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REVIEW

Computational repurposing of drugs for viral diseases and current and future pandemics

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Abstract

A large fraction of the world's population is directly impacted by acute or chronic viral infections, many of which have high mortality. As was brought home to us in 2020, viruses also have great potential to generate global pandemics that have killed millions and caused massive damage to economies. Clearly, we need cost-effective and rapid methods for finding drug treatments for poorly met infectious diseases and for responding effectively to the current and future pandemics. Repurposing or off-label use of existing drugs, whose safety and pharmacokinetics are well understood, is one useful way to provide fast drug therapies for patients. Computational methods have an important role to play because of their increasing effectiveness, high speed, and relatively low cost. Here we review the application of the main types of computational drug repurposing methods to discovery of therapies for viral diseases and for future pandemics highly likely to be caused by viral pathogens.

Extended author information available on the last page of the article

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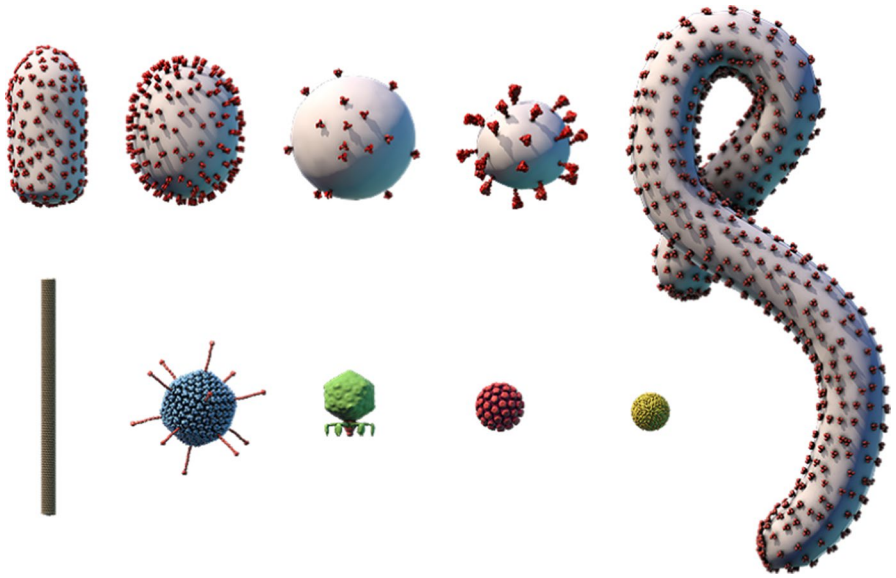
Graphical abstract



Keywords Drug repurposing · Viral diseases · Pandemics · Network models · Machine learning · Molecular dynamics

1 Introduction

The pandemic potential of viruses has been brought home, forcefully, starkly, and tragically in the first few years of the 2020s. Given that the current pandemic has generated 800 million cases, at least 7 million deaths, and massive social and economic disruption across the world, we ignore the potential danger of viruses at our peril. It must be remembered too that the 20 WHO-defined neglected tropical diseases (some of which are viral) affect nearly 2 billion people, including 0.5 billion children, and cause ~ 200,000 deaths per year, while TB and malaria kill 1.6 M and 600,000 respectively. They also cause considerable morbidity in hundreds of millions of people, largely in developing tropical countries in Africa, South America, and Asia. Climate change is already altering the spread of viral vectors such mosquitoes so we are likely see in increase in the range of viral, bacterial, and parasitic diseases in the coming years.

On the positive side, the coronavirus pandemic has shown how scientists can collaborate very quickly and effectively when emergencies arise. This has seen spectacular developments in vaccine generation and the roll out of fast-tracked, structure-based design of drugs targeting key proteins in pathogens, and a reawakening of the potential of drug repurposing.

Sadly, infectious diseases that affect people in developing countries attract little donor funding, because those diseases are very uncommon in wealthy, largely non-tropical countries. The large risk and high amortized cost of bringing a drug to market (~US$2 Bn) must be recovered by pharmaceutical companies through the sale price of the drug. Although the market is very large for NTDs, the ability of individuals or governments to pay for drugs is a major barrier to their development and sale.

Drugs rarely act at single molecular targets so exhibit polypharmacy, that is, hit multiple targets in the body. Although off-target effects can produce undesirable side effects, there can also be beneficial synergy between targets for specific illnesses. This have provided blockbuster drugs, e.g., Viagra for erectile dysfunction and Minoxidil for male pattern baldness [1]. How do we screen such compounds rapidly and efficiently for activity against new pandemic pathogens such as SARS-CoV-2? While selectivity for specific molecular targets can be 'designed in', this is never absolute. Thus, marketed drugs are sometimes used 'off-label' for treating other conditions. While understanding specificity and promiscuity of drugs is important for registering a drug for a specific application, it can also be very useful when poorly met medical needs can be addressed by the off-target properties of these drugs (Fig. 1). Deliberate investigation of the spectrum of drug activity has been undertaken only sporadically until recently. The SARS-CoV-2 pandemic saw

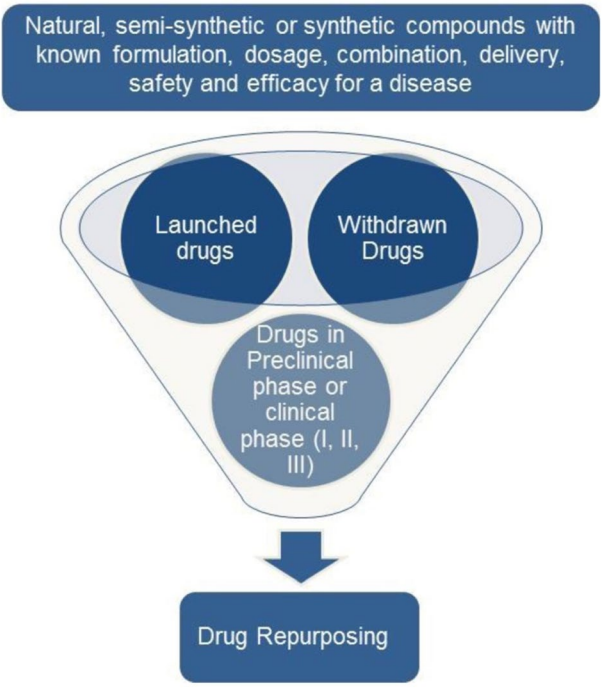


Fig. 1 The flow chart for drug repurposing. Marketed drugs or safe preclinical and clinical drugs are repurposed for treating a new disease. Used with permission from Kandwal and Fayne [2].

a massive increase in research on high throughput in vitro and in silico screens for identifying putative repurposed drugs for treating COVID-19 patients.

Drug purposing is a very attractive option because most of the cost of bringing useful drugs to the clinic has already been borne when the drug was first registered for its primary application. Thus, high throughput screening (in vitro or in silico) has the potential to identify candidates rapidly and cheaply for subsequent animal and human trials against viral infections for which few or no therapeutic options currently exist.

2 Computational methods used to repurpose drugs

Several diverse computational methods have been used to identify potential repurposing candidates using network theory, structure-based modelling, or ligand based methods.

2.1 Network models

Drug-target, target–target (protein–protein), and drug-target-drug networks describe the relationships between known (small molecule) drugs and their protein targets. Virtually all drugs exhibit polypharmacy (interact with more than one target), a phenomenon that underpins drug side effects and the repurposing of drugs for new medical indications. The interactions between the drugs and their targets, and between the targets and other targets can be represented by a network in which the drugs and targets are vertices and the connections are links or edges (Fig. 2) [3].

Network-based methods do not need 3D structures of targets and are derived from recommendation and link prediction algorithms in complex networks [4]. The degree or probability of the interactions (links or edges in network) can be encoded by assigning weights to the connections using algorithms such as Bayesian belief nets [5].

2.2 Machine learning

Machine learning (ML), a subset of artificial intelligence methods, has attracted enormous attention in the past decade due to the algorithms' very impressive performance in a wide range of medical, technical, and commercial spheres. Unlike hard coded expert systems and related algorithms, ML can learn patterns in very large, diverse, and complex data sets without recoding, and use these to make impressively accurate predictions. The most important issues affecting the accuracy and generalizability of ML models are the quality, quantity and diversity of training data, the quality of the features or descriptors used to encode molecules (mathematical entities that capture important molecular properties), and whether the relationship between the features and biological activity is linear or nonlinear (Fig. 3). Readers are encouraged to access recent reviews on the application of ML methods to

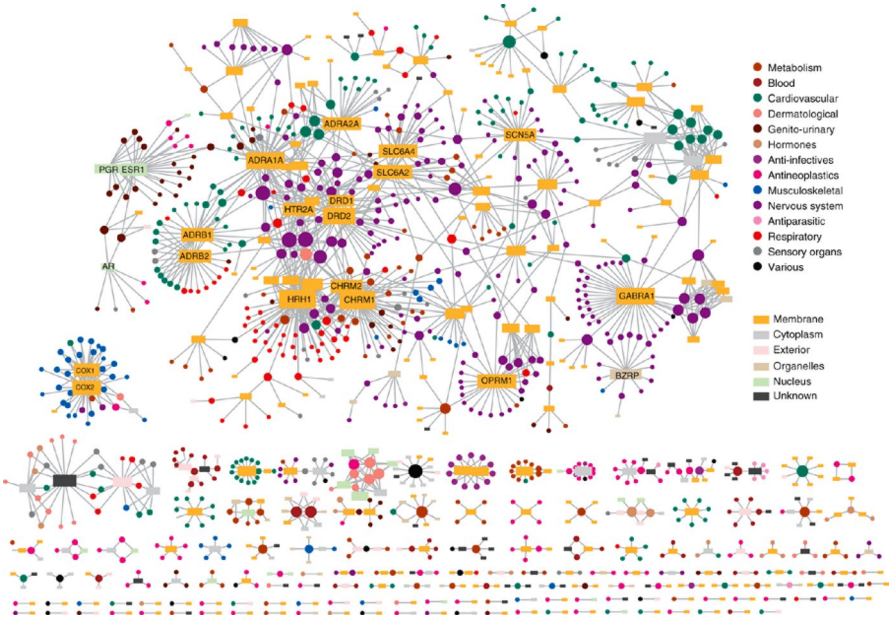


Fig. 2 A drug-target network representing interactions between FDA-approved drugs and their target proteins. Circles represent drugs and rectangles represent target proteins, their sizes denoting the number of targets for a drug (or number of drugs targeting the protein). Links denote whether a protein is a known target of a specific drug. Target proteins are colored by their cellular component and drugs by their Anatomical Therapeutic Chemical Classification. Used with permission from Yildirim et al. [3]

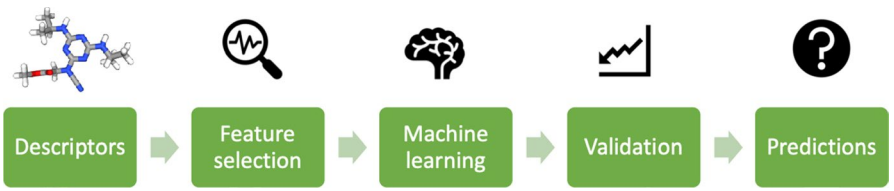


Fig. 3 Summary of operations for generating machine learning models of drug responses

discovery of infectious diseases for more details on their implementation, advantages, and disadvantages [6, 7].

2.3 Pharmacophores

Pharmacophores are regions in 3D space that define key functional group interactions in small organic ligands. They can be derived from real binding sites in protein pockets or, more commonly, from structure-activity relationship studies

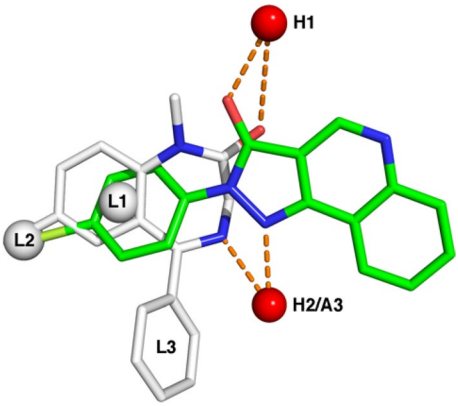


Fig. 4 Schematic of a pharmacophore model. White sticks represent carbon atoms of one drug, green represents carbon atoms of a related drug, red and blue sticks are oxygen and nitrogen atoms, red spheres labelled H1 and H2/A3 are hydrogen bond donating and accepting sites in the receptor respectively, and L1, L2, and L3 are lipophilic binding sites (Color figure online)

of ligands with measured activity (labels) against a molecular target. These interactions are of the general types: hydrogen bond donor; hydrogen bond acceptor; positive or negative formal or partial charge; hydrophobe (fat loving region); aromatic ring; or salt bridge (Fig. 4).

3D pharmacophores are derived from superimpositions of low energy conformation of a set of labelled molecules. Once a consistent pharmacophore model has been generated, it can be used to screen a large library of 3D structures of candidate molecules to identify those that best conform to the pharmacophore model. Common pharmacophore modelling tools are PharmDock, ZINCPharmer, and Pharmit. Recent reviews on pharmacophore modelling and other computational techniques for drug repurposing methods have been published by Sarvagalla et al. [8].

2.4 Docking

Molecular docking uses the 3D coordinates of a validated protein target to model the interactions of small drug-like organic molecules or drugs with putative binding sites Fig. 5. These interactions can modulate the function of these protein targets by, for example, inhibiting a key enzyme in the virus proteome. Protein structures can be obtained by structural biology, homology modelling, or by using very effective machine learning method exemplified by AlphaFold. Most docking algorithms define a grid around the protein target and calculate the energy of interaction of the target for the small molecule at each grid point. Favourable binding sites are those with the largest (negative) binding energy. Molecular mechanics-based energies are used to determine the most likely binding modes (poses) for each organic ligand. Generally, a separate scoring function is used to estimate the quality of the binding mode. Common docking algorithms are AutoDock, FETCH, Glide, Dock, and MOE. A recent paper critically reviewed the use of docking algorithms to discovery new inhibitors for pandemic viruses and for repurposing existing drugs, clinical trials candidates, and approved natural products [9].

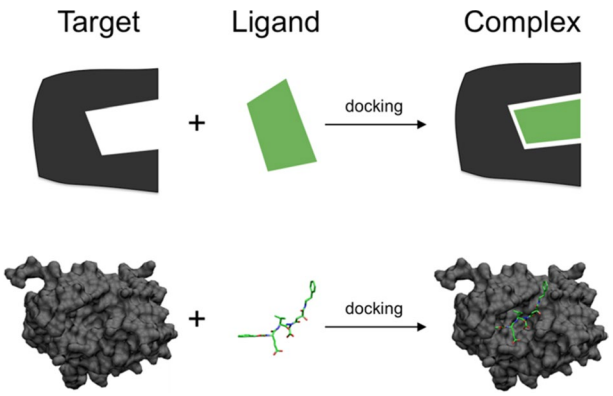


Fig. 5 Fundamentals of docking of a drug-like ligand to a protein target. CC-BY 4.0 from Wikipedia

2.5 Molecular dynamics

Molecular dynamics (MD) uses largely empirical force fields to describe the dynamic interactions of ligands with protein targets or between proteins and other proteins. Clearly, molecules and proteins are dynamic species that constantly change shape under the influence of the thermal bath in which they exist. MD calculations are complex and resource-intensive but, with the dramatic advances in computational resources, have now reached a stage where they can provide accurate and mechanistically useful information. MD calculations can provide information on stability of ligands in binding sites, on and off rates, effects of solvents on binding, and better estimates of binding free energies of ligands such as drugs. Common MD programs are GROMACS, Amber, and NAMD. The use of MD for drug repurposing has been reviewed recently by Sohraby et al. [10].

3 Review of computational repurposing studies for viruses

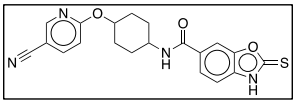
Computational methods have been used to investigate most families of viruses. Lack of suitable training drugs or protein coordinates for structure-based design has prevented these potentially valuable methods being applied to viruses with major impact on health now, or in the future.

3.1 Arboviruses—dengue fever, Japanese encephalitis, rift valley, Ross River, Murray Valley encephalitis, chikungunya, West Nile, and zika viruses

With almost half of the world population living at risk, tropical infectious diseases cause millions of deaths every year in developing countries. Lack of investment in this field means that as efficient structure-based drug discovery (SBDD), fragment screening, target-based drug discovery, and drug repurposing are of particular

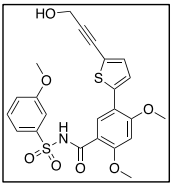
interest. As nearly all medicines impact more than one target, drug repurposing offers significant advantages in terms of speed to the clinic, as any candidates identified have already been through clinical trials and their toxicity and ADME properties are well understood. Drug repurposing is clearly an attractive option for vector-borne diseases that can quickly emerge or re-emerge worldwide to epidemic or pandemic levels. Mullins reviewed advances in in silico approaches to the challenge of drug repurposing, focusing particularly on development of generic platform technologies of broad value to researchers [11]. Recent advances in molecular docking methodologies and validation approaches, and their combination with machine learning or deep learning approaches are continually enhancing the precision of repurposing efforts. Elucidation of molecular mechanisms with molecular pathway data and knowledge of disease networks is enhancing discovery of repurposed drugs. AI is also being exploited to advance progress, although there are still challenges to be overcome in the successful integration of the new advances in useful platforms. De Godoy et al. reviewed the use of SBDD to targeting dengue, yellow fever, zika, and chikungunya enzymes in the RNA replication complex (RC) [12]. They highlighted successful examples of promising inhibitors and molecules already in preclinical/clinical phase trials.

Dengue virus (DENV) is a particularly important health concern with global warming extending the range of many tropical diseases. Currently, there is only one licensed vaccine against Dengue, Dengvaxia® but it is not suitable for children. Effective treatment of DENV is not possible due to lack of specific drugs.



1 ZINC92615064

The NS2B/NS3 protease is a key target for DENV rational drug design. Drug repurposing studies have previously identified bromocriptine as a potent anti-DENV compound. Fathima et al. used bromocriptine in a pharmacophore feature-based virtual screening campaign [13] using the ZINCPharmer pharmacophore online server and Argus Lab v4.0.1. Of 40,000 molecules similar to bromocriptine screened against the x-ray structure of the NS2B/NS3 protease complex using a pharmacophore model followed by docking with Autodock4.0, ZINC92615064 (**1**, N-(4-((5-cyanopyridin-2-yl)oxy)cyclohexyl)-2-thioxo-2,3-dihydrobenzo[d]oxazole-6-carboxamide) was identified as the best lead. It was predicted to bind substantially more strongly than bromocriptine. Molecular dynamics simulations of NS2B/NS3 protease in complex with bromocriptine and ZINC92615064 elucidated their modes of action.



2 novel biphenyl acetic acid

Yokokawa and co-workers reported the discovery of non-nucleoside dengue viral RNA-dependent-RNA polymerase (RdRp) inhibitors [14]. They used a structure-based fragment screen against the palm subdomain of RdRp to identify a promising and novel biphenyl acetic acid moiety (**2**). Subsequent medicinal chemistry optimization, guided by the target structure, generated a> 1000-fold improvement in potency in vitro. The optimized lead also exhibited low micromolar EC_{50} activity against all four dengue serotypes in cell-based assays.

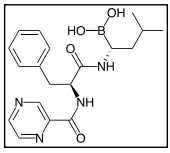
Direct-acting antivirals (DAAs) have been shown to be an effective approach. Non-structural protein 5 (NS5) is a highly conserved protein among Flaviviridae with an RNA-dependent RNA polymerase (DENV RdRp) domain at its C-terminal. An allosteric site has been targeted for anti-DENV drug development. Kumar et al. developed a pharmacophore model from 41 known inhibitors of the DENV RdRp domain and used it to screen the FDA approved drug database to identify potential drug repurposing candidates against DENV RdRp [15]. Screening hits were subsequently used in docking calculations and their RdRp binding poses were refined by MD simulations. Four potential drugs were identified: desmopressin; rutin; lypressin; and lanreotide. These drugs bound to the allosteric N-pocket of DENV RdRp but need to be validated by experimental assays.

Nascimento et al. reviewed studies of the NS5 RdRp, as this promising target has no human homolog [16]. While several NS5 RdRp inhibitors have been reported, none are currently in clinical trials. They reviewed RdRp inhibitors from natural, synthetic, and drug repurposing sources. summarizing structure-activity relationships (SAR), and proposed mechanisms of action. Natural products such as flavonoids, alkaloids, acetylenic acids, terpenes, and steroidal prohormones were all found to be potential RdRp inhibitors in in vitro assays. The review summarized computational studies identifying potential DENV agents from compound libraries, only one study cited screened FDA-approved drugs to identify a suitable repurposing candidate, the zinc salt of 10-undecenoic acid.

Naresh et al. also aimed to repurpose existing antiviral drugs for dengue disease [17]. They employed molecular docking to the dengue virus type 2 envelope protein (DENVE) to identify existing drugs that interact with the fusion process. To infect host cells, the DENVE protein must interact with host cell receptors, triggering receptor-mediated endocytosis. The relative binding modes and the affinities of all the selected drugs were predicted and compared with co-crystallized n-octyl-beta-D-glucopyranoside (beta OG) by MD simulations using CABS-flex-2.0. Two drugs, daclatasvir and famciclovir were identified as promising leads for combating

DENVE and exhibited good activity against surrogate viruses HSV-1 and HSV-2 with acceptable toxicity and showed useful larvicidal activity against mosquitoes. These leads need experimental in vitro and DENV animal model validation.

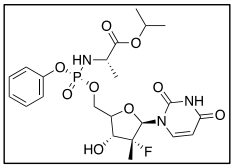
West Nile, dengue, yellow fever, and zika constitute a major global human and economic burden. No approved drugs are available for these viruses, stimulating efforts to identify of small molecules to fulfil that role. Again, RNA-dependent RNA polymerase (RdRp) is an essential enzyme for replication and transcription of the genome of flaviviruses and all RNA viruses. Indeed, the RdRps of the flaviviruses are all similar e.g., Japanese encephalitis and West Nile enzymes have 70% identity and dengue serotypes 2 and 3 have 76% and 81% identity with zika respectively. Murali et al. used the published X-ray crystal structures of Japanese encephalitis, west Nile, zika, and dengue RdRps to elucidate their interactions of repurposed drug molecules from the FDA-approved library of 1458 compounds using computational modelling [18]. They performed flexible docking using Glide and subjected the most promising hits to molecular dynamics simulations using the OPLS force field. The study identified four drugs, the bisphosfonates risedronic acid and tiludronic acid, the prostaglandins dinoprost tromethamine and epoprostenol as putative inhibitors of the viral protein in several viruses. They also proposed a possible mechanism of inhibition of the identified common small molecule toward RdRp of flavivirus. No experimental validation of these putative repurposed drugs was reported.



3 bortezomib

Japanese encephalitis is mainly epidemic in Asia where it affects 70,000 people annually. With climate change the range of the mosquito is increasing and isolated cases now occur in countries such as Australia. There are currently no approved drugs for JEV infection, and existing vaccines do not control all the strains. Lv et al. reported a study based on gene expression data of JEV infection and phenome-wide association study (PheWAS) data [19]. They used the HotNet2 (a topology-based method for finding significant subnetworks associated with disease) and GeneRank (gene ranking by the PageRank algorithm) methods to identify genes essential for JEV infection. They identified 286 genes implicated in the progress of JEV infection, with enrichment analysis suggesting these genes were largely related to viral infection and immune response. They validated the hit genes using the Gene Ontology and KEGG pathway enrichment databases to identify a drug that may be effective against JEV infection. Bortezomib was found to play a key role in the progress of JEV infection, so they investigated its effect using a JEV-infected mouse model. Bortezomib (**3**) reduced lethality in mice, alleviated suffering, and diminished brain damage caused by JEV infection.

Zika virus (ZIKV) is a mosquito-borne flavivirus for which there are no vaccines or effective drugs. The World Health Organization declared Zika a Public Health Emergency of International Concern in 2016. Over 1 million cases of ZIKV infection were reported in Brazil in 2015 and it rapidly spread to the Americas. As of February 2022, 89 countries have reported ZIKV infections (https://www.who.int/publications/m/item/countries-and-territories-with-current-or-previous-zika-virus-transmission). Although the symptoms of Zika fever are mild, infection can cause severe neurological disorders, principally microcephaly in newborns. Re-emerging viruses like ZIKV are a significant ongoing threat because of very easy transmission by air travel and possible mosquito range increases due to climate change. Experience over the past few years suggests that neurological complications of ZIKV present a long-term world-wide health challenge. For example, the incidence of adult Guillain-Barre syndrome has increased after Zika virus infection.



4 sofosbuvir

Gorshkov and co-workers reviewed the origin, pathology and treatment of ZIKV and reported progress toward design, re-purposing, and testing of candidates for prophylaxis and therapy (Fig. 6) [20]. Conspicuously, they state that brief, intensive, drug-repurposing work has identified several FDA-approved drugs that effectively inhibit the virus in infected adult mouse models. Importantly, they prevent maternal-foetal transmission and severe microcephaly in newborn mice. For example, sofosbuvir (**4**) and chloroquine prevented acute neurological effects and, as mother-to-child ZIKV transmission results in microcephaly, they prevented the vertical transmission of ZIKV in pregnant mice.

Mottin et al. have summarized current computational drug discovery research on anti-ZIKV drug discovery and exemplified successes of these methods [21]. They stated that computational drug repurposing "represents an opportunity that has been underutilized for ZIKV", as a number of existing drugs have been identified using in vitro ZIKV assays. However, general computational screening of very large chemical databases using structure-based and ligand-based methods is well in hand. The IBM World Community Grid (WCG) is collaborating with US and Brazilian universities on OpenZika project [22] (http://openzika.ufg.br). The project virtually screens millions of compounds against all the ZIKV protein structures and by ligand based QSAR modelling [22]. Docking calculations have involved the ZIKV proteins NS1, NS2B-NS3 protease, NS3 helicase, and NS5 polymerase. By 2020, almost 10 billion docking results have been obtained and many top hits virtually selected are undergoing experimental validation. As this work has not focused on repurposing of drugs specifically, most validated hits will still need to go through the full drug

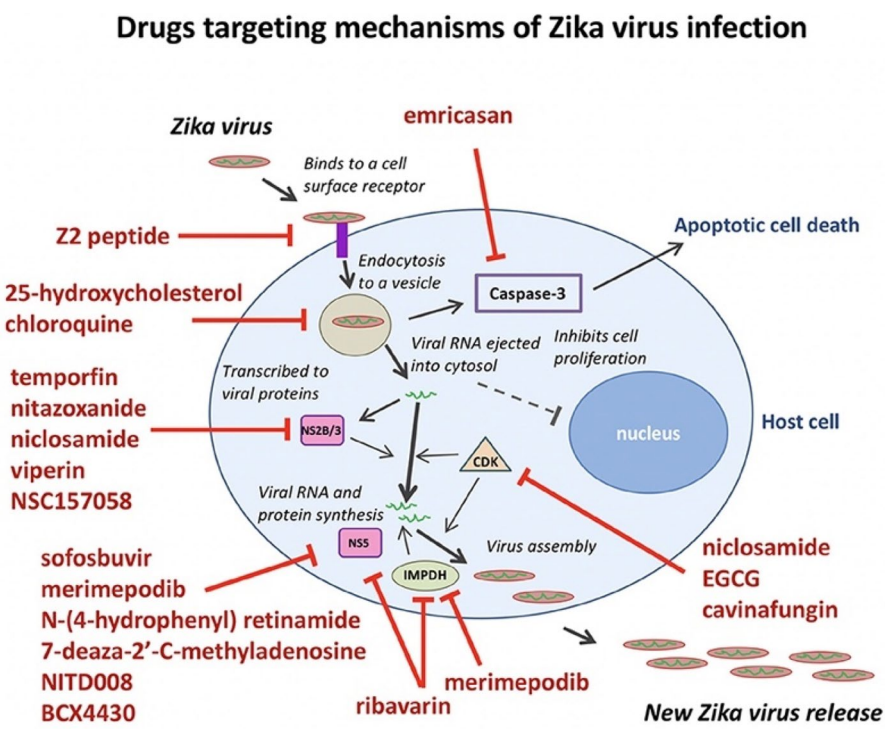
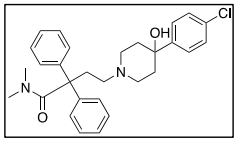


Fig. 6 Mechanisms of drugs targeting ZIKV. Creative Commons Attribution License (CC BY) from Gorshkov et al. [20]

development pipeline before registration. Devillers reviewed in vitro drug repurposing research for ZIKV infections, and highlighted a dozen drugs that showed promising activity as ZIKV therapies [23].

Using experimental data, Rajput et al. identified repurposed drugs against 14 viruses responsible for causing epidemics and pandemics including Zika [24]. They used a novel computational approach to identify targets for specific drugs, which were then experimentally validated in vitro or in vivo for useful antiviral activity. The targets were then used to identify novel FDA-approved drugs for each virus and prioritize them by calculating their confidence scores. Their *drug-target-drug* approach identified anti-cancer, antiviral, and immunosuppressant drugs as candidates for treating ZIKV infections. Notably, the anti-cancer drugs alvocidib, sorafenib, regorafenib, and lenvatinib were predicted to be putative therapies for ZIKV infection.



5 loperamide

Odhar et al. employed virtual screening of approved drugs against Zika virus NS2B/NS3 protease to identify new hits blocking viral replication and immune system evasion [25]. They screened 1615 FDA approved drugs using both molecular docking and molecular dynamics simulations. They identified the anti-muscarinic agent darifenacin and the anti-diarrheal agent loperamide (**5**) as potential inhibitors of Zika virus NS2B/NS3 protease. Both drugs remained stably bound to protease active site during simulation period. Experimental evaluation of these drugs against Zika virus NS2B/NS3 protease is required to confirm the computational predictions.

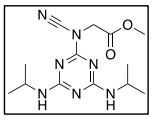
Bassetto et al. reported a structure-based design study using chikungunya NSP2 protease as a target for the discovery and development of selective inhibitors of CHIKV replication [26]. The NSP2 cysteine-protease activity plays a critical role in cleavage of the non-structural polyprotein precursor into the four mature NSPs. Their virtual screening campaign of a library of commercially available compounds used an optimised homology model of the CHIKV NSP2 protease and a pharmacophore model. This was followed by docking of the best candidates from the screen, and yielded several promising drug candidates that inhibited chikungunya virus-induced CPE formation in Vero cells at low μM concentrations. Although the molecules screened were not registered drugs, the study provided a useful proof-of-concept for the structure-based approach.

3.2 Filoviridae—Ebola, Marburg

Ebola Virus (EBOV) is extremely virulent, with a mortality rate up to 90%. Unfortunately, treatment is limited to quarantine and supportive care. It is mainly transmitted by contact with blood, sweat, saliva, and tears from infected wild animals. Recent EBOV outbreaks infected ~ 28,000 people, mainly in Liberia, Guinea, and Sierra Leone, with a mortality rate of 40–70%. The 2014 Ebola epidemic in West Africa caused over 11,000 fatalities [27]. Despite these alarming levels, there is still no FDA-approved drug for the effective treatment of these diseases. The most advanced drug to treat EBOV is remdesivir. However, it is a high-cost drug and is available only for intravenous use. Better antiviral drugs are urgently needed. Vaccine development for deadly zoonotic Nipah (NiV), Hendra (HeV), and Ebola (EBOV) viruses has targeted individual viruses. However, their bat reservoir host and geographic overlaps suggests multivalent vaccines are needed. Ithinji and coworkers used replication-incompetent pseudotyped vesicular stomatitis virus (VSV) virions or NiV-based virus-like particles (VLPs) as candidate multivalent vaccine

platforms [28]. They incorporated surface glycoproteins from NiV, HeV, and EBOV onto these virions and enhanced vaccine thermotolerance for use in regions lacking reliable refrigeration. This vaccine elicited safe, strong, and protective neutralizing antibody responses against NiV, HeV, or EBOV in a Syrian hamster model. This was the first proof-of-principle that replication-incompetent multivalent viral particle vaccines can protect against multiple deadly viruses with high pandemic potential. Clearly, multivalent drugs are also essential for treating patients already infected.

Nascimento and colleagues reviewed the major advances in computational drug discovery, drug repurposing, phenotypic screening assays, and classical medicinal chemistry techniques (bioisosterism, metabolism-based drug design, natural products) that can be applied to treat EBOV [29]. Ng and colleagues reviewed methods of drug repurposing for EBOV specifically [30]. Computational approaches such as machine learning and algorithms that model disease and drug interactions are discussed along with experimental approaches involving traditional wet-lab experiments. Schuler and co-workers also published a comprehensive systematic review of computational drug discovery, development, and repurposing methods for EBOV treatment prior to 2017 [31]. The review highlighted a variety of hypothesized and/or novel treatments as potential anti-Ebola activity. Approaches exploiting multiple targets or exhibiting polypharmacology were deemed to be the most promising for treating EVD.



6 Compound 5705213

Ebola and other viruses depend on cathepsin L, a lysosomal protease with many cellular functions, for entry into their target cells. Their viral glycoproteins must by cleavage before they can fuse with the host cell membrane. Elshabrawy and colleagues reported a high-throughput assay based on peptides containing the cathepsin L cleavage site derived from these viral glycoproteins [32]. A screen of 5000 small molecules (Chembridge Diverset Library) identified compound **6** that inhibited the cathepsin L cleavage of all viral peptides but not a host protein-derived peptide, pro-neuropeptide Y. This molecule inhibited the entry of all pseudotyped viruses in vitro. They further showed that the small molecule is a mixed inhibitor of cathepsin L with broad-spectrum antiviral lead activity. Although not a registered drug, is an ideal candidate for optimization and development into a potent antiviral against Ebola, and other deadly viruses.

Madrid and colleagues experimentally screened 21 FDA-approved drugs with broad-spectrum antiviral and/or antibacterial activities to identify those with in vitro activities against Ebola virus (EBOV) [33]. They did not use in silico screens to identify the candidates for EBOV in vitro testing. Compounds that showed some selective toxicity at a single concentration were tested in dose response assays in

Vero cells. Seven of these in vitro hits, chloroquine, amiodarone, prochlorperazine, benztropine, azithromycin, chlortetracycline, and clomiphene were tested for in vivo efficacy at a single dose administered intraperitoneally or orally. Although azithromycin and amiodarone initially demonstrated increases in survival in the mouse model, only chloroquine had confirmed efficacy. Conspicuously, no drug increased survival in a guinea pig model of EBOV infection at doses below the toxicity limit.

The antipsychotic chlorpromazine and the antidepressant fluvoxamine have been suggested as useful repurposed drugs against several viral diseases including Ebola. Golden et al. published a narrative review of psychotropic drugs in PubMed to the end of 2020 [34]. Of more than 100 agents, 37 were found to have activity against human pathogenic viruses. Some psychotropics drugs and drug classes had activity against multiple viruses, including Ebola, due to shared viral or cellular targets. However, translation of in vitro results to the clinical has been slow and these studies have not exploited computational drug repurposing methods yet.

Chopra et al. reported studies using the Computational Analysis of Novel Drug Opportunities (CANDO) platform to find putative EBOV drugs. CANDO is a suite of modelling packages such as CANDOCK (docking and accurate calculation of binding energies), and HHBLITS, ITASSER, and KoBaMIN (protein modelling, refinement, and dynamics), and COFACTOR (identification of ligand binding sites). The search was based on the idea that the multiple proteins that a given drug modulates creates a molecular interaction signature [27]. This can be used to repurpose drugs and natural products and discover new therapeutics. Docking and MD simulations ranked a library of 3733 FDA-approved drugs for their ability to inhibit proteins encoded by the genomes of five Ebola strains. The best repurposing candidates were compared to results of in vitro screening against Ebola virus-like particles and genetically engineered Ebola virus in cell viability studies to identify commonalities that highlight putative treatments for EVD. By integrating computational docking predictions with results from in vitro screening studies, the following compounds were prioritized for further in vivo and clinical testing—enfuvirtide, vancomycin, bleomycin, octreotide, lanreotide, somatostatin, ubidecarenone (CoQ10), and unoprostone.

Mohamed et al. recently reported a deep learning method LigDream (https://playmolecule.org/LigDream/), a shape decoding tool that decodes a voxelized molecule representation into SMILES strings, to identify novel and effective anti-EBOV inhibitors targeting VP24 [35]. This is the most important protein in EBOV because of its essential role in viral transcription, replication, and maturation of the nucleocapsid (Fig. 7) [9]. VP24 also binds karyopherin a that blocks accumulation of tyrosine phosphorylated STAT1 in the nucleus. Accordingly, VP24 protein was chosen as the drug target to be inhibited for Ebola therapy. Using galidesivir as a template, LigDream generated 100 close analogues were generated and analyzed using docking by AutoDock Vina. Molecular dynamics simulations of two lead molecules over 100 ns showed that both compounds bound more tightly to VP24 than galidesivir. This study exemplified the utility of deep learning for drug design relative to drug repurposing or database screening.

Muthaiyan et al. identified a lack of Ebola-Human-Drug interaction network models [36]. To address this deficiency, they compiled data for 270 human proteins

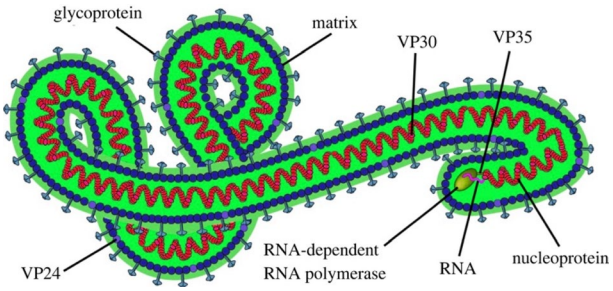
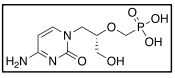


Fig. 7 Structure of EBV virion. CC BY 4.0 from Mohamed et al. 2022 [35]

interacting with EBOV from published experimental research. They used computational approaches to generate EBOV-human and EBOV-human-drugs interaction networks. This was achieved by Gene ontology (GO), pathway enrichment analysis using KEGG mapper, with the clustered network of genes being visualized using the Cytoscape. The results were used to find effective repurposed drug for EBOV treatment. They used 53 FDA-approved human-based drugs reported to identify genes targeted by these drugs that may be exploited to find other drugs that inhibit the Ebola entry. These drugs were microtubule inhibitors, estrogen receptor modulators, antihistamine and anticholinergic agents, pump/channel blockers. The established an integrated database, Ebolabase (http://ebola.bicpu.edu.in), that is linked to other repositories.

Rajput et al. also used drug-target network analysis to identify possible drug repurposing candidates for EBOV and other viruses with pandemic potential [24]. Using experimental data, they identified repurposed drugs against 14 viruses responsible for epidemics and pandemics—SARS-CoV-2, SARS, Middle East respiratory syndrome, influenza H1N1, Ebola, Zika, Nipah, chikungunya, among others. They developed a novel computational *drug-target-drug* method that uses drug targets for specific drugs that are validated in vitro or in vivo for antiviral activity. These drug-targets were used to identify FDA-approved drugs that may impact each virus and prioritize them by calculating using a confidence score. They found that most extracted targets involve cancer and signalling pathways. Prioritized drug candidates were validated using molecular docking. For EBOV they identified digoxin, diazoxide, bretylium, almitrine, and lenvatinib as potential drugs. For Marburg virus they identified chlorhexidine, citalopram, adalimumab, clemastine, and triprolidine as potential repurposed therapeutics.



7 cidofovir

Zhao et al. reported a study on identifying drug repurposing candidates from an integrated structural systems pharmacology pipeline combining proteome-scale

ligand binding site comparisons, protein-ligand docking, and MD simulations [37]. They screened 1766 FDA-approved drugs and 259 experimental drugs for their ability to inhibit the replication and virulence of Ebola and elucidated binding modes with EBOV molecular targets VP24 and the SAM-dependent 2′-O-methyltransferase (MTase) domain of RNA-directed RNA polymerase. A homology model of the MTase was constructed using Modeller v9.14. The docking studies employed Audodock4, Autodock Vina, PLANTS, and Surflex. The initial screens identified several interesting compounds such as the HIV protease inhibitor indinavir, an antifungal sinefungin, and several anti-viral drugs maraviroc, abacavir, telbivudine, and cidofovir (**7**). They postulated that these compounds inhibit Ebola RNA-directed RNA polymerase via the methyltransferase domain. These promising results from the in silico models and simulations suggest the hits would be strong candidates for in vitro and in vivo testing to evaluate the anti-Ebola activity of these drugs.

Veljkovic and colleagues proposed simple theoretical criterion for fast virtual screening of molecular libraries for candidate inhibitors of Ebola virus infection [38]. Their unusual approach used the average quasi valence number (AQVN) and the electron-ion interaction potential (EIIP) parameters that they state determine long-range interaction between biological molecules. For organic molecules, they are defined as:

$$EIIP = 0.25\ \frac{Z^{*}\sin(1.04\pi Z^{*})}{2\pi}$$

where Z* is the average quasi-valence number (AQVN),

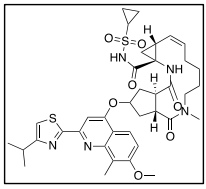
$$Z^{*} = \frac{1}{N}\sum\nolimits_{i=1}^{m} n_i Z_i$$

where Z_i is the valence number of the *i*-th atomic component, n_i is the number of atoms of the *i*-th component, *m* is the number of atomic components in the molecule, and N is the total number of atoms. The showed that 80% of known EBOV inhibitors lie in a similar space defined by these two metrics and used this to rapidly screen many potential repurposing candidates. Of 6438 drugs from DrugBank screened using this criterion they identified 267 approved and 382 experimental drugs as candidates for treatment of EVD. This included 15 antimalarial drugs and 32 antibiotics. An open-source Web server for screening chemical libraries for candidate anti-EBOV drugs was also established (http://www.biomedconsulting.info/ebola_screen.php).

3.3 Orthopoxviridae—smallpox and monkeypox (Mpox)

Smallpox is a devastating disease historically, being responsible for multiple large epidemics and many deaths, has now been eliminated by vaccination. However, monkeypox, a zoonotic disease whose primary hosts are rodents and non-human primates, has emerged as a disease of concern in the past few years. It is caused by a DNA virus (monkeypox virus (MPXV)), and is of increasing global concern, with a

2022 outbreak spreading to Europe during the COVID-19 pandemic. The new outbreak was due to novel mutations and variants. Currently, the only FDA approved poxvirus treatment is tecovirimat.



8 simeprevir

Surprisingly, there is limited research interest in monkeypox. Lam, Guan, and Mu used virtual screening (AutoDock Vina) and molecular dynamics simulations (Gromacs) to explore the repurposing of multiple drugs for poxviruses [39], exploiting several protein targets identified as promising in the literature. The structures of most protein targets were generated by the deep learning model, AlphaFold. They identified several drugs predicted to bind tightly to these viral protein targets critical for viral replication. These were NMCT and rutaecarpine for A48R, nilotinib for A50R, simeprevir for D13L, hypericin and naldemedine for F13L, and fosdagrocorat and lixivaptan for I7L. In particular, simeprevir (**8**) bound more strongly to the monkeypox D13L capsid protein than the in vitro active drug rifampin. D13L is a protein trimer complex that increases membrane rigidity and is important for viral particle morphogenesis. Inhibition of which has been reported to suppress viral replication.

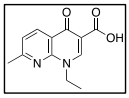
Mitoxantrone (MXN), an anthracycline derivative, an FDA-approved therapeutic for treating cancer and multiple sclerosis, was previously reported to exhibit antiviral activity against the vaccinia virus and monkeypox virus. Preet et al. used virtual screening, molecular docking, and ligand-based pharmacophore modelling of anthracene structures (1–13) closely related to MXN to identify potential repurposing of multiple compounds from the PubChem library [40]. Four chemical structures exhibited high predicted binding and may also suppress viral replication.

3.4 Orthomyxoviridae—influenza A, B, C

Although influenza is reasonably well controlled by vaccines, it clearly has large pandemic potential (1918 flu pandemic killed 50 million people). Additionally, treatment of those infected is limited, with only two neuraminidase inhibitor drugs available, oseltamivir (Tamiflu) and Zanamivir (Relenza), that can marginally shorten the duration of infections.

Many FDA-approved drugs have been repurposed to inhibit viruses, with several in clinical trials. As noted above, Rajput et al. identified repurposed drugs against 14 viruses responsible for causing epidemics and pandemics, including influenza H1N1 [24]. They used the drug-targets of specific drugs, experimentally validated in vitro or in vivo for antiviral activity. These targets were used to identify FDA-approved

drugs for each influenza and prioritize them. As noted above, pathway analysis showed most targets are in signalling pathways. For influenza A and B they identified 5 potential repurposed drugs—ceftriaxone, carfilzomib, nedocromil, paclitaxel, and zonisamide. No experimental validation of these drugs was undertaken.



9 nalidixic acid

The neuraminidase inhibitor, oseltamivir, is a common anti-influenza drug against which resistant H1N1 influenza viruses carrying the H275Y NA mutation have emerged. This and other potential mutations could generate a future pandemic influenza strain. Bao et al. reported a structure-based computational study that identified existing drugs that inhibit resistant viruses [41]. Two drugs, nalidixic acid (**9**) and dorzolamide, potently inhibited the neuraminidase activity of oseltamivir-resistant H1N1 viruses with the H275Y NA mutation. Interestingly, these drugs had no effect on the wild-type H1N1 enzyme at high concentrations, suggesting that the drugs specifically targeted the resistant mutation.

The high genetic variability of influenza A viruses complicates seasonal and pandemic vaccine development. Antiviral drugs are the first line of defence against antigenically different strains or new subtypes, although resistance against drugs targeting viral proteins emerges rapidly. Consequently, Enkirch et al. studied antiviral activity of existing, orally bioavailable drugs targeting cellular proteins required for virus replication [42]. Of 15 repurposed drugs, 4 inhibited in vitro infection 10- to 100-fold, without toxicity. Dextromethorphan and ketotifen exhibited ED_{50} values of 5 and 50 μM for the classic H1N1 PR8 strain, pandemic H1N1, and a seasonal H3N2 strain. In vivo experiments in mice showed that dextromethorphan reduced viral lung titres and was synergistic with oseltamivir. Paradoxically, dextromethorphan reduced clinical disease severity in ferrets infected a pandemic H1N1 strain but did not affect viral lung titres.

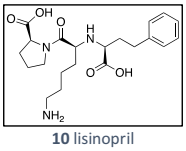
Simultaneous targeting of several immunomodulatory proteins is a potentially useful antiviral strategy that may lead to new indirect-acting pan-antiviral (IAPA) agents for treatment of viral respiratory illnesses. Kleandrova et al. reviewed the usefulness of computational multi-target drug discovery for the virtual screening of repurposed drugs as IAPA agents that the prime immune system by activating the toll-like receptor 7 (TLR7) and/or interferon genes (STING), while inhibiting inflammation-related proteins such as caspase-1 and tumor necrosis factor-alpha (TNF-alpha) [43].

Databases of system-wide phenotypic data of the host response to both drugs and pathogens, coupled with bioinformatics and computational methods, facilitate silico predictions of FDA-approved drugs as treatments against infection diseases [44]. These approaches can capture the complexity of the pathogen and drug host response as expression patterns or molecular interaction networks. For drug repurposing, they can identify new drug candidates against influenza and several parasitic

diseases. These methods can significantly reduce the time and cost for infectious diseases drug discovery.

The intrinsic ever-evolving nature of these viruses, the suboptimal efficacy of current influenza inactivated vaccines, and the emergence of resistance against the few available antiviral drugs provide an opportunity to novel therapeutics. Pizzorno and colleagues also reported an innovative strategy for drug repurposing targeting host factor rather than viral factors [45]. They used in vivo global transcriptomic signatures of infection from a patient cohort to generate a shortlist of existing drugs with novel, host-targeted inhibitory properties against influenza virus. They evaluated the antiviral potential of selected repurposing candidates in vitro, in vivo, and ex vivo. From a pool of 1309 FDA drugs, they identified a shortlist of 35 candidates using a computational screen, almost 90% of which had significant in vitro antiviral activity. Diltiazem, a calcium channel blocking antihypertensive drug, was a promising candidate for treating influenza infections. Transcriptomic signature analysis revealed that diltiazem modulated expression of specific host antiviral response and cholesterol metabolism genes. They also identified substantial synergy between diltiazem and oseltamivir, prompting rapid initiation of a Phase II clinical trial. These host-targeted drug repurposing strategies constitute a novel way of rapidly identifying new anti-infectious drugs for future epidemic or pandemic diseases that may slow the development of drug resistance.

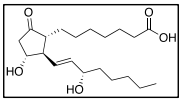
Radosevic et al. exploited a critical influenza A virus (IAV) matrix protein 2 (M2), an ion channel, to find repurposing candidates [46]. Although the existing influenza drugs, adamantanes, are M2 channel blockers, they have been discontinued because of drug resistance. These researchers used a in silico screening of drug space using the EIIP/AQVN filter followed by filtering of drugs by ligand based virtual screening and molecular docking. This allowed fast virtual screening of molecular libraries for candidate anti-influenza ion channel inhibitors for both wild type and adamantane-resistant influenza A viruses. Guanethidine, the best ranked drug from the virtual screen, showed significant anti-influenza activity in cell culture.)



10 lisinopril

Rohini and Shanthi exploited the surface glycoprotein of the influenza virus, neuraminidase (NA), to discover more potent repurposed drug inhibitors as novel influenza drugs [47]. NA plays a vital role in the release of new viral particles and spreads of infection in the respiratory tract. Although oseltamivir is used as a standard drug for the treatment of influenza, as mentioned, emergence of mutants with novel mutations has increased the resistance to it. In the present investigation, Rohini and Shanthi used computer-assisted combinatorial techniques to screen 8621 molecules from Drug Bank to find potent NA inhibitors. Initially, a 3D pharmacophore model was generated from 28 carbocylic influenza NA inhibitors plus

oseltamivir using PHASE module of Schrodinger. Subsequently, a 3D-QSAR model was built using the common pharmacophore. The model had an r^2 value of 0.99 and cross-validated q^2 value of 0.76. Finally, a three-stage docking process using the Glide algorithm was used to refine the best repurposing candidates. Their analyses identified lisinopril (**10**) to be a better binder to NA than currently approved drugs. In addition, it has the best match in binding geometry conformations with the existing NA inhibitor. Although the antiviral activity of lisinopril has been reported in the literature, this paper was the first report that lisinopril may be activity against influenza A infection.



11 alprostadil

Rohini and coworkers subsequently reported a drug repurposing strategy that integrates results from ligand-, energy-, receptor cavity-, and shape-based pharmacophore algorithms to identify new drugs for influenza [48]. This phased strategy initially selects repurposing candidates using pharmacophore hypotheses from the PHASE module of Schrodinger. The generated hypotheses for ligand-, e-pharmacophore, and receptor cavity were used to screen the DrugBank database. Models were evaluated by receiver operating curves and enrichment factors. Ultimately, the best pharmacophore model hits were subjected to molecular docking using the Glide module of Schrodinger. A consensus of all algorithms eliminated false positive hits and allowed reliable prediction. The interaction profile, pharmacokinetic, and pharmacodynamics properties were evaluated for the lead compounds. Alprostadil (**11**) exhibited better binding affinity toward neuraminidase than the existing inhibitor molecules. Predictions were validated using mutation analysis and molecular dynamics simulations. They suggested that integrative filtering exceeded the accuracy of other state-of-the-art methods for drug discovery.

Conventional influenza therapy is directed against the viral NA and the ion channel M2. Although these drugs are effective, the virus can readily acquire resistance. Most seasonal A/H1N1 viruses and the 2009 H1N1 virus are resistant to M2 inhibitors, and a significant proportion of the seasonal A/H1N1 viruses are resistant to the neuraminidase inhibitor oseltamivir. Sencanski et al. identified approved natural products as a promising source of low toxicity drug candidates for treatment of the influenza disease [49]. They showed that natural products combined with new therapeutic targets and drug repurposing techniques, can accelerate discovery of new influenza therapeutics.

3.5 Arenaviridae—Lassa and Junin mammarenavirus

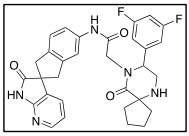
Lassa fever (LF) is a serious, rodent-borne disease in West Africa where the zoonotic host of Lassa virus (LASV) is common. Drugs for LF are very limited

and no preventive vaccine is available, resulting in high mortality in endemic areas. Aloke et al. reviewed the transmission and pathogenicity of LASV, and challenges of treatment options [50]. Large genetic diversity in different strains of LASV allows them to avoid the immune system, making development of LASV vaccines and drugs very challenging. They identified the LASV nucleoprotein (NP) as a novel drug target given its importance in the viral life cycle. Effective preventive measures, vaccines, target validation, and repurposing of existing drugs such as ribavirin using activity- or in silico-based methods are required to discover novel drugs for LF. Junin is also a pathogenic virus, found largely in regions of South America such as Argentina and Bolivia, which causes haemorrhagic fever in humans. It is transmitted to humans from mice causing chronic illness with high mortality. To date there has very been very little computational drug repurposing research published, although a recent ReFRAME study has screened a large number of drug candidates for in vitro activity against LASV and JUNV [51]. Aloke et al. recently highlighted the need for validation of druggable targets in LASV and JUNV and for drug repurposing to be pursued as a strategy [52].

As part of a computational study on drugs against multiple potential pandemic viruses, as discussed in detail above, Rajput and co-workers used a the "*drug-target-drug"* approach to predict the efficiency of repurposed drugs for LASV infections [24]. The repurposed drugs with the highest predicted activities were isoprenaline, loxapine, mycophenolate mofetil, ribavirin, and fostamatinib.

Minari et al. recently published a molecular docking study aiming to identify repurposed drugs and natural products that compare favourably with a standard drug, ribavirin, in potency against the virus [53]. They used AutoDock 4.2 to dock small molecules and natural products from PubChem against the 3D-structure of the endonuclease domain of LASV L polymerase. This protein target is presumed to cleave the cap structures of host mRNAs to initiate viral transcription. They also used computational models to assess the bioavailability and toxicology of the most promising natural product lead molecules α pinene, β pinene, and limonene.

A library of 200 drug-like compounds were assessed for binding to the alpha-dystroglycan receptor and blocking of LASV entry using computational docking by Arefin et al. [54]. Molecular docking was conducted using the PyRx 0.8 docking software. The most promising ligands were subsequently subjected to molecular dynamics simulations using the CABS-flex 2.0 (http://biocomp.chem.uw.edu.pl/CABSflex2) package and LARMD molecular dynamics simulator (http://chemyang.ccnu.edu.cn/ccb/server/LARMD/index.php). Amongst the six ligands with most favourable binding, chrysin, reticuline, and 3-caffeoylshikimic acid formed the the most stable interactions with the receptor. These three compounds should be investigated in both in-vitro and in-vivo studies to determine whether they suppress the entry of LASV to host cells.



12 MK-3207

There is very little reported research on computational repurposing of drugs and natural products for JUNV. Malhotra and colleagues reported a molecular docking analysis of FDA approved drugs with a glycoprotein from Junin (and Machupo) viruses [55]. The GP1 subunit of glycoprotein binds to the human receptor transferrin receptor 1 and initiates infection in humans, making it a potentially viable target for drug design and discovery. They docked 2115 FDA-approved drugs and 3754 investigational drugs into the target protein using the Autodock Vina software. The FDA-approved drugs with the best binding scores were Trypan Blue, lomitapide, eltrombopag, irinotecan, and dihydroergotamine. The leads from the investigational drugs were telomestatin, phthalocyanine, MK-3207 (**12**), and adozelesin. After consideration of possible toxicities and PAINS properties they focused on MK-3207 as having the optimal binding with the protein target and should be further validated for JUNV activity.

3.6 Coronaviridae—SARS, MERS, SARS-CoV-2

The current SARS-coronavirus 2 (SARS-CoV-2) pandemic, the most devastating for a century, has infected conservatively 800 million people and killed 7 million. The lack of vaccines to protect populations and drugs to treat COVID-19 patients highlighted the Impracticability of developing drugs using the traditional pathways—the time frames are just too long.

The pandemic caused by has raised important questions about its origins, mechanism of transfer to humans, and possible risk to companion or commercial animals. In silico structural homology modelling, protein-protein docking, and molecular dynamics simulation studies of the SARS-CoV-2 spike protein's ability to bind the target angiotensin converting enzyme 2 (ACE2) from relevant species were very useful [56]. ACE2 species in the upper half of the predicted affinity range (monkey, hamster, dog, ferret, cat) have been shown to be permissive to SARS-CoV-2 infection, and that the earliest SARS-CoV-2 isolates were surprisingly well adapted to human ACE2.

The pandemic stimulated great interest in computational drug repurposing as a fast strategy to find effective therapeutics. There was an unprecedented explosion of preprint and review papers on repurposing of drugs against a range of SARS-CoV-2 protein targets, principally the main protease, RdRP, and helicase. Clearly, it is impractical to review this enormous body of literature here and the reader is referred to more comprehensive reviews on computational drug repurposing for SARS-CoV-2 [9, 57–59]. Here, we provide a brief snapshot of this body of work.

Aherfi et al. reviewed drugs with in vitro antiviral activity against SARS-CoV-2, molecular docking data, and results from preliminary clinical studies [60]. By also considering pharmacokinetic properties they identified 11 molecules, nelfinavir, favipiravir, azithromycin, clofoctol, clofazimine, ivermectin, nitazoxanide, amodiaquine, heparin, chloroquine and hydroxychloroquine, of being of particular interest as possible repurposed drugs for COVID-19 treatment. Estrada reviewed the three main areas of modelling research for drugs useful against SARS CoV-2 and COVID-19: epidemiology; drug repurposing; and vaccine design [61]. He summarized most relevant literature on these modelling strategies to help researchers navigate this rapidly growing body of work. Mslati surveyed available biochemical and virtual screening studies against SARS-CoV-2 targets (spike, ACE2, RdRp, PLpro, and M-pro) and summarized repurposing candidates with consistent activity across diverse assays and predictive models [62]. They also examined repurposed drugs for efficacy against clinical COVID-19 infections and outcomes of their clinical trials.

RNA-dependent RNA-polymerase (RdRp) is an excellent target for drug development because it is not present in human cells. Vicenti, Zazzi and Saladini reviewed the available data on RdRp relevant to drug repurposing [63]. That noted that proofreading by SARS-CoV-2 exonuclease could limit the activity of drugs designed to be chain terminators and would favour drugs acting via delayed termination. Considering RdRp is highly conserved in coronaviruses, it is potentially useful pan-coronavirus target for drug development or drug repurposing for current and future pandemics. Xu et al. also reviewed therapeutic coronavirus targets and discussed existing small molecule drugs that may be repurposed for existing and emerging coronavirus infections [64]. They also described clinical progress in developing small molecule drugs for COVID-19.

Ben David et al. reported drug repurposing studies aimed to discover RBD-ACE2 binding inhibitors for COVID-19 therapy [65]. They developed an in vitro RBD-ACE2 binding assay and used it to identify inhibitors of this interaction via high-throughput screening of the LOPAC(R)1280 and DiscoveryProbeTM compound libraries. Sodium heparin, aurintricarboxylic acid, and ellagic acid, were had IC_{50} values between 0.6 and 5.5 μg/mL. A plaque reduction assay in Vero E6 cells infected with a SARS-CoV-2 surrogate virus confirmed the inhibition efficacy of heparin sodium and ATA. Molecular docking identified the relevant binding sites in the RBD.

Cavasotto and co-workers reported a docking-based screening study of a library of approved drugs and compounds undergoing clinical trials using quantum mechanical scoring [66]. They modelled three SARS-CoV-2 targets: the spike or S-protein; the main protease (Mpro); and the papain-like protease (PLpro). Their structure-based screening identified several structurally diverse compounds with potential antiviral activity against SARS-CoV-2. Repurposing candidates for Mpro included felypressin, brilacidin, samatasvir, eribaxaban, aplidin, candesartan, cilexetil and ritonavir. Drugs potentially active against PLpro included anatibant, pilaralisib, tiracizine, zabofloxacin, picotamide, cilazapril, and indisulam while pralatrexate, carumonam, aclerasteride and granotapide were predicted to inhibit the s protein.

Kandwal and Fayne used the pharmacophore-based drug design approach to repurpose existing drugs as inhibitors of SARS-CoV-2 viral proteins [2]. The Broad Institute's Drug Repurposing library of in-development and approved drugs and was computationally screened to identify potential inhibitors of SARS-CoV-2 protein targets.

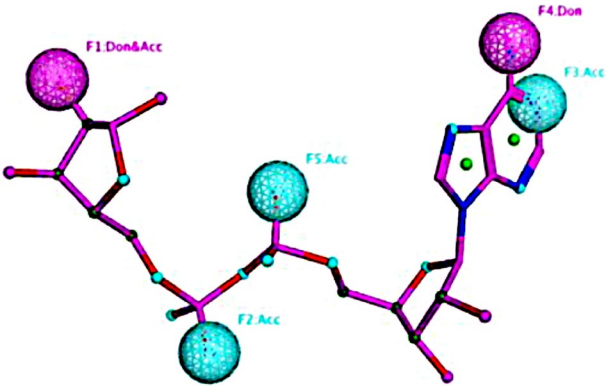
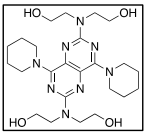


Fig. 8 Pharmacophore model for PLpro with adenosine-5-diphosphoribose (pink colour) generated from the PLPRO (6YWL) protein structure. Pink spheres denote hydrogen bond donors and blue spheres denote hydrogen bond acceptors (Color figure online)

Using rationally designed pharmacophore features, they identified molecules which could potentially be repurposed against viral nucleocapsid and several key non-structural proteins (Fig. 8). The pharmacophore features were generated from careful analysis of the interactions between co-crystalised ligands and the protein binding site. The pharmacophore features used were hydrogen bond donor and acceptor, hydrophobe, aromatic and charged groups such as cations and anions.

They screened the ChEMBL database to identify molecules with putative high inhibition of SARS-CoV-2. They subsequently correlated the predicted viral protein target properties with whole virus in vitro data. They identified four repurposing candidates for the SARS-CoV-2 PLpro—isepamicin, neohesperidin, tannic acid and streptomycin. They also identified repurposing candidates for several other protein targets e.g., for the RdRp complex: epigallocatechin-gallate, kuromanin, procyanidin-b-2, and rutin. Many of the hits generated by the pharmacophore searches were found to have modest to moderate activity against the targets and some inhibited the virus and were in clinical trials for COVID-19 treatment.



13 dipyridamole

Li et al. reported a virtual screening approach that employed accelerated, free energy perturbation-based absolute binding free energy (FEP-ABFE) predictions to identify drugs inhibiting SARS-CoV-2 main protease (Mpro) [67]. The predictions were based on a restraint energy distribution function. This allowed FEP-ABFE-based virtual screening of large drug libraries, otherwise relatively intractable using conventional methods. Of 25 drugs predicted to be good inhibitors, 15 were

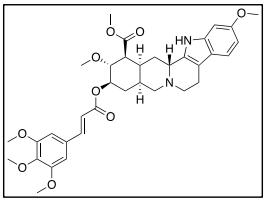
subsequently found to be potent inhibitors of SARS-CoV-2 Mpro in experiments. The drug with the tightest protease binding was dipyridamole (**13**) with a K_i value of 40 nM. It has shown promising therapeutic effects in clinical studies for treatment of patients with COVID-19. Hydroxychloroquine (K_i = 360 nM) and chloroquine (K_i = 560 nM) were 10x weaker inhibitors but constitute good lead compounds, however clinical evaluation of these two drugs to treat COVID-19 has been very disappointing [68].

Pauly et al. reviewed the lifecycle of SARS-CoV-2 and reviewed repurposing studies of the major protein targets such as 3CLpro, RdRp, ACE2, IL-6, and TMPRSS2 for drug development against COVID-19 [69]. They also reported a computational study in which 70 pre-existing clinical or clinical trial molecules were screened as RdRp inhibitors using molecular docking. Docking studies were performed using the Maestro 12.9 module of Schrodinger software with the experimental structure of RdRp as the target and remdesivir as the standard drug. Subsequent molecular dynamics simulation using GROMACS 2022.3 confirmed the binding mode and stability of the most potent compounds in the active site. The studies showed that many HIV protease inhibitors, such as lopinavir and ritonavir, bound well to the target RdRp. Additionally, AT-527, ledipasvir, bicalutamide, and cobicistat showed good docking scores.

Piplani and co-workers applied a robust virtual screening approach using Autodock Vina and molecular dynamics simulation in tandem to screen and calculate binding energies of repurposed drugs, clinical trials candidates and approved natural products as inhibitors of SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) [70]. They identified 80 promising compounds, consisting of antiviral drugs, natural products, and drugs with diverse modes of action. More than 30% of the 80 tightest binding compounds identified have been reported by others to have SARS-CoV-2 antiviral effects in vitro or in vivo. Of those not previously reported to have SARS-CoV-2 activity, eribulin, a macrocyclic ketone analogue of the marine compound halichondrin B and an anticancer drug, the AXL receptor tyrosine kinase inhibitor bemcentinib were of particular interest. These repurposing candidates may not only have utility in treating COVID-19 but provide a useful starting point for therapeutics against other coronaviruses. Their predictive in silico screening pipeline proved useful for repurposing existing drugs against other SARS-CoV-2 targets. Subsequently, these researchers used the pipeline to screen and calculate binding energies of repurposed drugs against the SARS-CoV-2 helicase protein (non-structural protein nsp13) [71]. Their top hits were antivirals, antihistamines, and antipsychotics. Of their shortlist of 87 drugs with the tightest helicase binding at least 30% had published evidence for in vivo or in vitro SARS-CoV-2 activity. The best repurposing candidates included: the antiviral agents, cabotegravir and RSV-604; the NK1 antagonist, aprepitant; the trypanocidal drug, aminoquinuride; the analgesic, antrafenine; the anticancer intercalator, epirubicin; the antihistamine, fexofenadine; and the anticoagulant, dicoumarol. They also used the methods to find repurposing candidates against the SARS-CoV-2 main protease, generating a shortlist of 84 candidates. [1] Again, $>25\%$ of these exhibited experimentally validated in vivo or in vitro activity against SARS-CoV-2, a very high hit rate. Their best candidates include

drugs and natural products such as rolitetracycline (antibiotic), disogluside (antifibrotic), zafirlukast (leukotriene antagonist), diosmin (venous disease), AZD-5991 (haematologic malignancies), and ruzasvir (hepatitis C), providing rational candidates for experimental validation.

Recently, these Piplani et al. virtually screened the recently discovered free fatty acid binding pocket in the spike protein [72]. The fatty acid, linoleic acid, binds to a hydrophobic pocket in the S protein near the receptor binding domain (RBD) [3], stabilizing its compact, closed conformation and blocking binding to human ACE2. Again, using the same docking and molecular dynamics protocol, this study provided novel insights into the SARS-CoV-2 spike protein and its potential regulation by endogenous hormones and drugs. Although some repurposing candidates have been demonstrated experimentally to inhibit SARS-CoV-2 activity, most candidate drugs have yet to be tested for SARS-CoV-2 activity. This study also provided a rationale for the effects of steroid and sex hormones and some vitamins on SARS-CoV-2 infection and COVID-19 recovery.



14 rescinnamine

Rajput et al. employed the SVM, random Forest, kNN, and deep and shallow neural network machine learning algorithms to repurpose drugs for use against SARS-CoV-2 [73]. They chose drugs and other chemicals with experimentally determined activity against coronaviruses from DrugRepV database. A merged data set of SARS-CoV-2, SARS, MERS, and overall coronavirus active molecules (over 400 molecules) were split into a training, validation, and test sets. Almost 18,000 structural, physicochemical and fingerprints descriptors were calculated for the molecules and a recursive feature selection algorithm was used to reduce their dimensionality. Models predicted the training and test set with correlation coefficients between 0.6–0.9. They also used an external independent validation dataset and decoy dataset to assess model robustness and determined model applicability domain. They employed the model to identify repurposed drug candidates against coronaviruses in DrugBank. Hits from this screen were validated by molecular docking against the spike protein/ACE2 complex structure. They predicted that verteporfin, leuprolide, alatrofloxacin, metergoline, rescinnamine (**14**), and telotristat ethyl would bind strongly to the the fatty acid pocket and inhibit infection by the virus.

Sadegh et al. developed CoVex, an interactive tool to explore the SARS-CoV-2 host interactome drug targets using virus-human protein, human protein-protein, and drug-target interactions (see https://exbio.wzw.tum.de/covex/) [74]. It allows network-based prediction of drug candidates and visual exploration of the virus-host interactome. The tool elucidated the mechanisms of virus life cycle drivers at the

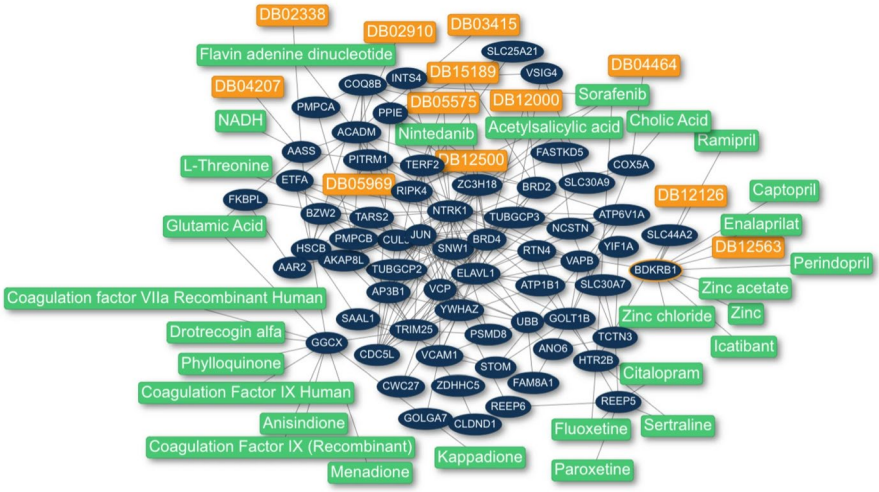


Fig. 9 Drug-protein–protein interaction network for viral proteins E, M, and spike. Blue nodes are protein targets, green are approved drugs, and orange are non-approved drugs. Lines represent interactions between proteins and drugs. Creative Commons Attribution 4.0 International Licence from Sadegh et al. [74] (Color figure online)

systems biology level, accelerating understanding of the molecular mechanisms of SARS-CoV-2 infection useful for predicting drug repurposing candidates (Fig. 9). The analysis identified chloroquine and deferoxamine, both which of which are in COVID-19 clinical trials, and methylene blue (approved for treatment of methemoglobinemia) as repurposing candidates.

Wu et al. analysed all proteins encoded by SARS-CoV-2 genes, compared them with other coronavirus proteins, and used homology modelling to build 19 protein structures [75]. These structures, plus two human targets, were used to computationally screen several compound libraries. These included the ZINC drug database 2924 compounds), a natural products database (1066 traditional Chinese herbal components and naturally occurring potential antiviral agents), and a small antiviral library (78 drugs). They reported screening results for the 3CLpro, spike, RdRp, and PLpro. The antivirals ribavirin, valganciclovir and thymidine, antibacterial drugs chloramphenicol, cefamandole and tigecycline, muscle relaxant chlorphenesin carbamate, and antitussive drug levodropropizine were predicted to have high binding affinity to PLpro. For Mpro, the antibacterial agents lymecycline, demeclocycline, doxycycline, and oxytetracycline, the anti-hypertensives nicardipine and telmisartan, and the hyponatremic conivaptan had highest predicted binding affinity. Betulonal, gnidicin and gniditrin, 2β,30β-dihydroxy-3,4-seco-friedelolactone-27-lactone, 14-deoxy-11,12-didehydroandrographolide, and 1,7-dihydroxy-3- methoxyxanthone were predicted to bind well to RdRp, They also used the structures to predict the likely targets for the drugs in the antiviral library.

Zhou et al. reported an integrative, antiviral drug repurposing platform based on systems pharmacology networks that describe the complex interactions between the virus-host interactome and drug targets in a human protein-protein interaction network [76]. They showed that 2019-nCoV/SARS-CoV-2 shares the highest nucleotide sequence identity with SARS-CoV (79.7%). Their envelope and nucleocapsid proteins share evolutionarily conserved regions with sequence identities of 96% and 90% relative to SARS-CoV. Their analyses identified 16 potential repurposable drugs for coronavirus infection. Several of these, melatonin, mercaptopurine, and sirolimus were validated by enrichment analyses of HCoV-induced transcriptomics data in human cell lines and drug-gene signatures. They identified synergistic combinations of drugs such as toremifene/emodin, sirolimus/dactinomycin, and mercaptopurine/melatonin, and) in which both drugs modulate the coronavirus-host subnetwork, but in different parts of the human interactome network.

3.7 Henipaviridae—Nipa and Hendra

This new genus was created for the highly pathogenic (Biosafety Level 4) paramyxovirus pathogens Hendra virus and Nipah virus. Both recently emerged from flying foxes to cause serious disease outbreaks in humans and livestock in the Asia-Pacific region [77]. It is concerning that they have an extraordinarily broad host range – flying foxes, horses, pigs, cats, dogs, and humans. Research on the henipaviruses is clearly restricted by their BSL4 status. However, henipavirus proteins expressed from cloned genes have increased our understanding of the attachment (G), fusion (F) and the phosphoprotein (P) gene products. Ephrin B2, the recently identified membrane receptor for the henipavirus G protein, is a widely distributed, conserved cell-surface glycoprotein in vertebrates. Its presence in neurons may explain virus growth in the brain and encephalitis in human patients. The F protein is a type I membrane protein that is cleaved by the ubiquitous cathepsin L. The P gene encodes the P, V and W proteins that allow henipaviruses to evade host antiviral defences by inhibiting both dsRNA signalling and interferon (IFN) signalling. The V and W proteins inhibit dsRNA signalling. The P, V and W proteins also inhibit IFN signalling via the STAT proteins. Some of these proteins constitute potential targets for drug therapies.

Nipah virus (NiV) causes severe encephalitis and respiratory diseases in humans. Despite its grave pathogenicity and pandemic potential, no drugs have yet been approved for human use. NiV attachment glycoprotein G (NiV-G), fusion glycoprotein (NiV-F), and nucleoprotein (NiV-N) are important in virus replication and spread so constitute attractive targets for anti-NiV drug discovery. Randhawa et al. screened potential multitarget chemical and phytochemical agents against NiV using a sequential molecular docking and MD-based approach [78]. Molecules were docked against NiV-G, NiV-F, and NiV-N and ranked by protein-binding affinity, interactions with critical binding-site residues, ADME properties, and stability in the binding site. They used a sequential molecular docking and molecular-dynamics-based approach that simultaneously targeted NiV-G, NiV-F, and NiV-N. Quickvina,

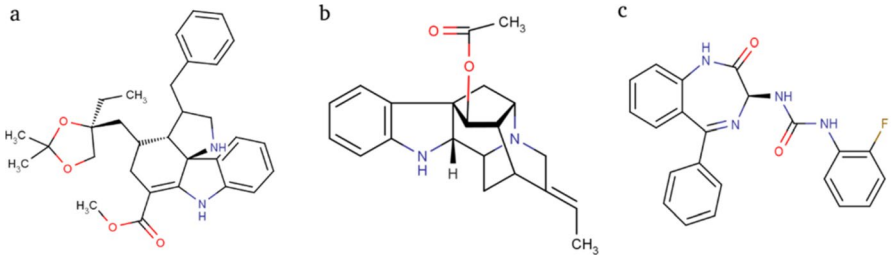


Fig. 10 Three most promising drug and natural products repurposing leads from Randhawa et al. **a** CARS0358, **b** 17-O-acetyl-nortetraphyllicine, and **c** RSV604. Creative Commons By Licence [78]

a variant of Autodock Vina was used for the docking calculations, and subsequent simulation of top hits was performed using GROMACS and the CHARMM36 force field. Molecules that bound to all the three proteins were of particular interest as possible multitarget inhibitors. The study identified phytochemical molecules 17-O-acetyl-nortetraphyllicine and CARS0358 (NA) as distinct multitarget inhibitors of all three viral proteins, and RSV604 as an inhibitor of NiV-G and NiV-N (Fig. 10). Validation of these predictions by in vitro and in vivo antiviral studies and animal model studies would enable them to proceed to clinical trials for treatment of NiV infections, and potentially provide the first treatments for Hendra infections.

As described above, Rajput et al., used a drug-target-drug computational approach to identify repurposed drugs against 14 viruses responsible for causing epidemics and pandemics, including Nipah, chikungunya, and others using a drug target network approach [24]. The putative drug targets they identified were used to screen a library of FDA-approved drugs for each virus and prioritize them. They identified 15 potential repurposed drugs against Nipah, including paroxetine, pindolol, methylephedrine, norepinephrine, and racepinephrine.

The NIV RdRp is also a promising target for antiviral drugs. Abduljalil et al. generated a computational homology modelling of the L protein [79]. Molecular docking using Autodock Vina was used to identify several nucleotide analogues, previously reported to inhibit viral RdRps, that may bind to the NIV RdRp domain. The most promising compounds were subjected to molecular dynamics simulation with GROMACS to validate their binding and estimate their binding free energies. Galidesivir, AT-9010, and Norov-29 bound most tightly to the RdRp (Fig. 11). Purine nucleotides were predicted to be the most effective scaffold for NIV drug optimization, constituting a starting point for medicinal chemistry and drug discovery campaigns for NiV therapeutics.

3.8 Hepatitis viruses

Zuckerman has summarized the essential properties of diverse hepatitis viruses, essentially defined by their ability to infect livers [80]. Hepatitis A virus (HAV), a hepatovirus, is a small, unenveloped symmetrical RNA virus that shares many of the characteristics of the picornavirus family. Hepatitis B virus (HBV), a hepadnavirus, is a double-stranded DNA virus that replicates by reverse transcription. Hepatitis

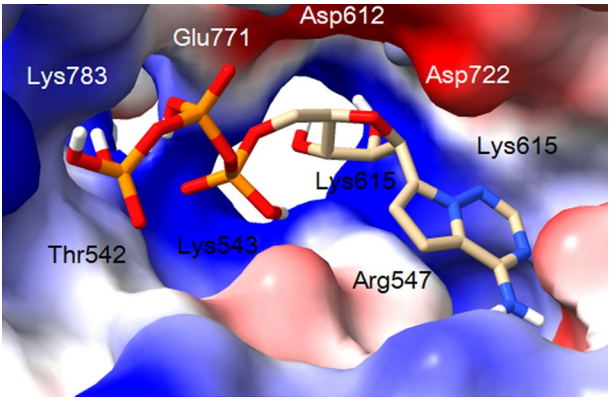


Fig. 11 Electrostatic surface for the binding site of NiV RdRp and docking pose for the top repurposing hit, Norov-29, Positively charged residues are blue, and negatively charged residues are red. Adapted with permission from Abduljalil et al. [79] (Color figure online)

C virus (HCV) is an enveloped single-stranded RNA virus related to flaviviruses not transmitted by arthropod vectors. Hepatitis D virus (HDV) is an unusual, single-stranded, circular RNA virus with similarities to some plant viral satellites and viroids. It requires a hepadnavirus helper for propagation in hepatocytes. It is an important cause of acute and severe chronic liver damage in many regions of the world. Hepatitis E virus (HEV), the cause of enterically-transmitted non-A, non-B hepatitis, is another non-enveloped, single-stranded RNA virus that shares many biophysical and biochemical features with caliciviruses.

Hepatitis C Virus (HCV) affects an estimated 71 million people around the world, with 400,000 deaths annually due to chronic cirrhosis and liver cancer. Although many drugs are available for HCV infections, drug resistance and toxicity are major issues. Tarannum and Nandi exploited RdRp, common to flaviviridae HCV, dengue, Zika, and yellow fever [81], to try to repurpose different tropical disease virus RdRp inhibitors for the HCV NS5B polymerase using structure-based molecular docking. They screened 87 compounds with dengue, yellow fever, and Zika RdRp inhibitory activity as potential RdRp leads using docking simulations. Only a N-sulfonylanthranilic acid derivative, 4′-azidocytidine (R1479), 5-benzenesulfonylmethyl-3-hydroxy-4-hydroxymethyl-pyridine-2-carboxylic acid hydroxyamide (DMB220), 2-(4-methoxy-3-thiophen-3-yl-phenyl)ethanoic acid (FD-83-KI26), 2-[(4-chloro-3-nitrobenzylidene)amino]-N-phenylbenzamide (CCG-7648), 4-[(2R,3R,4 S,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-3-oxopyrazine-2-carboxamide (T-1106), mycophenolic acid, and DMB213 exhibited good docking scores and had the same mode of interaction as the reference drug, sofosbuvir diphosphate, a standard HCV RdRp inhibitor. They bound strongly to the hepatitis C viral RdRp and thus may be potential leads for further testing of anti HCV activity and can be repurposed to combat HCV.

4 Conclusion and perspective

Clearly, there is a robust computational screening pipeline accessible to chemists and biologists that provides a fast, rational basis for identifying existing drugs, clinical trials candidates, and approved natural products for use against viruses responsible for current dangerous viral diseases and those with epidemic or pandemic potential. While these computational methods still have limitations and must be used carefully [9], their ability to identify molecules with experimentally validated activity against molecular targets and/or viruses suggests that the so-far unvalidated predictions are very strongly enriched in drugs likely to be active against the target viruses.

The impressive advances in computational docking methods, machine learning algorithms, and accurate prediction of protein target structures from sequence using AlphaFold and its competitors, is providing unprecedented opportunities for existing drugs and natural products to be repurposed to tackle serious viral disease responsible for massive morbidity and mortality worldwide. Given that much of this occurs in tropical and developing countries where the cost of drug development and treatments is a major factor in their discovery and accessibility rapid, cost-effective methods such a computational repurposing of drugs play an essential role in addressing these issues.

Author contributions As the sole author, my contribution is 100%.

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Declarations

Conflict of interest The author has no relevant financial or non-financial interests to disclose.

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