Here are the experimental results on 1957 compounds selected by 23 participants to Round #1 (hit identification round – no more than 100 compounds per participant) of CACHE challenge #2, targeting the RNA-binding site of SARS-CoV-2 NSP13. Forty-seven compounds selected by 18 participants are advancing to Round #2 (hit-expansion round – no more than 50 compounds per participant) of this CACHE challenge.

All compounds were tested for binding to NSP13 using an SPR assay, and for inhibiting the ATPase activity of NSP13 (which is coupled to the RNA-unwinding activity).

Our initial goal was to use the ATPase assay as an orthogonal validation assay, but we saw very little correlation between both assays, and decided to only use SPR to select compounds that are advancing to Round 2. Indeed, it is not clearly established that compounds occupying any cavity of the RNA binding site should inhibit the catalytic activity. Additionally, enzymatic assays are more prone to false positives than direct binding assays, such as SPR. To be completely transparent, we are still providing the data from the ATPase assay.

The structure of the compound will be shared at the end of Round #2. Here, we provide rounded molecular weight and cLogP values calculated with ICM (Molsoft

Below are details on the experimental data.

- Primary screen: adjusted relative 50uM

This is the relative binding signal to NSP13 measured by SPR with 50 µM compound, adjusted to a blank (no compound). The value represents a % of the expected binding signal based on the amount of protein immobilized on the chip. A value below 0.4 (< 40% of the expected binding signal) is not significant. A value >2 (> 200% of the expected binding signal) indicates that the binding is not specific. Compounds with 0.4 < value < 2. were advanced to dose-response experiments. Some compounds that did not meet these conditions were rescued because they were active in the ATPase assay and also advanced to dose-response by SPR.

- Primary screen: slope, Primary screen: slow dissociation, Primary screen: Rmax > Expected Rmax

These parameters are indicators of the validity of the binding signal (adjusted relative value above). If the slope of the SPR sensorgram or the dissociation rate are flagged with “Yes”, the compound is not coming off from the immobilized protein/reference surface, a potential sign of non-specific binding. Rmax > Expected Rmax indicates that the measured binding signal (RU or response unit) is greater than the expected signal. These flags are automatically raised by the SPR instrument.

If (Primary screen slope = Yes OR Primary screen slow dissociation = Yes OR Primary screen Rmax > Expected Rmax = Yes OR Solubility at 100 µM < 80%) compounds are dismissed.

A few compounds not meeting these conditions were also tested in dose response, based on ATPase assay data or other considerations.

The solubility data at 100 µM is in column AD and explained further down. Compounds that are not soluble at 100 µM are not tested in dose response to avoid clogging the SPR channels with precipitate.

-NSP13 KD (µM) [1]

This is the binding affinity of the compound for NSP13 measured by SPR in a dose-response experiment. We set the cut-off to KD < 150 µM.

-NSP13 % Binding [1]

This is the % of measured binding signal over the expected binding signal based on the amount of protein immobilized on the SPR chip, in the dose-response experiment. % Binding > 150 indicates non-specific binding.

- NSP13 KD (µM) [2]

Same as above. Some compounds were re-tested because the SPR sensorgram was not reliable, the compound precipitated at high concentration, or for some other reason.

-NSP13 % Binding [2]

Same as above.

**Compound where (NSP13 KD (µM) [1] < 150 AND NSP13 % Binding [1] < 150 AND NSP13 % Binding [1] > 30) OR (NSP13 KD (µM) [2] < 150 AND NSP13 % Binding [2] < 150 AND NSP13 % Binding [2] > 30) were advanced to Round 2 (“+” in last column).**

- NSP13 KD (µM) [3], NSP13 % Binding [3], NSP13 KD (µM) [4], NSP13 % Binding [4], WDR5 KD (µM) [1], WDR5 % Binding [1], WDR5 KD (µM) [2], WDR5 % Binding [2]

The selectivity of the hits where KD < 50 µM was evaluated by SPR: dose response was conducted in parallel and in duplicate against NSP13 and WDR5 (an unrelated protein). Very few compounds were found to bind WDR5. Note that even when the instrument did produce a good KD value for WDR5, the % binding was very low, indicating non-significant binding.

-ATPase % inhib at 50 µM [1]

Primary screening data in the ATPase assay at 50µM compound

- ATPase % inhib at 50 µM [2]

Some compounds (for ex. hits from the primary SPR assay) were re-tested in the ATPase assay

- ATPase IC50 (µM)

Hits from the 50µM ATPase screen were tested in dose response in the ATPase assay. “NI” : no inhibition

- ATPase IC50 Hill slope

Slope of the inhibition dose response. A slope < -2 is a red flag, suggestive of an artefact, such as compound aggregation.

- agg 200 µM, 100µM, 50 µM, 25 µM

Compound aggregation (in kCnts/sec) measured by dynamic light scattering (in the SPR buffer minus detergent, as detergent scatters light). As a rule of thumb, values above 3000 kCnts/sec are indicative of aggregation. A compound aggregating at concentrations ranges < 2 x KD is a red flag.

- sol 200 µM, sol 100 µM, sol 50 µM, sol 25 µM

Compound solubility (in % of laser power) measured by dynamic light scattering (in the SPR buffer minus detergent). A compound with solubility < 100% at concentrations < 2 x KD is a red flag.