Discovery of novel antimalarials through cell-based medicinal chemistry optimization of HTS hits

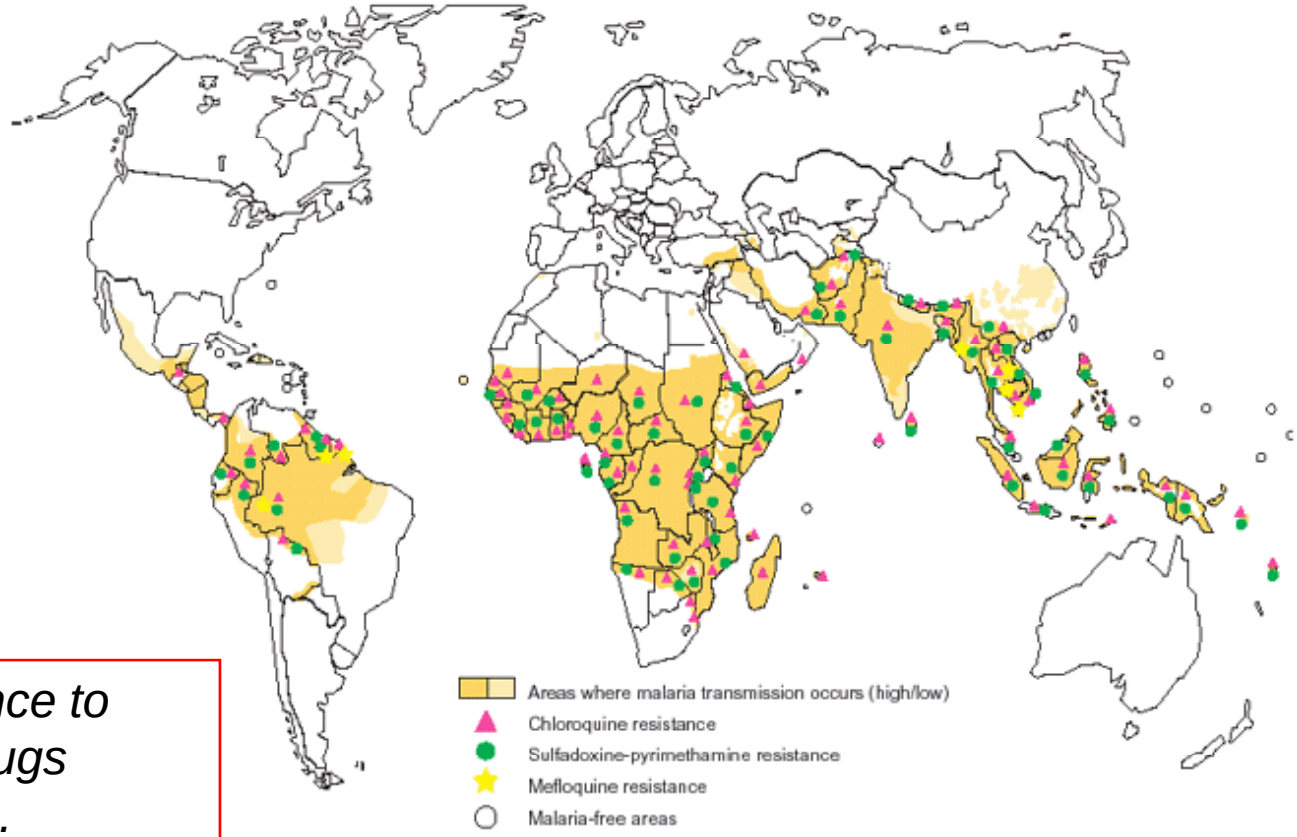
What is GNF?

–Funded by the Novartis Research Foundation
–Moved into a temporary 40,000 square foot laboratory facility in 3Q 1999
–Moved into permanent 260,000 square foot research campus in 1Q 2002
–Additional 24,000 square foot manufacturing facility
–Present headcount ~580 FTE
–Currently, up to preclinical toxicity studies done at site, integrated with Novartis development

Malaria: Medical Need

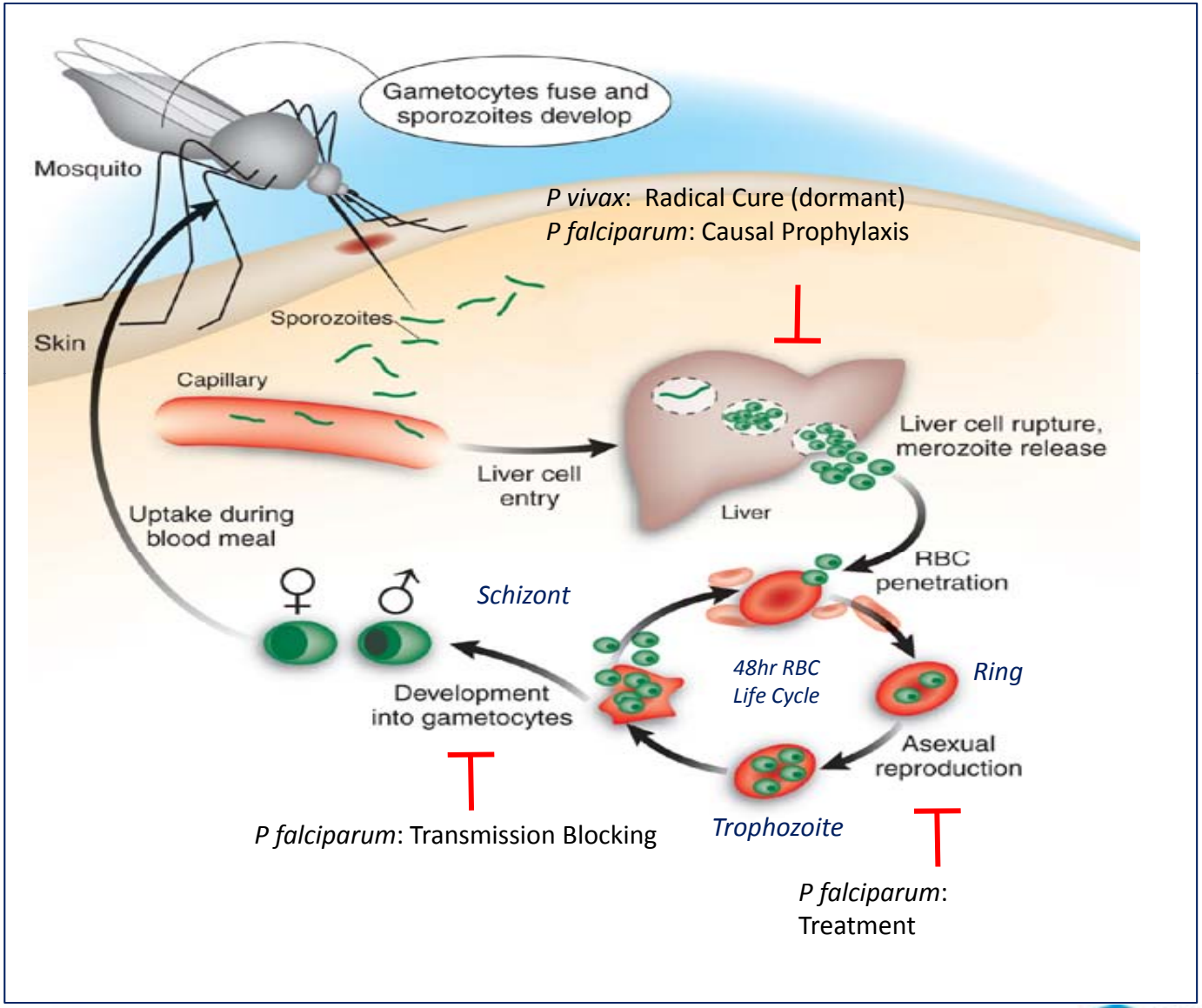
Growing resistance demands NCEs

– 500 million cases annually worldwide
– At least 1 million deaths per year, >75% under age of 5
– Resistance to current therapies widespread with exception of artemisinin derivatives
– Current artemisinin-based combination therapies (ACTs) contraindicated in 1st trimester



Drug resistance to marketed drugs widespread…

Complex Life-Cycle of the Parasite



Scope and Scientific Approaches

GNF scope is to use technology to develop novel assays with high throughput as well as translational component to develop novel antimalarial chemotypes through multiple parallel approaches

- **Cell-based screen approach**
  - Majority of anti-infectives discovered through cell-based screening by facilitating parallel interrogation of druggable targets and also addresses compound permeability issues
  - HTS on >2M compounds: *P. falciparum* infected human RBCs (proliferation inhibition)
  - Liver–stage infection assay (*P. vivax* infection)
- **Target identification methods**
  - Lab-evolved resistant strains upon compound treatment
  - Tiling array analysis and whole-genome sequencing to help MoA determination
  - Affinity chromatography/proteomics analysis
- **Target-based screen approach**
  - Plasmodium kinases: such as CDPK1, CDPK5, GSK3, CK2α
  - Additional biochemical targets screening at GNF on cell-active compounds

The NGBS consortium

- **Novartis Institute for Tropical Diseases (NITD)**, Singapore
  - Team leaders: Bryan Yeung, Zou Bin, Christophe Bodenreider
- **Genomic Institute of the Novartis Research Foundation (GNF)**
  - Team leaders: **Arnab Chatterjee, Kelli Kuhen and Elizabeth Winzeler**
- **Biomedical Primates Research Center (BPRC)**, Rijswijk (NL)
  - Team leader: Clemens Kocken
- **Swiss Tropical Institute (STI)**, Basel (CH) (now named Swiss TPH)
  - Team leader: Matthias Rottmann

- **The NGBS (NITD, GNF, BPRC, STI) consortium**
  - Program Head: Thierry Diagana (NITD)

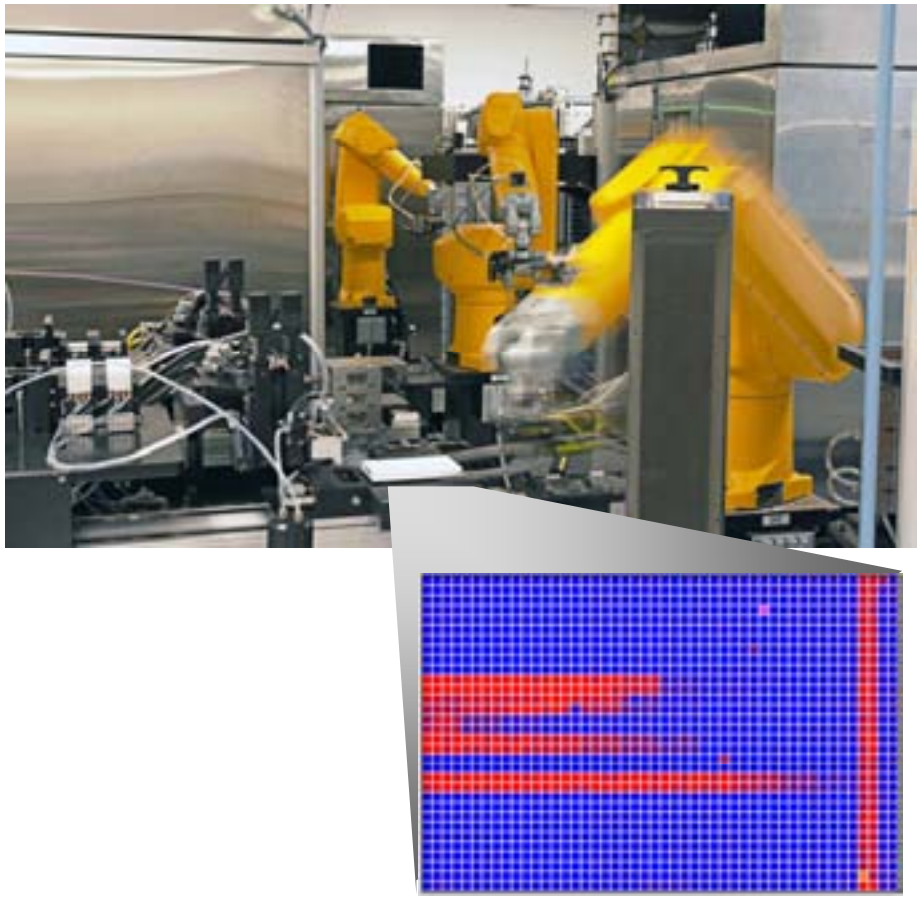
- NGBS is funded by:

wellcome trust

Medicines for Malaria Venture

EDB SINGAPORE

GNF Malaria Cell-based Screening Summary



- > 2M compounds screened at 1.25 μM (3d7)
- 4851 hits < 1.2 μM EC_{50} vs W2 and/or 3d7
  - "Malaria Box" public resource for malaria research community
  - https://www.ebi.ac.uk/chembldb/index.php/compound
- 1,256 < 200 nM EC_{50} vs 3d7
- >200 scaffolds represented in the reconfirmed compound set

Tremendous amount of chemical diversity from screen

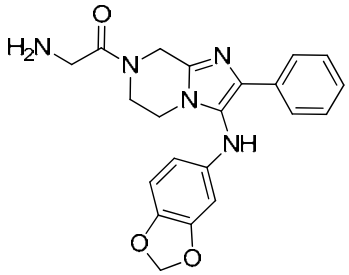
HTS reported in: Plouffe and co-workers *PNAS* **2008**, 9059.

Selection criteria for cell-based HLO at GNF

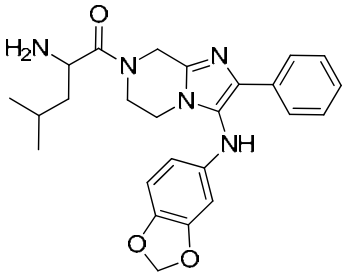
- ~5K reconfirmed blood-stage hits from HTS identified for further evaluation were determined by
  - Less than 1.25 µM EC_{50} with activity in 15 stain resistance panel
    - Less than 5-fold potency shift between strains
  - High selectivity in 6-cell line toxicity panel (SI > 20-fold)
  - Novel chemotype for malaria
  - Ease of synthesis (less than 7 steps)
  - Good solubility, metabolic stability and limited CYP450 inhibition
  - Multiple actives within a scaffold (if library diversity allows)
    - OPI algorithm used for clustering (developed at GNF) Yan, *et al. J. Chem. Inf. Model.* **2005** *45*, 1784 -1790
    - Integrates SAR information into analysis of HTS data to prioritize interesting singletons as well as lower activity compounds that have good SAR from the screen

This presentation will focus on 1 series prioritized from this clustering

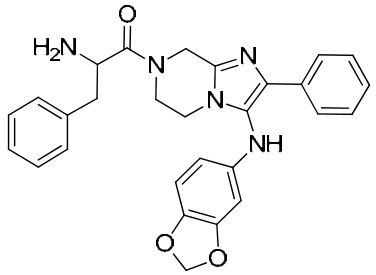
Imidazolopiperazines Hit series



GNF-Pf-5069



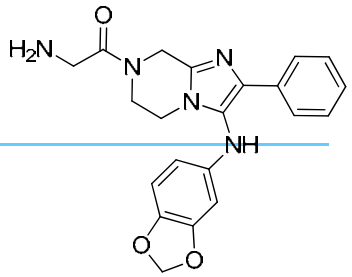
GNF-Pf-5179



GNF-Pf-5466

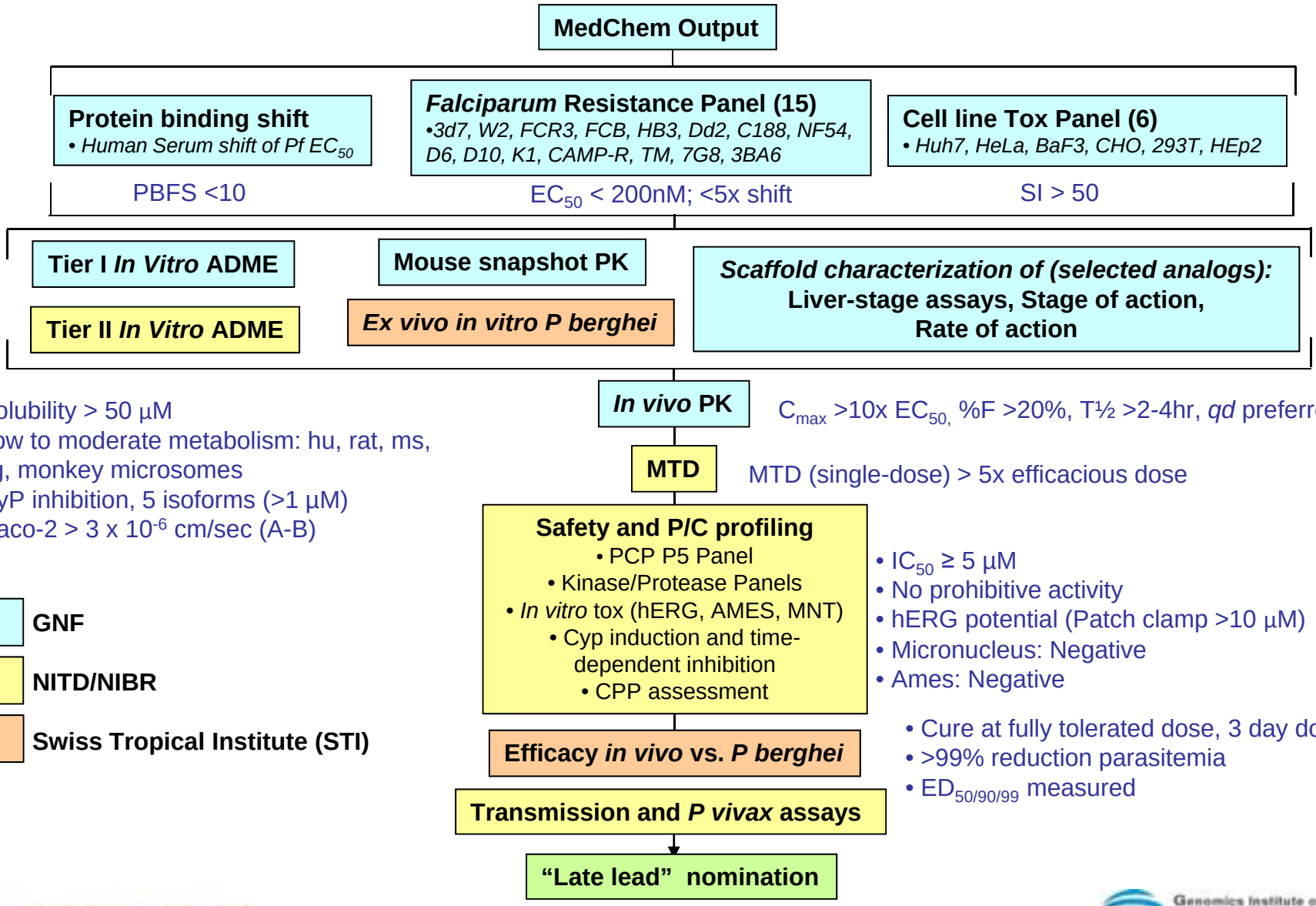
EBI ID	HTS W2 EC_{50} (µM)	HTS 3D7 EC_{50} (µM)	HTS Huh7 EC_{50} (µM)	Powder W2 EC_{50} (µM)	Powder 3D7 EC_{50} (µM)	Powder Huh7 EC_{50} (µM)
GNF-Pf-5069	0.097	0.063	>10	0.198, 0.473	0.161, 0.46	>100
GNF-Pf-5179	0.271	0.235	>10	0.122	0.119	>79
GNF-Pf-5466	0.119	0.116	>10	0.029	0.030	42.82

Imidazolopiperazines Hit Series



- Strengths
  - Unique scaffold for malaria, no biological activity annotation with structure
  - 4 step synthesis
  - Very good solubility; no activity on Cyp450s
  - Not identified in >100 HTS screens performed at GNF
- Issues
  - Moderate *in vitro* potency
  - *In vivo* snapshot PK: poor oral exposure
    - Likely due to catechol and glycinamide
  - hERG inhibition (35% at 10 μM, patch clamp)

Compound Progression Scheme



- Solubility > 50 µM
- Low to moderate metabolism: hu, rat, ms, dog, monkey microsomes
- CyP inhibition, 5 isoforms (>1 µM)
- Caco-2 > 3 x 10^{-6} cm/sec (A-B)

- IC_{50} ≥ 5 µM
- No prohibitive activity
- hERG potential (Patch clamp >10 µM)
- Micronucleus: Negative
- Ames: Negative

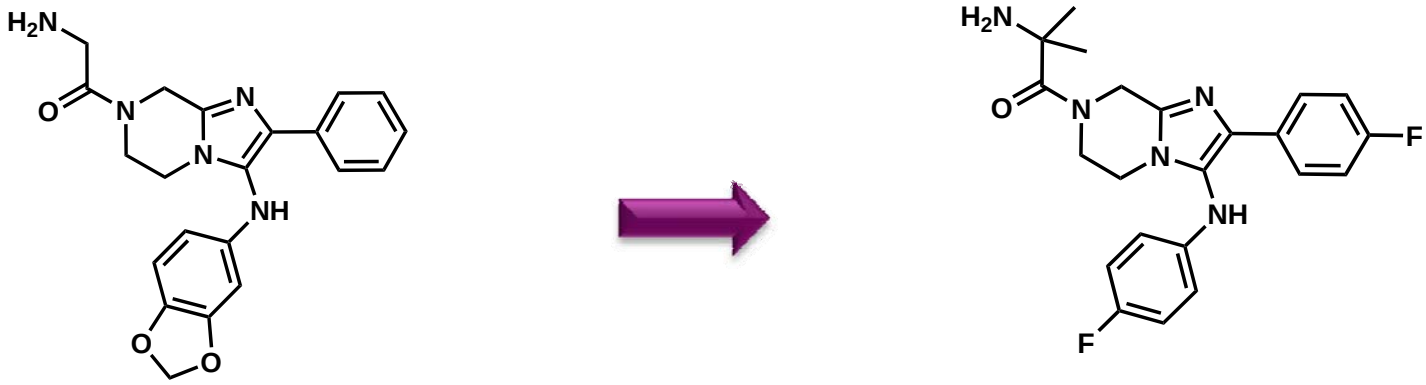
- Cure at fully tolerated dose, 3 day dosing
- >99% reduction parasitemia
- $ED_{50/90/99}$ measured

GNF

NITD/NIBR

Swiss Tropical Institute (STI)

Hit-to-lead optimization Summary



- GNF-Pf-5069
  - *Pf* 3D7 (EC_{50}): 460 nM
  - hERG (binding) IC_{50} = 19 µM
  - Mouse metabolic stability (ER): 0.24
  - Solubility (pH 6.8): > 175 µM
  - PO C_{max} (D.N.) = 16 nM / (mg/kg)
  - PO $AUC_{0\text{-}5h}$ (D.N.) = 48.6 h*nM / (mg/kg)

  Poor exposure limited any further work

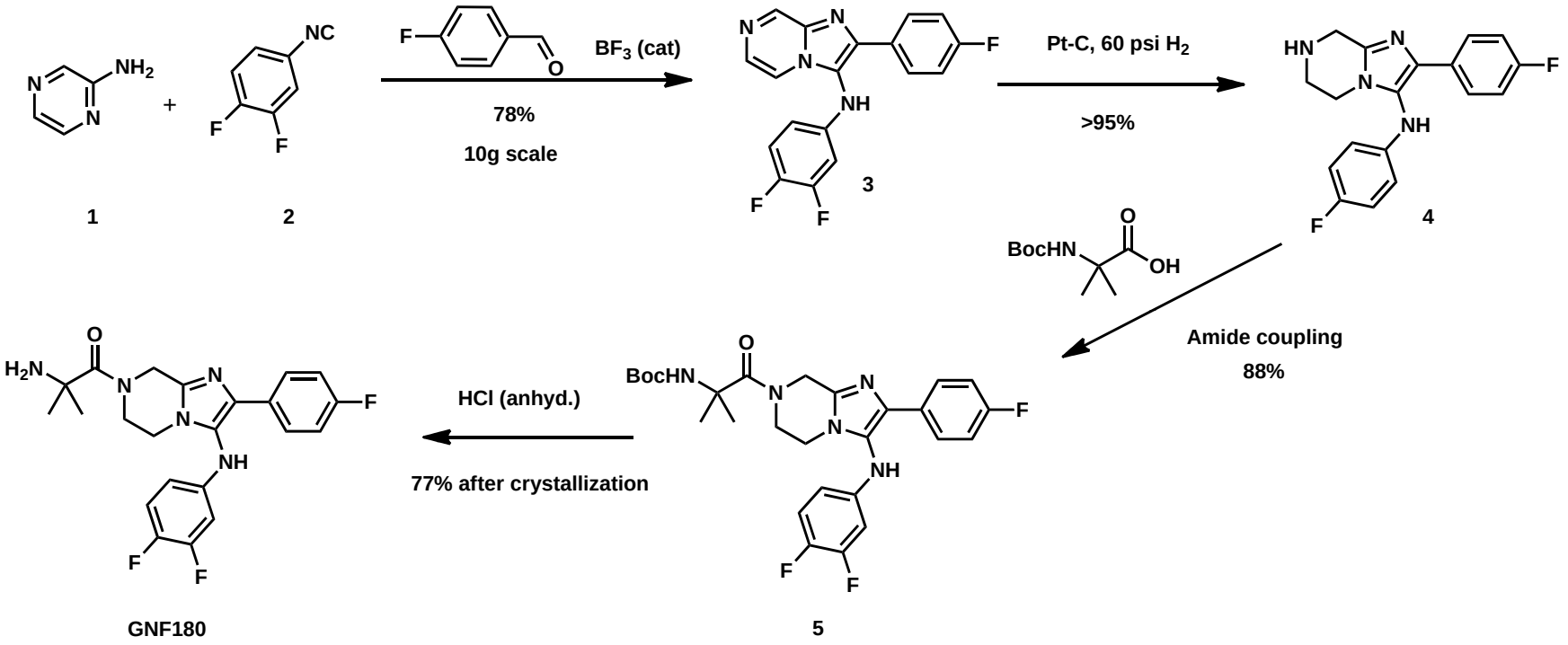
- Compound GNF776
  - *Pf* 3D7 (EC_{50}): 20 nM
  - hERG (binding) IC_{50} = 3.95 µM
  - CL = 60 mL/min/kg; V_{ss} = 23.7 L/kg; T½ = 6.2 h
  - PO C_{max} (D.N.) = 60 nM / (mg/kg)
  - PO $AUC_{0\text{-}inf}$ (D.N.) = 596.5 h*nM / (mg/kg)
  - F (%) = 87
  - Efficacy achieved in *p berghei* mouse model

(*D.N.: dose normalized)

Still need to improve hERG and potency

J. Med. Chem. **2011**, *54*, 5116.

Groebke-Blackburn Cyclization Route

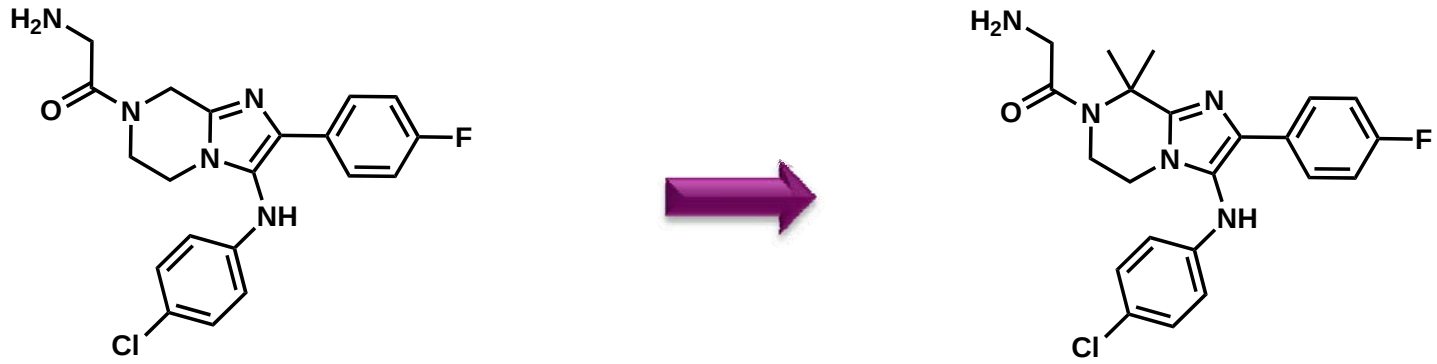


P falciparum 3D7
EC_{50} = 30 nM

- 4 steps and 53% overall yield
- No column chromatography

Imidazolopiperazine Core Optimization

8,8-dimethyl substitution significantly improves profile



- GNF268
  - *Pf* W2 (EC_{50}): 9 nM
  - hERG (binding) IC_{50} = 6.6 µM
  - CL = 1179 mL/min/kg; V_{ss} = 106.76 L/kg
  - Oral T½ = 2.2 h
  - PO C_{max} (D.N.) = 3.3 nM / (mg/kg)
  - PO AUC_{inf} (D.N.) = 30 hr*nM
  - F (%) = 17
  - 30x cross-resistance to GNF-Pf-5069 lab evolved resistance strain

- GNF179
  - *Pf* W2 (EC_{50}): 6 nM
  - hERG (binding) IC_{50} = 7.2 µM
  - CL = 21.92 mL/min/kg; V_{ss} = 11.8 L/kg
  - Oral T½ = 8.4 h
  - PO C_{max} (D.N.) = 60.5 nM / (mg/kg)
  - PO AUC_{inf} (D.N.) = 1035 hr*nM (mg/kg)
  - F (%) = 58
  - 7x cross-resistance to GNF-Pf-5069 lab evolved resistance strain

(*D.N.: dose normalized)

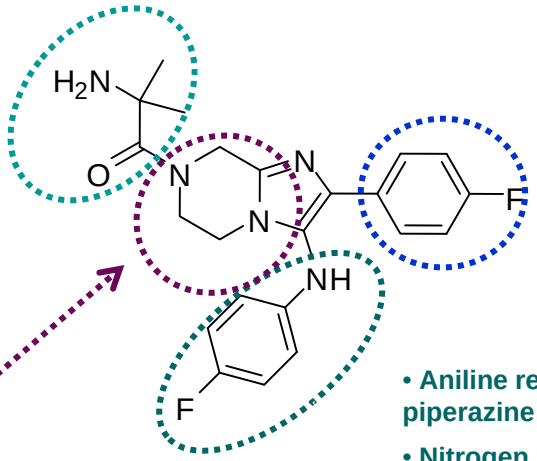
gem-Dimethyl group on piperazine improves potency and PK in mice

SAR Summary

- **Optimization of the Imidazolopiperazine scaffold:**
  - **Introduction of 8,8-dimethylgroup on piperazine improves *in vitro* and *in vivo* potency by 10-fold**
  - **Required glycine functionality is primary cause for hERG activity**

Amino acid SAR

- **NH_2 preferred; but also can use NHR, NR_2, OH and alkyl groups**
- **1-2 carbon spacer to NH_2 preferred**
- **Dimethylglycine improves *in vivo* PK relative to glycine (mouse and rat)**
- **Glycine least active on hERG (2-3 fold), while all other amino acids in similar range**



- **Aryl required**
- ***Para* and *meta* substituted aromatic preferred**
- ***Ortho* substituted aromatic not preferred**
- **Strong electron-withdrawing group not preferred**

Piperazine ring SAR

- **8-substitution helps with potency and mouse exposure (dimethyl > valine, alanine > hydrogen)**
- **5-dimethyl and 6-dimethyl moderately helps potency, no real effect on PK**
- **Cyclic lactams (6-oxo) derivatives active with 8,8-dimethyl substitution and reduces metabolism**

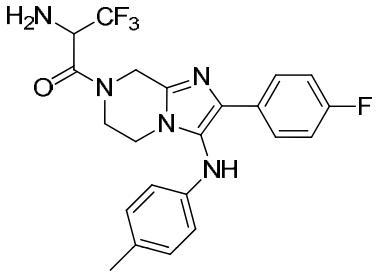
- **Aniline required with unsubstituted piperazine series**
- **Nitrogen required, but N-H is not in unsubstituted piperazines**
- **Variety of aromatics help potency (*para* and *meta* preferred)**
- **Heteroaromatics and cycloaliphatics not active in unsubstituted piperazine series, but active with 8-dimethyl groups**
- **Can modestly effect hERG activity**

hERG Optimization

Targeting the amine functionality

Modulate amine basicity:

GNF824



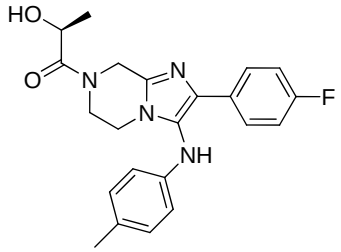
pKa = 3.3
Glycine amine pKa = 7.5

P. falciparum 3D7 EC_{50} = 50 nM

hERG (Dofetilide binding)
IC_{50} = 12.47 μM

Solubility (pH 6.8) = 34 mg/L

Replacement of amine with alcohol:



GNF557

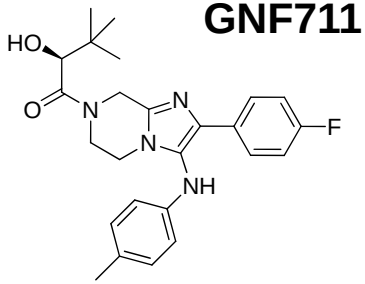
P falciparum 3D7 EC_{50} = 300 nM

hERG (Dofetilide binding)
IC_{50} > 30 μM

Solubility (pH 6.8) = 110 mg/L

Optimize
P. falciparum
potency

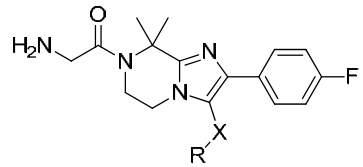
GNF711



P. falciparum 3D7 EC_{50} = 20 nM

Unable to progress due to poor metabolic stability and Log D

SAR of the 3-position (aniline group)

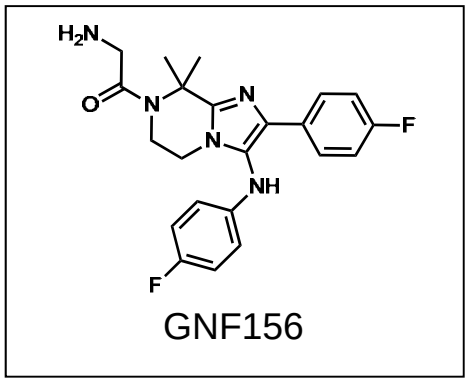


Cmpd	X	R	*Pf* 3D7 EC_{50} (nM)	pH 6.8 Solubility (µM)	Rat ER	hERG (binding) (µM)
GNF156	NH	4-F-Ph	10	>1000	0.52	6.4
GNF877	CH_2	4-F-Ph	8	>1000	0.49	2.7
GNF767	O	4-F-Ph	3	>175	0.13	4.0
GNF765	S	4-Me-Ph	8	42	ND	4.2
GNF772	SO	4-Me-Ph	42	>175	0.37	26.0
GNF771	SO_2	4-Me-Ph	5210 (NF54)	ND	ND	ND
GNF713	SO	3-F-Ph	481	ND	ND	12.0
GNF714	SO	4-F-Ph	70	ND	ND	>30
GNF715	SO	3,5-Me_2-Ph	381	ND	ND	15.0
GNF716	SO	3,4-F_2-Ph	89	ND	ND	27.0

Sulfoxide linkage is a good balance of potency, solubility and hERG

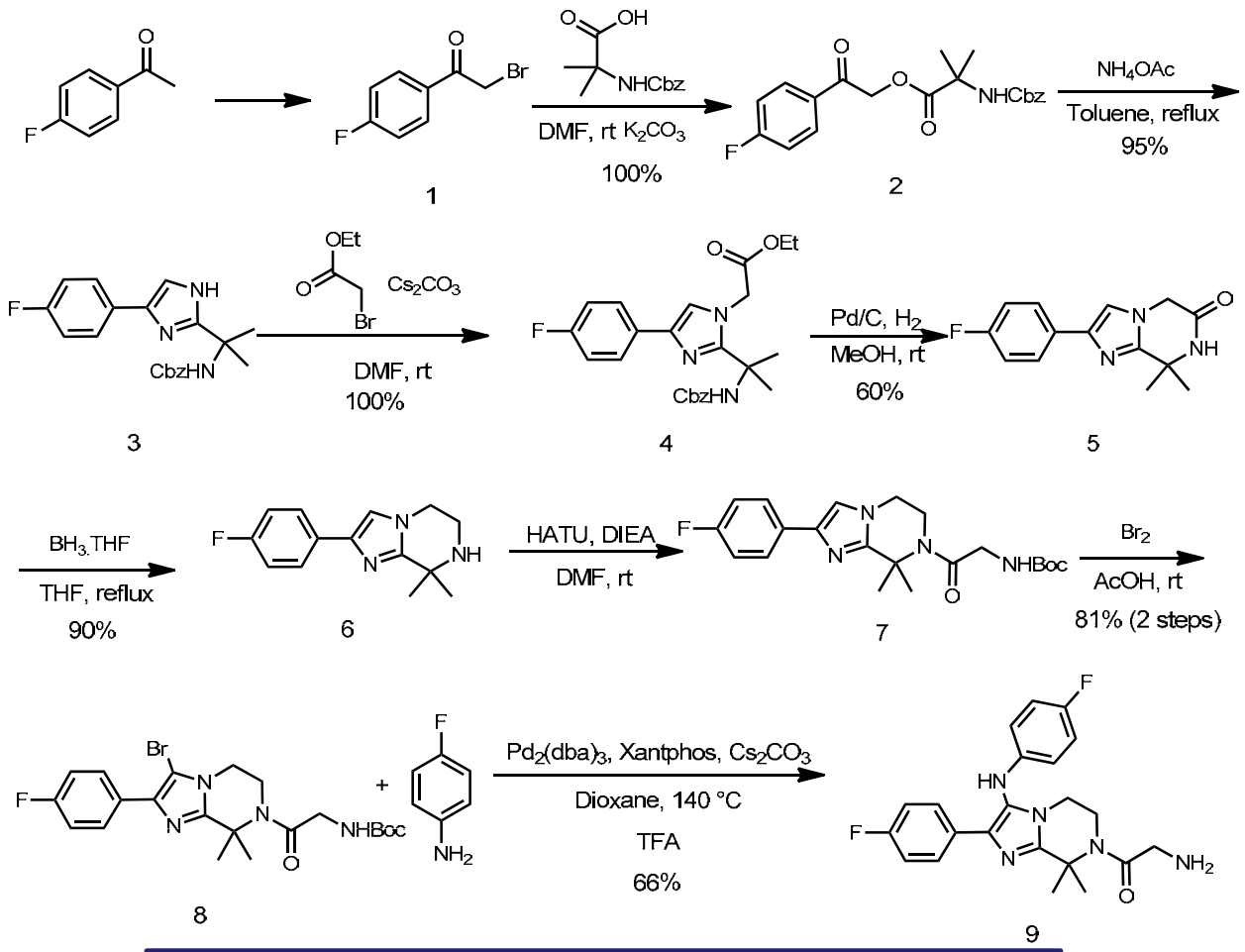
Profile of Preclinical candidate: GNF156

- ***In vitro* Activity Profile of Imidazolopiperazines**
  - *P. falciparum* W2 potency: EC_{50} = 0.006 μM
  - Compound exhibits < 5-fold shift across 15 strains
  - Selectivity vs. Huh7 cells: >10,000 fold
  - Complete transmission blocking at 500 nM
- ***In vivo* Profile**
  - Good oral PK/bioavailability and exposure; $T_{1/2}$ > 4 h
  - *In vivo* efficacy in *P. berghei* mouse model: ED_{99} = 2.2 mg/kg
  - Causal prophylaxis data: Fully protective at 20 mg/kg
- **ADME/Safety/Physicochemical Profile**
  - ADMET: solubility: >1000 μM @ pH1 and @ pH 6.8; no GSH trapping; no Cyp induction or phototoxicity flags
  - Clean PCP receptor panel, Ames, MNT and good metabolic stability
  - Formulation suitable for tox studies identified (35x exposure multiples achieved in rats and 16x in dogs)
  - hERG activity at 6.6 μM in binding assay; 13 μM in automated patch clamp assay
- **Unique activity for blood/liver-stage infections and transmission blocking**



GNF156

GNF156: Current Synthesis Route



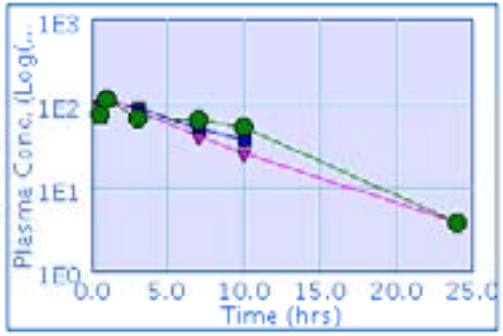
- 10 step route, but minimal purifications
- 27% overall yield
- Low cost of goods for starting materials

GNF156: *In vivo* efficacy in *P. berghei* model

Compound	# of animals per dose	ED_{50} (mg/kg)	ED_{90} (mg/kg)	ED_{99} (mg/kg)
Chloroquine	5	1.9	5.2	8.4
Mefloquine	5	3.8	5.0	8.6
Artesunate	5	5.0	20.5	119
Imidazolopiperazine GNF156	5	0.48	1.13	2.2

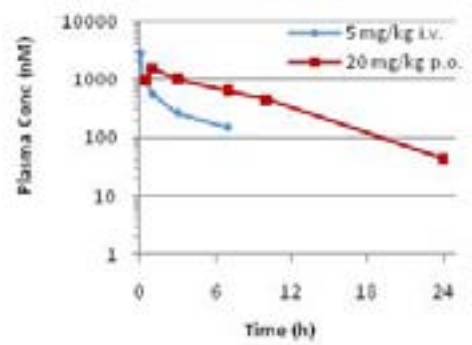
Oral Exposure in mouse at efficacious dose (2 mg/kg)

C_{max} = 112 ± 5 nM
T_{max} = 1 h
T1/2 = 4.8 ± 0.8 h
AUC_{inf} = 945 ± 110 nM*h
%F = 32 ± 4

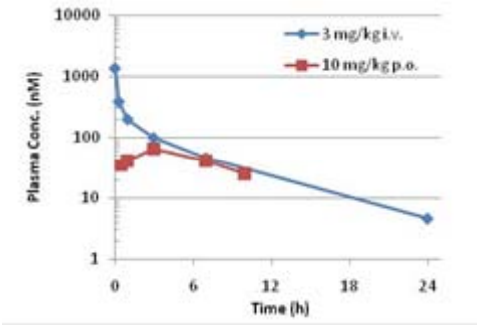


GNF156 PK Properties in Mouse, Rat and Dog

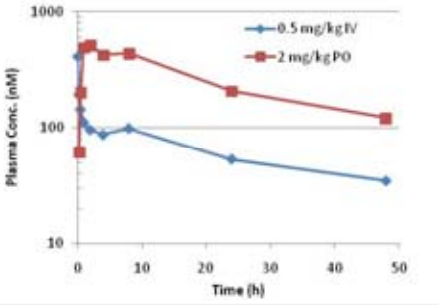
GNF156 Single Dose PK in Mice



GNF156 Single Dose PK in Rat



GNF156 Single Dose PK in Dog



Mouse PK (5 / 20 mg/kg, n=3)	
CL (ml / min•kg)	49 (mod)
V_{ss} (l/kg)	10 (high)
$t_{1/2term.}$ (h)	6.7
AUC (h•nM) p.o.	12156
C_{max} (nM) p.o.	1539
T_{max} p.o. (h)	1.0
Oral BA (%F)	74

Rat PK (3 / 10 mg/kg, n=3)	
CL (ml / min•kg)	24 (low)**
V_{ss} (l/kg)	26 (high)
$t_{1/2term.}$ (h)	4.9
AUC (h•nM) p.o.	975
C_{max} (nM) p.o.	91
T_{max} p.o. (h)	1.6
Oral BA (%F)	20

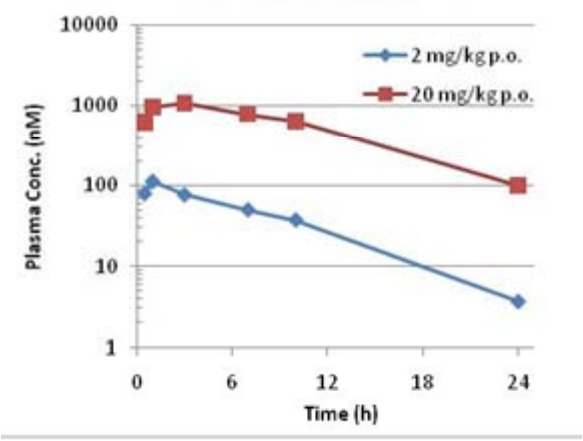
** Adjusted for RBC partitioning

Dog PK (0.5 / 2 mg/kg, n=3)	
CL (ml / min•kg)	5 (low)
V_{ss} (l/kg)	11 (high)
$t_{1/2term.}$ (h)	28.5
AUC (h•nM) p.o.	16142
C_{max} (nM) p.o.	553
T_{max} p.o. (h)	1.7
Oral BA (%F)	89

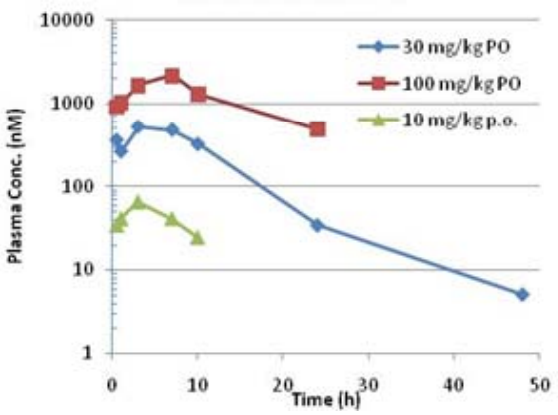
- In rat whole blood, GNF156 showed moderate RBC partition with blood to plasma ratio of 3.5.
- GNF156 exhibits low to moderate blood CL, high V_{ss}, and reasonable bioavailability across species
- Exposure multiple of 16 achieved in dogs (AUC_{eff} ~1000 h*nM at 2 mg/kg in mice). Long $T_{1/2}$ in dogs.

GNF156: Dose Linearity PK in Mouse and Rat

Mean Mouse Plasma Conc.



Mean Rat Plasma Conc



	Mouse		Rat		
Formulation	Solution		Solution		Suspension
Dose PO (mg/kg)	2	20	10	30	100
$t_{1/2}$, po (h)	4.9	4.4	4.7	6.2	8.4
AUC (h•nM) p.o.	945	12156	975	7338	34885
AUC fold change		12.9		11.6	35.8
C_{max} (nM)	112	1539	91	581	2233
C_{max} fold change		13.7		6.4	24.5
F (%)	32	74	20	40	57

- AUC increase more than dose proportional in mice
- AUC and C_{max} increase more than dose proportional in rats
- Exposure multiple of 35 achieved in rats at 100 mg/kg (AUC_{eff}~1000 h*nM at 2 mg/kg in mice)

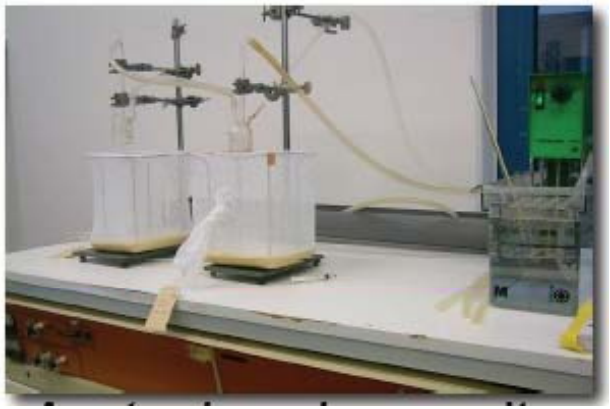
GNF156: Human efficacious dose projections

Species	Units	Mice	Rat	Dog	Human
Body weight	kg	0.025	0.25	8.7	70
Dose	mg/kg	5	3	0.5	1
Vss	L/kg	10.2	25.7	10.9	10.2 to 25.7
CL	mL/min/kg	49.2	84.4	4.8	0.9 to 4.5
T1/2	h	2	4.9	28.5	14.5 to 71
F	%	66	47	89	67

- Efficacy : ED_{99} in mice for KAF156 is 2.2 mg/kg.
- Bioavailability : F was estimated to be 67% in humans
- Clearance : 0.9 to 4.5 ml/min/kg using various methods.
- Human efficacious dose (to show similar efficacy to the mouse 99% parasitemia reduction) was predicted to be **2.3 to 48mg** based on the following assumption
  - **Efficacy is similar in P.b infected mice and P.f infected human**
  - **PK is similar in normal mice and P.b infected mice**

Imidazolopiperazines Block Transmission

Novel activity for a potent blood-stage antimalarial



Stage V gametocyte transmission
mosquito feeding experimental procedure:

1. The compounds are added to a bloodmeal with NF54 gametocytes
2. Six days after the fed mosquitoes are dissected and checked for oocysts
3. Compounds can have an effect on mosquitoes, therefore the mortality of the mosquitoes is checked every day

	% infected mosquitoes	# Oocysts/ mosquito
Control	95	49
GNF776 (0.7 μM)	60	1.5
GNF776 (7 μM)	0	0

Data furnished by Sauerwein lab

Conclusions and path forward:

- GNF776 (lead cmpd) inhibits oocyst development dose-dependently
- Optimized GNF156 blocks transmission completely at 500 nM
- This novel activity is critical for potential eradication goals

GNF156 preclinical safety summary

No significant tox at up to 30-45 (rat) or 135-250 (dog) fold over ED_{99} exposure

- **Genotoxicity**

  Mini Ames mutagenicity assay: negative

- **Phototoxicity**

  3T3 NRU assay: negative (PIF = 1.4)

- **2-week rat toxicity at 10, 30, and 100 mg/kg/day**

- 100 mg/kg/day: salivation, respiratory distress, ↑ lymphocyte counts and WBC, mild thyroid follicular hypertrophy/hyperplasia, and possibly testicular degeneration (1/5 rats but not in controls)

  ≥30 mg/kg/day: ↑ platelets (17-20%), in 3/5 rats at 30 and all rats at 100 mg/kg/day, mild-mod ↑ creatinine (up to 2-fold); mild (15%) ↑ globulin

  All doses: ↓ CK (-16- 56%) and glucose (-6 - 15%) in several rats per group

- **Dog rising dose with telemetry at 2, 10, 30, 100, 250 mg/kg**

  250 mg/kg: Transient heart rate increase without ECG changes

Lessons learned

- Molecular weights greatly effect the cellular optimization
  - Forces the chemistry to be very atom economical (high ligand efficiencies)
- SAR can be challenging, but getting hits from HTS with good SAR is critical
  - Better to have SAR than absolute potencies off the deck
  - Potencies can be optimized relatively quickly compared to other parameters
- Weekly cytotoxicity, protein binding shift potency, and cross-resistance data to other scaffold lab-evolved resistance strains really allows for better optimization of other parameters

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