Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

# 2,4-Diaminothienopyrimidines as Orally Active Antimalarial Agents

Diego González Cabrera,[†] Claire Le Manach,[†] Frederic Douelle,[†] Yassir Younis,[†] Tzu-Shean Feng,[†] Tanya Paquet,[†] Aloysius T. Nchinda,[†] Leslie J. Street,[†] Dale Taylor,[‡] Carmen de Kock,[‡] Lubbe Wiesner,[‡] Sandra Duffy,[§] Karen L. White,[∥] K. Mohammed Zabiulla,[⊥] Yuvaraj Sambandan,[⊥] Sridevi Bashyam,[⊥] David Waterson,[▽] Michael J. Witty,[▽] Susan A. Charman,[∥] Vicky M. Avery,[§] Sergio Wittlin,[▲,○] and Kelly Chibale*[,†,■]

[†]Department of Chemistry and [■]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

[‡]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Observatory 7925, South Africa

[§]Discovery Biology, Eskitis Institute, Griffith University (Nathan Campus), Don Young Road, Brisbane, Queensland 4111, Australia

[∥]Centre for Drug Candidate Optimisation, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia

[⊥]Syngene International Ltd., Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase, Jigani Link Road, Bangalore 560099, India

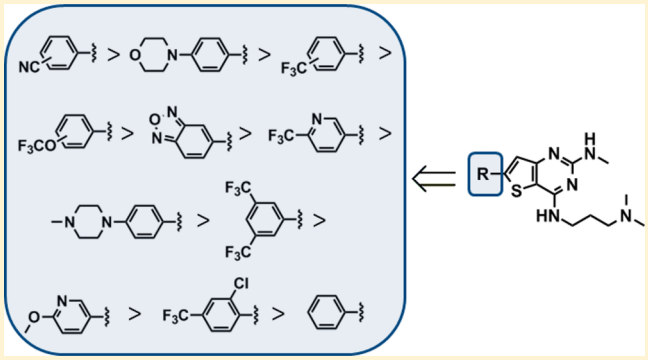
[▽]Medicines for Malaria Venture, International Center Cointrin, Route de Pré-Bois 20, Post Office Box 1826, 1215 Geneva, Switzerland

[▲]Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland

[○]University of Basel, 4002 Basel, Switzerland

Supporting Information

**ABSTRACT:** A novel series of 2,4-diaminothienopyrimidines with potential as antimalarials was identified from whole-cell high-throughput screening of a SoftFocus ion channel library. Synthesis and structure−activity relationship studies identified compounds with potent antiplasmodial activity and low in vitro cytotoxicity. Several of these analogues exhibited in vivo activity in the *Plasmodium berghei* mouse model when administered orally. However, inhibition of the hERG potassium channel was identified as a liability for this series.



## INTRODUCTION

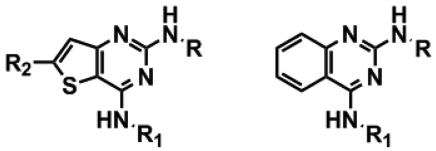
The rapid and widespread emergence of resistance to most marketed and commonly used antimalarial drugs has led to treatment failures and, as a consequence, to a rising concern over malaria control and/or eradication.[1] This is especially worrying in sub-Saharan Africa and southeast Asia where this infectious disease, typically caused by the *Plasmodium falciparum* organism, continues to inflict a tremendous burden. With ∼40% of the world's population being at risk of contracting this life-threatening parasitic disease,[2] there is an urgent need for novel and structurally diverse classes of compounds to develop effective and affordable drugs against malaria.[3]

One of the libraries identified from screening 36 608 compounds from the BioFocus DPI (BFDPI) SoftFocus library against 3D7 (sensitive) and Dd2 (multidrug resistant) strains of *P. falciparum*, in an image-based whole-cell assay,[4] was designated SFI06 (SoftFocus ion channel) and contained 554 analogues. Of these, 118 displayed greater than 80% inhibition of parasite growth at the 1.82 μM screening concentration. The overall hit rate from the SFI06 library was high at 21.3%. In comparison, screening libraries for the recently described thiazole[5] and aminopyridine series[6] exhibited significantly lower hit rates of 1.3% and 1.8%, respectively. As with other SoftFocus libraries, the rationale behind choosing the SoftFocus ion channel library for our high-throughput screening (HTS) campaign was that molecules contained in this target-focused library were designed with a particular protein target or protein family in mind. This is on the premise that, relative to conventional libraries, fewer compounds needed to be screened in a SoftFocus ion channel library in order to obtain hits. We have previously provided several advantages offered by SoftFocus libraries.[7]

**Received:** November 14, 2013
**Published:** January 21, 2014

The active compounds from the SFI06 series were identified as thienopyrimidines **1** (Figure 1). Analysis of the structure−activity relationship (SAR) from the HTS data as well as from synthesized representative thienopyrimidines **1** and the related quinazolines **2** (Figure 1) led to the conclusion that the 6-aryl-thienopyrimidine fragment was required for potent in vitro activity and that monosubstituted amines were the preferred substituents at the 2- and 4-positions. Despite this limitation, a range of aliphatic, aromatic, and heteroaromatic amine side chains were tolerated. However, more polar H-bonding groups (for example, amides) generally decreased activity.

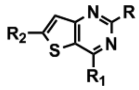


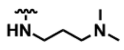
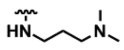
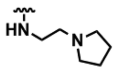
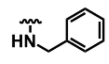
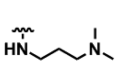
**1** **2**

**Figure 1.** Thienylpyrimidine (**1**) and quinazoline (**2**) structures.

Accordingly, a number of active analogues were selected for resynthesis and confirmatory testing against multidrug resistant (K1) and sensitive (NF54) strains of *P. falciparum* and counter-screened for cytotoxicity in the L6 mammalian cell line assay (Supporting Information). Several SAR trends were observed from the compounds tested (Table 1). First, the phenyl moiety at position 6 was essential for potent activity (**3** vs **4**). Second, replacement of the aminomethyl moiety at position 2 with other alkylamino substituents, such as an isopropylamino group, led to a significant loss of potency (**4** vs **8**), although basic alkylamino side chains were tolerated at this position and also at the 4-position. Finally, replacement of a benzyl group with an aniline moiety at position 4 resulted in a significant decrease in antiplasmodial activity (**7** vs **9**).

**Table 1. In Vitro Activity of Representative Compounds against Sensitive and Multidrug-Resistant Strains of *Plasmodium falciparum*[a]**



| compd | R | $R_1$ | $R_2$ | $IC_{50}$ (*n*M)[a] | |
|---|---|---|---|---|---|
| | | | | K1 | NF54 |
| chloroquine[b] | | | | 194 | 16 |
| artesunate[b] | | | | 3 | 4 |
| **3** |  |  | Br | 973 | 636 |
| **4** |  |  |  | 73 | 28 |
| **5** |  |  |  | 136 | 28 |
| **6** |  |  |  | 478 | 181 |
| **7** |  |  |  | 120 | 61 |
| **8** |  |  |  | 181 | 81 |
| **9** |  |  |  | 264 | 119 |

[a]Mean from *n* values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of *P. falciparum*. [b]Data from Gonzalez Cabrera et al.[5]

On the basis of good in vitro antiplasmodial potency and favorable absorption, distribution, metabolism, and excretion (ADME) properties, and in order to assess the antimalarial potential of this antiplasmodial chemotype, three analogues **4**, **5**, and **8** were selected for in vivo oral efficacy evaluation in the *Plasmodium berghei* mouse model.[8] Interestingly, **4** and **5** exhibited significant antimalarial activity at 4 × 50 mg/kg, with 87% and 95% reduction in parasitemia albeit with a modest survival time of 15 days. At this initial stage, these data were encouraging when it was considered that these analogues were chosen as representatives for hit validation with no additional optimization and/or chemical modification (Table 2).

Although related quinazoline[9] and pyrimidine[10] compounds with antiplasmodial activity were known, this was the first time that excellent in vivo antimalarial efficacy and mean survival time (MSD) of 2,4-diaminothienopyrimidines at low doses had been observed. It is noteworthy that 2,4-diaminothieno[2,3-*d*]pyrimidines have previously been reported as antifolates and antimalarials.[11] These differ structurally from the current SFI06 series compounds as they lack alkyl substituents on the 2- and 4-amino positions and contain a carbocyclic or heterocyclic ring fused at the 5,6-position. In addition, **4** displayed fast killing kinetics in the recently developed speed of action and stage-specificity assays.[12] This contrasts with the slower killing kinetics of dihydrofolate reductase inhibitors such as pyrimethamine. Fast-acting derivatives not only allow maximization of therapeutic efficacy but also potentially minimize the emergence of drug resistance, which is an ideal characteristic for new antimalarial drugs. On the basis of these initial promising results, it was concluded that 2,4-diaminothienopyrimidine compounds presented themselves as an attractive series for a hit-to-lead (H2L) and lead optimization (LO) program.

Herein we describe a comprehensive assessment of antiplasmodial activity, SAR analyses, antimalarial efficacy, and pharmacokinetic (PK) properties in parallel with human ether-à-go-go-related gene (hERG) inhibition studies to examine the potential cardiotoxicity risk posed by this novel antimalarial chemotype.

We began exploring the SAR based on **4**, by modifying the heterocyclic core to determine the optimum structural framework needed for potent antiplasmodial activity (Table 3). Variation in the position of the sulfur atom in the scaffold to give reverse thienopyrimidines (**10** and **11**) or replacement of the thiophene ring with the corresponding furan ring (**12**) led to a significant loss of activity, indicating the importance of the thieno[3,2-*d*]pyrimidine core for potency.

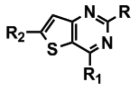
After investigating the consequence of varying the heterocyclic scaffold and using the initial hit compound **4** as starting point, we decided to explore the SAR around the 6-aryl substituent.

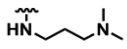
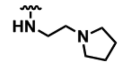
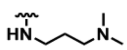
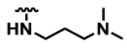
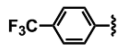
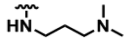
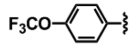
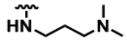
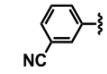
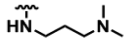
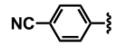
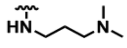
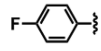
## RESULTS AND DISCUSSION

**Chemistry.** Scheme 1 summarizes the seven-step synthesis of 2,4-diaminothienopyrimidine compounds from commercially available reagents. Reaction of starting material **13** with trichloroacetyl isocyanate in tetrahydrofuran (THF) and


dx.doi.org/10.1021/jm401760c | J. Med. Chem. 2014, 57, 1014−1022

Table 2. In Vivo Antimalarial Efficacy for Single and Multiple Doses of Selected Compounds in *Plasmodium berghei*-Infected Mice[a]



| compd[a] | R | $R_1$ | $R_2$ | oral dose (mg/kg) | % reduction parasitemia (MSD)[b] |
|---|---|---|---|---|---|
| | | | | 4x30 | 99.9 (24)[c] |
| | | | | 100 | >99.9 (12)[c] |
| chloroquine | | | | 30 | 99.7 (9)[c] |
| | | | | 10 | 99.5 (7)[c] |
| | | | | 3 | 83 (7)[c] |
| 4 |  |  |  | 4x50 | 87 (15) |
| 5 |  |  |  | 4x50 | 95 (15) |
| 8 |  |  |  | 4x50 | 56 (7) |
| | | | | 4x50 | 99.8 (14) |
| 17 |  |  |  | 4x10 | 40 (<4)[d] |
| | | | | 4x3 | 40 (<4)[d] |
| | | | | 4x50 | 99.8 (23) |
| 23 |  |  |  | 4x10 | 40 (<4)[d] |
| | | | | 4x3 | 40 (<4)[d] |
| 20 |  |  |  | 4x50 | 84 (14) |
| 21 |  |  |  | 4x50 | 98 (7) |
| 25 |  |  |  | 4x50 | 86 (11) |

[a]Compounds were dissolved or suspended in a nonsolubilizing, standard suspension vehicle (hydroxypropyl methylcellulose, HPMC), except for **4**, **5**, and **8**, which were dosed in 70/30 Tween 80/ethanol, diluted before administration 10× with water. [b]MSD = mean survival time (in days). [c]Data from Gonzalez Cabrera et al.[5] [d]Mice were euthanized on day 4 in order to prevent death otherwise occurring at day 6.

subsequent bromination afforded substrate **14** in good yield (91%). Treatment of **14** with ammonia in $CH_3OH$ furnished intermediate **15**, which cyclized under basic conditions. Subsequent chlorination with $POCl_3$ produced key dichloro intermediate **16** in excellent yield (88%). Derivatives **4**–**9** and **17**–**43** were then accessed via two consecutive N-substitution reactions in the presence of the relevant amine, followed by a Suzuki cross coupling[13] reaction with commercially available boronic acids.

**Biology.** *In Vitro Antiplasmodial Activity.* The activity of the newly synthesized analogues was determined against multidrug-resistant (K1) and sensitive (NF54) strains, with chloroquine and artesunate as the reference drugs in all the experiments. Nineteen of these compounds showed potent antiplasmodial activity in the low nanomolar range ($IC_{50}$ NF54 < 100 nM), with five analogues having $IC_{50}$ values below 20 nM (Table 4).

Electron-withdrawing groups (EWG), such as -$CF_3$ (**17**) were well-tolerated and resulted in potent compounds. In contrast, groups such as methylsulfone (**29**) were less active compared to **4**. One of the most potent analogues, **21**, exhibited a 3-fold increase in potency compared to **4** and better activity than chloroquine against both NF54 and K1 strains. **25**, which contained a fluoro substituent, showed moderate potency.

Varying the position of the substituents from para to meta on the phenyl ring seemed to have relatively little effect on antiplasmodial activity (**17**, **21**, and **23** vs **18**, **20**, and **22**, respectively). Similarly, introducing an ortho substituent, as in **33**, had little adverse effect on potency.

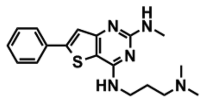
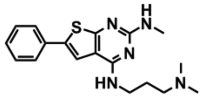
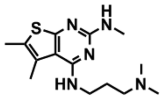
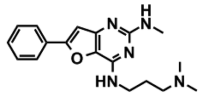
The potencies of **30**, **31**, and **32** indicated that aliphatic and heterocyclic rings were tolerated. It is noteworthy that the presence of the morpholino group in **30** gave rise to one of the most potent compounds of the series ($IC_{50}$ NF54 = 7 nM).

Disubstituted derivatives **33** and **34** retained good potency, while **35** significantly lost activity, presumably due to the presence of the polar EWG 4-methanesulfonyl substituent.

Attempts to replace the phenyl ring with heterocyclic or electron-deficient bicyclic groups led to a drop in potency (**36**, **39**, and **38**). However, antiplasmodial activity was regained by the introduction of a less polar benzoxadiazole group as shown


dx.doi.org/10.1021/jm401760c | J. Med. Chem. 2014, 57, 1014–1022

**Table 3. In Vitro Activity against Sensitive and Multidrug-Resistant Strains of *Plasmodium falciparum* and Solubility[a]**

| compd | structure | $IC_{50}$ (*n*M)[a] | | solubility (*μ*g/mL) | |
|---|---|---|---|---|---|
| | | K1 | NF54 | pH 2.0 | pH 6.5 |
| chloroquine[b] | | 194 | 16 | | |
| artesunate[b] | | 3 | 4 | | |
| **4** |  | 73 | 28 | >100 | >100[c] |
| **10** |  | 2193 | 1022 | 216 | 184[d] |
| **11** |  | - | >3408 | - | - |
| **12** |  | - | 533 | 214 | 203[d] |

[a]Mean from *n* values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of *P. falciparum*. [b]Data from Gonzalez Cabrera et al.[5] [c]Estimates from nephelometry. [d]Estimates from HPLC-DAD-MS. Dash indicates that the value was not measured.

for **37**, which exhibited a 2-fold improvement in activity compared to **4**.

Introduction of more polar carboxamide groups to the phenyl ring resulted in considerable loss of activity, albeit for the acetyl-containing analogue **40** the decrease was relatively moderate.

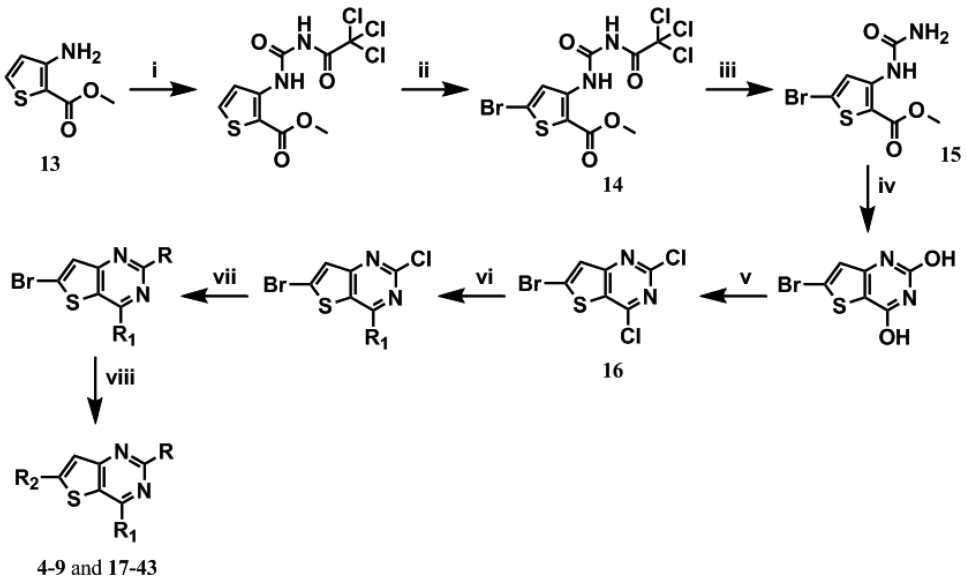
*In Vitro ADME Profiling.* Along with the extensive SAR studies toward optimizing the potency of this series, some of the most active analogues were evaluated for their physicochemical properties and in vitro metabolic stability in human liver microsomes. In general, most of the newly synthesized compounds had good solubility under acidic (pH 2) or neutral (pH 6.5) conditions, with the exception of **30**, which showed poor solubility at both pH values, and **19**, **26**, **31**, and **40**, which were poorly soluble at pH 6.5. The partition coefficients were generally moderate to high, with log $D_{7.4}$ values ranging from 2.1 to 4.3 (Table 4).

Most of the derivatives displayed low to moderate rates of degradation in human liver microsomes. On the basis of the in vitro intrinsic clearance values, low to moderate in vivo hepatic clearance would be expected (Table 5). Unfortunately **30**, one of the most potent of the series, showed a high rate of degradation in the microsomal incubations.

In addition to studies of their stability in human liver microsomes, key compounds were investigated in mouse and rat liver microsomes. Generally, metabolic stability of the derivatives was comparable across the three species (data not shown).

*In Vivo Efficacy Studies.* Compounds that displayed potent in vitro antiplasmodial activity ($IC_{50}$ < 100 nM) and good metabolic stability were evaluated for in vivo activity in the *P. berghei* mouse model.[8] Parasitemia reduction and MSD for multidose regimens are reported in Table 2. Following oral administration of 4 × 50 mg/kg, analogues **17** and **23** reduced parasitemia by more than 99% and prolonged MSD to 14 and 23 days, respectively. However, none of the treated animals were completely cured. Dosing the compounds at 4 × 3 mg/kg and 4 × 10 mg/kg was less effective, with <40% percent reduction in parasitemia compared to untreated infected mice. Despite their potent in vitro activity against *P. falciparum*, analogues such as **20** were inferior in terms of parasitemia reduction as well as MSD.

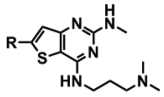
**Scheme 1. Synthetic Approach to 2,4-Diaminothienopyrimidine Analogues[a]**

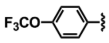
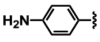
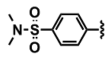
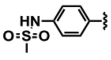
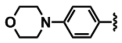
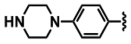
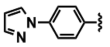
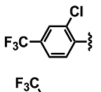
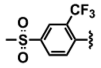
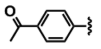
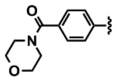
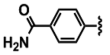
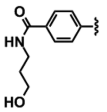



[a]Reagents and conditions: (i) trichloroacetyl isocyanate, THF, 0 °C to rt, 2 h, 91%; (ii) bromine, acetic acid, 0−80 °C, 14 h, 65%; (iii) ammonia, $CH_3OH$, 0 °C to rt, 30 min, 76%; (iv) *t*-BuOK, DMF, rt, 14 h, 99%; (v) $POCl_3$, *N*,*N*-dimethylaniline, 130 °C, 14 h, 88%; (vi) $R_1$ = appropriate amine, $Na_2CO_3$, ethanol, rt, 14 h, 93−99%; (vii) R = appropriate amine, in a sealed tube, THF, 100 °C, 14 h, 78−83%; (viii) $R_2$ = appropriate boronic acid, $Pd(PPh_3)_2Cl_2$, $K_2CO_3$, DMF, 90 °C, 14 h, 24−82%.


dx.doi.org/10.1021/jm401760c | J. Med. Chem. 2014, 57, 1014−1022

**Table 4. In Vitro Activity against Sensitive and Multidrug-Resistant Strains of *Plasmodium falciparum* and Physicochemical Properties[a]**



| compd | R | IC$_{50}$ (*n*M)[a] | | solubility (μg/mL) | | logD[b] |
|---|---|---|---|---|---|---|
| | | K1 | NF54 | pH 2.0 | pH 6.5 | pH 7.4 |
| chloroquine[c] | | 194 | 16 | | | |
| artesunate[c] | | 3 | 4 | | | |
| **4** |  | 73 | 28 | >100 | >100[d] | 2.2 |
| **17** |  | 32 | 29 | 240 | 177[e] | 4.1 |
| **18** |  | - | 13 | 180 | 160[e] | - |
| **19** |  | 39 | 21 | >100 | 12.5-25[d] | 3 |
| **20** |  | 17 | 4 | >100 | 25-50[d] | 2.9 |
| **21** |  | 19 | 9 | >100 | 50-100[d] | 2.9 |
| **22** |  | 20 | 16 | >100 | >100[d] | 4.3 |
| **23** |  | 26 | 28 | >100 | >100[d] | 4.6 |
| **24** |  | 38 | 40 | >100 | 25-50[d] | 2.8 |
| **25** |  | 92 | 42 | - | - | - |
| **26** |  | 87 | 26 | >100 | 12.5-25[d] | 2.3 |
| **27** |  | - | 89 | - | - | - |
| **28** |  | 150 | 161 | >100 | 25-50[d] | 2.1 |
| **29** |  | 172 | 143 | >100 | 25-50[d] | 2.1 |
| **30** |  | 12 | 7 | 12.5-25 | 12.5-25[d] | 3 |
| **31** |  | 59 | 94 | >100 | 12.5-25[d] | 3.5 |
| **32** |  | - | 74 | 217 | 191[e] | - |
| **33** |  | 47 | 16 | >100 | >100[d] | 4.5 |
| **34** |  | - | 33 | 186 | 130[e] | - |
| **35** |  | 800 | 271 | - | - | - |
| **36** |  | - | 159 | 214 | 194[e] | - |
| **37** |  | 29 | 12 | >100 | 25-50[d] | 3.3 |
| **38** |  | - | 192 | - | - | - |
| **39** |  | - | 194 | 221 | 203[e] | - |
| **40** |  | 81 | 39 | >100 | 12.5-25[d] | 2.9 |
| **41** |  | 359 | 312 | >100 | 25-50[d] | 2.2 |
| **42** |  | 458 | 195 | - | - | - |
| **43** |  | 1017 | 1225 | - | - | - |

[a]Mean from *n* values of ≥2 independent experiments with multidrug-resistant (K1) and sensitive (NF54) strains of *P. falciparum*. [b]Value measured by the chromatographic glogD technique. [c]Data from Gonzalez Cabrera et al.[5] [d]Estimates from nephelometry. [e]Estimates from HPLC-DAD-MS. Dash indicates that the value was not measured.

*In Vivo Pharmacokinetic Studies.* In an attempt to rationalize the in vivo efficacy data, PK studies on **4** were conducted in male Sprague-Dawley rats following oral (po) and intravenous (iv) administration. In addition, mouse exposure studies of **17** and **25** were performed at a single dose of 50 mg/kg. No sign of toxicity was observed when **4** was administered intravenously at a dose of 5 mg/kg or orally at a dose of 20 mg/kg. The in vivo plasma clearance in rats after iv dosing was high (154 mL·min$^{-1}$·kg$^{-1}$), much higher than the expected hepatic blood flow in rats (CL$_{plasma}$ 67.6 mL·min$^{-1}$·kg$^{-1}$). Ex vivo evaluation of the whole blood to plasma partitioning ratio (B/P) indicated that **4** distributes extensively into erythrocytes (B/P = 3.8). By use of this value to convert the plasma clearance to blood clearance, a value for CL$_{blood}$ was estimated to be 40 mL·min$^{-1}$·kg$^{-1}$, which is reasonably consistent with the microsome predicted clearance (predicted CL$_{blood}$ = 27.9 mL·min$^{-1}$·kg$^{-1}$) based on studies in rat liver microsomes. The high B/P is also one of the reasons for the extremely high plasma volume of distribution (139 L/kg); however, the blood volume of distribution (37 L/kg) was also very high. This trend for high volume of distribution is commonly seen with dibasic compounds and was presumably responsible for the long in vivo half-life of 21 h (Table 6). Furthermore, **4** had a moderate oral bioavailability (34%) likely limited by first-pass metabolism and consistent with the in vivo blood clearance being 41% of the hepatic blood flow in rats.

*Mouse Exposure Studies.* Plasma concentrations in *Plasmodium berghei*-infected mice were measured after a single oral dose of 50 mg/kg and followed for 24 h. Compound **17** showed significant in vivo exposure, with a higher plasma concentration and prolonged exposure profile compared to **25** (Table 7). Thus, the in vivo efficacy displayed by **17** can be rationalized on the basis of better drug exposure and higher in vitro potency [IC$_{50}$ = 32 nM (K1) and 29 nM (NF54)] compared to **25** [IC$_{50}$ = 92 nM (K1) and 42 nM (NF54)].


dx.doi.org/10.1021/jm401760c | J. Med. Chem. 2014, 57, 1014−1022

**Table 5. In Vitro Metabolic Stability in Human Liver Microsomes**

| compd | degrad half-life (min) | in vitro $CL_{int}$ [$\mu L \cdot min^{-1} \cdot (mg\ of\ protein)^{-1}$] | microsome-predicted $E_H$[a] (human) |
|---|---|---|---|
| **4** | 344.9 | 5 | 0.22 |
| **17** | 124 | 14 | 0.47 |
| **18** | 31.1 | 55.8 | 0.78 |
| **19** | 179 | 10 | 0.35 |
| **21** | 238 | 7 | 0.29 |
| **22** | 230 | 8 | 0.30 |
| **23** | >250 | <7 | <0.28[b] |
| **24** | 181 | 10 | 0.35 |
| **29** | 115 | 15 | 0.46 |
| **30** | 15 | 119 | 0.87 |
| **31** | 48 | 36 | 0.58 |
| **32** | 99.7 | 18.4 | 0.53 |
| **33** | 130 | 13 | 0.43 |
| **34** | >100 | <17 | <0.48[b] |
| **36** | >100 | <17 | <0.48[b] |
| **37** | >250 | <7 | <0.28[b] |
| **38** | 25.3 | 68.7 | 0.81 |
| **39** | 52.2 | 33.3 | 0.67 |
| **40** | 21 | 81 | 0.76 |
| **41** | 100 | 17 | 0.49 |

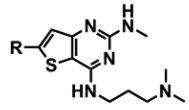
[a]Predicted hepatic extraction ratio based on in vitro intrinsic clearance ($CL_{int}$). [b]No measurable degradation of the parent compound was observed; hence, the clearance parameters could not be determined. $E_H$ was considered to be <0.28 or <0.48.

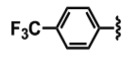
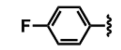
**Table 6. Pharmacokinetic Parameters for 4 in Male Sprague-Dawley Rats following Intravenous and Oral Administration**

| parameter | iv **4**[a] | oral **4**[a] |
|---|---|---|
| measured dose (mg/kg) | 4 | 19 |
| apparent $t_{1/2}$ (h) | 21 | 44 |
| plasma CL ($mL \cdot min^{-1} \cdot kg^{-1}$) | 154 | *c* |
| plasma $V_{ss}$ (L/kg) | 139 | *c* |
| blood CL[b] ($mL \cdot min^{-1} \cdot kg^{-1}$) | 41 | |
| blood $V_{ss}$[b] (L/kg) | 37 | |
| $C_{max}$ ($\mu M$) | *c* | 0.008 |
| $T_{max}$ (min) | *c* | 105 |
| $AUC_{0-\infty}$ ($\mu M \cdot min$) | 77 | 125 |
| bioavailability (%) | *c* | 34 |

[a]Values are the mean from two animals. [b]Calculated by dividing plasma CL or plasma $V_{ss}$ by the blood to plasma partitioning ratio (3.8). [c]Dash indicates that the value was not measured or was not relevant.

**Table 7. Plasma Concentrations of 17 and 25 in *Plasmodium berghei*-Infected Mice following Administration of a Single 50 mg/kg Oral Dose[a]**



| compd | R | plasma concentration ($\mu M$)[a] | | |
|---|---|---|---|---|
| | | 1h | 4h | 24h |
| **17** |  | 0.41 | 0.22 | 0.21 |
| **25** |  | 0.14 | 0.09 | 0.04 |

[a]Values are the mean from two animals. Sample time postdose is shown in hours.

The pharmacokinetic/pharmacodynamic (PK/PD) relationship for this series is difficult to interpret. As with many lipophilic dibasic compounds, the SFI06 series is characterized by very high volumes of distribution, which leads to very low blood levels but long apparent half-lives. In addition, compounds such as **4** are readily metabolized by demethylation on both side chains. However, the desmethyl compounds also retain intrinsic activity and would likely contribute to the overall efficacy of the compounds.

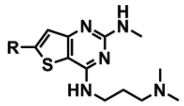
*In Vitro hERG Activity.* Six analogues of the series were tested for their activity against the hERG $K^+$ channel via in vitro IonWorks patch clamp electrophysiology. As observed in Table 8, **4** turned out to be a moderate inhibitor of the hERG channel ($IC_{50}$ = 8.5 $\mu M$). Unfortunately any changes made in the phenyl ring did not improve the hERG profile to desired levels, and all showed potential for hERG $K^+$ channel blockade at physiologically relevant concentrations ($IC_{50}$ values in 1−10 $\mu M$ range).

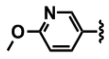
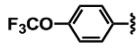
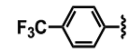
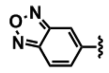
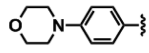
## CONCLUSION

From a high-throughput screen of 36 608 compounds, a novel series of 2,4-diamino-6-arylthienopyrimidines was identified. Nineteen of the newly synthesized compounds showed activity in the low nanomolar range ($IC_{50}$ < 100 nM) against multidrug-resistant (K1) and sensitive (NF54) strains of *P. falciparum*, with five analogues having an $IC_{50}$ below 20 nM. Compounds **4**, **17**, and **23** also suppressed the parasitemia and increased MSD in vivo in the *P. berghei* mouse model when administered orally at 4 × 50 mg/kg. However, no curative effect was observed at the tested dose. Pharmacokinetic studies on **4** revealed a high volume of distribution, with moderate


dx.doi.org/10.1021/jm401760c | J. Med. Chem. 2014, 57, 1014−1022

**Table 8. hERG Inhibition Data**



| compd | R | hERG (μM) |
|---|---|---|
| **4** |  | 8.5 |
| **24** |  | 6.8 |
| **23** |  | 2.8 |
| **17** |  | 2.7 |
| **37** |  | 2.0 |
| **30** |  | 1.8 |

blood clearance after iv administration and moderate bioavalability. Additionally, all of the more active compounds exhibited high affinity for the hERG $K^+$ channel. These liabilities will be addressed along with further investigation of the SAR in a lead optimization campaign.

## EXPERIMENTAL SECTION

**General Comments on Experimental Data.** Chloroform ($CHCl_3$) and tetrahydrofuran (THF) solvents were analytical-grade, without stabilizer; ethyl acetate, hexane, and dichloromethane were distilled. Unless stated otherwise, all other solvents and reagents were purchased from commercial sources and used without further purification. Column chromatography was carried out on silica gel 60 (Fluka), particle size 0.063−0.2 mm (70−230 mesh ASTM), as the stationary phase. Analytical thin-layer chromatograpjy (TLC) was performed on silica on TLC aluminum foils, 20 cm × 20 cm, with fluorescent indicator (200 μm thick, Fluka) visualized under UV light. Routine $^1H$ and $^{13}C$ NMR spectra were recorded on either a Varian Mercury-300 ($^1H$ 300.1 MHz, $^{13}C$ 75.5 MHz) or Bruker-400 ($^1H$ 400.2 MHz, $^{13}C$ 100.6 MHz) instrument. Spectra were recorded at ambient temperature, unless otherwise stated. Chemical shifts ($\delta$) are reported in parts per million from low to high field and referenced to residual solvent. Standard abbreviations indicating multiplicity are used as follows: br s = broad singlet, d = doublet, m = multiplet, q = quartet, quint = quintet, s = singlet, t = triplet. In many cases deuterated dimethyl sulfoxide (DMSO-$d_6$) was used as a solvent and $^1H$ was referenced to 2.500 ppm for the quintuplet downfield methyl signal. $^{13}C$ was referenced to the methyl carbon septuplet at 39.52 ppm. Atmospheric pressure chemical ionization (APCI) mass spectrometry was carried out by the services at the Centre for Drug Candidate Optimisation and Syngene. LC purity traces were performed by one of the methods shown in Supporting Information.

Purity was determined by HPLC or LC-MS, and all compounds were confirmed to have >95% purity.

**Synthesis of 6-Bromo-2,4-dichlorothieno[3,2-*d*]pyrimidine.** To a solution of **13** (20 g, 0.13 mol) in THF (200 mL) at 0 °C was added dropwise trichloroacetyl isocyanate (23.9 g, 0.13 mol), and then the mixture was stirred at rt for 2 h. The reaction mixture was concentrated under reduced pressure. The remaining residue was filtered and washed with diethyl ether to furnish methyl 3-[3-(2,2,2-trichloroacetyl)ureido)]thiophene-2-carboxylate (40 g, 91%) as a white solid. $^1H$ NMR (400.2 MHz, DMSO-$d_6$) $\delta$ = 12.04 (br s, 1H), 11.56 (br s, 1H), 7.95 (d, $J$ = 8.0 Hz, 1H), 7.92 (d, $J$ = 8.0 Hz, 1H), 3.84 (s, 3H); LC-MS (APCI) $m/z$ = 346.6 [$(M + H)^+$] (anal. calcd for $C_9H_7Cl_3N_2O_4S^+$, $m/z$ = 345.59); LC-MS 99.9%.

To a solution of methyl 3-[3-(2,2,2-trichloroacetyl)ureido]-thiophene-2-carboxylate (40 g, 0.12 mol) in acetic acid (400 mL) at 0 °C was added dropwise bromine (74.0 g, 0.46 mol), and then the mixture was stirred at 80 °C overnight. The reaction mixture was concentrated under reduced pressure, and the remaining residue was filtered and washed with diethyl ether (2 × 50 mL) to yield **14** (32 g, 65%) as a white solid. $^1H$ NMR (400.2 MHz, DMSO-$d_6$) $\delta$ = 11.67 (br s, 1H), 9.63 (br s, 1H), 8.16 (s, 1H), 3.78 (s, 3H); LC-MS APCI $m/z$ = 422.8 [$(M + H)^+$] (anal. calcd for $C_9H_6BrCl_3N_2O_4S^+$, $m/z$ = 421.83); LC-MS 92.9%.

To an ice-cooled suspension of **14** (32 g, 0.08 mol) in $CH_3OH$ (300 mL) was added ammonia, until the suspension became clear. The solution was then stirred at rt for 30 min, during which time a white solid precipitated. The white solid was filtered and washed with $CH_3OH$ (2 × 50 mL) to give **15** (16 g, 76%). $^1H$ NMR (400.2 MHz, DMSO-$d_6$) $\delta$ = 9.21 (s, 1H), 8.04 (s, 1H), 6.90 (s, 2H), 3.81 (s, 3H); LC-MS APCI $m/z$ = 278.8 [$(M + H)^+$] (anal. calcd for $C_7H_7BrN_2O_3S^+$, $m/z$ = 277.94); LC-MS 93.1%. To an ice-cooled solution of **15** (16 g, 0.06 mol) in *N*,*N*-dimethylformamide (DMF) (300 mL) was added potassium *t*-butoxide, and the mixture was stirred at rt overnight. The reaction mixture was concentrated under reduced pressure, and deionized water (500 mL) was added to the remaining residue. The solution was then acidified with 50% concentrated $H_2SO_4$, during which time a white solid precipitated. The solid was filtered and washed with deionized $H_2O$ (2 × 25 mL) and $CH_3OH$ (2 × 25 mL) to furnish 6-bromothieno[3,2-*d*]pyrimidine-2,4-diol (14 g, 99%). $^1H$ NMR (400.2 MHz, DMSO-$d_6$) $\delta$ = 11.60 (br s, 1H), 11.32 (br s, 1H), 7.03 (s, 1H); LC-MS APCI $m/z$ = 245.0 [$M^+$] (anal. calcd for $C_6H_3BrN_2O_2S^+$, $m/z$ = 245.91); LC-MS 99.0%.

To a solution of 6-bromothieno[3,2-*d*]pyrimidine-2,4-diol (14 g, 0.06 mol) in $POCl_3$ (300 mL) was added *N*,*N*-dimethylaniline (4.1 g, 0.03 mol), and the resulting solution was stirred at 130 °C overnight. The reaction mixture was concentrated under reduced pressure, and $CH_3OH$ (10 mL) was added to the remaining residue. The white solid was filtered and washed with $CH_3OH$ (2 × 5 mL) to give **16** (14 g, 88%). $^1H$ NMR (400.2 MHz, DMSO-$d_6$) $\delta$ = 8.06 (s, 1H); LC-MS APCI $m/z$ = 282.8 [$(M + H)^+$] (anal. calcd for $C_6HBrCl_2N_2S^+$, $m/z$ = 281.84); LC-MS >99.9%.

**General Procedure 1 for Synthesis of 6-Bromo-$N^4$-[3-(dimethylamino)propyl]-$N^2$-methylthieno[3,2-*d*]pyrimidine-2,4-diamine 3.** To a solution of **16** (5 g, 0.02 mol) in ethanol (150 mL) were added $Na_2CO_3$ (3.7 g, 0.04 mol) and 3-(dimethylamino)-propylamine (2 g, 0.02 mol). The solution was then stirred at rt for 4 h, after which time it was concentrated under reduced pressure. The remaining residue was dissolved in ethyl acetate, washed with brine (3 × 15 mL), dried over $Na_2SO_4$, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel, with $CH_2Cl_2$/$CH_3OH$ in a 9.8:0.2 (v/v) ratio as eluent, to furnish $N^1$-(6-bromo-2-chlorothieno[3,2-*d*]pyrimidin-4-yl)-$N^3$,$N^3$-dimethylpropane-1,3-diamine (4.4 g, 72%). $^1H$ NMR (400.2 MHz, DMSO-$d_6$) $\delta$ = 8.53 (br s, 1H), 7.57 (s, 1H), 2.91 (t, $J$ = 6.9 Hz, 2H), 2.53 (t, $J$ = 6.9 Hz, 2H), 2.34 (s, 6H), 1.79 (t, $J$ = 6.9 Hz, 2H).

$N^1$-(6-Bromo-2-chlorothieno[3,2-*d*]pyrimidin-4-yl)-$N^3$,$N^3$-dimethylpropane-1,3-diamine (4.4 g, 0.013 mol) was added into a sealed tube containing methylamine in THF, 2.0 M (150 mL), and the mixture was stirred at 95 °C overnight. The reaction mixture was concentrated under reduced pressure, and the remaining residue was subjected to column chromatography on silica gel, with $CH_2Cl_2$/$CH_3OH$ in a 9.8:0.2 (v/v) ratio as eluent, to give **3** (3.6 g, 83%) as a white solid. $^1H$ NMR (400.2 MHz, DMSO-$d_6$): $\delta$ = 7.12 (s, 1H), 3.61 (t, $J$ = 6.9 Hz, 2H), 3.24 (t, $J$ = 6.9 Hz, 2H), 3.05 (d, $J$ = 4.8 Hz, 3H), 2.93 (s, 6H), 2.04 (t, $J$ = 6.9 Hz, 2H); $^{13}C$ NMR (100.6 MHz, DMSO-$d_6$) $\delta$ = 161.2, 159.3, 156.0, 125.3, 120.7, 107.2, 55.5, 42.4, 36.8, 26.8, 25.2; Anal. RP-HPLC $t_R$ = 9.58 min (method 1A, purity 99.0%); LC-MS APCI $m/z$ = 344.2 [$M^+$] (anal. calcd for $C_{12}H_{18}BrN_5S^+$, $m/z$ = 344.28).

**General Procedure 2 for Synthesis of $N^4$-[3-(Dimethylamino)propyl]-$N^2$-methyl-6-phenylthieno[3,2-*d*]-**


dx.doi.org/10.1021/jm401760c | J. Med. Chem. 2014, 57, 1014−1022

**pyrimidine-2,4-diamine 4.** To a solution of **3** (0.2 g, 0.58 mmol) in 1,4-dioxane (2 mL) was added phenylboronic acid (0.1 g, 0.70 mmol). The mixture was thoroughly degassed with nitrogen for 15 min, after which time $Pd(PPh_3)_2Cl_2$ (20 mg, 0.03 mmol) was added, followed by (1 M) aqueous $K_2CO_3$ (0.61 mL, 0.61 mmol). The reaction mixture was stirred at 90 °C overnight and extracted with ethyl acetate (4 × 15 mL). The combined organic layers were washed with brine (3 × 10 mL), dried over $Na_2SO_4$, and concentrated in vacuo. The remaining residue was subjected to column chromatography on silica gel, with dichloromethane/methanol in a 8.5:1.5 (v/v) ratio as eluent, to furnish **4** as a white solid (0.13 g, 63%). $^1$H NMR (400.2 MHz, DMSO-$d_6$) $\delta$ = 8.28 (s, 1H), 7.75 (d, $J$ = 6.8 Hz, 2H), 7.53−7.36 (m, 2H), 6.32 (br s, 1H), 3.45−3.42 (m, 2H), 2.81 (s, 3H), 2.66−2.61 (m, 2H), 2.39 (s, 6H) 1.89−1.79 (m, 2H); $^{13}$C NMR (100.6 MHz, DMSO-$d_6$) $\delta$ = 165.1, 162.2, 157.2, 147.7, 133.9, 129.7, 129.5, 126.3, 120.1, 105.5, 56.5, 44.3, 38.6, 28.6, 26.2; Anal. RP-HPLC $t_R$ = 8.59 min (method 1B, purity 98.2%); LC-MS APCI $m/z$ 341.2 [$M^+$] (anal. calcd for $C_{18}H_{23}N_5S^+$, $m/z$ = 341.17).

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Additional text and one figure with details of characterization of selected compounds and procedures used for in vitro and in vivo antimalarial studies as well as in vitro metabolism and mouse exposure studies. This material is available free of charge via the Internet at http://pubs.acs.org.

## ■ AUTHOR INFORMATION


### Corresponding Author

*Phone +27-21-6502553; fax +27-21-6505195; e-mail Kelly.Chibale@uct.ac.za.


### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Medicines for Malaria Venture (MMV) for financial support for this research (Project MMV09/0002). We thank Dr. Jeremy N. Burrows (MMV) and Dr. David Hardick (BioFocus) for helpful discussions. The University of Cape Town, South African Medical Research, and South African Research Chairs initiative of the Department of Science and Technology, administered through the South African National Research Foundation, are gratefully acknowledged for support (K.C.). We thank Christian Scheurer, Petros Papastogiannidis, and Jolanda Kamber for assistance in performing the antimalarial assays.

## ■ ABBREVIATIONS USED

HTS, high throughput screen; SAR, structure−activity relationship; MSD, mean survival time; H2L, hit to lead; LO, lead optimization; PK, pharmacokinetics; hERG, human ether-à-go-go-related gene; rt, room temperature; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; EWG, electron-withdrawing group; EDG, electron-donating group; p.o, per os (oral administration); iv, intravenous administration; AUC, area under the curve; TLC, thin-layer chromatography; HPLC, high-pressure liquid chromatography; TMS, tetramethylsilane; MMV, Medicines for Malaria Venture

## ■ REFERENCES


(1) (a) Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. Artemisinin resistance in *Plasmodium falciparum* malaria. *New Engl. J. Med.* **2009**, *361*, 455−467. (b) Lim, P.; Wongsrichanalai, C.; Chim, P.; Khim, N.; Kim, S.; Chy, S.; Sem, R.; Nhem, S.; Yi, P.; Duong, S.; Bouth, D. M.; Genton, B.; Beck, H.-P.; Gobert, J. G.; Rogers, W. O.; Coppee, J.-Y.; Fandeur, T.; Mercereau-Puijalon, O.; Ringwald, P.; Le Bras, J.; Ariey, F. Decreased *in vitro* susceptibility of *Plasmodium falciparum* isolates to artesunate, mefloquine, chloroquine, and quinine in Cambodia from 2001 to 2007. *Antimicrob. Agents Chemother.* **2010**, *54*, 2135−2142. (c) Noedl, H.; Se, Y.; Schaecher, K.; Smith, B. L.; Socheat, D.; Fukuda, M. M.; Consortium, A. R. C. S. Evidence of artemisinin-resistant malaria in Western Cambodia. *New Engl. J. Med.* **2008**, *359*, 2619−2620.

(2) World Health Organization. *World Malaria Report 2011*; World Health Organization; Geneva, Switzerland, ISBN 9789241564403, 2011.

(3) (a) Miller, L. H.; Baruch, D. I.; Marsh, K.; Doumbo, O. K. The pathogenic basis of malaria. *Nature* **2002**, *415*, 673−679. (b) Burrows, N. J.; Chibale, K.; Wells, T. N. C. The state of the art in anti-malarial drug discovery and development. *Curr. Top. Med. Chem.* **2011**, *11*, 1226−1254. (c) Biot, C.; Chibale, K. Novel approaches to antimalarial drug discovery. *Infect. Disord.: Drug Targets* **2006**, *6*, 173−204.

(4) Duffy, S.; Avery, V. M. Development and optimization of a novel anti-malarial imaging assay validated for high throughput screening. *Am. J. Trop. Med. Hyg.* **2012**, *86*, 84−92.

(5) González Cabrera, D.; Douelle, F.; Feng, T.-S.; Nchinda, A. T.; Younis, Y.; White, K. L.; Wu, Q.; Ryan, E.; Burrows, J. N.; Waterson, D.; Witty, M. J.; Wittlin, S.; Charman, S. A.; Chibale, K. Novel orally active antimalarial thiazoles. *J. Med. Chem.* **2011**, *54*, 7713−7719.

(6) Younis, Y.; Douelle, F.; Feng, T.-S.; Gónzalez Cabrera, D.; Le Manach, C.; Nchinda, A. T.; Duffy, S.; White, K. L.; Shackleford, D. M.; Morizzi, J.; Mannila, J.; Katneni, K.; Bhamidipati, R.; Zabiulla, K. M.; Joseph, J. T.; Bashyam, S.; Waterson, D.; Witty, M. J.; Hardick, D.; Wittlin, S.; Avery, V.; Charman, S. A.; Chibale, K. 3,5-Diaryl-2-aminopyridines as a novel class of orally active antimalarials demonstrating single dose cure in mice and clinical candidate potential. *J. Med. Chem.* **2012**, *55*, 3479−3487.

(7) Paquet, T.; Gordon, R.; Waterson, D.; Witty, M. J.; Chibale, K. Antimalarial aminothiazoles and aminopyridines from phenotypic whole cell screening of a SoftFocus library. *Future Med. Chem.* **2012**, *4* (18), 2265−2277.

(8) Charman, S. A.; Arbe-Barnes, S.; Bathurst, I. C.; Brun, R.; Campbell, M.; Charman, W. N.; Chiu, F. C. K.; 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.

(9) (a) Madapa, S.; Tusi, Z.; Mishra, A.; Srivastava, K.; Pandey, S. K.; Tripathi, R.; Puri, S. K.; Batra, S. Search for new pharmacophores for antimalarial activity. Part II: Synthesis and antimalarial activity of new 6-ureido-4-anilinoquinazolines. *Bioorg. Med. Chem.* **2009**, *17*, 222−234. (b) Mishra, A.; Srivastava, K.; Tripathi, R.; Puri, S. K.; Batra, S. Search for new pharmacophores for antimalarial activity. Part III: Synthesis and bioevaluation of new 6-thioureido-4-anilinoquinazolines. *Eur. J. Med. Chem.* **2009**, *44*, 4404−4412. (c) Verhaeghe, P.; Azas, N.; Gasquet, M.; Hutter, S.; Ducros, C.; Laget, M.; Rault, S.; Rathelot, P.; Vanelle, P. Synthesis and antiplasmodial activity of new 4-aryl-2-trichloromethyl quinazolines. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 396−401. (d) Kabri, Y.; Azas, N.; Dumètre, A.; Hutter, S.; Laget, M.; Verhaeghe, P.; Gellis, A.; Vanelle, P. Original quinazoline derivatives displaying antiplasmodial properties. *Eur. J. Med. Chem.* **2010**, *45*, 616−622. (e) Verhaeghe, P.; Dumètre, A.; Castera-Ducros, C.; Hutter, S.; Laget, M.; Fersing, C.; Prieri, M.; Yzombard, J.; Sifredi, F.; Rault, S.; Rathelot, P.; Vanelle, P.; Azas, N. 4-Thiophenoxy-2-trichloromethy-quinazolines display *in vitro* selective antiplasmodial activity against the human malaria parasite *Plasmodium falciparum*. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6003−6006. (f) Malmquist, N. A.; Moss, T. A.; Mecheri, S.; Scherf, A.; Fuchter, M. J. Small-molecule histone

methyltransferase inhibitors display rapid antimalarial activity against all blood stage forms in *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 16708−16713.
(10) Morgan, J.; Haritakul, R.; Keller, P. A. Antimalarial activity of 2,4-diaminopyrimidines. *Lett. Drug Des. Discovery* **2008**, *5*, 277−280.
(11) (a) Rosowsky, A.; Chen, K. K. N.; Lin, M. 2,4-Diaminothieno-[2,3-*d*]pyrimidines as antifolates and antimalarials, 3. Synthesis of 5,6-disubstituted derivates and related tetracyclic analogs. *J. Med. Chem.* **1973**, *16*, 191−194. (b) Rosowsky, A.; Chaykousky, M.; Chen, K. K. N.; Lin, M.; Modest, E. J. 2,4-Diaminothieno[2,3-*d*]pyrimidines as antifolates and antimalarials, 2. Synthesis of 2,4-diaminopyrido-[4′,3′:4,5]thieno[2,3-*d*]pyrimidines and 2,4-diamino-8*H*-thiopyrano-[4′,3′:4,5]thieno[2,3-*d*]pyrimidines. *J. Med. Chem.* **1973**, *16*, 188−191. (c) Chaykousky, M.; Lin, M.; Rosowsky, A.; Modest, E. J. 2,4-Diaminothieno[2,3-*d*]pyrimidines as antifolates and antimalarials, 1. Synthesis of 2,4-diamino-5,6,4,8-tetrahydrothianaphtheno[2,3-*d*]-pyrimidines and related compounds. *J. Med. Chem.* **1973**, *16*, 185−188.
(12) Le Manach, C.; Scheurer, C.; Sax, S.; Schleiferböck, S.; González Cabrera, D.; Younis, Y.; Paquet, T.; Street, L. J.; Smith, P.; Ding, X.; Waterson, D.; Witty, M. J.; Leroy, D.; Chibale, K.; Wittlin, S. Fast *in vitro* methods to determine the speed of action and the stage-specificity of antimalarials in *Plasmodium falciparum*. *Malar. J.* **2013**, *12*, 424.
(13) (a) Miyaura, N.; Suzuki, A. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457−2483. (b) Thompson, A. E.; Hughes, G.; Batsanov, A. S.; Bryce, M. R.; Parry, P. R.; Tarbit, B. Palladium-catalyzed cross-coupling reactions of pyridylboronic acids with heteroaryl halides bearing a primary amine group: synthesis of highly substituted bipyridines and pyrazinopyr-idines. *J. Org. Chem.* **2005**, *70*, 388−390.