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Potent antimalarial 4-pyridones with improved physico-chemical properties

José M. Bueno *, Pilar Manzano, María C. García, Jesús Chicharro, Margarita Puente, Milagros Lorenzo, Adolfo García, Santiago Ferrer, Rubén M. Gómez, María T. Fraile, José L. Lavandera, José M. Fiandor, Jaume Vidal, Esperanza Herreros, Domingo Gargallo-Viola

Tres Cantos Medicines Development Campus, Diseases of the Developing World, GlaxoSmihKline, C/Severo Ochoa, 2, 28760-Tres Cantos, Madrid, Spain

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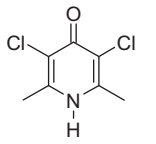
ABSTRACT

Antimalarial 4-pyridones are a novel class of inhibitors of the plasmodial mitochondrial electron transport chain targeting Cytochrome bc1 (complex III). In general, the most potent 4-pyridones are lipophilic molecules with poor solubility in aqueous media and low oral bioavailability in pre-clinical species from the solid dosage form. The strategy of introducing polar hydroxymethyl groups has enabled us to maintain the high levels of antimalarial potency observed for other more lipophilic analogues whilst improving the solubility and the oral bioavailability in pre-clinical species.

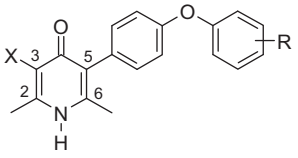
Malaria is still one of the major causes of death in the planet, with over half of the world's population exposed to the risk of infection. The World Health Organisation (WHO) has estimated the burden of malaria to be around 225 million clinical cases, leading to nearly 781,000 deaths every year, most of which are pregnant women and children under the age of five.[1]

Chloroquine and multidrug resistant *Plasmodium falciparum* strains have spread globally,[2] and there are a limited number of efficacious drug combinations available.[3] On top of this, the signs of emerging resistance to the artemisinins,[4] the basic component of the current gold standard combinations for the treatment of malaria, has led to renewed efforts to find novel antimalarial drugs.[5,6] The mitochondrial respiratory chain of *P. falciparum* makes an attractive target for chemotherapy, and is validated by atovaquone. Furthermore, it differs from the analogous mammalian system in a number of ways, suggesting that parasite specific agents should be achievable.[7–9]

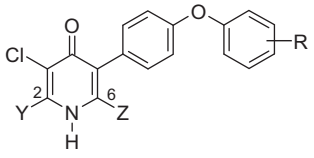
In our efforts to find novel and affordable antimalarials, scientists at the former Wellcome Laboratories and more recently at GlaxoSmithKline have developed a Medicinal Chemistry programme based on 4-pyridones related to the anti-coccidial drug clopidol (Fig. 1). As a result, a first series of potent diaryl-ether substituted 2,6-dimethyl-4-pyridones, have been reported.[10] In general, the most potent pyridones, exemplified by GW844520 and GW308678 were BCS class II compounds,[11] with very low solubility in aqueous media, good oral bioavailability in rodents from solution formulations at low doses (>50% at dose <1 mg/kg),[12] but low oral bioavailability at higher doses administered in suspension (<20%). The main issue for the development of these molecules is the lack of linearity



clopidol



GW844520, X= Cl, R= 4-OCF_3, *Pf*IC_{50}= 0.007 μM
GW308678, X= Cl, R= 3-CF_3, *Pf*IC_{50}= 0.003 μM



I: Z= CH_3; Y=CH_2OH, $CH_2NR_1R_2$,
II: Y=CH_3; Z= CH_2OH, $CH_2NR_1R_2$, COOH, $CON(CH_3)_2$
R=4-OCF_3, 3-CF_3

Figure 1.

* Corresponding author. Tel.: +34 91 807 0616; fax: +34 91 807 0550.
E-mail address: jose.m.bueno@gsk.com (J.M. Bueno).

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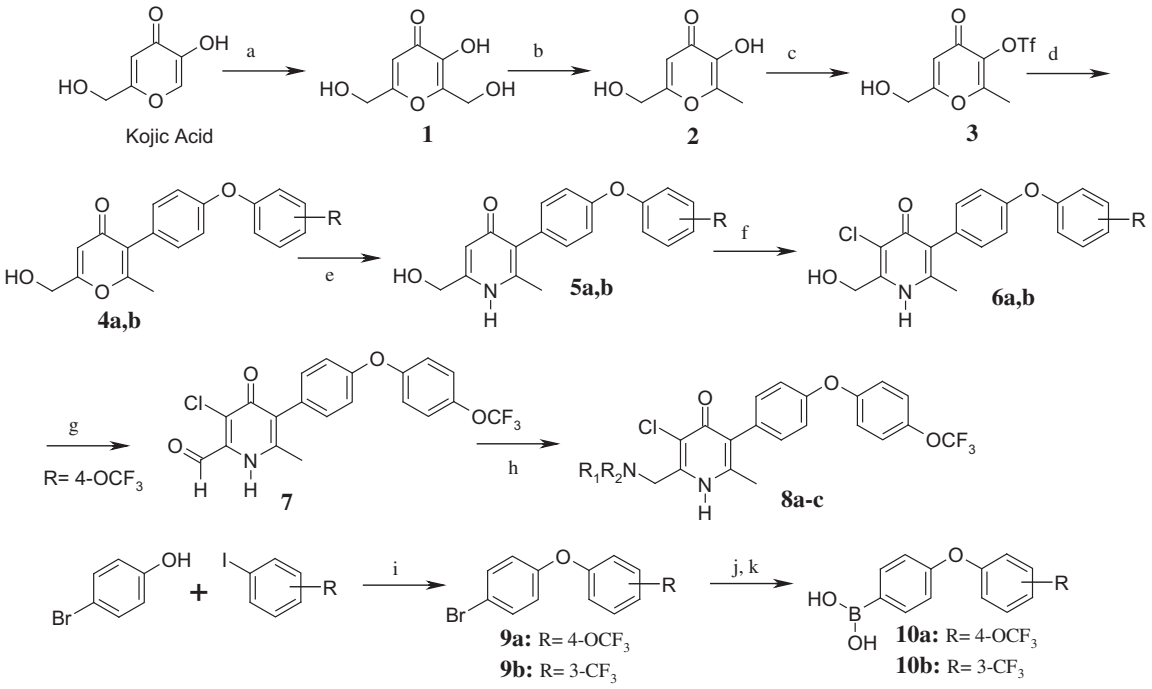
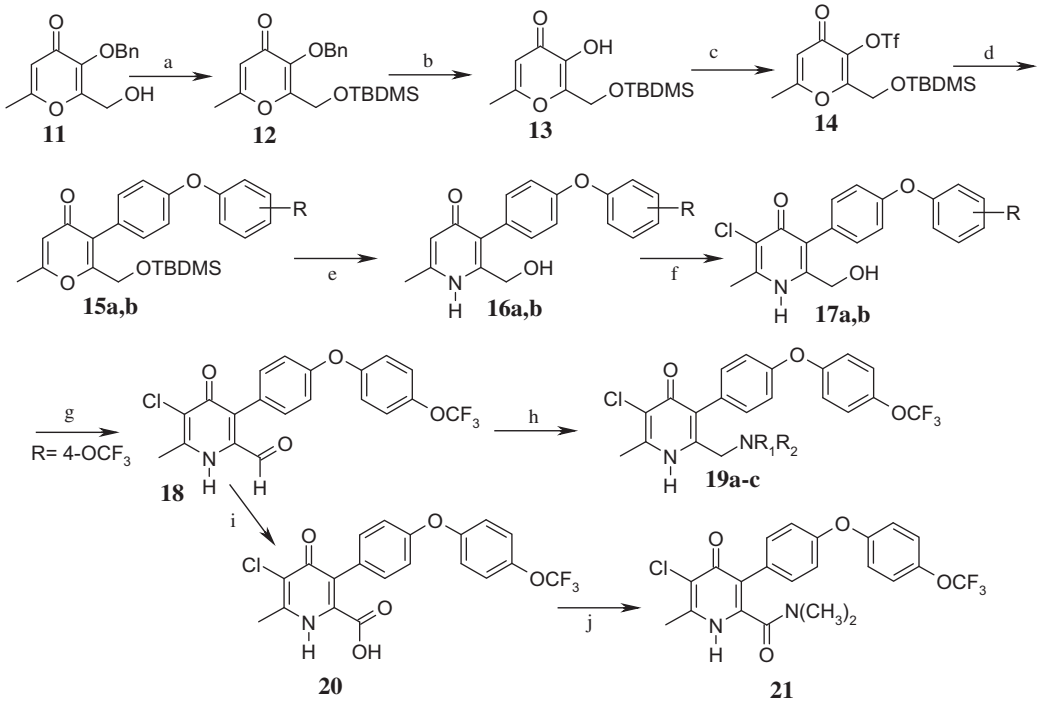


Scheme 1. Reagents and conditions: (a) 37% aq CH_2O, aq NaOH, rt 50%; (b) Zn, cc HCl, rt 53%; (c) Tf_2NPh, DMF, K_2CO_3, rt 50%; (d) **10a**,**b**, $PdCl_2(PPh_3)_2$, Toluene/EtOH, aq Na_2CO_3, 80 °C, 80% (**4a**), 90% (**4b**); (e) aq NH_3, EtOH, 140 °C, 50% (**5a**), 60% (**5b**); (f) trichloroisocyanuric acid, MeOH/DCM, 0 °C to rt 77% (**6a**), 75% (**6b**); (g) SO_3.Py, DMSO, TEA, DCM, 0 °C to rt 90%; (h) R_1R_2NH, $NaBH(AcO)_3$, AcOH, DCE, rt 60–70%; (i) CuCl, Cs_2CO_3, tetramethyl-heptanedione, NMP, 100 °C, 70% (**9a**), 90% (**9b**); (j) $(PriO)_3B$, *n*BuLi, THF, −78 °C; (k) 6N HCl, rt 80% (**10a**), 70% (**10b**).

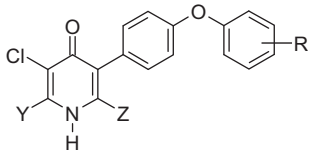


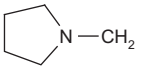
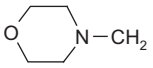
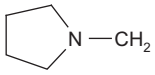
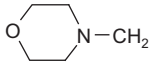
Scheme 2. Reagents and conditions: (a) TBDMSCl, DMF, imidazole, rt 96%; (b) H_2, 10% Pd(C), EtOAc; (c) Tf_2NPh, DMF, K_2CO_3, rt 90% (overall yield steps b + c); (d) **10a**,**b**, $PdCl_2(PPh_3)_2$, Toluene/EtOH, aq Na_2CO_3, 80 °C, 81% (**15a**), 75% (**15b**); (e) aq NH_3, EtOH, 140 °C, 60% (**16a**), 50% (**16b**); (f) trichloroisocyanuric acid, MeOH/DCM, 0 °C to rt 80% (**17a**), 78% (**17b**); (g) SO_3.Py, DMSO, TEA, DCM, 0 °C to rt 84%; (h) R_1R_2NH, $NaBH(AcO)_3$, AcOH, DCE, rt, 60–70%; (i) $NaClO_2$, NH_2SO_3H, acetone/water, rt, 82%; (j) $(CH_3)_2NH$, TOTU, TEA, DCE, 0 °C to rt 72%.

between doses and oral exposures that hinders the safety assessment studies in pre-clinical species.

GW844520 was progressed to pre-clinical studies and a back-up programme aimed at designing novel 4-pyridone derivatives with improved solubility and PK profile was started. In previous studies we have found that the introduction of tertiary amines,[10] esters or polar hydroxyl groups[13] at position C3 had deleterious effects on the antimalarial activity (*Pf* IC_{50} >0.25 μM). Herein we

Table 1
Antimalarial activity of compounds of general Formulae **I** and **II** (*P. falciparum* ^{3}H-hypoxanthine uptake)



Compound	Y	Z	R	*Pf* IC_{50} (mM)
6a	CH_2OH	CH_3	4-OCF_3	0.13
6b	CH_2OH	CH_3	3-CF_3	0.12
8a	Me_2NCH_2	CH_3	4-OCF_3	>0.25
8b		CH_3	4-OCF_3	>0.25
8c		CH_3	4-OCF_3	>0.25
17a	CH_3	CH_2OH	4-OCF_3	0.002
17b	CH_3	CH_2OH	3-CF_3	0.003
19a	CH_3	Me_2NCH_2	4-OCF_3	>0.25
19b	CH_3		4-OCF_3	>0.25
19c	CH_3		4-OCF_3	>0.25
20	CH_3	CO_2H	4-OCF_3	>0.25
21	CH_3	$CO_2N(Me)_2$	4-OCF_3	>0.25
GW844520	CH_3	CH_3	4-OCF_3	0.007
GW308678	CH_3	CH_3	3-CF_3	0.003

describe the synthesis and biological activity of a series of 4-pyridones bearing polar or ionizable moieties at positions C2 and C6 of the 4-pyridone ring (Fig. 1, Formulae **I** and **II**).

The classical approach for the construction of the 2,6-dimethyl-4-pyridone scaffold involves the condensation of the appropriate 2-propanone derivatives with acetic anhydride and polyphosphoric acid[10] or Eaton's reagent[14] followed by ammonolysis. However, the presence of substituents different from methyl at position C2 (Formula **I**) or at position C6 (Formula **II**), precludes the use of acetic anhydride, thus making the synthesis particularly challenging.

The synthesis of compounds of general Formula **I** was carried out from commercially available Kojic acid according to the synthetic route depicted in Scheme 1 (see Refs. 10 and 15 for experimental details). The introduction of the key phenyl-oxy-phenyl lipophilic moiety was carried out by Suzuki coupling between triflate **3** and the appropriate boronic acids **10a,b**[10] to afford pyrones **4a,b** which were subjected to ammonolysis and further chlorination by reaction with trichloroisocyanuric acid.[16] In general, the use of trichloroisocyanuric acid was found to provide a convenient substitute for *N*-chlorosuccinimide used previously in the chlorination of pyridones,[10] with advantages in shorter reaction times, lower reaction temperatures, cleaner reaction crudes and higher yields of final materials.

The synthesis of compounds of general Formula **II** (Scheme 2), has been carried out from Kojic acid derivative **11**[17] and involves the preparation of key intermediate **13**, in which the primary hydroxyl group is protected as TBDMS ether in order to prevent the migration of the reactive triflate group from C3–OH to the vicinal C2–CH_2OH group.[18] The introduction of the phenyl-oxy-phenyl lipophilic moiety and the preparation of the 4-pyridone nucleus have been accomplished in a similar way as described in Scheme 1.

Table 1 shows the in vitro antimalarial activity of the 4-pyridone derivatives prepared in terms of IC_{50} in the *P. falciparum* ^{3}H-hypoxanthine uptake assay, as described by Desjardins et al.[19] As shown, only compounds **17a**,**b**, with the hydroxymethyl group attached at position C6 of the 4-pyridone ring maintained the high level of antimalarial activity observed for their non hydroxylated counterparts GW844520 and GW308678, respectively. It is noteworthy that compounds **6a**,**b**, bearing the hydroxymethyl group at position C2 of the 4-pyridone ring are significantly less potent than their corresponding isomers **17a**,**b**. Concerning other polar or ionisable moieties, all the compounds having tertiary amines (**8a–c** and **19a–c**), carboxylic acid (**20**) or dimethyl-amide (**21**) showed no activity in the assay (>0.25 μM), irrespective of their position in the 4-pyridone ring.

A 3D theoretical model of the *P. falciparum* Cytochrome b:Rieske protein complex has been built by using the crystal structures of the bovine, chicken and yeast Cytochrome b as templates.[20–22] Compounds were docked within the active site and the conformations with best docking scores were then minimised. Figure 2 shows the best conformation identified for compound **17a**. From the analysis of these results we can conclude that whilst most of the interactions are hydrophobic, the most interesting one comes from a hydrogen bond formed between the C6–CH_2OH group and the carboxylic acid of Glu-261. This is a new interaction identified for **17a** which could explain the increase in potency seen for this

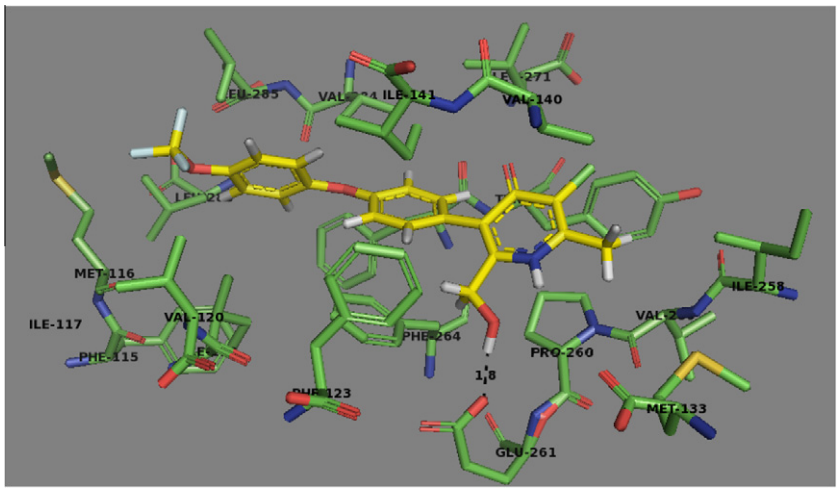


Figure 2. 3D-Theoretical model of the *P. falciparum* Cytbc1 active site showing the best conformation identified for compound **17a**. The program MOE (2008.09) was used for homology protein modelling and molecular calculations. Ligand docking studies were performed with program GOLD (v4.01) (Refs. 20 and 21).

Table 2
Physicochemical properties of compounds **17a** and GW844520

Compound	CHI log *D* (pH)			log *P*[a]	mp (°C)	PSA[b]	pK_a[a]	Solubility, mg/ml (pH)				
	2	7.4	10.5					S_0[c]	SGF (1.2)	FeSSIF (5.0)	FaSSIF (6.8)	PBS (7.4)
17a	2.43	2.42	2.2	4.4	272-274	71.55	9.73	4.6	372	2.34	0.69	<0.1
GW844520	2.75	2.79	2.77	4.8	296-298	51.32	10.1	1.4	287	0.7	0.5	<0.1

[a] Potentiometric titration measurements taken using a Gemini Profiler from pION.
[b] Calculated data (Adamantis software).
[c] Obtained from log *P* (Ref. 23).

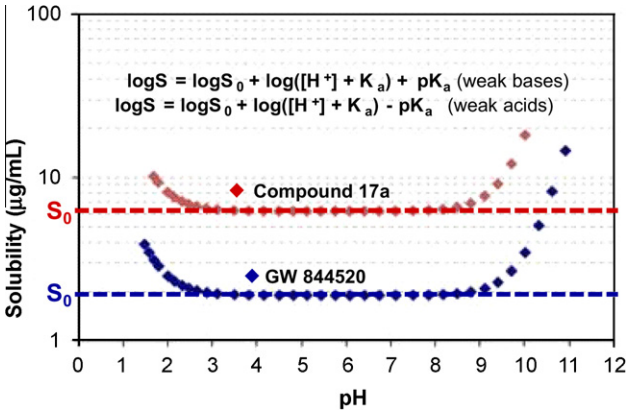


Figure 3. pH-Solubility profile obtained by using the Henderson–Hasselbach approach. Solubility profiles were calculated fitting a curve to potentiometric titration data in PBS, having previously measured pK_a and log *P* to estimate S_0 values (Ref. 23).

compound in comparison with those found for its isomer **6a** and GW844520 in similar docking studies. On the other hand, the drop of potency observed for compound **6a** is also in agreement with molecular modelling calculations, due to the hydrophobic nature of the aminoacids surrounding the position C2 of the pyridone ring (Ile-258, Val259 and Met-133).

Furthermore, the steric constrains around both positions C6, and C2 may explain why compounds with larger substituents such as di-alkyl-amines or amides were not active in the assay.

In order to study the influence of the 6-hydroxymethyl group present in compound **17a** on its physicochemical properties, in Table 2 are reported the chromatographic hydrophobicity indexes (CHI log *D*)[23] measured at pH 2.0, 7.4 and 10.5, log *P*, melting point, calculated polar surface area, pK_a, intrinsic solubility S_0, pH-solubility profile[24] and equilibrium solubility in different media, in comparison with GW844520.

As shown, compound **17a** is less lipophilic and, in general, it displays better physicochemical properties than its non hydroxylated analogue GW844520. Both molecules remained unionised at pH 2–10 as demonstrated by the CHI log *D* data and the pH-solubility curve (Fig. 3), obtained by using the Henderson–Hasselbach approach that uses the pK_a and the intrinsic solubility S_0 obtained from experimental log *P* values.[24] As shown in Table 2 and Figure 3, the intrinsic solubility S_0 obtained for compound **17a** is approximately three times higher than that for GW844520 over a wide range of pH.

The equilibrium solubility experiments were performed in PBS and bio-relevant media[25] (SGF, FaSSIF and FeSSIF) in order to gain insight about the potential absorption behaviour of these compounds after oral administration. Compound **17a** was more soluble than GW844520 in all the media tested except PBS (pH 7.4), where the equilibrium solubility measured for both compounds was extremely low. As expected, the best solubility was found in SGF at pH = 1.2, where the nitrogen atom of the pyridone ring is protonated (pK_a <1.5).

Melting points are indicative of the crystal-lattice energy, which for crystalline solids contribute significantly to solubility.[26] Although both **17a** and GW844520 are chemically stable solids with high melting points, the lower value measured for **17a** indicates a lower crystal-lattice energy, thus contributing to increase the solubility in comparison to the non hydroxylated analogue GW844520.

In order to evaluate the impact of the physico-chemical parameters measured on the in vivo behaviour, the pharmacokinetic profile of **17a** in comparison to GW844520 in CD1 mice and Beagle dogs was assessed (Table 3).

As shown, the increase in the solubility of **17a** in comparison with its nonhydroxylated analogue GW844520, particularly in SGF and FeSSIF, translated into a significant enhancement in the oral bioavailability from the solid dosage form (suspension in 1% methylcellulose) both in mice (50% vs 20%) and particularly in dogs (16% vs 4.4%). On the other hand, compound **17a** seems to be more sensitive to metabolic clearance, as demonstrated by the significant reduction in the half-lives in both species, possibly due to the oxidation of the hydroxyl group to carboxylic acid or by direct elimination via conjugation. In spite of its shorter half-life, compound **17a** has displayed excellent in vivo antimalarial efficacy in our murine model of *P. falciparum* malaria[27] (ED_{50} = 0.6 mg/kg).

In conclusion, the data reported suggest that it is possible to improve the physicochemical properties and pharmacokinetic profile of 4-pyridones by performing certain chemical transformations at the 4-pyridone ring. Although the presence of ionisable tertiary amines, carboxylic acid or amide groups are not allowed, hydroxymethyl groups show a very different behaviour, depending on their position. Thus, whilst the CH_2OH group induces a drop in

Table 3
Pharmacokinetic parameters of compounds **17a** and GW844520 in CD1 mice and Beagle dogs.

Compound	Mouse					Dog				
	po 10 mg/kg[a]		iv 0.2 mg/kg[b]			po 2 mg/kg[a]		iv 0.05 mg/kg[b]		
	C_{max} (mg/ml)	%*F*	V_d (L/kg)	Cl (ml/min/kg)	$t_{1/2}$ (h)	C_{max} (mg/ml)	%*F*	V_d (L/kg)	Cl (ml/min/kg)	$t_{1/2}$ (h)
17a	1.1	50	1	3	3.8	0.3	16	2.8	0.8	42
GW844520	0.9	20	1.2	0.6	24.1	0.03	4.4	2.9	0.2	143

[a] Oral gavage. Suspension in 1% methyl cellulose.
[b] Solution in 1% DMSO/7.5% PEG400/20% encapsin/saline, pH 6.

antimalarial activity in vitro when it is located at position C2 in comparison with its non hydroxylated counterpart, it is possible to obtain potent derivatives with improved solubility and pharmacokinetic properties by introducing the CH_2OH group at position C6 of the 4-pyridone ring. This behaviour offers new opportunities in the design of novel potent, more soluble and bioavailable antimalarial 4-pyridones.

Acknowledgement

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