Working towards pandemic preparedness:

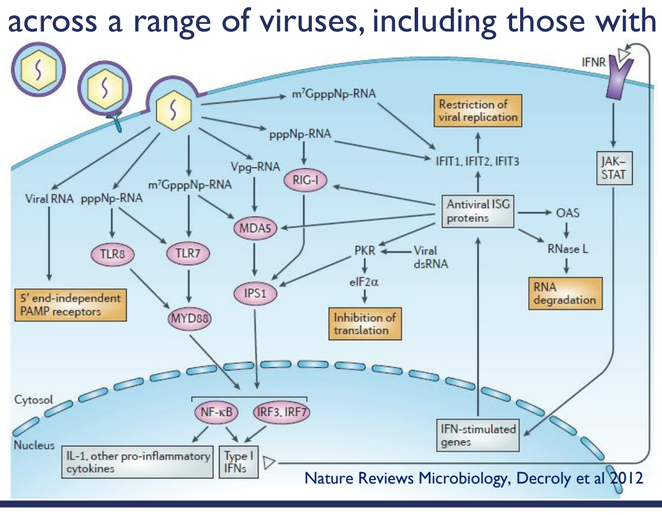
Inhibiting viral 5' RNA cap methylation with potent, selective, broad-spectrum anti-viral small molecules

Harrison H [1] , Glen A [1] , Andrews B [1] , Bayle E [1] , Duncan C [1] , Fyfe M [1] , Guest P [1] , Rausch O [1] , Swain C [1] , Thomas B [1] , Zemla-Brown A [1] , Baric R [2] , Chen Y [2] , Froggatt H [2] , Heise M [2] , Moorman N [2] , Morales N [2] , Scobey T [2] , Sheahan T [2] , Taft-Benz S [2] , Williams A [2] , McElwee M [3] , O'Connor L [3] , Patel AH [3] , Szemiel AM [3]

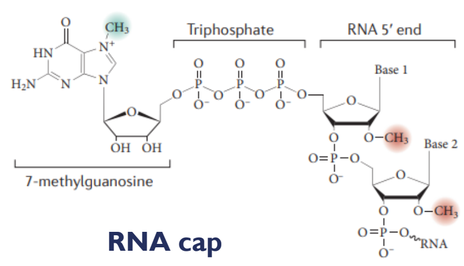
[1] STORM Therapeutics Limited, Babraham Research Campus, Cambridge, United Kingdom
[2] AViDD-READDI, University of North Carolina, Chapel Hill, North Carolina, USA
[3] CVR-CRUSH Drug-Screening and Resistance Hub, MRC-University of Glasgow Centre for Virus Research (CVR), Glasgow, United Kingdom

Introduction

The RNA genome of SARS-CoV-2 contains a 5′ cap that facilitates the essential functions of translation of viral proteins, protection from exonucleases and evasion of the host immune response. Viruses, including coronaviruses, produce their own capping enzymes which include RNA methyltransferases. We describe the development and characterisation of inhibitors of a coronavirus methyltransferase, NSP14. These agents prevent viral replication and expose the virus to the host immune response, leading to potent anti-viral cellular activity across a range of viruses, including those with pandemic potential.



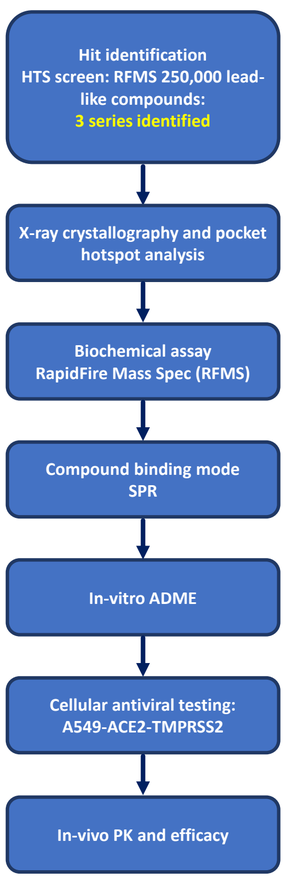
Nature Reviews Microbiology, Decroly et al 2012



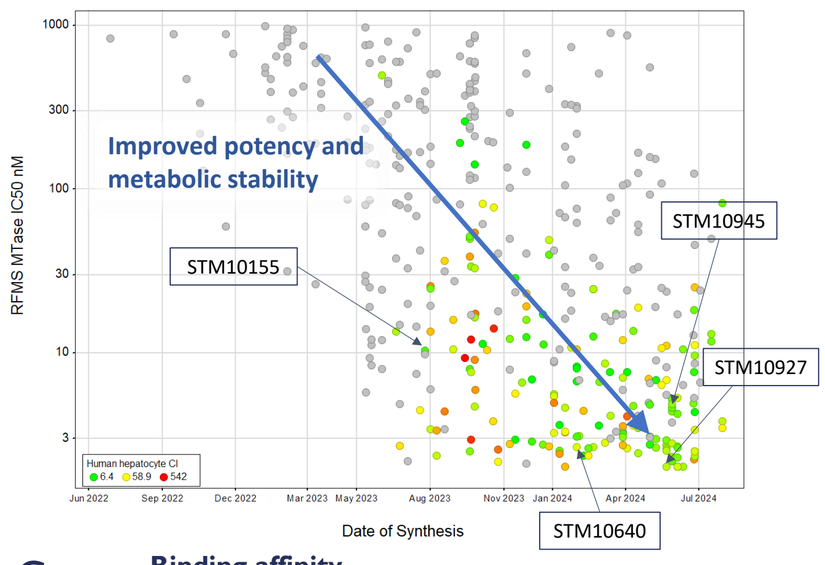
RNA cap

1) Identification & optimisation of viral-specific inhibitors

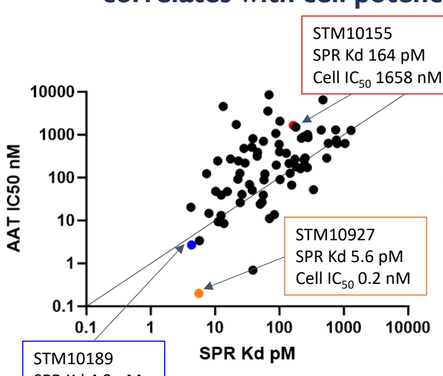
A Assay cascade



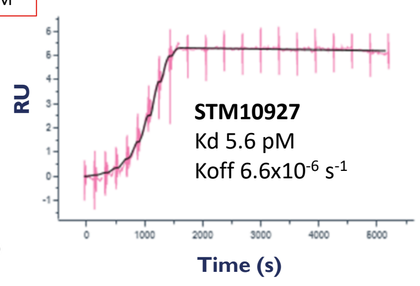
B Progress towards candidate



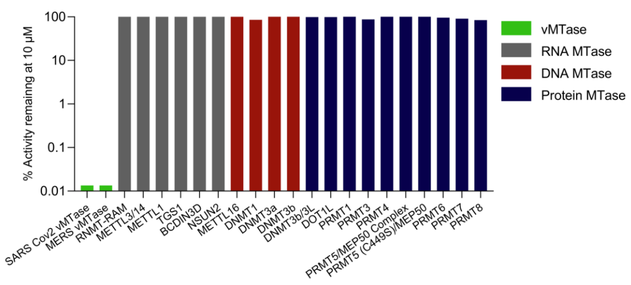
C Binding affinity correlates with cell potency



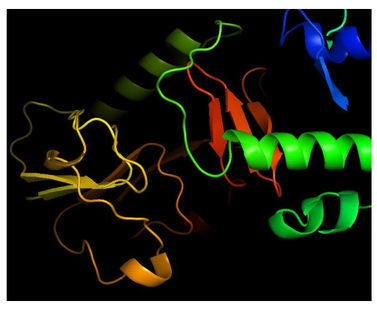
D Single digit picomolar binding by SPR



E Methyltransferase selectivity panel



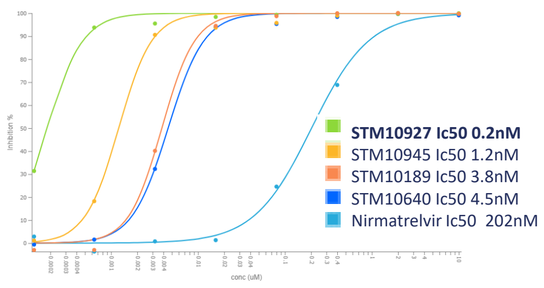
F Compound design is structurally-enabled



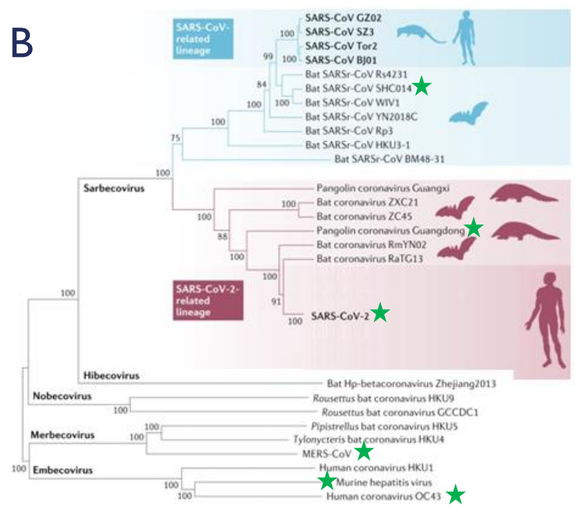
A. Schematic representation of the small molecule inhibitor profiling assay cascade.
B. Graph illustrating the progress to make potent and metabolically stable analogues.
C. Hit confirmation & routine design-make-test screening was performed using RapidFire Mass Spectrometry. Compounds too potent to accurately measure IC_{50}s in the RFMS assay were tested in single cycle kinetic surface plasmon resonance (SCK-SPR). SPR binding data has an excellent correlation with antiviral cell potency against SARS-CoV-2 in A549-ACE2-TMPRSS2 (AAT) cells.
D. STM10927 has single digit picomolar binding and a very slow off-rate by SCK-SPR.
E. STORM methyltransferase inhibitors are virus specific. The diagram shows profiling of STM10155 in a human methyltransferase panel (Reaction Biology and STORM). Data are displayed as percent enzyme activity relative to DMSO-treated controls.
F. Crystal structures of STORM compounds bound to the target are used for structure-based design.

2) Compounds demonstrate good drug-like properties & potent cellular activity across the coronavirus family

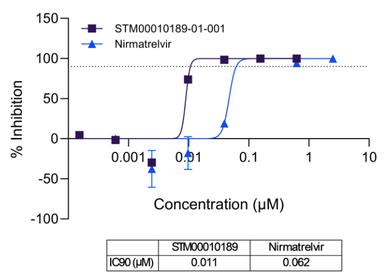
A Sub-nM cellular potency against SARS-CoV-2



B

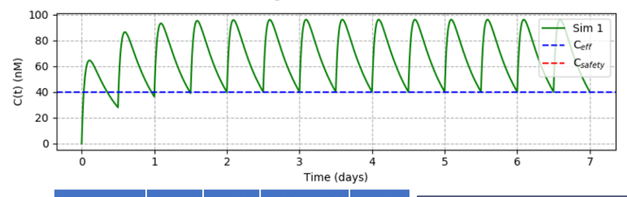


C STM10189 is >5-fold more potent than Nirmatrelvir in HAE-ALI assay



	STM00010189	Nirmatrelvir
IC90 (μM)	0.011	0.062

D STM10640, predicted human PK, dosed PO



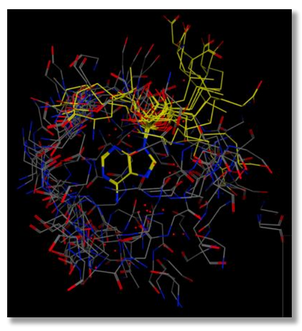
STM	MW	HBD	tPSA NO	logD
STM10640	478	2	110	3.2

------ 4x unbound IC_{90}

A. STORM compounds demonstrate significantly improved potency against SARS-CoV-2 in A549-ACE2-TMPRSS2 cells compared to Nirmatrelvir – the active component of Paxlovid (IF, SARS-CoV-2/human/GBR/CVR-GLA-1/2020)
B. Phylogenetic tree of Coronaviridae. STORM compounds demonstrate antiviral cellular potency for highlighted coronavirus subgenera: SARS-CoV-2, MERS, HuCoV-229E, HuCoV-OC43, PgCov/GD/2019, MHV and the emergent bat strain SHC14, with no cytotoxicity < 10uM (A549-ACE2-TMPRSS2, Huh7, MRC-5, DBT, VeroE6 & Vero81 cells). Adapted from https://www.nature.com/articles/s41579-020-00459-7
C. STM10189 inhibition of SARS-CoV-2 human/USA/USA-WA1/2020 reporter infection in human airway epithelial cells grown at an air-liquid interface (HAE-ALI).
D. STM10640 has a predicted oral dose of 8.5 mg/kg BID in human to cover 4x unbound IC_{90} at Cmin. Recent potent analogues have improved cell potency, human hepatocyte stability and fraction absorbed in rodent *in-vivo* PK. Physicochemical properties are in drug-like property space.

3) A novel platform for antiviral drug discovery with broad potential

A



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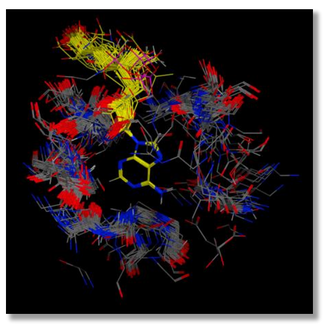

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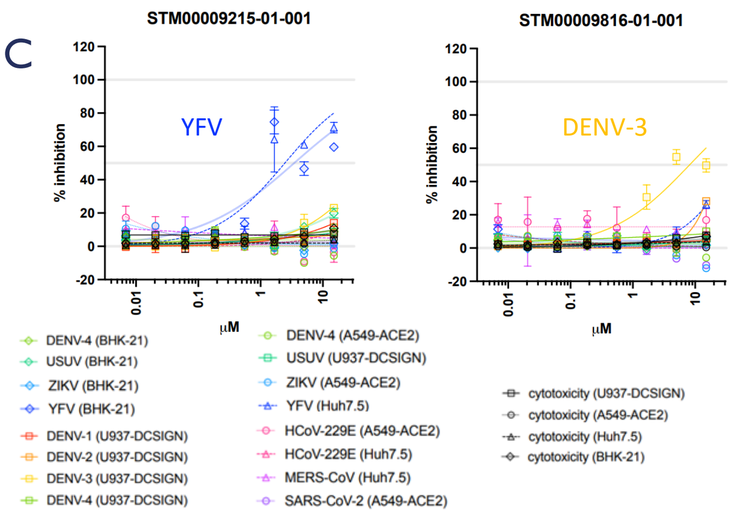

A. SAM/SAH, the methyltransferase cofactor/by-product, bound to a selection of non-viral human target proteins extracted from the PDB showing the diversity of conformations adopted in the proteins.
B. SAM/SAH bound to all the **viral** methyltransferases extracted from the PDB showing the similarity of the cofactor conformation and virus binding pocket, demonstrating the potential for pan-viral activity.
C. Preliminary data shows STM compounds specifically inhibiting viral replication of flaviviruses Yellow Fever and Dengue-3 in cell-based reporter assays, with no cytotoxicity up to 30uM.

Summary

- STORM has developed potent inhibitors of NSP14, exhibiting high selectivity for viral enzymes and 1000-fold greater cell potency versus the oral standard of care for COVID-19.
- Structural and functional evidence indicates STORM viral methyltransferase inhibitors could be active against additional viruses with pandemic potential and unmet medical need.
- *In-vitro* and *in-vivo* tool compounds are now available for further exploration of the immense potential of viral methyltransferase inhibitors for their unique dual mechanism of action; anti-replicative activity and activation of the host innate immune response to the virus.

Harnessing the power of RNA modification