- 1 The natural stilbenoid (–)-hopeaphenol inhibits cellular entry of SARS-CoV-2 USA-WA1/2020,
2 B.1.1.7 and B.1.351 variants
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21 Abstract

22 Antivirals are urgently needed to combat the global SARS-CoV-2/COVID-19 pandemic,
23 supplement existing vaccine efforts, and target emerging SARS-CoV-2 variants of concern.
24 Small molecules that interfere with binding of the viral spike receptor binding domain (RBD) to
25 the host ACE2 receptor may be effective inhibitors of SARS-CoV-2 cell entry. Here we screened
26 512 pure compounds derived from natural products using a high-throughput RBD/ACE2 binding
27 assay and identified (–)-hopeaphenol, a resveratrol tetramer, in addition to vatalbinoside A and
28 vaticanol B, as potent and selective inhibitors of RBD/ACE2 binding and viral entry. For
29 example, (–)-hopeaphenol disrupted RBD/ACE2 binding with a 50% inhibitory concentration
30 (IC₅₀) of 0.11 μM in contrast to an IC₅₀ of 28.3 μM against the unrelated host ligand/receptor
31 binding pair PD-1/PD-L1 (selectivity index = 257.3). When assessed against the USA-
32 WA1/2020 variant, (–)-hopeaphenol also inhibited entry of a VSVΔG-GFP reporter pseudovirus
33 expressing SARS-CoV-2 spike into ACE2-expressing Vero-E6 cells and *in vitro* replication of
34 infectious virus in cytopathic effect and yield reduction assays (50% effective concentrations
35 (EC₅₀s) = 10.2 – 23.4 μM) without cytotoxicity and approaching the activities of the control
36 antiviral remdesivir (EC₅₀s = 1.0 – 7.3 μM). Notably, (–)-hopeaphenol also inhibited two
37 emerging variants of concern including B.1.1.7 / “Alpha” and B.1.351 / “Beta” in both viral and
38 spike-containing pseudovirus assays with similar or improved activities over the USA-
39 WA1/2020 variant. These results identify (–)-hopeaphenol and related stilbenoid analogues as
40 potent and selective inhibitors of viral entry across multiple SARS-CoV-2 variants of concern.

41 Introduction

42 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of
43 Coronavirus Disease 2019 (COVID-19). Since crossing into humans in late 2019, SARS-CoV-2

44 has continued to cause substantial human morbidity and mortality worldwide. While SARS-
45 CoV-2 vaccines are in development with several approved for use, access to these vaccines
46 remains limited, particularly in low and middle-income countries. Moreover, vaccine hesitancy
47 and ongoing mutation of SARS-CoV-2 increase the risk of resistance to vaccine-induced
48 immune responses. Importantly, a significant gap in our therapeutic arsenal exists as no out-
49 patient chemotherapy strategy is currently licensed to prevent infection or to combat mild to
50 severe COVID-19. For example, while chemotherapy against the RNA polymerase and
51 neutralizing antibody infusions are available, they depend on access to patient infusion resources.
52 Thus, broadly-accessible SARS-CoV-2 antivirals to treat infections irrespective of
53 hospitalization are urgently needed to complement ongoing vaccination efforts.

54 One attractive therapeutic target of SARS-CoV-2 replication is its binding and entry into
55 host cells, which is induced by the trimeric viral spike glycoprotein (1). A primary cellular
56 receptor of SARS-CoV-2 entry is the angiotensin-converting enzyme II (ACE2) protein. Viral
57 entry is mediated by the receptor binding domain (RBD) of the S1 segment of spike, which
58 directly interacts with ACE2, while S2 mediates membrane fusion (2-3). Following RBD/ACE2
59 binding, SARS-CoV-2 gains entry to host cells through both an endosomal, clathrin-dependent
60 pathway as well as a clathrin-independent pathway which involves spike protein cleavage by
61 furin, TMPRSS2, and other host proteases (1-2). Antagonism of the RBD/ACE2 interaction
62 would therefore be expected to block SARS-CoV-2 entry and replication.

63 Since its worldwide spread in early 2020, SARS-CoV-2 variants have acquired mutations
64 within Spike that enhance binding to ACE2 and increase infectivity, as first detected by the
65 emergence of a D614G mutation which has since become dominant (4). More recently,
66 additional variants have emerged with further spike sequence divergence; these include B.1.1.7 /

67 “Alpha”, which originated in the United Kingdom, and B.1.351 (“Beta”, also called 501Y.V2)
68 from South Africa (5). The B.1.1.7 variant contains an additional N501Y mutation within the
69 RBD that causes reduced antibody neutralization by both convalescent and vaccine sera *in vitro*
70 (6). Concerningly, the B.1.351 variant, which along with N501Y has also acquired mutations
71 E484K and K417N within the RBD, has further reduced susceptibility to, or enhanced escape
72 from, neutralizing antibodies as well as sera from convalescent and vaccine-treated patients and
73 immunized mice (7-8). As these observations raise concerns about the emergence of variants that
74 become resistant to vaccine-induced immune responses, additional countermeasures with the
75 potential to target emerging SARS-CoV-2 variants are essential.

76 Pure compounds derived from natural products are a rich source of antivirals including
77 against coronaviruses (9). However, outside of computational studies, few natural products to
78 date have been demonstrated to substantially reduce SARS-CoV-2 replication (10). To identify
79 natural product compounds that may inhibit entry across multiple SARS-CoV-2 variants, we
80 developed an AlphaScreen-based RBD/ACE2 interaction assay to screen a pure compound
81 library containing 512 natural products and derivatives sourced predominantly from plants,
82 mushrooms and marine invertebrates of Australia, Papua New Guinea, and neighboring regions
83 (11-12). The top hits from this screen, which included the plant stilbenoids (–)-hopeaphenol,
84 vatalbinoside A, and vaticanol B (**Figure 1**), were then assessed for *in vitro* mechanisms of
85 action using SARS-CoV-2 pseudoviruses and antiviral activity against infectious SARS-CoV-2
86 variants encompassing parental and B.1.1.7 and B.1.351 variants.

87

88 **Results**

89 **Stilbenoids selectively inhibit the SARS-CoV-2 spike RBD / host ACE2 protein interaction**

90 To identify potential inhibitors of SARS-CoV-2 entry, we used AlphaScreen technology
91 (13) to develop a high-throughput, 384 well plate-based assay to monitor the interaction of
92 SARS-CoV-2 Spike RBD with host ACE2 (**Figure 2A**). Briefly, a SARS-CoV-2 RBD protein
93 derived from USA-WA1/2020 and containing a C-terminal His tag, in addition to a full-length
94 ACE2 peptide with a C-terminal Fc tag, were pre-bound to respective acceptor and donor beads
95 and co-incubated for 3 hours at room temperature. When a ligand/receptor binding event occurs,
96 excitation at 680 nm results in a singlet oxygen transfer between donor and receptor beads,
97 which results in luminescence at 615 nm. Compounds that inhibit binding of RBD to ACE2
98 should therefore inhibit luminescence. In the absence of compounds, we observed that
99 luminescence was highly dependent on concentrations of both RBD and ACE2 (**Figure 2A**) with
100 a > 200-fold signal to noise ratio and high reproducibility across experiments ($Z' > 0.8$) (14).
101 Further, when assessing the clinically-approved neutralizing antibody REGN10933
102 (Casirivimab), which acts by targeting Spike RBD (54), we found a dose-dependent inhibition
103 across 4 independent experiments, with a calculated average 50% inhibitory concentration
104 (IC₅₀) of 0.18 nM (**Figure 2B; Table 1**).

105 Using this assay, we then screened 512 pure compounds obtained from natural products
106 and semisynthetic derivatives available from Compounds Australia at Griffith University at 3
107 μ M, where 7 (1.4%) inhibited > 75% of fluorescence observed in the absence of compounds.
108 Activities of top compounds were then assessed for dose-response profiles, where the three most
109 active hits were a series of stilbenoid resveratrol tetramers including (–)-hopeaphenol,
110 vatalbinoside A, and vaticanol B (**Figure 1; Figure 2C**). These stilbenoids, exemplified by
111 hopeaphenol, are tetramers of resveratrol available from multiple plant sources (15). Using the
112 RBD/ACE2 AlphaScreen assay described above, dose-response profiles from 5 independent

113 experiments were obtained to calculate IC₅₀s of 0.11, 0.24, and 0.067 μ M for hopeaphenol,
114 vatalbinoside A, and vaticanol B, respectively (**Table 1**). In contrast, almost no activity was
115 observed in this assay by the resveratrol monomer (IC₅₀ > 100 μ M; **Figure 2C**), indicating that
116 inhibition requires a multimeric structure.

117 To confirm selectivity of hopeaphenol and analogues to disrupt the RBD/ACE2
118 interaction, we next assessed their ability to interfere with the unrelated host PD-1/PD-L1
119 ligand/receptor pair using a comparable and previously-described experimental approach (16),
120 where we observed a > 100-fold signal to noise ratio and $Z' > 0.75$ (**Figure 2D**). In this assay,
121 the control PD-1/PD-L1 antagonist BMS-1166 (17) disrupted bead proximity-based fluorescence
122 with an IC₅₀ of 0.0040 μ M (**Figure 2E**) but had no activity against the RBD/ACE2 interaction
123 (IC₅₀ > 100 μ M; data not shown). Conversely, hopeaphenol, vatalbinoside A, and vaticanol B
124 were all substantially less effective in disrupting PD-1/PD-L1, with IC₅₀s of 28.3, 23.3, and 16.6
125 μ M, respectively (**Figures 2E; Table 1**). From these two assays, the selectivity indices of
126 hopeaphenol, vatalbinoside A, and vaticanol B [i.e., IC₅₀ (PD-1/PD-L1) / IC₅₀ (RBD/ACE2)]
127 were calculated to be 257.3, 92.9, and 247.8, respectively, indicating high selectivity of these
128 compounds for disrupting the viral RBD/host ACE2 interaction over an unrelated host
129 ligand/receptor pair.

130

131 **Stilbenoids are weak inhibitors of viral main protease**

132 A recent high-throughput virtual screening study proposed that hopeaphenol may act as
133 an inhibitor of the SARS-CoV-2 main protease (M^{Pro}) by interfering with its active site (18),
134 thereby raising the possibility of hopeaphenol acting on multiple viral targets. To test this
135 possibility, we also developed an M^{Pro} enzymatic assay using an M^{Pro} peptide substrate

136 resembling those described previously (19), where a C-terminal 5-((2-
137 aminoethyl)amino)naphthalene-1-sulfonic acid (EDANS) fluorescent tag is quenched by an N-
138 terminal 4-([4-(dimethylamino)phenyl]azo)benzoic acid (DABCYL) tag. Following incubation
139 with recombinant M^{pro} , the cleaved substrate affords separation of the EDANS tag from the
140 DABCYL quencher and detection of fluorescence at 490 nm. Compounds that inhibit M^{pro}
141 activity are therefore expected to inhibit fluorescence. This assay also exhibited a > 10-fold
142 signal to noise ratio and $Z' > 0.6$ (**Figure 3A**) and was adaptable to 384-well screening format.

143 Using this assay, we observed that the control M^{pro} inhibitor GC-376 blocked enzymatic
144 activity with an IC_{50} of 0.0052 μM , consistent with previous observations (**Figure 3B**) (19-20).
145 In contrast, we observed that hopeaphenol, vatalbinoside A, and vaticanol B inhibited M^{pro}
146 activity with IC_{50} s of 42.5, 68.7 and 47.6 μM , respectively (**Figure 3B; Table 1**), suggesting
147 that these stilbenoids, while potentially capable of targeting M^{pro} , are to a first approximation
148 more effective against RBD binding to ACE2.

149

150 **Stilbenoids inhibit SARS-CoV-2 spike-dependent viral entry**

151 To assess whether hopeaphenol and analogues inhibit viral entry within a cellular
152 context, we generated a single-cycle pseudovirus consisting of a vesicular stomatitis virus (VSV)
153 backbone lacking the G fusion protein and expressing SARS-CoV-2 spike protein and green
154 fluorescent protein (GFP) reporter (VSV Δ G-S-GFP) (21). In our initial experiments, we
155 generated pseudovirus with spike sequence corresponding to the USA-WA1/2020 variant.
156 Pseudovirus was then incubated with cells in the presence or absence of stilbenoids in 384-well
157 format in duplicate. High-content imaging was then used to count total live and infected cells in
158 each culture across 15 non-overlapping images per well, as determined by Hoechst-stained

nuclei and cellular GFP fluorescence, respectively. This approach allowed us to image approximately 12,000 cells per well (**Supplemental Table 1**). Consistent with previous observations (22-23), VSV Δ G-S-GFP pseudovirus infected ACE2-expressing cells like Vero-E6 (**Figure 4A**) (24) but not cell lines lacking ACE2 like BHK-21 cells (data not shown). In this assay, we observed an average of $2.4 \pm 0.2\%$ GFP-positive cells (mean $\pm$ s.e.m.) across 3 independent experiments following 24 hours' incubation with pseudovirus and 0.1% DMSO vehicle control (**Figure 4A; Supplemental Table 1**) with no major changes in number of cell nuclei relative to uninfected cells (**Figure 4B**). Additionally, no major changes in total cell nuclei were observed in the presence of up to 50 μ M of any compound, indicating no overt effects on cell viability, with the exception of 50 μ M resveratrol which resulted in cultures with $60.3 \pm 7.0\%$ of nuclei observed in untreated, infected cells (**Figure 4B; Supplemental Table 1**).

However, when Vero-E6 cells were infected with pseudovirus in the presence of 50 μ M hopeaphenol, the number of GFP-positive cells was reduced, with only $28.2 \pm 1.2\%$ of infected cells observed as compared to infected cells treated with 0.1% DMSO ($p = 3.5 \times 10^{-3}$ vs. cells treated with no drug; **Figures 4A, C; Table 1; Supplemental Table 1**). Similar results were observed when infected cells were co-treated with 50 μ M vatalbinoside A, where only $40.4 \pm 16.7\%$ of GFP-positive cells were observed relative to infected, vehicle-treated cells, although this comparison did not reach statistical significance when compared to untreated, infected cells (**Figure 4C**). In contrast, 50 μ M vaticanol B resulted in GFP expression in $83.7 \pm 4.9\%$ of cells, indicating that the potent anti-RBD/ACE2 activity observed by AlphaScreen assay was not reproduced in the pseudotype assay. As expected, 50 μ M resveratrol had no effect on GFP-positive cells ($96.3 \pm 14.7\%$ infected cells relative to infected, vehicle-treated cells; **Figure 4C**). However, no compound inhibited GFP expression when incubated with infected cells at 15 μ M

182 (data not shown). Taken together, these results indicate that at least a subset of stilbenoids can
183 inhibit entry of pseudoviruses expressing SARS-CoV-2 spike protein *in vitro*, consistent with
184 AlphaScreen assay results, although this occurs at much higher concentrations.

185

186 **Stilbenoids inhibit infectious SARS-CoV-2 replication**

187 To assess cellular antiviral activity of hopeaphenol and analogues, we first used a
188 cytopathic effect (CPE) scoring-based assay with infectious virus in Vero-E6 cells (25-26).
189 Briefly, Vero-E6 cells were treated with agents for 2 hours in 8-fold replicates in 96-well format
190 before infection with 50x median tissue culture infectious dose (TCID₅₀) of SARS-CoV-2
191 (USA-WA1/2020 variant). Cells were then incubated for 4 days with daily scoring of CPE across
192 all wells by a user blinded to experimental conditions. Using this approach, we observed the
193 presence of CPE by 2 days post infection, as characterized by extensive cell rounding and
194 cellular debris that were observable by light microscopy (**Figure 5A**, arrows). By 4 days post-
195 infection, this CPE was observable across the cell culture and clearly distinguishable from
196 uninfected cell controls (**Figure 5A**). When low to sub-nanomolar concentrations of
197 REGN10933 were added 2 hours before infection, CPE was inhibited in these cultures after 4
198 days (**Figure 5B**) with a calculated average 50% effective concentration (EC₅₀) of 0.16 nM
199 across 3 independent experiments (**Figure 5C; Table 2**). We also observed no evidence of
200 cytotoxicity due to REGN10933 treatment, as measured by resazurin staining following 4 days
201 treatment of uninfected Vero-E6 cells (**Figure 5D**).

202 Similar to observations with REGN10933, when low micromolar concentrations of either
203 the control nucleoside analog remdesivir or the M^{pro} inhibitor GC-376 (27-28) were added 2
204 hours before infection, CPE was also completely inhibited in these cultures after 4 days (**Figures**

205 **5E-F)**, with calculated EC₅₀s of 2.5 and 3.9 μ M for remdesivir and GC-376, respectively
206 (**Figure 5C; Table 2**). Moreover, comparable activity was observed in the presence of
207 hopeaphenol (**Figure 5E**, bottom), which blocked SARS-CoV-2 replication after 4 days with a
208 calculated EC₅₀ of 10.2 μ M (**Figure 5F; Table 2**). Notably, while similar antiviral activity was
209 observed with vatalbinoside A (EC₅₀ = 13.8 μ M), we observed substantially less activity with
210 vaticanol B (EC₅₀ = 37.0 μ M; **Figure 5F; Table 2**), consistent with its reduced activity in
211 pseudovirus assays (**Figure 4C**). In contrast, no antiviral activity was observed by up to 100 μ M
212 resveratrol (**Figure 5F**). No evidence of cytotoxicity was observed by these compounds (**Figure**
213 **5G**).

214 To confirm antiviral activity of these compounds using more quantitative assessments,
215 we next pre-treated Vero-E6 cells with agents for 2 hours in 8-fold replicates followed by
216 infection with 150x TCID of USA-WA1/2020 virus. Four days following infection at this titer,
217 we observed widespread CPE (data not shown) which, when measured by resazurin staining,
218 indicated an average of only $17.1 \pm 0.2\%$ cell viability relative to uninfected cells cultured in
219 parallel across 3 independent experiments ($p < 10^{-4}$; **Figure 6A; Supplemental Table 2**). When
220 the same cells were treated with REGN10933, we observed a dose-dependent restoration of cell
221 viability, consistent with abrogation of virus-induced CPE, with an EC₅₀ of 0.25 nM (**Table 2**).
222 Comparable but higher EC₅₀s were observed in the presence of remdesivir (EC₅₀ = 7.3 μ M) as
223 well as hopeaphenol, vatalbinoside A, and vaticanol B (respective EC₅₀s = 23.4, 18.8, and > 50
224 μ M), while no evidence of restoration of cell viability occurred with up to 50 μ M resveratrol
225 (**Figure 6B; Table 2**).

226 To determine whether reduction in CPE also corresponded to virus yield reduction, cell
227 culture supernatants from 4 of 8 replicates from CPE scoring-based assays above were combined

228 and applied to uninfected Vero-E6 cells at 1:10 dilution in 384-well format in the absence of any
229 additional chemical intervention. Following incubation with infected supernatants for 48 hours,
230 cells were fixed and probed for cellular SARS-CoV-2 nucleocapsid expression by
231 immunostaining. Cell nuclei were counterstained with Hoechst, and high-content imaging was
232 used to count total live and infected cells across 9 non-overlapping images per well, which
233 allowed us to image and score approximately 2600 cells per well (**Supplemental Table 3**). No
234 nucleocapsid-positive cells were observed following treatment with uninfected culture
235 supernatants (**Figure 6C**). However, as shown in **Figure 6C**, widespread nucleocapsid-positive
236 cells (red) were observed following incubation with supernatants from USA-WA1/2020-infected
237 cultures without drug treatment, which was determined to include an average of $29.0 \pm 2.5\%$ of
238 all cells across 2 independent experiments (mean $\pm$ S.D.). In contrast, we observed few
239 nucleocapsid-positive cells following incubation with supernatants treated with 7 nM
240 REGN10933 or 50 μ M hopeaphenol (**Figure 6C**; **Supplemental Table 3**). We also observed
241 dose-dependent inhibition of secondary viral infection from initial cultures treated with
242 REGN10933 ($EC_{50} = 0.17$ nM) as well as remdesivir ($EC_{50} = 1.0$ μ M), hopeaphenol ($EC_{50} =$
243 13.5 μ M) and vatalbinoside A ($EC_{50} = 19.1$ μ M), although less so for vaticanol B ($EC_{50} > 50$
244 μ M; **Figure 6E**). Notably, as these EC_{50} s correlated well with those observed in CPE assays
245 above (**Table 2**), these observations suggest that antiviral activity observed here is unlikely to be
246 attributed to residual transfer and dilution of test agents.

247 Taken together, these results indicate that hopeaphenol and vatalbinoside A, and to a
248 lesser extent vaticanol B, inhibit SARS-CoV-2 replication *in vitro* in three separate infection-
249 based assays, with activities of hopeaphenol and vatalbinoside A at the same order of magnitude
250 as control SARS-CoV-2 antivirals remdesivir and/or GC-376.

251

252 **(-)-Hopeaphenol inhibits SARS-CoV-2 variants of concern with similar or improved**
253 **activities.**

254 To determine if hopeaphenol maintained activity against emerging SARS-CoV-2 variants
255 with accumulated mutations in the spike RBD, we first repeated the CPE scoring-based assay
256 using two SARS-CoV-2 variants of concern including B.1.1.7 (England/204820464/2020;
257 “Alpha”) and B.1.351 (KRISP-K005325/2020; “Beta”) (**Figure 7**). Similar to our previous
258 observations, infection of Vero-E6 cells with either strain resulted in detectable CPE across
259 cultures after 4 days (**Figure 7A**, top), which was completely abolished by pre-treatment with
260 0.7 nM REGN10933 in cells infected with B.1.1.7 but required higher concentrations in cells
261 infected with B.1.351 (**Figure 7A**, middle). These results corresponded to EC₅₀s of 0.013 and
262 9.2 nM in cells infected with B.1.1.7 or B.1.351, respectively (**Figure 7B-C; Table 2**), consistent
263 with previous reports indicating variable *in vitro* inhibitory activity for REGN10933 against
264 SARS-CoV-2 variants (55). We also observed dose-dependent inhibition of these two variants by
265 remdesivir, with calculated EC₅₀s of 1.5 and 1.4 μ M for B.1.1.7 and B.1.351 strains,
266 respectively (**Figures 7D-E**), which were in agreement with observations using USA-WA1/2020
267 virus (EC₅₀ = 2.5 μ M; **Table 2**). Notably, 15 μ M hopeaphenol also completely abrogated CPE
268 by both variants (**Figure 7A**, bottom). When assessed for dose-response profiles, we observed
269 that B.1.1.7 was inhibited by hopeaphenol with an EC₅₀ of 14.8 μ M (**Figure 7D**), which
270 approximated hopeaphenol’s activity against USA-WA1/2020 (EC₅₀ = 10.2 μ M; **Table 2**). In
271 contrast, B.1.351 was inhibited by hopeaphenol with an EC₅₀ of 2.3 μ M (**Figure 7E**), indicating
272 4.5-fold improved activity over USA-WA1/2020 (**Table 2**). Similar results were observed in the
273 presence of vatalbinoside A, which respectively inhibited B.1.1.7 and B.1.351 infection with

274 EC50s of 4.0 and 3.6 μM , as well as vaticanol B, which respectively inhibited infection with
275 EC50s of 32.4 and 25.3 μM (**Figures 7D-E; Table 2**).

276 To confirm these findings, we next repeated the quantitative viability restoration assay
277 described above. However, when cells were infected with 150x TCID₅₀ of either B.1.1.7 or
278 B.1.351, no significant reductions in viability were observed until 7 days post-infection, which
279 resulted in an average across 3 independent experiments of $85.5 \pm 1.7\%$ viability in B.1.1.7-
280 infected cells ($p = 0.007$) and $81.5 \pm 2.8\%$ viability in B.1.351-infected cells ($p = 0.026$) when
281 compared to uninfected cells tested in parallel (**Figures 8A-D; Supplemental Table 2**). The
282 source of this reduced CPE by 150x TCID₅₀ of B.1.1.7 and B.1.351 when compared to USA-
283 WA1/2020 is not immediately clear but could reflect more rapid adaptation of USA-WA1/2020
284 to Vero-E6 culture in these experiments. Regardless, treatment with REGN10933 was able to
285 restore viability in both sets of infected cells with respective EC50s of 0.10 and 0.97 nM (**Figure**
286 **8A-B; Table 2**). Hopeaphenol also restored viability in cells infected with B.1.1.7 or B.1.351,
287 with respective EC50s of 7.8 and 7.5 μM (**Figure 8C-D; Table 2**). In contrast, viability was only
288 somewhat restored with vatalbinoside A treatment: for example, while we determined an EC50
289 of 11.3 μM in B.1.351-infected cells, no more than $51.2 \pm 12.2\%$ restored viability at 20 μM was
290 observed in B.1.1.7-infected cells. Vaticanol B and remdesivir also did not restore viability at
291 any concentration up to 50 or 10 μM , respectively (**Figure 8C-D; Table 2**). These results suggest
292 that remdesivir and vaticanol B, and to a lesser extent vatalbinoside A do not maintain antiviral
293 activity over 7 days' culture in this context, perhaps due to reduced compound stability and/or
294 the adaptation of viruses to the presence of compounds over this extended time period.

295 In virus yield reduction assays where B.1.1.7 and B.1.351-infected supernatants from
296 CPE scoring-based assays were applied in 1:10 dilution to naïve Vero-E6 cells and scored 48

297 hours later for nucleocapsid protein by high-content imaging, we measured infection in $12.8 \pm$
298 1.1% of cells treated with B.1.1.7-infected supernatants and $18.1 \pm 0.7\%$ of cells treated with
299 B.1.351-infected supernatants (**Figure 8E**). We also observed dose-dependent inhibition from
300 supernatants previously treated with REGN10933 ($EC_{50}s = 0.011$ and 2.2 nM, respectively, for
301 cells treated with supernatants from B.1.1.7 or B.1.351 infection REGN10933; **Figure 8F-G**;
302 **Table 2**). These observations also extended to infected supernatants previously treated with
303 hopeaphenol, where $EC_{50}s$ of 11.4 and 8.8 μ M were observed from supernatants from B.1.1.7
304 and B.1.351-infected cultures, respectively (**Figure 8H-I**; **Table 2**). These results indicate that
305 hopeaphenol inhibits replication of two SARS-CoV-2 variants across the three infection-based
306 assays *in vitro*.

307 To confirm that the antiviral activities of stilbenoids against these variants of concern
308 corresponded to inhibition of viral entry, we generated VSV Δ G-S-GFP-based pseudoviruses
309 containing B.1.1.7 or B.1.351 spike sequences and infected Vero-E6 cells in the absence or
310 presence of 50 μ M hopeaphenol, vatalbinoside, or vaticanol B (**Figure 9**). Similar to previous
311 observations (**Figure 4B**), no major changes in total cell nuclei number were observed under any
312 experimental condition (**Supplemental Table 1**). Also broadly consistent with previous
313 observations with our original pseudovirus, we observed an average of 5.4 ± 1.6 and $4.6 \pm 0.6\%$
314 GFP-positive cells following 24 hours' incubation with pseudovirus containing B.1.1.7 or
315 B.1.351 spike, respectively (**Figure 9A**, top). Furthermore, infection of both pseudoviruses
316 continued to be inhibited by 50 μ M hopeaphenol (**Figure 9A**, bottom). For example, pseudovirus
317 containing B.1.1.7 spike was observed in only $34.1 \pm 13.6\%$ of cells relative to infected, vehicle-
318 treated cells treated with 0.1% DMSO ($p = 4 \times 10^{-4}$ **Figure 9B**; **Table 1**; **Supplemental Table**
319 **1**), similar to results from pseudovirus plus USA-WA1/2020 spike (i.e., $28.2 \pm 1.2\%$).

320 Additionally, pseudovirus containing B.1.351 spike was present in only $20.4 \pm 2.4\%$ of infected,
321 vehicle-treated cells when compared to infected cells without $50 \mu\text{M}$ hopeaphenol treatment ($p =$
322 0.01 ; **Figure 9C**). Cells infected with B.1.351 pseudovirus were reduced in the presence of 50
323 μM vatalbinoside A or vaticanol B which were comparable to observations with USA-
324 WA1/2020 pseudovirus (i.e., 41.1 ± 2.6 and $72.3 \pm 7.3\%$ of positive cells in no-drug, infected
325 cultures; **Figure 9C**). In contrast, vatalbinoside A and vaticanol B both inhibited B.1.1.7
326 pseudovirus infection at similar levels to what was observed for hopeaphenol (43.8 ± 3.4 and
327 $37.7 \pm 4.1\%$, respectively; **Figure 9B**).

328 Taken together, these results indicate that hopeaphenol, and to a lesser extent
329 vatalbinoside A and vaticanol B, inhibit both replication of infectious SARS-CoV-2 variants of
330 concern *in vitro* and entry of pseudoviruses containing divergent SARS-CoV-2 spike sequences.
331 In the case of hopeaphenol, its activities against B.1.1.7 and B.1.351 are similar or improved
332 when compared to activities against USA-WA1/2020.

333

334 Discussion

335 Antivirals that act across multiple SARS-CoV-2 variants are needed worldwide to
336 supplement ongoing vaccine efforts, to provide therapeutic options for mild to severe SARS-
337 CoV-2 infections irrespective of hospitalization, and to support COVID-19 treatment in
338 resource-limited regions with poor access to intravenous antibody infusions. Here we
339 investigated a library of pure compounds containing 512 pure natural products and derivatives
340 and identified three stilbenoids, exemplified by (–)-hopeaphenol, that disrupt the interaction of
341 viral spike RBD with its host ACE2 receptor, block viral entry of spike-containing
342 pseudoviruses, and antagonize infectious SARS-CoV-2 replication without cytotoxicity *in vitro*.

343 Importantly, hopeaphenol also inhibits two emerging variants of concern (B.1.1.7 and B.1.351)
344 which have acquired sequence variations that enhance SARS-CoV-2 infectivity and/or promote
345 reduced susceptibility or enhanced escape from neutralizing antibodies. Although the
346 concentrations of hopeaphenol needed to inhibit virus replication are much higher than those of
347 REGN10933 antibody, active concentrations of hopeaphenol do approximate those of small
348 molecule antivirals remdesivir and GC-376. Hopeaphenol and other stilbenoid analogues are
349 therefore promising leads for developing broad-spectrum SARS-CoV-2 entry inhibitors,
350 potentially for use as monotherapies or in combination with antiviral leads against other viral
351 targets; for example to improve efficacy, reduce toxicities, and/or raise genetic barriers to
352 potential viral resistance for clinical-stage nucleoside prodrugs and M^{pro} inhibitors when
353 compared to use as monotherapies (56, 57). These results also support studies to assess activity
354 against additional variants of concern (i.e., B.1.617.2 / “Delta”) as they continue to emerge and
355 spread.

356 (–)-Hopeaphenol, vatalbinoside A, vaticanol B, and related stilbenoids and their
357 stereoisomers have been isolated from a variety of plant sources including *Hopea*, *Vitis*, *Shorea*,
358 *Anisoptera*, and *Vatica* species, among others (29-37). These compounds have been reported to
359 exhibit several *in vitro* properties including antiproliferative (32-33, 37-38), antibacterial
360 (through inhibition of the type III secretion system of Gram-negative bacteria) (30, 39),
361 antifungal (40), anti-influenza and herpes simplex virus (41-42), and anti-inflammatory (34, 43)
362 activities, among others. Notably, hopeaphenol is additionally reported to inhibit plasma
363 triglyceride elevation in olive oil-treated mice and reduce plasma glucose in sucrose-loaded mice
364 at 200 mg/kg (44-45). It also exhibited hepatoprotective effects against LPS-induced liver injury
365 in mice at 100 mg/kg (34). These initial *in vivo* efficacy studies, which indicate tolerability at

366 high concentrations, support near-term *in vivo* studies of anti-SARS-CoV-2 efficacy by (–)-
367 hopeaphenol, although the concentrations of hopeaphenol that might be relevant *in vivo* as
368 compared to the *in vitro* concentrations identified here remain to be determined.

369 More recently, stilbenoids have been proposed as potential disruptors of SARS-CoV-2
370 spike protein with ACE2 in molecular docking studies (46). Another stilbenoid, kobophenol A,
371 was also recently reported to inhibit binding of RBD with ACE2 (IC₅₀ = 1.8 μM) and SARS-
372 CoV-2 replication (EC₅₀ = 71.6 μM) (47), and our data are consistent with these observations.
373 However, we observed no antiviral activity by resveratrol at up to 100 μM, which contrasts with
374 another recent study reporting an EC₅₀ of 10.7 μM in SARS-CoV-2-infected Vero-E6 cells
375 (BetaCov isolate) (48). However, this latter study measured supernatant viral RNA levels by
376 quantitative PCR after 48 hours' infection, and so disparate results could reflect differences in
377 sensitivity between quantitative PCR and assays used here. Another consideration is that the
378 three hit stilbenoid compounds, which are all polyphenolics, represent a structure class that has
379 been given a PAINS (Pan Assay Interference compounds) designation (49). Although we
380 observed that these compounds selectively disrupted RBD/ACE2 binding over an unrelated PD-
381 1/PD-L1 ligand/receptor pair, for example with 257.3-fold selectivity for hopeaphenol, caution
382 must still be taken when considering these compounds for further therapeutic development.
383 However, generation or isolation of stilbenoid analogues with potentially improved selectivity as
384 well as assessment of chemical leads in primary cell models remain warranted.

385 While the three stilbenoids identified here selectively disrupted RBD/ACE2 interactions
386 at sub-micromolar concentrations over an unrelated PD-1/PD-L1 ligand/receptor pair, inhibitory
387 activity against spike-containing pseudoviruses occurred only at 50 μM, although low
388 micromolar activity was generally observed for hopeaphenol and/or vatalbinside A in infectious

389 virus assays. These observations could partially reflect reduced activity against VSV-backbone
390 pseudoviruses in particular and/or reduced stability of these stilbenoids *in vitro*. For example,
391 reduced activity could be associated with assay-dependent structural differences in spike
392 proteins, particularly with regard to pseudovirus assays. However, stilbenoids are also reported
393 to be sensitive to oxidation due to the presence of the phenolic moieties and their ability to
394 delocalize an unpaired electron (50). They are also unstable to factors including oxygen, heat,
395 light, and pH changes (51-52). Consistent with the potential for reduced stability, vaticanol B,
396 while the most potent small molecule disruptor of RBD/ACE2 interactions by AlphaScreen, was
397 less effective than hopeaphenol and vatalbinoside A in both pseudovirus and across infectious
398 virus assays (**Tables 1, 2**). Moreover, vatalbinoside A (and remdesivir) were also less active after
399 7 days culture in virus restoration assays using SARS-CoV-2 variants as opposed to hopeaphenol
400 which maintained activity (**Table 2**). Although more study is required, these observations
401 suggest that hopeaphenol may have improved stability-dependent antiviral activity relative to
402 other investigated stilbenoids and perhaps remdesivir.

403 A recent report also describes use of a virtual screening approach which identified
404 hopeaphenol as a potential inhibitor of SARS-CoV-2 M^{pro} by interacting within its active site
405 (18). In contrast, we observed only weak inhibitory activity of hopeaphenol and analogues
406 against M^{pro} (e.g., IC₅₀s = ~40 – 70 μ M, compared to 0.0052 μ M for GC-376; **Table 1**). While
407 our studies do not rule out modest inhibition of M^{pro} by hopeaphenol, this activity is unlikely to
408 confer the primary antiviral activity observed *in vitro* (e.g., hopeaphenol EC₅₀s = 2.3 – 23.4 μ M
409 in infectious viral assays; **Table 2**). Nevertheless, these combined results do raise the intriguing
410 possibility of identifying stilbenoid derivatives that target both SARS-CoV-2 entry and M^{pro},

411 which in turn may improve antiviral activity and/or reduce the risk of eventual viral drug
412 resistance.

413 There are currently no licensed antivirals that reliably protect against COVID-19 in
414 outpatient settings. Recent reports of SARS-CoV-2 variants with accumulated spike mutations
415 and reduced susceptibility or escape from neutralizing sera from convalescent and vaccine-
416 treated patients (7-8) also raise the concern of emerging variants with resistance to pre-existing
417 immune responses. In contrast, we observe that hopeaphenol, despite acting as an inhibitor of
418 spike-mediated viral entry, inhibits *in vitro* replication of both an early SARS-CoV-2 isolate
419 (USA-WA1/2020) as well as two more recently emerging variants of concern (B.1.1.7 and
420 B.1.351) with comparable or improved activity. These results suggest that spike mutations that
421 promote vaccine-induced viral escape may be distinct from those that might arise from ongoing
422 treatment with hopeaphenol and potentially other stilbenoid-based entry inhibitors. Although
423 further studies are clearly needed, this possibility, in turn, raises the prospect of natural product-
424 based entry inhibitors that function as effective antiviral countermeasures in the absence of
425 available second-generation vaccines or therapies requiring inpatient administration.

426

427 **Materials and Methods**

428 **Chemical libraries and hit compounds**

429 The Davis Open Access Natural Product-Based Library consists of 512 distinct
430 compounds, the majority (53%) of which are natural products obtained primarily from Australian
431 fungal, plant, and marine invertebrate sources (11-12), as well as semi-synthetic natural product
432 analogues (28%) and known commercial drugs or synthetic compounds inspired by natural
433 products (19%). All compounds evaluated in this study were analyzed for purity prior to testing

434 and shown to be > 95% pure. Compounds were initially provided by Compounds Australia at
435 Griffith University in 5 mM stock solutions dissolved in dimethyl sulfoxide (DMSO; Sigma-
436 Aldrich, Sydney, Australia); as such, DMSO was used as the vehicle control in this study. The
437 three hit compounds identified following library screening, which are all known stilbenoids (30),
438 were re-supplied as dry powders for confirmation studies and further biological evaluation.

439

440 **Cells, viruses, and reagents**

441 Vero-E6 cells were obtained from the American Tissue Culture Collection. Vero-E6 cells
442 were cultured in D10+ media [Dulbecco's Modified Eagle Medium with 4.5 g/L glucose and L-
443 glutamine (Gibco, Gaithersburg, MD), 10% fetal bovine serum (Gemini Bio Products, West
444 Sacramento, CA, USA), 100 U of penicillin/mL, and 100 µg of streptomycin/mL (Sigma-
445 Aldrich, St. Louis, MO)] in a humidified incubator at 37 °C and 5% CO₂. BHK-21/WI-2 cells
446 were purchased from Kerafast (Boston, MA, USA) and cultured in D5+ media, which is identical
447 to D10+ media except for addition of 5% fetal bovine serum.

448 The following reagent was deposited by the Centers for Disease Control and Prevention
449 and obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-
450 WA1/2020, NR-52281. The following reagents were obtained through BEI Resources, NIAID,
451 NIH: SARS-Related Coronavirus 2, Isolate hCoV-19/England/204820464/2020, NR-54000,
452 contributed by Bassam Hallis and SARS-Related Coronavirus 2, Isolate hCov-19/South
453 Africa/KRISP-K005325/2020, NR-54009, contributed by Alex Sigal and Tulio de Oliveira.

454 Remdesivir and resveratrol were purchased from Sigma-Aldrich. GC-376 was purchased
455 from Selleckchem (Houston, TX, USA). REGN10933 was obtained from excess aliquot volumes
456 at the Perelman School of Medicine which could not be used for patients. Isolation, structural

457 confirmation, and purity of (-)-hopeaphenol, vatalbinoside A, and vaticanol B used in this study
458 were reported previously (30).

459

460 **Protein-protein interaction assays**

461 SARS-CoV-2 Spike-RBD binding to ACE2 was determined using AlphaScreen
462 technology. 2 nM of ACE2-Fc (Sino Biological, Chesterbrook, PA, USA) was incubated with 5
463 nM HIS-tagged SARS-CoV-2 Spike-RBD (Sino Biological) in the presence of 5 µg/mL nickel
464 chelate donor bead in a total of 10 µL of 20 mM Tris (pH 7.4), 150 mM KCl, and 0.05% CHAPS
465 in white, opaque, low-volume 384-well plates. Test compounds were diluted to 100x final
466 concentration in 100% DMSO. 5 µL of ACE2-Fc/Protein A acceptor bead was first added to the
467 plate, followed by 100 nL of test compounds and then 5 µL of CoV-Spike-RBD-HIS/Nickel
468 chelate donor beads. Test compounds were added to each well using a Janus Nanohead
469 (PerkinElmer, Waltham, MA, USA). For each experiment, all conditions were performed in
470 duplicate. Following 2 h incubation at room temperature, AlphaScreen fluorescent signals were
471 measured using a ClarioStar plate reader (BMG Labtech, Cary, NC, USA). Data were
472 normalized to percent inhibition, where 100% equaled the AlphaScreen signal in the absence of
473 SARS-CoV-2-Spike-RBD-His and 0% equaled AlphaScreen signal in the presence of both
474 proteins and DMSO alone.

475 PD-1 binding to PD-L1 was also determined using AlphaScreen technology. 0.5 nM of
476 human PD-L1-Fc (Sino Biological) was incubated with 5 nM HIS-tagged human PD-1 (Sino
477 Biological) in the presence of 5 µg/mL protein A AlphaScreen acceptor bead and 5 µg/mL nickel
478 chelate donor bead in a total volume of 10 µL of 20 mM HEPES (pH 7.4), 150 mM NaCl, and
479 0.005% Tween in white, opaque low-volume 384-well plates. 5 µL of PD-L1-Fc/protein A

480 acceptor bead was first added to the plate, followed by 100 nL of test compound prepared as
481 described above, followed by 5 μ L of PD-1-His/nickel chelate donor bead. For each experiment,
482 all conditions were performed in duplicate. Following 2 h incubation at room temperature, data
483 were collected as described above and normalized to percent inhibition, where 100% equaled the
484 AlphaScreen signal in the absence of PD-1-His, and 0% equaled AlphaScreen signal in the
485 presence of both proteins and 0.1% DMSO alone.

486

487 **Generation of M^{pro} protein**

488 The codon-optimized gene for SARS-CoV-2 M^{pro} (or 3CL^{pro}) (GenBank: QHD43415.1
489 aa 3264-3567) from strain BetaCoV/Wuhan/WIV04/2019 was ordered from IDT (Coralville, IA,
490 USA) and cloned into a HIS-SUMO expression vector (a modified pET-DUET; Novagen,
491 Madison, WI, USA). After transformation into BL21(DE3), the HIS-SUMO-M^{pro} fusion protein
492 was expressed using the autoinduction method (53) with 500 mL cultures at 22 °C overnight.
493 Cell pellets were resuspended in a buffer containing 25 mM Tris pH 8.5, 20 mM imidazole 200
494 mM NaCl, 5 mM β -mercaptoethanol and lysed using sonication and lysozyme and centrifuged at
495 high speed. The supernatant was applied to a Ni-NTA (nickel-nitrilotriacetic acid) column at 4
496 °C and washed with the resuspension buffer. The fusion protein was then eluted using a buffer
497 300 mM imidazole, 200 mM NaCl and 5 mM β -mercaptoethanol, concentrated and applied to a
498 gel filtration column (HiLoad 26/60 Superdex 75; Cytiva, Marlborough, MA) and equilibrated
499 with the resuspension buffer. Fractions with > 90% purity were pooled and incubated with
500 SUMO protease at 4 °C overnight. After cleavage, the digested protein solution was applied
501 twice to a 5 mL HIS-TRAP Ni-NTA column (Cytiva) to remove the HIS-SUMO and SUMO
502 protease, and the flow-through was collected. Finally, the protein was concentrated and applied

503 to a second gel filtration column (HiLoad 26/60 Superdex 75; Cytiva) equilibrated with 25 mM
504 HEPES pH 7.5, 150 mM NaCl, 2 mM TCEP. Purity (> 95%) was confirmed using an SDS-
505 PAGE gel.

506

507 **M^{pro} enzymatic assays**

508 Protease activity of recombinant M^{pro} was measured using the quenched fluorogenic
509 substrate {DABCYL}-Lys-Thr- Ser-Ala-Val-Leu-Gln-Ser-Gly-Phe-Arg-Lys-Met-Glu-
510 (EDANS)-NH₂ (Bachem, Vista, CA, USA). 5 µL of 25 nM M^{pro} diluted in assay buffer [25 mM
511 HEPES (pH 7.4), 150 mM NaCl, 5 mM DTT, 0.005% Tween) was dispensed into black, low-
512 volume 384-well plates. Test compounds were serially diluted into 100% DMSO, and 0.1 µL
513 was added to the assay using a Janus MDT Nanohead (PerkinElmer). Assays were initiated by
514 addition of 5 µL of 5 µM fluorogenic substrate, and fluorescence at 355 nm excitation and 460
515 nm emission was monitored every 5 minutes for 50 minutes using an Envision plate reader
516 (PerkinElmer). Rate of substrate cleavage was determined using linear regression of the raw data
517 values obtained during the time course. Slopes of these progress curves were then normalized to
518 percent inhibition, where 100% equaled rate in the absence of M^{pro} (which was typically 0), and
519 0% equaled rate of cleavage in the presence of M^{pro} and 0.1% DMSO.

520

521 **Generation of VSVΔG-S-GFP pseudoviruses**

522 SARS-CoV-2 SWE/01/2020 spike cDNA (containing 100% sequence identity to USA-
523 WA1/2020 in the RBD and 99.9% identity overall) was obtained as a gift from Dr. Stephen J.
524 Elledge. B.1.1.7 spike cDNA was generated from SWE/01/2020 spike cDNA by standard PCR
525 mutagenesis, and B.1.351 spike cDNA was synthesized (Genscript, Piscataway, NJ, USA).

526 Pseudoviruses were generated in BHK-21/WI-2 cells using a pseudotyped Δ G-GFP ($G^*\Delta$ G-
527 GFP) rVSV (Kerafast, Boston, MA, USA) in addition to spike sequences cloned into the paT7-
528 Spike plasmid as described previously (21). 2 hours following chemical transfection of spike
529 plasmid, cells were infected with Δ G-GFP ($G^*\Delta$ G-GFP) rVSV. Following 24 hours incubation,
530 supernatants were harvested, aliquoted, and stored at -80°C .

531

532 **Pseudovirus-based infectivity assays**

533 12,500 Vero-E6 cells resuspended in 12.5 μL D10+ were plated in 384 μL plates,
534 followed by addition of 6.25 μL of test agents diluted in D10+ at 4x desired final concentration
535 plus 6.25 μL of undiluted pseudovirus stock (total 25 μL reaction volumes). All experimental
536 conditions with test agents were performed in duplicate, and control cells with or without
537 pseudovirus in the presence of 0.1% DMSO vehicle control were tested 4-fold. Cells were
538 incubated at 37°C and 5% CO_2 for 24 hours. Cells were then stained with 25 μL of 5 $\mu\text{g/mL}$
539 Hoechst 33342 (Sigma Aldrich), incubated for 20 minutes, and fixed with paraformaldehyde to
540 2% final concentration. High content imaging was then performed across 15 non-overlapping
541 images per well using a Nikon Eclipse Ti Inverted Microscope and Nikon NIS Elements AR
542 Software Version 5.30.02 (Nikon Americas Inc., Melville, NY, USA). For each image, cell
543 nuclei and GFP-positive cells were counted, with GFP positive cells reported as percent of total
544 nuclei within each image.

545

546 **Resazurin cell viability assay in uninfected cells**

547 20,000 Vero-E6 cells were plated in 96-well plates and incubated overnight before
548 addition of compounds at defined concentrations. 0.1% DMSO vehicle control was added to

549 wells in the absence of test compounds. All experimental conditions were performed in
550 duplicate. Cells were then incubated at 37 °C and 5% CO₂ for 4 days before addition of resazurin
551 (Sigma Aldrich) to a final concentration of 20 µg/mL. Cells were incubated for an additional 4
552 hours before fluorescence intensity was measured using a ClarioStar plate reader (BMG
553 Labtech). Background fluorescence was subtracted from wells containing resazurin and D10+
554 media but no cells.

555

556 **Generation of SARS-CoV-2 viruses**

557 3 x 10⁶ Vero-E6 cells were incubated in 15 mL of D10+ media for 24 hours. Cells were
558 then washed and replaced with 10 mL of D10+ containing virus at a multiplicity of infection
559 MOI of 0.001. Cells were incubated for 5 – 7 days until clear CPE was observed throughout the
560 flask. Media was harvested and split into 250 µL aliquots for storage at -80 °C.

561 To titer virus stocks, Vero-E6 cells were first plated to 20,000 cells per well in 96-well
562 format in D10+ media and incubated for 24 hours. Following incubation, cells were incubated in
563 fresh D10+ containing 5-fold serial dilutions of a thawed virus aliquot (8 total dilutions, 5-fold
564 replicates) and incubated for an additional 4 days. Wells were then scored for the presence of
565 CPE. TCID₅₀s were calculated using the Reed-Muench method.

566

567 **Viral CPE scoring-based assays**

568 Vero-E6 cells were plated in D10+ to 20,000 cells per well in 96-well format and
569 incubated for 24 hours. Following incubation, compounds were added to final concentrations in
570 8-fold replicates and incubated for an additional 2 hours before addition of 50x TCID₅₀ of virus.
571 Each 96-well plate further contained uninfected cells and infected cells with 0.1% DMSO

572 vehicle control in 4-fold replicates. Cells were incubated for an additional 4 days, at which point
573 all wells were scored for the presence of CPE by a user blinded to the identity of the wells.

574

575 **Viral CPE quantitative assay (i.e., cell viability restoration)**

576 Assays were performed as described for viral CPE scoring-based assays but with addition
577 of 150x TCID₅₀ of virus at 2 hours following compound addition. Cells were then incubated for
578 an additional 4-7 days, at which point cells were treated with resazurin for 4 hours, fixed with
579 paraformaldehyde to a final concentration of 4%, and incubated at room temperature for at least
580 30 minutes to inactivate virus. Fluorescence intensity was then measured using a ClarioStar plate
581 reader. Background fluorescence was subtracted from wells containing resazurin and D10+
582 media but no cells.

583

584 **Virus yield reduction assays**

585 Vero-E6 cells were plated in D10+ to 5,000 cells per well in 384-well format and
586 incubated for 24 hours. Following incubation, supernatants from 4 of 8 replicates from viral CPE
587 scoring based-assay experimental conditions were combined and added to cells at 1:10 final
588 concentration dilutions. Each 384-well plate further contained supernatant dilutions from
589 uninfected cells and infected cells with 0.1% DMSO vehicle control. Cells were incubated for 48
590 hours and then fixed in a final concentration of 4% paraformaldehyde for at least 30 minutes to
591 inactivate virus. Immunostaining was then performed using primary anti-SARS-CoV-2
592 nucleocapsid primary antibody (HL344; GeneTex, Irvine, CA, USA) at 1:1000 concentration and
593 goat anti-rabbit IgG Alexa Fluor 555 secondary antibody at 1:2000 concentration (ThermoFisher,
594 Waltham, MA, USA). Cells were also counterstained with 1 µg/mL Hoechst. High content

595 imaging was performed across 9 non-overlapping images per well using a Nikon Eclipse Ti

596 Inverted Microscope as described above.

597

598 **Data analysis**

599 For all studies, 50% effective concentrations were calculated using nonlinear regression

600 of a one-side binding model using GraphPad Prism v. 8.4.3 (GraphPad, San Diego, CA, USA).

601 All data are presented as the mean $\pm$ s.e.m. from at least 3 independent experiments, with the

602 exception of data from virus yield reduction assays, which are presented as the mean $\pm$ S.D. from

603 2 independent experiments. Statistical significance was determined using the ratio paired t test

604 function in GraphPad Prism v. 8.4.3, with a p value < 0.05 considered significant.

605

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616 (<https://www.griffith.edu.au/institute-drug-discovery/unique-resources/naturebank>) from which

617 the majority of compounds found within the Davis Open Access Natural Product-based Library

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References

1. Xiu S, Dick A, Ju H, Mirzaie S, Abdi F, Cocklin S, Zhan P, Liu X. 2020. Inhibitors of SARS-CoV-2 entry: Current and future opportunities. *J Med Chem* 63:12256-12274.
2. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 181:271-280.
3. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367:1260-1263.
4. Hou YJ, Chiba S, Halfmann P, Here C, Kuroda M, Dinnon KH 3rd, Leist SR, Schäfer A, Nakajima N, Takahashi K, Lee RE, Mascenik TM, Graham R, Edwards CE, Tse LV, Okuda K, Markmann AJ, Bartlt L, de Silva A, Margolis DM, Boucher RC, Randell SH, Suzuki T, Gralinski LE, Kawaoka Y, Baric RS. 2020. SARS-CoV-2 D614G variant exhibits efficient replication *ex vivo* and transmission *in vivo*. *Science* 370:1464-1468.

641

642 5. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, Wang M, Yu J, Zhang B, Kwong PD,
643 Graham BS, Mascola JR, Chang JY, Yin MT, Sobieszczyk M, Kyratsous CA, Shapiro L, Sheng
644 Z, Huang Y, Ho DD. 2021. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7.
645 Nature doi: 10.1038/s41586-021-03398-2.

646

647 6. Supasa P, Zhou D, Dejnirattisai W, Liu C, Mentzer AJ, Ginn HM, Zhao Y, Duyvesteyn HME,
648 Nutalai R, Tuekprakhon A, Wang B, Paesen GC, Slon-Campos J, López-Camacho C, Hallis B,
649 Coombes N, Bewley KR, Charlton S, Walter TS, Barnes E, Dunachie SJ, Skelly D, Lumley SF,
650 Baker N, Shaik I, Humphries HE, Godwin K, Gent N, Sienkiewicz A, Dold C, Levin R, Dong T,
651 Pollard AJ, Knight JC, Klenerman P, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman A,
652 Bittaye M, Belij-Rammerstorfer S, Gilbert S, Hall DR, Williams MA, Paterson NG, James W,
653 Carroll MW, Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Srean GR. 2021. Reduced
654 neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. Cell doi:
655 10.1016/j.cell.2021.02.033.

656

657 7. Li Q, Nie J, Wu J, Zhang L, Ding R, Wang H, Zhang Y, Li T, Liu S, Zhang M, Zhao C, Liu H,
658 Nie L, Qin H, Wang M, Lu Q, Li X, Liu J, Liang H, Shi Y, Shen Y, Xie L, Zhang L, Qu X, Xu
659 W, Huang W, Wang Y. 2021. SARS-CoV-2 501Y.V2 variants lack higher infectivity but do
660 have immune escape. Cell doi: 10.1016/j.cell.2021.02.042.

661

662 8. Zhou D, Dejnirattisai W, Supasa P, Liu C, Mentzer AJ, Ginn HM, Zhao Y, Duyvesteyn HME,
663 Tuekprakhon A, Nutalai R, Wang B, Paesen GC, Lopez-Camacho C, Slon-Campos J, Hallis B,

- 664 Coombes N, Bewley K, Charlton S, Walter TS, Skelly D, Lumley SF, Dold C, Levin R, Dong T,
665 Pollard AJ, Knight JC, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman A, Bittaye M, Belij-
666 Rammerstorfer S, Gilbert S, James W, Carroll MW, Klenerman P, Barnes E, Dunachie SJ, Fry
667 EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR. 2021. Evidence of escape of SARS-CoV-2
668 variant B.1.351 from natural and vaccine-induced sera. Cell doi: 10.1016/j.cell.2021.02.037.
669
- 670 9. Islam MT, Sarkar C, El-Kersh DM, Jamaddar S, Uddin SJ, Shilpi JA, Mubarak MS. 2020.
671 Natural products and their derivatives against coronavirus: A review of the non-clinical and pre-
672 clinical data. *Phytother Res* 34:2471-2492.
673
- 674 10. Vougiotiannopoulou K, Corona A, Tramontano E, Alexis MN, Skaltsounis AL. 2021.
675 Natural and nature-derived products targeting human coronaviruses. *Molecules* 26:448.
676
- 677 11. Zulfiqar B, Jones AJ, Sykes ML, Shelper TB, Davis RA, Avery VM. 2017. Screening a
678 natural product-based library against kinetoplastid parasites. *Molecules* 22:1715.
679
- 680 12. Kuman R, Bidgood CL, Levrier C, Gunter JH, Nelson CC, Sadowski MC, Davis RA. 2020.
681 Synthesis of a unique psammaphysin F library and functional evaluation in prostate cancer cells
682 by multiparametric quantitative single cell imaging. *J Nat Prod* 83:2357-2366.
683
- 684 13. Yasgar A, Jadhav A, Simeonov A, Coussens NP. 2016. AlphaScreen-based assays: Ultra-
685 high-throughput screening for small-molecule inhibitors of challenging enzymes and protein-
686 protein interactions. *Methods Mol Biol* 1439:77-98.

687

688 14. Bray MA, Carpenter A. 2017. Advanced assay development guidelines for image-based high
689 content screening and analysis. In: Markossian S, Sittampalam GS, Grossman A, Brimacombe
690 K, Arkin M, Auld D, Austin CP, Baell J, Caaveiro JMM, Chung TDY, Coussens NP, Dahlin JL,
691 Devanaryan V, Foley TL, Glicksman M, Hall MD, Haas JV, Hoare SRJ, Inglese J, Iversen PW,
692 Kahl SD, Kales SC, Kirshner S, Lal-Nag M, Li Z, McGee J, McManus O, Riss T, Saradjian P,
693 Trask OJ Jr, Weidner JR, Wildey MJ, Xia M, Xu X, (eds), Assay Guidance Manual [Internet].
694 Bethesda (MD). Eli Lilly & Company and the National Center for Advancing Translational
695 Sciences.

696

697 15. Goufo P, Singh RK, Cortez I. 2020. A reference list of phenolic compounds (including
698 stilbenes) in grapevine (*Vitis vinifera* L.) roots, woods, canes, stems, and leaves. Antioxidants
699 (Basel) 9:398.

700

701 16. Lung J, Hung MS, Lin YC, Hung CH, Chen CC, Lee KD, Tsai YH. 2020. Virtual screening
702 and in vitro evaluation of PD-1 dimer stabilizers for uncoupling PD-1/PD-L1 interaction from
703 natural products. Molecules 25:5293.

704

705 17. Skalniak L, Zak KM, Guzik K, Magiera K, Musielak B, Pachota M, Szelazek B, Kocik J,
706 Grudnik P, Tomala M, Krzanik S, Pyrc K, Dömling A, Dubin G, Holak TA. 2017. Small-
707 molecule inhibitors of PD-1/PD-L1 immune checkpoint alleviate the PD-L1-induced exhaustion
708 of T cells. Oncotarget 8:72167-72181.

709

- 710 18. Sharma P, Vijayan V, Pant P, Sharma M, Vikram N, Kaur P, Singh TP, Sharma S. 2020.
711 Identification of potential drug candidates to combat COVID-19: A structural study using the
712 main protease (Mpro) of SARS-CoV-2. J Biomol Struct Dyn doi:
713 10.1080/07391102.2020.1798286.
714
- 715 19. Ma C, Sacco MD, Hurst B, Townsend JA, Hu Y, Szeto T, Zhang X, Tarbet B, Marty MT,
716 Chen Y, Wang J. 2020. Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2
717 viral replication by targeting the main protease. Cell Res 30:678-692.
718
- 719 20. Fu L, Ye F, Feng Y, Yu F, Wang Q, Wu Y, Zhao C, Sun H, Huang B, Niu P, Song H, Shi Y,
720 Li X, Tan W, Qi J, Gao GF. 2020. Both boceprevir and GC376 efficaciously inhibit SARS-CoV-
721 2 by targeting its main protease. Nat Commun 11:4417.
722
- 723 21. Whitt MA. 2010. Generation of VSV pseudotypes using recombinant Δ G-VSV for studies on
724 virus entry, identification of entry inhibitors, and immune responses to vaccines. J Virol Methods
725 169:365-374.
726
- 727 22. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL,
728 Luzuriaga K, Greenough TC, Choe H, Farzan M. 2003. Angiotensin-converting enzyme 2 is a
729 functional receptor for the SARS coronavirus. Nature 426:450-454.
730
- 731 23. Conceicao C, Thakur N, Human S, Kelly JT, Logan L, Bialy D, Bhat S, Stevenson-Leggett
732 P, Zagrajek AK, Hollinghurst P, Varga M, Tsirigoti C, Tully M, Chiu C, Moffat K, Silesian AP,

- 733 Hammond JA, Maier HJ, Bickerton E, Shelton H, Dietrich I, Graham SC, Bailey D. 2020. The
734 SARS-CoV-2 Spike protein has a broad tropism for mammalian ACE2 proteins. *PLoS Biol*
735 18:e3001016.
736
- 737 24. Ren X, Glende J, Al-Falah M, de Vries V, Schwegmann-Wessels C, Qu X, Tan L, Tschernig
738 T, Deng H, Naim HY, Herrler G. 2006. Analysis of ACE2 in polarized epithelial cells: surface
739 expression and function as receptor for severe acute respiratory syndrome-associated
740 coronavirus. *J Gen Virol* 87(Pt 6):1691-1695.
741
- 742 25. Case JB, Bailey AL, Kim AS, Chen RE, Diamond MS. 2020. Growth, detection,
743 quantification, and inactivation of SARS-CoV-2. *Virology* 548:39-48.
744
- 745 26. Gorshkov K, Chen CZ, Bostwick R, Rasmussen L, Xu M, Pradhan M, Tran BN, Zhu W,
746 Shamim K, Huang W, Hu X, Shen M, Klumpp-Thomas C, Itkin Z, Shinn P, Simeonov A,
747 Michael S, Hall MD, Lo DC, Zheng W. 2020. The SARS-CoV-2 cytopathic effect is blocked
748 with autophagy modulators. *ACS Infect Dis* doi: 10.1021/acsinfecdis.0c00349.
749
- 750 27. Pruijssers AJ, George AS, Schäfer A, Leist SR, Gralinski LE, Dinnon KH 3rd, Yount BL,
751 Agostini ML, Stevens LJ, Chappell JD, Lu X, Hughes TM, Gully K, Martinez DR, Brown AJ,
752 Graham RL, Perry JK, Du Pont V, Pitts J, Ma B, Babusis D, Murakami E, Feng JY, Bilello JP,
753 Porter DP, Cihlar T, Baric RS, Denison MR, Sheahan TP. 2020. Remdesivir inhibits SARS-
754 CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA
755 polymerase in mice. *Cell Rep* 32:107940.

756

757 28. Vuong W, Khan MB, Fischer C, Arutyunova E, Lamer T, Shields J, Saffran HA, McKay RT,
758 van Belkum MJ, Joyce MA, Young HS, Tyrrell DL, Vederas JC, Lemieux MJ. 2020. Feline
759 coronavirus drug inhibits the main protease of SARS-CoV-2 and blocks virus replication. Nat
760 Commun 11:4282.

761

762 29. Tanaka T, Ito T, Ido Y, Son TK, Nakaya K, Iinuma M, Ohyama M, Chelladurai V. 2000.
763 Stilbenoids in the stem bark of *Hopea parviflora*. Phytochemistry 53:1015-1019.

764

765 30. Davis RA, Beattie KD, Xu M, Yang X, Yin S, Holla H, Healy PC, Sykes M, Shelper T,
766 Avery VM, Elofsson M, Sundin C, Quinn RJ. 2014. Solving the supply of resveratrol tetramers
767 from Papua New Guinean rainforest *Anisoptera* species that inhibit bacterial type III secretion
768 systems. J Nat Prod 77:2633-2640.

769

770 31. Lin YS, Chen CR, Wu WH, Wen CL, Chang CI, Hou WC. 2015. Anti- α -glucosidase and
771 anti-dipeptidyl peptidase IV activities of extracts and purified compounds from *Vitis thunbergia*
772 var. *taiwaniana*. J Agric Food Chem 63:6393-6401.

773

774 32. Moriyama H, Moriyama M, Ninomiya K, Morikawa T, Hayakawa T. 2016. Inhibitory effects
775 of oligostilbenoids from the bark of *Shorea roxburghii* on malignant melanoma cell growth:
776 Implications for novel topical anticancer candidates. Biol Pharm Bull 39:1675-1682.

777

- 778 33. Chang CI, Chien WC, Huang KX, Hsu JL. 2017. Anti-inflammatory effects of vitisinol A
779 and four other oligostilbenes from *Ampelopsis brevipedunculata* var. *Hancei*. *Molecules*
780 22:1195.
781
- 782 34. Ninomiya K, Chaipech S, Kunikata Y, Yagi R, Pongpiriyadacha Y, Muraoka O, Morikawa
783 T. 2017. Quantitative determination of stilbenoids and dihydroisocoumarins in *Shorea*
784 *roxburghii* and evaluation of their hepatoprotective activity. *Int J Mol Sci* 18:451.
785
- 786 35. Schrader KK, Ibrahim MA, Abd-Alla HI, Cantrell CL, Pasco DS. 2018. Antibacterial
787 activities of metabolites from *Vitis rotundifolia* (Muscadine) roots against fish pathogenic
788 bacteria. *Molecules* 23:2761.
789
- 790 36. Prabha B, Sini S, Priyadarshini TS, Sasikumar P, Gopalan G, Joseph JP, Jithin MM, Sivan
791 VV, Jayamurthy P, Radhakrishnan KV. 2019. Anti-inflammatory effect and mechanism of action
792 of ellagic acid-3,3',4-trimethoxy-4'-O- α -L-rhamnopyranoside isolated from *Hopea parviflora* in
793 lipopolysaccharide-stimulated RAW 264.7 macrophages. *Nat Prod Res* doi:
794 10.1080/14786419.2019.1690486.
795
- 796 37. Aja I, Ruiz-Larrea MB, Courtois A, Krisa S, Richard T, Ruiz-Sanz JI. 2020. Screening of
797 natural stilbene oligomers from *Vitis vinifera* for anticancer activity on human hepatocellular
798 carcinoma cells. *Antioxidants (Basel)* 9:469.
799

- 800 38. Ohyama M, Tanaka T, Ito T, Iinuma M, Bastow KF, Lee KH. 1999. Antitumor agents 200.
801 Cytotoxicity of naturally occurring resveratrol oligomers and their acetate derivatives. Bioorg
802 Med Chem Lett 9:3057-3060.
803
- 804 39. Zetterström CE, Hasselgren J, Salin O, Davis RA, Quinn RJ, Sundin C, Elofsson M. 2013.
805 The resveratrol tetramer (-)-hopeaphenol inhibits type III secretion in the gram-negative
806 pathogens *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. PLoS One 8:e81969.
807
- 808 40. Schnee S, Queiroz EF, Voinesco F, Marcourt L, Dubuis PH, Wolfender JL, Gindro K. 2013.
809 *Vitis vinifera* canes, a new source of antifungal compounds against *Plasmopara viticola*,
810 *Erysiphe necator*, and *Botrytis cinera*. J Agric Food Chem 61:5459-5467.
811
- 812 41. Ito T, Hayashi K, Nishiguchi M, Hayashi T, Iinuma M. 2018. Resveratrol oligomer C-
813 glucosides and anti-viral resveratrol tetramers isolated from the stem bark of *Shorea uliginosa*.
814 Phytochem Lett 28:1-7.
815
- 816 42. Mattio LM, Catinella G, Pinto A, Dallavalle S. 2020. Natural and nature-inspired stilbenoids
817 as antiviral agents. Eur J Med Chem 202:112541.
818
- 819 43. Tabata Y, Takano K, Ito T, Iinuma M, Yoshimoto T, Miura H, Kitao Y, Ogawa S, Hori O.
820 2007. Vaticanol B, a resveratrol tetramer, regulates endoplasmic reticulum stress and
821 inflammation. Am J Physiol Cell Physiol 293:C411-418.
822

- 823 44. Morikawa T, Chaipech S, Matsuda H, Hamao M, Umeda Y, Sato H, Tamura H, Ninomiya K,
824 Yoshikawa M, Pongpiriyadacha Y, Hayakawa T, Muraoka O. 2012. Anti-hyperlipidemic
825 constituents from the bark of *Shorea roxburghii*. J Nat Med 66:516-524.
826
- 827 45. Morikawa T, Chaipech S, Matsuda H, Hamao M, Umeda Y, Sato H, Tamura H, Kon'I H,
828 Ninomiya K, Yoshikawa M, Pongpiriyadacha Y, Hayakawa T, Muraoka O. 2012.
829 Antidiabetogenic oligostilbenoids and 3-ethyl-4-phenyl-3,4-dihydroisocoumarins from the bark
830 of *Shorea roxburghii*. Bioorg Med Chem 20:832-840.
831
- 832 46. Wahedi HM, Ahmad S, Abbasi SW. 2020. Stilbene-based natural compounds as promising
833 drug candidates against COVID-19. J Biomol Struct Dyn doi: 10.1080/07391102.2020.1762743.
834
- 835 47. Gangadevi S, Badavath VN, Thakur A, Yin N, De Jonghe S, Acevedo O, Jochmans D,
836 Leyssen P, Wang K, Neyts J, Yujie T, Blum G. 2021. Kobophenol A inhibits binding of host
837 ACE2 receptor with spike RBD domain of SARS-CoV-2, a lead compound for blocking
838 COVID-19. J Phys Chem Lett 12:1793-1802.
839
- 840 48. Pasquereau S, Nehme Z, Ahmad SH, Daouad F, Van Assche JV, Wallet C, Schwartz C, Rohr
841 O, Morot-Bizot S, Herbein G. 2021. Resveratrol inhibits HCoV-229E and SARS-CoV-2
842 coronavirus replication *in vitro*. Viruses 13:354.
843
- 844 49. Baell JB. 2016. Feeling Nature's PAINS: Natural products, natural product drugs, and pan
845 assay interference compounds (PAINS). J Nat Prod 79:616-628.

846

847 50. Lü JM, Lin PH, Yao Q, Chen C. 2010. Chemical and molecular mechanisms of antioxidants:
848 Experimental approaches and model systems. *J Cell Mol Med* 14:840-860.

849

850 51. D'Archivio M, Filesi C, Vari R, Scazzocchio B, Masella R. 2010. Bioavailability of the
851 polyphenols: Status and controversies. *Int J Mol Sci* 11:1321-1342.

852

853 52. Marín L, Miguélez EM, Villar CJ, Lombó F. 2015. Bioavailability of dietary polyphenols
854 and gut microbiota metabolism: Antimicrobial properties. *Biomed Res Int* 2015:905215.

855

856 53. Studier FW. 2005. Protein production by auto-induction in high density shaking cultures.
857 *Protein Expr Purif* 41:207-234.

858

859 54. Starr TN, Greaney AJ, Addetia A, Hannon WW, Choudhary MC, Dingens AS, Li JZ, Bloom
860 JD. 2021. Prospective mapping of viral mutations that escape antibodies used to treat COVID-
861 19. *Science* 371:850-854.

862

863 55. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, Want M, Yu J, Zhang B, Kwong PD,
864 Graham BS, Mascola JR, Chang JY, Yin MT, Sobieszczyk M, Kyratsous CA, Shapiro L, Sheng
865 Z, Huang Y, Ho DD. 2021. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7.
866 *Nature* 593:130-135.

867

- 868 56. Painter GR, Natchus MG, Cohen O, Holman W, Painter WP. 2021. Developing a direct
869 acting, orally available antiviral agent in a pandemic: The evolution of molnupiravir as a
870 potential treatment for COVID-19. *Curr Opin Virol* 50:17-22.
871
- 872 57. Boras B, Jones RM, Anson BJ, Arenson D, Aschenbrenner L, Bakowski MA, Beutler N,
873 Binder J, Chen E, Eng H, Hammond H, Hammond J, Haupt RE, Hoffman R, Kadar EP, Kania R,
874 Kimoto E, Kirkpatrick MG, Lanyon L, Lendy EK, Lillis JR, Logue J, Luthra SA, Ma C, Mason
875 SW, McGrath ME, Noell S, Obach RS, O'Brien MN, O'Connor R, Ogilvie K, Owen D,
876 Pettersson M, Reese MR, Rogers TF, Rossulek MI, Sathish JG, Shirai N, Steppan C, Ticehurst
877 M, Updyke LW, Weston S, Zhu Y, Wang J, Chatterjee AK, Mesecar AD, Frieman MB,
878 Anderson AS, Allerton C. 2021. Discovery of a novel inhibitor of coronavirus 3CL protease for
879 the potential treatment of COVID-19. *bioRxiv* doi: 10.1101/2020.09.12.293498.
880

881 **Figure Legends**

882

883 **Figure 1.** Chemical structures of (–)-hopeaphenol (**A**), vatalbinoside A (**B**), vaticanol B (**C**), and
884 resveratrol (**D**).

885

886 **Figure 2.** Identification of stilbenoids as Spike-ACE2 inhibitors by AlphaScreen. **A**,
887 Demonstration of AlphaScreen-based luminescence due to interactions of His-tagged Spike RBD
888 (from USA-WA1/2020) and Fc-tagged ACE2 peptides. **B**, Dose-response curve of REGN10933
889 on luminescence due to disruption of RBD/ACE2 interactions. **C**, Dose-response curves of
890 stilbenoids and resveratrol on luminescence inhibition due to disruption of RBD/ACE2
891 interactions. **D**, Demonstration of AlphaScreen-based luminescence due to interactions of His-
892 tagged PD-1 and Fc-tagged PD-L1 peptides. **E**, Dose-response curves of stilbenoids and control
893 inhibitor BMS-1166 on luminescence inhibition due to disruption of PD-1/PD-L1 interactions.
894 Results in **B**, **C**, and **E**, denote the mean $\pm$ s.e.m. from at least 3 independent experiments.

895

896 **Figure 3.** Effects of stilbenoids on inhibition of SARS-CoV-2 M^{pro} activity. **A**, Demonstration of
897 recombinant M^{pro} enzymatic activity on a FRET-based fluorogenic peptide substrate. **B**, Dose-
898 response curves of stilbenoids and control inhibitor GC-376 on M^{pro} enzymatic activity. Results
899 in **B** denote the mean $\pm$ s.e.m. from at least 3 independent experiments.

900

901 **Figure 4.** Effects of stilbenoids on *in vitro* pseudovirus entry. **A**, Representative images of
902 uninfected Vero-E6 cells (left) or cells infected with VSVΔG-S-GFP pseudovirus containing
903 SARS-CoV-2 Spike (USA-WA1/2020) in the absence (center) or presence of 50 μ M

904 hopeaphenol (right). Blue denotes Hoechst-stained cell nuclei, and green indicates GFP-positive
905 infected cells. Scale bar = 100 μ m. **B**, Average number of total cell nuclei per cell field as
906 counted by high-content imaging. **C**, Percent of GFP-positive (i.e., pseudovirus-infected) cells as
907 measured by high-content imaging in the presence of stilbenoids. Results in **B** and **C** denote the
908 mean $\pm$ s.e.m. from 3 independent experiments. *, $p < 0.05$; ratio paired t test.

909
910 **Figure 5.** Effects of stilbenoids on infectious SARS-CoV-2 replication *in vitro* using a CPE
911 scoring-based assay. **A**, Representative images of Vero-E6 cells infected with 50x TCID₅₀ of
912 SARS-CoV-2 (USA-WA1/2020 variant) at 0 to 4 days post-infection. Arrows denote examples
913 of CPE. **B**, Representative images of infected cells in the presence of REGN10933 at stated
914 concentrations. **C**, Dose-response curve of REGN10933 on viral replication in Vero-E6 cells
915 after 4 days infection. **D**, Dose-response curve of REGN10933 on cell viability in uninfected
916 Vero-E6 cells after 4 days incubation. **E**, Representative images of infected cells in the presence
917 of remdesivir (top) and hopeaphenol (bottom) after 4 days incubation at stated concentrations. **F**,
918 Dose-response curves of stilbenoids, resveratrol, and remdesivir and GC-376 controls on viral
919 replication in Vero-E6 cells after 4 days infection. **G**, Dose-response curves of compounds on
920 cell viability in uninfected Vero-E6 after 4 days incubation. In **A**, **B**, and **E**, scale bars = 100 μ m.
921 Results in **C**, **D**, **F**, and **G** denote the mean $\pm$ s.e.m. from at least 3 independent experiments.

922
923 **Figure 6.** Quantitative effects of stilbenoids on infectious SARS-CoV-2 replication *in vitro*. **A**,
924 Dose-response curve of REGN10933 on cell viability in infected Vero-E6 cells after 4 days
925 infection with 150x TCID₅₀ of SARS-CoV-2 (USA-WA1/2020 variant). **B**, Dose response curve
926 of stilbenoids, resveratrol, and remdesivir on cell viability in infected Vero-E6 cells. Lower gray

927 dotted lines in **A** and **B** indicate infected cell viability plus 0.1% DMSO vehicle control. Results
928 in **A** and **B** represent the mean $\pm$ s.e.m. from 3 independent experiments. **C**, Representative
929 images of Vero-E6 cells treated with 1:10 dilutions of supernatants from CPE scoring-based
930 assays. Blue denotes Hoechst-stained cell nuclei, and red indicates SARS-CoV-2 nucleocapsid-
931 positive infected cells. Scale bar = 50 μ m. **D**, Dose-response curve of REGN10933 on
932 nucleocapsid-positive cells following supernatant treatments from CPE-scoring based assays. **E**,
933 Dose-response curves of stilbenoids and remdesivir on nucleocapsid-positive cells following
934 supernatant treatments from CPE-scoring based assays. Dotted lines in **D** and **E** denote percent
935 nucleocapsid-positive cells following treatment with infected supernatants plus 0.1% DMSO.
936 Results in **D** and **E** represent the mean $\pm$ S.D. from two independent experiments.

937 **Figure 7.** Effects of hopeaphenol and remdesivir on SARS-CoV-2 variant replication *in vitro*
938 using a CPE scoring-based assay. **A**, Representative images of Vero-E6 cells following 4 days
939 infection with either SARS-CoV-2 variants B.1.1.7 (left) or B.1.351 (right) in the presence of
940 0.1% DMSO vehicle control (top), 0.7 nM REGN10933 or 3 μ M remdesivir (middle), or 15 μ M
941 hopeaphenol (bottom). Scale bar = 100 μ m. **B-C**, Dose-response curves of REGN10933 in Vero-
942 E6 cells following 4 days infection with SARS-CoV-2 variant B.1.1.7 (**B**) or B.1.351 (**C**). **D-E**,
943 Dose-response curves of stilbenoids and remdesivir in Vero-E6 cells following 4 days infection
944 with SARS-CoV-2 variant B.1.1.7 (**D**) or B.1.351 (**E**). In **B** and **C**, data are presented relative to
945 infected cells treated with 0.1% DMSO vehicle control. Results in **B-E** denote the mean $\pm$ s.e.m.
946 from at least 3 independent experiments.

947

948 **Figure 8.** Quantitative effects of stilbenoids on infectious SARS-CoV-2 variant replication *in*
949 *vitro*. **A-B**, Dose-response curve of REGN10933 on cell viability in infected Vero-E6 cells after

950 4 days infection with 150x TCID₅₀ of SARS-CoV-2 variant B.1.1.7 (**A**) or B.1.351 (**B**). **C-D**,
951 Dose response curves of stilbenoids and remdesivir on cell viability in infected Vero-E6 cells
952 after 4 days infection with B.1.1.7 (**C**) or B.1.351 (**D**). Lower gray dotted lines in **A-D** indicate
953 infected cell viability plus 0.1% DMSO vehicle control. Results in **A-D** denote the mean $\pm$ s.e.m.
954 from at least 3 independent experiments. **E**, Representative images of Vero-E6 cells treated with
955 1:10 dilutions of supernatants from CPE scoring-based assays. Results are presented as described
956 in **Figure 6C**. Only single nucleocapsid positive cells were detected in the presence of 50 μ M
957 hopeaphenol. **F-G**, Dose-response curve of REGN10933 on nucleocapsid-positive cells
958 following treatment from supernatants infected with B.1.1.7 (**F**) or B.1.351 (**G**). **H-I**, Dose-
959 response curves of hopeaphenol on nucleocapsid-positive cells following treatment from
960 supernatants infected with B.1.1.7 (**H**) or B.1.351 (**I**). Dotted lines in **F-I** denote percent
961 nucleocapsid-positive cells following treatment with infected supernatants plus 0.1% DMSO.
962 Results in **F-I** represent the mean $\pm$ S.D. from two independent experiments.
963
964 **Figure 9.** Effects of hopeaphenol on entry of pseudoviruses containing SARS-CoV-2 spike
965 variants. **A**, Representative images of Vero-E6 cells infected with VSV Δ G-S-GFP pseudovirus
966 containing SARS-CoV-2 Spike from B.1.1.7 (left) or B.1.351 variants (right) in the presence of
967 0.1% DMSO (top) or 50 μ M hopeaphenol (bottom). Images are organized as described in Figure
968 4. **B-C**, Percent B.1.1.7 (**B**) or B.1.351 (**C**) pseudovirus-infected cells in the presence of 0.1%
969 DMSO or 50 μ M stilbenoids. Results in **B-C** represent the mean $\pm$ s.e.m. from at least 3
970 independent experiments. *, $p < 0.05$; ratio paired t test.

971 **Table 1.** Summary of total stilbenoid and control compound bioactivities in AlphaScreen and
 972 pseudovirus assays.

Compound	IC50				% infected cells at 50 μ M		
	Spike/ACE2	PD-1/PD-L1	Selectivity	Mpro	VSV Δ G-S-GFP pseudovirus		
	AlphaScreen	AlphaScreen	Index	Activity	WA1/2020	B.1.1.7	B.1.351
Hopeaphenol	0.11 μ M	28.3 μ M	257.3	42.5 μ M	28.2%	34.1%	20.4%
Vatالبinoside A	0.24 μ M	22.3 μ M	92.9	68.7 μ M	40.4%	43.8%	41.1%
Vaticanol B	0.067 μ M	16.6 μ M	247.8	47.6 μ M	83.7%	37.7%	72.3%
Resveratrol	> 100 μ M				96.3%		
REGN10933	0.18 nM						
BMS-1166	> 100 μ M	0.0040 μ M					
GC-376				0.0052 μ M			

973
 974

975 **Table 2.** Summary of total stilbenoid and control compound bioactivities in infectious SARS-
976 CoV-2 assays.

Compound	EC50			CC50	EC50			EC50		
	CPE scoring-based assay			Cell	Viability restoration in infected cells			Yield reduction assay		
	WA1/2020	B.1.1.7	B.1.351	Viability	WA1/2020	B.1.1.7	B.1.351	WA1/2020	B.1.1.7	B.1.351
Hopeaphenol	10.2 μ M	14.8 μ M	2.3 μ M	> 100 μ M	23.4 μ M	7.8 μ M	7.5 μ M	13.5 μ M	11.4 μ M	8.8 μ M
Vatambinoside A	13.8 μ M	4.0 μ M	3.6 μ M	> 100 μ M	18.8 μ M	> 50 μ M	11.3 μ M	19.1 μ M		
Vatambinol B	37.0 μ M	32.4 μ M	25.3 μ M	> 100 μ M	> 50 μ M	> 50 μ M	> 50 μ M	> 50 μ M		
Resveratrol	> 100 μ M			~ 100 μ M	> 50 μ M					
REGN10933	0.16 nM	0.013 nM	9.2 nM		0.25 nM	0.10 nM	0.97 nM	0.17 nM	0.011 nM	2.2 nM
Remdesivir	2.5 μ M	1.5 μ M	1.4 μ M	> 10 μ M	7.3 μ M	> 10 μ M	> 10 μ M	1.0 μ M		
GC-376	3.9 μ M			> 10 μ M						

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Figure 1

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