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Insilco discovery of promising antiviral drug Galidesivir against SARS-CoV2 RNA dependant RNA polymerase (RdRp) through molecular docking

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Abstract
Severe acute respiratory syndrome-corona virus 2 (SARS-CoV-2) is one important cause of global COVID-19 pandemic, resulting lakhs of infections and thousands of mortalities in the world. RNA dependant RNA polymerase (RdRp) is one important enzyme of corona virus, mediates both replication as well as the transcription of the virus, would be primary target for various antiviral drugs. Galidesivir is an adenosine analogue which inhibits the RNA polymerase promises one important antiviral drug candidate against SARS-CoV2.In this study, the binding interaction between protein target and Galidesivir was done under Auto Dock platform The best docked Galidesivir confirmation was found to have the lowest binding energy (-3.73 kcal/mol) and lowest inhibition constant (58.4 M), as well as several amino acid residues in close proximity, including Asp 623, Cys 622, Tyr 619, Lys 621, and Asp 760. This study was concluded that RdRp would be a potential drug target for the promising drug Galidesivir against SARS-CoV-2 in nearest future.

Keywords: Insilco, promising, drug, SARS-CoV2, RNA, RdRp

Introduction
Since 2019, the corona pandemic has had a catastrophic influence on the economy of the nation as well as on human mortality around the globe [1]. The World Health Organization (WHO) has received reports of over 412 million COVID-19 confirmed cases and more than 5 million fatalities to date. Important signs of SARS-CoV-2 infection include fever, cough, exhaustion, sore throat, expectoration, muscle pain, headache, pneumonia, dyspnoea, and multi-organ damage [2]. Moreover, various classes of drugs are being trailed to combat SARS-CoV2 viruses, including inhibitors of virus entry into cells, inhibitors of SARS-CoV-2 RNA, inhibitors of viral 3CL proteases, inhibitors of host factors required for virus infection, and also immunomodulators [3]. The SARS CoV2 RNA polymerase is a crucial enzyme that facilitates viral multiplication and transcription in the host cell, leaving an important therapeutic target [4]. Currently, different RNA polymerase inhibitors such as remdesivir, ribavirin, favipiravir, molnupiravir, and AT-527 have been approved by U.S. Food and Drug Administration (FDA) for treatment of hospitalized patients [5]. Nevertheless, the difficult intravenous administration technique and expensive cost of these drugs precluded their widespread use [6]. Although many SARS-CoV-2 vaccinations have been administered around the world, vaccine breakthrough infections have been commonly documented. Furthermore, immunocompromised individuals are not being fully protected after vaccination, and existing vaccines may also be incapable of combating the emerging SARS-CoV-2 variants [7]. So at this context, there is need of an effective therapeutics to mitigate the negative clinical consequences of SARS-CoV-2 infection.

Galidesivir (BCX4430, immucillin-A) is promising candidate against corona virus which is one an adenosine analogue, developed by BioCryst Pharmaceuticals, initially targeted for hepatitis C, but later used as a potential therapeutic regimen for deadly filovirus infections such as Ebola virus, Marburg virus as well as Zika virus [8]. Moreover, it exhibits broad-spectrum antiviral efficacy against a variety of different RNA virus such as, corona viruses, phlebo viruses, arena viruses, bunya viruses paramyxo viruses, flavi viruses [9]. It also shows good efficacy against Zika virus in mouse model.

Therefore, BioCryst began accepting patients for a randomised, double-blind, placebo-controlled clinical trial of galidesivir in patients with COVID-19 from April 9, 2020 and Galidesivir appears to be safe and well tolerated based on preliminary clinical exposure results [23]. The findings of this trial showed that Galidesivir was generally well tolerated and safe at all dose levels tested and the decline of SARS-CoV2 viral load in respiratory tract of the patient occurred in dose dependant manner. After administration either by IM injection or IV infusion, Galidesiviris is phosphorylated by cellular kinases being converted into active triphosphate (BCX4430-TP or BCX6870) form. After phosphorylation, BCX4430-TP is integrated into the viral RNA, that results in early chain termination [8].

Although the initial clinical trial phase of Galidesivir against corona virus gave satisfactory result, still it is needed for further testing in vivo as well as in vitro. Large sample must be tested in animal models before large scale human application across the world. Therefore, the insilco binding interaction between this drug and the target RdRp protein must be well defined before large clinical trial. There was still no report about the insilco physiochemical characteristics and the binding interaction between RdRp and Galidesivir. Therefore, this study was carried out with objective of unveiling the binding effect of Galidesivir with RdRp on treatment of SARS-CoV2 in nearest future.

Materials and Methods

The research was carried out in Biochemistry Division, Indian Veterinary Research Institute (IVRI), India from November 2022 to January 2023.

Retrieval of RdRp protein and drug compound

The RNA dependant RNA polymerase **(**RdRp) is one important enzyme for viral replication, was retrieved from RCSB protein database with accession number 6M71. The novel drug compound Galidesivir was also retrieved from PubChem database with CID 10445549.

Physiochemical analysis

Physiochemical characteristics of RdRp were performed with ProtoParam tool. The extinction coefficient, AA compositions, MW, pI, absorbance, instability index (II), aliphatic index (AI) and grand average of hydropathicity (GRAVY) were determined [11].

Secondary structure prediction

The secondary structure of RdRp (Alpha helix, Beta Bridge, Beta turn, extended strand and Random coil) was determined through GORIV prediction tool [12].

Molecular docking

In this study, Galidesivir and the drug target RNA dependant RNA polymerase (RdRp) were molecularly docked in AutoDock 4.2 (http://autodock.scripps.edu/) platform [13]. To prepare the macromolecule and the ligands for docking, the ADT tool was used to add Kollman charges and polar hydrogen atoms to the macromolecule and the ligands. The target protein's drug binding cavity was surrounded by a grid map with dimensions of 126, 126, 126 in the x, y, and z directions with a grid spacing of 0.375. The RdRp protein was kept rigid and the ligand molecules flexible within the drug binding pocket during docking, which was done using the Lamarckian Genetic Algorithm (LGA) of 10 runs (25000000 energy evaluation steps for each run).

Results and Discussion

Since 2019, there have been tens of thousands of illnesses and thousands of fatalities globally as a result of a novel coronavirus outbreak known as the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2). RNA dependant RNA polymerase is the core enzyme of this virus mediates the replication and the transcription machinery of the virus [14]. Therefore, new medications that target this enzyme can be used to treat Corona Virus. Although various antiviral drugs such as remdesivir, ribavirin, favipiravir, molnupiravir, and AT-527 have been tried against this virus, but all these drugs do not give the promising result. As a result, finding a new antiviral medication is prompted, i.e. Galidesivir which has tremendous antiviral activities [24]. With promise for broad-spectrum antiviral activity, Galidesivir is an adenosine analogue and RNA polymerase inhibitor. Galidesivir is metabolized into its monophosphate form after administration, and this form is subsequently changed into the triphosphate nucleotide, which is the active form. Further, premature chain termination is caused by the incorporation of Galidesivir triphosphate, which binds to viral RNA-dependent RNA polymerase (RdRp) and enters the developing viral RNA strand. This stops the replication and transcription of viruses [16]. So for the creation of an effective therapeutic regimen against the corona virus, detailed information regarding the physiochemical properties of RdRp and its binding interaction studies with Galidesivir is required.

Physiochemical characteristics of RdRp protein

The physiochemical characteristic of the RdRp protein is given in Table 1. The results indicated that the RdRp protein has an average molecular weight of 108 kDa and a predicted pI of 6.33, indicating that it is acidic in nature. It could be because this protein has a high amount of acidic amino acids such aspartic acid and glutamic acid (59%) [17]. The protein's instability index was determined to be 28.13, which is lower than 40, indicating that it is very stable in nature. This stability may be related to the presence of fewer dipeptides in the protein's composition. (Sahoo *et al*., 2018). The protein's aliphatic index, which indicates its thermal stability, was determined to be 77.60, which is close to 80. This finding suggests that the protein is stable in nature across a wide temperature range. It could be because this protein has a high proportion of aliphatic side chain amino acids, such alanine, valine, isoleucine, and leucine, occupying its space [19]. However, the Grand Average Hydropathy (GRAVY) values were discovered to be -0.256, which is less than zero, suggesting that this protein is more hydrophilic in nature and has a significant amount of polar amino acids on its surface [20].

Table 1: Physiochemical characteristics of RdRp protein Parameters

Physiochemical Parameters	Value
AA Length	942
MW	108031.62
pI	6.33
Extinction coefficient	137670
Instability index	28.13
Aliphatic index	77.60
Grand average of hydropathicity (GRAVY)	-0.256

Table 2: Showing the composition of different secondary structure of RdRp protein

Alpha helix (%)	Extended strands (%)	Random coil (%)
54.24	7.80	37.96

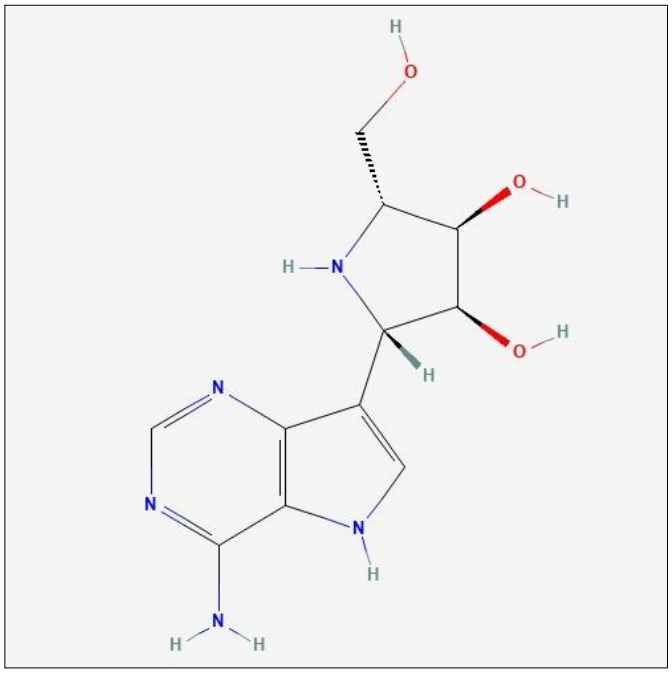

Fig 1: Chemical structure of drug Galidesivir

Table 3: Pharmacological Information about Galidesivir

Drug Name	Group approved	Molecular Weight	Accession Number	CAS Number	Molecular Formula
Galidesivir	Small	265.27 g/mol	10445549	249503-25-1	$C_{11}H_{15}N_5O_3$

Molecular Docking Studies

Eight distinct energetically advantageous binding conformations were obtained from the molecular docking of RdRp and Galidesivir within the active pocket of the suggested drug target. The structure with the lowest BE (binding energy) (-3.73 kcal/mol) among these was chosen for additional analysis because low binding energies are typically preferred for favorable binding modes during the docking of small compounds with target protein molecules, which may be a result of the docked structure's stability [21]. The lowest of all conformations for the binding inhibition constant, 58.4 M, indicated a potent inhibitor of the RdRp protein. It was discovered that the protein and ligand docked complex had a variety of interactions, including hydrophobic, electrostatic, and hydrogen interactions as shown in Fig 2 which could be because the binding pocket contains positively and negatively charged amino acid residues as well as hydrophilic and hydrophobic molecules [22] shown in Table 2.

Table 4: Polar contact information obtained from docking calculation

Ligand	Binding Energy	Inhibition constant (µM)	Residues	Atoms	Distance (Å)
Galidesivir	-3.73	58.4	Asp 623,	O8--H	1.7871
			Cys 622	S41--O	1.336
			Tyr 619	O8--H	1.540
			Lys 621	N-H	1.643
			Asp 760	O8--H	1.454

Our study resulted that the hydrogen bond was formed with the amino acid residue Asp 623 where strong hydrophobic interaction was existed due to presence of Cys 622 and Lys 621 within 2°A distance. However, presence of Tyr 619 and Asp 760 contributes the electrostatic interaction which may be conferred that Galidesivir can be used as a strong inhibitor against RdRp of Corona Virus. The 2 D structure of binding interaction was shown in Fig 2

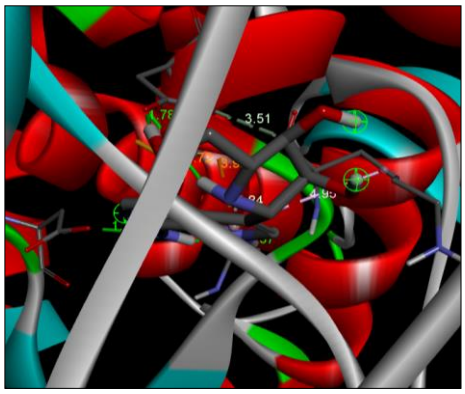

Fig 2: Interaction of Galidesivir with proposed drug target RdRp protein of Corona Virus visualized in Bio via Drug discovery Studio

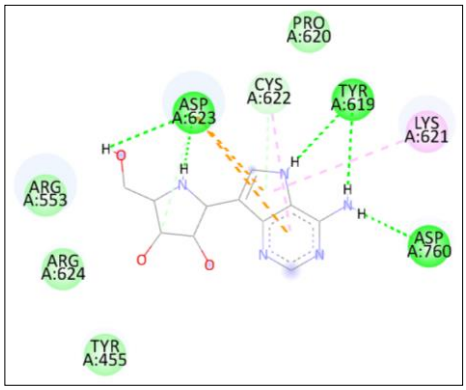

Fig 3: 2D structure of binding interaction between Galidesivir with proposed drug target RdRp protein of Corona Virus

Conclusion

This study was also concluded that RNA dependant RNA polymerase (RdRp) was an important enzyme for virus assembly and transcription process, can be considered as potential drug target for specific antiviral drug to combat this corona virus menace. The structural characterization of this protein revealed that this protein is very stable and hydrophilic in nature. Moreover the antiviral drug Galidesivir showed lowest binding energy (-3.73 kcal/mol) and least inhibition constant (58.4 µM) with RdRp during docking studies explaining its strong inhibitory effect upon Corona Virus. This prediction was also confirmed through the visualization of strong hydrophobic, electrostatic and hydrogen bonding interactions between protein-ligand complexes. However efficacy of this drug should be confirmed through *in vivo* and *in vitro* studies in nearest future. Therefore, Galidesivir could be a promising antiviral drug against RdRp of Corona Virus in nearest future.

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Conflict of Interest

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

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