated derivative, curdlan sulfate have numerous therapeutic applications. Curdlan sulfate has been reported to exhibit anti-malarial activity, anti-viral activity against HIV and dengue [17]. Curdlan sulfate has not been explored as an inhibitor for COVID-19. In this perspective, the current study was focused on molecular docking of receptor binding domains of SARS-CoV-2 target proteins with curdlan sulfate as ligand using a computational approach. It would be suggested to target the receptorbinding domains of the COVID-19 proteins rather than targeting the entire protein to reduce the viral pathogenesis.

2 Methods

2.1 Retrieval of Protein Target and Ligand

Three dimensional structures of four receptor binding domains of SARS-CoV-2 target proteins such as spike receptorbinding domain (PDB id: 7JVB), nucleocapsid protein N-terminal RNA binding domain (PDB id: 6M3M), non-structural protein -1(PDB id: 6ZLW), non-structural protein -5 (PDB id: 7JP1)

were retrieved from Protein Data Bank (PDB). The structure of ligand, curdlan sulfate was retrieved from the PubChem database.

2.2 Docking Tool

In this study, molecular docking was executed using AutoDock Vina (v1.5.6). It is an open-source tool for studying the molecular interactions via computational approach. This is done to predict the best ligand conformer for the particular target protein and to evaluate the binding affinity of the interacting ligands. Docking or binding scores are calculated in kcal/mol and the more negative value, the better binding of a ligand with the target protein.

2.3 Visualization Tool

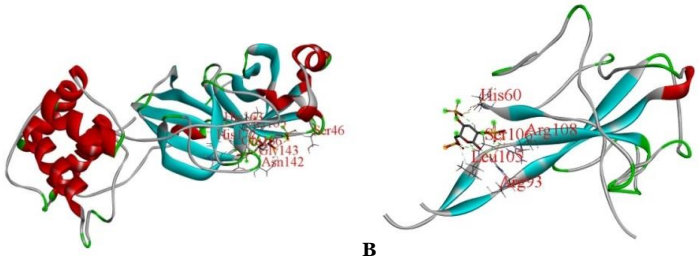
Discovery Studio was used as a tool to visualize the interacting amino acids between the receptor and ligand after docking.

2.4 Docking of Receptor Binding Domain of SARS-CoV-2 with Curdlan sulfate

Polar-H atoms were added before docking and were followed by the addition of Kollman charges [18]. Both the ligand and macromolecule files were then saved in pdbqt format. Then the grid box was created with a spacing of 0.375Å. The grid box (112Å × 166Å×164Å) centered at (12.054, -0.604, 6.602) Å for non-structural protein -5 RBD with curdlan sulfate. The grid box (126Å × 140Å×136Å) centered at (7.44, -23.999, -21.962) Å for nucleocapsid protein N-terminal RNA binding domain with curdlan sulfate. The grid box (160Å × 142Å×118Å) centered at (-20.624, 2.356, -9.763) Å for spike RBD with curdlan sulfate. The grid box (98Å × 100Å×102Å) centered at (239.11, 215.479, 197.672) Å for non-structural protein -1 RBD with curdlan sulfate. After docking, the result will be displayed in the command window where the binding affinity can be observed. Interacting amino acids and the type of interaction were visualized using Discovery Studio by opening receptor and output acquired in docking.

3 Results and Discussion

The ligand molecule, curdlan sulfate was docked against the receptor binding domains of SARS-CoV-2, and binding affinity was tabulated in Table 1. After running docking, it was found that curdlan sulfate has a better binding affinity towards receptor binding domains of SARS-CoV-2 such as spike receptorbinding domain, nucleocapsid protein N-terminal RNA binding domain, non-structural protein -1, and non-structural protein -5 (-6.8, -5.8, -5.7 and -5.0 kcal/mol respectively). Further, interacting amino acids were displayed in Table 1. Binding sites for four receptor binding domains of SARS-CoV-2 with curdlan sulfate were depicted in (Fig. 1).



A **B**

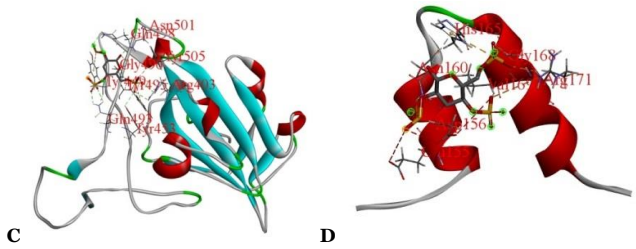


Fig. 1. 3D Visualization of protein-ligand interaction using Discovery Studio; (A) Non-structural protein-5 receptor binding domain with curdlan sulfate, (B) Nucleocapsid protein N-terminal RNA binding domain with curdlan sulfate, (C) Spike receptor binding domain with curdlan sulfate, (D) Non-structural protein-1 receptor binding domain with curdlan sulfate.

Ionizability observed regions were mostly neutral for all molecular interactions. Non-structural protein-5 and curdlan sulfate complex are stabilized by hydrophobic interaction at Met165 and hydrogen bonds at Glu166, Gly143, and Asn142 as presented in (Fig. 2 (A) and (B)) respectively. The docked result of nucleocapsid protein N-terminal RNA binding domain with curdlan sulfate indicated hydrophobic interaction at Leu105 and hydrogen bonding at Ser106 as viewed from (Fig. 3 (A) and (B)) respectively. Spike receptor binding domain interacts with curdlan sulfate through five hydrogen bond interactions at Tyr453, Gly496, Gln493, Gln498, and Asn501 are shown in (Fig. 4 (B)) with the absence of hydrophobic interaction (Fig. 4 (A)). Curdlan sulfate displayed hydrophobic interaction at Val169 non-structural protein-1 as shown in (Fig. 5(A)) while hydrogen bonding at Asn160 is shown in (Fig. 5(B)).

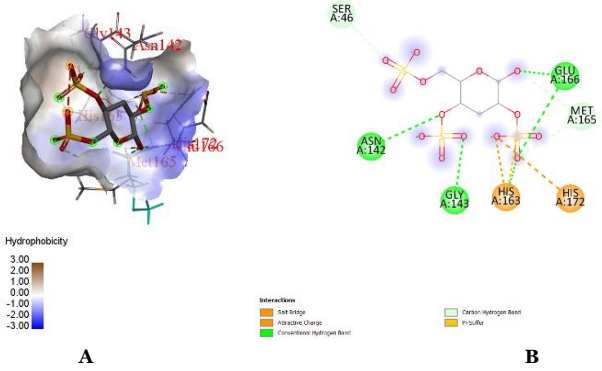


Fig. 2. Hydrophobic Interaction and Hydrogen Bonding between Non-structural Protein-5 Receptor Binding Domain of SARS-CoV-2 and Curdlan Sulfate

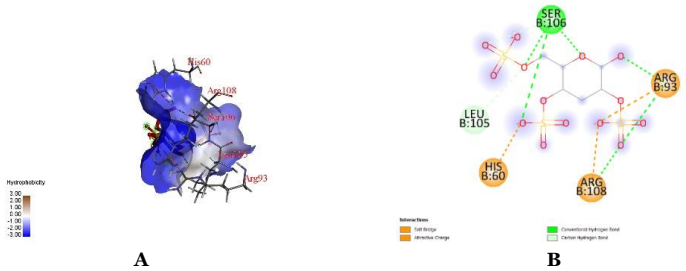


Fig. 3. Hydrophobic Interaction and Hydrogen Bonding between Nucleocapsid Protein N-terminal RNA Binding Domain of SARS-CoV-2 and Curdlan Sulfate

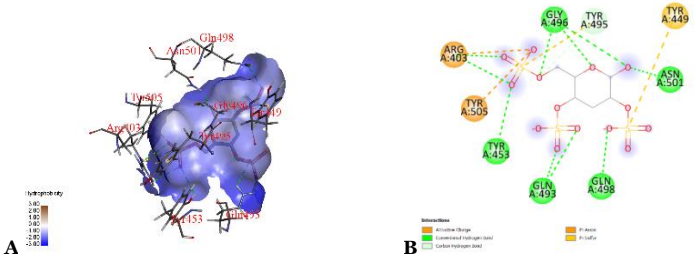


Fig. 4. Hydrophobic Interaction and Hydrogen Bonding between Spike Binding Domain of SARS-CoV-2 and Curdlan Sulfate

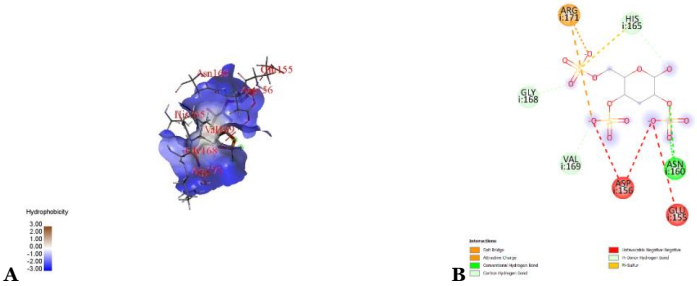


Fig. 5. Hydrophobic Interaction and Hydrogen Bonding Between Non-Structural Protein-1 Receptor Binding Domain of SARS-CoV-2 and Curdlan Sulfate

Table 1. Binding Energies and Amino acid Interaction of Receptor Binding Domain of SARS-CoV-2 with Curdlan Sulfate

S. No	Compound	Receptor binding domain of SARS-CoV-2	Binding Energy (kcal/mol)	Interacting Amino acids
1.	Curdlan Sulfate	Non-structural protein-5	-6.8	Ser46, Asn142, Gly143, His163, His172, Met165, Glu166
		Nucleocapsid protein N-terminal RNA binding domain	-5.8	Ser106, Arg93, Arg108, His60, Leu105
		Spike receptor binding domain	-5.7	Arg403, Tyr505, Tyr453, Gln493, Gln498, Asn501, Tyr449, Tyr495, Gly496
		Non-structural protein-1	-5.0	Arg171, His165, Gly168, Val169, Asp156, Asn160, Glu155

The above docking analysis results of the curdlan sulfate with different RBD of SARS-CoV-2 virussuggest the prospect of using these polysaccharide molecules as a potential inhibitor for COVID-19. Curdlan sulfate showed a good binding affinity with non-structural protein-5 with a docking score of -6.8 kcal/mol. Curdlan sulfate was reported as a potent inhibitor for HIV entry and dengue virus [27;28]. Table 2 shows the docking or binding scores of various molecules docked against different SARS-CoV-2 targets using bioinformatics tools. As reviewed from the literature, the binding affinity of curdlan sulfate is quite comparable with compounds such as curcumin, apigenin, piperine, genistein, daidzein, ritonavir, lopinavir, remdesivir, oseltamivir, ribavirin, lopinavir, ritonavir and ketoamide tested using *in silico* approach.

Table 2. Comparison of Binding Affinity of Various Compounds to COVID-19 Target Proteins docked using Different Computational Tools

S.No	Computational Tool used	Inhibitors	Target Protein	Binding Score (kcal/mol)	Refer ence
1.	AutoDock Vina	GR hydrochloride	ACE2 Protein of the Host Cell	-11.23	[18]
		RS504393 (CCR2 antagonist)		-8.32	
		TNP (Trinitrophenyl)		-7.42	
		GNF-5 (N-(2-hydroxyethyl)-3-[6-[[4-(trifluoromethoxy) phenyl] amino]-4-pyrimidinyl]-benzamide)		-7.57	
		Eptifibatide acetate		-6.05	
2	AutoDock Vina	EGCG. (Epigallocatechin gallate)	Spike Receptor Binding Domain (PDB id: 6XE1)	-9.7	[19]
		TF3 (Theaflavin-3,3'-digallate)		-11.6	
3	AutoDock Vina	Eltrombopag	NRP1 (Neuropilin-1)	-8.5	[20]
		Estradiol benzoate		-8.5	
		Lumacaftor		-8.4	
		Netupitant		-8.3	
		Epinastine		-8.3	

		Sorbinil		-8.3				
		Glimepiride		-8.2				
		Dutasteride		-8.2				
		Sitagliptin		-8.2				
		Trileptal		-8.2				
		Darifenacin		-8.1				
		Ergotamine		-8.1				
		Silibinin		-8.1				
		Dihydroergotamine		-8.0				
		Dicumarol		-8.0				
		EG01377 (dihydrochloride)		-6.0				
4	AutoDock Vina	Lopinavir	Spike Receptor-Binding Domain (PDB id: 2AJF)	-5.60				[21]
5	Lamarckian genetic algorithm	EGCG (Epigallocatechin gallate)	Prefusion 2019-nCoV Spike Glycoprotein (PDB id: 6VSB)	-7.26				[22]
		Curcumin		-5.05				
		Apigenin		-5.98				
		Beta Glucan		-3.20				
		Myricetin		-6.14				
		Quercetin		-6.14				
		Piperine		-6.05				
		Genistein		-6.54				
		Diadzein		-6.16				
		Ferulic acid		-5.44				
		Alliin		-4.57				
		Lipoic acid		-4.93				
		Resveratrol		-5.57				
		Glucosamine		-4.89				
		Gingerol		-4.46				
		Sulforaphane		-3.43				
		Allicin		-3.46				
		Remdesvir		-4.27				
		Chloroquine		-4.79				
6	PyRx 0.8		Spike Glycoprotein-SARS CoV 2 (PDB ids: 6VSBA, 6VSBB, 6VSBC) SARS Protein Receptor Binding Domain SARS CoV (PDB id:2GHV)	6VSBA	6VSBB	6VSBC	2GHV	[23]
		Ritonavir		-6.2	-5.6	-7.0	-7.6	
		Lopinavir		-6.9	-6.2	-6.6	-7.1	
		Remdesivir		-6.5	-6.8	-6.2	-6.9	
		Oseltamivir		-5.3	-6.0	-5.4	-5.8	
		Ribavirin		-5.5	-5.5	-5.4	-6.5	
		Mycophenolic acid		-4.7	-4.8	-5.4	-	

							6.6	
		Chloroquine		-4.8	-5.4	-4.8	-5.4	
		Hydroxychloroquine		-5.8	-5.6	-5.5	-5.8	
		Pemirolast		-6.6	-6.2	-6.3	-6.7	
		Eriodictyol		-7.0	-6.7	-7.6	-7.3	
		Isoniazid		-5.0	-4.4	-5.0	-5.1	
7	Schrodinger	Cangrelor	SARS-CoV-2 Spike Glycoprotein (PDB id: 2GHV)	-7.234				[24]
		Dpnh (NADH)		-7.038				
		Iomeprol		-7.687				
		Coenzyme A		-11.555				
		Tiludronate		-9.364				
		Dpnh (NADH)	3CLPRO Main Protease (PDB id: 1LVO)	-11.016				
		Zanamivir		-8.843				
		Bortezomib		-8.654				
		Saquinavir		-7.285				
		Flavin Adenine Dinucleotide (FAD) Adeflavin		-10.339				
		Cangrelor		-10.269				
		Carfilzomib		-8.924				
		Indinavir		-8.199				
		Remdesivir		-7.215				
8	AutoDock Vina	Remdesivir	COVID-19 Protease	-5.51				[5]
		Nelfinavir		-7.54				
		Lopinavir		-6.08				
		Ritonavir		-5.98				
		Ketoamide		-5.80				
9	AutoDock Vina	Oseltamivir	COVID-19 Main Protease with inhibitor N3	-4.7				[25]
		Ritonavir		-7.3				
		Remdesivir		-6.5				
		Ribavirin		-5.6				
		Favipiravir		-5.4				
		Chloroquine		-5.1				
		Hydroxychloroquine		-5.3				
		Chloroquine	SARS Spike Glycoprotein-Human ACE2 Complex	-7.1				
		Hydroxychloroquine		-6.8				
10	AutoDock Vina	Simeprevir	Main 3C-like protease	-10.0				[26]
		Saquinavir		9.3				
		Indinavir		-8.7				
		Tipranavir		-8.6				

		Faldaprevir		-8.4	
		Ritonavir		-8.1	
		Lopinavir		-8.1	
		Asunaprevir		-8.1	
		Atazanavir		-8.0	
		Nelfinavir		-7.9	
		Amprenavir		-7.7	
		Darunavir		-7.6	
		Fosamprenavir		-7.2	
		Umifenovir	Spike Envelope Glycoprotein	-7.7	
		Pleconaril		-7.1	
		Enfuvirtide		-5.9	

4 Conclusion

In this work, virtual screening of curdlan sulfate against target receptor-binding domains of coronavirus was carried using bioinformatics tools. Based on the molecular docking, curdlan sulfate is a potential molecule for the therapy of SARS-CoV-2. Further *in vitro and in vivo* evaluation and validation are necessary to confirm the antiviral activity of curdlan sulfate against COVID-19. However, this study using computational tools would serve as a foundation for the discovery of new carbohydrate-based drugs to lessen the transmission and symptoms of the SARS-CoV-2 virus.

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