Journal of
Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.0c00451 • Publication Date (Web): 28 Jul 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Development of potent *Pf*CLK3 inhibitors based on TCMDC-135051 as a new class of antimalarials

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Abstract

The protein kinase *Pf*CLK3 plays a critical role in the regulation of malarial parasite RNA splicing and is essential for the survival of blood stage *Plasmodium falciparum*. We recently validated *Pf*CLK3 as a drug target in malaria that offers prophylactic, transmission blocking and curative potential. Herein we describe the synthesis of our initial hit TCMDC-135051 **(1)** and efforts to establish a structure−activity relationship (SAR) with a 7-azaindole-based series. A total of 14 analogues were assessed in a TR-FRET assay against the full length recombinant protein kinase *Pf*CLK3 and 11 analogues were further assessed in asexual 3D7 (chloroquine sensitive) strains of *P. falciparum* parasites. SAR relating to rings A and B was established. These data together with analysis of activity against parasites collected from patients in the field suggest that TCMDC-135051 **(1)** is a promising lead compound for the development of new antimalarials with a novel mechanism of action targeting *Pf*CLK3.

Introduction

In the global fight against malaria the distribution of over 0.5 billion insecticide impregnated bed nets since 2015, together with artemisinin-based combination therapies have contributed to 20 million fewer cases of malaria being reported in 2017 compared to 2010.[1] Despite this success, the rate of reduction in malaria has stalled with no significant reduction seen over the last 3 years.[1] This, together with evidence of the emergence of resistance to both artemisinin[2,3] and partner drugs including piperaquine and mefloquine[4,5] means that there is a danger that the progress made in the reduction of malaria will be reversed unless new medicines with novel mechanisms of action are discovered. To address this, we have been testing the notion that targeting malaria parasite protein kinases, known to be essential for the survival of the parasite, might offer a novel strategy for the development of next generation anti-malarials.[6,7]

In 2011 we determined that 36 of the 65 eukaryotic protein kinases are essential for the survival of the blood stage of the most virulent species of human malaria, *Plasmodium falciparum*.[8] Among these protein kinases was *Pf*CLK3 (*PF*3D7_1114700), one of four members of the cyclin-dependent like protein kinase family (*Pf*CLK1-4). The plasmodium CLK-family are closely related to the mammalian CLK family and the

serine-arginine-rich protein kinase (SRPK) family[9] both of which are crucial mediators of multiple phosphorylation events on splicing factors necessary for the correct assembly and catalytic activity of spliceosomes.[10] Our *in vitro* studies showed that *Pf*CLK3 can phosphorylate parasite SR proteins[11] indicating that *Pf*CLK3, together with other members of the *Pf*CLK family[12], plays a role in the processing of parasite RNA.[9] We reasoned therefore that inhibitors to *Pf*CLK3 might have parasiticidal activity at any stage of the parasite life cycle where RNA splicing played an essential role.

Screening of ~25,000 compounds including all 13,533 compounds of the Tres Cantos Antimalarial Set (TCAMS) resulted in the discovery of TCMDC-135051 (**1**, Figure 1), a compound with nanomolar (nM) activity against *Pf*CLK3 *in vitro* kinase assays and submicromolar (µM) parasiticidal activity in asexual blood stage *P. falciparum* parasites (Figure 1).[13] Subsequent studies revealed that TCMDC-135051 rapidly killed *P. falciparum* at the trophozoite to schizont stages as well as preventing the development of stage V gametocytes and inhibiting the development of liver stage parasites. TCMDC-135051 also showed equivalent *in vitro* kinase activity at CLK3 from *P. falciparum*, *P. berghei* (mouse malaria), *P. vivax* (human malaria), *P. knowlesi* (monkey and human malaria).[13] Our recent studies have therefore validated *Pf*CLK3 as a target with the potential to deliver a curative treatment, be transmission blocking and act as a prophylactic target. [13]

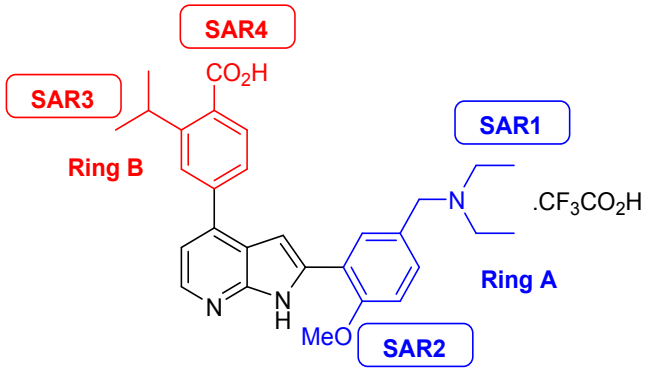


Figure 1. Structure and biological profile of hit *Pf*CLK3 inhibitor TCMDC-135051, **1**

Key Structural features of **1** include a core 7-azaindole scaffold with aromatic rings in the 2- and 4-positions (ring-A and –B respectively). These aromatic rings are further substituted with various functional groups, including a tertiary amine (ring A) and carboxylic acid (ring B) resulting in a zwitterionic compound at physiological pH.

7-Azaindoles are a widely studied pharmacophore incorporated in several therapeutic agents.[14] Kinases are the predominate target with the 7-azaindole moiety generally interacting at the ATP binding site within the kinase hinge region.[15,16] Interaction occurs between the kinase hinge region peptide backbone and the azaindole *via* two H-bonds. The first involving the pyridine nitrogen lone pair and peptide backbone NH,

and the second between the pyrrole NH and peptide backbone carbonyl. The resulting H-bond acceptor and donor bidentate 7-azaindole interaction with the hinge region of the kinase can occur in the more common "normal" orientation or the "flipped" orientation with the 2-position of the 7-azaindole projected out of the hinge region into the solvent exposed space.[17,18]

A number of small molecule kinase 7-azaindole inhibitors have progressed to different stages of clinical trials.[19] Potential drug candidate GSK1070916 is being developed as an Aurora kinase (Ser/Thr protein kinases family) inhibitor and has reached human clinical trials (Figure 2A).[20] The core scaffold is a 7-azaindole with aromatic substituents in the 2- and 4-positions. A X-ray crystal structure of the molecule:aurora kinase complex revealed a flipped hinge region binding mechanism, with 2-aryl projecting out of the hinge region into solvent and 4-aryl bound within the ribose pocket.[20]

Substructure analysis using Scifinder revealed several 7-azaindole analogues described as antimalarials. In 1972, Verbiscar *et al.* reported the first example of a 7-azaindole with antimalarial activity.[21] 1-*p*-Chlorobenzyl-7-azaindole-3-α-piperidylmethanol displayed antimalarial activity in mice against *Plasmodium berghei* (Figure 2B). More recently, Picard *et al.* reported 7-azaindole compounds that were identified from an *in silico* structure-based drug screen (10,13,483 compounds, Chembridge).[22] Compound IND31119 (Figure 2C) binds to the recombinant N-terminal domain of *Pf*Hsp90 and showed selectivity over human Hsp90 and *Pf*Hsp90 mutant.[22]

In addition to antimalarial activity, the 7-azaindole scaffold has been identified as having a wide variety of biological applications including antitumor activity and act as HIV-1 inhibitor in infected patients.[23,24] The 7-azaindole motif can be regarded as a privileged scaffold in medicinal chemistry as it is found in three clinical candidates (Vemurafenib, PLX3397, and AZD5363) (Figure 2D), which suggests its usefulness for developing novel pharmaceuticals.[25-27]

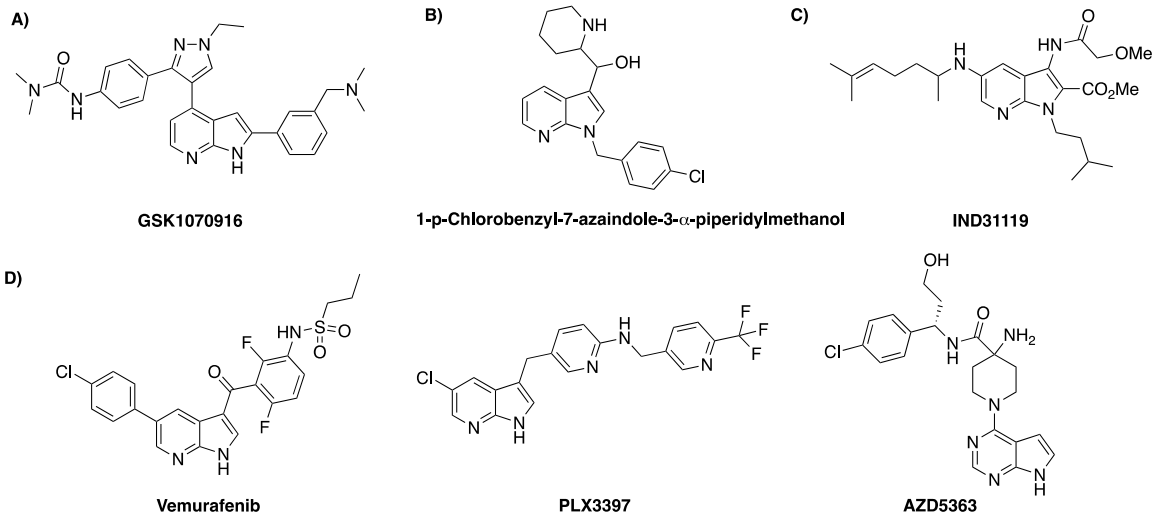


Figure 2. A) Aurora kinase inhibitor GSK1070916, B) 1-*p*-Chlorobenzyl-7-azaindole-3-α-piperidylmethanol has *in-vivo* activity against *Plasmodium berghei*, C) *Pf*Hsp90 inhibitor IND31119, D) Drug candidates incorporating a core 7-azaindole scaffold, Vemurafenib, PLX3397, and AZD5363.

To investigate the structure-activity relationship of hit compound **1**, a series of analogues were prepared through varying different substituents on ring-A and ring-B (Figure 1). The *N*-diethyl amine group (ring A) was initially replaced with different amine substituents to investigate lipophilicity and solubility (Figure 1, SAR1). Next, we replaced the methoxy moiety (ring A) with hydroxyl or hydrogen to modify polarity and to explore the role of the methoxy group on activity (Figure 1, SAR2). The isopropyl substituent (ring B) was replaced with other small hydrophobic substituents to probe non-coplanar conformations that could potentially lower the energy of crystal packing and consequently improve aqueous solubility and logP (Figure 1, SAR3). Finally, we exchanged the carboxylic acid group (ring B) with other substituents to investigate its role in binding, potentially improve metabolic stability and to explore the effect of increased lipophilic character (Figure 1, SAR4).

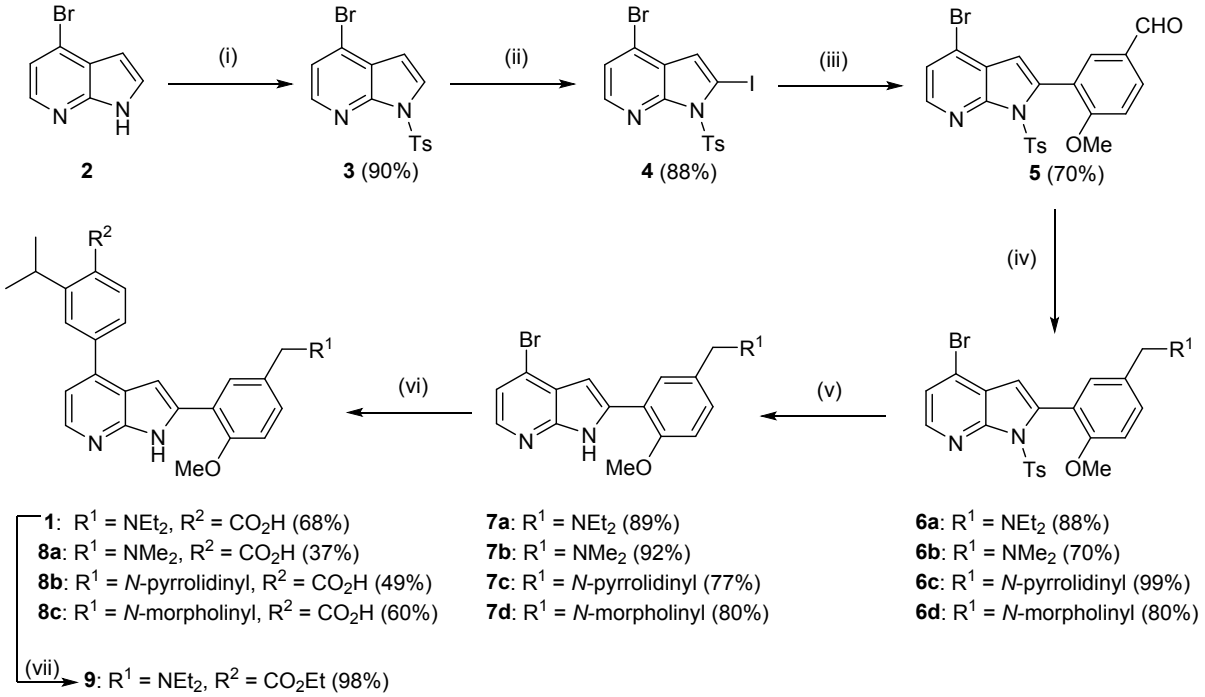
TCMDC-135051 is a promising hit compound for a medicinal chemistry programme to develop as a preclinical lead that meets many of the criteria set by the Medicines for Malaria Venture (capable of rapidly clearing the parasite, has multi-stage potency, killing multiple parasite species with action as a transmission blocker).[28,29] Here we describe the synthetic route to TCMDC-135051 and determine a structure-activity-relationship that will be key for lead development.

Chemistry

To investigate the effect of the *N*-diethyl group of ring-A on antimalarial activity, analogues of 4-(2-(5-((diethylamino)methyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoic acid (TCMDC-135051) **1**, **8a-c** were prepared as shown in scheme 1. Protection of 4-bromo-7-azaindole **2** was achieved using *p*-toluenesulfonyl chloride under basic conditions to provide *N*-tosyl-7-azaindole **3**. Regioselective iodination of **3** using lithium diisopropylamide (LDA) and iodine in THF at -78 °C provided 2-iodoazaindole **4**, which was subsequently subjected to Suzuki coupling with (5-formyl-2-methoxyphenyl)boronic acid to give 2-aryl substituted azaindole **5**. Reductive amination of the aldehyde functionality of **5** was performed with various amines in the presence of sodium triacetoxyborohydride to give amines **6a-d** in excellent yields. Next, the *N*-tosyl protecting group was removed under basic conditions at reflux for 18 h to yield **7a-d**. Finally, the desired analogues **1** and **8a-c** were obtained by Suzuki cross-coupling with 2-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (see SI for boronate ester synthesis).

Compound **9** was prepared as described in Scheme 1. The carboxylic acid of **1** was converted to its corresponding ethyl ester by refluxing in thionyl chloride and ethanol for 18h.

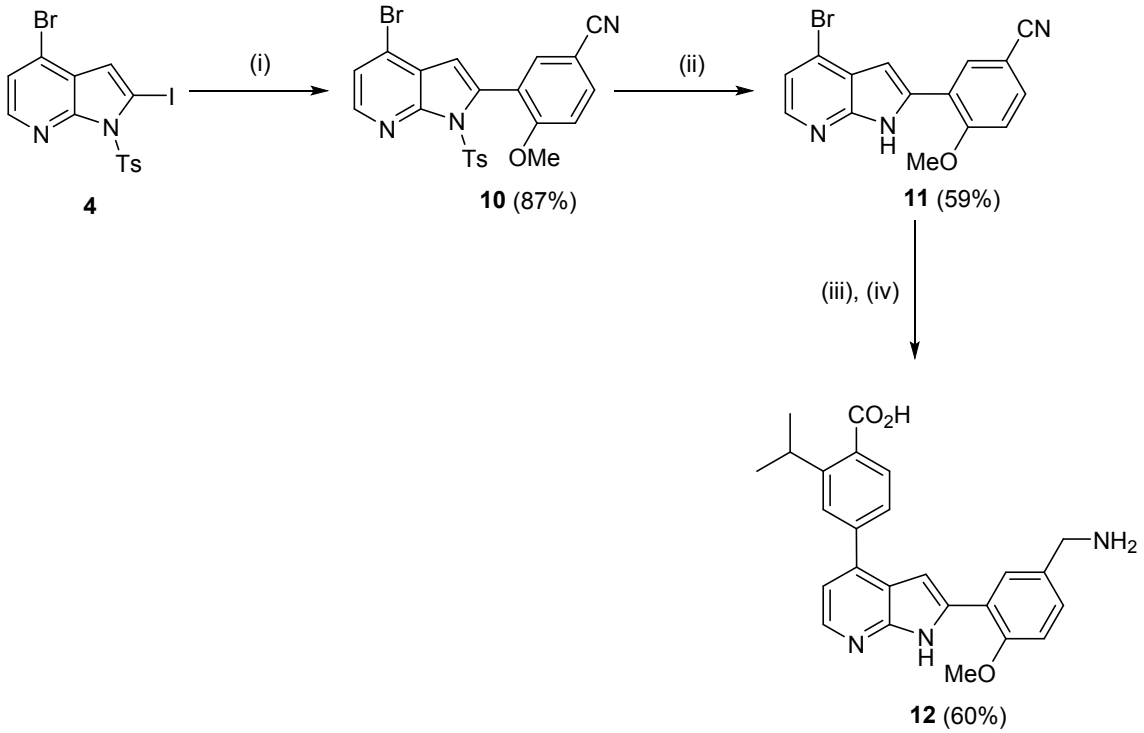
Scheme 1. Synthesis of TCMDC-135051 **1** and analogues **8a-c & 9**[a]



[a]Reagents and conditions: (i) TsCl, NaH, THF, 0 °C, 2 h; (ii) LDA, I_2, THF, -78 °C, 3 h; (iii) (5-formyl-2-methoxyphenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-Dioxane, 110 °C, 12 h; (iv) amine, $NaBH(AcO)_3$, 1,4-Dioxane, 20 °C, 12 h; (v) CH_3OH, K_2CO_3, 55 °C, 18 h; (vi) 2-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, $Pd(dppf)Cl_2.CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 110 °C, 0.5 h, MW; (vii) $SOCl_2$, CH_3CH_2OH, Reflux, 18 h.

The synthetic route outlined in Scheme 1 was modified to prepare primary amine **12**. The first step of this synthetic route involved coupling 2-iodo-azaindole **4** with the appropriate boronate ester under Suzuki conditions to yield nitrile **10** in 87% yield (Scheme 2). Tosyl deprotection was then achieved using K_2CO_3, followed by a Suzuki coupling at the 4-bromo-azaindole scaffold with the appropriate pinacol boronate ester**.** The crude material was reduced *in situ* using cobalt (II) chloride hexahydrate and sodium borohydride to provide the corresponding amine **12** (Scheme 2).

Scheme 2. Synthesis of 4-(2-(5-(aminomethyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoic acid **12**.[a]



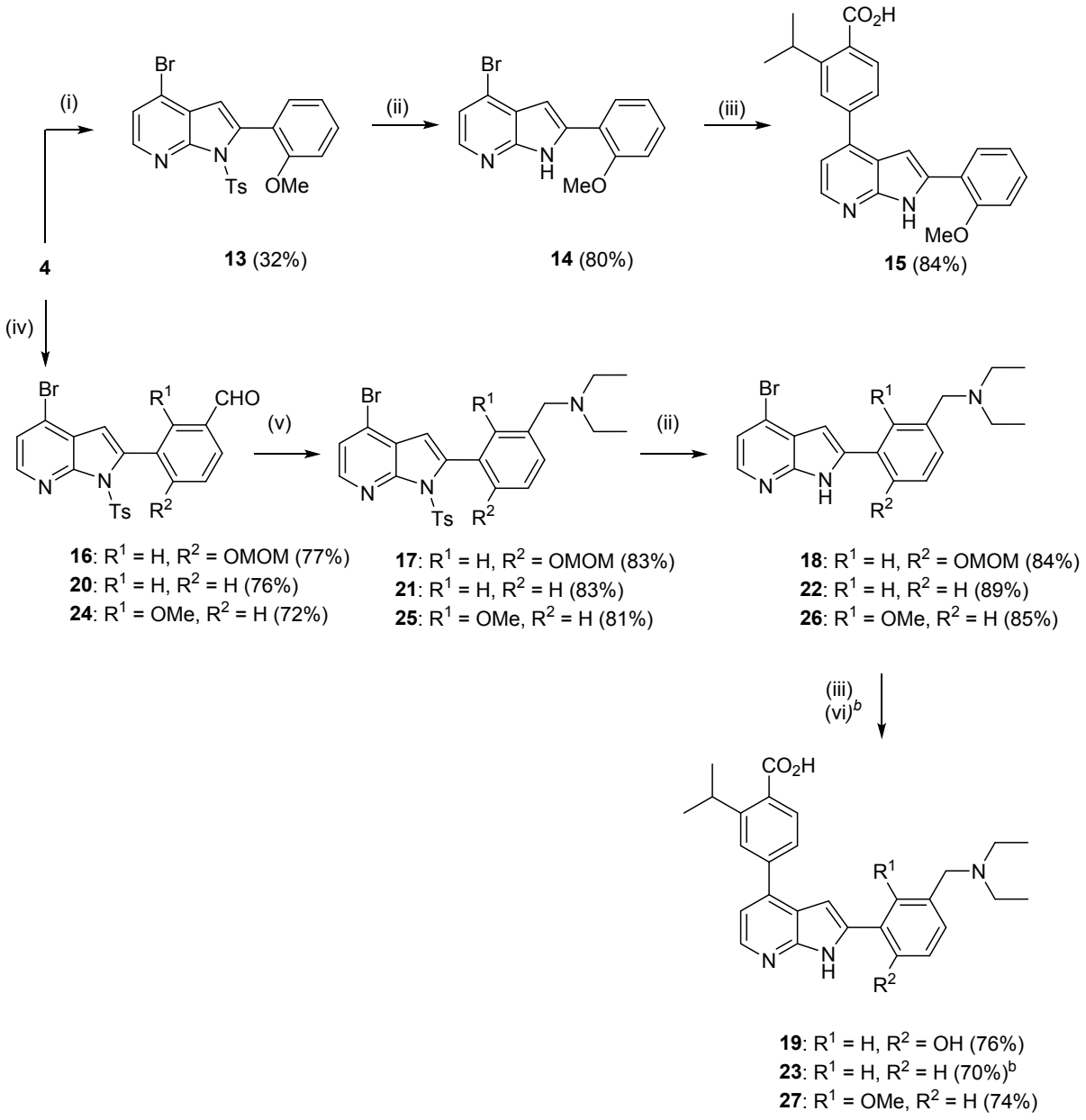
[a]Reagents and conditions: (i) 4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-Dioxane, 110 °C, 18 h; (ii) CH_3OH, K_2CO_3, 55 °C, 18 h; (iii) 2-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, $Pd(dppf)Cl_2.CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 110 °C, 0.5 h, MW; (iv) $CoCl_2{\cdot}6H_2O$, $NaBH_4$, CH_3OH.

Similarly, analogue **15** was synthesised *via* Suzuki coupling of **4** with 2-methoxyphenyl boronic acid, followed by tosyl deprotection and Suzuki coupling (Scheme 3).

To investigate the precise role of the methoxy group of ring-A on activity, we prepared analogues **19** and **23** in which the OMe is replaced by a hydroxy group (**19**) and OMe is removed (**23**) (Scheme 3). The synthetic route toward **19** starts with Suzuki coupling of **4** with methoxymethyl ether (MOM) protected boronate ester (see SI for boronate ester synthesis) to yield **16.** Reductive amination of **16** followed by removal of the tosyl group gave **18**. Finally, **18** was coupled with boronate ester under Suzuki conditions and the MOM protecting group was removed under acidic conditions to provide the desired analogue **19** in excellent yield. Analogue **23** was synthesized in 4 steps using the same methods.

To investigate whether the substitution pattern on ring A is important for antimalarial activity, we produced analogue **27** in which the methoxy group is moved from the para to ortho position relative to the methylene *N*-diethyl functionality (Scheme 3).

Scheme 3. Synthesis of 2-isopropyl-4-(2-(2-methoxyphenyl)-1*H*-pyrrolo[2,3-b]pyridin-4-yl)benzoic acid **15**, 4-(2-(5-((diethylamino)methyl)-2-hydroxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoic acid **19**, 4-(2-(3-((diethylamino)methyl)phenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoic acid **23** and [4-(2-(3-((diethylamino)methyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-b]pyridin-4-yl)-2-isopropylbenzoic acid] **27**.[a]



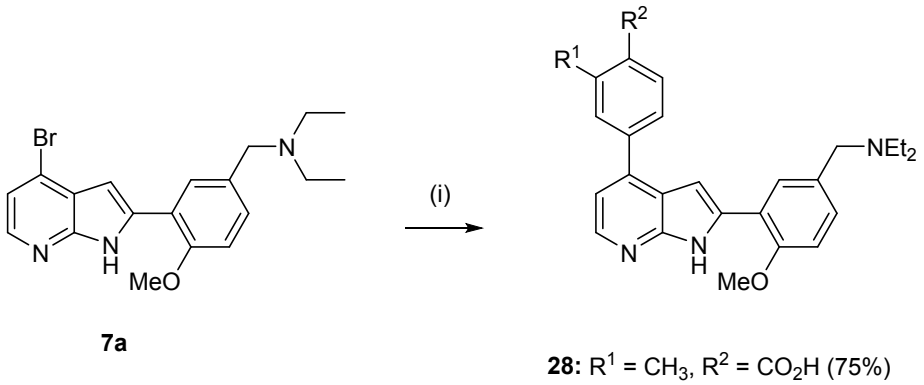
[a]Reagents and conditions: (i) (2-methoxyphenyl)boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-Dioxane, 110 °C, 12 h; (ii) CH_3OH, K_2CO_3, 55 °C, 18 h; (iii) 2-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, $Pd(dppf)Cl_2.CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 110 °C, 0.5 h, MW; (iv) 4-(methoxymethoxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde or 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-Dioxane, 110 °C, 18 h, (v) Et_2NH, $NaBH(AcO)_3$, 1,4-dioxane, rt, 18 h; (vi) HCl, $MeCN/H_2O$ 3:1.

To investigate the effect of the iso-propyl substituent (ring-B) on efficacy, we prepared analogues **28-29**, as shown in scheme 4. 4-Bromo-7-azaindole **7a** was coupled with various boronate esters under Suzuki conditions to yield the desired 4-aryl-7-azaindole analogues **28-29**.

To investigate the role of the carboxylic acid substituent of ring B on antimalarial activity, several analogues **30-32** were synthesised as depicted in scheme 4. Synthesis of analogues **30-32** was achieved by Suzuki coupling **7a** with various boronate esters (R^2 = 1*H*-tetrazole or H) to yield target compounds **30-31** in good yields.

An isomer in which the isopropyl and carboxylic acid were switched, **32** was synthesized to investigate the role of these functional groups on antimalarial activity (Scheme 4).

Scheme 4. Synthesis of 4-aryl-7-azaindole analogues,**28-32**.[a]



7a

28: R^1 = CH_3, R^2 = CO_2H (75%)
29: R^1 = H, R^2 = CO_2H (85%)
30: R^1 = $CH(CH_3)_2$, R_2 = 1*H*-tetrazole (62%)
31: R^1 = $CH(CH_3)_2$, R_2 = H (82%)
32: R^1 = CO_2H, R_2 = $CH(CH_3)_2$ (78%)

[a]Reagents and conditions: (i) boronate esters, $Pd(dppf)Cl_2.CH_2Cl_2$, Na_2CO_3, 1-4-dioxane, 110 °C, 0.5 h, MW.

Result and discussion

In this work, we have maintained the core 7-azaindole molecular scaffold of TCMDC-135051 and focused on the structure-activity relationships (SARs) of the substitutes on ring-A and –B to assess the requirement for this functionality. All synthesised analogues were assessed in a TR-FRET *Pf*CLK3 *in vitro* kinase assay against the full recombinant protein kinase (Table 1 and Table 2). Analogues which gave low nanomolar activity were then further assessed in live parasite viability (parasiticidal) assays using laboratory strain 3D7 (chloroquine sensitive) *P. falciparum* (Table 1 and Table 2). The synthesized analogues were evaluated for $LogD_{7.4}$ (Distribution co-efficient), metabolic stability, and the results are shown in Table 1 and 2.

SAR1 corresponding to analogues **8a-c, 12** and **15** were designed to examine the effect of the *N*-diethyl group of ring-A on antimalarial activity. In analogue **8a,** the *N*-diethyl group was replaced with an *N*-dimethyl group to investigate the effect of alkyl group size and molecular lipophilicity ($logD_{7.4}$ = 0.85). *In vitro* kinase activity of **8a** the half maximal inhibitory activity (IC_{50}) IC_{50} = 29 nM (pIC_{50} = 7.5 ± 0.224)

remains the same in recombinant *Pf*CLK3 kinase whereas a 2-fold decrease in the half maximal parasite growth inhibition effect (EC_{50}) EC_{50} = 457 nM (pEC_{50} = 6.3 ± 0.129) was observed (Table 1). To further investigate the steric requirements of this functionality and polarity, pyrrolidine (**8b**) and morpholino groups (**8c**) were introduced. Analogue **8b** shows comparable *in vitro* kinase activity, IC_{50} = 38 nM (pIC_{50} = 7.4 ± 0.113) and 2-fold decrease in parasite growth inhibition **8b** EC_{50} = 382 nM (pEC_{50} = 6.4 ± 0.081) was observed. Whereas the more polar **8c** shows a slight improvement *in vitro* kinase activity of IC_{50} = 9 nM (pIC_{50} = 8.0 ± 0.191), yet a 7-fold decrease in parasite growth inhibition was observed **8c,** EC_{50} = 1339 nM (pEC_{50} = 5.9 ± 0.118) (Table 1). To further investigate the polarity of this moiety we replaced the *N*-diethyl functionality with a more polar primary amine ($logD_{7.4}$ = 0.61). Analogue **12** incorporating a primary amine gave an IC_{50} = 76 nM (pIC_{50} = 7.1 ± 0.142), thus was 2-fold less potent *in vitro* and showed a dramatic loss of efficacy EC_{50} = 2801 nM (pEC_{50} = 5.6 ± 0.104) in parasites, indicating the need to decrease the polarity of the amine group for optimal parasite growth inhibition (Table 1). Analogue **15**, with the alkyl amine group removed ($logD_{7.4}$ = 2.45) was less potent *in vitro*, IC_{50} = 79.0 nM (pIC_{50} = 7.1 ± 0.132) against recombinant *Pf*CLK3 protein and in parasites, EC_{50} = 1456 nM (pEC_{50} = 5.8 ± 0.152). There was a 7-fold drop in the parasite growth inhibition, suggesting that the alkyl amine on ring A is important for antimalarial activity (Table 1).

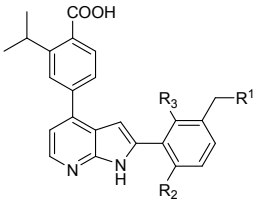


Table 1. Physicochemical properties and activity data of TCMDC-135051 ring A analogues.

Analogue	R^1	R^2	R^3	*Pf*CLK3		3D7		$logD_{7.4}$[c]	Clint (mL/min/g liver)[d]
				IC_{50} (nM)[a]	pIC_{50}	EC_{50} (nM)[b]	pEC_{50}		
1	NEt_2	OMe	H	40	7.4 (±0.221)	180	6.7 (±0.126)	0.93	1.12
8a	NMe_2	OMe	H	29	7.5 (±0.224)	457	6.3 (±0.129)	0.85	2.53
8b	N-pyrrolidinyl	OMe	H	38	7.4 (±0.113)	382	6.4 (±0.081)	2.43	1.94
8c	N-morpholinyl	OMe	H	9	8.0 (±0.191)	1339	5.9 (±0.118)	1.20	1.60
12	NH_2	OMe	H	76	7.1 (±0.142)	2801	5.6 (±0.104)	0.61	2.92
15	H	OMe	H	79	7.1 (±0.132)	1456	5.8 (±0.152)	2.45	2.54
19	NEt_2	OH	H	22	7.7 (±0.115)	3529	5.5 (±0.133)	0.59	1.94
23	NEt_2	H	H	25	7.6 (±0.089)	309	6.5 (±0.114)	0.80	0.85
27	NEt_2	H	OMe	17	7.7 (±0.116)	3167	5.6 (±0.109)	0.74	1.65

[a]IC_{50} (the concentration of an inhibitor where the response is reduced by half); [b]EC_{50} (the concentration of a drug that gives half-maximal response). IC_{50} and EC_{50} values are means ± standard error of the mean (S.E.M) of three independent experiments run in triplicates (n=3); [c]$LogD_{7.4}$ (Distribution co-efficient) was estimated using HPLC chromatography; [d]*In vitro* intrinsic clearance in mouse liver microsomes.

To explore the importance of the methoxy group (OMe) on ring-A, two analogues, hydroxyl **19** and **23**, with methoxy removed**,** were prepared. We first replaced the OMe group with a hydroxyl to investigate the role of polarity on activity ($logD_{7.4}$ = 0.59). Next, we replaced the OMe group with hydrogen (**23)** to investigate the importance of this functionality for activity. For both compounds **19** and **23** *in vitro* kinase potency was comparable IC_{50} = 22 nM (pIC_{50} = 7.7 ± 0.115) and IC_{50} = 25 nM (pIC_{50} = 7.6 ± 0.089) respectively (Table 1). When tested in parasites **19** shows significant loss of activity EC_{50} = 3529 nM (pEC_{50} = 5.5 ± 0.133).

Interestingly, replacing the OMe group with a hydrogen, **23** also shows 1.5-fold decrease in potency, EC_{50} = 309 nM (pEC_{50} = 6.5 ± 0.114) against parasites. These data demonstrated that the OMe group on ring A is required for *in vitro* kinase and parasite growth inhibition. Overall, analogues (**8a-c**, **12**, **15**, **19** and **23**) showed good metabolic stability in mouse liver microsomes (Table 1).

Next, we turned our attention to study the SAR of various functional groups on ring-B. First, we examined the role of binding through Van der Waals interactions *in vitro* and lipophilicity in parasites, imparted by the ring-B isopropyl group, on antimalarial activity. In analogue **28** ($logD_{7.4}$ = 0.66) the isopropyl group was replaced with methyl, whereas in analogue **29** ($logD_{7.4}$ = 0.65) the isopropyl was removed and replaced with hydrogen. Analogues **28** and **29** had a modest increase on *in vitro* kinase activity IC_{50} = 24 nM (pIC_{50} = 7.6 ± 0.10) and IC_{50} = 34 nM (pIC_{50} = 7.5 ± 0.089) respectively. However, a dramatic loss of potency was observed for both compounds **28** and **29** with EC_{50} = 1185 nM (pEC_{50} = 5.6 ± 0.097) and EC_{50} = 3272 nM (pEC_{50} = 5.5 ± 0.124) respectively, representing a 6-fold and 16-fold decrease in parasite growth inhibition (Table 2). Therefore, this group is not required for binding *in vitro*, however the effect of lipophilicity appears to be very important for parasite growth inhibition. Analogues **28** and **29**, were then evaluated for intrinsic clearance in mouse microsome. **28** (Cl_{int} = 1.33 mL/min/g liver) showed a similar clearance value to that of **1** (TCMDC-051) in mouse liver microsomes whereas **29** had a higher clearance under the same conditions (Cl_{int} = 11.01 mL/min/g liver) indicating the importance of isopropyl group.

At this point we decided to increase the lipophilicity and replace the isopropyl group to the larger *tert*-butyl group. However due to a restriction of the chemistry of boronate esters (instability of *tert*-butyl anion required for S_NAr reaction) we could not synthesize the proposed molecule. From these data, the alkyl substituent on ring-B appears not to be particularly important in the molecular recognition event with *Pf*CLK3, however is key for overall molecular lipophilicity that contributes to parasite growth inhibition.

To investigate the requirements (i.e. ionic and H-bonding) of the carboxylic acid (ring B) for antimalarial activity we employed a series of structural changes. The presence of a carboxylic acid functionality in a drug molecule has several potential drawbacks including limited permeability across biological membranes, metabolic instability, and potential idiosyncratic toxicities. A common isostere of a carboxylic acid, that overcomes many of these physicochemical limitations, is a tetrazole. We therefore designed tetrazole analogue **30**. This change was orchestrated to increase the lipophilicity of the molecule, retain H-bonding capability and investigate the role of potential ionic interactions with the enzyme. The tetrazole analogue **30** shows improved *in vitro* kinase potency with an IC_{50} = 19 nM (pIC_{50} = 7.7 ± 0.089), with comparable parasite growth inhibition, EC_{50} = 270 nM (pEC_{50} = 6.6 ± 0.158) (Table 2). The potency of analogue **30** was tested against resistant mutant *Pf*CLK3 (*Pf*CLK3_G449P) 3D7 parasites.[13] Against the mutant parasites (*Pf*CLK3_G449P) parasite growth inhibition of the analogue **30,** EC_{50} = 3494 nM (pEC_{50} = 5.5 ± 0.09) was 2-fold lower compared to **1** EC_{50} = 1806 nM (pEC_{50} = 5.7 ± 0.07).

Analogue **31** where the carboxylic acid group was replaced with a hydrogen shows significant loss of *in vitro* kinase activity IC_{50} = 1300 nM (pIC_{50} = 6.0 ± 0.091). Analogue **31** has high lipophilicity ($logD_{7.4}$ = 4.45) and *in vitro* high intrinsic clearance (Cl_{int} = 9.54 mL/min/g liver) demonstrating the importance of the carboxylic acid group (Table 2).

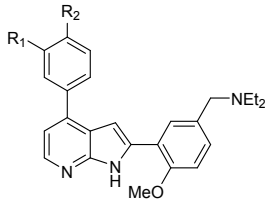


Table 2. Physicochemical properties and activity data of TCMDC-135051 ring B analogues.

Analogue	R^1	R^2	*Pf*CLK3		3D7		$logD_{7.4}$[c]	Cl_{int} (mL/min/g liver)[d]
			IC_{50} (nM)[a]	pIC_{50}	IC_{50} (nM)[b]	pIC_{50}		
28	CH_3	CO_2H	24	7.6 (±0.10)	1185	5.6 (±0.097)	0.66	1.33
29	H	CO_2H	34	7.5 (±0.089)	3272	5.5 (±0.124)	0.65	11.01
30	$CH(CH_3)_2$	1*H*-tetrazole	19	7.7 (±0.089)	270	6.6 (±0.158)	0.93	2.32
31	$CH(CH_3)_2$	H	1300	6.0 (±0.091)	ND[e]	ND[e]	4.45	9.54
9	$CH(CH_3)_2$	$CO_2CH_2CH_3$	390	6.4 (±0.087)	ND[e]	ND[e]	2.45	16.06
32	CO_2H	$CH(CH_3)_2$	1385	4.9 (±0.093)	ND[e]	ND[e]	ND[e]	ND[e]

[a]IC_{50} (the concentration of an inhibitor where the response is reduced by half); [b]EC_{50} (the concentration of a drug that gives half-maximal response). IC_{50} and EC_{50} values are means ± standard error of the mean (S.E.M) of three independent experiments run in triplicates (n=3); [c]$LogD_{7.4}$ (Distribution co-efficient) was estimated using HPLC chromatography; [d]*In vitro* intrinsic clearance in mouse liver microsomes; [e]ND, not determined

Analogue **9** has improved lipophilicity ($logD_{7.4}$ = 2.45) yet the *in vitro* kinase potency was low IC_{50} = 390 nM (pIC_{50} = 6.4 ± 0.087), indicating the presence of a functional group capable of donating a hydrogen bond is important for activity (Table 2). This analogue was not tested in parasites because of the low potency against recombinant *Pf*CLK3 kinase.

The final part of our SAR assessment was dedicated to exploring the possibility of orientation of key substituents. Varying the position of the OMe group from para to the ortho-position **27** resulted in 2-fold increase *in vitro* kinase activity IC_{50} = 17 nM (pIC_{50} = 7.7 ± 0.116). Whereas when **27** was tested in parasites the activity was lowered by 15-fold EC_{50} = 3167 nM (pEC_{50} = 5.6 ± 0.109) (Table 1).

We next investigated the position of the isopropyl and carboxylic acid substituents of ring B **32** on antimalarial activity. The change of orientation of the substituent is detrimental for the *in vitro* kinase activity IC_{50} = 1385nM (pIC_{50} = 4.9 ± 0.093) and therefore was not tested in parasites. These data suggested that the positioning of the isopropyl and carboxylic acid group was essential for binding to its cellular target.

No X-ray crystal structure has been reported for *Pf*CLK3 and so a homology model of *Pf*CLK3 was created using SWISS-MODEL to provide evidence of our proposed binding mechanism (i.e. hinge binder), which we hypothesise based on other literature examples of 7-azaindole scaffolds binding at the hinge region of the kinase domain. The structure of human PRPF4B kinase domain was selected as the template as the closest homologue (PDB 6CNH)(sequence identity 53.2%)).[30] Model accuracy was determined to be reasonable using SWISS-MODEL with QMEAN (Qualitative Model Energy Analysis) score = -2.26 and GMQE (Global

Model Quality Estimation) = 0.77. Overlay with Human Jnk1alpha kinase with 4-phenyl-7-azaindole IKK2 inhibitor bound (PDB 4AWI) facilitated identification of the proposed binding pocket.[31] Based on this model we propose that the 7-aza-indole scaffold interacts with the hinge region in the flipped conformation, H-bonding to the peptide backbone of the hinge region. The benzoic acid on ring B appears to occupy the ribose pocket and interacts with Lys-394. In the model, the ring-B iso-propyl group occupies a hydrophobic back pocket. Ring A projects into the solvent exposed space. The diethyl-amine and methoxy substituents are solvent exposed and may contribute to orientating the 7-azaindole in the flipped binding conformation.

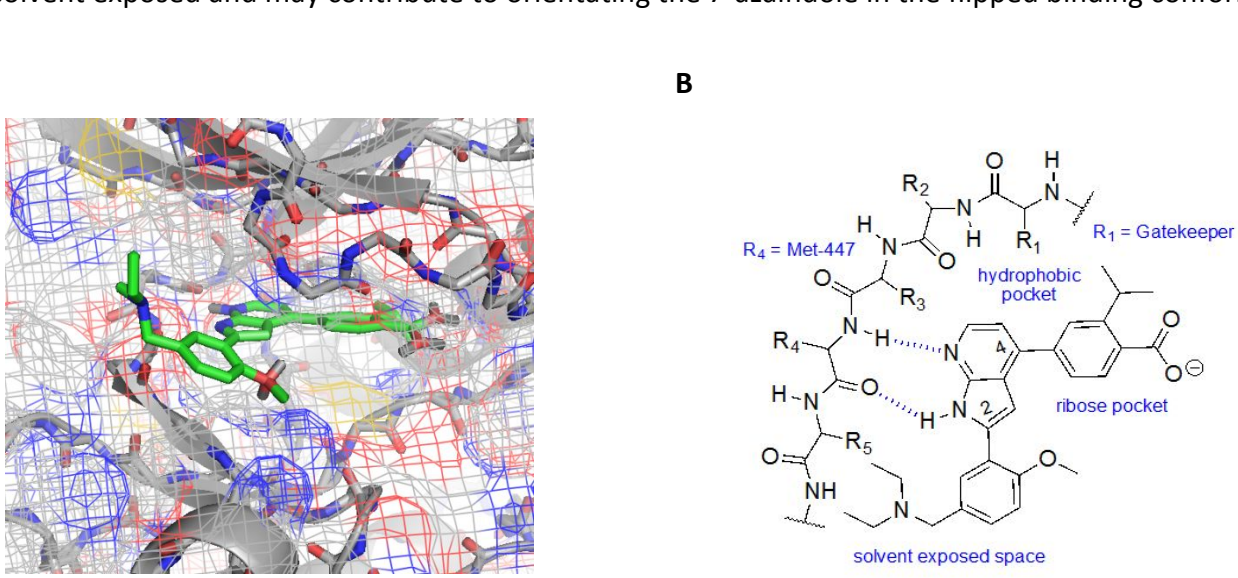


Figure 3. Putative binding mode of TCMDC-135051 **1** in a *Pf*CLK3 homology model.

We previously demonstrated that TCMDC-135051 showed selective inhibition of *Pf*CLK3 when compared against the closely related human kinases PRPF4B and CLK2, as well as the closest parasite kinase, *Pf*CLK1, and other parasite kinases (*Pf*PKG, *Pf*CDPK1).[13] To further assess kinase selectivity, and the potential for off target toxicity, TCMDC-135051 was screened against 140 human kinases at 1 µM concentration. Only 9 kinases were found to have less than 20% activity at this concentration (See Supporting Information).

TCMDC-135051 inhibits the growth of clinical field isolates

An important property for next generation anti-malarials is effectiveness against parasites that are resistant to currently used anti-malarials. To determine if the parent molecule TCMDC-135051 **1** might offer such activity we tested the efficacy of this compound against parasites that were collected from malaria patients in The Gambia. These parasite strains were sequenced and those that contained genetic markers of resistance were selected (Table 3). Parasites were then tested for resistance to pyrimethamine, a frontline antimalarial where resistance has been documented in parasites originating from The Gambia.[32]

This analysis identified nine parasite isolates from patients with varying degrees of resistance to pyrimethamine (Figure 4A, B) and which contained mutations in one or more of three genes; *Pf*CRT (*P. falciparum* chloroquine resistant transporter gene), *Pf*MDR1 (*P. falciparum* multidrug resistant gene 1), and *Pf*DHFR (*P. falciparum* dihydrofolate reductase gene). The mutations identified in these genes (Table 3)

have previously been associated with resistance to commonly used anti-malarials including chloroquine and pyrimethamine.[33] An EC_{50} concentration of pyrimethamine was then determined using the laboratory strain parasite 3D7 (EC_{50} = 18 nM). Using this concentration in parasite reduction rate (PRR) assays all isolates showed a reduction in parasitaemia by at least 70% except for three isolates (GB0020, GB002 and GB0026), which showed resistance to pyrimethamine treatment. These three isolates uniquely contained triple mutations in the *Pf*DHFR gene and a single mutation in *Pf*CRT (Table 3). For these isolates, resistance was evident after 24 hours of treatment with pyrimethamine where parasitaemia was only reduced by 45, 64, and 36% respectively (Figure 4A). Resistance to pyrimethamine of these three isolates was still evident after 72 hours of drug treatment (Figure 4A) and in parasites exposed to 10X the EC_{50} (180 nM) of pyrimethamine (Figure 4B).

In contrast, all isolates treated with the EC_{50} of TCMDC-135051, as determine in 3D7 parasites (EC_{50} = 180 nM), demonstrated total susceptibility including isolates GB0020, GB002 and GB0026 (Figure 4C). This was true both at the EC_{50} concentration and 10xEC_{50} for TCMDC-135051 (Figure 4D). Hence in the field isolates tested, TCDMDC-135051 showed equivalent activity against parasites that carried genetic markers of resistance and those that showed actual resistance to a front-line anti-malarial. This indicates the possibility that the parent molecule, and potentially analogues of this molecule, would be active against naturally circulating malaria parasites harbouring mutations that promoted resistance to current anti-malaria drugs. The PRR assay was setup using clinical isolates previously cryopreserved in liquid nitrogen (LN2). These were thawed and put in culture for at least one cycle (~48hour ± 2). The life cycle stage of the parasites was monitored using a blood film and greater than 95 % of the parasites are expected egress and form new invading rings. These new rings, mainly 0-3 hours old post invasion, were used as time point zero hours (t=0) for the assay. The parasites are then cultured with drugs for 24, 48 and 72 hours. For the 24 hours treatment, the drug was removed by washing twice with wash media and for longer treatments (48 and 72 hour time points) drug was replenished with fresh drug every 24 hours. After the treatment period, parasites were grown in fresh media for an additional 48 hours in freshly stained erythrocytes to allowed invasion from any viable parasites.

Table 3: Genotypes of field isolated parasite strains: Whole genome Sanger sequencing identified mutations in three parasite genes (Cholorquine resistant transporter (CRT), multi-drug resistant gene (*Pf*MDR1) and dihydrofolate reductase (*Pf*DHFR) associated with drug resistance. Shown are the amino acid changes associated with each of these three genes and whether the parasite strain contained the mutation.

	***Pf*CRT**	***Pf*MDR1**			***Pf*DHFR**			
Sample ID	K76T	N86Y	Y184F	N1042D	N51I	C59R	S108N	I164L
GB0006	X	X	X	N	X	X	N	L
GB0004	K	N	Y	N	N	R	N	L
GB0026	K	N	X	N	N	R	N	L
GB002	T	N	X	N	I	C	N	L
GB0021	K	N	F	N	X	X	N	L
GB0048	X	N	F	N	I	C	N	L

GB0071	X	N	Y	N	N	C	N	L
GB0087	T	N	Y	N	I	C	N	L
GB0020	T	Y	Y	N	I	C	N	L

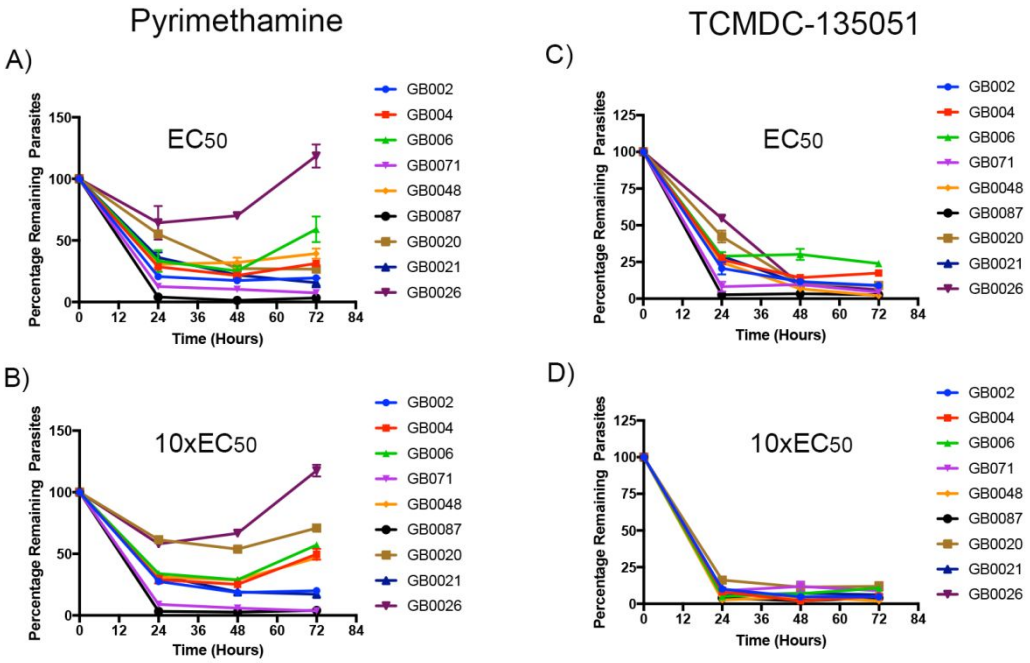


Figure 4: Parasite Reduction Rate of clinical isolates comparing pyrimethamine and TCMDC-135051 at EC_{50} and 10 times the EC_{50} of each drug. This represent the average of triplicate

Conclusions

In summary, we report the synthesis of hit *Pf*CLK3 inhibitor TCMDC-135051 **1** (*Pf*CLK3 IC_{50} = 40 nM, 3D7 EC_{50} = 180 nM) and a series of related 7-azaindole-based analogues. Of the 14 analogues, 11 had low nanomolar activity and were further assessed in a live parasite viability assays using the 3D7 (chloroquine sensitive) strain of *P. falciparum*. Tetrazole analogue **30** was identified with improved *in vitro* kinase activity (*Pf*CLK3, IC_{50} = 19 nM) and comparable activity in parasites 3D7 (EC_{50} = 271 nM). SAR was established for both ring A, highlighting the importance of H-bonding functionality in the 4-aryl position and for the alkyl amino group on ring B. Together these data provide a good starting point for the hit to lead development of novel *Pf*CLK3 inhibitors based on TCMDC-135051 (**1**).

Experimental Section

General information

Chemicals and solvents were purchased from standard suppliers and used without additional purification. All glassware was dried with a flame under flushing argon gas or stored in the oven and let cool under an inert atmosphere prior to use. Anhydrous solvents (THF, DCM and Et_2O) were obtained by passage through

solvent filtration systems (Pure Solv) and solvents were transferred by syringe. PET ether refers to petroleum (bp. 40-60 °C, reagent grade, Fisher Scientific). All reactions carried out under inert or dry atmosphere were carried out under a blanket of nitrogen. Thin-layer chromatography (TLC) was performed using aluminium plates precoated with silica gel (0.25 mm, 60 A° pore-size) impregnated with a fluorescent indicator (254 nm). Visualization on TLC was achieved by the use of UV light (254 nm). Flash column chromatography was undertaken on silica gel (400-630 mesh). Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on AVANCE III 400 Bruker (400 MHz). Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CDCl_3$, δ 7.26; CD_3OD, δ 3.31 and DMSO-d_6, δ 2.50). The following abbreviations were used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quadruplet, sept = septet, m = multiplet. Coupling constants, J, were reported in Hertz unit (Hz). Carbon 13 nuclear magnetic resonance spectroscopy (^{13}C NMR) was recorded on AVANCE III 400 Bruker (101 MHz) and was fully decoupled by broad band decoupling. Chemical shifts were reported in ppm referenced to the centre line of a triplet at 77.0, 49.0, 39.5 ppm of $CDCl_3$, CD_3OD and DMSO-d_6. Low-resolution mass spectrometry (LRMS) was performed on a Thermo Scientific LCQ Fleet quadrupole mass spectrometer using electrospray ionisation in positive mode (ESI^+), employing a 150 mm x 4 mm C18 column (Dr. Maisch Reprosil Gold). High-resolution mass spectrometry (HRMS) was performed on a Bruker microTOF-Q II (ESI^+). Preparative HPLC was carried out on a Dionex HPLC system equipped with Dionex P680 pumps and a Dionex UVD170U UV-vis detector (monitoring at 214 nm and 280 nm), using a Phenomenex, Gemini, C18, 5 µm, 250 x 21.2 mm column. Gradients were performed using solvents consisting of A (H_2O + 0.1% TFA) and B (CH_3CN + 0.1% TFA) and fractions were lyophilised on a Christ Alpha 2-4 LO plus freeze dryer. Final molecules were analysed on a Shimadzu reverse-phase HPLC (RP-HPLC) system equipped with Shimadzu LC-20AT pumps, a SIL-20A autosampler and a SPD-20A UV-vis detector (monitoring at 214 nm) using a Phenomenex, Aeris, 5 µm, peptide XB-C18, 150 x 4.6 mm column at a flow rate of 1 mL/min. RP-HPLC gradients were run using a solvent system consisting of solution A (5% CH_3CN in H_2O + 0.1% TFA) and B (5% H_2O in CH_3CN + 0.1% TFA). A gradient from 0% to 100% solution B over 20 min 0%-100% solution B. Purity of all final compounds is > 95% as determined by RP-HPLC.

Experimental procedures and characterisation data:

4-Bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridine (3)

To a solution of sodium hydride (1.83 g, 76.1 mmol, 3 equiv.) and tetrabutylammonium bromide (0.25 g, 0.76 mmol, 0.03 equiv.) in dichloromethane (80 mL) at 0 °C was added 4-bromo-1*H*-pyrrolo[2,3-*b*]pyridine, **2** (5 g, 25.4 mmol, 1 equiv.), the mixture was then left to stir at 0 °C for 15 mins. Toluene sulphonylchloride (5.81 g, 30.5 mmol, 1.2 equiv.) in dichloromethane (20 mL) was slowly added over 5 mins. The mixture was then left to warm up to room temperature and stirred for 1 hour. The reaction was quenched by addition

of water and extracted with dichloromethane. The organic layer was washed with brine and dried over magnesium sulphate. The residue was then purified by flash column chromatography (10% ethyl acetate-PET Ether) to give **3** as a colourless solid (8.83 g, 99%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (d, *J* = 5.2 Hz, 1H), 8.06 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 4.0 Hz, 1H), 7.35 (d, *J* = 5.3 Hz, 1H), 7.28 (d, *J* = 8.0 Hz, 2H), 6.64 (d, *J* = 4.0 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 146.8, 145.5, 145.0, 135.1, 129.7, 128.2, 127.0, 125.7, 124.4, 122.1, 104.9, 21.7; HRMS *m/z calcd for* $C_{14}H_{11}BrN_2NaO_2S$ $[M+Na]^+$ 372.9617 *found* 372.9608 (Δ = 2.3 ppm).

4-Bromo-2-iodo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridine (4)

n-Butyllithium (2.5 M; 6.3 mL, 15.6 mmol, 1.1 equiv.) was added dropwise to diisopropylamine (2.4 mL, 17.2 mmol, 1.2 equiv.) in diethyl ether (30 mL) at -78 °C over a period of 5 mins. The resulting solution was stirred at -78 °C for 60 mins and then slowly added *via* cannula to a solution of 4-bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridine, **3** (5.0 g, 14.2 mmol, 1 equiv.) and tetramethylethylenediamine (2.3 mL, 15.7 mmol, 1.1 equiv.) in diethyl ether (170 mL) over a period of 10 mins at -78 °C. The resulting solution was then stirred at -78 °C for 90 mins. Iodine (5.4 g, 21.4 mmol, 1.5 equiv.) was added in one portion, and the reaction mixture was stirred at -78 °C for 60 mins. The reaction was quenched with saturated ammonium chloride solution and the organic layer was washed with aqueous sodium thiosulphate and brine before drying over magnesium sulphate. The residue was then purified by column chromatography (20% ethyl acetate-PET ether) to give **4** as a colourless solid (5.59 g, 85 %; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.11 (d, *J* = 5.2 Hz, 1H), 8.01 (d, *J* = 8.5 Hz, 2H), 7.23 (d, *J* = 5.2 Hz, 1H), 7.22-7.19 (m, 2H), 6.96 (s, 1H), 2.30 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 149.1, 145.7, 144.7, 135.4, 129.8, 128.3, 125.3, 123.6, 122.4, 119.4, 21.7; HRMS *m/z calcd for* $C_{14}H_{10}BrIN_2NaO_2S$ $[M+Na]^+$ 498.8583 *found* 498.8602 (Δ = -3.8 ppm).

Method A: General Method of Suzuki Cross-Coupling

To a solution of desired aryl bromide (1.0 equiv.) and tetrakis(triphenylphosphine)palladium(0) (0.05 equiv.) in 1,4-dioxane was added boronic acid/ester (1.1 equiv.) under a nitrogen atmosphere. Aqueous sodium carbonate (2 M, 7.0 equiv.) was then added and the reaction mixture left to stir at 110 °C for 18 hrs. Solvent was removed under vacuum and the crude was dissolved in ethyl acetate and poured into water and extracted with ethyl acetate. The organic layer was washed with brine before drying over magnesium sulphate and purified by flash column chromatography as indicated.

Method B: Reductive Amination of Aldehydes

To a solution of aryl aldehyde (1.0 equiv.) in 1,4-dioxane was added amine (1.5 equiv.) and the solution was allowed to stir for 2 mins before the addition of sodium triacetoxyborohydride (2.5 equiv.). The reaction mixture was stirred at room temperature for 18 h before quenching with ammonium hydroxide.

The reaction mixture was extracted with ethyl acetate and washed with brine. The organic layer was dried over magnesium sulphate and the residue purified by flash column chromatography as indicated.

Method C: Deprotection of azaindole

To a solution of protected 7-azaindole (1 equiv.) in methanol was added potassium carbonate (3.5 equiv.) and refluxed for 18 h. Poured the reaction into a mixture of EtOAc (10 mL) and H_2O in a separatory funnel. Solvent was then removed under vacuum and the residue was then purified by flash column chromatography as indicated.

Method D: Suzuki Cross-Coupling with boronate ester

To a 10 mL microwave vial containing the required bromo-7-azaindole (1 equiv.) in 1,4- dioxane was added boronic acid/ester (1.1 equiv.), Pd(dppf)Cl_2·DCM complex (0.05 equiv.) under a nitrogen atmosphere. The solution was purged with nitrogen for 5 mins and the reaction microwaved at 110 °C for 0.5 h. The reaction was allowed to cool to room temperature and the mixture was filtered through celite eluting with methanol. The filtrate was evaporated and the resulting residue was purified by preparative HPLC: 10-95% acetonitrile in water + 0.1% TFA to give the desired products.

Method E: Synthesis of boronate ester

Boronate esters required for Suzuki coupling were prepared according to procedure reported in literature.[35] To a solution of aryl bromide (1 equiv.), bis(pinacolato)diboron (1.5 equiv.) and potassium acetate (3 equiv.) in 1,4-dioxane (20 ml), $PdCl_2$(dppf).CH_2Cl_2 complex (0.1 equiv.) were added under nitrogen and stirred at 100 °C for 3 hour. The reaction mixture was quenched with saturated $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness. The crude product was purified by chromatography (20% ethyl acetate-PET Ether) to give the desired boronate esters.

3-[4-Bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl]-4-methoxybenzaldehyde (5)

Prepared according to method A. Purification by flash chromatography (50% ethyl acetate-PET ether) afforded **5** as a yellow oil (1.72 g, 70%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.90 (s, 1H), 8.15 (d, *J* = 5.2 Hz, 1H), 7.95 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.84 (d, *J* = 2.1 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.28 (d, *J* = 5.3 Hz, 1H), 7.13 (d, *J* = 8.2 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.52 (s, 1H), 3.85 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 190.4, 163.5, 148.7, 145.1, 144.8, 137.6, 135.8, 134.4, 131.4, 129.4, 129.4, 128.1, 125.1, 123.3, 123.1, 122.3, 110.6, 107.9, 56.1, 21.6; HRMS m/z *calcd for* $C_{22}H_{17}BrN_2NaO_4S$ $[M+Na]^+$ 506.9985 *found* 506.9977 (Δ = 1.5 ppm).

***N*-(3-(4-bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)-4-methoxybenzyl)-*N*-ethylethanamine (6a)**

Prepared according to method B. Purification by flash chromatography (5% methanol-dichloromethane) afforded **6a** as a yellow oil (920 mg, 72%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.15 (d, *J* = 5.3 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.65 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.62 (d, *J* = 2.3 Hz, 1H), 7.47 (d, *J* = 5.3 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.65 (s, 1H), 4.45-4.35 (m, 2H), 3.81 (s, 3H), 3.31-3.23 (m, 4H), 2.36 (s, 3H), 1.38 (t, *J* = 7.3 Hz, 7H); ^{13}C NMR (101 MHz, CD_3OD) δ: 159.3, 148.4, 145.7, 144.3, 138.2, 135.5, 133.7, 133.6, 129.1, 127.6, 124.7, 123.3, 122.3, 122.3, 120.8, 111.1, 107.6, 55.2, 54.9, 20.1; HRMS *m/z calcd* for $C_{26}H_{28}BrN_3O_3S$ $[M+H]^+$ 541.1035 *found* 542.1093 (Δ = 2.7 ppm).

1-({3-[4-Bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl]-4-methoxyphenyl}methyl)-*N*,*N*-dimethylamine (6b)

Prepared according to method B. Purification by flash chromatography (5% methanol-dichloromethane) afforded **6b** as a yellow oil (225 mg, 70%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.17 (d, *J* = 5.3 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.65 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.61 (d, *J* = 2.3 Hz, 1H), 7.50 (d, *J* = 5.3 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.66 (s, 1H), 4.27 (d, *J* = 14.4 Hz, 2H), 3.83 (s, 3H), 2.84 (s, 7H), 2.38 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ: 156.2, 147.9, 142.1, 137.0, 131.0, 129.6, 129.5, 124.1, 122.6, 119.3, 118.8, 111.5, 98.8, 62.6, 55.0, 43.6; HRMS m/z *calcd for* $C_{24}H_{25}BrN_3O_3S$ $[M+H]^+$ 514.0795 *found* 514.0790 (Δ = 0.9 ppm).

1-({3-[4-Bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl]-4 methoxyphenyl}methyl)pyrrolidine (6c)

Prepared according to method B. Purification by flash chromatography (5% methanol-dichloromethane) afforded **6c** as a yellow oil (330 mg, 97%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.13 (d, *J* = 5.2 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.65-7.67 (m, 2H), 7.42 (d, *J* = 5.3 Hz, 1H), 7.28 (d, *J* = 8.3 Hz, 2H), 7.15 (d, *J* = 9.1 Hz, 1H), 6.63 (s, 1H), 4.44-4.29 (m, 1H), 3.78 (s, 3H), 2.32 (s, 3H), 2.10-2.06 (p, 4H), 1.95 (s, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ: 176.8, 159.1, 148.4, 145.6, 144.3, 138.6, 135.6, 129.2, 127.6, 124.6, 123.3, 122.8, 122.3, 122.1, 110.9, 107.5, 56.9, 54.9, 52.8, 22.5, 21.5, 20.2; HRMS *m/z calcd for* $C_{26}H_{27}BrN_3O_3S$ $[M+H]^+$ 540.0951 *found* 540.0934 (Δ; = 3.2 ppm).

4-({3-[4-Bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl]-4-methoxyphenyl}methyl)morpholine (6d)

Prepared according to method B. Purification by flash chromatography (5% methanol-dichloromethane) afforded **6d** as a yellow oil (270 mg, 80%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.03 (m, 3H), 7.71 (d, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.31 (d, *J* = 4.8 Hz, 1H), 7.23-7.18 (m, 3H), 6.96 (s, 1H), 4.36 (s, 2H), 4.04 (s, 3H), 3.34 (t, *J* = 6.2 Hz, 4H), 2.08 (t, *J* = 6.2 Hz, 4H), 1.99 (s, 3H); ^{13}C NMR (101 MHz, CD_3OD) δ: 157.5, 147.9, 142.5, 140.4, 136.2, 131.8, 130.4, 128.5, 125.6, 124.3, 123.6, 122.4, 120.1, 118.9, 112.2, 99.4, 62.9, 57.2, 55.2, 53.2, 22.5; HRMS m/z *calcd for* $C_{26}H_{27}BrN_3O_4S$ $[M+H]^+$ 556.0900 *found* 556.0902 (Δ = -0.2 ppm).

***N*-(3-(4-bromo-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)-4-methoxybenzyl)-*N*-ethylethanamine (7a)**

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **7a** as a colourless oil (560 mg, 79%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.00 (d, *J* = 5.3 Hz, 1H), 7.83 (d, *J* = 2.3 Hz, 1H), 7.36 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.29 (d, *J* = 5.3 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.91 (s, 1H), 4.00 (s, 3H), 3.75 (s, 2H), 3.31 (p, *J* = 1.6 Hz, 1H), 2.71 (q, *J* = 7.2 Hz, 4H), 1.15 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 156.3, 147.9, 142.1, 136.9, 131.2, 129.7, 124.11, 122.5, 119.4, 118.8, 111.6, 98.8, 56.1, 54.9, 9.4; HRMS *m/z calcd* for $C_{19}H_{22}BrN_3O$ $[M+H]^+$ 387.0946 *found* 388.1008 (Δ = 2.7 ppm).

1-[(3-{4-Bromo-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl}-4-methoxyphenyl)methyl]dimethylamine (7b)

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **7b** as a colourless oil (280 mg, 92%); ^{1}H NMR (400 MHz, CD_3OD) δ: 7.91 (d, *J* = 5.3 Hz, 1H), 7.70 (d, *J* = 2.2 Hz, 1H), 7.23 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.20 (d, *J* = 5.3 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.82 (s, 1H), 3.91 (s, 3H), 3.44 (s, 2H), 2.21 (s, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 156.3, 147.9, 142.1, 137.0, 131.0, 129.7, 129.5, 124.1, 122.6, 119.3, 118.8, 111.5, 98.8, 62.6, 54.9, 43.6; HRMS m/z *calcd for* $C_{17}H_{19}BrN_3OS$ $[M+H]^+$ 360.0706 *found* 360.0701 (Δ = 1.4 ppm).

1-[(3-{4-Bromo-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl}-4-methoxyphenyl)methyl]pyrrolidine (7c)

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **7c** as a yellow oil (165 mg, 77%); ^{1}H NMR (400 MHz, CD_3OD) δ: 7.88 (d, *J* = 5.2 Hz, 1H), 7.84 (d, *J* = 2.3 Hz, 1H), 7.36 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.16 (dd, *J* = 8.5, 3.4 Hz, 1H), 7.04 (d, *J* = 8.6 Hz, 1H), 6.81 (s, 1H), 4.06 (s, 2H), 3.89 (s, 3H), 3.04 (t, *J* = 6.8 Hz, 4H), 1.91 (p, *J* = 3.2 Hz, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ: 157.0, 148.0, 142.4, 136.5, 131.3, 130.0, 126.4, 124.2, 122.4, 119.9, 118.9, 112.0, 99.1, 58.1, 55.1, 53.4, 22.6; HRMS m/z *calcd for* $C_{19}H_{21}BrN_3O$ $[M+H]^+$ 386.0900 *found* 386.0903 (Δ = 0.8 ppm).

4-[(3-{4-Bromo-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl}-4-methoxyphenyl)methyl]morpholine (7d)

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **7d** as a yellow oil (269 mg, 80%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.18 (s, 1H), 8.08 (d, *J* = 5.1 Hz, 1H), 7.80 (d, *J* = 2.0 Hz, 1H), 7.65-7.60 (m, 2H), 7.58-7.55 (m, 1H), 7.33 (d, *J* = 5.1 Hz, 1H), 7.32 (d, *J* = 2.0 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 6.89 (d, *J* = 2.2 Hz, 1H), 3.94 (s, 3H), 3.58 (t, 4H), 3.47 (s, 2H), 2.38 (t, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 156.2, 149.1, 143.5, 136.8, 132.5, 130.9, 129.7, 129.2, 123.4, 122.1, 119.4, 119.1, 112.4, 100.2, 66.7, 62.3, 56.2, 53.6; HRMS m/z *calcd for* $C_{19}H_{21}BrN_3O_2$ $[M+H]^+$ 402.0812 *found* 402.0810 (Δ = 0.5 ppm).

4-(2-(5-((diethylamino)methyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoic acid, 1

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **1** in a (85 mg, 68%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.39 (d, *J* = 5.7 Hz, 1H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.97 (d, *J* = 8.0 Hz,

1H), 7.91 (d, *J* = 1.8 Hz, 1H), 7.74 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.58 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.52 (d, *J* = 5.8 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.31 (s, 1H), 4.37 (s, 2H), 4.07 (s, 3H), 3.94 (p, *J* = 6.9 Hz, 1H), 3.25 (dq, *J* = 14.2, 7.1 Hz, 4H), 1.39-1.34 (m, 12H); ^{13}C NMR (101 MHz, CD_3OD) δ: 169.8, 157.9, 150.1, 145.6, 143.9, 140.0, 137.8, 136.8, 132.9, 131.8, 131.0, 130.3, 126.4, 125.6, 122.1, 121.8, 119.6, 114.9, 112.5, 100.3, 55.3, 55.2, 29.4, 23.0, 7.6; HRMS *m/z calcd* for $C_{29}H_{33}N_3O_3$ $[M+H]^+$ 471.2522 *found* 472.2576 (Δ = 2.1 ppm).

4-(2-{5-[(Dimethylamino)methyl]-2-methoxyphenyl}-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-(propan-2-yl)benzoic acid (8a)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **8a** in a (11 mg, 29%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.27 (d, *J* = 5.4 Hz, 1H), 7.89 (d, *J* = 2.3 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.79 (d, *J* = 1.7 Hz, 1H), 7.63 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.44 (dd, *J* = 8.6, 2.3 Hz, 2H), 7.32 (d, *J* = 5.5 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.13 (s, 1H), 4.23 (s, 2H), 3.97 (s, 3H), 3.89-3.81 (m, 1H), 2.79 (s, 6H), 1.27 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 169.9, 158.1, 150.2, 146.7, 142.6, 139.6, 138.4, 135.1, 133.2, 132.2, 131.1, 130.4, 126.5, 125.7, 122.3, 120.4, 119.2, 115.0, 112.5, 100.5, 60.1, 55.3, 41.4, 29.5, 23.0; HRMS m/z *calcd for* $C_{27}H_{29}N_3O_3$ $[M+H]^+$ 470.2438 *found* 470.2433 (Δ = 1.1 ppm).

4-(2-{2-Methoxy-5-[(pyrrolidin-1-yl)methyl]phenyl}-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-(propan-2-yl)benzoic acid (8b)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **8b** in a (27 mg, 37%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.27 (br s, 1H), 7.90 (s, 1H), 7.86 (d, *J* = 8.0 Hz, 1H), 7.79 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 7.36 (s, 1H), 7.21-7.15 (m, 2H), 4.29 (s, 2H), 3.96 (s, 3H), 3.85-3.81 (m, 1H), 3.42 (br. s., 2H), 3.14 (br s, 2H), 2.08 (br. s., 2H), 1.93 (br. s., 2H), 1.26 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 169.9, 157.9, 150.1, 144.5, 140.5, 137.4, 132.1, 131.5, 130.4, 130.3, 126.3, 125.5, 123.4, 119.9, 112.4, 100.0, 57.2, 55.2, 53.3, 29.4, 23.0, 22.4; HRMS m/z *calcd for* $C_{29}H_{32}N_3O_3$ $[M+H]^+$ 470.2438 *found* 470.2433 (Δ = 1.1 ppm).

4-(2-{2-Methoxy-5-[(morpholin-4-yl)methyl]phenyl}-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-(propan-2-yl)benzoic acid (8c)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **8c** in a (34 mg, 60%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.43 (d, *J* = 5.8 Hz, 1H), 8.04 (d, *J* = 2.3 Hz, 1H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.93 (d, *J* = 1.8 Hz, 1H), 7.78 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.61 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.56 (d, *J* = 5.7 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.32 (s, 1H), 4.41 (s, 2H), 4.10 (s, 3H), 4.07 (s, 2H), 3.98-3.93 (m, 1H), 3.76 (s, 2H), 3.44 (s, 2H), 3.26 (s, 2H), 1.39 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz DMSO-d_6) δ: 170.3, 169.7, 149.6, 147.1, 136.0, 133.0, 133.0, 132.2, 132.1, 131.6, 131.3, 130.6, 128.3, 126.5, 125.9, 121.7, 120.6, 112.9, 100.5, 63.8, 59.4, 56.4, 51.2, 49.1, 29.4, 24.4, 24.3; HRMS m/z *calcd for* $C_{29}H_{32}N_3O_4$ $[M+H]^+$ 486.2387 *found* 486.2384 (Δ = 0.8 ppm).

Ethyl 4-(2-(5-((diethylamino)methyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoate (9)

Purification by preparative HPLC 10-95% MeCN/H_2O to give **9** in a (36 mg, 98%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.39 (d, *J* = 5.6 Hz, 1H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.92 (d, *J* = 8.2 Hz, 2H), 7.77-7.72 (m, 1H), 7.57 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.49 (d, *J* = 5.7 Hz, 1H), 7.33 (d, *J* = 8.6 Hz, 1H), 7.28 (s, 1H), 4.42 (q, *J* = 7.1 Hz, 2H), 4.37 (s, 2H), 4.07 (s, 3H), 3.82 (p, *J* = 6.9 Hz, 1H), 3.25 (p, *J* = 7.4 Hz, 4H), 1.50-1.29 (m, 16H); ^{13}C NMR (101 MHz, CD_3OD) δ: 168.1, 157.9, 149.9, 140.7, 138.8, 137.2, 132.6, 131.1, 130.9, 130.1, 126.4, 125.6, 122.0, 120.1, 114.9, 112.5, 99.9, 60.9, 55.3, 29.6, 22.9, 13.2, 7.7; HRMS *m/z calcd* for $C_{31}H_{37}N_3O_3$ $[M+H]^+$ 499.2835 *found* 500.2913 (Δ = 4.1 ppm)

3-[4-Bromo-1-(4-methylbenzenesulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl]-4-methoxybenzonitrile (10)

Prepared according to method B. Purification by flash chromatography (50% ethyl acetate-PET ether) afforded **10** as a yellow oil (586 mg, 87%; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.23 (d, *J* = 5.2 Hz, 1H), 8.03-7.99 (m, 2H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.61 (d, *J* = 5.2 Hz, 1H), 7.41-7.37 (m, 2H), 7.34-7.29 (m, 2H), 6.81 (s, 1H), 3.84 (s, 3H), 2.34 (s, 3H); ^{13}C NMR (101 MHz, DMSO- d_6) δ: 161.8, 148.3, 145.9, 145.7, 143.5, 137.1, 136.4, 135.5, 134.8, 130.2, 128.2, 125.0, 123.0, 119.3, 112.4, 109.5, 108.3, 103.1, 56.6, 21.6; HRMS m/z *calcd for* $C_{22}H_{16}BrN_3NaO_3S$ $[M+Na]^+$ 503.9988 f*ound* 503.9973 (Δ = 3.0 ppm).

3-{4-Bromo-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl}-4-methoxybenzonitrile (11)

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **11** as a yellow oil (327 mg, 59%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.24 (s, 1H), 8.29 (d, *J* = 2.1 Hz, 1H), 8.06 (d, *J* = 5.1 Hz, 1H), 7.81 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.33 – 7.29 (m, 2H), 6.96 (s, 1H), 3.98 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 207.0, 160.3, 149.1, 144.4, 134.3, 132.5, 124.1, 121.1, 119.4, 119.3, 113.7, 103.8, 101.8, 56.9, 31.2; HRMS m/z *calcd for* $C_{15}H_{11}BrN_3O$ $[M+H]^+$ 328.0080 *found* 328.0076 (Δ = 1.1 ppm).

4-{2-[5-(aminomethyl)-2-methoxyphenyl]-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl}-2-(propan-2-yl)benzoic acid (12)

Prepared according to method D. The filtrate residue was dissolved in methanol (10 mL), $CoCl_2{\cdot}6H_2O$ (3 equiv) was then added before cooling to 0 °C. $NaBH_4$ (10 equiv) was slowly added to the reaction and the mixture was allowed to stir at room temperature for a further 1 hour before filtration through celite and purification by preparative HPLC 10-95% MeCN/H_2O to give **13** (26 mg, 60%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.01 (s, 1H), 8.30 (d, *J* = 5.0 Hz, 1H), 8.17 (br. s., 2H), 7.94 (d, *J* = 2.3 Hz, 1H), 7.82- 7.80 (m, 2H), 7.62 (dd, *J* = 8.0, 1.8 Hz, 1H), 7.41 (dd, *J* = 8.6, 2.2 Hz, 1H), 7.24 (d, *J* = 5.0 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.09 (s, 1H), 3.98 (q, *J* = 5.6 Hz, 2H), 3.89 (s, 3H), 3.83- 3.76 (m, 1H), 1.25 (d, *J* = 6.9 Hz, 6H).; ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.7, 157.0, 149.6, 149.2, 142.9, 141.4, 140.4, 136.6, 131.4, 130.6, 130.6, 129.7, 126.7,

126.6, 125.9, 120.1, 119.0, 115.2, 112.7, 100.0, 56.3, 42.4, 29.4, 24.3; HRMS m/z *calcd for* $C_{25}H_{26}N_3O_3$ $[M + H]^+$ 416.1969 *found* 416.1968 (Δ = 0.2 ppm).

4-Bromo-2-(2-methoxyphenyl)-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridine (13)

Prepared according to method A. Purification by flash chromatography (50% ethyl acetate-PET ether) afforded **14** as a colourless oil (960 mg, 32%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 5.3 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.41 (td, *J* = 8.2, 1.7 Hz, 1H), 7.29-7.24 (m, 2H), 7.12 (d, *J* = 8.1 Hz, 2H), 6.99 (t, *J* = 7.5 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 1H), 6.45 (s, 1H), 3.73 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 158.3, 148.7, 144.8, 144.4, 139.4, 136.1, 131.1, 130.9, 129.2, 128.2, 124.8, 123.5, 122.1, 121.8, 120.0, 110.4, 107.3, 55.5, 21.6; HRMS m/z *calcd for* $C_{21}H_{17}BrN_2NaO_3S$ $[M+Na]^+$ 479.0035 *found* 479.0035 (Δ = 0.0 ppm).

4-Bromo-2-(2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridine (14)

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **15** as a colourless oil (300 mg 80%), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.38 (s, 1H), 8.09 (d, *J* = 5.2 Hz, 1H), 7.92 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.41-7.34 (m, 1H), 7.30-7.25 (m, 2H), 7.14-7.07 (m, 2H), 6.89 (s, 1H), 4.04 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 156.1, 148.1, 143.0, 137.0, 129.9, 128.4, 124.3, 122.2, 121.6, 119.4, 119.3, 112.0, 97.8, 55.9; HRMS m/z *calcd for* $C_{14}H_{12}BrN_2O$ $[M+H]^+$ 303.0128 *found* 303.0122 (Δ = 1.8 ppm).

4-[2-(2-Methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl]-2-(propan-2-yl)benzoic acid (15)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **16** in a (30 mg, 59%); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.05 (s, 1H), 8.32 (d, *J* = 5.0 Hz, 1H), 7.92 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.90 (d, *J* = 1.8 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.68 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.38 (ddd, *J* = 8.7, 7.4, 1.7 Hz, 1H), 7.27 (d, *J* = 5.0 Hz, 1H), 7.20-7.18 (m, 2H), 7.08 (td, *J* = 7.6, 1.0 Hz, 1H), 3.92 (s, 3H), 3.90-3.83 (m, 1H), 1.32 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 169.7, 157.1, 149.9, 149.6, 141.7, 136.5, 131.1, 130.7, 129.9, 126.5, 125.9, 121.2, 100.3, 45.4, 29.4; HRMS m/z *calcd for* $C_{24}H_{23}N_2O_3$ $[M+H]^+$ 387.17034 *found* 387.1710 (Δ = -1.7 ppm).

3-(4-Bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)-4-(methoxymethoxy)benzaldehyde (16)

Prepared according to method A. Purification by flash chromatography (50% ethyl acetate-PET ether) afforded **17** as a colourless oil (850 mg, 77%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.92 (s, 1H), 8.16 (d, *J* = 5.3 Hz, 1H), 7.92 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.84 (d, *J* = 2.1 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.29 (d, *J* = 5.3 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 3H), 6.53 (s, 1H), 5.26-5.22 (m, 2H), 3.46 (s, 3H), 2.27 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 190.6, 161.7, 148.6, 145.2, 144.9, 137.7, 135.5, 134.4, 131.0, 130.0, 129.4, 128.0, 125.1, 123.6, 122.3, 113.9, 108.0, 95.1, 56.9, 21.6; HRMS m/z *calcd for* $C_{23}H_{19}BrN_2NaO_5S$ $[M+Na]^+$ 537.0090 *found* 537.0098 (Δ = -1.7 ppm).

({3-[4-bromo-1-(4-methylbenzenesulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl]-4-(methoxymethoxy)phenyl}methyl)diethylamine (17)

Prepared according to method B. Purification by flash chromatography (5% methanol-dichloromethane) afforded **18** as a colourless oil (672 mg, 87%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.16 (d, *J* = 5.3 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 7.9 Hz, 2H), 7.50 (d, *J* = 5.3 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.31 (d, *J* = 8.2 Hz, 2H), 6.68 (s, 1H), 5.18 (br. s., 1H), 5.28 (br. s., 1H), 4.20 (d, *J* = 5.4 Hz, 2H), 3.43 (s, 3H), 3.10 (q, *J* = 7.1 Hz, 4H), 2.37 (s, 3H), 1.33 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 156.7, 148.4, 145.7, 144.3, 138.7, 135.5, 133.1, 132.9, 129.2, 127.6, 124.7, 123.3, 122.8, 122.4, 114.1, 107.5, 94.7, 55.4, 55.4, 46.2, 20.1, 8.4; HRMS m/z *calcd for* $C_{27}H_{31}BrN_3O_4S$ $[M+H]^+$ 572.1213 *found* 572.1208 (Δ = 0.9 ppm).

[(3-{4-Bromo-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl}-4-(methoxymethoxy)phenyl)methyl]diethylamine (18)

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **19** as a yellow oil (420 mg, 69%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.03 (d, *J* = 5.2 Hz, 1H), 7.84 (d, *J* = 2.1 Hz, 1H), 7.34 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.31-7.28 (m, 2H), 6.96 (s, 1H), 5.39 (s, 2H), 4.88 (s, 3H), 3.73 (s, 2H), 3.50 (s, 3H), 2.70 (q, *J* = 7.2 Hz, 4H), 1.15 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 153.9, 148.0, 142.3, 136.7, 130.9, 130.5, 129.7, 124.2, 122.6, 120.4, 118.9, 115.2, 99.3, 94.7, 56.1, 55.4, 46.1, 9.5; HRMS m/z *calcd for* $C_{21}H_{25}BrN_2O_2$ $[M+H]^+$ 418.1113 *found* 418.1112 (Δ = -0.2 ppm).

4-[2-(2-Methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl]-2-(propan-2-yl)benzoic acid (19)

Prepared according to method D. The filter residue was then stirred in a mixture of acetonitrile/HCl (3:1) for 30 mins before filtering through celite and purification by preparative HPLC 10-95% MeCN/H_2O to give **21** in a (72 mg, 70%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.30 (d, *J* = 5.3 Hz, 1H), 7.92 (d, *J* = 2.2 Hz, 1H), 7.89 (d, *J* = 8.1 Hz, 1H), 7.84 (d, *J* = 1.6 Hz, 1H), 7.68 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.46 (d, *J* = 5.7 Hz, 1H), 7.3-7.31 (m, 2H), 7.04 (d, *J* = 8.4 Hz, 1H), 4.24 (s, 2H), 3.85 (sept, *J* = 6.9 Hz, 1H), 3.19-3.12 (m, 4H), 1.30-1.27 (m, 12H); ^{13}C NMR (101 MHz, CD_3OD) δ: 169.8, 156.5, 150.2, 139.7, 138.9, 135.4, 132.7, 132.1, 130.8, 130.4, 126.6, 125.7, 121.0, 117.4, 117.1, 115.0, 99.7, 55.4, 46.4, 29.5, 23.0; HRMS m/z *calcd for* $C_{28}H_{32}N_3O_3$ $[M + H]^+$ 458.2438 *found* 458.2430 (Δ = 1.8 ppm).

3-(4-bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)benzaldehyde (20)

Prepared according to method A. Purification by flash chromatography (50% ethyl acetate-PET ether) afforded **22** as a colourless oil (500 mg, 76%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.04 (s, 1H), 8.23 (d, *J* = 5.2 Hz, 1H), 7.96 (t, *J* = 1.8 Hz, 1H), 7.93 (d, *J* = 7.7 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.33 (d, *J* = 5.3 Hz, 1H), 7.14 (d, *J* = 8.3 Hz, 2H), 6.56 (s, 1H), 2.29 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 191.8, 149.5, 145.5, 145.2, 141.0, 136.0, 135.9, 135.2, 133.3, 130.6, 130.3, 129.6, 128.5, 127.9,

125.3, 123.6, 122.9, 109.1, 21.7; HRMS m/z *calcd for* $C_{21}H_{15}BrN_2NaO_3S$ $[M+Na]^+$ 476.9879 *found* 476.9884 (Δ = -1.0 ppm).

({3-[4-Bromo-1-(4-methylbenzenesulfonyl)-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl]-4-(methoxymethoxy)phenyl}methyl)diethylamine (21)

Prepared according to method B. Purification by flash chromatography (5% methanol-dichloromethane) afforded **23** as a colourless oil (154 mg, 83%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.22 (d, *J* = 5.3 Hz, 1H), 7.87 (d, *J* = 1.9 Hz, 1H), 7.75-7.70 (m, 2H), 7.68 (d, *J* = 7.4 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 2H), 7.53 (d, *J* = 5.3 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 2H), 6.79 (s, 1H), 4.50 (s, 2H), 3.35-3.30 (m, 4H), 2.35 (s, 3H), 1.42 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 156.7, 148.4, 145.7, 144.3, 138.7, 135.5, 133.1, 132.9, 129.2, 127.6, 124.7, 123.3, 122.8, 122.4, 114.1, 107.5, 94.7, 55.4, 55.4, 46.2, 20.1, 8.4; HRMS m/z *calcd for* $C_{25}H_{27}BrN_3O_2S$ $[M+H]^+$ 512.1002 *found* 512.1013 (Δ = -2.3 ppm).

[(3-{4-Bromo-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl}phenyl)methyl]diethylamine (22)

Prepared according to method C. Purification by flash chromatography (10% methanol-dichloromethane) afforded **24** as a colourless oil (95 mg, 89%); ^{1}H NMR (400 MHz, CD_3OD) δ: 7.92 (d, *J* = 5.3 Hz, 1H), 7.78 (s, 1H), 7.71 (d, *J* = 7.8 Hz, 1H), 7.37 (t, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 5.3 Hz, 1H), 6.76 (s, 1H), 3.70 (s, 2H), 2.62 (q, *J* = 7.2 Hz, 4H), 1.06 (t, *J* = 7.1 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 148.9, 142.4, 139.7, 131.5, 129.7, 128.9, 126.8, 124.7, 124.2, 123.2, 118.9, 96.9, 56.8, 46.3, 9.6; HRMS m/z *calcd for* $C_{18}H_{21}BrN_3$ $[M+H]^+$ 358.0913 *found* 358.0907 (Δ = 1.7 ppm).

4-(2-{3-[(Diethylamino)methyl]phenyl}-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-(propan-2-yl)benzoic acid (23)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **25** in a (30 mg, 70%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.25 (s, 1H), 7.94 (s, 1H), 7.91 (d, *J* = 6.9 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.73 (s, 1H), 7.62 (s, 2H), 7.53 (s, 2H), 7.45 (d, *J* = 7.7 Hz, 1H), 7.28 (d, *J* = 5.2 Hz, 1H), 7.04 (s, 1H), 4.32 (s, 2H), 3.83 (sept, *J* = 13.9, 6.9 Hz, 1H), 3.16 (br. s., 4H), 1.27 (q, *J* = 7.2 Hz, 12H); ^{13}C NMR (101 MHz, CD_3OD) δ: 170.0, 150.1, 132.3, 130.8, 130.6, 130.3, 129.9, 128.1, 127.0, 126.2, 125.5, 115.1, 111.9, 97.8, 55.7, 46.7, 29.4, 23.0, 7.6; HRMS m/z *calcd for* $C_{28}H_{32}N_3O_2$ $[M + H]^+$ 442.2489 *found* 442.2476 (Δ = 3.0 ppm).

3-(4-bromo-1-tosyl-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)-2-methoxybenzaldehyde (24)

Prepared according to method A. Purification by flash chromatography (50% ethyl acetate-PET ether) afforded **26** as a colourless oil (990 mg, 72%); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.49 (s, 1H), 8.28 (d, *J* = 5.3 Hz, 1H), 8.07-7.90 (m, 3H), 7.59 (dd, *J* = 7.4, 1.8 Hz, 1H), 7.41 (d, *J* = 5.3 Hz, 1H), 7.37-7.30 (m, 1H), 7.25 (d, *J* = 6.7 Hz, 2H), 6.66 (s, 1H), 3.75 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ: 189.6, 162.0, 148.7, 145.4, 145.0, 137.1, 137.1, 135.6, 130.2, 129.4, 129.0, 128.6, 127.3, 125.2, 123.7, 123.1, 122.5, 108.1, 63.6, 21.7; HRMS *m/z calcd* for $C_{22}H_{17}BrN_2O_4SNa$ $[M+Na]^+$ 507.9990 *found* 508.0068 (Δ = 0.2 ppm).

_N_-(3-(4-bromo-1-tosyl-1_H_-pyrrolo[2,3-_b_]pyridin-2-yl)-2-methoxybenzyl)-_N_-ethylethanamine (25)

Prepared according to method B. Purification by flash chromatography (50% ethyl acetate-PET ether) afforded **27** as a yellow oil (765 mg, 81%; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.18 (d, *J* = 5.3 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.69 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.63 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.51 (d, *J* = 5.3 Hz, 1H), 7.41 (t, *J* = 7.7 Hz, 1H), 7.33-7.27 (m, 2H), 6.85 (s, 1H), 4.56 (d, *J* = 13.3 Hz, 1H), 4.28 (d, *J* = 13.3 Hz, 1H), 3.58 (s, 3H), 2.35 (s, 3H), 1.39 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 178.1, 158.6, 148.6, 145.9, 144.8, 138.4, 135.2, 133.7, 132.8, 129.2, 127.8, 126.4, 124.9, 124.2, 124.1, 123.2, 122.6, 108.0, 60.3, 50.2, 22.4, 20.2, 8.1; HRMS *m/z calcd* for $C_{26}H_{28}BrN_3O_3S$ $[M+H]^+$ 541.1035 *found* 542.1095 (Δ = 3.6 ppm).

_N_-(3-(4-bromo-1_H_-pyrrolo[2,3-_b_]pyridin-2-yl)-2-methoxybenzyl)-_N_-ethylethanamine (26)

Prepared according to method C. Purification by flash chromatography (5% methanol-dichloromethane) afforded **28** as a yellow oil (630 mg, 85%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.12 (d, *J* = 5.4 Hz, 1H), 7.92 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.59 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.46-7.37 (m, 2H), 7.03 (s, 1H), 4.43 (s, 2H), 3.69 (s, 3H), 3.32-3.28 (m, 4H), 1.43 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 157.0, 147.6, 142.1, 135.7, 132.8, 131.9, 125.6, 125.4, 125.3, 124.5, 123.2, 119.2, 100.5, 60.6, 50.9, 7.7; HRMS *m/z calcd* for $C_{19}H_{22}BrN_3O$ $[M+H]^+$ 387.0946 *found* 388.1008 (Δ = 2.7 ppm).

4-(2-(3-((diethylamino)methyl)-2-methoxyphenyl)-1_H_-pyrrolo[2,3-_b_]pyridin-4-yl)-2-isopropylbenzoic acid (27)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **29** in a (30 mg, 74%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.41 (s, 1H), 7.98-7.92 (m, 2H), 7.89 (d, *J* = 1.7 Hz, 1H), 7.73 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.58 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.47-7.43 (m, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.23 (s, 1H), 4.43 (s, 2H), 3.94 (p, *J* = 6.8 Hz, 1H), 3.72 (s, 3H), 3.31-3.25 (m, 5H), 1.42 (t, *J* = 7.3 Hz, 6H), 1.36 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 169.9, 157.1, 150.1, 140.6, 136.3, 132.8, 131.9, 131.4, 130.3, 126.3, 125.5, 125.4, 124.6, 100.5, 60.7, 50.8, 29.4, 23.0, 7.7; HRMS *m/z calcd* for $C_{29}H_{33}N_3O_3$ $[M+H]^+$ 471.2522 *found* 472.2579 (Δ = 3.4 ppm).

4-(2-(5-((diethylamino)methyl)-2-methoxyphenyl)-1_H_-pyrrolo[2,3-_b_]pyridin-4-yl)-2-methylbenzoic acid (28)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **30** in a (50 mg, 75%); ^{1}H NMR (101 MHz, CD_3OD) δ: 8.27 (d, *J* = 5.6 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 1H), 7.94 (d, *J* = 2.3 Hz, 1H), 7.70 – 7.61 (m, 2H), 7.46 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.36 (d, *J* = 5.6 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.16 (s, 1H), 4.27 (s, 2H), 3.99 (s, 3H), 3.15 (p, *J* = 7.2 Hz, 4H), 2.63 (s, 3H), 1.27 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (400 MHz, CD_3OD) δ: 168.9, 157.8, 140.9, 140.3, 136.5, 133.0, 131.5, 131.3, 131.2, 125.9, 122.2, 119.6, 115.1,

112.5, 99.7, 55.3, 20.6, 7.6; HRMS *m/z calcd* for $C_{27}H_{29}N_3O_3$ $[M+H]^+$ 443.2209 *found* 444.2214 (Δ = 1.4 ppm).

4-(2-(5-((diethylamino)methyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)benzoic acid (29)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **31** in a (100 mg, 85%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.28 (d, *J* = 5.5 Hz, 1H), 8.16 (d, *J* = 8.5 Hz, 2H), 7.95 (d, *J* = 2.3 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 2H), 7.46 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.38 (d, *J* = 5.6 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.17 (s, 1H), 4.27 (s, 2H), 3.99 (s, 3H), 3.15 (p, *J* = 7.3 Hz, 4H), 1.27 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 168.0, 157.6, 148.6, 143.1, 142.4, 140.9, 136.3, 132.0, 130.9, 130.6, 129.9, 129.3, 128.3, 122.1, 120.8, 114.9, 112.4, 98.6, 55.5, 55.2, 7.6; HRMS *m/z calcd* for $C_{26}H_{27}N_3O_3$ $[M+H]^+$ 429.2052 *found* 430.2056 (Δ = 1.1 ppm).

***N*-ethyl-N-(3-(4-(3-isopropyl-4-(1H-tetrazol-5-yl)phenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)-4-methoxybenzyl)ethanamine (30)**

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **32** in a (27 mg, 62%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.39 (d, *J* = 5.8 Hz, 1H), 8.01 (d, *J* = 2.3 Hz, 1H), 7.97 (d, *J* = 8.0 Hz, 1H), 7.91 (d, *J* = 1.8 Hz, 1H), 7.74 (dd, *J* = 8.0, 1.9 Hz, 1H), 7.58 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.52 (d, *J* = 5.8 Hz, 1H), 7.32 (d, *J* = 8.7 Hz, 1H), 7.31 (s, 1H), 4.37 (s, 2H), 4.07 (s, 3H), 3.94 (p, *J* = 6.9 Hz, 1H), 3.25 (dd, *J* = 12.2, 7.0 Hz, 4H), 1.42-1.33 (m, 12H); ^{13}C NMR (101 MHz, CD_3OD) δ: 169.8, 157.9, 150.1, 145.6, 143.9, 140.0, 137.8, 136.8, 132.9, 131.8, 131.0, 130.3, 126.4, 125.6, 122.1, 121.8, 119.6, 114.9, 112.5, 100.3, 55.3, 55.2, 29.4, 23.0, 7.6; HRMS *m/z calcd* for $C_{29}H_{33}N_7O$ $[M+H]^+$ 495.2747 *found* 496.2829 (Δ = 0.6 ppm).

***N*-ethyl-*N*-(3-(4-(3-isopropylphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-2-yl)-4-methoxybenzyl)ethanamine (31)**

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **33** in a (67 mg, 82%); ^{1}H NMR (101 MHz, CD_3OD) δ: 8.37 (d, *J* = 5.9 Hz, 1H), 8.03 (d, *J* = 2.2 Hz, 1H), 7.75 -7.68 (m, 2H), 7.61-7.54 (m, 2H), 7.50 (dd, *J* = 16.9, 6.8 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 4.37 (s, 2H), 4.08 (s, 3H), 3.25 (p, *J* = 7.5 Hz, 4H), 3.08 (p, *J* = 6.9 Hz, 1H), 1.43-1.31 (m, 12H); ^{13}C NMR (400 MHz, CD_3OD) δ: 157.9, 149.9, 137.2, 132.8, 131.1, 128.9, 127.7, 126.5, 126.1, 122.1, 119.8, 114.9, 112.5, 100.3, 55.3, 34.1, 23.1, 7.6; HRMS *m/z calcd* for $C_{28}H_{33}N_3O$ $[M+H]^+$ 427.2624 *found* 428.2624 (Δ = 3.8 ppm).

5-(2-(5-((diethylamino)methyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoic acid (32)

Prepared according to method D. Purification by preparative HPLC 10-95% MeCN/H_2O to give **34** in a (55 mg, 78%); ^{1}H NMR (400 MHz, CD_3OD) δ: 8.41 (d, *J* = 5.7 Hz, 1H), 8.26 (d, *J* = 2.0 Hz, 1H), 7.99 (ddd, *J* = 9.3, 8.0, 1.9 Hz, 2H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.60 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.53 (d, *J* = 5.7 Hz, 1H), 7.42 (t, *J* = 7.7

Hz, 1H), 7.36 (s, 1H), 4.43 (s, 2H), 3.92 (p, *J* = 6.9 Hz, 1H), 3.73 (s, 3H), 3.31-3.25 (m, 4H), 1.42 (t, *J* = 7.3 Hz, 6H), 1.34 (d, *J* = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ: 169.8, 157.0, 151.0, 145.4, 144.8, 137.8, 136.9, 134.4, 133.1, 131.9, 131.6, 131.3, 129.8, 127.2, 125.4, 124.9, 124.7, 121.6, 114.9, 100.8, 60.7, 50.9, 29.4, 22.8, 7.7; HRMS *m/z calcd* for $C_{29}H_{33}N_3O_3$ $[M+H]^+$ 471.2522 *found* 472.2579 (Δ = 3.4 ppm).

Assay

P. falciparum culture and synchronisation

P. falciparum cultures were maintained in RPMI-1640 media (Invitrogen) supplemented with 0.2% sodium bicarbonate, 0.5% Albumax II, 2.0 mM L-glutamine (Sigma) and 10 mg/L gentamycin. For continuous culture, the parasites were kept at 4% haematocrit in human erythrocytes from 0+ blood donors and between 0.5 - 3% parasitaemia maintained in an incubator at 37 °C, 5% carbon dioxide (CO_2), 5% oxygen (O_2) and 90% nitrogen (N_2). To obtain highly synchronous ring stage parasites for the drug assays, cultures were double synchronised using Percoll and Sorbitol synchronisation as previously described.[35-36] First, highly segmented schizonts were enriched by centrifugation on a 70% Percoll (GE Healthcare) cushion gradient. The Schizont pellet was collected and washed before fresh erythrocytes were added to a final haematocrit of 4%. The schizonts were incubated for about 1-2 hours shaking continuously to allow egress and re-invasion of new erythrocytes. Residual schizonts were then removed by treating the pellet with sorbitol to generate highly synchronous 1-2 hours old ring-stage parasites.

Determining the IC_{50} of compound inhibitors and drugs-*ex vivo*

To determine the IC_{50} of the molecules in parasites (*P. falciparum* 3D7) *ex vivo*, the molecules were diluted 1 in 3 from a starting concentration of 100 µM for 12 dilution points. Fifty microliters of freshly diluted drugs, at twice the required final concentrations were aliquoted into black 96-well plates. To the drug plates, 50 µl of parasites prepared at 8% haematocrit at a parasitaemia (0.3 - 0.5%) were added and mixed by pipetting up and down several times giving a final culture volume of 100 µl at the required drug concentration (top concentration of 100 µM) and 4% haematocrit. To the 'no drug' control, growth media was added and uninfected erythrocytes were included on the plate as blank. The outer wells were filled with media to reduce evaporation from the experimental wells and the plates incubated for 50 hours (± 2 hours) to allow the parasites sufficient time to re-invade before they are collected and frozen. To quantify growth inhibition, the plates were thawed at room temperature for at least 1 hour and 100 µl of lysis buffer (20 mM Tris-HCl; 5 mM EDTA; 0.004% saponin and triton X-100) in PBS containing Sybr Green I (1µl in 5 ml) was added to each well and mixed by pipetting up and down several times and incubated for 1 hour in the dark shaking. Using a Fluroskan/ClarioStar plate reader at excitation of 485 nm and emission of 538 nm, plate absorbances were acquired. The data was normalised against the controls and graphs were

generated using Graph Pad Prism 8 to determine the IC_{50} values using the non-linear regression log (inhibitor) versus response (three parameter) curve.

Time Resolve Florescence Energy Transfer (TR-FRET) to determine the IC_{50} of the inhibitors with full-length *Pf*CLK3 recombinant protein

The TR-FRET assays, a high-throughput inhibition assay, as described previously[13] was used to determine the potency of the small molecules generated against full-length *Pf*CLK3 recombinant protein in a kinase buffer (containing 50 mM HEPES, 10 mM $MgCl_2$, 2 mM DTT, 0.01% Tween 20, and 1 mM EGTA), U*Light*-labeled peptide substrate (MBP peptide (sequence: CFFKNIVTPRTPPPSQGK). First, in a 10µL reaction volume, 5µl of twice the required enzyme concentration (50 nM) and 2.5µl of 4 times the required substrate concentration mix containing cold ATP, and the serially diluted drugs were mixed in a black 384-plate well plate and incubated at 37 °C for 1 hour. The reaction was stopped after incubation by adding the stopping/detection solution (containing 30 mM EDTA in 1X Lance detection buffer and 3 nM Europium-labeled anti-phospho specific antibody) and incubated for another hour at RT before phosphorylation signals were measured using the ClarioStar.

For each test compound, percent inhibition (response) which was calculated using the formula:

$$\text{Percentage inhibition (response)} = \left[\frac{(Kinase\ activy - blank)}{(Maximum\ kinase\ activity - blank)}\right] * 100$$

was plotted against log molar concentration of compound to calculate the IC_{50} (potency) of each inhibitor molecule and plotted using GraphPad prism software. All experiments were done in triplicates and the data presented is the S.E.M of three independent experiments run in triplicates.

*Pf*CLK3 phosphorylation of substrate results in the Europium-labeled anti-phospho specific antibody recognizing the phosphorylated site on the substrate. The Europium donor fluorophore is excited at 320 nm or 340 nm and energy is transferred to the U*Light* acceptor dye on the substrate, which finally results in the emission of light at 665 nm. The level of *ULight* peptide phosphorylation correlates with the intensity of the emission. For normalization, a no kinase and a no inhibitor reaction wells were included and all experiments conducted in triplicates. Drug dilutions, protein concentrations and incubation times were the same for easy comparison of results.

Microsomal stability

Compounds were incubated at 37 °C at a concentration of 1 µM with CD1 mouse liver microsomes (GIBCO™, Thermo Fisher Scientific) in a suspension of 50 mM potassium phosphate buffer (pH 7.4) with a final protein concentration of 0.5 mg/mL. The reaction was started by the addition of excess NADPH and then quenched at several time points starting from time zero then at 3, 6, 9, 15 and 30 min addition of

acetonitrile to an aliquot of the sample. Internal standard was added to each sample before centrifugation to remove any precipitates before monitoring loss of parent compound by HPLC analysis using Shimadzu LC-20A (Shimadzu, UK). Prism (Graphpad, USA) was used to fit an exponential decay for substrate depletion and subsequently rate constant (k) from the peak area of the parent compound to internal standard at each time point. Rate of intrinsic clearance (CL_{int}) was then calculated according to the methods of Obach using the equation:[37]

CL_{int} (mL/min/g liver) = k x v x microsomal protein yield

Where V is the incubation volume (volume/mg protein) and microsomal protein yield is assumed to be 52.5 mg protein/ g liver. With verapamil used as a positive control.

Distribution coefficient ($LogD_{7.4}$)

Distribution coefficient ($LogD_{7.4}$) was estimated by correlation of the compounds chromatographic retention properties to those of 10 standard compounds with known distribution coefficients ranging from -0.5 to 5.5 at pH 7.4. A fast gradient HPLC methodology was used based on the method developed by Valkó *et al*.[38]

Kinase Screen Method

Each enzyme is assayed in its linear range with 0.3 µM substrate in 50 mM Tris pH 7. 5, 0.1 mM EGTA, 0.01 mM DTT, relevant Mg/ATP (5, 20 or 50 µM) for 30 min at room temp. Assays are stopped by the addition of 3% orthophosphoric acid and harvester onto p81 filter paper using the Perkin elmer unifilter harvester. Once dried, they are read on a Perkin elmer Topcount NXT scintillation counter for 30 sec/well.[39]

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXXX.

Experimental procedures and characterization data for all compounds, copies of ^{1}H, ^{13}C, NMR spectra for all compounds, $LogD_{7.4}$ (Distribution co-efficient), metabolic stability and analytical HPLC traces for final compounds. *P. falciparum* culture, synchronisation and TR-FRET assay data. Molecular formula strings (CSV) file

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Acknowledgments

#A.M. and O.J. have contributed equally to this work. We thank the University of Glasgow (A.G.J. and A.B.T.), EPSRC (Research Project Grant EP/N034295/1) (A.M.) and MRC Developmental Gap Fund (A.S.-A.) for financial support of this research. O.J. thanks MRC Toxicology Unit and MRC Unit the Gambia for a PhD studentship. K.M. thanks the University of Glasgow for a Lord Kelvin Adam Smith Studentship. M.M.A. thanks the University of Glasgow for a Lord Kelvin Adam Smith Fellowship. The authors also thank Andrew Monaghan (high-resolution mass spectrometry, University of Glasgow) for technical assistance. *Corresponding authors.

Abbreviations Used

*Pf*CLK3, *Plasmodium falciparum* cyclin-dependent like kinase; TCMDC, Tres cantos antimalarial set; TR-FRET, Time resolve florescence energy transfer; SRPK, Serine-arginine-rich protein kinase; S_NAr, Nucleophilic aromatic substitution; *Pf*PKG, *Plasmodium falciparum* Protein Kinase G; *Pf*CDPK1, *Plasmodium falciparum* Calcium dependent protein kinase 1; *Pf*CRT, *P. falciparum* chloroquine resistant transporter gene; *Pf*MDR1, *Plasmodium falciparum* multidrug resistant gene 1; *Pf*DHFR, *Plasmodium falciparum* dihydrofolate reductase gene.

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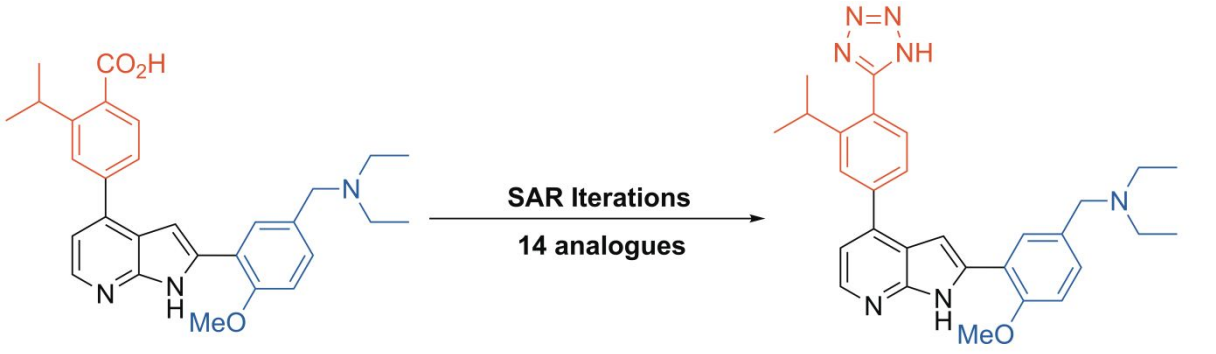
Table of Contents Graphic



TCMDC-135051:TCAMS Hit

Early lead, ***30***

*Pf*CLK3 (IC_{50}) = 19 nM (pIC_{50} = 7.7)
3D7 (EC_{50}) = 270 nM (pEC_{50} = 6.6)