ORIGINAL RESEARCH ARTICLES

PHYSICOCHEMICAL AND PHARMACOKINETIC ANALYSIS AND DOCKING OF DRUG REPOSITIONING AGAINST SARS-COV-2: AN *IN SILICO* STUDY

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ABSTRACT

Studies on the development of effective and cost-effective oral drugs are the new priority of the pharmaceutical industry for the prevention and treatment of COVID-19. This work was based on the computational analysis of physicochemical parameters, pharmacokinetic and toxicological measurements, molecular docking and *in silico* measurement of the antiviral activity of 12 repositionable drugs. The Molinspiration platform (physical-chemical parameters), pkCSM® (absorption, distribution, metabolism and excretion), OSIRIS Property Explorer® (toxicological measurements), Seam® (Docking with the RdRp protein) and AVCpred server® (antiviral activity) were used. Considering the 12 selected repositionable drugs, molecular anchoring data with the RdRp protein, only the drug tilorone had lower binding energy than the control used in this study (Molnupiravir). Ledipasvir, daclatasvir and piperaquine showed the best percentage of antiviral inhibition considering the control pattern. ADME-Tox data showed that piperaquine has a high toxicological potential for mutagenesis, tumorigenesis and irritant effects. The findings of this study indicate that ledipasvir and daclatasvir showed greatest potential for inhibition RdRp and action against COVID-19.

Keywords: Coronavirus, drug repositioning, viral RNA polymerase, ADME-Tox, SARS-CoV-2, *in silico* tools, docking

INTRODUCTION

The pandemic caused by the coronavirus SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) had a huge financial and social impact on global public health[1,2]. Over 641 million cases of this infectious disease have been confirmed and nearly 6.6 million deaths have been reported worldwide[3].

Several vaccines for COVID-19 have been developed by the pharmaceutical industry and are effective in reducing the number of hospitalizations and deaths of patients[4–6]. However, some regions around the world still have little access to vaccine doses. Additionally, some outbreaks of infections are reappearing, related to the emergence of new variants that may not be responsive to vaccines developed so far[7]. Likewise, the clinical application of remdesivir, a drug used to treat COVID-19, has been very restricted due to the need for intravenous administration[8,9]. The monoclonal antibodies approved so far are high-cost medications that also require intravenous administration[10]. Treatment with another orally administered COVID-19 drug, Molnupiravir, a prodrug for a nucleoside analog, is highly priced at US$750[11]. The economic and health costs of COVID-19 have impacted the world, treatments that can reduce this burden are eagerly sought[11,12]. Therefore, effective and cost-effective oral drugs are the priority for the prevention and control of COVID-19, as they can be used after exposure to SARS-CoV-2 or at the first sign of the disease[13].

In silico methods have been an important tool for the analysis of the structures of the SARS-CoV-2[14,15]. Computational methods that investigate the properties of absorption, distribution, metabolism, excretion and toxicity of new drug candidates are part of the current paradigm of drug discovery by the pharmaceutical industry[16]. Additionally, docking has emerged as one of the most important computational methods for screening chemical compounds for therapeutic potential[17].

Traditional drug discovery methods are arduous, expensive and with a high risk of failure. A potential

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alternative solution to this process is the reuse of "old" drugs as a way of identifying new therapeutic options for existing or already marketed drugs[18].

Several studies have evaluated the potential of repositionable drugs for treating viral infections, such as elbasvir[19], ledipasvir[20], daclatasvir[21], ivermectin[22], digoxin[23], cobicistat[24], piperaquine[25], tilorone[26], dasatinib[27], digitoxin[28], darunavir[29] and sildenafil[30].

Thus, in this work, we carried out a physical-chemical analysis, ADME attributes, *in silico* toxicity and docking of drugs approved by the Food and Drug Administration (FDA) with application for treating SARS-CoV-2.

COVID-19 pandemic has already claimed many lives, and its high transmissibility continues to have compromising effects on the health of the world's population. Therapeutic vaccines have proved to be a useful tool for containing severe cases and the death of countless individuals[9]. However, the development of orally administered anti-Covid drugs, accessible to the population, is still an important demand to be considered by the pharmaceutical industry and by researchers around the world[31].

MATERIALS AND METHODS

Literature Search

A literature search was performed in the PubMed and Clinicaltrials.gov databases to identify potential drugs for new therapeutic purposes. From a wide search, the following drugs were selected to be part of the scope of this study: elbasvir, ledipasvir, daclatasvir, ivermectin, digoxin, cobicistat, piperaquine, tilorone, dasatinib, digitoxin, darunavir and sildenafil. Molnupiravir, an orally administered drug for COVID-19 in its prodrug or active form, was used as a comparison parameter for the analyses (standard control).

Evaluation of the physicochemical, pharmacokinetic and toxicological properties of repositionable drugs

The physical-chemical properties of the selected compounds were evaluated using the Molinspiration server tool (http://www.molinspiration.com). This tool makes it possible to calculate the physicochemical properties of chemical structures based on the "Rule of Five" developed by Lipinski *et al*[32]. This rule states that drug candidates that violate one of the following rules are likely to have low oral bioavailability: log P≤ 5; molecular weight ≤ 500; number of hydrogen bond acceptors (nON) ≤ 10; the number of hydrogen bond donors (nOHNH) ≤ 5; topological polar surface area (TPSA) ≤140 Å^2 and number of rotatable bonds (nRot) ≤ 10[33,34]. Absorption, distribution, metabolism and excretion (ADME) characteristics of repositionable drugs were predicted using the online server pkCSM: predicting small-molecule pharmacokinetic properties using graph-based signatures https://biosig.lab.uq.edu.au/pkcsm/[35]. Toxicity prediction of selected compounds was carried out by using the OSIRIS Property Explorer® tool (https://www.organic-chemistry.org/prog/peo/)[36,37].

Docking

RNA-dependent RNA polymerase (6M71) was retrieved from the protein database (PDB)[38]. A total of 12 repositionable drugs were collected in SDS (3D) format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[39]. Other molecules were subjected to a 3D structure generation on the CORINA website (https://www.mn-am.com/) using its SMILE. Additionally, the pdbqt files for the binders were generated by OpenBabel[40]. Docking is an efficient computational tool that classifies anchored drugs on the binding affinity of ligand-receptor complexes[41,42]. The SeamDock server was used to measure the binding affinity of the 12 repositionable drugs to RNA polymerase-dependent de RNA of SARS-CoV-2[43]. The fitting procedure involved the Vina software platform. The coordinates of the box from the center and size were, respectively, (X -3 Å, Y 4Å, Z 5 Å) and (X 65 Å, Y 67 Å, Z 83 Å).

Antiviral activity prediction

The AVCpred server (http://crdd.osdd.net/servers/avcpred/) was used to evaluate the antiviral potential of all repositionable drugs. This tool is capable of predicting the antiviral potential of different viruses, including the SARS coronavirus and other respiratory viruses[44].

RESULTS AND DISCUSSION

Structural and physicochemical properties

The emergency nature of the COVID-19 pandemic made the pharmaceutical industries and research laboratories resort to other means to enable efficient therapies in less time. A shorter and safer path to adopt is a drug repositioning[45]. Drug repositioning consists of identifying new therapeutic uses for drugs already approved and studied[46,47]. High-performance computational approaches have strengthened the development of drug repositioning approaches[48]. Drug discovery and development is a complex and time-

Table I: Physicochemical properties of repositionable drugs with potential action against COVID-19

Drugs	Molecular mass (g moL^{-1})	LogP	Polar surface area (PSA) (Å2)	nON	nrotb	nOHNH	Violations of Lipinski's rule
Elbasvir	882.03	8.85	188.82	16	13	4	3
Ledispavir	889.02	9.21	174.65	14	12	4	3
Daclatasvir	738.89	7.77	174.65	14	13	4	3
Ivermectin	875.11	4.58	170.09	14	8	3	2
Digoxin	780.95	1.12	203.08	14	7	6	3
Cobicistat	776.04	7.45	138.02	12	20	3	3
Piperaquine	535.52	5.60	38.74	6	6	0	2
Tilorone	410.56	4.85	42.02	5	12	0	0
Dasatinib	488.02	3.13	106.50	9	7	3	0
Digitoxin	764.95	2.03	182.85	13	7	5	2
Darunavir	547.67	4.32	140.43	10	12	4	1
Sildenafil	474.59	2.51	113.43	10	7	1	0
Molnupiravir (EIDD-2801) prodrug	329.31	-0.26	143.15	10	6	4	0
Molnupiravir (EIDD-1931) Active drug	259.22	-1.87	137.07	9	3	5	0

consuming process that requires the interrelation of several areas[49]. The traditional process of developing new molecules with pharmacotherapeutic activity generally has a success rate of only 2.01%, and the number of approved drugs has been decreasing since the 1990s[50]. Additionally, it is an expensive and time-consuming process[18].

The initial emphasis is based on physicochemical properties and rules to reduce attrition related to the oral bioavailability of drug candidates. In 1997, Christopher *et al* analyzed physiological characteristics of approved drugs and drug candidates in clinical trials at the time and proposed Lipinski's rule or "rule of 5"[51]. This rule could predict the probability that a given compound is orally active and prioritized compounds that have a molecular mass less than 500 Daltons, octanol-water partition coefficient (LogP) less than 5, hydrogen bond donors ≤5, and hydrogen bond acceptors less than or equal to 10[52,53]. Some works also considered it important to analyze the molecular polar surface area (PSA) less than or equal to 140Å^2 and the number of rotating flexibilities that must be between 0 and 10. Table I represents the physicochemical parameters of the selected anti-Covid drug candidates.

The MW (molecular weight) range of the repositionable drugs selected in this study is wide and ranges from 410.56 to 889.02. The control band of the standard drug molnupiravir (prodrug and active drug) presented data of 329.31 and 259.22, respectively. Tilorone, dasatinib and sildenafil molecules are the only ones with a cutoff value lower than that defined by the Lipinski rule for molecular weight.

The logP range of compounds varies from 1.12 to 9.2. Molnupiravir (prodrug and active drug) logP values showed data of -0.26 and -1.87, respectively. LogP is probably an important feature to assess oral absorptive capacity. The molecules digoxin, tilorone, dasatinib, digitoxin, darunavir and sildenafil are structures with a cut-off value within the range established by the rule of 5 for lipophilicity. Values above 5 for logP are unfavorable for oral absorption.

It appears that molnupiravir in its active form (EIDD-1931) has a very polar character. In this structural form, the molecule presents a low percentage of oral absorption. The pro-drug molnupiravir (EIDD-2901) promoted a more lipophilic character to the molecule, which allowed this

Table II: Computational evaluation of important absorption and distribution properties for some repositionable drug candidates for COVID-19 action

Drug	Intestinal absorption (human) (% absorbed)	Caco-2 Cell permeability (log Papp in 10^{-6} cm s^{-1})	P-gp substrate (yes/no)	VDss (human) (log L kg^{-1})	Fraction unbound (Fu, human)
Elbasvir	78.995	-0.203	Yes	0.086	0.36
Ledispavir	79.443	0.007	Yes	0.184	0.344
Daclatasvir	59.275	-0.553	Yes	0.185	0.32
Ivermectin	87.6	0.637	Yes	0.213	0.115
Digoxin	68.501	0.596	Yes	0.199	0.32
Cobicistat	76.503	0.6	Yes	0.668	0
Piperaquine	92.89	1.101	Yes	1.903	0.079
Tilorone	92.213	1.04	Yes	2.189	0.193
Dasatinib	83.217	0.878	Yes	1.441	0.216
Digitoxin	74.287	0.601	Yes	0.259	0.276
Darunavir	75.477	0.493	Yes	0.602	0.055
Sildenafil	81.256	0.135	Yes	1.091	0.172
Molnupiravir (EIDD-2801) Prodrug	53.464	0.531	No	0.581	0.67
Molnupiravir (EIDD-1931) Active drug	50.392	0.366	No	0.313	0.916

Table III: Computational evaluation of important permeability, metabolism and excretion properties for some repositionable drug candidates for COVID-19 action

Drug	BBB permeability	CYP2D6 inhibitor	CYP3A4 inhibitor	Total clearance
Elbasvir	-2.328	No	Yes	-0.155
Ledispavir	-1.672	No	No	0.095
Daclatasvir	-1.945	No	Yes	0.116
Ivermectin	-1.823	No	No	0.513
Digoxin	-1.397	No	No	0.479
Cobicistat	-1.761	No	Yes	0.845
Piperaquine	0.555	Yes	Yes	0.638
Tilorone	-0.111	Yes	Yes	1.014
Dasatinib	-1.53	No	Yes	0.477
Digitoxin	-1.364	No	No	0.445
Darunavir	-1.111	No	Yes	0.622
Sildenafil	-1.416	No	Yes	0.251
Molnupiravir (EIDD-2801) Prodrug	-1.057	No	No	0.203
Molnupiravir (EIDD-1931) Active drug	-1.12	No	No	No

Table IV: Computational evaluation of the toxicological properties of repositionable drugs for COVID-19 action

Drug	Mutagenesis	Tumorigenesis	Reproductive effects	Irritants effects
Elbasvir	high risk	high risk	low risk	low risk
Ledispavir	low risk	low risk	low risk	low risk
Daclatasvir	low risk	low risk	low risk	low risk
Ivermectin	low risk	low risk	low risk	low risk
Digoxin	low risk	low risk	low risk	low risk
Cobicistat	high risk	low risk	low risk	low risk
Piperaquine	high risk	high risk	low risk	high risk
Tilorone	high risk	high risk	low risk	low risk
Dasatinib	low risk	low risk	high risk	high risk
Digitoxin	low risk	low risk	low risk	low risk
Darunavir	low risk	low risk	low risk	low risk
Sildenafil	low risk	low risk	low risk	low risk
Molnupiravir (EIDD-2801) Prodrug	high risk	low risk	low risk	high risk
Molnupiravir (EIDD-1931) Active drug	high risk	low risk	low risk	low risk

drug to improve its oral bioavailability profile. This was probably a key strategy for molnupiravir to succeed as a drug used for treating COVID-19[54].

Predicted polarity (TPSA) also shows various variations from 38.74 to 203.0 among the repositionable drugs selected in this study. According to the defined cut-off values for TPSA (≤140 Å^2), the molecules cobicistat, piperaquine, tilorone, dasatinib and sildenafil are likely to have a high probability of oral bioavailability. The predicted polarity (TPSA) for the molnupiravir active form or prodrug has values within the range of Lipinski's rule.

Among all repositionable drugs, only digoxin was not within the range of number of hydrogen bond donors (nOHNH) ≤ 5. The drugs piperaquine, tilorone, dasatinib, darunavir and sildenafil are within the Lipinski rule considering the range for a number of hydrogen bond acceptors (nON) ≤ 10. Digoxin, piperaquine, dasatinib, digitoxin and sildenafil do not violate the number of rotational bonds.

Of the 12 molecules investigated, only 3 compounds (tilorone, dasatinib and sildenafil) are within the permitted range of physicochemical properties and satisfy all key parameters of the physicochemical properties of Lipinski's rule. The drug darunavir violates the rule by only 1 point. Ivermectin, piperaquine, and digitoxin violate the 2-point rule, and the other molecules violate Lipinski's rule by 3-points. Violation of 2 or more criteria of this rule predicts that the molecule will not be orally bioavailable.

Lipinski's rule has established itself as a cornerstone in the decision-making process for drug development screening, both in research centers and within the pharmaceutical industry. Despite this, the violation of the criteria established in this rule should not prevent further investigation of drug candidates[55]. This parameter should only serve as a guideline in the conduct of other experimental techniques, and in obtaining more data that can clarify the oral bioavailability of therapeutic molecules.

Prediction of ADME-Tox and related properties

Studies have shown that about 90% of the development of new clinical drug candidates fail, despite the implementation of several successful strategies by the pharmaceutical industry[56]. Computational methods of analyzing pharmacokinetic properties have become a crucial strategy for the early stages of the drug discovery process[57]. When the absorption, distribution, metabolism and excretion criteria are well established, they serve to guide future studies with more promising results.

To predict the characteristics of permeation, absorption and distribution, the analyses of the percentage parameters of intestinal absorption were considered; Caco-2 permeability; P-gp substrate; volume of distribution (VDss) and fraction unbound. Additionally, the role of CYPs in drug metabolism, the ability to cross the blood-brain barrier and renal clearance were also evaluated as pharmacokinetic parameters (Table II).

Among the ADME properties calculated in that study for repositionable drugs was the total human intestinal absorption percentage (%). Orally administered drugs have the advantages of convenience, patient preference, cost-effectiveness, and ease of large-scale manufacturing of oral dosage forms. The absorption capacity of a drug in the gastrointestinal tract depends on multiple factors that include physicochemical characteristics of drugs, solubility, partition coefficients, ionization and passive transport mechanisms[58]. The biopharmaceutical classification system (BCS) was created in 1995 and since then, has been an important tool for predicting the intestinal absorption of drugs after oral administration[59]. To demonstrate the suitability of a method, drug candidates can represent a range of scores based on low (e.g., < 50%), moderate (e.g., 50 - 89%) and high (≥ 90%) absorption[60,61].

Considering this proposed classification, only piperaquine and tilorone have a high rate of oral absorption. All other drugs would fit into the group of drugs with moderate oral absorption. No drug would be considered to have poor oral absorption. It is also noteworthy that all repositionable drugs selected here have a higher intestinal absorption percentage than molnupiravir in its active form or in its prodrug form.

Caco-2 cells are a lineage of epithelial cells that have become a *de facto* standard in the study of drug transport and identification of substrates, inhibitors and inducers of intestinal transporters, especially related to P-glycoprotein (P-gp)[62–64]. This model of permeability to the Caco-2 cell monolayer has been used as a gold technique to assess the bioavailability of drugs in human beings[65]. Based on *in vitro*/*in vivo* correlation studies, clear permeability coefficient log Papp rate (10^{-6} cm/s), is considered high if log Papp > 0.9, and considered low if log Papp < 0.9[66]. The Caco-2 cell permeability of the repositionable drugs is listed in Table II. The drugs piperaquine and tilorone are the only molecules that have a value above the high absorption rate for Caco-2. All other drugs have, according to the Caco-2 test, low cell permeability and low oral absorption.

All repositionable drugs from this experimental design found a substrate for P-gp efflux: P-glycoprotein, a 170 kDa membrane protein, expressed in different cell types, and which can alter drug absorption, distribution, metabolism and elimination[67]. P-glycoprotein plays a key role in drug transport in many organs. In the intestine, P-glycoprotein pumps drug back into the lumen, decreasing their absorption.

After the drug is absorbed from the administration site, it is distributed to extracellular fluids. In the circulation, almost all drugs are in equilibrium between the bound and unbound states with serum proteins at different affinities. Only free molecules can interact with macromolecular targets. Thus, the efficiency of a drug is altered by the drug's binding efficiency with plasma proteins.

Using the pkCSM online tool, we could assess the unbound fraction (Fu) and steady-state volume of distribution (VDSs) of all repositionable drugs. VDS is a fundamental parameter that helps to define the total dose of a drug. The values for these two parameters are provided in Table II.

Among the repositionable drugs, only piperaquine and tilorone inhibit the CYP2D6 enzyme. Among the same group, only the drugs ledispavir, ivermectin, digoxin and digitoxin do not inhibit the CYP3A4 enzyme[68] (Table III).

Cytochromes P450 (CYPs) are a superfamily of constitutive and inducible proteins. They have a group of hemoproteins – responsible for the oxidative metabolism of several drugs[69]. Data show that this superfamily of enzymes metabolizes approximately 90% of all marketed drugs. In the drug discovery process, one of the important issues is to avoid CYP inhibition leading to toxic drug accumulation and adverse drug interactions.

Piperaquine and tilorone can cross BBB. SARS-CoV-2 has the ability to infect the central nervous system in addition to the respiratory system. The blood-brain barrier (BBB) as part of absorption, protects the central nervous system (CNS) by separating the brain bloodstream tissue[70]. Renal clearance was also assessed for all repositionable drugs. Clearance quantifies the rate of irreversible removal of a drug from the body.

Table IV shows the prediction of toxicological results for possible candidates for anti-Covid drugs. The prediction evaluates the ability of mutagenesis, tumorigenesis, irritant effects and effects on the reproductive system. Data were obtained using the OSIRIS Property Explorer®. Ledispavir, daclatasvir, ivermectin, digoxin, digitoxin, darunavir and

Table V: Docking of the RdRp (PDB ID = 6M71) to repositionable drugs

Molecule	Binding affinity (kcal mol^{-1})	Amino acids hydrogen bonds	Amino acids hydrophobic interactions	Weak hydrogen bond	Cation-pi interaction	ionic interaction
Elbasvir	-9.5	Thr394(A); Asn140(B); Thr141(B); Thr48(B)	Pro323(A); Thr324(A); Phe396(A); Arg457(A); Val675(A); Pro677(A) Leu122(B); Thr141(B); Phe147(B)	Thr148 (B); Phe147	None	None
Ledipasvir	-9.6	Thr409(A); Asp684(A); Ala 685(A); Tyr689(A)	Val410(A); Lys 500(A) Lys545(A); Tyr546(A); Val557(A); Ala685(A); Ala688(A); Tyr689(A)	Asp684(A); Ala685(A)	Lys500(A)	None
Daclatasvir	-8.8	Thr409(A); Leu544(A); Ser682(A); Asp684(A); Thr409(A)	Val410(A); Lys545(A); Tyr546(A); Val557(A); Thr687(A); Ala688(A)	Thr409(A)	None	None
Ivermectin	-9	Asn136(B); Ser177(B); Gln34(C); Thr135(B); Ser177(B); Gln31(C); Ser177(B)	Asp134(B); Pro178(B); Trp182(B); Gln31(C)	Ser177(B)	None	None
Digoxin	-9.4	Pro169(A); Ser255(A); Ile266(A); Thr319(A); Thr394(A); N459(A); Ser255(A); Arg249(A); Thr394(A)	L172(A); Y265(A); P323(A); L460(A)	None	None	None
Cobicistat	-7.9	Y129(A) N138(A) K714(A) Y129(A) K47(A) D711(A)	His133(A) Asp140(A) Ala706(A) Lys780(A)	Asn138(A) Thr710(A)	None	None

Piperaquine	-8.6	Ileu266(A) Pro677(A)	Tyr265(A) Thr319(A) Pro461(A)	None	Tyr265(A)	None
Tilorone	-6.2	Ser255(A) Lys267(A)	Thr252(A) Tyr265(A) Trp268(A) Leu270(A) Pro322(A) Leu122(B)	Phe321(A)	None	None
Dasatinib	-7.8	Thr252(A) Thr319(A) Thr252(A)	Arg249(A) Tyr265(A)	Thr252(A) Pro461(A) Pro323(A)	None	None
Digitoxin	-9.4	Ser255(A) Thr319(A) Cys395(A) Ser397(A) Lys267(A) Ser255(A) Tyr149(B) Ser397(A)	Tyr265(A) Pro322(A) Pro322(A) Leu389(A) Phe396(A)	None	None	None
Darunavir	-7.5	Tyr129(A) Ser709(A) Ala771(A) S772(A) Asn781(A) Thr710(A) Asp711(A)	Lys47(A) Tyr129(A) His133(A) Asp135(A) Lys780(A)	Ala706(A) Thr710(A)	Lys780(A)	None
Sildenafil	-8	Thr252(A) Ser255(A) Thr319(A)	Tyr265(A) Pro461(A)	Thr246(A) Thr319(A)	None	None
Molnupiravir (EIDD-2801) Prodrug	-7.7	Tyr129(A); His133(A); Ala706(A); Ser709(A); Ser784(A); Lys47(A)	Tyr129(A)	His133(A)	None	None
Molnupiravir (EIDD-1931) Active drug	-6.9	Glu811(A) Asp761(A) Asp760(A) Ser759(A) Thr617(A)	None	Asp761(A) Glu811(A) Gly616(A)	None	Asp761(A)

sildenafil present low risk for mutagenesis, tumorigenesis, reproductive effects and irritant effects.

In view of the set of data obtained, we can say that initially the compounds ledispavir, daclatasvir, ivermectin, digoxin, digitoxin, darunavir and sildenafil would be the most apt to move to later stages of the virtual screening performed in this work because all these 7 compounds present low risk of toxicity considering the *in silico* analysis.

Molecular docking studies

Docking studies are used to evaluate the interaction of macromolecules and possible drug candidates[71]. The characterization of possible interactions between molecular targets and drugs is of great importance in the field of drug discovery and development[72].

In this work, the activities of 12 repositionable drugs were selected against the target with SARS-CoV-2 RdRp (PDB ID = 6M71). Severe acute respiratory syndrome 2 (SARS-CoV-2) viral RNA-dependent RNA polymerase (RdRp) targeting. RNA polymerase is a key enzyme for the replication and transcription of this positive-sense single-stranded RNA virus. This target has become a promising one for treating COVID-19 with the drugs remdesivir and molnupiravir for this infection[73]. The active site of RdRps in ss(+)RNA viruses is characterized by structural motifs A to G, forming a crucial "common core." Motifs A, B and C make up the palm, while motif F is in the fingers. Motifs D and E are in the palm, and G is in the fingers, sharing a similar three-dimensional space across species. The fingers interact with the major groove of the template RNA. Each motif plays a specific role in RNA positioning and entry NTP for replication[74]. Motifs A, B, and C collectively form the active site's floor. Motif B secures the ribose of the RNA template, and motif F arches over the active site. Aspartates in motif A interact with the metal ion of incoming nucleotides. Motif D aids chain elongation, and motif E provides a "primer grip." Motif F is crucial for RNA template geometry, and its structure varies among species. In flaviviridae, F motif may be truncated. *Thosea asigna* exhibits motif order switching (F, C, A, and B). The G motif contributes to the input channel[75].

Table VI: Antiviral activity of repositionable drugs with a percentage of general inhibition *in silico* evaluation

Molecule	General antiviral activity (%)
Elbasvir	48.978
Ledipasvir	69.561
Daclatasvir	74.899
Ivermectin	33.854
Digoxin	32.418
Cobicistat	50.239
Piperaquine	99.413
Tilorone	50.924
Dasatinib	50.118
Digitoxin	32.268
Darunavir	33.201
Sildenafil	56.713
Molnupiravir (prodrug)	6.635
N(4)-Hydroxycytidine (Molnupiravir active drug)	66.537

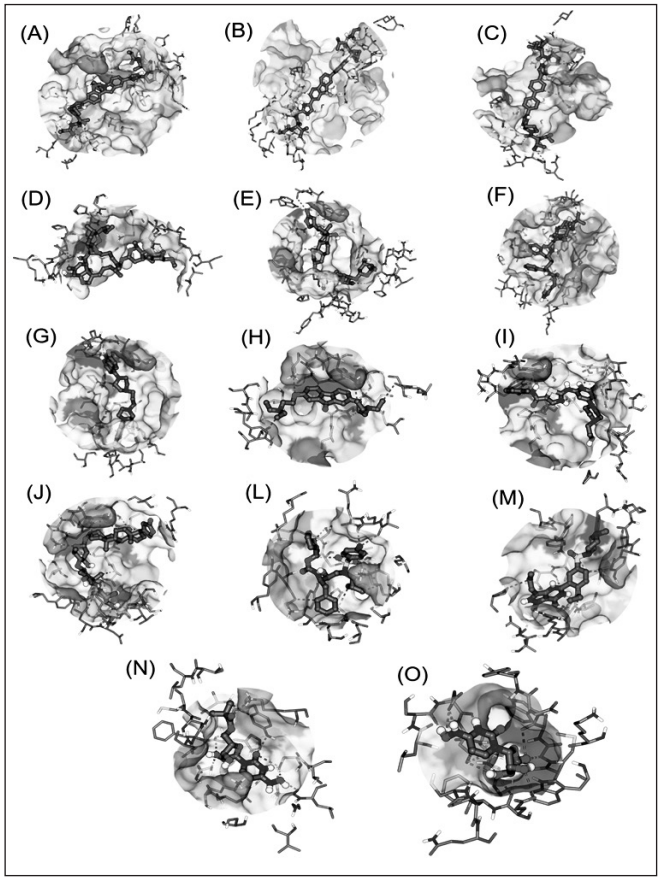

Fig. 1: Target-16M71-anchored ligands: (A) Elbasvir (B) Ledipasvir (C) Daclatasvir (D) Ivermectin (E) Digoxin (F) Cobicistat (G) Piperaquine (H) Tilorone (I) Dasatinib (J) Digitoxin (L) Darunavir, (M) Sildenafil and Molnupiravir in its (N) prodrug and (O) active form

Using SeamDock, we measured binding affinity energy parameters and hydrogen bonding, hydrophobic, ionic and cation-pi interactions (Table V).

The binding energies of the repositionable drugs ranged between -6.2 and -9.6 kcal mol^{-1}. Molnupiravir prodrug and drug active binding energies of -7.7 and -6.9, respectively. Considering that molnupiravir in its active form (-6.9 kcal mol^{-1}) is the form that interacts with the target of COVID-19 in the human body, its value was considered a cutoff for better affinities of repositionable drugs with macromolecules. Considering that molnupiravir in its active form (-6.9 kcal mol^{-1}) is the form that interacts with the target of COVID-19 in the human body, its value was considered a cutoff for better affinities of repositionable drugs with macromolecules. Elbasvir, ledipasvir, daclatasvir, ivermectin, digoxin, cobicistat, dasatinib, digitoxin, darunavir and sildenafil show better binding affinity with the anti-Covid target. Only tilorone has a lower binding energy than the active form. The anchored conformations of the 12 repositionable drugs plus molnupiravir in its prodrug form (EIDD-2801) and in its active form (EIDD-1931) are shown in Fig. 1.

Finally, the AVCpred platform was used to measure the general antiviral activity of repositionable drugs. The data achieved here show that molnupiravir in its active form has an antiviral action of 66.54% as shown in Table VI. Considering this value as a cutoff point, it is considered that the drugs ledipasvir, daclatasvir and piperaquine have greater antiviral activity. AVCpred is a tool that uses the QSAR strategy to predict the antiviral potential of drugs using relationships that connect molecular descriptors and inhibition.

Inhibition of viral growth via drugs occurs through different targets of the viral life cycle phase, such as fusion, integration, replication and maturation and must preserve the host organism[76].

The emergence of new variants of SARS-Cov-2 and the need for more effective pharmacotherapeutic options to be available make it necessary to develop a wide variety of antiviral pharmacotherapeutic options. As future perspectives, studies in cells and in animal models should be conducted with the drugs ledipasvir and daclatasvir in order to elucidate the potential of these molecules against COVID-19. Research should include SARS-Co-2 infected patients to elucidate these benefits in humans.

CONCLUSION

For the design and development process of drugs administered orally, low cost and with action against COVID-19, the physicochemical, pharmacokinetic, interactions via docking and antiviral actions were evaluated. In this work, 12 repositionable drugs were evaluated in an *in silico* screening procedure. The data obtained here showed that considering the molecular docking values, only the drug tilorone has lower binding energy than the control used in this study (molnupiravir), considering the RdRp target. The drugs ledipasvir, daclatasvir and piperaquine have the best percentage of antiviral inhibition. Pharmacokinetic and toxicological data showed that piperaquine has a high toxicological potential for mutagenesis, tumorigenesis and irritants effects. Therefore, considering the set of data achieved, the drugs ledipasvir and daclatasvir proved to be the most promising drugs for inhibiting RdRp (PDB ID = 6M71) and action against COVID-19. Computational strategies and repositionable drugs have shown promise for evaluating the action against COVID-19. Based on these findings, ledipasvir and daclatasvir could be used to design effective antiviral drugs against SARS-CoV-2.

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