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\textsubscript{50}: 0.6 µM) of human coronavirus 229E (HCoV-229E) replication.

**Introduction**

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.

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 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**