Friedrich-Alexander-Universität
Medizinische Fakultät

Orally Bioavailable RORγ/DHODH Dual Host-Targeting Small Molecules with Broad-Spectrum Antiviral Activity

Annual meeting of the Society for Virology 2025

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Retinoic acid receptor-related orphan receptor γ isoform 1 (RORγ1)

Target 1

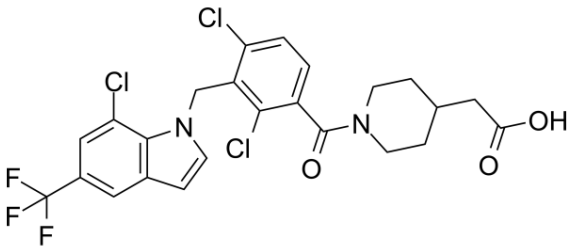
- Nuclear receptor and transcription factor: “master regulator of cholesterol biosynthesis”

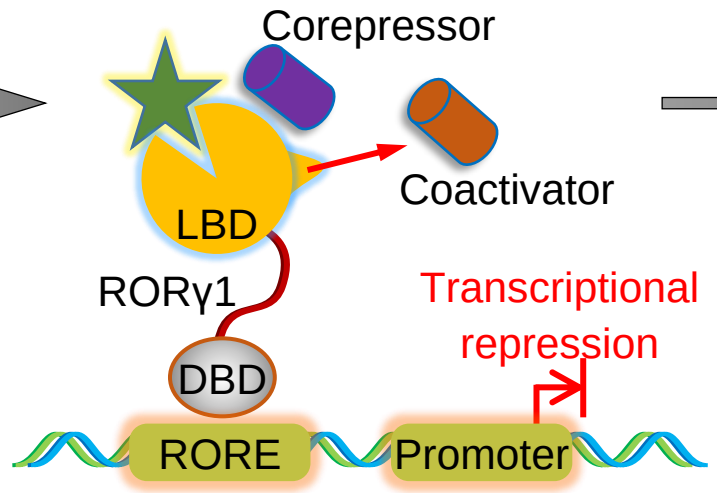
→ Viral replication often depends on cholesterol

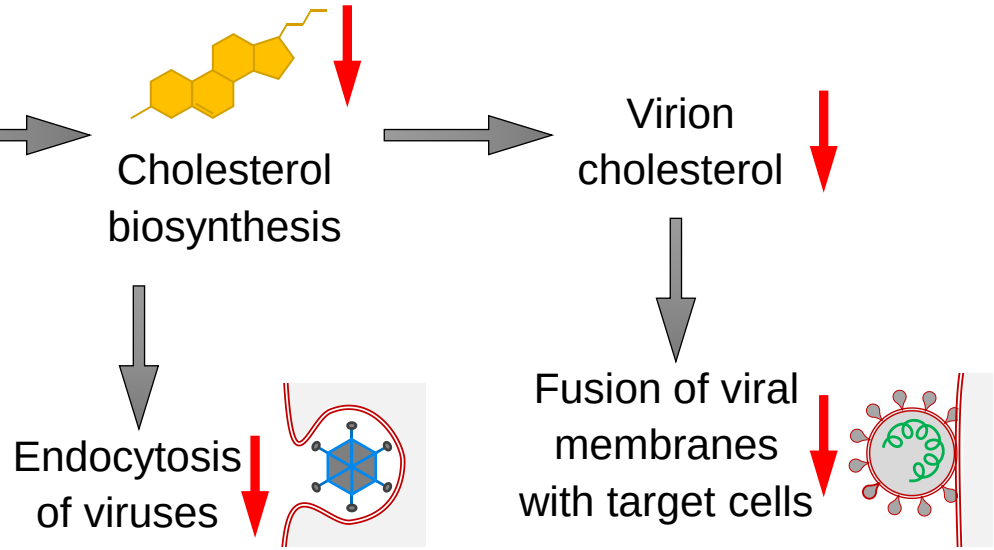
- RORγ inverse agonists induce conformational changes in the ligand binding domain (LBD)

→ Consequently, transcriptional corepressors are recruited and coactivators are displaced

Cedirogant
(inverse agonist,
„inhibitor“)





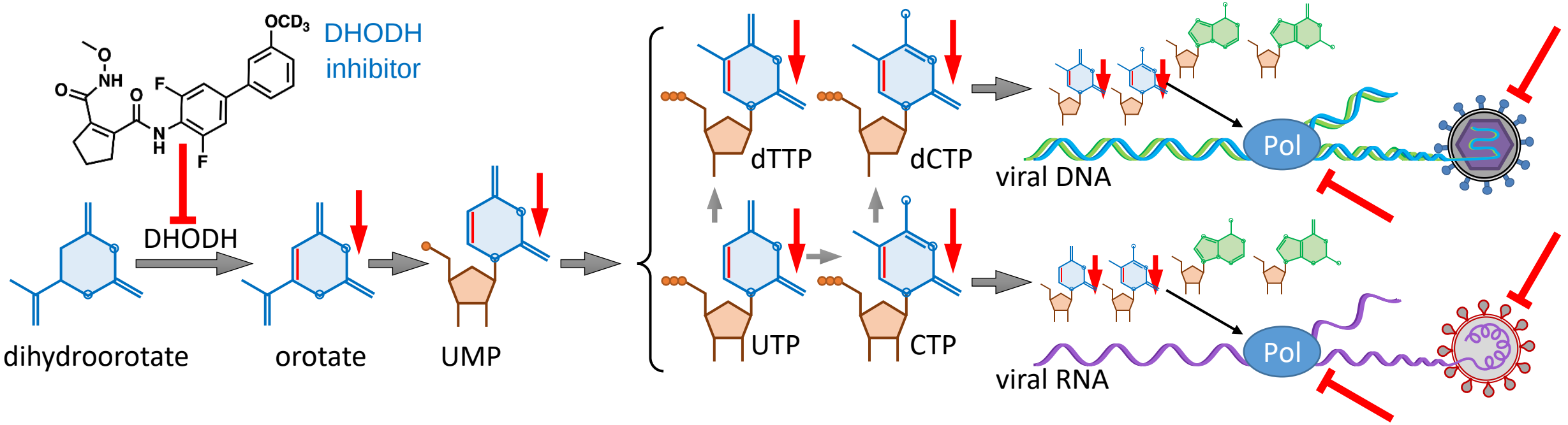


- **Activity against human cytomegalovirus (HCMV) and severe acute respiratory syndrome corona virus type 2 (SARS-CoV-2) in cell culture models presumably by depletion of cellular cholesterol**

Dihydroorotate dehydrogenase (DHODH)

Target 2

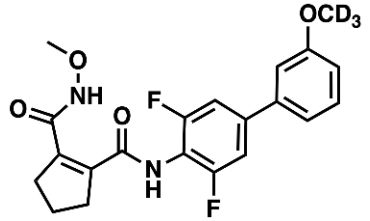
- DHODH catalyzes the rate-limiting step of the *de novo* pyrimidine biosynthesis
- UMP = precursor of pyrimidine (deoxy)nucleotide triphosphates
- …required for viral genome replication



- **Broad antiviral activity in cell culture models by depletion of pyrimidine (deoxy)nucleotide which in turn interferes with viral genome replication**

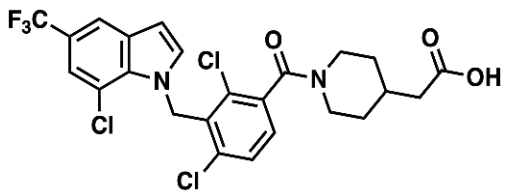
Novel RORγ/DHODH dual inhibitors

DHODH



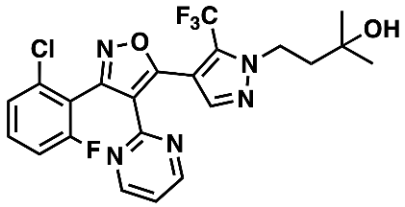
1414

RORγ

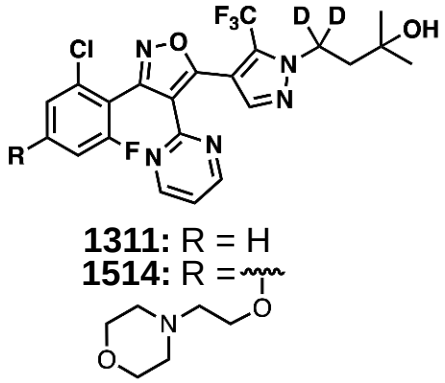


cedirogant

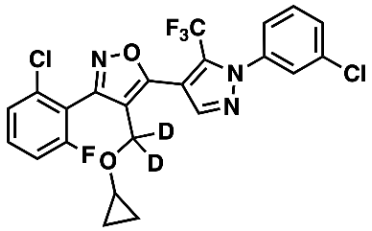
RORγ/DHODH



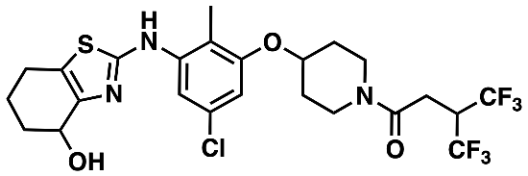
izumerogant



1311: R = H
1514: R =



1404



1797

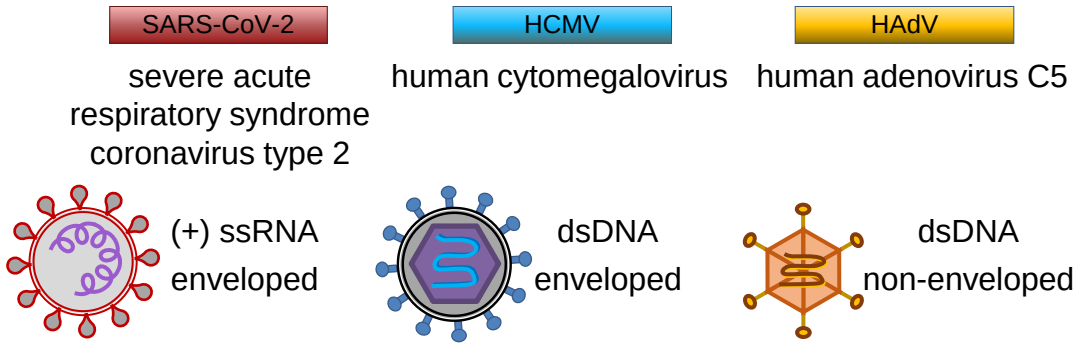
	DHODH (nM)		RORγ (nM)	
1414	150 ± 30	4	3310 ± 822	7
cedirogant	inactive (>100 µM)	7	19.6 ± 7.4	4
izumerogant	98 ± 9	2	10.0 ± 2.8	2
1311	90 ± 6	1	11.9 ± 2.9	3
1514	2200 ± 260	6	952 ± 693	6
1404	100 ± 4	3	9.7 ± 3.6	1
1797	520 ± 40	5	33.4 ± 8.1	5

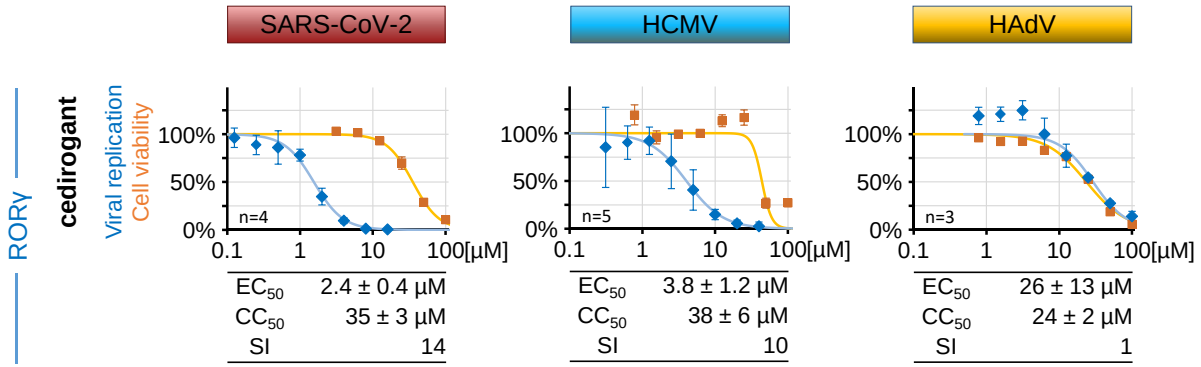
- **1414 and cedirogant are only active on their respective target**
- **Izumerogant, 1311 and 1404 display single target activities comparable to selective DHODH inhibitor 1414 or selective RORγ inhibitor cedirogant**
- **1514 and 1797 display lower activities on both targets**
- **Izumerogant is well-tolerated in humans and reaches peak plasma level of ~1 µM after multiple oral doses (Polasek *et al.*, 2023)**

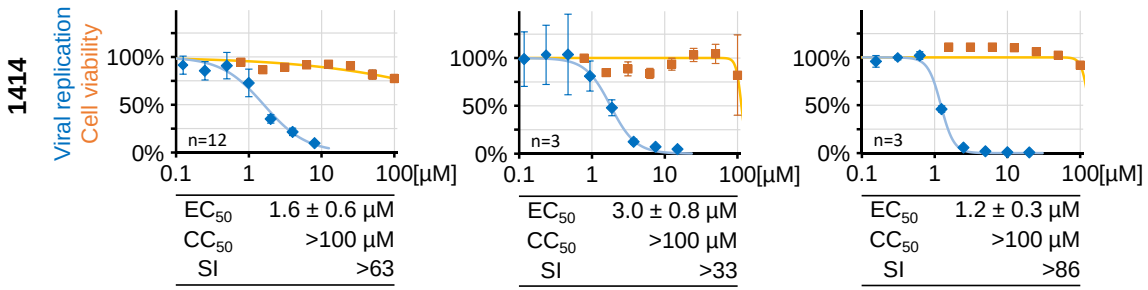
Compounds designed and provided by Christian Gege/Immunic AG

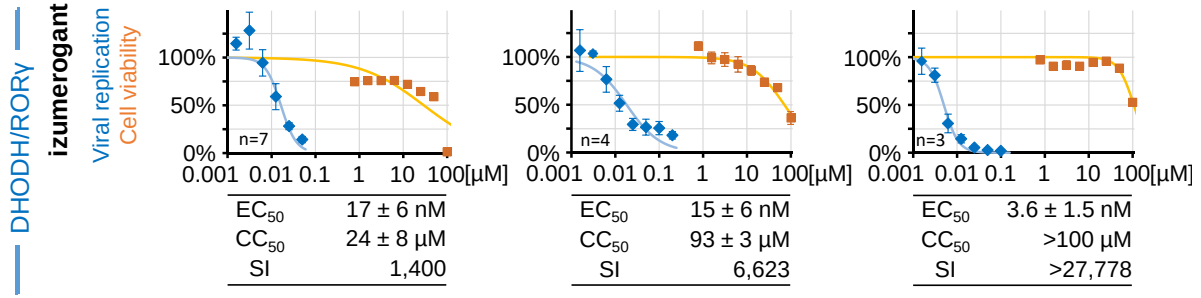
Effect of RORγ/DHODH dual inhibitors on viral replication

Antiviral assays using permissive cells infected with recombinant reporter viruses



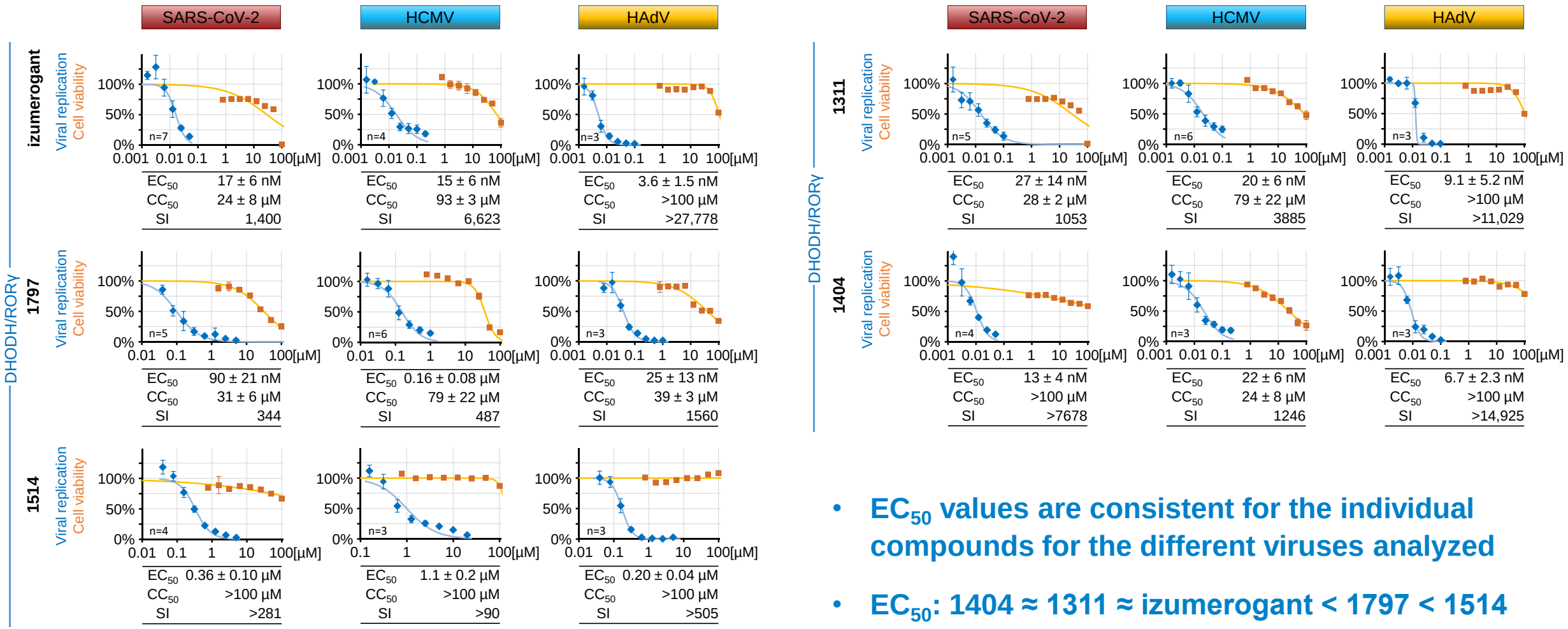






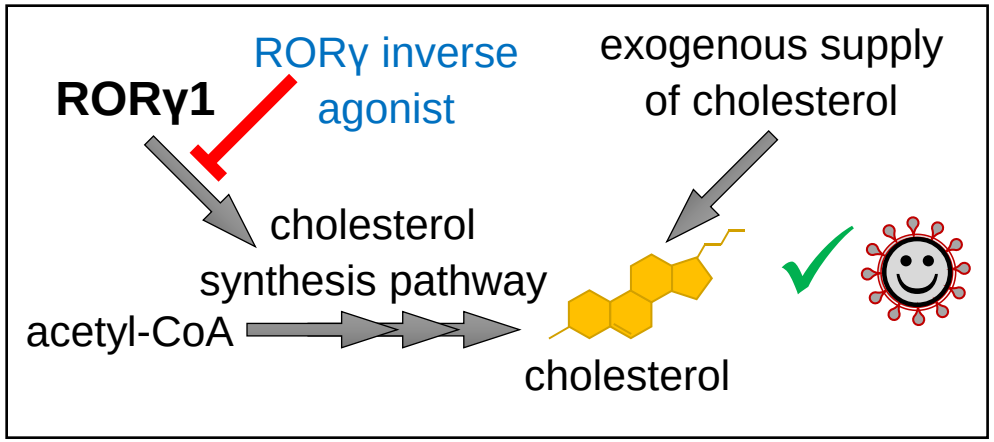
- **1414 inhibits all viruses analyzed**
- **Cedirogant is active against HCMV and SARS-CoV-2, but has no specific activity against HAdV**
- **Izumerogant is highly effective against all three viruses analyzed**

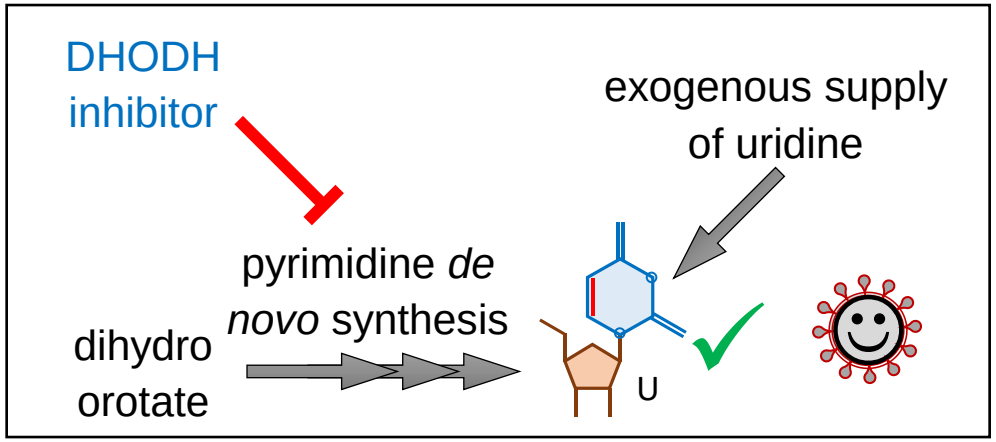
Comparative antiviral analyses of RORγ/DHODH dual inhibitors

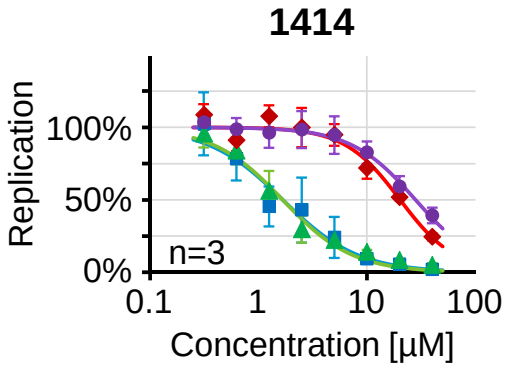


- **EC_{50} values are consistent for the individual compounds for the different viruses analyzed**
- **EC_{50}: 1404 ≈ 1311 ≈ izumerogant < 1797 < 1514**

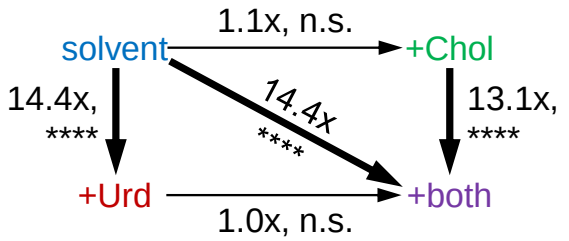
Dissecting the contribution of the single targets of dual targeting inhibitors to the overall activity of dual targeting compounds

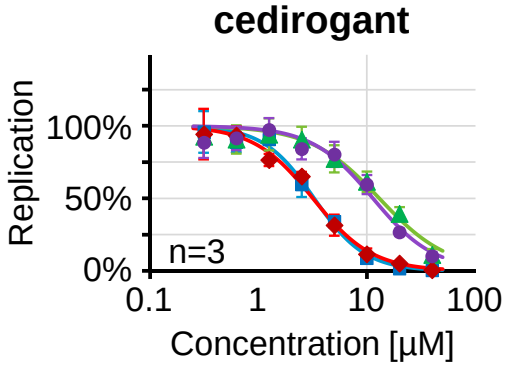




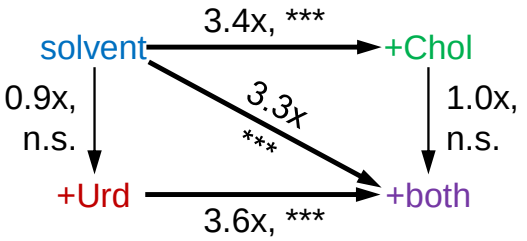


EC_{50}
2.1 ± 1.3 µM
2.0 ± 0.8 µM
19 ± 3 µM
20 ± 5 µM





EC_{50}
3.8 ± 0.7 µM
13 ± 0 µM
3.6 ± 0.9 µM
13 ± 2 µM



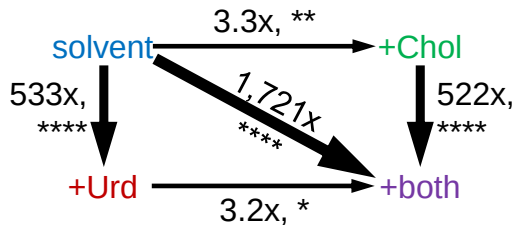
supplement added	EC_{50}	fold EC_{50} increase
solvent	solvent	<1.5x
+Chol	+Chol	1.5x – 10x
+Urd	+Urd	10x – 100x
+both	+both	100x – 1,000x
		>1,000x

- **Cholesterol (+Chol) and uridine (+Urd) supplementation clearly discriminate DHODH inhibitors from RORγ inverse agonists**

Metabolite supplementation on dual targeting compounds

izumerogant

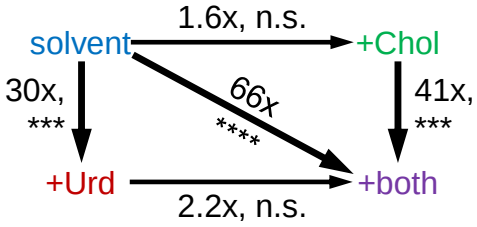
EC_{50}
6.6 ± 2.5 nM
22 ± 5 nM
3.5 ± 0.6 µM
11 ± 1 µM



EC_{50}	fold EC_{50} increase
solvent	
+Chol	→ <1.5x
+Urd	→ 1.5x – 10x
+both	→ 10x – 100x
	→ 100x – 1,000x
	→ >1,000x

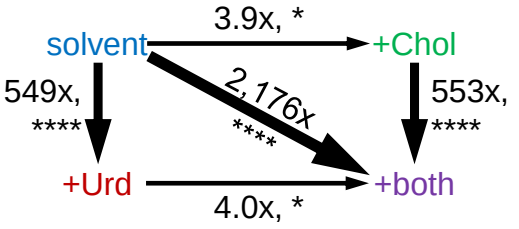
1797

EC_{50}
0.11 ± 0.03 µM
0.17 ± 0.05 µM
3.2 ± 2.5 µM
7.1 ± 2.4 µM



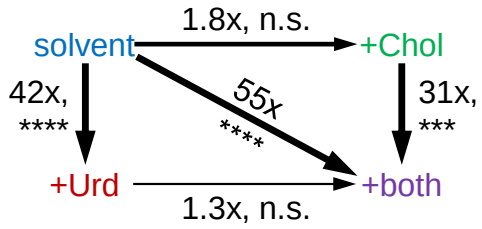
1311

EC_{50}
19 ± 14 nM
42 ± 16 nM
3.7 ± 0.2 µM
14 ± 2.1 µM



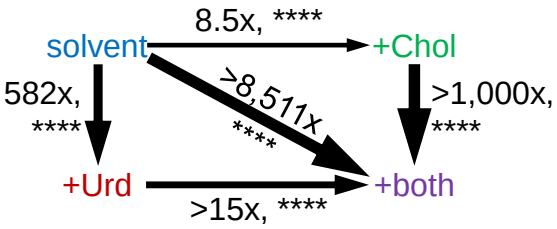
1514

EC_{50}
0.25 ± 0.03 µM
0.45 ± 0.09 µM
10 ± 6 µM
14 ± 7 µM



1404

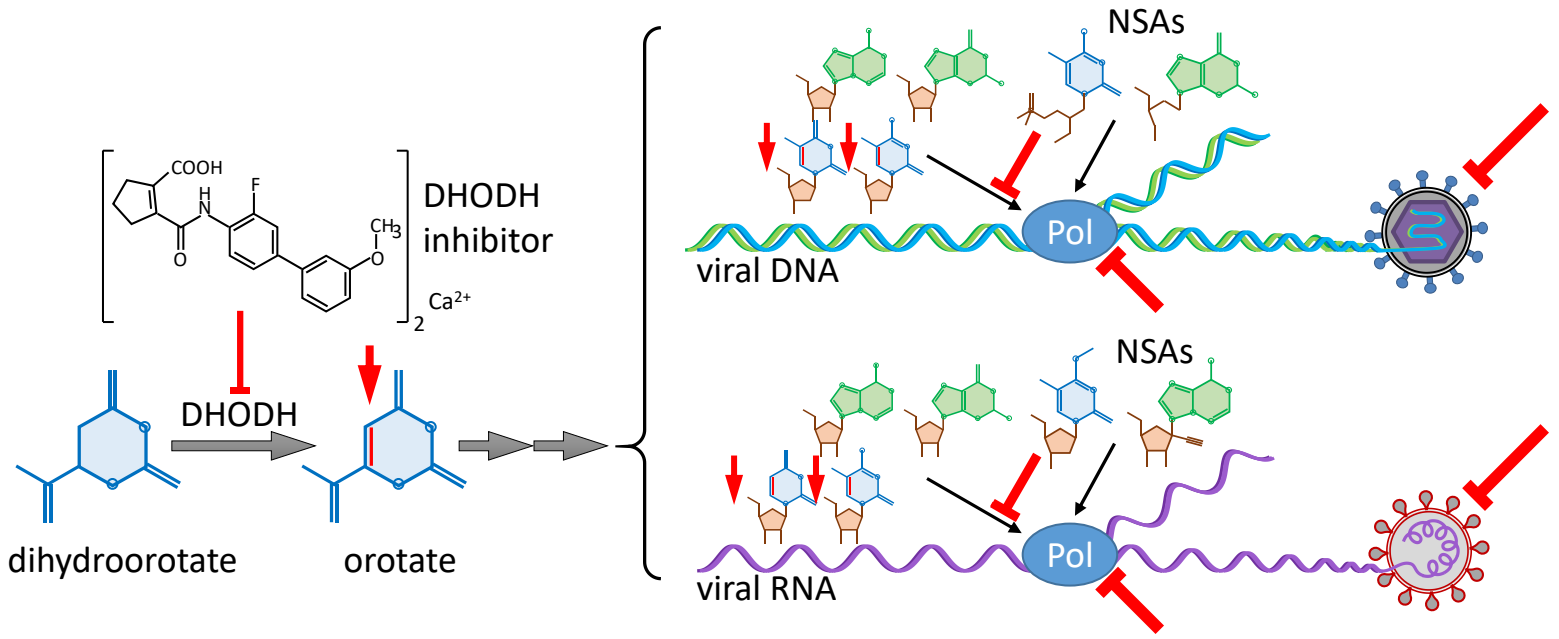
EC_{50}
4.7 ± 0.6 nM
40 ± 10 nM
2.7 ± 0.6 µM
>40 µM



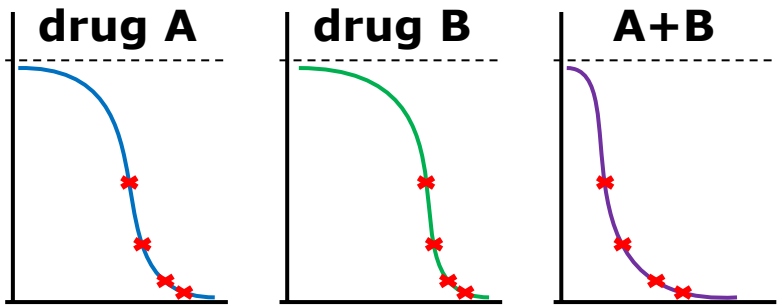
- **Inhibition of both targets contributes to the antiviral effect**
- **Supplementation affects compounds to a variable extent**
- **Effect correlates with the antiviral activity and single target inhibition**

Synergistic drug interaction of DHODH inhibitors with nucleoside analogs (NSAs)

- Synergistic combinations of diverse DHODH inhibitors and NSAs have been described in multiple instances
- **Is this synergism maintained for RORγ/DHODH dual inhibitors?**



- **Loewe additivity fixed dose ratio assay**



A	B	A+B	A+B		
EC_{50}	EC_{50}	EC_{50}	CI_{50}	×0.1	
EC_{75}	EC_{75}	EC_{75}	CI_{75}	×0.2	
EC_{90}	EC_{90}	EC_{90}	CI_{90}	×0.3	CI_{wt}
EC_{95}	EC_{95}	EC_{95}	CI_{95}	×0.4	

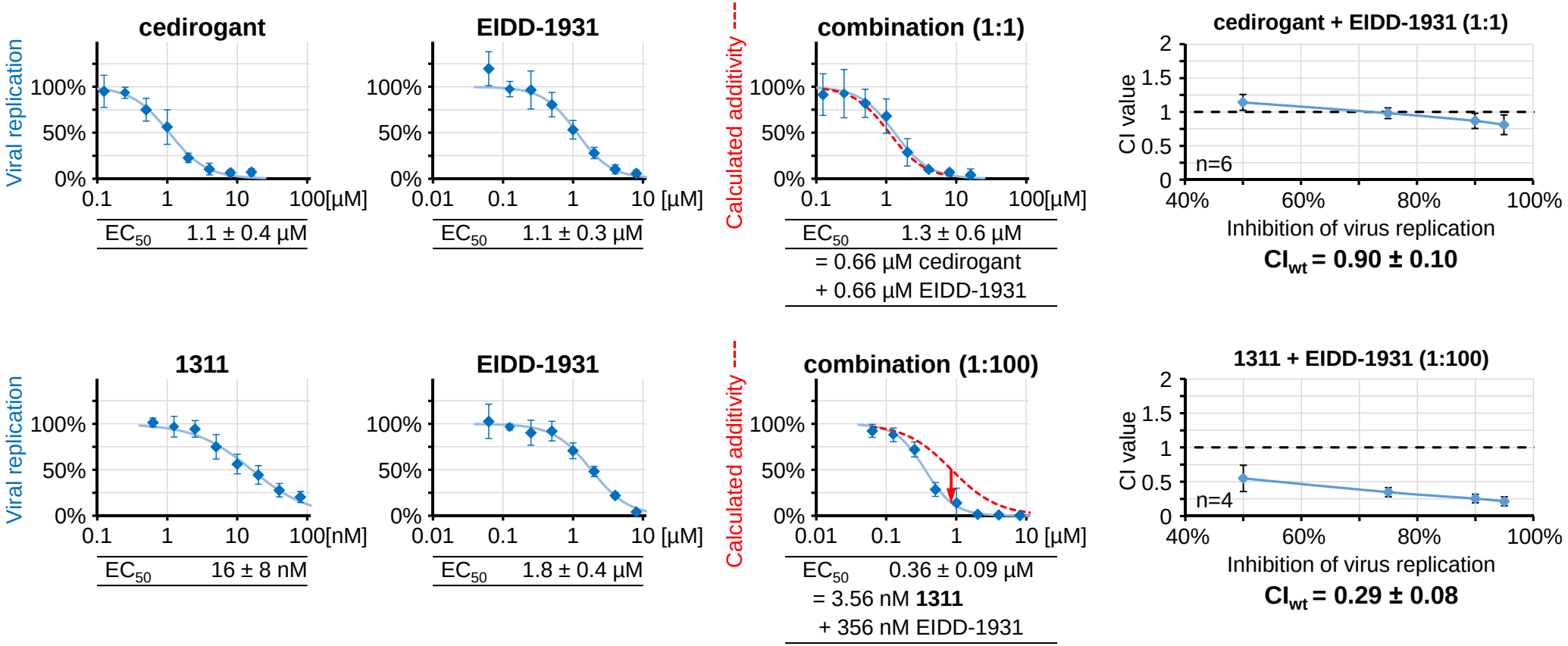
>1.2	antagonistic
0.8-1.2	additive
<0.8	synergistic

CI, combination index

Drug interaction of a RORγ/DHODH dual inhibitor with an nucleoside analog

>1.2	antagonistic
0.8-1.2	additive
<0.8	synergistic

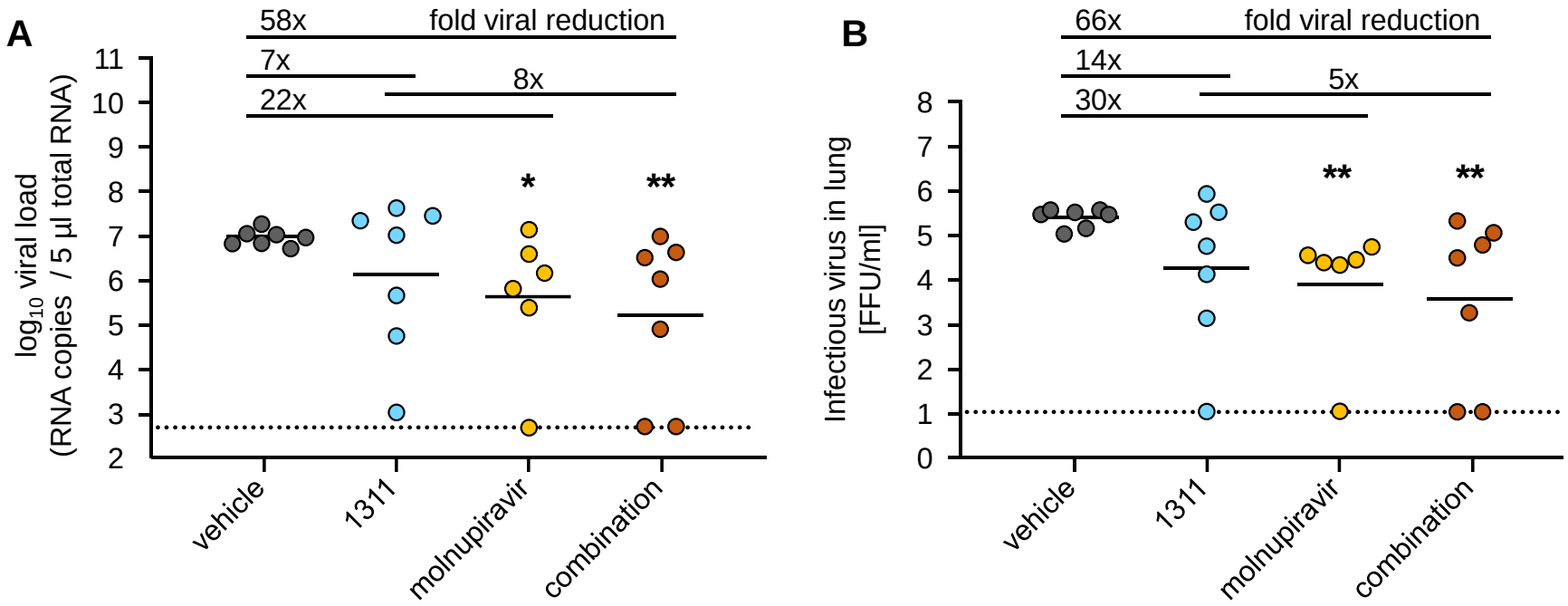
SARS-CoV-2



- **The RORγ/DHDOH dual compound 1311 reveals a synergistic interaction with a nucleoside analog as previously described for selective DHODH inhibitors**

Evaluation of 1311 in an *in vivo* mouse model

- K18-hACE2 mice intranasally infected with SARS-CoV-2, oral administration of drugs twice per day
- Viral load quantitation in the lungs by RT-qPCR and infectious titer determination 4 days post-infection



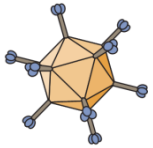
- **1311 reduced viral load in lungs of SARS-CoV-2 infected mice**
- **Combination with molnupiravir further increases efficacy of 1311**

Experiments performed by the Lab of T. Grunwald, Mann-Whitney U-test in comparison to vehicle control

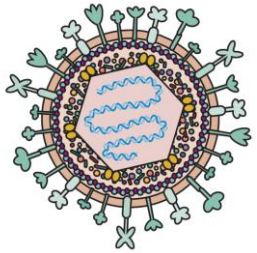
And what about other viruses?

Antiviral EC_{50} values of 1404

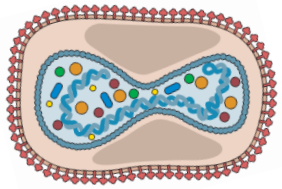
Baltimore I, dsDNA viruses



HAdV (6.2 nM)
non-eveloped

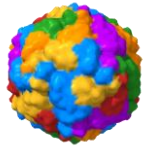


HCMV (22 nM)

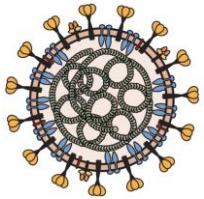


MPXV (3.2 nM)

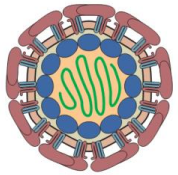
Baltimore IV, ssRNA (+) viruses



HRV-14 (1.0 nM)
non-eveloped

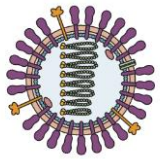


SARS-CoV-2 (13 nM)
CoV 229E (6.5 nM)
CoV OC43 (7.5 nM)

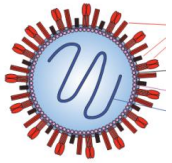


ZIKV (18 nM)

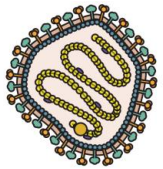
Baltimore VI, ssRNA (-) viruses



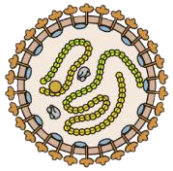
IAV (110 nM)



RSV (3.3 nM)



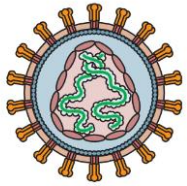
MeV (7.5 nM)



LASV (17 nM)*

* Kim et al., (2020) Viruses, Jul 29;12(8):821.

Baltimore V, retroviruses



HIV-1 (1.2 nM)

- **Broad *in vitro* antiviral activity against RNA and DNA viruses, either enveloped or non-enveloped**

Additional experiments performed by the groups of A. Ensser, M. Tenbusch and K. Sparrer

Summary

- RORγ/DHODH dual compounds reveal potent and broad antiviral activity in cell culture
- The inhibition of both targets, RORγ and DHODH, contributes to the antiviral effect
- Synergistic drug-interaction of **1311** with a nucleoside analog
- Efficacy of **1311** in single and nucleoside analog combination treatment in a SARS-CoV-2 mouse model

➔ **Orally bioavailable RORγ/DHODH dual compounds might have great potential as a strategy for pandemic preparedness**

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Thomas Grunwald, Nadja Uhlig, Valentina Eberlein, Leila Issmail

Thank you for your attention!