Denovo designing, retrosynthetic analysis, and combinatorial synthesis of a hybrid antiviral (VTAR-01) to inhibit the interaction of SARS-CoV2 spike glycoprotein with human angiotensin-converting enzyme 2.

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Keywords: Hybrid Antiviral molecule; SARS-CoV2 spike glycoprotein; Angiotensin-converting enzyme 2; Denovo designing; retrosynthetic analysis; Molecular dynamics simulation

Summary Statement:
SARS CoV2 has caused an outbreak globally and responsible for high mortality and morbidity. Interaction of the receptor-binding domain of spike protein of this virus with human ACE-2 is vital for the infection. Hence, denovo designed hybrid antiviral molecule (VT-AR-01) targeting RBD-ACE2 interaction may play a very significant role in controlling the COVID19 disease.
Abstract

SARS-like coronavirus (SARS-CoV2) has emerged as a global threat to humankind and is rapidly spreading. The infectivity, pathogenesis, and infection of this virus are dependent on the interaction of SARS-CoV2 spike protein with human ACE2 (hACE2). Spike protein contains a receptor-binding domain (RBD) that recognizes hACE-2. In the present study, we are reporting a denovo designed novel hybrid antiviral 'VT-AR-01' molecule that binds at the interface of RBD-hACE2 interaction. A series of antiviral molecules were tested for binding at the interface of RBD-hACE2 interaction. In-silico screening, molecular mechanics, molecular dynamics simulation (MDS) analysis suggest ribavirin, ascorbate, lopinavir, and hydroxychloroquine have strong interaction at RBD-hACE2 interface. These four molecules were used for denovo fragment-based antiviral design. Denovo designing, docking, and MDS analysis identified a 'VTAR' hybrid molecule that has better interaction with this interface as compared to all antiviral used to design it. We have further used retrosynthetic analysis and combinatorial synthesis to design 100 variants of VT-AR molecules. Retrosynthetic analysis and combinatorial synthesis, along with docking and MDS, identified VT-AR-01 that interact with the interface of the RBD-ACE2 complex. MDS analysis confirmed its interaction with the RBD-ACE2 interface by involving Glu35 and Lys353 of ACE2, as well as Gln493 and Ser494 of RBD. Interaction of spike protein with ACE2 is essential for pathogenesis and infection of this virus; hence, this in-silico designed hybrid antiviral molecule (VT-AR-01) that binds at the interface of RBD-hACE2 may be further developed to control the infection of SARS-CoV2.
1. Introduction

The emergence of novel coronavirus and the rapid outbreak of SARS-COV-2 infection has cause endangering global health and the economy (Wu et al., 2020) (Huang et al., 2020). On March 11, 2020, the world health organization (WHO) declared the outbreak a pandemic. On 07 Aug, 2020 there are > 1,90,00,000 cumulative cases globally and > 7,00,000 death with ~ 5% mortality rate in outcome cases. The main risk factors include older age, comorbidities, low lymphocyte count and high RALE (radiographical assessment of lung edema) (Ciceri et al., 2020). The average incubation period of this virus is 2 to 14 days (mainly 3 to 7 days), but it may be enhanced in person with long term use of glucocorticoids (Han et al., 2020).

Additionally, there is a decrease in CD8$^+$ T cells and NK cells in COVID-19 patients (Jiang et al., 2020) that is associated with worse prognosis and systemic inflammation (Urra et al., 2020). Receptor recognition by coronavirus is a determinant for its infection, pathogenicity, and human interaction (Perlman and Netland, 2009). Virus infection is initiated with the binding of viral particles to the host surface receptors. The receptor recognition by coronavirus involves the receptor-binding domain (RBD) of the spike protein of SARS-COV-2 (Shang et al., 2020) and human angiotensin-converting enzyme 2 (Letko et al., 2020). It was reported that the ACE-2 gene is hypomethylated and overexpressed in lupus T cells, and epigenetic dysregulation of ACE2 might suggest COVID-19 susceptibility and severity in lupus patients (Sawalha et al., 2020). As the receptor recognition is an important determinant for infection of coronavirus. RBD of this protein contains a core and a receptor-binding motif (RBM) that mediates its contact with the human ACE-2 (Shang et al., 2020). The hACE2 contains two virus-binding hotspots that are important for SARS-CoV2 binding (Wu et al., 2012). The recent study (Shang et al., 2020) have shown the residues Leu455, Phe486, Gln493, Ser495, Asn501 of RBD of SARS-CoV2, and Met82, Lys31, Glu35, Asp38, and Lys353 of ACE-2 as important for the interaction of this virus with the human host. This shows that these residues can play a vital role in host-pathogen interaction and pathogenesis of this virus. An inhibitor targeted at the interface of this interaction can play a significant role in preventing the pathogenicity of this virus.

Recently different non-specific antiviral and other therapeutics repurposing approaches were used to minimize the losses due to infection by SARS-CoV2, but their possible mechanism of action has not investigated yet. Broad-spectrum antiviral molecules like remdesivir, ribavirin, lopinavir, and hydroxychloroquine, etc. are currently used against the COVID-19 (Singh et al., 2020). Hydroxychloroquine has been proposed to inhibit the virus entry, but its mechanism of action is unclear (Liu et al., 2020). Regulators have different opinions on the use of some of
the recommended molecules (Jaffe, 2020). The glycation of spike protein is also important for the interaction (Walls et al., 2016) and spike protein of SARS-CoV-2 and ACE-2 have shown to be glycosylated (Wrapp et al., 2020) (Zhou et al., 2020) and hence it is also essential to include glycans in studies to have a better picture of host-pathogen interaction.

There is no specific medicine available for the treatment of COVID-19; hence it is an urgent requirement of the current time to develop novel specific novel antiviral that interferes in the interaction of SARS-coV2 with the human host. In the present study, we have used denovo designing, retrosynthetic analysis, combinatorial synthesis, and molecular dynamics simulations to design hybrid molecules that target the interaction of spike's RBD and hACE2 receptor. The designed antiviral VT-AR-01 may inhibit the interaction of RBD of Spike protein of SARS-coV2 with the human hACE2.

2. Results

2.1 Selection of interface of SARS-CoV2 spike protein and human ACE2 interaction.

The PDB structure of the SARS-CoV2 receptor-binding domain (RBD) of spike protein complexed with angiotensin-converting enzyme 2 (ACE-2) was taken from RCSB (PDB number 6VW1, 2.68Å). This PDB structure consists of four subunits with two subunits (A, B) of ACE2 and two subunits (E, F) of RBD, metal ion (zinc, chloride), and carbohydrate moiety (beta-D-mannose, N-acetyl-glucosamine). The subunit A and B of ACE2, and E and F of RBD in the PDB structure are identical; hence one identical subunit (B and F) were removed from the PDB structure. The glycation is shown to be important for the interaction between spike protein and ACE2, hence beta-D-mannose and N-acetyl-glucosamine of A and E subunits of the complex have been considered for the present study. The RBD-ACE2 complex was prepared, optimized, and minimised using OPLS-2005 force fields. The recent study (Shang et al., 2020) have shown that Leu455, Phe486, Gln493, Ser495, Asn501 of RBD of the spike protein of SARS-CoV-2, and Met82, Lys31, Glu35, Asp38, and Lys353 of ACE-2 are present at the interface of the interaction and play a significant role in the host-pathogen interaction. The present study aims to design an inhibitor that targets this interface of RBD and ACE-2 interaction; hence we have used these amino acid residues for the grid generation for docking and considered for further studies.
2.2 Selection of antiviral molecules

The antiviral molecules are selected based on the available literature search and their efficacy on the SARS-CoV-2. The umifenovir and its analogs, lopinavir, ribavirin, hydroxychloroquine, ascorbate, remdesivir, oseltamivir, ritonavir, and tamoxifen are selected for the present study. The ligand preparation resulted in 139 selected tautomer of these antiviral molecules that were used for its interaction with the RBD-ACE-2 interface. The selection of novel antiviral molecules has been discussed in the coming sections.

2.3 Docking at XP mode identified an antiviral molecule that binds at the interface of the RBD-ACE-2 complex.

We have performed docking studies using Glide’s Extra Precision XP mode. XP docking suggests that all the selected molecules docked with negative binding energy. Ascorbate, ribavirin, hydroxychloroquine, umifenovir, favipiravir, and lopinavir have shown better interaction than other antiviral molecules studied. The docking poses are shown in figure 1, and docking scores, along with binding energies, are shown in table 1. The interacting residues of the RBD-ACE-2 interface that interact with individual antiviral molecules are shown in table 2. It was observed that some molecules have better interaction with residues of RBD while others have better interaction with the human ACE-2, but they all docked at the interface of the RBD-ACE-2 complex.

2.4 Binding free energy calculations using molecular mechanics confirms the interaction of antiviral molecules with the RBD-ACE-2 complex.

All the antivirals were undergone molecular mechanics analysis with generalized born and surface area solvation (MM-GBSA) methods to calculate the Gibbs free energy of binding. The result of this analysis is listed in table 1. It was found that all the molecules have favorable Gibbs free energy change for binding to the RBD-ACE-2 complex. Lopinavir has the best binding energy (-40.8Kcal/mol), followed by Ribavirin (-38.72Kcal/mol). Based on docking and binding free energy results, ascorbate, ribavirin, hydroxychloroquine, umifenovir, and lopinavir are selected for molecular dynamics analysis (MDS) and further study.

2.5 Molecular dynamics simulation analysis confirms that ascorbate, ribavirin, lopinavir, and hydroxychloroquine interact at the interface of the RBD-ACE-2 complex.

Molecular dynamics simulation (MDS) was performed for different ligand-protein complexes such as RBD-ACE2-ascorbate, RBD-ACE2-ribavirin, RBD-ACE2-lopinavir, RBD-ACE2-umifenovir RBD-ACE2-hydroxychloroquine, up to 5ns, and results were analysed for RMSD,
RMSF, ligand stability, bonding, etc. MDS analysis of the RBD-ACE2-ascorbate complex showed that RMSD of protein and ligands are <3 Å that showed stable complex (Figure 2). RMSF study showed that the most of the protein (except terminal) has the RMSF <2Å that showed less flocculation in protein conformation hence a stable structure. Interaction between ascorbate and RBD-ACE2 complex involves His34, Glu37, Lys353, Lys403, Tyr453, Ser494, Gly496, Phe497, Asn501, and Tyr505 and at least seven contacts always exist with more than 30% simulation time (Figure 2D). It forms six hydrogen bonds in the same simulation time that showed that the interaction is specific.

Similarly, MDS analysis of the RBD-ACE2-ribavirin complex has RMSD <1.6Å at 5ns, which suggests a very stable complex (Figure 3). RMSF study showed that the most of the protein (except terminal) has the RMSF <2Å that showed stable structure with some flocculation in protein conformation at the terminal. Amino acids involved in the interaction between ribavirin and RBD-ACE2 complex are His34, Glu35, Glu37, Lys403, Tyr453, Ser494, Gly496, and Tyr505, and have six contacts that always exist between ribavirin and RBD-ACE2 in more than 30% simulation time (Figure 3D). Similarly, MDS analysis of RBD-ACE2-lopinavir complex showed that RMSD of protein and ligands have RMSD <2Å at 5ns that showed a very stable complex (Supplementary Figure S1). RMSF of this complex was found to be <2Å that showed less flocculation in protein conformation hence a stable structure. Lopinavir interacts with RBD-ACE2 complex via Lys31, Glu35, Leu79, Tyr449, Glu484, Tyr489, Phe490, with at least nine contacts that always exist more than 30% simulation time (Supplementary Figure S1-D). Although the strength of the interaction is good, but it only forms three hydrogen bonds at the same time that makes this interaction less specific.

Similarly, MDS analysis of RBD-ACE2-umifenovir complex showed protein RMSD of <3Å (Supplementary Figure S2), but ligand has an RMSD of <5 Å that showed its limited stability in the complex. RMSF analysis showed an RMSF <2Å that showed less flocculation in protein conformation. Interaction between umifenovir and RBD-ACE2 complex involved involves Lys31, Glu35, Lys68, Phe72, Tyr489, and at least four contacts always exist more than 30% simulation time (Supplementary Figure S2-D) with one hydrogen bond. This showed that this complex is relatively less stable than other complexes. In addition, MDS analysis of RBD-ACE2-hydroxychloroquine complex showed that RMSD of protein has an RMSD of <3Å that showed stable complex (Supplementary Figure S3), but ligand has RMSD of <15 Å that showed its limited stability in the complex. RMSF analysis showed that the most protein (except terminal) has the RMSF <2Å that showed less flocculation in protein conformation hence a stable protein structure. Interaction between hydroxychloroquine and RBD-ACE2
Comparative interacting residues analysis in the MDS results showed that the ascorbate and ribavirin have the best interaction and interacts with both RBD as well as ACE-2. Similarly, lopinavir has strong interaction with ACE-2. In addition, hydroxychloroquine forms a very strong interaction with Glu484 residue of RBD, which is in the binding pocket but not involved in physical interaction with ACE2. Hence, from MDS result analysis, ascorbate, ribavirin, lopinavir, and hydroxychloroquine are selected for denovo fragment-based drug design.

2.6 Denovo fragment-based designing and MDS analysis identified VT-AR as a hybrid antiviral targeted at the RBD-ACE-2 interface.

Denovo fragment-based drug designing was started with the fragmentation of the selected molecules i.e., ascorbate, ribavirin, lopinavir, and hydroxychloroquine. A total of 414 fragments was produced that includes 386 fragments of lopinavir, 21 fragments of hydroxychloroquine, five fragments of ribavirin, and two fragments of ascorbate. All the 414 fragments were docked (in SP mode) to the grid of the RBD-ACE2 complex. On the basis of a manual analysis of docked complexes, fragment 1 and 4 of ribavirin, fragment 1, 2 of ascorbate, fragment 6, 8, 15, 17, 21 of the hydroxychloroquine and fragment 122, 214, 350, 361, 373 of the lopinavir were selected for denovo fragment-based design. This produces 14 combinations, which along with four original molecules, were prepared via LigPrep that produces a total of 139 tautomers. All 139 tautomers are undergone virtual screening for their binding to the grid at the RBD-ACE2 interface. The two fragments (a hybrid of fragment 4 of ribavirin, and fragment 1 of the ascorbate) showed the best docking. Their docking score (-7.04) was found to be higher than their parent molecules (Supplementary Table S1) and named as 'VTAR', and chemically it is (2S)-2-{1-[(2S,3S,4S)-3,4-dihydroxyoxolan-2-yl]-1H-1,2,4-triazol-3-yl}-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate (Figure 4 A and B). To further confirm the interaction, the MDS analysis of the RBD-ACE2-VTAR complex was performed that showed that RMSD of both protein and ligands are <2 Å that showed stable complex (Figure 5). RMSF analysis showed that the most of the protein (except terminal) has the RMSF <3Å that showed stable complex some flocculation in protein conformation at terminals. Interaction between VT-AR and RBD-ACE2 complex involved involves His34, Glu35, Asp38, Lys353, Lys403, Tyr 449, Tyr453, Gln493, Ser494, Gly496, and at least ten contacts always exist with more than 30% simulation time (Figure 5D) and eight hydrogen bonds between VTAR and RBD-ACE2 complex that showed a very strong and specific interaction.
2.7 Retrosynthetic analysis, combinatorial synthesis, and MDS analysis identified VTAR-01 as a hybrid antiviral targeted to the RBD-ACE-2 interface

To produce synthetically tractable lead-like compounds, retrosynthetic analysis, and combinatorial synthesis was performed that produces 100 products of VT-AR with more than 100 modifications. The 100 products were prepared and docked to the RBD-ACE2 complex. Docking and binding energy calculation identified VTAR-01 as best docked molecule (-7.8) and identified as (3R,4R)-3-hydroxy-4-{2-[(3S,4S,5S)-4-hydroxy-5-{3-[(2S)-4-hydroxy-3-oxido-5-oxo-2,5-dihydrofuran-2-yl]-1H-1,2,4-triazol-1-yl]oxolan-3-yl}oxy]-2-oxoethyl)sulfanyl]pyrrolidin-1-ium (Figure 4C and D). To further confirm the interaction, the MDS analysis of the RBD-ACE2-VTAR-01 complex was performed up to 25ns, and the result showed that RMSD of both protein and ligands are <2.5 Å that showed a very stable complex (Figure 6). RMSF analysis showed that the most of the protein (except terminal) have the RMSF <3Å at most of the simulation time that showed less flocculation in protein conformation. Interaction between VTAR-01 and RBD-ACE2 complex involves His34, Glu35, Lys353, Lys403, Tyr453, Glu484, Gln493, Ser494, Gly496, and at least nine contacts always exist more than 30% simulation time (Figure 5D). The comparative analysis showed that VTAR-01 form eight hydrogen bonds with the RBD-ACE2 complex that shows specific binding with VTAR-01. Comparative analysis of all the results showed that VTAR-01 has the highest docking score (-7.8), has eight hydrogen bonds, and interacts with Glu35 and Lys353 of ACE2, as well as Gln493 and Ser494 of RBD confirm its strong interaction with RBD-ACE2 interface. It is also noted in MDS analysis that interaction of VTAR-01 with the Glu35 and Gln493 exists at 100% simulation time. This further confirms its strong interactions at the interface of the RBD-ACE2 complex.

2.8 Designed VTAR-01 has favourable ADMET properties

ADMET (absorption, distribution, metabolism, excretion, and toxicity) analysis of VTAR-01 was performed, and the result showed that most of its ADMET parameters are in the acceptable limits (Supplementary Table S2) that further support the designed leads. QPlogPw, QPlogPo/w, QPlogS showed that VTAR-01 is water-soluble. The availability of free drugs in blood is essential to cross the membrane and binding to molecular targets. The QPlogKhasa showed that the VTAR-01 has very low interaction with plasma proteins.
2.9 Designed VTAR-01 has no human off-targets and no predicted cytotoxicity in the cell line.

Presence of lead's off-targets in human may reduce the efficacy and causes cytotoxicity. Hence, human off-targets and cytotoxicity of VTAR-01 in cell lines were predicted. The result showed no human off-targets of this VTAR-01. Similarly, the predicted cytotoxicity of the VTRRT13-V2.1 has been demonstrated that it is non-cytotoxic to tumor and non-tumor cell lines (at Pa>0.6). This further enhances the possibility of the development of VTAR-01 as a possible lead.

3. Discussion

Infection caused by SARS-CoV2 has emerged as a global pandemic. The spike protein of coronavirus facilitates viral entry into target cells. Human receptor recognition by this protein is a vital infection determinant for coronavirus. The receptor recognition by coronavirus involves the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 (Shang et al., 2020) and human angiotensin-converting enzyme 2 (Letko et al., 2020). The recent study (Shang et al., 2020) showed that Leu455, Phe486, Gln493, Ser495, Asn501 of RBD of spike protein, and Met82, Lys31, Glu35, Asp38, and Lys353 of ACE-2 directly interact with each other. Currently, no specific FDA approved treatment is available for the infection caused by SARS-CoV2. There are different non-specific treatments for COVID-19 that are presently used, such as remdesivir, ribavirin, lopinavir, and hydroxychloroquine, etc. (Singh et al., 2020) but their mechanism of action on SARS-CoV2 has not been investigated till date. Ribavirin is a guanosine analog that has reported to have multiple mechanisms of action (Loustaud-Ratti et al., 2016) and has also shown to have an inhibitory effect against SARS-coV2 (Khalili et al., 2020). Lopinavir, an HIV type 1 Aspartate protease inhibitor, have activity against other coronaviruses like SARS-CoV (Chu et al., 2004). It is reported that lopinavir-ritonavir with ribavirin and interferon alfa resulted in the survival of MERS-CoV infection (Kim et al., 2016). Remdesivir (GS-5734) is currently used with lopinavir against COVID-19 without knowing its targets in SARS-CoV (Choy et al., 2020). Ritonavir, Lopinavir, and Remdesivir have been used to control other coronaviruses (Sheahan et al., 2020). Recent clinical trials showed that broad-spectrum antiviral lopinavir and ritonavir alone were ineffective against COVID-19 (Cao et al., 2020). Recently, hydroxychloroquine is found to have activity against SARS-coV2 (Gao et al., 2020), and it was found to be more effective than chloroquine (Yao et al., 2020). Recent reports also suggest hydroxychloroquine did not prevent illness when used as postexposure prophylaxis within 4days after exposure (Boulware et al., 2020). Ascorbate has
shown a positive effect on other SARS coronavirus, and it has shown multiple mechanisms of action (Hemilä, 2003). In the present study, the interaction between RBD of the spike protein of SARS-CoV2 with human ACE2 is targeted by novel hybrid antiviral VTAR-1, which was designed using denovo designing, retrosynthetic analysis, and combinatorial synthesis, and molecular dynamic simulation.

PDB structure of the RBD-ACE2 complex was retrieved from the RCSB, and the grid was prepared at the interface of this interaction. The docking, binding energy calculation, and molecular dynamic simulation calculation have shown that ascorbate, ribavirin, hydroxychloroquine, umifenovir, and lopinavir have good interactions with this interface. It has been shown that absorbate has shown some preventive effect in infection by the other coronavirus (Hemilä, 2003), but its preventive role in the infection of SARS coV2 is under Phase 2 clinical trial (clinicaltrails.gov identifier: NCT04264533) and its outcome is expected by September 2020. The present study highlighted the molecular mechanism of the ascorbate (Vitamin C) to prevent the infection by SARS-CoV2. In addition to that, this study also explains the probable molecular mechanism of chloroquine in preventing the infection by SARS-CoV2. Experimental studies have shown that hydroxychloroquine is effective against SARS-CoV2 (Yao et al., 2020), but its mechanism of action is not studied. Various in-vitro experimental data suggest that the ribavirin has shown an inhibitory effect on SARS-CoV2 (Khalili et al., 2020), but a possible mechanism of action is not clear to date. The present study also gives the molecular explanation for some success of ribavirin, ascorbate, and lopinavir against the SARS-CoV2, but these broad-spectrum antiviral molecules are not specific to the SARS-CoV2. Therefore, in the present study, we are reporting a specific hybrid inhibitor VTAR-01 targeted at the interaction point of the receptor-binding domain of spike protein with the human ACE2 receptor (hACE2). It is also seen that the expression of the ACE2 receptor is not only limited to lungs, but its presence is also involved in extrapulmonary spread (Gu et al., 2005) that further enhances the importance of this study.

To find a novel inhibitor, we have used denovo fragment-based drug design using FDA approved antiviral molecules like ascorbate, ribavirin, hydroxychloroquine, and lopinavir, which showed good integration with RBD-ACE2 complex. A total of 414 fragments were generated from these molecules. They were docked to the interface of RBD-ACE2 complex, and best-docked fragments were manually analyzed of its interaction at the interface of this complex. The selected fragments were joined via denovo fragment-based drug design. This approach joined the different fragments based on the structural similarity to the active molecules and their scaffolds, hence used to navigate a huge chemical space (Kawai et al.,
The hybrid molecules were further confirming its interaction interface of RBD-ACE2 complex using XP-mode of docking and molecular dynamics simulations. This select VT-AR as a possible denovo synthesized hybrid molecule that can interact at the interface via Gln493 and Ser 494 of RBD of SARS-CoV2 and Glu35, Asp38 and Lys 353 of hACE2.

Lead optimization required the design, synthesis, and profiling of thousands of leads analogs prior to clinical candidate nomination that takes a longer time. Further, to explore possible improvement in the VTAR, we have used in-silico based retrosynthetic analysis, combinatorial synthesis, and MDS analysis to design a new hybrid molecule. Retrosynthetic analysis followed by combinatorial synthesis generated 100 analogs of the denovo designed hybrid molecules (VTAR) in synthetically accessible chemical space and investigated for its interaction with the RBD-ACE2 complex. All the analysis identified VTAR-01 as a better molecule than VTAR and have shown the best interaction at the interface of the RBD-ACE2 complex. MDS analysis confirms the interaction of VTAR-01 at the interface of the RBD-hACE2 complex via Gln493 and Ser 494 of RBD of SARS-CoV2 and Glu35 and Lys 353 of hACE2. This showed the modification in VTAR-01 by retrosynthetic analysis, combinatorial synthesis further enhances the docking and binding energy without changing its interacting positions at the interface of RBD-hACE2 complex.

In addition to the inhibition of virus entry, it is also essential to investigate the other complication of SARS-CoV2 infection. During infection, there is a burst of massive inflammation in the lung and other organs like kidney, cardiovascular, and neurological systems, which is associated with cytokine release syndrome know as 'cytokine storm'. The blockage of this cytokine storm is also vital along with inhibition of virus entry to other cells. Different approaches are investigated, such as blockage cytokine storm by rapamycin (Omarjee et al., 2020), and blood purification therapy (Ma et al., 2020). Similarly, blockage of IL-6 and granulocyte macrophage-colony stimulating factor to inhibit monocyte-macrophage recruitment and differentiation to lung and block inflammatory response (Gómez-Rial and Martinón-Torres, 2020). In addition to that, deposition of immune complexes (ICs) inside vascular walls can also induce cytokine storm (Roncati et al., 2020) hence status of ICs may be too critical for design and immune-based treatments like plasma therapy and vaccine (Vuitton et al., 2020). To make the situation more complicated, there is an activation of complement C3 during lung injury, and C3-targeted intervention by compstatin-based complement C3 inhibitor AMY-101 prevents complement-mediated inflammatory damage in COVID-19 patients (Mastaglio et al., 2020). Antibody-based therapies also represent valuable treatment approaches to treat symptomatic patients and prophylactically in at-risk individuals.
(Yager, 2020). One such vaccine, i.e. recombinants adenovirus type-5 (Ad5) vectored COVID19 vaccine, is tolerable and immunogenic at 28 days post-vaccination (Zhu et al., 2020). Presence of different antigens in connective tissue, cardiovascular, gastrointestinal, and nervous systems that have cross-reaction with SARS-CoV2 antibodies further complicate the condition and cause autoimmunity (Vojdani and Kharrazian, 2020). Therefore, along with the inhibitor that blocks the interaction between spike protein with ACE-2, the other factors like cytokine storm and autoimmunity may also be taken care. A combination of different approaches may be suitable to control COVID-19.

Therefore, the present study concludes that denovo designing, retrosynthetic analysis, and combinatorial synthesis, molecular dynamic simulation study designed a novel hybrid antiviral VT-AR-1 that can bind at the interface of RBD of the spike protein of SARS-CoV2 with human ACE2 receptor. This molecule VTAR-01 needs to be synthesized and experimentally tested against SARS-CoV2 in animals and humans before using as therapeutics.

4. Methods

4.1 Retrieval of the structure of SARS-CoV2 complexed with ACE-2

The PDB structure of the SARS-CoV2 receptor-binding domain (RBD) of spike protein with Angiotensin-converting enzyme 2 (ACE-2) is available hence retrieved from RCSB (PDB number 6VW1, resolution 2.68Å). This PDB structure also has Zinc ion, Chloride ion, ethylene glycol, beta-D-mannose, N-acetyl-glucosamine in A chain, and beta-D-mannose, N-acetyl-glucosamine in E-chain. This PBD is a tetramer (ACE-2, A, and B; RBD, E, and F) with two identical sets. The B and F subunit was removed using Maestro. The selected RBD-ACE2 complex (A and E unit) was pre-processed by assigning bond orders, adding hydrogens, created zero-order bonds for metals, creates disulphide bonds, minimised optimised for water orientation and pH, and minimised for converge heavy atoms to 0.3RMSD using OPLS_2005 force field. We have identified the interacting residues of RBD and ACE-2 as per published article (Shang et al., 2020) and used for receptor grid generation using the Leu455, Phe486, Gln493, Ser495, Asn501 of RBD and Met82, Lys31, Glu35, Asp38, and Lys353 of ACE-2 as binding residues. This receptor grid was used to screen a number of the antiviral molecule that is targeted to the interface of the interaction of RBD and ACE-2.
4.2 Ligand preparation

The antiviral currently prescribed for COVID-19, as well as targeting the virus membrane fusion, were selected in the present study. The selected antiviral drugs were umifenovir, and its analogs, lopinavir, ribavirin, hydroxychloroquine, ascorbate, remdesivir, oseltamivir, ritonavir, and tamoxifen. The SDF structure of the antiviral molecules was downloaded from the PubChem database. These SDF structures, along with denovo design hybrid antiviral molecules, were prepared by using LigPrep modules of the Schrodinger as per published protocol (Tiwari et al., 2018b).

4.3 Docking of antiviral on the interface of the RBD-ACE-2 complex

All the prepared ligands were docked to the docking grid of RBD-ACE-2 complex in XP (Extra Precision) mode as per published protocol (Verma and Tiwari, 2018).

4.4 Molecular mechanics/generalized born surface area (MM-GBSA) calculations of selected library compounds

To get more accurate interaction, XP docked antiviral molecule with RBD-ACE-2 complexes were further subjected to molecular mechanics with generalized born surface area (MM-GBSA) calculations using the Prime module of Schrodinger as per published methods (Tiwari et al., 2019).

4.5 Molecular Dynamics Simulation (MDS) analysis

MDS was performed using Desmond modules of the Schrodinger 2019-4 per published methods (Wright et al., 2020). The protein-ligand complex was prepared and subjected to the system builder using the OPLS3e force field. The system was built for the protein-ligand complex using the TIP3P solvent model; sodium ion was added to make charge-neutral, 0.15M NaCl was added to make the system close to the natural system. The simulation was run for 5ns or 25ns (VTAR01-protein complex), with 5ps trajectory recording intervals. System energy was set to be 1.2, and the ensemble class used was NPT. The simulation was set to run at 300k at 1.01325bar. The option to relax the system before simulation was selected. The simulated system was analysed for the simulation interaction diagram.

4.6 Denovo fragment-based drug design

De-novo fragment-based drug designing is a newly emerged approach (Kawai et al., 2014). The different fragments of selected antiviral molecules were generated. The different fragments of ligands were docked into the binding site of the RBD-ACE-2 complex. The docked complex was manually analysed for its position of docking in the binding site. Different fragments (fragment 1 and 4 of ribavirin; fragment 122, 214 350, 361 and 373 of lopinavir; fragment 1 of Ascorbate; fragment 6, 8, 15, 17, 21 of hydroxychloroquine) were selected based on their
binding to the binding interface of RBD-ACE-2 complex. The selected fragments were joined using the Breed module to generate newly designed hybrid antiviral molecules. The designed antiviral molecules were further confirmed for their interaction with the binding interface of the RBD-ACE-2 complex using XP-docking and molecular dynamics simulations analysis.

4.7 Retrosynthetic analysis and combinatorial synthesis
To produce synthetically tractable lead-like compounds, retrosynthetic analysis, and combinatorial synthesis were performed for the denovo synthesised 'VT-AR' hybrid antiviral molecule as per published method using PathFinder (Konze et al., 2019). In-silico enumeration provides a more exhaustive exploration of available chemical space than traditional empirical SAR studies. PathFinder can incorporate more than 100 reactions like C-C bond formation, alkylation, ether formation, amide coupling, and heterocyclic systems like imidazole, triazoles, oxazole, indole, pyridine that are required for the molecular scaffolds and drug discovery (Bemis and Murcko, 1996). A total of 100 number of pathways are investigated to produce 100 products. The top 10% product that is similar to the input molecule VT-AR are selected for further analysis. The selected 100 products were analysed by docking and molecular dynamics simulations.

4.8 ADMET analysis
Absorption, distribution, metabolism, excretion, and toxicity analysis were carried out for the selected lead VT-AR01 using QikProp analysis as per published protocol (Tiwari et al., 2020).

4.9 Identification of human off-targets of designed lead
The human off-targets of the designed lead was predicted using Swiss Target Prediction (Daina et al., 2019) using the published protocol (Tiwari et al., 2018a).

4.10 Prediction of cytotoxicity of designed lead on cell lines
In-silico cytotoxicity screening was performed by CLC-Pred (Cell line Cytotoxicity Predictor) as per published protocol (Lagunin et al., 2018).

5. Acknowledgments: I would like to thank the Central University of Rajasthan for providing the Schrodinger suite. I would like to thank Dr. Monalisa Tiwari for proofreading the revised manuscript.

6. Ethical approval: The present study does not involve human and animal samples.

7. Competing interests: The author has declared that no competing interests exist.

8: Funding: The experiment was performed in the absence of any financial support.

9. Data availability: All the data are available in the manuscript and its supplementary data.
10. References:

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Figure 1. Interaction diagram showing docking pose of Lopinavir (A), hydroxychloroquine, ribavirin (C), Umifenovir (D), Ascorbate (E) and Remdesivir (F) at the interface of the receptor-binding domain of SARS-CoV2 spike glycoprotein and human angiotensin-converting enzyme 2. Docking grid used generated using binding residues at the interface of interaction.
Figure 2: Root-mean-square deviation (A) and Root mean square fluctuations (B), Interacting residues (C), and the interacting fraction (D) during molecular dynamics simulation analysis of RBD-hACE2-ascorbate complex.
Figure 3: Root-mean-square deviation (A) and Root mean square fluctuations (B), Interacting residues (C), and the interacting fraction (D) during molecular dynamics simulation analysis of RBD-hACE2-Ribavirin complex.
**Figure 4**: Interaction diagram showing interacting amino acid residues and their docking pose in RBD-hACE2-VTAR complex (A and B), RBD-hACE2-VTAR01 complex (C and D).
Figure 5: Root-mean-square deviation (A) and Root mean square fluctuations (B), Interacting residues (C), and the interacting fraction (D) during molecular dynamics simulation analysis of RBD-hACE2-V TAR complex.
Figure 6: Root-mean-square deviation (A) and Root mean square fluctuations (B), Interacting residues (C), and the interacting fraction (D) during molecular dynamics simulation analysis of RBD-hACE2-VTAR01 complex. Experimental was performed till 25ns, and 1000frame was recorded throughout the simulation.
Table 1- Result showing outcome of GLIDE molecular docking in XP mode and Binding free energies result from Prime analysis using the MMGBSA approach. The docking was performed using Grid at the interface of the RBD-ACE2 complex.

| Molecules           | Pubchem ID | Glide G-Score (in Kcal/Mol) | Glide E-Model (in Kcal/mol) | dG Binding (in Kcal/mol) |
|---------------------|------------|-----------------------------|-----------------------------|--------------------------|
| Ascorbate           | 54678501   | -6.71*                      | -36.36                     | -17.01                   |
| Ribavirin           | 37542      | -6.58*                      | -35.58                     | -38.72                   |
| Hydroxychloroquine  | 3652       | -6.44*                      | -41.65                     | -26.25                   |
| Umifenovir          | 131411     | -5.73*                      | -42.30                     | -19.69                   |
| Favipiravir         | 492405     | -5.65                       | -34.34                     | -3.092                   |
| Lopinavir           | 92727      | -4.95                       | -63.55                     | -40.82*                  |
| Oseltamivir         | 65028      | -4.72                       | -40.77                     | -28.74                   |
| Remdesivir          | 121304016  | -4.45                       | -73.35                     | -19.53                   |
| Ritonavir           | 392622     | -4.35                       | -73.04                     | -23.01                   |
| Oseltamivir         | 65028      | -3.82                       | -35.80                     | -21.97                   |
| Tamoxifen           | 2733526    | -1.95                       | -32.38                     | -26.23                   |

*Basis of selection for next steps MDS analysis

Table 2- Interacting residues of the selected ligand from docking in XP mode. The interaction diagram was prepared using Maestro software.

| Residue     | Ascorbate | Ribavirin | Hydroxychloroquine | Umifenovir | Lopinavir |
|-------------|-----------|-----------|--------------------|------------|-----------|
| SARS-       | Leu455    |           |                    |            |           |
| CoV2        | Phe486    | -         | Yes                | Yes        | Yes       |
|             | Gln493    | Yes       |                    | Yes        | Yes       |
|             | Ser494    | Yes       |                    | Yes        | Yes       |
|             | Asn501    | -         |                    | Yes        |           |
| ACE-2       | Met82     | -         |                    |            |           |
|             | Lys31     | -         | Yes                |            | Yes       |
|             | Glu35     | -         | Yes                | Yes        | Yes       |
|             | Asp38     | Yes       |                    | Yes        | Yes       |
|             | Lys353    | -         |                    |            |           |
Figure S1: Root-mean-square deviation (A) and Root mean square fluctuations (B), Interacting residues (C), and the interacting fraction (D) during molecular dynamics simulation analysis of RBD-hACE2-lopinavir complex.
Figure S2: Root-mean-square deviation (A) and Root mean square fluctuations (B), Interacting residues (C), and the interacting fraction (D) during molecular dynamics simulation analysis of RBD-hACE2-umifenovir complex.

Figure S3: Root-mean-square deviation (A) and Root mean square fluctuations (B), Interacting residues (C) and the interacting fraction, (D) during molecular dynamics simulation analysis of RBD-hACE2-hydroxychloroquine complex.
Table S1: Result showing outcome of GLIDE molecular docking in XP mode and Binding free energies result from Prime analysis using MMGBSA approach for the denovo designed hybrid molecule ‘VTAR’ and retrosynthesised hybrid molecule ‘VTAR-01’

| Name (Chemical Name) | Glide G-Score (in Kcal/Mol) | Glide E-Model (in Kcal/mol) | MMGBSA dG Binding (in Kcal/mol) |
|----------------------|-----------------------------|-----------------------------|---------------------------------|
| VT-AR                | -7.04                       | -45.56                      | -21.19                          |
| (2S)-2-{1-[(2S,3S,4S)-3,4-dihydroxyoxolan-2-yl]-1H-1,2,4-triazol-3-yl}-4-hydroxy-5-oxo-2,5-dihydrofuran-3-olate |                           |                                |                                 |
| VT-AR-01             | -7.80                       | -66.22                      | -29.00                          |
| (3R,4R)-3-hydroxy-4-{(2-[[3S,4S,5S)-4-hydroxy-5-[(2S)-4-hydroxy-3-oxido-5-oxo-2,5-dihydrofuran-2-yl]-1H-1,2,4-triazol-1-yl]oxolan-3-yl]oxy]-2-oxoethyl)sulfanyl]pyrrolidin-1-ium | |                                |                                 |

Table S2: Result showing outcome of ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis of VT-AR-01. The prediction was performed using QikProp analysis.

| ADMAT Properties | Value for VT-AR-01 |
|------------------|--------------------|
| Molecular Weight | 444.415            |
| H-bond Donor     | 5.0                |
| H-bond Acceptor  | 16.6               |
| Rotatable bonds  | 10                 |
| QP polrz         | 38.773             |
| QPlogP16         | 13.973             |
| QPlogPoct        | 30.714             |
| QPlogPw          | 26.114             |
| QPlogPo/w        | -2.676             |
| QPlogS           | -1.691             |
| QPlogHERG        | -5.844             |
| QPlogKhasa       | -1.197             |
| Metab            | 7                  |