Generate, Repurpose, Validate: A Receptor-Mediated Atom-by-Atom Drug Generation for SARS-Cov-2 Spike Protein and Similarity-Mapped Drug Repurposing for COVID-19 with Rigorous Free Energy Validation Using Well-Tempered Metadynamics

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Finding a cure for Covid-19 is of immediate and paramount importance. In this study, we propose new and repurpose drugs to prevent SARS-Cov-2 (Covid-19) viral attack on human cells. Our study comprises three steps: generation of new molecules, structural similarity mapping to existing approved and investigational drugs, and validation of their binding strengths to the viral spike proteins based on rigorous all-atom well-tempered metadynamics free energy calculations. We show that some of our new molecules and some of the existing drugs bind more strongly than human ACE2 protein to the viral spike protein. Therefore, these drug molecules may have the potential to be repurposed as a preventive therapy for Covid-19, subject to further experimental verifications.

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Generate, repurpose, validate: a receptor-mediated atom-by-atom drug generation for SARS-Cov-2 spike protein and similarity-mapped drug repurposing for COVID-19 with rigorous free energy validation using well-tempered metadynamics

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ABSTRACT: Covid-19 has wreaked havoc over mankind. To find potential therapeutics, we have developed a method to generate de novo molecules that grow atom-by-atom optimizing their interaction energy with the receptor. With this method, we obtained several ligands that bind strongly to the receptor binding domain (RBD) of the viral spike protein and chose top 35 molecules (~0.3% percent of the number generated) based on the interaction energy. We also mapped the de novo molecules to approved and investigational drugs and chose 20 best candidates based on the docking score. We validated our molecules by calculating the computationally most accurate, albeit expensive, all-atom explicit-water free energy surfaces for all the 55 molecules along with hACE2 with RBD using well-tempered metadynamics simulations. We also show that our calculated free energy estimate of hACE2 and RBD is in close agreement with experiments. Additionally, we found several molecules (4 de novo and 5 drugs) that have comparable or stronger binding affinity to the RBD than that of the hACE2. This implies that these molecules and drugs could have the potential to block the RBD-hACE2 interaction and thereby prevent further infection in human cells. While the de novo molecules provide us with novel viral-entry inhibitors and will require extensive toxicological and pharmacological screenings, the drugs could be tested immediately for their efficacies against Covid-19. Among the drugs we obtained, the strong binding ones are Danoprevir, Glecaprevir, Paritaprevir (hepatitis C protease inhibitor), Saquinavir (HIV protease inhibitor) and Solithromycin (ketolide antibiotic used against community acquired pneumonia). This pool of drugs will allow strategic repurposing against Covid-19 for a particular prevailing condition.

INTRODUCTION

SARS-CoV-2, commonly known as the "Novel Coronavirus" or "COVID-19", is a positive strand RNA beta-coronavirus with large sequence similarities to the SARS-CoV and BatCov RATG13 RNA viruses.¹ Despite being known from at least early last year² to as far back as 2006 as found in a report by Tang *et al.*¹, the virus, seemingly incurable and unstoppable, is in an exponential growth phase all across the world with numbers increasing every day. The approximate number of COVID-19 cases is ~3.1 million worldwide with ~0.22 million deaths by the end of April 2020.³ Without a cure or prevention, this virus could very well become much more than the highly morbid pandemic it currently is.

Therefore, it is imminent to find a cure for the virus. Coronavirus is encapsulated by a membrane full of trimeric spike proteins. This spike protein interacts with the peptidase domain (PD) of the human angiotensin-converting enzyme 2 (hACE2).⁴ Consequently, this spike protein has been alluded to as a potential target to design preventive and curative therapeutics.⁵ An alternate common target for viruses is the Main protease (^MPro) and the Non-Structured Proteins (NSPs). For SARS-Cov-2 (Cov2), the high resolution crystal

structure of CL3 protease, also known as ^MPro, was resolved recently.⁶ However, simulation studies have indicated that the enzyme active site (the putative drug design target) of SARS-CoV-2 ^MPro is highly flexible making it less prone to be inhibited by common viral protease inhibitors.⁷ To find other potential targets, Zhou *et al.*⁸ mapped protein-protein interaction network for proteins involved in Cov-2 and came up with many targets to be used for drug repurposing by docking the drugs from the DrugBank⁹.

The detailed structural elucidation of Cov2 and hACE2 (hACE2) interface by Shang *et al.*¹⁰ shows that the spike protein's RBD region remains to be the most important target for drug design for Cov2. The study identifies residues responsible for the interaction and tagged it as receptor binding motif (RBM). RBM is a part of a binding domain called RBD, which again is a part of the S1 region of the spike protein, whose entire structure was solved recently by Wrapp *et al.*¹¹ This protein is composed of three subunits. Each of these subunits contains a receptor binding domain (RBD). In two of the subunits, the RBD is in the so-called "down" configuration and in one it is in the "up" configuration. However, the exact structure of the RBD complexed to hACE2 and the interactions involved in the site were

redetermined very recently by Yan *et al.* by X-ray diffraction.¹²

Although the spike protein is trimeric¹¹ and exists in both open and closed forms¹³, it is the RBD of the monomer that is responsible for cellular recognition, its movement is also independent of the rest of the protein.¹⁴ Figure 1a shows the structure of the spike protein, where RBD is in the "up" configuration, in contact with hACE2. Figure 1b shows the RBD separately to highlight the interaction hotspot (i.e., RBM¹⁰). The structure of RBM serves as the hotspot for a potential inhibitor of the interaction between spike protein and hACE2 peptidase domain.

Figure 1. (a) The trimeric structure of the S1 region of the spike protein obtained from protein data bank with ID 6VSB¹¹; RBD is shown in red and the hACE2 shown in green. The arrow indicates the target for a new inhibitor design. (b) The magnified version of the modelled RBD (in red) with the RBM (hotspot) shown as mesh surface in cyan.

The recent havoc created by the virus resulted in an upsurge of studies towards a remedy. Smith and co-workers performed ensemble docking of several drugs and identified some important ones that would bind to the RBD.¹⁵ This was followed by several docking studies targeting spike protein¹⁵⁻¹⁶, Cov2 protease¹⁷, and hACE2¹⁸, etc. All these studies resulted into a great number of possible drugs which could be repurposed for the treatment of Covid-19, pending a rigorous theoretical or an experimental tests.

Although docking is a quick method to sieve plausible candidate for binding, it often fails to ascertain the correct binding constant due to lack of water, ions, and entropic effects due to fluctuations of proteins, ligands and water molecules.¹⁹ Therefore, docking gives a wide range of molecules to try and often it becomes difficult to choose the best from them. Despite these pitfalls, docking gives a quick method for finding repurpose drugs.²⁰ The most reliable and accurate approach to estimate binding constant, computationally, is to calculate it from an all-atom, explicit water simulation.²¹ To the best of our knowledge, no attempt has been made to find out the binding constants of any of the ligands through such rigorous methods for any of the target proteins for Covid-19.

The competitive inhibition by the drug will work if the RBD binds to the drug stronger than it does to hACE2. Therefore, estimating the binding constant accurately is essential. Using surface plasmon resonance experiments, Shang *et al.* reported a value of 44.2 nM (-10.2 kcal/mol at 300K) binding affinity for the his-tagged RBD by covalently immobilizing the protein to the sample substrate.¹⁰ However, noncovalent association methods, which underestimates the K_d , provide a lower value of 2.9 nM¹³ and 1.2nM¹³. The K_d measured by Wrapp *et al.* using biolayer interferometry is 34.6 nM.¹¹ The above values when converted to free energy at 300K temperature will yield a range between -10.3 and -12.3 kcal/mol. We will show later that our computational estimate of the above is very close to the above experimental result.

Therefore, our primary aim for this study is to find a molecule that can bind to the hotspot of RBD with binding free energy lower than -10.3 kcal/mol. For that, we have developed a de novo molecule generation program, called 'DeNovo', that creates molecules atom-by-atom in the protein's hotspot (a defined structural region of a biomolecule) to optimize the interaction energy between the two. The idea of such a de novo generation stems from the fact that the chemical space is infinite²² and there are molecules in our chemical space that would strongly bind to a given receptor, if only we can find them. Although the program is quite general and can grow molecules for any hotspot, we apply it for the first time here to grow inhibitors for the RBD by targeting the RBM region (Fig. 1b). Our approach will be particularly helpful for creating inhibitors where existing drugs have already started facing resistance and a completely new design for a drug is essential.²³ The current problem of Covid-19, however, demands a different solution, where we need to provide molecules worthy of immediate clinical trials.

Here we have generated several de novo molecules and found analogous drugs from the DrugBank⁹ that could be repurposed for Covid-19 using a similarity-based mapping. We have validated our approach by performing the computationally rigorous all-atom, explicit water well-tempered metadynamics²⁴ free energy calculations and show that 9 molecules (4 de novo and 5 drugs) have free energy of binding to the RBD lower than -10.3 (lower than the cut-off set above), implying their highly promising potential to inhibit the viral attack on human protein. As the benchmark, we calculated the free energy of binding between RBD-hACE2 in good agreement with experiments.

Therefore, our study provides a new, viable, and successful approach for finding novel and repurposed drugs for Covid-19, although it has the potential to be used for any other receptor-mediated drug design as well.

RESULT AND DISCUSSION

A. Generation of specific binders for a receptor. Our DeNovo program (see Method), schematically shown in Fig. S1 of the supporting information (SI), generated 13516 molecules having 10 to 50 heavy atoms to cover a broad spectrum of molecular scaffolds. The distribution of interaction energy between the molecule and the RBD is shown in Fig. 2. Once fitted to a standard Gaussian function, the mean comes at -27 kcal/mol and standard deviation at 11.5 kcal/mol. We have chosen ~0.3% of the molecules by selecting those that have interaction energy less than a cut-off

value of -61.5 kcal/mol (Fig. 2) set at three standard deviations lower than the mean energy.

Figure 2. Distribution of interaction energies with RBD for 13516 DeNovo generated molecules. The mean and standard deviations of the fitted Gaussian distribution is -27.0 kcal/mol and 11.5 kcal/mol, respectively. The cut-off (redline) is set at three standard deviations lower than the mean, i.e., at -61.5 kcal/mol.

Thus we gathered 35 molecules, plotted in Fig. S2 of SI. The names of the molecules are chosen based on an internal criteria of numbering. At this point, we have a set of good molecules to work with. However, our molecule generation had a few caveats: (1) our hotspot was rigid, (2) there were no water molecules (partially accounted for by using dielectric constant), (3) and the entropy contributions could not be taken into account. Therefore, to calculate the exact binding free energy, to the extent that a classical force field can provide, we have subjected the aforementioned drugs to a state-of-the-art rigorous well-tempered²⁴ version of metadynamics²⁵ simulation for all these molecules as described later.

B. Route to repurposing. As we will see later, the DeNovo generated molecules are indeed strong binders and some of which could be candidate drugs. However, synthesis and

toxicology study for these molecules may take much longer. Therefore, under the current state of urgency, we need readily available drugs that can be repurposed for Covid-19. Relying on the capability of our de novo molecules and building on the concept that similar molecules have similar chemistry²⁶, we looked for similar molecules in the DrugBank⁹ using Tanimoto (or Jaccard)²⁷ similarity search which uses bitwise fragment comparison to accurately match structures based on chemical fragments and motifs present in the input structures². This similarity algorithm generally preserves relative positions of functional groups. We have chosen drugs for each of our 35 molecules with the Tanimoto coefficient ≥ 0.4 , following the recommendation of Baldi *et al.* who showed that a Tanimoto score of 0.4 is significant for a database of over 10,000 molecules.²⁸ This similarity search led to several drug molecules for each of the 35 de novo molecules, listed in Table S1 with their similarity score in brackets. After removing the irrelevant drugs (shown in red), such as the ones in the category of narcotics, anaesthetics, illicit substances, etc. (see the full list in SI), we ended up with 123 unique drugs. Given the difficulty and time required to perform free energy calculations for all these molecules, we docked these all the drugs to the same hotspot (Fig. S3 of SI), using docking score purely as a sieving criterion, and selected molecules with a docking score < -8.0 kcal/mol. This narrowed down our list of potentially repurposable drugs to 20 molecules. Figure S4 of SI shows the chemical structures of the chosen drug molecules and their docking scores are given in Table S2. As docking score varies even with the same parameters based on the representation of the drug (see Table S3 of SI and related discussion), we carried out the free energy simulations of these drug molecules with well-tempered²⁴ metadynamics method, as well.

To capture the relationships between the de novo and drug molecules, we plot the similarity matrix of all the 55 molecules in Fig. 3. This matrix obtained by measuring similarity-based clustering of each pair of molecules. This matrix provides valuable information on the structural diversity of molecules that have led to favourable properties. We find that there are two separate clusters, one dominated by repurposed drugs and the other dominated by de novo molecules.

Figure 3. Heat map of the Tanimoto similarity amongst the chosen list of de novo and drug molecules. The hierarchical diagram also depicts the similarity between different molecules. Two clusters of de novo molecules and drugs are clearly visible. However, there are cross similarity also present.

We note that the molecular similarity reflects on the similarity in binding free energy as well. The best-binding de novo molecules (as scored based on DeNovo's interaction energy) are in themselves quite similar, as are the best-binding repurposed drugs in themselves. This is particularly noticeable once the molecules have been mapped to their free energies, which is done for those molecules with < -10.0 kcal/mol in the left panel of Fig. 4. Furthermore, even though it may seem at the first glance that the cross-similarity is less, there are marked, and sporadically distributed regions of cross-similarity observed between the de novo molecules and repurposed drugs, which can be attributed to appreciable similarities in their maximal common substructures.

The left panel of Fig. 3 shows the structures of some of the best molecules along with their free energy (see below). It is truly reassuring that we obtained several molecules, both from our de novo generation and repurposing strategy, that are comparable or stronger in binding to RBD than hACE2.

Figure 4. Free energy surface of RBD binding to hACE2 against two collective variables, native contact (N_C) and distvec (Å). The structures along the path of dissociation are shown along the free energy bar diagram.

C. Free Energy Surfaces of Protein-Protein and Protein-Drug Complexes: An induced fit Mechanism. Free energy calculation with all-atom explicit water system is by far the most accurate, albeit expensive, estimate of binding among various computational methods.²¹ Metadynamics is an extremely popular state-of-the-art method to calculate free energy surface for complex systems²⁹ and it has been shown to reproduce experimental observations closely³⁰⁻³¹. Recently, attempts to estimate free energy of binding was done for the protease, albeit with an approximate method called MM/PBSA³², which takes water as continuum, thus leading to an inaccurate estimation of entropy, and furthermore due to its lack of entropic effects it is also not ideally suited for providing mechanistic insights. All-atom with explicit water free energy calculation not only estimates the free energy more accurately, it also captures the mechanism of the binding. To the best of our knowledge, we are the first to perform the free energy calculations for the spike protein's interaction with hACE2 and other ligands. Here we have covered a huge list of 55 ligand molecules (35 are de novo and 20 are known drugs).

Before calculating the free energy for all the 55 drugs molecules, we validated our approach by calculating the free energy surface for RBD and hACE2 for comparison. Starting with the available crystal structure of the spike protein and hACE2 (PDB id 6VSB¹¹), we first selected only the RBD region, modelled the loop region (see method), solvated the system with water and physiological concentrations of ions and performed multiple, long (~230 ns) well-tempered metadynamics simulations against multiple collective variables such as native contact (Fig. S5 of SI) and distvec (Fig. S6 SI) to study the binding free energy surface of these two proteins. While native contact helps to untangle the interactions between two proteins, distvec is a vectorial distance that helps move the proteins apart. These coordinates were used successfully in our previous studies of drug

intercalation³³⁻³⁴ and protein-protein interactions³⁵ in our group.

Figure 4 shows the free energy surface which verifies the crystal structure configuration as the global free energy minimum. A few snapshots of the configuration of both proteins are shown along the unbinding pathway which depicts that the major reason for stability is due to direct

protein-protein interaction. As soon as the proteins detach themselves, the free energy increases pushing the proteins apart, reflected in the increase in distvec. From this free energy surface, we get the binding free energy to be -13.3 kcal/mol which is in close agreement with the experimental results. A second metadynamics simulation of the same system yielded free energy estimate of -12.7 kcal/mol making the average -13.0 kcal/mol.

Figure 5. Free energy surfaces (FES) of three best de novo molecules and three best drug molecules in terms of free energy of binding. The chemical structure and the name of each molecule are shown in the inset. The free energy bar used to plot FES is shown in the inset of 37_42. The structures of the free energy minimum of each molecule, made using chimera³⁶, are shown above/below the corresponding FES's. The protein is drawn in surface representation while the drug is drawn with both stick and surface (with 80% transparency). Note the deep cavity formed by each of these molecules in the protein.

Using the same set of collective variables and starting with either the docked state for drugs or the DeNovo generated configurations for the novel molecules, we performed metadynamics for all the 55 molecules and calculated their free energy surfaces.

Figure S7 to S10 in SI show the two-dimensional free energy surfaces of binding of all the molecules. In Fig. 5, we show the FES of three top de novo molecules and 3 top drugs. A

representative structure of the most stable configuration obtained from the metadynamics simulation is shown either above or below the FES of the respective molecule. Figure 5 shows that the molecules are nicely packed inside the hotspot region.

Since each molecule behaves differently, it is not possible to find a unifying trend in their binding mechanism. However, most of the strong binding molecules have a narrow free

energy profile along the native contact. Once the contacts (the short distance between the ligand and the protein) are broken, the separation between the ligand and protein quickly increases.

We summarize the results from our metadynamics simulations of all the systems (55 molecules and hACE2) in Fig. 6. The details of the system size and the runtime are given in Table S4 of SI. The free energies of the molecules are shown as vertical bar, where the RBD-hACE2 free energy values (experimental and computational) are shown as horizontal bars. We provided error estimates for the free energy for the hACE2 and some of the strong binding drug

molecules by performing multiple free energy calculations. Different experimental techniques provide different estimates for the strength of hACE2 binding to the spike protein, as represented by the grey bar in Fig. 3. However, the lower limit of the interaction is -12.3 kcal/mol which is close to our calculated value of -13.0 kcal/mol. The vertical bars show the binding free energy of 35 de novo molecules and 20 drug molecules. We can see that at least 9 molecules touch or cross the experimental bar indicating that RBD would bind to these molecules comparably or stronger than hACE2. Three molecules (47_68, danoprevir and solithromycin) supersede even the lowest estimate of binding strength of hACE2 with RBD.

Figure 6. Free energy of binding for all the 35 de novo molecules and 20 drugs to RBD calculated using all-atom, explicit water, well-tempered metadynamics simulations. The horizontal grey bar indicates the experimental range of free energy of binding of RBD and hACE2. The orange bar is the free energy estimate from multiple metadynamics simulations of RBD and hACE2. Error bars, obtained using multiple metadynamics simulations, are shown for some of the strong binding molecules. Note that, with RBD, many molecules bind comparably or stronger than hACE2.

Figure 5 shows that the strong binding molecules create a cavity within the protein. To understand this better, we investigated the flexible loop (residues 445 to 468) around the hotspot region. We have introduced an angle θ (see Fig. S11 of SI) that categorizes the configuration of the loop near the hotspot into three distinct conformations – close, open, and semi-open. We calculated the distribution of θ from the metadynamics simulations for all the systems including the free protein and hACE2 bound states. We plot the distribution for the most stable ligand, free protein and hACE2 bound protein in Fig. 7. This distribution shows that peaks at high

value (155°) in the hACE2 bound RBD configuration, attributed to the open configuration. The distribution of θ is around 80° in the free state, characterized here as the semi open state while the distribution of θ for the most stable ligand bound RBD is around 60° . The loop configurations for the other ligand bound states lie between the two extremes of $55^\circ < \theta < 155^\circ$ (between hACE2 and danoprevir bound states). The θ for all the other ligand-bound states are shown in Fig. S12 of SI.

Figure 7. Distribution of loop configuration in terms of the angle between the red and green vectors (see Fig. S11 of SI) from the metadynamics simulation. The top panel shows three categories of loop configurations: open, semi open and close states. Representative images of the most stable ligand-bound states are shown where the structures are arranged from open to close configurations in clockwise fashion. Note that, while free protein belongs to the semi open configuration, the hACE2-bound and danoprevir (and 47_68)-bound RBD lie in the two extremes -- open and close states, respectively.

The above distribution indicates that binding of RBD to hACE2 and the ligands discussed here generate very different protein configurations. Therefore, upon binding of these ligands, the RBD configuration will be different and it will not be able to interact with the hACE2 effectively, achieving the competitive inhibition.

CONCLUSION

In this study, we have targeted a particular receptor, namely the receptor binding domain (RBD) of the spike protein, and designed several molecules through an atom-by-atom de novo generation protocol that tries to optimize the interaction of those molecules with the receptors. We selected only top 0.3% molecules (35 in number) and carried out free energy calculations using all-atom models of protein, ligand, water and ions using state-of-the-art well-tempered metadynamics method. The success of our de novo method is validated when 10 molecules have binding free energy to RBD < -9 kcal/mol. We then mapped each of the molecules to approved and

investigational drugs in the DrugBank⁹ based on structural similarity and picked up the top 20 based on docking score. The all-atom free energy calculations were also performed with these drug molecules to the RBD. Out of the 20 molecules chosen, 11 of them bind to RBD with binding free energy below -9 kcal/mol. To validate our free energy method, we also calculated free energy of hACE2 with RBD and found it (-13.0 kcal/mol) in close agreement with experiment (-12.3 kcal/mol). Overall, we found at least four de novo molecules and five drugs molecules that bind to RBD comparable or stronger than the hACE2. This implies that these molecules could in principle inhibit the interaction of the RBD with hACE2 and prevent the viral attack on the human cell.

These repurposed drugs are molecules which seem to have by themselves a rich history as drugs for several threatening and even currently looming diseases. *Ciluprevir*, *Glecaprevir* and *Paritaprevir* are all hepatitis virus NS3/4A inhibitors, designed based on the transition state intermediates in the

enzymatic active site with promising remedial properties as a small molecule inhibitor of the hepatitis infection. The possible binding mechanism of these drugs are generally mediated by α -keto or sulfonate-based electrophilic warheads³⁷⁻³⁸, disrupting key interactions in the nucleophilic NS3/4A enzymatic cavity. Apart from Glecaprevir, both Ciluprevir and Paritaprevir are administered alongside CYP3 inhibitors to improve their cell permeability and lower their IC50 dosage. On similar lines, *Danoprevir* and *Saquinavir* were also transition state analogue-based inhibitors for the NS3 protease, and are made to competitively inhibit the gag-pol substrate.³⁹⁻⁴⁰ Solithromycin is an anti-microbial⁴¹, anti-inflammatory⁴² drug that has been used in several occasions for decades⁴³; its role as an antiviral is known for respiratory tract infections⁴⁴, apart from being a potent inhibitor to a host of other diseases⁴³. This multi-functionality that we report for the aforementioned drugs is something be expected due to the immense number of secondary and even tertiary roles a drug molecule has once it enters the human body. As a study by Baker *et al.* eloquently shows, around 35,000 of all known drugs and drug-like substances worldwide (belonging to different chemical classes) have been used in more than one disease, while roughly 189 such molecules have been used as drugs for over 300 diseases.⁴⁵

Therefore, in this study, we not only established a protocol of finding new molecules for any given receptor, we also provide a definitive way to find and repurpose drugs in-silico. We have also validated our finding to the widely accepted and rigorous free energy protocol currently present in the literature. We hope that experiments carried out with the molecules presented here will further validate our results and it will be useful for our ongoing endeavour to fight against COVID-19 pandemic.

METHODS

A. DeNovo: The atom-by-atom synthesis of a strong binder.

Here we describe our algorithm of de novo atom-by-atom construction of molecules in the hotspot of a receptor. The algorithm is based on the fact that any molecule can be represented as a graph where nodes are atoms and the bonds are edges. The first evidence of graph representation of the molecules dates back to 1867 by Kekule⁴⁶. Recently, graph based algorithm was used to create 166 billion molecules in vacuum from only 17 atoms⁴⁷ of C, N, O, S, and halogens, which reinforces the fact that the chemical space is infinite and therefore it is possible to get possibly multiple strong binders for each hotspot.

We, however, grow the molecules in the protein hotspot from the similar set of atoms (C, N, O, S, etc.). The classical nonbonding interaction energy (both van der Waals and electrostatic) between the drug and the hotspot is calculated using CHARMM27⁴⁸ force field at each stage and the generation proceeds following an algorithm similar to that of the configuration bias Monte Carlo (CBMC)⁴⁹. Prior to molecular generation, the incomplete residues were completed using xleap of AMBERTools⁵⁰ and the missing residues of the protein were modelled by Modeller 9.21⁵¹. We targeted a prefixed atom number for the molecule and tried to improve the interaction of the molecule using CBMC criteria. This way, we obtained molecules having between 10 to 50 heavy atoms. Unlike in CBMC, our molecule is made of many different atoms. We used geometric criteria (equilibrium distance and angles) for the formation of rings. Finally, even when a molecule reaches the desired number of atoms, CBMC protocol was used to create different molecules of similar size. The program is fully CPU-parallelised and runs efficiently over several cores (here we used 48 cores for our molecule generation). The schematic growth profile is shown in S1 of the supporting information (SI) and a video of a selected section of the generation

suitable for conveying all the key points of the algorithm is provided as SV1 of SI.

B. Similarity Measurement. We have chosen all de novo generated molecules that interact with the protein with energy lower than our cut-off -61.5 kcal/mol (see Fig. 2). Then we performed a similarity search using the DrugBank⁹ search engine employing Tanimoto algorithm⁵². All approved and investigational drug molecules with similarity above 0.4, a cutoff based on the studies of Baldi *et al.*²⁸, were considered. Table S1 of SI list all the drug molecules for each of the 35 de novo molecule that match the criterion.

C. Docking of the matched drugs. Based on the similarity measurement, we came up with a list of 123 unique drug molecules from our 35 de novo molecules. We then docked these drug molecules using AutoDock Vina version 1.1.2⁵³, in the hotspot. A box centered around SER443 is created with dimensions 36.7 Å x 26.1 Å x 40.9 Å and default vina parameters. The docking setup is shown pictorially in Fig. S3. Input files of the drugs are created by converting PDB files to PDBQT using OpenBabel 3.0.0⁵⁴. The docking scores for all these molecules are shown in bracket in Table S1. The drug molecules with docking score less than -8.0 kcal/mol were considered for free energy calculations.

D. Force Field Generation. All drug molecules were optimized using HF (Hartree Fock) theory with 6-31G* basis set using Gaussian 09 software⁵⁵. Thereafter, antechamber⁵⁶ module of AMBER18⁵⁰ is used for the RESP charge calculation of drug atoms. The forcefield was generated using GAFF forcefield⁵⁷. The topology and coordinates were then converted into the GROMACS format by using a python script acpype.py (available at <https://github.com/t-acpype>).

E. System Setup for Simulation. The starting structure of the SARS-CoV-2 spike glycoprotein (PDB ID: 6VSB) was obtained from the Protein Data Bank. Modeller 9.21⁵¹ was used for modeling the missing residues, which predicted five number of three-dimensional structural forms using chain A of protein as the template. The best possible structure was predicted considering the DOPE (Discrete Optimized Protein Energy) score⁵⁸. All of the simulations were performed using molecular dynamics software GROMACS 2019.6⁵⁹. For the study of the protein-ligand complex, we only considered the RBD region of the protein (residues 302 to 506) and topology was prepared using AMBER99SB force field⁶⁰. Each complex system was solvated by ~23000 TIP3P water molecules⁶¹ in a box of dimension 70X70X180 Å³. The physiological concentration (150 mM) of Na⁺ and Cl⁻ ions along with extra Cl⁻ ion were used to neutralize the system.

F. Equilibration and Simulation. Initially each system was energy minimized using steepest descent⁶² method for 10000 steps, followed by heating it to 300K in 200 ps using Berendsen thermostat and barostat⁶³ with coupling constant of 0.6 ps. Restraints of 25 kcal/mol/Å² were applied on heavy atoms during the heating process. Thereafter, equilibration was carried out for 2 ns at constant temperature (300 K) and pressure (1 bar) without any restraints using same thermostat and barostat with coupling constants of 0.2 ps each. The last 100 ps of NPT simulation was used to calculate the average volume the same, which was used in the final 5 ns unrestrained NVT equilibration using the Nosé-Hoover⁶⁴ thermostat with coupling constant of 0.2 ps. During the simulation, LINCS algorithm⁶⁵ was used to constrain all the bonds and Particle Mesh Ewald (PME) method⁶⁶ was used for electrostatics. The distance cut-offs for the van der Waals (vdW) and electrostatic long-range interaction was kept at 10 Å. The time step for each simulation was taken to be 2 fs.

G. Free energy calculation using metadynamics. The equilibrated Ligand-bound protein structure was initially simulated for 5 ns. If the ligand was found to be bound after 5 ns simulation, we subjected the system for free energy calculation. To calculate the binding free energy of drugs, well-tempered metadynamics²⁴ simula-

tions were performed after equilibration using native contacts as collective variables (see SI for full description). We performed a long (~100 ns). metadynamics simulation with a hill height of 0.2 kJ/mol and a bias factor of 10, and hills deposition rate of 2 ps. Gaussian widths for distvec and native contacts were taken to be 0.6 Å and 5, respectively. An upper wall restraint was applied at 45° on the angle between two vectors $\hat{b}$ and $\hat{d}$. For free-energy calculations, PLUMED 2.6⁶⁷ was used along with GROMACS. The system size and run lengths of all the systems are provided in the Table S4 of SI.

ASSOCIATED CONTENT

SUPPORTING INFORMATION

DeNovo flow chart, image containing all the selected de novo molecules, table showing list of similar drugs, image showing docking region, table of docking scores for selected drugs, table for docking variations, image of all the selected drug molecules, definition and picture of the collective variables used, table showing system size and other information for all the systems, and free energy surfaces of binding to RBD for all the 55 molecules.

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ABBREVIATIONS

FES, free energy surface; Cov2, SARS-COV-2.

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Supporting information for

Generate, repurpose, validate: a receptor-mediated atom-by-atom drug generation for SARS-Cov-2 spike protein and similarity-mapped drug repurposing for COVID-19 with rigorous free energy validation using well-tempered metadynamics

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A. DE NOVO PROGRAM FLOWCHART.

Figure S1. The algorithmic flowchart of de novo drug generation protocol (left panel) and the molecular growth subroutine (right panel).

Figure S2. The 35 de novo molecules that made the energy cutoff criteria discussed in the method. Binding free energy of all these molecules were calculated using all-atom explicit water simulations using metadynamics¹.

B. TABLE OF SIMILAR DRUG MOLECULES

Table S1. List of similar drug molecules retrieved from DrugBank² based on Tanimoto coefficient cutoff of 0.4 for each of the chosen de novo molecules. All narcotics, anesthetics, illicit substances, anti-depressants, schizophrenia/Alzheimer's/Parkinson's related hyper specific molecules, contrasting agents, neuromuscular blocking agents, immune-depressants, and pain relievers have been discarded due to their biochemical and pharmacological irrelevance to this problem. These rejects have been marked in red, all others that were acceptable were passed on to docking to RBD and are marked in blue. The docking score and similarity score to the respective de novo molecules are shown in bracket. For the rejected molecules, only similarity score is shown.

SR. No.	DeNovo Id	Similar Drugs with DrugBank Id with similarity and docking scores
1	29_19	DB06200(0.456,-6), DB04878(0.449,-5.6), DB09031(0.444,-5.5), DB01337(0.432), DB01339(0.432), DB00657(0.422), DB11807(0.422), DB00728(0.418,-6.8), DB05513(0.416), DB11860(0.407,-7.4), DB00419(0.402), DB14872(0.402), DB04834(0.401,-6)
2	32_26	DB06614(0.557,-5.6), DB11682(0.522,-6.6), DB15286(0.5), DB00312(0.489), DB08873(0.483,-6.1), DB11860(0.479,-7.4), DB00786(0.478,-6.5), DB06345(0.478,-7.6), DB06293(0.471,-5.8), DB01351(0.468), DB01353(0.468), DB06335(0.463,-5.9), DB00418(0.463), DB00237(0.46), DB00790(0.446,-6.2), DB00599(0.445), DB13253(0.442), DB06573(0.441,-6.1), DB06827(0.437,-8.2), DB00306(0.436), DB11762(0.433,-6.2), DB01154(0.429), DB06124(0.429,-6.4), DB00241(0.426), DB00248(0.419,-6.2), DB14932(0.419,-6.5), DB12613(0.419,-6.7), DB05541(0.418,-5.5), DB00474(0.417), DB11879(0.414), DB05434(0.413,-7), DB06641(0.413,-6), DB01627(0.412,-5.9), DB11938(0.41,-7.5), DB00178(0.409,-6.8), DB12416(0.407,-7.3), DB01216(0.406), DB15412(0.406,-5.8), DB00519(0.405,-6.7), DB04845(0.404,-7.1), DB06127(0.401), DB01190(0.401,-6)
3	37_42	DB00657(0.5), DB11807(0.5), DB06200(0.47,-6), DB12493(0.465), DB06714(0.463), DB13012(0.463,-6.2), DB15264(0.452), DB00302(0.443), DB04545(0.438,-4.5), DB12032(0.438), DB01074(0.426), DB04878(0.424,-5.6), DB00996(0.422), DB00230(0.419), DB05513(0.415), DB12105(0.409), DB01337(0.408), DB01339(0.408), DB11684(0.407), DB01637(0.404), DB11860(0.404,-7.4)
4	39_32	DB09421(0.536,-7.6), DB12370(0.507,-7), DB11944(0.503), DB15188(0.5,-6.6), DB11695(0.47), DB12199(0.461,-7.9), DB06763(0.457,-7.4), DB12631(0.45,-7.5), DB12099(0.448), DB01085(0.447), DB04896(0.446), DB08918(0.446), DB11720(0.437), DB05521(0.436,-7.9), DB01437(0.433), DB12833(0.433,-8), DB00357(0.43), DB06471(0.429), DB15299(0.429), DB14870(0.428), DB06335(0.425,-5.9), DB15091(0.421), DB00561(0.418), DB00490(0.418), DB00248(0.417,-6.2), DB06448(0.416), DB14813(0.408), DB01618(0.406), DB04872(0.406), DB14854(0.406), DB05796(0.404), DB00117(0.404), DB09102(0.403), DB06645(0.403), DB14955(0.402), DB09290(0.4)
5	39_57	DB06335(0.546,-5.9), DB12795(0.471,-6.4), DB11860(0.462,-7.4), DB00786(0.453,-6.5), DB04878(0.442,-5.6), DB04348(0.438,-8.3), DB08834(0.438), DB00790(0.436,-6.2), DB04876(0.432,-6.5), DB15286(0.429), DB05541(0.424,-5.5), DB02691(0.42), DB06127(0.411), DB06614(0.408,-5.6), DB15412(0.408,-5.8), DB06657(0.408), DB11684(0.405), DB00141(0.403), DB12536(0.403), DB01338(0.403,-7.3), DB12677(0.401,-6), DB01627(0.4,-5.9)
6	40_35	DB00790(0.593,-6.2), DB01337(0.557), DB01339(0.557), DB06124(0.54,-6.4), DB04834(0.539,-6), DB01338(0.536,-7.3), DB11860(0.528,-7.4), DB00728(0.525,-6.8), DB06335(0.514,-5.9), DB06614(0.514,-5.6), DB00178(0.512,-6.8), DB08965(0.507), DB00519(0.506,-6.7), DB13288(0.504), DB00479(0.5,-6.1), DB06696(0.485), DB11684(0.484), DB03567(0.483), DB01197(0.481), DB12232(0.481,-6.1), DB11682(0.476,-6.6), DB13087(0.476), DB12677(0.474,-6), DB05645(0.472,-6.5), DB12613(0.47,-6.7), DB14932(0.47,-6.5), DB11762(0.467,-6.2), DB01627(0.466,-5.9), DB12839(0.463), DB06345(0.456,-7.6), DB15286(0.455), DB06045(0.453), DB05814(0.453), DB01083(0.452), DB00786(0.45,-6.5), DB11879(0.449), DB01190(0.444,-6), DB06580(0.442), DB12655(0.441), DB04876(0.441,-6.5), DB09291(0.44), DB06472(0.437), DB05434(0.435,-7), DB05541(0.434,-5.5), DB12416(0.431,-7.3), DB13106(0.43), DB08873(0.43,-6.1), DB11929(0.43,-8.2), DB12287(0.43), DB04878(0.429,-5.6), DB06127(0.429), DB12704(0.428), DB00141(0.425), DB12536(0.425), DB06354(0.425,-7.9), DB08818(0.424), DB09271(0.424), DB00584(0.423), DB11876(0.422), DB11781(0.422), DB00899(0.421), DB02691(0.42), DB12615(0.417), DB15412(0.417,-5.8), DB11797(0.415), DB00517(0.413), DB09292(0.412,-8.4), DB00207(0.412,-7), DB01340(0.411), DB05633(0.408), DB09026(0.408,-6.5), DB00198(0.407), DB01232(0.407,-8.7), DB06293(0.407,-5.8), DB11801(0.407), DB06497(0.407,-8.1), DB13262(0.406), DB06573(0.406,-6.1), DB00708(0.404), DB14783(0.403), DB12795(0.403,-6.4), DB00747(0.401), DB09300(0.401), DB11315(0.401), DB12086(0.401), DB12178(0.401,-8), DB04863(0.4)
7	41_31	DB06705(0.52), DB06293(0.503,-5.8), DB06124(0.497,-6.4), DB12677(0.495,-6), DB06614(0.489,-5.6), DB05434(0.489,-7), DB00786(0.486,-6.5), DB06827(0.484,-8.2), DB08993(0.48,-8.2), DB01627(0.48,-5.9), DB01190(0.479,-6), DB08873(0.474,-6.1), DB15286(0.473), DB11801(0.47),

		DB12791(0.47,-6.5), DB12791(0.465,-6.5), DB06497(0.46,-8.1), DB00790(0.456,-6.2), DB08889(0.455,-7.5), DB12613(0.455,-6.7), DB14911(0.452,-7.5), DB00141(0.452), DB12536(0.452), DB11784(0.451,-6.7), DB12730(0.45,-6.8), DB11762(0.449,-6.2), DB06335(0.449,-5.9), DB15412(0.448,-5.8), DB06045(0.448), DB12615(0.448), DB11879(0.445), DB06573(0.444,-6.1), DB12704(0.441), DB09291(0.438), DB11890(0.435,-6.7), DB00207(0.435,-7), DB11471(0.434), DB00479(0.431,-6.1), DB05645(0.431,-6.5), DB12416(0.43,-7.3), DB13034(0.43,-7.3), DB14932(0.43,-6.5), DB00337(0.43,-8.7), DB00178(0.428,-6.8), DB06696(0.427), DB15188(0.427,-6.6), DB15205(0.425), DB00519(0.424,-6.7), DB01082(0.423), DB11938(0.422,-7.5), DB04876(0.422,-6.5), DB14941(0.422), DB11929(0.421,-8.2), DB12538(0.421,-8.7), DB01590(0.42), DB06641(0.42,-6), DB00864(0.419), DB13067(0.418), DB00877(0.418,-7.9), DB11797(0.418), DB00558(0.416), DB12508(0.416,-8.4), DB12279(0.416), DB06233(0.415,-7.6), DB11273(0.415), DB11274(0.415,-7.4), DB11275(0.415,-7.8), DB06478(0.412), DB05633(0.412), DB13345(0.411), DB00224(0.411,-7.5), DB14760(0.411,-8.4), DB11781(0.411), DB11586(0.411,-7.3), DB00305(0.409), DB12989(0.407), DB05541(0.407,-5.5), DB00320(0.407), DB12232(0.406,-6.1), DB11512(0.406), DB08818(0.405), DB11682(0.405,-6.6), DB09271(0.405), DB01319(0.404), DB11996(0.404), DB15251(0.404), DB01601(0.403,-7.7), DB12069(0.403,-8), DB04878(0.402,-5.6), DB09059(0.402), DB11709(0.402), DB00955(0.402), DB06419(0.401), DB15601(0.401), DB11783(0.401), DB11779(0.401,-9), DB01232(0.40,-8.7)
8	41_37	DB01190(0.483,-6), DB08873(0.48,-6.1), DB01627(0.478,-5.9), DB06573(0.478,-6.1), DB00760(0.471,-6.2), DB06293(0.463,-5.8), DB06211(0.451,-6.5), DB11586(0.45,-7.3), DB12791(0.45,-6.5), DB09297(0.45,-9.1), DB12791(0.449,-6.5), DB06233(0.443,-7.6), DB11929(0.443,-8.2), DB00303(0.441,-7.4), DB12876(0.439,-8.7), DB00503(0.438,-8), DB15249(0.438,-8), DB06827(0.437,-8.2), DB08993(0.437,-8.2), DB11779(0.436,-9), DB11273(0.436), DB11274(0.436,-7.4), DB11275(0.436,-7.8), DB11575(0.435,-7.5), DB12069(0.434,-8), DB12037(0.433,-7.9), DB00248(0.432,-6.2), DB13345(0.432), DB01598(0.432,-5.4), DB12730(0.431,-6.8), DB06335(0.431,-5.9), DB13028(0.428,-6.1), DB12587(0.428,-6.5), DB05868(0.427,-8.1), DB11808(0.426,-7.3), DB11682(0.426,-6.6), DB01601(0.425,-7.7), DB00790(0.425,-6.2), DB00320(0.424), DB00696(0.423,-8.7), DB01200(0.423,-7.9), DB13034(0.422,-7.3), DB12538(0.42,-8.7), DB00739(0.42), DB14911(0.42,-7.5), DB06614(0.419,-5.6), DB12677(0.419,-6), DB06124(0.418,-6.4), DB00337(0.418,-8.7), DB09065(0.418), DB05434(0.418,-7), DB01180(0.418), DB01992(0.418), DB01764(0.417,-7.2), DB00319(0.417,-7.2), DB14932(0.417,-6.5), DB00948(0.416,-7.5), DB00224(0.416,-7.5), DB14760(0.416,-8.4), DB05796(0.416), DB08889(0.416,-7.5), DB13879(0.416,-8.3), DB12026(0.416,-7.2), DB01590(0.415), DB00786(0.415,-6.5), DB09308(0.415,-9), DB12785(0.415), DB01301(0.413), DB12416(0.413,-7.3), DB00877(0.412,-7.9), DB15284(0.412), DB11961(0.412), DB15040(0.411), DB06497(0.411,-8.1), DB12279(0.41), DB00256(0.41), DB04845(0.41,-7.1), DB12228(0.41,-8.3), DB15188(0.41,-6.6), DB06290(0.409,-8.3), DB12691(0.409), DB00864(0.409), DB06604(0.409), DB12615(0.408), DB00206(0.408), DB11691(0.408), DB11762(0.407,-6.2), DB01089(0.407), DB12190(0.407), DB12190(0.407), DB12939(0.406), DB15205(0.404), DB12486(0.404), DB11783(0.402), DB12329(0.402), DB12795(0.402,-6.4), DB00558(0.401), DB00541(0.401), DB01061(0.4), DB01346(0.4), DB05521(0.4,-7.9), DB13253(0.4), DB01232(0.4,-8.7)
9	41_46	DB06641(0.482,-6), DB09031(0.467,-5.5), DB00312(0.449), DB01351(0.436), DB01353(0.436), DB00599(0.434), DB00418(0.433), DB13253(0.432), DB00237(0.427), DB01154(0.426), DB00306(0.413), DB00241(0.411)
10	44_15	DB06641(0.482,-6), DB09031(0.467,-5.5), DB00312(0.449), DB01351(0.436), DB01353(0.436), DB00599(0.434), DB00418(0.433), DB13253(0.432), DB00237(0.427), DB01154(0.426), DB00306(0.413), DB00241(0.411)
11	44_16	DB06345(0.557,-7.6), DB00312(0.556), DB08873(0.542,-6.1), DB11879(0.538), DB01351(0.528), DB01353(0.528), DB05541(0.527,-5.5), DB06614(0.522,-5.6), DB01107(0.52), DB00237(0.519), DB00418(0.517), DB11682(0.515,-6.6), DB11860(0.513,-7.4), DB05645(0.5,-6.5), DB06335(0.5,-5.9), DB00599(0.496), DB15199(0.495,-6.4), DB00593(0.49), DB15286(0.489), DB00306(0.483), DB00786(0.474,-6.5), DB01154(0.472), DB00241(0.47), DB15601(0.469), DB08958(0.465), DB06573(0.459,-6.1), DB05434(0.457,-7), DB12833(0.455,-8), DB12839(0.455), DB12613(0.454,-6.7), DB06657(0.45), DB00490(0.448), DB00790(0.446,-6.2), DB12692(0.444), DB12991(0.444), DB13087(0.444), DB13018(0.44), DB01216(0.439), DB06045(0.435), DB13288(0.434), DB05814(0.433), DB06200(0.433,-6), DB00474(0.432), DB13253(0.431), DB01197(0.429), DB01252(0.427,-7.9), DB01437(0.424), DB13221(0.421), DB04896(0.419), DB08918(0.419), DB12444(0.419), DB12951(0.418), DB06124(0.417,-6.4), DB12184(0.417), DB09026(0.407,-6.5), DB11801(0.406), DB11890(0.405,-6.7), DB00155(0.404), DB00746(0.404), DB11938(0.404,-7.5), DB00357(0.401), DB11876(0.4)
12	44_18	DB00312(0.56), DB01107(0.556), DB01351(0.532), DB01353(0.532), DB00237(0.523), DB00418(0.521), DB15286(0.514), DB00593(0.51), DB11860(0.504,-7.4), DB06335(0.493,-5.9), DB13253(0.492), DB05541(0.491,-5.5), DB00786(0.489,-6.5), DB00306(0.487), DB00241(0.475), DB11682(0.474,-6.6), DB02691(0.462), DB06200(0.454,-6), DB06614(0.451,-5.6), DB00599(0.45), DB06293(0.449,-5.8), DB12833(0.448,-8), DB12839(0.446), DB06657(0.441), DB06345(0.44,-7.6),

		DB00474(0.436), DB08873(0.435,-6.1), DB05513(0.432), DB00490(0.431), DB01252(0.431,-7.9), DB00790(0.43,-6.2), DB01154(0.43), DB01437(0.429), DB01783(0.429), DB05645(0.426,-6.5), DB13087(0.425), DB11190(0.423), DB04348(0.42,-8.3), DB08834(0.42), DB06274(0.419), DB09026(0.419,-6.5), DB12712(0.418), DB05814(0.415), DB15412(0.412,-5.8), DB01197(0.411), DB12951(0.41), DB12613(0.408,-6.7), DB11938(0.407,-7.5), DB12677(0.405,-6), DB01216(0.403), DB00561(0.403), DB13288(0.402), DB11879(0.402), DB12184(0.4), DB15199(0.4,-6.4)
13	44_43	DB01154(0.563), DB00418(0.546), DB00599(0.526), DB00306(0.513), DB00312(0.491), DB00241(0.487), DB11825(0.469), DB01351(0.466), DB01353(0.466), DB08873(0.464,-6.1), DB00237(0.457), DB13253(0.447), DB12513(0.438), DB01216(0.434), DB13221(0.427), DB12020(0.416), DB15601(0.409), DB00786(0.407,-6.5), DB05541(0.407,-5.5), DB06657(0.402)
14	44_81	DB06614(0.509,-5.6), DB11860(0.459,-7.4), DB15286(0.448), DB06345(0.447,-7.6), DB06293(0.445,-5.8), DB00786(0.442,-6.5), DB08873(0.441,-6.1), DB06335(0.437,-5.9), DB11762(0.433,-6.2), DB11682(0.431,-6.6), DB13253(0.424), DB00479(0.42,-6.1), DB14542(0.419,-7.6), DB14631(0.417,-7.4), DB12704(0.416), DB00418(0.416), DB06696(0.408), DB12416(0.407,-7.3), DB06124(0.406,-6.4), DB09026(0.405,-6.5), DB11471(0.405), DB04878(0.403,-5.6), DB06641(0.403,-6)
15	45_23	DB01107(0.7), DB06657(0.6), DB01351(0.59), DB01353(0.59), DB00593(0.571), DB00312(0.57), DB00237(0.523), DB00418(0.521), DB05541(0.517,-5.5), DB11860(0.516,-7.4), DB00241(0.511), DB02691(0.495), DB00306(0.479), DB00599(0.479), DB12839(0.477), DB12692(0.475), DB12991(0.475), DB06200(0.472,-6), DB12951(0.472), DB01783(0.471), DB00746(0.468), DB06345(0.467,-7.6), DB15199(0.462,-6.4), DB01154(0.451), DB13288(0.449), DB01437(0.449), DB12521(0.446), DB13253(0.443), DB11190(0.443), DB05513(0.443), DB13099(0.433,-5.1), DB09357(0.432), DB13087(0.432), DB08873(0.431,-6.1), DB00490(0.425), DB04348(0.425,-8.3), DB08834(0.425), DB06335(0.424,-5.9), DB15601(0.422), DB00786(0.419,-6.5), DB15286(0.416), DB12444(0.415), DB01074(0.412), DB09210(0.41), DB01202(0.41), DB11868(0.41), DB00357(0.407), DB01216(0.403), DB11879(0.402), DB12833(0.4,-8)
16	45_24	DB01107(0.633), DB06345(0.56,-7.6), DB05541(0.559,-5.5), DB11860(0.541,-7.4), DB00593(0.538), DB01074(0.536), DB13288(0.527), DB00312(0.526), DB06200(0.526,-6), DB01351(0.511), DB01353(0.511), DB06657(0.506), DB00237(0.5), DB05513(0.5), DB11682(0.5,-6.6), DB00786(0.492,-6.5), DB01216(0.487), DB00418(0.486), DB06614(0.484,-5.6), DB06335(0.483,-5.9), DB00657(0.471), DB11807(0.471), DB11879(0.468), DB08873(0.463,-6.1), DB00306(0.462), DB15286(0.461), DB00241(0.461), DB15601(0.46), DB05645(0.457,-6.5), DB12951(0.45), DB00599(0.447), DB06714(0.441), DB01437(0.435), DB05832(0.432,-7.1), DB04876(0.431,-6.5), DB00746(0.43), DB01197(0.427), DB01202(0.427), DB11868(0.427), DB01154(0.423), DB12513(0.423), DB12712(0.422), DB01783(0.421), DB08958(0.42), DB13087(0.417), DB15199(0.416,-6.4), DB00790(0.415,-6.2), DB12839(0.412), DB12833(0.412,-8), DB00141(0.411), DB12536(0.411), DB01255(0.409), DB05814(0.407), DB05434(0.403,-7), DB13221(0.402), DB11801(0.402), DB05885(0.402), DB04878(0.4,-5.6), DB09357(0.4), DB12613(0.4,-6.7), DB06045(0.4)
17	45_74	DB04878(0.4,-5.6)
18	46_12	DB11860(0.554,-7.4), DB15264(0.53), DB02691(0.516), DB12032(0.515), DB00230(0.5), DB00996(0.5), DB13099(0.492,-5.1), DB06335(0.491,-5.9), DB12105(0.486), DB00302(0.478), DB04348(0.471,-8.3), DB08834(0.471), DB06200(0.459,-6), DB01107(0.458), DB00312(0.457), DB05541(0.457,-5.5), DB12444(0.457), DB12493(0.456), DB00593(0.455), DB00599(0.454), DB04878(0.451,-5.6), DB06657(0.443), DB13087(0.438), DB12839(0.433), DB05513(0.431), DB11825(0.429), DB00149(0.426), DB14187(0.426), DB01351(0.426), DB01353(0.426), DB00786(0.424,-6.5), DB11684(0.418), DB00657(0.418), DB11807(0.418), DB01154(0.415), DB00237(0.415), DB00513(0.413), DB00167(0.412), DB12521(0.412), DB05814(0.411), DB04876(0.411,-6.5), DB00418(0.41), DB15286(0.409), DB13288(0.409), DB15412(0.408,-5.8), DB06714(0.406), DB01337(0.405), DB01339(0.405), DB09357(0.405), DB00130(0.4)
19	46_13	DB08873(0.492,-6.1), DB06573(0.443,-6.1), DB14941(0.441), DB11682(0.434,-6.6), DB14075(0.429), DB15286(0.426), DB00790(0.425,-6.2), DB06124(0.424,-6.4), DB06045(0.423), DB12613(0.422,-6.7), DB14173(0.422), DB04876(0.42,-6.5), DB06293(0.416,-5.8), DB00786(0.412,-6.5), DB12416(0.41,-7.3), DB06614(0.41,-5.6), DB13018(0.409), DB06335(0.408,-5.9), DB15251(0.408), DB06827(0.407,-8.2), DB01601(0.407,-7.7), DB05645(0.405,-6.5), DB11783(0.405), DB13034(0.4,-7.3)
20	46_22	DB00657(0.714), DB11807(0.714), DB06714(0.612), DB01074(0.554), DB06200(0.516,-6), DB13012(0.508,-6.2), DB12105(0.5), DB04878(0.494,-5.6), DB12032(0.484), DB11860(0.483,-7.4), DB00478(0.471), DB04926(0.469), DB05513(0.46), DB11924(0.455), DB15264(0.452), DB00915(0.451), DB01043(0.451), DB12449(0.446), DB00302(0.443), DB06333(0.43,-6.3), DB06461(0.43,-6.6), DB11684(0.422), DB00996(0.422), DB01337(0.422), DB01339(0.422), DB00230(0.419), DB04545(0.419,-4.5), DB13288(0.412), DB00149(0.41), DB12493(0.405), DB01637(0.404), DB01245(0.4), DB01718(0.4)

21	46_24	DB14631(0.451,-7.4), DB14542(0.444,-7.6), DB05456(0.434), DB11471(0.425), DB12655(0.422), DB09031(0.419,-5.5), DB01256(0.417), DB14669(0.417), DB09123(0.413), DB06705(0.409), DB14096(0.407), DB01627(0.406,-5.9), DB06293(0.406,-5.8), DB09091(0.405), DB11921(0.404), DB11762(0.402,-6.2), DB01190(0.402,-6), DB01590(0.4)
22	46_37	DB06641(0.487,-6), DB00312(0.467), DB09031(0.459,-5.5), DB00599(0.452), DB06293(0.45,-5.8), DB00418(0.45), DB01154(0.443), DB01351(0.442), DB01353(0.442), DB13253(0.437), DB15286(0.434), DB00237(0.433), DB06614(0.424,-5.6), DB11860(0.422,-7.4), DB14542(0.42,-7.6), DB00306(0.419), DB14631(0.409,-7.4), DB00241(0.406), DB15412(0.405,-5.8), DB11848(0.403), DB06335(0.403,-5.9)
23	47_11	DB05541(0.508,-5.5), DB06335(0.497,-5.9), DB11879(0.496), DB05645(0.486,-6.5), DB06573(0.468,-6.1), DB12833(0.465,-8), DB13018(0.464), DB08873(0.461,-6.1), DB00786(0.455,-6.5), DB15286(0.451), DB05434(0.449,-7), DB12692(0.448), DB12991(0.448), DB15199(0.447,-6.4), DB12613(0.447,-6.7), DB00490(0.441), DB00790(0.44,-6.2), DB06124(0.438,-6.4), DB00312(0.43), DB15601(0.43), DB06657(0.429), DB06127(0.425), DB15412(0.423,-5.8), DB12677(0.422,-6), DB11709(0.418), DB12795(0.417,-6.4), DB01107(0.416), DB06614(0.415,-5.6), DB12184(0.413), DB00593(0.412), DB01351(0.407), DB01353(0.407), DB00418(0.407), DB06045(0.403), DB01597(0.402), DB00237(0.4)
24	47_26	DB08873(0.506,-6.1), DB06573(0.477,-6.1), DB11879(0.465), DB00599(0.46), DB06335(0.459,-5.9), DB15412(0.458,-5.8), DB00312(0.452), DB01154(0.451), DB13018(0.444), DB15601(0.44), DB00418(0.438), DB00790(0.432,-6.2), DB05541(0.431,-5.5), DB01351(0.43), DB01353(0.43), DB05645(0.429,-6.5), DB00474(0.428), DB12795(0.427,-6.4), DB11682(0.425,-6.6), DB00237(0.422), DB06127(0.418), DB15286(0.417), DB15199(0.416,-6.4), DB06124(0.415,-6.4), DB05434(0.415,-7), DB00786(0.41,-6.5), DB00306(0.41), DB12613(0.404,-6.7), DB04876(0.4,-6.5), DB12692(0.4), DB01627(0.4,-5.9), DB12991(0.4)
25	47_27	DB06641(0.515,-6), DB14981(0.48,-5.1), DB14542(0.445,-7.6), DB14631(0.433,-7.4), DB09031(0.426,-5.5), DB04878(0.418,-5.6), DB11848(0.407)
26	47_68	DB11860(0.579,-7.4), DB06335(0.533,-5.9), DB00786(0.53,-6.5), DB15286(0.521), DB06614(0.511,-5.6), DB05541(0.5,-5.5), DB01107(0.477), DB06293(0.476,-5.8), DB00141(0.475), DB12536(0.475), DB13087(0.471), DB04878(0.47,-5.6), DB05814(0.469), DB00790(0.467,-6.2), DB04876(0.466,-6.5), DB08873(0.461,-6.1), DB02691(0.458), DB00517(0.458), DB06641(0.457,-6), DB12839(0.456), DB11684(0.455), DB01197(0.453), DB12677(0.453,-6), DB09357(0.449), DB13288(0.448), DB12232(0.448,-6.1), DB12704(0.447), DB05645(0.445,-6.5), DB12613(0.445,-6.7), DB00731(0.442), DB11801(0.442), DB14932(0.441,-6.5), DB01216(0.441), DB00419(0.439), DB01783(0.439), DB14872(0.439), DB05434(0.438,-7), DB11797(0.438), DB15412(0.438,-5.8), DB06345(0.437,-7.6), DB00593(0.433), DB00491(0.43), DB04348(0.429,-8.3), DB08834(0.429), DB06045(0.427), DB11784(0.425,-6.7), DB05805(0.425), DB11938(0.423,-7.5), DB06156(0.423), DB09292(0.423,-8.4), DB00178(0.422,-6.8), DB11879(0.421), DB11682(0.421,-6.6), DB01252(0.419,-7.9), DB11762(0.418,-6.2), DB06354(0.417,-7.9), DB00519(0.417,-6.7), DB03424(0.417), DB15601(0.416), DB00312(0.415), DB00207(0.414,-7), DB06127(0.414), DB00907(0.413), DB04896(0.411), DB08918(0.411), DB06657(0.411), DB12013(0.411), DB00422(0.409), DB06701(0.409), DB09026(0.409,-6.5), DB01337(0.409), DB01339(0.409), DB06497(0.408,-8.1), DB08824(0.407), DB12712(0.407), DB01437(0.406), DB05137(0.406), DB01255(0.405), DB06124(0.402,-6.4), DB01627(0.402,-5.9), DB00418(0.402), DB04834(0.401,-6), DB00479(0.401,-6.1), DB09271(0.401), DB13004(0.401), DB08889(0.401,-7.5), DB04947(0.4), DB06472(0.4), DB04947(0.4)
27	48_44	DB05541(0.641,-5.5), DB01107(0.627), DB00593(0.595), DB11860(0.57,-7.4), DB11879(0.566), DB05645(0.563,-6.5), DB06335(0.559,-5.9), DB00786(0.529,-6.5), DB15601(0.527), DB13288(0.526), DB06657(0.524), DB08873(0.519,-6.1), DB01216(0.512), DB06345(0.51,-7.6), DB00312(0.51), DB06200(0.506,-6), DB15199(0.505,-6.4), DB13087(0.505), DB05434(0.496,-7), DB12833(0.492,-8), DB12613(0.492,-6.7), DB00490(0.487), DB01437(0.487), DB05814(0.486), DB00746(0.483), DB01197(0.482), DB01351(0.48), DB01353(0.48), DB04896(0.479), DB08918(0.479), DB12839(0.474), DB01074(0.474), DB15286(0.473), DB06614(0.473,-5.6), DB00418(0.473), DB00790(0.47,-6.2), DB00237(0.47), DB11801(0.468), DB05513(0.462), DB06045(0.46), DB12184(0.453), DB12444(0.451), DB01255(0.45), DB00599(0.449), DB01202(0.446), DB11868(0.446), DB05832(0.445,-7.1), DB00357(0.444), DB11682(0.441,-6.6), DB05885(0.441), DB01252(0.438,-7.9), DB00561(0.438), DB00306(0.436), DB11876(0.436), DB12951(0.435), DB12712(0.435), DB00657(0.434), DB11807(0.434), DB04876(0.433,-6.5), DB12521(0.429), DB00141(0.427), DB12536(0.427), DB01154(0.426), DB12513(0.425), DB01783(0.424), DB06573(0.423,-6.1), DB12692(0.422), DB12991(0.422), DB00241(0.422), DB00731(0.421), DB02691(0.421), DB09357(0.419), DB12677(0.418,-6), DB11684(0.417), DB13099(0.417,-5.1), DB09026(0.415,-6.5), DB11938(0.412,-7.5), DB13221(0.406), DB05521(0.406,-7.9), DB06714(0.405), DB11784(0.405,-6.7), DB11797(0.403), DB00178(0.403,-6.8)

28	48_59	DB06705(0.489), DB06641(0.481,-6), DB09031(0.48,-5.5), DB11860(0.475,-7.4), DB13615(0.472), DB14932(0.443,-6.5), DB00803(0.442), DB06335(0.441,-5.9), DB06293(0.44,-5.8), DB00728(0.44,-6.8), DB11693(0.439), DB00091(0.438), DB12139(0.438), DB13068(0.438), DB15259(0.438), DB14631(0.437,-7.4), DB04933(0.433), DB11882(0.431), DB14542(0.43,-7.6), DB02691(0.429), DB04878(0.426,-5.6), DB06614(0.425,-5.6), DB09114(0.423), DB11283(0.423), DB14099(0.423), DB14829(0.423), DB00479(0.422,-6.1), DB05456(0.42), DB00786(0.42,-6.5), DB06087(0.419), DB00207(0.419,-7), DB06696(0.418), DB15286(0.418), DB04834(0.417,-6), DB15412(0.416,-5.8), DB01337(0.416), DB01339(0.416), DB11471(0.413), DB00517(0.412), DB01111(0.411), DB14669(0.411), DB06447(0.41), DB09026(0.407,-6.5), DB11797(0.405), DB04660(0.405), DB00141(0.404), DB12536(0.404), DB11762(0.403,-6.2), DB11332(0.403), DB12655(0.402), DB05814(0.401), DB12232(0.401,-6.1), DB01627(0.401,-5.9), DB00790(0.4,-6.2), DB11813(0.4)
29	49_24	DB06573(0.586,-6.1), DB08873(0.52,-6.1), DB05645(0.513,-6.5), DB11879(0.503), DB05541(0.483,-5.5), DB12613(0.482,-6.7), DB15601(0.478), DB00786(0.473,-6.5), DB12795(0.471,-6.4), DB05434(0.468,-7), DB06124(0.463,-6.4), DB00312(0.463), DB12677(0.462,-6), DB06045(0.458), DB13018(0.455), DB11682(0.453,-6.6), DB12833(0.449,-8), DB00418(0.449), DB15286(0.444), DB11709(0.443), DB01351(0.442), DB01353(0.442), DB06335(0.442,-5.9), DB15188(0.439,-6.6), DB13090(0.437), DB00237(0.435), DB01282(0.435), DB00490(0.435), DB00790(0.434,-6.2), DB00599(0.431), DB12692(0.431), DB12991(0.431), DB15199(0.43,-6.4), DB06497(0.429,-8.1), DB11801(0.428), DB04898(0.427), DB09059(0.425), DB01154(0.425), DB00306(0.423), DB00474(0.422), DB11938(0.422,-7.5), DB11890(0.422,-6.7), DB06614(0.419,-5.6), DB15412(0.418,-5.8), DB05885(0.418), DB04842(0.417), DB11784(0.416,-6.7), DB13034(0.415,-7.3), DB01601(0.414,-7.7), DB14911(0.413,-7.5), DB01058(0.413), DB11749(0.413), DB00241(0.413), DB12377(0.413), DB05633(0.411), DB01041(0.411), DB01627(0.41,-5.9), DB12184(0.41), DB05521(0.409,-7.9), DB15205(0.407), DB06127(0.406), DB09421(0.404,-7.6), DB00143(0.403), DB12279(0.403), DB09060(0.402), DB09291(0.402)
30	49_38	DB06335(0.548,-5.9), DB06573(0.51,-6.1), DB06124(0.498,-6.4), DB11709(0.493), DB01190(0.492,-6), DB08873(0.49,-6.1), DB13034(0.488,-7.3), DB01627(0.487,-5.9), DB12613(0.487,-6.7), DB12677(0.481,-6), DB00786(0.478,-6.5), DB15286(0.474), DB06614(0.474,-5.6), DB00790(0.464,-6.2), DB05645(0.464,-6.5), DB05541(0.461,-5.5), DB06127(0.458), DB04876(0.456,-6.5), DB11879(0.455), DB00143(0.451), DB15412(0.449,-5.8), DB06045(0.449), DB01282(0.448), DB12279(0.447), DB14911(0.447,-7.5), DB15188(0.446,-6.6), DB12795(0.446,-6.4), DB05434(0.443,-7), DB06211(0.441,-6.5), DB12730(0.439,-6.8), DB09059(0.439), DB13018(0.438), DB00760(0.436,-6.2), DB06827(0.435,-8.2), DB11890(0.43,-6.7), DB00143(0.427), DB11762(0.426,-6.2), DB00178(0.423,-6.8), DB06604(0.42), DB05446(0.42), DB06293(0.42,-5.8), DB00519(0.419,-6.7), DB06497(0.418,-8.1), DB00303(0.417,-7.4), DB15297(0.416), DB00886(0.414), DB05633(0.414), DB01598(0.413,-5.4), DB05796(0.412), DB11938(0.41,-7.5), DB04898(0.41), DB12380(0.409), DB11929(0.408,-8.2), DB15299(0.408), DB13028(0.408,-6.1), DB05521(0.406,-7.9), DB01000(0.406), DB09291(0.405), DB12292(0.404), DB12541(0.403), DB15601(0.403), DB01348(0.403), DB08889(0.4,-7.5)
31	50_20	DB06705(0.459), DB12416(0.429,-7.3), DB12791(0.429,-6.5), DB08873(0.429,-6.1), DB12704(0.427), DB06614(0.426,-5.6), DB06827(0.42,-8.2), DB12791(0.42,-6.5), DB15286(0.413), DB11586(0.411,-7.3), DB06641(0.41,-6), DB01346(0.409), DB14941(0.409), DB12730(0.406,-6.8), DB06124(0.405,-6.4), DB14911(0.402,-7.5), DB01190(0.401,-6)
32	50_21	DB11879(0.716), DB08873(0.655,-6.1), DB05645(0.651,-6.5), DB05541(0.643,-5.5), DB15601(0.606), DB12613(0.59,-6.7), DB05434(0.583,-7), DB15199(0.583,-6.4), DB00786(0.581,-6.5), DB06573(0.561,-6.1), DB06045(0.551), DB12692(0.547), DB12991(0.547), DB00790(0.544,-6.2), DB11801(0.543), DB06335(0.539,-5.9), DB15286(0.537), DB11682(0.532,-6.6), DB06614(0.527,-5.6), DB06124(0.525,-6.4), DB11890(0.524,-6.7), DB00490(0.522), DB12833(0.514,-8), DB11938(0.513,-7.5), DB05633(0.504), DB05832(0.503,-7.1), DB13018(0.496), DB06345(0.496,-7.6), DB05521(0.495,-7.9), DB05885(0.493), DB12184(0.493), DB06657(0.491), DB12677(0.486,-6), DB11860(0.484,-7.4), DB00312(0.484), DB01202(0.482), DB11868(0.482), DB15251(0.48), DB13087(0.48), DB14940(0.477), DB11784(0.476,-6.7), DB04876(0.475,-6.5), DB11981(0.474), DB11709(0.473), DB00178(0.471,-6.8), DB13247(0.469), DB06497(0.468,-8.1), DB00418(0.466), DB00519(0.466,-6.7), DB11702(0.465), DB01197(0.463), DB04898(0.461), DB01351(0.46), DB01353(0.46), DB14911(0.459,-7.5), DB11876(0.458), DB05814(0.456), DB00722(0.456), DB05155(0.455), DB01058(0.454), DB01058(0.454), DB00237(0.452), DB12795(0.451,-6.4), DB15188(0.45,-6.6), DB01216(0.45), DB01107(0.448), DB00143(0.448), DB06293(0.446,-5.8), DB12501(0.446), DB11996(0.446), DB13034(0.441,-7.3), DB06127(0.44), DB14941(0.439), DB01282(0.438), DB06827(0.437,-8.2), DB00306(0.436), DB00593(0.432), DB15412(0.429,-5.8), DB01601(0.428,-7.7), DB12377(0.428), DB01255(0.426), DB08958(0.426), DB12199(0.425,-7.9), DB00241(0.424), DB00599(0.424), DB12128(0.423), DB12239(0.42), DB09210(0.42), DB09421(0.42,-7.6), DB01154(0.417), DB00731(0.414), DB09059(0.413), DB13090(0.413), DB00143(0.411), DB00584(0.411), DB01041(0.411), DB05446(0.41), DB08382(0.409), DB12839(0.408), DB15205(0.407), DB12122(0.407), DB11821(0.406), DB05426(0.406), DB00380(0.405), DB12013(0.403), DB01627(0.403,-5.9), DB06645(0.403),

		DB13067(0.402), DB09026(0.402,-6.5), DB08889(0.402,-7.5), DB01000(0.402), DB12054(0.402), DB13288(0.402), DB12492(0.401), DB09060(0.401), DB00895(0.401), DB09291(0.401), DB01232(0.4,-8.7), DB03424(0.4), DB09286(0.4), DB15460(0.4)
33	50_23	DB13099(0.508,-5.1), DB01107(0.488), DB00312(0.484), DB00599(0.479), DB06657(0.474), DB00593(0.468), DB01351(0.452), DB01353(0.452), DB00237(0.441), DB01154(0.438), DB00418(0.433), DB06335(0.425,-5.9), DB05541(0.408,-5.5), DB04348(0.4,-8.3), DB08834(0.4)
34	50_76	DB11860(0.592,-7.4), DB06335(0.564,-5.9), DB04878(0.543,-5.6), DB06614(0.524,-5.6), DB04876(0.513,-6.5), DB06200(0.513,-6), DB01337(0.482), DB01339(0.482), DB00657(0.479), DB11807(0.479), DB13288(0.469), DB12287(0.468), DB02691(0.466), DB04545(0.466,-4.5), DB00491(0.463), DB01338(0.463,-7.3), DB00419(0.458), DB14872(0.458), DB13012(0.452,-6.2), DB05513(0.45), DB00786(0.448,-6.5), DB00141(0.444), DB04834(0.444,-6), DB12536(0.444), DB15286(0.444), DB04348(0.443,-8.3), DB08834(0.443), DB05541(0.442,-5.5), DB01074(0.442), DB06345(0.441,-7.6), DB00728(0.44,-6.8), DB06333(0.44,-6.3), DB06461(0.44,-6.6), DB12449(0.44), DB11684(0.434), DB13004(0.428), DB03206(0.426), DB05018(0.426), DB13262(0.422), DB00790(0.42,-6.2), DB00517(0.415), DB12704(0.414), DB00942(0.414), DB13087(0.411), DB06714(0.411), DB15412(0.41,-5.8), DB01197(0.409), DB00387(0.407), DB11797(0.407), DB12839(0.406), DB06787(0.405), DB00593(0.404), DB01227(0.403), DB00376(0.402)
35	50_82	DB06335(0.496,-5.9), DB04876(0.473,-6.5), DB13288(0.431), DB05541(0.421,-5.5), DB06345(0.408,-7.6), DB15601(0.401)

C. DOCKING RELATED INFORMATION.

Figure S3. The docking region with the hotspot on SARS-CoV-2 spike RBD that binds to the hACE2 peptidase domain.

Table S2. Docking scores for the final list of drugs.

DrugBank ID	Drug Name	Docking Score (kcal/mol)	DrugBank ID	Drug Name	Docking Score (kcal/mol)
DB09297	Paritaprevir	-9.1	DB14760	Narlaprevir	-8.4
DB09308	Solithromycin	-9.0	DB04348	Taurocholic acid	-8.3
DB11779	Danoprevir	-9.0	DB06290	Simeprevir	-8.3
DB00337	Pimecrolimus	-8.7	DB12228	Telcagepant	-8.3
DB00696	Ergotamine	-8.7	DB13879	Glecaprevir	-8.3
DB01232	Saquinavir	-8.7	DB06827	Viomycin	-8.2
DB12538	Nepadutant	-8.7	DB08993	Enviomycin	-8.2
DB12876	GS-9256	-8.7	DB11929	Vaniprevir	-8.2
DB09292	Sacubitril	-8.4	DB05868	Ciluprevir	-8.1
DB12508	E7107	-8.4	DB06497	Aplaviroc	-8.1

Variability in the docking scores: It is important to mention here that several recent studies have used docking for finding repurpose drugs.³⁻⁹ While docking is good as a relative measure of the strength of binding, it cannot provide an accurate estimate of binding strength. The scores vary depending on the scoring function, grid size, the region selected etc. More importantly, even when same program and other parameters are used, the scores vary based on the molecular representation of the drugs. Table S3 shows that even when using the exact same parameters are used (grid, center, receptor area, etc.) docking scores in AutoDock Vina1.1.2¹⁰ vary for the same drug taken directly from the DrugBank² (which does not have nonpolar hydrogen atoms), or after adding nonpolar hydrogens to the same drug obtained from the DrugBank² or after obtaining a 3D optimized configuration of the same drugs using OpenBabel¹¹.

Table S3. Docking scores for some drugs of different structures under different levels of hydrogenation and geometric optimization

Molecular Code	Docking Score (polar-H, kcal/mol)	Docking Score (all-H, kcal/mol)	Docking Score (all-H + 3D optimized, kcal/mol)
DB11929(Vaniprevir)	-9.3	-7.4	-8.1
DB14760(Narlaprevir)	-8.7	-6.5	-7.6
DB04348(Turocholic Acid)	-8.6	-7.3	-8.0
DB01232(Saquinavir)	-8.5	-7.3	-8.7
DB14631(Prednisolone Phosphate)	-8.5	-7.1	-7.3
DB14542(Hydroxycortisone Phosphate)	-8.4	-7.1	-7.5
DB00503(Ritonavir)	-8.3	-7.4	-8.0

Figure S4. The chemical structures of the drug molecules selected based on the criteria mentioned in the manuscript. Binding free energy of all these molecules were calculated using all-atom explicit water simulations using well-tempered metadynamics¹.

D. FREE ENERGY RELATED INFORMATION

(1) Definition of Collective Variables. We used two collective variables X and native contact for the well-tempered metadynamics¹ simulations. We discuss the construction of each of the variables below.

(a) Reaction coordinate distvec is defined by two vectors $\vec{b}$ and $\vec{d}$. While $\vec{b}$ is the unit vector from the COM of residues ARG331, VAL369, TYR370, ALA371, LEU487, SER488, GLU490 to the COM of residues SER323, VAL324, TYR325, VAL375, ILE376, ARG377, GLY378, ASP379, GLU380, VAL381, ARG382, GLN383, GLY390, LYS391, ILE392, TYR395, ASN396, SER412, ASN413, ASN414, LEU415, ASP416, SER417, TYR423, ASN424, TYR425, LEU426, TYR427, ARG428, PRO465, LEU466, GLN467, SER468, TYR469, GLY470, PHE471, GLN472, PRO473, THR474, VAL477, GLY478, TYR479, GLN480, PRO481, TYR482 that lie more toward the hotspot region. $\vec{d}$ is the vector from the COM of ARG331, VAL369, TYR370, ALA371, LEU487, SER488, GLU490 to the COM of drug molecule. $X = \vec{b} \cdot \vec{d}$, $\theta = \cos^{-1}(\vec{b} \cdot \vec{d} / |\vec{d}|)$. Figure S5 shows a schematic diagram of distvec (X).

Figure S5. The collective variable distvec (X) and its components are defined in the picture. The protein is shown in grey and ligand is shown in red.

(b) The Native contact (N_c) is defined by the spatial proximity of groups of atoms in the native state. The native contact between one group (gA) of atoms to the other group (gB) of atoms is defined as,¹²

$$N_c = \sum_{i \in gA} \sum_{j \in gB} s_{ij},$$

where, s_{ij} given by,

$$s_{ij} = \begin{cases} 1 & \forall \quad r_{ij} \leq 0 \\ \left(1 - \left(\frac{r_{ij}}{r_0}\right)^n\right) / \left(1 - \left(\frac{r_{ij}}{r_0}\right)^m\right) & \forall \quad r_{ij} > 0 \end{cases}$$

and $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j| - d_0$. The user-defined parameters were chosen to be $n=6$, $m=0$, $r_0=5.5$ Å and $d_0=0$ Å. Above equation ensures the variation of s_{ij} is continuous and differentiable. Also, the cut off value being large allows N_c to be higher than in the native state if more atoms come close. The heavy atoms of the ligand constitute gA while the heavy atoms of part of the protein (residues 376-377, 379, 382-383, 389-392, 394-396, 427-430, 440-470, 475, 477, 479) constitute gB . We have chosen the residues of the protein that either belong to the loop (residues 442 to 468) or reside within 5.5 Å from the ligand in the bound state. Figure S6 shows the protein's region in yellow and the ligand in orange.

Figure S6. The collective variable native contact is shown. gA (ligand) is shown in orange and gB (part of the protein) is shown in yellow.

Table S4. The system size and simulation lengths for the metadynamics simulation for all the systems. A general optimization, heating and 5 ns normal molecular dynamics simulations are applied to all the systems. The metadynamics simulations were not carried out for the unstable system and runtime is denoted for them as “NA”.

System	Runtime (ns)	System Size (number of atoms)	System	Runtime (ns)	System Size (number of atoms)
hACE2 (run 1)	234	236699	hACE2 (run 2)	51	236699
47_68 (run 1)	154	86305	Danoprevir	236	114053
47_68 (run2)	381	86305	Danoprevir	99	114053
37_42 (run 1)	41	86337	Solithromycin	149	114129
37_42 (run2)	64	86337	Solithromycin	157	114129
45_23 (run 1)	115	114066	Saquinavir	44	86314
45_23 (run 2)	126	114066	Saquinavir (run 2)	104	86314
41_46 (run 1)	64	114089	Glecaprevir	166	86308
41_46 (run 2)	67	114089	Paritaprevir	106	114087
49_24	48	86318	Ciluprevir	118	86330
50_76	88	86329	Simeprevir	56	114028
50_21	156	114066	Paritaprevir	48	114087
45_24	72	86323	E7107	124	114121
44_18	109	114091	Ergotamine	240	114037
50_23	75	86324	GS-9256	156	86284
50_20	30	86318	Enviomycin	62.9	86351
48_44	92	86318	Nepadutant	53	86294
44_16	20	114068	Vaniprevir (run 1)	97	86309
47_27	63	114028	Vaniprevir (run 2)	151	86309
44_43	37	99557	Telcagepant	88	86316
46_24	16	114066	Pimecrolimus	73	114056
39_32	14	86356	Narlaprevir	10.4	86376

46_13	24	86310	Sacubitril	29	86338
46_12	13	86310	Taurocholic acid	24	86352
29_19	14	114079	Aplaviroc	52	86307
46_37	11	114066	Viomycin	6	86309
47_11	18	86318	49_38	NA	86318
47_26	23	86305	45_74	NA	114074
41_37	16	86283	44_15 (run 1)	NA	86298
50_82	14	86316	44_15 (run 2)	NA	86298
41_31	8	114039	39_57	NA	86330
44_81	3	86293	32_26	NA	86300
46_22	18	114066	40_35	NA	86327
48_59	7	114066			

(2) Free Energy Surfaces. The two-dimensional free energy surfaces of binding of all stable the de novo and drug molecules are shown, except some of. All the plots are made using the software MetadynMiner¹³.

Figure S7. Free Energy surfaces of de novo and drug molecules with free energy stability between -14.4 kcal/mol and -9.9 kcal/mol. The chemical structure of the molecule is shown in the inset of the respective plot. Free energy color bar for this plot is shown.

Figure S8. Free Energy surfaces of de novo and drug molecules with free energy stability between -9.8 kcal/mol and -8.7 kcal/mol. The chemical structure of the molecule is shown in the inset of the respective plot. Free energy color bar for this plot is shown.

Figure S9. Free Energy surfaces of de novo and drug molecules with free energy stability between -8.6 kcal/mol and -5.6 kcal/mol (both ends are included). The chemical structure of the molecule is shown in the inset of the respective plot. Free energy color bar for this plot is shown.

Figure S10. Free Energy surfaces of de novo and drug molecules with free energy stability between -5.5 kcal/mol and -3.4 kcal/mol (both ends are included). The chemical structure of the molecule is shown in the inset of the respective plot. Free energy color bar for this plot is shown.

Figure S11. Representation of angle θ between vectors $\hat{V}_1$ and $\hat{V}_2$. $\hat{V}_1$ is a vector from the point A to point C, whereas $\hat{V}_2$ goes from point A to point B. The point A is the center of mass (COM) of residues 423 to 429 and 465 to 470. The point C is the COM of residues 415 to 422 and 470 to 479. The point B is the COM of residues 454 to 459. Only carbon, nitrogen and oxygen atoms were used to calculate COM calculation.

Figure S12. The values of θ for the most stable ligand bound RBD configurations for all the 35 de novo molecules and 20 drugs. Note that, although the θ is low for 48_44, the molecule is not encapsulated by the loop.

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