Glucose antimetabolite 2-Deoxy-D-Glucose and its derivative as promising candidates for tackling COVID-19: Insights derived from *in silico* docking and molecular simulations

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Summary

A novel respiratory pathogen, SARS-CoV-2 has recently received worldwide attention and has been declared a public health emergency of global concern. Entry of SARS-CoV-2 is mediated through the viral spike glycoprotein (S2). Afterwards, the virus gets hold of the host cell machinery by employing the use of viral main protease 3CLpro and NSP15 endoribonuclease. In the present *in silico* study, active site mapping of the viral virulence factors was rendered by means of DoG Site Scorer. The possibility of repurposing of 2-deoxy-D-glucose (2-DG), a radio-chemo-modifier drug used for optimizing cancer therapy, and one of its derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose, has been investigated by conducting ligand-receptor docking. Binding pose depictions of ligands and viral receptors were assessed by employing molecular dynamics analysis. Molinspiration and Toxicity Estimation Software tools were used to assess the drug likeliness, bioactivity indices and ADMETox values. 2-DG can dock efficiently with viral main protease 3CLpro as well as NSP15 endoribonuclease, thus efficiently inactivating these viral receptors leading to incapacitation of the SARS-CoV-2 virus. Such incapacitation was possible by means of formation of a hydrogen bond between 2-DG and proline residues of viral protease. The 2-DG derivative formed a hydrogen bond with the glutamine amino acid residues of the viral spike glycoprotein. The present *in silico* study supports the potential benefits of using 2-DG and its glucopyranose derivative as repurposed drugs/prodrugs for mitigating the novel COVID-19 infection. Since both these moieties present no signs of serious toxicity, further empirical studies on model systems and human clinical trials to ascertain effective dose-response are warranted and should be urgently initiated.

Keywords: 2-deoxyglucose, binding free energy, corona virus, drug repurposing, molecular docking, SARS-CoV-2.

Abbreviations: 2-DG: 2-deoxy-D-glucose; ADMETox: Adsorption, Distribution, Metabolism, Toxicity; CoV: Coronavirus; DARS: Decoys as Reference State; FFT: Fast Fourier Transform; GPCR: G-protein coupled receptor; MERS: Middle East Respiratory Syndrome; NSP: Non structural protein; O.E.C.D.: Organisation for Economic Co-operation and Development; PDB: Protein Data Bank; QSAR: Quantitative Structure Activity Relationship; RCSB: Royal

Collaborative Structural Biology; SARS: Severe Acute Respiratory Syndrome; T.E.S.T.: Toxicity Estimation Software Tool; TPSA: Total polar surface area; VIF: Viral infectivity factor

Running title: 2-deoxy-D-glucose holds promise for combating COVID-19.

1. Introduction

The Corona virus (COVID-19), which sprung up in China during the late November, 2019, has shown a burgeoning spread since then as it has been known to infect more than 8,03,011 people around the world, resulting in nearly 39,025 deaths as of 31 March, 2020 (Shao, 2020; WHO, 2020). It has been found to spread in about 201 countries within a short time span of three months and hence, has been declared a pandemic by the World Health Organization on 11th of March, 2020 (Cucinotta & Vanelli, 2020).

Coronaviruses presents a large family of enveloped RNA (non-segmented, positive sense) viruses that cause zoonotic respiratory or occasional gastrointestinal infections in humans, wherein camels, cattle, bats and cats may serve as reservoirs of viral transmission (Ye et al., 2020). The earlier timeline of spread of Coronaviruses have suggested that mainly 3 outbreaks of deadly pneumonia have been caused by Coronaviruses in the 21st Century. These pathogenic serotypes of Coronaviruses have been named as SARS-CoV (Severe Acute Respiratory Syndrome causing Coronavirus, outbreak in 2002); MERS-CoV (Middle East Respiratory Syndrome causing Coronavirus, outbreak in 2012); and SARS-CoV-2 (Novel Beta-Coronavirus, outbreak in 2019) (Guarner, 2020). Genomic analysis have delineated the phylogenetic similarity between SARS-CoV and SARS-CoV-2, however, the latter shows a mutational degree of genomic diversification, mainly in the NSP domains (16 non-structural protein domains). Such mutations in the NSP domains of SARS-CoV-2 may be responsible for the differences in the host responsiveness, transmissibility and fatality of COVID-19 (Fung et al., 2020).

Analyzing the early history of SARS-CoV-2, it has been found that the virus got transmitted from animals to humans as several cases of COVID-19 disease transmission were directly linked to seafood and live animal ingestion in Wuhan, China (Jiang et al., 2020; Ward et al., 2020). It has also been found that the SARS-CoV-2 bears nearly 96.2% similarities with that of the bat CoV RaTG13, thereby indicating bats to be the natural reservoir of this virus (Zhou et al., 2020). Consequently, person-to-person spread of infection began through direct contact with the infected individuals and via respiratory droplets (Carlos et al., 2020). Some investigations have also suggested that SARS-CoV-2 may be present in feces of infected individuals and even after the patient is cured, thereby indicating a feco-oral route of viral transmission as well (Yeo et al., 2020).

There are different stages of transmission of this virus, *i.e.*, contracting the disease upon travelling to the virus-hit countries (Stage 1); local transmission by coming in contact with patients with a foreign travel history (Stage 2); community transmission with difficulty in tracing the actual source of infection (Stage 3); and ultimately occurrence of an epidemic, wherein the disease spreads at an alarmingly high rate and hence becomes unlikely to be controlled. Italy and

China have unfortunately reached the stage 3 of transmission, wherein the death tolls are constantly increasing with rapidly rising new cases of infection. India is still at stage 2 of COVID-19 outbreak and hence the disease transmission can be restricted by adopting proper quarantine and isolation measures (WHO, 2020; Jiang et al., 2020).

SARS-CoV-2 possesses a high magnitude of risk owing to its massive transmission rate (~3%), high case fatality rate (~4.3 − 11%, however the fatality rate may change), longer half life of virus (4-72 hours), nosocomial mode of transmission (~79% transmission in hospitals) and asymptomatic mode of transmission (~2-14 days of incubation). The most common symptoms of COVID-19 include fever, malaise, nasal congestion, dry cough, sore throat, dyspnoea, diarrhoea and multiple organ complications. However, some people serve as asymptomatic carriers of the disease. Such asymptomatic cases of COVID-19 are the most difficult to diagnose and thereupon treat. Although the defined symptoms appear to be mild, however, there have been reported illnesses ranging from mild to severe conditions, and even death (Huang et al., 2020; Kim, 2020; Ralph et al., 2020). Despite several research efforts, there are yet no specific antiviral medications and vaccines available for fighting with COVID-19. Many ongoing clinical trials are currently being conducted to identify the most propitious drug candidate against COVID-19. The most acclamatory way of identifying the propitious drug candidates for COVID-19 depends on understanding the pathophysiology of SARS-CoV-2 (Guo et al., 2020).

The first step of attachment and entry of Coronaviruses is dependent on the binding of SARS-CoV-2 spike glycoprotein (S2) to cellular receptors (Angiotensin converting enzyme 2, ACE2) of the host. Secondly, after entry into the host cell, the virus starts replicating with the aid of viral nuclease (NSP15 endoribonuclease) and protease (Main Protease 3CLpro). All these said viral virulence factors are vital for the viral life cycle (Liu et al., 2020). Hence, unraveling the pathogenesis of these virulence factors might provide insights into the etiology of COVID-19 and reveal therapeutic targets (**Fig. 1**).

Although, the structure and sequence of these viral virulence factors are known and drug screening is continuously being conducted by targeting these virulence factors. However, yet there are no approved drugs for effectively managing COVID-19 infection. WHO has recently announced restricted use permission for repurposed anti-HIV, anti-malarial, anti-flu and anti-Ebola drugs (Guo et al., 2020; Senathilake et al., 2020). Considering such a considerable emergency of this outbreak, the current *in silico* study is aimed at investigating the possibilities of a glucose anti-metabolite, 2-deoxy-D-glucose (2-DG) as a repurposed drug for the treatment of novel SARS-CoV-2 virus. Post entry of virus, the host cells have been observed to undergo metabolic reprogramming to meet the increased demand of nutrients and energy for replication of the virus, wherein 2-DG might serve as a probable drug candidate as it acts as a dual inhibitor of glycolysis as well as glycosylation (Gualdoni et al., 2018). 2-DG has already been granted permission for clinical trials, as evidenced from previously published results (Mohanti et al., 1996; Vijayaraghavan et al., 2006; Dwarkanath et al., 2009).

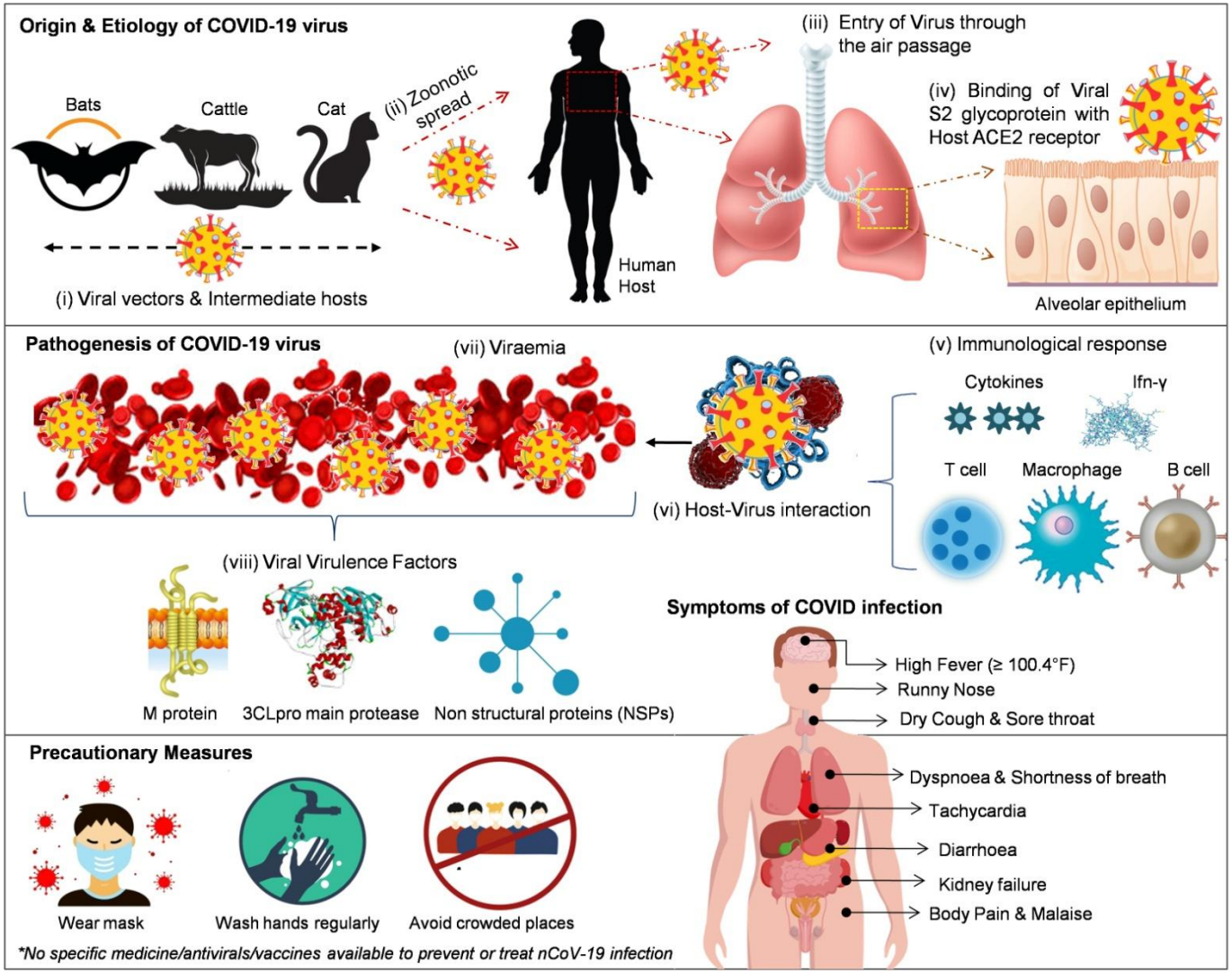


Fig. 1. Etiology, Transmission Patterns and Pathogenesis of Novel Coronavirus (nCoV-19).

(i) Bats are possibly the primary reservoir of nCoV-19. Cattle and cats also may serve as intermediate hosts; (ii) SARS-CoV-2 may originate from bats or unknown intermediate hosts and zoonotic disease spread occurs wherein the virus crosses the species barrier and enters human system; (iii) Virus infects the tracheal and bronchial epithelial cells and spreads to lung parenchymal cells, bronchioles and pneumocytes; (iv) Viral entry is facilitated by binding of viral envelope glycoprotein (S2) with host ACE2 receptor; (v) Viral infection elicits immunological response, wherein inflammatory cytokines and interferon gamma are produced. Additionally, T cell, macrophage and b cell gets activated; (vi) However, nCoV-19 interacts with the host cell and gets hold of its machinery and ultimately destructs the host cell; (vii) nCoV-19 replicates and spreads via the blood endothelial system, ultimately causing viraemia (high viral load in blood and bodily fluids); (viii) nCoV-19 produces and expresses virulence factors, namely, M protein (transmembrane protein for nutrient transport), 3CLpro main protease (enzyme for cleaving viral proteins, mainly responsible for capsid formation), and non structural proteins (NSP1-NSP16 responsible for other viral functions).

In the present study, the drug-like potential of 2-DG will be studied by targeting SARS-CoV-2 spike glycoprotein (S2), viral nuclease (NSP15 endoribonuclease) and protease (Main Protease 3CLpro). The binding mechanism of 2-DG with the said viral virulence factors will be assessed by means of *in silico* molecular docking as well as pharmacophore modeling. Moreover, another tetra-acetate glucopyranose derivative of 2-DG (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) has also been assessed for studying its binding affinities with the said viral virulence factors. The rationale for selecting this tetra-acetate glucopyranose derivative as probable antiviral drug is dependent on its activity of impairing glycolysis and glycosylation. Hence, this derivative can possibly be used as a prodrug for 2-DG (Jeon et al., 2020; Pajak et al., 2020). One such prodrug of 2-DG, namely, 3,6-di-O-acetyl-2-deoxy-d-glucose has been developed in Dr. Waldemar Priebe's laboratory. This compound is currently being tested as an antiviral drug for targeting the novel Coronavirus (Priebe et al., 2018; Keith et al., 2019; Pajak et al., 2020). Similar plan of repositioning 2-deoxy-D-glucose and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose has been presented in the present study, wherein all the molecular interactions of 2-DG and 2-DG derivative have been compared with the currently used anti-retroviral drugs, *i.e.*, lopinavir; anti-flu drug, *i.e.*, favipiravir; and anti-malarial drug, *i.e.*, hydroxychloroquine. The detailed molecular interactions and probable modes of action of 2-DG and its prodrug have also been discussed in the present manuscript.

2. Materials and Methods

Conduction of the present *in silico* study has been made possible by the assistance of several databases including PubChem (https://pubchem.ncbi.nlm.nih.gov/), RCSB Protein Data Bank (https://www.rcsb.org/) and Proteins Plus Server (https://proteins.plus/); and softwares like Argus lab (http://www.arguslab.com/arguslab.com/ArgusLab.html), Molinspiration (https://www.molinspiration.com/), Open Babel (http://openbabel.org), Hex (http://hex.loria.fr/), and Toxicity Estimation Software Tool (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test). PubChem is an open chemistry database that provides two-dimensional chemical information about the ligands being used in this study (Butkiewicz et al., 2013). The RCSB Protein Data Bank is a global archive of three-dimensional structural data of biomolecules, per say viral receptors in this study (Rose et al., 2015). Proteins Plus server is a common online server for computational drug modeling, wherein one of its counterparts, namely, Pose View is used to visualize receptor structures and create pose depictions of ligand-receptor binding. Moreover, another counterpart of Proteins Plus server, namely, DoG Site Scorer is used to predict the active binding sites and druggability of binding pockets of receptors (Fährrolfes et al., 2017; Volkamer et al., 2012). Argus lab is molecular modeling software which is mainly used to visualize the receptors as well as ligands and customize both of them for docking (Joy et al., 2006). Molinspiration is online chemiinformatics software focused on calculating the molecular properties of ligands and predicting their bioactivity properties (Jarrahpour et al., 2012). OpenBabel is an open platform for inter-converting chemical file formats, thereby aiding in converting the 2D structure of ligands to 3D pdb structure and hence

customizing them for molecular docking (Samdani & Vetrivel, 2018). Hex is an interactive molecular docking program for calculating the binding energies of interaction between receptors and ligands (Ritchie & Venkatraman, 2010). Toxicity Estimation Software Tool is a Quantitative Structure Activity Relationships (QSAR) which is used to estimate the toxicity of ligands based on the molecular descriptors of the ligands (Barron et al., 2012).

2.1 Preparation of 3D structure of viral virulence factors as receptors

The crystal structures of SARS-CoV-2 spike glycoprotein (S2; PDB code: 6VSB), viral nuclease (NSP15 endoribonuclease; PDB code: 6VWW) and protease (Main Protease 3CLpro; PDB code: 1Q2W) were obtained from RCSB Protein Data Bank (https://www.rcsb.org/). Hydrogen atoms were introduced in all these 3D structures using Argus Lab (4.0.1), so as to customize the viral receptors for rigid docking (http://www.arguslab.com/arguslab.com/ArgusLab.html).

2.2 Preparation of 3D structure of 2-DG and 2-DG derivative as ligands

The structure of 2-deoxy-D-glucose and 2-DG derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) were downloaded in xml format from PubChem database and structures were validated (Butkiewicz et al., 2013). Hydrogen atoms were introduced into the ligands structure using Argus lab (4.0.1), so as to customize them for rigid docking. The hydrogenated ligand molecules were then converted into pdb format using Open Babel (2.4) interface (openbabel.org/docs/dev/OpenBabel.pdf), as required for rigid docking. Similarly, 3D structures of standard chemotherapeutic agents (lopinavir, favipiravir, hydroxychloroquine) were also customized for docking.

2.3 Active site analysis of viral virulence factors

DoG Site Scorer, a web based tool (https://proteins.plus/), was used to predict the possible binding sites in the 3D structure of spike glycoprotein, viral nuclease and viral main protease. Predictions with DoG Site Scorer were based on the difference of gaussian filter to detect potential pockets on the protein surfaces and thereby splitting them into various sub-pockets. Subsequently, global properties, describing the size, shape and chemical features of the predicted pockets were calculated so as to estimate simple score for each pocket, based on a linear combination of three descriptors, *i.e.*, volume, hydrophobicity and enclosure. For each queried input structure, a druggability score between 0-to-1 was obtained. Higher the druggability score, higher the physiological relevance of the pocket as potential target (Volkamer et al., 2012).

2.4 Molecular Docking and Ligand Receptor Binding analysis

The docking analysis of pdb structures of 2-deoxyglucose and its analogue (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) with viral receptors (spike glycoprotein, viral nuclease and viral main protease) was carried by Hex Cuda 8.0.0 software. Receptor and Ligand files were imported in the software (Harika et al., 2017). The grid dimension of docking was defined

according the to the binding site analysis of DoG Site Scorer (Volkamer et al., 2012). Graphic settings and Docking parameters were customized so as to calculate the binding energies (E values) of ligand receptor docking. The parameters used for the docking process were set as (i) Correlation type: Shape + Electro + DARS, (ii) FFT mode: 3D fast lite, (iii) Grid Dimension: 0.6, (iv) Receptor range: 180°, (v) Ligand range: 180°, (vi) Twist range: 360°. The best docked conformations with lowest docking energy were selected for further MD simulations using Pose View for creating pose depictions of selected ligand-receptor binding (Ezat et al., 2014). Molecular Docking and MD simulations for the standard chemotherapeutic agents (lopinavir, favipiravir, hydroxychloroquine) were also conducted. The MM-PBSA method was used to compute the binding free energy of receptor-ligand docking during simulation. In this study, the binding free energy of the receptors to ligands was calculated using the GROMACS tool, wherein the binding free energy of the receptor and ligand was defined as

$\Delta G_{binding} = \Delta G_{complex} - (\Delta G_{receptor} + \Delta G_{ligand})$

For each subunit, the free energy, G, can be presented as summation of mechanical potential energy (Electrostatic and Vander Waals interaction) and solvation free energy ($G_{polar} + G_{nonpolar}$), wherein the total entropy is excluded from the total value (Weis et al., 2006).

2.5 Molinspiration based Molecular property and Bioactivity analysis

Molinspiration software was used to analyze molecular descriptors and bioactivity scores of the ligands and standard chemotherapeutic agents, namely, MiLog P, Total polar surface area (TPSA), molecular weight, number of atoms, number of rotatable bonds, number of hydrogen bond donors and acceptors. Bioactivity of the ligands was also checked by using Molinspiration which can analyze the activity score of GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors. Ligands were loaded in the Molinspiration software in SMILES format and the molecular descriptor as well as bioactivity analysis was conducted (Jarrahpour et al., 2012).

2.6 *In silico* Toxicity estimation

Ligands (2-DG and 2-DG derivative) and standard chemotherapeutic agents (lopinavir, favipiravir, and hydroxychloroquine) were uploaded in the Toxicity Estimation Software Tool in sdf format. Oral rat LD_{50}, Bioconcentration Factor, Developmental Toxicity and Ames Mutagenicity were estimated using consensus method of QSAR analysis (Barron et al., 2012).

3. Results & Discussion

3.1 Active site analysis

Active site analysis of SARS-CoV-2 spike glycoprotein (S2), viral nuclease (NSP15 endoribonuclease) and protease (Main Protease 3CLpro) as conducted by DoG Site Scorer indicated that there are various active pockets within the studied viral virulence factors with druggability ranging from 0.12 to 0.86 (**Table 1**).

Table 1. Identified active pockets and corresponding pocket area, volume, enclosure and druggability score for Coronavirus SARS-CoV-2 virulence factors.

Viral Virulence Factor (PDB ID)	Total Pockets	Active pockets†	Area ($Å^2$)	Volume ($Å^3$)	Enclosure (Å)	Hydrophobicity (Kcal/ $Å^2$)	Drug Score
Spike glycoprotein (6VSB)	106	P_11	807.13	662.19	0.06	0.27	0.847823
		P_7	1027.43	885.73	0.16	0.31	0.831111
		P_9	792.72	781.17	0.08	0.24	0.824138
		P_10	987.15	728.29	0.08	0.44	0.822958
		P_6	1194.95	1024.53	0.17	0.42	0.815125
NSP15 endoribonuclease (6VWW)	31	P_1	787.38	683.48	0.06	0.24	0.860743
		P_0	712.51	685.95	0.08	0.25	0.851172
		P_3	561.74	467.99	0.23	0.46	0.782467
		P_2	832.74	507.64	0.14	0.54	0.765538
		P_5	621.35	344.13	0.07	0.45	0.70914
Main Protease 3CLpro (1Q2W)	11	P_0	2867.97	2443.71	0.12	0.33	0.805165
		P_1	589.09	364.2	0.2	0.59	0.753192
		P_2	341.62	297.14	0.25	0.48	0.479914
		P_4	430.51	222.15	0.25	0.44	0.478243
		P_3	373.5	237.35	0.25	0.25	0.464209

†Only the most active pockets have been presented with a high druggability score; Shaded rows indicate the most druggable pockets which will be further employed for docking studies.

It was found that pockets P_11 (Drug score: 0.847), P_1 (Drug score:0.860) and P_0 (Drug score: 0.805) were energetically favourable for performing further molecular docking studies with the viral receptors being spike glycoprotein, NSP15 endoribonuclease and Main Protease 3CLpro, respectively. While conducting the active site analysis, the DoG Site Scorer tool analysed the heavy atom coordinates on the surface of the 3D structure of the respective viral receptors. Depending on these atomic coordinates, a hypothetical grid was spanned by outruling the chances of any spatial overlap of the grid with the heavy atoms. Furthermore, the tool engages in applying a Gaussian filter to the defined grids, so as to identify spherical pockets of binding. Druggability score (0-1) of the selected spherical pockets are deduced on the basis of their surface area, volume, enclosure and hydrophobicity. As a general rule, higher druggability score is indicative of a more druggable pocket (Volkamer et al., 2012). The most druggable pockets of SARS-CoV-2 spike glycoprotein, NSP15 endoribonuclease and main protease 3CLpro have been elucidated in **Fig. 2**.

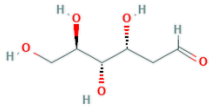
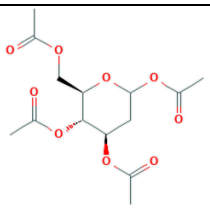
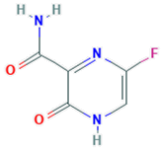
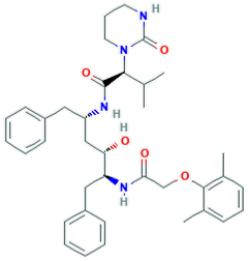
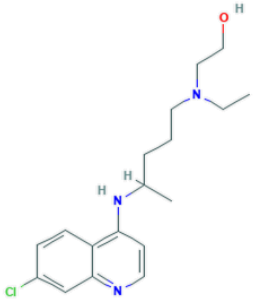
3.2 Molecular Docking

Docking results of the viral virulence factors, namely, spike glycoprotein, NSP15 endoribonuclease and Main Protease 3CLpro; and the drug 2-deoxy-D-glucose (2-DG) as well as 2-DG derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) are shown in **Table 2**. These docking based E values have also been compared with that of the standard drugs (lopinavir, favipiravir and hydroxychloroquine). The Hex based docking results reveal that the E-value of docking of 2-DG with viral main protease 3CLpro ($E\ value_{2\text{-}DG\ +\ Protease}$ = -140.05

Kcal/mol) was found to be better than that of the standard drug lopinavir (E value$_{\text{Lopinavir + Protease}}$ = -124.00 Kcal/mol). Similarly, the docking of 2-DG with viral endoribonuclease also yielded significantly better binding energies (E value$_{\text{2-DG + Endoribonuclease}}$ = -168.65 Kcal/mol) as compared to that of the standard drug favipiravir (E value$_{\text{Favipiravir + Endoribonuclease}}$ = -128.00 Kcal/mol). However, the binding energy of 2-DG with that of spike glycoprotein (E value$_{\text{2-DG + Spike glycoprotein}}$ = -118.31 Kcal/mol) was found to be moderately lower as compared to that of the tested standard drugs. It is obvious from the E-values that 2-deoxy-D-glucose binds spontaneously and irreversibly to main protease 3CLpro and viral endoribonuclease, wherein the binding efficiency of 2-DG has been found to be exceedingly better than that of lopinavir and favipiravir. Such significant binding affinity of 2-DG with that of SARS-CoV-2 viral receptors presumably indicates the probable mechanism of action of 2-deoxy-D-glucose as viral protease and endoribonuclease inhibitor. Viral protease is fundamental for continuing the viral life cycle of SARS-CoV-2 as it is required by the virus to catalyze the cleavage of viral polyprotein precursors which are ultimately necessary for viral capsid formation and enzyme production (Anand et al., 2003). Similarly, viral endonucleases are necessary for catalyzing the processing of viral RNAs and hence are required for enduring the process of viral replication (Ward et al., 2020). Henceforth, the 2-deoxy-D-glucose moiety contingently inactivates the viral protease, thereby inhibiting the process of viral capsid formation. Furthermore, 2-DG may also be responsible for withholding the action of viral endoribonuclease, thereby halting the process of viral replication altogether.

Moreover, the 2-DG derivative, namely, 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose also showed an increase in the free energy of the complex with the viral receptors. The E-value of docking of 2-DG derivative with viral main protease 3CLpro (E value$_{\text{2-DG derivative + Protease}}$ = -187.64 Kcal/mol) was found to be better than that of the standard drug lopinavir (E value$_{\text{Lopinavir + Protease}}$ = -124.00 Kcal/mol). Similarly, the docking of 2-DG derivative with viral endoribonuclease (E value$_{\text{2-DG derivative + Endoribonuclease}}$ = -208.33 Kcal/mol) as well as spike glycoprotein (E value$_{\text{2-DG derivative + Spike glycoprotein}}$ = -173.89 Kcal/mol) yielded significantly better results as compared to that of favipiravir, wherein its E value is lower in both cases, *i.e.*, E value$_{\text{Favipiravir + Endoribonuclease}}$ = -128.00 Kcal/mol; and E value$_{\text{Favipiravir + Spike glycoprotein}}$ = -118.31 Kcal/mol. The 2-DG derivative exhibited significantly better binding values as compared to that of 2-DG itself. The derivative displayed spontaneous binding efficiencies while docking with viral protease, viral endonuclease and spike glycoprotein. The binding energy of 2-DG derivative was found to be comparable to that of hydroxychloroquine which has been proposed as the cornerstone for COVID-19 therapy. Hence, 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose could presumably mitigate the virus completely as it could restrict viral entry into the host cell by inactivating the spike glycoprotein; halt viral capsid formation by inactivating the viral main protease; and cease viral replication by inactivating the viral endoribonuclease. Earlier studies have also indicated that glucopyranose derivatives are glycolysis inhibitors and cause mitochondrial oxidative phosphorylation, thereby indicating a probable antiviral role of 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose (Jeon et al., 2020).

Table 2. Molecular Docking of 2-deoxy-D-glucose and its derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) with SARS-CoV-2 viral virulence factors.

Ligand / Standard	Chemical structure†	E Value (Kcal/mol)		
		Main Protease 3CLpro	Spike glycoprotein	NSP15 endoribonuclease
2-Deoxy-D-Glucose		-140.05	-118.31	-168.65
1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose		-187.64	-173.89	-208.33
Favipiravir		-267.00	-127.33	-128.00
Lopinavir		-124.00	-277.34	-305.84
Hydroxychloroquine		-235.48	-207.47	-213.54

†Chemical structures have been derived from PubChem (https://pubchem.ncbi.nlm.nih.gov/); $\Delta G_{binding} = \Delta G_{complex} - (\Delta G_{receptor} + \Delta G_{ligand})$

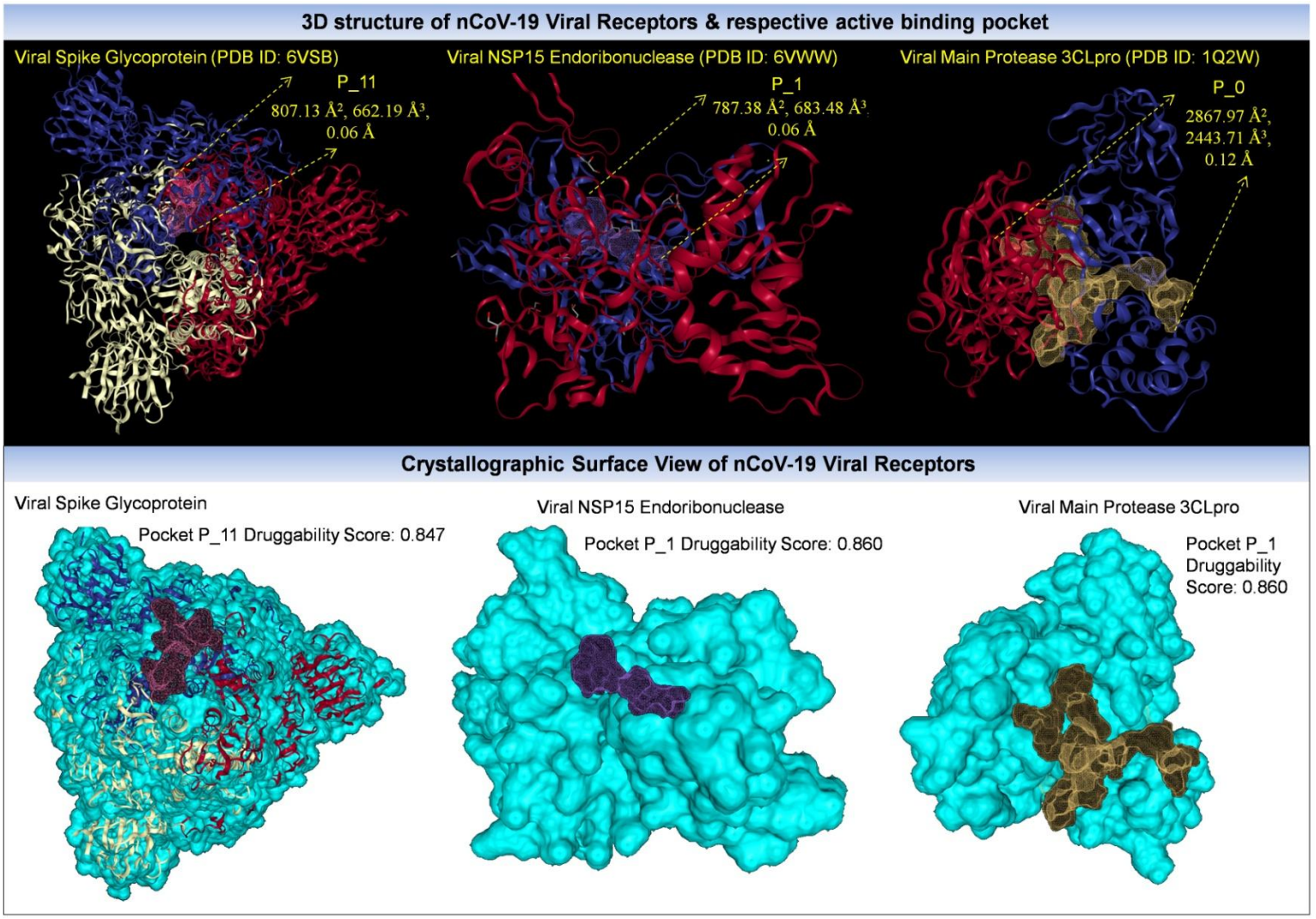


Fig. 2. Active site analysis of nCoV-19 viral receptors as deduced using DoG Site Scorer
Active drug binding pockets of viral spike glycoprotein (Pocket P_11), NSP15 endoribonuclease (Pocket P_1) and main protease 3CLpro (Pocket P_0). Druggability scores and crystallographic structure of the binding pocket has also been elucidated.

3.3 Ligand Receptor binding pose depictions

The best docking pose of 2-DG, and its derivatives with SARS-CoV-2 viral receptors was also identified using Pose View tool so as to visualize the interactions of the ligands with that of the residues present in the active sites of the viral receptors. Both 2-deoxy-D-glucose and its derivative were found to form salt bridges with the amino acid residues of the viral receptors, namely, main protease 3CLpro and viral spike glycoprotein, respectively. The orientational binding of the ligands and the viral receptors showing the pose view and residue interactions have been depicted in **Fig. 3**. It was observed that the hydroxyl group of 2-deoxy-D-glucose formed a hydrogen bond with the carbonyl residue of Proline amino acid (108^{th} position) found in the viral main protease. In earlier studies it has been found that the proline amino acid residues

are found in the conserved domains of HIV viral infectivity factor (Vif) and these proline-rich motifs are therapeutic targets for neutralizing the human immunodeficiency virus (Yang et al., 2003; Ralph et al., 2020). Chemical bridging of 2-deoxy-D-glucose and proline residues of viral main protease 3CLpro present a similar case where proline residues were invariably bound and neutralized, thereby possibly neutralizing the COVID-19 virus. Similarly, the 2-DG derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) formed a hydrogen bond with the amide group of Glutamine amino acid (804^{th} position) found in the viral spike glycoprotein. Reynard and Volchkov have also previously highlighted that mutation or any change in the glutamine residues of Ebola virus spike glycoprotein causes viral neutralization (Reynard & Volchkov, 2015). In conclusion, the binding interactions of 2-deoxy-D-glucose with viral main protease and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose with viral spike glycoprotein is now evident, as analysed by using Pose View tool.

2-deoxy-D-glucose and its derivative can influence several cellular pathways, including glycolysis, glycosylation, endoplasmic stress response (ER), phagocytosis and apoptosis. Both the moieties inhibit the processes of glucose transport and glycolysis by competing with glucose. Competitive uptake of 2-DG or its derivative in the infected cell leads to the formation of 2-deoxy-d-glucose-6-phosphate (2-DG-6-P) by means of hexokinase enzyme. 2-DG-6-P cannot be further metabolized, thereby hampering the bioenergetic process of ATP production by glycolysis (Sharma et al., 1996); inactivating the glycolytic enzymes; inducing cell cycle arrest and ultimately leading to inactivation of nCoV-19 in infected cells (Maher et al., 2004; Pajak et al., 2020). The depletion of ATP levels leads to activation of AMP-activated protein kinase (AMPK). Such activation will lead to phosphorylation of proteins of the mTOR kinase complex (mammalian target of rapamycin kinase, mTORC). As a consequence, expression of p53 is induced which ultimately promotes cell cycle arrest (G1 phase arrest) in virus infected cells. All these factors (glycolysis inhibition, ATP depletion and cell cycle arrest) cause a sensitized response leading to the upregulation of TNF expression, ultimately leading to an apoptotic response. Moreover, both 2-DG and its tetra-acetate glucopyranose derivative escalate the production of reactive oxygen species, ultimately leading to virus infected cell death (**Fig. 4**) (Zhang et al., 2015; Pajak et al., 2020).

3.4 Molecular property analysis

After analyzing the binding energies and ligand-receptor binding pose depictions, it was requisite to evaluate the drug likeliness of the ligands. Analysis of molecular descriptors is necessary in elucidating the pharmacokinetic parameters of the drugs such as absorption, distribution, metabolism, and excretion. Molinspiration software was used to analyze the Lipinski Rule of Five, including the Log P value (partition coefficient), molecular weight, polar surface area, number of hydrogen bond donor and number of hydrogen bond acceptor. According to the Lipinski's rule, a drug like moiety should have a low molecular weight ($\leq$ 500 D), log P value $\leq$ 5, number of hydrogen bond acceptors $\leq$10, and number of hydrogen bond donors $\leq$5. A bioactive druggable molecule should ensue to at least 4 of the 5 Lipinski rules (Zhang &

Wilkinson, 2007). In the present study, it was found that 2-deoxy-D-glucose and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose befalls within the said permissible limits of Lipinski rules and hence, both these drugs can be said to possess satisfactory oral bioavailability (**Table 3**).

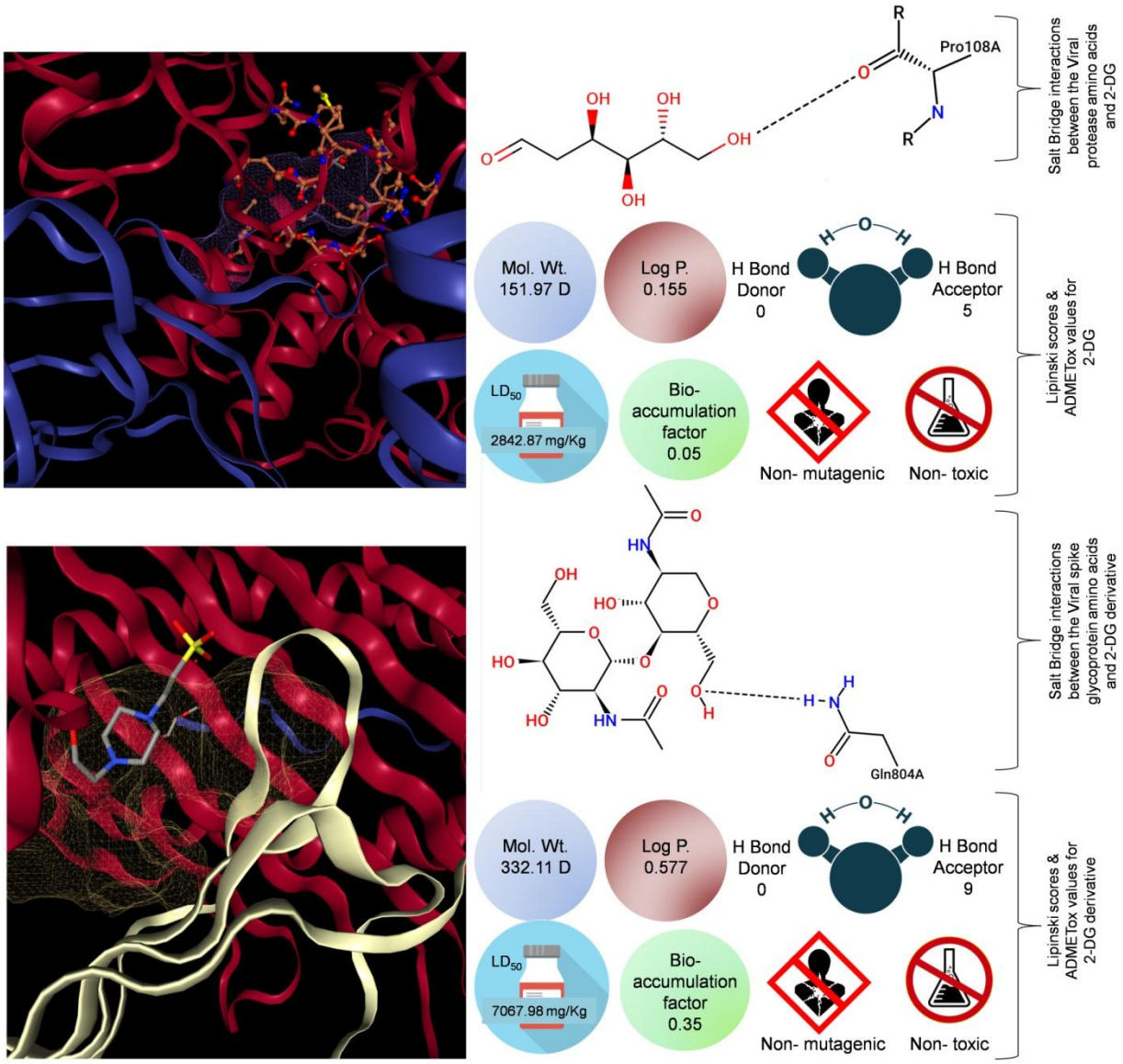


Fig. 3. Binding pose depictions, Lipinski scores and *in silico* ADMETox values for probable drug candidates (2-deoxy-D-glucose and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) targeting nCoV-19 viral receptors

Hydroxyl group of 2-deoxy-D-glucose (2-DG) formed a hydrogen bond with the carbonyl residue of Proline amino acid (108th position) found in the viral main protease 3CLpro. 2-DG sufficed to all the Lipinski rules and showed no signs of toxicity. Moreover, 2-DG derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) formed a hydrogen bond with the amide group of Glutamine amino acid (804th position) found in the viral spike glycoprotein. 2-DG derivative also sufficed to all the Lipinski rules and showed negligible signs of toxicity.

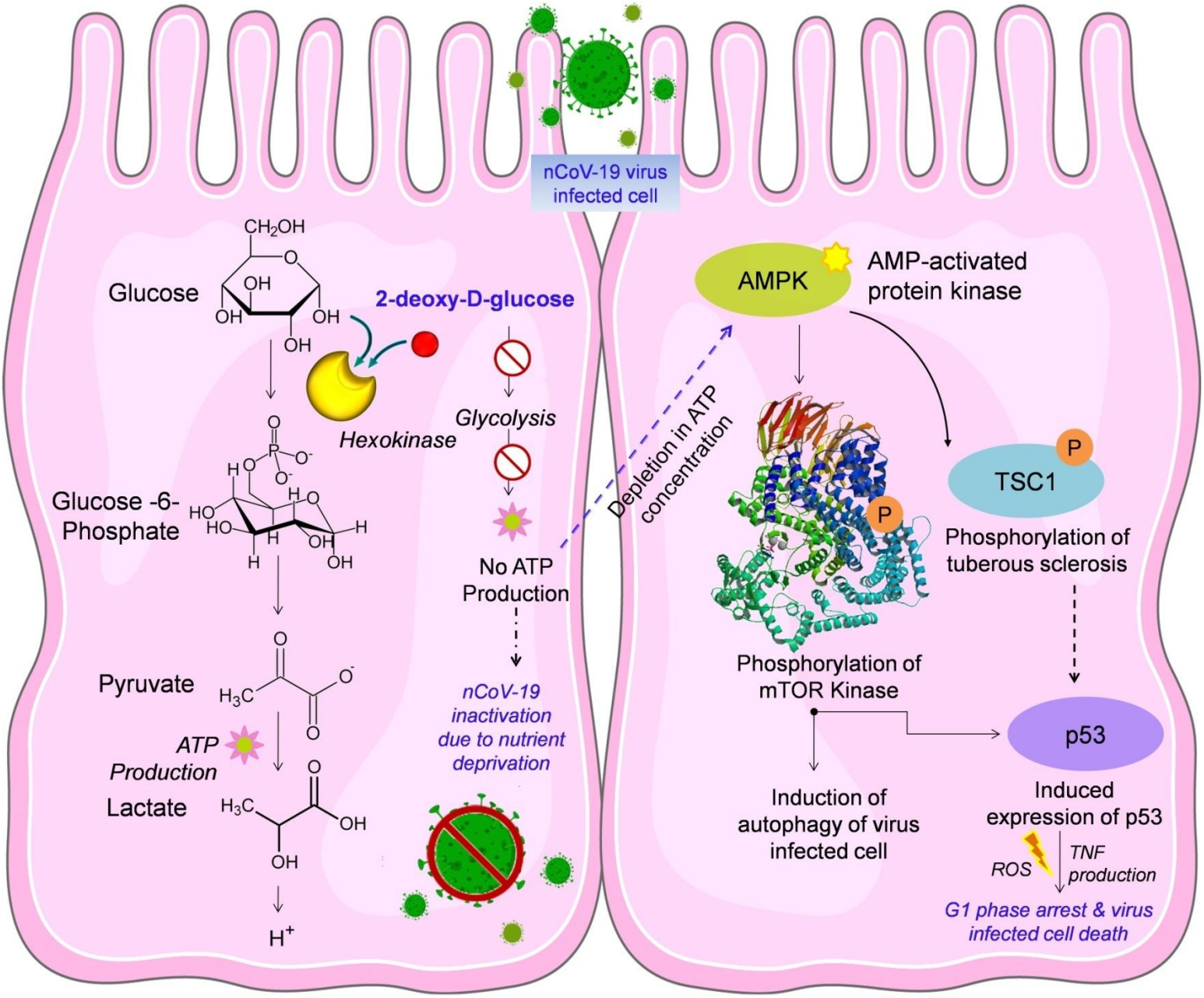


Fig. 4. Molecular mode of action of 2-deoxy-D-glucose as an anti-COVID-19 remedy

2 deoxy-D-glucose (2-DG) enters the cell where it competitively replaces glucose and binds hexokinase, thereby inhibiting the process of glycolysis and further ATP production in virus infected cells. Such nutrient and ATP deprivation causes the inactivation of nCoV-19. Moreover, the depletion of ATP and excess of AMP in virus infected cells triggers the activation of AMP activated protein kinase, which in turn causes phosphorylation of mTOR (mammalian target of rapamycin) kinase and tuberous sclerosis proteins (TSC1). This also results in inducing the expression of p53 which arrests the cell cycle at G1 checkpoint. Moreover, 2-DG also activates the production of reactive oxygen species (ROS) and tumour necrosis factor (TNF) mainly responsible for eliciting autophagy of virus infected cells, thereby controlling the spread of infection.

Table 3. Physicochemical properties of 2-deoxy-D-glucose and its derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) in comparison with the standard chemotherapeutic agents.

Ligand/ Standard	Physicochemical Properties				
	Mol. Wt. (≤ 500 D)	Log P (≤ 5)†	H-Bond Donor (≤ 5)	H-Donor Acceptor (≤ 10)	Lipinski violations (if any)
2-Deoxy-D-Glucose	151.97	0.155	0	5	0
1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose	332.11	0.577	0	9	0
Favipiravir	157.03	-0.822	2	5	0
Lopinavir	628.36	3.688	4	9	1
Hydroxychloroquine	335.88	4.00	4	2	0

†Logarithm of compound partition coefficient between *n*-octanol and water.

3.5 Bioactivity analysis

Molinspiration was used to virtually screen the biological activity of drug moieties, per say 2-DG and 2-DG derivative in the present study. The fundamental principle behind this *in silico* bioactivity screening is the identification of substructure(s) responsible for endowing pharmacological features (GPCR binding ability, ion channel modulation potential, kinase inhibition activity, nuclear receptor binding potential, and protease inhibition) to the drug molecules being studied. The bioactivity score of the ligands and standard chemosynthetic moieties is presented in **Table 4**. In general, if the bioactivity score for a particular target is more than 0.0, then the said drug moiety is considered to be highly active. Additionally, a bioactivity score of a ligand lying between -5.0 and 0.0 is considered to be moderately active. However, bioactivity scores of ligands below -5.0 render it to be inactive (Singh et al., 2013). As observed in Table 4, the bioactivity scores of 2-DG for most of the bioactivity descriptors were below -0.5, thereby indicating its inactivity towards those targets. However, 2-DG possessed moderate bioactivity as ion channel modulator (Bioactivity score $_{\text{Ion channel modulator}}$ ~ -0.14) and protease inhibitor (Bioactivity score $_{\text{Protease inhibitor}}$ ~ -0.37). This bioactivity score of 2-DG is in corroboration with the molecular docking results which also suggest 2-DG to be a significant protease inhibitor ($E_{\text{2-DG + Protease}}$ = -140.05 Kcal/mol). The antiviral effect of 2-DG has also been recognized in previous studies. Inhibition of multiplication has been reported for some enveloped viruses such as influenza virus, sindbis virus, semliki forest virus, herpes simplex virus, respiratory syncytial virus and measles virus (Kang & Hwang, 2006; Krol et al., 2017). Furthermore, 2-DG eliminated genital herpes from most of the tested patients. It also alleviated the severity of infection of calves with respiratory syncytial virus and infectious of bovine rhino-tracheitis virus (Leung et al., 2012). According to all these earlier studies, inhibition of viral envelope biosynthesis and virion assembly due to blocked glycosylation of membrane proteins appears to be the major mechanism of 2-DG for virus attenuation. This has been supported by altered gel electrophoresis profiles of membrane proteins as well as denuded appearance of

budding particles shown by electron microscopy. Studies also suggest that 2- DG can also suppress viral gene expression or viral replication (Camarasa et al., 1986; Kang & Hwang, 2006; Leung et al., 2012; Krol et al., 2017).

Furthermore, the bioactivity score of 2-DG derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) suggested that it mainly acts as a GPCR ligand (Bioactivity score $_{\text{GPCR ligand}}$ ~ 0.13), ion channel modulator (Bioactivity score $_{\text{Ion channel modulator}}$ ~ 0.04) and protease inhibitor (Bioactivity score $_{\text{Protease inhibitor}}$ ~ 0.17). In alliance with the bioactivity analysis, molecular docking data of 2-DG derivative has also suggested it to be a significant protease inhibitor ($E_{\text{2-DG derivative + Protease}}$ = -187.64 Kcal/mol).

Table 4. Bioactivity score of 2-deoxy-D-glucose and its derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) in comparison with the standard chemotherapeutic agents.

Ligand/ Standard	Bioactivity Descriptors				
	GPCR ligand	**Ion channel modulator**	**Kinase inhibitor**	**Nuclear receptor ligand**	**Protease inhibitor**
2-Deoxy-D-Glucose	-0.72	-0.14	-1.01	-0.78	-0.37
1,3,4,6-Tetra-O-acetyl-2-deoxy-D-glucopyranose	0.13	0.04	-0.11	-0.04	0.17
Favipiravir	-0.62	-0.44	-0.31	-1.50	-0.91
Lopinavir	0.04	-0.78	-0.55	-0.66	0.42
Hydroxychloroquine	0.35	0.30	0.44	-0.12	0.12

Grey Shaded rows indicate the highly active status of the respective moiety for a given descriptor.

Blue shaded rows indicate the moderately active status of the respective moiety for a given descriptor

3.6 Toxicity estimation

In silico toxicity estimation of the drug moieties (2-DG and 2-DG derivative) was conducted by using Toxicity Estimation Software Tool (T.E.S.T) which predicts the key toxicity parameters (Rat acute dose LD_{50}, bioaccumulation factor, developmental toxicity and Ames mutagenicity) on the basis of the chemical structure. The fundamental principle behind such toxicological assessment is quantitative structure activity relationship (QSAR) as generated on the basis of OECD datasets. Computational assessment of these toxicity parameters also aids in predicting the probable side effects of the test compounds (Barron et al., 2012). In the present study, the tested ligands (2-deoxy-D-glucose and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose did not show any major signs of toxicity or side effects as shown in **Table 5**. In earlier studies that has been carried out in animals and humans have proved that 2-deoxy-D-glucose and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose are safe to be administered by all routes of administration (Mohanti et al., 1996; Singh et al., 2005; Vijayaraghavan et al., 2006). Moreover, 2-DG has already been tested for its efficacy as radio-therapeutic and cytotoxic chemotherapeutic targeting pancreatic, breast, ovarian and lung cancer. However, its short half life and very low bioaccumulation factor limits the utility of 2-DG. Certain contraindications and adverse drug reactions have also been found to be associated with higher doses of 2-DG. These

complications include fatigue, dizziness, nausea and hypoglycemia. However, in previous studies conducted on brain tumor patients (glioblastoma), it was observed that most of the side effects of oral administration of 2-DG (upto doses of 250 mg /kg b.w.) are transient and reversible (Singh et al., 2005). Additionally, the said contraindications can be surmounted by using 2-DG derivatives as prodrugs. Taking this into account, tetra-acetate glucopyranose derivative of 2-DG can be used as a prodrug to improve 2-DG's pharmacokinetics, bioavailability and its drug-like properties (Pajak et al., 2020).

Table 5. ADMETox prediction for 2-deoxy-D-glucose and its derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) in comparison with the standard chemotherapeutic agents.

Ligand/ Standard	**Toxicity Descriptors**				
	Oral Rat LD_{50} (≥ 100 mg/Kg)	**Bioaccumulation Factor (≤1)**	**Developmental Toxicity (≤ 0.5)**	**Mutagenicity (≤ 0.5)**	**Inference**
2-Deoxy-D-Glucose	2842.87	0.05	0.42	0.43	Non Toxicant
1,3,4,6-Tetra-O-acetyl-2-deoxy-D-glucopyranose	7067.98	0.35	0.69	0.32	Very Mild Developmental Toxicant
Favipiravir	1310.93	0.34	0.54	0.04	Very Mild Developmental Toxicant
Lopinavir	1825.46	0.43	0.93	0.08	Developmental Toxicant
Hydroxychloroquine	1240	0.37	0.62	0.04	Very Mild Developmental Toxicant

4. Conclusion

The biggest challenge in battling the novel coronavirus (nCoV-19) pandemic is the dearth of effective therapeutic regimens. The present *in silico* study was aimed to assess the probable utility of bioactive compounds, 2-deoxy-D-glucose and its derivative 1,3,4,6-Tetra-O-acetyl-2-deoxy-D-glucopyranose against nCoV-19. The most pertinent viral physiological targets (viral spike glycoprotein S2, viral NSP15 endoribonuclease and viral main protease 3CLpro) were selected as receptors for conducting molecular docking analysis. The computer based pharmacophore modeling approach has generated interesting insights into the underlying binding mechanisms of the above-mentioned viral receptors with 2-DG and 2-DG derivative. It is noteworthy that 2-deoxy-D-glucose have shown significant activity towards inactivating the SARS-CoV-2 viral receptors, wherein, the E-value of docking of 2-DG with viral main protease 3CLpro and NSP15 endoribonuclease is significantly better than that of the standard drug lopinavir and favipiravir. Such significant binding affinities of 2-DG result from formation of a salt bridge between the hydroxyl group of 2-deoxy-D-glucose and carbonyl residue of Proline (108^{th} position) found in the viral protease. Similarly, 2-DG tetra-acetate glucopyranose

derivative (prodrug of 2-DG) displayed exceptional binding efficiencies while docking with viral protease, viral endonuclease and spike glycoprotein. The *in silico* bioactivity analysis suggest that both these molecules act mainly as protease inhibitors. Present results also indicate that both 2-DG and 2-DG derivative possess adequate oral bioavailability without any major signs of toxicity or side effects,

In sum, present *in silico* results, taken together with the published empirical findings on the effects of 2-DG on retrovirus infected cell lines and murine model systems, suggest that 2-DG may considerably reduce the infectivity and virulence of nCOVID-19 by inhibiting both the entry and the replication of the virus inside the host cells. To verify this possibility, further basic studies on model systems infected with nCOVID-19 are necessary before human clinical trials can be conducted. In view of the huge global devastation caused by the current viral pandemic and lack of any effective therapy, research work to explore the therapeutic potential of 2-deoxy-D-glucose and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose should be urgently undertaken with well coordinated multi-institutional collaborations.

Author Contributions

AB, VJ and RKS conceived the presented research. PT, SS and SND analyzed the information, generated the artwork, and co-wrote the manuscript. RKS and AV investigated and supervised the findings of the work. VJ and RKS provided critical revision of this article, and approved the manuscript for submission. All authors agreed with the final version of this manuscript.

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Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is an *in silico* study.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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