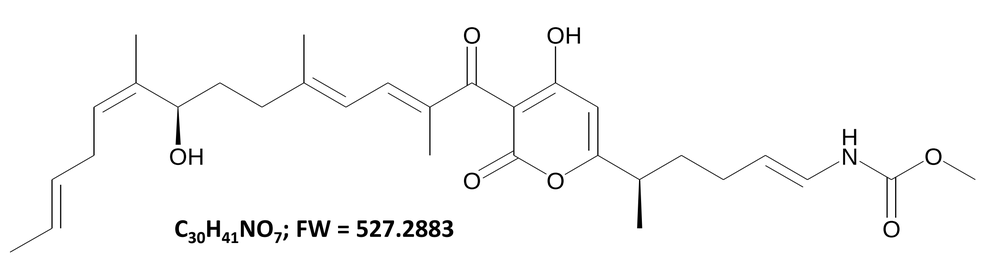


$C_{30}H_{41}NO_7$; FW = 527.2883

Pre-clinical development of the natural compound antibiotic Corallopyronin A

K Pfarr, A Schiefer, A Krome, T Becker, S Kehraus, T Aden, H Neufeld, C Lämmer, R Jansen, G König, M Hübner, S Alt, R Müller, M Unemo, G Bierbaum, W Shafer, B Henrichfreise, K Wagner, T Hesterkamp, M Stadler, A Hoerauf

Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Bonn, Germany; German Center for Infection Research (DZIF), partner site Bonn-Cologne, Bonn, Germany; Department of Pharmaceutical Technology and Biopharmacy, University of Bonn, Bonn, Germany; Institute for Pharmaceutical Biology, University of Bonn, Bonn, Germany; Department Microbial Drugs, Helmholtz Center for Infection Research, Braunschweig, Germany; DZIF, partner site Hanover-Braunschweig, Braunschweig, Germany; Translational Project Management Office, German Center for Infection Research, Braunschweig, Germany; Department Microbial Natural Products, Helmholtz Institute for Pharmaceutical Research Saarland, Saarland, Germany; DZIF, partner site Hanover-Braunschweig, Braunschweig, Germany; WHO Collaborating Centre for Gonorrhoea and Other STIs, Örebro, Sweden; Emory Antibiotic Resistance Center, Emory University School of Medicine, Atlanta, GA, USA; Institute for Pharmaceutical Microbiology, University of Bonn, Bonn, Germany

Abstract

Introduction: Corallopyronin A (CorA) inhibits bacterial DNA-dependent RNA polymerase. With its different mode of action, binding to the switch region, it is effective against rifampicin-resistant *Staphylococcus aureus*. CorA also kills Gram-negative *Wolbachia*, endobacteria of filarial nematodes, depletion of which results in worm sterility and death. Within the German Center for Infection Research (DZIF, www.dzif.de) we are developing CorA to treat filarial infections.

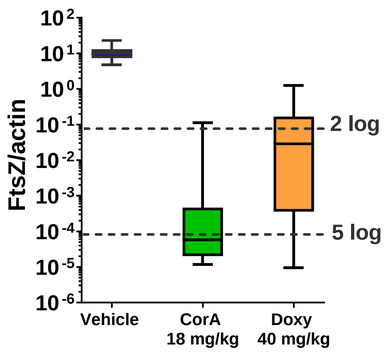
Methods: CorA has successfully completed *in vitro* and *in vivo* efficacy studies and most standard *in vitro* and *in vivo* non-GLP ADMET studies.

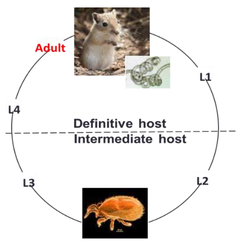
Results: CorA does not alter the expression of CYP450s and CYP3A4 induction via PXR is eight-fold lower than rifampicin. CorA has protein binding comparable to ibuprofen and is stable in plasma >240 min. Human and dog microsomes metabolize CorA slowly: $t_{1/2}$ >45 min (phase I reactions producing oxidation metabolites, minimal glucuronidation). The CorA EC_{50} for three off-target hits (A3 and PPARγ receptors, COX1 enzyme) are 170-1500-fold higher than the CorA *in vitro* effective concentration against *Wolbachia*. CorA does not inhibit hERG. No chromosomal damage in human lymphocytes was observed and CorA was AMES test negative. These results indicate that CorA is non-toxic and pharmacologically safe. Oral formulations using the amorphous solid dispersion principle increased the stability (>95% after 3 months at 30 °C) and solubility of CorA that are well tolerated by rodents and canines. We have shown that CorA has efficacy against active *Chlamydia trachomatis* infection and now demonstrated that it also impairs penicillin induced persistent *C. trachomatis*. CorA also has activity against the WHO and CDC panels of antimicrobial resistant *Neisseria gonorrhoeae*. The frequency of mutation in *N. gonorrhoeae* is low and no spontaneous mutants at 4X MIC have been selected. The frequency of mutation to resistance in *S. aureus* (strain HG001) is significantly lower (2.8 - 4.4-fold) than the rate determined with rifampicin.

Conclusions: CorA is a novel solution to several Global Health targets in the UN Sustainable Development Goals and WHO Priority Pathogen List requiring new antibiotics. We have funding from DZIF, BMBF and the EU to finalize the pre-clinical package, including formulation development and *in vivo* toxicity in two species to guide planning of the regulatory conform GLP studies. The manufacturing protocol - heterologous expression in *Myxococcus xanthus* and optimized DSP – has been transferred to a Contract Manufacturing Organization for up-scaling to produce pre-GMP and cGMP-grade material for the GLP studies.

Efficacy

➢ *Wolbachia* endosymbionts of filarial worms

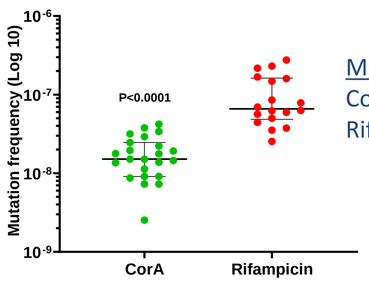




Depletes >98% of *Wolbachia* blocking development and killing worms

➢ *Staphylococcus aureus*

Strains	Characteristics	MIC [µg/ml]
HG001	Lab strain	0.25
Mu50	MRSA, VISA +Rif resistance	0.25
N315	MRSA	0.25
ATCC 33591	MRSA	0.125



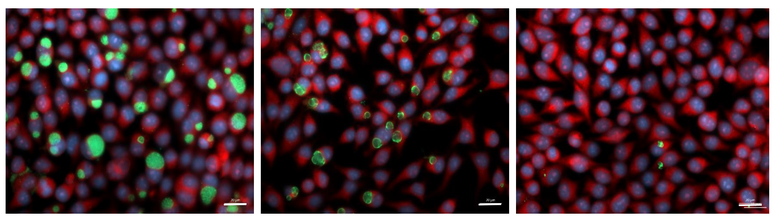
Mutation frequency:
CorA: $3.8x10^{-8}$ (mutations in *rpoB* or *rpoC*)
Rifampicin: $1.3x10^{-7}$

➢ Sexually transmitted infections (STIs)

Chlamydia trachomatis

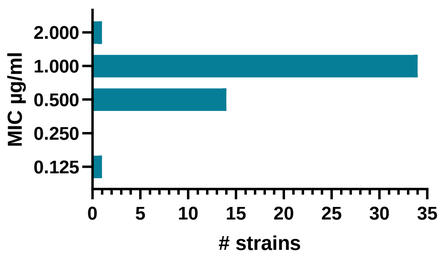
Penicillin-induced persistent infection

Control | **Mock treatment** | **CorA (2 µg/mL)**



➢ CorA is active against persistent *C. trachomatis*

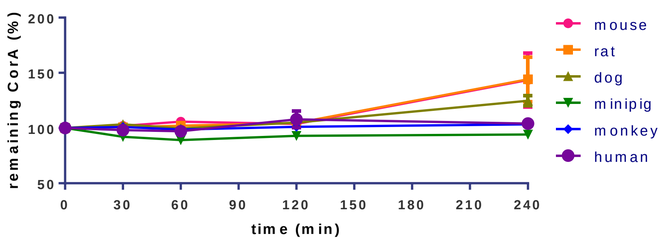
Neisseria gonorrhoeae



➢ *N. gonorrhoeae* are susceptible to CorA MIC 0.5-1 µg/ml
➢ No spontaneous resistance seen in *N. gonorrhoeae* at 4X MIC. Predict a rate of $<10^{-10}$ (*S. aureus* rate is 10^{-8})

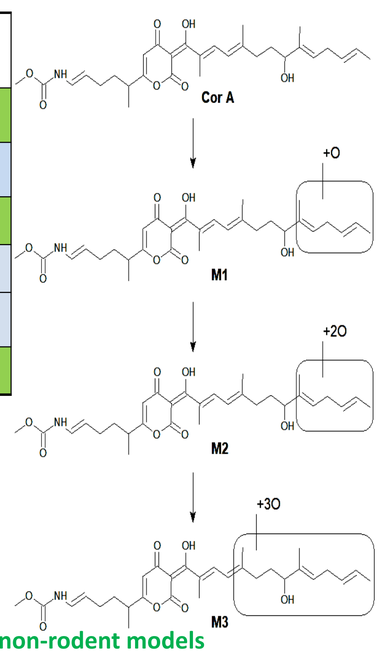
ADME

CorA plasma stability



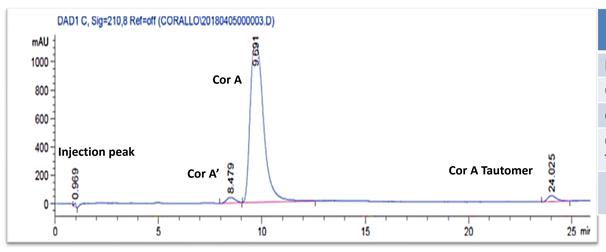
CorA metabolism in microsomes

Species	Intrinsic CL (µL/min/mg protein)	t ½ (min)	Identified Metabolites (45min)
Mouse	84.4	32.9	CorA, M1, (M2)
Rat	220.7	12.6	CorA, M1, (M2)
Dog	42.0	>45	CorA, M1
Mini-pig	1602.7	1.7	M1-M4
Monkey	146	19	CorA, M1, (M2)
Human	35.2	>45	CorA, M1



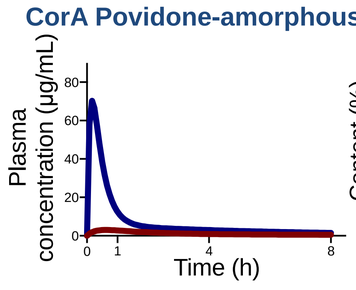
➢ Mouse and dog are best rodent and non-rodent models
➢ Metabolized via phase I reactions

CorA purity after new DSP

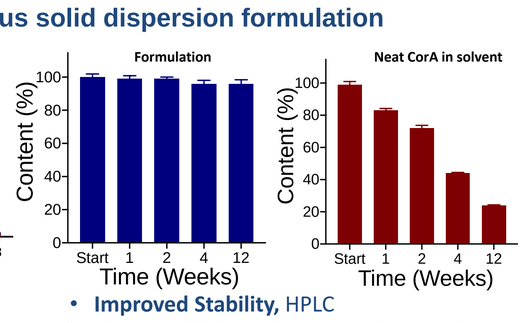


Peak	RetTime [min]	Area %	*In vitro* EC_{50}
Injection	0.97	0.289	--
CorA'	8.48	2.144	0.622 µM
CorA	9.69	95.176	0.013 µM
CorA Tautomer	24.03	2.391	
	Total CorA	97.6%	CorA + Tautomer

CorA Povidone-amorphous solid dispersion formulation



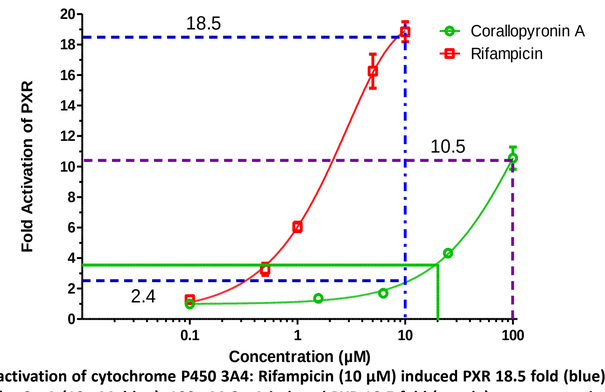
- **Improved Bioavailability**
  PK Study in BALB/c Mice
  ➢ Oral bioavailability 59%
  vs 11% of neat CorA



- **Improved Stability,** HPLC
  ➢ Stable (>95%) during test period of 12 weeks at 30 °C/ 65% RH vs >24% of neat CorA

Toxicology

Induction of CYP450 3A4



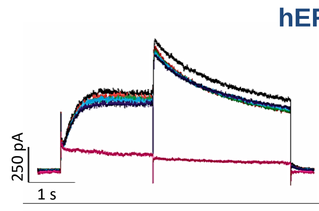
PXR activation of cytochrome P450 3A4: Rifampicin (10 µM) induced PXR 18.5 fold (blue) vs. 2.4 fold for CorA (10 µM, blue). 100 µM CorA induced PXR 10.5 fold (purple), a concentration >500-times the effective *in vitro* concentration 189.5 nM. Mouse PK studies measured 21.8 µM CorA in plasma that might result in a 3.5 fold activation of the PXR receptor (green line) *in vivo*.

➢ Drug-drug interactions similar to rifampicin are not expected

Toxicity studies

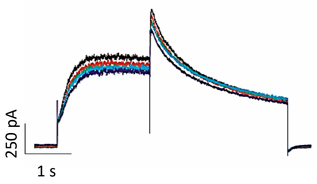
- **Micronucleus:** no chromosomal damage up to cytotoxicity [100 µg/ml]
- **AMES:** no evidence for genotoxicity [0.1-1000 µg/ml]
- **Liver toxicity:** no toxicity in rat and human hepatocytes [200 µM]
- **Phototoxicity:** no phototoxicity up to the limit of solubility [38 µM]

hERG Assay



Cell: ctrl_E8-7
Bath Solution (Black)
0.5% DMSO:
- time-matched with 1st application (Red)
- time-matched with 2nd application (Green)
- time-matched with 3rd application (Blue)
- time-matched with 4th application (Purple)

100nM E-4031 (Pink)



Cell: CorA_E15-14
Bath Solution (Black)
0.3 µM CorA (Red)
1 µM CorA (Green)
3 µM CorA (Blue)
10 µM CorA (Purple)

➢ Inhibition <6% at 10 µM
➢ Predicted IC_{50} > 10 µM

Off-target profiling

Inhibition or stimulation	Receptor / Enzyme	Function of the receptor	Location	Other drugs with same target
55.5 %	A3 Adenosine receptor	Intracellular signaling, CNS	Lung, liver	Drugs against rheumatoid arthritis
83.7 %	PPARγ Peroxisome proliferator-activated receptor	Regulates fatty acid storage and glucose metabolism	Adipose tissue, colon, macrophages	Drugs against diabetes, obesity, atherosclerosis, cancer
53.8 %	COX1 Cyclooxygenase 1	Prostaglandin biosynthesis	In all tissues	Aspirin, ibuprofen
27.8 %	Cl^- channel GABA-gated	Most important inhibitory receptor in the CNS - various functions	CNS	Benzodiazepines, barbiturates, anesthetic steroids
30.8 %	I_2	Uncertain – modulatory function on monoamine oxigenases	Brain (neurons and astorcytes)	In Phase II/III development for chronic pain, neurological diseases

71 receptors and 27 enzymes were incubated with 1 µM CorA. Inhibition/stimulation was calculated. CorA significantly altered (>50%) the activity of 3 proteins (orange background) and weakly altered (25-50%) the activity of 2 (blue background). The IC_{50} values of these receptors / enzymes are 170-1500 fold higher than the IC_{50} of CorA against *Wolbachia* endobacteria *in vitro*.

➢ CorA has no relevant safety issues

Future plans

- PK/PD from Murine Thigh Infection Model (*S. aureus/N. gonorrhoeae*)
- Determine activity/toxicity of CorA metabolites
- Feasibility study, CorA production with GMP- certified CMO
- Dose range finding to identify the maximal tolerated dose in rats
- GLP safety pharmacology and *in vivo* toxicology in rodents and dogs
- Plan phase 1 trial with advice from BfArM

Achievements
- US Patent: US 9168244 B2, granted 2015 (treatment of filariasis)
- US Patent: US 9687470 B2, granted 2017 (prevention of filariasis)
- EU Patent: EP 2704708 B1, granted 2017 and validated in: DE, GB, NL, CH, IT, ES, FR and HR
- Pat. Pend.: WO 2014/181000 A1 (heterologous production)

Third party funding
- BMBF - Wirkstoffentwicklung auf Basis von Naturstoffen zur Bekämpfung von Infektionskrankheiten – "Corals"
- This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 815628

Publications
- Krome et al. (2020) Pharmaceutics 12
- Schiefer et al. (2020) PLOS NTD 14
- Loeper et al. (2019) Front Microbiol 10
- Shima et al. (2018) Int J Antimicrob Agents 52
- Kock et al. (2018) Antimicrob Agents Chemother 27
- Schäberle et al.(2015) J Nat Prod 78
- Schäberle et al. (2014) Int J Med Microbiol 304
- Schiefer et al. (2012) J Infect Dis 206

Contact: Prof. Achim Hoerauf (Achim.Hoerauf@ukbonn.de); Dr. Kenneth Pfarr (Kenneth.Pfarr@ukbonn.de)