Betulonic Acid Derivatives Interfering with Coronavirus Replication via the Nsp15 Endoribonuclease

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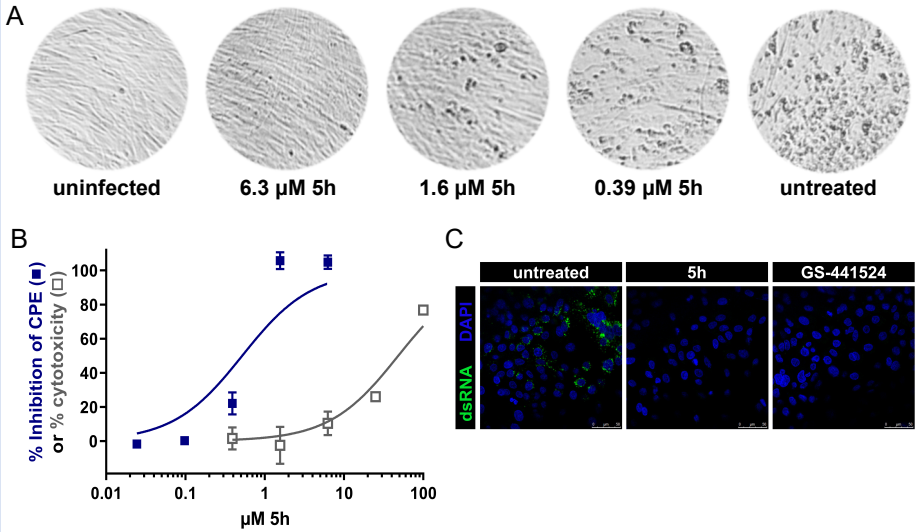
Introduction

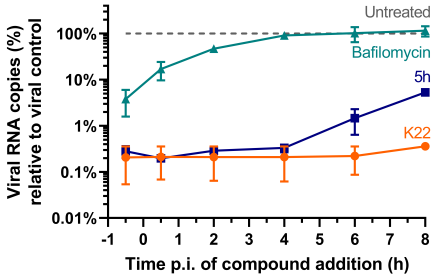
In 2007, betulonic acid was reported by Wen et al. [1] to suppress SARS-CoV replication in cell culture, but no follow-up was thus far conducted. Here we describe the discovery of a structurally related class of **coronavirus inhibitors** acting on **nsp15**, a hexameric protein component of the viral replication-transcription complexes that is endowed with immune evasion-associated endoribonuclease (**EndoU**) activity. SAR exploration of these **1,2,3-triazolo fused betulonic acid derivatives** yielded lead molecule **5h** as a strong inhibitor (antiviral EC_{50}: 0.6 µM) of **human coronavirus 229E** (HCoV-229E) replication.

5h blocks HCoV-229E replication



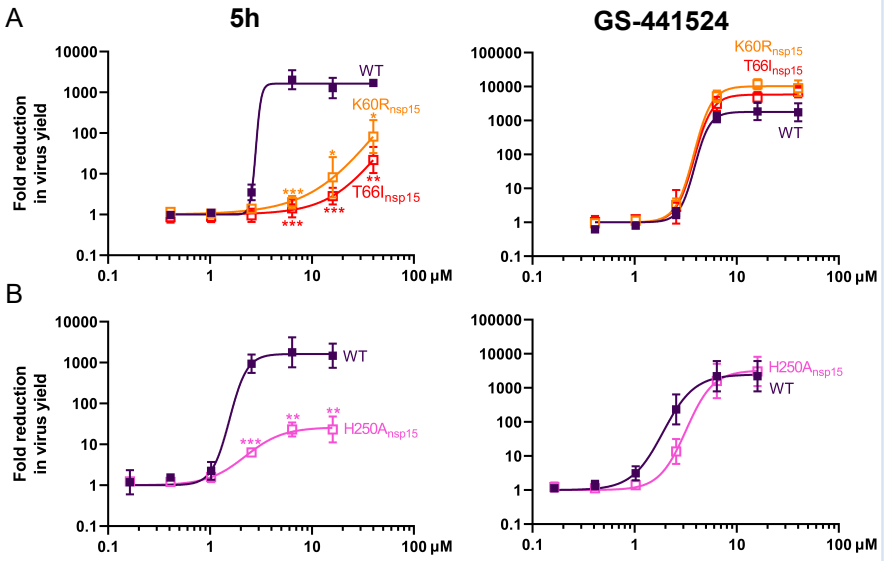
(A) Protection against virus-induced cytopathic effect (CPE) in human embryonic lung (HEL) cells.
(B) % Inhibition of virus-induced CPE or % cytotoxicity in HEL cells, both determined by **MTS cell viability assay**.
(C) Immunofluorescence detection of **viral dsRNA** in HCoV-229E-infected human airway derived 16HBE cells at 24 h p.i. GS-441524, the nucleoside form of remdesivir [2], was included as reference.

5h acts at an early stage in viral RNA synthesis



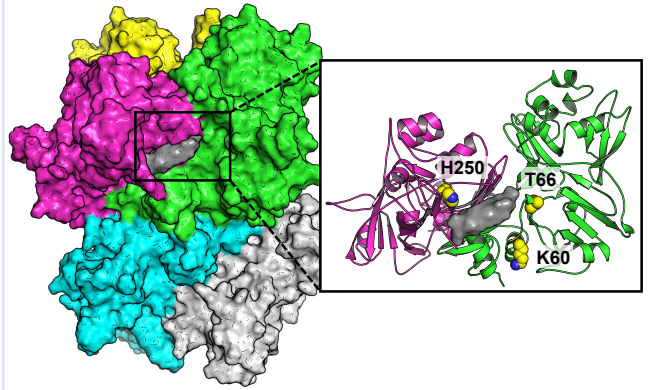
Time-of-addition profile of **5h** and reference compounds **K22** (RNA synthesis inhibitor [3]) and **bafilomycin** (entry inhibitor). Compound addition was delayed until different time points p.i. and viral RNA was quantified at 16 h p.i. **5h** acts **post-entry at an early stage in viral RNA synthesis**.

Mutations in nsp15 confer resistance to 5h



Viruses carrying **mutations in nsp15** are resistant to **5h** (left panels), but not to **GS-441524** (right panels). **(A) 5h-resistant mutants** obtained by **virus passaging** under 5h carry substitution K60R or T66I in nsp15. **(B) EndoU deficient mutant virus**, carrying substitution $H250A_{nsp15}$ and constructed by reverse genetics [4]. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

5h binds at nsp15 dimer interface



Docking model of the **5h** binding pocket, at the **interface of two nsp15 dimers**. **5h** interacts with the catalytic H250 in one monomer, and with K60 and T66 in the neighbouring monomer.

Conclusion

Resistance studies combined with *in silico* analyses established that **5h** targets an **nsp15 dimer interface**. Although **5h** exhibits restricted activity towards HCoV-229E, its chemical scaffold should be amenable to **structure-guided modifications** to broaden its **CoV activity spectrum**. Besides, by identifying a unique mechanism to interfere with CoV replication, our findings set the stage to further explore this **nsp15 pocket** for anti-coronavirus **drug design**.

References

[1] Wen et al. 2007. J. Med. Chem. 50, 4087-95.
[2] Sheahan et al. 2017. Sci. Transl. Med. 9, eaal3653.
[3] Lundin et al. 2014. PLoS Pathog. 10, e1004166.
[4] Kindler et al. 2017. PLoS Pathog. 13, e1006195.