

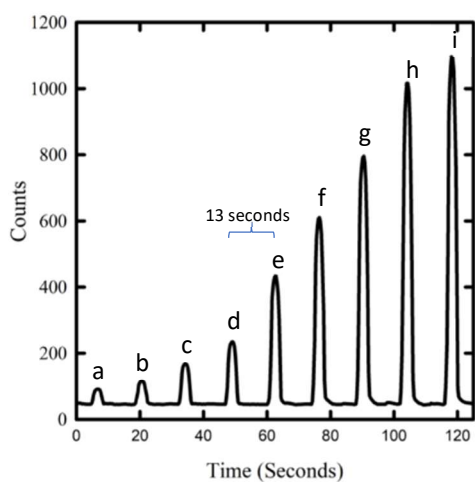
Supplementary Material

Development of a high-throughput screening assay to identify inhibitors of the SARS-CoV-2 guanine-N7 methyltransferase using RapidFire mass spectrometry

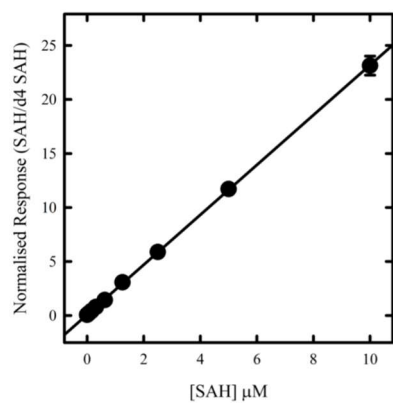
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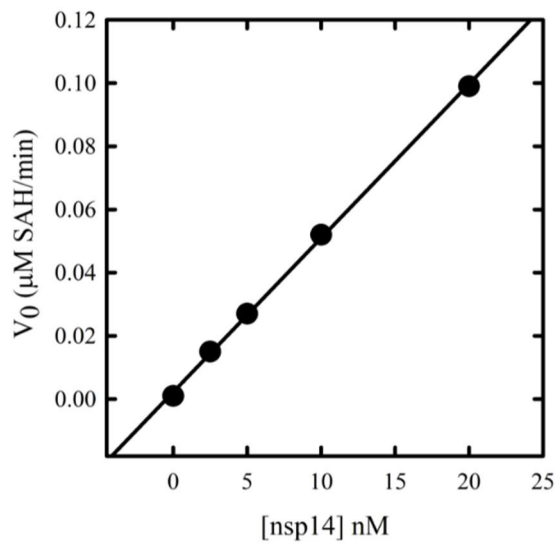


Supplemental Figure 1:
Representative chromatogram for RF-MS/MS (MRM transition 385.1/134) of SAH formation in the reaction of 5 nM nsp14, 1 μ M SAM and 0.7 μ M cap at various time points. (a) 0 min; (b) 2 min; (c) 5 min; (d) 10 min; (e) 20 min; (f) 30 min; (g) 45 min; (h) 60 min; (i) 90 min. The injection cycle time is 13 seconds.

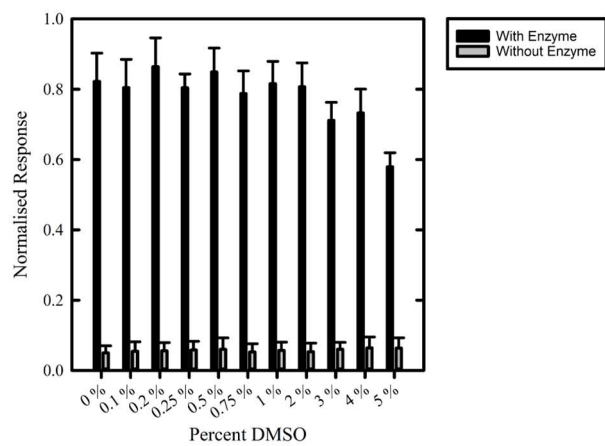


Supplemental Figure 2:

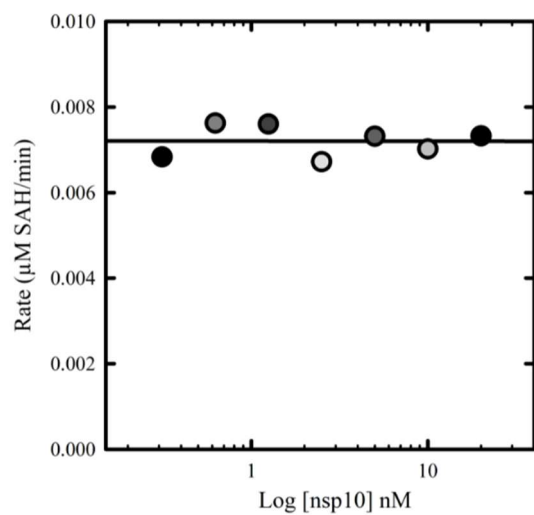
Representative calibration curve (standard curve) for SAH by RF-MS/MS. SAH was spiked at 0.017 – 10 μM in a background of assay buffer and quenching solution containing 0.08 μM internal standard (d4 SAH) and measured by RF-MS/MS. Values plotted are mean \pm standard deviation (n=4)



Supplemental Figure 3:
Plot of initial velocity against
nsp14 concentration. Initial
velocity determined up to 30
minutes.

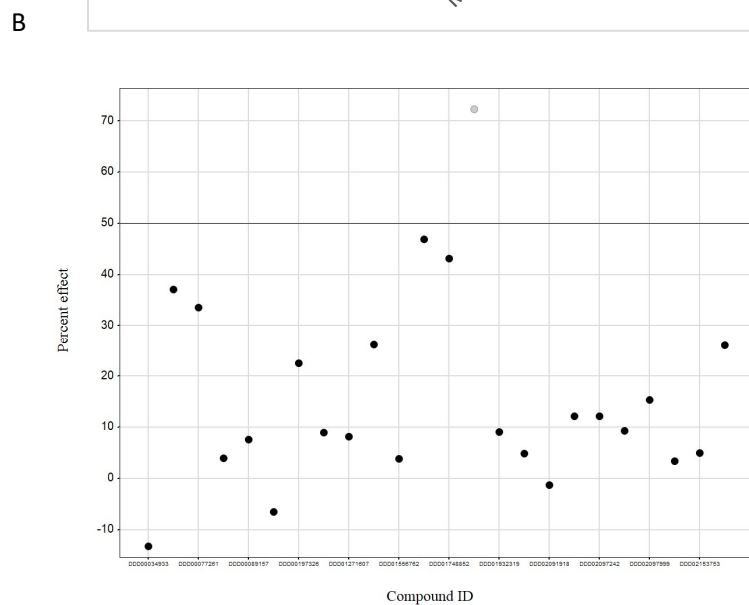
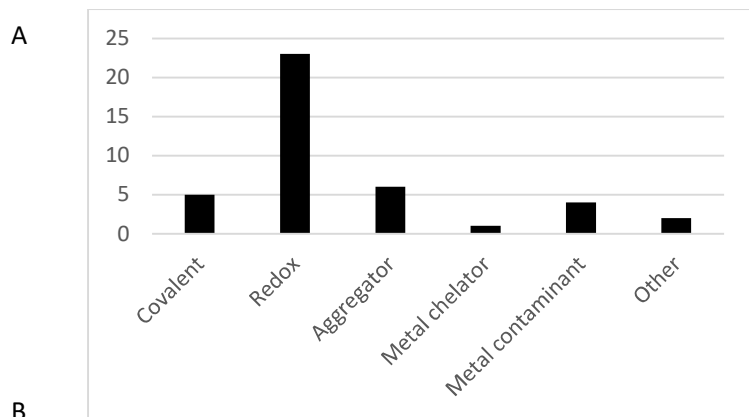


Supplemental Figure 4:
 Determination of nsp14 sensitivity to DMSO. Nsp14, cap and SAM tested at identified screening concentrations (5 nM, 0.7 μ M and 1 μ M respectively) for 60 minutes.

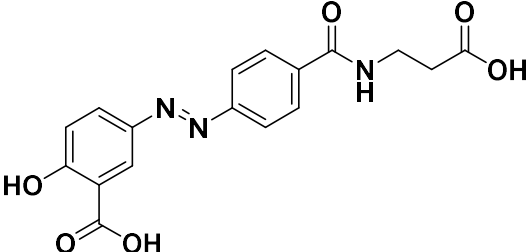
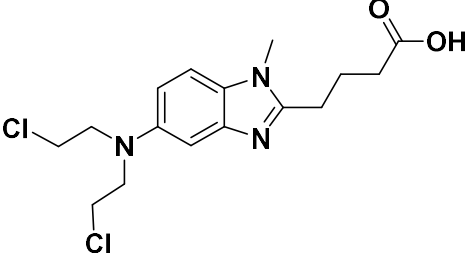
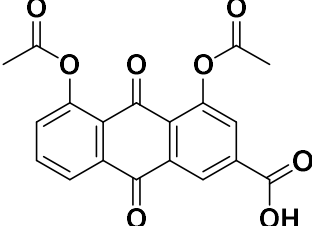
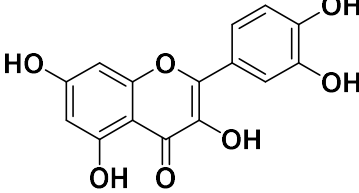
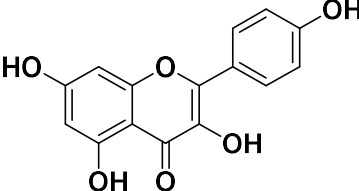
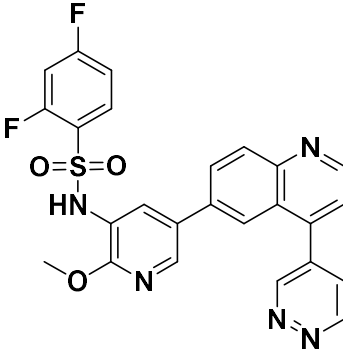


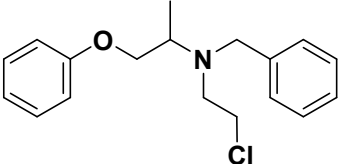
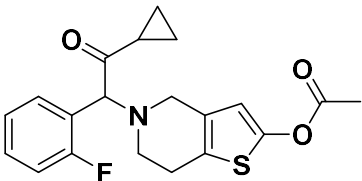
Supplemental Figure 5

Determination of effect of increasing nsp10 concentrations on reaction rate. Nsp10 titrated from a top concentration of 20 nM with identified screening concentrations of all other assay components. Rates determined from reaction progress curves.



Supplemental Figure 6:
 A) Breakdown on the number of compounds for each assay interference mechanism and B) Plot of results from interference set testing. Cut off was set at 50 % (grey line).

Name	Structure	SARS-CoV-2 nsp14 IC ₅₀ (n = 2)
Balsalazide		22.8 - >100 μM
Bendamustine		26.6 - 40.1 μM
Diacerein		40.7 - 80.4 μM
3'-hydroxykaempferol		16.9 - 22.8 μM
Kaempferol		22.4 - 28.5 μM
Omipalisib		5.5 - 55.0 μM

Phenoxybenzamine		58.2 - 91.0 μM
Prasugrel		25.8 - 36.6 μM

Supplemental Table 1:
Names, structures and nsp14 IC_{50} for statistically significant hits