A broad inhibitor of coronavirus replication acting via the nsp15 endoribonuclease

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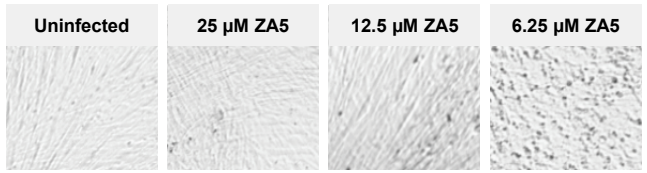
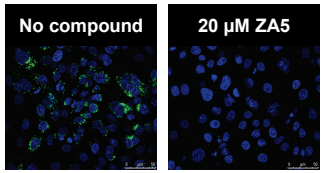
Introduction

The SARS-CoV-2 pandemic proves that pan-coronavirus (CoV) inhibitors are urgently needed, since it is very likely that highly pathogenic CoVs will continue to arise from zoonotic reservoirs. We here report the discovery of a small molecule inhibitor of coronavirus replication, designated ZA5, possessing potent and broad anti-coronavirus activity. ZA5 acts via nsp15, a hexameric endoribonuclease that is part of the replication-transcription complex. Since nsp15 is considered to have a role in CoV replication [1] and immune evasion [2], inhibition of nsp15 may have a dual outcome: a direct suppressive effect on CoV replication and an indirect effect via reversal of viral immune evasion and boosting of host antiviral immunity. This dual pharmacological effect appears an obvious asset to treat CoV infections.

ZA5 shows robust and broad anti-coronavirus activity



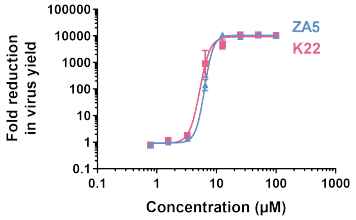
CPE reduction assay with HCoV-229E in human embryonic lung (HEL) cells. ZA5 dose-dependently inhibits the virus, giving full inhibition at 25 µM without any cytotoxicity.



ZA5 prevents dsRNA formation in CoV-infected human airway 16HBE cells.

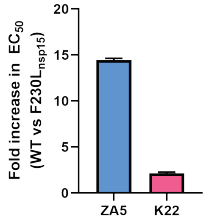
Virus	Cells	Cytotoxicity (µM)	Antiviral activity (µM)	
			EC_{50}	EC_{90}
HCoV-229E	HEL	≥100	9.0	4.1
HCoV-NL63	Vero118	>50	ND	7.0
MHV-A59	L2	>100	ND	5.6
FIPV	CRFK	>100	13	ND
SARS-CoV-2	VeroE6-GFP	>100	9.6	ND

ZA5 inhibits all five CoV strains tested thus far.



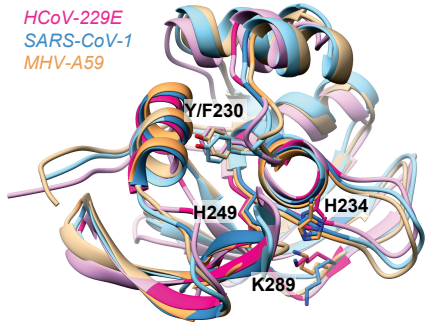
ZA5 produces up to 4-log10 reduction in virus yield, similarly as the reference compound K22 [3].

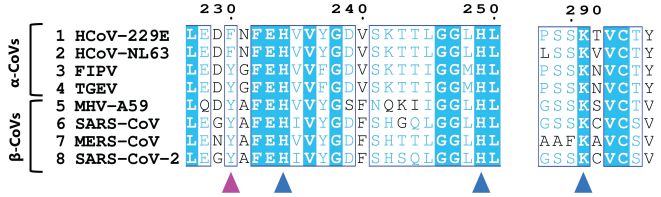
ZA5 acts via nsp15

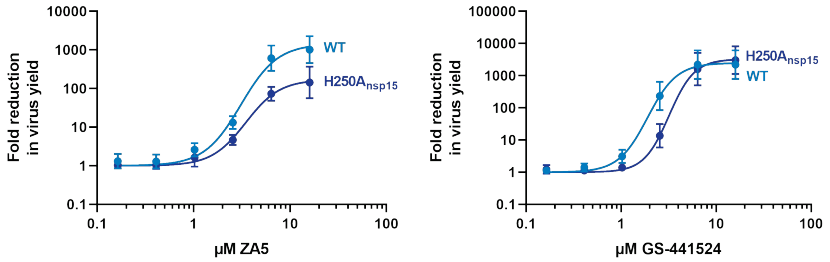


Resistance selection by serial virus (HCoV-229E) passaging indicated that ZA5-resistance is associated with substitution F230L, located near the CoV nsp15 EndoU domain. This mutation resulted in a 14-fold increase in EC_{50} value.

Superposition of nsp15 crystal structures (right) and alignment of c-terminal part of nsp15 (below) showing ZA5-resistance mutation (pink triangle) and catalytic triad (blue triangles). Colored in darker colors are fully conserved residues. The fact that residue 230-F/Y is conserved in all human and animal CoVs aligns with the finding that ZA5 has broad CoV activity.

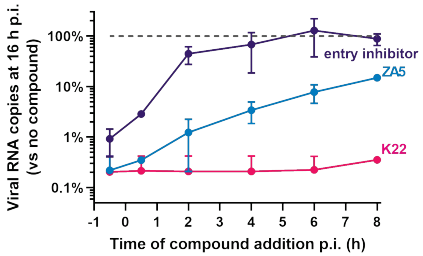






The $H250A_{nsp15}$ catalytic site mutant form of HCoV-229E [2] is 7-fold less sensitive to ZA5, but equally sensitive to the polymerase inhibitor GS-441524. This supports that ZA5 acts via nsp15.

ZA5 executes prolonged inhibition of CoV replication



Time-of-addition profile of ZA5, an entry inhibitor and the RNA synthesis inhibitor K22. ZA5 gradually loses its activity when added after virus infection, suggesting a prolonged action mechanism.

Conclusion

ZA5 is active against SARS-CoV-2 and several other CoVs and therefore qualifies as a pan-CoV inhibitor. Resistance studies showed that ZA5 acts via nsp15, revealing an unexplored druggable target. Structure-aided design with the nsp15 crystal structure is ongoing to further improve the activity of this lead compound.

References

[1] Guarino LA et al. *J Mol Biol* 2005, **353**(5): 1106.
[2] Kindler E et al. *PLoS Pathog* 2017, **13**(2): e1006195.
[3] Lundin A et al. *PLoS Pathog* 2014, **10**(5): e1004166.