

'Physicochemical and in vitro assays as PKPD predictors for New Antimalarial Pyridones'



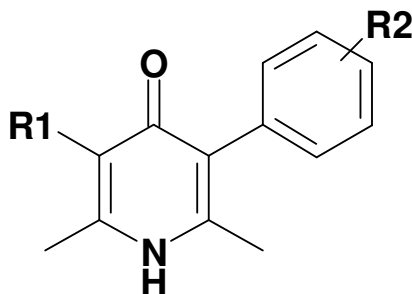
José Luis Lavandera
GlaxoSmithKline R&D.
Deseases of the Developing World (DDW)
Tres Cantos Medicines Development Campus.

Agenda

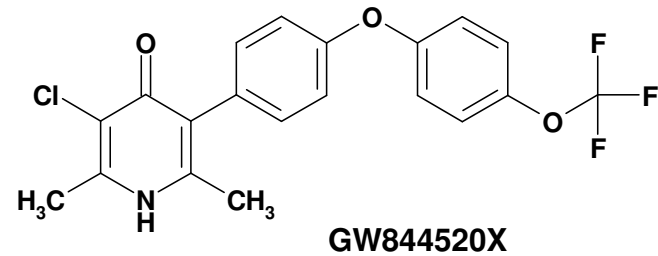
- Rational background
- Physicochemical assays
- *in vitro* ADME assays
- PKPD properties
- Summary

Rational background

- Malaria remains a major infective disease in man with over 40% of world's population exposed to the risk of infection, and estimated hundred million clinical cases every year.
- *Plasmodium falciparum* (*Pfalc.*) is the major causative agent for Malaria.
- The spread of Chloroquine and multidrug resistant *Pfalc.* strains.
- Electron transport chain is one of the most interesting targets, i.e. mitochondrial respiration (Atovaquone, Malarone)
- Pyridones are compounds active against *Plasmodium falciparum* which kill the parasite through the inhibition of the bc1 complex.

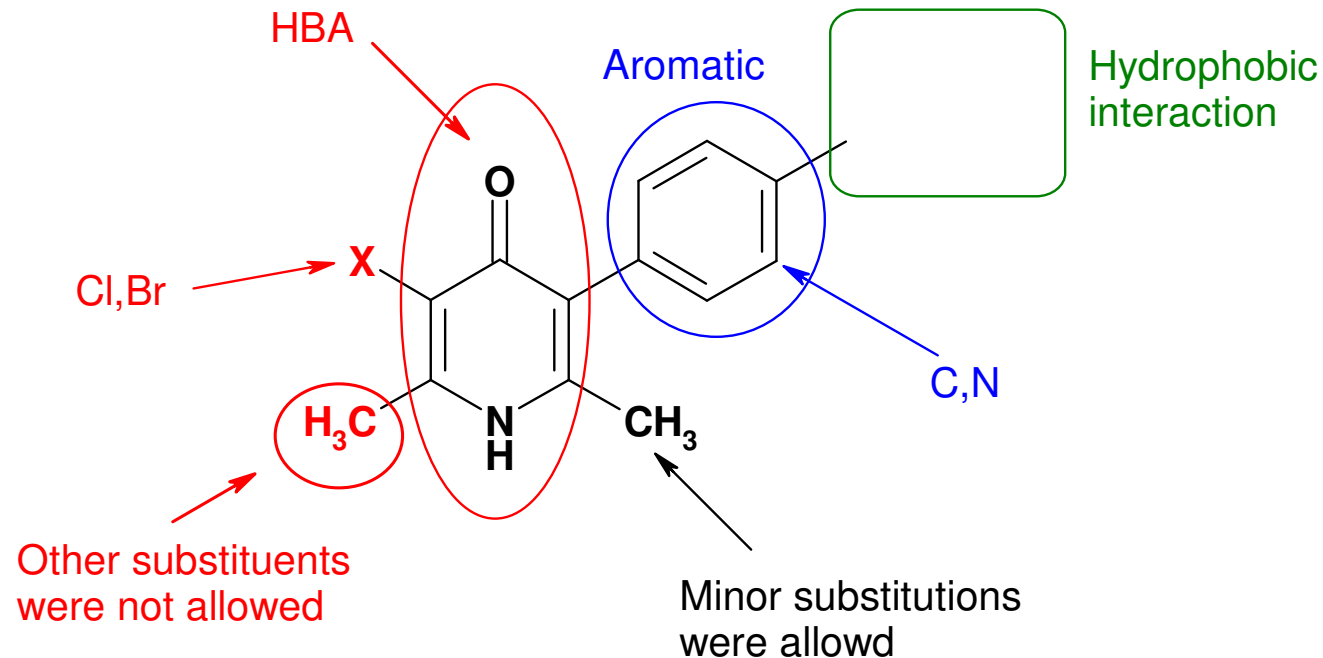


Initial hit from the HTS



- Potent and selective inhibitor of the *P.falc.* Bc1 enzyme complex in the biochemical assay.
- Good potency against the parasite.
- Lack of cytotoxicity.
- Developability properties:
 - good *in vitro* clearance in microsomal fractions from different species
 - Good stability in hepatocytes.
 - Very High plasma protein binding
 - Low permeability
- Poor solubility < 0.1 ug/ml PBS

Antimalarial Pyridones pharmacophore



Goal → to improve PKPD properties based on physicochemical properties.

Physicochemical assays

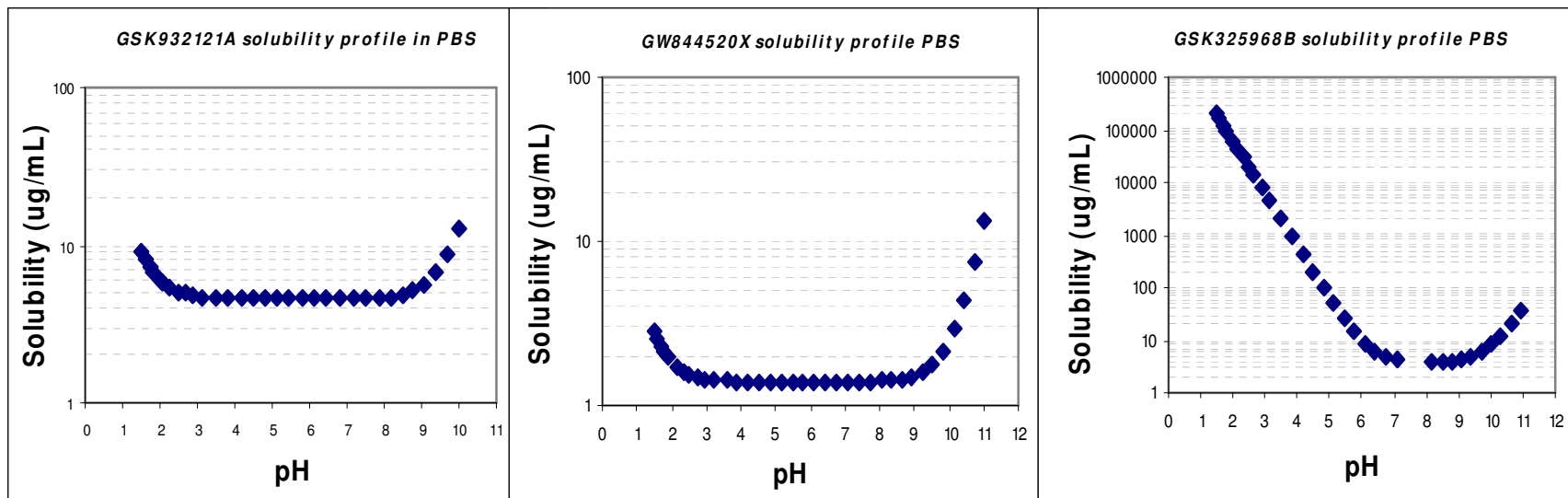
- Hydrophobicity
 - CHlogD at pH: 2.0, 7.4, 10.2
 - Chromatographic Hydrophobicity Indexes*, (Valko, K. et al)
- pH-solubility profile.
- Solubility screening in biorelevant media
 - Simulated Gastric Fluid (SGF) pH: 1.2
 - Phosphate Based Buffer (PBS) pH: 7.4
 - Fasted State Simulated Intestinal Fluid (FaSSIF) pH: 6.8
 - Fed State Simulated Intestinal Fluid (FeSSIF) pH: 5.0

Physicochemical assays

- Hydrophobicity
 - CHlogD at pH: 2.0, 7.4, 10.2
 - Chromatographic Hydrophobicity Indexes*, (Valko, K. et al)
- pH-solubility profile.
- Solubility screening in biorelevant media
 - Simulated Gastric Fluid (SGF) pH: 2.0
 - Phosphate Based Buffer (PBS) pH: 7.4
 - Fasted State Simulated Intestinal Fluid (FaSSIF) pH: 6.8
 - Fed State Simulated Intestinal Fluid (FeSSIF) pH: 5.0

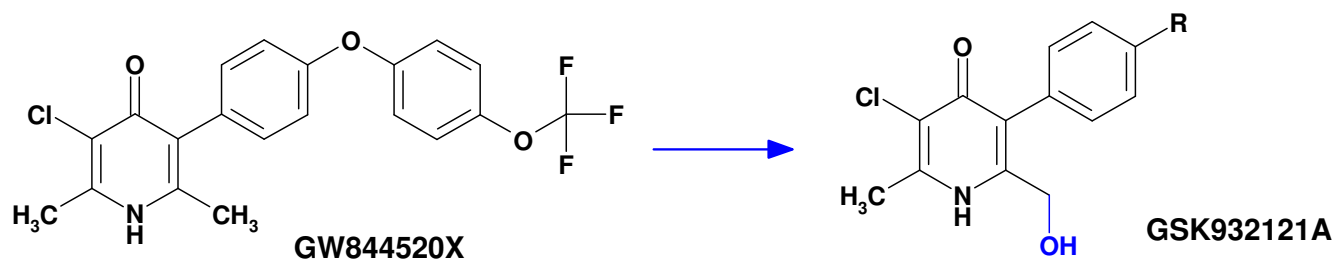
pH- solubility profiles.

- The logS values as a function of pH. The intrinsic solubility S_0 and pKa values were accurately measured in PBS for these compounds in order to obtain the solubility-pH profile using the Henderson-Hasselbalch approach to derive equations (I) and (II) which were used to draw.
 - $\log S = \log S_0 + \log([H^+] + K_a) + pK_a$ for weak bases (I)
 - $\log S = \log S_0 + \log([H^+] + K_a) - pK_a$ for weak acids (II)



Physicochemical profiling (I).

Introduction of polar groups



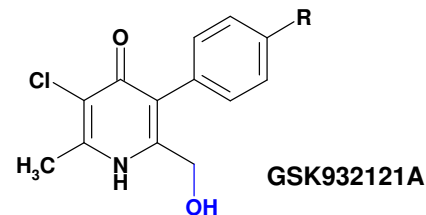
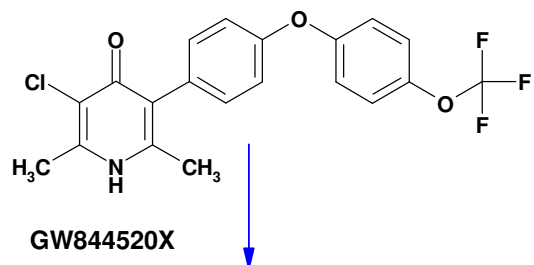
Lipophilicity properties

	<i>CHIlogD2.0</i>	<i>CHIlogD7.4</i>	<i>CHIlogD10.5</i>	<i>clogP</i>
GW844520X	2.75	2.79	2.77	4.9
GSK932121A	2.43	2.42	2.20	3.81

pK_a and pH solubility profile in PBS

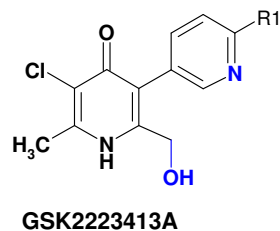
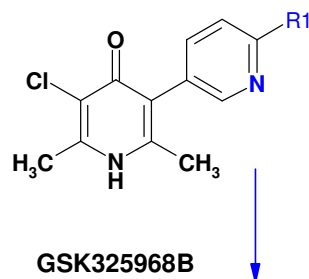
	<i>pKa</i>	<i>So (ug/ml)</i>
GW844520X	10.10	1.4
GSK932121A	9.73	4.6

Physicochemical profiling (II). Introduction of heteroatoms



Lipophilicity properties

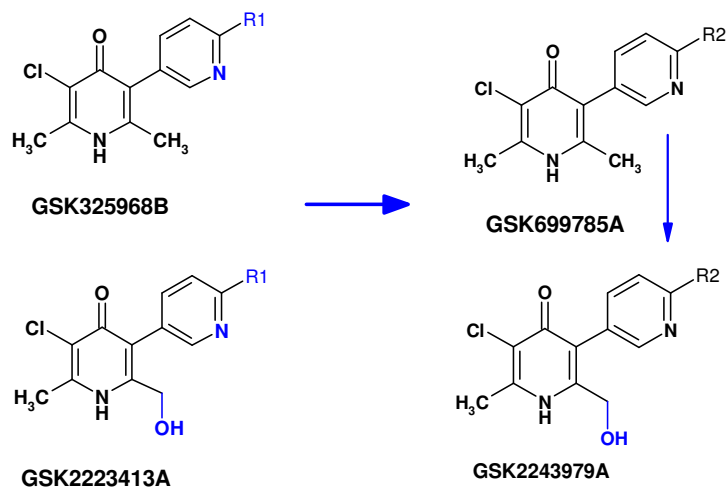
	<i>CHIlogD2.0</i>	<i>CHIlogD7.4</i>	<i>CHIlogD10.5</i>	<i>clogP</i>
GW844520X	2.75	2.79	2.77	4.9
GSK932121A	2.43	2.42	2.20	3.81
GSK325968B	1.47	2.13	1.99	3.52
GSK2223413A	1.31	1.84	1.32	2.44



pK_a and *pH* solubility profile in PBS

	<i>pK_a</i>	<i>S₀</i> (ug/ml)
GW844520X	10.10	1.4
GSK932121A	9.73	4.6
GSK325968B	9.59; 6.21	3.8
GSK699785A	9.74; 5.96	6.83

Physicochemical profiling (III).



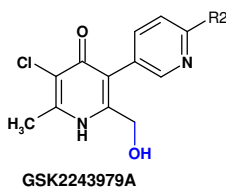
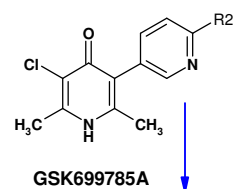
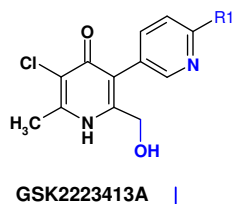
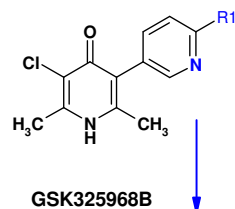
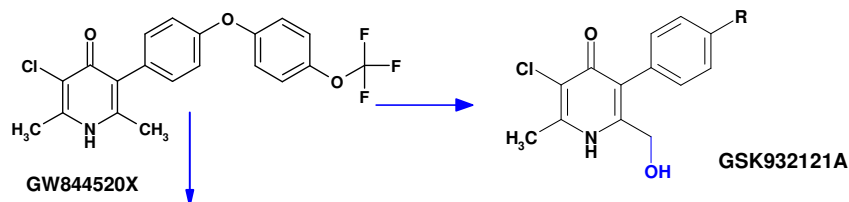
	<i>pK_a</i> and pH solubility profile in PBS	
	<i>pK_a</i>	<i>S₀</i> (ug/ml)
GW844520X	10.10	1.4
GSK932121A	9.73	4.6
GSK325968B	9.59; 6.21	3.8
GSK699785A	9.74; 5.96	6.83
GSK2223413A	9.3; 5.71	14.75
GSK2243979A	9.59; 5.86	13.39

Lipophilicity properties

	<i>CHIlogD2.0</i>	<i>CHIlogD7.4</i>	<i>CHIlogD10.5</i>	<i>clogP</i>
GW844520X	2.75	2.79	2.77	4.9
GSK932121A	2.43	2.42	2.20	3.81
GSK325968B	1.47	2.13	1.99	3.52
GSK2223413A	1.31	1.84	1.32	2.44
GSK699785A	2.07	2.24	1.97	3.44
GSK2243979A	1.64	1.79	1.23	2.35

Physicochemical assays

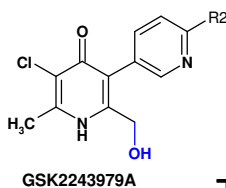
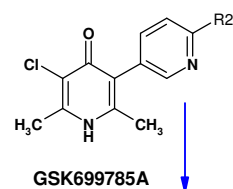
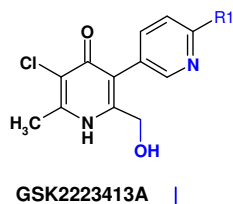
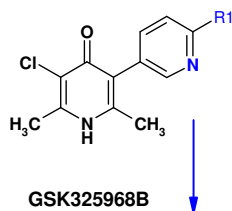
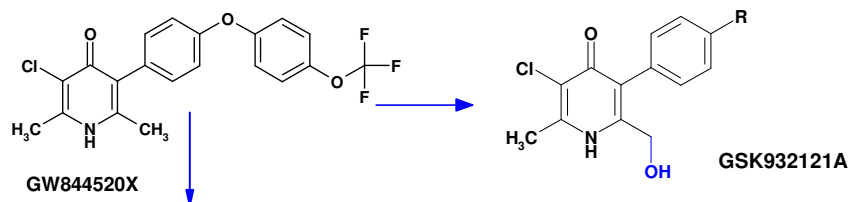
- Hydrophobicity
 - CHlogD at pH: 2.0, 7.4, 10.2
 - Chromatographic Hydrophobicity Indexes, (Valko, K. et al)*
- pH-solubility profile.
- Solubility screening in biorelevant media
 - Simulated Gastric Fluid (SGF) pH: 2.0
 - Phosphate Based Buffer (PBS) pH: 7.4
 - Fasted State Simulated Intestinal Fluid (FaSSIF) pH: 6.8
 - Fed State Simulated Intestinal Fluid (FeSSIF) pH: 5.0



Equilibrium solubility in biorelevant media.

	Equilibrium solubility $\mu\text{g/ml}$ (pH)			
	SGF (1.2)	FeSSIF (5.0)	FaSSIF (6.8)	PBS (7.4)
GW844520X	286.94	0.70	0.50	<0.1
GSK932121A	372.1	2.34	0.69	<0.1
GSK325968B	218.79	1.40	0.50	<0.1
GSK699785A	340.00	12.50	0.10	<0.1
GSK2223413A	348.21	23.4	0.13	<0.1
GSK2243979A	455.0	18.88	0.22	0.2

Equilibrium solubility experiments were performed in bio-relevant media, SGF, FaSSIF, FeSSIF and PBS, in order to get insight about the potential absorption behaviour of these compounds after oral administration. The best solubility was found in SGF at pH 1.2, where they are protonated at the nitrogen atom of the pyridone ring ($\text{pK}_a < 1.5$).



Solubility in biorelevant media vs. S_0

	Equilibrium solubility $\mu\text{g/ml}$ (pH)				
	SGF (1.2)	FeSSIF (5.0)	FaSSIF (6.8)	PBS (7.4)	S_0 (ug/ml)
GW844520X	286.94	0.70	0.50	<0.1	1.4
GSK932121A	372.1	2.34	0.69	<0.1	4.6
GSK325968B	218.79	1.40	0.50	<0.1	3.8
GSK699785A	340.00	12.50	0.10	<0.1	6.83
GSK2223413A	348.21	23.4	0.13	<0.1	14.75
GSK2243979A	455.0	18.88	0.22	0.2	13.39

These S_0 values and the pH-solubility profiles are in general agreement with those equilibrium solubility measures in SGF, FaSSIF, FeSSIF and PBS.

in vitro ADME profiling assays


- Primary assays:
 - Clearance in microsomes (mouse, rat, human)
 - Plasma Protein Binding (mouse, human).
 - Permeability in Caco-2 and MDCK cell lines.
- Secondary assays:
 - Metabolic stability in hepatocytes (rat)
 - hERG

in vitro ADME profiling assays

- Primary assays:
 - Clearance in microsomes (mouse, rat, human)
 - Plasma Protein Binding (mouse, human).
 - Permeability in Caco-2 cell lines.
- Secondary assays:
 - Metabolic stability in hepatocytes (rat)
 - hERG

in vitro ADME profiling: plasma protein binding

	Conc. μM	Mouse CD1	Human
GW 844520X	1 μM	99,2 \pm 0,1	99,7 \pm 0,1
	10 μM	99,1 \pm 0,1	99,7 \pm 0,1
GSK932121A	1 μM	97,6 \pm 0,2	99,0 \pm 0,1
	10 μM	98,1 \pm 0,1	99,4 \pm 0,1
GSK325968B	1 μM	96,8 \pm 0,2	98,3 \pm 0,1
	10 μM	97,6 \pm 0,1	98,9 \pm 0,1
GSK2223413A	1 μM	96,5 \pm 0,2	98,9 \pm 0,1
	10 μM	96,0 \pm 0,2	99,1 \pm 0,1
GSK699785A	1 μM	95,8 \pm 0,9	98,3 \pm 0,1
	10 μM	96,1 \pm 0,1	98,2 \pm 0,1
GSK2243979A	1 μM	95,0 \pm 0,6	97,7 \pm 0,1
	10 μM	93,8 \pm 0,7	98,6 \pm 0,2



Plasma protein binding measures obtained from equilibrium dialysis experiments in two species. In general those values obtained in mouse and human plasma follow the same trend seen for the hydrophobicity of these compounds.

Permeability in Caco-2 cell lines

	<i>P_{app}</i> (nm/s)	
GW844520X	4.60	
GSK932121A	39.20	
GSK325968B	46.45	
GSK2223413A	62.25	
GSK699785A	78.00	
GSK2243979A	83.52	
Amprenavir	114.07	moderate
Antipirina	244.84	hight

PKPD properties

Mouse Pharmacokinetics (p.o. 10 mg/Kg)

	<i>C_{max} (ug/ml)</i>	<i>T_{max}</i>	<i>AUC(0-t)</i>	<i>DNAUC(0-t)</i>	<i>% F(0-t)</i>
GW844520X	0.95	10.0	50.1	4.32	19.40
GSK932121A	1.11	6.0	59.4	2.77	50.00
GSK325968B	5.29	10.0	166.8	15.50	49.80
GSK2223413A	12.4	5.3	194	20.3	99.9
GSK699785A	6.38	10	204.1	17.73	30.3
GSK2243979A	1.85	6	21.2	2.14	22

PK parameters measured in whole blood after p.o. administration to CD1 mice, as suspensions in 1% Methyl Cellulose at 10 mg/Kg. The influence of lipophilicity and solubility in SGF of these compounds on their PK properties, seems to be in agreement with the relative C_{max} and AUC observed for these derivatives.

Summary

- Physicochemical properties were the key drivers of PK properties of antimalarial pyridones.
- Lipophilicity reduction and solubility increase in biorelevant media have shown a good correlation with exposure improvement.