Supporting information

Discovery of a quinoline-4-carboxamide derivative with a novel mechanism of action, multi-stage antimalarial activity, and potent *in vivo* efficacy

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Biology Methodology

Plasmodium falciparum screening

Assays against *P. falciparum* were conducted as previously described.[1] Cultures of the widely-used malaria reference strain of chloroquine-sensitive *Plasmodium falciparum* strain 3D7 were maintained in a 5% suspension of human red blood cells (obtained from East of Scotland Blood Transfusion Service, Ninewells Hospital, Dundee) cultured in RPMI 1640 medium (pH 7.3) supplemented with 0.5% Albumax II (Gibco Life Technologies, San Diego, CA), 12 mM sodium bicarbonate, 0.2 mM hypoxanthine and 20 mg/L gentamicin at 37°C, in a humidified atmosphere of 1% O_2, 3% CO_2 with a balance of nitrogen. Growth inhibition was quantified using a fluorescence assay utilising the binding of SYBR green to double stranded DNA, which emits a fluorescent signal at 528 nm after excitation at 485nm.[2] Mefloquine (potency range 30-60 nM) was used as a drug control to monitor the quality of the assay (Z' = 0.6 to 0.8, Signal to background $\geq$3, where Z' is a measure of the discrimination between the positive and negative controls on a screen plate). A 96-well [^{3}H]-Hypoxanthine incorporation assay was also developed as a secondary assay in order to validate compounds from the initial screen in an orthogonal platform.[3] Compound bioactivity from both assays was expressed as EC_{50}, the effective concentration of compound causing 50% inhibition of parasite growth.

P. falciparum (panel of resistant strains) in vitro assay. (Swiss TPH)

All Plasmodium strains were cultured according to Trager and Jensen and are described at www.beiresources.org (resistant strains: K1, W2, 7G8, TM90C2A, D6 and V1/S, sensitive strain: NF54). EC_{50} values were determined in vitro by measuring incorporation of the nucleic acid precursor [^{3}H]hypoxanthine.[1c]

Mammalian Cell Growth Inhibition assay

A counter-screen against normal diploid human fibroblasts (MRC-5 cell line) was carried out to exclude non-selective, and toxic compounds. The assay was essentially carried out as described previously.[4] Cells were plated and incubated overnight to allow them to adhere as monolayers. A working stock of each test compound was transferred to an intermediate 384-well plate and pre-diluted with minimum essential media (MEM). The pre-diluted stocks were then transferred onto the cell monolayers, and the plates were incubated for 68 h. Resazurin, to a final concentration of 50 µM, was added to each well, after which plates were incubated for a further 3 h and measured for fluorescence (λ_{ex}=528 nm, λ_{em}=590 nm)

In vitro Cell Assay Data Analysis.

All data was processed using IDBS ActivityBase® raw data was converted into per cent inhibition through linear regression by setting the high inhibition control as 100% and the no inhibition control as 0%. Quality control criteria for passing plates were as follows: Z'> 0.5, S:B> 3, $\%CV_{(\text{no inhibition control})} < 15$. The formula used to calculate Z' is $1 - \frac{3 \cdot (StDev\_high + StDev\_low)}{ABS(Mean\_high - Mean\_low)}$.

All EC_{50} Curve fitting was undertaken using XLFit version 4.2 using Model 205 with the following 4 parametric equation: $y = A + \frac{B - A}{1 + (C/x)^D}$ $A + \frac{(B-A)}{(1+(C/x)^D)}$,where A=% inhibition at bottom, B=%

inhibition at top, C= EC_{50}, D= slope, *x*= inhibitor concentration and y= % inhibition. If curve did not reach 100% of inhibition, B was fixed to 100 only when at least 50% of inhibition was reached.

***Plasmodium falciparum* late stage (IV-V) gametocyte high content imaging assay**

Plasmodium falciparum NF54$^{-pfs16\text{-}LUC\text{-}GFP}$ stage IV-V Gametocytes were produced as described previously.[5] Compounds were solubilized in DMSO and serially diluted in three concentrations per log standard curves in DMSO. One µl of compound was subsequently diluted in 25µl of water (4% DMSO) and 5µl transferred into the base of 384 well PerkinElmer CellCarrier plates. Forty-five µl of stage IV-V gametocytes (10%P in 0.1%H) were added to the imaging plates which were in turn sealed with a gas permeable membrane and incubated at 37°C, 5% CO_2, 5% O_2, 90% N_2 and 60% humidity for 72hours.

Post incubation, 5µl of MitoTracker® Red CM-H2Xros (MTR) solution (0.66ug/ml) was added to all wells and the plates incubated for a further 16hours. The assay plates were then imaged and the number of viable gametocytes determined for test compounds plus 0.4%DMSO and 5µM Puromycin. All data was normalized to % inhibition using the positive (5uM puromycin) and negative (0.4%DMSO) control values. Percent inhibition was plotted against log compound concentration using a 4 parameter log dose, non-linear regression analysis, with sigmoidal dose response (variable slope) curve fit using Prizm 4.0. IC50 values were obtained from this curve fit. The compound testing was performed in duplicate point within two separate experiments.

***Plasmodium berghei* ookinete development assay**

Assay was performed as previously reported.[6] Briefly blood containing *P. berghei* CTRPp-GFP gametocytes was rapidly harvested from infected mice and introduced to test compounds pre-diluted in ookinete medium (to induce gamete formation and support ookinete development) in 96-well plates. Plates were incubated in the dark at 21°C for 22 hr before GFP fluorescence intensity measured in a plate reader. % inhibition of ookinete formation was calculated with reference to the fluorescence intensity values of a positive control (20 µM cycloheximide) and a solvent-only negative control (DMSO). All assays were performed in triplicate independent experiments and EC_{50} values calculated in Graphpad Prism.

Aqueous solubility

The aqueous solubility of the test compounds was measured using laser nephelometry, as described previously.[7] Compounds were subject to serial dilution from 10 mg/mL to 0.5 mg/mL in DMSO. An aliquot was then mixed with MilliQ water to obtain an aqueous dilution plate with a final concentration range of 100 – 5 µg/mL, with a final DMSO concentration of 1.0%. Triplicate aliquots were transferred to a flat bottomed polystyrene plate which was immediately read on the NEPHELOstar (BMG Lab Technologies). The amount of laser scatter caused by insoluble particulates (relative nephelometry units, RNU) was plotted against compound concentration using a segmental regression fit, with the point of inflection being quoted as the compounds aqueous solubility (µg/mL; reported in µM).

Intrinsic Clearance (Cli) experiments

The procedure was carried out as reported previously.[7] Test compound (0.5 µM) was incubated with female CD1 mouse, male sprague-dawley rat or pooled human mixed gender liver microsomes (Xenotech LLC ^{TM} ; 0.5 mg/mL 50 mM potassium phosphate buffer, pH 7.4) and the reaction initiated

by the addition of excess NADPH (8 mg/mL 50 mM potassium phosphate buffer, pH 7.4). Immediately, at time zero, then at 3, 6, 9, 15 and 30 min an aliquot (50 µL) of the incubation mixture was removed and mixed with acetonitrile (100 µL) to stop the reaction. Internal standard was added to all samples, the samples centrifuged to sediment precipitated protein and the plates then sealed prior to UPLC-MS/MS analysis using a Quattro Premier XE (Waters Corporation, USA).

XLfit (IDBS, UK) was used to calculate the exponential decay and consequently the rate constant (k) from the ratio of peak area of test compound to internal standard at each timepoint. The rate of intrinsic clearance (CLi) of each test compound was then calculated using the following calculation:

CLi (mL/min/g liver) = k x V x Microsomal protein yield

Where V (mL/mg protein) is the incubation volume/mg protein added and microsomal protein yield is taken as 52.5 mg protein per g liver. Verapamil (0.5 µM) was used as a positive control to confirm acceptable assay performance. Experiments were performed using a single time-course experiment.

Plasma Protein Binding (PPB) experiments

This was based on a previously described method, with the exception that NMRI mouse plasma was used here.[8] In brief, a 96 well equilibrium dialysis apparatus was used to determine the free fraction in plasma for each compound (HT Dialysis LLC, Gales Ferry, CT). Membranes (12-14 kDA cut-off) were conditioned in deionised water for 60 min, followed by conditioning in 80:20 deionised water:ethanol for 20 min, and then rinsed in isotonic buffer before use. Female CD1 mouse plasma was removed from the freezer and allowed to thaw on the day of experiment. Thawed plasma was then centrifuged (Allegra X12-R, Beckman Coulter, USA), spiked with test compound (final concentration 10 µg/mL), and 150 µL aliquots (n=6 replicate determinations) loaded into the 96-well equilibrium dialysis plate. Dialysis against isotonic buffer (150 µL) was carried out for 5 h in a temperature controlled incubator at ~37ºC (Barworld scientific Ltd, UK) using an orbital microplate shaker at 100 revolutions/minute (Barworld Scientific Ltd, UK). At the end of the incubation period, 50 µL aliquots of plasma or buffer were transferred to micronic tubes (Micronic B.V., the Netherlands) and the composition in each tube balanced with control fluid (50 µL), such that the volume of buffer to plasma is the same. Sample extraction was performed by the addition of 200 µL of acetonitrile containing an appropriate internal standard. Samples were allowed to mix for 1 min and then centrifuged at 3000 rpm in 96-well blocks for 15 min (Allegra X12-R, Beckman Coulter, USA) after which 150 µL of supernatant was removed to 50 µL of water. All samples were analysed by UPLC-MS/MS on a Quattro Premier XE Mass Spectrometer (Waters Corporation, USA). The unbound fraction was determined as the ratio of the peak area in buffer to that in plasma. Experiments were run in triplicate.

Parallel Artificial Membrane Permeability Assay (PAMPA)

PAMPA was performed using a 96-well pre-coated BD Gentest™ PAMPA plate (BD Biosciences, U.K.). Each well was divided into two chambers; donor and acceptor, separated by a lipid-oil-lipid tri-layer constructed in a porous filter. The effective permeability, P_e, of the compound was measured at pH 7.4. Stock solutions (5 mM) of the compound were prepared in DMSO. The compound was then further diluted to 10 µM in phosphate buffered saline at pH 7.4. The final DMSO concentration did not exceed 5 % v/v. The compound dissolved in phosphate buffered saline was then added to the donor side of the membrane and phosphate buffered saline without compound was added to the acceptor side. The PAMPA plate was left at room temperature for 5 h. After which time, an aliquot

(100 µl) was then removed from both acceptor and donor compartments and mixed with acetonitrile (80 µl) containing an internal standard. The samples were centrifuged (10 min, 5^{0}C, 3270 g) to sediment precipitated protein and sealed prior to UPLC-MS/MS analysis using a Quattro Premier XE (Waters Corp, USA). P_e was calculated as shown in the below equation:

$$P_e \text{ (nm/sec)} = 10^7 \times \left[\frac{- \ln [1 - C_A(t) / C_{equi}]}{A * (1/V_D + 1/V_A) * t} \right]$$

Where:

$C_A(t)$ = peak area of compound present in acceptor well at time t = 18000 sec

C_{equi} = $[C_D(t) * V_D + C_A(t) * V_A] / (V_D + V_A)$

A = filter area

V_D = donor well volume

V_A = acceptor well volume

t = incubation time

$C_D(t)$ = peak area of compound present in donor well at time t = 18000 sec

Recovery of compound from donor and acceptor wells was calculated and data was only accepted when recovery exceeded 70 %.

Human ether-à-go-go related gene (hERG) K^+ assay (Outsourced)

Compounds were tested for inhibition of the human ether-à-go-go-related gene (hERG) K^+ channel using IonWorks patch clamp electrophysiology. Eight-point concentration-response curves were generated on 2 occasions using 3-fold serial dilutions from the maximum final assay concentration.

Mouse pharmacokinetics

Compound was dosed as a bolus solution intravenously (**12**) at 3mg free base/kg (dose volume: 5 mL/kg; dose vehicle: 10% DMSO, 90% saline) to female NMRI mice (n=3) or dosed orally (**12** and **13**) by gavage as a solution at 10 mg free base/kg (dose volume: 10mL/kg; Dose vehicle: 5 or 10% DMSO; 40% PEG400; 50 or 55% distilled water) to female NMRI mice (n=3/dose level). Female NMRI mice were chosen as these represent the sex and strain used for the P.berghei mouse model of malaria. Blood samples (10 µl) were taken from each mouse at 5, 15 and 30 minutes, 1, 2, 4, 6, and 8 hours post-dose, mixed with two volumes of distilled water and stored frozen until UPLC-MS/MS analysis. The level of each compound in mouse blood was determined by UPLC-MS/MS as previously reported.[9] Pharmacokinetic parameters were derived from the blood concentration time curve using PKsolutions software v 2.0 (Summit Research Services, USA).

Rat Pharmacokinetics:

Compound **2** was dosed as a bolus solution intravenously at 1 mg free base/kg (dose volume: 5 mL/kg; dose vehicle: 5% DMSO;saline) or by gavage orally as solution at 5 mg free base/kg (dose volume: 10 mL/kg; Dose vehicle: 0.5% w/v hydroxypropylmethylcellulose with 0.4% v/v Tween 80 and 0.5% v/v benzyl alcohol) to male Sprague Dawley rats (n=3/dose route). Blood samples were taken from each rat at 5, 15, 30 min, 1, 2, 4, 6, 8, 24, 30 (PO only) and 48 (PO only) h post-dose, mixed with two volumes of distilled water and stored frozen until UPLC/MS/MS analysis.

Compound **41** was dosed as a bolus solution intravenously at 1 mg free base/kg (dose volume: 5 mL/kg; dose vehicle: 5% DMSO;saline) or by gavage orally as solution at 3 mg free base/kg (dose volume: 10 mL/kg; Dose vehicle: 0.5% w/v hydroxypropylmethylcellulose with 0.4% v/v Tween 80 and 0.5% v/v benzyl alcohol) to male Sprague Dawley rats (n=3/dose route). Blood samples were taken from each rat at 5, 15, 30 min, 1, 2, 4, 6, 8, 24 and 30 h post-dose, mixed with two volumes of distilled water and stored frozen until UPLC/MS/MS analysis.

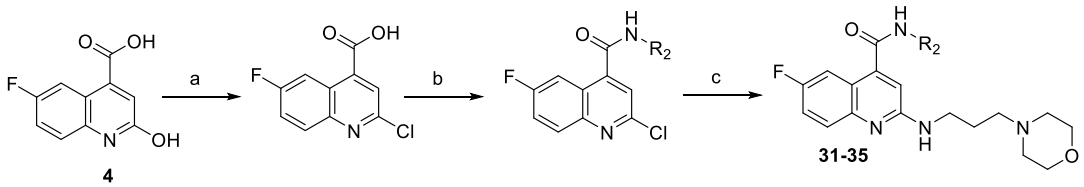
Pharmacokinetic parameters were derived from the blood concentration time curve for each compound using PKsolutions software v 2.0 (Summit Research Services, USA).

***In vivo* antimalarial efficacy studies in *P. berghei*. (Swiss TPH)**

In vivo efficacy was conducted as previously described[10] with the modification that female NMRI mice (n = 3) were infected with a GFP-transfected *P. berghei* ANKA strain (donated by A. P. Waters and C. J. Janse, Leiden University, The Netherlands), and parasitaemia determined (96 h after infection) using standard flow cytometry techniques. Compounds **1, 25, 27, 30** and **49** were prepared in 10% DMSO, 40% PEG 400 and 50% water prior to administration orally, once daily for 4 days. Compounds **40, 41, 43** and **44** were prepared in a non-solubilizing, standard suspension vehicle called HPMC (0.5% [wt/vol] hydroxypropylmethylcellulose, 0.5% [vol/vol] benzyl alcohol, 0.4% [vol/vol] Tween 80, and 0.9% [wt/vol] sodium chloride in water) prior to administration orally, once daily for 4 days. Blood samples were collected on day 4 (96 h after infection) in two volumes water. Animals were observed for signs of overt toxicity/poor tolerability every 15 min for the first hour post dosing and then hourly up to 4 h after dosing each day. The animals were selected randomly for each group but were not blinded.

Chemistry Experimental Section

Scheme S1



Conditions: (a) $POCl_3$, reflux, 52%; (b) amine, EDC, HOBt, DMF or amine, CDMT, NMO, DCM, 16-25%; (c) 3-morpholinopropylamine, acetonitrile, 170°C, microwave, 2h or 3-morpholinopropylamine, NMP, 200°C, microwave, 10 min, 29-42%

2-Chloro-6-fluoroquinoline-4-carboxylic acid. A solution of 6-fluoro-2-hydroxy-quinoline-4-carboxylic acid (**4,** R_1=F) (10.6 g) in $POCl_3$ (70 mL) was refluxed for 2h. Reaction mixture was allowed to cool down to room temperature and $POCl_3$ was evaporated under reduced pressured. Reaction crude was poured over ice water (400 mL) and stirred for 30 minutes at room temperature. The resulting precipitate was filtered and washed with water (200 mL). Solid was then stirred in 100 mL of 0.5 M KOH until fully dissolved and pH was adjusted to pH 2 with 3N HCl. Precipitate was filtered and dried to the desired product as an off white solid. Product was used for the next step without further purification. Yield, 52% (6 g) ^{1}H NMR (500 MHz, DMSO) δ 8.46 (dd, *J* = 3.0, 10.9 Hz, 1H), 8.11 (dd, *J* = 5.7, 9.3 Hz, 1H), 7.97 (s, 1H), 7.85-7.80 (m, 1H) ppm; LC-MS m/z 226 $(M+H)^+$.

General procedure D: Preparation of 2-chloro-6-fluoroquinoline 4-carboxamides intermediates. 2-Chloro-6-fluoroquinoline-4-carboxylic acid (1 eq), 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (1.2 eq) and *N*-methylmorpholine (2 eq) in DCM (3 mL) was stirred at room temperature for 1h. The corresponding amine (1.5 eq) was added and the reaction mixture was stirred at room temperature overnight. Reaction was partitioned between DCM (50 mL) and $NaHCO_3$ sat. aq. solution (10 mL). Organic phase was dried over $MgSO_4$ and solvents were removed under reduce pressure. Solvents were removed and product was purified by column chromatography on 12 g silica cartridges using A: DCM and B: MeOH.

2-Chloro-6-fluoro-*N*-(2-(4-fluoropiperidin-1-yl)ethyl)quinoline-4-carboxamide. Prepared using general procedure **D** starting from 2-chloro-6-fluoroquinoline-4-carboxylic acid (200 mg, 0.89 mmol) and 2-(4-fluropiperidine)ethylamine[11] (175 mg) to afford the desired product as an off-white solid. Yield, 70% (220 mg) ^{1}H NMR (500 MHz, DMSO) δ 8.82 (t, J=5.8 Hz, 1H), 8.12 - 8.03 (m, 2H), 7.84 - 7.79 (m, 1H), 7.68 (s, 1H), 4.77 - 4.62 (m, 1H), 3.46 (q, J=6.1 Hz, 2H), 2.63 (s, 2H), 2.53 (t, *J* = 6.2 Hz, 2H), 2.39 (s, 2H), 1.94 - 1.84 (m, 2H), 1.79 - 1.70 (m, 2H) ppm; LCMS (ES+) m/z 354 $[M+H]^+$.

2-Chloro-*N*-(2-(4,4-difluoropiperidin-1-yl)ethyl)-6-fluoroquinoline-4-carboxamide. Prepared using general procedure **D** starting from 2-chloro-6-fluoroquinoline-4-carboxylic acid (173 mg, 0.8 mmol) and 2-(4,4-difluropiperidine)ethylamine[11] (190 mg) to afford the desired product as a white solid. Yield, 16% (46 mg); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 5.3, 9.2 Hz, 1H), 7.87 (dd, *J* = 2.8, 9.6 Hz, 1H), 7.51 (ddd, *J* = 2.8, 7.9, 9.3 Hz, 1H), 7.40 (s, 1H), 6.82 (s, 1H), 3.65 - 3.61 (m, 2H), 2.71 - 2.63 (m, 6H), 2.07 - 1.97 (m, 4H) ppm; ^{19}F NMR (407.5 MHz; $CDCl_3$) δ -109.74 ppm; LC-MS m/z 372 $(M+H)^+$.

2-Chloro-*N*-(2-(3,3-difluoropyrrolidin-1-yl)ethyl)-6-fluoroquinoline-4-carboxamide. Prepared using general procedure **D**, starting from 2-chloro-6-fluoroquinoline-4-carboxylic acid (200 mg, 0.89 mmol) and 2-(3,3-difluropyrrolidin)ethylamine[11] (180 mg) to afford the desired product (273 mg, 86% yield), as an off-white solid: ^{1}H NMR (500 MHz, DMSO) d 8.88 (t, J=5.8 Hz, 1H), 8.10 (dd, *J* = 5.4, 9.2 Hz, 1H), 7.98 (dd, *J* = 2.8, 10.1 Hz, 1H), 7.81 (dt, *J* = 2.8, 8.7 Hz, 1H), 7.68 (s, 1H), 3.46 (ddd, *J* = 6.4, 6.4, 6.4 Hz, 2H), 3.00 (dd, *J* = 13.6, 13.6 Hz, 2H), 2.80 (t, *J* = 6.9 Hz, 2H), 2.67 (t, *J* = 6.2 Hz, 2H), 2.32 - 2.22 (m, 2H) ppm; LCMS (ES+) m/z 358 $[M+H]^+$.

2-Chloro-6-fluoro-*N*-(2-morpholinoethyl)quinoline-4-carboxamide. A solution of 2-chloro-6-fluoroquinoline-4-carboxylic acid (310 mg, 1.4 mmol) in DMF (10 mL), EDC (528 mg, 2.7 mmol, 2 eq) and HOBt (371 mg, 2.7 mmol, 2 eq) was added. 2-morpholinoethylamine (0.36 mL, 2.75 mmol, 2 eq) and DIPEA (0.5 mL, 2.75 mmol, 2 eq) were added to the reaction mixture. The reaction was stirred for 6 h at room temperature. Reaction was quenched with 5% LiCl (10 mL) and extracted with DCM (2 x 100 mL). Organic phase was dried over $MgSO_4$ and solvents were removed under reduce pressure. Product was purified by column chromatography on a 12 g silica cartridge using A: DCM and B: 10% MeOH-NH_3 in DCM. Fractions containing product were pooled together to obtain the desired product as a white solid. Yield, 25% (119 mg); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (dd, *J* = 5.3, 9.2 Hz, 1H), 7.86 -7.83 (m, 1H), 7.47-7.43 (m, 1H), 7.37 (s, 1H), 6.76 (s, 1H), 3.64 (t, *J* = 4.6 Hz, 4H), 3.56 (q, *J* = 5.6 Hz, 2H), 2.57 (t, *J* = 5.9 Hz, 2H), 2.46 (s, 4H) ppm; LC-MS m/z 338 $(M+H)^+$.

General procedure E: Preparation of 2-amino-4-carboxamides (31, 34 and 35). A solution the corresponding quinoline carboxamide (1eq) and 3-morpholinopropylamine (3 eq) in *N*-methyl pyrrolidinone (NMP) (2.0 mL) in a microwave vial was heated at 200°C for 10 minutes, under microwave irradiation. Mixture diluted with DCM (20 mL), organics washed with water (10 mL) and filtered through a phase separator. Mixture purified by SCX-2 (amine capture) solid-phase extraction followed by column chromatography purification (0-10% 7M ammonia in methanol/dichloromethane).

6-fluoro-*N*-(2-(4-fluoropiperidin-1-yl)ethyl)-2-((3-morpholinopropyl)amino)quinoline-4-carboxamide (31). Prepared using general procedure **E** starting from 2-chloro-6-fluoro-*N*-(2-(4-fluoropiperidin-1-yl)ethyl)quinoline-4-carboxamide (100mg, 0.28 mmol), to afford the desired product (53 mg, 41% yield), as an off-white solid; ^{1}H NMR (500 MHz, DMSO) δ 8.55 (t, J=5.5 Hz, 1H), 7.65 (dd, *J* = 2.9, 10.3 Hz, 1H), 7.53 (dd, *J* = 5.5, 9.1 Hz, 1H), 7.38 (td, *J* = 8.9, 3.0 Hz, 1H), 7.17 (t, *J* = 5.4 Hz, 1H), 6.80 (s, 1H), 4.76 - 4.61 (m, 1H), 3.60 - 3.54 (m, 4H), 3.41 (q, *J* = 6.3 Hz, 4H), 2.65 - 2.58 (m, 3H), 2.40 - 2.29 (m, 9H), 1.93 - 1.83 (m, 2H), 1.79 - 1.69 (m, 4H) ppm; LCMS (ES+) m/z 462 $[M+H]^+$.

***N*-(2-(3,3-difluoropiperidin-1-yl)ethyl)-6-fluoro-2-((3-morpholinopropyl)amino)quinoline-4-carboxamide (34).** Prepared using general procedure **E** starting from intermediate 2-chloro-*N*-(2-(4,4-difluoropiperidin-1-yl)ethyl)-6-fluoroquinoline-4-carboxamide (100 mg, 0.28 mmol), to afford the desired product (55 mg, 42% yield), as an off-white solid; ^{1}H NMR (500 MHz, DMSO) δ 8.58 (t, J=5.7 Hz, 1H), 7.58 - 7.51 (m, 2H), 7.37 (dt, J=3.0, 8.7 Hz, 1H), 7.16 (t, J=5.5 Hz, 1H), 6.80 (s, 1H), 3.59 (t, J=4.7 Hz, 4H), 3.45 - 3.38 (m, 4H), 2.73 (t, J=11.7 Hz, 2H), 2.59 (t, J=6.5 Hz, 2H), 2.51 - 2.49 (m, 2H), 2.40 - 2.34 (m, 6H), 1.92 - 1.83 (m, 2H), 1.77 - 1.66 (m, 4H) ppm; LCMS (ES+) m/z 480 $[M+H]^+$.

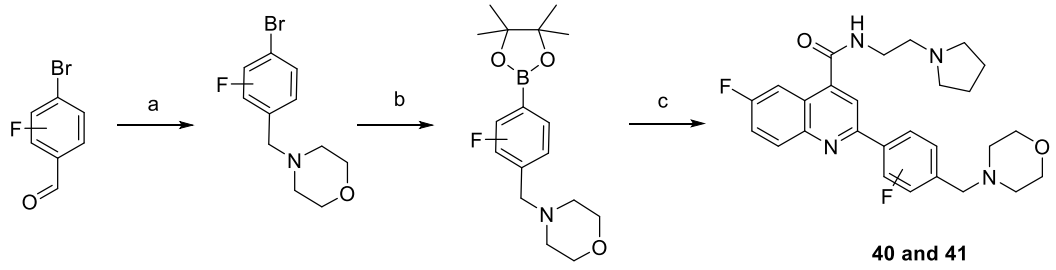
***N*-(2-(3,3-difluoropyrrolidin-1-yl)ethyl)-6-fluoro-2-((3-morpholinopropyl)amino)quinoline-4-carboxamide (35).** Prepared using general procedure **E** starting from 2-chloro-*N*-(2-(3,3-difluoropyrrolidin-1-yl)ethyl)-6-fluoroquinoline-4-carboxamide (100mg, 0.28 mmol), to afford the

desired product (43 mg, 33% yield), as an off-white solid; ^{1}H NMR (500 MHz, DMSO) 8.63 (t, J=5.8 Hz, 1H), 7.60 - 7.51 (m, 2H), 7.37 (td, J=8.8, 3.0 Hz, 1H), 7.17 (t, J=5.4 Hz, 1H), 6.80 (s, 1H), 5.75 (s, 1H), 3.59 (t, J=4.6 Hz, 4H), 3.41 (q, J=6.1 Hz, 4H), 2.98 (t, J=13.6 Hz, 2H), 2.78 (t, J=6.9 Hz, 2H), 2.64 (t, J=6.4 Hz, 2H), 2.39 - 2.34 (m, 5H), 2.30 - 2.20 (m, 2H), 1.77 - 1.70 (m, 2H) ppm; LCMS (ES+) m/z 466 $[M+H]^+$.

6-Fluoro-*N*-(2-morpholinoethyl)-2-((3-morpholinopropyl)amino)quinoline-4-carboxamide (32). A solution of 2-chloro-6-fluoro-*N*-(2-morpholinoethyl)quinoline-4-carboxamide (119 mg, 0.35 mmol) and 3-morpholinopropylamine (0.15 mL, 1.1 mmol, 3 eq) in acetonitrile (3 mL) in a microwave vial was heated at 170°C for 2 h under microwave irradiation. Reaction was partitioned between DCM (50 mL) and $NaHCO_3$ sat. aq. solution (20 mL). Organic phase was dried over $MgSO_4$ and solvents were removed under reduce pressure. Solvents were removed and product was purified by column chromatography on 4g silica cartridges using A: DCM and B:20% MeOH-NH_3 in DCM and the following gradient: 2 min hold A, 18 min ramp to 30%B, 2 min hold at 30%B. Fractions containing product were pooled together to obtain **32** as a white solid. Yield, 29% (45 mg); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66-7.63 (m, 2H), 6.83 (dd, *J* = 4.7, 4.7 Hz, 1H), 6.64 (s, 1H), 6.00-5.95 (m, 1H), 3.77-3.70 (m, 8H), 3.64 (q, *J* = 5.7 Hz, 2H), 3.52 (t, *J* = 6.3 Hz, 2H), 2.65 (t, *J* = 5.8 Hz, 2H), 2.57-2.45 (m, 10H), 1.86-1.79 (m, 2H) ppm; ^{19}F NMR (407.5 MHz; $CDCl_3$) δ -118.92 ppm; LC-MS m/z 445 $(M+H)^+$.

***N*-(2-(4,4-difluoropiperidin-1-yl)ethyl)-6-fluoro-2-((3-morpholinopropyl)amino)quinoline-4-carboxamide (33).** A solution of 2-chloro-*N*-(2-(4,4-difluoropiperidin-1-yl)ethyl)-6-fluoroquinoline-4-carboxamide (46 mg, 0.12 mmol) and 3-morpholinopropylamine (0.05 mL, 0.37 mmol, 3 eq) in acetonitrile (2 mL) in a microwave vial was heated at 170°C for 2 h under microwave irradiation. Reaction was partitioned between DCM (50 mL) and $NaHCO_3$ sat. aq. solution (20 mL). Organic phase was dried over $MgSO_4$ and solvents were removed under reduce pressure. Solvents were removed and product was purified by column chromatography on 4g silica cartridges using A: DCM and B:20% MeOH-NH_3 in DCM and the following gradient: 2 min hold 100%A, 15 min ramp to 20%B, 4 min hold at 20%B. Fractions containing product were pooled together to obtain **33** as a white solid. Yield, 30% (18 mg); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69-7.65 (m, 2H), 7.35-7.30 (m, 1H), 6.68 (s, 1H), 6.60 (t, *J* = 5.0 Hz, 1H), 5.95 (s, 1H), 3.78-3.49 (m, 8H), 2.72-2.47 (m, 12H), 2.06 - 1.99 (m, 4H), 1.88-1.81 (m, 2H) ppm; ^{19}F NMR (407.5 MHz; $CDCl_3$) δ -118.95 ppm; LC-MS m/z 480 $(M+H)^+$.

Scheme S2



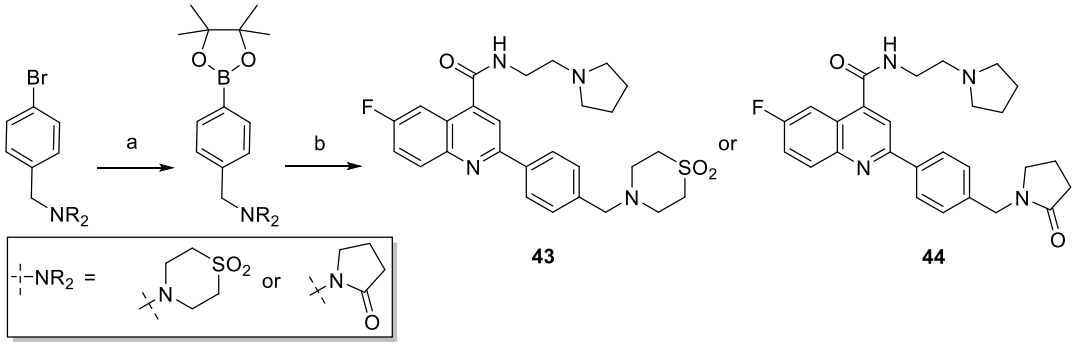
Conditions: (a) morpholine, $Na(OAc)_3BH$, chloroform, 60°C, 17h; (b) pinacol diborane, KOAc, $Pd(dppf)Cl_2$, dioxane, mw, 0.5h, 120°C; (c) aryl chloride (**5**), K_3PO_4 in H_2O, Pd tetrakis, mw, 0.5h, 120°C

6-Fluoro-2-(3-fluoro-4-(morpholinomethyl)phenyl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (40) (Procedure applied to compounds 40 and 41). A mixture of 4-bromo-2-fluoro benzaldehyde (500 mg, 2.5 mmol, 1 eq) and morpholine (0.22 mL, 215 mg, 2.5 mmol) was prepared

in CHCl3 (10 mL) at rt and heated to 58-60°C in a sealed tube for 1h. The mixture was then cooled to room temp and sodium triacetoxyborohydride (783 mg, 3.69 mmol) was added and the mixture heated again to 58-60°C in a sealed tube for 16h. The mixture was then concentrated *in vacuo* and then diluted with DCM (20 mL), washed with H_2O (2 x 5 mL), filtered through a phase separator and the filtrate concentrated *in vacuo*. Crude 1HNMR in CDCl3 showed the substituted benzyl morpholine intermediate in quantitative yield. Crude product was dissolved in dioxane (10 mL) and to it added dipinacol borane (751 mg, 2.96 mmol), KOAc (532 mg, 5.42 mmol), and Pd(dppf)Cl_2 (90 mg, 0.12 mmol) and the mixture heated in mw, 30 mins, 120°C. Mixture concentrated *in vacuo*, diluted with DCM (10 mL), washed with water (5 mL) and filtered through a phase separator. Filtrate concentrated *in vacuo* to afford crude boronic ester intermediate by LCMS and 1HNMR in CDCl3. Crude product was dissolved in DMF (10 mL) and to it added aryl chloride (**5**) (396 mg, 1.2 mmol), K_3PO_4 (522 mg, 2.46 mmol) in H_2O (2 mL), Pd tetrakis (71 mg, 0.06 mmol) and the mixture heated in mw, 30 mins, 120°C. Mixture then diluted in EtOAc (50 mL), washed with 5% LiCl aq. (3 x 20 mL), brine (20 mL) and mixture concentrated *in vacuo* and purified by column (0-10% 7M NH3 in MeOH/DCM). Required further purification. Filtered through a celite pad using DCM/MeOH as eluent. Purified by HPLC to afford **40** an off-white solid (102 mg, 0.21 mmol, 9% over 3 steps). ^{1}H NMR (500 MHz, CDCl3) δ 8.18 (dd, *J* = 5.5, 9.2 Hz, 1H), 7.98 - 7.94 (m, 2H), 7.90 - 7.88 (m, 2H), 7.58 - 7.52 (m, 2H), 6.78 (t, *J* = 4.3 Hz, 1H), 3.73 (t, *J* = 4.7 Hz, 4H), 3.71 - 3.65 (m, 4H), 2.78 (t, *J* = 5.9 Hz, 2H), 2.61 - 2.57 (m, 4H), 2.53 (t, *J* = 4.3 Hz, 4H), 1.81 - 1.77 (m, 4H); LCMS (ES+) m/z 481 $[M+H]^+$.

6-Fluoro-2-(2-fluoro-4-(morpholinomethyl)phenyl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (41) ^{1}H NMR (500 MHz, CDCl3) δ 8.18 (dd, *J* = 5.5, 9.1 Hz, 1H), 8.09 - 8.01 (m, 3H), 7.55 - 7.51 (m, 1H), 7.30 - 7.22 (m, 3H), 3.78 (d, *J* = 3.9 Hz, 2H), 3.74 (t, *J* = 4.7 Hz, 4H), 2.99 - 2.85 (m, 6H), 2.49 (t, *J* = 4.3 Hz, 4H), 1.93 (s, 4H), 1.59 (s, 2H); LCMS (ES+) m/z 481 $[M+H]^+$.

Scheme S3



Conditions: (a) 1,1-dioxide pinacol diborane, KOAc, Pd(dppf)Cl_2, dioxane, mw, 0.5h, 120°C; (b) aryl chloride (**5**), K_3PO_4 in H_2O, Pd tetrakis, mw, 0.5h, 120°C

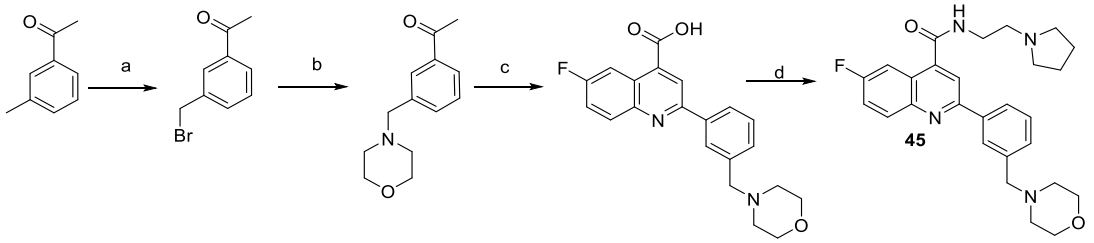
2-(4-((1,1-Dioxidothiomorpholino)methyl)phenyl)-6-fluoro-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (43) (General procedure applied to compounds 43 and 44).

4-(4-bromobenzyl)thiomorpholine (283 mg, 0.93 mmol) in dioxane (3 mL) was prepared at rt and pinacol borane (284 mg, 1.12 mmol), KOAc (201 mg, 2.05 mmol) and finally, Pd(dppf)Cl_2 (34 mg, 0.05 mmol) added and the mixture heated in a mw at 120°C for 1h. The mixture was then concentrated *in vacuo* and then diluted with DCM (20 mL), washed with H_2O (2 x 5 mL), filtered through a phase separator and the filtrate concentrated *in vacuo*. Crude 1HNMR showed boronic ester intermediate in quantitative yield. Crude product was dissolved in DMF (4 mL) and to it added aryl

chloride **5** (150 mg, 0.47 mmol), K_3PO_4 (198 mg, 0.93 mmol) in H_2O (1 mL), Pd tetrakis (27 mg, 0.02 mmol) and the mixture heated in mw, 30 mins, 120ºC. Mixture then filtered through a celite pad and washed with EtOAc (4 x 10 mL). Organics then washed with 5% LiCl aq. (3 x 10 mL), brine (10 mL), dried over MgSO4 and concentrated *in vacuo*. Mixture purified by HPLC autoprep to afford **43** as an off-white solid (64 mg, 0.13 mmol, 14% yield over 2 steps). ^{1}H NMR (500 MHz, CDCl3) δ 12.57 (s, 1H), 9.11 (s, 1H), 8.54 (s, 1H), 8.37 (d, *J* = 8.0 Hz, 2H), 8.22 - 8.16 (m, 2H), 7.53 - 7.45 (m, 2H), 4.02 - 3.93 (m, 4H), 3.73 (d, *J* = 0.9 Hz, 2H), 3.42 (dd, *J* = 5.4, 9.8 Hz, 2H), 3.06 (d, *J* = 20.3 Hz, 8H), 2.91 (t, *J* = 12.9 Hz, 2H), 2.35 - 2.30 (m, 2H), 2.16 (q, *J* = 4.5 Hz, 2H) ppm; LCMS (ES+) m/z 511 $[M+H]^+$.

6-Fluoro-2-(4-((2-oxopyrrolidin-1-yl)methyl)phenyl)-N-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (44). ^{1}H NMR (500 MHz, CDCl3) δ 8.20 - 8.13 (m, 3H), 8.04 - 7.97 (m, 2H), 7.52 (ddd, *J* = 2.8, 8.0, 9.2 Hz, 1H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.09 (s, 1H), 4.53 (s, 2H), 3.72 (q, *J* = 5.6 Hz, 2H), 3.29 (t, *J* = 7.1 Hz, 2H), 2.87 (t, *J* = 5.7 Hz, 2H), 2.70 (s, 4H), 2.47 (t, *J* = 8.1 Hz, 2H), 2.05 - 1.98 (m, 2H), 1.85 (s, 4H); LCMS (ES+) m/z 461 $[M+H]^+$.

Scheme S4



Conditions: (a) NBS, benzoyl peroxide cat., dichlorobenzene, 140°C, 16h, 49% yield; (b) morpholine, K_2CO_3, acetonitrile, 40°C, 16h, 68% yield; (e) 5-fluoroisatin, KOH, EtOH, 120°C, microwave 20 min, 28% yield; (f) amine, CDMT, NMO, DCM, 66% yield.

1-(3-(Bromomethyl)phenyl)ethanone. To 1-(m-tolyl)ethanone (2 g, 14.9 mmol) and NBS (2.9 g, 16.4 mmol) in dichlorobenzene (100 mL), benzoyl peroxide (wet with 25% water, 0.2 g, 0.7 mmol) was added and the reaction was refluxed overnight. The reaction was allowed to cool down to room temperature and filtered through Celite. The filtrated was concentrated under reduced pressure and the product was purified by column chromatography using 40 g silica cartridge, A: hexane, B: ethyl acetate and the following gradient: 1 min hold 100% A, 18 min ramp to 30% B, 3 min hold 100% B. Fractions containing product were pooled together and the solvent was removed to obtain the desired product as a yellow oil with a purity of 64% by LC-MS. Product was used for the next step without further purification. Yield, 49% (2.44 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 1.7 Hz, 1H), 7.91 (d, *J* = 7.7 Hz, 1H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.48 (dd, *J* = 7.7, 7.7 Hz, 1H), 4.55 (s, 2H), 2.66-2.64 (m, 3H) ppm; LC-MS m/z 214 $(M+H)^+$.

1-(3-(Morpholinomethyl)phenyl)ethanone. To a suspension of 1-(3-(bromomethyl)phenyl)ethanone (2.4 g, 11.4 mmol) and morpholine (1.2 g, 13.7 mmol) in acetonitrile (40 mL), potassium carbonate (2.4 g,17.2 mmol) was added. The reaction was stirred at 60 ºC for 3h. The reaction crude was filtered and the filtrated was concentrated under reduced pressure. The product was purified by column chromatography using a 40 g silica cartridge and A: hexane, B: ethyl acetate and the following gradient: 1 min hold 100% A, 18 min ramp to 100% B, 3 min hold at 100%B. Fractions containing product were pooled together to obtain the desired product. Yield, 68% (1.74 g); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (s, 1H), 7.86 (d, *J* = 7.7 Hz, 1H), 7.57 (d, *J*

= 7.5 Hz, 1H), 7.43 (dd, *J* = 7.6, 7.6 Hz, 1H), 3.72 (t, *J* = 4.7 Hz, 4H), 3.56 (s, 2H), 2.62 (s, 3H), 2.47-2.43 (m, 4H) ppm; LC-MS m/z 220 $(M+H)^+$.

6-Fluoro-2-(3-(morpholinomethyl)phenyl)quinoline-4-carboxylic acid. To a suspension of 1-(3-(morpholinomethyl)phenyl)ethanone (1.6 g,7.3 mmol) in ethanol (10 mL) in microwave vial, 5-fluoroisatin (1.2 g,7.3 mmol) and water (10 mL) were added. After the addition of KOH (4.1 g, 73.4 mmol) the reaction mixture was heated at 120 ^oC for 20 min under microwave irradiation. The reaction crude was diluted with water (50 mL) and pH adjusted to 7-8 with 10% HCl. The resulting precipitate was filtrated, washed with water (50 mL) and ethyl acetate (20 mL) and dried under reduced pressured to obtain the desired product was an off white solid. Yield, 28% (0.77 g); ^1H NMR (500 MHz, DMSO) δ 8.53-8.48 (m, 2H), 8.25-8.17 (m, 3H), 7.80-7.75 (m, 1H), 7.57-7.49 (m, 2H), 3.69 (s, 2H), 3.62 (t, *J* = 4.5 Hz, 4H), 2.52-2.50 (m, 4H) ppm; LC-MS m/z 367 (M +H)$^+$.

6-Fluoro-2-(3-(morpholinomethyl)phenyl)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinoline-4-carboxamide (45). A solution of 6-fluoro-2-(3-(morpholinomethyl)phenyl)quinoline-4-carboxylic acid (769 mg, 2.1 mmol), 2-chloro-4,6-dimethoxy-1,3,5-triazine CDMT (442 mg, 2.5 mmol, 1.2 eq) and *N*-methylmorpholine (0.47 mL, 4.2 mmol, 2 eq) in DCM (30 mL) was stirred at room temperature for 1h. 2-Pyrrolidin-1-ylethanamine (0.321 mL, 2.5 mmol, 1.5 eq) was added and the reaction mixture was stirred at room temperature for 2h. Reaction was partitioned between DCM (100 mL) and $NaHCO_3$ sat. aq. solution (10 mL). Organic phase was dried over $MgSO_4$ and solvents were removed under reduce pressure. Reaction crude was purified by column chromatography on a 24 g silica cartridge using A: DCM and B: 10% MeOH-NH_3 in DCM and the following gradient: 1 min hold 100%A, 13 min ramp to 35%B, 5 min hold at 35%B. Fractions containing product were pooled together and solvents were removed under reduced pressure. The sample was suspended in acetonitrile (100 mL) and DCM (10 mL) was added and cooled down (-18°C) for 4h. The suspension was filtered to obtain **45** as a white solid. Yield, 66% (665 mg); ^1H NMR (500 MHz, $CDCl_3$) δ 8.22 (dd, *J* = 5.5, 9.3 Hz, 1H), 8.12 (br s, 1H), 8.07-8.05 (m, 1H), 8.02 (br s, 1H), 7.99 (dd, *J* = 2.8, 9.9 Hz, 1H), 7.57-7.47 (m, 3H), 6.93 (br s, 1H), 3.76-3.74 (m, 4H), 3.71 (dd, *J* = 5.4, 11.5 Hz, 2H), 3.64 (s, 2H), 2.82 (t, *J* = 5.9 Hz, 2H), 2.63-2.62 (m, 4H), 2.52 (br s, 4H), 1.83-1.81 (m, 4H) ppm; LC-MS m/z 463 M +H)$^+$.

References

1. (a) Trager, W.; Jensen, J. B., Human malaria parasites in continuous culture. *Science* **1976,** *193*, 673-675; (b) Bennett, T. N.; Paguio, M.; Gligorijevic, B.; Seudieu, C.; Kosar, A. D.; Davidson, E.; Roepe, P. D., Novel, rapid, and inexpensive cell-based quantification of antimalarial drug efficacy. *Antimicrob. Agents Chemother.* **2004,** *48*, 1807-1810; (c) Snyder, C.; Chollet, J.; Santo-Tomas, J.; Scheurer, C.; Wittlin, S., In vitro and in vivo interaction of synthetic peroxide RBx11160 (OZ277) with piperaquine in *Plasmodium* models. *Exp. Parasitol.* **2007,** *115*, 296-300.
2. Plouffe, D.; Brinker, A.; McNamara, C.; Henson, K.; Kato, N.; Kuhen, K.; Nagle, A.; Adrian, F.; Matzen, J. T.; Anderson, P.; Nam, T. G.; Gray, N. S.; Chatterjee, A.; Janes, J.; Yan, S. F.; Trager, R.; Caldwell, J. S.; Schultz, P. G.; Zhou, Y.; Winzeler, E. A., In silico activity profiling reveals the mechanism of action of antimalarials discovered in a high-throughput screen. *Proc. Natl. Acad. Sci. USA* **2008,** *105*, 9059-9064.
3. Chulay, J. D.; Haynes, J. D.; Diggs, C. L., *Plasmodium falciparum*: assessment of in vitro growth by [3H]hypoxanthine incorporation. *Exp. Parasitol.* **1983,** *55*, 138-146.
4. Brand, S.; Cleghorn, L. A.; McElroy, S. P.; Robinson, D. A.; Smith, V. C.; Hallyburton, I.; Harrison, J. R.; Norcross, N. R.; Spinks, D.; Bayliss, T.; Norval, S.; Stojanovski, L.; Torrie, L. S.; Frearson, J. A.; Brenk, R.; Fairlamb, A. H.; Ferguson, M. A.; Read, K. D.; Wyatt, P. G.; Gilbert, I. H., Discovery of a

novel class of orally active trypanocidal N-myristoyltransferase inhibitors. *J. Med. Chem.* **2012,** *55*, 140-152.

5. Duffy, S.; Avery, V. M., Identification of inhibitors of *Plasmodium falciparum* gametocyte development. *Malar. J.* **2013,** *12*, 408.

6. Delves, M. J.; Ramakrishnan, C.; Blagborough, A. M.; Leroy, D.; Wells, T. N.; Sinden, R. E., A high-throughput assay for the identification of malarial transmission-blocking drugs and vaccines. *Int. J. Parasitol.* **2012,** *42*, 999-1006.

7. Patterson, S.; Wyllie, S.; Stojanovski, L.; Perry, M. R.; Simeons, F. R.; Norval, S.; Osuna-Cabello, M.; De Rycker, M.; Read, K. D.; Fairlamb, A. H., The R enantiomer of the antitubercular drug PA-824 as a potential oral treatment for visceral Leishmaniasis. *Antimicrob. Agents Chemother.* **2013,** *57*, 4699-4706.

8. Jones, D. C.; Hallyburton, I.; Stojanovski, L.; Read, K. D.; Frearson, J. A.; Fairlamb, A. H., Identification of a k-opioid agonist as a potent and selective lead for drug development against human African trypanosomiasis. *Biochem. Pharmacol.* **2010,** *80*, 1478-1486.

9. Sokolova, A. Y.; Wyllie, S.; Patterson, S.; Oza, S. L.; Read, K. D.; Fairlamb, A. H., Cross-resistance to nitro drugs and implications for treatment of human African trypanosomiasis. *Antimicrob. Agents Chemother.* **2010,** *54*, 2893-2900.

10. Charman, S. A.; Arbe-Barnes, S.; Bathurst, I. C.; Brun, R.; Campbell, M.; Charman, W. N.; Chiu, F. C.; Chollet, J.; Craft, J. C.; Creek, D. J.; Dong, Y.; Matile, H.; Maurer, M.; Morizzi, J.; Nguyen, T.; Papastogiannidis, P.; Scheurer, C.; Shackleford, D. M.; Sriraghavan, K.; Stingelin, L.; Tang, Y.; Urwyler, H.; Wang, X.; White, K. L.; Wittlin, S.; Zhou, L.; Vennerstrom, J. L., Synthetic ozonide drug candidate OZ439 offers new hope for a single-dose cure of uncomplicated malaria. *Proc. Natl. Acad. Sci. U S A* **2011,** *108*, 4400-4405.

11. Haerter, M.; Beck, H.; Ellinghaus, P.; Berhoerster, K.; Greschat, S.; Thierauch, K. H. Aminoalkyl-substituted compounds as hif inhibitors. 2010.