Supplemental Materials

Figure S1. (Related to Figure 1) Purification of PLpro in bacteria and baculovirus.
A. Purification of His-TEV-PLpro from bacteria. Lanes 11-13 contain monomer of PLpro from the last step of the purification on a gel filtration column and these were pooled.
B. Purification of Flag-His-PLpro from baculovirus. Lane 2-4 contain monomer of PLpro from a gel filtration column and were pooled. The tag remains intact with protein.

Figure S2. (Related to Figure 2) Optimalization of the FRET assay for PLpro.
A. PLpro was able to cleave substrate Pro2 (nsp2/3) but not Pro1 (nsp1/2) (see Material & Methods)
B. PLpro was able to cleave substrate Pro3 (12 amino acids) more efficiently than Pro2 (10 amino acids).
C. Comparison between the TEV-tagged and Sumo-tagged versions of PLpro enzyme activities.
D. Comparison between the baculovirus and bacterial (TEV-tagged) purified PLpro.

Figure S3. (Related to Figure 4) Gel-based assay.
A. Purification of the substrate (67 kDa) with Superdex S200. Fractions 14-18 were pooled and used for the gel-based assay.
B. Dihydrotanshione I, beta-lapachone, cryptotanshinone, tanshinone IIA, Cdk4 inhibitor III, GLR-0617, and Ro 08-2750 did not inhibit 3CLpro (nsp5) activity. Z-VAD-FMK is a novel inhibitor discovered against nsp5 (reference nsp5 paper in this issue)

Figure S4. EC50 of beta-lapachone (A), Cdk4 inhibitor III (B), cryptotanshinone (C), and tanshinone IIA (D) from the cell culture-based viral proliferation assay.

Figure S5. (Related to Figure 7) (A and C) Viral proliferation assay for dihydrotanshinone I, Ro 08-2750, GRL-0617, beta-lapachone, cryptotanshinone, tanshinone IIA, and Cdk4 inhibitor III. B. There is no obvious additional effect of
combining remdesivir with compounds in (A). These compounds either do not stop COVID-19 viral proliferation or are cytotoxic.

**Table S1.** Compound list of over 5000 compounds with scores from low and high concentration.

**Table S2.** Oligomers and PLpro optimized sequence.
Supplementary Figure S1

A. Bacteria-His-TEV-PLpro: Gel Filtration fractions

Peak fractions

100  70  55  35  25  15  10

Lane:  1  2  3  4  5  6  7  8  9  10  11  12  13  14

PLpro

B. Baculovirus-Flag-His-PLpro: Gel filtration fractions

Peak fractions

100  70  55  35  25  15  10

Lane:  1  2  3  4  5  6

His-Flag-PLpro
Supplementary Figure S2

A) Substrate Pro1 vs Pro2

B) Substrate: Pro2 vs Pro3

C) Bacteria TEV- vs Sumo-tagged

D) Bacteria vs Baculovirus
**A**

Gel filtration elution fractions

<table>
<thead>
<tr>
<th>Lane</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
</table>

Substrate for gel-based assay

**B**

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|

- Cleavage product
**Supplementary Figure S4**

**A**

Beta-lapachone

- EC<sub>50</sub> = 4.39 µM

**B**

Cdk4 inhibitor III

- EC<sub>50</sub> = 3.402 µM

**C**

Cryptanshinone

- EC<sub>50</sub> = 222.9 µM

**D**

Tanshinone IIA

- EC<sub>50</sub> = 1153 µM
Supplementary Figure S5C

No virus

Vehicle

1 µM

3 µM

10 µM

30 µM

100 µM

300 µM

beta-lapachone
Cryptotanshinone
Tanshinone IIA
Cdk4 inhibitor III