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FULL-LENGTH TITLE: Pipeline for drug repurposing of FDA-approved drugs against SARS-CoV-1 and SARS-CoV-2

SHORT TITLE (FOR THE RUNNING HEAD): Drug repurposing for COVID-19

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ABSTRACT

Since the beginning of the COVID-19 pandemics, large *in silico* screening studies and numerous *in vitro* studies have assessed the antiviral activity of various drugs on SARS-CoV2. In the context of health emergency, drug repurposing represents the most relevant strategy, due to the reduced time for approval by international medicines agencies, the low cost of development, and the advantage that toxicity is already well known compared to the *de novo* design of new molecules. In this paper, we aimed to review as exhaustively as possible, drugs which have shown an *in vitro* antiviral activity against SARS-CoV-2 and that could be considered as therapeutics options. For some of them, antiviral efficacy was also predicted by *in silico* studies.

MAIN TEXT

INTRODUCTION

CoVs are enveloped viruses which belong to the *Nidovirales* order and are divided into four genera based on phylogeny (https://talk.ictvonline.org/p/coronavirus-genomes). CoVs have been detected in a wide spectrum of mammals and avian species such as dogs, cats, pigs, chickens, cows, camels, bats, mink and/or pangolins, and cause severe diseases including gastroenteritis and respiratory tract diseases. Seven human coronaviruses (HCoVs) have been identified to date (HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, MERS-CoV and Severe Acute Respiratory Syndrome Coronavirus 1 and 2). Their virions are about 120–160 nm in diameter and contain a linear, positive, single-stranded RNA genome of ≈26-32 kilobases, which encodes 16 non-structural proteins (nsp1 to nsp16), and four or five structural proteins including the spike (S), envelope (E), membrane (M), nucleocapsid (N) and, for HCoV-OC43 and HCoV-HKU1, the hemagglutinin (HE) [1-4].

Currently, there are no specific antiviral drugs which target HCoV viruses. However large *in silico* screening studies and numerous *in vitro* studies have assessed the antiviral activity of various drugs on HCoV. These studies have accelerated substantially since the beginning of the COVID-19 pandemic at the end of 2019. Drug repurposing represents the most pertinent strategy, due to the reduced time for approval by international medicines agencies, the low cost of development, and low risk, in comparison with the *de novo* design of new molecules for which clinical trials to test efficacy and safety are not possible within the context of a health emergency. Drug repurposing offers an opportunity to by-pass pre-clinical tests, by using molecules whose toxicity is already well known.

MATERIAL & METHODS

1/ BIBLIOGRAPHIC SEARCHES

On 8 November 2020, searches were carried out on Pubmed, and Google scholar using the keywords “coronaviruses”, “human coronaviruses”, “Severe Acute Respiratory Syndrome Coronavirus”, “SARS-CoV”, “SARS-CoV-2”, “in vitro”, “cell culture”, “sensitivity assay”, “drug repurposing”, “drug repositioning”, “in silico”, “computational”. Clinical trials were excluded. The results of these searches were completed with data collected from the Stanford coronavirus antiviral research database also on 8 November 2020 (https://covdb.stanford.edu).

The sensitivity assays carried out on interferons and monoclonal antibodies were excluded. We reviewed the following: 1) the molecules tested in at least four sensitivity assays and 2) the most potent molecules based on effective concentration 50 (EC_{50}), which is the compound concentration found in different studies that is required to inhibit viral RNA replication by 50%.

The main data provided on pharmacodynamics and drug toxicity were collected from the DrugBank database (https://go.drugbank.com/drugs/DB00836) [1].

2/ ANALYSIS METHODS USED IN *IN VITRO* SENSITIVITY ASSAYS PERFORMED ON CELL CULTURES

Several essential parameters used for *in vitro* test sensitivity assays varied according to studies. These parameters include cell lines, the multiplicity of infection (MOI: infectious virus titre divided by the number of cells), the time between addition of the drug and incubation until addition of viruses, drug concentration, end-point for evaluation of viral replication and the read-out system for the assessment of viral replication.

The cells used in each *in vitro* sensitivity assay have to be adapted according to the virus tested: the condition to be used is that cells have to be permissive for the virus tested, implying that they harbour the virus cell receptor specific for the virus tested.

Most of the studies that assessed the antiviral activity of molecules against SARS-CoVs used Vero-E6 cells (kidney epithelial cells from African green monkeys), a cell line largely used to propagate many viruses, mainly due to their IFN-deficiency, and thus have a high permissivity to SARS-CoV-1 and SARS-CoV-2. For SARS-CoV-2, a large panel of other cells were used, either naturally expressing the cellular receptor for the virus, or transfected with the human ACE2 gene. All the cell lines that were used in the sensitivity assays are summarised in Figure 1. Primary cell lines have been little used for coronavirus sensitivity assays. Primary cell culture is the *ex vivo* culture of cells derived from tissue explants. The Human Airway Epithelial (HAE) primary cell line is a pseudostratified mucociliary epithelium that was used for SARS-CoV-1 and SARS-CoV-2 [2-4]. In addition, human embryonic stem cell-derived cardiomyocytes and *ex vivo* lung cultures have also been used for sensitivity assays [5]. All the discrepancies on the other parameters are summarised in Figure 2.

The multiplicity of infection (MOI) of the virus was not standardised and varied greatly depending on the virus and the study, ranging from 0.0001 to 10. The MOI mostly used for

SARS and SARS-CoV-2 was 0.01. It should be noted that some studies do not mention the MOI used.[6]

The time of drug incubation was also a variable parameter (from 48 hours before to five hours post virus inoculation). However, most of the studies used a one hour incubation of cells with the compounds before virus inoculation. In most assays, dose response curves were performed with the aim of calculating the EC_{50}, which requires the assessment of compound dilutions. Most studies also evaluate the CC_{50}, which is the concentration that reduces the total cell number by 50%. However, for some experiments that evaluated only one compound concentration, the EC50 could not be calculated, although the percentage of virus inhibition with the compound concentration used is generally reported.

Viral replication could be assessed through an evaluation of cytopathic effects with CellTiter-Blue (PROMEGA), neutral red, MTT (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium) or visually. CellTiter-Blue is a commercial fluorescent assay to monitor cell viability. Neutral red is taken up by viable cells, the ability to incorporate neutral red decreasing as lysis occurs. A colorimetric quantitation will be inversely correlated to the cytopathic effects. MTT enters cells, is reduced by NAD(P)H-dependent oxidoreductase in formazan and turns purple; this coloration will be quantified by a spectrophotometric evaluation. Importantly, it should be noted that the sensitivity thresholds of the detection of cytopathic effects could be different with a visual inspection rather than a fluorescence detection performed with the CellTiterGlo. This difference according to the method of detection had already been reported [7].

Other studies assessed infectious viruses by determining the viral titre at the end of the experiment. Viral replication could also be assessed by virus expression: either by RNA quantification, or the expression of a viral protein. RNA quantification performed by quantitative RT-PCR made it possible to compare the viral load in control cells infected by the virus with those in the presence of the tested compound. The differences in the viral loads makes it possible to assess the percentage of inhibition of the viral replication.

Some studies used green fluorescent protein (GFP) or nanoluciferase-expressing recombinant viral strains, the viral expression being quantified by fluorometry at the end-point evaluation.

Finally, a few teams still use plaque reduction assays.

Another parameter should also be considered. The viral strain was not the same in all the studies, despite the fact that these viruses are known to accumulate mutations and are present as quasi-species in patients. Only very few studies were found to test several strains [5].

3- MOLECULAR DOCKING

Interestingly, these approaches can supplement *in vitro* cell sensitivity assays by predicting the interactions of drugs in pathways important in the viral replication cycle. They are complementary and have the added value of targeting the most interesting repositioned drug candidates. Targeted candidates can subsequently be tested by sensitivity assays on cell cultures.

The study of protein-protein interactions could be useful in terms of targeting possible therapeutic options. The objective is to clone, tag and express SARS-CoV-2 proteins in human cells to identify human proteins that are physically associated with them, using affinity-purification mass spectrometry. On the high confidence protein-protein interactions that are identified, sensitivity assays are then carried out on cell cultures that were used to confirm these results [8].

The strategy could be purely based on digital predictions. Computational tools have also been used to better understand the interaction between drugs and viral or host proteins. Molecular docking and molecular dynamic simulations have been used to find binding energies resulting from the simulated interactions of several thousands of compounds, with the viral or host proteins.[9]

Other studies investigated whether SARS-CoV-2 proteins could interact with targeted pathways previously shown to be essential for coronaviruses replication cycles, which were the unfolded protein response (UPR), the mitochondrial permeability transition pores (MPTP), NLRP-3 inflammasome and autophagy of the host cells. After that, the objective was to identify drugs known to modulate these pathways.[10]. The most widely used strategy was to screen a database of several hundreds or thousands of compounds and to predict binding affinity with the viral or host proteins involved in the viral replication cycle.

RESULTS

Although a few anti-HCoV drugs can act directly on the viral proteins, most drugs interfere with the cell metabolism and the molecular crosstalk taking place between the virus and the target cell. Alongside sensitivity assays in cell culture, dozens of molecular docking studies have been carried out. Among the viral proteins involved in molecular docking studies, the 3-chymotrypsin-like protease (3CL-PR), the RNA-dependent-RNA polymerase (RdRp) and the spike protein have been deeply explored as druggable targets. To our knowledge, 228 main compounds have been found by computational studies to be drug candidates.

1. Antivirals

Remdesivir

Remdesivir (GS-5734) is a parenteral phosphate prodrug of an adenosine analogue acting as a chain terminator of the RNA polymerase, at position i+3. The 1'-cyano group of remdesivir sterically clashes with the complex RdRp, inhibiting further enzyme translocation and terminating replication. Remdesivir was initially described as a potential treatment for Ebola[11-13]. It displays *in vitro* activity against several viral families including *Arenaviridae, Flaviviridae, Filoviridae, Paramyxoviridae, Pneumoviridae,* and *Coronaviridae*. Its anti-coronal activity was largely tested in *in vitro* cell cultures with four sensitivity assays performed on SARS-CoV [14-16] and 49 on SARS-CoV-2 [3;16-43]. The EC50 varied from 0.002µM when tested with a MOI=1 on Huh7.5 cells and an evaluation by a visual inspection of the cytopathic effects [27] and 27µM when tested with a MOI=0.02 on Vero E6 cells and an evaluation based on virus performed by RNA expression quantification. [17]. Two studies were carried out on Human Airway Epithelial cells [3;44]. One of them used an MOI of 0.5 and provided an EC_{50} of 0.01 µM and a selectivity index >1000 [22].

After a single two-hour intravenous infusion of 75mg of remdesivir, the plasmatic concentration of the prodrug remdesivir peaks at 2.5µM (2229 ng/mL), which is expected to achieve an antiviral efficacy according to the EC50 obtained *in vitro* [45].

Combinations using remdesivir were also found to have antiviral efficacy. Choy *et al* found a synergy between remdesivir at 6.25 µM and emetine at 0.195 µM with a 64.9% inhibition in viral yield [17]. In addition, one study reported that the combination of remdesivir and diltiazem revealed a synergistic antiviral activity against SARS-CoV-2 across a wide dose range that remained below reported remdesivir therapeutic plasma concentrations [46;47].

Diltiazem is a Ca^{2+} channel blocker commonly used in the treatment of hypertension for which antiviral activity was recently demonstrated against the influenza virus in A549 human lung epithelial cells with a very low EC_{50} of 0.84nM and which was confirmed in primary cells of Reconstituted Human Airway Epithelia [48]. Its antiviral properties may be based on the capacity to induce type I and III interferon antiviral responses [48].

Remdesivir has been predicted to bind to the RNA-dependent RNA polymerase of SARS-CoV-2 with a binding energy of -7.6kcal/mol, which possibly may inhibit its function [49], but also with the main viral protease with a binding energy ranging from -6.4 to -7.2kcal/mol [50-52].

Lopinavir

Lopinavir was with remdesivir, among the first antiviral tested on SARS-CoV-2. It is an inhibitor of the HIV protease. It is marketed in association with another protease inhibitor,

ritonavir, which acts an enzymatic inhibitor, thus boosting the plasmatic concentration of lopinavir. Lopinavir showed antiviral activity against SARS-CoV *in vitro*, with EC_{50} varying from 1.7 on VeroE6/TMPRSS2 at MOI 0.01 [53] to >50µM on Vero E6 and does not mention the MOI [54], moreover with a low specificity index (<0.1). Choy *et al.* observed antiviral activity of lopinavir against SARS-CoV-2 *in vitro* with an EC_{50} = 26 µM on Vero E6 cells [17], but with a low selectivity index. The antiviral effect could therefore be due to cytotoxicity. Moreover, the EC_{50} could not be expected to be lower than the plasmatic concentration obtained with 400/100mg of lopinavir/ritonavir dosing and thus may not achieve the antiviral activity *in vivo*. It was virtually predicted that lopinavir would exhibit a low free binding energy to the active site of the SARS-CoV-2 main protease, ranging from -7.57 to -9.9 kcal/mol, by binding near the crucial catalytic residues, HIS41 and CYS145. [51;55-58], but also with Thr26, Gly143 and Ser144 [52].

Atazanavir

Atazanavir, an inhibitor of the HIV protease, exhibits a EC_{50} =0.2µM against SARS-CoV-2 at MOI=0.01 on A549 cells, evaluated by virus expression. In addition, antiviral activity was reduced when using the combination of atazanavir and ritonavir with an EC_{50} = 0.6µM in the same experimental conditions [59]. The EC_{50} was considerably increased on Vero E6/TMPRSS2 with an EC_{50} of 9.4µM at an MOI of 0.01 [60].To note, the EC50 on Vero cells (>50µM) was more than 5- to 50-fold higher than those in other cells [18;59], [60].

Favipiravir

Favipiravir is a direct acting antiviral with a structure of a modified pyrazine analogue. This prodrug requires activation by ribosylation and phosphorylation to provide the active favipiravir-RTP. The active metabolite acts as a nucleotide analogue, binds and inhibits the RNA-dependent RNA polymerase, thus preventing viral replication, through a combination of chain termination and lethal mutagenesis by causing C-to-U and G-to-A transitions in the SARS-CoV-2 genome. It was initially developed to treat influenza infections. Maximal plasmatic concentration reaches 51.5 µg/mL.

Given that the catalytic domain RdRp of the influenza virus may be similar to those of other RNA viruses, favipiravir has been investigated for the treatment of infections with Ebola virus, Lassa virus, and SARS-CoV-2. Although it is currently being investigated through clinical trials, *in vitro* sensitivity assays have revealed contradictory results. An antiviral effect was concluded by Driouich *et al.* with a strong dose effect. The sensitivity assay on VeroE6 cells was based on a reduction of the cytopathic effect, with an MOI of 0.001, an EC_{50} was 32µg/mL, and with an MOI of 0.01, it was 70µg/mL (CC_{50} >78.5µg/mL) [61].

Shannon *et al.* also drew conclusions as to the efficacy of favipiravir, although noted a high EC_{50} of 207µM when the evaluation was based on the visualisation of cytopathic effects and 118µM when it was based on an evaluation of RNA quantification [62].

With similar results, Wang *et al.* were more cautious, concluding that the reduction of viral infection in VeroE6 at an MOI of 0.05 was shown only with high concentrations (EC_{50} = 61.88 µM, CC_{50} > 400 µM, SI > 6.46). Efficacy was evaluated by viral quantification in the cell supernatant by qRT-PCR and confirmed by virus nucleoprotein expression visualised through immunofluorescence microscopy [19].

In contrast, Choy *et al.* showed that favipiravir at a high concentration (100 µM) had no antiviral efficacy against SARS-CoV-2 when MOI = 0.02 on the same cell lines (Vero E6), with an evaluation by both quantitation of infectious virus and RNA quantification [17]. Zandi *et al.*, Jeon *et al.*, and Liu and Ohashi also concluded that there was no antiviral efficacy [18;36;53;63].

Ribavirin

Ribavirin is a guanosine analogue that needs to be activated by an adenosine kinase, and which blocks viral RNA synthesis and viral mRNA capping. It displays a broad antiviral spectrum against several RNA and DNA viruses. It was initially indicated in the treatment of HCV infections. It is currently used as an adjunct therapy to several new direct acting antivirals targeting HCV. The main toxicity is the risk of anaemia.

Ribavirin clearly does not inhibit SARS-CoV-replication in Vero, Vero E6 or Vero76 cells at high concentrations, and the EC_{50} ranges from 82µM to >4095µM [64-69].

However, one study concluded that ribavirin could inhibit SARS-CoV replication in other cells (PK15, Caco-2, CL14 and HPEK), as EC_{50}s, ranging from 2.2 to 9.4 µg/ml, were found in cells by determination of virus yield (MOI = 0.01), and that high concentrations of ribavirin (50 µg/ml) completely suppressed the formation of cytopathic effects in all cell lines infected with two strains of SARS-CoV.

Although some clinical trials are currently evaluating the efficacy of ribavirin against COVID-19, no antiviral activity of ribavirin against SARS-CoV-2 has been clearly demonstrated. Pizzorno *et al.* concluded that there was an absence of antiviral efficacy of this compound with an IC_{50}>10µM tested on Vero E6 cells with a MOI of 0.01. [3] Similarly, Choy *et al.* found that no viral inhibition was found at 100 µM [17].

Wang observed that only a high concentration of ribavirin (EC_{50} = 109.50 µM, CC_{50} > 400 µM, SI > 3.65) was required to reduce viral infection [19]. Congruently, Zandi *et al.* found that 20 µM showed only 12% inhibitory effect with concomitant cytotoxicity [63].

Nelfinavir

Nelfinavir is a protease inhibitor that is effective against HIV. It could also act against the SARS-CoV 3CL protease. With an MOI of 0.01, nelfinavir has an activity against SARS-CoV with low EC_{50} of 0.05 and 1.1 on Vero E6 and VeroE6/TMPRSS2, respectively [70]. Its activity has also been shown against SARS-CoV-2 on the same cell lines and on HeLa-ACE2 [24;32;36;53;70]. Docking studies have predicted that nelfinavir may inhibit the main protease with a binding energy of -7kcal/mol [71], but also unexpectedly binds near the SARS-CoV-2 spike protein [72].

Daclatasvir

Daclatasvir is an inhibitor of the NS5a protein of HCV used in combination with other direct acting antiviral agents in HCV-induced hepatitis. The EC_{50} of daclatasvir against SARS-CoV-2, tested on three different cell lines (Huh7, Vero E6 and Calu3), with an end point assessment at 24 or 48 hours by infectious virus evaluation was low, varying from 0.6 to 1.1µM at an MOI of 0.1 or 0.01 [73].

Entecavir

Entecavir is a nucleoside analogue used in the treatment of Hepatitis B viral hepatitis. The EC_{50} of entecavir against SARS-CoV-2 on Huh7 cells was 0.04µM at MOI 0.2 with an evaluation by viral expression (N protein) [23].

2. Antiparasitic agents

2.1- Antimalarial drugs

Quinolines

Quinolines, including chloroquine, amodiaquine, quinine, mefloquine, lumefantrine, piperaquine, pyronaridine and tafenoquine, remain the drugs that are the most recommended in the treatment of malaria. Chloroquine and hydroxychloroquine, an analogue of chloroquine, are indicated in the treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and chronic discoid lupus erythematosus.

The median effective concentration (EC_{50}) of chloroquine on SARS-CoV, which was assessed in four *in vitro* studies on Vero E6 cells [74-77], ranged from 4.1 to 8.8 µM. The selectivity index, i.e., the ratio between the 50% cytotoxicity concentration (CC_{50}) and EC_{50}, was assessed in two assays and provided medium values of >11 and >31 [75;77]. The EC_{50} was also evaluated on Vero76 cells by Barnard *et al.*, revealing similar activity with EC_{50} ranging from 3 to 5 µM, but with lower selectivity indexes ranging from 2 to 3.3 [78].

SARS-CoV-2 was also susceptible to chloroquine and hydroxychloroquine. *In vitro* effects of chloroquine on infected VeroE6 cells showed discrepant EC_{50} ranging from 0.1 to >50µM with an MOI of 0.01 [59;79]. Wang *et al.* found an EC_{50} of 1.1 µM and an SI > 88.5 at an MOI of 0.05 [19]. Gendrot *et al.* estimated the EC_{50} at 2.1 µM and an SI > 47 at an MOI of 0.25 [80]. Liu *et al.* demonstrated that EC_{50} increased when the MOI increased (2.7 µM at an MOI of 0.01; 3.8 µM at an MOI of 0.02; 7.1 µM at an MOI of 0.2 and 7.3 at an MOI of 0.8) [81]. Moreover, EC_{50} on infected Vero cells ranged from 0.5 to 7.3µM with an MOI of 0.01 and even higher than 100µM in a study where the MOI was not mentioned [18;82;83]. These concentrations under 10 µM were consistent with concentrations observed in human plasma and lungs. Chloroquine given at 100 mg day in the prophylaxis of malaria leads to a plasma concentration of 0.01 to 0.4 mg/l, i.e., 0.03 to 1.25 µM [84]. Chloroquine has an excellent diffusion and tissue concentration which would lead to chloroquine levels 200 to 700 times higher in the lung than in the blood (a concentration which can go up to 280 mg/kg in the lung) [85].

The EC_{50} of hydroxychloroquine assessed on SARS-CoV-2 infected Vero E6 cells ranged from 1.5 µM to 17.3 µM [20;79] and from 0.7 to 6.3µM on Vero cells [82]. These concentrations were consistent with concentrations observed in human plasma and lungs. An oral uptake of 400 mg of hydroxychloroquine led to a maximum blood concentration (C_{max}) of 1.22 µM [86]. Moreover, hydroxychloroquine accumulated 30 times more in the lungs than in the blood (around 0.3 µM vs 7.8 µM at six hours) [87]. However, one study used as cell support TMPRSS2 (transmembrane serine protease 2) expressing human lung cell line (Calu-3) and TMPRSS2-Vero resulted in the absence of antiviral efficacy of chloroquine against SARS-CoV-2 [83]. Another work also described the absence of an antiviral effect of hydroxychloroquine on SARS-CoV-2 at an MOI=0.1 on infected human airway epithelia reconstituted from human primary cells obtained from nasal or bronchial biopsies [88].

Chloroquine and hydroxychloroquine are understood to act on the viral post-entry step. Viral entry occurs after the interaction between the S1 subunit with the ACE2 cell surface receptor and a cleavage at the S1/S2 junction by TMPRSS2. This leads to an interaction with cell surface phospholipid bilayers. The nucleocapsid of the virus then gets into endosomal vesicle. After acidification of the late endosome, the cathepsin enables the release of viral genomic RNA. Cathepsin B and cathepsin L are endosomal cysteine proteases, whose activation requires a low pH [89], prevented in this case by chloroquine or hydrochloroquine. It has been demonstrated that both molecules could alter the function of lysosomes, by increasing their pH, while late endosome cathepsins are active at low pH only. This mechanism has been well

demonstrated in the SARS-CoV-2 infection for hydroxychloroquine. The two molecules may also inhibit the terminal glycosylation of ACE2, the host receptor of SARS-CoV and SARS-CoV-2, rising with an alteration of the interaction with the viral spike protein and thus, virus entry in cells [19;76;81]. The spike viral protein of SARS-CoV-2 used the ACE-2 receptor for entry, but also sialic acids and gangliosides. *In silico* analyses showed that the viral spike protein was not able to bind gangliosides in the presence of chloroquine or hydroxychloroquine [90].

It is notable that methylene blue also exhibited an *in vitro* antiviral activity against SARS-CoV-2 with a mean EC50 of 0.30µM at an MOI of 0.25. [80]

Amodiaquine revealed an antiviral activity against SARS-CoV on VeroE6 and Vero76 and against SARS-CoV-2 on Vero, Vero E6 [18;74;78;79]. It is notable that in one study, the EC_{50} assessed on Calu-3 cells was found to be >50µM i.e., 10 times higher than in Vero cells [34]. Desethylamodiaquine, the metabolite of amodiaquine, showed high *in vitro* efficacy with an EC_{50} of 0.52 µM and an SI of 166 [80]. A fixed dose of artesunate-amodiaquine (200 mg/ 540 mg), the dose recommended in malaria treatment, led to a plasma C_{max} of desethylamodiaquine around 879 ng/ml (around 4 µM) [91]. About 0.07% of the administered oral dose (8.6 mg/kg) of amodiaquine was found in rat lungs [92]

Mefloquine is a methanolquinoline with an antimalarial activity that is effective against *Plasmodium falciparum* and *Plasmodium vivax.* It is a blood schizonticide which interacts with the phospholipid bilayer, altering the membrane cell stability leading to cell lysis. It is an antagonist of Fe(II)-protoporphyrin IX of *P. falciparum* and Adenosine receptor A2a in humans and an inhibitor of the parasite 80S ribosomal subunit. The EC_{50} of mefloquine against SARS-CoV-2 varied between 1.8 and 8.1 µM on Vero E6 cells (MOI 0.002 to 0.25) with low selectivity indexes between 2.3 and 8 [79;93] while on Calu-3 cells, no antiviral activity was observed [34]. At 10 µM, mefloquine completely inhibited the cytopathic effect on Vero E6 cells infected by SARS-CoV-2 [94]. Mefloquine administered at the malaria therapeutic dose (1250 mg) led to a blood concentration of 1,648 ng/ml (around 4 µM) in healthy males [95]. A study on post-mortem cases showed that mefloquine levels are 10 times higher in the lung than in the blood (a concentration which can go up to 180 mg/kg in the lung) [96].

Pyronaridine, whose chemical structure is based on 9-aminoacridine with an amodiaquine-like side chain, exhibits antimalarial activity and is used in combination with artesunate in this indication. It acts by modifying the food vacuoles of the parasites, by inhibiting the formation of beta-haematin, leading to the accumulation of toxic heme in *Plasmodium* and inhibiting the

glutathione-dependent degradation of haematin, resulting in parasite death. The antiviral activity of pyronaridine was previously demonstrated against the Ebola virus and more recently, against SARS-CoV-2 [27;97]. Bae *et al.* showed that pyronaridine at an MOI=0.01 could inhibit SARS-CoV-2 replication in Vero cells with an EC_{50} of 1.1 and 2.2 µM, after 24 and 48 hours of culture, respectively. Gendrot *et al.* showed that pyronaridine exerted effective antiviral activity against infected Vero E6 cells at an MOI of 0.25 after 48 hours contact with an EC_{50} of 0.72 µM and an SI of 22 [80]. Pyronaridine tetraphosphate given at 720 mg day led to a plasma concentration of 271 ng/ml (around 0.3 µM) in humans and a $t_{1/2}$ of 33.5 days [98]. A single oral dose of 2 mg (10 mg/kg) in rats led to a blood C_{max} of 223 ng/ml and a lung C_{max} of 36.4 µg/g (165 more concentrated) [99]. The antiviral activity of pyronaridine against SARS-CoV-2 is compatible with malaria oral therapeutic doses.

Quinine, the second line treatment for severe malaria after artesunate IV, showed medium antiviral *in vitro* activity with an EC_{50} of 10.7 µM and an EC_{90} of 38.8 µM [100]. A 600 mg single oral dose of quinine sulphate led to blood C_{max} around 3.5 mg/l (around 8.5 µM) [101]. However, after intravenous doses of 10 mg/kg of quinine in rats, the observed concentration of the lung/blood ratio was 246 [102]. The *in vitro* effectiveness of the concentration in the lungs to cure SARS-CoV-2 is achievable in humans.

Halofantrine is a synthetic antimalarial which is effective against multi drug resistant *P. falciparum*, and which belongs to the phenanthrene class, together with quinine and lumefantrine. Its antiviral activity against SARS-CoV-2, evaluated by viral expression (GFP) was demonstrated at an MOI=2.2 with an EC_{50}=0.3µM on HeLa ACE2. [24]

Tafenoquine was approved by the FDA in 2018 for use in the treatment and prevention of the relapse of malaria caused by *Plasmodium vivax*. The maximal plasmatic concentration of tafenoquine exhibited interindividual variability, its bioavailability being greatly influenced by high-fat meals. Preliminary studies demonstrated that tafenoquine had an antiviral activity against SARS-CoV-2 in Vero E6 cells in two studies that found an EC_{50} from 2.5 to 16µM [103;104] .

Artemisinin and derivatives

Artemisinin ("qinghaosu") is an ancient medical drug used in traditional Chinese medicine, used as for the treatment of malaria, traces of which were found in emergency prescriptions issued by the physician Ge Hong (284-363). It is extracted from the plant qinghao, which is the wormwood *Artemisia annua.* Its potent activity against *Plasmodium*, including chloroquine-resistant *Plasmodium falciparum*, was recently rediscovered with a large-scale promotion of its use in 2004. Artemisinin-based combination therapies are

currently recommended by the World Health Organization (WHO) as first-and second-line therapies for malaria. The antiviral activity of *Artemisia annua* derivatives has been explored in *in vitro* cell cultures against SARS-CoV-2. [97;105;106]. Gilmore *et al.* showed that artesunate was more potent than the *Artemisia annua* plant extracts, artemisinin and artemether (which was found not to be effective against the virus) with an EC_{50} of 7 µg/mL (3.4 µM), 128-260 µg/mL (7.3 µM), 151 µg/mL (535 µM), and > 179 µg/mL (> 600 µM) on Vero E6 cells, and similar results on human hepatoma Huh7.5 cells. It is notable that close to complete inhibition of the viral replication was obtained for 15µg/mL and 22 µg/mL on Vero E6 and Huh 7.5 cells, respectively. However, dihydroartemisinin is the active metabolite of all artemisinin derivatives (artemisinin, artemether, artesunate). Dihydroartemisinin showed low antiviral activity with an EC_{50} of 20.1 µM at an MOI of 0.25 [100]. Artesunate, which displays a better oral bioavailability compared to artemether, [107] with a maximal plasmatic peak concentration of 29.5µM after an IV bolus of 120 mg and 2.6µM after an oral dose of 100 mg, could be an effective antiviral *in vivo* [108].

Since 2002, the WHO has recommended the use of artemisinin-based combination therapy in the treatment of uncomplicated falciparum malaria (artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperaquine and artesunate-mefloquine). A study showed that concentrations of fixed-doses of artemisinin-based combination therapy equivalent to C_{max} of the two partners at commonly recommended doses for uncomplicated malaria, were able to inhibit 27.1 to 72.1% of the Vero E Cells infected with SARS-CoV-2 [109]. Treatment with artesunate-mefloquine (expected blood C_{max} at 8.3 and 1 µM) leads to replication inhibition of 72.1%.

2.2. Other antiparasitic agents

Nitazoxanide

Nitazoxanide is a broad spectrum anti-infective drug that belongs to the class of *thiazolides*. It was approved in 2002 for the treatment of *Cryptosporidium* and *Giardia lamblia* infections. Its antiviral activity was previously shown *in vitro* against a wide range of RNA and DNA viruses including influenza, respiratory syncytial virus, rotavirus, hepatitis B, hepatitis C, dengue, human immunodeficiency virus, and more recently against SARS-CoV-2 [110]. Indeed, in one study its EC_{50} was 2.1 µM on Vero E6 cells with a high selectivity index (>17) [19]. One pharmacokinetic analysis suggested that nitazoxanide plasma levels could reach concentrations above the reported EC_{50} level[43]. As of July 2020, it is being studied in five ongoing and nine planned clinical trials. A single 500mg dose treatment of nitazoxanide

achieves within one to four hours, tizoxanide (active metabolite of nitazoxanide) plasma concentrations greater than 10 μM, with a half-life of 1.3 to 1.8 hours and good tolerance [111].

Niclosamide

Niclosamide is an anthelmintic developed in 1953 by Bayer laboratories, approved by the FDA in 1982 for human use, and currently included in the WHO's list of essential medicines. It acts as an anticestodal by uncoupling the oxidative phosphorylation, thus inhibiting the production of ATP, which is essential for the energetic metabolism of the parasite [112]. It is effective against *Taenia saginata* (beef tapeworm), *Taenia solium* (pork tapeworm), *Diphyllobothrium latum* (fish tapeworm) and *Hymenolepis nana*. Drug repurposing screening studies identified niclosamide as a multifunctional drug which displays a large range of clinical applications such as bacterial and viral infections, metabolic diseases, neuropathic pain, rheumatoid arthritis, and even cancer. Among the proposed mechanisms of action, it may regulate several signalling pathways and biological processes including notably mTOR (mammalian target of rapamycin), STAT3 (signal transducer and activator of transcription 3), and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signalling pathways, and may also reduce endosomal acidification and viral dsRNA replication [113;114].

Its antiviral activity against SARS-CoV was demonstrated on Vero E6 cells [115;116] and against SARS-CoV-2 on the same cell lines and also Vero, Huh7, and Vero [6;18;23;117]

Ivermectin

Ivermectin is a semisynthetic broad spectrum anthelmintic agent, that is orally administered in the treatment of intestinal strongyloidiasis due to *Strongyloides stercoralis*, onchocerciasis due to *Onchocerca volvulus* and scabies due to *Sarcoptes scabiei*.

It showed an antiviral activity against SARS-CoV-2 on Vero E6 cells with a EC50 at 1.7μM with an MOI of 0.01 evaluated by viral expression (N protein) [118]. Congruently, computational studies predicted that ivermectin could dock in two specific regions of the SARS-CoV-2 spike and of the ACE2 receptor, with a binding energy with the complex spike-ACE2 of -18 kcal/mol [119].

Emetine

Emetine is a toxic alkaloid of ipecac, extracted from the root of the plant *Psychotria Ipecacuanha* (Rubiaceae) used in phytomedicine for centuries and known to be the main component of ipecac syrup used as an emetic. Emetine is a BANM-approved drug for the

treatment of intestinal amebiasis but is not currently FDA-approved. In eukaryotic cells, emetine irreversibly blocks ribosome movement along the mRNA thus preventing protein synthesis strands and inhibiting DNA replication. It has also been shown that it may up- and down-regulate several genes [120]. It also exhibits many biological properties including as antimalarial, antineoplastic, antiamoebic, contraceptive and antiviral activities against vaccinia, dengue, Zika, Ebola and SARS-CoV-2 viruses [17;120-124]. It was reported that emetine could inhibit dengue virus at an early stage of the replication cycle, possibly by blocking the translation of the polyprotein precursor, a key step for the formation of viral proteins and further RNA replication [121]. It also inhibits Zika and Ebola virus infections by inhibiting viral replication and decreasing viral entry [123]. It is no longer currently marketed in medical specialties but has long been used in the past for the treatment of intestinal amebiasis and for emptying the stomach in the cases of acute intoxication.

Adverse events after its ingestion include cardiac and hepatic events, renal toxicity, diarrhoea and vomiting. Emetine has been shown to inhibit multiple SARS-CoV and SARS-CoV-2 *in vitro*, with an EC_{50}s < 1.0 µM in Vero cells and in Caco2 cell lines, respectively (EC_{50}=0.05µM for SARS-CoV and 0.5µM for SARS-CoV-2). Interestingly, 8 and 24 hours after oral administration, the concentration of emetine in the lungs is ~173 and ~294 times higher than in plasma [122].

3. Antibiotics

Clofazimine

Clofazimine is a highly lipophilic antimicrobial agent that acts on the respiratory chain of bacteria and on ion transporters. By oxidising the reduced form of clofazimine, the intracellular cycle of redox reactions provides ROS with an antimicrobial activity. Moreover, clofazimine interacts with the phospholipid bilayer of the membrane, generating antimicrobial lysophospholipids that favour membrane dysfunction, which raises anomalies in potassium K^+ recapture. Clofazimine is used in the treatment of leprosy. It also displays an anti-inflammatory activity due to the suppression of T-lymphocyte activity. The main adverse event is the orange-pink to brownish-black discoloration of the skin, conjunctivae, and body fluids. Antiviral activity has been observed on SARS-CoV-2 on Huh7, Vero E6 with low EC50 ranging from 0.08 to 0.5 µM [23;125].

Yuan *et al.* also demonstrated this antiviral effect on primary cells. First, on human embryonic stem cell-derived cardiomyocytes, clofazimine at 10µM could reduce viral titres in the cell lysate by >3-log10 compared with the DMSO control. Second, an *ex vivo* lung culture system

infected with SARS-CoV-2 for 24 hours and clofazimine treatment starting two hours post-inoculation revealed a potent inhibition of viral replication [5]. In this study, the authors conclude that the effective antiviral concentration of clofazimine of 310nM may be achievable in patients, the peak serum concentration being 861 nM.

A docking study using a virtual screening procedure of the 1,615 FDA-approved drugs selected clofazimine as one of the top 25 compounds with lowest docking score with SARS-CoV-2 main protease [126].

Azithromycin

Azithromycin is a broad-spectrum macrolide antibiotic, approved by the FDA in 1991. As is the case for other macrolides, it inhibits bacterial protein synthesis and translation, in addition to an additional immunomodulatory effect [127]. It has an extensive uptake in tissue, particularly in the lungs, tonsils and prostate [127]. It exhibits an antiviral activity against SARS-CoV-2 with an EC_{50} of 2.1µM on VeroE6 cells at an MOI of 0.002 [20]. It is notable that the combination of hydroxychloroquine at 5µM and azithromycin at 5µM tested on Vero-E6 cells resulted in a relative inhibition of SARS-CoV-2 of 97.5%. [128] It has been predicted that azithromycin could interact with a conserved amino acid triad Q-134/F-135/N-137, located at the tip of the SARS-CoV-2 spike protein, but also displays strong interactions with the viral main protease, and two host proteins involved in the replications cycle of SARS-CoV-2: the receptor ACE2 and the host cathepsin L [129;130].

Doxycycline

Doxycycline, a second-generation tetracycline with broad-spectrum antimicrobial, antimalarial and anti-inflammatory activities, showed an EC_{50} of 4.5 µM against SARS-CoV-2 infected Vero E6 cells at an MOI of 0.25 [131]. Doxycycline interacted at both entry and post-entry stages of SARS-CoV-2 infection in Vero E6 cells. A daily oral uptake of 100 mg or 200 mg of doxycycline in healthy volunteers led to a C_{max} value of 1.7 and 5 µg/mL (around 3.4 and 10 µM) [132;133]. The C_{max}/EC_{50} and C_{max}/EC_{90} ratios for doxycycline in plasma ranged from 0.75 to 2.21. The C_{max}/EC_{50} ratios in plasma would appear low to reach effective concentrations to inhibit SARS-CoV-2 in humans. However, in the lungs, doxycycline was two to four times higher than in plasma [134]. A daily oral uptake of 100 or 200 mg of doxycycline led to a C_{max} value from 3.4 to 20 µg/g in the lungs.

The inhibition of both entry and viral replication after SARS-CoV-2 entry is consistent with results from combinatorial computational approaches. Docking analysis showed that doxycycline could strongly bind to the spike protein (S) of SARS-CoV-2 [135]. Moreover, doxycycline and, more generally, tetracyclines could bind to the main protease (M^{pro}) of

SARS-CoV-2, also known as 3C-like protease, which is essential for the replication cycle of SARS-CoV-2 by leading to the formation of non-structural proteins (NSPs) [136;137].

Teicoplanin

Teicoplanin, a glycopeptide antibiotic used to treat Gram-positive bacterial infections prevents the early step of the viral life cycle by inhibiting cathepsin L in the late endosome/lysosome and blocking the entry of pseudo-typed viruses for Ebola, MERS-CoV and SARS-CoV-1 [138]. For SARS-CoV-2, docking studies also showed that this molecule harboured a relatively high affinity with the $3CL^{Pro}$ protease, with ten to twenty times greater potency in inhibiting protease activity than other drugs such as atazanavir, chloroquine, hydroxychloroquine, azithromycin, or lopinavir [139]. To date, the antiviral activity on SARS-CoV-2 has only been assessed with spike-pseudoviruses, and shows an IC_{50} of only 1.66 µM, which is lower than the routine serum drug concentration (~7–8 µM) [140]. Further *in vitro* studies are needed to assess the antiviral activity of this molecule on SARS-CoV-2.

4. Antipsychotics

4.1. Phenothiazine derivatives

Promazine

Promazine is a dopaminergic antagonist, a H1-receptor antagonist, a muscarinic antagonist, and a serotonergic antagonist. It is a phenothiazine antipsychotic drug with antiemetic properties and acts as a prolyl oligopeptidase inhibitor. It does not exhibit antiviral efficacy against SARS-CoV on Vero76 cells with EC_{50} ranging from 7.4 to 28µM.[64;141]

Chlorpromazine

Chlorpromazine is an antipsychotic agent with anti-emetic activity. It antagonises dopamine receptors. It is active against SARS-CoV on Vero E6 cells [74;75] and also recently showed an antiviral effect against SARS-CoV-2 [79]. A sensitivity assay against SARS-CoV-2 on Vero E6 found an EC_{50} between 3.1µM with an evaluation by CPE and an MOI of 0.004 [79].

4.2. Thioxanthene derivatives: Chlorprothixene

Chlorprothixene is a dopamine receptor antagonist (D1, D2, D3) used as an antipsychotic drug. Chlorprothixene also strongly blocks the 5-HT2, histamine H1, muscarinic and alpha1 adrenergic receptors. It has shown antiviral activity against SARS-CoV-2 with an EC_{50} of 8.9µM on Vero E6 at an MO I0.002. [142]

5. Antihistaminics

Desloratadine

Desloratadine is synthetic piperidinyl-benzimidazole derivative, and a reversible competitive inhibitor of histamine H1 receptors, with antiallergic properties. It inhibits SARS-CoV-2 replication at an MOI of 0.5 on A549/ACE2 cells with an EC_{50} of 0.9µM, evaluated by viral expression of the S protein [28].

Ebastine

Ebastine is a second-generation piperidine H1-antihistamine which potently antagonises H1-histamine receptors. It demonstrates an antiviral activity against SARS-CoV-2 with an EC_{50} ranging from 1.2 to 6.9µM on 4 different cell lines: Calu3, Huh7.5, Vero, and Vero CCL81 [18;27].

6. Kinase inhibitors

Tyrosine kinase inhibitors are an orally-administered targeted treatment of malignancies. They are competitive inhibitors of ATP at the catalytic binding site of tyrosine kinase. They target different kinases, and cause skin toxicity, mainly folliculitis, in addition to myelosuppression (anaemia, thrombopenia, neutropenia) and compound specific adverse events.

Imatinib was the first labelled tyrosine kinase inhibitor, indicated for chronic myeloid leukaemia with the Philadelphia chromosome. On SARS-CoV-1 infected Vero E6 cells, its EC_{50} ranged from 5 to > 20µM. [74;143]. A lower EC_{50} (3.2µM) was observed for SARS-CoV-2 on the same cells in two different assays with an MOI of 0.004 and 0.01 [79].

Lapatinib is used to treat breast cancer with an overexpression of ErbB2 receptors. It showed an antiviral effect on SARS-CoV-2 at an MOI=0.5 on A549/ACE2 cells with an EC_{50} of 1.6 µM [28].

Dacomitinib is indicated for the treatment of metastatic non-small cell lung cancer. On Huh7.5 cells, with an MOI of 1, an EC_{50} against SARS-CoV-2 was 0.8µM when the effect was evaluated by visual inspection of cytopathic effects, with a selectivity index of 15. [27]

On Calu-3 and with an MOI of 0.5, the EC_{50} was 0.04µM ,with a high selectivity index of 226 and an evaluation by visual inspection of cytopathic effects [27].

Bosutinib is indicated for Philadelphia+ chronic myeloid leukaemia. On Huh7 cells, SARS-CoV-2 at an MOI of 0.2 was inhibited by Bosutinib with an EC_{50} of 0.02µM with a high selectivity index >100 [23].

Fedratinib, used for treatment of myelofibrosis, exhibits an antiviral activity against SARS-CoV-2 at an MOI of 0.2 on Huh7 cells with an EC_{50} of 0.02µM and a selectivity index of 83 [23].

Gilteritinib is indicated for the treatment of acute myeloid leukaemia with FLT3 receptor mutation. FDA approved in 2018 [18;23;34], it shows a heterogenous profile of activity against SARS-CoV-2 depending on the cells used in the different sensitivity assays. The most potent activity was observed on Huh7 cells, at an MOI=0.2 with a low EC_{50} of 0.2µM and a selectivity index of 8.9. Intermediately, on Vero cells with an MOI=0.01, the EC_{50} was 6.8µM with a selectivity index of 5.5. Finally, on Calu-3 cells, at an MOI of 0.1, no antiviral activity could be observed with an EC_{50}>50µM**.**

Nilotinib is a Bcr-abl Kinase inhibitor that was approved by the FDA in 2007 for the treatment of Ph+ chronic myeloid leukaemia. Adverse events include myelosuppression and prolongation of the QT interval. Interstitial pneumonia has also been reported. It seems to have a potent antiviral activity against SARS-CoV-2 at an MOI=0.1 with an EC_{50} of 0.08µM on Vero E6 cells [144] and <0.01µM on Vero cells with a selectivity index ≈ 3000 [145]. This antiviral activity operates by an unknown mechanism. A virtual screening of the ZINC database showed that Nilotinib could interact with the NSP12-NSP7-NSP8 interface of SARS-CoV-2, which is the essential component of the replication complex of SARS-CoV-2 with NSP12 consisting in the catalytic subunit with RNA-dependent RNA polymerase activity, and NSP7 and NSP8 being cofactors that stimulate this polymerase activity [146]. Another molecular docking study that screened 15,000 molecular candidates from DrugBank and natural compounds from the Traditional Chinese Medicine Systems Pharmacology Database showed that nilotinib was among the top ten RBDs of the viral spike-binding molecules from DrugBank with a free energy of -7.9 kcal/mol [147].

7. Immunosuppressive agents

Mycophenolate is an immunosuppressant and antiproliferative drug used to treat prophylaxis or organ rejection in renal transplant recipients in combination with cyclosporin and corticosteroids. It acts as a selective and competitive inhibitor of inosine monophosphate

dehydrogenase and thus inhibits *de novo* guanosine nucleotide synthesis. It also displays a potent inhibitory effect on proliferative T and B lymphocytes responses. It has also been shown that it could inhibit TMPRSS2, which is involved in SARS-CoV-2 entry. On SARS-CoV-2 infected Vero E6 /TMPRSS2, an EC_{50} of 0.9µM was found at an MOI of 0.01 [148].

Cyclosporin is a calcineurin inhibitor which was FDA-approved in 1983, with potent immunosuppressive properties on T cells for preventing organ rejection and preventing and treating graft versus host disease in bone marrow transplantation. Its immunomodulatory properties are also used for various autoimmune conditions such as rheumatoid arthritis, and inflammatory diseases such as severe psoriasis.

Its antiviral effect explored on SARS-CoV-2 infected Calu-3 cells showed an EC_{50} of 0.2µM with an MOI of 0.5 [27] and an EC_{50} of 4.7µM with an MOI of 0.1 [34].

The highest EC_{50} was observed on Vero cells and was 5.8µM at an MOI of 0.01 with an evaluation by viral expression of N protein [18].

Although immunosuppressant drugs are not an option for therapeutic use in COVID-19, it is worthwhile exploiting the antiviral profile that has been shown *in vitro*, in organ transplant recipients receiving long term treatment with these molecules.

8. Cardiac glycosides

Cardiac glycosides are organic compounds that potently inhibit the Na+/K+ exchanging ATPase, leading to the increase of Na+ intracellular concentration, and to an increase in intracellular Ca2+ via the Na+/Ca2+ pump. This increased intracellular calcium concentration is the basis of the inotropic property of these drugs. Although they cannot reasonably be an option in the treatment of COVID-19, some sensitivity assays have been performed to assess their potential antiviral activity.

Digoxin originated from *Digitalis purpurea* is indicated for atrial fibrillation and heart failure. It has been shown to have an antiviral activity against SARS-CoV-2 with an EC_{50} ranging from 0.04 (at an MOI of 0.1) to 0.2µM (at an MOI of 0.01) on Vero cells [18;145].

Digitoxin has a longer half-life than digoxin and is used for congestive cardiac insufficiency, arrythmias and heart failure. EC_{50} for SARS-CoV-2 ranges from 0.1 (at an MOI of 0.1) to 0.2µM (at an MOI of 0.01) à on Vero and Calu-3 cells [18;145;149].

Ouabain is a glycoside obtained from the seeds of *Strophanthus gratus* and is indicated for atrial fibrillation flutter and heart failure, with a potent antiviral activity against SARS-CoV-2 at an MOI of 0.01 with an EC_{50}<0.1µM on Vero and Calu-3 cells with high selectivity indices [18;83].

9. Antineoplastics

Antineoplastic agents do obviously not represent a clinical option for SARS-CoV-2 infection, however their potent antiviral activity shown in a few *in vitro* studies could be interesting to explore in patients currently treated by these molecules.

Gemcitabine is a cytidine analogue that blocks the enzyme that converts cytosine into deoxycytosine. It also blocks thymidylate synthetase, resulting in blocking DNA replication and in premature apoptosis and arrested tumour growth. It is labelled as a chemotherapeutic agent in various carcinomas. Its antiviral activity against SARS-CoV-2 was assessed at an MOI of 0.005 on Vero E6 cells at an EC_{50} of 1.2µM with a selectivity index >32 [150].

Thioguanine is a 6-thiopurine analogue, competing with hypoxanthine and guanine, and also belongs to the family of antimetabolite agents. It is used in acute non-lymphocytic leukaemias. It displays an additional cytotoxic action due to its incorporation into RNA. On SARS-CoV-2 infected Huh7 cells, at an MOI of 0.2, the EC_{50} of thioguanine was 0.2µM with a selectivity index >9.3 [23].

10. Anti-oestrogens

Tamoxifen is a non-steroidal anti-oestrogen compound that competitively inhibits oestrogen binding to its receptor and is used for the treatment of oestrogen-receptor positive breast cancer. It was FDA-approved in 1977. Toremifene is a non-steroidal selective oestrogen receptor modulator with a structure that is closely related to tamoxifen and which is also indicated in the treatment of breast cancers. Tamoxifen citrate and Toremifene were respectively tested in three and four sensitivity assays against SARS-CoV-2 with an EC_{50} ranging from 1.8 to 34µM and 4.8 to 12µM on Vero E6 respectively [18;74;79;142]. It should be noted that tamoxifen citrate does not exhibit an antiviral effect against SARS-CoV with an EC_{50} of 93µM on SARS-CoV infected Vero E6 cells [74].

However, the EC_{50} of raloxifene against SARS-CoV-2 was 3.8µMon A549/ACE2 at an MOI of 0.5 [28].

11. Calcium channel blocker with an action on the cardiovascular system

Amlodipine is an antihypertensive drug belonging to the family of dihydropyridine calcium channel blockers, used for the treatment of hypertension, coronary artery disease and chronic

stable angina. It does not exhibit an antiviral activity as assessed in six cell culture assays with an imprecisely defined EC_{50} but <50µM [151] on Vero E6 and Calu-3 cells and <10µM on HPSC-derived organoids and A549/ACE2 [152], moreover with low selectivity indexes.

Verapamil is an old drug belonging to the family of non-dihydropyridine calcium channel blockers such as diltiazem, for which an antiviral activity was demonstrated in combination with remdesivir in one study [46]. It is used for hypertension, arrythmias and angina. On SARS-CoV-2-infected Huh7 cells (MOI 0.2), the EC_{50} of verapamil was low, at 0.5µM with a selectivity index >3.8µM [23].

12. Drugs acting on the alimentary tract and metabolism

Loperamide is a long-acting synthetic antidiarrheal by inhibiting peristaltic activity. It is an opioid µ receptor agonist and a non-selective calcium channel blocker. Although it is not absorbed from the gut, its antiviral affect was assessed against SARS-CoV-2 and revealed EC_{50} varying from 9.3µM at an MOI of 0.01 [18;18] on Vero cells to 13µM at an MOI of 0.1 on Calu-3 cells [34].

Metoclopramide is a dopamine D2 antagonist with prokinetic and antiemetic effects that treats nausea, vomiting and gastro-oesophageal reflux disease. It was FDA-approved in 1980. On Huh7 cells, it shows an antiviral activity against SARS-CoV-2 at an MOI of 0.2 with an EC_{50} of 0.5µM [23].

13. Others

Lomitapide is a microsomal triglyceride transfer protein inhibitor indicated for homozygous familial hypercholesterolemia, largely used to decrease LDL-cholesterol and total cholesterol levels. An antiviral activity against SARS-CoV-2 at an MOI of 0.2 was shown on Huh7 cells, with an EC_{50} of 0.5µM, efficacy being evaluated by the viral expression of N protein [23].

Proteinase inhibitor antifibrinolytics

Aprotinin, a single chain polypeptide isolated from bovine lung with antifibrinolytic and anti-inflammatory activities, camostat and nafamostat, two serine protease inhibitors, are three antifibrinolytic drugs tested in numerous sensitivity assays for SARS-CoV-2. Camostat and nafamostat may act by inhibiting the host TMPRSS2. Given the thrombotic risk in SARS-CoV-2 infection, these drugs, although they showed a potential antiviral activity especially for camostat and nafamostat with a respective EC_{50} ranging from 0.3µM (on Calu-3) and >50µM

(on Vero) [18;27] and from 0.002 (on Calu-3) and >100µM (on VeroE6/TMPRSS2) [153;154], cannot be considered as therapeutic options.

Apilimod is an inhibitor of the production of IL12 and IL23, initially developed for the treatment of Crohn's disease and rheumatoid arthritis but which was ultimately not effective in these indications. It also inhibits the lipid kinase enzyme PIKfyve. It was subsequently repurposed for Ebola virus disease and Lassa fever. It is not currently FDA-approved.

The EC_{50} obtained on SARS-CoV-2-infected Vero E6 cells was 0.02µM at an MOI of 0.002 with an evaluation of cytopathic effects and viral expression. Other sensitivity assays carried out on HeLa-ACE2, A549/ACE2, 293T/ACE2 and Huh-7/ACE2 showed low EC_{50} below 0.9µM.[21;21;26] [24] [25]

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Table 1 summarises the main studies of sensitivity assays i.e., where at least three tests were performed for each compound, regardless of the virus. The main results of molecular docking studies are summarised in Supplementary Table 1.

Finally, when comparing the results of molecular docking with those of the most potent compounds found in *in vitro* sensitivity assays based on an EC_{50}<3µM, 18 compounds were found by both approaches.

Fourteen of these are currently FDA-approved. These include digitoxin, a cardiac glycoside, two antivirals (remdesivir, nelfinavir and lopinavir), three antimalarial drugs (amodiaquine, chloroquine and hydroxychloroquine), one immunosuppressant (Cyclosporin A), two antineoplastics (nilotinib and tretinoin), one anti-inflammatory (celecoxib), two antibiotics (azithromycin and clofazimine) and one anthelminthic (ivermectin). As possible therapeutic options in COVID-19, when considering the possibility of oral administration, and the adverse events inherent to their pharmacological properties, nelfinavir, lopinavir, azithromycin, clofazimine, ivermectin, amodiaquine, chloroquine and hydroxychloroquine represent possible drug candidates for COVID-19 treatment.

DISCUSSION

In this study, we attempted to review as exhaustively as possible the different molecules that have been tested *in vitro* on SARS-CoV-2. In recent months, we have seen a competition between drugs recently produced by the pharmaceutical industry (Lopinavir, Remdesivir, Ritonavir) and older molecules. It is clear that the significant paradigm that is emerging is that older molecules, known to be active in a certain area, are likely to be active in other functions. Thus, among the usable molecules that are effective *in vitro* on SARS-CoV-2 are molecules belonging to therapeutic classes as different as anti-malarial drugs, other anti-parasitic agents, antibiotics, anti-psychotics, immunosuppressive agents, cardiotonic glycosides and many other families of molecules. Some of these molecules have been known for a long time, all of them are available and are extremely low cost, which poses the problem of managing therapeutic trials using these molecules in pathologies requiring a reinforcement of the therapeutic arsenal. It is likely that it is in the poorest countries or countries where there is no pharmaceutical industry, that clinical trials involving these molecules will develop,

where there are no conflicts of interest with new molecules that are likely to be extremely profitable. This is already the case in Pakistan, Iraq, and Bangladesh where clinical trials evaluating the efficacy of such molecules in COVID-19 are already taking place (https://clinicaltrials.gov). In any case, we are experiencing a turning point in infectious disease therapeutics, given that there are a considerable number of molecules of natural origin or produced or improved by humans, which have multiple activities and have the advantage of being both inexpensive and having a level of toxicity that is perfectly known and identified, which can save several years and considerable sums of money to achieve for entirely new products. However, the economic model allowing molecules of this nature to be repositioned is currently lacking in Western countries and will have to be the subject of major political reflection.

SUMMARY POINTS :

- A considerable number of existing molecules of natural origin or produced or improved by humans display multiple activities.
- In the current context of health emergency, drug repurposing constitutes the most relevant approach to get therapeutics options. It allows bypassing long pre-clinical steps, and uses molecules for which the security and the toxicity are well known and have been for a long time, in addition to the advantage of the low cost of the use of old molecules.
- Since the beginning of the COVID-19 pandemics at the end of 2019, numerous studies including large scale screening assessed the *in vitro* antiviral activity of available FDA-approved compounds against the SARS-CoV2.
- When considering the possibility of oral administration, and the adverse events inherent to their pharmacological properties, the review of these studies allow to finally consider as possible therapeutic options in COVID-19, a total of 8 possible drug candidates for COVID-19 treatment.

FUTURE PERSPECTIVE:

Drug repurposing is a different view of pharmaceutical research, undoubtedly marks a turning point in infectious disease therapeutics, and deserves be the subject of major political reflection.

FIGURE LEGENDS

Figure 1. Cell lines that can be used in sensitivity assays for human coronaviruses, according to their cell receptors

Figure 2. Representation of the discrepant parameters used in sensitivity assays.

Figure 3. Schematic of the replicative cycle of SARS-CoV-2 and evidenced or putative sites of action of drugs

TABLES

TABLE 1 MOST POTENT COMPOUNDS ON SARS-COV-2 ACCORDING TO *IN VITRO* SENSITIVITY ASSAYS THAT OBTAINED EC50<3µM, AND CORRESPONDING COMPUTATIONAL PREDICTIONS

Compound	Status	Sensitivity assays				Predicted by molecular docking
		Minimal EC_{50} (µM)	Cells	Maximal EC_{50} (µM)	Cells	
Anthelminthic						
Niclosamide	**Approved, Investigational, Vet Approved**	0.09	Vero E6	0.3	Vero	No
Ivermectin	**Approved**	1,7	Vero E6	2	Vero/hSLAM	Yes - Kadioglu (https://www.who.int/bulletin/online_first/20-255943.pdf)
Antiarrythmics						
Amiodarone	**Approved, Investigational**	0.05	Huh7	>100	Vero	No
Verapamil	**Approved**	0.5	Huh7			No
Antibacterial						
Nigericin	*Experimental*	0.09	VeroE6/TMPRSS2			No
Brilacidin	*Investigational*	0.6	Calu-3	~5	Vero	No
Azithromycin	**Approved**	2.1	Vero E6			Yes - El-hoshoudy, J Mol Liquids 2020; Fantini, Int J Antimicrob Agents, 2020 ; Bezerra Braz, International Journal of Antimicrobial Agents, 2020
Clofazimine	**Approved, Investigational**	0.01	Vero	<5	Cardiomyocytes	Yes - Hosseini, Life Science 2020
Lasalocid	*Vet Approved*	0.4	VeroE6/TMPRSS2			No
Salinomycin	*Vet Approved*	0.003	Calu-3	0,2	Vero CCL81	No
Monensin	*Experimental, Vet Approved*	0.1	VeroE6/TMPRSS2			No
Monensin sodium salt	*Experimental, Vet Approved*	0.6	Vero			No
Narasin	*Experimental, Vet Approved*	0.07	VeroE6/TMPRSS2			No
Indanomycin	*Not listed*	0.6	VeroE6/TMPRSS2			No
Antidepressant						
Clomipramine	**Approved**	2	A549/ACE2	14	Vero E6	No
Trimipramine	**Approved**	1.5	A549/ACE2	10	Vero E6	No
Indatraline	*Not listed*	1.6	VeroE6/TMPR			No

			SS2			
Antifibrinolytic						
Aprotinin	*Approved, Investigational, Withdrawn*	0.5	Caco-2			No
Aprotinin/Omp	*Approved, Investigational, Withdrawn*	0.2	Caco-2			No
Alpha-1 antitrypsin	*Investigational*	0.8	Vero E6	>20	Caco-2	No
Nafamostat	*Investigational*	0.002	Calu-3	>100	VeroE6/TMPRSS2	No
Camostat	*Experimental*	0.3	Calu-3	>50	Vero	No
Antifungal						
Ketoconazole	**Approved, Investigational**	2.4	Caco-2			No
Cycloheximide	*Not listed*	0.6	Caco-2			No
Antihistaminic						
Desloratadine	**Approved, Investigational**	0.9	A549/ACE2			No
Ebastine	**Approved, Investigational**	1.2	Huh7.5	6,9	Vero	No
Astemizole	*Withdrawn*	0.9	293T/ACE2	~1,2	Vero E6	No
Azelastine	**Approved**	2.4	A549/ACE2			No
Antiinflammatory						
Celecoxib	**Approved**	0.04	Vero			Yes - Gimeno, Int J Mol Sci 2020
Auranofin	**Approved Investigational**	1.4	Huh7			No
Antineoplastic						
Brequinar	*Experimental*	0.3	Vero E6			No
Gemcitabine	**Approved**	1.2	Vero E6			No
Thioguanine	**Approved**	0.2	Huh7			No
Tamoxifen citrate	**Approved**	1.8	Vero E6	34	Vero E6	No
Bemcentinib	*Investigational*	0.1	Huh7.5	>50	Vero E6	No
Naquotinib	*Investigational*	0.06	Huh7.5			No
Tamibarotene	*Investigational*	2.5	Vero E6			
Tretinoin	**Approved, Investigational, Nutraceutical**	1	Vero E6			Yes -Dey, Comput Biol Med, 2020
Raloxifene HCl	**Approved, Investigational**	0.02	Vero			No
Bafetinib	*Investigational*	2.2	A549/ACE2			No
Bosutinib	**Approved**	0.02	Huh7			No
Fedratinib	**Approved, Investigational**	0.02	Huh7			No
Gilteritinib	**Approved, Investigational**	0.2	Huh7	>50	Calu-3	No
Dacomitinib	**Approved, Investigational**	0.04	Calu-3	0,8	Huh7.5	No
Lapatinib	**Approved, Investigational**	1.6	A549/ACE2			No
Nilotinib	**Approved, Investigational**	0.08	Vero E6			Yes - Ruan J Med Virol 2020; Wei, Chin J Integr Med 2020
Abiraterone acetate	**Approved**	1.9	Vero E6	7,1	Vero E6	No

Berzosertib	*Investigational*	0.005	Vero E6	0,7	Vero E6	No
Temsirolimus	**Approved**	2.9	Vero			No
Vistusertib	*Investigational*	0.02	Vero E6			No
Antiparkinson agent						
Benztropine	**Approved**	1.8	A549/ACE2			No
Antiprotozoal						
Nitazoxanide	**Approved**	1	Vero E6	4,9	Vero E6	No
Emetine	*Experimental; not Approved*	0.5	Vero E6			Yes - Das Journal of Biomolecular Structure and Dynamics 2020;
Suramin	*Investigational*	2.9	Vero E6	20	Vero E6	No
Diiodohydroxyquinoline	**Approved**	1.4	Vero E6			No
Pyronaridine	*Investigational*	0.2	Huh7.5	8,6	Calu-3	Yes - Hosseini Life Sciences 2020
Piperaquine (in combination with dihydroartemisinin)	*Experimental, Investigational* -	2.1	Huh7.5			No
Maduramycin	*Vet Approved*	0.06	VeroE6/TMPRSS2			No
Amodiaquine	**Approved, Investigational**	0.6	Huh7.5	>50	Calu-3	Yes - Peele, Inform Med Unlocked. 2020
Hydroxychloroquine	**Approved**	0.2	Huh7.5	<10	Vero E6	El-hoshoudy, J Mol Liquids 2020; Bezerra Braz, International Journal of Antimicrobial Agents, 2020; Chitranshi, J Transl Med. 2020; Fantini, Int J Antimicrob Agents, 2020
Tafenoquine	**Approved, Investigational**	2.5	Vero E6	16	Vero E6	No
Chloroquine	**Approved, Investigational, Vet Approved**	0.1	Vero E6	>50	Vero E6	Yes - Li et al (preprint) 2020; El-hoshoudy, J Mol Liquids 2020; Bezerra Braz, International Journal of Antimicrobial Agents, 2020; Chitranshi, J Transl Med. 2020
Artesunate	**Approved, Investigational**	0,5	Calu-3	53	Vero	No
Halofantrine	**Approved**	0,3	HeLa-ACE2			No
Antipsychotic						
Flupenthixol	*Approved, Investigational, Withdrawn in USA*	0.6	A549/ACE2			No
Thioridazine HCl	*Withdrawn*	2.2	Vero			No

Elopiprazole	*Not listed*	1.6	Vero E6	2,7	Huh-7/ACE2	No
Metoclopramide	**Approved Investigational**	0.5	Huh7			No
Antiseptic (topical)						
Hexachlorophene	*Withdrawn*	0.9	Vero	1,5	Calu-3	No
Antiviral						
Atazanavir/r	**Approved**	0.5	Vero E6	0,6	A549	No
Daclatasvir	**Approved**	0.6	Huh7	1,1	Calu-3	No
Remdesivir	**Approved Investigational**	0.002	Huh7.5	<20	HAE	Yes - Hall Travel Medicine and Infectious Disease 2020; El-hoshoudy, J Mol Liquids 2020; Chitranshi, J Transl Med. 2020; Elfiky, Life Sciences 2020;
Diltiazem + Remdesivir	**Approved Investigational**	0.3	Vero	0,7	Vero	No
Remdesivir/Omp	**Approved Investigational**	0.02	Caco-2			No
Entecavir	**Approved, Investigational**	0.04	Huh7	>20	Vero	No
Boceprevir	*Approved, Withdrawn*	1.9	Vero 76			Yes - Eleftheriou, Molecules, 2020
Lopinavir	**Approved**	1.7	VeroE6/TMPRSS2	>50	Vero E6	Das Journal of Biomolecular Structure and Dynamics 2020; Hakmi, Bioinformation, 2020; El-hoshoudy, J Mol Liquids 2020; Eleftheriou, Molecules, 2020; Chitranshi, J Transl Med. 2020; Peele, Inform Med Unlocked. 2020
Atazanavir	**Approved**	0.2	A549	>50	Vero	No
Nelfinavir	**Approved**	0.8	VeroE6/TMPRSS2	>50	Vero E6	Yes - Musarrat, J Med Virol, 2020; Huynh, J Phys Chem Lett, 2020
Cardiac glycoside						
Ouabain octahydrate	**(ouabain Approved)**	0.02	Vero			No
Ouabain	**Approved**	0.02	Vero	<0,1	Vero	No
Digoxin	**Approved- cardiac glycoside**	0.04	Vero	0,2	Vero	No
Digitoxin	**Approved, Investigational**	0.1	Vero	0,2	Vero	Yes - Wei, Chin J Integr Med 2020
Hemostatic						
Polidocanol	**Approved**	0.2	Caco-2			No
Hypolipidemic agent						
Lomitapide	**Approved, Investigational**	0.8	Huh7			No

Immunosuppressant						
Cyclosporin	**Approved, Investigational, Vet Approved**	0.2	Calu-3	5,8	Vero	Yes - El-hoshoudy, J Mol Liquids 2020
Mycophenolate	**Approved**	0.9	VeroE6/TMPRSS2			No
Interleukin inhibitor						
Apilimod	*Investigational*	0.007	A549/ACE2	~0,01	Vero E6	No
Pancreatic lipase inhibitor						
Cetilistat	*Investigational*	1.1	Vero E6			No
Antispasmodic						
Ethaverine	**Approved (France, Germany, Spain)**	0.6	Caco-2			No
Others						
Hanfangchin A (Tetrandrine)	*Experimental*	0.6	Huh-7/ACE2	1,2	Vero E6	No
Almitrine	**Approved**	1.4	Caco-2			No
Acitretin	**Approved**	2.5	Vero E6			
Pristimerin	*Not listed*	0.1	Vero E6			No
Lycorine	*Not listed*	0.3	Vero E6			No
Cepharanthine	*Not listed*	0.01	Huh7.5	30	Calu-3	Yes - Ruan J Med Virol 2020
Homorringtonine	**Approved, Investigational**	2.1	Vero E6	2,5	Vero E6	No
Leupeptin Hemisulfate	*Not listed*	0.03	Huh7.5			No
Nanchangmycin	*Not listed*	0.01	Vero CCL81	0,07	VeroE6/TMPRSS2	No
Lactoferrin	*Not listed*	0.3	Huh7			No
Griffithsin	*Not listed*	0.06	Vero E6			No
Liquiritin	*Not listed*	2.4	Vero E6			No

Compound	SARS-CoV	SARS-CoV-2	Status
Amlodipine		6	Approved
Amodiaquine		7	Approved, Investigational
Artesunate		8	Approved, Investigational
Atazanavir		4	Approved
Chloroquine		33	Approved, Investigational, Vet approved
Chlorpromazine		4	Approved, Investigational, Vet approved
Ciclesonide		4	Approved, Investigational
Clofazimine		6	Approved, Investigational
Cyclosporin		4	Approved, Investigational, Vet approved
Ebastine		4	Approved, Investigational
Favipiravir		11	Approved, Investigational
Hydroxychloroquine		21	Approved
Loperamide		6	Approved
Lopinavir		12	Approved
Mefloquine		5	Approved, Investigational
Nelfinavir		5	Approved
Niclosamide		4	Approved, Investigational, Vet approved
Remdesivir	1	51	Approved Investigational
Ribavirin		6	Approved
Ritonavir		4	Approved, Investigational
Sofosbuvir		4	Approved
Tafenoquine		4	Approved, Investigational
Trifluoperazine		4	Approved, Investigational
Alpha-1 antitrypsin		4	Investigational
Apilimod		8	Investigational
AZD8055		4	Investigational
Pyronaridine		5	Investigational
Nafamostat	1	11	Investigational
Beta-D-N4-Hydroxycytidine	1	6	Experimental
Camostat	1	7	Experimental
GS-441524		11	Experimental
Aprotinin	1	7	Approved, Investigational then Withdrawn
Terfenadine		4	Approved then Withdrawn
Berbamine		4	Not listed
Cepharanthine		7	not listed
GC376		7	Not listed
K11777	2	7	Not listed
RS-504393		4	Not listed
VBY-825		4	Not listed
Z-FA-FMK		5	Not listed
Salinomycin		4	Vet approved
Total général	7	326	

Table 2. List of the compounds tested at least four times in sensitivity assays on cell cultures for SARS-CoV-1 and SARS-CoV-2. The status mentioned in the third column was based on the DrugBank site (https://go.drugbank.com/drugs)

SUPPLEMENTARY TABLE 1:

List of druggable compounds for which a molecular docking study predicted an interaction with a viral protein of SARS-CoV-2.

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