4(1H)-Pyridones as putative antimalarials

Domingo Gargallo
Diseases of Developing World R&D Center, GlaxoSmithKline

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Medicines for Malaria Venture

4(1H)-Pyridones as putative antimalarials

- **Project Background**
  - Mode of Action
  - Critical Pathway
  - Chemical approach
- **Reference compound**
  - Biology
  - DMPK
  - Safety Assessment
  - Resistance
  - Chem. Dev & Pharm. Dev
- **Summary and upcoming steps**

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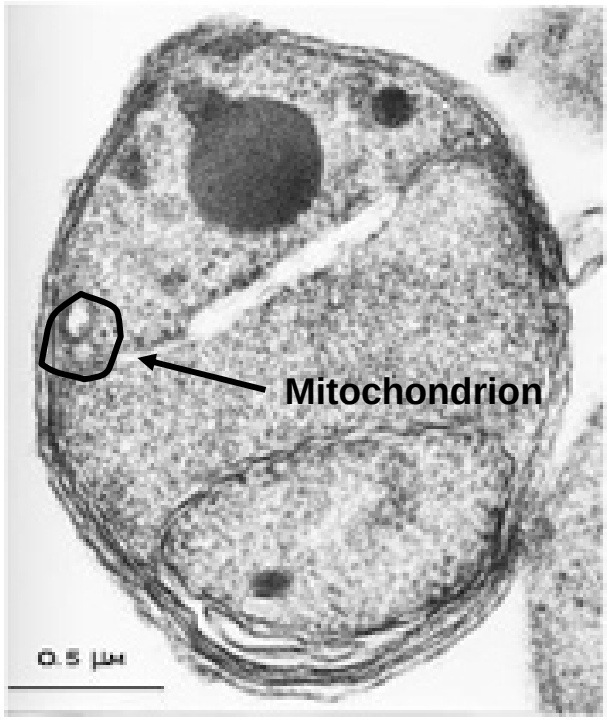
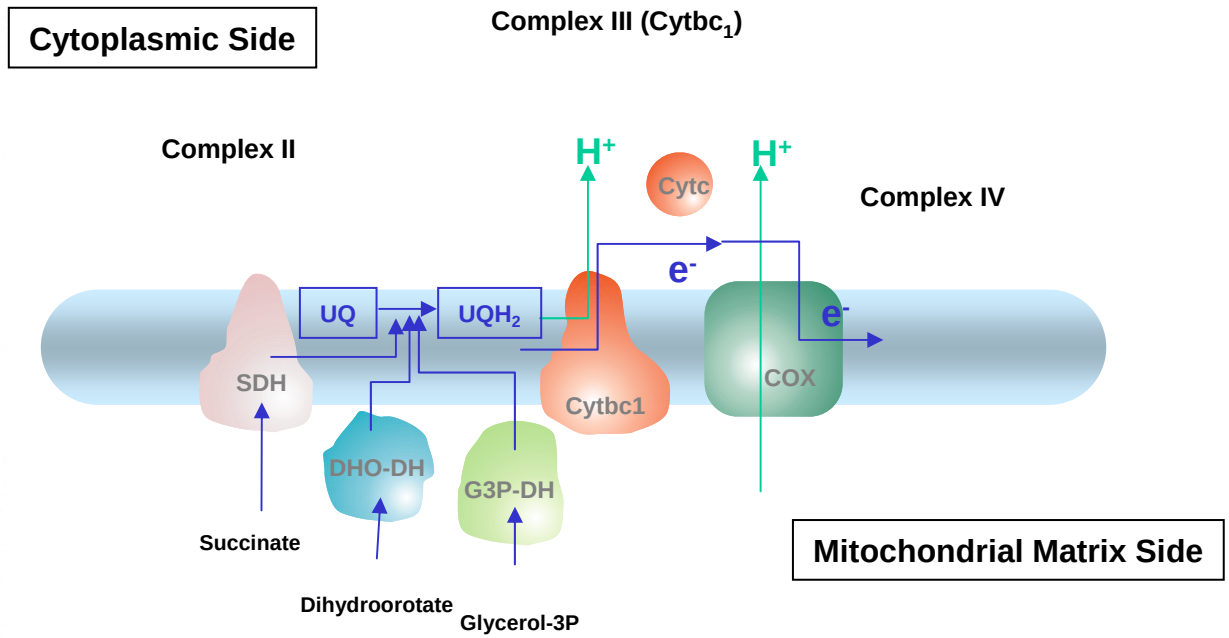
4(1H)-Pyridones Mode of Action

Inhibition of *P. falciparum* mitochondrial Electron Transport Chain.





Reasons for selectivity :

- ***Plasmodium*** **uses little oxygen. ATP synthesis mainly through fermentation**
- ***Plasmodium*** **mitochondria is essential for pyrimidine and haem synthesis**
- **Mitochondrial electron transport in *Plasmodium* acts as a sink for reduced equivalent (i.e. succinate or orotate)**

(Electron micrograph kindly provided by Dr. Peter David, Unité d'Immunologie Moléculaire des Parasites, I.P.)
http://www.pasteur.fr/recherche/unites/ImmStr/en/projects/malaria.html

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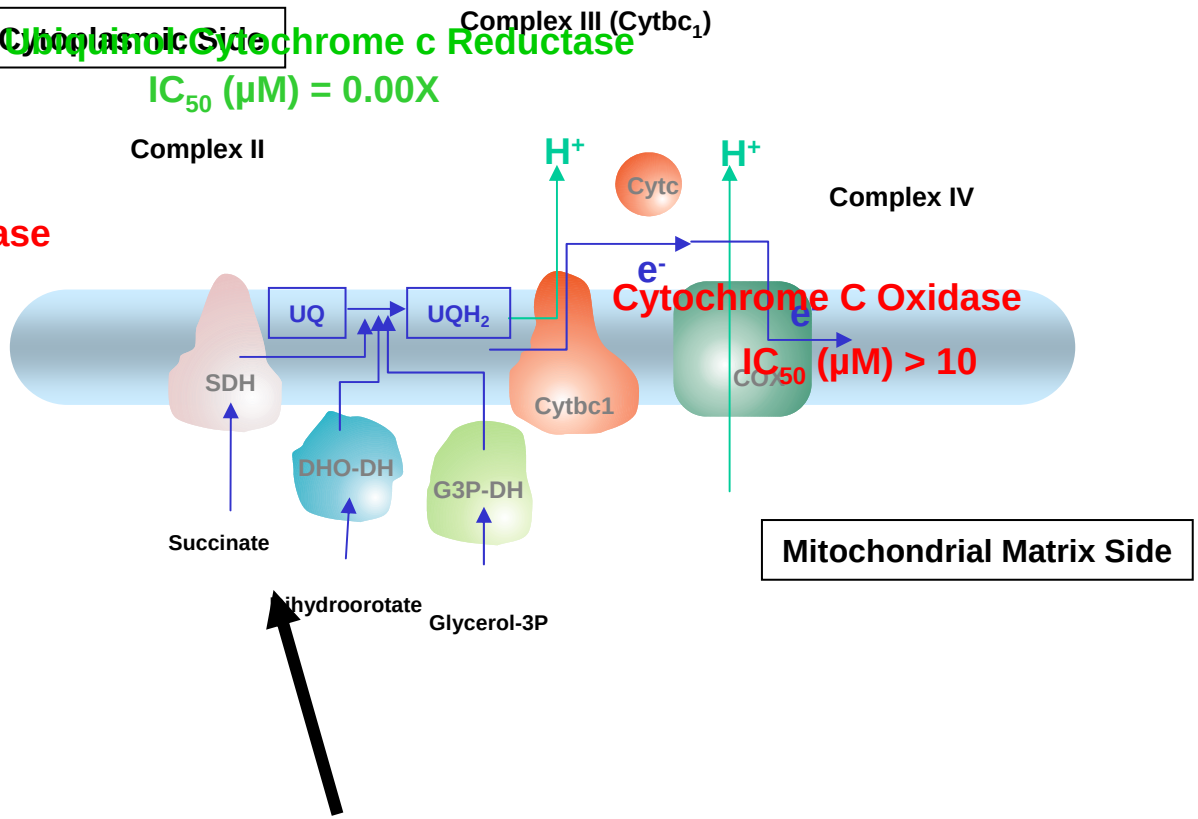
4(1H)-Pyridones Mode of Action

Inhibition of *P. falciparum* mitochondrial Electron Transport Chain.

Succinate:Ubiquinone Reductase

IC_{50} (µM) > 10



4-(1H) PYRIDONES

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4(1H)-Pyridones as putative antimalarials

Target Product Profile: Treatment of uncomplicated malaria

- Efficacy against *Plasmodium spp*. including multi-drug resistant strains
- No antagonism against potential combination drugs
- Maximum 3-days treatment, orally administered (1-day is optimal)
- Safe and well tolerated
- Low generation of resistance
- Inexpensive, easy to manufacture, transport and store

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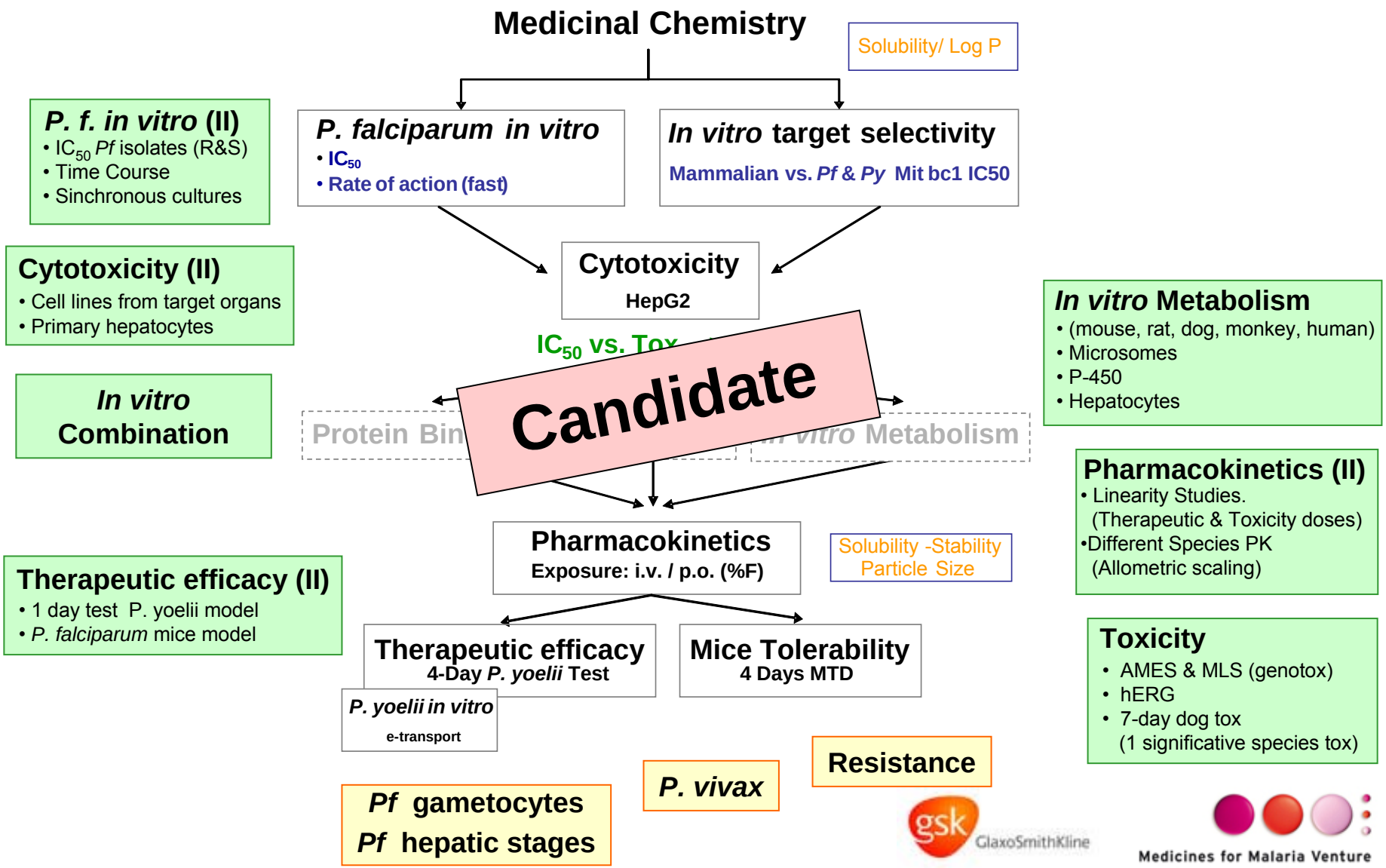
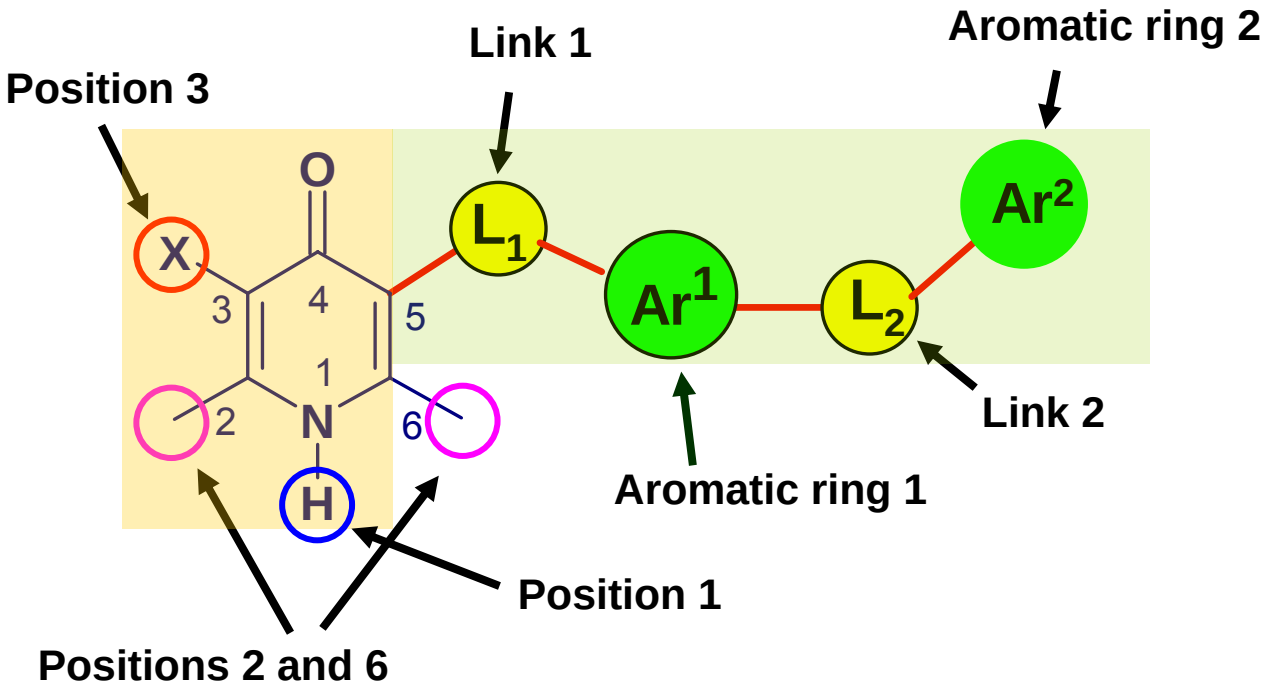
4(1H)-Pyridones Critical Pathway



4(1H)-Pyridones: Chemical approach

Structural features of antimalarial 4(1H)Pyridones



Hydrophilic moiety (Pyridone): Responsible for the antimalarial activity

Lipophilic tail: Modulation of antiparasitic potency, PK

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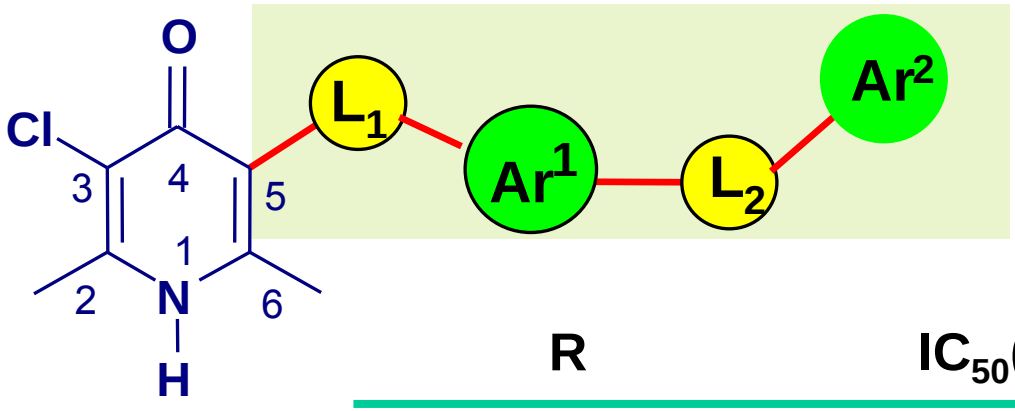
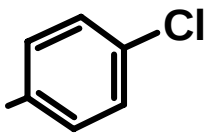
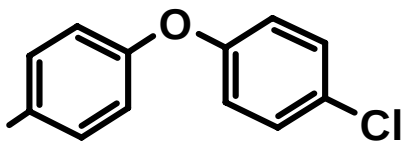
4(1H)-Pyridones: Chemical approach



R	$IC_{50}(\mu M)$
-Cl	20
	4
	0.9
	0.06

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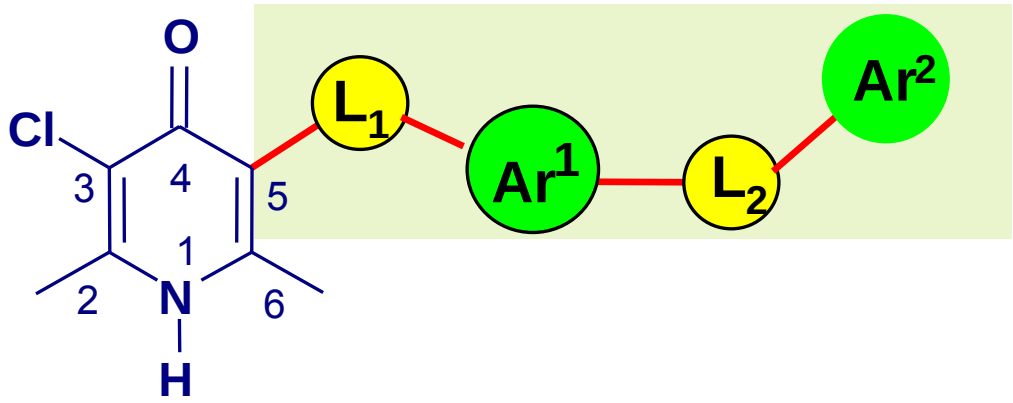
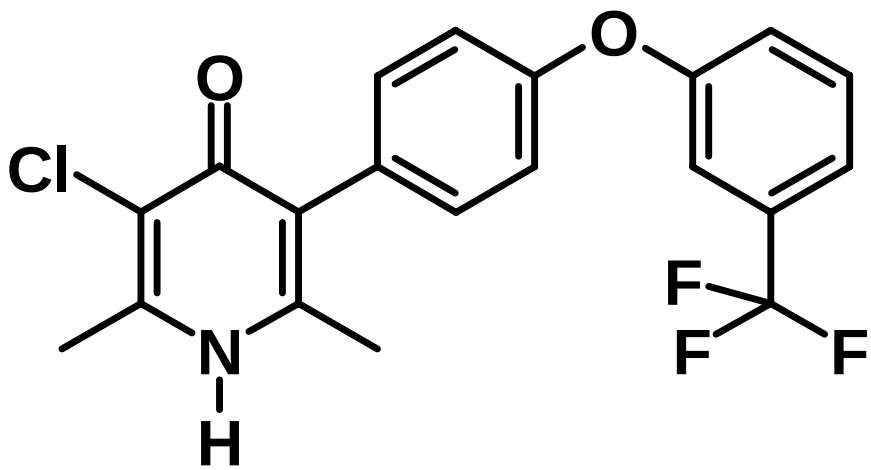
4(1H)-Pyridones: Reference compound (RC)





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4(1H)-Pyridones: RC Biology

Target Activity and Selectivity

Inhibition of Mitochondrial electron transport chain Complexes IC50 (µM)	
P. falciparum 3D7A Complex II	> 3
P. falciparum 3D7A Complex III	0.002
P. falciparum 3D7A Complex IV	> 3
Plasmodium yoelii 17X Complex III	0.003
Human HEK293 cells Complex II	> 3
Human HEK293 cells Complex III	0.51
Human HEK293 cells Complex IV	> 3
Dog MDCK1 cells Complex III	0.61
Mouse L1210 cells Complex III	0.69

Target Selectivity Ratio **x255**

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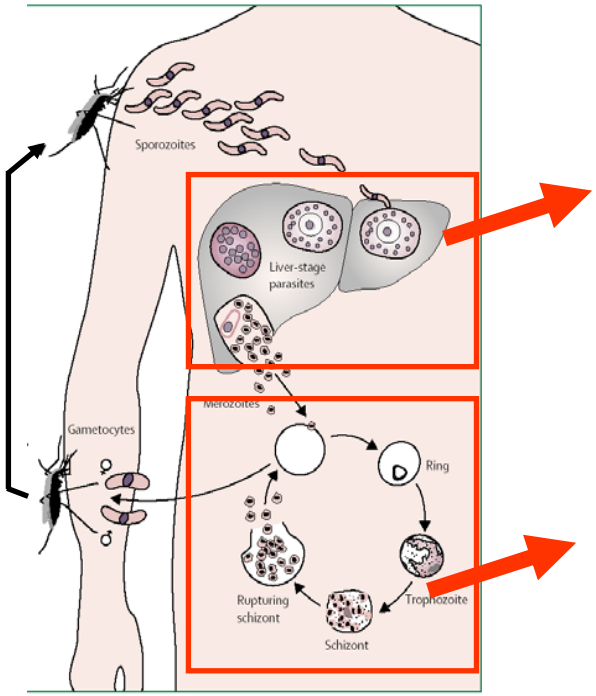
4(1H)-Pyridones: RC Biology

Whole cell activity and selectivity



Cytotoxicity (human cell lines)

IC_{50} > 2.5 µg/ml

P.falciparum liver stages.

IC_{50} 0.020 µg/ml
IC_{90} 0.050 µg/ml

P. falciparum blood stages

IC_{50} 0.002 µg/ml
IC_{90} 0.008 µg/ml

P. vivax blood stages

IC_{50} < 0.002 µg/ml

The selectivity index, cytotoxicity vs activity against *P. falciparum* x1000

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4(1H)-Pyridones: RC Biology

Activity against *P.falciparum* resistant strains

P. falciparum strains	*P. falciparum* whole-cell inhibition IC_{50} (µg ml^{-1})			
	RC	Chloroquine	Pyrimethamine	Atovaquone
3D7A	0.002	0.01	0.005	0.0002
FCR3	0.001	**0.15**	0.009	**0.9**
K1	0.002	**0.20**	**4.7**	0.0001
Dd2	0.002	**0.10**	**14.4**	0.0001
Hb3	0.003	0.02	**0.8**	0.0001
W2	0.002	**0.10**	**5.7**	0.0002

RC was active against *P.falciparum* resistant strains to the most affordable and widely-used drugs.

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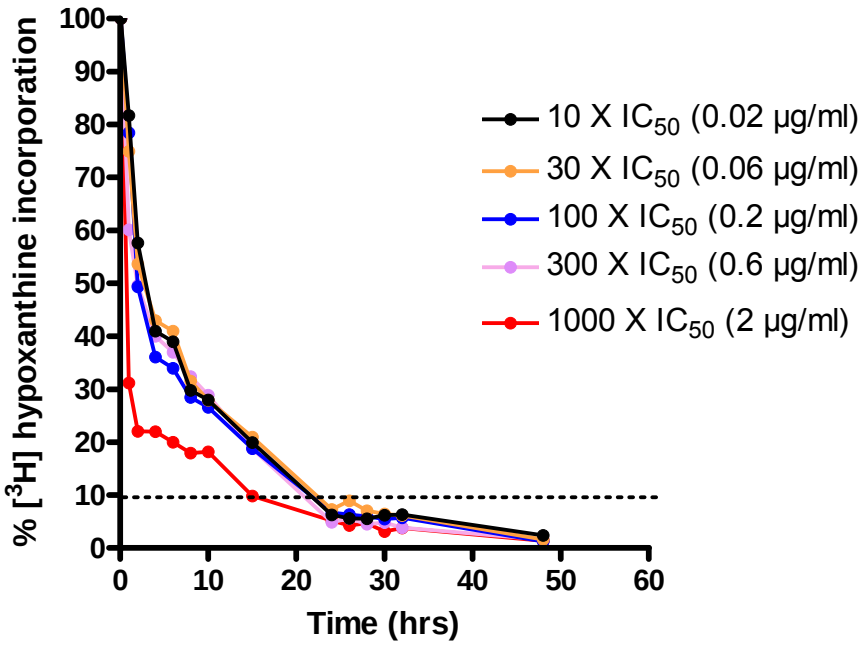
4(1H)-Pyridones: RC Biology

Time Inhibition Curves



- 90% inhibition of growth was achieved after 15-24hrs exposure
- The anti-plasmodium effect seems to be time-dependent

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4(1H)-Pyridones: RC Biology

In vitro Combinations with Antimalarial Drugs

Partner Drug	Strains
atovaquone	3D7 (standard sensitive strain)
artemisinin	K1 (CQ^R, Pyr^R)
chloroquine	FCR3 (ATV^R, CQ^R)
pyrimethamine	Dd2 (CQ^R, Pyr^R)
proguanil	HB3 (Pyr^R)

- **All the combinations investigated were additive or indifferent.**
- **Antagonistic effects were not observed with any of the combinations tested**

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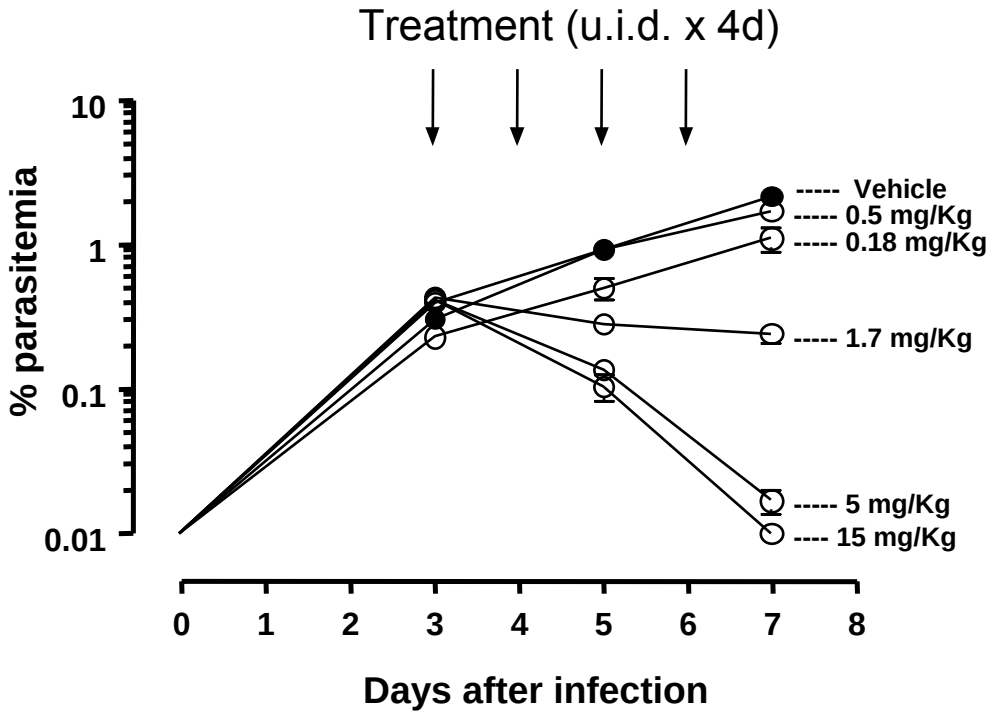
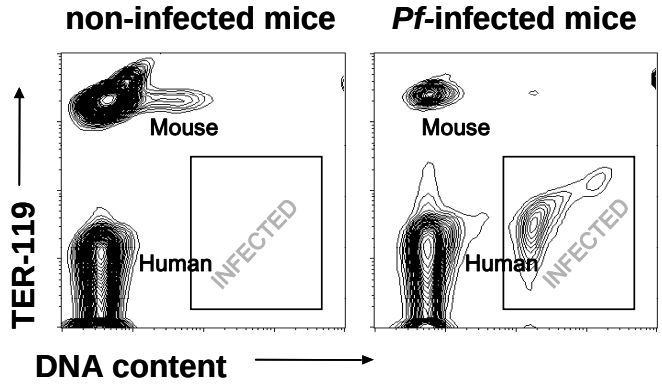
4(1H)-Pyridones: RC Biology

Therapeutic Efficacy against *P. falciparum*





	P. yoelii	*P. falciparum*
ED_{50} (mg/Kg)	0.3	0.6
ED_{90} (mg/Kg)	0.5	1.5

- Good correlation *P. yoelii* and *P. falciparum*
- Non Recrudescence Dose (NRD) *P.yoelii* 4 mg/Kg

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4(1H)-Pyridones: RC Safety Assessment

Biochemical Serum Analysis- Toxicity markers

Total Protein (g/dl)

Albumin (

Ch

T

G

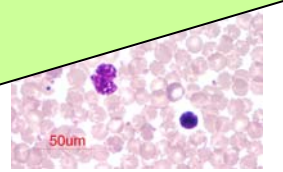
C

Urein Nitrogen (mg/ml)

Creatinine (mg/ml)

Mouse 4-day oral toxicity study up to 1000 mg/kg/day

No dose-limiting toxicity up to 1000 mg/kg/day



Red cells (x10^9/ml)

White cells (x10^6/ml)

Platelets (x10^6/ml)

Dog 7-day oral toxicity study up to 300 mg/kg/day

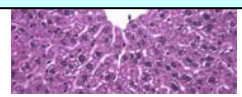
No dose-limiting toxicity up to 300 mg/kg/day. Only emesis observed and slight body weight decrease (7-8%)

Histopathology

Heart

Brain

Liver



Kidneys

Spleen

No Genotox alerts (AMES, MLS, Micronucleus)

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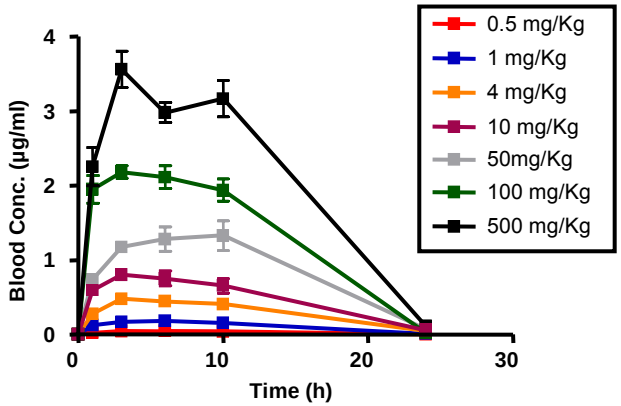
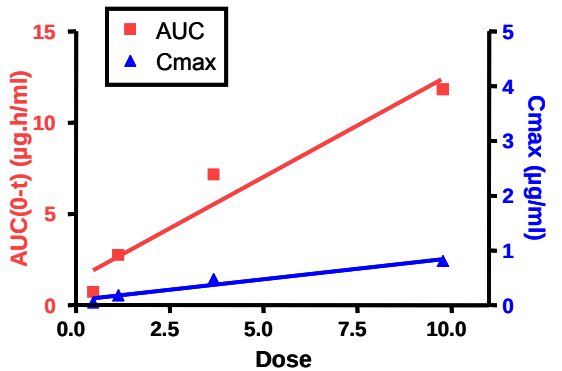
4(1H)-Pyridones: RC DMPK

Linearity studies in mice

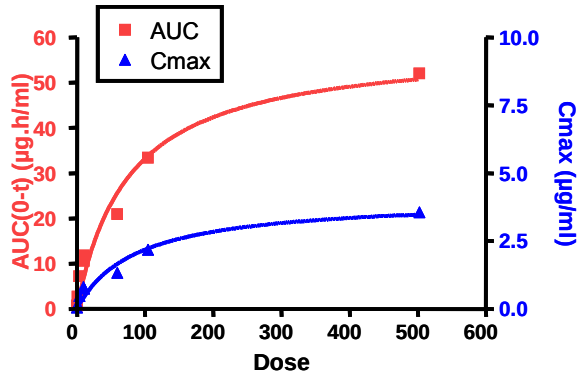
Dose[a] (mg/Kg)	C_{max} (µg/ml)	T_{max} (h)	$AUC_{(0-t)}$ (µg.h/ml)	$AUC_{(0-\infty)}$ (µg.h/ml)	$DNAUC_{(0-\infty)}$ [b] (µg.h/ml per mg/Kg)
0.5 **ED90**	0.05	6	0.74	0.74	1.60
1	0.18	6	2.75	2.82	2.43
4 **NRD**	0.48	3	7.17	7.52	1.95
10	0.81	3	12.65	12.75	1.29
50	1.33	10	20.97	21.38	0.35
100	2.18	3	33.27	33.29	0.32
500	3.56	3	51.98	52.51	0.103





	AUC	Cmax
r^2	0.9393	0.9432

10 mg/Kg



	AUC	Cmax
R^2	0.9595	0.9505

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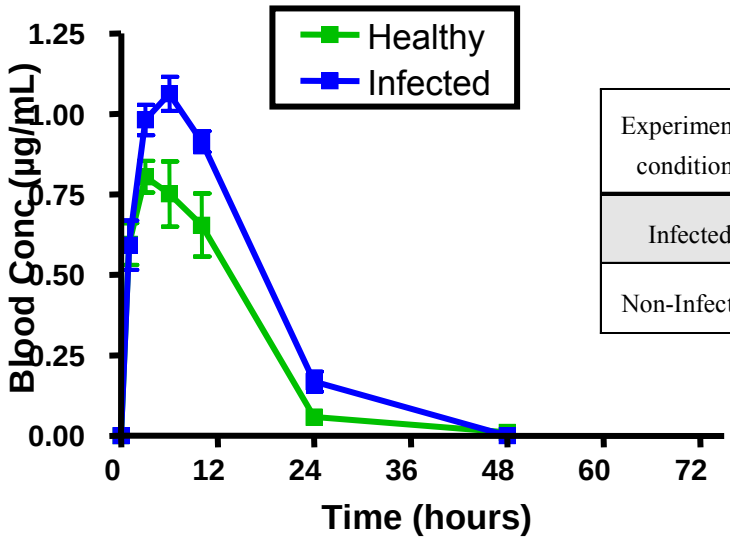
4(1H)-Pyridones: RC DMPK

Pharmacokinetics in infected mice



Experimental conditions	Dose (mg/Kg)	Cmax (µg/ml)	Tmax (hours)	AUC(0-t) (µg.h/ml)	AUC(0-inf) (µg.h/ml)	DNAUC(0-inf) (µg.h/ml per mg/Kg)
Infected	10	1.06	6	16.46	18.03	1.7
Non-Infected	10	0.81	3	12.65	12.75	1.3

The comparison of the pharmacokinetic profile of RC in healthy and infected mice showed no statistically significant differences in absorption, distribution or clearance

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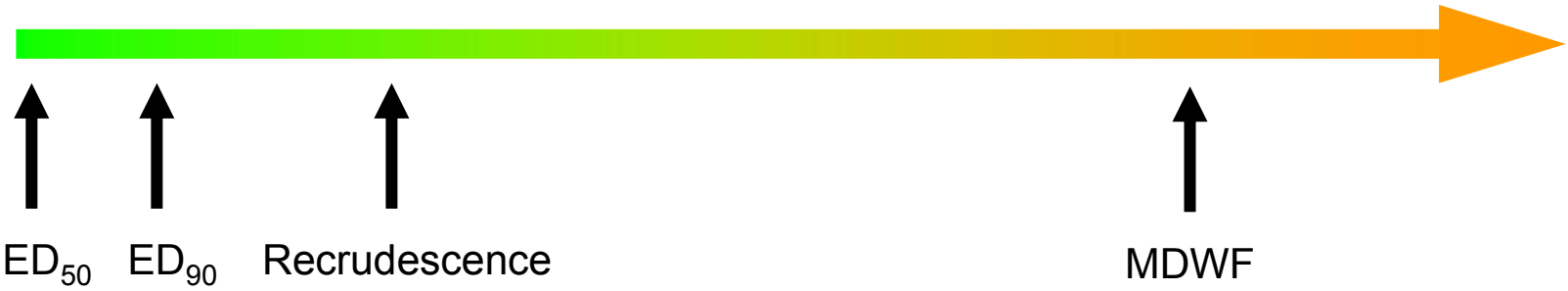
4(1H)-Pyridones: RC Therapeutic Index in mice

Therapeutic Efficacy

According to '4-days test'

Safety Assessment

> 500 mg/Kg (51.98)



0.26	**(0.35)**	**> 1900x**	**> 140x**
0.54	**(0.74)**	**> 900x**	**> 70x**
4	**(7.17)**	**> 125x**	**> 7x**
		Dose based	**Exposure** based

4(1H)-Pyridones: RC DMPK

Intravenous Pharmacokinetics in pre-clinical species (Mouse, Rat, Dog and Monkey)

Parameter	Mouse	Rat	Monkey	Dog
CL_b (mL/min/kg)	4.0	12.5 ± 1.8	6.8 ± 1.1	0.6 ± 0.1
Vdss (L/kg)	1.3	1.4 ± 0.1	1.5 ± 0.2	2.6 ± 0.2
$T_{1/2}$ (h)	4.0	1.5 ± 0.3	2.8 ± 0.3	61.4 ± 18.4
MRT (h)	5.5	1.9 ± 0.3	3.7 ± 0.2	67.0 ± 20.6

Low clearance in all species

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4(1H)-Pyridones: RC DMPK

In vitro Metabolite ID

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Mouse	✓		✓		✓	✓		
Rat	✓	✓	✓	✓	✓	✓	✓	✓
Dog		✓			✓	✓		
Monkey	✓		✓		✓	✓		✓
Human					✓	✓		

PC1-PC4: mono-oxygenation metabolites;
PC5: methyl-hydroxylation metabolite;
PC6: N-oxide metabolite;
PC7-PC8: di-oxygenation metabolite.

- **Intrinsic clearance was low in all species (moderate in rat and monkey hepatocytes)**
- **Metabolites detected in human were also detected in all preclinical species**
- **PB and blood partitioning conserved across species**

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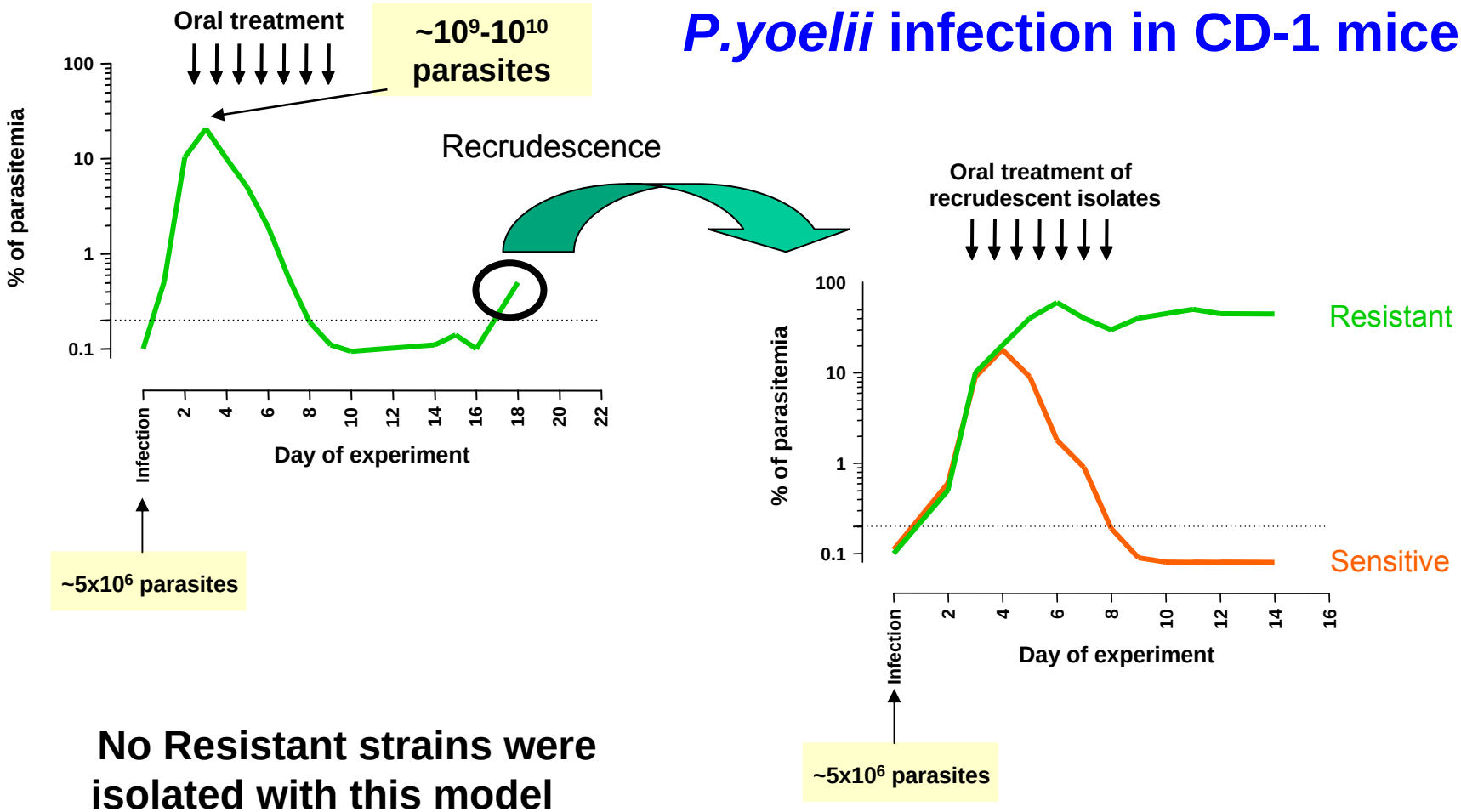
4(1H)-Pyridones: RC Resistances *in vivo*

P.yoelii infection in CD-1 mice



No Resistant strains were isolated with this model

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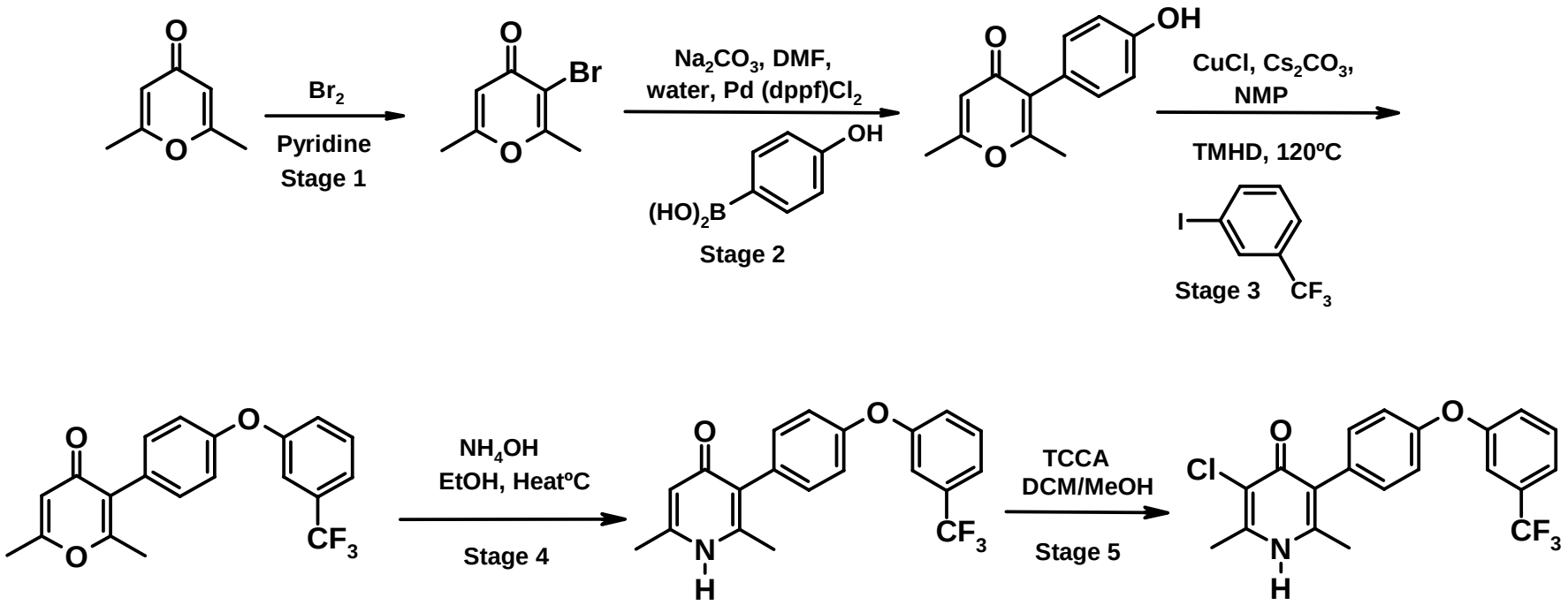
4(1H)-Pyridones: RC Chemical Development



- Five stages involving readily available materials
- Robust, scalable and straightforward until hundreds of gram scale

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4(1H)-Pyridones: RC Pharmaceutical Develop.

Alternative versions (salts)	Not available (weak acid/base character)
Solid state form	Crystalline
Hydration	Anhydrate
Polymorphic forms	2 identified (one dominant polymorph)
Hygroscopicity	Non-hygroscopic
Moisture pick-up (5-90% RH)	< 0.2%, No form change
Solid state stability	Stable at 1 month, Chemically & Physically
Solubility	<0.1 µg/mL pH 2-10 <0.1 µg/mL in SGF 0.9 µg/mL in Fasted SIF 4.8 µg/mL in Fed SIF

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4(1H)-Pyridones: Summary

Target Product Profile: Treatment of uncomplicated malaria

- Efficacy against *Plasmodium spp*. including multi-drug resistant strains ✓
- No antagonism against potential combination drugs ✓
- Maximum 3-days treatment, orally administered (1-day is optimal) ✓
- Safe and well tolerated ✓
- Low generation of resistance ✓
- Inexpensive, easy to manufacture, transport and store ✓

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4(1H)-Pyridones: upcoming steps

FTIH: October 2008

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Acknowledgements

4(1H)-Pyridones as [illegible] Antimalarials

Domingo G
Diseases o

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