Molecular Omics

ROYAL SOCIETY OF CHEMISTRY

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Mol. Omics*, 2020, **16**, 474

Repurposing drugs against the main protease of SARS-CoV-2: mechanism-based insights supported by available laboratory and clinical data†

Sohini Chakraborti, Sneha Bheemireddy and Narayanaswamy Srinivasan *

The ongoing global pandemic of COVID-19 has brought life to almost a standstill with the implementation of lockdowns and social distancing as some of the preventive measures in the absence of any approved specific therapeutic interventions. To combat this crisis, research communities worldwide are falling back on the existing repertoire of approved/investigational drugs to probe into their anti-coronavirus properties. In this report, we describe our unique efforts in identifying potential drugs that could be repurposed against the main protease of SARS-CoV-2 (SARS-CoV-2 M^{pro}). To achieve this goal, we have primarily exploited the principles of 'neighbourhood behaviour' in the protein 3D (workflow-I) and chemical 2D structural space (workflow-II) coupled with docking simulations and insights into the possible modes of action of the selected candidates from the available literature. This integrative approach culminated in prioritizing 29 potential repurpose-able agents (20 approved drugs and 9 investigational molecules) against SARS-CoV-2 M^{pro}. Apart from the approved/investigational anti-viral drugs, other notable hits include anti-bacterial, anti-inflammatory, anti-cancer and anti-coagulant drugs. Our analysis suggests that some of these drugs have the potential to simultaneously modulate the functions of viral proteins and the host response system. Interestingly, many of these identified candidates (12 molecules from workflow-I and several molecules, belonging to the chemical classes of alkaloids, tetracyclines, peptidomimetics, from workflow-II) are suggested to possess anti-viral properties, which is supported by laboratory and clinical data. Furthermore, this work opens a new avenue of research to probe into the molecular mechanism of action of many drugs, which are known to demonstrate anti-viral activity but are so far not known to target viral proteases.

Received 15th May 2020,
Accepted 25th June 2020

DOI: 10.1039/d0mo00057d

rsc.li/molomics

Introduction

In December 2019, the first cluster of cases of pneumonia caused by an 'unknown' microbe was reported in Wuhan, which soon became a global threat. This 'unknown' microbe was eventually identified to be a novel coronavirus (a positive stranded RNA virus) that shares 79.6% sequence identity with SARS-CoV and has therefore been named as SARS-CoV-2.[1] The disease caused by SARS-CoV-2 has been named COVID-19.[2] As of April 2020, there are reports of ~3 million confirmed cases of COVID-19 worldwide with more than ~0.2 million deaths.[3] At the time of preparing this document, there are no approved drugs/vaccines available specifically for the treatment of SARS-CoV-2 infection. It is unlikely that, within the next few months, any novel therapeutic interventions (inhibitors of SARS-CoV-2 proteins/vaccine/other therapeutic agents) to treat SARS-CoV-2 infection would be approved and launched in the market. This is because any new therapy must undergo rigorous evaluations with regard to safety and efficacy, which is time-consuming. The immediate alternative solution to combat this pandemic could be adopting repurposing approaches, which are expected to be faster and require less economic investment.[4] As of now, for the treatment of COVID-19 patients, medical experts around the globe are generally resorting to known antivirals and adjunctive therapy involving drugs that target the signalling pathways in the host which are perturbed in response to viral infections.[5] To place drug repurposing on fast track, research communities worldwide are trying to tap the potential of the existing approved/investigational drugs using various computational and/or experimental techniques to understand which of these drugs could be used for the treatment of SARS-CoV-2 infection.[6–8]

The popular drug targets of SARS-CoV-2 include one of the structural proteins, the Spike surface glycoprotein (S protein:

Molecular Biophysics Unit, Indian Institute of Science, Bengaluru 560012, India. E-mail: ns@iisc.ac.in

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0mo00057d

responsible for viral entry into the host cell), and a few non-structural proteins (Nsp): Nsp3/papain-like protease (cleaves the N-terminus of the replicase polyprotein), Nsp5/main protease (also referred to as the 3-chymotrypsin-like protease $3CL^{pro}$: cleaves the C-terminus of replicase polyprotein at 11 sites), and RNA-dependent RNA polymerase (RdRp: responsible for the replication and transcription of the viral RNA genome).[6,9–11] Recent reports on the comparative genome analysis of SARS-CoV-2 indicate that the sequences sampled from different geographical regions show variations in three of the four mentioned drug targets: S protein, Nsp3 and RdRp. Variants of few other SARS-CoV-2 proteins, such as Nucleoprotein, Nsp2, Nsp4, Nsp6, orf8, orf7a *etc.*, have also been observed.[12–15] Since we are still in the initial stages of understanding this virus, it is difficult to comprehend how such mutations would affect the response of the variant proteins to the targeting inhibitors. However, to the best of our knowledge, no mutation in the main protease (M^{pro}) has been reported so far in any of the variants of the SARS-CoV-2 genome analysed to date. Moreover, the primary substrate binding site of M^{pro} has been found to be conserved in related coronaviruses, implying the importance of this protein in the critical maintenance of viral life-cycle.[16] These observations are helpful in translating the learning from previous efforts to design a novel SARS-CoV-2 M^{pro} inhibitor.[17–21] Earlier reports suggest that no human proteases have a cleavage specificity similar to that of the proteases of coronaviruses, indicating that the inhibitors of these proteins are unlikely to cause adverse side effects.[22] Also, our preliminary attempt to check the presence of a closely related homologue of SARS-CoV-2 M^{pro} in humans by means of a sequence-based search[23] did not yield any significant hit (data not shown). Taken together, this evidence suggests that SARS-CoV-2 M^{pro} is an attractive drug target and inhibitors targeting this protein could be developed as broad spectrum anti-coronavirus drugs.

Our understanding, from the available data as discussed above, prompted us to initiate a hunt for potential repurpose-able candidates considering SARS-CoV-2 M^{pro} as the target of foremost importance. M^{pro}s of coronaviruses are cysteine-proteases comprising three domains: I (residues 10 to 99), II (residues 100 to 182) and III (residues 198 to 303). Domain I and II are made up of 6-stranded β barrels, resembling the architecture of chymotrypsin and of picornavirus 3C proteinases. The substrate-binding site lies in a cleft between these two domains and a long loop (residues 183 to 197) connects domain II to the C-terminal of domain III. A globular cluster of five helices from domain III has been implicated in the proteolytic activity of M^{pro}.[22] Unlike serine proteases or other cysteine proteases, M^{pro}s of related coronaviruses harbour only two catalytic residues, H41 and C145, instead of three residues, thus forming a catalytic dyad. A buried water molecule (which is hydrogen-bonded to H41 and at least two other residues in the substrate-binding site) is found in the structures of SARS-CoV and SARS-CoV-2 M^{pro} at the site which would generally be occupied by the third member in the case of a catalytic triad.[17] Apart from the two catalytic residues, several residues in their proximity which are present in the substrate binding site, such as M49, S139, F140, N142, H163, E166 and S147, have been experimentally shown to be functionally or structurally important.[24–27] At the time of writing this manuscript, 170 structures of different SARS-CoV-2 proteins were released in RCSB PDB.[28] More than 60% of the structures are of M^{pro}. This spectacular effort of structural biologists at this critical time, has accelerated the research on the design and development of novel inhibitors against the protein[22,27,29] and is immensely helpful in the structure-guided drug repurposing ventures.

In this work, we have employed a repurposing strategy which is based on the fundamental principle of 'neighbourhood behaviour' implemented through two different workflows involving (i) protein three-dimensional (3D) space and (ii) two dimensional (2D) chemical space of small molecules. Recognition between the biological molecules is governed by complementarity in different fingerprints, which are likely to be similar among neighbours in the protein 3D (proteins with similar structures) and chemical 2D (chemically similar compounds) spaces. In general, most protein structural neighbours are known to perform similar functions, which are facilitated mainly *via* the conserved/semi-conserved fingerprints (shape, volume, electrostatics) of the molecular recognition sites between the proteins and their binding partner/s (ligands: proteins, small molecules, peptides *etc.*).[30–32] In favourable cases, a structure comparison can reveal distant evolutionary relationships, which are otherwise difficult to capture by sequence comparison and hence can be helpful in understanding the molecular recognition pattern. On a similar note, small molecules which share similar chemical scaffolds (chemical neighbours) generally possess similar topological fingerprints and are known to elucidate similar pharmacological responses in many instances. As the number of common features between the two small molecules increases, the chances that they will demonstrate similar biological activities increase.[33–36] These analyses helped us in identifying a handful of known drugs that can be considered for repurposing against SARS-CoV-2 M^{pro}. Some of these drugs have already been reported to possess anti-viral (and in a few cases, specifically, anti-coronavirus) properties by laboratory and/or clinical investigation. To the best of our knowledge, many drugs such as gabexate, zoliflodacin, mitomycin, foretinib and freselestat are reported for the first time in this paper with strong structural support. Given the success of our approach in identifying known antivirals, we feel it is worthwhile to try the newly identified potential repurpose-able drugs in our work through laboratory and, subsequently, clinical explorations.

Materials and methods

In this work, we implement two independent workflows which are based on the principle of 'neighbourhood behaviour'. The first workflow (workflow-I) involves identifying potential repurpose-able candidates by recognizing structurally similar proteins. The second workflow (workflow-II) involves the identification of potential repurpose-able candidates by analysing

the chemical similarity of the approved drugs/investigational drug candidates with two known non-covalent inhibitors of SARS-CoV-2 M^{pro} as described below. In the past, we have implemented analogous computational drug repurposing pipelines to identify potential repurpose-able candidates for anti-malarial, anti-tuberculosis and anti-fungal therapies.[37–39]

Workflow-I

This approach can be simplified into five basic steps and has been pictorially represented in Fig. 1.

Identification of the structural neighbours of SARS-CoV-2 M^{pro}. This step involved the search for structural neighbours of SARS-CoV-2 M^{pro} (PDB code: 6Y84[40]) using the DALI[41] server

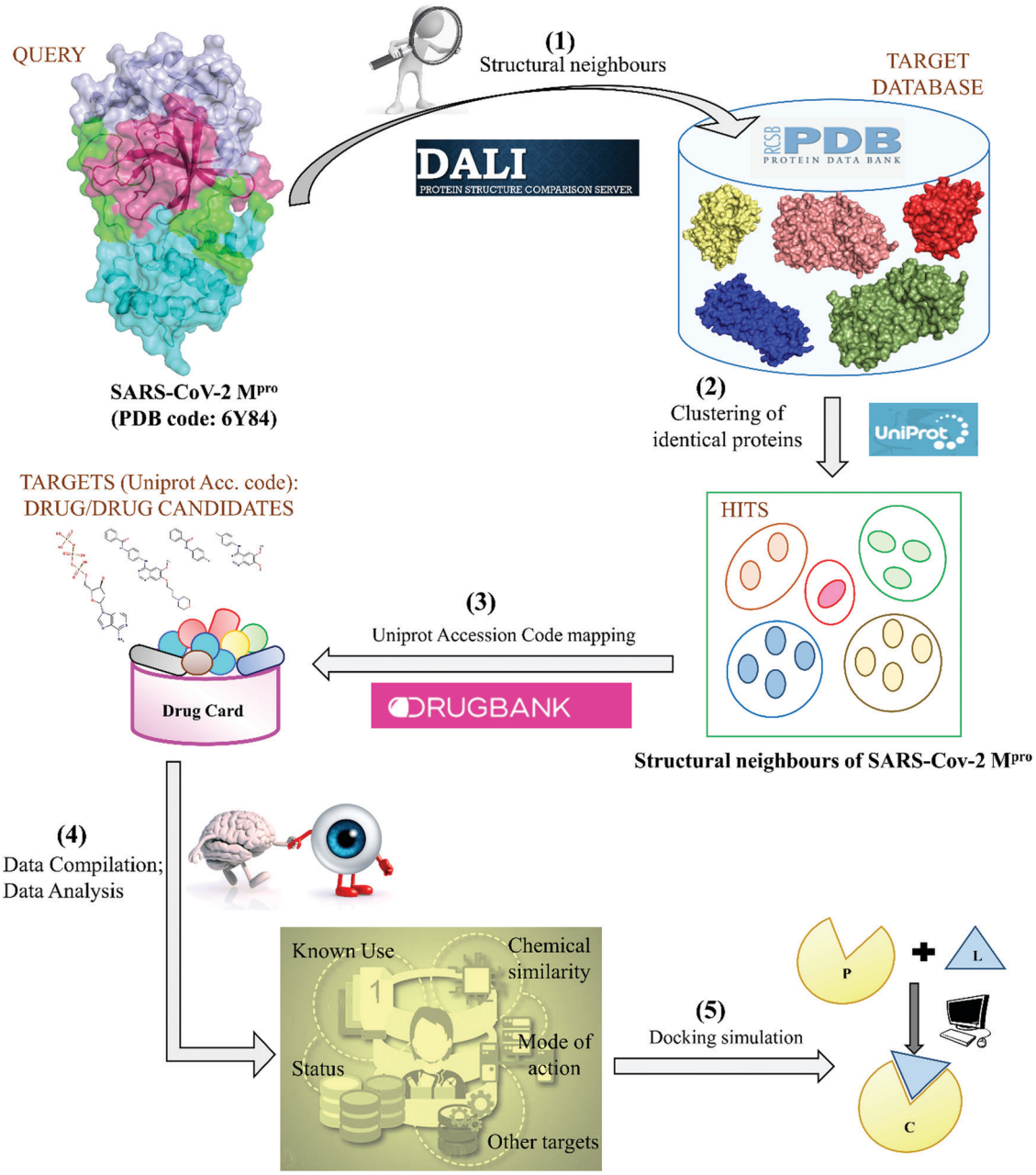

Fig. 1 Workflow-I. The steps (1 to 5) involved in this workflow as discussed in the text have been pictorially represented depicting the search for structural neighbours of SARS-CoV-2 main protease in Protein Data Bank and thereafter finding if any DrugBank molecule could be associated with those structural neighbours using different computational tools. Once such association is made, the data are analysed mainly manually (due to availability of limited computational resources at home and occasional remote access to desktop in our laboratory) to prioritize the compounds for testing. Selected compounds are subjected to docking simulation in step 5. In the depiction of step 5, P indicates protein, L indicates ligand and C indicates predicted complex. The three domains in the SARS-CoV-2 main protease structure are highlighted in different colours (left topmost protein structure in surface representation: light blue – domain I: residue 10–99; purple-domain II: residue 100–182; cyan-domain III: residue: 198–303, green-disordered regions). The images of the protein in surface representations have been generated using PyMOL (Schrödinger, LLC) freely available for academic usage. The flow has been prepared using Microsoft PowerPoint and collecting clipart from Google search.

(http://ekhidna2.biocenter.helsinki.fi/dali/). DALI is a protein structure comparison server that takes the 3D coordinates of a protein as input in a PDB format and then searches the PDB for similar structures (structural neighbours) following which it returns a list of structural neighbours (hits), structural alignments and coordinate sets of superimposed structures. The hits (indicated by a corresponding PDB code, chain code and protein name) are sorted by *Z*-score in the output file. Similarities with a *Z*-score lower than 2 are spurious.

Grouping/clustering of proteins. Next, the reliable hits (Z-score $\geq$ 2) obtained from the previous step were clustered based on their protein identity. The PDB entries of the hits were mapped on to their corresponding Uniprot accession code.[9] An identical Uniprot code corresponding to multiple PDB entries indicates that the sequence of the concerned set of proteins is identical (or the parent protein from which the constructs have been derived are identical, in cases when a mutation is introduced in the experimentally determined structure deposited in the PDB). Also, the information on the folds of these proteins was obtained from SCOPe[42] (or superfamily information was retrieved from CATH,[43,44] whenever the fold information was not available in SCOPe).

Search for DrugBank molecules. The hits for which the Uniprot accession codes could be obtained were then searched in the DrugBank[45] (version 5.1.5) database to check if there is a known molecule associated with the corresponding protein. DrugBank is a curated hub of comprehensive information (description, targets, chemical structure, known use, pharmacodynamic and pharmacokinetic profiles, *etc.*) on drugs/drug candidates which may fall in any one or many of the following categories: 'Approved' (the molecules which have successfully cleared the clinical trial for an indication and are approved for treatment for that particular indication), 'Investigational' (the molecules which are under clinical trial for at least one indication), 'Experimental' (these are generally the molecules which are in the pre-clinical development stage), 'Vet Approved' (molecules which are approved for treatment against the indicated veterinary disease), 'Withdrawn' (molecules which were once approved but have been withdrawn due to toxicity related or commercial reasons). Each molecule in the DrugBank is identified with a unique code that starts with 'DB' followed by numbers.

Data compilation and analysis. The drug card/s (detailed record of each molecule in the DrugBank) of the molecules associated with the proteins shortlisted from the above step were then analysed to extract relevant details. Following this, we have assessed the chemical similarity of the selected small molecules (obtained from DrugBank) with reported SARS-CoV-2 M^{pro} non-covalent inhibitors, *viz.*, 'O6K' bound to SARS-CoV-2 M^{pro} structure (PDB codes: 6Y2F and 6Y2G[22]) and 'X77' bound to SARS-CoV-2 M^{pro} structure (PDB code: 6W63[46]). Tanimoto coefficients (TC1 and TC2, respectively) between each pair of shortlisted DrugBank molecules and 'O6K' followed by 'X77' were calculated using RDKit (RDKit: Open-source cheminformatics; http://www.rdkit.org/) implemented in an in-house python code. The Tanimoto coefficients range between 0 and 1. Higher the value of Tanimoto coefficients, greater is the chemical similarity between the two compounds in comparison.[47] The information on current clinical trials has been retrieved from ClinicalTrial.gov, a database of privately and publicly funded clinical studies being conducted worldwide (available at https://ClinicalTrials.gov). Each registered clinical study is identified with a unique code which starts with the letters 'NCT' followed by a few numbers (this code is commonly referred to as the NCT number). The COVID-19 Drug Repurposing Database hosted by Excelra at https://www.excelra.com/ and LitCovid[48] available at https://www.ncbi.nlm.nih.gov/research/coronavirus/ were also consulted for literature survey pertinent to our hits. Promals3D[49] was used for the structure-guided sequence alignment and the ProBiS[50] webserver was used to perform the local structural alignment of the binding sites.

Docking simulation and analysis. 'Approved/Investigational' molecules with a molecular weight $<$850 Dalton were selected for docking simulations using Autodock Vina[51] to predict whether these molecules could be favourably accommodated in the binding pocket of SARS-CoV-2 M^{pro} where 'O6K' and 'X77' are shown to bind in the respective crystal structures. The only 'experimental' molecule which was considered for docking study was DB07275 due to the reason which is explained later. The protein co-ordinates from the PDB entry 6W63 were used for docking. The crystallographic quality of the bound ligand and the protein binding site was verified using the EDIA server,[52] which has been found to be satisfactory for conducting docking studies. The raw co-ordinate file as obtained from RCSB PDB[53] was prepared using the PDB2PQR server (version 2.0.0)[54] at pH 7.4 ensuring that the prepared protein had an optimized hydrogen bonding network. The resulting 'pqr' file was converted to 'pdbqt' format using the default settings of prepare_receptor4.py, a Python script which can be found in the MGLTools package.[55] The 3D-coordinate files of the ligands were obtained from either DrugBank/ChemSpider/PubChem[45,56,57] and were then converted to the 'mol2' format using OpenBabel[58] by adding explicit hydrogens at pH 7.4. These 'mol2' files of the ligands were then converted to the 'pdbqt' format using the default settings of prepare_ligand4.py, a Python script which can be found in the MGLTools package.[55] The dimension of the grid box used for docking was set as 14 Å, 18 Å and 16 Å in the x, y and z directions, respectively. The grid spacing was set at 1 Å and the x, y and z co-ordinates for the centre of the grid boxes were chosen as -20.925, 18.403 and -28.117. 20 binding modes per ligand were generated with an energy range of 9 kcal mol^{-1}. Except for the co-ordinates of the conserved water in the catalytic site, all other water co-ordinates were deleted prior to grid generation. In all the docking runs, only the flexibility of the ligands was considered. The binding site residues were considered as rigid. The docking protocols were validated through re-docking experiments to ensure that the docking algorithm is able to reproduce the bound pose of the native ligands ('O6K' and 'X77') present in the respective crystal structures. Upon obtaining satisfactory re-docking results (data not shown), the docking runs for the compounds in our dataset

were initiated. The docked poses were analysed for detecting the non-covalent interactions using the default settings of Maestro GUI available for academic usage (Schrödinger, LLC). The best pose for each docked ligand has been considered for reporting in this article. The best pose was selected based on visual inspection, which involved analysis with respect to the consistency of binding modes and the consideration of the possibility of interactions with important binding site residues (which are mentioned earlier) in different subsites of the SARS-CoV-2 M^{pro} binding pocket.

Workflow-II

Like workflow-I, this workflow is also a five step process and has been shown in Fig. 2. Each step is described below.

Identification of chemical neighbours and filtering. These steps involve the calculation of the Tanimoto coefficients (TC1 and TC2, as mentioned in workflow-I) of DrugBank molecules, which are 'Approved' and/or 'Investigational', with respect to 'O6K' and 'X77'. A cut-off of 0.55 for Tanimoto coefficients was chosen to select molecules for further analysis. Thus, the molecules that have TC1 $\geq$ 0.55 and TC2 $\geq$ 0.55 have

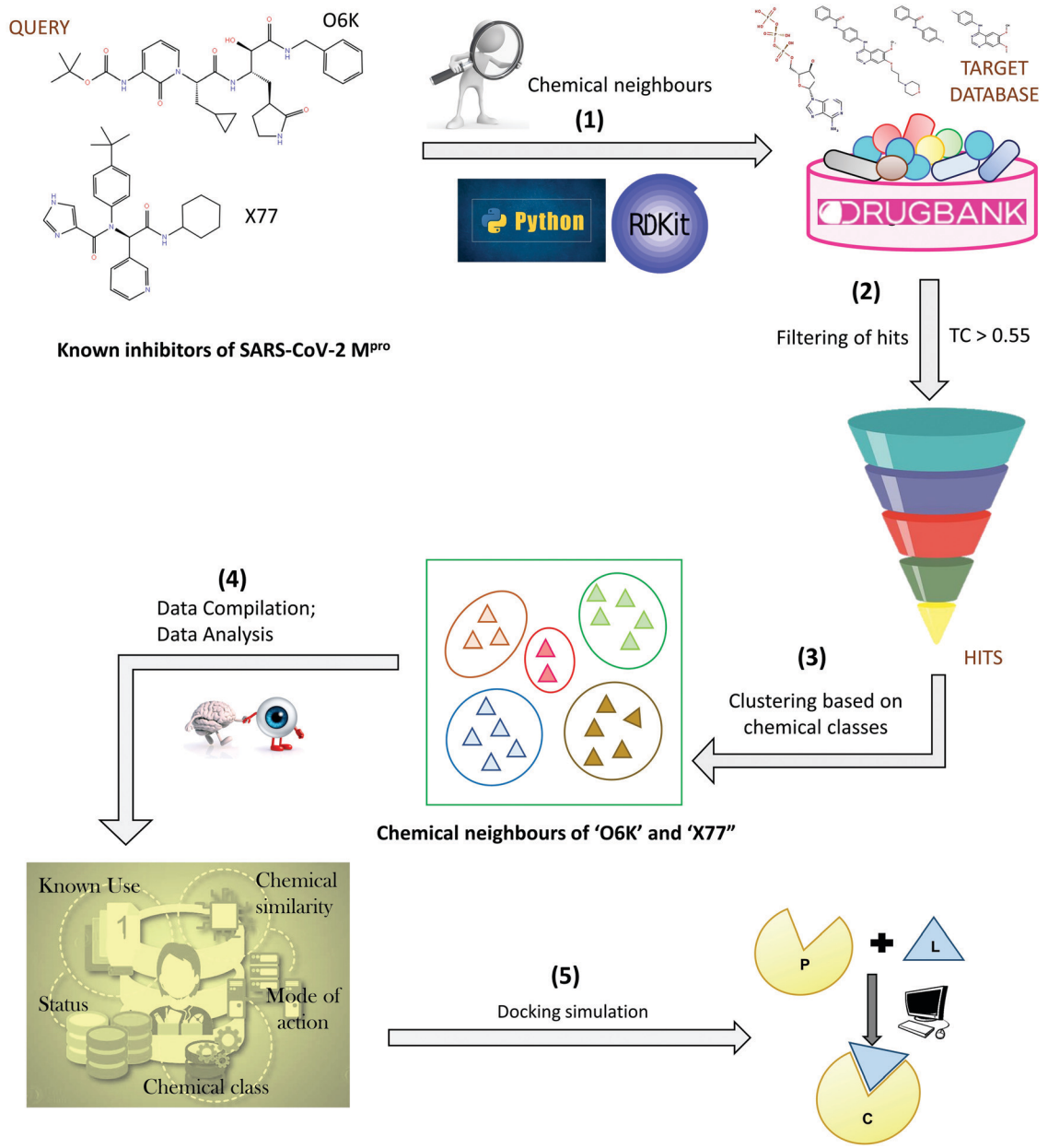

Fig. 2 Workflow-II. The steps (1 to 5) involved in this workflow as discussed in the text have been pictorially represented depicting the search for chemical neighbours of the reported inhibitors of SARS-CoV-2 main protease in DrugBank. A cut-off of 0.55 for TC (Tanimoto coefficient) is chosen to filter the hits which are then clustered based on their chemical class and at least one representative from each chemical class is chosen for docking simulation followed by data analysis as explained in the legend of Fig. 1. The 2D image of O6K and X77 has been obtained from the PDB. The flow has been prepared in Microsoft PowerPoint using clipart from Google search.

been considered for detailed analysis and discussion in this article.

Clustering/grouping. The selected molecules were then clustered/grouped based on their chemical class as denoted in the 'Taxonomy' field of the respective drug card available in the DrugBank database (wherever such information was not available in the 'Taxonomoy' field, the 'Description'/'Category' fields were inspected for relevant information).

Data compilation and analysis. This step involves the analysis and compilation of data similar to workflow-I.

Molecular docking. At least one molecule from each chemical class, which has a molecular weight $<$ 850 Dalton, was selected for docking simulation. The docking protocol used here is the same as that mentioned in workflow-I.

Lastly, a set of 44 hits combined from the results of workflow-I and II were considered for calculating the 2D chemical similarities (Tanimoto coefficient) with respect to a panel of 26 drugs that are already reported to be potential antiviral agents and/or specifically effective in anti-SARS-CoV-2 therapy. The higher the Tanimoto coefficient between a pair of compounds, the higher the extent of similarity in the 2D chemical fingerprints and *vice versa*.

The 2D chemical structures of the compounds considered for docking from workflow-I and workflow-II can be visualized in the ESI.†

Results and discussion

The computational approach followed in this work through the implementation of two different workflows helped in identifying several potential inhibitors, which could be considered for further probing to understand their fitness for repurposing against SARS-CoV-2. The findings from the application of each of the workflows are elaborated below.

Workflow-I

The search for structural neighbours of SARS-CoV-2 M^{pro} yielded 3001 protein chains as reliable hits (Table S1a, ESI†). The self-hits, *i.e.*, hits comprising PDB entries of SARS-CoV-2 M^{pro} were discarded for the analysis. The remaining PDB entries could be mapped to 229 unique Uniprot entries corresponding to 109 different organisms as per the Uniprot nomenclature (Table S1b, ESI†). 82 out of these 229 proteins belong to various RNA viruses (Alphavirus: 2; Flaviviridae: 39; Nidovirales: 31; Norwalk virus: 2; Picornaviridae: 5; Potyvirus: 2; Sesbania mosaic virus: 1). The remaining 147 proteins belong to cellular organisms (Bacteria: 33; Eukaryota: 114). Notably, 56 out of the 114 eukaryotes are humans. As expected, the majority of the proteins ($\sim$96%) for which fold/superfamily information could be obtained belong to the trypsin-like serine protease fold (Table S1b and Fig. S1, ESI†), to which the M^{pro} of coronaviruses also belong. Out of the 229 proteins, we could find at least one DrugBank molecule associated with 51 proteins. These 51 cases have been taken forward for further analysis.

The above statistics suggests that the SARS-CoV-2 M^{pro} is structurally similar to 229 proteins (encoded in the genome of 109 different organisms) for which at least one 3D structure is available in the PDB. Furthermore, although the sequence search using BLAST did not yield any significant human relative of SARS-CoV-2 M^{pro}, the structural search against the PDB has yielded some human proteins as hits. This hints that while the sequence of SARS-CoV-2 M^{pro} is very diverse from any protein encoded by the human genome, its global structure is similar to some of the human proteins. However, global structural similarities might not always imply the conservation of local structural features at the binding sites, which drives the molecular recognition phenomenon. Therefore, we also investigated the binding site (local) structural similarity of SARS-CoV-2 M^{pro} with all the protein structures available in PDB using ProBis as mentioned in the methodology section. The results of a ProBis search yielded a list of 186 proteins with recognizable similarity in the binding sites with respect to SARS-CoV-2 M^{pro} (Table S1c and d, ESI†). However, only 9 out of these 186 proteins are highly confident associations (Z-score $\geq$ 2) and they do not include any human proteins. Except for the SARS-CoV main protease, there are no common hits between the DALI and ProBis results. On one hand, this implies that all human protein hits obtained from the DALI search might not have enough local resemblance with the binding site of SARS-CoV-2 M^{pro}. Therefore, a molecule targeted at SARS-CoV-2 M^{pro} has only a very low chance of cross-reactivity with the human proteins. On the other hand, this also implies that all of the Drugbank molecules that we have fetched out based on the global protein structural similarity associations made by DALI might either not be able to bind to SARS-CoV-2 M^{pro} or show a different binding mode due to differences in the binding site features. Predicting how far the chemistry and flexibility of ligands coupled with a gross resemblance of protein structures at the fold level would influence the favourable accommodation of the selected DrugBank molecules in the SARS-CoV-2 M^{pro} binding pocket, we have performed a case-by-case analysis. This involved the calculation of chemical similarity coefficients and docking simulations as detailed later. It is to be mentioned here that although the highly confident hits obtained from the ProBis search do not involve any human proteins, there are 30 human proteins which have been indicated to possess a detectable level of similarity in their binding sites ($1 \leq Z$-score ≤ 1.73) as that of SARS-CoV-2 M^{pro}. These 30 proteins could be considered as a list of probable off-targets for SARS-CoV-2 M^{pro} inhibitors in human hosts and can be probed further for toxicity analysis (Table S1d, ESI†).

In this study, we mainly focussed on those structural neighbours of SARS-CoV-2 M^{pro} (as obtained from the DALI search) which are known as a target for at least one small molecule listed in DrugBank. Biotech products, such as antibodies or any protein-based therapies, nutraceuticals, dietary supplements and vitamins, if indicated in the DrugBank as targeting a protein structurally related to SARS-CoV-2 M^{pro}, are not considered further in this work. However, such hits might be interesting to explore in a separate study. Analysis of the details

of the molecules of interest revealed that these molecules belong to diverse therapeutic areas: antiviral, anticoagulant, anti-cancer, anti-inflammatory agents *etc.* (Table S2a, ESI†). It is to be noted that for a few of the molecules (especially the molecules belonging to the 'experimental' group), detailed information is not available. Interestingly, 7 molecules which possess higher chemical similarity with 'O6K' and 'X77' (TC1 $\geq$ 0.5 and TC2 $\geq$ 0.5) than most other molecules in our dataset are all known antiviral agents (Table S2a, ESI†). These 7 molecules along with other approved/investigational molecules (mol. wt. $<$ 850 Dalton) known to be used in different therapeutic areas, resulting in a set of 25 molecules, were considered for docking analysis. The predicted binding affinity as calculated from the docked poses indicates that all of these 25 molecules could be favourably accommodated in the SARS-CoV-2 M^{pro} binding pocket which is occupied by the inhibitors 'O6K' in 6Y2F and 6Y2G and 'X77' in 6W63 (Table S2b, ESI†). 11 molecules from our hit list have been reported to have benefits in the treatment of viral infections through laboratory and/or clinical experiments. Some of these have been specifically reported to possess anti-coronavirus properties. Additionally, a 12th molecule (foretinib, a tyrosine kinase inhibitor) has indirect evidence of anti-coronavirus properties as discussed later (Table S2b, ESI†). We suggest that the 17 molecules (out of the 25 shortlisted molecules which were considered for docking studies) with a predicted binding affinity of ≤ -7 kcal mol^{-1} could be prioritized for the experimental testing of their inhibitory action against SARS-CoV-2 M^{pro} (Table 1).

Table 1 List of drugs/drug candidates prioritized from workflow-I

Sl no.	Name of DrugBank molecule	DrugBank ID	Drug group	Known category/description/ indication	Category of available support for anti-viral property*	Predicted binding affinity (Autodock Vina score of best poses; kcal mol^{-1})
1	Simeprevir	DB06290	Approved	Antiviral agents/protease inhibitors	II$^{@}$	−9.1
2	Danoprevir	DB11779	Investigational	NS3/4A protease inhibitor/ cytochrome P-450 enzyme inhibitors	III$^{\$}$	−8.8
3	Nafamostat	DB12598	Investigational/ approved in Asian countries	Anticoagulant/antirheumatic agents/protease inhibitors	II$^{\$}$	−8.3
4	Remdesivir	DB14761	Investigational	Antiviral agents	III$^{\$}$	−8.0
	Remdesivir triphosphate	Active metabolite of Remdesivir				−7.2
5	Ciluprevir	DB05868	Investigational	HCV NS3 protease inhibitor	II$^{@}$	−8.0
6	3-(1,1-Dioxido-4*H*-1,2,4-benzothiadiazin-3-yl)-4-hydroxy-1-(3-methylbutyl)-quinolin-2(1*H*)-one	DB07275	Experimental	Antiviral agent	N/A	−8.0
7	Edoxaban	DB09075	Approved	Anticoagulants/serine protease inhibitors	N/A	−8.0
8	Glecaprevir	DB13879	Approved, investigational	Antiviral agents/NS3/4A protease inhibitors	N/A	−7.9
9	Ribavirin triphosphate	N/A (active metabolite of ribavirin, DB00811)	Approved	Antiviral agent, antimetabolite	III$^{\$}$	−7.8
10	Apixaban	DB06605	Approved	Anticoagulants/serine protease inhibitors	N/A	−7.7
11	Foretinib	DB12307	Investigational	Anti-cancer/tyrosine kinase inhibitor	II$^{\#}$	−7.7
12	Freselestat	DB03925	Experimental, investigational	Leukocyte elastase, antagonists & inhibitors (COPD treatment)	N/A	−7.5
13	Argatroban	DB00278	Approved, investigational	Anticoagulant/protease inhibitors	II$^{\$}$	−7.4
14	Iloprost	DB01088	Approved, investigational	Anticoagulant/ hypotensive Agents	N/A	−7.4
15	Asunaprevir	DB11586	Approved, investigational, withdrawn	HCV NS3 protease inhibitor/ HIV protease inhibitors	II	−7.3
16	Dabigatran etexilate (prodrug)/Dabigatran (active metabolite)	DB06695/ DB14726	Approved/ investigational	Anticoagulant/protease inhibitors	N/A	−7.2
17	Betrixaban	DB12364	Approved, investigational	Anticoagulant/protease inhibitors	N/A	−7.0

N/A: not available. * The Roman numerals indicate the type of available support for anti-viral property. I: *in silico*; II: lab experiment and/or clinical study; III: all three categories (*in silico*, lab and clinical experimental support) are available; @: anti-coronavirus specific property suggested through *in silico* experiments; $: anti-coronavirus specific property suggested through *in silico* as well as lab and/or clinical experiments; #: evidence of anti-coronavirus property is available from lab/clinical results for a similar molecule, *i.e.*, imatinib (P.S.: both imatinib and foretinib are TK inhibitors). For details regarding the evidences, kindly refer to text.

Interestingly, besides the antiviral agents (like simeprevir, danoprevir, remdesivir, DB07275, ciluprevir), some of the high scoring hits (a predicted binding affinity of ≤ -8 kcal mol^{-1}) among the 17 prioritized molecules include approved anti-coagulants like nafamostat and edoxaban. All the molecules which were considered for docking analysis are either approved and/or investigational drugs except DB07275 (which is an experimental molecule with known antiviral properties). This experimental molecule was considered for docking because its chemical similarity with 'O6K' and 'X77' is comparable to that of the approved/investigational antiviral drugs in our hit list. Docking studies predicted that this molecule could bind to SARS-CoV-2 M^{pro} with an affinity of -8.0 kcal mol^{-1} involving E166 in hydrogen bonding and several other functional residues in hydrophobic interactions (Table 1 and Table S2b, ESI†). Our analysis suggests that besides the 17 prioritized molecules, some of the 8 remaining molecules with a predicted binding affinity against SARS-CoV-2 M^{pro} of > -7 kcal mol^{-1} (for *e.g.*, gabexate, camostat, ribavirin, ibuprofen, nesbuvir, and tranexamic acid) would also be equally interesting for further probing as they are potential SARS-CoV-2 M^{pro} binders and are also likely to offer benefits in SARS-CoV-2 treatment through modulating the host response system as discussed in the following sub-section.

Hits with support from available laboratory and clinical data

In this section and the corresponding section under results of workflow-II, while the references to the availability of laboratory data are included in a standard literature citation format, the references to clinical data are cited by providing the respective NCT numbers (which have been described in the methodology section). Most of these clinical studies are on-going or not yet recruited. Therefore, any published literature on the outcomes of clinical studies is unavailable at the moment. Readers are encouraged to track the developments of the clinical trial findings using the corresponding NCT numbers.

Remdesivir and other anti-viral agents. Remdesivir (which was originally developed for treating Ebola infection[59]) is one of the most promising antiviral agents which is reported to be effective against SARS-CoV-2 infection.[60,61] This molecule is enrolled for clinical studies under several conditions related to COVID-19 treatment (NCT04335123, NCT04292899, NCT04292730, NCT04349410, NCT04252664, NCT04321616, NCT04257656, NCT04323761, NCT04315948, NCT04280705, NCT04302766). Interestingly, it has been picked up in our analysis too. Remdesivir is indicated to target the SARS-CoV replicase polyprotein 1ab (Uniprot code: P0C6X7) and the RNA-directed RNA polymerase L of Zaire ebolavirus (strain Mayinga-76) (Uniprot code: Q05318) in DrugBank. It is to be noted that the replicase polyprotein 1ab is a multifunctional protein which is $\sim$7000 residues long.[9,10,62] It contains the proteinases responsible for the cleavages of the polyprotein including the SARS-CoV M^{pro} (which is a $\sim$300 residues long protein, corresponding to the sequence range 3264–3569). The PDB entries corresponding to Uniport code P0C6X7 (*i.e.*, SARS-CoV replicase polyprotein 1ab), which have been picked up as structural neighbours of SARS-CoV-2 M^{pro}, cover only the sequence region of the replicase polyprotein 1ab that belongs to SARS-CoV M^{pro} (a stretch of $\sim$300 residues in the $\sim$7000 residues long sequence) (Table S1a, ESI†). The sequence of SARS-CoV M^{pro} is 96% identical to SARS-CoV-2 M^{pro} and the overlay of SARS-CoV M^{pro} structure onto SARS-CoV-2 M^{pro} shows that the binding sites of these two proteins are conserved (Fig. S2, ESI†). This is also evident from the fact that the SARS-CoV M^{pro} structure has been picked by both the DALI search and the ProBis search with high confidence *Z*-scores indicating a highly similar local (binding site) and global structure (Tables S1a–d, ESI†). Therefore, by virtue of a high structural and sequence similarity of the binding sites, it appears that a molecule which inhibits SARS-CoV M^{pro} is likely to bind to SARS-CoV M^{pro} and subsequently arrest its activity. It is to be noted that another important protein encoded in the replicase polyprotein 1ab sequence is the RNA-directed RNA polymerase (RdRp; sequence range 4393–5324), which is responsible for the replication and transcription of the viral RNA genome. The earlier literature shows that remdesivir is known to interfere with the RdRp mediated RNA synthesis of MERS-CoV and SARS-CoV. The proposed mechanism of action of remdesivir is through the incorporation of the active triphosphate into viral RNA.[63,64] There is no available experimental evidence suggesting remdesivir to be binding to SARS-CoV/SARS-CoV-2 M^{pro}. However, it is known that the nucleoside analogues have multiple mechanisms of viral inhibition and there are data which suggest that remdesivir may exhibit its antiviral activity through multiple modes which are yet to be unveiled.[65] Agostini *et al.* have suggested that further experiments are required to precisely define the mechanism of action of remdesivir against CoVs.[66] This intrigued us to investigate whether remdesivir and its active metabolite remdesivir triphosphate could bind to the SARS-CoV-2 M^{pro} active site. Interestingly, the binding affinity for both remdesivir and remdesivir triphosphate against SARS-CoV-2 M^{pro} has been predicted to be favourable in engaging the important residues in non-covalent interactions (Table S2b; Fig. 3 and Fig. S3, ESI†). Docking studies performed by other investigators also indicate that remdesivir has the potential to bind to SARS-CoV-2 M^{pro}.[67] It is to be noted that if our predictions are correct, it is most likely that the active metabolite (*i.e.*, remdesivir triphosphate) of remdesivir (which itself is a prodrug) would be predominantly targeting M^{pro} under physiological conditions. However, the previous computational study has not taken this factor into consideration.[67] Our docking results suggest that remdesivir triphosphate and remdesivir might have a comparable affinity against M^{pro}. These results demand extensive experimental investigation into the detailed molecular mechanism of action of remdesivir and explore the potential of remdesivir to target CoV M^{pro}s which are highly conserved across related species. Consequently, development of remdesivir as a broad spectrum anti-CoV agent should also be probed.

Other interesting hits involve ribavirin and danoprevir. Both these molecules are registered for COVID-19 clinical trials (ribavirin: NCT04335123, NCT04276688; danoprevir: NCT04345276, NCT04291729). Ribavirin is known to act through several mechanisms of action that lead to the inhibition of viral RNA

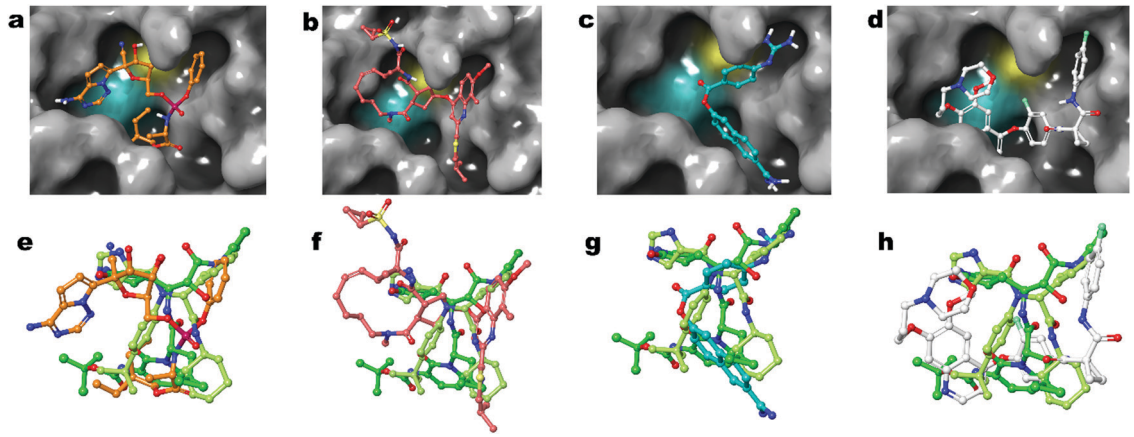

Fig. 3 Docked poses of four representative molecules in the SARS-CoV-2 main protease binding pocket obtained from workflow-I. (Upper) The protein binding site is shown in surface representation (grey) and the ligands are shown as ball and stick models. The protein surface shown in yellow and teal are C145 and H41, respectively. (a) Remdesivir (orange), (b) Simeprevir (faded red), (c) Nafamostat (teal), and (d) Foretinib (white). (Lower) The overlay of docked compounds (which are shown in the upper panel) on to the bound pose of O6K (as seen in 6Y2F; faded green) and X77 (as seen in 6W63; green). The order of the docked compounds in (e)–(h) is identical to that in the upper panel. To maintain visual clarity, in the upper panel only polar hydrogens of the ligands are shown whereas in the lower panel, no hydrogens have been shown. The images have been generated using Maestro GUI (Schrödinger, LLC) freely available for academic usage.

and protein synthesis. One of the mechanisms involves viral mRNA polymerase inhibition by its active metabolites: mono-, di-, and predominantly triphosphosphate ribavirin.[68] Therefore, besides ribavirin, we were also interested to probe if ribavirin triphosphate (RTP) has the potential to bind to SARS-CoV-2 M^{pro}. In fact, RTP (-7.8 kcal mol^{-1}) is predicted to be a slightly better binder than the parent compound, ribavirin (-6.3 kcal mol^{-1}). Ribavirin is also known to modulate the host response system that contributes to benefits in anti-viral therapy.[68] A clinical report from a study (NCT04291729) carried out in China on 11 COVID-19 patients suggests that danoprevir (a potent hepatitis C virus protease inhibitor) boosted by ritonavir (a CYP3A4 inhibitor to enhance plasma concentration of danoprevir while it also acts as a human immunodeficiency virus protease inhibitor at high doses) is a promising therapeutic option.[69] Notably, among the other antivirals, simeprevir and ciluprevir have been indicated as potential *in silico* hits against SARS-CoV-2 M^{pro} by other investigators in a recent publication.[70]

Nafamostat and other anticoagulant agents. Nafamostat is a known human serine protease inhibitor and is approved in Asian countries as an anticoagulant drug.[71] Nafamostat is also known for its anti-inflammatory properties and is used in treating pancreatitis.[72] Excitingly, Hoffmann and co-workers have showed that the human transmembrane protease serine 2 receptor (TMPRSS2) primes SARS-CoV-2 Spike protein for entry and the serine protease inhibitor camostat (an analogue of nafamostat) effectively blocks SARS-CoV-2 infection of lung cells.[73] The potential of nafamostat to block MERS-CoV infection has been demonstrated in the past.[74] The known human targets of nafamostat (as per DrugBank record) which have been picked up as structural neighbours of SARS-CoV-2 M^{pro} are prothrombin, kallikrein and coagulation factor XII. For camostat, the target that has been associated in our study is trypsin-1. All these known targets of nafamostat and camostat belong to the trypsin-like serine proteases fold. A structure-guided sequence alignment of prothrombin (selected as one of the representatives among all the mentioned associated targets based on the best *Z*-score from DALI search), TMPRSS2 and SARS-CoV-2 M^{pro} showed that the active sites of the former two proteins are highly diverged from the later with only two residues conserved at key positions (position corresponding to H41 and G143 of SARS- CoV-2 M^{pro}) (Fig. S4 and S5, ESI†). To predict whether a molecule (such as camostat and nafamostat) which binds to prothrombin and TMPRSS2 can also bind to SARS-CoV-2 M^{pro}, we performed docking simulation. Indeed, the predicted binding poses of camostat and nafamostat in the SARS-CoV-2 M^{pro} active site indicate favourable interactions between the protein and these two ligands (Table 1, Table S2b, Fig. 3 and Fig. S3, ESI†). This suggests that, although the binding site sequence is very diverged, the conservation of a few key residues coupled with gross structural similarity of the proteins (by virtue of similar fold) and ligand's flexibility may aid in the binding of the serine protease inhibitors to the active site of SARS-CoV-2 M^{pro} (which is a cysteine protease). Both camostat (NCT04338906, NCT04355052, NCT04353284, NCT04321096) and nafamostat (NCT04352400) have been registered for clinical trials in the treatment of COVID-19. Furthermore, our analysis hints that apart from nafamostat and camostat, other anticoagulants like dabigatran (which is the active form of dabigatran etexilate), argatroban, betrixaban, iloprost, apixaban, and edoxaban might also possess the potential for binding to SARS-CoV-2 M^{pro} (Table 1 and Table S2b, ESI†). It has been reported that COVID-19 patients often develop disseminated intravascular coagulation and treatment with anticoagulants can decrease the mortality in severe cases.[75,76] Our results show that several human proteins recognized as structural neighbours of SARS-CoV-2 M^{pro} are involved in the regulation of blood clotting and all these proteins adopt a trypsin-like serine protease fold like the

SARS-CoV-2 M^{pro} (Tables S1b and S2a, ESI†). Some of these proteins (like hepatocyte growth factor and coagulation factor X) are known to be targeted by heparin (a natural anticoagulant released from mast cells) or its derivatives.[77] It is to be noted that heparin (and its derivatives like tinzaparin; NCT04344756) and other anticoagulants like fondaparinux (NCT04359212) are currently being explored in clinical trials for treating SARS-CoV-2 infections. Non-heparin anticoagulants like argatroban and bivalirudin (two anticoagulants in our hit list, Table S2b, ESI†) have been recommended by medical experts on thrombosis and hemostasis for the management of disease conditions in COVID-19 patients who are allergic to heparin or have a high risk of heparin-induced thrombocytopenia.[78] However, direct-acting oral anticoagulants (DOACs) like apixaban, edoxaban and dabigatran have been recently reported to cause bleeding complications when concomitantly administered with antiviral agents for treating SARS-CoV-2 infections. In order to prevent such complications, medical experts have suggested to opt for either dosage adjustments of the DOACs or switch to alternative parenteral antithrombotic strategies for as long as the patient is under treatment with antiviral drugs.[79] Another notable hit among the anticoagulants is gabexate, a synthetic serine protease inhibitor, known to decrease the production of inflammatory cytokines.[80] Clinical reports suggest that severe deterioration has been observed in some COVID-19 patients due to a cytokine storm in their bodies.[81] Although gabexate is predicted to be a slightly weaker binder of SARS-CoV-2 M^{pro} than the previously mentioned anticoagulants in our hit list (Table S2a and b, ESI†), however, given its role in decreasing cytokine productions and anti-coagulation, it can be explored further for anti-COVID-19 therapy. Taken together, our analysis indicates that anticoagulant therapeutics could serve as important agents for the management of SARS-CoV-2 infections through multiple modes of action that involve modulation of the harmful host responses to the pathogenic conditions and arresting the activity of one of the most important viral targets (*i.e.*, SARS-CoV-2 M^{pro}).

Anti-inflammatory agents. Our analysis also shows that some anti-inflammatory agents can be potential binders of SARS-CoV-2 M^{pro}. Although there has been some confusion that the usage of anti-inflammatory drugs during coronavirus infections may aggravate the disease conditions, there is no scientific experimental basis to it and this has been explained in detail in a recent article[82] by Garret A. FitzGerald. Interestingly, benefits of combining antiviral and anti-inflammatory agents (like baricitinib which modulates the functions of several kinases that interfere with cytokine mediated inflammation or involve in endocytosis) to treat COVID-19 have been reported.[83,84] Mining the available data on the on-going clinical trials revealed that baricitinib has already been registered for clinical trials in both mono and combinatorial therapy to treat SARS-CoV-2 infection (NCT04358614, NCT04321993, NCT04340232, NCT04346147, NCT04345289, and NCT04320277). Also, there are publications that emphasize that ibuprofen can be helpful in lung infections caused by bacteria/viruses by reducing the amount of inflammation, which causes damage to the lungs.[85] Interestingly, ibuprofen has been predicted in our study to bind to SARS-CoV-2 M^{pro}, and this molecule is also registered for clinical trial to evaluate the reduction in the severity and progression of lung injury in COVID-19 patients through its administration (NCT04334629). Freselestat, another anti-inflammatory agent in our hit list which is a known serine-protease inhibitor (neutrophil elastase antagonist), has also been predicted to bind to SARS-CoV-2 M^{pro} (Table 1 and Table S2a, b, ESI†).

Other hits. The other interesting hits involve an anti-cancer drug, foretinib, a tyrosine kinase (TK) inhibitor, and tranexamic acid, an antifibrinolytic agent. Using cell-based assay techniques, Dyall *et al.*[86] have observed earlier that TK inhibitors like imatinib and dasatinib are effective in MERS-CoV or SARS-CoV infections with no or low cytotoxicity. Based on their study, they have concluded that the TK inhibitors appear to target host factors rather than viral proteins. Additionally, imatinib is known to possess anti-inflammatory properties through the inhibition of TNF-α production[87] and elevated levels of this pro-inflammatory cytokine have been observed in severe COVID-19 patients.[81] Due to the known antiviral and anti-inflammatory properties of imatinib, it has been registered for multiple clinical trials (NCT04346147, NCT04356495, and NCT04357613) for the treatment of SARS-CoV-2 infection. Our results indicate that foretinib which is also a TK inhibitor (like imatinib and dasatinib), can possibly bind to the SARS-CoV-2 M^{pro} and may also elucidate a beneficial response by modulation of host signalling pathways (Table 1, Table S2b, Fig. 3 and Fig. S2, ESI†). Notably, the critical role of dual-specificity tyrosine phosphorylation-regulated kinases (DYRKs) during viral replication and the high antiviral potential of DYRK inhibitors have been reported by Hutterer *et al.* in 2017.[88] Thus, our results demand further research on the possibilities of a dual mechanism of action of TK inhibitors with respect to the modulation of host and viral proteins. Interestingly, tranexamic acid which is predicted to be a weak binder (-4.1 kcal mol^{-1}) of SARS-CoV-2 M^{pro} in our study has also been found to be registered for clinical trials of COVID-19 treatment (NCT04338074 and NCT04338126). It has been suggested in a recent report[89] that endogenous protease plasmin acts on SARS-CoV-2 virus by cleaving a newly inserted furin site in the S protein of the virus resulting in increased infectivity and virulence. Patients with hypertension, diabetes, coronary artery disease, cerebrovascular illness, lung disease and kidney dysfunction commonly have higher levels of plasmin/plasminogen. This mechanism can be attributed to poorer outcomes in such patients with these co-morbidities. Therefore, drugs that inhibit the conversion of plasminogen to plasmin, like tranexamic acid, might prove useful. Amiloride (another predicted binder of SARS-CoV-2 M^{pro} from our study), an approved diuretic and hypotensive agent, is known to increase the cytosolic pH by acting on the Na^+/K^+ exchanger. A low cytosolic pH has been found to aid viral entry into the host cell. Drugs like hydroxychloroquine (HQ) are therefore proving to be useful in the management of COVID-19 pathological conditions. Given the higher potency of amiloride than HQ and its effectiveness in lung tissue, a team of medical experts speculated in a recent report that amiloride could be used alone or with HQ in the prophylaxis and treatment of COVID-19.[90]

Workflow-II

The hunt for molecules chemically similar to the known SARS-CoV-2 M^{pro} inhibitors ('O6K' and 'X77') using the computational protocol as described earlier resulted in the identification of 85 approved and/or investigational DrugBank molecules (Table S3, ESI†). Like in workflow-I, biotech products and dietary supplements were not considered for this analysis. Information on the chemical class could be obtained for 76 molecules which revealed that the majority of the hits belong to the class of alkaloids (~37%), followed by tetracyclines (~23%), carboxylic acid derivates/peptide analogues (~13%) and others (~27%) (Fig. S6, ESI†). Docking simulation of at least one compound from each chemical class indicated that these compounds have the potential to be favourably accommodated within the active site of SARS-CoV-2 M^{pro} (Table S3, ESI†). We suggest that the docked molecules whose binding affinities are predicted to be ≤ -7 kcal mol^{-1} can be prioritized for experimental probing (Table 2). Our detailed analyses on the classes of hits (alkaloids, tetracyclines) with reported anti-viral properties and some other promising hits are presented below.

Hits with support from available laboratory and clinical data

Alkaloids. These are naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Among the alkaloids, camptothecin derivatives and vinca alkaloids are the major subclasses of alkaloids which we have obtained as hits in our search for chemical neighbours of two known non-covalent inhibitors of SARS-CoV-2 M^{pro} ('O6K' and 'X77'). This is particularly exciting to us as the benefits of natural products in several therapeutic areas have been demonstrated through time-tested traditional medicinal practices and folklore. Moreover, natural products have inspired the development of most of the modern day synthetic drugs.[91] Vinca and camptothecin alkaloids have cytotoxic properties and are known for their anticancer properties. They are the active ingredients in many semi-synthetic anticancer formulations.[92,93] Vinca alkaloids are obtained from several species of Vinca genus and periwinkle (*Catharanthus roseus*) plant. While Vinca plants are native to Europe, Northwest Africa and southwest Asia, periwinkle is native to Madagascar. However, periwinkle is widely cultivated and is naturalised in subtropical and tropical areas of the world like Australia, Malaysia, India, Pakistan and Bangladesh (source of information: Wikipedia). A recent literature report on repurposing drugs (using *in silico* techniques) against the interface between SARS-CoV-2 S-protein and human ACE2 protein identified vidarabine (a vinca alkaloid) as a potential candidate.[94] Camptothecin is isolated from the bark and stem of *Camptotheca acuminata*, a tree native to China used for cancer treatment in Traditional Chinese Medicine (source of information: Wikipedia). Plants of the genus Ophiorrhiza which grow in the South-Western Ghats of India show the presence of a significant amount of camptothecin. *Ophiorrhiza mungos* is traditionally used in anticancer treatment in Ayurveda.[95] It is interesting to note that the evidence of potent inhibition of herpes virus by camptothecins could be found in the earlier literature.[96] Furthermore, a recent *in vitro* study by Choy *et al.* demonstrated that natural alkaloids like, homoharringtonine and emetine, which are known to possess

Table 2 List of drugs selected from among the representative hits as obtained from workflow-II

Sl No.	Drug name	DrugBank ID	Status	Use	Chemical class	Predicted binding affinity (Autodock Vina score of best poses; kcal mol^{-1})
1	Beclabuvir	DB12225	Investigational	Antiviral agents	Indoles and derivatives[a]	−9.9
2	Zoliflodacin	DB12817	Investigational	Anti-bacterial (gonorrhoea treatment)	Quinolines and derivatives	−9.8
3	Bromocriptine	DB01200	Approved, investigational	Anti-Parkinson agents (dopamine Agonist)	Ergoline and derivatives (Alkaloids)[a]	−9.3
4	UK-432,097	DB12691	Investigational	Pulmonary disease, chronic obstructive	Purine nucleosides[a]	−9.2
5	Ergotamine	DB00696	Approved	Sympatholytic (adrenergic blocking) agents: antimigraine preparations	Ergoline and derivatives (alkaloids)[a]	−8.8
6	Bictegravir	DB11799	Approved, investigational	Antiviral agents	Pyridines and derivatives[a]	−8.6
7	Oxytetracycline	DB00595	Approved, investigational, vet approved	Antibacterial agents	Tetracyclines[a]	−8.2
8	Tigecycline	DB00560	Approved	Antibacterial agents	Tetracyclines[a]	−8.0
9	Ceftolozane	DB09050	Approved, Investigational	Anti-Bacterial Agents	Lactams[b]	−7.4
10	Vinflunine	DB11641	Approved, investigational	Antineoplastic and immunomodulating agents	Vinca alkaloids[a]	−7.2
11	Vindesine	DB00309	Approved, investigational	Antineoplastic and immunomodulating agents	Vinca alkaloids[a]	−7.1
12	Topotecan	DB01030	Approved, investigational	Antineoplastic and immunomodulating agents	Camptothecins (alkaloids and derivatives)[a]	−7.1

[a] Molecules for which anti-viral properties are known either specifically or for the chemical class to which they belong as discussed in the text. [b] Molecules which belong to a therapeutic class (for *e.g.*, anti-bacterial agents) for which benefits in anti-viral therapy (particularly to treat co-infections) are indicated in the literature.

anti-herpes virus properties are also effective in inhibiting SARS-CoV-2 replication.[97] An obvious question that may arise is how safe these alkaloids would be for SARS-CoV-2 treatment since these alkaloids are known to kill cancerous cells by interfering with their cell division and such a mechanism may pose a risk to the body's normal dividing cells. This question can be effectively answered only by conducting experiments in clinical set ups. However, it is worth mentioning again that earlier studies by Dyall *et al.*[86] indicate that anti-cancer drugs targeting tyrosine-kinases like imatinib and dasatinib (known anticancer drugs) are effective in MERS-CoV or SARS-CoV infections with no or low cytotoxicity. The said study also reports similar observation with gemcitabine hydrochloride, another anti-cancer drug that interferes with DNA metabolism and affects cell division. Moreover, the role of anti-cancer targets like DYRK in the inhibition of viral replication and antiviral properties of DYRK inhibitors which include harmine (a harmala alkaloid) has also been reported.[88] Besides the vinca alkaloids and camptothecins, our results suggest that alkaloids like ergoline and its derivatives, opiate alkaloids and harmala alkaloids which have been picked up as hits in our study could be potential binders of SARS-CoV-2 M^{pro} (Table 2, Table S3, Fig. 4 and Fig. S7, ESI†) and based on the available evidence, the possibilities of anti-SARS-CoV-2 properties of these alkaloids cannot be negated.

Tertacyclines. These compounds are polyketides having an octahydrotetracene-2-carboxamide skeleton, substituted with many hydroxy and other groups. Tetracyclines form the second most populated class of chemicals in our hit list obtained from workflow-II. These compounds are known for their antibacterial properties. Two of the compounds, oxytetracycline and tigecycline, selected for docking from this class show favourable binding scores and the predicted poses are engaged in interactions with important binding site residues, such as H41, T190 and E166 (Table 2, Table S3, Fig. 4 and Fig. S7, ESI†). Interestingly, virtual screening of a library of compounds against SARS-CoV-2 M^{pro} by researchers from another group has also identified tetracyclines as potential hits.[98] Furthermore, an experimental study aiming to map the interactions between SARS-CoV-2 and human proteins has shown that human mitochondrial ribosome components MRPS5, MRPS27, MRPS2, and MRPS25 interact with SARS-CoV-2 Nsp8 protein.[99] Therefore, it has been suggested that antibiotics which have an off-target effect on mitochondrial ribosomes, such as tigecycline, might prove helpful in the management of SARS-CoV-2 infections.[99,100] Taken together, our results indicate the potential of tigecycline to be beneficial for SARS-CoV-2 treatment, probably mediated by its effect on mitochondrial ribosomes and viral main protease. The potential of doxycycline (another candidate in our hit list belonging to the class of tetracycline antibiotics) in the management of SARS-CoV-2 infection due to its anti-inflammatory and anti-aging properties has also been suggested in the literature.[101–103] Multiple modes of viral inhibition by tetracyclines owing to their roles in anti-inflammatory pathways and arresting of viral RNA replication are known.[103–107] These factors coupled with their long-standing safety profile and high lung-tissue penetration property[108] demand thorough experimental investigation on the potential of all the molecules belonging to the tetracycline class (especially those shortlisted from our analysis based on chemical similarity with known inhibitors of SARS-CoV-2 M^{pro}, Table S3, ESI†) to treat SARS-CoV-2 infection.

Other hits. The other interesting hits involve the known antivirals, like bictegravir, beclabuvir, baloxavir, ruzasvir, setrobuvir, furaprevir, and GS-9256. While bictegravir and beclabuvir are approved antiviral drugs, the remaining are investigational drugs (Table 2 and Table S3, ESI†). A purine nucleoside, UK-432,097 (DB12691), which acts as an adenosine A2 receptor agonist and has been used in clinical trials for the treatment of chronic pulmonary lung disease, is predicted to bind to SARS-CoV-2 M^{pro} with a comparatively higher affinity (-9.2 kcal mol^{-1}). The importance of nucleoside analogues in anti-viral medicinal chemistry has been established over decades.[109]

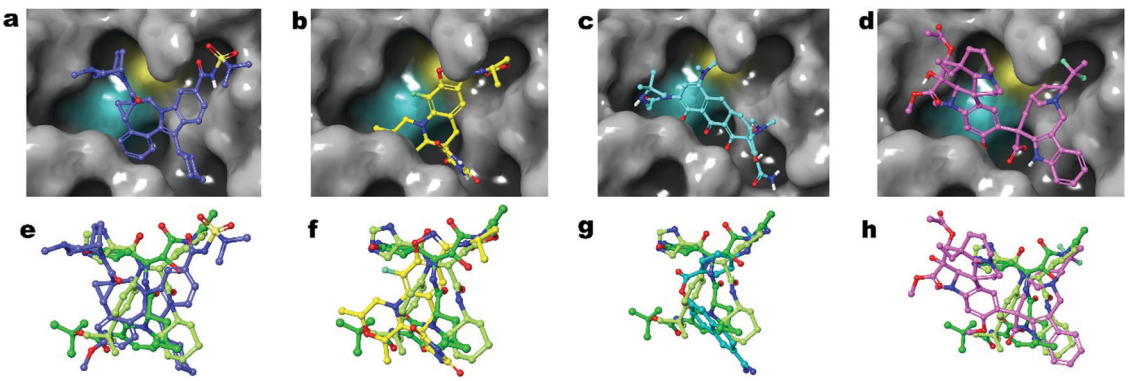

Fig. 4 Docked poses of four representative molecules in SARS-CoV-2 main protease binding pocket obtained from workflow-II. (Upper) The protein binding site is shown in surface representation (grey) and the ligands are shown as ball and stick models. The protein surface shown in yellow and teal are C145 and H41, respectively. (a) Beclabuvir (blue), (b) Zoliflodacin (yellow), (c) Tigecycline (cyan) (d) and Vinflunine (pink). (Lower) The overlay of docked compounds (which are shown in the upper panel) on to the bound pose of O6K (as seen in 6Y2F; faded green) and X77 (as seen in 6W63; green). The order of the docked compounds in (e)–(h) is identical to that in the upper panel. To maintain visual clarity, in the upper panel only polar hydrogens are shown whereas in the lower panel, no hydrogens have been shown. The images have been generated using Maestro GUI (Schrödinger, LLC) freely available for academic usage.

Therefore, our result strongly suggests further probing of UK-432,097 as an anti-coronavirus agent. Zoliflodacin, an investigational anti-bacterial drug, is among the top ranking ligands predicted to bind to SARS-CoV-2 M^{pro} with an affinity of -9.8 kcal mol^{-1} (Table 2 and Table S3, ESI†). Our analysis hints that the anti-bacterial agents belonging to the lactam and macrolactam class could also be promising SARS-CoV-2 M^{pro} inhibitors and need further probing. Another notable hit from our study is dactinomycin, a peptidomimetic drug, that acts as an anti-bacterial and anti-neoplastic agent. Dactinomycin has been shown to inhibit the growth of feline enteric coronavirus strain in feline embryo cells.[110] A recent computational study employing a network-based drug repurposing approach suggested sirolimus and dactinomycin as one of the potential drug combinations which could be effective in treating SARS-CoV-2 infection.[111] It is to be noted that most FDA-approved antiviral agents that target viral proteases are peptidomimetics and macrocyclic compounds.[112] This strongly urges the experimental testing of the other peptidomimetics (bleomycin, quinpristin, ramoplanin) as well which are identified in our study as a potential binder of SARS-CoV-2 M^{pro}. Furthermore, like many other viral infections, some of the COVID-19 patients are also reported to suffer from bacterial and fungal co-infection. Therefore, rational usage of antibiotics and antifungal agents has been suggested in severe cases.[113–115] Notably, many of the compounds obtained as hits in workflow-II are anti-bacterial agents (*e.g.* vancomycin, ceftolozane, tetracyclines, dactinomycin, mitomycin *etc.*). Known anti-fungal agents, micofungin and nikkomycin Z, have also been picked up as hits in workflow-II.

Analysis on the chemical similarity of the hits identified from workflow I and II with anti-coronavirus compounds already reported

In the previous sections, we have discussed from a pharmacological perspective, similarities between hits proposed in this work and drugs which are either already in clinical trials or reported in the literature to be potential antiviral (or specifically anti-coronavirus) compounds. To understand the 2D chemical similarities between the already discussed hits (44 compounds) as identified in this study and the molecules (26 selected compounds) reported in the literature, we have calculated the Tanimoto coefficients (TC) between the pairs of compounds (Table S4, ESI†). The extent of the similarities between a pair of molecules at the level of chemical fingerprints hints the likelihood of similarities in their biological activity as mentioned before. Interestingly, this analysis reveals that two alkaloids: vinflunine and lurbinectedin show the maximum chemical similarities across the selected panel of compounds which are already reported. Both these compounds exhibit reasonable chemical similarities (TC $>$ 0.70) with known antivirals (remdesivir, simeprevir and ciluprevir), antibacterial agents (tetracyclines) and natural alkaloids like homoharringtonine. The potential of remdesivir, tetracyclines, and homoharringtonine in anti-SARS-CoV-2 therapy has already been discussed. This finding strongly advocates for the need to test the potential of vinflunine, lurbinectedin and other alkaloids identified in our study for their effectiveness in anti-SARS-CoV-2 treatment. Investigational drugs like GS-9256 and UK-432,097 also show reasonable similarities with many of the reference compounds selected for this analysis. Compounds such as gabexate, iloprost and amiloride show the least similarities across the panel of the selected set of reported molecules that we have considered for this analysis. This suggests that gabextae, iloprost and amiloride have chemical scaffolds which are diverse from reported compounds. This might indicate a dissimilar biological activity profile of these compounds with respect to the reference set of compounds that we have chosen. However, our docking results indicate favourable accommodation of these compounds in the SARS-CoV-2 M^{pro} binding pocket. Furthermore, it should be noted that the diverse chemical scaffold of these hits compared to the known drugs could be exploited as an advantage to tackle the emergence of resistance.[116] Based on the similarities and dissimilarities of chemical fingerprints of the compounds proposed in our study with the already reported molecules, one could prioritize compounds for experimental validation.

Conclusions

The approach used in this study is a generic and a simple one which could ideally be applied for any protein against which the repurposed drugs are aimed to be targeted. The basic principle of our analysis with respect to workflow-I lies in understanding the 'neighbourhood behaviour' in the protein 3D structural space which is dependent on the protein evolutionary relationships. Similarly, the fundamental science of workflow-II involves understanding the 'neighbourhood behaviour' in the 2D chemical space of bioactive compounds. Such an understanding coupled with a rational implementation of *in silico* techniques helped us to identify two sets of drug/drug-candidates from two independent workflows. From workflow-I, we have prioritized 17 drug/drug-candidates which might have the potential to bind to SARS-CoV-2 M^{pro} and we advocate for their rigorous experimental testing. From workflow-II, 12 representative molecules have been suggested. Out of these 29 (= 17 + 12) molecules, 20 molecules are approved by the FDA for treating one or multiple therapeutic indications. Two of these 20 are not truly FDA approved molecules: one of these two, asunaprevir, has been withdrawn from the market post approval due to commercial reasons and another, nafamostat, is not yet approved by the USFDA but is approved in Asian countries and widely prescribed in Japan indicating the availability of safety data.[117] Many of our predicted drugs are known antiviral drug/drug-candidates (like remdesivir, ribavirin, simeprevir, beclabuvir *etc.*, Table S5, ESI†). Some of the many other notable hits include anticoagulants (nafamostat, edoxaban, gabexate *etc.*), anti-cancer drugs (foretinib, vinca alkaloids, camptothecins *etc.*), anti-bacterial agents (belonging to the class of tetracyclines, lactam and macrolactams, and peptidomimetics), and anti-inflammatory agents (like ibuprofen and freselestat). The results discussed in this report are solely from *in silico* approaches

involving minimal computational resources and demand validation in experimental and clinical set ups. Given the necessity to meet the global crisis of combating COVID-19, we believe these findings could serve as an initial set of reasonable hits to the experimentalists to start out with testing. In accordance with our vision, we are providing access to the ESI† (Tables S2–S5) containing the entire list of molecules analysed by us with details relevant to medicinal chemists and biochemists that might be helpful in procuring these compounds from established vendors and designing the assay protocols. The SMILES code[118] of all the compounds are provided along with a list (or link to the list) of all known targets of each compound. Furthermore, the drugs/drug candidates which are known to inhibit/induce cytochrome P450 enzymes and hence are likely to influence drug-drug interactions are also indicated in the compiled data so that appropriate measures could be incorporated in the experimental and/or clinical study protocols. Additionally, any special observation noted while analysing the details of each drug as provided in DrugBank, has also been documented. For *e.g.*, we have indicated if any molecule is known to be a prodrug. Prodrugs (irinotecan, baloxavir marboxil, dabigatran etexilate *etc.*) are most often available in

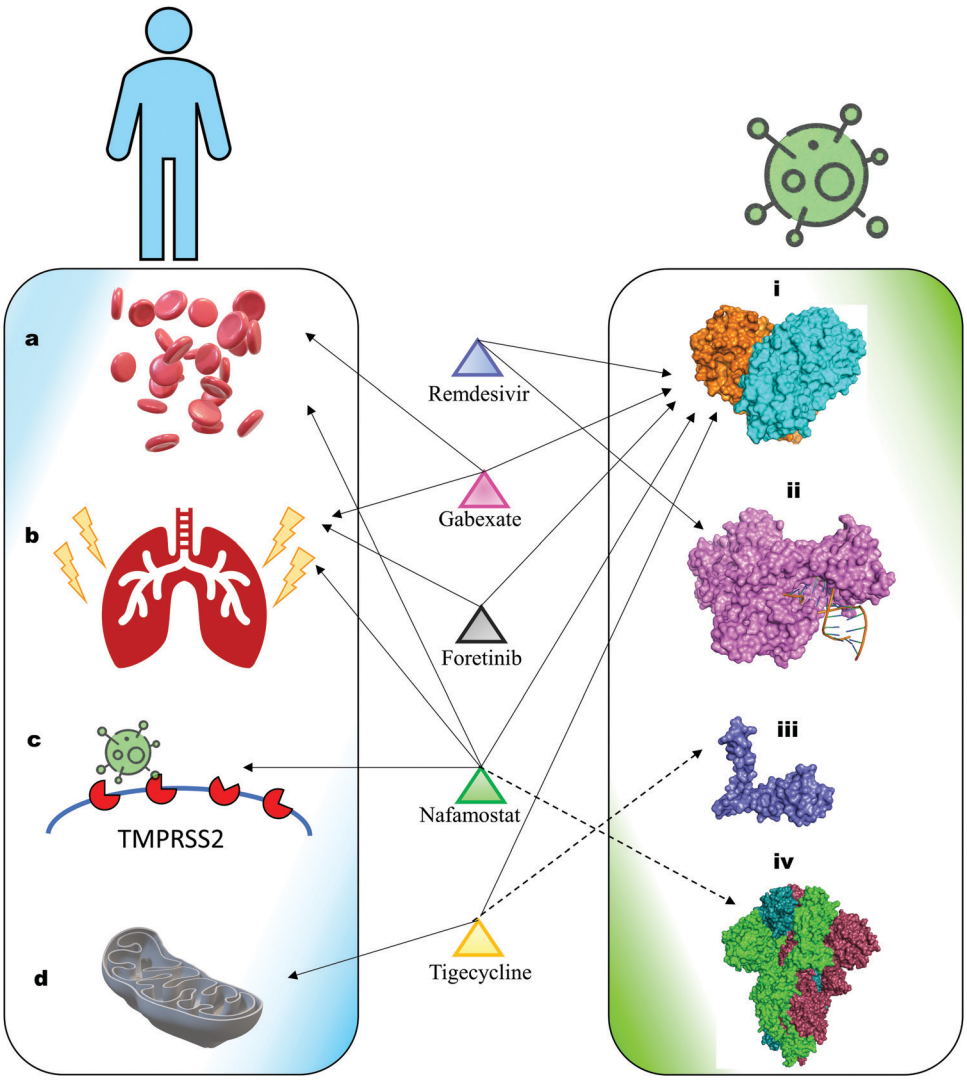

Fig. 5 Pictorial representation of the proposed multiple mode of viral inhibition by representative hits. The figure indicates the possible mechanisms of action of representative hits from our analysis which would aid in the management of SARS-CoV-2 infection. The right vertical panel shows the various host response systems which are known to be targeted by the respective drugs in the middle column. These host physiological pathways are involved in SARS-CoV-2 infection: (a) blood coagulation regulation, (b) inflammatory response (mediated by cytokines especially in the lungs), (c) TMPRSS2 mediated viral entry into the host cell, and (d) interaction with human mitochondria (involving the mitochondrial ribosome components MRPS5, MRPS27, MRPS2, and MRPS25). The left vertical column contains the experimental structure of various SARS-CoV-2 proteins which are either proven (in case of remdesivir's target RdRp) or indicated targets of the drugs picked up in our analysis. The targets are (i) M^{pro} (PDB code: 6LU7), (ii) RdRp (obtained from PDB code: 7BV2, chain A bound to template primer RNA in orange), (iii) Nsp8 (obtained from PDB code: 7BV2, chain B), and (iv) Spike glycoprotein (PDB code: 6VXX). While the solid arrows indicate the possibilities of direct modulation of the functions of the viral targets/host response system, the arrows with broken lines indicate the possibilities of indirect interferences. Details regarding each mechanism have been discussed in the text.

their inactive form. However, under physiological conditions, the prodrugs undergo metabolic changes to form their active metabolites that elucidate the desired pharmacological response.[119] Therefore, while setting out to test these drugs *in vitro*, the design of the experimental protocol should ensure the conversion of the prodrug to its active form.

Some of the drugs like foretinib, mitomycin, gabexate, freselestat *etc.* (Table S5, ESI†) have not been identified in earlier studies as potential binders of SARS-CoV-2 M^{pro} and are suggested for the first time based on our work. The strength of our predictions advocates for trying out and testing these molecules to understand their potential in anti-COVID-19 therapy. It is exciting to note that many of our predicted molecules are reported to possess anti-viral properties and some of these are already enrolled for clinical trials to study their benefits in the treatment of COVID-19 as discussed already. 12 molecules shortlisted from workflow-I (Table S2b, ESI†) and several molecules from workflow-II belonging to the class of alkaloids, tetracyclines and peptidomimetics (in addition to some approved/investigational anti-viral drugs) hold direct/indirect evidence of anti-viral properties. Although such molecules are already known to possess anti-viral properties, the mechanism of action of none of these drugs as available from published literature indicates that SARS-CoV-2 M^{pro} could be a target of the concerned drugs. However, in some cases, the possibility of multiple modes of action (such as, remdesivir[65,66]) has been indicated and the need for further investigation to unravel detailed mechanisms has also been realized by the research community. Thus, besides suggesting a handful of drugs which could possibly target SARS-CoV-2 M^{pro}, our analysis also opens up a new avenue of research to probe if SARS-CoV-2 M^{pro} could be one of the targets of those drugs which are already known to be effective in corornavirus infections. To the best of our knowledge, this is perhaps one of the few reports on computational drug repurposing against SARS-CoV-2 which deals with an extensive literature survey to provide insights into the possible mechanisms of action of the short-listed candidates with hints on drugs which are likely to demonstrate dual mode of action by modulating the host response and functions of viral proteins (Fig. 5). It is to be noted that the drugs which elicit their antiviral property by modulating the host response system might be helpful in tackling the problem of resistance. Such a strategy has already been demonstrated to be beneficial.[120,121] Our study also opens the possibilities to probe into the benefits of synergism for developing a strategic therapeutic regimen. A recent computational study has shown that the binding energy of a combination of three antiviral drugs: lopinavir, oseltamivir and ritonavir against SARS-CoV-2 M^{pro} is stronger than that of each drug docked against the said protein individually.[122] Based on such reports and our own findings, we suggest that a cocktail of drugs which target SARS-CoV-2 M^{pro}, besides eliciting their already known pharmacological functions should be exploited as potential drug combinations to treat COVID-19. Such cocktail of drugs may involve antiviral drugs, host-response modulating agents (anti-coagulants, anti-inflammatory agents *etc.*) and antibiotics (to prevent secondary infections) which are indicated in our study.

Lastly, as any drug repurposing approach is based on the principle of polypharmacology, the effects of the predicted drugs on undesired targets need to be monitored with utmost importance.[4,123] The results of our DALI search and ProbiS search (Table S1, ESI†) suggest some of the possible host off-targets for the molecules that can target SARS-CoV-2 M^{pro}. These findings provide clues in designing the toxicity profiling studies. Overall, our predictions show some promising results which could contribute toward meeting the global challenge of the COVID-19 pandemic and urge experimental validations.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Research in our group is generally supported by the Mathematical Biology program and FIST program sponsored by the Department of Science and Technology (DST) and the Department of Biotechnology (DBT), Government of India in the form of IISc-DBT partnership programme. Support from UGC, India -- Centre for Advanced Studies and Ministry of Human Resource Development, India is gratefully acknowledged. SC acknowledges the financial support by DST-INSPIRE towards her research fellowship. SB acknowledges the financial support by CSIR, Government of India towards her research fellowship. NS is a J. C. Bose National Fellow. The authors are thankful to Ms Yazhini Arangasamy for providing remote technical assistance. All the group members, Dr. TJ Narwani and Prof. R. Sowdhamini are acknowledged for useful discussions. The authors are also grateful to Mr. Koustav Purkait for assistance in data compilation and proof reading.

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