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Novel inhibitors of *Plasmodium falciparum* based on 2,5-disubstituted furans

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TITLE: Novel Inhibitors of Plasmodium Falciparum based on 2,5-disubstituted Furans.

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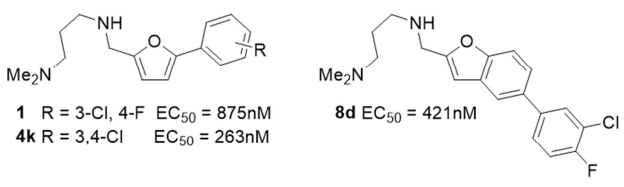
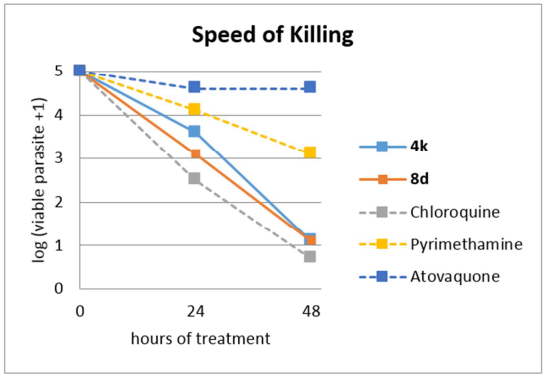
ABSTRACT.

Phenotypic HTS campaigns with a blood stage malaria assay have been used to discover novel chemotypes for malaria treatment with potential alternative mechanisms of action compared to existing agents. N^1-(5-(3-Chloro-4-fluorophenyl)furan-2-yl)-N^3,N^3-dimethylpropane-1,3-diamine, **1** was identified as a modest inhibitor of *P.falciparum* NF54 (IC_{50} = 875nM) with an apparent long plasma half-life after high dose oral administration to mice, although the compound later showed poor metabolic stability in liver microsomes through ring- and side chain-oxidation and *N*-dealkylation. We describe here the synthesis of derivatives of **1**, exploring the influence of substitution patterns around the aromatic ring, variations on the alkyl chain and modifications in the core heterocycle, in order to probe potency and metabolic stability, where **4k** showed a long half-life in rats.

GRAPHICAL ABSTRACT.





KEYWORDS. Malaria, Furan, Diamines, Synthesis, nM Inhibitors

1. Introduction.

The WHO estimated that 198 million cases of malaria occurred in 2013 with a death toll of 584,000 [1]. The disease, social and economic burdens are highest in Africa, where 90% of all malaria deaths occur and of those, 78% are accounted for by children under the age of 5 years. Globally, 3.3 billion people are at risk of contracting malaria, with 1.2 billion living with a high risk. While access to vector control, preventive therapy and drug treatment have improved and mortality has been reduced, the development of resistant parasites is still a major concern for the future.

To diminish the likelihood of developing resistance with current antimalarials, combination therapies with artemisinins and a second longer half-life drug have displaced single agents such as chloroquine. Even so, the continued development of drug candidates with novel chemotypes and mechanisms of action against resistant strains is still necessary to respond to the significant clinical challenges. In particular, recent studies show declining rates of parasite clearance with artemisinin treatment in Southeast-Asia which is alarming [2-4], and as previously observed with chloroquine, resistance could spread across Asia to Africa and thus threaten global efforts to control and eliminate malaria. Coinciding with the development of resistance, the pharmaceutical industry started to screen their compound libraries against *P. falciparum* [5-7]; a process aided by the development of phenotypic HTS, and hits have been made publicly available by the Medicines for Malaria Venture (MMV) by a number of means, including the MMV Malaria Box [8]. In addition, the decoding of the *P. falciparum* genome sequence and analysis of new pathways that identify novel biological targets offers an opportunity to rationally design improved antimalarial scaffolds [9]. For maximum efficiency, new drugs should adhere to MMV's objective of single dose treatments which requires compounds with long duration of action.

1.1. Initial lead. Furans have recently been described with anti malarial activity [10]. A similar compound, **1** (Fig. 1), was identified from high throughput screening [6] at GlaxoSmithKline and showed modest activity against *Plasmodium falciparum* NF54 cell culture (EC_{50}, 875nM). Profiling

under Malaria Box protocols showed the compound had a long plasma half-life of greater than 6 h after oral administration (40 mg/kg) to mice, low cytotoxicity in MRC5 cells (EC_{50}, 17μM) and weak hERG inhibition (48% inhibition at 10 μM) [11]. Unfortunately, later studies revealed poor metabolic stability of **1** in human, rat and mouse liver microsomes through oxidation, which suggests that this high dose, long oral half-life in mice probably results from saturation of clearance pathways. Therefore, our goal was to enhance antiparasitic activity and improve metabolic stability of this lead structure. Three main structural elements were considered for optimization: the aryl-ring, the central furan-template and the diamine side-chain. The main problems with metabolic stability were associated with dehydrogenation and dealkylation of the amine side-chain, and oxidation of the furan and aryl rings. Herein, we describe the synthesis and systematic modification of all three subunits, and effects on biological activity and metabolic stability.

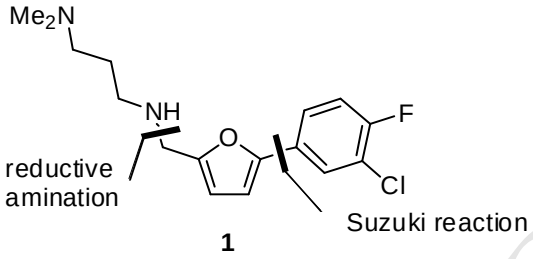
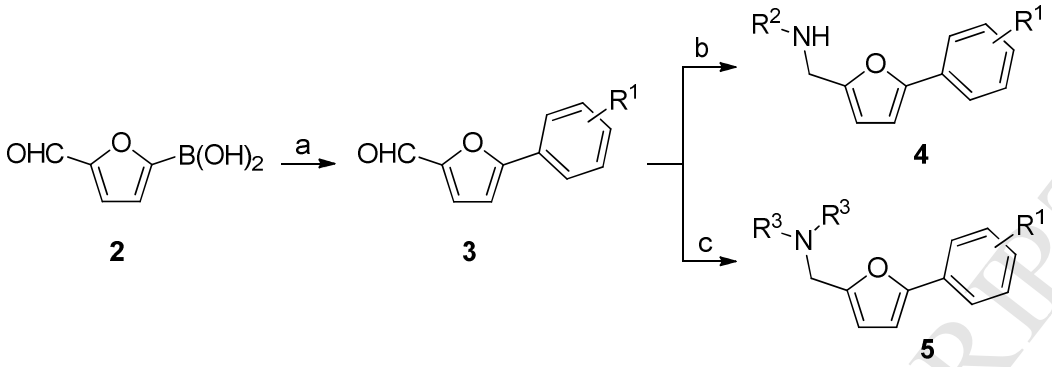


Fig. 1. Lead structure.

2. Results and discussion

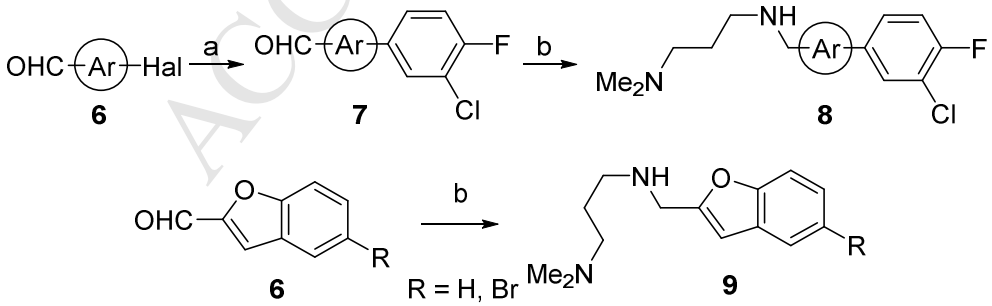
2.1. Chemistry. The synthesis of aryl and amine derivatives of **1** are outlined in Scheme 1 where boronic acid **2** was obtained in two steps from furfural using known conditions [12, 13]. This was then coupled with a number of aryl-halides via a Suzuki reaction to obtain intermediates of type **3** [14]. The substitution pattern of the aryl moiety was changed systematically from mono- to tri-substitution with a variety of combinations. Products were typically obtained with moderate to very good yields, with the exception of sterically hindered derivatives. Reductive aminations with primary amines were conducted in two steps, with confirmation of the identity of the imine-intermediates either by ^{1}H-NMR or IR. For secondary amines, reactions were carried out in one pot with delayed addition of sodium triacetoxyborohydride, and products **4** and **5** were obtained after purification. In some cases, free

amine products were converted into solid hydrochloride salts which are moderately hydroscopic in air, but stable if kept in a closed container.



Scheme 1. Synthesis of **4** and **5;** Reagents and conditions: (a) Ar-Hal, $PdCl_2(PPh_3)_2$ (5mol%), 2M $Na_2CO_{3\ (aq)}$, DME: EtOH (3:2), 60-75°C, 1-3h (36-95%); (b) primary amine, CH_2Cl_2 or Et_2O, then $NaBH_4$, MeOH (41-96%); (c) secondary amine, CH_2Cl_2, then $NaBH(OAc)_3$ (83-94%).

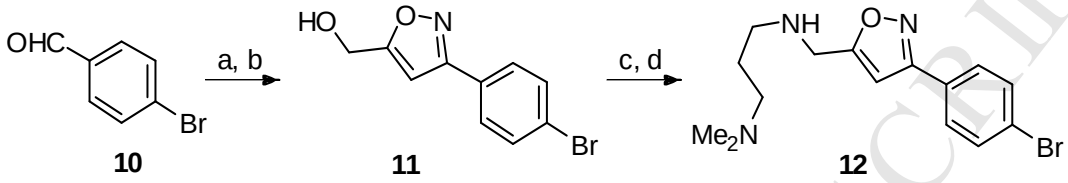
The majority of compounds where the furan template is replaced by different aryl rings were obtained from Suzuki coupling of the corresponding bromo-substituted aldehydes **6** with (3-chloro-4-fluorophenyl)boronic acid (Scheme 2), which was prepared under standard conditions [15]. In the case of the pyridine derivative **7b**, conditions using tetrakis(triphenylphosphine)palladium were applied to improve the yield significantly [16]. In the Suzuki coupling with the benzofurancarboxaldehyde, the temperature was increased to 100°C, maintaining the same palladium catalyst as for the furan series. The previously established reductive amination of intermediate **7** led to the derivatives of type **8**. Three benzofurans (**8d**, **9a** and **9b**) were prepared to investigate the potential for the introduction of fused ring systems.



Scheme 2. Synthesis of **8a-8d** and **9a-b;** Reagents and conditions: (a) (3-chloro-4-fluorophenyl)boronic acid, $PdCl_2(PPh_3)_2$ (5mol%), 2M $Na_2CO_{3\ aq}$, DME, EtOH, 60-100°C, 1-3h or

$Pd(PPh_3)_4$, 2M $Na_2CO_{3\ (aq)}$, toluene, MeOH, 110°C, 7h (75-88%). (b) $(CH_3)_2N(CH_2)_3NH_2$, Et_2O or CH_2Cl_2, then $NaBH_4$, MeOH (39-96%).

Finally, we were interested in replacing the furan ring by other five-membered heterocycles and we selected the isoxazole system described in Scheme 3. 4-Bromo benzaldehyde was converted into intermediate **11** through two steps [17], followed by oxidation with pyridinium chlorochromate and reductive amination to obtain the isoxazole derivative **12**.



10 **11** **12**

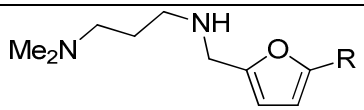
Scheme 3. Synthesis of **12;** Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, Na_2CO_3, MeOH, H_2O, rt; (b) propargyl alcohol, NEt_3, NCS, CH_2Cl_2, reflux (46% over two steps); (c) PCC, CH_2Cl_2, 4h, rt; (d) $(CH_3)_2N(CH_2)_3NH_2$, Et_2O, then $NaBH_4$, MeOH (65% over two steps).

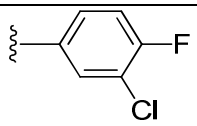
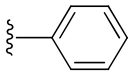
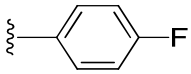
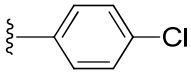
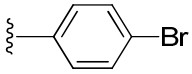
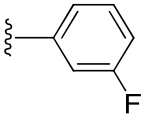
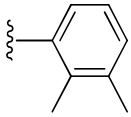
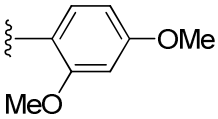
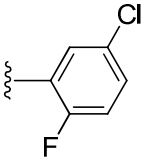
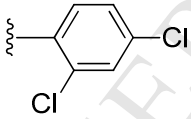
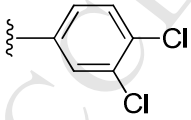
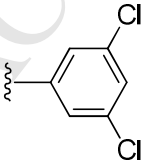
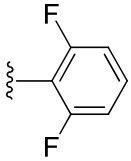
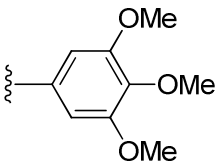
2.2. Structure-Activity Relationships. The biological activities of compounds were determined against the drug sensitive *P. falciparum* NF54 strain *in vitro* (EC_{50}, NF54) [18]. *In vitro* intrinsic clearance was assessed by substrate depletion upon incubation with human (HLM) and mouse (MLM) liver microsomes in the presence of the co-factor, NADPH. Data are summarized in Tables 1-4.

2.2.1. Modification of the aryl moiety. Of utmost importance for antiparasitic activity is *para* halogenation of the aryl moiety at the 2-position of the furan template (Table 1, **4d**: Cl or **4e**: Br), as removal (**4b**) or replacement with a smaller fluoro substituent (**4c**) leads to a dramatic decrease in potency. Additional *meta*-chloro substitution (**1** and **4k**) is tolerated as long as the *para*-position is also halogenated. Substitution in both *meta*-positions (**4l**) leads to a decrease in activity. Similar results were observed for the introduction of *ortho*-substituents as exemplified by comparison of **4d** with **4j**. With respect to rapid clearance, only the two methoxy-substituted derivatives (**4h** and **4n**) show low intrinsic clearance in human liver microsomes (HLM), which is likely to result from very low $cLogD_{7.4}$ (-0.5 and -0.7 respectively), but both derivatives showed a complete loss of antiparasitic activity.

Table 1.

Substitution of the Aryl Moiety.

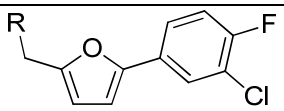


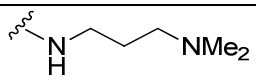
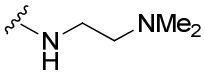
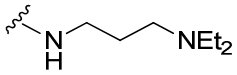
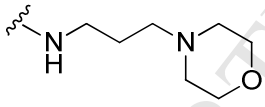
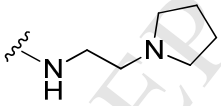
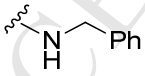
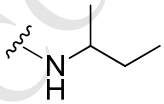
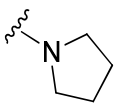
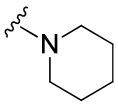
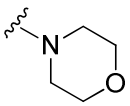
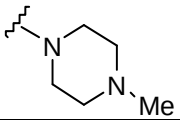
Compound number	R	NF54 EC_{50} (nM)	*In vitro* CL_{int} (µL/min/mg protein) HLM	MLM	$cLogD_{7.4}$
1		875	93	84	0.5
4a	H	>10000			0.7
4b		7206	42	110	-0.2
4c		2622	34	63	-0.1
4d		342	15	67	0.4
4e		442	19	84	0.6
4f		3599	N/A	N/A	0.0
4g		7224	24	200	0.8
4h		6311	< 7	74	-0.5
4i		1912	19	159	0.6
4j		1465	16	100	1.0
4k		263	28	95	1.0
4l		1190	16	89	1.0
4m		> 10000	34	285	0.1
4n		> 10000	< 7	16	-0.7

2.2.2. Modification on the amine side chain. An especially tight SAR was observed for modification on the amine side chain (Table 2). Complete activity loss followed from shortening the chain length (**5a** and **5d**), cyclisation to a morpholino derivative (**5c**) and the absence of the terminal tertiary amine (**5e**-**5i**). The only modifications that showed any activity came from replacement of the dimethylamine (**1**) with a diethylamine (**5b**), and incorporation of an N-methylpiperazine (**5j**), but without significant influence on the metabolic stability.

Table 2.

Replacement of the Amine Side Chain.



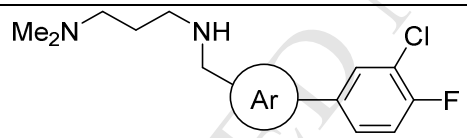
Compound number	R	NF54 EC_{50} (nM)	*In vitro* CL_{int} (µL/min/mg protein) HLM	MLM	$cLogD_{7.4}$
1		875	93	84	0.5
5a		5360	61	377	1.5
5b		1210	61	100	0.8
5c		9729	31	101	1.3
5d		6238	49	147	2.0
5e		> 10000	56	306	3.8
5f		> 10000	29	122	2.6
5g		> 10000	72	655	2.1
5h		> 10000	90	371	2.6
5i		> 10000	72	854	3.0
5j		1868	25	542	2.4

2.2.3. Modification of the furan ring. Various studies covered the structure activity relationship of heterocycles in relation to therapeutic properties, including anti malaria activity [19-21].

In an attempt to exchange the core heterocycle, several cyclic replacements were investigated. Exchange of the furan template with *meta*- or *para*-substituted phenyl rings or a 2,6-substituted pyridine was tolerated and resulted in activity comparable to the lead compound **1** (Table 3, **8a**-**8c)**. Interestingly, the 2,5-disubstituted benzofuran **8d** also showed good activity and a significant decrease in the microsomal intrinsic clearance. Moving the amine side chain to the *para* position of the phenyl ring (**8c**) gave an improvement in potency of 546 nM and also resulted in a significant improvement in metabolic stability in both HLM and MLM. Further testing of benzofuran analogs identified **9a** with only slightly decreased activity, but introducing a 5-bromo substituent (**9b**) led to a complete loss of potency (Table 4). Similarly, the isoxazole derivative **12** was inactive.

Table 3.

Replacement of the Furan Ring.



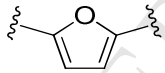
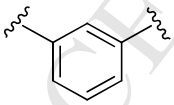
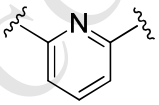
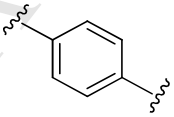
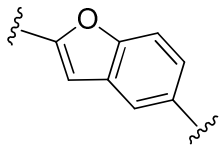
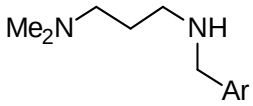
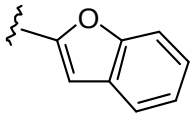
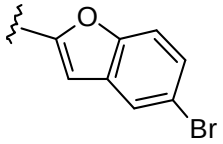
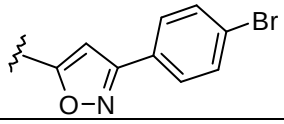
Compound number	Ar	NF54 EC_{50} (nM)	*In vitro* CL_{int} (µL/min/mg protein) HLM	MLM	$cLogD_{7.4}$
1		875	93	84	0.5
8a		475	113	50	0.7
8b		822	170	52	0.8
8c		546	20	41	0.8
8d		421	13	69	1.6

Table 4.

Replacement of the Furan Ring.



Compound number	Ar	NF54 EC_{50} (nM)	*In vitro* CL_{int} (µL/min/mg protein) HLM	MLM	$cLogD_{7.4}$
9a		804	N/A	N/A	-0.8
9b		5849	35	105	0.0
12		> 5000	24	53	0.3

2.3. Stability and clearance. Compound **1** was stable in freshly collected rat plasma *in vitro* and demonstrated extensive distribution into erythrocytes with a whole blood to plasma concentration ratio of approximately 5.

On dosing **1** to Sprague Dawley rats (1 mg/kg IV, 20 mg/kg po, Fig. 2A), the compound rapidly and extensively distributed from plasma (at least partially into erythrocytes) with a long apparent terminal half-life. Plasma concentration versus time profiles were not well defined, and there was considerable variability in the IV plasma concentrations at later time points precluding the assessment of pharmacokinetic parameters (clearance, volume of distribution, half-life and oral bioavailability). Following administration to mice (Fig. 2B), plasma concentrations again showed rapid distribution and concentrations were higher than seen in rats even after accounting for the difference in dose. *In vitro* studies suggested that intrinsic clearance in rat liver microsomes (>500 µL/min/mg) was considerably higher than that in HLM or MLM (Table 1) which may at least partially account for these differences. Overall, plasma concentrations of **1** after oral dosing to mice were considerably below what was likely to be needed for efficacy given the EC_{50} of ~0.9 µM.

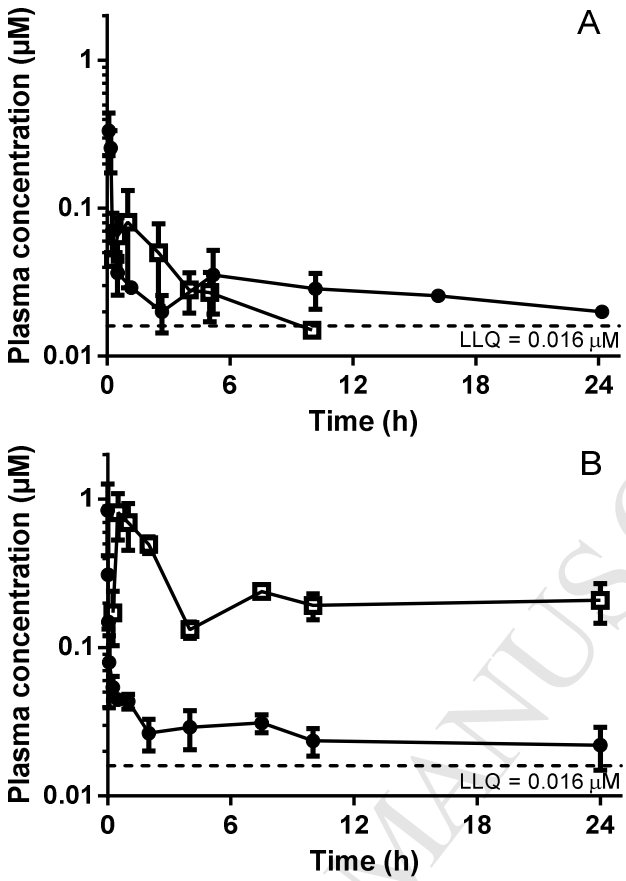


Fig. 2. Plasma concentration versus time profiles of **1** in (A) male Sprague Dawley rats (0.9 mg/kg IV (filled circles) and 19 mg/kg oral (open squares) administration) and (B) male Swiss outbred mice (1.1 mg/kg IV (filled circles) and 48 mg/kg oral (open squares) administration). Data points represent the mean and range for n=2 rats or n=2 mice at each time point.

Metabolite ID was performed by incubation of **1** with human, mouse and rat liver microsomes over a period of 60 min. Several metabolites were detected (Table 5) including a mono-oxygenation or furan ring opened metabolite (M+16) seen in each species, two distinct M-2 metabolites, one present in RLM (M-2 (I)) and the other in MLM and HLM (M-2 (II)), an M-14 metabolite likely corresponding to N-demethylation (HLM and MLM only), and two secondary M+32 bis-oxygenation metabolites in RLM. Even though full structure elucidation was not conducted, these preliminary results suggest significant species differences in the metabolic pathways and major metabolites formed.

Table 5.

Metabolite profile in liver microsomes.

Species	% Substrate Consumed	Relative Peak Area (as % of Total Metabolite Peak Area)					
		M+16	M-2 (I)	M-2 (II)	M-14	M+32 (I)	M+32 (II)
Human	88	12	ND	57	31	ND	ND
Rat	99	5	85	ND	ND	5	5
Mouse	86	89	ND	7	4	ND	ND

ND = not detected

As **4k** showed good activity and slightly better metabolic stability in HLMs, it was also tested *in vivo* to determine pharmacokinetic properties (Fig. 3). As with **1**, a rapid decline in plasma concentrations after IV administration was seen, at least partially due to high partitioning into erythrocytes (*in vitro* whole blood to plasma partitioning ratio ~ 6.9). Blood clearance (~17 mL/min/kg) was moderate and volume of distribution (Vss, 16 L/kg) was high. Oral bioavailability for **4k** was ~16%, with maximum plasma concentrations detected 30 min post-dose suggesting rapid absorption. Plasma profiles after both oral and IV administration exhibited a prolonged terminal phase with half-lives ranging from 9-25 h (oral) and 10-17 h (IV). Collectively, the pharmacokinetic parameters for **4k** represent a moderate improvement compared to those seen for **1**.

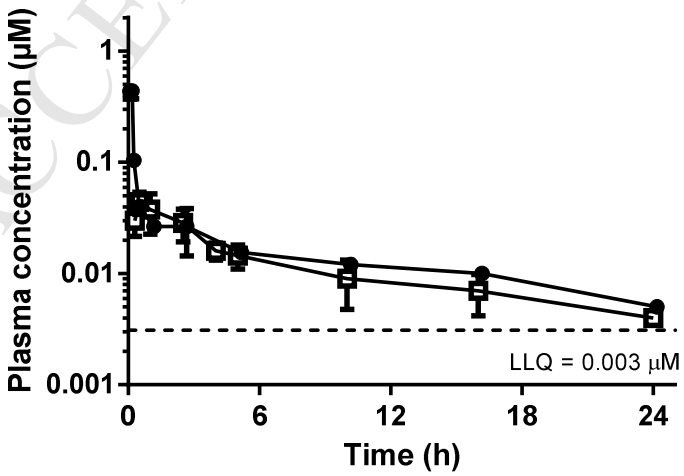


Fig. 3. Plasma concentrations of **4k** in male Sprague Dawley rats following IV (1.2 mg/kg, filled circles) and oral (5.5 mg/kg, open squares) administration. Data points represent the mean and range for n=2 animals.

2.4. Malaria assays.

The parasite reduction ratio (PRR) quantifies the speed of killing observed *in vitro* by measuring the number of viable parasites after treatment of the *Pf* 3D7A strain at a concentration of 10 times the IC_{50} of the selected drug [22]. Both **4k** and **8d** showed a chloroquine-like profile, which is consistent with a fast kill rate (Fig. 4). As the rate of killing is believed to be related to the mode of action, this suggests that the furan and benzofuran disrupt a common target. Compound **8d** also maintained potency when tested on the *Pf* K1 cell line which is resistant to the antimalarial drugs chloroquine, pyrimethamine and sulfadoxine (EC_{50}, 490 nM vs drug sensitive *Pf* NF54 strain EC_{50}, 309 nM) [23], but showed no efficacy in a *P. berghei* infected mouse efficacy model after 4 oral daily doses at 50 mg/kg [24, 25]. The same derivatives (**4k** and **8d**) showed no *in vitro* activity against *P. berghei* liver stage parasites at a concentration of 10 µM [26, 27], and no cytotoxicity to HepG2 cells at the same concentration.

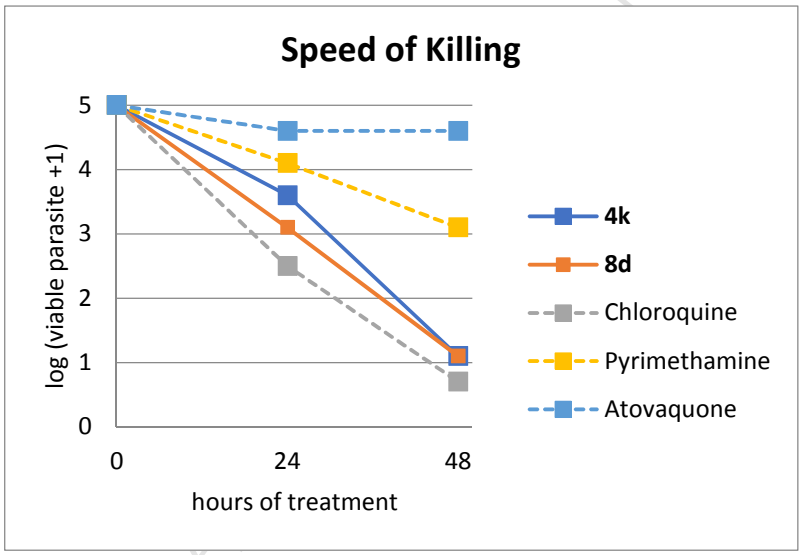


Fig. 4. Speed of killing.

To assess the potential of the series to generate resistance and to give insights into the mode of action, Dd2 parasites were incubated with sub-lethal concentrations of **1** for extended periods [28]. After 125 days incubation, no resistant parasites were obtained, suggesting that this series has a low propensity to generate resistance.

3. Conclusion. A novel chemotype with blood stage antimalarial activity has been discovered with nanomolar antiparasitic activity, but with tight SAR limitations. Several analogues showed lower *in vitro* intrinsic clearance compared to **1** in HLMs and one derivative (**4k**) demonstrated reasonable pharmacokinetic properties *in vivo* in rats. The low propensity to generate resistance suggests further work is warranted to determine the mode of action and further characterize the series.

4. Experimental section.

4.1. Materials and Methods. Dichloromethane (CH_2Cl_2) was distilled from CaH_2. Flash column chromatography was conducted using silica gel (230−400 mesh) or aluminum oxide (activated, neutral, Brockmann I, ~150 mesh). Analytical thin-layer chromatography was performed on silica gel 60 F_{254} plates, and the visualization was accomplished using UV light and phosphomolybdic acid followed by heating. ^{1}H and proton-decoupled ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD at 250 MHz (^{1}H) and 60 MHz (^{13}C), 400 MHz (^{1}H) and 100 MHz (^{13}C), 500 MHz (^{1}H) and 125 MHz (^{13}C), or 600 MHz (^{1}H) and 150 MHz (^{13}C). The chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) at 0.00 ppm or the solvent signal as an internal standard ($CDCl_3$ at 7.26 ppm and CD_3OD at 3.30 ppm for ^{1}H NMR spectra and $CDCl_3$ at 77.0 ppm and CD_3OD at 49.0 ppm for ^{13}C NMR spectra). Data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet, dd = doublet of doublets, ddd = doublet of doublet of doublets, m = multiplet; coupling constant(s) in Hz; integration. High-resolution mass spectrometry (HRMS) was performed using the electrospray ionization (ESI) technique. The parent ions ([M + H]+ or [M + Na]+) are cited. The purity (≥95%) of all compounds evaluated in this work was determined by ^{1}H NMR.

4.2. Chemistry

4.2.1. Method A - Suzuki Reaction: 1.00 mmol Arylhalide, 1.30 mmol furfural-boronic acid and 0.05 mmol Bis(triphenylphosphine)palladium(II) dichloride were treated with 0.30 ml dimethoxyethane, 0.50 ml ethanol and 0.30 ml aqueous 2M sodium carbonate solution. The reaction was heated to 65°C for 1h or until the TLC showed no remaining starting material. The mixture was evaporated and extracted three times with ethyl acetate. The combined organic layers were washed

with brine, dried over MgSO4, filtered and concentrated. The crude product was purified by column chromatography using hexanes/ethyl acetate (9:1).

4.2.2. Method B - Reductive amination with primary amines: 1.00 mmol Aldehyde and 1.20 mmol primary amine were stirred in 3.33 ml dichloromethane or diethyl ether for 20h at room termperature. Conversion into the imine was confirmed by ^{1}H-NMR or IR. The reaction was dried over $MgSO_4$, filtered and concentrated. The crude intermediate was dissolved in 2 ml methanol, treated with 2.00 mmol sodium borohydride and stirred over night at room temperature. The solvent was evaporated and the residue was stirred with 1M aqueous sodium hydroxide solution and dichloromethane for 20min. Afterwards, the layers were separated and it was extracted 3 times with dichloromethane. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. If necessary, the product was purified by column chromatography on neutral alumnia with methanol in dichloromethane.

In the case of hydrochlorides, the crude product was dissolved in dichloromethane and dropwise added to a 0.5M solution of hydrochloric acid in diethylether. The precipitate was recrystalized from methanol with diethylether and dried in vacuum.

4.2.3 Method C - Reductive amination with secondary amines: 0.40 mmol Aldehyde and 0.52 mmol secondary amine were stirred in 2.50 ml dichloromethane for 24h, then 0.45 mmol sodium triacetoxyborohydride were added and it was stirred additional 24h. The mixture was washed with saturated aqueous sodium bicarbonate solution and brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography on neutral alumnia with methanol in dichloromethane.

4.2.4. 6-(3-Chloro-4-fluorophenyl)picolinaldehyde (7b): To a mixture of 6-bromo-2-pyridinecarboxaldehyde (37 mg, 0.20 mmol) in degassed toluene (0.18 mL) and degassed MeOH (0.07 mL), 3-chloro-4-fluorophenylboronic (49 mg, 0.29 mmol), Tetrakis(triphenylphosphine)palladium(0) (9 mg, 0.008 mmol) and degassed Na_2CO_3 2M aqueous (0.09 mL) were added. The reaction was heated at 110^{o}C for 7h under argon. Then, it was cooled and stirred with 0.35 mL aqueous 2M Na_2CO_3 for 5min. The layers were separated and it was extracted 4 times with dichloromethane (2.5 mL). The combined organic layers were washed with brine (0.5 mL),

dried over MgSO4, filtered and concentrated. The crude product was purified by column chromatography using hexanes/ethyl acetate (7:1) and then by recrystallization with CH_2Cl_2/hexanes. The procedure provided 45 mg of the title compound as a white solid. **Yield**: 96%. **^{1}H NMR** (250 MHz, $CDCl_3$): δ = 10.15 (s, 1H), 8.20 (dd, *J* = 2.3, 7.1 Hz, 1H), 8.04-7.86 (m, 4H), 7.28 (t, *J* = 8.6 Hz, 1H). **^{13}C** NMR (60 MHz, $CDCl_3$): δ = 193.5, 159.1 (d, *J*(C,F) = 252,3 Hz), 155.5, 152.8, 138.1, 135.2 (d, *J*(C,F) = 3.8Hz), 129.4, 126.7 (d, *J*(C,F) = 7.5 Hz), 124.0, 121.9 (d, *J*(C,F) = 18.1 Hz), 120.2, 117.0 (d, *J*(C,F) = 21.4 Hz).

4.2.5. (3-(4-Bromophenyl)isoxazol-5-yl)methanol (11): To a mixture of 4-bromobenzaldehyde (1.85 g, 10 mmol) in of methanol (5 mL) and of water (15 mL), $NH_2OH{\cdot}HCl$ (836 mg, 12 mmol) was added at room temperature. After 40min, Na_2CO_3 (634 mg, 6 mmol) was carefully added to the mixture containing a white precipitate. The reaction mixture was stirred overnight and then filtered. The white solid was dried under vacuum and resulted in the desired oxime (1817 mg) which was used in the next step without purification. Yield: 91%. Under argon, *N*-chlorosuccinimide (480 mg, 3.6 mmol) was added to a solution of 4-bromobenzaldehyde oxime (600 mg, 3.0 mmol) in dry dichloromethane (4.5 mL) After 50min, propargyl alcohol (0.22 mL, 3.6 mmol) and dry Et_3N (0.46 mL, 3.3 mmol) were added to the green reaction. (CAUTION! The solution fumes). The mixture was refluxed for 4.5h and then washed with 2 mL of brine. The layers were separated and it was extracted 3 times with dichloromethane (15 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography using hexanes/ethyl acetate (1:1) and provided of the title compound as a white solid (392 mg). **Yield**: 51%. **^{1}H NMR** (250 MHz, $CDCl_3$): δ = 7.54-7.72 (m, 4H), 6.54 (s, 1H), 4.83 (d, *J* = 4.5 Hz, 1H), 2.28 (bs, 1H).

4.2.6. 3-(4-Bromophenyl)isoxazole-5-carbaldehyde: To a mixture of of PCC (32 mg, 0.15 mmol) in dry dichlorometane (0.5 mL), (3-(4-bromophenyl)isoxazol-5-yl)methanol (25 mg, 0.10 mmol) was added at room temperature. The dark brown mixture was stirred for 5h and then filtrated in a short silica pad with diethyl ether (15 mL). The ethereal phase was concentrated, purified by column chromatography using hexanes/ethyl acetate (7:3) and provided the title compound as a white solid (43 mg). **Yield**: 86%. **^{1}H NMR** (250 MHz, $CDCl_3$): δ = 10.04 (s, 1H), 7.77-7.69 (m, 2H), 7.69-7.60

(m, 2H), 7.26 (s, 1H). **^{13}C NMR** (60 MHz, $CDCl_3$): δ = 178.3, 166.5, 162.3, 132.4, 128.3, 126.6, 125.3, 106.2.

4.2.7. *N*^{1}-((3-(4-Bromophenyl)isoxazol-5-yl)methyl)-*N*^{3},*N*^{3}-dimethylpropane-1,3-diamine hydrochloride (12): Using method C with 3-(4-bromophenyl)isoxazole-5-carbaldehyde (22 mg, 0.09 mmol), the crude product was dissolved in a small amount of dichlorometane and converting it in the dihydrochloride salt by adding excess of HCl 0.5M in Et_2O. The precipitate was filtered and dried under vacuum to give the title compound as a white solid (25 mg). **Yield**: 71%. **^{1}H NMR** (500 MHz, MeOD): δ = 7.84-7.79 (m, 2H), 7.71-7.67 (m, 2H), 7.18 (s, 1H), 4.60 (s, 2H), 3.32-3.24 (m, 4H), 2.94 (s, 6H), 2.28-2.19 (m, 2H). **^{13}C NMR** (125 MHz, MeOD): δ = 164.8, 163.6, 133.5, 129.6, 128.7, 125.8, 105.7, 55.5, 45.7, 43.6, 42.6, 22.6. **HRMS**: ($M+H^+$) calc for C15H21BrN3O, 338.08625; found, 338.08566.

ASSOCIATED CONTENT

Supporting Information. Additional details of the synthesis and characterization of the described compounds, and the procedures used for the metabolism and PK studies, as well as in the *in vitro* and *in vivo* antimalarial studies. This material is available free of charge via the Internet.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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ABBREVIATIONS

Ar, aromatic ring; DME, dimethoxyethane; HLM, human liver microsomes; HTS, high throughput screening; MLM, mouse liver microsomes; NCS, N-chlorosuccinimide; PCC, pyridinium chlorochromate; PK, pharmacokinetics; PRR, parasite reduction ratio.

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List of caption:

TITLE: Novel Inhibitors of Plasmodium Falciparum based on 2,5-disubstituted Furans

Highlights

- Furans as new chemotype for Plasmodium falciparum inhibition
- Examples classified as fast killers in parasite reduction ratio assay
- No resistance induced in Dd2 parasites