IMI2 Project ID 101005077

CARE – Corona Accelerated R&D in Europe

WP3 – WP Hits to leads

D3.3 Optimised Lead 2

Short report

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Document History

Version	Date	Description
V1	20/03/2025	First version

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Motivation

The compound series 08 targeting the Membrane protein of SARS-Cov-2 is close to an "optimized lead" stage based on the criteria as put forward at the start of CARE.

This compound series has been identified in a phenotypic screen performed under the SCORE consortium and was transferred to CARE for further development. Over 950 analogues of this series were made, which resulted in the development of two subseries (subseries C and subseries D). Several lead compounds were developed and showed promising profiles allowing animal proof-of-concept. Subsequently, this series entered the lead optimisation phase and delivered optimised compounds. In the table below, we have included two representative compounds that we believe are close to an "optimized lead" based on the criteria as put forward at the start of CARE, although multiple criteria have not yet been evaluated due to lack of budgets and time restraints.

- CIM573991, a compound from subseries C. It was identified in a lead optimisation process showing significantly improved potency (~25 nM), very good microsomal stability and good activity against SARS-CoV-1. In vivo proof of concept has been demonstrated for this subseries and we have shown no/weak hERG inhibition in the series.

- CIM575729 is a compound from subseries D. This subseries has compounds with in vitro potencies in the 5-10 nM range against SARS-CoV-2 and in the same range against SARS-CoV-1. This particular compound has a good potency (~32 nM), excellent microsomal stability and a broader spectrum of activity, including against OC43 activity.

In the last months of the CARE project, additional compounds were being synthesized in subseries D. We recently started to understand the SAR of the subseries for potentially unlocking broader spectrum of activity. Compounds with low to potentially sub-nM antiviral activity extending beyond the Sarbecovirus family have also already been identified.

Optimized lead criteria

Key Activity	Optimized Lead/Candidate	Details	CIM573991 (subseries C)	CIM575729 (subseries D)
Efficacy (in vitro)				
Activity against COVID-19 (cytopathy HTS assay and direct viral activity assays)	EC50 <100 nM (confirmed activity in >2 cell types, at least 1 human)	B.1.1.7	0.025 µM	0.032 µM
Selectivity over primary host cell line	>1000-fold, (CC50 > 10 µM)	SI	SI > 307	SI > 145
Cellular toxicity (Hek293, HepG2, Huh7, Hela, Vero, MDCK, Calu3 or A459 cells)	CC50 >10uM	A549	7.7 µM	5.7 µM
		Huh7	2.3	0.7
		Hela	24.5	3.5
Clear SAR for series	Clear MoA (in vitro or in vivo)		Achieved	Achieved
EC50 variation for geographical isolates	<10-fold variation	SARS-CoV-2 Wuhan	0,023 µM	Achieved in series
		SARS-CoV-2 BA.5	Achieved in series	Achieved in series
		SARS-CoV-2 B1.1.7	Achieved in series	Achieved in series
		SARS-CoV-2 B1.1.7 + 40%HS	Achieved in series	Achieved in series
EC50 against other coronaviruses	<10-fold variation	SARS-CoV-1	0.242 µM	0.168 µM
		MERS	7.4	>30
		OC43	2.9	0.222
		229E	>50	>50
Frequency of resistance	determined		Low	Low
Mechanims of action	determined		M protein	M protein
Characterisation of resistance mechanism	resistance mutations characterised		Achieved	Achieved
Biochemical activity				
Activity against target protein	Clear MoA (in vitro or in vivo)		Cryo-EM obtained in series	Cryo-EM obtained in subseries
Target selectivity vs. mammalian homolog	>100-fold (preferably >1000 if a mitochondrial target)		No homolog	No homolog
Clear SAR for series			Achieved in subseries	Achieved in subseries
Efficacy (in vivo)				
Animal Model	> 3 log unit reduction of pfu in animal model: dose response completed	log reduction log_{10} $TCID_{50}$/mg lung	Achieved in subseries	Achieved in subseries
DMPK (in vitro)				
Solubility	> 0.1 mg/ml @ pH 2, 6 and 7.4		Kinetic solubility: 5.6 µM	Kinetic solubility: 16.7 µM
FASSIF solubility	> 0.1 mg/ml			
Permeability MDCK, (CaCo2)	>100 nm/sec			
Efflux ratio ((Papp)B-A/(Papp)A-B))	<2			
Mouse/Rat microsomal turnover	< 20 uL/min/mg		17/10	6.2/3.7
Human microsomal turnover	< 20 uL/min/mg		17	<2.3

Mouse/Rat hepatocyte turnover	< 20 uL/min/mg		11/29	27/OG
Human hepatocyte turnover	< 20 uL/min/mg		45	5
Microsomal binding across species	complete		Achieved	Achieved
cLogP	≤ 3.5 (preferably <3)		3.5	3.6
cLogD	≤ 3.5 (preferably <3)		2.9	2.7
Mol Wt	<400		603.75	567.64
Induction: PXR	< 70% Rifampin at 10 uM			
CYP450s (1A2, 2C9, 2C19, 2D6 and 3A4) inhibition	IC50 >10 µM for 5 isoforms		> 10 µM 6 isoforms, 2D6 = 6.8 µM	
CYP450s (Human hepatocytes:1A2, 2B6, 3A4) induction	IC50 >10 µM for 5 isoforms			
CYP450s irreversible inhibition	No irreversible inhibition (<2-fold IC50 shift)			
Drug transporters: P-glycoprotein and OATP1B1	No significant inhibition			
Blood:plasma partitioning across species	Done			
Preliminary metabolism studies (reaction phenotyping)	Indications that more than one enzyme responsible for metabolism. Reduced cDDi risk as victim			
Preliminary metabolite ID (Hepatocytes – preclinical species and man)	Major metabolites identified. No evidence of major unique metabolites in humans			
Protein binding (PD, tox. species and human)	<95%		98f,90%	
Reactive metabolites	No GSH adducts; No metabolism dependent inhibition (CYP3A4)			
Major metabolites identified in tox species and humans	determined			
Simulated gastric fluid, plasma and whole blood stability	80% remaining after 4 hours			
DMPK (in vivo)				
Mouse (PD model) IV clearance	< 50% liver blood flow		9%	
Mouse (PD model) volume of distribution	> 0.5 L/Kg; >2.5L/Kg: consistent with site of action - lungs		4.0	
PD model (mouse) plasma t½	>1 hr		3.3	
PD model (mouse) PO bioavailability	> 30% to support in-vivo mouse studies and anticipated human PK			
Oral bioavailability in rats*	> 30%			

IV Clearance (rat)	< 30% liver blood flow			
Elimination half-life (oral) - rat	> 2 hours			
Oral bioavailability in dogs*	> 30%			
IVClearance (dog)	<30% liver blood flow			
Elimination half-life (oral) - dog	> 3 hours			
Volume of distribution (rat and dog)	Vd consistent with proposed site of action (lungs)			
Tissue distribution	Exposure in relevant tissue compartment demonstrated		Higher concentration in lungs than in plasma for series	Higher concentration in lungs than in plasma for series
PK/PD relationship	PK/PD relationship defined to predict uid, bid or tid requirement. TPP supports tid schedule			
Dose linearity	dose linearity to >30 fold therapeutic dose			
Blood exposure of compound	>(5-10x) EC50		Achieved for subseries	Achieved for series
Toxicity (in vitro)				
Reactive functionality	no structural alerts		No alerts	No alerts
Genotoxicity	no structural alerts			
AMES	Clean in full AMES test (5 strains) w/wo S9 fraction			
in vitro micronucleus test	Clean in in vitro micronucleus test w/wo S9 fraction			
Cardiotoxicity: hERG assay	IC20/efficacious dose Cmax, unbound >30		Achieved in series	Achieved in series
Specificity evaluation on a selected panel	> 100-fold, No major adverse event indicators			
Tolerability	Efficacious dose is at or below the maximum tolerated dose			
CEREP/ kinase panel	No overt inhibition at <10 uM			
Phototoxicity	UV scan			
Chemistry				
Measures of drug-likeness / drug-quality LE			0.24	0.26
Measures of drug-likeness / drug-quality LLE	> 4.5		4.7	4.8
Measures of drug-likeness / drug-quality LELP	< 10		15	14
Stereochemistry	Contribution to activity/toxicity/metabolism established		2 chiral centers of known absolute configuration	2 chiral centers of known absolute configuration
Chemical Tractability	scale up to 100g complete			

Chemical optimization strategy				
IP Status	No IP or license issues		No IP issues/FTO	