DIVERSITY-ORIENTED SYNTHESIS YIELDS NOVEL MULTISTAGE ANTIMALARIAL INHIBITORS

Kato, N. *et al*, *Nature,* **2016**, Accelerated Article Preview

Celeste Alverez

Current Literature

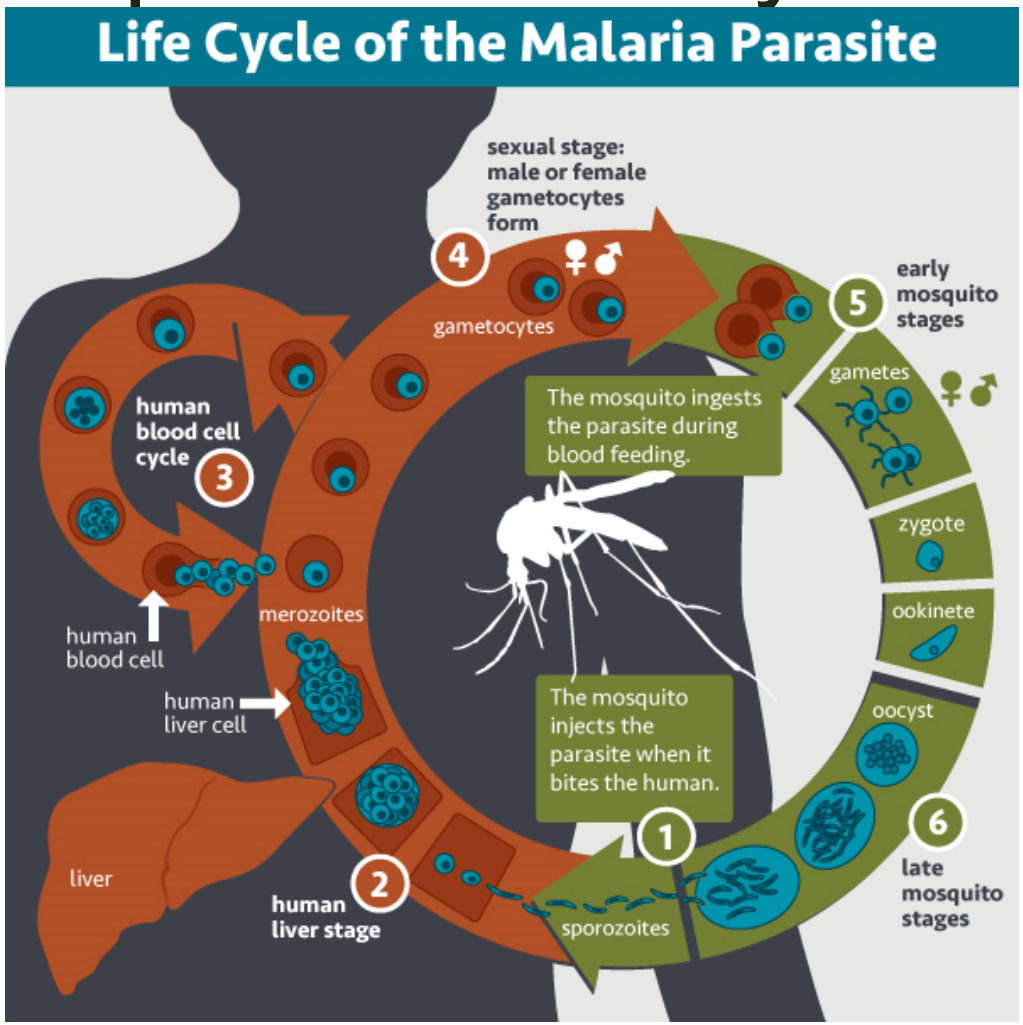
9/10/2016

Malaria

- Caused by *Plasmodium* parasites
- Carried by 30-40 species of the *Anopheles* genus mosquitos
- 2 types:
  - Uncomplicated
    - Symptoms are flu-like including fever, sweats, neausea/vomiting, chills, headaches, bodyaches
  - Severe
    - Complicated by organ failure, blood or metabolism abnormalities
      - Including anemia, kidney failure, cerebral malaria, hypotension, acute respiratory distress syndrome, hyperparasitemia (>5% of blood cells are infected with parasite
  - Can relapse depending on the type of parasite causing the initial infection (due to dormant parasite living in liver)

"Malaria" CDC, https://www.cdc.gov/malaria/index.html

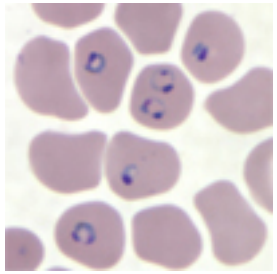
Malaria parasite life cycle



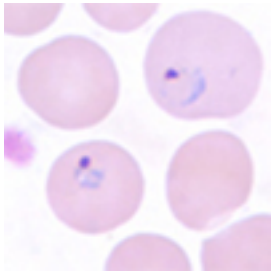
"Malaria" NIAID, https://www.niaid.nih.gov/topics/Malaria/Pages/lifecycle.aspx

Parasites

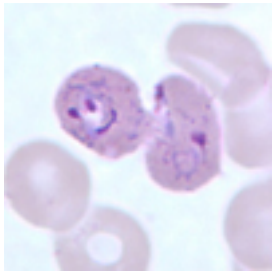
- 5 malaria causing parasites that infect humans
  - *P. falciparum* (causes severe form, found worldwide in tropical/subtropical areas)
  - *P. vivax* (can lead to relapse, found mostly in South America and Asia, some in Africa)
  - *P. ovale* (can lead to relapse, found mostly in Africa and western Pacific islands)
  - *P. malariae* (can lead to chronic malaria, found worldwide)
  - *P. knowlesi* (typically infects macaques, but can infect humans; found in Southeast Asia, 24-replication cycle)



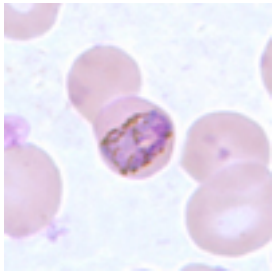
P. falciparum



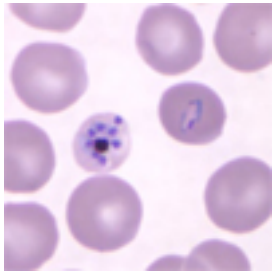
P. vivax



P. ovale



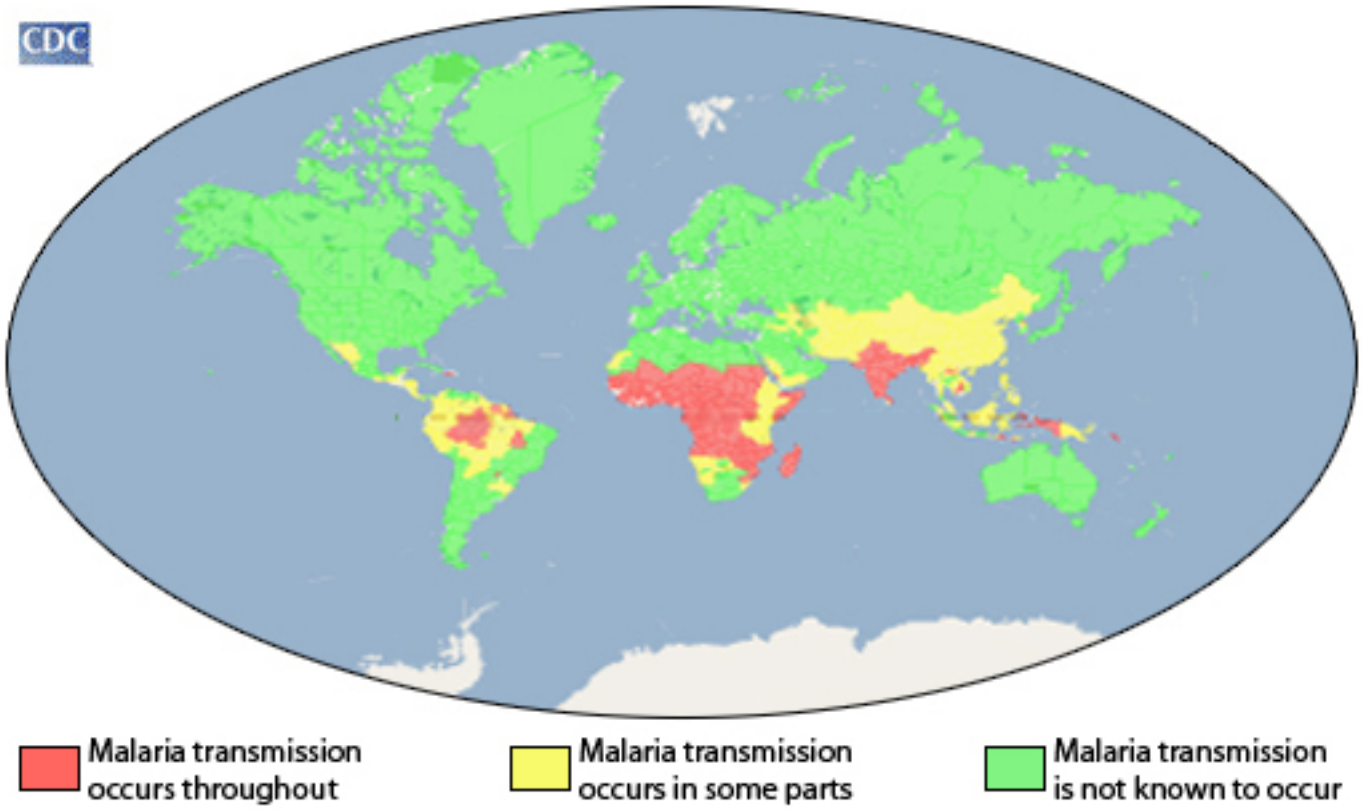
P. malariae



P. knowlesi

"Malaria" CDC, https://www.cdc.gov/malaria/about/biology/parasites.html

World Distribution

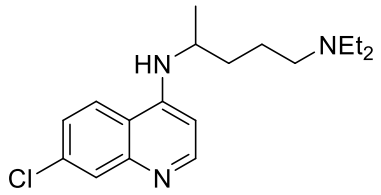


- Estimated 214 million cases in 2015

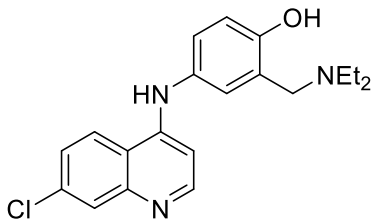
"Malaria" CDC, https://www.cdc.gov/malaria/about/distribution.html

Current Standard of Care

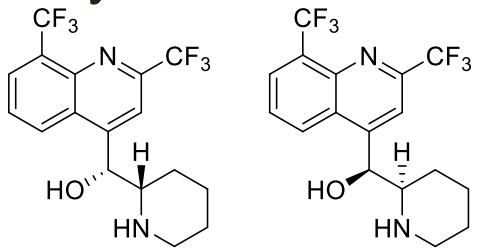
- Typically effective against blood-stage parasite (the form causing active disease)
- Need drugs effective at all stages of life cycle



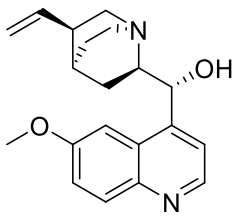
Chloroquine
discovered 1934
P. falciparum widely resistant



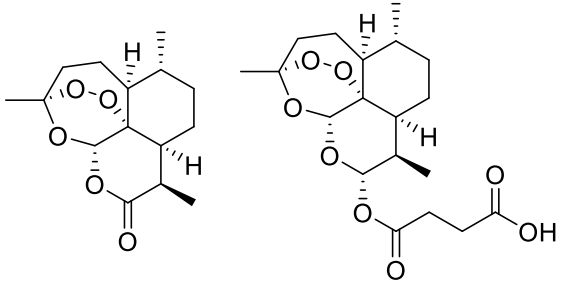
Amodiaquine
can be used to treat chloroquine-resistant *P. falciparum* malaria



Mefloquine
discovered 1970s
Potentially serious
neurological/psychological side effects



Quinine
extracts containing quinine have been used to treat malaria since the 1600's



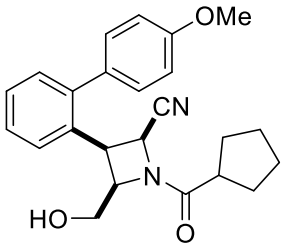
Artemisinin and derivatives
suggested to be used in combination vs. as a singular treatment by WHO

"Malaria" CDC, https://www.cdc.gov/malaria/diagnosis_treatment/treatment.html

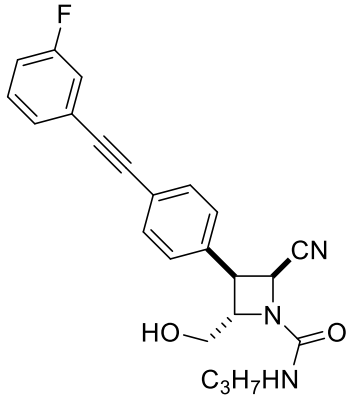
Compound Identification

1. ~100,000 compounds screened against a multi-drug resistant strain of *P. falciparum* (Dd2) for inhibition of blood-stage parasite growth
2. Counter-screened against a panel of parasite isolates and drug-resistant clones to deprioritize compounds with known mechanisms of action
   - Including screening efficacy in liver-stage and transmission-stages

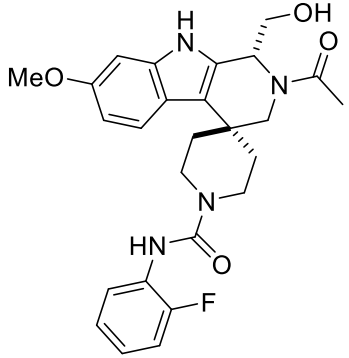
- 4 series were found:



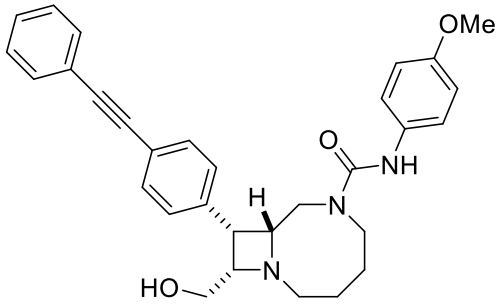
BRD0026



BRD7539



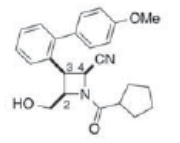
BRD73842



BRD3444

Activity of Compounds with Known Targets

a



BRD0026
Stereochemistry R, S, S

b

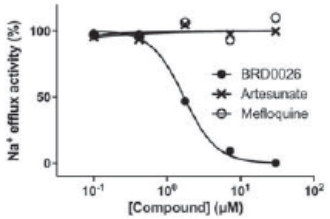
Stereochemistry C_2, C_3, C_4
Pf, Dd2 EC_{50}

S, S, R > 5.00 µM	R, R, S > 5.00 µM
S, R, S > 5.00 µM	R, S, R 0.532 µM
S, S, S 0.867 µM	R, R, R > 5.00 µM
R, S, S 0.346 µM	S, R, R > 5.00 µM

c

Assay (µM)	BRD0026
Pf, Dd2, EC_{50}	0.346
*Pf*NITD609^R, EC_{50}	1.77
Pf gametocyte, IV-V, EC_{50}	1.98
Pb liver stage, EC_{50}	> 20
PBS solubility	~20
HepG2, CC_{50}	> 50

d



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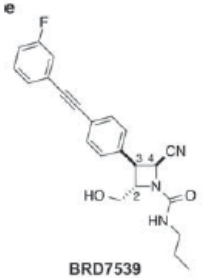


BRD7539
Stereochemistry S, S, S

f

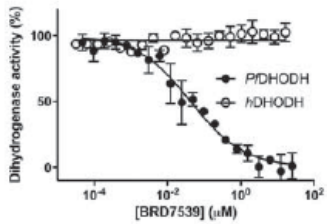
Stereochemistry C_2, C_3, C_4
Pf, Dd2 EC_{50}

R, S, S 0.035 µM	S, R, R > 5.00 µM
R, R, S > 5.00 µM	S, S, R > 5.00 µM
R, S, R > 5.00 µM	S, R, S > 5.00 µM
R, R, R 3.01 µM	S, S, S 0.010 µM

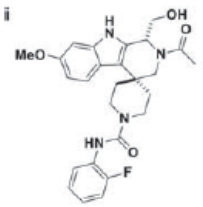
g

Assay (µM)	BRD7539
Pf, Dd2, EC_{50}	0.010
*Pf*scDHODH, EC_{50}	8.910
Pf, TM90C6B*, EC_{50}	0.011
*Pf*CYTb:G33V†, EC_{50}	0.003
*Pf*DHODH:E182D‡, EC_{50}	0.637
*Pf*rDHODH, IC_{50}	0.033
Pf gametocyte, IV-V, EC_{50}	> 20.0
Pb liver stage, EC_{50}	> 15.0
PBS solubility	< 1.0
HepG2, CC_{50}	> 50.0

h



i



BRD73842
Stereochemistry R

j

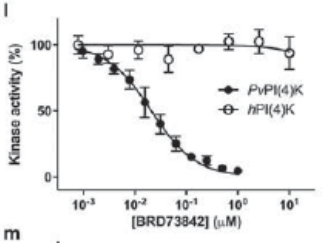
Stereochemistry
Pf, Dd2 EC_{50}

R 0.069 µM	S 2.31 µM

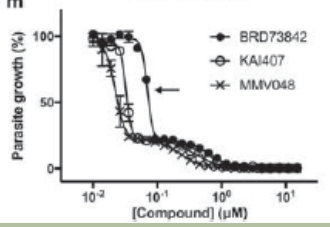
k

Assay (µM)	BRD73842
Pf, Dd2, EC_{50}	0.069
Pf gametocyte, IV-V, EC_{50}	0.643
Pb liver stage, EC_{50}	0.459
Pv PI(4)K, IC_{50}	0.021
PBS solubility (µM)	66
HepG2 CC_{50} (µM)	49
hERG IC_{50} (µM)	>10

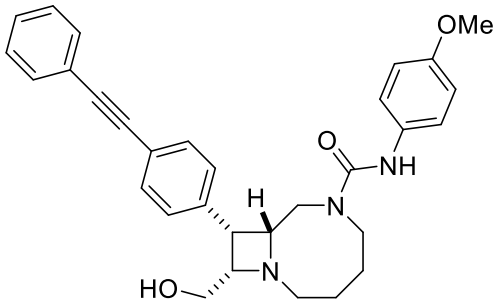
l



m



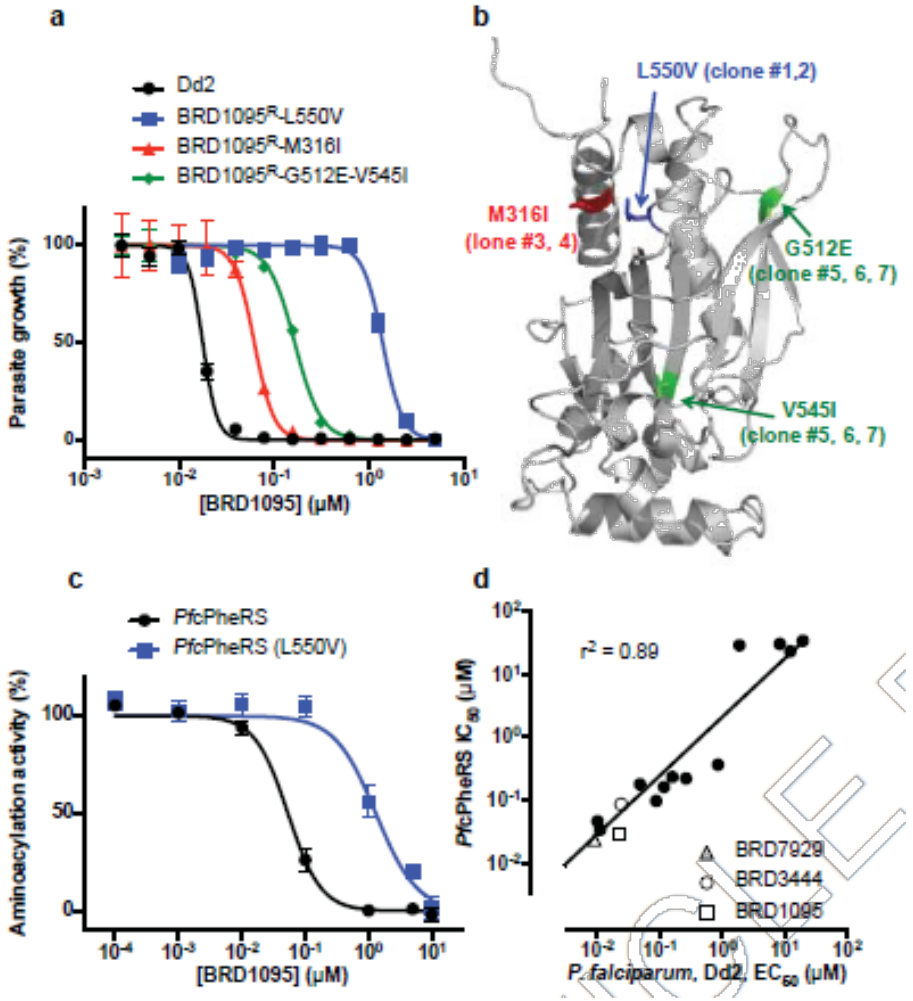
BRD3444



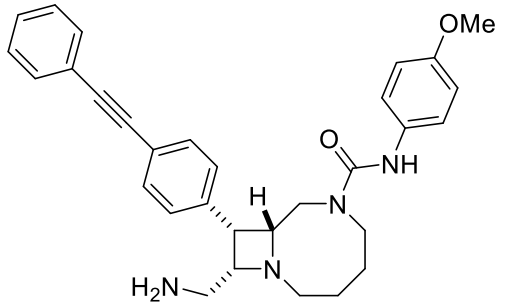
BRD3444
Pf, Dd2 EC_{50} = 9 nM
Pf gametocyte (IV-V) EC_{50} = 663 nM
Pb liver stage EC_{50} = 140 nM

- Shows submicromolar potency at 3 stages of *P. falciparum* life cycle

BRD3444 MOA



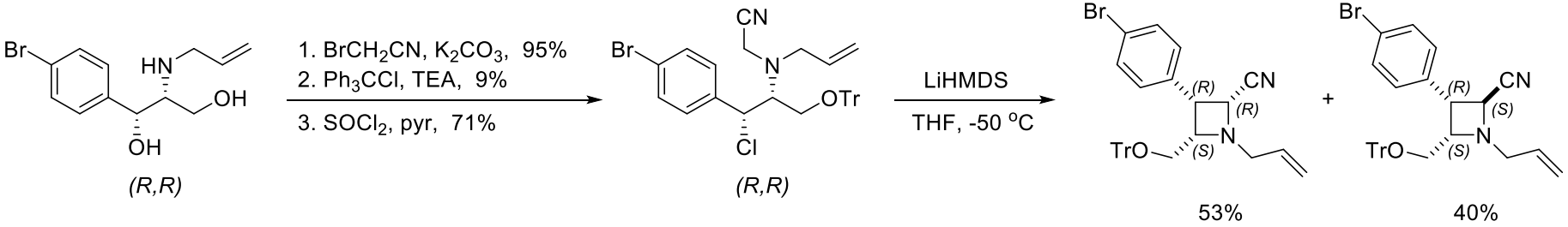
- Developed mutant lines resistant to BRD1095

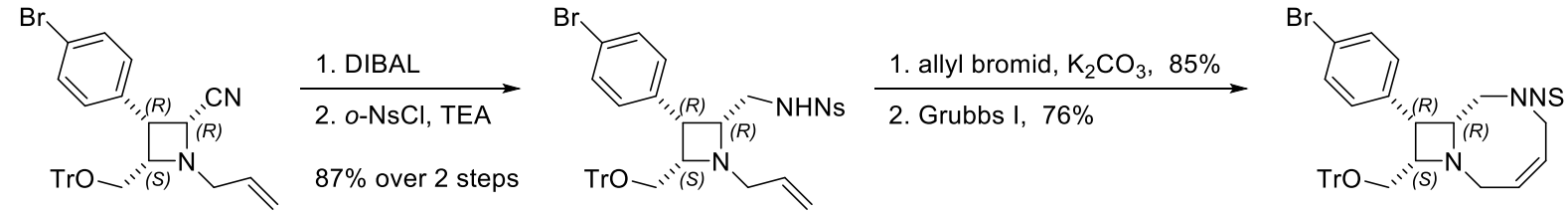


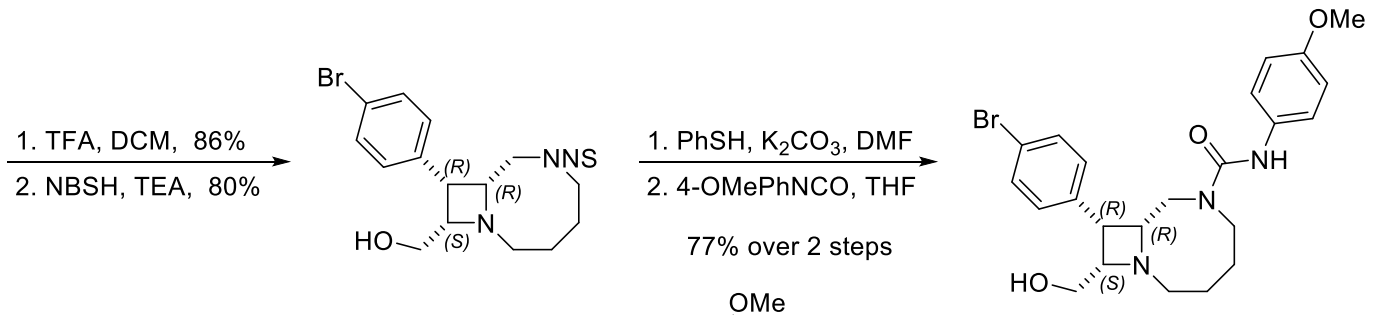
BRD1095

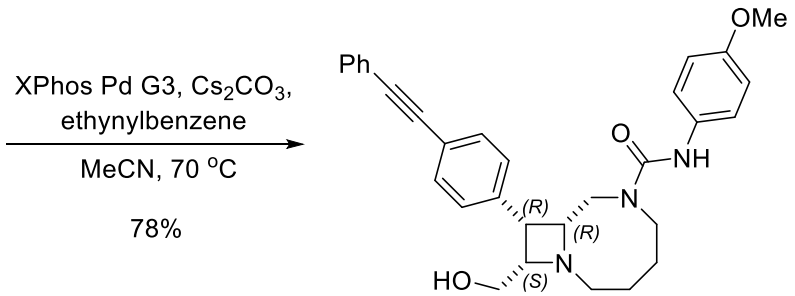
- Found a single-nucleotide variant in the α-subunit of phenylalanyl-tRNA synthetase (*Pfc*PheRS)
- **PheRS is new target for antimalarial treatment**

BRD3444 Synthesis

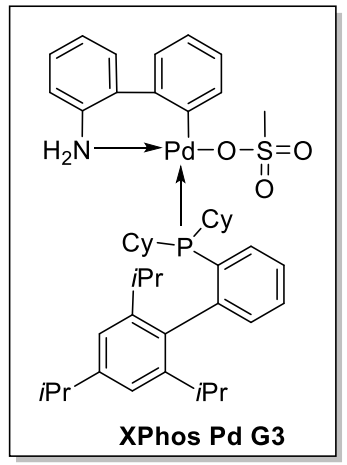








BRD3444



9/10/2016

J. Org. Chem. 2012, 77, 7187−7211

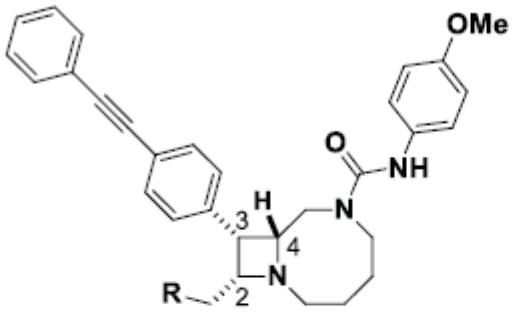
Kato, N. *et al*, *Nature,* **2016**, Accelerated Article Preview

SAR

	BRD3444*
Pf, Dd2 EC_{50} (nM)	9
PBS solubility (μM)	< 1
Mouse Plasma protein binding (%)	99.9
Mouse Cl_{int} (μL/min/mg)	248
Human Cl_{int} (μL/min/mg)	142
HepG2 CC_{50} (μM)	> 50
hERG IC_{50} (μM)	5.2

Route (mg/kg)	IV (3)	PO (10)
Cmax (μM)		0.6
Tmax (hr)		0.5
$T_{1/2}$ (hr)	3.7	3.2
AUC_{0-t} (μM*hr)	1.2¶	4¶
AUC_{0-inf} (μM*hr)	1.4	4
MRT_{0-inf} (hr)	2.8	
Vss (L/kg)	12	
F (%)	86	
CL (mL/min/kg)	72	

a



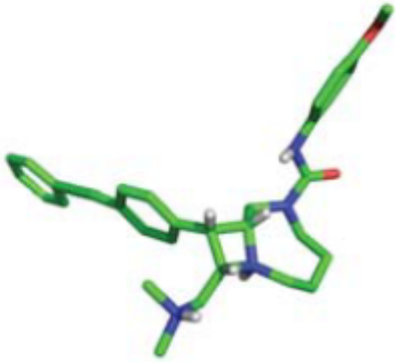
Stereochemistry *S, R, R*

b

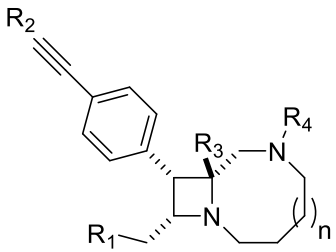
Stereochemistry C_2, C_3, C_4 *Pf*, Dd2 EC_{50}

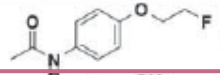
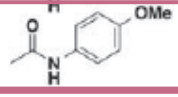
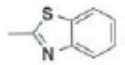
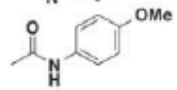
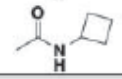
S, S, R 6.840 μM	*R, R, S* 1.370 μM
S, R, S 1.640 μM	*R, S, R* 4.650 μM
S, S, S 3.440 μM	*R, R, R* 0.017 μM
R, S, S 4.970 μM	*S, R, R* 0.009 μM

c

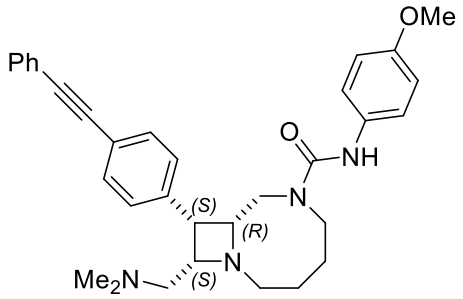


SAR



	*Pf*Dd2 EC_{50} (μM)	*Pfc*PheRS IC_{50} (μM)	R¹	R²	R³	R⁴	n
BRD8805	0.003	0.033	-NMe_2	-Ph	-H		1
BRD7929	0.009	0.023	"	"	"		1
BRD1095	0.010	0.046	-NH_2	"	"	"	1
BRD3444	0.011	0.033	-OH	"	"	"	1
BRD3316	0.022	0.029	-$O(CH_2)_2CO_2H$	"	"	"	1
BRD4716	0.024	0.086	-NMe*i*Pr	"	"	"	1
BRD2132	0.048	0.179	-$NMe(CH_2)_2F$	"	"	"	1
BRD0185	0.087	0.097	-OH	"	"	"	2
BRD8493	0.116	0.162		"	"	"	1
BRD6479	0.158	0.233		"	"	"	1
BRD4873	0.261	0.221	-OH	-2-CNPh	"	"	1
BRD9599	0.850	0.366	"	-Ph	"	"	0
BRD2936	1.87	29.4	"	"	-CH_2OH	"	1
BRD5349	8.32	30.9	"	"	-H		1
BRD5774	12.2	23.4	"		"		1
BRD8260	19.5	34.6	"	-Ph	"		1

BRD7929 vs. BRD3444



BRD7929

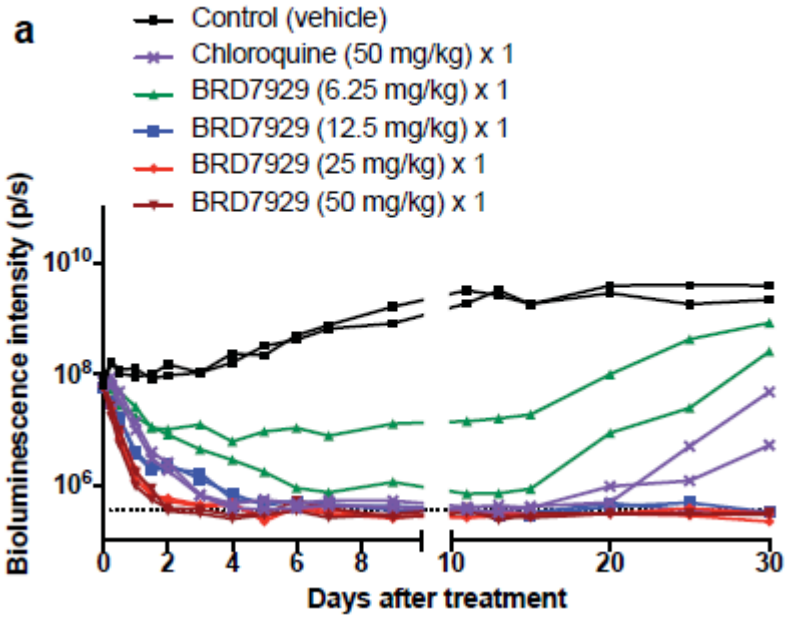
Species (strain)	Stage	EC_{50} (μM)	
		BRD3444	BRD7929
P. falciparum (Dd2)	Blood	0.009	0.005
P. falciparum ($3D7^{HLH/BRD}$)	Blood		0.009
P. falciparum (3D7)	Gametocyte (IV-V)	0.663	0.160
P. falciparum (NF54)	Gametocyte (ID / D)*		0.270 / < 10
P. falciparum (NF54)	Gametocyte (E / L)†	0.282 / 1.44	
P. falciparum (NF54)	Gamete formation (M / F)‡	~1.00 / 0.804	
P. falciparum (NF54)	Liver	1.31	0.340
P. berghei (ANKA)	Liver	0.140	0.162
P. cynomolgi (M)	Liver (SF / LF)¶	3.34 / 2.86	0.933 / 1.04

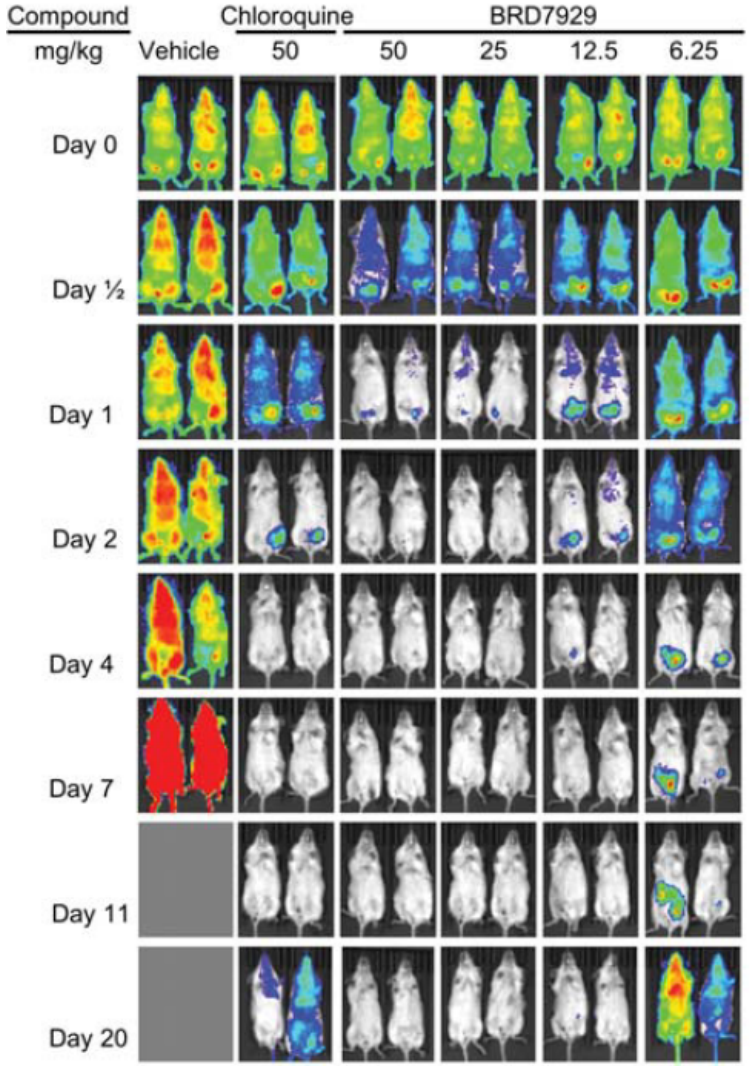
BRD7929 vs. BRD3444

	BRD3444*		BRD1095*		BRD7929*			BRD7929†	
Pf, Dd2 EC_{50} (nM)	9		10		9				
PBS solubility (μM)	< 1		25		15				
Mouse Plasma protein binding (%)	99.9		99.3		99.9				
Mouse Cl_{int} (μL/min/mg)	248		< 20		21				
Human Cl_{int} (μL/min/mg)	142		< 20		31				
HepG2 CC_{50} (μM)	> 50		15.6		9				
hERG IC_{50} (μM)	5.2		5.2		2.1				
Route (mg/kg)	IV (3)	PO (10)	IV (3)	PO (10)	IV (2.5)	IV (2.5)‡	PO (10)	PO (3)	PO (9)
Cmax (μM)		0.6		0.6			0.54	0.21	0.6
Tmax (hr)		0.5		4			8	12	12
$T_{1/2}$ (hr)	3.7	3.2		28.8	N.C	32			
AUC_{0-t} (μM*hr)	1.2¶	4¶	7¶	11.7¶	3.5¶	9#	11¶	6.4¶	19.7¶
AUC_{0-inf} (μM*hr)	1.4	4	14.9			11.2		7.2	22.6
MRT_{0-inf} (hr)	2.8		39.2		40.5	45		35.4	37.8
Vss (L/kg)	12		16		24	19			
F (%)	86		50				80§		
CL (mL/min/kg)	72		6.7		9.9	7.1			

* PK in CD-1 mice
†PK in *P. falciparum* (3D7HLH/BRD) infected NSG mice
‡IV determined in a separate assay over 72 h to determine T1/2
¶t = 24 h
#t = 72 h
§F (%) based on initial IV study at 24 h

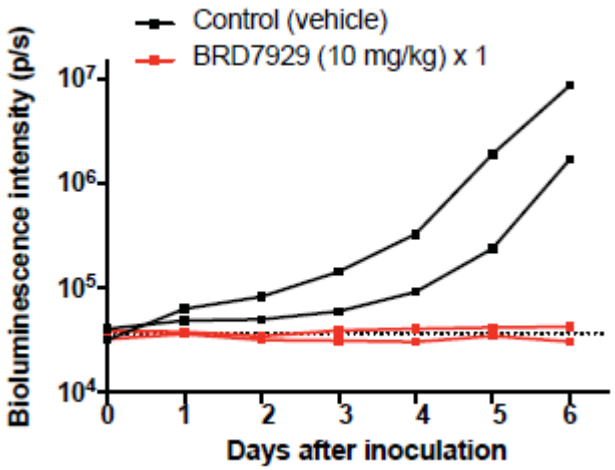
Efficacy of BRD7929 in blood-stage





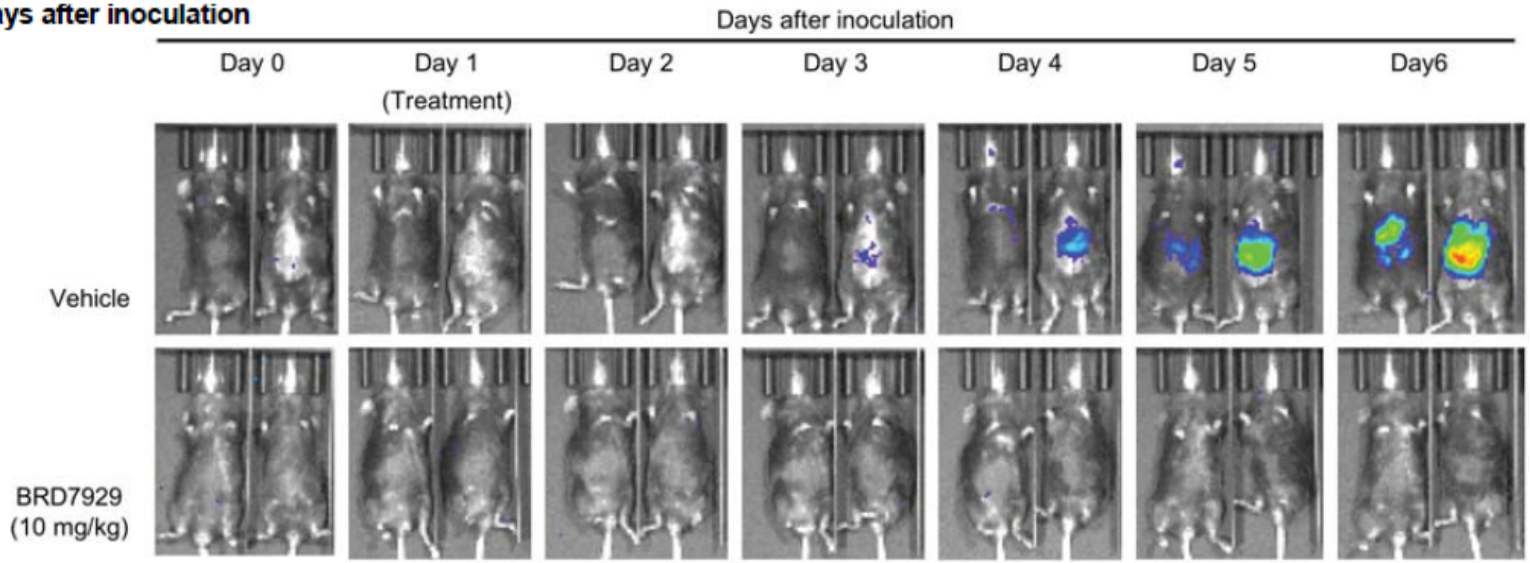
Single dose curing in a humanized mouse model of blood-stage infection

Efficacy of BRD7929 in liver-stage

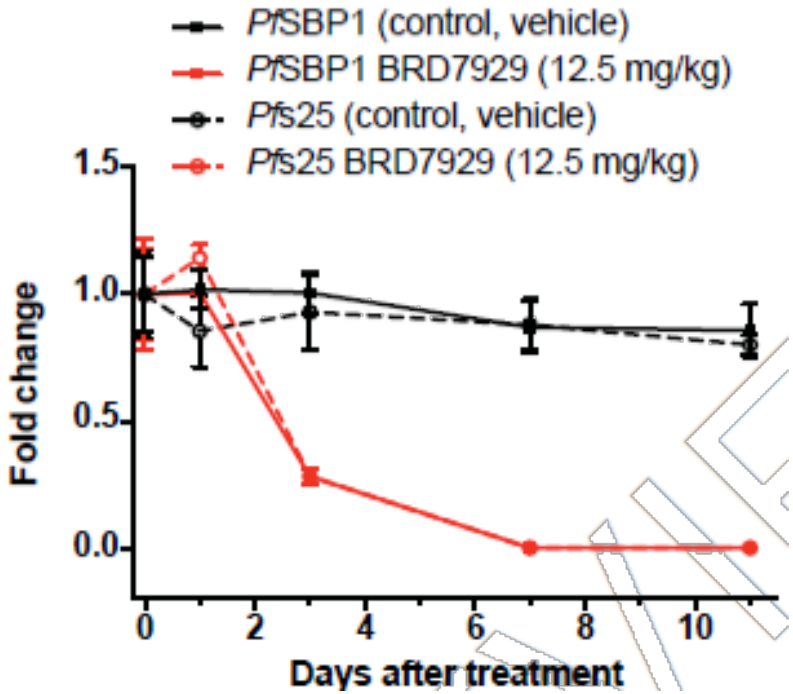


- Blood-stage biomarker also not present in blood

Suggests elimination of dormant liver-stage infection



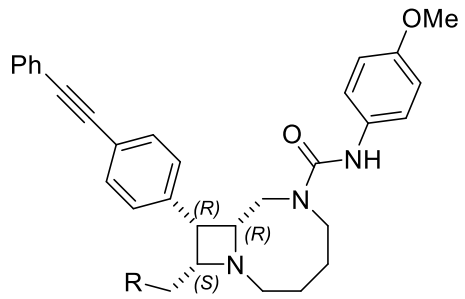
Efficacy of BRD7929 in transmission-stage



- Marker not detectable after 7 days

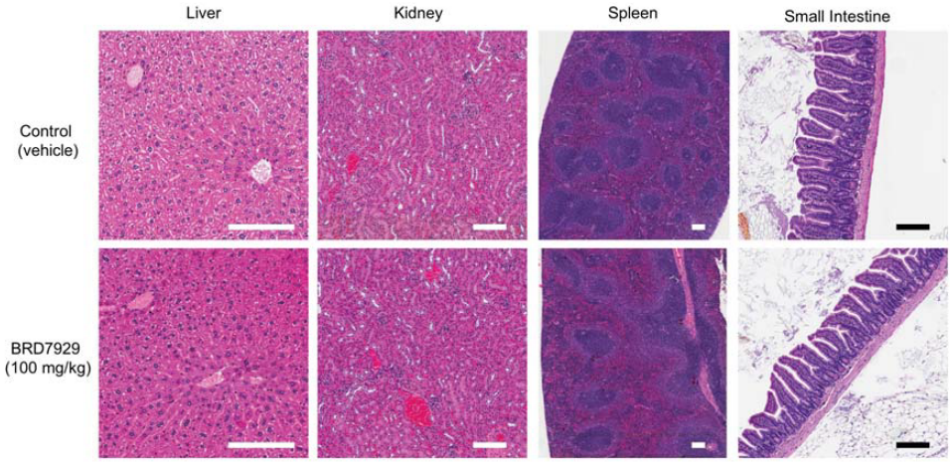
Suggests the use as prophylaxis to prevent transmission of malarial parasites

Safety of BRD7929



Compound		BRD3444	BRD1095	BRD7929	BRD3316
	R=	OH	NH_2	NMe_2	$O(CH_2)_2CO_2H$
HepG2; CC_{50} (μM)		> 50	16	9	> 50
A549; CC_{50} (μM)		18	10	6	> 50
HEK 293; CC_{50} (μM)		45	16	10	> 50
Phototoxicity 3T3 NRU[a*]		Non-phototoxic		Non-phototoxic	
Reversible CYP inhibition[†]; IC_{50} (μM)		> 10 (all)	4 (CYP1A)	> 10 (all)	> 10 (all)
Time-dependent CYP inhibition; k_{inact}/KI ($\mu M^{-1}L^{-1}min^{-1}$)[‡]		0.0158 (CYP3A)	negative (all)	negative (all)	negative (all)

- BRD7929 shows moderate cytotoxicity in select cell lines and hERG inhibition at 2.21 uM
- BRD3316 shows much better safety profile but is 4-fold less potent against *Pf* Dd2 (no other data shown)



- High dose study of BRD7929 (100 mg/kg) shows no adverse effects in organs shown

Conclusions

- Utilizing a diversity-oriented synthesis generated 4 new inhibitors of malarial parasites
- A bicyclo azetidine (BRD3444) was found to inhibit parasite growth through a previously unknown malarial target (*Pf*cPheRS)
- After SAR exploration BRD7929 was found to eliminate blood- and liver-stage *P. falciparum* infection in a humanized mouse model with a single dose
- BRD7929 was also found to apparently prevent transmission possibly allowing for use as a prophylactic against *P. falciparum* with a single dose
- The safety profile needs to be further explored, however initial high dose tests suggest tolerability
- Resistance was unable to be forced over 60 days while the known antimalarial atovaquone acquired resistance during this same time