Short communication

Evaluation of drugs for potential repurposing against COVID-19 using a tier-based scoring system

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Background: As the coronavirus disease 2019 (COVID-19) pandemic grows daily, we remain with no prophylactic and only minimal therapeutic interventions to prevent or ameliorate severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2). Prior to SARS-CoV-2 emergence, high throughput screens utilizing clinically developed drugs identified compounds with in vitro inhibitory effect on human coronaviruses that may have potential for repurposing as treatment options for COVID-19. However, caution should be applied to repurposing of these drugs when they are taken out of context of human pharmacokinetic parameters associated with normal therapeutic use.

Methods: Our aim was to provide a tier-based scoring system to interrogate this data set and match each drug with its human pharmacokinetic criteria, such as route of administration, therapeutic plasma levels and half-life, tissue distribution and safety.

Results: Our analysis excluded most previously identified drugs but identified members of four drug classes (anti-malarial amino-quinolones, selective estrogen receptor modulators [SERMs], low potency tricyclic antipsychotics and tricyclic antidepressants) as potential drug candidates for COVID-19. Two of them, the tricyclic antipsychotics and tricyclic antidepressants were further excluded based on a high adverse event profile.

Conclusions: In summary, our findings using a new pharmacokinetic-based scoring system supports efficacy testing of only a minority of candidates against SARS-CoV-2 infection.

Introduction

Currently, there is no licensed treatment for COVID-19 worldwide, but the FDA has recently given emergency approval for the use of remdesivir in COVID-19 patients [1]. In addition, over 1,000 clinical trials are currently ongoing or in set-up mode in different countries, including drugs such as lopinavir/ritonavir, dexamethasone, hydroxychloroquine and inhaled interferon beta-1a [2]. Overall, the current situation is far less than satisfying and there is an urgent need for additional treatment options, especially as the pandemic moves into lower resourced countries.

Over the past decade, several studies have used high throughput screens (HTS) to identify clinically developed drugs with in vitro inhibitory capacity against human coronaviruses (hCoVs) that may have potential for repurposing as prophylactic or therapeutic treatment options for hCoV infections. These HTS have identified >60 drugs with inhibitory effect as measured by reduction of replication of multiple hCoVs in a variety of different mammalian cell types in vitro. However, candidates are rarely considered in light of their pharmacokinetic parameters associated with normal therapeutic dosing. The aim of our study was to use a tier-based scoring system to interrogate this data set by matching drugs with their respective human pharmacokinetic criteria as well as their safety and systemic side effects relevant within the COVID-19 patient setting, allowing us to exclude identified HTS candidates based on these defined pharmacokinetic criteria. Remaining candidates were then further considered
based on potential for adverse effects within the COVID-19 patient treatment environment.

**Methods**

Screening clinically approved pharmaceuticals for repurposing removes the substantial time burden associated with movement of experimental drugs from preclinical stage through the regulatory pathway to approval. Repurposing can be especially important for the rapid identification of candidate drugs against emerging infectious diseases such as the present pandemic COVID-19. With notable exceptions [3], only a few drugs have been successfully repurposed, and none for the prevention or treatment of virus infection. With this in mind, our tier-based analysis used three HTS studies as a source of clinically developed drugs with inhibitory effect against in vitro replication of multiple hCoVs [4–6]. Drug candidates were critically examined based on the following key pharmacokinetic parameters: route of normal administration, therapeu
tic plasma levels and half-life, tissue distribution, and safety and adverse reactions. Availability and cost were additional important parameters given the anticipated need for treatment options within low- and middle-income countries. These characteristics were used to remove candidates based on pharmacokinetic parameters and potential for adverse events not consistent with prophylactic/therapeutic use for COVID-19, and prioritize remaining drugs for possible in vitro confirmation of SARS-CoV-2 inhibitory activity and movement into preclinical animal models (Tier 1 and Tier 2; Tables 1 and 2). Tier 1 represents drug candidates with administration, pharmacokinetic and safety parameters suitable for movement into preclinical models; Tier 2 represents similarly suitable candidates, but with a higher adverse event profile. Drugs in Tier 3 have a lower priority due to higher risk of complications in the COVID-19 patient setting, and Tier 4 drugs are those with low prophylactic/therapeutic potential against COVID-19 and/or with high potential for adverse effects (Additional file 1).

**Results**

In the study of de Wilde et al. [5], a 348 FDA-approved drug library was screened for inhibitory activity against Middle East respiratory syndrome CoV (MERS-CoV), with those identified with high inhibitory effect (half-maximal effective concentration \( EC_{50} \) at low micro-molar concentrations) being tested further for activity against SARS-CoV-1 and hCoV-229E. This HTS resulted in identification of four candidates with low micro-molar \( EC_{50} \) concentrations against these three hCoVs and low cellular toxicity. The Dyall et al. [6] study screened by HTS a library of 290 FDA-approved or experimental drugs with defined molecular targets that had previously shown activity against RNA viruses [7,8]. The study identified 27 compounds with inhibitory activity against MERS-CoV and SARS-CoV-1 with \( EC_{50} \) levels in the low micro-molar range with minimal cytotoxicity. The 2019 HTS of Shen et al. [4] screened a 2,000-component library of FDA-approved and pharmacologically active compounds. Seven compounds were identified with an \( EC_{50} \) of <5 \( \mu M \) against four distinct hCoVs. For the purpose of our study, we expanded the inclusion criteria of Shen et al. [4] to include a total of 36 compounds with an \( EC_{50} \) of <20 \( \mu M \) for the four hCoVs and low cytotoxicity. Together, our analysis comprised a total of 58 compounds. We excluded 26 compounds and identified 11 and 21 compounds with high and medium priority, respectively, with potential for therapeutic intervention against COVID-19. In addition to the single HIV protease inhibitor, lopinavir, the high priority (Tier 1 and Tier 2) compounds represented multiple members from four key drug classes: antimalarial quinolones, selective estrogen receptor modulators (SERMs), amine tricyclic antipsychotics and amine tricyclic antidepressants.

After removal of primarily experimental agents or those with high toxicity, pharmacokinetic parameters of therapeutic plasma levels of normal dosing and plasma half-life were used as an initial measure to assess whether hCoV inhibitors reach levels required for virus inhibition within the patient. For example, loperamide, an antidiarrhoeal agent identified as an attractive candidate for repurposing in two HTS screens [4,5] has therapeutic plasma levels >3 orders of magnitude lower than its \( EC_{50} \) against any hCoV tested [9]. This assessment resulted in removal of 26 candidates; drugs applied topically were also removed. Although multiple antipsychotics were identified by HTS as broad inhibitors of hCoV replication in vitro, only the low potency 1st generation tricyclics reached the necessary therapeutic plasma levels for inclusion (Tier 2), with the more potent later generation tricyclics commonly orders of magnitude below their \( EC_{50} \).

Four distinct but structurally related members of the antimalarial quinolone class displayed inhibitory effects on multiple hCoVs. Normal therapeutic levels of these agents reached plasma levels approximating the identified \( EC_{50} \) in the HTS studies. Lung tissue distribution (when available) was used as a further parameter, wherein lung tissue-specific accumulation was regarded as a positive indicator for potential therapeutic effect. Based on available data, the quinolone compounds have been shown to accumulate at high (approximately 1,000-fold) levels in the lung compared with plasma. Consistent with the prophylactic use of quinolones against malaria, most members exhibit a long plasma half-life, with oral administration being the preferred...
remdesivir (GS-5734; hCoV polymerase inhibitor). Studies using disease management when used early therefore expected to be more effective for COVID-19 on inhibition of hCoV replication. Drug candidates are HTS studies detailed above identify compounds based pathological being host immune response-driven. The of virus replication at early times, whilst later lung changing over time: disease being a more direct effect of SARS-CoV-2 appears to be critical, with disease aetiology may be contraindicated for use in COVID-19 patients, drugs can result in a level of immune suppression that mechanisms. Depending on the dosage, these anti-neoplastic agents were identified that interfere with DNA and RNA replication. The prodrug mycophenolate moftel, through its mycophenolic acid active metabolite, is an inhibitor of guanosine synthesis through an inhibitory effect on inosine monophosphate dehydrogenase; gemcitabine and hycanthone both inhibit DNA and RNA synthesis directly through distinct mechanisms. Half of treated patients may report somnolence and dizziness aside of other adverse events. Patients with high the protease inhibitor lopinavir, the SERMs tamoxifen and toremifene and the anti-malarials chloroquine and hydroxychloroquine have wider therapeutic indices than the tricyclic drugs and have decades of widespread clinical use across geographies, patient demographics and comorbidities. Furthermore, they are contraindicated in patients with multiple comorbidities which place these patients into the COVID-19 vulnerable category. The amine tricyclic antidepressants clomipramine and desipramine face similar challenges. Half of treated patients may report somnolence and dizziness aside of other adverse events. Patients with underlying medical conditions are either more likely to experience some of these class toxicities (for example, glaucoma, urinary retention from their anti-cholinergic properties) or be contraindicated. Furthermore, they show potential for overdose misuse and suicidal ideation as well as withdrawal symptoms even after short courses of treatment. The key importance of timing may be a possible explanation for inconsistent results from recent and ongoing studies investigating repurposing of drugs such as hydroxychloroquine, which again emphasizes the need for preclinical animal challenge models before progression to human clinical trials.

To be useful clinically, drugs will need to have a minimal adverse event profile (particularly for prophylaxis), not be contraindicated in patients who have underlying medical conditions and achieve therapeutic drug concentrations rapidly. The low potency tricyclic antipsychotics do not possess these attributes; both chlorpromazine and promazine need to be carefully titrated to optimal therapeutic doses, and many patients report a plethora of adverse events. Furthermore, they are contraindicated in patients with multiple comorbidities which place these patients into the COVID-19 vulnerable category. The amine tricyclic antidepressants clomipramine and desipramine face similar challenges. Half of treated patients may report somnolence and dizziness aside of other adverse events. Patients with underlying medical conditions are either more likely to experience some of these class toxicities (for example, glaucoma, urinary retention from their anti-cholinergic properties) or be contraindicated. Furthermore, they show potential for overdose misuse and suicidal ideation as well as withdrawal symptoms even after short courses of treatment.

Timing of therapeutic intervention against SARS-CoV-2 appears to be critical, with disease aetiology changing over time: disease being a more direct effect of virus replication at early times, whilst later lung pathology being host immune response-driven. The HTS studies detailed above identify compounds based on inhibition of hCoV replication. Drug candidates are therefore expected to be more effective for COVID-19 disease management when used early. Studies using remdesivir (GS-5734; hCoV polymerase inhibitor) highlight the altering course of disease over time and the importance of instigating antiviral measures early. Later drug administration reduced virus replication but failed to improve lung function or disease outcome in the immunopathologic-driven stage of disease [12]. This key importance of timing may be a possible explanation for inconsistent results from recent and ongoing studies investigating repurposing of drugs such as hydroxychloroquine, which again emphasizes the need for preclinical animal challenge models before progression to human clinical trials.

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The protease inhibitor lopinavir, the SERMs tamoxifen and toremifene and the anti-malarials chloroquine and hydroxychloroquine have wider therapeutic indices than the tricyclic drugs and have decades of widespread clinical use across geographies, patient demographics and comorbidities. Lopinavir’s recommended daily dose for HIV-1 infection (800 mg) produces plasma levels covering the EC_{50} values for pathogenic hCoVs. Tamoxifen and toremifene are customarily used at daily doses of 20 mg and 60 mg, respectively, for breast cancer. However, higher daily doses (approximately 600 mg and 680 mg, respectively) are relatively well tolerated under short durations reaching plasma concentrations after a single dose at antiviral EC_{50} levels and evidence of greater concentrations within tissue.

**Discussion**

COVID-19 is a rapidly evolving situation. During review of the manuscript some of the drugs under consideration were tested for *in vitro* inhibitory activity against SARS-CoV-2, which is shown in the
Table 1. Tier 1

<table>
<thead>
<tr>
<th>Name of drug</th>
<th>Plasma levels and lung distribution</th>
<th>Half-life</th>
<th>EC\textsubscript{50}, CC\textsubscript{50} (µM)</th>
<th>Reason for tier designation</th>
<th>Key acute toxicities of potential concern during short durations of treatment in COVID-19 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quinolones: antimalarial</strong></td>
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<tr>
<td>Chloroquine diphosphate(^b) (515.9)</td>
<td>Oral administration of 310 mg (daily) or 500 mg (weekly) results in peak plasma concentrations of 0.125 µg/ml (0.24 µM) and 0.15 µg/ml (0.29 µM) to 0.25 µg/ml (0.49 µM), respectively. Concentration in lung 200–700 times plasma level.(^d)</td>
<td>40 to 90 days.(^i) Major metabolite of chloroquine is desethylchloroquine, which retains antiplasmodial activity.(^c)</td>
<td>MERS (3.0, 58.1); SARS (4.1, &gt;128); 229E (3.3, &gt;50); MERS (6.278); SARS (6.538), low CC\textsubscript{50}; OC43 (0.33, &gt;20); NLGI (4.89, &gt;20); MERS (16.44, &gt;20); AS9 (15.92, &gt;20)(^a)</td>
<td>Pro: lung levels within range of EC\textsubscript{50}, very high lung accumulation; extremely long half-life; extensive clinical experience; low cost</td>
<td>Contraindicated in patients with retinal disease/visual field defects as eye toxicity associated with both of high dose and prolonged use; may worsen psoriasis and porphyria. Haemolysis in patients with G6PD.(^e)</td>
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<tr>
<td>Hydroxychloroquine sulfate(^c) (434.0)</td>
<td>Therapeutic plasma range for SLE: 0.5 µg/ml (1.15 µM) to 2.0 µg/ml (4.6 µM); lung approximately 800-fold higher than plasma level.(^k) Similar pharmacokinetics as chloroquine.(^d)</td>
<td>40 days(^i)</td>
<td>MERS (8.279); SARS (7.966), low CC\textsubscript{50}; SARS-CoV-2 (11.17, &gt;50)(^a)</td>
<td>Pro: lung levels within range of EC\textsubscript{50}; very high lung accumulation; extremely long half-life; extensive clinical experience; low cost</td>
<td>QT prolongation, hypoglycaemia, lowering of seizure threshold, haemolysis in patients with G6PD deficiency.(^d)</td>
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<td><strong>Antineoplastic: selective estrogen receptor modulators</strong></td>
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<tr>
<td>Tamoxifen citrate(^c) (563.6)</td>
<td>Therapeutic levels at 0.067 µg/ml (0.12 µM) to 0.183 µg/ml (0.325 µM), Doses as high as 200 mg have been used. Metabolized to multiple active metabolites with longer half-lives than parent. High levels (&gt;100-fold) accumulate in lungs based on human and rat data, which are comparable.(^f)</td>
<td>4 to 11 days(^i)</td>
<td>MERS (10.117); SARS (9.286), low CC\textsubscript{50}; SARS-CoV-2 (11.17, 37.96)(^a)</td>
<td>Pro: lung levels within range of EC\textsubscript{50}; high lung accumulation; long half-life; extensive clinical experience; affordable and widely available in LMIC(^d)</td>
<td>Reversible transaminitis. Contraindicated in patients with a history of DVT/PE and/or taking coumarin-type anticoagulants(^d)</td>
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<tr>
<td>Toremifene citrate (triphenylethylene derivative of tamoxifen)(^c) (598.1); N-Desmethyltoremifene (391.9)</td>
<td>Therapeutic steady-state concentrations 1.1 µg/ml (1.84 µM) to 1.3 µg/ml (2.17 µM), with 2.7 µg/ml (4.89 µM) to 5.8 µg/ml (14.8 µM)(^j). Toremifene and DMT accumulates in lung to high levels.(^l)</td>
<td>6 days; N-desmethyloctamoxifen (DMT) is active metabolite with half-life of 21 days.(^i)</td>
<td>MERS (12.915); SARS (11.969), low CC\textsubscript{50}; SARS-CoV-2 (11.30, 20.51)(^a)</td>
<td>Pro: lung levels within range of EC\textsubscript{50}; high lung accumulation; long half-life; extensive clinical experience</td>
<td>QT prolongation, auto-induction of 3A4, contraindicated in patients with thromboembolic diseases.(^d)</td>
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</tbody>
</table>

\(^{a}\)Shen et al, 2019 [4]; \(^{b}\)de Wilde et al, 2014 [5]; \(^{c}\)Dyall et al., 2014 [6]; \(^{d}\)AHFS Drug Information, 2020 [9]; \(^{e}\)Regenthal et al, 1999 [25]; \(^{f}\)McChesney et al, 1967 [27]; \(^{g}\)Weston et al, 2020 [28]; \(^{h}\)Brunton et al, 2017 [11]; \(^{i}\)Lien et al, 1991 [29]; \(^{j}\)Martei et al, 2018 [30]; \(^{k}\)Sippo et al, 1997 [31]; \(^{l}\)Atzori et al, 2003 [32]; \(^{m}\)Yamamoto et al, 2020 [19]. EC\textsubscript{50}, half-maximal effective concentration; ELF, epithelial lining fluid; LMIC, low to middle-income countries; MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome; SLE, systemic lupus erythematosus.
### Table 1. Continued

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<tr>
<td>Antiretroviral: Lopinavir(^b) (628.8) protease inhibitor</td>
<td>Formulated with ritonavir as Kaletra(^b). Following 400/100 mg lopinavir/ritonavir oral dose twice daily results in peak concentration of 9.8 µg/ml (15.58 µM): Analysis of ELF from patient on standard regimen reported 8.1 µg/ml (12.88 µM) in plasma compared with 14.4 µg/ml (22.90 µM) in ELF(^m)</td>
<td>3 to 8 h(^i)</td>
<td>MERS (8.0, 24.4); SARS (17.1, &gt;32); 229E (6.6, 37.6); SARS-CoV-2 (5.73, 74.44)(^h)</td>
<td>Pros: lung levels within range of EC_{50}; suitable half-life; good clinical history; Cons: high cost</td>
<td>Hyperglycaemia, QT and other ECG changes, rarely pancreatitis and hepatotoxicity(^d)</td>
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\(^{a}\) Analysis of ELF from patient on standard regimen reported 8.1 µg/ml (12.88 µM) in plasma compared with 14.4 µg/ml (22.90 µM) in ELF.

\(^{b}\) Formulated with ritonavir as Kaletra. Following 400/100 mg lopinavir/ritonavir oral dose twice daily results in peak concentration of 9.8 µg/ml (15.58 µM).

\(^{c}\) Pros: lung levels within range of EC_{50}; suitable half-life; good clinical history; Cons: high cost.

\(^{d}\) Hyperglycaemia, QT and other ECG changes, rarely pancreatitis and hepatotoxicity.

\(^{e}\) Analysis of ELF from patient on standard regimen reported 8.1 µg/ml (12.88 µM) in plasma compared with 14.4 µg/ml (22.90 µM) in ELF.
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<td>Amodiaquine dihydrochloride dihydrate (464.8)</td>
<td>Oral 600 mg results in peak concentration of 0.032 µg/ml (0.07 µM) after 30 min. Peak concentration of main active metabolite (desethylamodiaquine) 0.181 µg/ml (0.39 µM) by 2 h. High tissue retention.</td>
<td>30 min</td>
<td>MERS (6.212); SARS (1.274), Low CC₅₀; SARS-CoV-2 (4.94, 34.42)</td>
<td>Pros: lung levels within range of EC₅₀; very high lung accumulation; extremely long half-life; extensive clinical experience; cost. Cons: higher toxicity than chloroquine and hydroxychloroquine and no longer recommended for use in US</td>
<td>Common side effect reports of somnolence and dizziness with caution therefore for driving/operating machinery. Rarely causes severe hepatotoxicity, agranulocytosis, and retinopathy especially with prolonged use; thus, contraindicated in patients with known retinopathy or liver pathology including HIV patients taking zidovudine and efavirenz where risk of hepatitis is higher.</td>
</tr>
<tr>
<td><strong>Mefloquine</strong> (378.3)</td>
<td>Oral administration of 250 mg once weekly (recommended prophylaxis) results in average peak plasma levels of 0.42 µg/ml (1.1 µM). Accumulates to high levels in lung.</td>
<td>20 days</td>
<td>MERS (7.416); SARS (15.553), Low CC₅₀</td>
<td>Pros: lung levels within range of EC₅₀; very high lung accumulation; extremely long half-life; extensive clinical experience. Cons: relatively high cost</td>
<td>Impairment of coordination (for example, driving) during and for some weeks after cessation of mefloquine, ECG abnormalities; risk of mental alterations and should not be used in patients with history of depression, generalised anxiety disorder, psychosis, schizophrenia, major psychiatric illness, epilepsy.</td>
</tr>
<tr>
<td><strong>Neurotransmitter inhibitors:</strong> Chlorpromazine therapeautic levels</td>
<td>Therapeutic levels of 0.03 µg/ml (0.08 µM) to 0.15 µg/ml (0.42 µM). Moderate, but variable lung accumulation for tricyclic variable, but ranges at steady state in a variety of animals from 4.4-fold to 53-fold accumulation in lung compared with blood.</td>
<td>23 to 37 h</td>
<td>MERS (4.9, 21.3); SARS (8.8, 24.3); 229E (2.5, 23.5); MERS (9.514); SARS (12.971), Low CC₅₀; SARS-CoV-2 (4.03, 11.88)</td>
<td>Pros: lung levels within range of EC₅₀; suitable half-life; extensive clinical experience</td>
<td>Hypotension, hyperglycaemia, temperature dysregulation, contraindicted in circulatory collapse and CNS depression. Requirement to slowly titrate dose upwards and high inter-subject PK variability.</td>
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*Dyall et al., 2014 [6]; Winstanley et al., 1987 [10]; Weston et al., 2020 [28]; "AHFS Drug Information, 2020 [9]; "Brunton et al., 2017 [11]; "de Wilde et al., 2014 [5]; "Regenthal et al., 1999 [25]; "Potts, 1962 [33]; Shen et al., 2019 [4]; "Aitchison et al., 2010 [34]. CC₅₀, half-maximal cytotoxic concentration; CNS, central nervous system; ECG, electrocardiogram; EC₅₀, half-maximal effective concentration; MAO, monoamine oxidase inhibitor; MERS, Middle East respiratory syndrome; PK, pharmacokinetic; SARS, severe acute respiratory syndrome.
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<th>Name of drug (mol. wt. g/mol)</th>
<th>Plasma levels and lung distribution</th>
<th>Half-life</th>
<th>EC$<em>{50}$, CC$</em>{50}$ (µM)</th>
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<tr>
<td><strong>Promazine</strong> (284.4)</td>
<td>Therapeutic dose 0.01 µg/ml (0.035 µM) to 0.06 µg/ml (0.18 µM)</td>
<td>5 to 41 h$^2$</td>
<td>OC$_{43}$ (0.41, &gt;20); NL63 (1.37, &gt;20); MERS (13.72, &gt;20); A59 (0.51, &gt;20)$^1$</td>
<td>Pros: lung levels within range of EC$_{50}$; suitable half-life; extensive clinical experience</td>
<td>Hypotension, temperature dysregulation, contraindicated in CNS depression. Requirement to slowly titrate dose upwards.$^d$</td>
</tr>
<tr>
<td><strong>Neurotransmitter inhibitors:</strong></td>
<td>Clomipramine HCl (351.3)</td>
<td>Therapeutic levels 0.15 µg/ml (0.43 µM) to 0.5 µg/ml (1.42 µM)$^c$ Clomipramine and active metabolite 4- to 8-fold accumulation in lung in rats after oral dosage over 14 days to reach steady state.$^3$</td>
<td>32 h (70 h for active metabolite)$^f$</td>
<td>MERS (8.332); SARS (13.238), Low CC$_{50}$; SARS-CoV-2 (7.59), &gt;29.68)$^1$</td>
<td>Pros: lung levels within range of EC$_{50}$; suitable half-life; extensive clinical experience</td>
</tr>
<tr>
<td><strong>Desipramine</strong> (302.8)</td>
<td>Therapeutic levels of 0.125 µg/ml (0.41 µM) to 0.3 µg/ml (0.99 µM)$^c$ Lung accumulation data not available. Assume comparable to clomipramine.</td>
<td>30 h$^3$</td>
<td>OC$_{43}$ (1.67, &gt;20); NL63 (6.68, &gt;20); MERS (11.59, &gt;20); A59 (8.75, &gt;20)$^1$</td>
<td>Pros: lung levels within range of EC$_{50}$; suitable half-life; extensive clinical experience</td>
<td>Somnolence, dizziness, hypotension and potential for suicidal ideation, seizures and misuse as overdose. Should not be given to patients following recent myocardial infarction or taking MAO drugs. Caution in patients with ECG abnormalities, glaucoma or urinary retention. Highly variable PK and doses should not exceed 300 mg/day.$^9$</td>
</tr>
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accompanying tables (Tables 1 and 2; Additional file 1). During this time, two pre-clinical animal studies have also reported the absence of any effect of hydroxychloroquine when used either prophylactically or therapeutically against SARS-CoV-2 replication or associated disease [13,14]. Hydroxychloroquine treatment was also recently removed from the WHO Solidarity COVID-19 clinical study based on evidence from the Solidarity trial, a Cochrane review of the drug as well as on the release of a report from the UK-based RECOVERY trial where hydroxychloroquine showed no effect on mortality rate of COVID-19 patients [15,16]. Similarly, a post-exposure prophylaxis trial showed no effect of hydroxychloroquine on the incidence of infection from high and moderate-risk exposure to SARS-CoV-2 [17]. Multiple pre-exposure prophylaxis trials remain ongoing [18]. New recent data has also shed light on a possible mechanism behind the apparent divergence in inhibitory effect of hydroxychloroquine between in vitro and in vivo studies; wherein, the virus uses a distinct entry pathway in the Vero cells standardly used for in vitro determination of drug sensitivity, compared with the pathway utilized in lung epithelium in vitro and presumably in vivo. Notably, only the entry pathway in Vero cells is susceptible to inhibition by endosomal pathway inhibitors such as hydroxychloroquine [19,20].

In summary, caution should be applied to repurposing of drugs when they are taken out of context of human pharmacokinetic parameters associated with normal therapeutic use. Our tier-based scoring system to analyse drugs identified through HTS with in vitro efficacy against one or more hCoVs resulted in the exclusion of the majority of compounds for further consideration. Similar to the quinolones, SERMs (that is, tamoxifen and toremifene) are a class of drugs that have characteristics of low micro-molar hCoV inhibitory activity, attractive human pharmacokinetics, favourable tissue accumulation and good safety profile for use in COVID patients [9]. The next step for all potential candidates will be preclinical efficacy testing in animal models against SARS-CoV-2 challenge. Repurposing of clinically approved drugs helps remove the concern of overt drug toxicity. However, animal infection models are critical as they place the treatment within the context of the kinetics of virus infection within the host. They can also identify unexpected enhancement of disease by a drug in context of viral infection, as experienced with mycophenolate mofetil against MERS-CoV in nonhuman primates [21]. A similar enhancement effect was seen for chloroquine prophylaxis, but not treatment of mosquito-transmitted chikungunya, which corresponded with an immunomodulatory effect of the drug, and again emphasizes the importance of timing in therapeutic intervention [22].

Finally, combinations of drugs are often far more effective than single compounds [23]. Therefore, these Tier 1 drugs should be considered for combined use to take advantage of possible synergy between drugs with differing modalities of virus inhibition. However, unpredicted antagonism can also result from such combinations, as was recently observed between chloroquine and remdesivir [24]. Again, such studies initially need to be performed using in vitro cell systems and, importantly, preclinical animal models prior to considering movement into humans.

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Disclosure statement
AH is a former employee of AstraZeneca and current shareholder of AstraZeneca who licenses tamoxifen. The remaining authors declare no competing interests.

Additional file

References


