OXFORD

A comprehensive drug repurposing study for COVID19 treatment: novel putative dihydroorotate dehydrogenase inhibitors show association to serotonin–dopamine receptors

Burak Berber and Osman Doluca

Corresponding author: Osman Doluca, Department of Biomedical Engineering, Izmir University of Economics, Izmir 35330, Turkey.
E-mail: osman.doluca@ieu.edu.tr

Abstract

Dihydroorotate dehydrogenase (DHODH) is a key enzyme required for *de novo* pyrimidine synthesis and it is suggested as a target for COVID19 treatment due to high pyrimidine demand by the virus replication in the infected host cells as well as its proven effect of blocking of cytokine release by the immune cells to prevent inflammation leading to acute respiratory distress. There are a number of clinical trials underway for COVID19 treatment using DHODH inhibitors; however, there are only a small number of known DHODH antagonists available for testing. Here, we have applied a methodology to identify DHODH antagonist candidates, and compared them using *in silico* target prediction tools. A large set of 7900 FDA-approved and clinical stage drugs obtained from DrugBank were docked against 20 different structures DHODH available in PDB. Drugs were eliminated according to their predicted affinities by Autodock Vina. About 28 FDA-approved and 79 clinical trial ongoing drugs remained. The mode of interaction of these molecules was analyzed by repeating docking using Autodock 4 and DS Visualiser. Finally, the target region predictions of 28 FDA-approved drugs were determined through PASS and SwissTargetPrediction tools. Interestingly, the analysis of *in silico* target predictions revealed that serotonin–dopamine receptor antagonists could also be potential DHODH inhibitors. Our candidates shared a common attribute, a possible interaction with serotonin–dopamine receptors as well as other oxidoreductases, like DHODH. Moreover, the Bruton Tyrosine Kinase-inhibitor acalabrutinib and serotonin–dopamine receptor inhibitor drugs on our list have been found in the literature that have shown to be effective against Sars-CoV-2, while the path of activity is yet to be identified. Identifying an effective drug that can suppress both inflammation and virus proliferation will play a crucial role in the treatment of COVID. Therefore, we suggest experimental investigation of the 28 FDA-approved drugs on DHODH activity and Sars-CoV-2 virus proliferation. Those who are found experimentally effective can play an important role in COVID19 treatment. Moreover, we suggest investigating COVID19 case conditions in patients using schizophrenia and depression drugs.

Key words: COVID19; Sars-CoV-2; DHODH; target prediction; molecular docking

Burak Berber has completed his MSc in the field of Molecular Biology. He worked on a short-term project at UNAM University (Mexico) about Alzheimer's disease with the Mevlana Exchange Programme funded by the Council of Higher Education (Turkey). He is a PhD candidate at Eskisehir Technical University. Also, working at Eskisehir Technical University, Department of Biology as research assistant.
Osman Doluca has completed his PhD on alternative DNA structures and design of DNA probes in Massey University, New Zealand. Since then, he has been working on developing bioinformatics tools for prediction of DNA structure and investigation of the biological role of G-quadruplexes and their function–structure relationship. He is currently working at Izmir University of Economics as assistant professor.

Introduction

In December 2019, it was announced that a fatal pneumonia disease occurred in Wuhan, China. Clinicians in China have found that the cause of this disease was a new coronavirus. This virus, called Sars-CoV-2, has been determined as a result of the genome sequencing analysis and was 79.5% similar genetically to multiple acute respiratory syndrome (SARS) virus [1, 2]. This virus, which spread from Wuhan to the whole world, has infected more than 41 million as of 21 October 2020, causing more than 1 million deaths. The Sars-CoV-2, which the World Health Organization has declared as a pandemic as of 11 February 2020, causes acute lung injury, as the main cause of death.

More than 3611 clinical trials are underway (according to ClinicalTrials.gov, 19 October 2020), such as drug development, vaccination studies and serum/plasma treatment [3]. Considering patient serum amount is low as a recently spreading disease and the vaccine development is expected to take time, it is of great importance to test FDA-approved drugs in the treatment of COVID19, especially for the rapid control of the disease [4]. Current drug discovery studies focus on RNA polymerase inhibition [5], angiotensin converting enzyme-2 (ACE-2) and Spike Protein Blockers [5–7], transmembrane serine protease-2 (TMPRSS2) inhibitors [8]. These target regions are focused for preventing the virus from binding to the target protein, entry into the cell and replication.

Beside similarity of the genomic sequences of Sars and Sars-CoV-2, their biochemical interactions and pathogenesis have been shown to utilize similar pathways [9]. The binding to the ACE-2 receptor of Sars-CoV-2 lung type-2 endothelial cells, inflammation triggers the cascade, results in respiratory failure due to acute respiratory distress syndrome (ARDS) [9, 10]. One of the most important factors of ARDS formation is due to the overwhelming release of proinflammatory cytokines (such as IFN-a, IFN-γ, IL-1B, IL-6, IL-12, IL-18, TNF-α). Following virus entry and infection of cells, host immune response and inflammation cascade begin via antigen-presenting cells (APC) and macrophages [4, 9]. This process takes place due to two functions of APC: (1) It provides antigen against foreign pathogen to CD4 + T cells (Th1) and (2) releases Interleukin-12 to stimulate Th1 cells. Stimulated Th1 cells stimulate CD8 + T-killer cells (Th2) and attack all cells with a foreign antigen against this pathogen [11]. It also triggers B cells to produce antigen-specific antibodies when Th1 cells are activated. Interferon-1 (IFN-1) has a protective effect especially in SARS and Middle East Respiratory Syndrome (MERS) infection. It has been detected in animal studies that IFN-1 signal transduction is suppressed in cells with infection of the SARS virus [12]. Also, antigen creation is suppressed by the virus. It has been determined that non-structural (nsp) proteins located in the ORF regions of the virus genome play a role in these suppression mechanisms [13].

Most common drug treatment methods aim treatment of the newly infected or about preventing the infection at the first place [9]. However, since patients admit to hospitals with symptoms of cough and fever, at this stage the inflammation has already started. The use of remdesivir and hydroxychloroquine at the time of the outbreak has been common for the treatment of COVID19. However, with increasing number of clinical studies, it has been revealed that these two drugs do not show statistically significant effect against this disease [14].

Favalli *et al.* [15] stated that similar pathophysiological findings were found between COVID19 and Rheumatoid Arthritis (RA) disease. They suggested that anti-rheumatic drugs can also be used in COVID19 [15]. Although RA is painful and quite debilitating, it is a chronic inflammatory disease characterized by gradual destruction of joints, deformity, disability and premature death in infants [16].

Anti-inflammatory drugs, in particular, anti-cytokines have been used to prevent inflammatory formation [17]. One of these treatment methods has been the suppression of the dihydroorotate dehydrogenase (DHODH) enzyme [18]. Pyrimidines play roles in the formation of phosphodiester bonds with purines in double helix DNA, glycoprotein, phospholipids, RNA and DNA [19, 20]. There are two important ways in the synthesis of pyrimidines in most conserved organisms; salvage and *de novo* pathways [19]. Under normal conditions, along with differentiated cells, inactive lymphocytes prefer salvage pyrimidine synthesis [21, 22]. However, upon stimulation, they need *de novo* pyrimidine synthesis where the DHODH enzyme takes part. With the suppression of pyrimidine synthesis, activated lymphocytes undergo metabolic stress and the release of pro-inflammatory cytokines such as IL-17 and IFN-γ decreases leading to apoptosis [22–24].

DHODH is a flavoenzyme localized in the inner membrane of the mitochondria, involved in the fourth step of *de novo* pyrimidine synthesis [21, 25]. Using Ubiquione as a cofactor, it catalyzes dihyrooratate to oratate. It is necessary for the cell as it is the only enzyme that can perform this process during the formation of uridine monophosphate used in RNA synthesis [20].

DHODH enzyme is proven to play an active role in cancer and immunological disorders such as acute myeloid leukemia, RA, multiple sclerosis [18, 21, 26–28]. When the pathogenesis of COVID19 on lung injury was examined, similar immune reactions were detected in autoimmune diseases such as RA [15]. Suppression of the DHODH enzyme in humans is a proven treatment method for immune disorders, especially cancer, RA (Leflunomide) and multiple sclerosis (Teriflunomide).

The viruses are in need of a high amount of nucleic acid to complete their life cycle in the host cell. Therefore, inhibition of nucleotide biosynthesis was previously considered a potential anti-viral strategy [6]. In accordance, DHODH inhibitors used in the treatment of autoimmune diseases have also been identified by studies that have broad spectrum anti-viral effects [29]. DHODH inhibition has been found to have antiviral effects against rotavirus, dengue virus, foot and mouth disease virus [29–33]. Liu *et al.* [34] showed that suppression of *de novo* pyrimidine synthesis in antiviral treatment strategies, apart from RNA-dependent RNA polymerase inhibition, may be the target mechanism for many viral pathogens including coronavirus. Indeed, drug targets in the treatment of COVID19 have been determined by a multi-omics study and DHODH has been identified as one of the three target candidates [35].

Infact, a recent study showed the significance of DHODH in proliferation of the virus. DHODH+ and DHODH-A549 cells, which were created with the CRISPR technique, were infected with the Sars-CoV-2 and the proliferation of the viruses. It was reported that there was no significant change in cell proliferation of DHODH-A549 compared to DHODH+ cells after 72 h, yet, in DHODH-A549 cells, the virus was found to grow 1000 times slower. With this study, it was determined that DHODH enzyme is important for the virus replication [33].

Recently, new DHODH inhibitors discovered in a number of studies have been demonstrated to suppress both virus infection and cytokine storm [33, 36, 37]. It has been reported that the clinical trials of IMU-838 and PCT299 against COVID19 are ongoing (ClinicalTrials Code: NCT04379271, ClinicalTrials Code: NCT04439071). *In vitro* and clinical studies utilizing DHODH inhibitors has been increasing against COVID19 (Chictr2000030058, ClinicalTrials Code: NCT04361214, Clinical
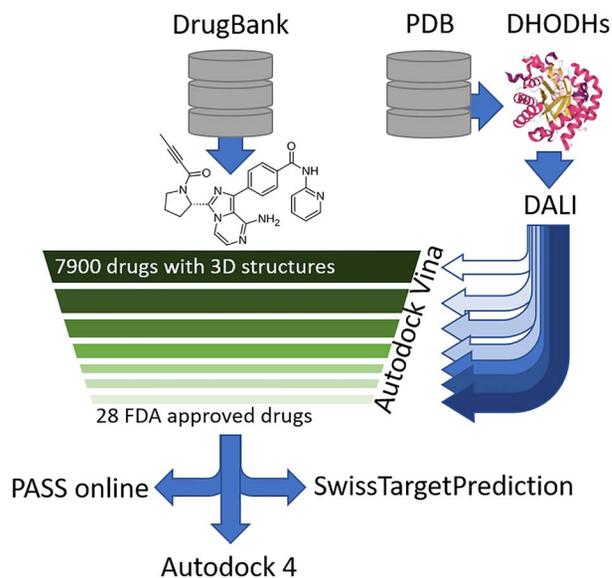
Figure 1. A graphical summary of the methods employed in this study.

Trials Code: NCT04379271, ClinicalTrials Code: NCT04439071, ClinicalTrials Code: NCT04516915). Yet, considering the fast pace of the pandemic, and low rate of drugs passing the clinical stage, more candidates are necessary for testing. The aim of our study is to determine potential candidates among approximately 7900 candidate compounds, including FDA-approved drugs, to target the DHODH enzyme using serial molecular docking analysis on 20 different DHODH crystal structures. Those with the high binding energy in all DHODH enzymes tested were distinguished. Among the identified drugs, the FDA-approved drugs further analyzed with *in silico* target-prediction tools, PASS online and SwissTargetPrediction. The target similarities of the molecules determined by comparing the FDA-approved DHODH inhibitor, Leflunomide, are discussed (Figure 1).

Related work

There have been a number of *in silico* studies focusing molecular docking for discovering drug candidates for treatment of COVID19. Cavasotto and Filippo have utilized docking of 11 552 molecules onto three different proteins of COVID19 virus, the spike protein, 3-chymotrypsin-like protease and papain-like protease. To obtain conformational diversity, additional structures were also included for papain-like protease. The lowest energy poses were taken for each molecule and were subject to further energy optimization by regarding amino-acids within 4 Å of any docked molecule were flexible. The compounds were then ranked according to a novel method utilizing the docking and optimization scores [38].

Another more high-throughput docking study, evaluates a total of 34 500 drug or drug-like molecules were analyzed with molecular docking for their affinity towards a single crystal structure of 3-chymotrypsin-like protease. Using a threshold score, the molecules with high affinity were identified and further eliminated by reperforming molecular docking with a second algorithm [39]. While using a much larger candidate database, this approach again had the disadvantage of being tested for only a single structure.

Another study focusing only 61 reported antiviral agents and targeted 5 co-crystalized structures of COVID19 virus main

protease. Out of 61, only one showed significant binding to all structures despite the fact that all structures belonging to the same protease. These show that re-crystallization or ligand-dependent changes can affect the docking scores. Remdesivir, a drug already used for COVID19 treatment, [40] showed significant docking but for only three of the structures [41].

DHODH has seen relatively less interest for COVID19 treatment. In one study published in 2012 focusing drug discovery with molecular docking, the researchers identified 18 candidate molecules with a high binding score that could target the active site of the DHODH enzyme from 280 000 molecules via docking to a crystal structure of DHODH, 1D3G [42]. A recent study published after the beginning of the pandemic showed two of the 18 candidate molecules to suppress Sars-CoV-2 proliferation 17 times stronger ($IC_{50} = 17$ μM) compared to FDA-approved DHODH inhibitors ($IC_{50} = 300$ μM) [33]. Unfortunately, while this study provided immensely valuable candidates, since its publication in 2012, none of the 18 original candidates has been listed as FDA approved to the best of our knowledge. In the urgency of the pandemic, it became apparent that instead of extended clinical trials, drug repurposing may provide the key drugs to the patients sooner.

For that reason, in this work we focused on FDA-approved drugs available in Drugbank. In addition, deviating from previous docking studies for treating COVID19, we have employed multiple DHODH structures as a means to filter drug candidates. It is normal that the number of crystalized protein structures of this virus is low since it was recently discovered, and remains a significant obstacle in front of *in silico* studies. However, understanding the viral mechanisms help us exploit alternative targets. Especially considering any virus uses the hosts systems, which are often well characterized, enables us to focus on the host protein structures. In this particular case, DHODH remains to be a putative target to deal with the virus. In that sense, the most important novelty of this work in comparison to recent drug-repurposing molecular docking studies targeting COVID19 would be the use of a well-established DHODH as our target and consecutively using a much larger number of structures as a way of inclusion the conformational variety of the protein.

Methods

Obtaining ligands

All drug structures of 7900 ligands with a 3D structure are downloaded from DrugBank [43] and are converted from sdf format into individual PDBQT files using an in-house Python code and openbabel (version 3.0.0) [44]. The hydrogens were added/removed in accordance to simulate pH 7.0 and gasteiger charges were added to PDBQT files. Only the largest and unique fragments were taken in each drug file.

Obtaining receptor files

From Protein Databank, (www.wwpdb.org) [45] among all structures of Human DHODH, only the asymmetric X-ray crystallography structures with a maximum resolution of 2.0 Å and published after 2010 are selected. Among these structures, some are also eliminated due to reading errors. The following 20 PDB structures were remained: PDB ID's: 2wv8 [46], 3kvj [47], 3kvl [47], 4igh [48], 4jtu [49], 4zl1 [50], 4zmg [51], 5h2z [52], 5h73 [53], 5hqe [54], 5k9c [27], 5k9d [27], 5mut [55], 5mvc [55], 5mvd [55], 6idj [56], 6j3b [57], 6j3c [57], 6jmd [58] and 6lzl [59]. Only the initial

structure of each PDB was used if more than one structure was present in each file.

Receptor preparation

The remaining 20 PDB files were then aligned to 6j3c PDB file as a reference using pairwise structure comparison tool on DALI Server [60]. Only the first chain in each file (chain A) was used for the alignment (see Table S1 available online at https://academic.oup.com/bib). The aligned protein structures were converted to PDBQT files, using MGL Tools [61]. All non-polar hydrogens were removed merging charges and polar hydrogens were added where necessary. The charges were recalculated using Kollman charges. All non-standard residues were removed. All water and other molecules except the main chain are removed.

Autodock Vina docking analysis

Since the all PDB structures were aligned, the active sites also were aligned. A single gridbox with a center at $x = 34.569$, $y = -15.848$, $z = -18.938$ Å) and sizes of 18, 28 and 22 Å, at respective edges, encapsulated the active site and was used for all receptors.

Using an in-house Python code and Vina (Autodock), an automated docking was performed for all filtered ligands and receptor files [62]. An exhaustiveness of 16 was used. All Vina docking analysis were performed on a computer with 12 core CPU and 32 GB ECC Ram. Each ligand that received an affinity equal or higher than −11.0 (kcal/mol) for any of the receptors was removed from future docking analysis. All dockings were started using 6j3c and continued with the rest, in alphabetical order according to PDB IDs.

Autodock 4.2 docking analysis

Same gridbox used for Vina with a center at $x = 34.569$, $y = -15.848$, $z = -18.938$ Å) and sizes of 18, 28 and 22 Å, at respective edges, was used for docking using Autodock 4. Since Autodock 4.2 requires a step site, the default step size of 0.345 Å was used.

Identifying interacting aminoacids

Autodock docking analysis was displayed with MGLtools 1.5.6, and the protein and ligand were recorded in a single pdb file by selecting the best binding affinity from 10 different binding results. Receptor–ligand interactions were analyzed with the DS visualizer program of the obtained pdb.

In silico target predictions

SMILE codes of the drug candidates were obtained from Pub-chem and entered in http://new.swisstargetprediction.ch/ and http://www.pharmaexpert.ru/passonline to search for probable targets in humans based on the similarity to known chemicals in the database [63, 64].

Results

Sequential molecular docking

Our study employed around 7900 structures from DrugBank [43] with 3d structures. In our effort to identify possible inhibitor candidates, we have performed molecular docking of these
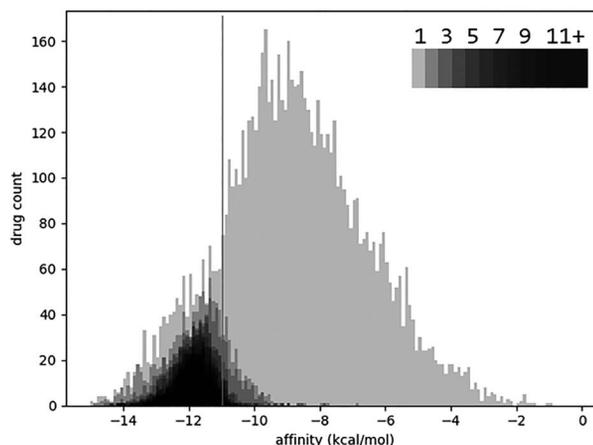
Figure 2. The distributions of drug affinities for each DHODH structure tested. The darker shades indicate the increased number of structures that recoded a drug at corresponding affinity. Red line indicates −11 kcal/mol, the threshold used to eliminate low affinity drug candidates.

molecules to 20 DHODH crystal structures using a gridbox selected based on the known active site binding inhibitors. A distribution of all binding affinities towards to the first DHODH structures tested (6j3c) was obtained. Of this distribution, structures with affinity better than −11.0 kcal/mol, corresponding to top 12%, was selected for docking on the next DHODH structure. As can be seen in Figure 2, the initial distribution obtained with 6j3c was centered around −9 kcal/mol. After the initial docking with 6j3c, the remaining structures showed a distribution with a mean of around −12 kcal/mol (Table 1). This process continued for other crystal structures with more drugs eliminated with each cycle. The decrease in the remaining drugs indicated that the majority of the drugs were filtered out after the docking with the third structure (Figure 3). However, a small portion of the drug candidates continued to spill above the threshold, resulting in their elimination as well. The continued docking and elimination process decreased the ratio of the drugs eliminated.

As a result of the sequential molecular docking 243 drugs were identified, of which 28 were FDA-approved and 79 were clinically tested candidates (Table 1 and see Table S2 available online at https://academic.oup.com/bib). These drugs are shown to be used effectively in a range of diseases such as RA, myeloid leukemia, schizophrenia, depression, HIV infection, spinal muscular atrophy (SMA) and many types of cancer (Table 2). Among the drugs detected, Sorafenib [65, 66], Regorafenib [67, 68], Pexidartinib [69, 70], Capmatinib [71, 72] are used as active drugs in many types of cancer as multiple kinase inhibitors. Raltegravir and Dolutegravir are used as anti-HIV drugs and clinical studies are continuing in COVID19 [73–75]. Glyburide, Glipizide and Canagliflozin are used in various type 2 diabetes diseases [76–78]. Droperidol [79], Risperidone [80], Domperidone [81], Aripiprazole [82, 83], Paliperidone [84], Sertindole [85], Vilazodone [86], Brexpiprazole [87, 88], Lasmiditan [89] are used as serotonin, dopamine receptor antagonist in schizophrenia and depression diseases.

The FDA-approved drugs were then docked again to 6j3c using Autodock 4.2 docking tool. This yielded affinity as well as inhibition constant (Ki) for all 28 FDA-approved drugs (Table 1). The Autodock 4.2 docking results were then analyzed for their interaction with amino acids using DS Visualizer (Table 3) HIS56, VAL143 and ALA59 were the common amino acids that showed
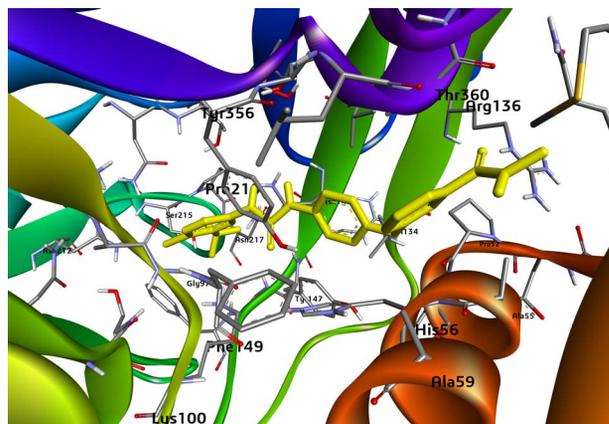
Table 1. Autodock vina and autodock 4.2 results of FDA-approved drugs obtained by extensive molecular docking screening against DHODH structures

DrugBank code	Drug name	PDB:6J3C Autodock Vina ΔG (kcal/mol)	PDB:6J3C Autodock 4.2 ΔG (kcal/mol)	Average of Autodock Vina ΔG results (kcal/mol)	PDB:6J3C Autodock 4.2 constant of inhibition (Ki)
DB00398	Sorafenib	−12.7	−9.3	−12.365	152.27 nM
DB00450	Droperidol	−12.0	−7.83	−11.995	1.84 μM
DB00619	Imatinib	−11.9	−9.8	−12.53	65.63 nM
DB00734	Risperidone	−12.7	−10.16	−13.25	35.64 nM
DB01016	Glyburide	−12.4	−9.41	−12.27	126.51 nM
DB01067	Glipizide	−11.7	−9.53	−12.15	102.66 nM
DB01184	Domperidone	−12.2	−9.22	−12.155	174.27 nM
DB01238	Aripiprazole	−12.1	−8.81	−11.745	349.33 nM
DB01267	Paliperidone	−12.7	−9.84	−13.145	61.03 nM
DB06144	Sertindole	−11.6	−10.65	−12.17	15.71 nM
DB06684	Vilazodone	−12.3	−10.12	−12.195	38.36 nM
DB06817	Raltegravir	−11.9	−7.48	−12.09	3.31 μM
DB08883	Perampanel	−11.9	−9.87	−12.02	58.22 nM
DB08896	Regorafenib	−13.1	−9.17	−12.675	191.04 nM
DB08907	Canagliflozin	−11.9	−9.75	−11.79	71.00 nM
DB08930	Dolutegravir	−11.8	−8.9	−12.11	300.44 nM
DB09042	Tedizolid phosphate	−12.1	−9.38	−12.18	132.42 nM
DB09128	Brexpiprazole	−12.4	−12.1	−11.975	1.36 nM
DB11526	Masitinib	−12.6	−7.31	−13.145	4.37 μM
DB11703	Acalabrutinib	−12.0	−9.94	−12.15	52.01 nM
DB11732	Lasmiditan	−12.4	−7.2	−11.885	5.31 μM
DB11791	Capmatinib	−14.1	−9.68	−13.275	80.50 nM
DB11793	Niraparib	−11.2	−8.64	−11.745	462.16 nM
DB12836	Grapiprant	−12.2	−10.66	−12.28	15.41 nM
DB12867	Benperidol	−11.8	−8.67	−11.94	439.62 nM
DB12978	Pexidartinib	−12.4	−8.75	−12.35	388.56 nM
DB13931	Netarsudil	−12.7	−9.67	−12.65	81.36 nM
DB15305	Risdiplam	−11.8	−10	−11.815	46.83 nM
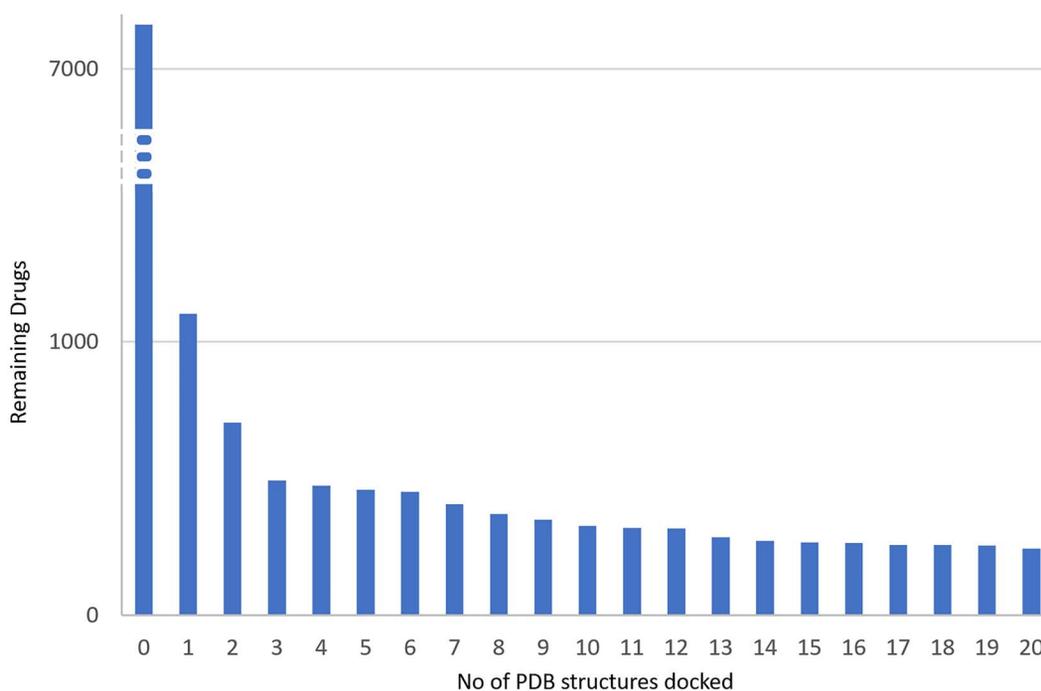
Figure 3. The number of drug candidates remained after each cycle of docking.

Table 2. Drugbank codes, common names, known targets and associated disease of 28 FDA approved drugs disocvered in this study.

DrugBank code	Drug name	Target	Diseases
DB00398	Sorafenib	Multiple intracellular (CRAF, BRAF and mutant BRAF) and cell surface kinases (KIT, FLT-3, VEGFR-2, VEGFR-3, and PDGFR-ß)	Advanced Renal Cell Carcinoma Gastrointestinal Stromal Tumors (GISTs) Hemangiosarcoma Unresectable Hepatocellular Carcinoma Locally recurrent refractory to radioactive iodine treatment Thyroid carcinoma Metastatic refractory to radioactive iodine treatment Thyroid carcinoma
DB00450	Droperidol	Dopamine(2) receptor antagonism with minor antagonistic effects on alpha-1 adrenergic receptors	Agitation Chemotherapy-induced nausea and vomiting Delirium Nausea and vomiting
DB00619	Imatinib	Inhibits the Bcr-Abl tyrosine kinase	Treating chronic myelogenous leukemia, GISTs and a number of other malignancies.
DB00734	Risperidone	Inhibition of dopaminergic D2 receptors and serotonergic 5-HT2A receptors	Acute Mania Irritability Mixed manic depressive episode Schizophrenia Agitated psychotic state
DB01016	Glyburide	The closure of ATP-sensitive potassium channels on beta cells	Gestational Diabetes Mellitus (GDM) Glycemic control Type 2 Diabetes Mellitus
DB01067	Glipizide	A sulfonylurea medication used in Type 2 Diabetes to sensitize pancreatic beta cells and stimulate insulin release	Type 2 Diabetes Mellitus
DB01184	Domperidone	A specific blocker of dopamine receptors	Diabetic Gastroparesis Gastrointestinal symptoms Upper gastrointestinal motility disorders
DB01238	Aripiprazole	Agonism of dopaminic and 5-HT1A receptors and antagonism of alpha adrenergic and 5-HT2A receptors	Agitation Bipolar 1 disorder Irritability Major Depressive Disorder (MDD) Mixed manic depressive episode Psychosis Psychotic Depression Schizophrenia Tourette's Disorder (TD) Acute Manic episode
DB01267	Paliperidone	Central dopamine Type 2 (D2) and serotonin Type 2 (5HT2A) receptor antagonism. Paliperidone is also active as an antagonist at alpha 1 and alpha 2 adrenergic receptors and H1 histaminergic receptors	Delusional Parasitosis Schizoaffective Disorders Schizophrenia
DB06144	Sertindole	Affinity for dopamine D2, serotonin 5-HT2A and 5-HT2C, and alpha1-adrenoreceptors	Schizophrenia
DB06684	Vilazodone	High affinity and selectivity for the 5-hydroxytryptamine (5-HT) transporter and 5-HT(1A) receptors	MDD
DB06817	Raltegravir	Antiretroviral drug produced by Merck & Co., used to treat HIV infection	Human Immunodeficiency Virus Type 1 (HIV-1) Infection
DB08883	Perampanel	A noncompetitive AMPA glutamate receptor antagonist	Grand mal Generalized tonic–clonic seizure Partial-onset seizures
DB08896	Regorafenib	An orally administered inhibitor of multiple kinases	Metastatic GIST Locally advanced GIST Refractory, metastatic colorectal cancer Unresectable GIST
DB08907	Canagliflozin	A sodium-glucose cotransporter 2 (SGLT2) inhibitor	Cardiovascular events Cardiovascular mortality End Stage Renal Disease (ESRD) Type 2 Diabetes Mellitus Elevated serum creatinine Hospitalization due to cardiac failure
DB08930	Dolutegravir	A HIV-1 intergrase inhibitor	HumanImmunodeficiencyVirusType1(HIV-1)Infection
DB09042	Tedizolid phosphate	Generally effective against multidrug-resistant Gram-positive bacteria	Acute bacterial skin and skin structure infections
DB09128	Brexpiprazole	A novel D2 dopamine and serotonin 1A partial agonist	MDD Schizophrenia
DB11703	Acalabrutinib	Bruton Tyrosine Kinase inhibitor	Chronic Lymphocytic Leukemia (CLL) Mantle Cell Lymphoma (MCL) Small Lymphocytic Lymphoma
DB11732	Lasmiditan	Selective agonism of the 5-HT1F receptor	Migraine headache, with or without Aura
DB11791	Capmatinib	A small molecule kinase inhibitor	Metastatic Non-Small Cell Lung Cancer
DB11793	Niraparib	Orally active PARP inhibitor	Fallopian Tube Cancer Ovarian Epithelial Cancer Primary Peritoneal Cancer
DB12836	Grapiprant	Prostaglandin receptor antagonists	The effects of grapiprant have been reported to be effective in the relief from arthritic pain in canine patients
DB12867	Benperidol	Mechanism not clearly known	Dementia, Depression, Schizophrenia, Anxiety Disorders, and Psychosomatic Disorders, among others.
DB12978	Pexidartinib	A selective tyrosine kinase inhibitor (CSF1)	SymptomaticTenosynovialGiantCellTumor
DB13931	Netarsudil	A Rho kinase inhibitor	Increased intraocular pressure
DB15305	Risdiplam	Orally bioavailable mRNA splicing modifier	SMA

Figure 4. Molecular docking of Sorafenib to DHODH crystal structure (PDB:6j3c) showing interaction with amino acids TYR356, ALA55, ARG136, VAL143, ALA59, HIS56, THR360, MET43, LEU46, LYS100, PRO216 and PHE149.

interaction with the majority of the drugs (20, 19 and 19 drugs, respectively). These were followed by ALA55, TYR356 (17 and 15 drugs, respectively). The amino acids showed to be accumulated at the active site cavity, with ability to inhibit the enzyme at this position through competitive inhibition mechanism (Figure 4).

Target prediction analysis

The possible targets were discovered with the SwissTargetPrediction and PASS prediction tools [63, 64]. In the SwissTarget-Prediction, the interactions of 376 342 compounds with 3068 proteins consist of an experimentally proven data set. The querry molecule is matched according to similarity among 376 342 compounds and the estimated target proteins are determined. The 2D similarity is measured based on Tanitomo index and 3D similarity is measured based on Manhattan distance similarity quantity between Electoroscape 5D flat vector [63]. This tool searches among known ligands based on 2D and 3D similarities to a given querry to predict possible targets. In addition to SwissTargetPrediction the prediction of activity spectra for substances (PASS) web tool was also used for comparison. PASS web tool calculates using Bayesian approach by considering 2D structural similarities, only. PASS calculates probability for the query to be biologically active (Pa) or inactive (Pi) on target regions. This tool analyzes over 20 000 principal compounds and 180 000 biological related compounds using the MDDR database. In addition, PASS can analyze 3678 pharmacological activity [64].

In silico target prediction analysis of potential DHODH inhibitor candidates determined by comparing to FDA-approved DHODH inhibitor, Leflunomide. Leflunomide is shown as probable inhibitor to other oxidoreductates, xanthine dehydrogenase, monoamine oxidase B, arachidonate-5-lipogenase, cylclooxygenase-2 according to SwissTargetPrediction. Similarly, a number of drug candidates were also shown as probable inhibitors to the same oxidoreductases (Table 4). While only acalabrutinib and raltegravir was found directly associated to DHODH, Aripiprazole, Benperidol, Brexpiprazole, Canagliflozin, Capmatinib, Domperidone, Droperidol, Grapiprant, Lasmiditan, Perampanel, Raltegravir, Risdiplam, Sertindole and Thedizolid phosphate have been found to be putative targets for other various oxidoreductases.

When compared using PASS, 4 out of 28 number of drugs were found directly associated to Dihydroorotase inhibition,

as well as Leflunomide, suggesting a sound the experimental design. Capmatinib, Netarsudil, Regorafenib and Sorafenib are determined as Dihydroorotase inhibitor by PASS, contradicting SwissTargetPrediction results. However, an anti-inflammatory activity was associated to a larger set including Dolutegravir, Lasmiditan, Mastinib, Netarsudil, Paliperidone, Perampanel, Pexidartinib, Risdiplam and Risperidone, most of which were not associated to DHODH inhibition directly (see Table S3 available online at https://academic.oup.com/bib).

Discussion

Association with serotonin–dopamine receptors

As mentioned previously, eight of the candidates, were found to be used as serotonin–dopamine receptor antagonists using SwissTargetPrediction. Oddly, in agreement with it, Leflunomide, as the known DHODH inhibitor but not being listed in our drug candidates, also showed high similarity to dopamine and norepinephrine transporter antagonists as well as the DHODH effectors. In addition, antagonists of other serotonin-receptors, 5HT-6, 5HT-2a, 5HT-2b, 5HT-2c, also show a degree of similarity with Leflunomide (Table 5).

The serotonin function is regulated by 7 members (15 subtypes) of the serotonin receptor in mammals [90]. Immune cells express 5HT-1, 5HT-2, 5HT-3, 5HT-4 and 5HT-7 class serotonin receptor, serotonin transporter (SERT), important enzyme for serotonin synthesis (TPH) and monoamine oxidase (MAO) for serotonin degradation [91].

Remarkably, among our candidates, Aripirazole, Domperidone, Lasmiditan, and Sertindole showed to be among broad serotonin receptor antagonists as well as oxidoreductase inhibitors. Sertindole and Aripiprazole also have structural similarity with monoamine oxidase enzyme inhibitors. The fact that 5-hydroxytrypamine (5-HT) antagonists are also shown as probable inhibitor for monoamine oxidase, which plays a role in serotonin degradation, and show high binding affinity to DHODH according to molecular docking analysis raises the question whether DHODH plays a role in the anti-inflammatory effect of 5-HT antagonists. In structural similarity analyzes, similar targets were found between Leflunomide and 5-HT antagonist drugs, and 5-HT antagonist drugs were among the molecules with the highest binding affinity to the DHODH enzyme among the 7900 molecules. It has been demonstrated by other studies that 5-HT antagonists cause suppression of cytokine release in immune cells by causing serotonin blocking [90, 92–96]. However these studies did not consider or tested for DHODH inhibition that may be on the main pathway to the anti-inflammatory effect.

The aforementioned SwissTargetPrediction results are also supported by the PASS analysis. Accordingly, besides our reference molecule, Leflunomide, Acalabrutinib, Aripiprazole, Benperidol, Brexipipirazole, Domperidone, Droperidol, Glipizide, Glyburide, Imatinib, Lasmiditan, Masitinib, Netarsudil, Nirapanib, Paliperidone, Risperidone, Sertindole and Vilazodone are predicted as antagonists for various members of 5-HTs. Ten of these drugs were also associated to mood disorder treatment.

Briefly, an apparent similarity between serotonin–dopamine receptor inhibitors and DHODH inhibitors was supported by both *in silico* analysis tools we have utilized. Despite the anti-inflammatory effects of 5-HT antagonist drugs used in diseases such as schizophrenia and depression have not been fully elucidated. For this reason, it should be taken into consideration that

Table 3. The interactions between amino acids and identified drugs predicted through the analysis of Autodock 4 docking results using DS visualizer.

Amino acids	acalabrutinib	aripiprazole	benperidol	brexipiprazole	canagliflozin	capmatinib	dolutegravir	domperidone	droperidol	glyburide	glypizide	graplipant	imatinib	lasmiditan	masitinib	netarsudil	niraparib	paliperidone	perampanel	pexidartinib	regorafenib	risdiplam	risperidone	sertindole	sorafenib	tedizolid phosphate	vilazodone	Total
THR285												X							X									2
ALA96										X									X									2
VAL333												X							X									2
ALA95								X			X							X	X									4
LYS255																			X									1
TYR356	X	X			X			X		X		X	X	X	X	X		X	X			X		X	X			15
ALA55	X	X			X	X		X		X		X	X	X	X	X	X	X		X	X		X		X			17
ARG136	X		X			X	X					X						X							X	X	X	9
PRO364	X	X	X	X	X	X						X	X				X			X		X						11
VAL143	X	X		X	X	X		X			X	X	X	X	X	X	X	X			X	X	X	X	X			19
ALA59	X		X	X		X		X			X	X	X	X	X	X	X			X	X	X	X	X	X		X	19
HIS56	X	X	X	X	X	X	X	X	X			X	X			X				X	X	X	X	X	X	X	X	20
THR360	X			X		X	X	X		X		X					X	X			X	X		X				12
SER305	X																											1
PHE62				X					X				X	X		X				X		X						7
TYR147				X																X		X					X	4
MET111				X																X								2
PRO52				X	X					X			X					X		X								6
VAL134				X		X								X		X	X				X	X		X			X	9
LEU359			X	X		X			X		X		X				X			X	X		X		X	X	X	13
MET43			X	X		X		X	X				X			X	X				X				X	X	X	12
LEU46										X			X			X		X		X	X				X	X		8
LYS100												X										X				X		3
THR357					X				X	X			X								X							5
ASN145									X							X												2
ASN284					X																							1
LEU67														X														1
LEU58									X	X			X							X								4
TYR38						X										X												2
LEU42									X											X								2
PHE98																												1
PRO216																									X			1
PHE149																									X			1
THR63																							X					1

Table 4. Those with similarity to various oxidoreductase inhibitors among the FDA approved drugs identified in this study.

Drug name	Target	Common name	ChEMBL ID	Target class	Probability (%)	Known actives (3D/2D)
Leflunomide	Dihydroorotate dehydrogenase (by homology)	DHODH	CHEMBL1966	Oxidoreductase	100	12/4
	Xanthine dehydrogenase	XDH	CHEMBL1929	Oxidoreductase	11.2	4/0
	Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	11.2	40/0
	Arachidonate 5-lipoxygenase	ALOX5	CHEMBL215	Oxidoreductase	11.2	6/0
	Cyclooxygenase-2	PTGS2	CHEMBL230	Oxidoreductase	11.2	78/0
Acalabrutinib	Dihydroorotate dehydrogenase (by homology)	DHODH	CHEMBL1966	Oxidoreductase	11	18/0
	Cyclooxygenase-2	PTGS2	CHEMBL230	Oxidoreductase	11	343/0
	Inosine-5′-monophosphate dehydrogenase 2	IMPDH2	CHEMBL2002	Oxidoreductase	11	89/0
	Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	11	319/0
Aripiprazole	Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	10.6	62/15
	Egl nine homolog 1	EGLN1	CHEMBL5697	Oxidoreductase	10.6	20/0
	Arachidonate 5-lipoxygenase	ALOX5	CHEMBL215	Oxidoreductase	10.6	53/0
Benperidol	Egl nine homolog 1	EGLN1	CHEMBL5697	Oxidoreductase	11.5	51/0
Brexpiprazole	Arachidonate 5-lipoxygenase	ALOX5	CHEMBL215	Oxidoreductase	11.8	80/0
Canagliflozin	Glyceraldehyde-3-phosphate dehydrogenase liver	GAPDH	CHEMBL2284	Oxidoreductase	11.8	10/0
	Inosine-5′-monophosphate dehydrogenase 2	IMPDH2	CHEMBL2002	Oxidoreductase	11.8	34/0
Capmatinib	Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	11.8	278/0
	Inosine-5′-monophosphate dehydrogenase 2	IMPDH2	CHEMBL2002	Oxidoreductase	11.8	173/0
Domperidone	Inosine-5′-monophosphate dehydrogenase 2	IMPDH2	CHEMBL2002	Oxidoreductase	10.6	99/0
	Cyclooxygenase-2	PTGS2	CHEMBL230	Oxidoreductase	10.6	502/0
Droperidol	Inosine-5′-monophosphate dehydrogenase 2	IMPDH2	CHEMBL2002	Oxidoreductase	11.5	155/0
Grapiprant	HMG-CoA reductase (by homology)	HMGCR	CHEMBL402	Oxidoreductase	11	161/0
	Arachidonate 5-lipoxygenase	ALOX5	CHEMBL215	Oxidoreductase	11	167/0
Lasmiditan	Cyclooxygenase-2	PTGS2	CHEMBL230	Oxidoreductase	11.3	18/0
Perampanel	Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	11.3	116/0
	Dihydrofolate reductase	DHFR	CHEMBL202	Oxidoreductase	11.3	9/0
Raltegravir	Egl nine homolog 1	EGLN1	CHEMBL5697	Oxidoreductase	10.6	67/0
	4-hydroxyphenyl pyruvate dioxygenase	HPD	CHEMBL1861	Oxidoreductase	10.6	74/0
	Dihydroorotate dehydrogenase (by homology)	DHODH	CHEMBL1966	Oxidoreductase	0	12/0
	Monoamine oxidase A	MAOA	CHEMBL1951	Oxidoreductase	0	1/0
	Cyclooxygenase-2	PTGS2	CHEMBL230	Oxidoreductase	0	1/0
Risdiplam	Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	10.6	9/0
Sertindole	Monoamine oxidase A	MAOA	CHEMBL1951	Oxidoreductase	11.8	56/0
Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	11.8	70/0	
Thedizolid phosphate	Monoamine oxidase A	MAOA	CHEMBL1951	Oxidoreductase	15	0/11
	Monoamine oxidase B	MAOB	CHEMBL2039	Oxidoreductase	15	0/1

Notes: The data were obtained from the SwissTargetPrediction tool. The score (probability) between 0 and 1 was converted as a percentage. DHODH inhibitor, Leflunomide, is listed as reference.

possible DHODH inhibitory activities of the specified molecules may be responsible for the observed anti-inflammatory effect.

Support by clinical findings

Since the beginning of the pandemic, many different drug target regions have been investigated for the treatment of COVID19, and studies on the DHODH target continue to increase. In addition, there are studies in which the drugs we obtained in our list were tested against the virus. On 20 February 2020, the clinical study of Leflunomide (FDA-approved DHODH inhibitor) was used in Phase-2 (240 patients) COVID19, however, results on 28 patients were recently shared as pre-print (Chictr2000030058).

In April, Immun Therapeutics proved that their drug candidate, called IMU-838, acts as DHODH inhibitors. After their announcement that they will quickly try this drug in COVID19 patients, in which they show that they have antiviral and anti-inflammatory properties, Phase-2 and Phase-3 clinical studies were started in 600 patients in May 2020 (ClinicalTrials Code: NCT04379271). On 20 October 2020, Immun Therapeutics announced that IMU-838 has been supported by European Investment Bank for the possible treatment of COVID19. On 10 August 2020, Luban et al. announced as pre-print, novel DHODH inhibitor PTC299 inhibits SARS-CoV-2 replication at low dose (EC50 range, 2.0–31.6 nM with selectivity index > 3.8). Also, this drug has been found to suppress the release of important cytokines that play a role in ARDS (IL-6, IL17) and vascular endothelial growth factor (VEGF). In addition, a clinical study started on July 9 to investigate the effect of PTC299 on COVID19 (Clinical Code: NCT04439071) [36].

Table 5. List of discovered drugs showing similarity to serotonin–dopamine receptor antagonists

Drug name	Target	Common name	ChEMBL ID	Target class	Probability (%)	Known actives (3D/2D)
Leflunomide	Norepinephrine transporter	SLC6A2	CHEMBL222	Electrochemical transporter	100	20/1
	Dopamine transporter	SLC6A3	CHEMBL238	Electrochemical transporter	100	15/1
	Serotonin 6 (5-HT6) receptor	HTR6	CHEMBL3371	Family A G proteincoupled receptor	11.2	13/0
	Androgen Receptor	AR	CHEMBL1871	Nuclear receptor	11.2	176/0
	Serotonin 2a (5-HT2a) receptor	HTR2A	CHEMBL224	Family A G proteincoupled receptor	11.2	33/0
	Serotonin 2c (5-HT2c) receptor	HTR2C	CHEMBL225	Family A G proteincoupled receptor	11.2	35/0
	Serotonin 2b (5-HT2b) receptor	HTR2B	CHEMBL1833	Family A G proteincoupled receptor	0	16/0
	Dopamine D2 receptor	DRD2	CHEMBL217	Family A G proteincoupled receptor	0	1/0
Aripiprazole	Serotonin 2b (5-HT2b) receptor	HTR2B	CHEMBL1833	Family A G proteincoupled receptor	100	175/29
	Serotonin 1b (5-HT1b) receptor	HTR1B	CHEMBL1898	Family A G proteincoupled receptor	100	492/35
	Serotonin 3a (5-HT3a) receptor	HTR3A	CHEMBL1899	Family A G proteincoupled receptor	100	74/1
	Serotonin 1d (5-HT1d) receptor	HTR1D	CHEMBL1983	Family A G proteincoupled receptor	100	471/14
	Serotonin 1a (5-HT1a) receptor	HTR1A	CHEMBL214	Family A G proteincoupled receptor	100	1215/127
	Serotonin 2a (5-HT2a) receptor	HTR2A	CHEMBL224	Family A G proteincoupled receptor	100	1507/160
	Dopamine D1 receptor	DRD1	CHEMBL2056	Family A G proteincoupled receptor	100	196/18
	Dopamine D2 receptor	DRD2	CHEMBL217	Family A G proteincoupled receptor	100	3320/423
	Dopamine D3 receptor	DRD3	CHEMBL234	Family A G proteincoupled receptor	100	1610/162
	Dopamine D4 receptor	DRD4	CHEMBL219	Family A G proteincoupled receptor	100	647/181
	Serotonin 2c (5-HT2c) receptor	HTR2C	CHEMBL225	Family A G proteincoupled receptor	100	484/47
	Serotonin transporter	SLC6A4	CHEMBL228	Electrochemical transporter	100	1188/102
	HERG	KCNH2	CHEMBL240	Voltage-gated ion channel	100	1035/39
	Serotonin 6 (5-HT6) receptor	HTR6	CHEMBL3371	Family A G proteincoupled receptor	100	371/43
	Serotonin 7 (5-HT7) receptor	HTR7	CHEMBL3155	Family A G proteincoupled receptor	100	602/55
	Serotonin 5a (5-H5a) receptor	HTR5a	CHEMBL3426	Family A G proteincoupled receptor	100	34/1
	Prostanoid EP2 receptor	PTGER2	CHEMBL1881	Family A G proteincoupled receptor	10.6	9/0
Domperidone	Serotonin 2b (5-HT2b) receptor	HTR2B	CHEMBL1833	Family A G proteincoupled receptor	100	60/4
	Serotonin 1a (5-HT1a) receptor	HTR1A	CHEMBL214	Family A G proteincoupled receptor	58.9	277/20
	Serotonin 2a (5-HT2a) receptor	HTR2A	CHEMBL224	Family A G proteincoupled receptor	100	413/35
	Serotonin 6 (5-HT6) receptor	HTR6	CHEMBL3371	Family A G proteincoupled receptor	10.6	204/13
	Serotonin 7 (5-HT7) receptor	HTR7	CHEMBL3155	Family A G proteincoupled receptor	10.6	128/45
	Serotonin 2c (5-HT2c) receptor	HTR2C	CHEMBL225	Family A G proteincoupled receptor	100	291/6
	Dopamine D2 receptor	DRD2	CHEMBL217	Family A G proteincoupled receptor	100	529/73
	Dopamine D3 receptor	DRD3	CHEMBL234	Family A G proteincoupled receptor	100	245/28
	Dopamine D4 receptor	DRD4	CHEMBL219	Family A G proteincoupled receptor	10.6	280/16
	Serotonin transporter	SLC6A4	CHEMBL228	Electrochemical transporter	100	128/25
	HERG	KCNH2	CHEMBL240	Voltage-gated ion channel	100	378/69
	Norepinephrine transporter	SLC6A2	CHEMBL222	Electrochemical transporter	100	10/31
Lasmiditan	Serotonin 1f (5-HT1f) receptor	HTR1F	CHEMBL1805	Family A G proteincoupled receptor	67.3	93/50
Sertindole	Serotonin 1b (5-HT1b) receptor	HTR1B	CHEMBL1898	Family A G proteincoupled receptor	100	535/241
	Serotonin 1d (5-HT1d) receptor	HTR1D	CHEMBL1983	Family A G proteincoupled receptor	11.8	488/218
	Serotonin 1a (5-HT1a) receptor	HTR1A	CHEMBL214	Family A G proteincoupled receptor	100	1452/128
	Serotonin 2a (5-HT2a) receptor	HTR2A	CHEMBL224	Family A G proteincoupled receptor	100	1689/119
	Dopamine D1 receptor	DRD1	CHEMBL2056	Family A G proteincoupled receptor	100	244/14
	Dopamine D2 receptor	DRD2	CHEMBL217	Family A G proteincoupled receptor	100	3894/335
	Dopamine D3 receptor	DRD3	CHEMBL234	Family A G proteincoupled receptor	100	1715/60
	Dopamine D4 receptor	DRD4	ChEMBL219	Family A G proteincoupled receptor	100	851/41
	Serotonin 2c (5-HT2c) receptor	HTR2C	CHEMBL225	Family A G proteincoupled receptor	100	649/41
	Serotonin transporter	SLC6A4	CHEMBL228	Electrochemical transporter	11.8	1598/275
	HERG	KCNH2	CHEMBL240	Voltage-gated ion channel	100	1071/49
	Serotonin 6 (5-HT6) receptor	HTR6	CHEMBL3371	Family A G proteincoupled receptor	100	600/59
	Serotonin 7 (5-HT7) receptor	HTR7	CHEMBL3155	Family A G proteincoupled receptor	11.8	842/22

Notes: The data were obtained from the SwissTargetPrediction tool. The score (probability) between 0 and 1 was converted as a percentage. DHODH inhibitor, Leflunomide, is listed as reference.

On April 28, the Phase-1 study started on 20 COVID19 patients for the drug Leflunomide in the USA (ClinicalTrials Code: NCT04361214). The first clinical trial results of leflunomide (Chictr2000030058), an FDA-approved DHODH inhibitor, in Covid19 disease, Wang *et al*. published by preprint [97]. In an open-label controlled study conducted in Wuhan between 13 March and 17 April, leflunomide was found to be an effective drug in Covid19 disease. Twelve patients were determined as standard of care (SOC) group and 15 patients as SOC + leflunomide group. After 14 days, leflunomide treatment showed that patients returned to a highly negative rate compared to the SOC group (80% versus 16.7% SOC). In consequence of the study, no adverse effects or deaths were detected.

Ellinger *et al*. screened 5632 candidate drugs on caco-2 cells against Sars-CoV-2. The multikinase inhibitors Sorafenib and Regorafenib in our list have been stated in the study published as that study and are among the effective molecules against Sars-CoV-2 [98]. Klann *et al*. investigated by phosphoproteome analysis after caco-2 cell infection how Sars-CoV-2 changed the signaling network in the host cell. In this study, after infection 2197 phosphopeptide increased and 799 phosphopeptide decreased. As a result of detailed bioinformatic analysis on viral proteins residues, it was revealed that viral proteins can be

phosphorylated by kinases. Therefore, different kinase inhibitors were preferred and the effects of these molecules on Sars-CoV-2 were revealed. It has been reported that sorafenib, which is also on our list, is effective against Sars-CoV-2 at a very low dose (EC50: 4.85 μM). Considering the cytotoxic effect of the molecule, it appears that it can be an important virus inhibitor. In addition to sorafenib, there are multikinase inhibitors such as capmatinib, regorafenib, pexidartinib on our list. With this study, the effective role of multi-kinase inhibitors against Sars-CoV-2 infection suggests that the activities of these molecules may be related to possible DHODH activation [99]. In another study by Weston *et al*. published in another pre-print, 20 FDA-approved drugs were analyzed against Sars-CoV-2 *in vivo* and *in vitro*. In this study, it is stated that imatinib plays a highly effective role against the virus with a selectivity index value of more than 9.5 [100].

The results were followed up with ongoing clinical studies (Clinical code: NCT04357613, NCT04394416, EudraCT2020-001236-10). On the other hand, a cell culture study showed that a high imatinib concentration would be required for demonstrated viral inhibition in the host cells [101]. However, the same study also suggests the potential of İmatinib for its anti-inflammatory effect for COVID19 treatment which may explain the low incidence of COVID19 among chronic myeloid leukemia patients who take imatinib [102–104].

The effect of Acalabrutinib, known as Bruton Tyrosine Kinase (BTK) inhibitor, which is one of the drugs that we have identified as a possible DHODH inhibitor in our list, has been clinically researched and published by Roschewski *et al*. on June 5 [105]. As a hypothesis, the researchers predicted that acalabrutinib's BTK inhibitor activity can prevent ARDS in covid19 disease by blocking cytokine release in macrophages. Along with acalabrutinib treatment, a significant increase was observed in the oxygen respiration capacities of the patients and dramatic decrease was detected in IL-6 levels. Researchers suggest that acalabrutinib can be used effectively to suppress inflammation leading to a clinical study started (Clinical Code: NCT04497948).

A study published on 24th of July by Riva *et al*., is quite remarkable when compared to our own data. Researchers have created a library called ReFRAME, which consists of 12 000 FDA-approved drugs and others in clinical trials. Molecules with an effective EC_{50} value were selected, 21 were selected and 13 drugs were found to be effective to suppress Sars-CoV-2. When these experimentally identified drugs were compared to our findings, remarkable similarity can be seen. The fact that known target regions of these 13 selected molecules include serotonin and dopamine antagonists, coincides with the fact that 8 of 28 FDA-approved drugs we have identified are too serotonin and dopamine antagonists. Especially, it was determined that Elopiprazole, a serotonin 1a-Dopamine D2 receptor antagonist, did not cause any changes in the number of cells by curb the infection in Vero6-Sars-CoV-2 infected cells at very low doses (EC_{50}: 1.6 μM). Elopiprazole is not included in Drugbank and, ergo, this study. Another effective molecule found in the study, Apilimod, was found effective in Phase II clinical studies against diseases such as RA, common variable immune deficiency (CVID). In addition, it has been reported by researchers that this drug has been found to suppress reproduction of EBOV, Lassa virus, Marburg viruses in human cells. Apilimod showed similarities with serotonin 2b-2c antagonists. It is noteworthy that the information obtained about the apilimod and the given effects are similar to the expected effects as a result of DHODH inhibition, raising the question whether an interaction between DHODH and the apilimod exists. When we analyzed this drug, which

was eliminated only after seventh docking analysis (against 6j3c, 2wv8, 3kvj, 3kvl, 4igh, 4jtu and finally 4zl1) showed affinity in a range of −11.0 and −12.9 kcal/mol according to Autodock Vina. In accordance, our extensive potential DHODH inhibitor screening shows strong association between serotonin–dopamine receptor antagonists and possible DHODH inhibitors compared to this study [106].

Conclusion

With the help of *in silico* analyzes, drug repurposing studies are increasing day by day, targeting such as Sars-CoV-2 spike protein structure or RNA polymerases [98, 100, 107–109]. However, considering that DHODH is a possible target for COVID19 treatment, there is no *in silico* analysis available focusing FDA-approved drugs. It is our hope that these pre-approved drugs may provide a quicker response to the pandemic than other drug discovery approaches.

Here, we have analyzed 7900 molecules with 3D structures for their putative binding at the active site of DHODH using docking analysis as a potential target candidate for COVID19 treatment. Uniquely, we have provided a straightforward method to filter and identify the most probable antagonist candidates by sequentially docking for several DHODH structures available. Any candidate that provided an affinity above the threshold is filtered until 28 FDA-approved and 79 clinically tested drugs were left. Next, the discovered drugs were analyzed using target prediction tools and the literature was scanned for their effect against Sars-CoV-2. We have compared our candidates with FDA-approved DHODH inhibitor, Leflunomide.

In summary, a number of FDA-approved drugs that may be more effective in DHODH inhibition than Leflunomide were identified by the sequential molecular docking analysis, and their potential for COVID19 treatment was discussed. The target regions and structural similarities of these drugs with high binding energy are also discussed in detail. Interestingly, it is quite remarkable that the drugs that show high binding energy against DHODH enzyme on our list have similarities to other oxidoreductase inhibitors as well as the antagonist of dopamine and serotonin receptors. Infact the anti-inflammatory effect shown by many serotonin–dopamine receptor inhibitors may be associated with DHODH inhibition. We suggest that the COVID19 prevalence among patients with diseases treated with these antagonists should be followed. After determining the DHODH enzyme inhibition by the 28 FDA-approved drugs and *in vitro* demonstration of Sars-CoV-2 inhibition, we recommend that the active drugs may be tested clinically on COVID19 patients.

Key Points

- DHODH inhibition may play an important role in the treatment of COVID19.
- Both the syndrome of excessive cytokine release and the replication of the virus can be prevented by DHODH inhibition.
- 28 FDA-approved drugs were discovered to be potential new DHODH inhibitors.
- The majority (8/28) of potential DHODH inhibitory drugs are serotonin–dopamine receptor antagonists, suggesting that other serotonin–dopamine receptor antagonists may be associated with DHODH inactivation.

- It has been predicted that the anti-inflammatory effect of serotonin–dopamine receptor antagonists may be related to DHODH inactivation. It may also be an important finding to follow up those who use these drugs with COVID19.

Supplementary data

Supplementary data are available online at *Briefings in Bioinformatics*.

References

1. Zhou P, Lou YX, Wang XG, *et al*. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;**579**:270–3.
2. Lu R, Zhao X, Li J, *et al*. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020;**395**:565–74.
3. Maxmen A. More than 80 clinical trials launch to test coronavirus treatments. *Nature* 2020;**578**(7795):347–8.
4. Rabi FA, Al Zoubi MS, Al-Nasser AD, *et al*. Sars-cov-2 and coronavirus disease 2019: what we know so far. *Pathogens* 2020;**9**:1–14.
5. Wang M, Cao R, Zhang L, *et al*. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020;**30**:269–71.
6. Murithi JM, Owen ES, Istvan ES, *et al*. Combining stage specificity and metabolomic profiling to advance antimalarial drug discovery. *Cell Chem Biol* 2020;**27**:158–171.e3.
7. Vincent MJ, Bergeron E, Benjannet S, *et al*. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virol J* 2005;**2**(69).
8. Hoffmann M, Kleine-Weber H, Krüger N, *et al*. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020;**181**(2):271–280.e8. doi: https://doi.org/10.1016/j.cell.2020.02.052.
9. Li X, Geng M, Peng Y, *et al*. Molecular immune pathogenesis and diagnosis of COVID-19. *J Pharm Anal* 2020;**19**:1–7.
10. Imai Y, Kuba K, Rao S, *et al*. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005;**436**:112–6.
11. Channappanavar R, Fehr AR, Vijay R, *et al*. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. *Cell Host Microbe* 2016;**19**(2):181–93.
12. Channappanavar R, Fehr AR, Zheng J, *et al*. IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. *J Clin Invest* 2019;**129**(9):3625–39.
13. Yang Y, Zhang L, Geng H, *et al*. The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory syndrome coronavirus (MERS-CoV) are potent interferon antagonists. *Protein Cell* 2013;**4**(12):951–61.
14. Wang Y, Zhang D, Du G, *et al*. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020;**395**:1569–78.
15. Favalli EG, Ingegnoli F, De Lucia O, *et al*. COVID-19 infection and rheumatoid arthritis: faraway, so close! *Autoimmun Rev* 2020;**19**(5):102523.
16. Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. *Lancet* 2010;**376**(9746):1094–108.
17. Zhang W, Zhao Y, Zhang F, *et al*. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): the experience of clinical immunologists from China. *Clin Immunol* 2020;**214**:108393.
18. Breedveld FC, Dayer JM. Leflunomide: mode of action in the treatment of rheumatoid arthritis. *Ann Rheum Dis* 2000;**59**:841–9.
19. Löffler M, Zameitat E. Pyrimidine biosynthesis and degradation (catabolism). Lennarz WJ, Lane MD(eds) *Encycl Biol Chem Second Ed*, Academic Press, 2013, pp. 712–8. doi: 10.1016/B978-0-12-378630-2.00178-X.
20. Löffler M, Carrey EA, Zameitat E. Orotic acid, more than just an intermediate of pyrimidine de novo synthesis. *J Genet Genomics* 2015;**42**(5):207–19.
21. Sykes DB. The emergence of dihydroorotate dehydrogenase (DHODH) as a therapeutic target in acute myeloid leukemia. *Expert Opin Ther Targets* 2018;**22**:893–8.
22. Tan JL, Fogley RD, Flynn RA, *et al*. Stress from nucleotide depletion activates the transcriptional regulator HEXIM1 to suppress melanoma. *Mol Cell* 2016;**62**(1):34–46.
23. Fitzpatrick LR, Deml L, Hofmann C, *et al*. 4SC-101, a novel immunosuppressive drug, inhibits IL-17 and attenuates colitis in two murine models of inflammatory bowel disease. *Inflamm Bowel Dis* 2010;**16**(10):1763–77.
24. Fitzpatrick LR, Small JS, Doblhofer R, *et al*. Vidofludimus inhibits colonic interleukin-17 and improves hapten-induced colitis in rats by a unique dual mode of action. *J Pharmacol Exp Ther* 2012;**342**(3):850–60.
25. Mascia L, Turchi G, Bemi V, *et al*. Uracil salvage pathway in PC12 cells. *Biochim Biophys Acta Gen Subj* 2000;**1524**(1):45–50.
26. Sykes DB, Kfoury YS, Mercier FE, *et al*. Inhibition of dihydroorotate dehydrogenase overcomes differentiation blockade in acute myeloid leukemia. *Cell* 2016;**167**:171–186.e15.
27. Lewis TA, Sykes DB, Law JM, *et al*. Development of ML390: a human DHODH inhibitor that induces differentiation in acute myeloid leukemia. *ACS Med Chem Lett* 2016;**7**(12):1112–7.
28. Singh A, Maqbool M, Mobashir M, *et al*. Dihydroorotate dehydrogenase: a drug target for the development of antimalarials. *Eur J Med Chem* 2017;**125**:640–51.
29. Mei-jiao G, Shi-fang L, Yan-yan C, *et al*. Antiviral effects of selected IMPDH and DHODH inhibitors against foot and mouth disease virus. *Biomed Pharmacother* 2019;**118**:1–7.
30. Chen S, Ding S, Yin Y, *et al*. Suppression of pyrimidine biosynthesis by targeting DHODH enzyme robustly inhibits rotavirus replication. *Antiviral Res* 2019;**167**:35–44.
31. Wang Q-Y, Bushell S, Qing M, *et al*. Inhibition of dengue virus through suppression of host pyrimidine biosynthesis. *J Virol* 2011;**85**(13):6548–56.
32. Hoffmann HH, Kunz A, Simon VA, *et al*. Broad-spectrum antiviral that interferes with de novo pyrimidine biosynthesis. *Proc Natl Acad Sci USA* 2011;**108**(14):5777–82.
33. Xiong R, Zhang L, Li S, *et al*. Novel and potent inhibitors targeting DHODH are broad-spectrum antivirals against RNA viruses including newly-emerged coronavirus SARS-CoV-2. *Protein Cell* 2020;**11**:723–39.
34. Liu Q, Gupta A, Okesli-Armlovich A, *et al*. Enhancing the antiviral efficacy of RNA-dependent RNA polymerase inhibition by combination with modulators of pyrimidine metabolism. *Cell Chem Biol* 2020;**27**:668–677.e9.

35. Zheng J, Zhang Y, Liu Y, *et al*. Multi-omics study revealing tissue-dependent putative mechanisms of SARS-CoV-2 drug targets on viral infections and complex diseases. *medRxiv* 2020; 2020.05.07.20093286. doi: 10.1101/2020.05.07.20093286.

36. Luban J, Sattler R, Mühlberger E, *et al*. The DHODH inhibitor PTC299 arrests SARS-CoV-2 replication and suppresses induction of inflammatory cytokines. *bioRxiv* 2020; 2020.08.05.238394. doi: 10.1016/j.virusres.2020.198246.

37. Immunic, Inc. *Reports that IMU-838, a Selective Oral DHODH Inhibitor has Demonstrated Preclinical Activity Against SARS-CoV-2 and Explores Plans for a Phase 2 Clinical Trial in COVID-19 Patients*. Immunic Therapeutics, 2020, Press release. https://www.immunic-therapeutics.com/2020/04/21/immunic-inc-reports-that-imu-838-a-selective-oral-dhodh-inhibitor-has-demonstrated-preclinical-activity-against-sars-cov-2-and-explores-plans-for-a-phase-2-clinical-trial-in-covid-19-patients/.

38. Cavasotto CN, Di Filippo JI. In silico drug repurposing for COVID-19: targeting SARS-CoV-2 proteins through docking and consensus ranking. *Mol Inform* 2020;**39**:2000115.

39. Elmezayen AD, Al-Obaidi A, Şahin AT, *et al*. Drug repurposing for coronavirus (COVID-19): in silico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. *J Biomol Struct Dyn* 2020;1–13. doi: 10.1080/07391102.2020.1758791.

40. Liu C, Zhou Q, Li Y, *et al*. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. *ACS Cent Sci* 2020;**6**(3):315–31.

41. Shah B, Modi P, Sagar SR. In silico studies on therapeutic agents for COVID-19: drug repurposing approach. *Life Sci* 2020;**1**(252):117652.

42. Diao Y, Lu W, Jin H, *et al*. Discovery of diverse human dihydroorotate dehydrogenase inhibitors as immunosuppressive agents by structure-based virtual screening. *J Med Chem* 2012;**55**:8341–9.

43. Wishart DS, Feunang YD, Guo AC, *et al*. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res* 2018;**46**(D1):D1074–82.

44. O'Boyle NM, Banck M, James CA, *et al*. Open babel: an open chemical toolbox. *J Chem* 2011;**3**:33.

45. Berman H, Henrick K, Nakamura H. Announcing the worldwide protein Data Bank. *Nat Struct Biol* 2003;**10**:980.

46. Fritzson I, Svensson B, Al-Karadaghi S, *et al*. Inhibition of human dhodh by 4-hydroxycoumarins, fenamic acids, and n-(alkylcarbonyl)anthranilic acids identified by structure-guided fragment selection. *ChemMedChem* 2010;**5**(4):608–17.

47. McLean LR, Zhang Y, Degnen W, *et al*. Discovery of novel inhibitors for DHODH via virtual screening and X-ray crystallographic structures. *Bioorg Med Chem Lett* 2010;**20**(6):1981–4.

48. Das P, Deng X, Zhang L, *et al*. SAR-based optimization of a 4-quinoline carboxylic acid analogue with potent antiviral activity. *ACS Med Chem Lett* 2013;**4**(6):517–21.

49. RCSB PDB ID: 4JTU; Zhu L, Zhu J, Ren X, Li H. Crystal structure of human dihydroorotate dehydrogenase (DHODH) with brequinar analogue. 2003. doi: 10.2210/pdb4JTU/pdb.

50. RCSB PDB ID: 4ZL1; Huang J, Wu D, Ouyang P, *et al*. Crystal structure of human dihydroorotate dehydrogenase (DHODH) with 18X at 1.86 A resolution. 2015. doi: 10.2210/pdb4ZL1/pdb.

51. RCSB PDB ID: 4ZMG; Ren XL, Zhu JS, Zhu LL, Li HL. Crystal structure of human dihydroorotate dehydrogenase (DHODH) with DH03A338. 2016. doi: 10.2210/pdb4ZMG/pdb.

52. RCSB PDB ID: 5H2Z; Wu D, Huang J. Crystal structure of Human Dihydroorotate Dehydrogenase (DHODH) with 7GF. 2017. doi: 10.2210/pdb5H2Z/pdb.

53. RCSB PDB ID: 5H73; Huang J, Wu D. Crystal structure of human DHODH with 18F. 2017. doi: 10.2210/pdb5H73/pdb.

54. RCSB PDB ID: 5HQE; Huang J, Wu D, Lu Q. Crystal structure of human dihydroorotate dehydrogenase (DHODH) with compound 18T. 2016. doi: 10.2210/pdb5HQE/pdb.

55. Sainas S, Pippione AC, Giorgis M, *et al*. Design, synthesis, biological evaluation and X-ray structural studies of potent human dihydroorotate dehydrogenase inhibitors based on hydroxylated azole scaffolds. *Eur J Med Chem* 2017;**129**:287–302.

56. RCSB PDB ID: 6IDJ; Shiba T, Inaoka DK, Sato D, *et al*. Crystal structure of human DHODH in complex with ferulenol. doi: 10.2210/pdb6IDJ/pdb.

57. Zeng T, Zuo Z, Luo Y, *et al*. A novel series of human dihydroorotate dehydrogenase inhibitors discovered by in vitro screening: inhibition activity and crystallographic binding mode. *FEBS Open Bio* 2019;**9**(8):1348–54.

58. Zuo Z, Liu X, Qian X, *et al*. Bifunctional Naphtho[2,3-d][1,2,3]triazole-4,9-dione Compounds Exhibit Antitumor Effects In Vitro and In Vivo by Inhibiting Dihydroorotate Dehydrogenase and Inducing Reactive Oxygen Species Production. *J of Med Chem* 2020;**63**(14):7633–52.

59. RCSB PDB ID: 6LZL; Liu ZH, Wu D, Lu WQ, Huang J. Crystal structure of human dihydroorotate dehydrogenase (DHODH) with Piperine. 2020. doi: 10.2210/pdb6LZL/pdb.

60. Holm L, Elofsson A. Benchmarking fold detection by DaliLite v.5. *Bioinformatics* 2019;**35**(24):5326–7.

61. Morris GM, Goodsell DS, Pique ME, *et al*. Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity. *J Comput Chem* 2009;**30**(16):2785–91.

62. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2009;**31**(2):455–61.

63. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res* 2019;**47**:W357–3664.

64. Parasuraman S. Prediction of activity spectra for substances. *J Pharmacol Pharmacother* 2011;**2**:52–3.

65. Abdelgalil AA, Alkahtani HM, Al-Jenoobi FI. Sorafenib. *Profiles Drug Subst Excipients Relat Methodol* 2019;**44**:239–66.

66. Wilhelm SM, Adnane L, Newell P, *et al*. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther* 2008;**7**(10):3129–40.

67. Bruix J, Qin S, Merle P, *et al*. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2017;**389**(10064):56–66.

68. Li J, Qin S, Xu R, *et al*. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2015;**16**(6):619–29.

69. Roskoski R. Properties of FDA-approved small molecule protein kinase inhibitors: a 2020 update. *Pharmacol Res* 2020;**152**:104609.

70. Tap WD, Gelderblom H, Palmerini E, *et al*. Pexidartinib versus placebo for advanced tenosynovial giant cell

tumour (ENLIVEN): a randomised phase 3 trial. *Lancet* 2019;**394**:478–87.

71. Baltschukat S, Engstler BS, Huang A, et al. Capmatinib (INC280) is active against models of non–small cell lung cancer and other cancer types with defined mechanisms of MET activation. *Clin Cancer Res* 2019;**25**(10):3164–75.

72. Wolf J, Seto T, Han J-Y, et al. Capmatinib (INC280) in METΔex14 -mutated advanced non-small cell lung cancer (NSCLC): efficacy data from the phase II GEOMETRY mono-1 study. *J Clin Oncol* 2019;**37**(15_suppl):9004–4.

73. Beck BR, Shin B, Choi Y, et al. Predicting commercially available antiviral drugs that may act on the novel coronavirus (SARS-CoV-2) through a drug-target interaction deep learning model. *Comput Struct Biotechnol J* 2020;**18**:784–90.

74. Raffi F, Jaeger H, Quiros-Roldan E, et al. Once-daily dolutegravir versus twice-daily raltegravir in antiretroviral-naive adults with HIV-1 infection (SPRING-2 study): 96 week results from a randomised, double-blind, non-inferiority trial. *Lancet Infect Dis* 2013;**13**(11):927–35.

75. Blanco JL, Ambrosioni J, Garcia F, et al. COVID-19 in patients with HIV: clinical case series. *Lancet HIV* 2020;**7**(5): e314–6.

76. Kahn SE, Haffner SM, Heise MA, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006;**355**(23):2427–43.

77. Nauck MA, Del Prato S, Meier JJ, et al. Dapagliflozin versus glipizide as add-on therapy in patients with type 2 diabetes who have inadequate glycemic control with metformin: a randomized, 52-week, double-blind, active-controlled non-inferiority trial. *Diabetes Care* 2011;**34**(9):2015–22.

78. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med* 2017;**377**(7):644–57.

79. Khokhar MA, Rathbone J. Droperidol for psychosis-induced aggression or agitation. *Cochrane Database Syst Rev* 2016;**12**(12):CD002830.

80. Lieberman JA, Scott Stroup T, McEvoy JP, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *N Engl J Med* 2005;**353**(12):1209–23.

81. Barone JA. Domperidone: a peripherally acting dopamine2-receptor antagonist. *Ann Pharmacother* 1999;**33**(4): 429–40.

82. Burris KD, Molski TF, Xu C, et al. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D2 receptors. *J Pharmacol Exp Ther* 2002; **302**(1):381–9.

83. Hirsch LE, Pringsheim T. Aripiprazole for autism spectrum disorders (ASD). *Cochrane Database Syst Rev* 2016;**2016**(6):CD009043.

84. Hough D, Gopal S, Vijapurkar U, et al. Paliperidone palmitate maintenance treatment in delaying the time-to-relapse in patients with schizophrenia: a randomized, double-blind, placebo-controlled study. *Schizophr Res* 2010; **116**(2–3):107–17.

85. Kasper S, Möller HJ, Hale A. The European post-marketing observational sertindole study: an investigation of the safety of antipsychotic drug treatment. *Eur Arch Psychiatry Clin Neurosci* 2010;**260**(1):59–68.

86. Khan A, Cutler AJ, Kajdasz DK, et al. A randomized, double-blind, placebo-controlled, 8-week study of vilazodone, a serotonergic agent for the treatment of major depressive disorder. *J Clin Psychiatry* 2011;**72**(4):441–7.

87. Fleischhacker WW, Hobart M, Ouyang J, et al. Efficacy and safety of brexpiprazole (OPC-34712) as maintenance treatment in adults with schizophrenia: a randomized, double-blind, placebo-controlled study. *Int J Neuropsychopharmacol* 2017;**20**(1):11–21.

88. Maeda K, Sugino H, Akazawa H, et al. Brexpiprazole I: in vitro and in vivo characterization of a novel serotonin-dopamine activity modulator. *J Pharmacol Exp Ther* 2014;**350**(3):589–604.

89. Färkkilä M, Diener HC, Géraud G, et al. Efficacy and tolerability of lasmiditan, an oral 5-HT 1F receptor agonist, for the acute treatment of migraine: a phase 2 randomised, placebo-controlled, parallel-group, dose-ranging study. *Lancet Neurol* 2012;**11**(5):405–13.

90. Shajib MS, Khan WI. The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol* 2015;**213**:561–74.

91. Herr N, Bode C, Duerschmied D. The effects of serotonin in immune cells. *Front Cardiovasc Med* 2017;**4**:48.

92. Stratz C, Anakwue J, Bhatia H, et al. Anti-inflammatory effects of 5-HT3 receptor antagonists in interleukin-1beta stimulated primary human chondrocytes. *Int Immunopharmacol* 2014;**22**:160–6.

93. Nau F, Yu B, Martin D, et al. Serotonin 5-HT2A receptor activation blocks TNF-α mediated inflammation in vivo. *PLoS One* 2013;**8**:2–9.

94. Domínguez-Soto Á, Usategui A, de Las Casas-Engel M, et al. Serotonin drives the acquisition of a profibrotic and anti-inflammatory gene profile through the 5-HT7R-PKA signaling axis. *Sci Rep* 2017;**7**:1–15.

95. Kato S. Role of serotonin 5-HT3 receptors in intestinal inflammation. *Biol Pharm Bull* 2013;**36**:1406–9.

96. Andrade C. Nonsteroidal anti-inflammatory drugs and 5-HT3 serotonin receptor antagonists as innovative antipsychotic augmentation treatments for schizophrenia. *J Clin Psychiatry* 2014;**75**:707–9.

97. Wang Q, Guo H, Li Y, et al. Efficacy and safety of Leflunomide for refractory COVID-19: an open-label controlled study. *medRxiv* 2020; 2020.05.29.20114223. doi: 10.1101/2020.04.21.054387.

98. Ellinger B, Bojkova D, Zaliani A, et al. Identification of inhibitors of SARS-CoV-2 in-vitro cellular toxicity in human (Caco-2) cells using a large scale drug repurposing collection. 2020;1–19.

99. Klann K, Bojkova D, Tascher G, et al. Growth factor receptor signaling inhibition prevents SARS-CoV-2 replication. *Mol Cell* 2020;**80**:164–174.e4.

100. Weston S, Haupt R, Logue J, et al. FDA approved drugs with broad anti-coronaviral activity inhibit SARS-CoV-2 in vitro. *bioRxiv* 2020. doi: 10.1101/2020.03.25.008482.

101. Zhao H, Mendenhall M, Deininger MW. Imatinib is not a potent anti-SARS-CoV-2 drug. *Leukemia* 2020;**34**:3085–7.

102. Ector GICG, Huijskens EGW, Blijlevens NMA, et al. Prevalence of COVID-19 diagnosis in Dutch CML patients during the 2020 SARS-CoV2 pandemic. A prospective cohort study. *Leukemia* 2020;**34**:2533–5.

103. Breccia M, Abruzzese E, Bocchia M, et al. Chronic myeloid leukemia management at the time of the COVID-19 pandemic in Italy. A campus CML survey. *Leukemia* 2020;**34**:2260–1.

104. Li W, Wang D, Guo J, et al. COVID-19 in persons with chronic myeloid leukaemia. *Leukemia* 2020;**34**:1799–804.

105. Roschewski M, Lionakis MS, Sharman JP, *et al*. Inhibition of Bruton tyrosine kinase in patients with severe COVID-19. *Sci Immunol* 2020;**5**:1–19.

106. Riva L, Yuan S, Yin X, *et al*. Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. *Nature* 2020;**586**:113–119.

107. Cheke RS. The molecular docking study of potential drug candidates showing anti-COVID-19 activity by exploring of therapeutic targets of SARS-CoV-2. *Eurasian J Med Oncol* 2020;**4**:185–95.

108. Touret F, Gilles M, Barral K, *et al*. In vitro screening of a FDA approved chemical library reveals potential inhibitors of SARS-CoV-2 replication. *Sci Rep* 2020;**10**:13093. doi: 10.1038/s41598-020-70143-6.

109. Heiser K, McLean PF, Davis CT, *et al*. Identification of potential treatments for COVID-19 through artificial intelligence-enabled phenomic analysis of human cells infected with SARS-CoV-2. *BioRxiv* 2020. doi: 10.1101/2020.04.21.054387.