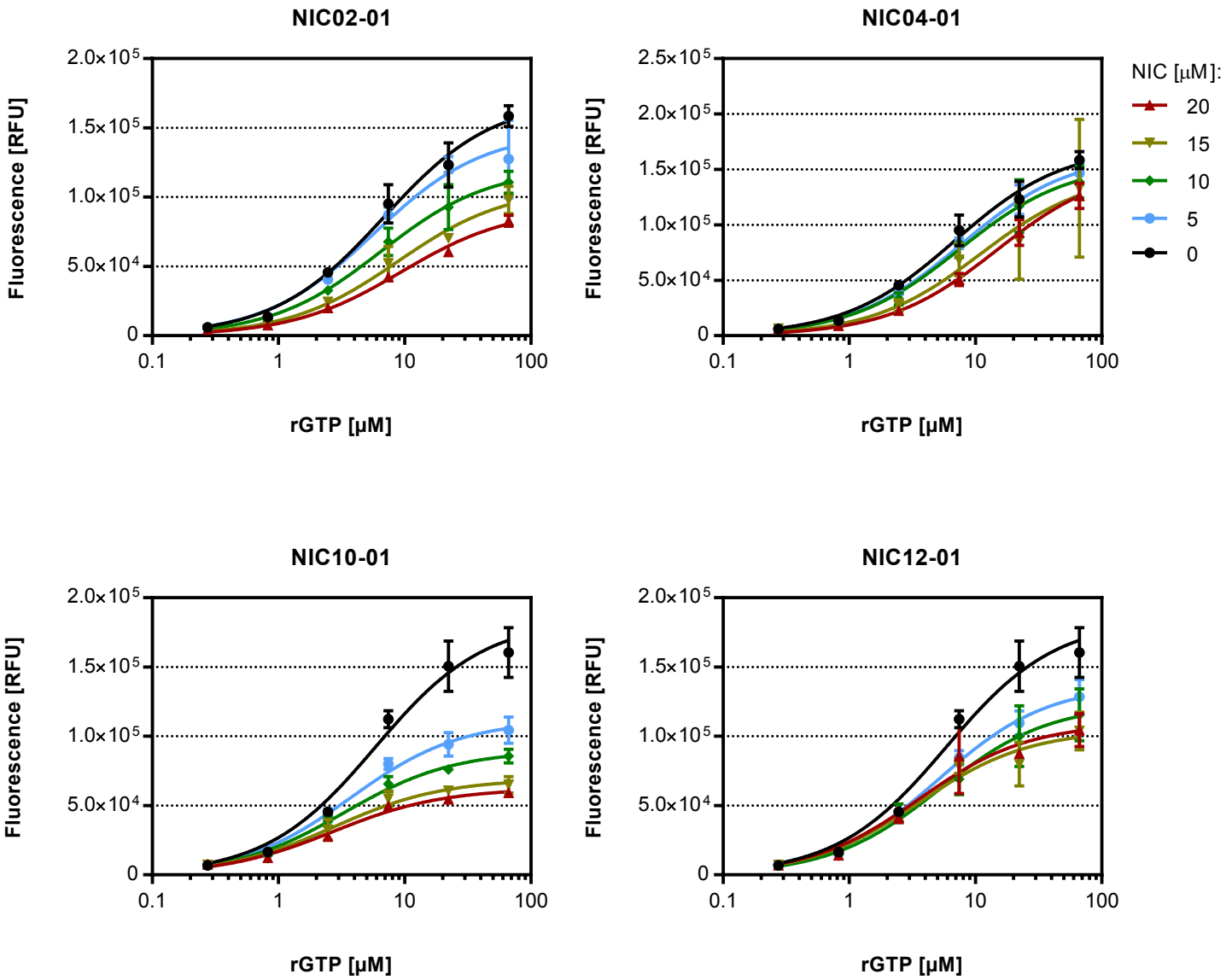
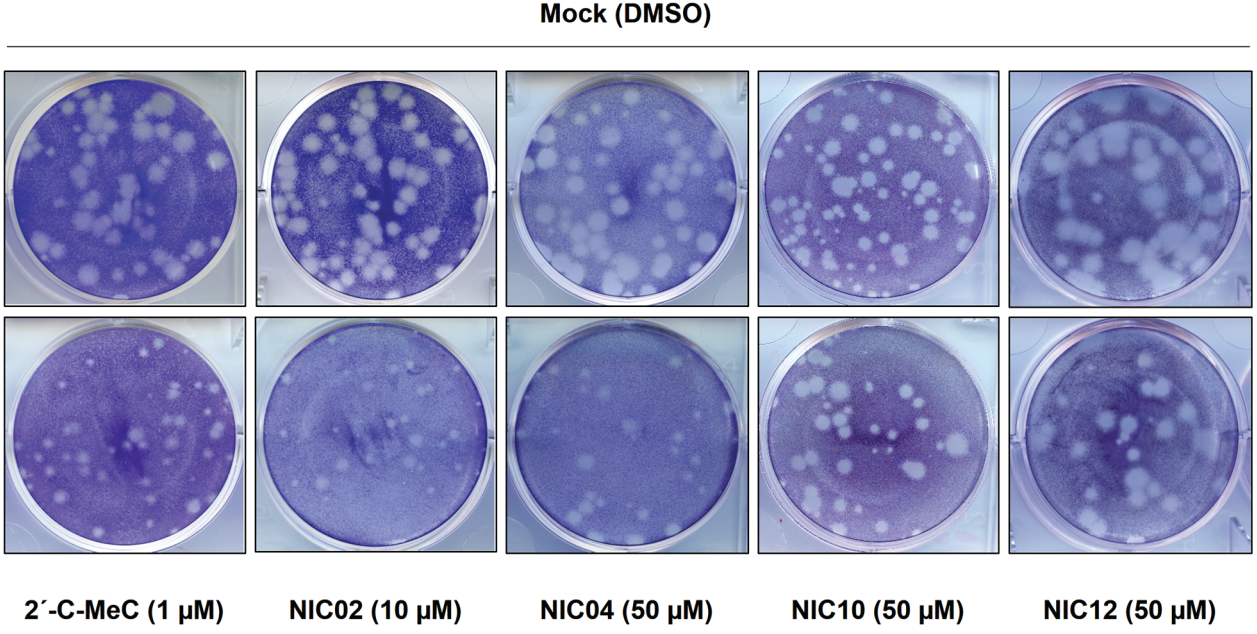
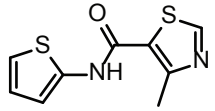


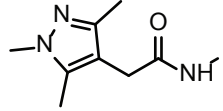
Supplementary Figure 1. Kinetics of NoV GII.4 RdRp activity in the presence of NIC inhibitors. The activity of the NoV RdRp was tested at various concentrations of GTP (substrate) and inhibitor. For each of the substrate concentrations (0.2 to 66 µM), the inhibitor concentration was also varied from 0 to 20 µM. RdRp assays were performed in triplicate and presented as the average with standard deviations. The data was used to generate double reciprocal Lineweaver–Burk plots (Figure 3).



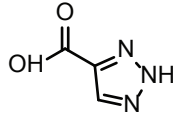
Supplementary Figure 2. MNV plaque reduction by lead NoV NNIs. Plaque formation was visualised 48 h after treatment with different compounds and subsequent infection with MNV. Control wells for each experiment, treated with DMSO (vehicle), are shown in the top panel. Inhibitor treatment is shown in the bottom panel where compounds were used at a concentration that was not toxic to RAW 264.7 cells.



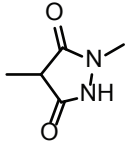
C1



C2



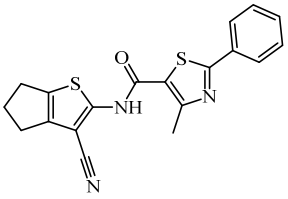
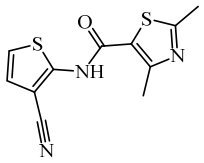
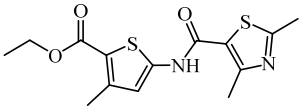
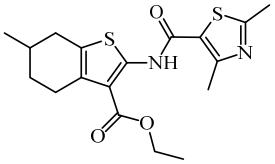
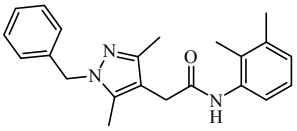
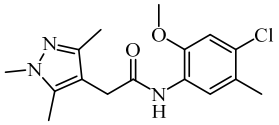
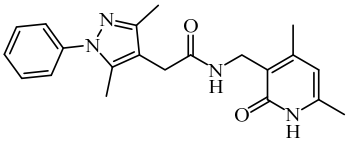
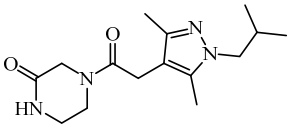
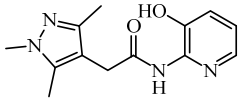
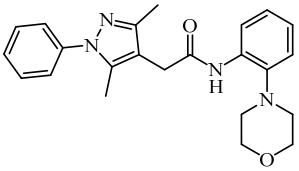
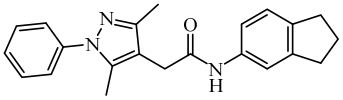
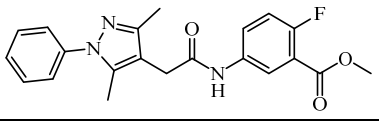
C3

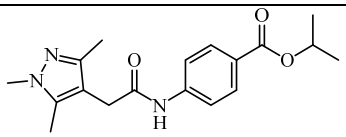
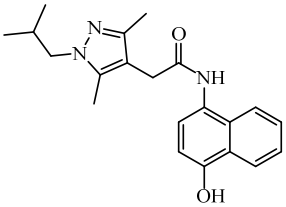
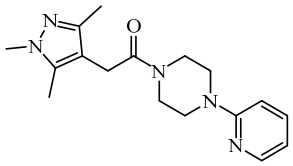
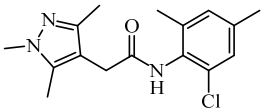
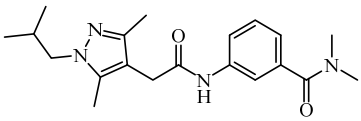
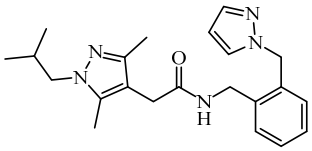
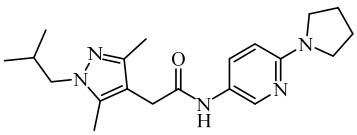
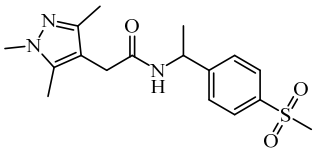
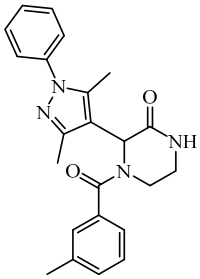
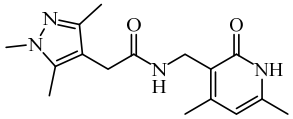
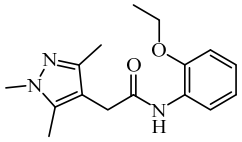


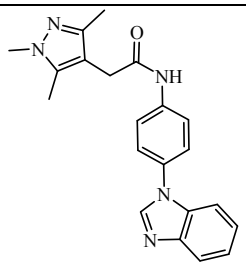
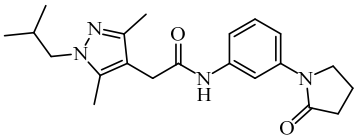
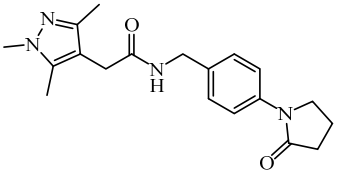
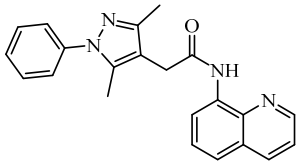
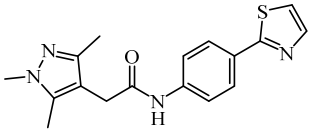
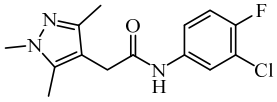
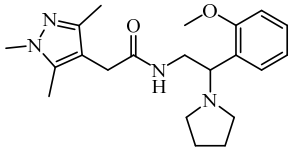
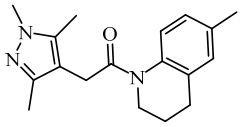
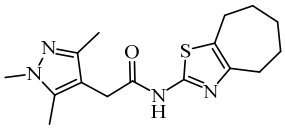
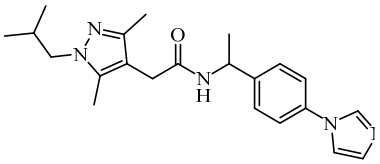
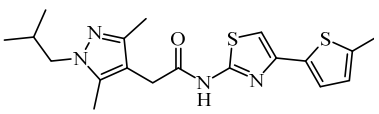
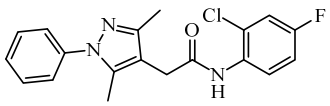
C4

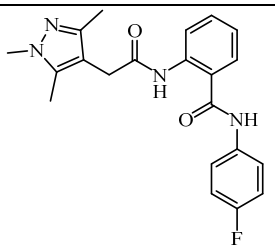
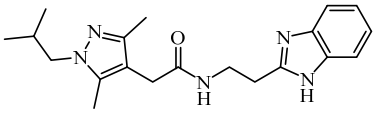
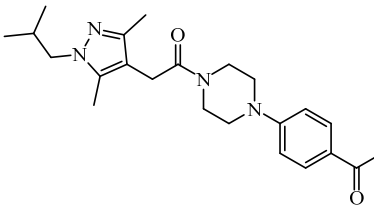
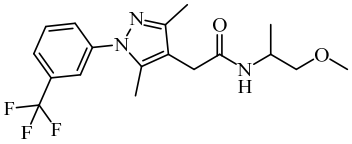
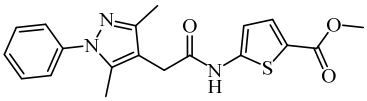
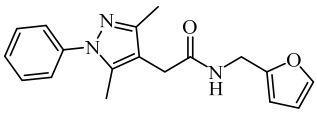
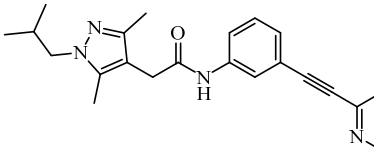
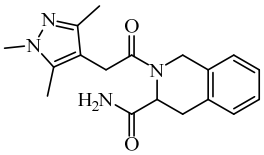
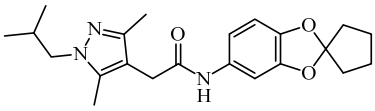
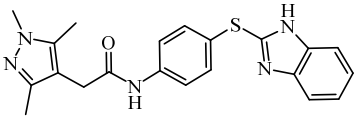
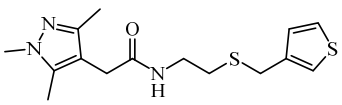
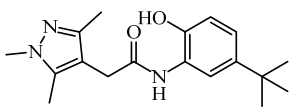
Supplementary Figure 3. Core structures used for SAR analysis. Structures of the four leading compounds, without their functional groups, were used to identify analogue molecules from the compound library. These core structures are shown for NIC02 (C1), NIC04 (C2), NIC10 (C3) and NIC12 (C4).

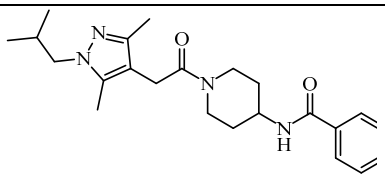
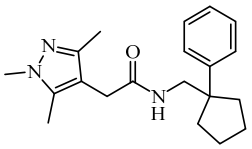
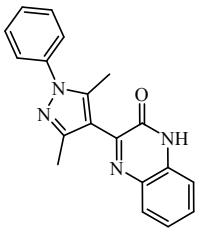
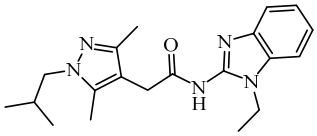
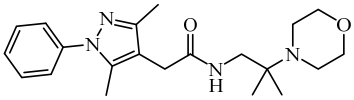
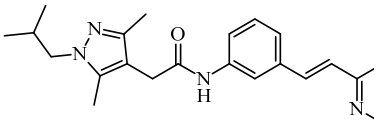
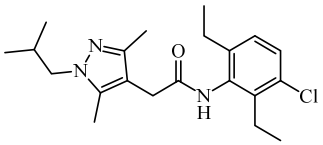
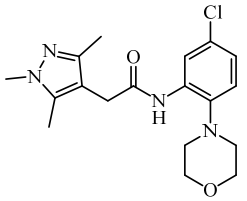
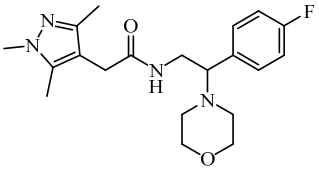
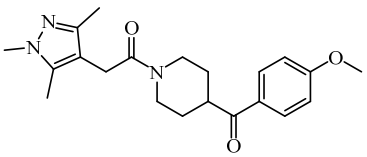
Supplementary Table 1. Inhibitory activity of NoV NNI analogues

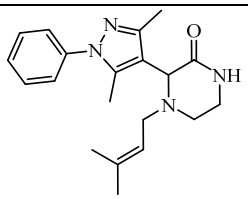
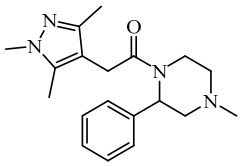
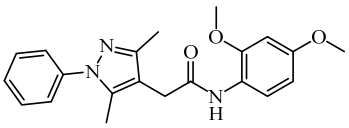
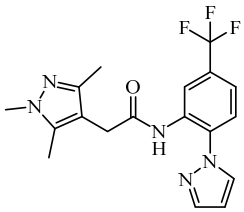
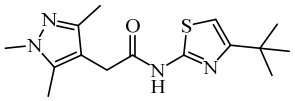
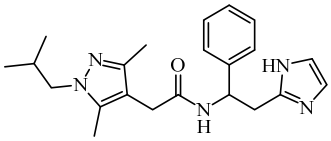
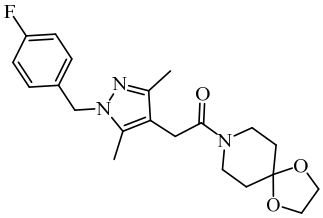
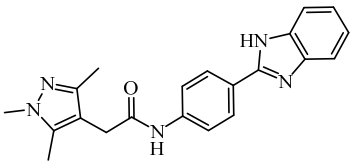
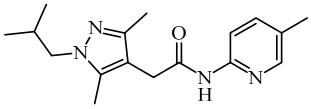
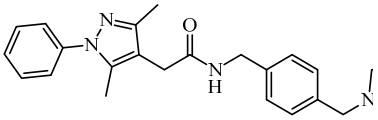
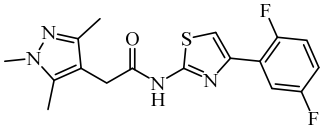
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
NIC02			64.1 ± 1.0
WECC-0147676		NIC02	17.1 ± 0.4
WECC-0124682		NIC02	-1.6 ± 1.8
WECC-0000826		NIC02	-3.5 ± 4.5
NIC04			30.4 ± 7.5
WECC-0121840		NIC04	10.8 ± 16
WECC-0119984		NIC04	9.8 ± 9.7
WECC-0155998		NIC04	9.3 ± 0.8
WECC-0155058		NIC04	8.0 ± 2.8
WECC-0129793		NIC04	7.8 ± 9.2
WECC-0131157		NIC04	7.5 ± 10.2
WECC-0130545		NIC04	7.4 ± 1.0

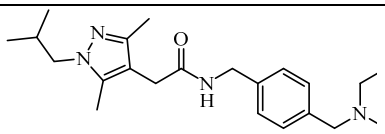
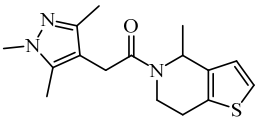
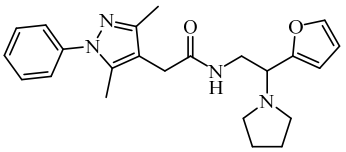
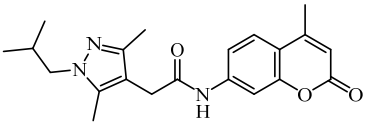
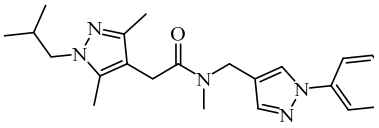
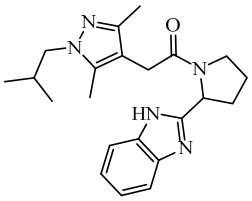
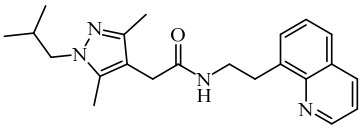
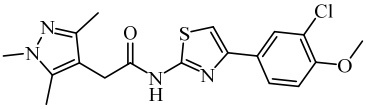
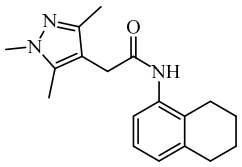
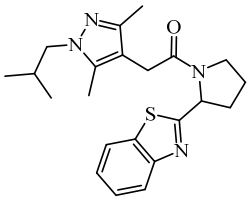
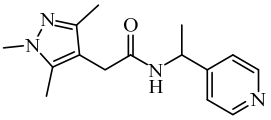
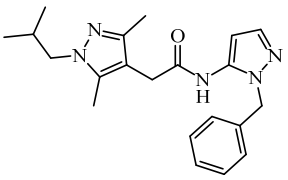
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0159854		NIC04	7.1 ± 4.0
WECC-0131506		NIC04	6.8 ± 5.1
WECC-0133030		NIC04	6.1 ± 8.6
WECC-0157421		NIC04	5.8 ± 5.4
WECC-0155007		NIC04	5.3 ± 3.4
WECC-0124111		NIC04	5.2 ± 10.2
WECC-0151645		NIC04	5.2 ± 4.2
WECC-0141628		NIC04	5.0 ± 7.6
WECC-0135652		NIC04	4.7 ± 13
WECC-0153396		NIC04	4.6 ± 2.3
WECC-0132604		NIC04	4.3 ± 6.1

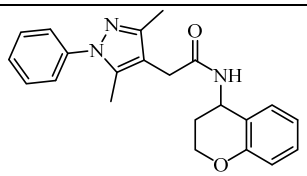
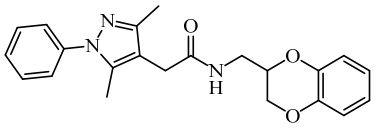
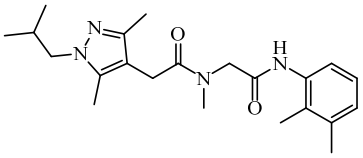
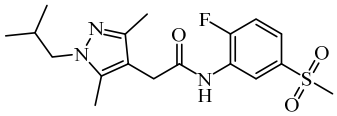
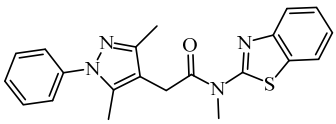
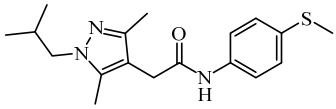
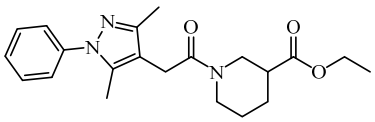
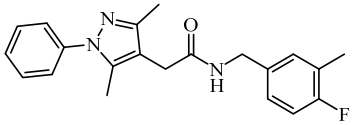
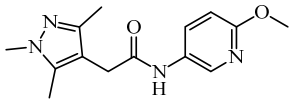
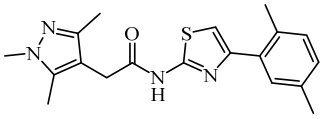
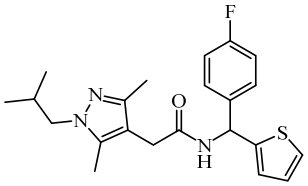
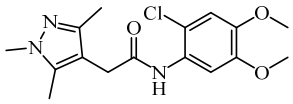
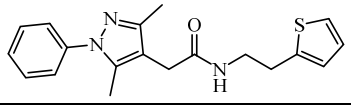
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0136981		NIC04	4.2 ± 6.7
WECC-0127545		NIC04	4.0 ± 6.4
WECC-0127616		NIC04	3.9 ± 9.0
WECC-0129123		NIC04	3.8 ± 4.6
WECC-0145342		NIC04	3.8 ± 4.6
WECC-0157113		NIC04	3.6 ± 2.7
WECC-0153684		NIC04	3.6 ± 5.3
WECC-0154797		NIC04	3.5 ± 1.9
WECC-0088299		NIC04	3.5 ± 9.5
WECC-0126967		NIC04	3.3 ± 6.3
WECC-0084573		NIC04	3.1 ± 7.4
WECC-0138117		NIC04	3.0 ± 7.3

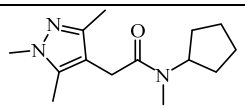
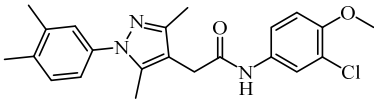
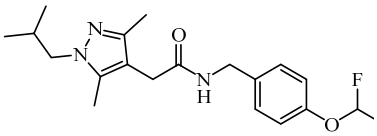
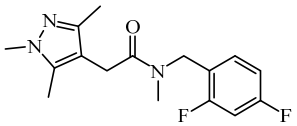
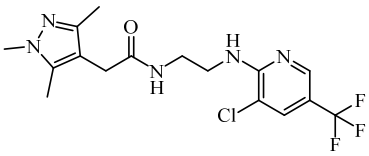
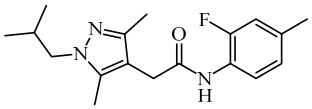
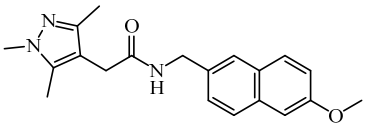
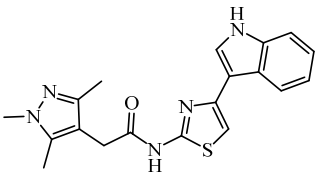
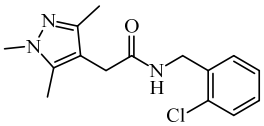
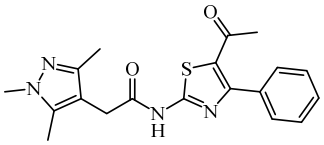
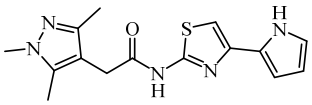
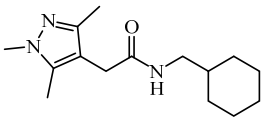
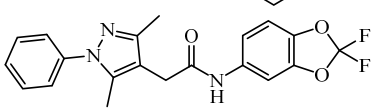
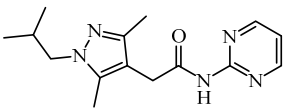
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0124815		NIC04	2.9 ± 7.9
WECC-0138218		NIC04	2.7 ± 16.3
WECC-0141598		NIC04	2.6 ± 2.4
WECC-0158058		NIC04	2.5 ± 7.8
WECC-0155892		NIC04	2.1 ± 2.5
WECC-0124388		NIC04	2.0 ± 2.8
WECC-0141334		NIC04	2.0 ± 3.7
WECC-0154307		NIC04	1.9 ± 2.5
WECC-0118923		NIC04	1.7 ± 6.4
WECC-0087233		NIC04	1.5 ± 12.0
WECC-0087236		NIC04	1.1 ± 16.4
WECC-0152270		NIC04	0.9 ± 10.8

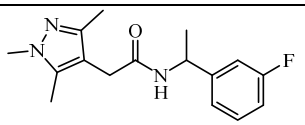
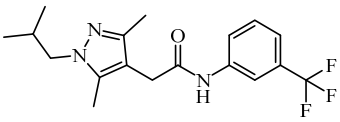
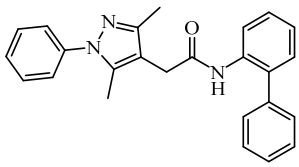
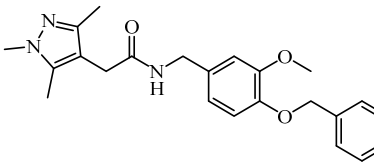
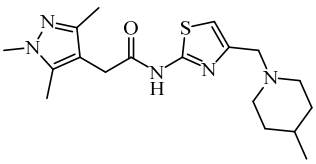
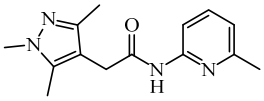
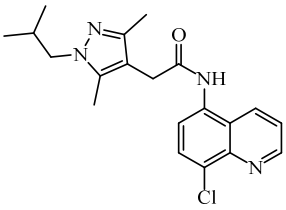
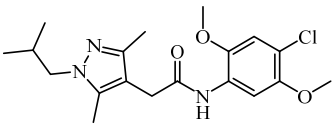
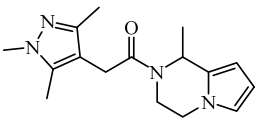
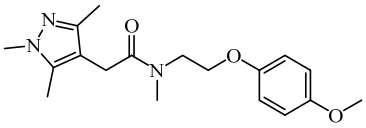
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0139047		NIC04	0.7 ± 11.8
WECC-0158431		NIC04	0.7 ± 3.5
WECC-0114628		NIC04	0.6 ± 7.3
WECC-0130765		NIC04	0.5 ± 11.7
WECC-0129673		NIC04	0.2 ± 7.1
WECC-0137813		NIC04	0 ± 3.3
WECC-0148245		NIC04	0 ± 0.8
WECC-0127985		NIC04	-0.3 ± 5.7
WECC-0157037		NIC04	-0.3 ± 5.3
WECC-0154971		NIC04	-0.3 ± 4.2

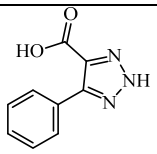
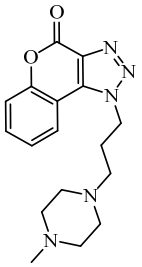
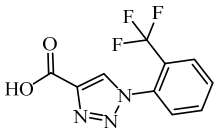
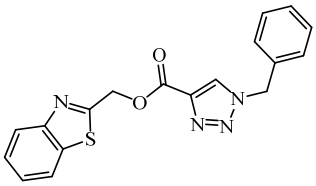
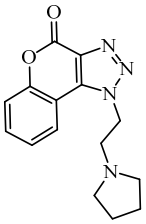
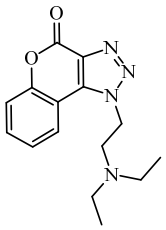
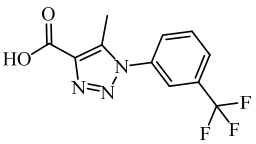
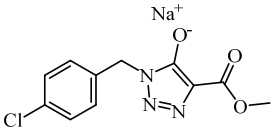
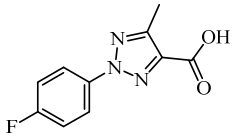
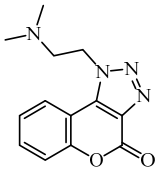
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0138748		NIC04	-0.4 ± 2.0
WECC-0159453		NIC04	-0.5 ± 5.1
WECC-0127208		NIC04	-0.7 ± 6.4
WECC-0137534		NIC04	-0.7 ± 7.2
WECC-0087169		NIC04	-0.7 ± 5.5
WECC-0133646		NIC04	-0.8 ± 5.9
WECC-0004464		NIC04	-0.8 ± 5.9
WECC-0122129		NIC04	-0.9 ± 2.5
WECC-0140492		NIC04	-0.9 ± 2.7
WECC-0153988		NIC04	-1.0 ± 9.3
WECC-0087065		NIC04	-1.1 ± 3.5

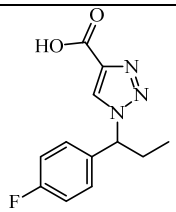
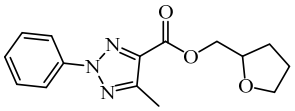
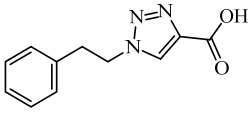
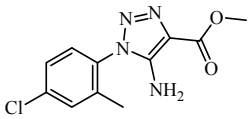
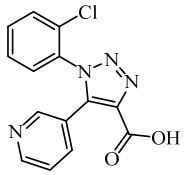
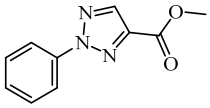
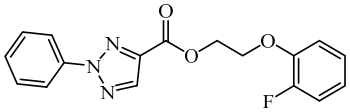
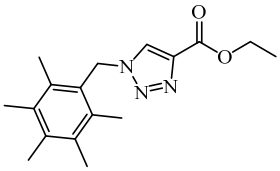
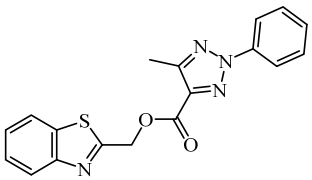
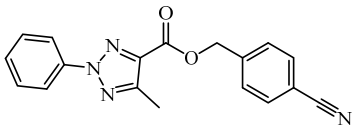
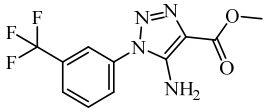
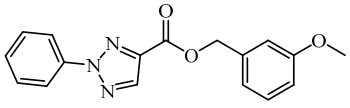
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0136288		NIC04	-1.2 ± 4.4
WECC-0153838		NIC04	-1.2 ± 11.8
WECC-0121462		NIC04	-1.2 ± 6.4
WECC-0084669		NIC04	-1.7 ± 8.0
WECC-0138256		NIC04	-1.8 ± 1.3
WECC-0137579		NIC04	-2.0 ± 9.1
WECC-0130415		NIC04	-2.4 ± 5.4
WECC-0087067		NIC04	-2.5 ± 1.6
WECC-0143711		NIC04	-2.7 ± 5.0
WECC-0122543		NIC04	-2.7 ± 5.8
WECC-0152365		NIC04	-2.9 ± 4.3
WECC-0151606		NIC04	-3.0 ± 3.3

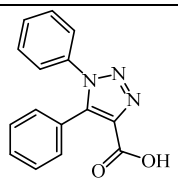
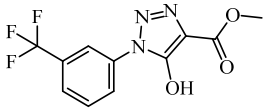
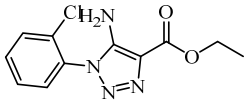
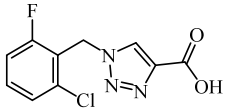
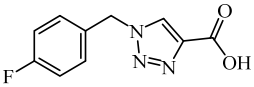
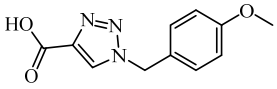
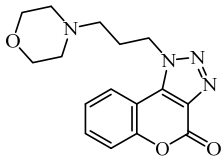
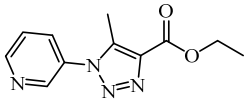
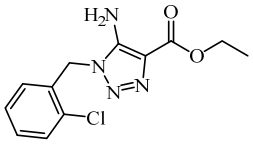
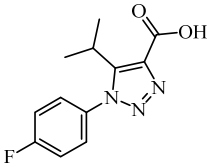
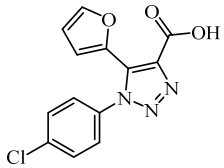
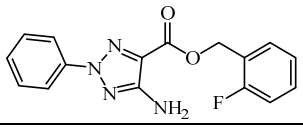
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0159049		NIC04	-3.1 ± 3.1
WECC-0154458		NIC04	-3.1 ± 3.2
WECC-0123651		NIC04	-3.4 ± 9.5
WECC-0149289		NIC04	-3.4 ± 4.0
WECC-0125513		NIC04	-3.4 ± 2.6
WECC-0084725		NIC04	-3.6 ± 7.9
WECC-0156093		NIC04	-3.9 ± 4.9
WECC-0147229		NIC04	-4.1 ± 5.1
WECC-0133556		NIC04	-4.2 ± 5.1
WECC-0086989		NIC04	-4.3 ± 5.9
WECC-0148560		NIC04	-4.3 ± 1.6
WECC-0158829		NIC04	-4.6 ± 4.7
WECC-0142703		NIC04	-4.7 ± 5.1

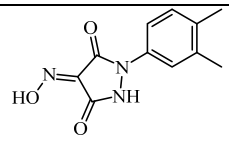
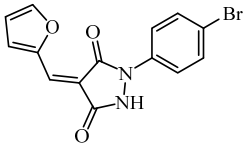
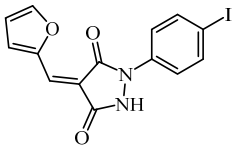
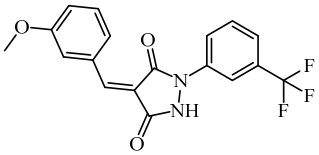
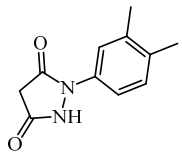
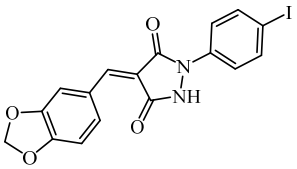
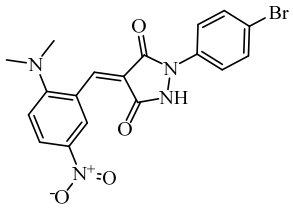
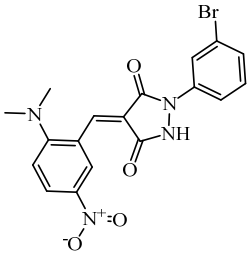
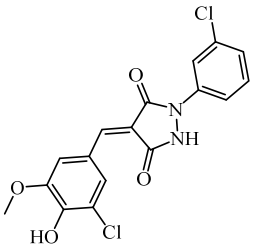
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0142013		NIC04	-5.1 ± 4.3
WECC-0003117		NIC04	-5.2 ± 3.1
WECC-0123846		NIC04	-5.2 ± 3.7
WECC-0151472		NIC04	-5.6 ± 3.0
WECC-0121988		NIC04	-5.6 ± 3.2
WECC-0149206		NIC04	-5.7 ± 6.0
WECC-0131954		NIC04	-6.3 ± 2.4
WECC-0086990		NIC04	-6.4 ± 2.0
WECC-0143716		NIC04	-6.4 ± 10.5
WECC-0087232		NIC04	-7.1 ± 8.2
WECC-0086991		NIC04	-7.3 ± 2.1
WECC-0147453		NIC04	-7.6 ± 0.8
WECC-0149778		NIC04	-8.5 ± 12.1
WECC-0145176		NIC04	-8.5 ± 9.7

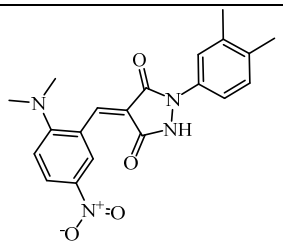
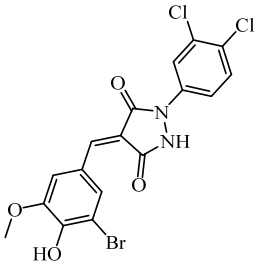
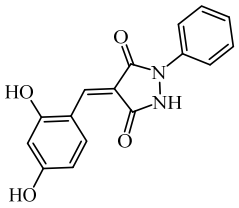
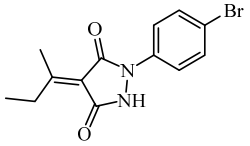
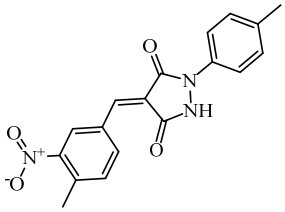
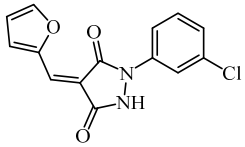
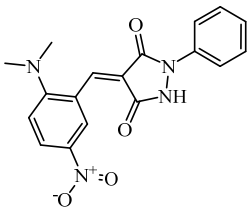
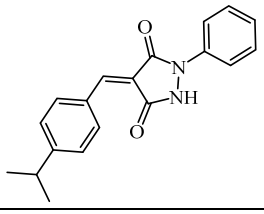
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0150495		NIC04	-8.5 ± 1.1
WECC-0150513		NIC04	-8.5 ± 5.8
WECC-0149135		NIC04	-8.9 ± 4.0
WECC-0120518		NIC04	-9.1 ± 0.8
WECC-0087380		NIC04	-9.4 ± 8.3
WECC-0146702		NIC04	-10.4 ± 7.8
WECC-0143988		NIC04	-11.1 ± 5.8
WECC-0157612		NIC04	-11.4 ± 2.0
WECC-0144033		NIC04	-11.5 ± 2.4
WECC-0151320		NIC04	-13.5 ± 4.4

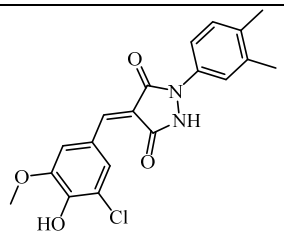
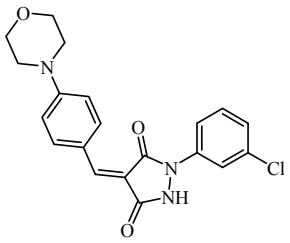
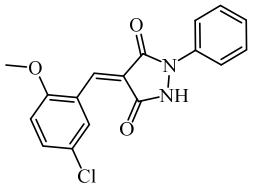
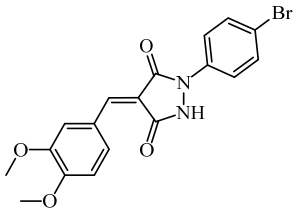
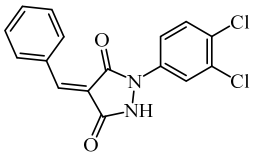
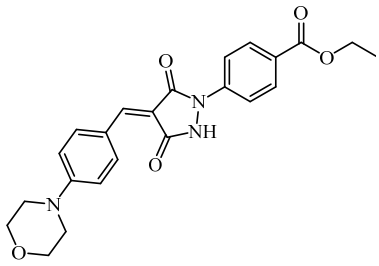
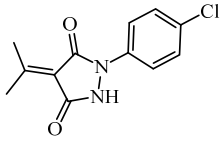
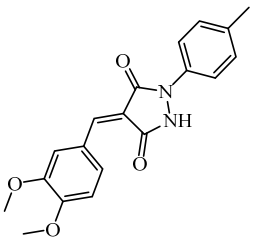
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
NIC10			41.2 ± 0.5
WECC-0029330		NIC10	22.1 ± 13.2
WECC-0141606		NIC10	15.5 ± 14.8
WECC-0159361		NIC10	15.2 ± 18.2
WECC-0029329		NIC10	12.5 ± 6.1
WECC-0029328		NIC10	12.1 ± 16.4
WECC-0131788		NIC10	11.7 ± 6.4
WECC-0022218		NIC10	8.0 ± 4.5
WECC-0111306		NIC10	7.4 ± 4.2
WECC-0029327		NIC10	6.7 ± 11

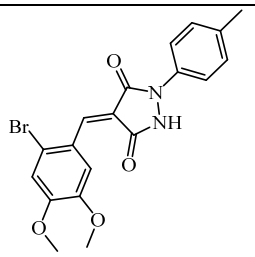
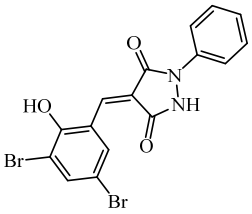
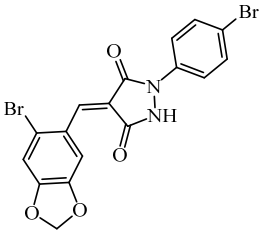
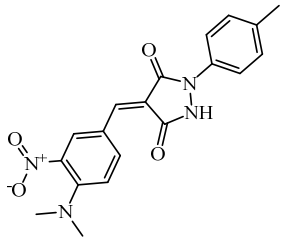
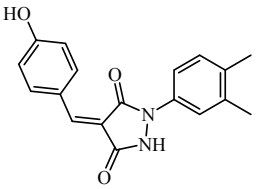
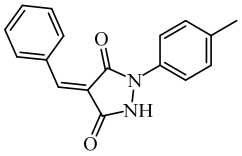
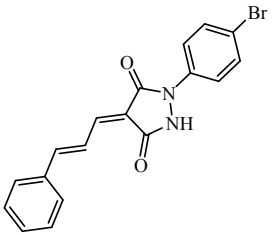
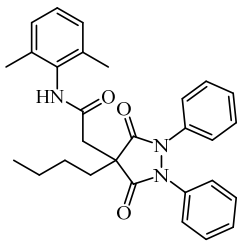
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0007325		NIC10	5.8 ± 10.9
WECC-0155904		NIC10	5.2 ± 7.1
WECC-0007329		NIC10	3.8 ± 2.8
WECC-0020694		NIC10	3.5 ± 2.5
WECC-0000368		NIC10	1.8 ± 7.9
WECC-0150391		NIC10	1.8 ± 9.2
WECC-0140134		NIC10	1.7 ± 3.2
WECC-0018094		NIC10	1.6 ± 13.0
WECC-0115190		NIC10	0.9 ± 1.2
WECC-0110971		NIC10	-0.2 ± 2.7
WECC-0017416		NIC10	-0.2 ± 5.4
WECC-0158809		NIC10	-0.7 ± 7.0

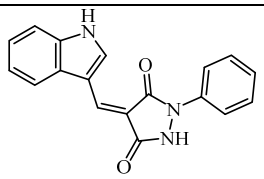
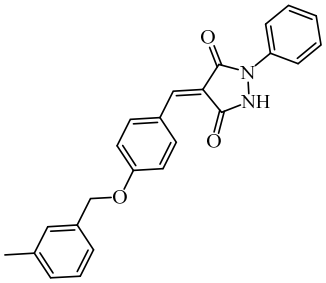
Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0015626		NIC10	-1.0 ± 2.3
WECC-0020931		NIC10	-2.2 ± 5.2
WECC-0009462		NIC10	-3.0 ± 6.8
WECC-0007328		NIC10	-3.7 ± 5.3
WECC-0007327		NIC10	-4.4 ± 3.0
WECC-0007320		NIC10	-4.7 ± 3.0
WECC-0056532		NIC10	-7.0 ± 4.9
WECC-0019921		NIC10	-9.1 ± 1.2
WECC-0018026		NIC10	-10.5 ± 18
WECC-0144671		NIC10	-10.6 ± 5.4
WECC-0015501		NIC10	-14.2 ± 16
WECC-0003882		NIC10	-22.1 ± 13.7

Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
NIC12			47.8 ± 4.6
WECC-0056153		NIC12	75.7 ± 1.2
WECC-0056152		NIC12	61.8 ± 6.6
WECC-0041278		NIC12	54.5 ± 5.0
WECC-0055263		NIC12	35.2 ± 8.6
WECC-0037505		NIC12	32.7 ± 11.6
WECC-0041308		NIC12	32.0 ± 20.6
WECC-0041226		NIC12	31.4 ± 6.1
WECC-0041162		NIC12	30.3 ± 9.5

Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0041342		NIC12	30.1 ± 7.0
WECC-0038991		NIC12	28.7 ± 6.2
WECC-0036746		NIC12	25.0 ± 2.5
WECC-0056747		NIC12	24.2 ± 4.7
WECC-0038666		NIC12	23.1 ± 4.8
WECC-0037742		NIC12	22.8 ± 5.4
WECC-0041320		NIC12	19.4 ± 9.5
WECC-0037399		NIC12	18.4 ± 4.6

Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0041293		NIC12	17.9 ± 0.0
WECC-0037895		NIC12	17.7 ± 1.1
WECC-0041194		NIC12	16.2 ± 7.8
WECC-0036860		NIC12	15.9 ± 4.9
WECC-0038046		NIC12	15.6 ± 5.5
WECC-0038578		NIC12	13.4 ± 7.0
WECC-0056154		NIC12	13.1 ± 3.3
WECC-0038695		NIC12	12.4 ± 7.1

Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0038714		NIC12	12.3 ± 0.4
WECC-0028330		NIC12	12.2 ± 4.9
WECC-0036826		NIC12	11.7 ± 1.1
WECC-0038507		NIC12	9.9 ± 9.1
WECC-0037083		NIC12	8.5 ± 2.7
WECC-0038688		NIC12	6.8 ± 4.3
WECC-0037573		NIC12	4.7 ± 1.2
WECC-0046425		NIC12	3.2 ± 8.8

Entry number	Structure	Parent compound	NoV RdRp Inhibition [% of control]*
WECC-0036872		NIC12	2.8 ± 4.4
WECC-0045626		NIC12	-1.7 ± 11.6

* Inhibition of the NoV GII.4 RdRp at a compound concentration of 10 μM