



Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Potent antimalarial 4-pyridones with improved physico-chemical properties

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ARTICLE INFO

Article history:

Received 15 June 2011

Revised 11 July 2011

Accepted 12 July 2011

Available online 23 July 2011

Keywords:

Malaria

Plasmodium falciparum

4-Pyridones

Solubility

Pharmacokinetic

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.

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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 Glaxo-SmithKline have developed a Medicinal Chemistry programme based on 4-pyridones related to the anti-coagulant drug clopidogrel (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

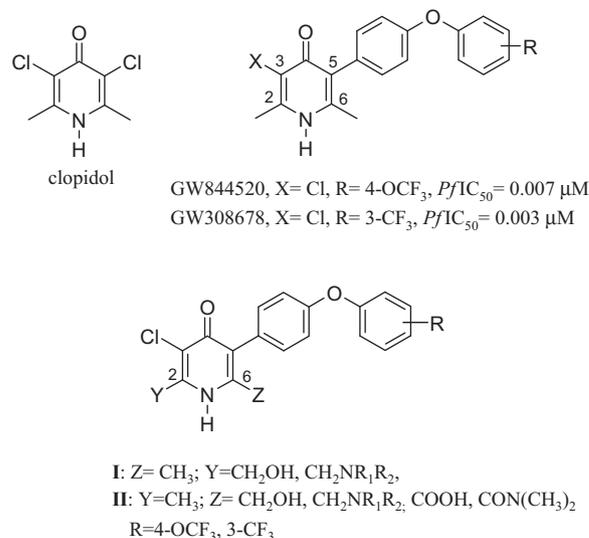
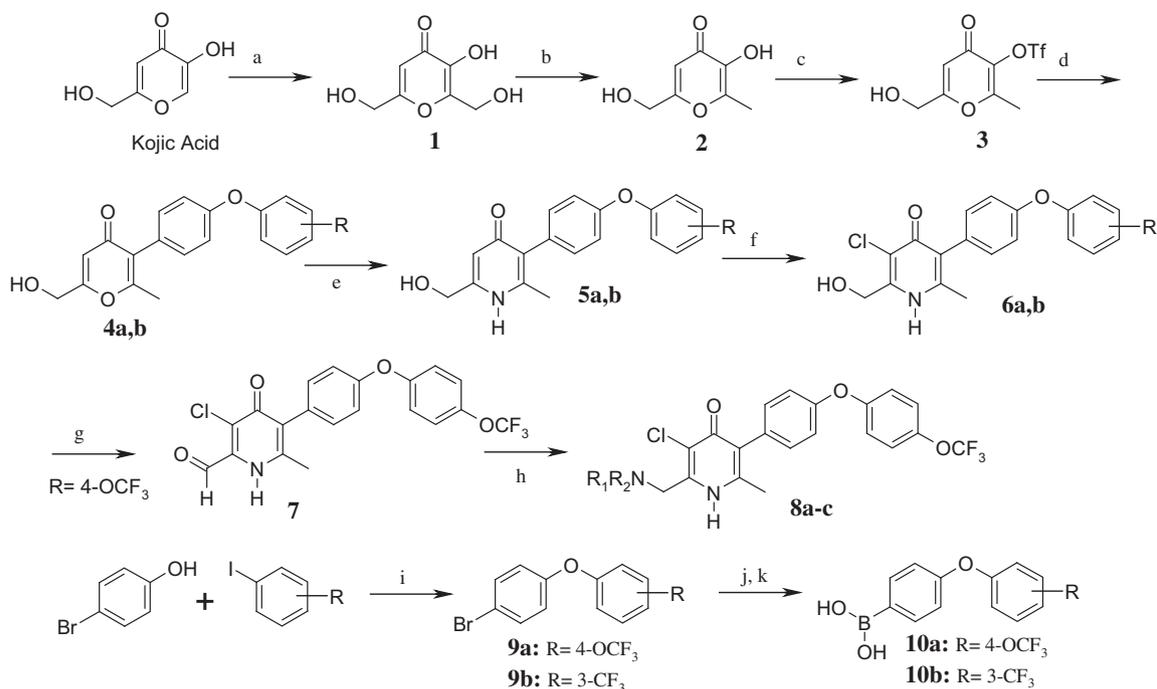


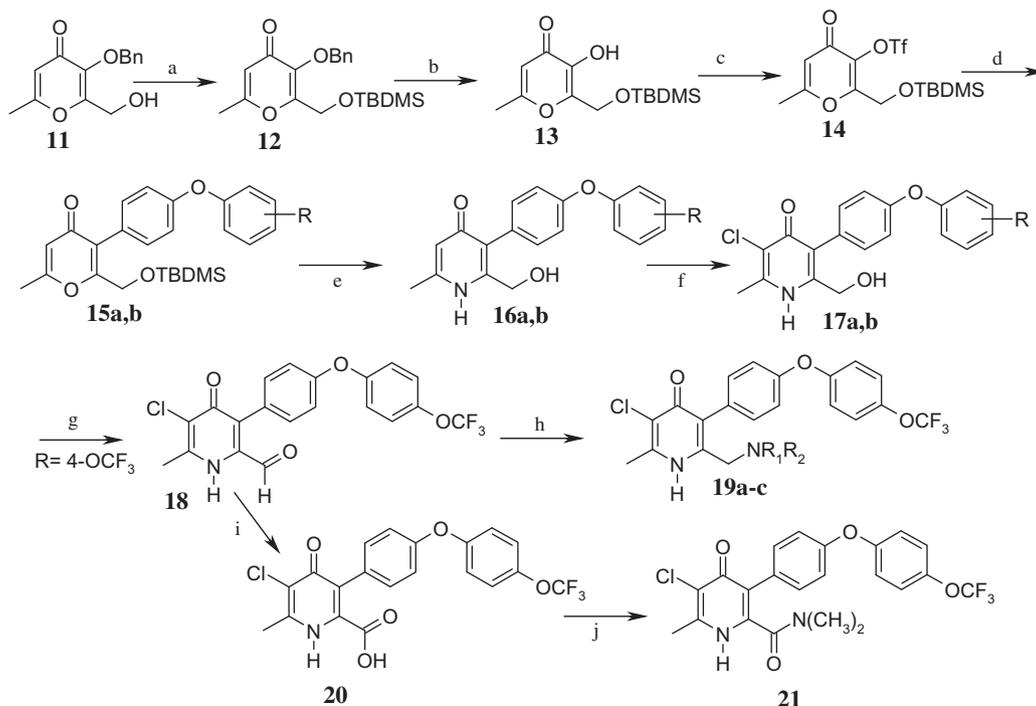
Figure 1.

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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) Ti_2NPh , DMF, K_2CO_3 , rt 50%; (d) **10a,b**, $\text{PdCl}_2(\text{PPh}_3)_2$, 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) $\text{SO}_3\cdot\text{Py}$, DMSO, TEA, DCM, 0 °C to rt 90%; (h) $\text{R}_1\text{R}_2\text{NH}$, $\text{NaBH}(\text{AcO})_3$, AcOH, DCE, rt 60–70%; (i) CuCl , Cs_2CO_3 , tetramethyl-heptanedione, NMP, 100 °C, 70% (**9a**), 90% (**9b**); (j) $(\text{PriO})_3\text{B}$, $n\text{BuLi}$, THF, –78 °C; (k) 6N HCl, rt 80% (**10a**), 70% (**10b**).



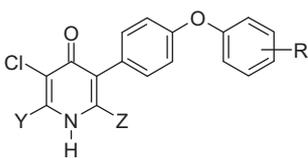
Scheme 2. Reagents and conditions: (a) TBDMSCl, DMF, imidazole, rt 96%; (b) H_2 , 10% Pd(C), EtOAc; (c) Ti_2NPh , DMF, K_2CO_3 , rt 90% (overall yield steps b + c); (d) **10a,b**, $\text{PdCl}_2(\text{PPh}_3)_2$, Toluene/EtOH, aq Na_2CO_3 , 80 °C, 81% (**15a**), 75% (**15b**); (e) aq NH_3 , EtOH, 140 °C, 60% (**16a**), 50% (**16b**); (f) trichloroisocyanuric acid, MeOH/DCM, 0 °C to rt 80% (**17a**), 78% (**17b**); (g) $\text{SO}_3\cdot\text{Py}$, DMSO, TEA, DCM, 0 °C to rt 84%; (h) $\text{R}_1\text{R}_2\text{NH}$, $\text{NaBH}(\text{AcO})_3$, AcOH, DCE, rt, 60–70%; (i) NaClO_2 , $\text{NH}_2\text{SO}_3\text{H}$, acetone/water, rt, 82%; (j) $(\text{CH}_3)_2\text{NH}$, TOTU, TEA, DCE, 0 °C to rt 72%.

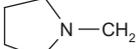
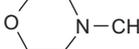
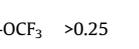
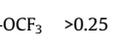
between doses and oral exposures that hinders the safety assessment studies in pre-clinical species.

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 groups¹³ 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* ³H-hypoxanthine uptake)



Compound	Y	Z	R	Pf IC ₅₀ (mM)
6a	CH ₂ OH	CH ₃	4-OCF ₃	0.13
6b	CH ₂ OH	CH ₃	3-CF ₃	0.12
8a	Me ₂ NCH 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 ₂ OH	4-OCF ₃	0.002
17b	CH ₃	CH ₂ OH	3-CF ₃	0.003
19a	CH ₃	Me ₂ NCH 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 ₂ H	4-OCF ₃	>0.25
21	CH ₃	CO ₂ N(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 acid¹⁰ or Eaton's reagent¹⁴ 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–CH₂OH 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 IC₅₀ in the *P. falciparum* ³H-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–CH₂OH 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

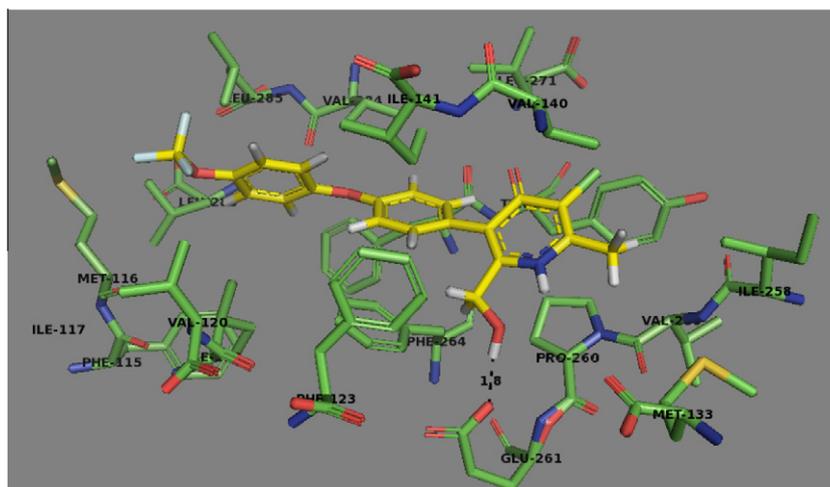


Figure 2. 3D-Theoretical model of the *P. falciparum* Cytbc1 active site showing the best conformation identified for compound **17a**. The program MOE (2008.09) was used for homology protein modelling and molecular calculations. Ligand docking studies were performed with program GOLD (v4.01) (Refs.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

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

Compound	CHI log <i>D</i> (pH)			log <i>P</i> ^a	mp (°C)	PSA ^b	p <i>K</i> _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).