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Synthesis and Structure-Activity Relationships of Novel Antimalarial 5-Pyridinyl-4(*1H*)-Pyridones.

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Abstract

Malaria is still one of the most prevalent parasitic infection in the world with half of the world's population at risk of malaria. The effectiveness of current antimalarial therapy is under continuous threat through the spread of resistant *Plasmodium* strains, even to the most recent class of antimalarial drugs (ACT: artemisinin-combination-therapies). As a consequence, there is still an urgent requirement for new antimalarial drugs. We previously reported the identification of 4(1H)-pyridones as a novel series with potent antimalarial activity. The low solubility was identified as an issue to address. In this paper, we

describe the synthesis and biological evaluation of 4(*1H*)-pyridones with potent antimalarial activity *in vitro* and *in vivo* and improved pharmacokinetic profile.Their main structural novelty is the presence of polar moieties such as hydroxyl groups and the replacement of lipophilic phenyl rings by pyridine at the lipophilic side chain.

Introduction

Malaria continues to be a leading cause of death on the planet, with 50% of the population being exposed to the risk of infection. More than 200 million new clinical cases are estimated annually that leads to approximately 800,000 deaths each year primarily to children under five and expectant mothers[1].

Although the control of the disease still depends on chemotherapy, the spread of resistant *P.falciparum* strains to most of the antimalarials currently in use, including chloroquine[2], the limited number of drugs currently available against malaria[3] and the alarming evidences pointing to the appearance of resistant or "less sensitive" *P. falciparum* strains to artemisinin derivatives[4], the basic components of the current gold standard combinations for the treatment of malaria (ACT), has fueled the research effort to find novel drugs for the treatment of malaria[5,6]. In this way, the mitochondrial respiratory chain of *P. falciparum* is an attractive target for chemotherapy due to its differences from the analogous mammalian system[7-10] and its essential caracter for the parasite. In the erythrocytic stages, mitochondria are involved in several essential metabolic processes, which interesting enough do not include ATP production and vary depending on parasitre species. Therefore, proteins involved in mitochondrial physiology have the potental for antiparasitic drug discovery[8].

The election of mitochondrial electron transport chain of *P. falciparum* as a putative target in antimalarial drug discovery has been validated and has been succesful, as demonstrated with the development of atovaquone (Figure 1), introduced into therapy in 1997 as a combination with proguanil.

The combination Atovaquone-Proguanil is currently used in treating multidrug-resistant malaria and for prophylaxis in areas with chloroquine resistance[11].

Atovaquone inhibits the *P. falciparum* mitochondrial respiratory chain at the cytochrome bc_1 level (Complex III), demonstrating that it is a proven drug target in the prevention and treatment of malaria.

The bc1 complex is central to mitochondrial function in *P. falciparum*, and it is composed by two binding sites, named Qo and Qi, for oxidation of Ubiquinol and reduction of Ubiquinone, respectively. Most of the antimicrobial drugs, such as Atovaquone, target the Qo site[12]. Unfortunately, the rapid rise of resistances, mainly caused by mutations at the binding site, has compromised the use of Atovaquone as a first line antimalarial.

In our efforts to find novel antimalarials, scientists at GSK have developed a Medicinal Chemistry programme based on the 4(1*H*)-pyridone core present at the known anti-coccidial drug clopidol (Figure 1). Although initially it was presumed that 4(1*H*)-pyridones were binding the Qo site at the cytochrome bc_1[13], recent studies seem to point to Qi as the binding site for these compounds[14].

Clopidol displayed weak antimalarial activity in animal models[15] and there is evidence indicating that its anticoccidial action is related to mitochondrial impairment[16]. The strategy for increasing the antimalarial activity of clopidol was the replacement of one of the chlorine atoms by different lipophilic side chains. As a result, a series of potent 2,6-dimethyl-4(1*H*)-pyridones such as GW844520 and GW308678, bearing que diphenyl-ether moiety, have been prepared (Figure 1). The novel compounds have demonstrated to be superior to chloroquine *in vitro* against erythrocytic stages of the parasite and *in vivo* in the *P. yoelii* mouse model[17]. Compared to clopidol, the most active 4(*1H*)-Pyridones prepared showed >500 fold improvement in the *in vitro* activity (IC_{50} for inhibition of *P. falciparum*) and ca. 100 fold improvement in the *in vivo* efficacy (ED_{50} in a murine model of *P. yoelii)*. In general, the most potent compounds were characterized as class II compounds according to the Developability Classification System[18], with high permeability and low solubility in aqueous media. They display high oral bioavailability in mouse and rat at low doses administered from solution formulations (50-100% at doses <1 mg/Kg)[19,20] and low oral bioavailability when administered as a solid form in suspension (4-

20%)[21], clearly suggesting that the absorption by oral route is hampered by low solubility and/or dissolution rate of the solid sample. In this regard, it is expected that the physicochemical properties of the ultimate solid dosage form and the particle size could be a key determinants of the preclinical and clinical studies. In a continued effort to overcome the issues related to the poor solubility and oral bioavailability, we have focused our attention on designing novel potent antimalarial 4(*1H*)-pyridones with improved solubility and physicochemical properties.

Given the great influence of the lipophilic tail on the antimalarial activity of 4(*1H*)-pyridones[17], we first tried to increase the solubility of diphenyl-ether derivatives by attaching polar groups and ionisable tertiary amines and polar hydroxyl groups at the 4-pyridone ring. Although the introduction of hydroxyl groups, esters and tertiary amines at both positions C2 and C6 of the pyridone ring has led to a complete loss of the antimalarial activity, the introduction of polar CH_2OH groups exerted different effects on the antimalarial activity depending on their position[21]. As shown in Figure 1, while the hydroxymethyl group induces a significant drop in antimalarial activity *in vitro* in comparison with its non hydroxylated analogue when it is located at position C2, it is possible to maintain high levels of activity by attaching the hydroxymethyl group at position C6 of the 4-pyridone ring, thus leading to potent and more polar compounds with improved PK properties.

Within the strategies aimed at improving physicochemical properties and oral bioavailability, in addition to the introduction of CH_2OH groups at position C6, we have also explored the effect of replacing phenyl rings by more polar heterocycles into the lipophilic side tail. In this paper, we report the synthesis and biological evaluation of a new series of antimalarial 4(1*H*)-pyridone derivatives bearing substituted pyridine rings at position C5 (Figure 2). As shown, we have investigated the effect of both flexible and rigid linkers between the aromatic rings (**Ia-d)** as well as the absence of any linker between them (**IIa,b**).

In summary, according to BCS[(18)], antimalarial 4(1H)Pyridones have traditionally behave as Class II compounds, displaying fair permeability and very low solubility in PBS and other aqueous media. For

this reason, since the very beginning of our Medicinal Chemistry Programme, we have tried to increase the solubility by introducing polar/ionizable groups into the molecules. In this way, as commented previously, while most of these changes have normally lead to a complete loss of *in vitro* antimalarial activity, we have observed that the introduction of an OH group at position C6 maintain a good level of antimalarial activity, thus offering new opportunities for both novel compounds or the synthesis of appropriate prodrugs with improved solubility.

Following this strategy, in this paper we describe the preparation of novel "hybrid" more polar 4(*1H*)-pyridone derivatives that join the presence of pyridine rings at position C5 with the effect of the polar hydroxymethyl groups at position C6 of the 4-pyridone core (**IIb**) described previously. As expected these structural change has led compounds with better physicochemical and PK profiles, maintaining excellent antimalarial activity.

The novel compounds described in this paper have allowed us to wide the scope of antimalarial 4(*1H*)Pyridiones available, thus offering new opportunities to increase the current antimalarial pipeline.

Chemistry

In general, the 4(1*H*)-pyridone core has been traditionally obtained by ammonolysis of the corresponding 4-pyranones. All the compounds described in this paper (except compounds of general formulae **Ib** and **IIb)** have been obtained from key intermediate 4-pyranone **6**, which was prepared from commercially available 5-formyl-2-methoxypyridine **1**, in a similar way as described previously for other pyridones prepared by us[17] (Scheme 1).

Alternatively, intermediate ketone **4** has been obtained in only one step via Stille C-C coupling by reaction of 5-bromo-2-methoxypyridine **2** with isopropenyl acetate and tributyltin methoxide in the presence of the tandem Palladium Acetate/Tri-o-tolylphosphine (TOTP) as catalysts[22]. Condensation of methyl ketone **4** with acetic anhydride was carried out by using either polyphosphoric acid (PPA)[17,23] or more conveniently the less viscous and commercially available Eaton's reagent[24] (mixture of

phosphorous pentoxide in methanesulphonic acid), with advantages in ease of handling and aqueous work-up.

4-pyranone **6**, obtained by acidic hydrolysis of **5**, was used directly in the synthesis of 6-(benzyloxy)pyridine derivatives (**Ia**) as indicated in Scheme 2.

Selective O-benzylation with a series of bencyl halides was accomplished in the presence of silver carbonate[25] to afford 4-pyranones **7-13**, which were subjected to ammonolysis under high pressure and temperature to furnish the corresponding 4-pyridones **14-20**. Subsequent halogenation at position C3 with either N-bromosuccinimide[17,23] or with trichloroisocyanuric acid[26], led to the corresponding 3-bromo or 3-chloro derivatives **21-27** and **28-34**, respectively. The use of cheap and readily available trichloroisocyanuric acid was found to provide a convenient substitute for other chlorinating agents such as N-chlorosuccinimide[23], with advantages in shorter reaction times, atom economy and lower reaction temperature, leading to higher yields of chlorinated material, particularly large scale preparations.

Phenyloxy-pyridin-3-yl derivative **40** was prepared following a completely different synthetic approach, starting from commercially available 2,6-dimethyl-4(1*H*)-pyridone **38** (Scheme 3). Careful monochlorination of **38** with a slight excess of N-chlorosuccinimide at room temperature (trichloroisocyanuric acid proved to be unsuccessful for the monochlorination step due to its high reactivity), followed by iodination in basic solution afforded intermediate **39**, which was used as a suitable starting material for the introduction of the lipophilic side chain via Suzuki C-C coupling. The synthesis of the boronic acid **37** was performed from commercially available 2,5-dibromopyridine **35** by selective displacement of the more reactive 2-bromine atom under Ullmann conditions and subsequent lithiation and boronic acid formation under classical conditions to afford **37**. Finally, reaction of **39** with **37** in the presence of a suitable Palladium catalyst led to phenyloxy-pyridinyl derivative **40**.

With regard to the introduction of carbon substituents at position C6´of the pyridine ring (compounds **Ic,d** and **IIa,** Scheme 4), while the activation of position C6´ hydroxyl group by reaction of 4-pyranone **6** with either $POBr_3$ or $POCl_3$ led to complex mixtures of reaction, treatment with N-phenyl-trifluoromethane-sulfonimide[27] cleanly afforded the chemically stable and crystalline triflate **41** in high

yield and purity. Intermediate **41** has proved to be an ideal starting material for Sonogashira and Suzuki coupling reactions that finally have led to final 4(*1H*)-pyridones **47-49** (formula **Ic**), **54-55** (formula **Id**) and **106-125** (formula **IIa**), respectively, according to the synthetic routes depicted in Scheme 4.

As mentioned previously, the 2,6-dimethyl-4-pyridone scaffold is accesible by condensation of the appropriate 2-propanone derivatives with acetic anhydride in the presence of a dehydrating agent such as polyphosphoric acid or Eaton´s reagent (Scheme 1). However, the presence of any substituent different from methyl at positions C6 like in compounds of Formula **IIb**, precludes the use of the symmetrical acetic anhydride, thus making their synthesis particularly challenging. Therefore, the preparation of C6CH_2OH derivatives of general formula **IIb**, has been tackled through a completely different synthetic approach, described previously by us, starting from commercially available Kojic acid[21] (Scheme 5).

The synthesis starts from benzyl derivative **130**, available from Kojic acid[28], and involves the protection of the primary hydroxyl group as TBDMS ether, removal of benzyl group by hydrogenolysis and subsequent triflylation of C3-hydroxyl group to afford **133**. It is noteworthy that in the absence of the hindered TBDMS ether, migration of the reactive triflyl group from C3-OTf to the vicinal C2-CH_2OH group[29] takes place. Suzuki coupling between triflate **133** and the appropriate boronic acids **128** and **129** led to the corresponding 4-pyranones **134, 135** which were subjected to ammonolysis with concurrent loss of the TBDMS protective group, and further chlorination of the resulting 4-pyridones **136, 137** to afford the target hydroxymethyl derivatives **138, 139**.

Results and discussion

With regard to compounds bearing rigid linkers between the aromatic rings, from the data shown in Table 1 it seems that, although compounds 47 and 48 are quite active, the presence of a rigid linker is related to a lose of potency in comparison with their flexible analogs (compounds 22, 29 and 54). This effect could be explained on the basis of the higher freedom degrees for flexible side chains, which could help making more hydrophobic favored interactions, adopting the active site topology.

On the other hand, it is noteworthy that the presence of two lipophilic groups such as CF_3 in compounds **25, 32** or Fluorine atoms in compounds **27, 34** led to a decrease of the antimalarial activity, which is in contrast with the general correlation observed between lipophilicity and potency in other antimalarial 4(*1H*)-pyridones. This effect could also be explained on the basis of some clashing contacts within the active site[30]

In general, as shown previously in other pyridones[17], the effect of the type of the halogen at position C3 on the antimalarial activity is negligible as demonstrated the good correlation between the IC_{50} values of bromoderivatives **21-27** and chloroderivatives **28-34**, with the only exception of 4-Fluoro-phenyl derivative **33** which displays slightly lower activity than its brominated counterpart **26**.

In Table 2 the biological data for phenyl-pyridinyl derivatives are reported. The main structural feature of this large family of molecules is the rigidity of the lipophilic side tail caused by the absence of any linker between the pyridine and the phenyl rings. From the data shown some observations on SAR can be made.

Concerning substitution at the phenyl ring, it seems that the presence of lipophilic and electron withdrawing groups such as CF_3 or OCF_3 is required to have high antimalarial activity, in a similar way as observed in other pyridones previously reported[17]. The extreme sensitivity of the lipophilic tail to the presence of polar moieties is also observed in the drop in the antimalarial activity displayed by compounds **112-114** with less lipophilic substituents such as CN,SO_2Me and the hydrophilic CH_2OH group. With regard to the effect of halogens (Cl and F), it is noteworthy that the antimalarial activity is higher in compounds 1**09**, **121** and **111**, **123** bearing the halogen atom at position ortho (chlorine and fluorine, respectively) in contrast with their isomers having the halogen atoms attached at positions meta or para.

However, the most interesting effect observed in this series is the clear increase in the antimalarial activity observed for di-substituted derivatives, in contrast with the series of flexible O-benzyl derivatives.

With regard to physicochemical properties, in Table 3 is shown the lipophilicity in terms of Chromatographic Hydrophobicity Indexes (CHIlogD)[32] at pH=2, 7.4 and 10.5, protein binding (HSA)[33] and solubility in simulated biorelevant media (FaSSIF and FeSSIF)[34] for a series of selected compounds.

As shown, benzyloxy derivatives **21** and **28** are lipophilic compounds, with similar $CHIlogD_{7.4}$ values to other analogous pyridones having phenyl ring instead of pyridine[21]. Both compounds remained unionized at pH=2 and pH=10.5 and highly protein bound (%HSA=97). Removal of the OCH_2 linker led to rigid, less lipophilic and less protein bound derivatives **106** and **116**. Furthermore, from the CHIlogD data obtained at different pH, it seems the conjugation of the phenyl ring contributes to increase the basicity of the pyridine ring, as demonstrated the lower values of CHIogD obtained at pH=2 in comparison to neutral phenyl ring, which is in accordance with a decrease of the lipophilic character of these molecules due to protonation of the pyridine ring in the acidic media. As expected, substitutions by 2-Cl,4-CF_3 and 2-F,4-CF_3 at phenyl ring in compounds **124** and **125** not only slightly increased the lipophilicity, but also decrease the basicity of the pyridine in comparison to the monosubstituted derivatives. This effect could be explained on the basis of the strong electron-withdrawing effect exerted by the 4-trifluoromethyl-phenyl moiety into the pyridine ring[35] and the steric effect exerted by the substituents at the ortho position,that prevents the conjugation between the two aromatic rings.

As expected, C6-CH_2OH derivatives **138** and **139** are less lipophilic derivatives, with lower CHIlogD values than their non hydroxylated counterparts **106** and **125** and higher solubilities, particularly in FeSSIF. Furthermore, the introduction of the hydroxyl group decreases significantly the $CHIlogD_{10.5}$ related to deprotonation of the 4(1H)-pyridone ring, in comparison to the $CHIlogD_{7.4}$, suggesting a higher degree of ionization at pH=10.5 than the non hydroxylated derivatives.

In general, the introduction of a more polar pyridine ring instead of phenyl does not significantly increase the solubility profile of the compounds prepared at physiologically relevant media in comparison to compounds described previously[21]. The extremely low solubility showed by the antimalarial 4(*1H*)pyridones in aqueous media has been attributed not only to their relatively high

lipophilicity but also to intrinsic structural features of the 4(1H)-pyridone ring, in particular its marked tendency to form strong hydrogen bonding networks. In this way, it has been reported in the literature that clopidol and other 4(*1H*)pyridones[36-38] adopt predominantly the 4-keto tautomer form in the solid state, forming structures supported by strong hydrogen bonding networks. This is in accordance with the high melting points (m.p.>250ºC) measured for some of the 4(*1H*)pyridones synthetized (data not reported here).

In Table 4 the main PK parameters measured for a series of selected antimalarial 4(*1H*)pyridones are shown. In general, the oral bioavailabilities in mouse for compounds **106**, **116, 118, 119, 124, 125** and **138**, bearing substituted phenyl-pyridinyl moieties attached to the pyridone ring are higher than that observed for other more lipophilic phenyloxyphenyl-4(*1H*)pyridones described previously when administered as a suspension in 1% Methyl Cellulose (~20%)[20, 21]. It is noteworthy the significant increase in the oral bioavailability observed for the C6CH_2OH derivative **138** (>100%) in comparison with its non hydroxylated analog **116** (54.9%), which is in accordance with previous PK data reported by us for the phenyloxy-phenyl series.

Concerning clearance and related half-lives, it seems that they are mainly influenced by factors such as the type of substituent attached to the pyridine ring and the relative position of the substituents at the phenyl ring. With regard to the type of substituent, as mentioned previously, compounds having flexible linkers such as **28**, showed significantly shorter half-life ($t_{1/2}$=5.3h) than its rigid counterpart **116** ($t_{1/2}$=18.7), presumably due to the metabolic oxidation at the labile bencylic position and f urther degradation, which could also explain its lower oral bioavailability. With regard to the substitution at the pendant phenyl ring present at the end of the side chain, it seems that reducing metabolism by use of carefully placed blocking groups is important, as demonstrated by the longer half-life displayed in para substituted derivatives such as **118** ($t_{1/2}$=23.4h) in comparison to its meta substituted isomer **119** ($t_{1/2}$=5.1h).

The presence of a polar C6-CH_2OH group in compound **138** decreased the lipophilicity but increased the clearance and shorten the half-life in comparison to its non hydroxylated and more lipophilic analog

116. This unusual behavior is in accordance with metabolic studies carried out for other 2,6-dimethyl-pyridones in which it has been observed that the hydroxylation at methyl groups is probably the main Phase I metabolic pathway[19], normally followed by conjugation with glucuronic acid (data not shown here). This fact points to Phase II metabolism as the most likely route of elimination of the 4(*1H*)pyridones bearing CH_2OH groups attached at position C6 of the pyridone ring.

Table 5 shows the *in vivo* antimalarial efficacy determined in a mouse *P. yoelii* model[39,40], administering test samples in four oral doses by gavage over four days. The *in vivo* data are expressed as ED_{50} (mg/kg) values, representing the dose (estimated from the dose-response curve) for parasite reduction of 50% relative to untreated controls.

As shown, with the only exception of compound **28**, which displayed an ED_{50}=16.3 mg/Kg, all the pyridones tested have been highly efficacious *in vivo*, with ED_{50} values ≤ 1 mg/Kg. In general, it seems that the compound exposure and duration of cover *in vivo*, along with the *in vitro* antimalarial potency (*Pf*IC_{50}) correlates fairly well with the *in vivo* efficacy. Thus, the most efficacious compound (**125**) displays high antimalarial activity *in vitro*, long half-live and high AUC. This observation is in accordance with a study[41] concerning the viability of the intra-erythrocytic forms of *P. falciparum*, indicating that the effect of the inhibition of the Mitochondrial Electron Transport chain in *P. falciparum* depends upon both the erythrocytic stage of the parasites and the duration of exposure. Thus for example, as the ring-stage parasites appear to remain viable for up to 48h with an inhibited Mitochondrial Electron Transport Chain, the drug needs to maintain inhibitory concentrations in plasma for at least that length of time to achieve high levels of efficacy.

Conclusions

A new series of potent antimalarial 4(*1H*)pyridones *in vitro* and *in vivo* have been obtained by introducing pyridine rings instead of phenyl into the lipophilic side chain attached to the 4(1H)-pyridone core. In general, the new compounds display improved pharmacokinetic profiles in comparison with the more lipophilic phenoxyphenyl derivatives previously described by us whilst maintaining similar levels

of antimalarial efficacy *in vitro* and *in vivo*. This improvement in the pharmacokinetic profile has been particularly relevant in compounds that combine the effect of the pyridine ring with the presence of polar hydroxymethyl groups attached at position C6 of the 4(1H)-pyridone ring, leading to a significant increase of solubility in biorelevant media and oral bioavailability in mice. The results obtained demonstrate that novel potent and more developable antimalarial 4(*1H*)pyridones can be prepared by joining chemical transformations at both the very sensitive lipophilic tail and the 4(1H)-pyridone scaffold, thus opening new ways for the chemical exploration of this exciting family of antimalarials.

Experimental section

Biology

Determination of activity in vitro against P.falciparum

The activity of the test compounds against *P. falciparum in vitro* was determined using a modification of the semi-automated microdilution technique of Desjardins.[42] Further experimental details can be found in the supporting information available.

Pharmacokinetics

Measurement of pharmacokinetic parameters in CD1 mice was carried out by using Specific pathogen-free female CD1 (Hsd:ICR) mice of 8 to 10 weeks of age were obtained from Harlan Laboratories (Udine, Italy). The mice were maintained in the GlaxoSmithKline Laboratory Animal Science animal facilities at Tres Cantos (Spain). Further experimental details are available in the supplementary information provided.

In vivo efficacy in 4d P. yoelii murine model

The blood schizontocidal activity of test compounds was determined using a modified 4-day suppressive assay[39] following the method previously described by us[40]. Further experimental details can be found in the supplementary information available.

Physicochemical parameters

Determination of CHIlogD, %HSA and equilibrium solubility in FaSSIF and FeSSIF have been carried out following procedures reported in the literature[32,33]. Further experimental details are available in the supplementary information provided.

Chemistry

Starting materials were obtained from commercial suppliers and used without further purification unless otherwise indicated. Flash chromatography was carried out using prepacked Isolute Flash or Biotage silica gel columns as the stationary phase and analytical grade solvents as the eluent unless otherwise stated. Preparative TLC purification was carried out in 20x20 cm plates Merck (silica gel 60, F254, 2 mm), by using mixtures of analytical grade solvents as eluents. ^{1}H-NMR spectra were determined on a Varian Unity spectrometer (300 MHz). Chemical shifts are reported as δ values (ppm) downfield from tetramethylsilane, used as an internal standard in the solvent indicated. Total ion current traces were obtained for electrospray positive and negative ionization (ES+/ES-) on a Waters ZMD 2000. Analytical chromatographic conditions used for the LC/MS analysis were as follows. The column was ACE, 4.6 mm _ 30 mm, and the stationary phase particle size was 3 μm. Solvent A was an aqueous solvent consisting of water + 0.1% formic acid. Solvent B was an organic solvent consisting of acetonitrile + 0.1% formic acid. The composition of the solvent over 5 min is shown in Table 6. Additional chromatographic parameters were as follows: flow rate, 1 mL/min; injection volume, 5 μL; column temperature, 30 _C; UV wavelength range, 220-330 nm.

Table 6. Analytical Chromatography: composition of eluent mixture over 5 min.

Time (min)	% solvent B
0	10
0.2	10
3.5	90
4	90

4.01	90
5	10

The purity of all tested compounds was >95% by using the analytical method described above. ^{1}H-NMR spectra of all the compounds listed not included here are available in the supplementary information provided.

2-Methoxy-5-[(2-nitroprop-1-en-1-yl]pyridine (3)

A solution of 5-formyl-2-methoxypyridine **1** (14.56g, 0.1 mol) and butylamine (23.1 ml, 0.2 mol) in dry toluene (90 ml) was heated to reflux in a flask equipped with a Dean-Stark trap in order to remove water. After heating for 3h, the mixture was concentrated to dryness and the residue dissolved in acetic acid (45 ml). To the solution was added nitroethane (11.4 ml, 0.15 mol) and the mixture was heated to 100ºC. After 3h of heating the mixture was cooled to room temperature and poured onto 400 ml of a mixture ice/water with vigorous stirring. The mixture was extracted with ethyl acetate (400 ml) and the organic layer washed successively with water (2x300 ml), 10% $NaHCO_3$ (2x300 ml), water (300 ml) and brine (300 ml), then dried over $MgSO_4$. Elimination of the solvent under vacuum gave a yellow powder which was purified by column chromatography on silica gel, eluting with mixtures hexane/ethyl acetate (v/v 20:1 and 10:1) to afford 16.65g of the title compound as a yellow powder (85% yield). ^{1}H-NMR(δ, ppm, $CDCl_3$): 8.30(m, 1H); 8.02(bs, 1h)7.68(dd,1H); 6.83(d, 1H); 3.99(s, 3H); 2.47(s, 3H)

1-(6-Methoxypyridin-3-yl)acetone (4)

Method A:

In a 1L round bottom flask equipped with mechanical stirring were placed iron powder (55g, 1 mol) and AcOH (220ml). A solution of Intermediate **3** (16.65g, 0.08 mol) in acetic acid (120ml) was added dropwise over this suspension. The mixture was heated to reflux with vigorous stirring under nitrogen. After 2 h of heating the mixture was cooled to room temperature and water (750ml) was added. The mixture was extracted with dichloromethane (3x300ml) and the combined organic layers washed with water (400 ml), saturated $NaHCO_3$ (400 ml), water (400 ml) and brine (400 ml), then dried over $MgSO_4$.

Elimination of the solvent to dryness gave 12.5g of the title compound (93% yield) as a pale brown oil which was used for the next step without further purification.

Method B

A solution of 5-bromo-2-methoxypyridine (16 ml, 23.2g, 0.12 mol) and dry toluene (625ml) was deoxygenated by bubbling argon for 20 min. Tri-o-tolylphosphine (2.99g, 0.009 mol), palladium acetate (1.65g, 0.007 mol), isopropenyl acetate (20.3 ml, 0.18 mol) and tributyltin methoxide (49.6 ml, 0.17 mol) were added successively under argon atmosphere and the mixture heated to 80ºC. After 2.5 h the mixture was cooled to room temperature and filtered. The filtrate was concentrated to dryness under vacuum and treated with acetonitrile (800 ml). The solid precipitated was removed by filtration and the filtrate washed with hexane (3x800 ml). The acetonitrile solution was dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel, eluting with mixtures hexane/ethyl acetate (v/v 9:1 and 3:1). 13.78g of the title compound were obtained as a yellow oil (70% yield). ^{1}H-NMR(δ, ppm, $CDCl_3$): 7.97(bd, 1H); 7.41(dd,1H); 6.73(d, 1H); 3.92(s, 3H); 3.63(s, 2H); 2.19(s, 3H)

3-(6-Methoxypyridin-3-yl)-2,6-dimethyl-4*H*-pyran-4-one (5)

Method A:

In a 1L round bottom flask equipped with mechanical stirring were placed polyphosphoric acid (117 g) and acetic anhydride (55 ml) and the thick mixture heated to 95ºC. To this mixture was added dropwise a solution of Intermediate **4** (17.26g, 0.1 mol) in acetic anhydride (75 ml). The dark mixture was heated at 95ºC under inert atmosphere for 1.5h. The mixture was cooled to room temperature and water (700 ml) was added carefully (exotherm). The suspension obtained was taken-out the flask and neutralized at 0ºC (ice/water bath) by adding solid NaOH and extracted with dichloromethane (3x300 ml). The organic layer was washed successively with 10% Na_2CO_3 (500 ml), water (500 ml) and brine (500 ml), dried over $MgSO_4$ and concentrated to dryness. The dark brown oily residue was purified by column chromatography on silica gel, eluting with mixtures hexane/acetone (v/v 5:1 and 4:1). 15g of the title compound were obtained as a orange powder (64% yield).

Method B:

To a mixture of Eaton´s reagent (4.5 ml) and acetic anhydride (3 ml) pre-heated at 50ºC was added dropwise a solution of Intermediate **4** (1g, 6 mmol) in acetic anhydride (3 ml). After heating overnight the mixture was allowed to cool to room temperature, then water (50 ml) was added carefully. The mixture was extracted with toluene (50 ml) and the aqueous layer was placed in an ice-water bath, then neutralized carefully with solid NaOH (3g). The resulting aqueous mixture was extracted with DCM (2x50 ml) and the combined organic layers dried over $MgSO_4$. Solvent was ecaporated and the crude purified by silica gel column chromatography, eluting with a mixture Hexane/EtOAc v/v 3:1 to afford 0.8g of the title compound as a brown powder (58% yield).

^{1}H-NMR(δ, ppm, $CDCl_3$): 8.01(bd, 1H); 7.51(dd, 1H); 6.81(d, 1H); 6.19(s, 1H); 3.95(s, 3H); 2.29(s, 3H); 2.23(s, 3H)

3-(6-Hydroxypyridin-3-yl)-2,6-dimethyl-4*H*-pyran-4-one (6)

A solution of Intermediate **5** (15g, 0.064 mol) in 6N Hydrochloric acid (330 ml) was heated at 110ºC for 48h, then cooled to room temperature. The resulting solution was carefully neutralized at 0ºC (ice/water bath) by slow addition of 6N NaOH. The aqueous solution was passed through a column of resin DIAION HP20 (250-600°C) in order to eliminate inorganics and the organics were eluted with methanol. Elimination of the solvent gave a brown powder which was dissolved in a mixture dichloromethane/10% methanol and filtered through a pad of celite. Elimination of the solvent gave 13.4g of the title compound as a pale brown powder which was used for the next step without further purification (95% yield). ^{1}H-NMR(δ, ppm, CD_3OD): 7.47(dd, 1H); 7.37(d, 1H); 6.59(d, 1H); 6.24(s, 1H); 2.34(s, 3H); 2.30(s, 3H)

2,6-dimethyl-5-(6-{[4-(trifluoromethoxy)benzyl]oxy}pyridin-3-yl)-4*H*-pyran-4-one (7)

To a solution of Intermediate **6** (0.326 g, 1.5 mmol) in dry toluene (15 ml) were added consecutively 4-trifluoromethoxybenzyl bromide (0.6 ml, 3 mmol) and silver carbonate (0.31 g, 1.12 mmol). The mixture was heated at 60ºC in the dark for 30h, then cooled to room temperature. The remaining solids were separated by filtration and the solid washed with ethyl acetate. The combined organics were

washed with 10% $NaHCO_3$ (75 ml) and water (2x50 ml), then dried over Na_2SO_4. Elimination of the solvent under vacuum gave an oil which was purified by column chromatography on silica gel, eluting with mixtures hexane/ethyl acetate (v/v 2:1 and 1:1), to afford 0.423g (72% yield) of the title compound as a pale brown powder. ^{1}H-NMR(δ, ppm, DMSO-d_6): 8.02(d, 1H); 7.61-7.58(m, 3H); 7.38(bd, 2H); 6.93(d, 1H); 6.21(s, 1H); 5.39(s, 2H); 2.27(s, 3H); 2.18(s, 3H)

Compounds **8-13** were prepared in a similar manner to **7** by using the appropriates substituted benzyl halides (spectroscopical data available in the supporting material).

2,6-Dimethyl-6'-{[4-(trifluoromethoxy)benzyl]oxy}-3,3'-bipyridin-4(1*H*)-one (14)

To a solution of Intermediate **7** (0.85g, 2.17 mmol) in ethanol (30 ml) was added commercial 30% aqueous ammonia (110 ml) with stirring. The suspension thus obtained was placed into a steel reactor and heated to 140ºC. After eight hours of heating, the reactor was allowed to cool to room temperature overnight with mechanical stirring. The precipitate was filtered and washed with ethyl acetate. 0.495g of the title compound were obtained as a pale brown powder after drying under vacuum (59% yield). ^{1}H-NMR(δ, ppm, DMSO-d_6): 11.15(bs, 1H); 7.94(dd, 1H); 7.58(d, 2H); 7.54(dd,1H); 7.37(d, 2H); 6.88(d, 1H); 5.92(s, 1H); 5.38(s, 2H); 2.18(s, 3H); 2.08(s, 3H)

Compounds **15-20** were prepared in a similar manner to **14** from the appropriate 4-pyranones (spectroscopical data available in the supporting material).

5-Bromo-2,6-dimethyl-6'-{[4-(trifluoromethoxy)benzyl]oxy}-3,3'-bipyridin-4(1*H*)-one (21)

To a solution of Intermediate **14** (0.211g, 0.54 mmol) in a mixture dichloromethane/methanol v/v 2:1 (30 ml) was added portionwise N-bromosuccinimide (0.106g, 0.6 mmol). The mixture was stirred at room temperature under inert atmosphere for 1h. Removal of the solvent under vacuum gave a crude white powder which was triturated with acetonitrile (20 ml) and filtered. The solid obtained was washed with acetonitrile, then dried under vacuum to afford 0.217 g of the title compound as a white powder (85% yield). ^{1}H-NMR(δ, ppm, DMSO-d_6): 11.15(bs, 1H); 7.97(bd, 1H); 7.62-7.55(m,3H); 7.37(d, 2H); 6.90(d, 1H); 5.39(s, 2H); 2.41(s, 3H); 2.1(s, 3H); [ES MS] m/z 470 (MH+)

Compounds **22-27** were prepared in a similar manner to **21** from the appropriate 4(*1H*)pyridones (spectroscopical data available in the supporting material).

5-Chloro-2,6-dimethyl-6'-{[4-(trifluoromethoxy)benzyl]oxy}-3,3'-bipyridin-4(1*H*)-one (28)

To a cooled solution (0-4ºC, ice/water bath) of Intermediate **14** (0.276g, 0.71 mmol) in a mixture dichloromethane/methanol v/v 2:1 (24 ml) was added portionwise under inert atmosphere trichloroisocyanuric acid (67 mg, 0.29 mmol). The mixture was stirred at low temperature under inert atmosphere for 1.5h, then diluted with a mixture dichloromethane/methanol v/v 2:1 (30 ml). The solid precipitated was filtered and the filtrate was concentrated to dryness under vacuum to afford a solid residue which was suspended in a mixture of aqueous 0.5N NaOH (30 ml) and methanol (10ml). After stirring for 1h, the mixture was diluted by adding water (20 ml) and the remaining precipitate was filtered and washed successively with water (3x10 ml), mixture methanol/water v/v 1:1 (2x10 ml) and acetonitrile (3x10ml). 0.193g of the title compound were obtained as a white powder after drying under vacuum (64% yield). ^{1}H-NMR(δ, ppm, DMSO-d_6): 11.65(bs, 1H); 7.98(bd, 1H); 7.61-7.55(m,3H); 7.38(d, 2H); 6.91(d, 1H); 5.39(s, 2H); 2.37(s, 3H); 2.11(s,3H); [ES MS] m/z 425 (MH+)

Compounds **29-34** were prepared in a similar manner to **28** from the appropriate 4(*1H*)pyridones (spectroscopical data available in the supporting material).

5-Bromo-2-({4-[(trifluoromethyl)oxy]phenyl}oxy)pyridine (36)

To a mixture of 4-trifluoromethoxyphenol (4.4ml, 0.033 mol) and sodium hydride (60% dispersion in mineral oil, 1.35g, 0.033 mol) in dry DMF (3ml) was added a solution of 2,5-dibromopyridine **35** (1g, 0.004 mol) in dry DMF (3mL). The resulting mixture was heated at 60ºC for 8h. Upon cooling, ethyl acetate was added and the mixture was washed with NH_4Cl 1N.The organic layer was dried over Na_2SO_4 and the solvent removed by evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel (eluent: AcOEt/Hexane 1:10) to afford 1.08g of the final compound as a colorless oil (80% yield). ^{1}H-NMR(δ, ppm, $CDCl_3$): 8.21 (d, 1H), 7.80 (dd, 1H), 7.25 (d, 2H), 7.14 (d, 2H), 6.87 (d, 1H).

[6-({4-[(Trifluoromethyl)oxy]phenyl}oxy)-3-pyridinyl]boronic acid (37)

A solution of **36** (0.60g, 1.88 mmol) and tri-isopropyl borate (0.54 mL, 2.33 mmol) in dry THF (6 mL) was added n-BuLi 1.6M (1.24mL, 1.98 mmol) at -78ºC. The reaction was allowed to warm to room temperature and stirred for 8h, then quenched by addition of HCl 2N until pH=2. After stirring for 1h, the aqueous phase was extracted with EtOAc, the organic phase was dried over Na_2SO_4, evaporated under reduced pressure and the crude was washed with pentane to yield 0.330 g of the title compound as a white solid (59% yield). ^{1}H NMR (δ, ppm, CD_3OD): 8.45 (d, 1H), 8.16 (dd, 1H), 7.41 (d, 2H), 7.25 (d, 2H), 7.03 (d, 1H)

3-Chloro-5-iodo-2,6-dimethyl-4(1*H*)-pyridinone (39)

A suspension of 2,6-dimethyl-4(*1H*)Pyridinone **38** (10g, 0.081 mol) and N-chlorosuccinimide (14.3g, 0.107 mol) in a mixture of methanol (100 ml) and dichloromethane (250ml) as solvent was stirred under inert atmosphere at room temperature overnight. The remaining precipitate was filtered and discarded, then the filtrate was concentrated to dryness to afford a residue which was triturated with acetonitrile. The insoluble material was filtered and washed with acetonitrile, then dried under vacuum to afford 8.6g of material, which was added portionwise onto a solution of sodium hydroxide (2.2g, 0.07 mol) in water (340ml). To the resulting solution was added iodine (13.8g, 0.054 mol) and the mixture stirred at room temperature for 1h. The precipitate obtained was filtered-off and washed with water and acetonitrile (2x100 ml) to afford 11.5g of the title compound (50% yield). ^{1}H-NMR(δ, ppm, CD_3OD): 2.59(s, 3H); 2.45(s, 3H)

5-Chloro-2,6-dimethyl-6'-({4-[(trifluoromethyl)oxy]phenyl}oxy)-3,3'-bipyridin-4(1*H*)-one (40)

A mixture of boronic acid **37** (0.100 g, 0.33mmol), potassium carbonate (0.290g, 2.1mmol), palladium acetate (0.008g, 0.03mmol) and **39** (0.211g, 0.7mmol) in DMF (2mL) was heated at 140ºC, for 30 minutes under microwave irradiation. The reaction mixture was filtered through a pad of celite, evaporated under reduced pressure and purified by column chromatography on silica gel (eluent CH_2Cl_2/CH_3OH, 10:1) to afford 0.087g of the title compound as a white solid (60% yield). ^{1}H NMR (δ,

ppm, DMSO-d_6): 11.69 (s, 1H), 7.95 (d, 1H), 7.72 (dd, 1H), 7.42 (d, 2H), 7.30 (d, 2H), 7.10 (d, 1H), 2.38 (s, 3H), 2.12 (s, 3H); [ES MS] m/z 411 (MH^+)

2,6-Dimethyl-3-(6-trifluoromethanesulfonyloxypyridin-3-yl)-4*H*-pyran-4-one (41)

To a suspension of Intermediate **6** (4g, 0.018 mol) in dry N,N-dimethylformamide (120 ml) was added powdered potassium carbonate (7.62g, 0.07 mol). After stirring at room temperature for 2 minutes, solid N-phenyltrifluoromethanesulfonimide (6.58g, 0.018 mol) was added portionwise. After 1h of stirring the suspension was diluted with ethyl acetate (300 ml) and washed with ammonium chloride (3x250 ml). The solvent was removed to dryness under vacuum and the residue dissolved in diethyl ether (200 ml). The ethereal solution was washed with 1N NaOH (4x200 ml) and brine (200 ml), dried over Na_2SO_4 and concentrated to dryness to afford 5g of the title compound as a pale yellow powder (78% yield), which was used for the next step without further purification. ^{1}H-NMR(δ, ppm, $CDCl_3$): 8.24(d, 1H); 7.86(dd, 1H); 7.23(d, 1H); 6.23(s, 1H); 2.32(s, 3H); 2.26(s,3H)

2,6-Dimethyl-3-(6-{[4-(trifluoromethyl)phenyl]ethynyl}-3-pyridinyl)-4*H*-pyran-4-one (42)

To a solution of Intermediate **41** (0.25g, 0.72 mmol) in a mixture of dry N,N-dimethylformamide (5 ml) and triethylamine (2 ml) were added consecutively CuI (0.051g, 0.27 mmol)), $(PPh_3)_2PdCl_2$(0.050 g, 0.07 mmol) and PPh_3 (0.111g, 0.42 mmol). The mixture was deoxygenated by bubbling argon and 4-ethynyl-α,α,α-trifluorotoluene (0.23 ml, 1.35 mmol) was added, then heated to reflux for 1h. The mixture was cooled to room temperature, diluted with ethyl acetate and washed with 1N NH_4Cl. The solvent was evaporated and the resulting crude was dissolved in diethyl ether. The ethereal solution was washed with 1N HCl until no product was detected in the organic layer. The acidic aqueous solution was basified by adding 2N NaOH and extracted with tert-butyl-methyl-ethyl. Elimination of the solvent gave 0.14g of the title compound (52% yield). ^{1}H-NMR(δ, ppm, CD_3OD): 8.47(s,1H); 7.79(m, 6H); 6.30(s, 1H); 2.37(s, 3H); 2.29(s,3H)

Compounds **43** and **44** were prepared in a similar manner to **42** by using the appropriate ethynyl derivatives (spectroscopical data available in the supporting material).

2,6-Dimethyl-6'-{[4-(trifluoromethyl)phenyl]ethynyl}-3,3'-bipyridin-4(1*H*)-one (45)

To a solution of Intermediate **42** (0.07g, 0.19 mmol) in MeOH (1 ml) was added commercial 30% aqueous ammonia (3ml) and the suspension heated to 140ºC for 1h under microwave radiation. Upon cooling, the solid precipitated was filtrated and washed with acetonitrile. 0.035g of the title compound were obtained (50% yield). ^{1}H-NMR(δ, ppm, CD_3OD): 8.45(m,1H); 7.75(m, 6H); 6.30(s, 1H); 2.36(s, 3H); 2.23(s, 3H)

Compound **46** was prepared in a similar manner to **45** from the appropriate 4-pyranone **43** (spectroscopical data available in the supporting material).

5-Bromo-2,6-dimethyl-6'-{[4-(trifluoromethyl)phenyl]ethynyl}-3,3'-bipyridin-4(1*H*)-one (47)

To a solution of Intermediate **45** (0.012 g, 0.032 mmol) in a mixture of dichloromethane/methanol v/v 5:1 (6 ml) was added portionwise at room temperature N-bromosuccinimide (0.0064g, 0.035 mmol). The suspension was stirred at room temperature for 30 min, then concentrated to dryness under vacuum. The residue thus obtained was triturated with acetonitrile and filtered. The solid was washed with acetonitrile and dried under vacuum. 0.010 g of the title compound were obtained as a white powder (69% yield). ^{1}H-NMR(δ, ppm, CD_3OD): 8.47(m,1H); 7.77(m, 6H); 2.56(s, 3H); 2.23(s, 3H); [ES MS] m/z 447 (MH+)

5-Chloro-2,6-dimethyl-6'-{[4-(trifluoromethyl)phenyl]ethynyl}-3,3'-bipyridin-4(1*H*)-one (48)

To a solution of Intermediate **45** (0.027g, 0.073 mmol) in a mixture of dichloromethane/methanol v/v 5:1 (6 ml) was added portionwise at room temperature trichloroisocyanuric acid (0.0068g, 0.029 mmol). The suspension was stirred at room temperature for 30 min, then concentrated to dryness under vacuum. The residue thus obtained was purified by preparative TLC chromatography, eluting with a mixture dichloromethane/5% methanol. 0.015 g of the title compound were obtained (50% yield).^{1}H-NMR(δ, ppm, CD_3OD): 8.47(m,1H); 7.77(m, 6H); 2.51(s, 3H); 2.23(s, 3H); [ES MS] m/z 401 (MH+)

Compound **49** was prepared in a similar way described for **48** from 4(1H)-pyridone **46** (spectroscopical data available in the supporting material).

2,6-Dimethyl-3-(6-{2-[4-(trifluoromethyl)phenyl]ethyl}-3-pyridinyl)-4*H*-pyran-4-one (50)

A solution of Intermediate **42** (0.2g, 0.54 mmol) in a mixture of ethyl acetate/methanol v/v 3:1 (20 ml) was deoxygenated by bubbling argon for 5 minutes, then Palladium 10% w/w on activated charcoal (0.050 g) and 1H HCl (0.541 ml) were added. The mixture was hydrogenated at 30 psi for 4 hours. The catalyst was removed by filtration and the solvent evaporated to dryness under vacuum to afford 0.168g of the expected compound (83% yield). ^{1}H-NMR(δ, ppm, $CDCl_3$): 8.41(m,1H); 7.70-7.64(m, 1H); 7.56-7.52(m, 2H); 7.33(d, 2H); 7.17(d, 1H); 6.21(m, 2H); 3.14(m, 4H); 2.30(s, 3H); 2.23(s,3H)

Compound **51** was prepared in a similar manner to **50** from compound **43**(spectroscopical data available in the supporting material).

2,6-Dimethyl-6'-{2-[4-(trifluoromethyl)phenyl]ethyl}-3,3'-bipyridin-4(1*H*)-one (52)

To a solution of Intermediate **50** (0.164g, 0.44 mmol) in MeOH (1 ml) was added commercial 30% aqueous ammonia (3ml) and the suspension heated to 145ºC for 45 minutes under microwave radiation. Upon cooling, the solid precipitated was filtrated and washed with acetonitrile. 0.092g of the title compound were obtained (56% yield). ^{1}H-NMR(δ, ppm, DMSO-d_6): 11.18(bs, 1H); 8.28(m, 1H); 7.63(d, 2H); 7.51-7.46(m, 3H); 7.25(d, 1H); 5.93(s, 1H); 3.08(m, 4H); 2.19(s, 3H); 2.06(s, 3H).

Compound **53** was synthetized in a similar way to **52** from the intermediate **51** (spectroscopical data available in the supporting material).

5-Chloro-2,6-dimethyl-6'-{2-[4-(trifluoromethyl)phenyl]ethyl}-3,3'-bipyridin-4(1*H*)-one (54)

To a solution of Intermediate **52** (0.038g, 0.1 mmol) in a mixture dichloromethane/methanol v/v 2:1 (6 ml) was added portionwise Trichloroisocyanuric acid (9.3 mg, 0.04 mmol). After 30 min of stirring at room temperature the solvent was removed under vacuum and the residue purified by preparative TLC chromatography, eluting with a mixture dichloromethane/10% methanol. 0.025g of the title compound were obtained (61% yield). ^{1}H-NMR(δ, ppm, CD_3OD): 8.34(m,1H); 7.60(dd, 1H); 7.47(m, 2H); 7.30(d, 1H); 3.14(m,4H); 2.50(s,3H); 2.17(m, 3H); [ES MS] m/z 405 (MH^-)

Compound **55** was prepared in a similar manner to **54** from the appropriate 4(1H)-pyridone **53** (spectroscopical data available in the supporting material).

2,6-dimethyl-3-(6-{4-[(trifluoromethyl)oxy]phenyl}-3-pyridinyl)-4*H*-pyran-4-one (56).

To a solution of Intermediate **41** (2.27 g, 6.49 mmol) in a mixture of toluene (120 ml) and ethanol (60 ml) were added successively $(PPh_3)_2PdCl_2$(0.456 g, 0.39 mmol) and 4-trifluoromethoxyphenyl boronic acid (1.6g, 7.76 mmol) and the resulting suspension was deoxygenated by bubbling argon for 5 minutes. Saturated $NaHCO_3$ (23 ml) was added dropwise and the mixture heated to 95ºC for 2.5 h. The mixture was cooled to room temperature and the mixture concentrated to dryness under vacuum. The oily residue thus obtained was dissolved in tert-butyl-methyl ether (400 ml) and washed with 1N NaOH (2x250ml). The organic layer was washed with 1N HCl (7x100 ml) until no remaining material was detected in the organic layer. The aqueous layer was carefully basified by slow addition of 6N NaOH (80 ml) and 2N NaOH (150ml), then extracted with tert-butyl-methyl ether (2x300 ml). The combined organic layers were washed with brine (500 ml), dried over Na_2SO_4 and concentrated to dryness to afford the title compound (2g) as a yellowish powder (85% yield). ^{1}H-NMR (δ, ppm, $CDCl_3$): δ 8.54(m, 1H); 8.06-8.01(m, 2H); 7.79-7-72(m, 2H); 7.32(bd, 2H); 6.24(s, 1H); 2.32(s, 3H); 2.29(s, 3H).

Compounds **57-79** were prepared in a similar manner to **56**, by coupling between triflate **41** and the appropriate boronic acids (spectroscopical data available in the supporting material).

2,6-dimethyl-3-(6-{4-[(2,2,2-trifluoroethyl)oxy]phenyl}-3-pyridinyl)-4*H*-pyran-4-one (80).

In a sealed tube, to a mixture of intermediate **71** (0.1 g, 0.34 mmol) and 2-iodo-1,1,1-trifluoroethane (0.12 ml, 0.1.19 mmol) dissolved in dry DMF (1.40 ml) cesium carbonate (0.22 g, 0.68 mmol) was added under argon atmosphere and the mixture was heated to 50ºC overnight. The reaction was quenched with water and extracted with EtOAc. The aqueous layer was extracted with EtOAc (2x15 ml) and the combined organic layers were washed with 1N NH_4Cl and dried over Na_2SO_4. Column chromatography (eluent: CH_2Cl_2/EtOAc) of the crude afforded the title compound (0.067g) as a yellow solid (52% yield). ^{1}H-NMR (δ, ppm, CD_3OD): δ 8.50(m, 1H); 8.12-8.05(m,2H); 7.94-7.91(m,1H); 7.81-

7.77(m,1H); 7.36-7.12(m, 2H); 6.30(s, 1H); 4.87(m, 2H); 2.38(s, 3H); 2.30(s, 3H); [ES MS] m/z 376 (MH+).

3-(6-{4-[(difluoromethyl)oxy]phenyl}-3-pyridinyl)-2,6-dimethyl-4H-pyran-4-one (81).

Intermediate **72** (0.036 g, 0.118 mmol) was placed in a teflon round botton flask and diethylaminosulfur trifluoride (DAST) (1.0 ml, 5.0 mmol) was added dropwise at 0ºC under argon atmosphere. The mixture was stirred at room temperature overnight. Then reaction was cooled in an ice bath and carefully quenched with water. The mixture was diluted with CH_2Cl_2 (15 mL) and the layers partitioned. The aqueous layer was extracted with CH_2Cl_2 (3x15 ml) and the combined organic layers washed with 10% $NaHCO_3$ (1x15mL) and brine, dried over Na_2SO_4, filtered and concentrated under vacuo. The crude was purified by column chromatography (eluent: MeOH/CH_2Cl_2) to afford the title compound (0.104 g) as a brown solid (32% yield). ^{1}H-NMR (δ, $CDCl_3$): δ 7.61-8.55(m, 1H); 8.11(d, 2H); 7.84-7.78(m,2H); 7.64-7.61(m,2H); 6.71(m, 1H); 6.24(m, 1H); 4.87(m, 2H; 2.32(s, 3H); 2.30(s, 3H); [ES MS] m/z 328 (MH+).

2,6-dimethyl-6´-{4-[(trifluoromethyl)oxy]phenyl}-3,3´-bipyridin-4(1*H*)-one (82).

To a solution of Intermediate **56** (2.115 g, 5.87 mmol) in ethanol (13 ml) was added commercial 30% aqueous ammonia (44 ml, 610 mmol) with stirring. The suspension thus obtained was placed into a steel reactor and heated to 140ºC. After eight hours of heating, the reactor was allowed to cool to room temperature overnight. The precipitate was filtered and washed successively with ethyl acetate and dichloromethane to give compound **82** (1.38 g, 65%) as a pale brown powder after drying under vacuum. ^{1}H-NMR (δ, DMSO-d_6): δ 11.24(bs, 1H); 8.48(m,1H); 8.23(m, 2H); 7.99(d, 1H); 7.73(dd,1H); 7.48(m, 2H); 5.97(s, 1H); 2.21(s, 3H); 2.14(s, 3H).

Compounds **83-105** were prepared by a similar method to that described for compound **82** from the corresponding 4-pyranones (spectroscopical data available in the supporting material).

5-Bromo-2,6-dimethyl-6'-{4-[(trifluoromethyl)oxy]phenyl}-3,3'-bipyridin-4(1*H*)-one (106).

To a solution of intermediate **82** (0.064 g, 0.18 mmol) in a mixture of dichloromethane/methanol v/v 1:1 (2 ml) was added portionwise at room temperature N-bromosuccinimide (0.026 g, 0.15 mmol). The

suspension was stirred at room temperature for 30 min, then concentrated to dryness under vacuum. The residue thus obtained was triturated with ethyl acetate and filtered. The solid was washed with ethyl acetate and dried under vacuum. 0.062g of compound **106** was obtained as a white powder (78% yield). ^{1}H-NMR (δ, DMSO-d_6): δ 11.7-11.6(bs, 1H); 8.49(m,1H); 8.27-8.22(m, 2H); 8.03(d, 1H); 7.75(dd,1H); 7.48(m, 2H); 2.44(s, 3H); 2.15(s, 3H); [ES MS] m/z 439 (MH+).

Compounds **107-115** were prepared in a similar manner to compound **106** from the corresponding 4(1H)-pyridone (spectroscopical data available in the supporting material).

5-Chloro-2,6-dimethyl-6'-{4-[(trifluoromethyl)oxy]phenyl}-3,3'-bipyridin-4(1*H*)-one (116).

To a cooled solution (0-4ºC, ice/water bath) of **82** (1.39 g, 3.86 mmol) in a mixture of dichloromethane/methanol v/v 2:1 (120 ml) was added portionwise at room temperature trichloroisocyanuric acid (0.365 g, 1.57 mmol) and stirring continued for 1h. The mixture was filtered and the precipitate washed with a mixture dichloromethane/methanol v/v 2:1. The filtrate was evaporated and the solid thus obtained suspended in aqueous 0.5N NaOH (60 ml) and diluted with methanol (20 ml) and water (140 ml) and the precipitate formed was isolated by filtration. The solid was washed successively with water (3x20 ml) and acetonitrile (3x20 ml). 1,1g of compound **116** were obtained as a white powder after drying under vacuum (72% yield). ^{1}H-NMR (δ, ppm, DMSO-d_6): δ 11.7-11.6(bs, 1H); 8.50(m,1H); 8.24(m, 2H); 8.02(d, 1H); 7.75(m,1H); 7.48(m, 2H); 2.39(s, 3H); 2.15(s, 3H); [ES MS] m/z 393 (MH+).

Compounds **117-125** were prepared by a similar way followed to achieve compound **116** starting from the corresponding 4(*1H*)pyridones (spectroscopical data available in the supporting material).

5-bromo-2-{4-[(trifluoromethyl)oxy]phenyl}pyridine (126)

A mixture of 2,5-dibromopyridine **35** (5.00g, 21.11 mmol), cesium carbonate (7.56g, 23.22 mmol) and 4-(trifluoromethoxy)benzeneboronic acid (4.13g, 20.05 mmol) in Tol/EtOH 10/1 (165 ml) was stirred and deoxygenated with argon for 15 min. $(PPh_3)_2PdCl_2$ (0.741 g, 1.055 mmol) was added over this mixture and the reaction was heated at 80 ºC for 1h. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed with brine, dried over sodium sulfate and the

solvent evaporated under vacuum. The residue obtained was purified by flash column chromatography (silica gel, 15:1 Hex:AcOEt) to afford 5.8 g of the title compound (90% yield). ^{1}H NMR (δ, ppm, DMSO-d_6): 8.80 (d, 1H); 8.25-8.10 (m, 3H); 7.99 (d, 1H); 7.48 (d, 2H).; [ES MS] m/z: 318 $[M+H]^+$.

5-bromo-2-[2-fluoro-4-(trifluoromethyl)phenyl]pyridine (127)

To a stirred mixture of 2,5-dibromopyridine (3.76g, 15.8 mmol), [2-fluoro-4-(trifluoromethyl)phenyl]boronic acid (3g, 14.4 mmol) and 2M Na_2CO_3 (21.6 ml, 43.3 mmol) in 108 ml of toluene and 36 ml of ethanol (3 : 1), were added $Pd(PPh_3)_4$ (0.83g, 0.7 mmol) under nitrogen. The mixture was heated at 80ºC overnight. The suspension obtained was diluted with 100 ml of AcOEt and washed with water (3x50 ml). The resulting organic layer was dried over anhydrous $MgSO_4$ and evaporated to afford 4.1g of crude material which was used for the next step without any further purification (83% yield). ^{1}H NMR (δ, ppm, DMSO-d_6) 8.89 (d, 1 H); 8.22 (ddd, 1 H); 8.13 (t, 1 H); 7.8-7.7 (m, 2 H); 7.73 (m, 1 H).

(6-{4-[(trifluoromethyl)oxy]phenyl}-3-pyridinyl)boronic acid (128)

Compound **128** was prepared following an analogous procedure to that used for compound **129** starting from bromoderivative **126**. ^{1}H-NMR (δ, ppm, DMSO-d_6): 8.96 (s, 1H); 8.38 (s,2H); 8.29-8.16 (m, 3H); 7.97 (d, 1H); 7.47 (d, 2H); [ES MS] m/z: 284 [M+H]

{6-[2-fluoro-4-(trifluoromethyl)phenyl]-3-pyridinyl}boronic acid (129)

A solution of crude **127** (3.75g, 11.72 mmol) in 1,4-dioxane (60mL) was deoxygenated with argon, then bis(pinacolato)-diboron (3.57g, 14.06 mmol), $(PPh_3)_2PdCl_2$ (0.164 g, 0.234 mmol) and potassium acetate (3.45g, 35.1 mmol) were added. The mixture was heated to 100 ºC for approx 5 h then allowed to cool to room temperature with stirring overnight, filtered through celite, and concentrated to dryness. The crude was suspended in a 120 ml of a mixture acetone/water v/v 1:1 and ammonium acetate (2.0 g, 26.4 mmol) was added followed by $NaIO_4$ (5.6g, 26.4 mmol). The mixture was stirred at room temperature overnight. The organic solvent was evaporated under reduced pressure and 2N NaOH (30 mL) was added. The mixture was stirred at room temperature for 15 min then extracted with CH_2Cl_2 (2x

60 mL). The aqueous layer was cooled to 0 ºC, and 2N HCl was added drop-wise until pH was adjusted to 5. The white solid precipitated was filtered and dried under vacuum to afford 1.2g of the title compound (36% yield). ^{1}H-NMR (δ, ppm, DMSO-d_6): 9.03 (s, 1H); 8.26-8.15 (m, 2H); 7.85-7.82 (m, 2H); 7.74-7.71 (d, 1H).

3-Benzyloxy-6-methyl-2-(O-tert-butyldimethylsilyl)hydroxymethyl-pyran-4(1H)-one (131)

In a 2L jacketed reactor were introduced 4-pyranone **130** (66.65 g, 0.27 mol, Ref. 26) and dry N,N-dimethylformamide (500 mL). Imidazole (55.28g, 0.81 mol) and a solution of tert-butyldimethylsilyl chloride (48.94g, 0.32 mol) in dry N,N-dimethylformamide (50 mL) were added. After stirring for 3 h, ethyl acetate (900 mL) and 1N NH_4Cl (700 mL) were added. The two layers were partitioned and the organic layer washed with 1N NH_4Cl (2x900 mL) and brine (900 mL), dried over Na_2SO_4, filtered and concentrated to dryness under vacuum to give 100.68 g of the title compound as a pale yellow oil (100% yield). ^{1}H-NMR(δ, ppm, $CDCl_3$): 7.35(m, 5H); 6.20(s,1H); 5.16(s, 2H); 4.39(s, 2H); 2.26(s, 3H); 0.87(s, 9H); 0.04 (s, 6H)

3-Hydroxy-6-methyl-2-(O-tert-butyldimethylsilyl)hydroxymethyl-pyran-4(1H)-one (132)

Over a solution of **131** (23.71g, 65 mmol) in ethyl acetate (600 mL) under N_2 atmosphere was added palladium 10% w/w on activated charcoal (700.9 mg). The mixture was hydrogenated at 30 psi for 3 hours, after that time the catalyst was removed by filtration. The solvent was evaporated to dryness to afford 16.72 g of the title compound (94% yield). ^{1}H-NMR (δ, ppm, $CDCl_3$): 6.43(bd, 1H); 6.23(s,1H); 4.70(s, 2H); 2.32(s, 3H); 0.91 (s, 9H); 0.12 (s, 6H)

6-Methyl-2-(O-tert-butyldimethylsilyl)hydroxymethyl-3-trifluoromethanesulfonyloxy-pyran-4(1H)-one (133)

In a 500 mL round bottom flask was introduced compound **132** (16.72g, 61 mmol) in dry N,N-dimethylformamide (170 mL) and the solution stirred under N_2 atmosphere for 10 min. Then N-phenyltrifluoromethanesulfonimide (23.85g, 66 mmol) and powdered potassium carbonate (10.68g, 77 mmol) were added in portionwise. After stirring for 1.5 h, potassium carbonate was removed by

filtration and washed with tert-butyl-methyl-ether (300 mL). The filtrate was diluted with 100 mL of tert-butyl-methyl-ether and washed with 1N NH_4Cl (2x400mL). The aqueous layer was extracted with tert-butyl-methyl-ether (200 mL). The organic layers were washed with Na_2CO_3 (3x400 mL) and brine (400 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give 24.7 g of the title compound as a pale orange solid (100% yield). ^{1}H-NMR (δ, ppm, $CDCl_3$): 6.29(s, 1H); 4.6624(s, 2H); 2.34(s, 3H); 0.92 (s, 9H); 0.13 (s, 6H)

2-({[(1,1-dimethylethyl)(dimethyl)silyl]oxy}methyl)-6-methyl-3-(6-{4-[(trifluoromethyl)oxy]phenyl}-3-pyridinyl)-4*H*-pyran-4-one (134)

To a deoxygenated solution of boronic acid **128** (0.736g, 2.60 mmol) and triflate **133** (1.361g, 3.38 mmol) in a mixture of anhydrous ethanol (21 ml) and anhydrous toluene (7 ml) were added solid Na_2CO_3 (1.103g, 10.4 mmol) and $(PPh_3)_2PdCl_2$ (0.183g, 0.260 mmol). The reaction mixture was flushed with argon for 10 min, then heated to 80 °C for 2h. Additional boronic acid **128** (0.45g, 1.6 mmol) and $(PPh_3)_2PdCl_2$(0.183g, 0.26 mmol) were added and the mixture stirred at 80ºC for 1h. The reaction mixture was filtered, solvent removed by evaporation and the residue obtained was purified by flash chromatography on silica gel (Hex:AcOEt 0-50%) to afford 0.850 g of the title compound (66% yield). ^{1}H-NMR (δ, ppm, CD_3OD): 8.58-8.51(m, 1H); 8.17-8.07(m, 2H); 8.00-7.91(m, 1H); 7.88-7.79(m, 1H); 7.45-7.35(m, 2H); 6.38-6.32(m, 1H); 4.53-4.46(m, 2H); 2.44-2.36(m, 3H); 0.91-0.83(m, 9H); 0.08-0.01(m, 6H); [ES MS] m/z: 492 $[M+H]^+$.

2-({[(1,1-dimethylethyl)(dimethyl)silyl]oxy}methyl)-3-{6-[2-fluoro-4-(trifluoromethyl)phenyl]-3-pyridinyl}-6-methyl-4*H*-pyran-4-one (135)

Compound **135** was prepared following an analogous procedure to that used for compound **134**, starting from boronic acid **129**.

^{1}H-NMR (δ, ppm, CD_3OD) 8.58(s, 1 H), 8.08(t, 1 H), 7.9-7.8(m, 2 H), 7.59(m, 2 H), 6.31(s, 1 H), 4.46 (s, 2 H), 2.36(s, 3 H), 0.87(s, 9 H), 0.05(s, 6 H); [ES MS] m/z: 494 $[M+H]^+$.

2-(hydroxymethyl)-6-methyl-6'-{4-[(trifluoromethyl)oxy]phenyl}-3,3'-bipyridin-4(1H)-one (136)

In a 20 mL vessel microwave was introduced compound **134** (0.5g, 1.017 mmol) dissolved in ethanol (8ml). Ammonia (32% in water, 5.78 ml, 85 mmol) was added and the reaction vessel sealed and heated in a microwave oven to 140 ºC for 50 min. After cooling, reaction was evaporated to dryness. The solid residue was triturated with diethyl ether, filtered and washed with diethyl ether, dried under vacuum to afford 355 mg of the title compound (94% yield). ^{1}H NMR (δ, ppm, DMSO-d_6): 8.48(dd, 1H); 8.21(d, 2H); 7.93(dd, 1H); 7.72(dd, 1H); 7.46(d, 2H); 5.97 (s, 1H); 4.17(s, 2H); 2.18(s, 3H); [ES MS] m/z: 377 $[M+H]^+$.

6'-[2-fluoro-4-(trifluoromethyl)phenyl]-2-(hydroxymethyl)-6-methyl-3,3'-bipyridin-4(1*H*)-one (137)

Compound **137** was prepared following an analogous procedure to that used for compound **136**, starting from compound **135**. ^{1}H NMR(δ, ppm, DMSO-d_6) 11.22(s, NH); 8.58(s, 1 H); 8.21(t, 1 H); 7.8-7.7(m, 4 H); 6.03(s, 1 H); 5.61(s, OH); 4.27(s, 2 H); 2.27(s, 3 H).

5-chloro-2-(hydroxymethyl)-6-methyl-6'-{4-[(trifluoromethyl)oxy]phenyl}-3,3'-bipyridin-4(*1H*)-one (138)

To a stirred solution of compound **136** (4.85g, 12.89 mmol) in a mixture of dichloromethane (150 ml) and methanol (75 ml) were added at 0ºC under nitrogen trichloroisocyanuric acid (1.225g, 5.27 mmol). The reaction mixture was stirred at 0 °C for 1h, then filtered and concentrated, The residue was stirred with 5% Na_2CO_3 solution for 1h, filtered and washed with H_2O, this solid was dried at vacuum overnight. The solid was triturated with AcOEt to afford 4.24 g of the title compound (80% yield).
^{1}H NMR (δ, ppm, DMSO-d_6): 11.69(bs, 1H); 8.54-8.50(m, 1H); 8.25(d, 2H); 8.03(d, 1H); 7.78(dd, 1H); 7.49(d, 2H); 5.67(s, 1H); 4.28(s, 2H); 2.49(s, 3H); [ES MS] m/z: 411 $[M+H]^+$

5-chloro-6'-[2-fluoro-4-(trifluoromethyl)phenyl]-2-(hydroxymethyl)-6-methyl-3,3'-bipyridin-4(1*H*)-one (139)

Compound **139** was prepared following an analogous procedure to that used for compound **138**, starting from compound **137**. ^{1}H NMR (δ, ppm, DMSO-d_6): 11.73(bs, 1H); 8.60(d, 1H); 8.22(t, 1H); 7.96-7.79(m, 3H); 7.74(d, 1H); 5.68(t, 1H); 4.29(d, 2H); 2.48(s, 3H); [ES MS] m/z: 413 $[M+H]^+$.

ASSOCIATED CONTENT

Supporting Information. Spectroscopic data for compounds **8-13**, **15-20**, **22-27**, **29-34**, **43-44**, **46**, **49**, **51**, **53**, **55**, **57-79**, **83-105**, **107-115**, **117-125**. Detailed information about determination of physicochemical properties and procedures for *in vitro* activity, *in vivo* PK and efficacy assays. Smiles structural list (CSV)

Animal care and biological samples

All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals.

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

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The authors declare no conflict of interest

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ABBREVIATIONS

ACT, Artemisinin combination therapy; CF3, Trifluoromethyl; CHIlogD, Chromatographic hydrophobicity index distribution coefficient; Cl, Clearance; Cmax, Maximum concentration; DAST, Diethylaminosulfur trifluoride; EtOAc, Ethyl acetate; EtOH, Ethanol; GSK, GlaxoSmithKline; KOAc, Potassium Acetate; MMV, Medicines for Malaria Venture; MW, Microwave; NCS, N-Chloro Succinimide; $PhN(SO_2CF_3)_2$, N-Phenyl-trifluoromethanesulfonimide; *P. berghei, Plasmodium berghei;* Pd/C, Palladium on carbon; $Pd(PPh3)_4$, Tetrakis(triphenylphosphine)palladium(0); $Pd(PPh3)_2Cl_2$, Palladium(II)bis(triphenylphosphine) dichloride; *P. falciparum, Plasmodium falciparum; Pf Cyt bc1, P. falciparum cytochrome bc1;* R.T., Room temperature; Tmax, maximun time; TBDMS, Tert-butyl dimethylsilyl ether; TBDMSCl, Tert-butyl dimethylsilyl chloride; TEA, Triethylamine; Tol, Toluene; TOTP, Tris(o-tolyl)phosphine; Vd, Volume of distribution;

REFERENCES

(1) World malaria report. Accessed on December, 2015. WHO Geneva, Switzerland. http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/

(2) Wells, T. N.; Poll, E. M. When is enough enough? The need for a robust pipeline of high-quality antimalarials, *Discov. Med*. **2010**, *9*, 389-398

(3) Olliaro, P.; Wells, T.N. The global portfolio of new antimalarial medicines under development, *Clin. Pharm. Ther*. **2009**, *85(6)*, 584-595

(4) Woodrow, C.J.; White, N.J. The clinical impact of artemisinin resistance in Southeast Asia and the potential for future spread drug resistance, *Microbiology Reviews*. **2017**, *41*(*1*), 34-48

(5) Blasco, B.; Leroy,D.;White, L.; Fidock, D.A. Antimalarial drug resistance: linking *Plasmodium falciparum* parasite biology to the clinic, *Nature Medicine*. **2017**, *8*, 917-928

(6) Burrows, J. N. Antimalarial drug discovery: where next? *Future Medicinal Chemistry,* **2012**, *4*, 2233-2235

(7) Rodrigues, T.; Lopes, F.; Moreira, R. Inhibitors of the mitochondrial electron transport chain and the novo Pyrimidine biosynthesis as antimalarials: The present status, *Curr. Med. Chem.* **2010**, *17*, 929-956

(8) Mather, M.W., Henry, K. W.; Vaidya, A.B. Mitochondrial drug targets in apicomplexan parasites, *Curr. Drug Targets* **2007**, *8*, 49-60

(9) Painter, H.J.; Morrisey, J.M.; Mather, M.W.; Vaidya, A.B. Specific role of mitochondrial electron transport in blood-stage *Plasmodium falciparum*, *Nature* **2007**, *446*, 88-91

(10) Mi-Ichi, F.; Takeo, S.; Takashima, E.; Kobayashi, T.; Kim, H.S.; Wataya, Y.; Matsuda, A.; Torrii, M.; Tsuboi,T.; Kita, K. Unique properties of respiratory chain in Plasmodium falciparum mitochondria, *Adv. Exp. Med. Biol*. **2003**, *531*, 117-133

(11) Looareesuwan, S.; Chulay, J.D.; Canfield, C.J., Hutchinson, D.B. Malarone (Atovaquone and Proguanil Hydrochloride): A review of its clinical development for treatment of malaria. Malarone clinical trials study group, *Am. J. Trop Med. Hyg*. **1999**, *60*, 533-541

(12) Kessl, J.J.; Lange, B.B.; Merbitz-Zahradnik, T.; Zwicker, K.; Hill, P.; Meunier, B.; Palsdottir, H.; Hunte, C.; Meshnick, S.; Trumpower, B.L. Molecular basis for atovaquone binding to the cytochrome bc1 complex., *J. Biol Chem.* **2003**, *278*, 31312-31318

(13) Bueno, J.M.; Herreros, E.; Angulo, I.; Ferrer, S.; Fiandor, J.M.; Gamo, F.; Gargallo-Viola, D; Derimanov, G. Exploration of 4(1H)-pyridones as a novel family of potent antimalarial inhibitors of the plasmadial cytochrome bc1, *Future Med. Chem.* **2012**, 2311-2323.

(14) Capper, M.J.; O'Neill, P.M.; Fisher, N.; Strange, R.W.; Moss, D.; Ward,S.A.; Berry, N.G.; Lawreson, A.S.; Hasnain, S.S.; Biagini, G.A.; Antonyuk, S.V. Antimalarial 4(1H)-pyridones bind to the Qi site of cytochrome bc1, *PNAS.* **2015**, *112*, 755-760

(15) Markley, L.D.; Van Heertum, J.C.; Dooreubos, H.E., Markley, L. D.; Van Heertum, J. C.; Doorenbos, H. E. Antimalarial activity of Clopidol, 3,5-Dichloro-2,6-dimethyl-4-pyridinol, and its esters, carbonates, and sulfonates, *J. Med. Chem.* **1972**, *15*, 1188-1189

(16) Fry, M.; Williams, R.B. Effects of Decoquinate and Clopidol on electron transport in mitochondria of Eimeria tenella (Apicomplexa: Coccidia), *Biochem. Pharmacol.* **1984**, *33*, 229-240

(17) Yeates, C.L., Batchelor, J.F.; Capon, E.C.; Cheesman, N.J.; Fry, M.; Hudson, A.T.; Pudney, M.; Trimming, H.; Woolven, J.; Bueno, J.M.; Chicharro, J.; Fernández, E.; Fiandor, J.M., Gargallo-Viola, D.; Gómez de las Heras, F.; Herreros, E.; León, M.L. Synthesis and structure-activity relationships of 4-pyridones as potential antimalarials, *J. Med. Chem.* **2008**, *51*, 2845-2852

(18) Butler, J.; Dressman, J.B. The developability classification system: Application of biopharmaceutics concepts to formulation development, *J. Pharm. Sci.* **2010**, *99(12)*, 4940-4954

(19) Xiang, H.; Surdy-Freed, J.; Moorthy, G.S.; Hugger, E.; Bambal, R.,; Han, C.; Ferrer, S.; Gargallo-Viola, D.; Davis, C.B. Preclinical drug metabolism and pharmacokinetic evaluation of GW844520, a novel anti-malarial mitochondrial electron transport inhibitor*, J. Pharm. Sci.* **2006**, *95*, 2657

(20) Xiang, H.; Surdy-Freed, J.; Subbanagounder, G.; Hugger, E.; Han, C.; Bambal, R.; Ferrer, S.; Gargallo-Viola, D.; Davis, C.B. Preclinical pharmacokinetics and metabolism of GW308678, a second generation 4(1*H*)-Pyridone anti-malarial mitochondrial electron transport inhibitor. In: Program and abstracts of the 55th annual meeting of the ASTMH. *Am. J. Trop. Med. Hyg.* **2006**, *75* (Suppl. 5). 248

(21) Bueno, J.M.; Manzano, P.; García, M.C.; Chicharro, J.; Puente, M.; Lorenzo, M.; García, A.; Ferrer, S.; Gómez, R.M., Fraile, M.T.; Lavandera, J.L.; Fiandor, J.M.; Vidal, J.; Herreros, E.; Gargallo-Viola, D. Potent antimalarial 4-pyridones with improved physico-chemical properties, *Bioor. Med. Chem. Lett.* **2011**, *21*, 5214

(22) Nair, V.; Turner, G.A.; Buenger, G.S.; Chamberlain, S.D. New methodologies for the synthesis of C-2 functionalized hypoxanthine nucleosides, *J. Org. Chem.* **1988**, *53*, 3051

(23) Batchelor, J.F.; Yeates, C.L. Preparation of Substituted 4-Pyridinones as Antimalarials. Eur. Pat. Appl. EP 447164, 1991

(24) Eaton, P.E.; Carlson, G.R.; Lee, J.T. Phosphorus pentoxide-methanesulfonic acid. Convenient alternative to polyphosphoric acid, *J. Org. Chem.* **1973**, *38(23)*, 4071-4073

(25) Collina, G.; Forlani, L.; Mezzina, E.; Sintoni, M.; Todesco, P.E. Alkylation of 2-hydroxybenzothiazole salts , *Gazzetta Chimica Italiana* **1987**, *117(5)*, 281-286

(26) Tilstam, U.; Winmann, H. Trichloroisocyanuric acid: A safe and efficient oxidant, *Org. Proc. Res. Dev.* **2002**, *6(4)*, 384-393

(27) Bengtson, A.; Hallberg, A.; Larhed, M. Fast synthesis of aryl triflates with controlled microwave heating, *Org. Lett.* **2002**, *4*, 1231-1233

(28) Liu, D.Z.; Piyamongkol, S.; Liu, D.Y.; Khodr, H.H.; Lu, S.L.; Hider, R.C. Synthesis of 2-amido-3- hydroxypyridin-4(*1H*)-ones: novel iron chelators with enhanced pFe^{3+} values, *Bioorg. Med. Chem.* **2001**, *9*, 563

(29) Pankiewicz, K.W.; Nawrot, B.C.; Watanaba, K.A. (Trifluoromethyl)sulfonyl (triflyl) migration. Synthesis of 6,3'-anhydro-3-benzyl-1-(5-chloro-5-deoxy-.beta.-D-xylofuranosyl)barbituric acid from the 2'-trifluoromethanesulfonate (triflate) of 6,5'-anhydro-3-benzyl-1-.beta.-D-ribofuranosylbarbituric acid, *J. Org. Chem*. **1986**, *51(9)*, 1525

(30) José Luis Lavandera. Unpublished results. GlaxoSmithKline laboratories.

(31) Ismail, F.M.D.; Drew, M.G.B.; Dascombe, M.J. Modulation of drug pharmacokinetics and pharmacodynamics by fluorine substitution, *Chemistry Today*, **2009**, *27(3)*, 16

(32) Valkó, K.; Du, D.M.; Bevan, Ch.; Reynolds, D.P., Abraham, M.H. Rapid method for the estimation of octanol/water partition coefficient (LogPoct) from gradient RP-HPLC retention and hydrogen bond acidity term (Sigma alpha2H), *Curr. Med. Chem.* **2001**, *8(9)*, 1137

(33) Valkó, K. ; Nunhuck, S.; Bevan, C.; Abraham, M.H.; Reynolds, D.P. Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. *J. Pharm. Sci.* **2003**, *92*, 2236-2248

(34) Dressman, J.B.; Reppas, Ch. *In vitro–in vivo* correlations for lipophilic, poorly water-soluble drugs, *Eur. J. Pharm. Sci.* **2000**, *11*, Suppl. 2 S73

(35) Grandberg, I.I.; Faizova, G.K.; Kost, A.N. Comparative basicities of substituted pyridines and electronegativity series for substituents in the pyridine series, *Chemistry of Heterocyclic Compounds* **1966**, *2(4)*, 421-425

(36) Besso H.; Imafuku, K.; Matsumura, H. Tautomerism of 4-pyridones, *Bull. Chem. Soc. Japan.* **1977**, *50(3)*, 710

(37) Katritzky, A. R.; Karelson, M.; Harris, P. A. Prototropic tautomerism of heteroaromatic compounds., *Heterocycles* **1991,** *22,* 329-367.

(38) Boer, F. P. The crystal structure of Clopidol *, Acta. Crystallogr.* **1972,** *28,* 3200-3206.

(39) Peters, W.; Robinson, B.L. **1999,** Malaria. In *Handbook of Animal Models of Infection*; Zak O., Sande M. A., Eds.; Academic Press: New York, 1999; Vol.1 , pp 757–773

(40) Jiménez-Díaz, M.B.; Rullas, J.; Mulet, T.; Fernandez, L.; Bravo, C.; Angulo-Barturen, I. Improvement of detection specificity of Plasmodium-infected murine erythrocytes by flow cytometry using autofluorescence and YOYO-1. *Cytometry A* **2005**, *67*, 27–36

(41) Painter, H.J.; Morrisey, J.M.; Vaidya A.B. Mitochondrial electron transport inhibition and viability of intraerythrocytic *Plasmodium falciparum, Antimicrob. Agents Chemother.* **2010**, *54(12)*, 5281-5287

(42) Desjardins, R.E.; Canfield, C.J.; Haynes, J.D.; Chulay, J.D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique, *Antimicrob. Agents Chemother.* **1979**, *16(6)*, 710

Figure 1. Structure of Atovaquone, Clopidol, previous pyridone hits and new polar derivatives

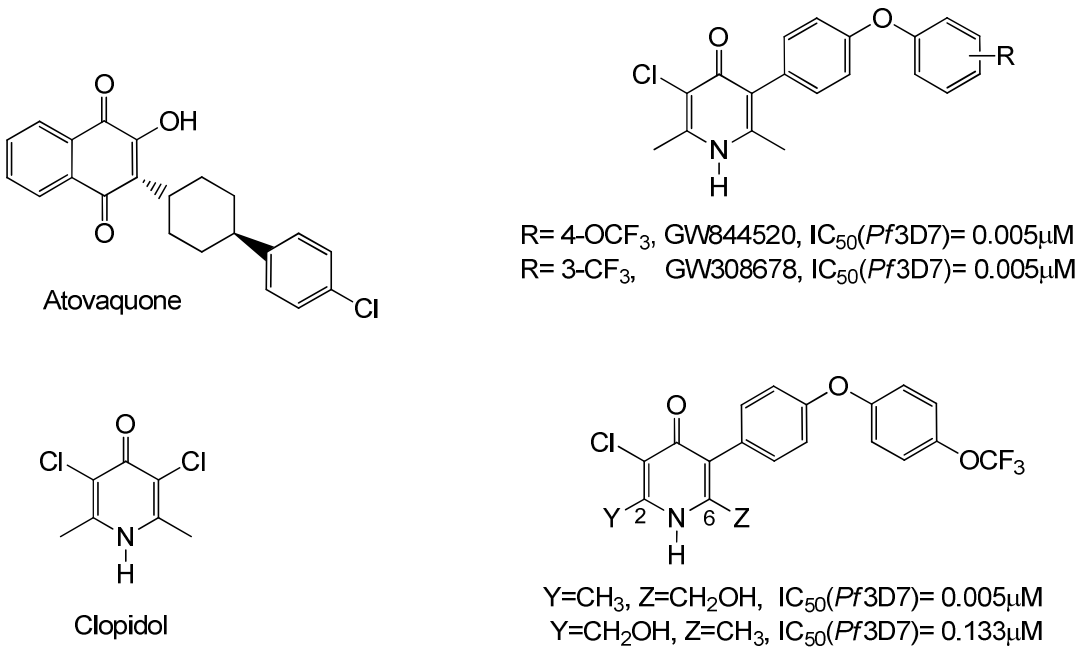
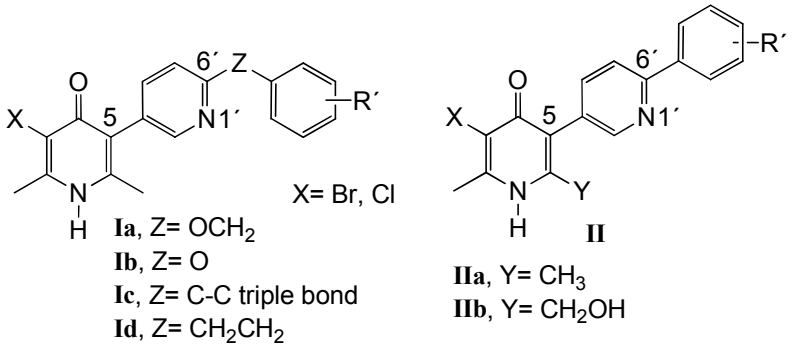
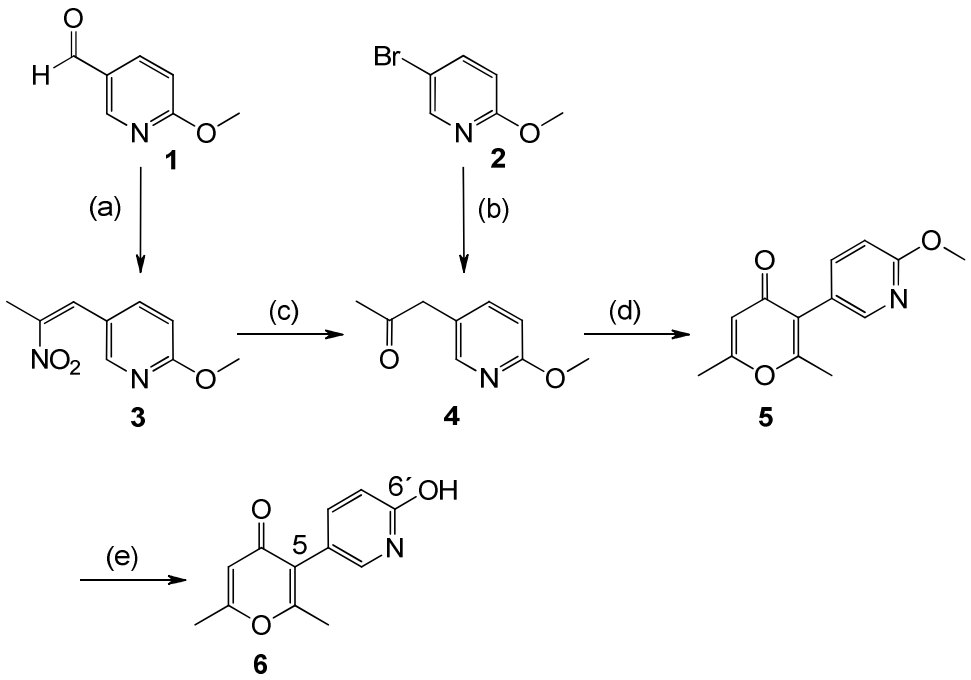


Figure 2. Novel antimalarial 5-Pyridinyl-4(1H)-pyridone derivatives

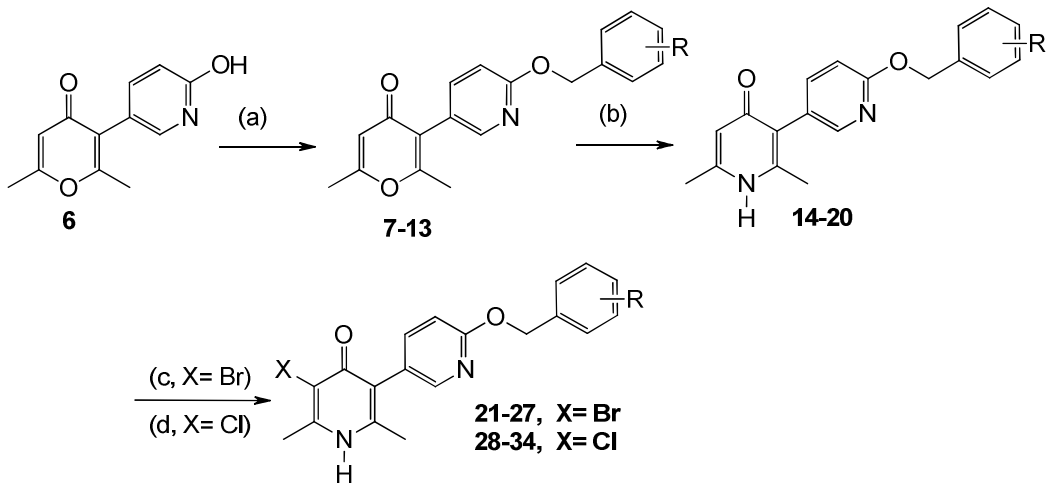


Scheme 1. Synthesis of 4-pyranone **6**[a].



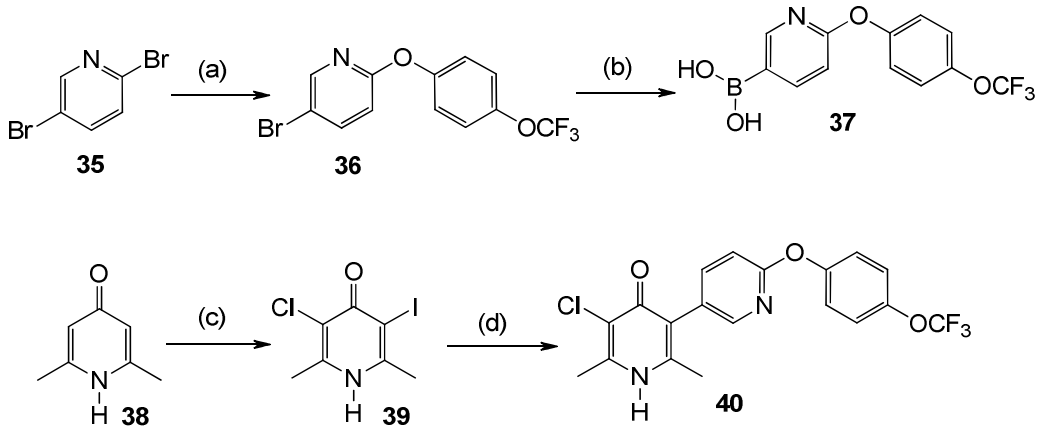
[a] Reagents and conditions: (a) i) *n*-$BuNH_2$, toluene, reflux, ii) $EtNO_2$, AcOH, 100ºC, 85% (b) Isopropenyl acetate, *n*-Bu_3SnOMe, $Pd(OAc)_2$, TOTP, toluene, 80 ^oC,70% (c) Fe, AcOH, reflux, 94% (d) Ac_2O, PPA, 95 ^oC, 64% or Ac_2O, Eaton´s reagent, 50 ^oC, 58% (e) 6N HCl, 110ºC, 95%

Scheme 2. Synthesis of O-Benzyl-substituted derivatives **Ia**[a].



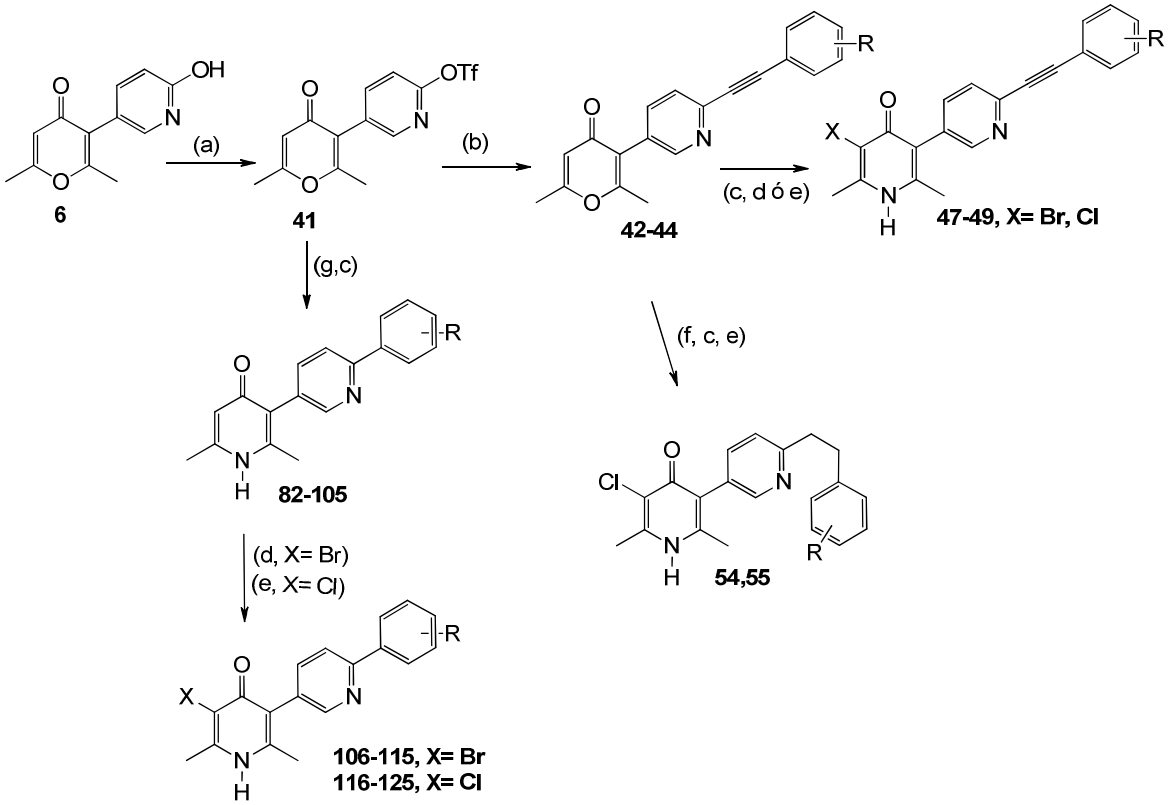
[a] Reagents and conditions: (a) Benzyl halide, Ag_2CO_3, 60ºC, dark, 72% (b) aq NH_3, EtOH, 140ºC, 59% (c) N-bromosuccinimide, DCM/MeOH, r.t.,85% (d) Trichloroisocyanuric acid, DCM/MeOH, 0ºC,64%

Scheme 3. Synthesis of phenyloxy derivative **40 (Ib)**[a]



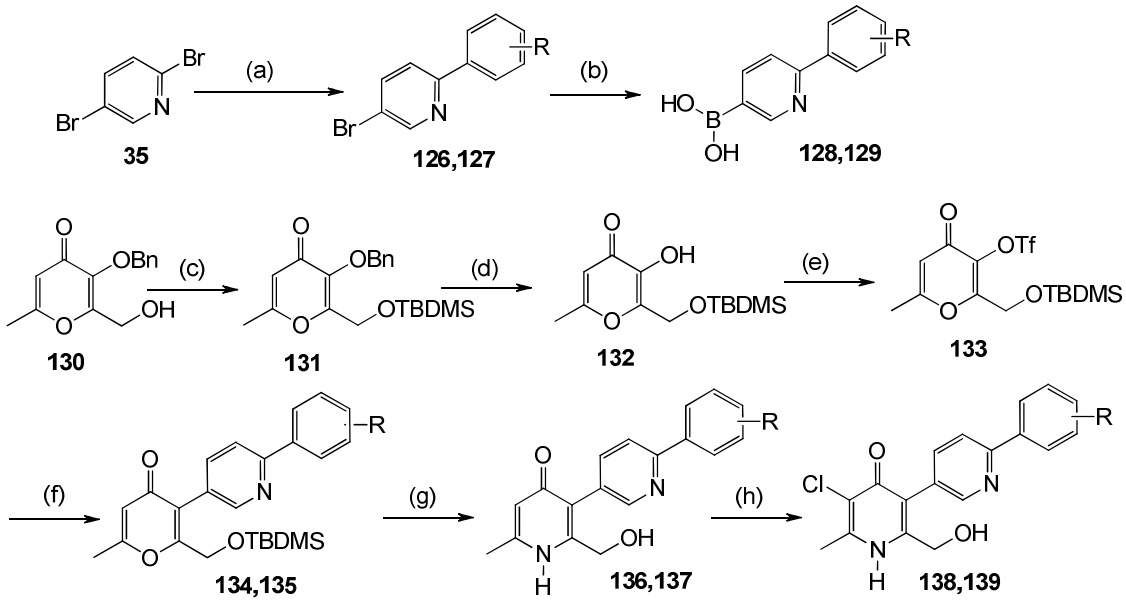
[a] Reagents and conditions: (a) *i)*NaH, DMF, 60ºC, *ii)* 4-trifluoromethoxy-phenol, 80% (b) *i)* $B(OPr^i)_3$, THF, n-BuLi, -78ºC, *ii)* 2N HCl, r.t., 59% (c) *i)*NCS, DCM/MeOH, r.t., *ii)* I_2, NaOH, water, r.t., 50% (d) **37**, $Pd(OAc)_2$, DMF, K_2CO_3, 140ºC, MW, 60%

Scheme 4. Synthesis of C6´-substituted derivatives of general formulae **Ic,d** and **IIa**[a].



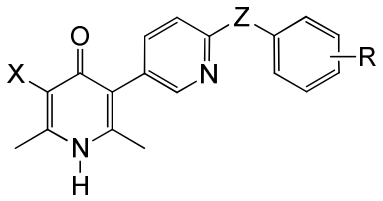
[a] Reagents and conditions: (a) $PhN(SO_2CF_3)_2$, K_2CO_3, DMF, r.t.; (b) CuI, $(PPh_3)_2PdCl_2$, PPh_3, Alkyne, TEA, DMF, reflux,52% (c) aq NH_3, EtOH, 140ºC; (d) N-bromosuccinimide, DCM/MeOH, r.t.; (e) trichloroisocyanuric acid, DCM/MeOH, 0ºC; (f) H_2, 10% Pd(C), EtOAc/MeOH, HCl; (g) Boronic acid, $(PPh_3)_2PdCl_2$, Toluene/EtOH, aq $NaHCO_3$, 95ºC; (h) ICH_2CF_3, Cs_2CO_3, DMF, 50ºC; (i) DAST (neat), 0ºC.

Scheme 5. Synthesis of 6-hydroxymethyl-4(1H)-pyridone derivatives of general formula **IIb**[a]



[a] Reagents and conditions: (a) Boronic Acid, Toluene/EtOH, Palladium catalyst, Cs_2CO_3, 80ºC; (b) *i)* Bis(pinacolato)diboron, $(PPh_3)_2PdCl_2$, KOAc, 100ºC, 1,4-dioxane, *ii)* NH_4Ac, $NaIO_4$, Acetone/Water, r.t.; (c) TBDMSCl, DMF, Imidazole, r.t.; (d) H_2, 10% Pd(C), EtOAc; (e) $PhN(SO_2CF_3)_2$, K_2CO_3, DMF, r.t.; (f) **128** or **129**, $(PPh_3)_2PdCl_2$, Toluene/EtOH, aq. Na_2CO_3, 80ºC; (g) aq. NH_3, EtOH, 140ºC (h) trichloroisocyanuric acid, MeOH/DCM, 0ºC-r.t.

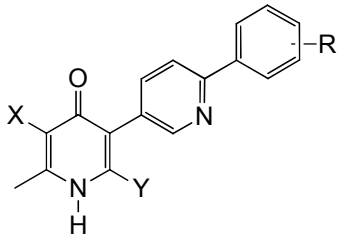
Table 1. Biological activity of compounds of general formula **Ia-d**.



Ia, Z= OCH_2
Ib, Z= O
Ic, Z= C-C triple bond
Id, Z= CH_2CH_2

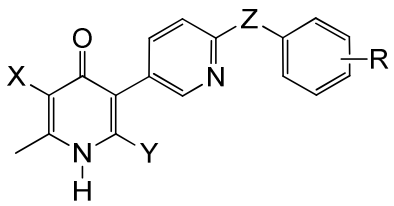
Compound	Z	R	X	*Pf*3D7A $IC_{50}(\mu M)$
21	OCH_2	4-OCF_3	Br	0.019
22	OCH_2	4-CF_3	Br	0.002
23	OCH_2	3-CF_3	Br	0.004
24	OCH_2	2-CF_3	Br	0.011
25	OCH_2	3,5-diCF_3	Br	0.033
26	OCH_2	4-F	Br	0.010
27	OCH_2	3,5-diF	Br	0.040
28	OCH_2	4-OCF_3	Cl	0.014
29	OCH_2	4-CF_3	Cl	0.005
30	OCH_2	3-CF_3	Cl	0.005
31	OCH_2	2-CF_3	Cl	0.010
32	OCH_2	3,5-diCF_3	Cl	0.029
33	OCH_2	4-F	Cl	0.033
34	OCH_2	3,5-diF	Cl	0.080
40	O	4-OCF_3	Cl	0.033
47	CΞC	4-CF_3	Br	0.058
48	CΞC	4-CF_3	Cl	0.097
49	CΞC	2,4-diF	Cl	0.472
54	CH_2CH_2	4-CF_3	Cl	0.005
55	CH_2CH_2	3-CF_3	Cl	0.007

Table 2. Biological activity of phenyl-pyridinyl derivatives.



Compound	Y	R	X	*Pf*3D7A IC_{50}(μM)
106	CH_3	4-OCF_3	Br	0.020
107	CH_3	3-CF_3	Br	0.007
108	CH_3	4-Cl	Br	0.110
109	CH_3	2-Cl	Br	0.044
110	CH_3	4-F	Br	0.129
111	CH_3	2-F	Br	0.064
112	CH_3	3-CH_2OH	Br	0.324
113	CH_3	4-CN	Br	0.329
114	CH_3	4-SO_2Me	Br	0.288
115	CH_3	2,4-diCF_3	Br	0.006
116	CH_3	4-OCF_3	Cl	0.020
117	CH_3	2-OCF_3	Cl	0.035
118	CH_3	4-CF_3	Cl	0.016
119	CH_3	3-CF_3	Cl	0.040
120	CH_3	3-Cl	Cl	0.116
121	CH_3	2-Cl	Cl	0.026
122	CH_3	4-F	Cl	0.082
123	CH_3	2-F	Cl	0.049
124	CH_3	2-Cl,4-CF_3	Cl	0.002
125	CH_3	2-F,4-CF_3	Cl	0.010
138	CH_2OH	4-OCF_3	Cl	0.034
139	CH_2OH	2-F,4-CF_3	Cl	0.005

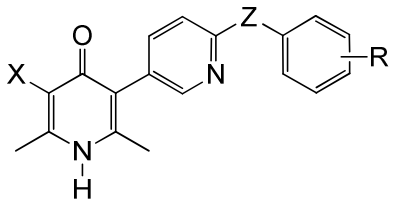
Table 3. Physico-chemical parameters for a series of selected compounds



Cpd	Y	Z	R	X	CHIlogD (pH)[a]			% HSA[a]	Equilibrium Solubility (µg/ml)[a]	
					2	7,4	10,5		FaSSIF	FeSSIF
21	CH_3	OCH_2	4-OCF_3	Br	2.97	3.05	3.00	97	2.08	2
28	CH_3	OCH_2	4-OCF_3	Cl	2.84	2.95	2.89	97	3.76	3.95
106	CH_3	---	4-OCF_3	Br	1.99	2.66	2.50	96	3.06	0.66
116	CH_3	---	4-OCF_3	Cl	1.88	2.56	2.38	96	1.42	4.71
118	CH_3	---	4-CF_3	Cl	1.99	2.43	2.21	95	1.99	4.18
119	CH_3	---	3-CF_3	Cl	2.0	2,36	2.19	95	4.16	4
124	CH_3	---	2-Cl,4-CF_3	Cl	2.08	2.3	2.24	95	<1	<1
125	CH_3	---	2-F,4-CF_3	Cl	2.07	2.24	1.97	95	<1	1.33
138	CH_2OH	---	4-OCF_3	Cl	1.55	2.05	1.48	95	0.1	23.4
139	CH_2OH	---	2-F,4-CF_3	Cl	1.86	1.99	1.34	94	0.24	1.88

a) Experimental details can be found in References 32-34 and supporting information provided.

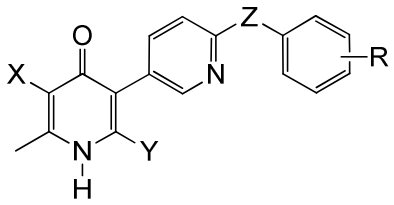
Table 4. Pharmacokinetic parameters for a series of selected compounds in CD1 mice.



	iv (mg/Kg)[a]			po (mg/Kg)[b]				
Cpd (*i.v* dose/p.o dose)	Vd (L/Kg)	Cl (ml/min/Kg)	$t_{1/2}$ (h)	C_{max} (μg/ml)	T_{max} (h)	AUC 0-t (μg.h/ml)	AUC ∞ (μg.h/ml)	%F
28 (0.256/8.3)	1.26	2.74	5.32	0.3	6	5.6	5.6	11
106 (0.192/ 10.36)	0.91	0.72	14.59	4.1	10	108.7	117.6	49.15
116 (0.17/ 10.757)	0.85	0.53	18.7	2.29	10	166.8	186.8	54.9
118 (0.1987/ 4.42)	1.41	0.69	23.45	3.31	10	100.6	106.3	100
119 (0.195/9.354)	0.68	1.52	5.13	2.47	3	39.5	39.5	38.67
124 (0.211/8.366)	1.24	1.11	12.8	1.69	10	37.4	37.5	29.98
125 (0.219/11.512)	0.75	0.26	33.76	6.38	10	204.1	225.5	30
138 (1.45/ 8.7)	1.11	1.76	7.4	8.54	1	109.77	110.99	132.7

a) *iv* route: solution in 20% Encapsin/5% DMSO/PEG/saline pH=6
b) oral route: suspension in 1% Methyl Cellulose

Table 5. Therapeutic efficacy of selected compounds in the 4day *P. yoelii* mouse model[a]



Cpd	Y	Z	R	X	ED_{50} (mg/Kg)
28	CH_3	OCH_2	4-OCF_3	Cl	16.3
116	CH_3	---	4-OCF_3	Cl	0.15
118	CH_3	---	4-CF_3	Cl	0.25
119	CH_3	---	3-CF_3	Cl	1.2
124	CH_3	---	2-Cl,4-CF_3	Cl	0.33
125	CH_3	---	2-F,4-CF_3	Cl	0.08
138	CH_2OH	---	4-OCF_3	Cl	0.4
139	CH_2OH	---	2F,4-CF_3	Cl	0.2

[a] suspension in 1% Methyl Cellulose

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