

1 **Identification of five antiviral compounds from the Pandemic Response Box targeting SARS-**
2 **CoV-2**

3 **Running title:** Identification of five compounds targeting SARS-CoV-2.

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19

20 **Abstract**

21 With currently over 4 million confirmed cases worldwide, including more than 300'000 deaths, the
22 current Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic has a major
23 impact on the economy and health care system. Currently, a limited amount of prophylactic or
24 therapeutic intervention options are available against SARS-CoV-2. In this study, we screened 400
25 compounds from the antimicrobial ‘Pandemic Response Box’ library for inhibiting properties against
26 SARS-CoV-2. We identified sixteen compounds that potently inhibited SARS-CoV-2 replication, of
27 which five compounds displayed equal or even higher antiviral activity compared to Remdesivir. These
28 results show that five compounds should be further investigated for their mode of action, safety and
29 efficacy against SARS-CoV-2.

30 **Highlights:**

- 31 • 400 compounds from the pandemic response box were tested for antiviral activity against
32 SARS-CoV-2.
- 33 • 5 compounds had an equal or higher antiviral efficacy towards SARS-CoV-2, compared to the
34 nucleoside analogue Remdesivir.

35

36 **Introduction**

37 In December 2019, a new zoonotic coronavirus emerged in Wuhan, Hubei Province, China
38 named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which is the etiological
39 agent of Coronavirus Disease 2019 (COVID-19) [1–3]. The clinical features of SARS-CoV-2-infected
40 patients range from mild cold-like symptoms to severe illness ultimately leading to acute respiratory
41 distress syndrome (ARDS) [2,4]. Patients at older age and with underlying comorbidities are at higher
42 risk for developing severe courses of COVID-19 [5]. Despite unprecedented international public health
43 response measures to contain SARS-CoV-2 transmissions, the viral outbreak is currently categorized
44 as a pandemic with over 4 million confirmed-laboratory cases reported worldwide, including over
45 300'000 deaths as of 8th of May 2020 [6]. At present, and despite earlier outbreaks by SARS-CoV and
46 Middle East Respiratory Syndrome (MERS)-CoV, there are limited approved antiviral treatment
47 options such as antiviral drugs, vaccines and immuno-prophylaxis that can be used prophylactically or
48 therapeutically to halt the current SARS-CoV-2 infections.

49 Vaccine development takes multiple years until it can be administered to patients, and
50 although these processes are expedited during outbreaks of emerging viruses, the eventual worldwide
51 vaccine distribution may be delayed for several months after the initial outbreak [7]. Moreover, while
52 vaccines are used prophylactically, antiviral drugs can be employed both prophylactically and
53 therapeutically. For SARS-CoV-2, several antiviral compounds are currently evaluated, such as the
54 nucleoside analogue Remdesivir, the TMPRSS2 protease inhibitor cameostat mesylate, and the
55 antimalaria drug (Hydroxy-) chloroquine, that target different stages of the viral replication cycle [8,9].
56 These three antiviral drugs have been recently tested clinically in small cohorts, and their efficacy
57 against SARS-CoV-2 infections is currently evaluated [10]. Nevertheless, RNA viruses, including
58 coronaviruses, are known to rapidly evade antiviral drug inhibition by developing resistance mutations
59 and subsequent selection of drug-resistant viral populations [11–13]. Therefore, the use of multiple
60 drug regimes as well as expanding the repertoire of available antiviral treatment options are of crucial
61 importance to combat the SARS-CoV-2 pandemic.

62 Since the beginning of the 21st century, the world has encountered multiple epidemics that
63 were caused by a viral or bacterial agent, like the Ebola-, Measles-, Zika-viruses and cholera [14].
64 Some epidemics even reached pandemic proportions, like the Influenza A/H1N1 virus and the
65 currently circulating SARS-CoV-2. As a rapid response to these virulent agents, the Medicines for
66 Malaria Venture (MMV, mmv.org) and Drugs for Neglected Diseases initiative (DNDi, dndi.org)

67 developed the pandemic response box (PRB), a compound library containing 400 compounds with
68 antibacterial, antifungal and antiviral properties. This compound library contains drugs that are already
69 marketed or are at various stages of development and analysis and allows for the rapid investigation
70 of repurposing drugs against newly emerging pathogens.

71 To this end, we performed an *in vitro*-based screen of 400 preselected compounds with
72 antibacterial, antifungal and antiviral properties contained in the PRB and assessed their antiviral
73 activity against SARS-CoV-2. A stringent large-scale screen in Vero-E6 cells highlighted sixteen
74 compounds that prevented virus-induced cytopathogenic effects (CPE) while displaying low
75 cytotoxicity and no effect on cell viability. Further evaluation revealed that five compounds had an
76 equal or higher antiviral efficacy against SARS-CoV-2, compared to the nucleoside analogue
77 Remdesivir. Together, these data demonstrate that stringent *in vitro* screening of preselected
78 compounds leads to the rapid identification of potent antiviral candidate drugs targeting SARS-CoV-2.
79

80 **Material and methods**

81 **Cells, viruses and compounds.**

82 Vero-E6 cells (kindly provided by M. Müller/C. Drosten, Charité, Berlin, Germany) were propagated in
83 Dulbecco's Modified Eagle Medium-GlutaMAX, 10% (v/v) heat-inactivated fetal bovine serum, 100
84 mg/ml streptomycin, 100 IU/ml penicillin, 1% (w/v) non-essential amino acids and 15 mM HEPES
85 (Gibco). Cells were maintained at 37°C in a humidified incubator with 5% CO₂. SARS-CoV-2 (SARS-
86 CoV-2/München-1.1/2020/929 [15], stocks were produced on Vero-E6 cells, aliquoted and stored at -
87 80°C. Viral titers were determined by tissue culture infectious dose 50 (TCID₅₀) on Vero-E6 cells.

88 **Compound preparation of the pandemic response box.**

89 All compounds were dissolved and diluted in dimethylsulfoxide (DMSO, Sigma) to 1 mM aliquots in
90 96-well plates (TPP) that were kept at -20°C until further use. Compounds were diluted at the
91 indicated concentration in cell culture medium.

92 **Survival screening.**

93 Vero-E6 cells were seeded in 96-well clear bottom, black plates (Costar), 20'000 cells per well one
94 day prior to the experiment. Cells were pretreated for 2 hours with 1 µM of each compound contained

95 in the PRB. Remdesivir [8], K22 [12] and DMSO controls were included in each plate. Subsequently,
96 cells were infected with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.01 in compound-
97 containing medium and incubated at 37°C in a humidified incubator with 5% CO₂. Uninfected (mock)
98 controls were included in each plate. At 48 hours post-infection (hpi), cells were visually inspected for
99 virus-induced CPE prior to fixation with 4% (v/v) neutral buffered formalin (Formafix AG) and stained
100 with crystal violet. Cell viability and cytotoxicity were assessed in parallel, in identically treated,
101 uninfected plates. Two independent experiments were performed, each including a technical
102 duplicate. Wells containing an intact cell layer without apparent CPE after infection and displaying high
103 cell viability and low cytotoxicity were considered as hits.

104 **Cell cytotoxicity and cell viability.**

105 Cell cytotoxicity and viability were assessed using CellTox™ green cytotoxicity assay (Promega) and
106 Cell-Titer Glo 2.0 assay (Promega), respectively, according to manufacturer's protocols. Readout was
107 performed on a Cytaion 5 Cell Imaging Multi-Mode Reader (Biotek).

108 **IC₅₀ determination of selected compounds.**

109 Vero-E6 cells were seeded in 96-well clear bottom, black plates (Costar), 20'000 cells per well one
110 day prior to the experiment. Cells were pretreated for 2 hours with 2-fold serial dilutions of selected
111 compounds, ranging from 4 µM to 0.063 µM. Cells were infected with SARS-CoV-2 (MOI of 0.01) in
112 compound-containing medium and incubated at 37°C in a humidified incubator with 5% CO₂. Cells
113 were fixed with 4% (v/v) neutral buffered formalin at 24 hours post-infection and processed for
114 immunofluorescence analysis. Briefly, cells were permeabilized with 0.1% (v/v) Triton X-100 (Merck)
115 for 5 minutes and blocked in PBS supplemented with 50 mM NH₄Cl, 0.1% (w/v) Saponin (Sigma) and
116 2% (w/v) Bovine serum albumine (IgG-free, Jackson immunoresearch). SARS-CoV-2 antigen-positive
117 cells were detected using a rabbit polyclonal anti-SARS-CoV nucleocapsid protein (Rockland, 200-
118 401-A50) and a secondary Alexa Fluor® 488-labeled donkey anti-Rabbit IgG (H+L) (Jackson
119 Immunoresearch). Samples were counterstained using 4',6-diamidino-2-phenylindole (DAPI, Thermo
120 Fisher Scientific) to visualize the nuclei and finally washed with PBS.

121 Images were acquired on a Cytaion5 Cell Imaging Multi-Mode Reader (Biotek) equipped with a 4x air
122 objective (NA: 0.13). Four images per well were acquired to cover the entire surface of the well and
123 processed and stitched using the Gen5 Image prime software package (v3.08.01). Mean intensity

124 ratios of the GFP (SARS-Nucleocapsid) and DAPI (Nuclei) signals were calculated for each individual
125 well. Cell viability and cytotoxicity were assessed in parallel, in identically treated, uninfected plates.

126 **Data representation.**

127 Graphs were generated using GraphPad Prism software version 8.4.2 and the final figures were
128 assembled in Adobe Illustrator CS6. Brightness and contrast of microscopy picture were minimally
129 adjusted and processed identically to their corresponding control using FIJI. Images were assembled
130 using the FigureJ plugin in FIJI [16].

131 **Results**

132 **Survival screen with compounds included in the pandemic response box against SARS-CoV-2.**

133 To identify potential compounds that can be used as intervention option against SARS-CoV-2
134 replication, we screened 201 antibacterial, 46 antifungal and 153 antiviral molecules included in the
135 PRB for their antiviral activity. We initially screened the 400 compounds at a conservative
136 concentration of 1 μ M. Based on the documented inhibition of coronavirus replication, Remdesivir and
137 K22 were included as a positive control [8,12]. Vero-E6 cells were pretreated for 2 hours and
138 subsequently infected with SARS-CoV-2 (MOI of 0.01) for 48 hours in drug-containing medium. Cell
139 survival was arbitrarily scored, from 0 to 2, upon visual inspection and evaluation of SARS-CoV-2-
140 induced CPE (**Supp. Fig. 1a**). This screen resulted in a total of five compounds that completely
141 inhibited SARS-CoV-2-induced CPE (white), while twelve other compounds showed an intermediate
142 CPE reduction (Grey). In parallel, we performed cell viability and cell cytotoxicity assays to exclude
143 any detrimental effect of each compound on the cells. Only for one compound (Plate B, D10) we
144 observed low cell viability and high cytotoxicity. The sixteen remaining compounds are antifungal
145 (three), antibacterial (six) and antiviral (seven) compounds (**Table 1**), which, similarly to their vehicle
146 control (DMSO), Remdesivir and K22, did not influence cell cytotoxicity and cell viability (**Supp. Fig.**
147 **1b, c**). These results provide evidence for the relevance of a conservative and rapid screening of
148 libraries containing compounds that target viral replication.

149

150 **Antiviral efficacy against SARS-CoV-2.**

151 To further confirm and evaluate the extent of antiviral activity of the previously highlighted sixteen
152 compounds, cells were pretreated with the selected compounds at dosages ranging from 4 μ M to
153 0.063 μ M and infected with SARS-CoV-2 (MOI of 0.01). After 24 hours of infection, cells were fixed
154 and processed for immunofluorescence analysis using the anti-SARS-CoV nucleocapsid protein
155 antibody and DAPI. The efficacy of the selected compounds to inhibit SARS-CoV-2, as well as their
156 individual effects of cell viability and cytotoxicity, were compared to Remdesivir. The IC₅₀ values for
157 each compound were inferred by calculating the ratio of the total intensity of the nucleocapsid protein
158 and DAPI signals (infected cells / total cells). This indicated that five of sixteen selected candidate
159 compounds, *N*-Nonyldeoxyojirimycin (NN-DNJ), PDNJ0803, Chloroquine, Retro-2.1 and URMC-099-
160 C, inhibited the virus at least as potently as the reference compound Remdesivir (**Figure 1**).

161 Moreover, these five compounds showed equal or lower IC₅₀ values against SARS-CoV-2 replication
162 than the reference compound Remdesivir (IC₅₀ = 1.842), without increased cytotoxicity or decreased
163 cell viability compared to the vehicle control on the analyzed concentrations (**Fig. 2 A-G, Table 2**). The
164 remaining eleven compounds showed little or no inhibition when compared to the reference
165 compound, and therefore no reliable IC₅₀ could be calculated (**Supp. Fig 2**).

166 In parallel to the efficacy, we determined the half-maximum cytotoxicity concentration (CC₅₀) of
167 each respective compound at a concentration range from 0.04 µM to 50 µM using uninfected Vero-E6
168 cells. This demonstrated that the previously tested compounds were all well tolerated at 2 or 4-fold
169 higher concentrations than the initial screening concentration of 1 µM (**Figure 2H, table 2**). At
170 concentrations up to 50 µM, only URMC-099-C displayed moderate cell cytotoxicity, resulting in a
171 CC₅₀ of 13.1 µM, while all other compounds had a CC₅₀ above 50 µM (**Table 2**). Further, the resulting
172 selectivity indexes (SI) showed that Retro-2.1 (SI = 101.9) is the most effective and less cytotoxic
173 inhibitor, followed by chloroquine (SI = 48.5), URMC-099-C (SI = 39.1), whereas NN-DNJ (SI = 29.82),
174 and PDNJ0803 (SI = 24.68) had comparable SI to that of Remdesivir (SI = 27.14) (**Table 2**).
175 Combined these results demonstrate that Retro-2.1 is the most potent antiviral candidate that should
176 be further evaluated on its mode of action, efficacy and safety in pre-clinical models such as human
177 airway epithelial cultures, as well as appropriate *in vivo* models for SARS-CoV-2.

178 **Discussion**

179 In this study we demonstrate that a conservative *in vitro* screening approach of 400
180 compounds from the PRB resulted in the identification of five compounds with potent antiviral activity
181 against SARS-CoV-2. This included the anti-malaria drug chloroquine and antibacterial Retro-2.1, as
182 well as antiviral compounds NN-DNJ, PDNJ0803, and URMC-099-C. Antiviral efficacy testing revealed
183 that Chloroquine, NN-DNJ, PDNJ0803, and URMC-099-C, all have a comparable characteristics to
184 that of the reference compound Remdesivir, while the antimicrobial compound Retro-2.1 displayed
185 almost a 4-fold higher selectivity index profile over that of Remdesivir in Vero-E6 cells. These results
186 indicate that conservative *in vitro* screening approaches of preselected compounds can lead to the
187 rapid identification of potent antiviral candidates targeting SARS-CoV-2. We and others have
188 previously screened different compound libraries against a variety of coronaviruses in a range of 10 to
189 50 μ M that identified several compounds with antiviral activity against SARS-CoV and MERS-CoV.
190 [12,17,18]. However, in the current study we employed a stringent survival screening approach to
191 expedite the identification of candidate compounds effective against SARS-CoV-2. This comes with
192 the expense that compounds effective at higher molar concentrations are missed.

193 We used the nucleoside analogue Remdesivir as a reference compound against SARS-CoV-2
194 and observed an IC₅₀ of 1.842 μ M and a CC₅₀ of >50 μ M on Vero-E6 cells, in agreement with previous
195 studies describing the inhibitory effect of Remdesivir against SARS-CoV-2 [8]. In line with this, we also
196 identified Chloroquine as a potent antiviral compound against SARS-CoV-2, which has also been
197 shown to effectively inhibit SARS-CoV-2 *in vitro* [8]. In contrast, K22 neither inhibited SARS-CoV nor
198 SARS-CoV-2 (**Figure 1**) replication at low concentrations [12]. This exemplifies the robust
199 reproducibility of the antiviral screen presented here and its consistency with previously published
200 reports. Further, it establishes Remdesivir and chloroquine as reliable reference compounds during
201 antiviral efficacy assessments of novel compounds against SARS-CoV-2 and other coronaviruses.

202 The five candidate compounds identified here, namely chloroquine, NN-DNJ, PDNJ0803,
203 URMC-099-C, and Retro-2.1 have previously also been demonstrated to inhibit the replication of other
204 RNA-viruses such as *Filoviruses*, *Flaviviruses* and *Picornaviruses* [19–21]. Although we did not
205 evaluate the mode of action of the identified compounds, it has been described that Chloroquine
206 interferes with the endosomal viral entry and release pathways of coronaviruses, including SARS-
207 CoV-2, whereas the immunomodulatory compound URMC-099-C might influence important Mitogen-
208 activated protein kinases (MAPK) associated biological processes [8,22,23]. Interestingly, Retro-2.1

209 remodels the intracellular distribution of syntaxins, which consequently alters vesicular retrograde
210 transport between endosomes and the Golgi apparatus [24,25]. Of note, we previously identified several
211 syntaxins (*stxbp1*, *stxbp3*, *stx5a*, and *stx18*) in close proximity of the replication and transcription
212 complexes during coronavirus replication [26]. Since coronaviruses strongly rely on cellular
213 endomembranes and trafficking pathways for efficient replication, Retro-2.1 might directly target the
214 Achilles heel of coronavirus replication. Further, NN-DNJ and its derivative PBDNJ0803 are categorized
215 into the same class of inhibitors that target the cellular α -glucosidase I and II, which support the
216 maturation, secretion and function of viral glycoproteins [27]. NN-DNJ and PBDNJ0803 interfere with
217 the interactions between glycoproteins and the ER-chaperones Calnexin and Calreticulin, thereby
218 disturbing the removal of terminal glucose residues from the *n*-glycans of glycoproteins [28].
219 Moreover, NN-DNJ has been described to effectively inhibit the replication of a wide range of RNA
220 viruses such as Hepatitis C virus and Flaviviruses, as well as SARS-CoV [27–29]. Lastly, URMC-099-
221 C has mainly been described as a suppressor of the inflammatory response and has been suggested
222 for antiretroviral therapy [30]. Investigations deciphering its effect during the viral life cycle are limited,
223 however, it is involved in the MAPK-pathway and is a positive regulator of the c-jun N-terminal kinase
224 signaling pathway [23]. In summary, further evaluation on the mode of action and *in vivo* efficacy of
225 each compound against SARS-CoV-2 and other coronaviruses is warranted to establish whether
226 conserved host or viral features can be efficiently targeted by the selected antiviral compounds.

227 In summary, the stringent *in vitro* screening of 400 compounds identified several candidate
228 compounds possessing potent antiviral properties against SARS-CoV-2 that vindicate further efficacy
229 and safety testing in pre-clinical *in vitro* and *in vivo* models as novel intervention strategies against
230 SARS-CoV-2 and other coronaviruses.

231

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236 pandemic response box and supplying the compounds. We also would like to thank Gary Prescott of
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238 analysis.

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243 **Declaration of Interests**

244 The authors declare no competing interests.

245 **Figures and tables**

246 **Figure 1: Immunofluorescence staining of the dilution series of the hits.** To determine the
247 efficiency of inhibition of each specific compound that were selected after the survival screen, dilution
248 series ranging from 4 to 0.063 µM were prepared followed by infection with SARS-CoV-2 (MOI of
249 0.01). Vero-E6 cells were pretreated with the compound 2 hours before infection at 37°C with an CO₂-
250 humidity of 5% and fixed 24 hours post infection, followed by immunostaining with the cross-reactive
251 SARS-CoV Nucleoprotein antigen (SARS-N) and DAPI. Remdesivir and K22 included were included
252 as controls. The acquisition was performed with a 4x air objective whereby four pictures per well were
253 stitched with the build-in Gen5-software. The image are representative of the results of three individual
254 experiments. Scale bar represent 1 µM.

255 **Figure 2: IC₅₀ determination of the five compound hits that showed inhibition against SARS-
256 CoV-2.** The cell cytotoxicity (**A**) and cell viability (**B**) were assessed from each compound dilution of
257 the five hits after 24 hours of incubation on Vero-E6 cells at 37°C with a 5% CO₂-humidity. The cells
258 were pretreated for 2 hours with the compound dilution prior to infection. To determine the inhibition of
259 the five compounds, the measured total fluorescence signal intensity of GFP (infected cells) per well
260 was divided by the total fluorescence signal intensity of DAPI (presence of cells) per well, indicated as
261 the GFP/DAPI ratio. The GFP/DAPI ratio of each compound dilution of Retro-2.1 (**C**), Chloroquine (**D**),
262 n-Nonyldeoxyojirimycin (NN-DNJ) (**E**), PBDNJ0803 (**F**) and URMC-099 (**G**) are compared to
263 Remdesivir, K22 and DMSO. Results are displayed as means and SD of three individual experiments.
264 To control for cytotoxicity, the half maximum cytotoxicity concentration 50% (CC₅₀) per compound
265 were determined (**H**). Results of the CC₅₀ are shown as means and SD from two individual
266 experiments.

Plate position:	Section	MMV-number	CHEMBL ID	Websitelink or literaturelink:	Name	Clinical stage according CHEMBL	Chemical formula
Plate A, D2	Antifungals	MMV1634386	CHEMBL3311228	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL3311228/	Oteseconazole	3 (Phase III)	C23 H16 F7 N5 O2
Plate A, E2	Antifungals	MMV637528	CHEMBL64391	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL64391/	Itraconazole	4 (approved)	C35 H38 Cl2 N8 O4
Plate B, A2	Antibacterials	MMV1483032	CHEMBL243644	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL243644/	AC1MTT7T	0 (research)	C22 H18 N4 O4
Plate B, A7	Antibacterials	MMV637945	CHEMBL403	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL403/	Sulbactam	4 (approved)	C8 H11 N O5 S
Plate B, D9	Antibacterials	MMV1582492	CHEMBL3109593	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL3109593/	Retro-2.1	0 (research)	C23 H18 F N3 O S2
Plate B, F3	Antibacterials	MMV1582487	CHEMBL198796	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL198796/	Decylphosphinate	0 (research)	C13 H28 N O4 P
Plate C, A5	Antibacterials	MMV1578576	CHEMBL1568820	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL1568820/	-	0 (research)	C15 H12 F N3 O
Plate C, E7	Antibacterials	MMV000008	CHEMBL76	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL76/	Chloroquine	4 (approved)	C18 H26 Cl N3
Plate D, A11	Antivirals	MMV1593513	CHEMBL408500	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL408500/	N-Nonyl Deoxynojirimycin (NN-DNJ)	0 (research)	C15 H31 N O4
Plate D, C7	Antivirals	MMV690621	-	Patent: WO2006118607A2	NA for racemic	-	C18 H16 Cl N3 O
Plate D, E10	Antivirals	MMV1634401	CHEMBL1652119	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL1652119/	PBDNJ0803	0 (research)	C20 H33 N O5
Plate D, E11	Antivirals	MMV002780	CHEMBL402487	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL402487/	Noscapine	0 (research)	C22 H23 N O7
Plate E, A6	Antivirals	MMV1580482	CHEMBL2436978	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL2436978/ Patent: WO 2014085795 A1	URMC-099-C	0 (research)	C27 H27 N5
Plate E, B7	Antivirals	MMV1593544	CHEMBL3752642	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL3752642/	-	0 (research)	C36 H43 N3 O5
Plate E, E11	Antifungals	MMV002350	CHEMBL561	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL561/	Lomefloxacin	4 (approved)	C17H19F2N3O3
Plate E, F11	Antivirals	MMV1580483	CHEMBL3960662	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL3960662/	AZD0156	0 (research)	C26 H31 N5 O3
-	Control	-	CHEMBL4065616	https://www.ebi.ac.uk/chembl/compound_report_card/CHEMBL4065616/	Remdesivir	2 (Phase II)	C27 H35 N6 O8 P

Table 1: The sixteen compounds that showed inhibition of the SARS-CoV-2 after the survival screen. Remdesivir was included as reference drug control.

Name	IC ₅₀	CC ₅₀	SI	Class	Tested against other viruses	Literature reference	Mode of action	Administration	Tested on:	Bioavailable
Retro-2.1	0.4909	50	101.9	Retrograde transport inhibitor	<ul style="list-style-type: none"> ● Enterovirus, ● Herpes simplex virus ● Filoviruses ● Vaccina virus ● Polyomavirus 	[19,31–34]	<ul style="list-style-type: none"> ● Modulate intracellular vesicle transport (Enterovirus 71), ● Blocks entry (Herpes Simplex Virus 2) ● Blocks entry that follows by glycoprotein proteolysis (Filoviruses) ● Localization towards the ER (Polyomavirus) ● endosome-to-Golgi apparatus trafficking (Vaccina virus) ● induce cellular STX5 displacement from the Golgi upon treatment, leading to an inhibition of retrograde transport (Cytomegalovirus) 	<ul style="list-style-type: none"> ● Prophylactic ● Therapeutic 	<ul style="list-style-type: none"> ● Vero ● HeLa ● 293S ● Mice 	No
Chloroquine	1.03	50	48.5	Autophagic proteolysis inhibitor Endosomal acidification inhibitor	<ul style="list-style-type: none"> ● Herpes simplex virus ● Picornaviruses ● Poliovirus ● SARS-CoV ● HIV-1 ● Hepatitis A virus ● Hepatitis B virus ● Hepatitis C virus ● Influenza A virus ● Influenza B virus ● Bovine viral diarrhea virus ● 229E-CoV ● OC43-CoV ● Chikungunya virus ● Dengue virus ● Crimean-Congo Hemorrhagic fever ● Ebola virus ● Nipah virus ● Hendra virus ● Lassa virus ● Rabies virus 	[21,22,35–55]	<ul style="list-style-type: none"> ● Raise endosomal pH (Picornavirus) ● Terminal glycosylation of the ACE2-receptor (SARS-CoV) ● Alter the glycosylation of the glycoprotein (HIV-1) ● Interference with endosomal acidification (Hepatitis C virus) ● Endosomal acidification (BVDV) ● P38 MAPK and ERK activation (229E) 	<ul style="list-style-type: none"> ● Prophylactic ● Therapeutic 	<ul style="list-style-type: none"> ● VeroE6 ● Vero 76 ● L132 ● MDBK ● Huh7 ● NS20 murine neuroblastoma cells ● B-SC-1 ● A549 ● Humans ● U937 ● MRC5 ● Guinea pigs 	Yes

N-Nonyl Deoxyno Jirimycin (NNDNJ)	1.677	50	29.8	ER α-glucosidase inhibitors	<ul style="list-style-type: none"> ● Bovine viral diarrhea virus ● West nile virus ● Dengue virus ● Hepatitis B virus ● Hepatitis C virus ● Flaviviruses ● Woodchuck hepatitis virus ● Ebola virus ● Tacaribe virus ● Junin virus ● SARS-CoV 	[20,27–29,56–60] US patent: 9040488	<ul style="list-style-type: none"> ● Protein folding (Hepatitis B virus, Bovine viral diarrhea virus) ● Protein trafficking (Woodchuck hepatitis virus) 	<ul style="list-style-type: none"> ● Prophylactic ● Therapeutic 	<ul style="list-style-type: none"> ● MDBK ● BHK (-21) ● HepG2 ● Huh7.5 ● Woodchucks 	Yes
PBDNJ0803	2.026	50	24.7	ER α-glucosidase inhibitors	<ul style="list-style-type: none"> ● Dengue virus ● Bovine viral diarrhea virus ● West nile virus ● Ebola virus ● Tacaribe virus ● Junin virus 	[27,29] US Patent: 9040488	<ul style="list-style-type: none"> ● Protein folding (Bovine viral diarrhea virus) 	<ul style="list-style-type: none"> ● Therapeutic 	<ul style="list-style-type: none"> ● MDBK ● BHK ● Vero 	No
URMC-099-C	0.335	13.1	39.1	Mixed-lineage kinase 3 inhibitor	<ul style="list-style-type: none"> ● HIV-1 ● Zika virus 	[23,61–63]	<ul style="list-style-type: none"> ● Anti-inflammatory ● Blocks phosphorylation of MLK3 ● Inhibits viral maturation in the endosome ● pro-viral effect in replication 	<ul style="list-style-type: none"> ● Prophylactic ● Therapeutic 	<ul style="list-style-type: none"> ● BV-2 microglial ● Mice ● Macrophages ● SNB-19 	Yes
Remdesivir	1.842	50	27.1	Nucleoside analogue	<ul style="list-style-type: none"> ● Ebola virus ● Marburg virus ● SARS-CoV ● MERS-CoV ● Respiratory Syncytial Virus ● Junin virus ● Lassa virus ● Hendra virus ● Nipah virus ● Rift Valley Fever Virus ● Mumps virus ● Measles virus ● Coronaviruses ● Murine hepatitis virus 	[13,64–68]	<ul style="list-style-type: none"> ● Interferes with the RNA dependent RNA polymerase activity 	<ul style="list-style-type: none"> ● Prophylactic ● Therapeutic 	<ul style="list-style-type: none"> ● Primary macrophages ● HeLa ● HFF-1 ● HMVEC-TERT ● Rhesus monkeys ● Huh7 ● Hep-2 ● U2OS ● Vero ● Mice ● Human airway epithelial 	Yes

269 **Table 2:** The IC₅₀ and CC₅₀ values of the 5 compounds that showed inhibition of SARS-CoV-2 during the compound dilution series, including references to the

270 mode of action of these compounds against other viruses. Remdesivir is included as a reference drug control

271 **Literature:**

- 272 1. Wang, C.; Horby, P.W.; Hayden, F.G.; Gao, G.F. A novel coronavirus outbreak of global health
273 concern. *Lancet* **2020**, *395*, 470–473, doi:10.1016/S0140-6736(20)30185-9.
- 274 2. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y.; Zhang, L.; Fan, G.; Xu, J.; Gu, X.; et al.
275 Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*
276 **2020**, *395*, 497–506, doi:10.1016/S0140-6736(20)30183-5.
- 277 3. Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.;
278 et al. A novel coronavirus from patients with pneumonia in China, 2019. *N. Engl. J. Med.* **2020**,
279 doi:10.1056/NEJMoa2001017.
- 280 4. Zhou, F.; Yu, T.; Du, R.; Fan, G.; Liu, Y.; Liu, Z.; Xiang, J.; Wang, Y.; Song, B.; Gu, X.; et al.
281 Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China:
282 a retrospective cohort study. *Lancet* **2020**, *395*, 1054–1062, doi:10.1016/S0140-
283 6736(20)30566-3.
- 284 5. Yang, J.; Zheng, Y.; Gou, X.; Pu, K.; Chen, Z.; Guo, Q.; Ji, R.; Wang, H.; Wang, Y.; Zhou, Y.
285 Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a
286 systematic review and meta-analysis. *Int. J. Infect. Dis.* **2020**, *0*, doi:10.1016/j.ijid.2020.03.017.
- 287 6. Dong, E.; Du, H.; Gardner, L. An interactive web-based dashboard to track COVID-19 in real
288 time. *Lancet Infect. Dis.* **2020**, *20*, 533–534, doi:10.1016/S1473-3099(20)30120-1.
- 289 7. Lurie, N.; Saville, M.; Hatchett, R.; Halton, J. Developing Covid-19 Vaccines at Pandemic
290 Speed. *N. Engl. J. Med.* **2020**, doi:10.1056/nejmp2005630.
- 291 8. Wang, M.; Cao, R.; Zhang, L.; Yang, X.; Liu, J.; Xu, M.; Shi, Z.; Hu, Z.; Zhong, W.; Xiao, G.
292 Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-
293 nCoV) in vitro. *Cell Res.* **2020**, *30*, 269–271, doi:10.1038/s41422-020-0282-0.
- 294 9. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Krüger, N.; Herrler, T.; Erichsen, S.;
295 Schiergens, T.S.; Herrler, G.; Wu, N.-H.; Nitsche, A.; et al. SARS-CoV-2 Cell Entry Depends on
296 ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**,
297 doi:10.1016/j.cell.2020.02.052.

- 298 10. Wang, Y.; Zhang, D.; Du, G.; Du, R.; Zhao, J.; Jin, Y.; Fu, S.; Gao, L.; Cheng, Z.; Lu, Q.; et al.
299 Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled,
300 multicentre trial. *Lancet* **2020**, doi:10.1016/S0140-6736(20)31022-9.
- 301 11. Smith, E.C.; Blanc, H.; Vignuzzi, M.; Denison, M.R. Coronaviruses Lacking Exoribonuclease
302 Activity Are Susceptible to Lethal Mutagenesis: Evidence for Proofreading and Potential
303 Therapeutics. *PLoS Pathog.* **2013**, doi:10.1371/journal.ppat.1003565.
- 304 12. Lundin, A.; Dijkman, R.; Bergström, T.; Kann, N.; Adamiak, B.; Hannoun, C.; Kindler, E.;
305 Jónsdóttir, H.R.; Muth, D.; Kint, J.; et al. Targeting membrane-bound viral RNA synthesis
306 reveals potent inhibition of diverse coronaviruses including the middle East respiratory
307 syndrome virus. *PLoS Pathog.* **2014**, *10*, e1004166, doi:10.1371/journal.ppat.1004166.
- 308 13. Agostini, M.L.; Andres, E.L.; Sims, A.C.; Graham, R.L.; Sheahan, T.P.; Lu, X.; Smith, E.C.;
309 Case, J.B.; Feng, J.Y.; Jordan, R.; et al. Coronavirus susceptibility to the antiviral remdesivir
310 (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. *MBio*
311 **2018**, *9*, 1–15, doi:10.1128/mBio.00221-18.
- 312 14. Bloom, D.E.; Cadarette, D. Infectious Disease Threats in the Twenty-First Century:
313 Strengthening the Global Response. *Front. Immunol.* **2019**, *10*, 549,
314 doi:10.3389/fimmu.2019.00549.
- 315 15. Tran Thi Nhu Thao, Fabien Labroussaa, Nadine Ebert, Philip V'kovski, Hanspeter Stalder,
316 Jasmine Portmann, Jenna Kelly, Silvio Steiner, Melle Holwerda, Annika Kratze, Mitra Gultom,
317 Laura Laloli, Linda Hüsser, Manon Wider, Stephanie Pfaender, Dagny Hirt, Va, V.T.; 9 Rapid
318 reconstruction of SARS-CoV-2 using a synthetic genomics platform. *Nature* **2020**,
319 doi:10.1038/s41586-020-2294-9.
- 320 16. Mutterer, J.; Zinck, E. Quick-and-clean article figures with FigureJ. *J. Microsc.* **2013**, *252*, 89–
321 91.
- 322 17. De Wilde, A.H.; Jochmans, D.; Posthuma, C.C.; Zevenhoven-Dobbe, J.C.; Van Nieuwkoop, S.;
323 Bestebroer, T.M.; Van Den Hoogen, B.G.; Neyts, J.; Snijder, E.J. Screening of an FDA-
324 approved compound library identifies four small-molecule inhibitors of Middle East respiratory
325 syndrome coronavirus replication in cell culture. *Antimicrob. Agents Chemother.* **2014**, *58*,

- 326 4875–4884, doi:10.1128/AAC.03011-14.
- 327 18. Dyall, J.; Coleman, C.M.; Hart, B.J.; Venkataraman, T.; Holbrook, M.R.; Kindrachuk, J.;
328 Johnson, R.F.; Olinger, G.G.; Jahrling, P.B.; Laidlaw, M.; et al. Repurposing of clinically
329 developed drugs for treatment of Middle East respiratory syndrome coronavirus infection.
330 *Antimicrob. Agents Chemother.* **2014**, *58*, 4885–4893, doi:10.1128/AAC.03036-14.
- 331 19. Shtanko, O.; Sakurai, Y.; Reyes, A.N.; Noël, R.; Cintrat, J.C.; Gillet, D.; Barbier, J.; Davey, R.A.
332 Retro-2 and its dihydroquinazolinone derivatives inhibit filovirus infection. *Antiviral Res.* **2018**,
333 *149*, 154–163, doi:10.1016/j.antiviral.2017.11.016.
- 334 20. Gu, B.; Mason, P.; Wang, L.; Norton, P.; Bourne, N.; Moriarty, R.; Mehta, A.; Despande, M.;
335 Shah, R.; Block, T. Antiviral profiles of novel iminocyclitol compounds against bovine viral
336 diarrhea virus, West Nile virus, dengue virus and hepatitis B virus. *Antivir. Chem. Chemother.*
337 **2007**, *18*, 49–59, doi:10.1177/095632020701800105.
- 338 21. Feng, Z.; Hensley, L.; McKnight, K.L.; Hu, F.; Madden, V.; Ping, L.; Jeong, S.H.; Walker, C.;
339 Lanford, R.E.; Lemon, S.M. A pathogenic picornavirus acquires an envelope by hijacking
340 cellular membranes. *Nature* **2013**, *496*, 367–371, doi:10.1038/nature12029.
- 341 22. Vincent, M.J.; Bergeron, E.; Benjannet, S.; Erickson, B.R.; Rollin, P.E.; Ksiazek, T.G.; Seidah,
342 N.G.; Nichol, S.T. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread.
343 *Virol. J.* **2005**, *2*, 1–10, doi:10.1186/1743-422X-2-69.
- 344 23. Xu, H.; Cheng, M.; Chi, X.; Liu, X.; Zhou, J.; Lin, T.; Yang, W. High-Throughput Screening
345 Identifies Mixed-Lineage Kinase 3 as a Key Host Regulatory Factor in Zika Virus Infection. *J.*
346 *Virol.* **2019**, *93*, 1–16, doi:10.1128/jvi.00758-19.
- 347 24. Gupta, N.; Pons, V.; Noël, R.; Buisson, D.A.; Michau, A.; Johannes, L.; Gillet, D.; Barbier, J.;
348 Cintrat, J.C. (S)-N-methyldihydroquinazolinones are the active enantiomers of retro-2 derived
349 compounds against toxins. *ACS Med. Chem. Lett.* **2014**, *5*, 94–97, doi:10.1021/ml400457j.
- 350 25. Stechmann, B.; Bai, S.K.; Gobbo, E.; Lopez, R.; Merer, G.; Pinchard, S.; Panigai, L.; Tenza, D.;
351 Raposo, G.; Beaumelle, B.; et al. Inhibition of retrograde transport protects mice from lethal
352 ricin challenge. *Cell* **2010**, *141*, 231–242, doi:10.1016/j.cell.2010.01.043.

- 353 26. V'kovski, P.; Gerber, M.; Kelly, J.; Pfaender, S.; Ebert, N.; Braga Lagache, S.; Simillion, C.;
354 Portmann, J.; Stalder, H.; Gaschen, V.; et al. Determination of host proteins composing the
355 microenvironment of coronavirus replicase complexes by proximity-labeling. *eLife* **2019**, *8*,
356 doi:10.7554/eLife.42037.
- 357 27. Chang, J.; Wang, L.; Ma, D.; Qu, X.; Guo, H.; Xu, X.; Mason, P.M.; Bourne, N.; Moriarty, R.;
358 Gu, B.; et al. Novel imino sugar derivatives demonstrate potent antiviral activity against
359 flaviviruses. *Antimicrob. Agents Chemother.* **2009**, *53*, 1501–1508, doi:10.1128/AAC.01457-08.
- 360 28. Fukushi, M.; Yoshinaka, Y.; Matsuoka, Y.; Hatakeyama, S.; Ishizaka, Y.; Kirikae, T.; Sasazuki,
361 T.; Miyoshi-Akiyama, T. Monitoring of S Protein Maturation in the Endoplasmic Reticulum by
362 Calnexin Is Important for the Infectivity of Severe Acute Respiratory Syndrome Coronavirus. *J.
363 Virol.* **2012**, *86*, 11745–11753, doi:10.1128/jvi.01250-12.
- 364 29. Qu, X.; Pan, X.; Weidner, J.; Yu, W.; Alonzi, D.; Xu, X.; Butters, T.; Block, T.; Guo, J.T.; Chang,
365 J. Inhibitors of endoplasmic reticulum α-glucosidases potently suppress hepatitis C virus virion
366 assembly and release. *Antimicrob. Agents Chemother.* **2011**, *55*, 1036–1044,
367 doi:10.1128/AAC.01319-10.
- 368 30. Zhang, G.; Guo, D.; Dash, P.K.; Araínga, M.; Wiederin, J.L.; Haverland, N.A.; Knibbe-Hollinger,
369 J.; Martinez-Skinner, A.; Ciborowski, P.; Goodfellow, V.S.; et al. The mixed lineage kinase-3
370 inhibitor URMC-099 improves therapeutic outcomes for long-acting antiretroviral therapy.
371 *Nanomedicine Nanotechnology, Biol. Med.* **2016**, *12*, 109–122,
372 doi:10.1016/j.nano.2015.09.009.
- 373 31. Dai, W.; Wu, Y.; Bi, J.; Lu, X.; Hou, A.; Zhou, Y.; Sun, B.; Kong, W.; Barbier, J.; Cintrat, J.C.; et
374 al. Antiviral effects of Retro-2cycl and Retro-2.1 against Enterovirus 71 in vitro and in vivo.
375 *Antiviral Res.* **2017**, *144*, 311–321, doi:10.1016/j.antiviral.2017.07.001.
- 376 32. Desai, D.; Lauver, M.; Ostman, A.; Cruz, L.; Ferguson, K.; Jin, G.; Roper, B.; Brosius, D.;
377 Lukacher, A.; Amin, S.; et al. Inhibition of diverse opportunistic viruses by structurally optimized
378 retrograde trafficking inhibitors. *Bioorganic Med. Chem.* **2019**, *27*, 1795–1803,
379 doi:10.1016/j.bmc.2019.03.026.
- 380 33. Maru, S.; Jin, G.; Desai, D.; Amin, S.; Shwetank; Lauver, M.D.; Lukacher, A.E. Inhibition of

- 381 Retrograde Transport Limits Polyomavirus Infection In Vivo. *mSphere* **2017**,
382 doi:10.1128/mspheredirect.00494-17.
- 383 34. Harrison, K.; Haga, I.R.; Pechenick Jowers, T.; Jasim, S.; Cintrat, J.-C.; Gillet, D.; Schmitt-
384 John, T.; Digard, P.; Beard, P.M. Vaccinia Virus Uses Retromer-Independent Cellular
385 Retrograde Transport Pathways To Facilitate the Wrapping of Intracellular Mature Virions
386 during Virus Morphogenesis. *J. Virol.* **2016**, *90*, 10120–10132, doi:10.1128/jvi.01464-16.
- 387 35. Dai, W.; Wu, Y.; Bi, J.; Wang, S.; Li, F.; Kong, W.; Barbier, J.; Cintrat, J.C.; Gao, F.; Gillet, D.;
388 et al. Antiviral effects of abma against herpes simplex virus type 2 in vitro and in vivo. *Viruses*
389 **2018**, *10*, 1–15, doi:10.3390/v10030119.
- 390 36. Bishop, N.E. Examination of potential inhibitors of hepatitis A virus uncoating. *Intervirology*
391 **1998**, *41*, 261–271, doi:10.1159/000024948.
- 392 37. Mizui, T.; Yamashina, S.; Tanida, I.; Takei, Y.; Ueno, T.; Sakamoto, N.; Ikejima, K.; Kitamura,
393 T.; Enomoto, N.; Sakai, T.; et al. Inhibition of hepatitis C virus replication by chloroquine
394 targeting virus-associated autophagy. *J. Gastroenterol.* **2010**, *45*, 195–203,
395 doi:10.1007/s00535-009-0132-9.
- 396 38. Eng, E.O.; Chew, J.S.W.; Jin, P.L.; Chua, R.C.S. In vitro inhibition of human influenza A virus
397 replication by chloroquine. *Virol. J.* **2006**, *3*, 3–5, doi:10.1186/1743-422X-3-39.
- 398 39. Yan, Y.; Zou, Z.; Sun, Y.; Li, X.; Xu, K.F.; Wei, Y.; Jin, N.; Jiang, C. Anti-malaria drug
399 chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal
400 model. *Cell Res.* **2013**, *23*, 300–302, doi:10.1038/cr.2012.165.
- 401 40. Paton, N.I.; Lee, L.; Xu, Y.; Ooi, E.E.; Cheung, Y.B.; Archuleta, S.; Wong, G.; Smith, A.W.
402 Chloroquine for influenza prevention: A randomised, double-blind, placebo controlled trial.
403 *Lancet Infect. Dis.* **2011**, *11*, 677–683, doi:10.1016/S1473-3099(11)70065-2.
- 404 41. De Lamballerie, X.; Boisson, V.; Reynier, J.C.; Enault, S.; Charrel, R.N.; Flahault, A.; Roques,
405 P.; Grand, R. Le On chikungunya acute infection and chloroquine treatment. *Vector-Borne*
406 *Zoonotic Dis.* **2008**, *8*, 837–839, doi:10.1089/vbz.2008.0049.
- 407 42. Khan, M.; Santhosh, S.R.; Tiwari, M.; Lakshmana Rao, P. V.; Parida, M. Assessment of in vitro

- 408 prophylactic and therapeutic efficacy of chloroquine against Chikungunya virus in Vero cells. *J.*
409 *Med. Virol.* **2010**, *82*, 817–824, doi:10.1002/jmv.21663.
- 410 43. Farias, K.J.S.; Machado, P.R.L.; de Almeida Junior, R.F.; de Aquino, A.A.; da Fonseca, B.A.L.
411 Chloroquine interferes with dengue-2 virus replication in U937 cells. *Microbiol. Immunol.* **2014**,
412 *58*, 318–326, doi:10.1111/1348-0421.12154.
- 413 44. Delvecchio, R.; Higa, L.M.; Pezzuto, P.; Valadão, A.L.; Garcez, P.P.; Monteiro, F.L.; Loiola,
414 E.C.; Dias, A.A.; Silva, F.J.M.; Aliota, M.T.; et al. Chloroquine, an endocytosis blocking agent,
415 inhibits zika virus infection in different cell models. *Viruses* **2016**, *8*, 1–15,
416 doi:10.3390/v8120322.
- 417 45. Porotto, M.; Orefice, G.; Yokoyama, C.C.; Mungall, B.A.; Realubit, R.; Sganga, M.L.; Aljofan,
418 M.; Whitt, M.; Glickman, F.; Moscona, A. Simulating Henipavirus Multicycle Replication in a
419 Screening Assay Leads to Identification of a Promising Candidate for Therapy. *J. Virol.* **2009**,
420 *83*, 5148–5155, doi:10.1128/jvi.00164-09.
- 421 46. Barnard, D.L.; Day, C.W.; Bailey, K.; Heiner, M.; Montgomery, R.; Lauridsen, L.; Chan, P.K.S.;
422 Sidwell, R.W. Evaluation of immunomodulators, interferons and known in vitro SARS-CoV
423 inhibitors for inhibition of SARS-CoV replication in BALB/c mice. *Antivir. Chem. Chemother.*
424 **2006**, *17*, 275–284, doi:10.1177/095632020601700505.
- 425 47. Freiberg, A.N.; Worthy, M.N.; Lee, B.; Holbrook, M.R. Combined chloroquine and ribavirin
426 treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. *J.*
427 *Gen. Virol.* **2010**, *91*, 765–772, doi:10.1099/vir.0.017269-0.
- 428 48. Ferraris, O.; Moroso, M.; Pernet, O.; Emonet, S.; Ferrier Rembert, A.; Paranhos-Baccalà, G.;
429 Peyrefitte, C.N. Evaluation of Crimean-Congo hemorrhagic fever virus in vitro inhibition by
430 chloroquine and chlorpromazine, two FDA approved molecules. *Antiviral Res.* **2015**, *118*, 75–
431 81, doi:10.1016/j.antiviral.2015.03.005.
- 432 49. Dowall, S.D.; Bosworth, A.; Watson, R.; Bewley, K.; Taylor, I.; Rayner, E.; Hunter, L.; Pearson,
433 G.; Easterbrook, L.; Pitman, J.; et al. Chloroquine inhibited ebola virus replication in vitro but
434 failed to protect against infection and disease in the in vivo guinea pig model. *J. Gen. Virol.*
435 **2015**, *96*, 3484–3492, doi:10.1099/jgv.0.000309.

- 436 50. Kono, M.; Tatsumi, K.; Imai, A.M.; Saito, K.; Kuriyama, T.; Shirasawa, H. Inhibition of human
437 coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: Involvement of
438 p38 MAPK and ERK. *Antiviral Res.* **2008**, *77*, 150–152, doi:10.1016/j.antiviral.2007.10.011.
- 439 51. Lecot, S.; Belouzard, S.; Dubuisson, J.; Rouillé, Y. Bovine Viral Diarrhea Virus Entry Is
440 Dependent on Clathrin-Mediated Endocytosis. *J. Virol.* **2005**, *79*, 10826–10829,
441 doi:10.1128/jvi.79.16.10826-10829.2005.
- 442 52. Blanchard, E.; Belouzard, S.; Goueslain, L.; Wakita, T.; Dubuisson, J.; Wychowski, C.; Rouillé,
443 Y. Hepatitis C Virus Entry Depends on Clathrin-Mediated Endocytosis. *J. Virol.* **2006**, *80*,
444 6964–6972, doi:10.1128/jvi.00024-06.
- 445 53. Tsiang, H.; Superti, F. Ammonium chloride and chloroquine inhibit rabies virus infection in
446 neuroblastoma cells. *Arch. Virol.* **1984**, doi:10.1007/BF01310010.
- 447 54. Kronenberger, P.; Vrijen, R.; Boeyé, A. Chloroquine induces empty capsid formation during
448 poliovirus eclipse. *J. Virol.* **1991**, *65*, 7008–7011, doi:10.1128/jvi.65.12.7008-7011.1991.
- 449 55. Romanelli, F.; Smith, K.; Hoven, A. Chloroquine and Hydroxychloroquine as Inhibitors of
450 Human Immunodeficiency Virus (HIV-1) Activity. *Curr. Pharm. Des.* **2005**, *10*,
451 doi:10.2174/1381612043383791.
- 452 56. Mehta, A.; Ouzounov, S.; Jordan, R.; Simsek, E.; Lu, X.; Moriarty, R.M.; Jacob, G.; Dwek, R.A.;
453 Block, T.M. Imino sugars that are less toxic but more potent as antivirals, in vitro, compared
454 with N-n-nonyl DNJ. *Antivir. Chem. Chemother.* **2002**, *13*, 299–304,
455 doi:10.1177/095632020201300505.
- 456 57. Wu, S.-F.; Lee, C.-J.; Liao, C.-L.; Dwek, R.A.; Zitzmann, N.; Lin, Y.-L. Antiviral Effects of an
457 Iminosugar Derivative on Flavivirus Infections. *J. Virol.* **2002**, *76*, 3596–3604,
458 doi:10.1128/jvi.76.8.3596-3604.2002.
- 459 58. Zitzmann, N.; Mehta, A.S.; Carrouée, S.; Butters, T.D.; Platt, F.M.; McCauley, J.; Blumberg,
460 B.S.; Dwek, R.A.; Block, T.M. Imino sugars inhibit the formation and secretion of bovine viral
461 diarrhea virus, a pestivirus model of hepatitis C virus: Implications for the development of broad
462 spectrum anti-hepatitis virus agents. *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 11878–11882,
463 doi:10.1073/pnas.96.21.11878.

- 464 59. Steinmann, E.; Whitfield, T.; Kallis, S.; Dwek, R.A.; Zitzmann, N.; Pietschmann, T.;
465 Bartenschlager, R. Antiviral effects of amantadine and iminosugar derivatives against hepatitis
466 C virus. *Hepatology* **2007**, *46*, :330-8, doi:10.1002/hep.21686.
- 467 60. Block, T.M.; Lu, X.; Mehta, A.S.; Blumberg, B.S.; Tennant, B.; Ebling, M.; Korba, B.; Lansky,
468 D.M.; Jacob, G.S.; Dwek, R.A. Treatment of chronic hepadnavirus infection in a woodchuck
469 animal model with an inhibitor of protein folding and trafficking. *Nat. Med.* **1998**, *4*, 610–614,
470 doi:10.1038/nm0598-610.
- 471 61. Goodfellow, V.S.; Loweth, C.J.; Ravula, S.B.; Wiemann, T.; Nguyen, T.; Xu, Y.; Todd, D.E.;
472 Sheppard, D.; Pollack, S.; Polesskaya, O.; et al. Discovery, synthesis, and characterization of
473 an orally bioavailable, brain penetrant inhibitor of mixed lineage kinase 3. *J. Med. Chem.* **2013**,
474 doi:10.1021/jm401094t.
- 475 62. Marker, D.F.; Tremblay, M.È.; Puccini, J.M.; Barbieri, J.; Gantz Marker, M.A.; Loweth, C.J.;
476 Chris Muly, E.; Lu, S.M.; Goodfellow, V.S.; Dewhurst, S.; et al. The new small-molecule mixed-
477 lineage kinase 3 inhibitor URMC-099 is neuroprotective and anti-inflammatory in models of
478 human immunodeficiency virus-associated neurocognitive disorders. *J. Neurosci.* **2013**, *33*,
479 9998–10010, doi:10.1523/JNEUROSCI.0598-13.2013.
- 480 63. Zhang, G.; Guo, D.; Dash, P.K.; Araíga, M.; Wiederin, J.L.; Haverland, N.A.; Knibbe-Hollinger,
481 J.; Martinez-Skinner, A.; Ciborowski, P.; Goodfellow, V.S.; et al. The mixed lineage kinase-3
482 inhibitor URMC-099 improves therapeutic outcomes for long-acting antiretroviral therapy.
483 *Nanomedicine Nanotechnology, Biol. Med.* **2016**, doi:10.1016/j.nano.2015.09.009.
- 484 64. Warren, T.K.; Jordan, R.; Lo, M.K.; Ray, A.S.; Mackman, R.L.; Soloveva, V.; Siegel, D.; Perron,
485 M.; Bannister, R.; Hui, H.C.; et al. Therapeutic efficacy of the small molecule GS-5734 against
486 Ebola virus in rhesus monkeys. *Nature* **2016**, *531*, 381–385, doi:10.1038/nature17180.
- 487 65. Lo, M.K.; Jordan, R.; Arvey, A.; Sudhamsu, J.; Shrivastava-Ranjan, P.; Hotard, A.L.; Flint, M.;
488 McMullan, L.K.; Siegel, D.; Clarke, M.O.; et al. GS-5734 and its parent nucleoside analog
489 inhibit Filo-, Pneumo-, and Paramyxoviruses. *Sci. Rep.* **2017**, *7*, 1–7, doi:10.1038/srep43395.
- 490 66. Sheahan, T.P.; Sims, A.C.; Leist, S.R.; Schäfer, A.; Won, J.; Brown, A.J.; Montgomery, S.A.;
491 Hogg, A.; Babusis, D.; Clarke, M.O.; et al. Comparative therapeutic efficacy of remdesivir and

- 492 combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat. Commun.* **2020**,
493 11, doi:10.1038/s41467-019-13940-6.
- 494 67. Sheahan, T.P.; Sims, A.C.; Zhou, S.; Graham, R.L.; Pruijssers, A.J.; Agostini, M.L.; Leist, S.R.;
495 Schäfer, A.; Dinnon, K.H.; Stevens, L.J.; et al. An orally bioavailable broad-spectrum antiviral
496 inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in
497 mice. *Sci. Transl. Med.* **2020**, 5883, doi:10.1126/scitranslmed.abb5883.
- 498 68. Sheahan, T.P.; Sims, A.C.; Graham, R.L.; Menachery, V.D.; Gralinski, L.E.; Case, J.B.; Leist,
499 S.R.; Pyrc, K.; Feng, J.Y.; Trantcheva, I.; et al. Broad-spectrum antiviral GS-5734 inhibits both
500 epidemic and zoonotic coronaviruses. *Sci. Transl. Med.* **2017**, 9,
501 doi:10.1126/scitranslmed.aal3653.
- 502



