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De novo Drug Design of Potential Inhibitors of the Receptor Binding Domain of SARS-CoV-2 Variants †

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Abstract: Here, novel potential inhibitors of SARS-CoV-2 variants were designed *de novo* using generative neural networks. The top-performing ligand based on docking performance and ADMET profile is CID #526. It forms several hydrogen bonds with the wild SARS-CoV-2, indicating its potential as an inhibitor of the receptor binding domain. Mutated variants of the RBD also showed good interactions with CID #526, implying the inhibitory properties of our top-performing compound against various variants. Molecular dynamics analysis showed a stable ligand-RBD complex. CID #526 can easily be synthesized using low-cost starting molecules. Overall, the generated ligands merit further investigation to determine their efficacy and safety as a treatment against COVID-19.

Keywords: SARS-CoV-2; COVID-19; *de novo* drug design; molecular dynamics

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1. Introduction

COVID-19, a disease caused by the coronavirus SARS-CoV-2 [1], continues to spread over millions of people across all nations one year after it was first reported in Wuhan, China. Several vaccines, such as those developed by Pfizer/BioNTech [2] and Moderna [3], among others, have already been deployed after showing promising efficacy rates in their clinical trials. However, the rise of new variants of SARS-CoV-2 could threaten the efficacy of the current vaccines, which have been developed from the parent variant [4,5].

A viral isolate is named a variant of concern (VOC) if there is sufficient evidence that the emerging variant causes increased transmissibility, increased disease severity, or reduction/failure of existing diagnostics, treatments, and vaccines. The first VOC identified is the B.1.1.7 lineage, first reported in the United Kingdom. It contains the N501Y, A570D, D614G, and P681H mutations and 69/70/144 deletions in the spike protein. These mutations caused a significant surge in COVID-19 cases in the UK and then the EU in the last quarter of 2021 and the early months of 2021, owing to the replicative advantage acquired by the new variant [6,7].

Meanwhile, the B.1.351 lineage, first reported in Nelson Mandela Bay, South Africa, also involves multiple mutations in the spike protein, including K417N, E484K, N501Y, and D614G, resulting in increased transmissibility and evasion of immunity [8,9]. The P.1 lineage also contains several mutations in the spike protein: K417T, E484K, N501Y, and D614G. This variant was first reported by the National Institute of Infectious Diseases (NIID) in Japan from isolates obtained from Brazilian travelers. These mutations are shown to potentially escape immunity with increased transmissibility compared to the wild-type coronavirus [10,11].

Several other mutations in the SARS-CoV-2 spike protein have been recorded from genomic analysis of isolates worldwide. The top substitutions in the SARS-CoV-2 spike

protein are N501Y, E484K, L452R, S477N, N439K, T478K, K417N, S464P, N501T, are A520S [12]. E484K is thought to be associated with an increased probability of evading the immune response, while the N501Y and D614G mutations are shown to be associated with increased transmissibility [13,14]. Together with the slow pace of immunization programs and vaccine inequality, these variants could further evolve the virus, which could hamper our collective progress in COVID-19 management. As such, it remains imperative that further research be done to develop other vaccines and therapeutics against COVID-19. However, drug development for COVID-19 was side-tracked due to the disappointing results of the solidarity trials conducted by the World Health Organization (WHO) [15].

Inhibiting the critical proteins involved in the viral life cycle of SARS-CoV-2 is the primary strategy currently used for developing new drugs against COVID-19. Viral entry can be prevented by inhibiting the spike protein or by ACE2 modulation, such as ACE2 inhibition and RAAS inhibition. The main protease and the papain-like protease, responsible for the cleavage of polyproteins translated from viral RNA into functional or effector proteins for virus replication and packaging within the host cells, could also be drug targets to block viral replication. Inhibiting the RNA-dependent RNA polymerase, which assists in the translation and replication of the virus, could interfere with the replication, transcription, and translation of viral genomic material leading to the termination of viral reproduction. Other drug targets are envelope proteins, membrane proteins, and nucleoproteins [16–18].

Several reviews summarize the use of potential drugs against COVID-19 [19–23]. Nevertheless, despite the influx of studies on drug therapies against COVID-19, only a few have gained the approval of the U.S. Foods and Drugs Administration under their Emergency Use Authorization (EUA). These include using remdesivir, bamlanivimab, andasirivimab/imdevimab to manage severe COVID-19 cases [24]. The WHO has approved only remdesivir as a treatment for patients requiring hospitalization. Despite only a few, these drugs represent considerable progress in drug development, considering the long pipeline involved in drug discovery up to the approval process by local and international regulators.

Computational approaches can be used to perform high-throughput screening of drug libraries and small molecule databases for candidate drugs against their affinities with the target binding site. This strategy is called structure-based drug design. This is, however, limited by the quality of the database used [25,26]. Another approach is to design new drug-like molecules *de novo* following a fragment-based design concept (ligand-based design). This uses the structural information of the biological target as a design guide which is advantageous for it allows an ample chemical space of virtual structures to be explored without actual synthesis of such a large number of compounds. However, this also presents a challenge on how to handle the infinitely large number of theoretically possible topologies and the variety of conformations for a single topology [27,28]. This requires efficient optimization algorithms and high-quality QSAR descriptors to navigate the chemical search space [27,29]. For both *in silico* strategies, the feasibility of the candidate drugs to be synthesized further slows down the drug discovery process.

Here, novel drug molecules were designed that target the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, which is known to be involved in the viral attachment to its host cell. D*e novo* drug design was performed against the original variant, the B.1.1.7 variant, the B.1.351 variant, and the B.1.427 variants of SARS-CoV-2. These drug-like molecules could inhibit viral replication for possible use as new therapies against COVID-19.

2. Computational Details

2.1. Target Preparation

The high-resolution crystal structure of the receptor-binding domain (RBD) of SARS-CoV-2 spike protein (PDB 6MOJ) [30] was obtained from the Protein Data Bank. Its

structure was mutated with the corresponding amino acid substitutions for each SARS-CoV-2 variant. All four structures are then pre-processed to get their minimum-energy configuration which was used in subsequent docking calculations. Specifically, missing hydrogens were added, correct bond orders were checked and assigned, correct protonation states were predicted, and hydrogen bonds were optimized through systematic and cluster-based approaches. Restrained minimization was also applied to relax bonds, angles, and overlaps within each structure.

2.2. De novo Drug Design by Generative Neural Networks

De novo drug design was also implemented using the LIGANN web server to generate a library of drug-like molecules that target the RBD of each SARS-CoV-2 variant through generative neural networks. In particular, a generative adversarial network was used to produce complementary ligand shapes in a multimodal fashion. Then a shape captioning network decodes the ligand shapes into SMILES strings [31] which were then converted to a structure file using Open Babel v. 3.1.1 [32]. Next, the chemical library generated for each SARS-CoV-2 variant was combined to produce the main library. This main library, consisting of 2,334 molecules, was then docked against the RBD of each SARS-CoV-2 variant using the same docking protocol described above as implemented in the LEA3D web server. Finally, the docking scores of the drug molecules against each SARS-CoV-2 variant were averaged and then ranked to determine the best-performing drugs that could inhibit all four variants.

2.3. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Study

All performing drug candidates against the RBD of SARS-CoV-2 were analyzed for their ADMET profiles using the ADMETlab 2.0 platform. ADMETlab 2.0 is based on 53 predictive models from a comprehensively collected database of 288,867 molecules. The library of input molecules is fed into a Multi-task Graph Attention (MGA) framework to generate the ADMET profiles of each entry based on the trained regression models [33]. Only those that pass the following criteria were deemed promising candidate molecules: good human intestinal absorption ($p < 0.7$), low probability of passing through the blood-brain barrier ($p < 0.7$), good drug clearance ($CL \geq 5$), low hERG toxicity ($p < 0.3$), low hepatoxicity ($p < 0.3$), low drug-induced liver damage probability ($p < 0.3$), low mutagenicity ($p < 0.3$), low acute toxicity ($p < 0.3$), and low carcinogenicity ($p < 0.3$).

2.4. Molecular Dynamics

Ligand-protein interactional binding mode and the dynamical unbinding process by performing molecular dynamics calculations on the top-performing ligand obtained from *de novo* drug design. The calculations were implemented using the Ligand and Receptor Molecular Dynamics (LARMD) webserver [34] for each protein-ligand complex structure. The antechamber module and the Tleap module of the AMBER16 program [35] were used to assign the bcc charges for the ligand atoms and construct the complex's coordinate and topology files. The AMBER ff14SB force field [36] and gaff force field [37,38] were used for amino acid residues and ligands. The structures are solvated using an octahedron box of TIP3P waters [39] extended at least 10 Å in each direction from the solute [40]. Na^+ and/or Cl^- ions are added to the system as counter ions. Four-step minimization of the system was achieved using the Sander module in AMBER16. Two thousand steps steepest descent method and 3000 steps conjugated gradient method were used for each minimization step. The system was then heated from 10 to 300 K in 30 ps using an NVT ensemble followed by dynamics run at 300 K and 1 atm for four ns. All dynamics runs were done using the Pmemd module of AMBER16. The MD trajectories are then analyzed using the Cpptraj module of AMBER16. MDTraj was used to calculate nonnative contact [41], and Bio3d was utilized to analyze PCA and residue cross-correlation [42] as implemented in the LARMD server.

3. Results and Discussion

3.1. Structure and Mutations in the Receptor Binding Domain

The minimized structure of the receptor-binding domain (RBD) of the original wild-type SARS-CoV-2 is shown in Figure S1. Structurally, the RBD consists of 195 amino acid residues. Accordingly, the single amino acid substitutions in the RBD of SARS-CoV-2 (N501, Q498, E484, T470, L452, and N439) against SARS-CoV-1 resulted in the loss of favorable interactions with hACE2. Meanwhile, five RBD substitutions (P499, Q493, F486, A475, and L455) led to enhanced SARS-CoV-2 RBD-hACE2 binding activity [43]. In our subsequent docking experiments, these amino acid residues are then used to define the binding site region. Interestingly, these mutations are involved in the new SARS-CoV-2 variants discovered. N501Y mutation is present in the B.1.1.7, B.1.351, and P.1 variants. This mutation is associated with increased transmissibility of the new VOCs. Meanwhile, the E484K mutation is present in the B.1.351 and P.1 variants. The E484K mutation is associated with the increased ability of the said VOCs to evade innate and acquired immunity. So far, only the B.1.351 variant contains the K417N mutation in the RBD.

3.2. De novo Drug Design

A chemical library of potential inhibitors of the RBD of SARS-CoV-2 using generative neural networks was generated *de novo*. This is more robust than other *de novo* drug design techniques since it captures the structure of the binding site and then populates a library of complementary ligand shapes in a multimodal fashion. Here, we used 50 ligand shape generations and 20 decodings per shape to generate 539 molecules based on wild-type, 631 molecules based on B.1.1.7 variant, 615 molecules using the B.1.351 variant, and 572 molecules using the P.1 variant. We combined these molecules into a single chemical library of 2,357 ligands and then docked them against each COVID-19 variant. The average docking scores for each ligand were calculated and then ranked to determine the best-performing ligands that can inhibit all four variants of SARS-CoV-2. The top-performing ligands based on the averaged docking scores are shown in Figures S1 and S2.

Among the generated top-performing ligands, CID #526 or [3-(4-ethylpiperazin-1-yl)propyl](1-{3-[3-(morpholin-4-yl)propyl]-1,2,4-oxadiazol-5-yl}-4-phenylbutyl)amine, has excellent ADMET profile compared to others; hence we further investigated this molecule. CID #526 obeys all parameters set by Lipinski's Rule of Five. Moreover, it has good human intestinal absorption, good MDCK permeability (log P_{app} = 8.02 x 10^{-6}), low plasma protein binding (53.56%), desirable volume distribution (1.069 L/kg), high clearance (7.05 mL/min/kg), and doesn't inhibit the Cytochrome 450 metabolic pathway. It also has low cardiotoxicity (p = 0.399), hepatoxicity (p = 0.565), Ames mutagenicity (p = 0.037), and carcinogenicity (0.067).

Two hydrogen bonds with Gly 496, one with the amine group and one with the oxygen atom of the five-membered heterocycle of CID #526, are formed with the Wild SARS-CoV-2. It also forms a hydrogen bond with Ser 494 and has a π-π interaction with Tyr 505 of the Wild SARS-CoV-2. Only the hydrogen bond with the oxygen atom is conserved with the B.1.1.7 variant. CID #596 interacts differently with the B.1.351 variant. It forms a hydrogen bond with the nitrogen atom in the five-membered heterocycle and interacts with Phe 490 via π-π interaction. Only the π-π interaction is conserved in the P.1 variant, but it forms two different hydrogen bonds, one for Gln 493 and Ser 494, respectively.

3.3. Molecular Dynamics Analysis

Molecular dynamics simulation was done on the docked protein-ligand structure to elucidate further the inhibitory effect of CID #526 against the four variants of SARS-CoV-2 investigated in this study. The results are shown in the Supplementary Materials. Strong binding energy was calculated for each variant, showing the good inhibitory property of CID #526. It performs the best for the wild SARS-CoV-2, as evidenced by the lowest

binding energy, indicating the most stable ligand-protein complex. Meanwhile, it performs the least against the B.1.1.7 variant, having the highest binding energy.

We also performed principal component analysis and dynamic cross-correlation analysis to evaluate the inhibitory effect of CID #526 against the SARS-CoV-2 RBD based on the MD trajectory. Because the top three principal components are sufficient to capture 50% of the total variance in a given family of structures, only the top three principal components were analyzed [34]. The stability of the docked CID #526 was confirmed based on our results, confirming our hypothesis that CID #526 could be an inhibitor of SARS-CoV-2's receptor binding domain.

4. Conclusions

De novo drug design using generative neural networks was done to generate a library of possible inhibitors of each variant of SARS-CoV-2. These ligands were docked against the receptor binding domain of SARS-CoV-2 variants and were ranked according to the average values of their docking scores. The top-performing ligands were evaluated for their ADMET profiles. Among these compounds, CID #526 emerged as a top candidate with excellent docking performance against all four variants and a good ADMET profile. It forms several hydrogen bonds and interacts with the RBD via π-π interaction. Molecular dynamics analysis revealed the stability of the docked compound in the RBD of SARS-CoV-2 and with comparable binding energies against all four variants. The organic retrosynthesis study showed that CID #526 could be synthesized using five major reaction steps. Further studies are needed to ascertain these compounds' efficacy, safety, and tolerability for the treatment of COVID-19.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, **Figure S1.** Structure of the receptor-binding domain of the wild-type SARS-CoV-2 (RBD-W); **Figure S2.** Structures of top-performing *de novo* – generated drug-like molecule (Compound ID 526) docked in the RBD of SARS-CoV-2 and their corresponding ligand interaction diagrams; **Figure S3.** Top-performing (1-15) drug-like molecules to inhibit the RBD of SARS-CoV-2 variants.; **Figure S4.** Top-performing (16-30) drug-like molecules to inhibit the RBD of SARS-CoV-2 variants.; **Table S1.** Comparative performance of top-performing de novo - designed drugs as potential inhibitors of the RBD of SARS-CoV-2 variants.; **Table S2.** ADMET properties of top-performing de novo - designed drugs as potential inhibitors of the RBD of SARS-CoV-2 variants.; **Figure S5.** RMSD profile of docked CID #526 designed using generative neural networks in RBD, RMSD histogram of the ligand, and RMSD histogram of the receptor for (a-c) Wild-type, (d-f) B.1.1.7 variant, (g-i) B.135 variant, and (j-l) P.1 variant SARS-CoV-2.; **Figure S6.** Principal Component Analysis (PCA) for the MD trajectory of CID # 526 binding into the RBD of Wild SARS-CoV-2.; **Figure S7.** Principal Component Analysis (PCA) for the MD trajectory of CID # 526 unbinding into the RBD of Wild SARS-CoV-2.; **Figure S8.** Dynamical residue cross-correlation map for the MD trajectory of the (a) binding process and (b) unbinding process of the receptor-ligand complex involving CID #526 docked in the RBD of Wild SARS CoV-2.; **Figure S9.** Residue-wise loading for (a) PC1, (b) PC2, (c) PC3 of the binding process of the receptor-ligand complex involving CID #526 docked in the RBD of Wild SARS-CoV-2. Residue-wise loading for (d) PC1, (e) PC2, (f) PC3 of the unbinding process of the receptor-ligand complex involving CID #526 docked in Wild SARS-CoV-2.; **Figure S10.** Principal Component Analysis (PCA) for the MD trajectory of CID # 526 binding into the RBD of B.1.1.7 variant SARS-CoV-2.; **Figure S11.** Principal Component Analysis (PCA) for the MD trajectory of CID # 526 unbinding into the RBD of B.1.1.7 variant SARS-CoV-2.; **Figure S12.** Dynamical residue cross-correlation map for the MD trajectory of the (a) binding process and (b) unbinding process of the receptor-ligand complex involving CID #526 docked in the RBD of B.1.1.7 variant SARS CoV-2.; **Figure S13.** Residue-wise loading for (a) PC1, (b) PC2, (c) PC3 of the binding process of the receptor-ligand complex involving CID #526 docked in the RBD of B.1.1.7 variant SARS-CoV-2. Residue-wise loading for (d) PC1, (e) PC2, (f) PC3 of the unbinding process of the receptor-ligand complex involving CID #526 docked in B.1.1.7 variant SARS-CoV-2.; **Figure S14.** Principal Component Analysis (PCA) for the MD trajectory of CID # 526 binding into the RBD of B.1.351 variant SARS-CoV-2.; **Figure 15** (a) Principal Component Analysis (PCA) for the MD trajectory of CID # 526 unbinding into the RBD of B.1.351 variant SARS-CoV-2.; **Figure S16.** Dynamical residue cross-correlation map for the MD trajectory of the (a) binding process and (b) unbinding process of the receptor-ligand

complex involving CID #526 docked in the RBD of B.1.351 variant SARS CoV-2.; **Figure S17.** Residue-wise loading for (a) PC1, (b) PC2, (c) PC3 of the binding process of the receptor-ligand complex involving CID #526 docked in the RBD of B.1.351 variant SARS-CoV-2. Residue-wise loading for (d) PC1, (e) PC2, (f) PC3 of the unbinding process of the receptor-ligand complex involving CID #526 docked in B.1.351 variant SARS-CoV-2.; **Figure S18.** Principal Component Analysis (PCA) for the MD trajectory of CID # 526 binding into the RBD of P.1 variant SARS-CoV-.; **Figure S19.** Principal Component Analysis (PCA) for the MD trajectory of CID # 526 unbinding into the RBD of P.1 variant SARS-CoV-2.; **Figure S20.** Dynamical residue cross-correlation map for the MD trajectory of the (a) binding process and (b) unbinding process of the receptor-ligand complex involving CID #526 docked in the RBD of P.1 variant SARS CoV-2.; **Figure S21.** Residue-wise loading for (a) PC1, (b) PC2, (c) PC3 of the binding process of the receptor-ligand complex involving CID #526 docked in the RBD of P.1 variant SARS-CoV-2. Residue-wise loading for (d) PC1, (e) PC2, (f) PC3 of the unbinding process of the receptor-ligand complex involving CID #526 docked in P.1 variant SARS-CoV-2.

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