Development of potent and selective *Pf*CLK3 inhibitors based on GSK-TCMDC151 as a new class of antimalarials

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Abstract

The 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 SAR with a 7-azaindole-based series. A total of 14 analogues were assessed in a TR-FRET assay against the full recombinant protein kinase *Pf*CLK3 and 10 were further assessed in parasites 3D7 (chloroquine sensitive) strains of *P. falciparum*. SAR relating to rings A and B was established. These data 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 billon insecticide impregnated bed nets since 2015, together with artemisinin-based combination therapies have contributed to 20 million few cases of malaria being reported in 2017 compared to 2010.[1] Despite this success, the rate of reduction in malaria has flattened 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 over the last several years will be reversed unless new medicines with novel mechanisms of action are discovered. To this end we have been testing the notion that targeting malaria parasite

protein kinases might offer a novel strategy for the development of next generation anti-malarials.[6,7]

In 2011 we determined that 36 or 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 showing that *Pf*CLK3 can phosphorylate parasite SR proteins[11] indicates 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 nM activity against *Pf*CLK3 in in vitro kinase assays and sub µM parasiticidal activity in blood stage *P. falciparum* (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 inhibition the development of liver stage parasites. 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.

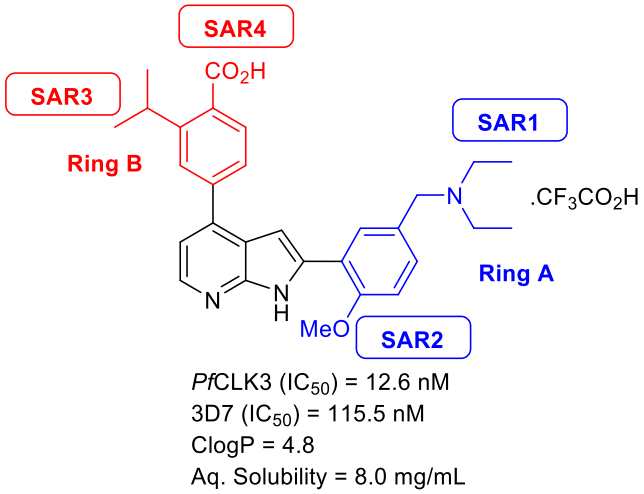


Figure 1. Structure and biological profile of hit *Pf*CLK3 inhibitor TCMDC-135051, **1**

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.

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 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 with the molecule bound 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.

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 (IC_{50} = 31.1 µM ; Kd = 14.1 µM) and showed selectivity over human Hsp90 and *Pf*Hsp90 mutant.

In addition to antimalarial activity, the 7-azaindole scaffold has been identified as having a wide variety of biological applications including antitumor activity and for the reduction of antiviral activity in HIV-1-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 2C), which suggests its usefulness for developing novel pharmaceuticals.[25-27]

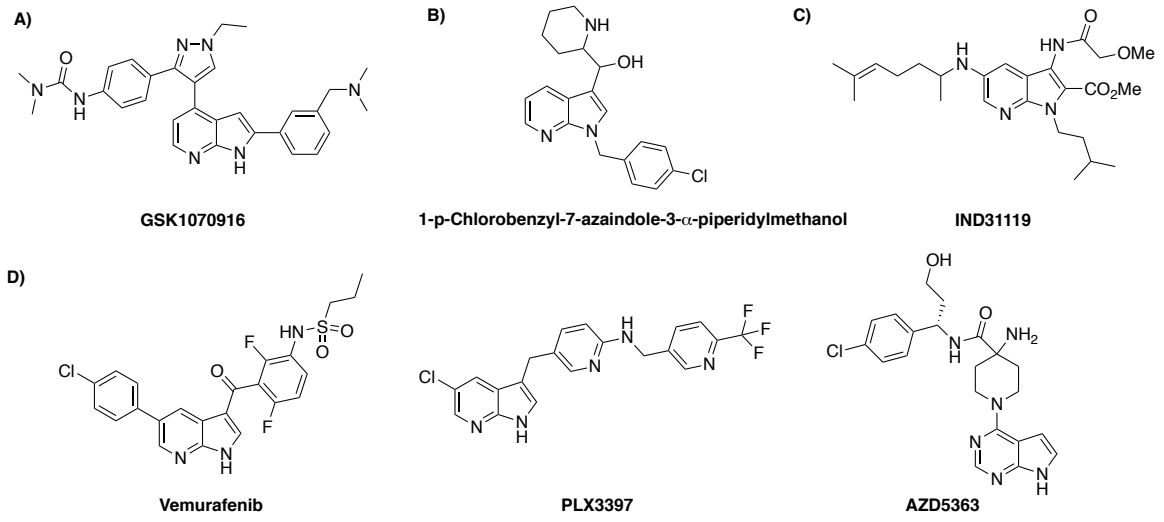


Figure 2. A) Aurora kinase inhibitor GSK1070916, B) 1-p-Chlorobenzyl-7-azaindole-3-a-piperidylmethanol has *in-vivo* activity against *Plasmodium berghei*, C) PfHsp90 inhibitor IND31119, D) Drug candidates incorporating a core 7-azaindole scaffold, Vemurafenib, PLX3397, and AZD5363.

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 improve polarity and to explore the role of the

methoxy group on activity (Figure 1, SAR2). 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 improving aqueous solubility and ClogP (Figure 1, SAR3). Finally, we exchanged the carboxylic acid group (ring B) with other substituents to investigate the role in binding, potentially improve metabolic stability and to explore the effect of increase lipophilic character (Figure 1, SAR4).

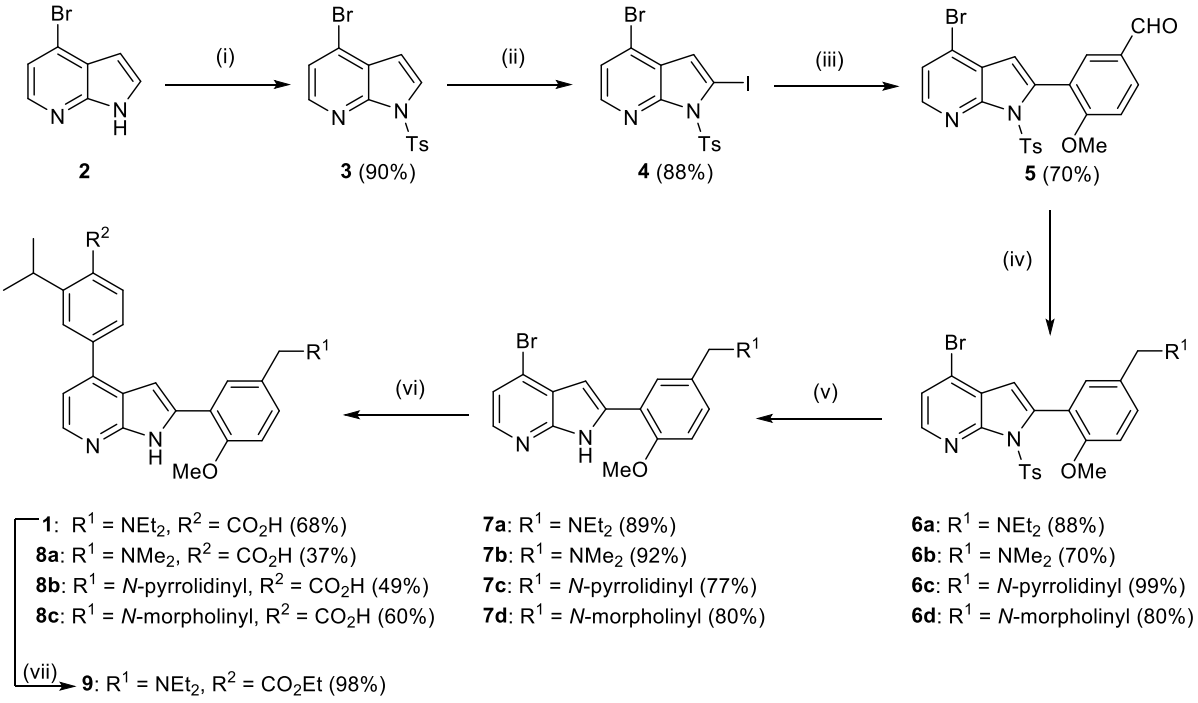
TCMDC-135051 is a promising hit compound for a medicinal chemistry programme to develop as a preclinical lead that meets many of the criteria set by the Medicines for Malaria Venture (capable of rapidly clearing the parasite, multi-stage, parasite killing across multiple species with action as a transmission blocker).[28,29] Here we describe the synthetic route to TCMDC-135051 and determine a structure-activity-relationship that resulted in an improvement in *in vitro* and *in vivo* potency together with improved drug-like properties.

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.

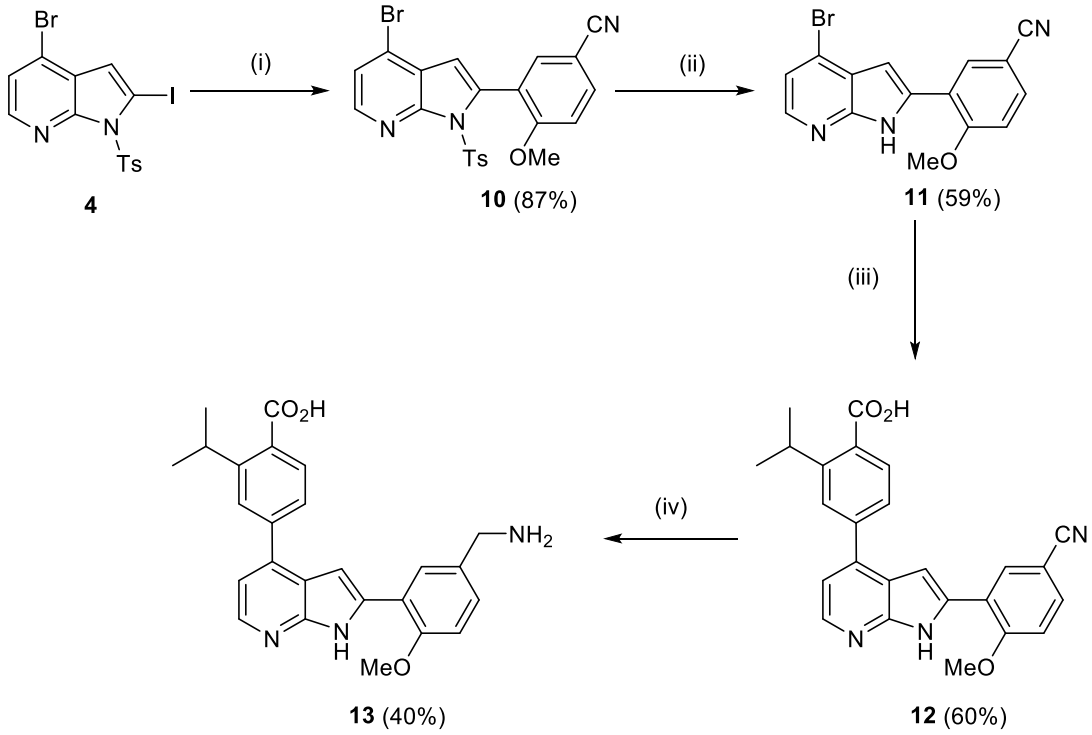
Scheme 1. Synthesis of TCMDC-135051 **1** and analogues **8a-c & 9**[a]



[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, $Pd(PPh_3)_3$, 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 **13**. 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 to yield **12.** The final step was nitrile reduction using cobalt (II) chloride hexahydrate and sodium borohydride to provide the corresponding amine **13** (Scheme 2).

Scheme 2. Synthesis of 4-(2-(5-(aminomethyl)-2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-4-yl)-2-isopropylbenzoic acid **13**.[a]



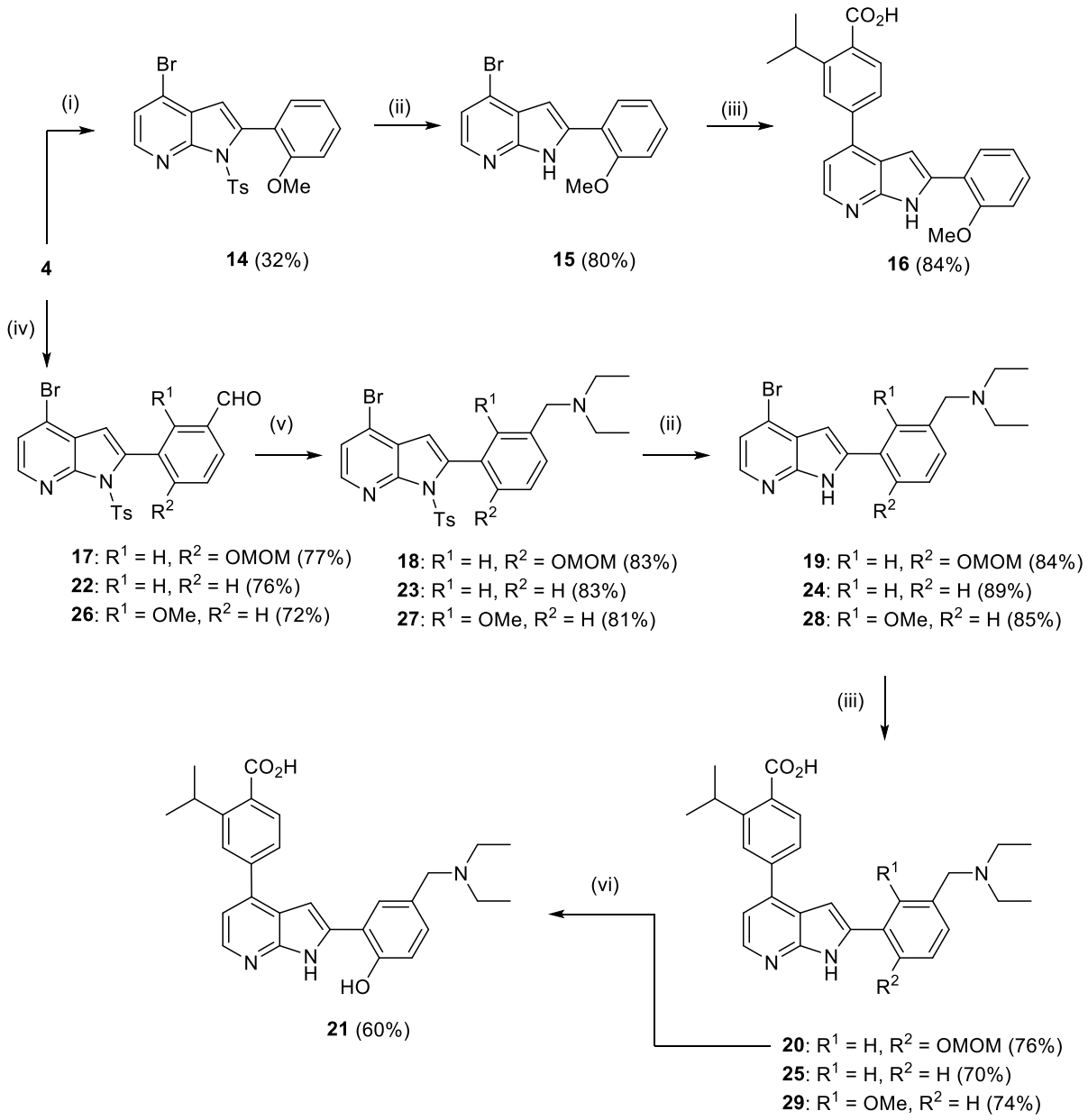
[a]Reagents and conditions: (i) 4-methoxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-Dioxane, 110 °C, 18 h; (ii) CH_3OH, K_2CO_3, 55 °C, 18 h; (iii) 2-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, $Pd(dppf)Cl_2.CH_2Cl_2$, Na_2CO_3,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

Similarly, analogue **16** 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 **21** and **25** in which the OMe is replaced by a hydroxy group (**21)** and OMe is removed (**25)** (Scheme 3). The synthetic route toward **21** starts with Suzuki coupling of **4** with methoxymethyl ether (MOM) protected boronate ester (see SI for boronate ester synthesis) to yield **17.** Reductive amination of **17** followed by removal of the tosyl group gave **19**. Finally, **19** was coupled with boronate ester under Suzuki conditions and the MOM protecting group was removed under acidic conditions to provide the desired analogue **21** in excellent yield. Analogue **25** 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 **29** in which the methoxy group is moved from the ortho to para position relative to the methylene N-diethyl functionality (Scheme 3).

Scheme 3. Synthesis of 2-isopropyl-4-(2-(2-methoxyphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)benzoic acid **16**, 4-(2-(5-((diethylamino)methyl)-2-hydroxyphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-2-isopropylbenzoic acid **21**, 4-(2-(3-((diethylamino)methyl)phenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-2-isopropylbenzoic acid **25** and [4-(2-(3-((diethylamino)methyl)-2-methoxyphenyl)-1H-pyrrolo[2,3-b]pyridin-4-yl)-2-isopropylbenzoic acid] **29**.[a]



[a]Reagents and conditions: (i) (2-methoxyphenyl)boronic acid, $Pd(PPh_3)_3$, Na_2CO_3, 1,4-Dioxane, 110

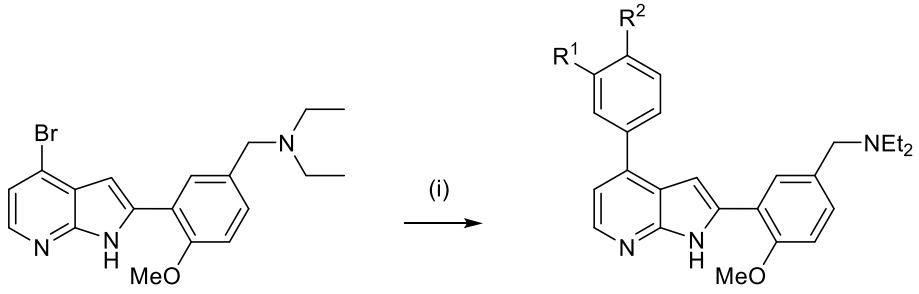
°C, 12 h; (ii) CH_3OH, K_2CO_3, 55 °C, 18 h; (iii) 2-isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid, $Pd(dppf)Cl_2.CH_2Cl_2$, Na_2CO_3, 1,4-dioxane, 110 °C, 0.5 h, MW; (iv) 4-(methoxymethoxy)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde or 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzaldehyde, $Pd(PPh_3)_4$, Na_2CO_3, 1,4-Dioxane, 110 ^{O}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 **30-31**, 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 **30-31**.

To investigate the role of the carboxylic acid substituent of ring B on antimalarial activity, several analogues **32-34** were synthesised as depicted in scheme 4. Synthesis of analogues **32-34** was achieved by Suzuki coupling **7a** with various boronate esters (R^2 = H or 1*H*-etrazole) to yield target compounds **32-33** in good yields.

An isomer in which the isopropyl and carboxylic acid were switched, **34** was synthesized to investigate the role of these functional groups on antimalarial activity (Scheme 4).

Scheme 4. Synthesis of 4-aryl-7-azaindole analogues, **30-34**.[a]



7a

30: $R^1 = CH_3$, $R^2 = CO_2H$ (75%)
31: R^1 = H, $R^2 = CO_2H$ (85%)
32: $R_1 = CH(CH_3)_2$, R_2 = 1*H*-tetrazole (62%)
33: $R_1 = CH(CH_3)_2$, R_2 = H (82%)
34: $R_1 = CO_2H$, $R_2 = CH(CH_3)_2$ (78%)

[a]Reagents and conditions: (i) boronate esters, $Pd(dppf)Cl_2$, 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. All synthesised analogues were assessed in a TR-FRET assay against the full recombinant protein kinase *Pf*CLK3 (Table 1 and Table 2). Analogues which gave low nanomolar activity were then further assessed in parasites 3D7 (chloroquine sensitive) strains of *P. falciparum* (Table 1 and Table 2).

SAR1 corresponding to analogues **8a-c, 13** and **16** 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 *N*-dimethyl group to investigate the effect of alkyl group size and molecular lipophilicity. In vitro activity of **8a** IC_{50} = 28.7 nM (pIC_{50} = 7.5) remains the same whereas 2-fold decrease in the parasiticidal activity IC_{50} = 456.8 nM (pIC_{50} = 6.3) was observed (Table 1). Further, to increase the sp^3 character and solubility of the hit molecule pyrrolidine (**8b**) and morpholino group (**8c**) was introduced. Analogue **8b** IC_{50} = 38.3 nM (pIC_{50} = 7.4) shows comparable activity whereas **8c** IC_{50} = 9.4 nM (pIC_{50} = 8.0) shows a slight improvement in vitro activity (Table 1). Surprisingly when tested in parasites, **8b** IC_{50} = 446.3 nM (pIC_{50} = 6.4) shows a slight decrease in parasiticidal activity whereas analogue **8c** IC_{50} = 2032 nM (pIC_{50} = 5.7) shows a 10-fold decrease in parasiticidal activity (Table 1). To increase the solubility of the hit molecule we replaced the N-diethyl functionality with a primary amine, **13**. The substitution was detrimental for the potency of the hit molecule. Analogue **13** incorporating a primary amine gave IC_{50} = 75.5 nM (pIC_{50} = 7.1), was 3-fold less potent in vitro and showed dramatic loss of efficacy IC_{50} = 3445 nM (pIC_{50} = 5.5) in parasites indicating the need to sterically block the amine group for activity (Table 1). Analogue **16** IC_{50} = 79.0 nM (pIC_{50} = 7.1) was less potent in vitro and in parasites IC_{50} = 1207 nM (pIC_{50} = 5.9). There was a 6-fold drop in the activity, suggesting that the methylene linking the N-diethyl group to ring A is important for antimalarial activity (Table 1).

Table 1. Activity results of SAR of ring-A

Analogue	clogP	TPSA (A°)	LipE		*Pf*CLK3		3D7	
			*Pf*CLK3	3D7	IC_{50} (nM)	pIC_{50}	IC_{50} (nM)	pIC_{50}
8a	1.6	82.5	5.9	4.7	28.7	7.5	456.8	6.3
8b	2.1	82.5	5.3	4.2	38.3	7.4	446.8	6.4
8c	1.4	91.7	6.7	4.3	9.4	8.0	2032	5.7
13	1.7	105.7	5.4	3.6	75.5	7.1	3445	5.5
16	5.7	7.8	1.4	0.2	79.0	7.1	1456	5.8
21	2.1	93.5	5.6	3.2	21.6	7.7	3529	5.5
25	2.4	73.3	5.2	4.1	24.8	7.6	317.5	6.5
29	2.4	82.5	5.4	3.1	17.4		3167	

To explore the importance of the methoxy group (OMe) on ring-A, two analogues **21** and **25** were prepared and examined for in vitro antimalarial activity. We first replaced the OMe group with a hydroxyl to investigate the role of polarity on activity. Next, we replaced the OMe group with hydrogen (**25)** to investigate the importance of this functionality for activity. For both compounds **21** IC_{50} = 21.56 nM (pIC_{50} = 7.7) and **25** IC_{50} = 24.78 nM (pIC_{50} = 7.6) in vitro potency was retained (Table 1). However, when tested in parasites **21** IC_{50} = 3529 nM (pIC_{50} = 5.5) shows complete loss of activity. Interestingly, while replacing the OMe group by hydrogen in **25** retained potency IC_{50} = 317.5 nM (pIC_{50} = 6.5), in parasites. This result demonstrated that OMe group on ring A could be replaced with hydrogen while retaining the activity in vitro and in parasites.

Next we turned our attention to study the SAR of various functional groups on ring-B. First, we examine the role of binding through van der waals interactions in vitro and lipophilicity in parasites imparted by isopropyl group on antimalarial activity. In analogue **30** the isopropyl group was replaced with methyl, whereas in analogue **31** the isopropyl was removed and replaced with hydrogen. Analogues **30** IC_{50} = 24.09 nM (pIC_{50} = 7.5) and **31** IC_{50} = 34.35 nM (pIC_{50} = 7.5) retained activity in vitro. However, a dramatic loss of potency was observed for both compounds **30** IC_{50} = 1743 nM (pIC_{50} = 5.7) and **31** IC_{50} = 3972 nM (pIC_{50} = 5.4), 9-fold and 20-fold respectively against parasites (Table 2). Therefore this group is not required for binding in vitro, however the effect of lipophilicity is very important for parasiticidal activity.

At this point we decided to increase the lipophilicity and replace the isopropyl group to the larger *tert*-butyl group. However due to restriction of chemistry of boronate esters (instability of *tert*-butyl anion required for S_NAr reaction; see SI for boronate ester synthesis) we could not synthesize the proposed molecule. From these data, the alkyl substituent on ring-B appears not to be involved in the recognition event with *Pf*CLK3, however is key in terms of overall molecular lipophilicity for parasiticidal activity.

To investigate the requirements (ie. 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, **32**. This change was orchestrated to increase lipophilicity of the molecule, retain H-bonding capability and investigate the role of potential ionic interactions with the enzyme.

Tetrazole analogue **32** shows comparable potency with TCMDC-135051 IC_{50} = 18.5 nM (pIC_{50} = 7.7) in vitro, as well as in IC_{50} = 271.3 nM (pIC_{50} = 6.6) parasites (Table 2). This may be because **32** has improved lipophilicity and resulted in efficient penetration of parasite infected erythrocytes. Analogue **33** IC_{50} = 1.03 µM (pIC_{50} = 6.0) was completely inactive in vitro and demonstrates the importance of the carboxylic acid group (Table 2).

Table 2. Activity results of SAR of ring-B

Analogue	clogP	TPSA (A°)	LipE		*Pf*CLK3		3D7	
			*Pf*CLK3	3D7	IC_{50} (nM)	pIC_{50}	IC_{50} (nM)	pIC_{50}
30	1.6	82.5	6.1	4.2	24.1	7.6	1743	5.7
31	1.3	82.5	6.2	4.1	34.4	7.5	3972	5.4
32	6.2	91.8	1.5	0.4	18.5	7.7	271.3	6.6
33	6.9	42.3	-0.9	ND	10300	6.0	ND	ND
9	7.0	68.7	-0.6	ND	390.4	6.4	ND	ND
34	2.4	82.5		ND	13850		ND	ND

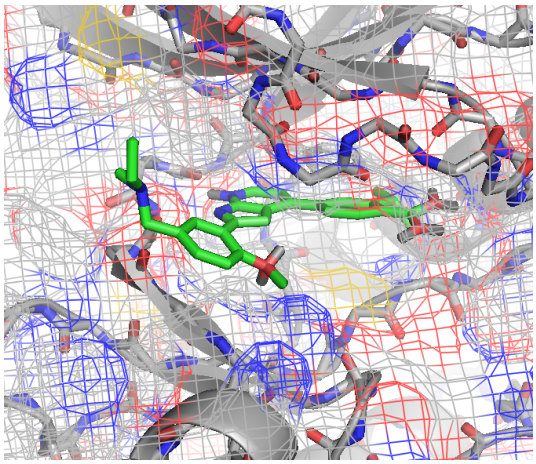
Analogue **9** (ester prodrug) has improved lipophilicity yet the IC_{50} = 390.4 nM (pIC_{50} = 6.4) in vitro activity was not high, indicating the presence of a functional group capable of donating a hydrogen bond is important for activity (Table 2).

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 **29** IC_{50} = 17.47 nM (pIC_{50} = 7.9) had retained activity in vitro. Whereas when **29** IC_{50} = 3167 nM (pIC_{50} = 7.5) was tested in parasites activity was lowered by 15-fold magnitude (Table 1). The observed loss of potency in parasites may be due to the steric clash/spatial interaction of OMe with NEt_2 group resulting in loss of lipophilicity imparted by ethyl groups.

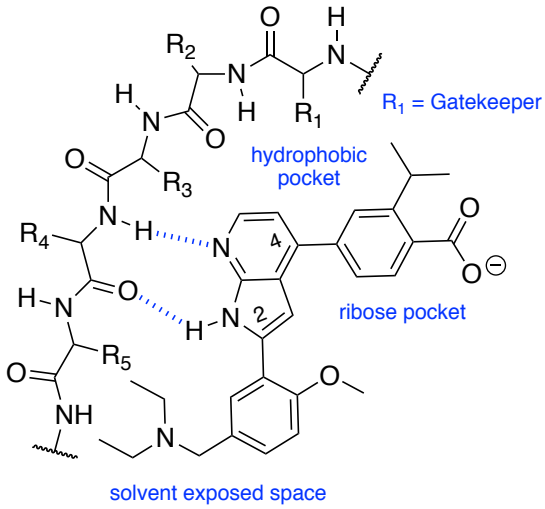
We next investigated the position of the isopropyl and carboxylic acid substituents of ring B **34** on antimalarial activity. The change of orientation of the substituent is detrimental for the activity in vitro IC_{50} = 13,850 nM (pIC_{50} = 7.9) as well as in parasites IC_{50} = ND (Table 2). This 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 PfCLK3 and so a homology model of PfCLK3 was created using the structure of human PRPF4B kinase domain as the closest homologue (PDB 6CNH). Overlay with Human Jnk1alpha kinase with 4-phenyl-7-azaindole IKK2 inhibitor bound (PDB 4AWI) facilitated identification of the proposed binding pocket. 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 occupies the ribose pocket and the Ring A diethyl-amine occupies the solvent exposed space.

A



B



Conclusions

In summary, we report the synthesis of hit PfCLK inhibitor TCMDC-135051 **1** (PfCLK3 IC_{50} = 13 nM, 3D7 IC_{50} = 303 nM) and a series of related 7-azaindole-based analogues. Of the 14 analogues, 10 had low nanomolar activity and were further assessed in parasites 3D7 (chloroquine sensitive) strains of *P. falciparum*. Tetrazole analogue **32** was identified with comparable activity ((*Pf*CLK3, IC_{50} = 18.5 nM and 3D7 IC_{50} = 271.3 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 PfCLK inhibitors based on TCMDC-135051 **1**.

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