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Novel Orally Active Antimalarial Thiazoles

Diego González Cabrera,[†] Frederic Douelle,[†] Tzu-Shean Feng,[†] Aloysius T. Nchinda,[†] Yassir Younis,[†] Karen L. White,[‡] Quoc Wu,[‡] Eileen Ryan,[‡] Jeremy N. Burrows,[§] David Waterson,[§] Michael J. Witty,[§] Sergio Wittlin,[∥,⊥] Susan A. Charman,[‡] and Kelly Chibale*[,†,#]

[†]Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

[‡]Centre for Drug Candidate Optimisation, Monash University (Parkville campus), 381 Royal Parade, Parkville, VIC 3052, Australia

[§]Medicines for Malaria Venture, ICC, Route de Pré-Bois 20, PO Box 1826, 1215 Geneva, Switzerland

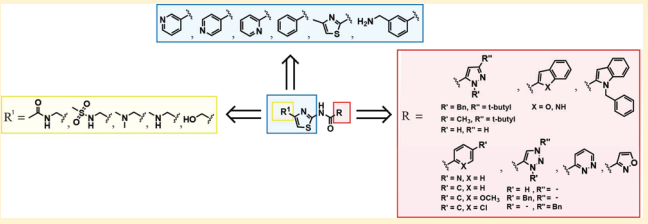
[∥]Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland

[⊥]University of Basel, Socinstrasse 57, 4002 Basel, Switzerland

[#]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

Supporting Information

ABSTRACT: An aminomethylthiazole pyrazole carboxamide lead **3** with good in vitro antiplasmodial activity [IC_{50}: 0.08 μM (K1, chloroquine and multidrug resistant strain) and 0.07 μM (NF54, chloroquine sensitive strain)] and microsomal metabolic stability was identified from whole cell screening of a SoftFocus kinase library. Compound **3** also exhibited in vivo activity in the *P. berghei* mouse model at 4 × 50 mg/kg administration via the oral route, showing 99.5% activity and 9 days survival and showed low in vitro cytotoxicity. Pharmacokinetic studies in rats revealed good oral bioavailability (51% at 22 mg/kg) with a moderate rate of absorption, reasonable half-life ($t_{1/2}$ 3 h), and high volume of distribution with moderately high plasma and blood clearance after IV administration. Toward toxicity profiling, **3** exhibited moderate potential to inhibit CYP1A2 (IC_{50} = 1.5 μM) and 2D6 (IC_{50} = 0.4 μM) as well as having a potential hERG liability (IC_{50} = 3.7 μM).



INTRODUCTION

According to the World Health Organization 2010 report, malaria is responsible for 225 million clinical cases and 781000 deaths annually, especially among children and pregnant women.[1] It is transmitted to humans by four species of protozoan parasites of the genus *Plasmodium*, namely *falciparum*, *vivax*, *malariae*, and *ovale*.[2] Of these, *Plasmodium falciparum* is the most virulent and causes more than 95% of malaria-related morbidity and mortality.[2,3b]

Various antimalarial drugs are presently used for the treatment of this tropical disease; however, the rapid spread of resistance has seriously compromised their efficacy.[2,4] Therefore, efforts continue to be dedicated to the search for novel and structurally diverse compounds with potent antiplasmodial activity while also being affordable.

Screening of a BioFocus DPI (BFDPI) SoftFocus library of 35000 compounds identified a range of hits with greater than 80% inhibition at an average primary and retest concentration of 1.82 μM against the malaria parasite *Plasmodium falciparum* sensitive (3D7) or resistant (Dd2) strains and which also showed no cytotoxicity at that concentration. The library of 35000 compounds represented more than 200 scaffolds that had primarily been designed as potential kinase inhibitors, G-protein coupled receptor (GPCR) antagonists or ion channel modulators. One of the libraries, SFK48, comprised around 170 compounds, primarily ureas (**1**) and aminomethyl thiazole amides (**2**), Figure 1. The objective was to explore the SAR to evaluate this series potential for a late-lead optimization program and systematic exploration of the requirement for the aminomethylthiazole moiety of aminothiazole carboxamides. Although there were a range of examples with alkyl and aryl substituents on the methyl group of the aminomethyl moiety and a range of amides and ureas with a lipophilic "right-hand-side", none of these were as potent as aminomethyl thiazole **3**. Representative compounds from the active series were selected for resynthesis and/or confirmatory testing against *Plasmodia*, and cytotoxicity was determined in the L6 mammalian cell line cytotoxicity assay. A literature search revealed no prior art relating to the antiplasmodial activity of aminomethylthiazoles. However, related compounds have been reported to possess a range of pharmacological properties. These include carboxamido-benzyl pyrazoles as activators of soluble guanylate cyclase[5] and pyrazole amide derivatives as inhibitors of protein kinases.[6] From analogues tested, aminomethylthiazole pyrazole carboxamide **3** was identified as the most promising with good in vitro antiplasmodial activity IC_{50} 0.08 μM (K1, chloroquine and multidrug resistant strain) and 0.07 μM (NF54, chloroquine

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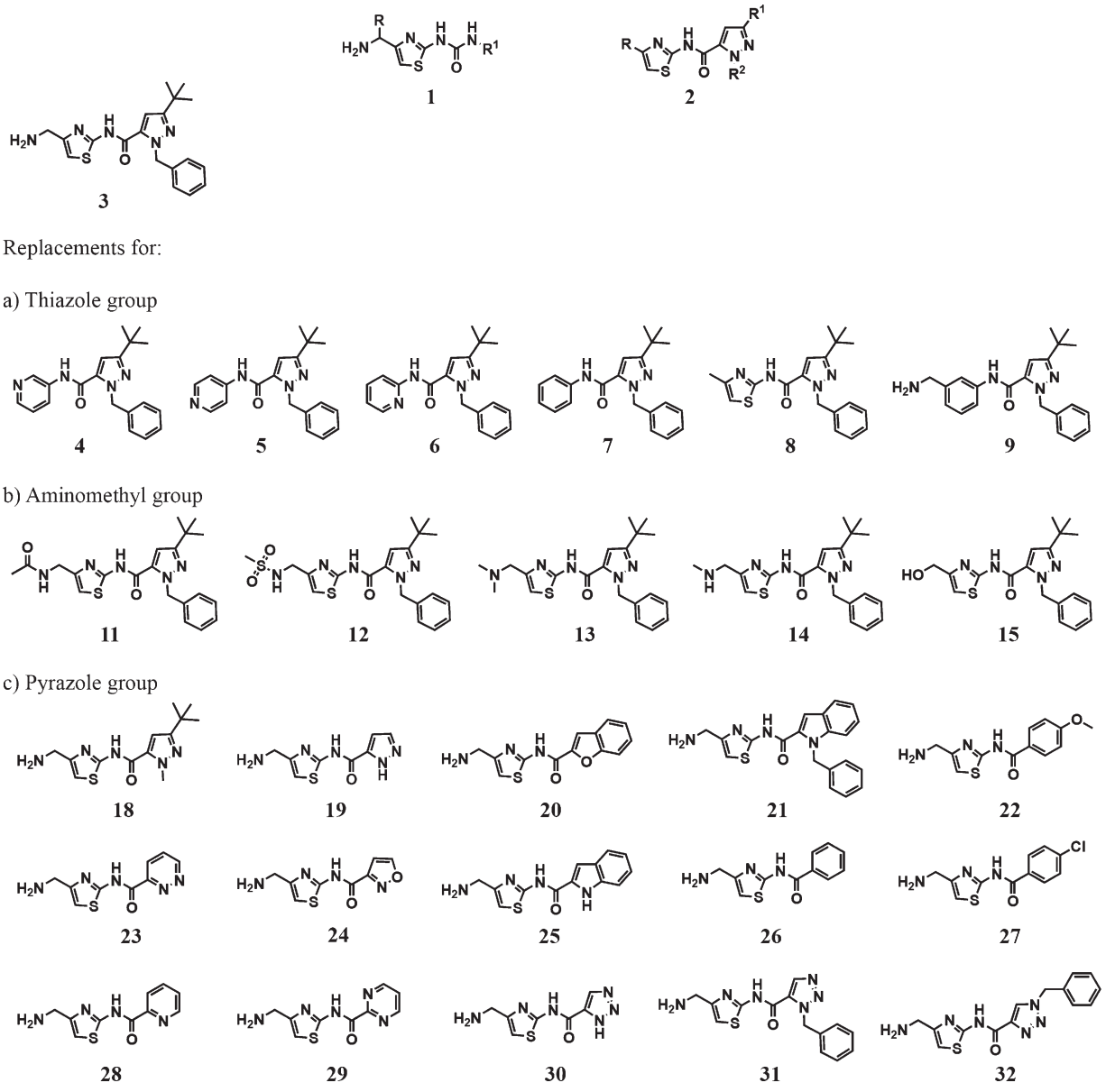

Figure 1. Chemical structures of thiazole ureas and carboxamides.

sensitive strain) vs chloroquine IC_{50} 0.194 μM (K1) and 0.016 μM (NF54) and low cytotoxicity with at least >10-fold selectivity as determined in the L6 mammalian cells. On the basis of these initial results, it was concluded that the series of aminothiazole carboxamides (**2**) offered the combination of novelty, potential clean pharmacology, and in vitro antiplasmodial potency to warrant further investigation as potential antimalarial leads. Initially focusing on aminomethylthiazole pyrazole carboxamide **3**, we set about to investigate the in vitro microsomal metabolic stability using human, rat, and mouse microsomes as well as in vivo efficacy and pharmacokinetic (PK) studies to determine oral bioavailabilities, half-lives, and clearance values. In parallel, we examined potential liabilities of **3** with respect to inhibition of the hERG channel and the major drug metabolizing cytochrome P450s as well as investigation of broader SAR and selectivity vs mammalian kinases. Herein we describe the results of our studies focused on SAR exploration and detailed profiling of the frontrunner compound **3**.

RESULTS AND DISCUSSION

Chemistry. Target compounds for SAR exploration were synthesized by a relatively straightforward synthetic approach (Scheme 1). Compounds **4**−**9** were synthesized via amidation of commercially available pyrazole carboxylic acid, **10**, in the presence of the appropriate amine, to yield analogues where the thiazole group was replaced by pyridyl, phenyl, methylthiazole, and (aminomethyl)phenyl moieties, respectively (Scheme 1a). The synthesis of **3** and related compounds (**11**−**15**) involved the preparation of key starting material **16**,[7] followed by amidation to give key chloro intermediate **17**, which was then converted to **3** by reduction of the azide precursor. Amidation and sulfonylation of compound **3** then delivered amide **11** and sulfonamide **12**, respectively. On the other hand, nucleophilic substitution of the chloro group in intermediate **17** by appropriate nucleophiles resulted in target compounds **13**−**15** (Scheme 1b).

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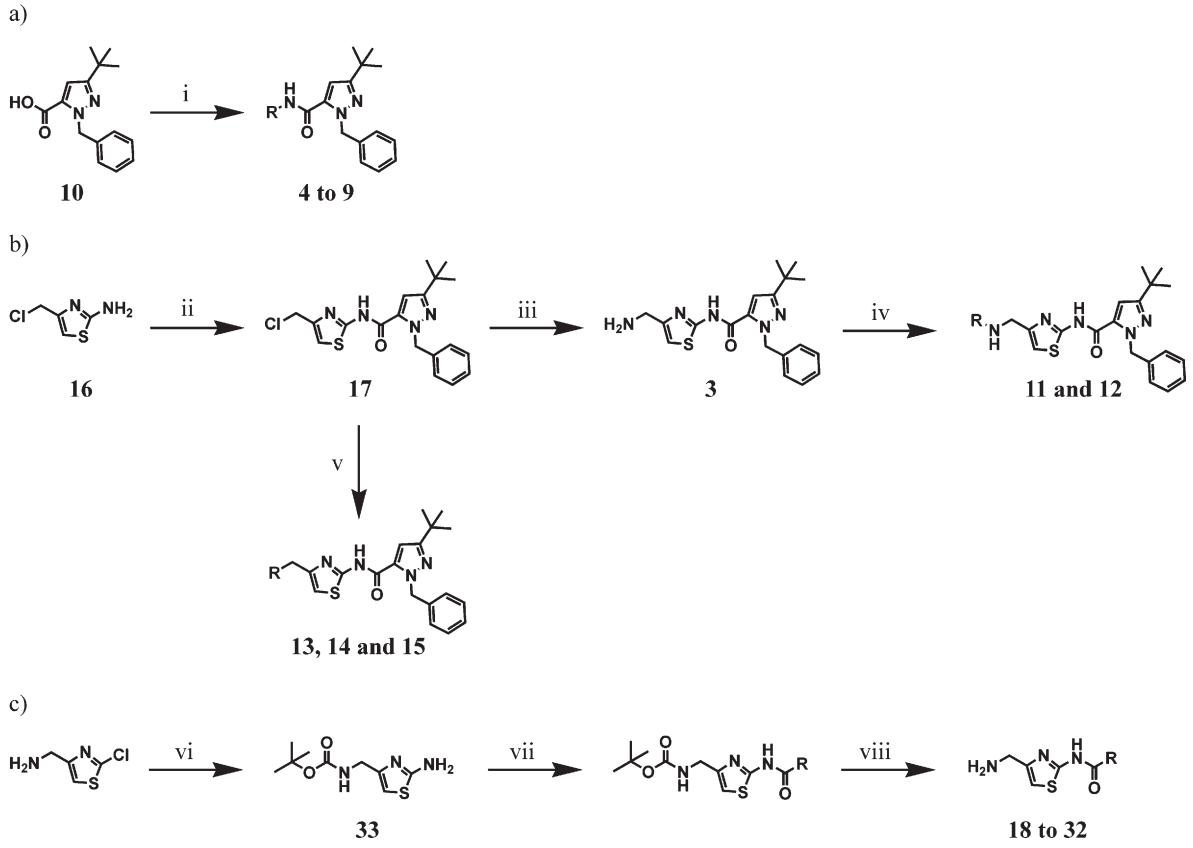
Scheme 1. General Synthetic Approach to Thiazoles Carboxamides[a]



[a] Reagents and conditions: (i) **R** = appropriate amine, EDCI, HOBt, *N*,*N*-diispropylethylamine, CH_2Cl_2, 12 h; (ii) **10**, EDCI, HOBt, *N*,*N*-diispropylethylamine, CH_2Cl_2, 12 h; (iii) NaN_3, DMF, 80 °C, 24 h; H_2O, triphenylphosphine, THF, 24 h; (iv) compound **11**, acetic acid, EDCI, DMAP, CH_2Cl_2, 14 h, compound **12**, methanesulfonyl chloride, triethylamine, CH_2Cl_2, 0 °C to rt, 1 h; (v) compounds **13** and **14**, dimethylamine or methylamine, respectively, CH_2Cl_2, 0 °C to rt, 12 h, compound **15**, TFA, sodium nitrate, 0 °C to rt, 3 h, H_2O; (vi) NH_3 (gas), −15 °C for 30 min, rt for 16 h; triethylamine, *tert*-butyloxycarbonyl anhydride, 0 °C to rt, 16 h; (vii) **R** = appropriate carboxylic acid, EDCI, DMAP, **33**, CH_2Cl_2, 14 h; (viii) trifluoroacetic acid, CH_2Cl_2, rt, 2 h, Amberlyst A-21, CH_2Cl_2/CH_3OH, 30 min.

Analogues from **18**−**32** were synthesized according to Scheme 1c by coupling commercially available or prepared carboxylic acids with amine **33**. Quantitative deprotection of the N-Boc group was accomplished using trifluoroacetic acid, which was then removed with weakly basic Amberlyst A-21 ion-exchange resin.[8]

Biology. *In Vitro Antiplasmodial Activity.* To study the SAR, we first synthesized analogues based on the replacement of the three main substructure motifs in analogue **3**; thiazole, aminomethyl, and pyrazole groups (Figure 1). The in vitro antiplasmodial SAR initially involved evaluation of analogues using a sensitive (NF54) and multidrug resistant (K1) strain of *P. falciparum*[9] with cytotoxicity evaluation (against the murine L-6 mammalian cell line). Chloroquine and artesunate were used as the reference drugs in all the experiments. The in vitro antiplasmodial activities of the compounds, as indicated by their IC_{50} values, are shown in Table 1.

In general, all analogues were equally active against both strains (K1 and NF54). Most compounds were significantly less active with $IC_{50} > 1$ μM. Functionalization or removal of the primary amine generally reduced or lost antiplasmodial activity, with only analogue **14** retaining some potency. Coupling with the potency of **3** demonstrates the significance of the basic amine group, with the unsubstituted aminomethyl group being critical to potent in vitro activity. In general, derivatives in which the pyrazole moiety was replaced by other aromatic and heteroaromatic groups were poorly potent against both strains of the parasite compared to compound **3**, with the suggestion that more lipophilic groups are better tolerated but any change significantly reducing activity. Thus, it appears that a very limited range of structural modifications can be tolerated with the aminomethylthiazole moieties being critical (Figure 2). At this juncture, it is noteworthy that our initial goal was to explore preliminary SAR aimed at establishing the minimum structural requirement for in vitro antiplasmodial activity and microsomal metabolic stability in order to inform future broader SAR studies aimed at identifying compounds, which have the potential for improved potency and other desirable profiles.

Compound **3** was further profiled for potential cardiovascular (hERG) and drug−drug interaction risks. It was found to exhibit moderate potential to inhibit CYP1A2 ($IC_{50} = 1.5$ μM) and 2D6 ($IC_{50} = 0.4$ μM) as well as having a potential hERG liability ($IC_{50} = 3.7$ μM), Table 2.

In Vitro Metabolic Stability. Metabolic stability of the new analogues was assessed in vitro in human, rat, and mouse microsomal preparations.[10] The metabolic stability parameters and microsome-predicted hepatic extraction ratios (E_H) are shown in the Supporting Information. In general, the metabolic stability values were reasonably consistent across the three species. The results

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Table 1. In Vitro Activity against Drug-Sensitive and Drug-Resistant Strains of *Plasmodium falciparum* Solubility and Partition Coefficients Values

	IC_{50} (μM)[a]		solubility (μg/mL)[b]		log D[c]	
compd	K1	NF54	pH 2.0	pH 6.5	pH 3.0	pH 7.4
chloroquine	0.194	0.016				
artesunate	0.003	0.004				
3	0.08	0.07	4931[d]	142.2[d]	2.4	3.5
4	6.45	4.99	>100	6.3−12.5	3.5	3.8
5	7.05	3.88	>100	6.3−12.5	2.6	4.0
6	10.23	10.46	25−50	6.3−12.5	4.5	4.5
7	10.96	7.92	1.6−3.1	1.6−3.1	4.7	4.7
8	4.94	4.12	<1.6	<1.6	4.7	4.7
9	1.47	2.15	>100	25−50	2.2	2.9
11	4.27	6.24	12.5−25	12.5−25	3.3	3.3
12	2.93	4.68	3.1−6.3	3.1−6.3	3.7	3.7
13	1.48	2.41	>100	25−50	2.6	4.2
14	0.19	0.16	25−50	12.5−25	2.6	3.8
15	0.67	0.71	12.5−25	3.1−6.3	3.5	3.4
18	34.11	>34.11	>100	50−100	1.2	1.6
19	>44.83	>44.83	>100	>100	0.0	0.1
20	36.62	>36.62	>100	50−100	1.1	1.5
21	6.98	10.33	25−50	1.6−3.1	2.4	3.0
22	11.10	13.10	50−100	25−50	0.9	1.2
23	42.54	>42.54	>100	50−100	0.0	0.2
24	>44.64	>44.64	50−100	50−100	0.0	−0.1
25	22.21	30.40	>100	25−100	1.1	1.4
26	42.87	>42.87	>100	25−100	0.7	1.0
27	37.45	>37.45	>100	25−100	1.2	1.6
28	>42.73	>42.73	>100	>100	0.3	0.7
29	>42.54	>42.54	>100	>100	−0.2	−0.1
30	>44.63	>44.63	>100	>100	−0.2	−0.3
31	>3.18	>3.18	>100	50−100	1.3	1.6
32	>3.18	>3.18	50−100	25−50	1.1	1.4

[a] Mean from n values of ≥2 independent experiments. [b] Estimates using nephelometry. [c] Value measured using the chromatographic glogD technique. [d] Equilibrium solubility at 37 °C.

indicated that compound **3** was metabolically stable, whereas analogues **4**−**15** exhibited high to moderate rates of metabolic degradation across the three species, with the exception of compounds **9** and **14**, which were the most metabolically stable compounds of the series. It is conceivable that compounds **13** and **14** may undergo metabolic *N*-demethylation reactions. Generally, rates of degradation for the pyrazole containing compound were low to moderate.

Physicochemical Properties. Experimental techniques were used to evaluate the physicochemical properties of the compounds. With the exception of compound **3** for which equilibrium solubility was determined, kinetic solubility was determined for all other compounds. The aqueous solubility of the compounds were quite varied at pH 6.5, with values spanning the range of the kinetic solubility assay. Solubility trends under acidic conditions (pH 2) were as expected; compounds with ionizable basic groups showed improved solubility, whereas the neutral compounds had similar solubility to that at pH 6.5. Compounds generally showed poor to moderate kinetic solubility at pH 6.5, with only compound **13** showing good solubility under neutral conditions. This suggests at least partial ionization of the dimethylamino group of compound **13** at pH 6.5, hence the enhanced solubility. The equilibrium solubility of the frontrunner compound **3** was assessed in pH 2, 6.5, and 7.4 buffers at 37 °C. This compound was found to have very good solubility (>4 mg/mL) at pH 2, reflecting partial ionization due to the basic character of the compound. Under more neutral conditions (pH 6.5), solubility (142.2 μg/mL) was lower but still good. The partion coefficients (log $D_{7.4}$) values were in the range 3.3−4.7. Under acidic conditions (pH 3), some of the compounds demonstrated a drop in partition coefficient, consistent with predicted ionization characterisitics.

In Vivo Studies. Antimalarial Activity against Plasmodium berghei in NMRI Mice. The most active antiplasmodial and metabolically stable analogue (**3**) in the library was tested against *Plasmodium berghei* infected mice.[9] Mean survival results are summarized in Table 3. Low single oral (po) doses (10 and 30 mg/kg) were ineffective, with less than 40% percent reduction in parasitemia compared to untreated, infected animals. Compound **3** was active at single oral doses of 100 and 200 mg/kg, resulting in 94% and 99.4% suppression of parasitemia, respectively. To put these results in perspective, the activity of chloroquine in the *P. berghei* model starts to drop off at 3 mg/kg (83% activity). Determination of the effective doses where 50% and 90% reduction in parasitemia is observed (ED_{50}/ED_{90}) indicated that compound **3** is more than 10-fold less potent compared to chloroquine with ED_{50}/ED_{90} values of 37 and 86 mg/kg versus 1.9 and 4.2 mg/kg (single oral dosing). No significant increase in survival could be achieved with a 4 × 50 mg/kg dosing regimen.

In Vivo Pharmacokinetic Studies. The in vivo pharmacokinetics of compound **3** were assessed following intravenous (IV) and oral administration to male Sprague−Dawley rats (Table 4). The apparent terminal half-life of **3** was approximately 3.7 h after IV administration; volume of distribution was high and plasma and blood clearances were moderate. The blood clearance was consistent with the results of the in vitro metabolic stability studies in rat liver microsomes, which predicted that **3** would be subjected to moderate in vivo hepatic clearance. The rate of absorption after oral administration was relatively slow, with maximum plasma concentrations occurring at 195 min postdose, and the apparent oral bioavailability was approximately 50%. Direct urinary excretion of parent compound was negligible following both intravenous and oral administration. Analogue **3** exhibited moderately high protein binding in human, rat, and mouse plasma at 200 ng/mL with fraction bound values of 94.0%, 97.1%, and 97.9%, respectively.

CONCLUSION

A novel series of aminomethylthiazole pyrazole carboxamides derivatives have been identified which combine good in vitro activity against *P. falciparum* with oral efficacy in a *P. berghei* mouse model. The lead compound **3** shows good stability in rat and mouse microsomes and has a reasonably long half-life in rats with a good pharmacokinetic profile and oral bioavailability. Further work is needed to identify compounds which have the potential for improved potency and a reduced hERG risk profile.

EXPERIMENTAL SECTION

General Comments on Experimental Data. Chloroform ($CHCl_3$) and tetrahydrofuran (THF) solvents were analytical grade, without stabilizer; ethyl acetate, hexane, and dichloromethane (CH_2Cl_2)

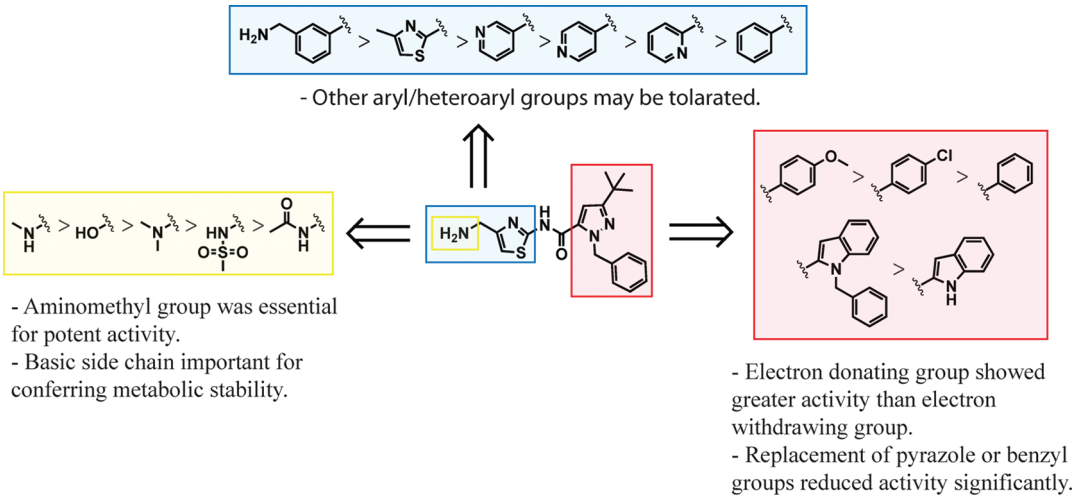

Figure 2. SAR studies on compound **3**.

Table 2. In Vitro Mammalian Cytotoxicity, Cytochrome P450, and hERG Inhibition of Compound 3

cytochrome P450 inhibition (μM)							
1A2	2C9	2C19	2D6	3A 4/5 (midazolam)	3A 4/5 (testosterone)	hERG (μM)	cytotox (L6) (μM)
1.5	10	15	0.4	>30 (16%)	>30 (49%)	3.7	4.9

Table 3. Antimalarial Efficacy Using Single- and Multidose of Compound 3 in *Plasmodium berghei*-Infected Mice[a]

		% reduction parasitemia (MSD)[d]
compd	dose (mg/kg)[c]	oral
3	200	99.4 (8)
	100	94 (6)
	30	<40 (6)
	10	<40 (3)[b]
	4 × 50	99.5 (9)[c]
chloroquine	100	> 99.9 (12)
	30	99.7 (9)
	10	99.5 (7)
	3	83 (7)
	4 × 30	99.9 (24)

[a] Compounds were dissolved or suspended in 70/30 Tween 80/ethanol and diluted 10× with water. [b] Mice were euthanized on day 3, in order to prevent death otherwise occurring at ~day 6. [c] Average of 2 independent experiments. [d] MSD = mean survival time (in days).

were distilled. Unless stated otherwise, all other reagents were purchased from commercial sources and used without further purification. Column chromatography was carried out using silica gel 60 (Fluka) particle size 0.063−0.2 mm (70−230 mesh ASTM) as the stationary phase. Analytical TLC was performed on silica on TLC aluminum foils, H × W 20 cm × 20 cm, with fluorescent indicator (200 μm thick, Fluka) and visualized under UV light. Melting points were determined on a Reichert−Jung

Table 4. Pharmacokinetic Parameters for Compound 3 in Male Sprague Dawley Rats Following Intravenous and Oral Administration

	3	
parameter	IV[a]	oral[a]
measured dose (mg/kg)	4.5	21.8
apparent $t_{1/2}$ (h)	3.7	3.1
plasma CL_{total} (mL/min/kg)	42.6	–[b]
blood CL_{total} (mL/min/kg)	39.1	–
B/P ratio	1.1	–
V_{ss} (L/kg)	10.1	–
C_{max} (μM)	–	1.6
T_{max} (min)	–	195
$AUC_{0-\infty}$ (μM·min)	289	706
bioavailability (%)	–	50.4

[a] Values are the mean from two animals. [b] Dash indicates that the value was not measured or was not relevant.

Thermovar hotstage microscope and are uncorrected. Routine ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Mercury-300 (^{1}H 300.1, ^{13}C 75.5 MHz) or 400 MHz on a Bruker AV 400 (^{1}H 400.0, ^{13}C 100.6 MHz) instrument. Spectra were recorded at ambient temperature unless otherwise stated. Chemical shifts (δ) 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, d = doublet, m = multiplet, q = quartet, quint. = quintet, s = singlet, t = triplet. In many cases [D_6]DMSO was used as a solvent and the ^{1}H was referenced to 2.500 ppm for the quintuplet downfield methyl signal. ^{13}C was reference 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 using one of the methods shown in Supporting Information.

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

General Procedure 1 for the Synthesis of 3-*tert*-Butyl-1-benzyl-*N*-(pyridin-3-yl)-1*H*-pyrazole-5-carboxamide (4). To a solution of 3-*tert*-butyl-1-methyl-1*H*-pyrazole-5-carboxylic acid (0.30 g, 1.16 mmol), 3-aminopyridine (0.11 g, 1.16 mmol), EDCI (0.22 g, 1.16 mmol),

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and HOBt (0.16 g, 1.16 mmol) in dry dichloromethane (5 mL), *N*,*N*-diisopropylethylamine (0.15 g, 1.16 mmol) was added. The resulting reaction mixture was stirred for 12 h and then washed with saturated aqueous sodium hydrogen carbonate (3 × 5 mL), saturated aqueous sodium chloride (2 × 5 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a colorless solid. The remaining residue was subjected to column chromatography on silica gel using dichloromethane/methanol in a 9.9:0.1 v/v ratio (0.21 g, 54%); mp 141−143 °C. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 8.54−8.20 (m, 4H, ArH), 7.22 (br s, 5H, NH), 6.65 (s, 1H, ArH), 5.74 (s, 2H, −CH_2Ph), 1.31 (s, 9H, *t*-butyl). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 161.0, 158.2, 145.7, 141.6, 137.9, 134.8, 134.7, 128.6, 128.0, 127.7 (×2), 123.9, 104.3, 54.8, 32.3, 30.7. Anal. RP-HPLC t_R = 6.82 min (method 1C, purity 97.7%). LRMS (APCI): *m*/*z* = 335.1300 [(M + H)$^+$] (anal. calcd for $C_{20}H_{23}N_4O^+$: *m*/*z* = 335.1872).

1-Benzyl-3-*tert*-butyl-*N*-(4-chloromethylthiazol-2-yl)-1*H*-pyrazole-5-carboxamide (17). EDCI (0.78 g, 4.08 mmol) was added in small batches to a solution of 3-*tert*-butyl-1-methyl-1*H*-pyrazole-5-carboxylic acid (0.55 g, 2.99 mmol) and HOBt (0.55 g, 4.08 mmol) in dichloromethane (15 mL). The reaction mixture was stirred for 10 min, at which time a solution of 4-chloromethyl thiazol-zylamine hydrochloride (0.50 g, 2.72 mmol) and *N*,*N*-diisopropylethylamine (0.70 g, 5.44 mmol) in dichloromethane (5 mL) was added in small portions and then the mixture was stirred for an additional 14 h at room temperature. The solution was washed with saturated aqueous sodium hydrogen carbonate (3 × 10 mL), saturated aqueous sodium chloride (2 × 10 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The remaining residue was subjected to column chromatography on silica gel using hexane/dichloromethane in a 8.5:1.5 v/v ratio as eluent to give the title compound as a white solid (0.90 g, 87%); mp 94−96 °C. ^{1}H NMR (400.0 MHz, $CDCl_3$): δ = 7.27−7.20 (m, 5H, ArH), 6.86 (s, 1H, ArH), 6.49 (s, 1H, ArH), 5.78 (s, 2H, −CH_2Ph), 4.02 (s, 2H, −CH_2Cl), 1.27 (s, 9H, *t*-butyl). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 161.4, 158.9, 157.4, 146.8, 137.5, 133.1, 128.7, 127.8 (×2), 112.4, 105.2, 54.9, 40.8, 32.3, 30.6. LRMS (APCI): *m*/*z* = 388.0496 [M$^+$] (anal. calcd for $C_{19}H_{21}ClN_4OS^+$: *m*/*z* = 388.1125).

3-*tert*-Butyl-*N*-(4-(aminomethyl)thiazol-2-yl)-1-benzyl-1*H*-pyrazole-5-carboxamide (3). NaN_3 (1.34 g, 20.62 mmol) was slowly added to a solution of 1-benzyl-3-*tert*-butyl-*N*-(4-chloromethyl thiazol-2-yl)-1*H*-pyrazole-5-carboxamide (0.40 g, 1.03 mmol) in DMF (5 mL). The reaction mixture was stirred at 80 °C for 24 h and was then concentrated under reduced pressure to give 3-*tert*-butyl-*N*-(4-(azidomethyl)thiazol-2-yl)-1-benzyl-1*H*-pyrazole-5-carboxamide, which was used without further purification. To a solution of 3-*tert*-butyl-*N*-(4-(azidomethyl)thiazol-2-yl)-1-benzyl-1*H*-pyrazole-5-carboxamide (322 mg, 0.82 mmol) in THF (3 mL) was added distilled water (15 mg, 0.82 mmol), followed by triphenylphosphine (235 mg, 0.90 mmol). The reaction mixture was stirred for 24 h and then washed with saturated aqueous sodium hydrogen carbonate (3 × 5 mL), saturated aqueous sodium chloride (2 × 5 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a colorless solid. The remaining residue was subjected to column chromatography on silica gel using hexane/ethyl acetate in a 8:2 v/v ratio as eluent to furnish **3** as a light-yellow solid (202 mg, 67%); mp 163−165 °C. ^{1}H NMR (400.0 MHz, $CDCl_3$): δ = 7.24−7.15 (m, 5H, ArH), 6.82 (s, 1H, ArH), 6.61 (s, 1H, ArH), 5.76 (s, 2H, −CH_2Ph), 3.79 (s, 2H, −CH_2NH$_2$), 1.29 (s, 9H, *t*-butyl). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 161.3, 158.5, 157.4, 152.8, 137.7, 133.4, 128.6, 127.9, 127.7, 108.2, 105.1, 54.8, 42.4, 32.3, 30.6. Anal. RP-HPLC t_R = 7.86 min (method 1A, purity 98. 2%). LRMS (APCI): *m*/*z* = 370.1404 [(M + H)$^+$] (anal. calcd for $C_{19}H_{24}N_5OS^+$: *m*/*z* = 370.1702).

General Procedure 2 for the Synthesis of 3-*tert*-Butyl-*N*-(4-(acetamidomethyl)thiazol-2-yl)-1-benzyl-1*H*-pyrazole-5-carboxamide (11). EDCI (68 mg, 0.35 mmol) was added in small batches to a solution of acetic acid (9 mg, 0.16 mmol) and DMAP (43 mg, 0.35 mmol) in dichloromethane (2 mL). The reaction mixture was stirred for 10 min, at which time 3-*tert*-butyl-*N*-(4-(aminomethyl)-thiazol-2-yl)-1-benzyl-1*H*-pyrazole-5-carboxamide (52 mg, 0.14 mmol) was added in small portions and then the mixture was stirred for an additional 14 h at room temperature. The solution was washed with saturated aqueous sodium hydrogen carbonate (3 × 5 mL), saturated aqueous sodium chloride (2 × 5 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a colorless solid. The remaining residue was subjected to column chromatography on silica gel using dichloromethane/methanol in a 9.8:0.2 v/v ratio as eluent to furnish **11** as a white solid (37 mg, 63%); mp 121−123 °C. ^{1}H NMR (300.1 MHz, $CDCl_3$): δ = 7.31−7.20 (m, 5H, ArH), 6.77 (s, 1H, ArH), 6.74 (s, 1H, ArH), 6.05 (t, *J* = 5.7 Hz, 1H, −N*H*−), 5.81 (s, 2H, −CH_2Ph), 4.37 (d, *J* = 5.7 Hz, 2H, −CH_2−), 1.99 (s, 3H, −CH_3), 1.34 (s, 9H, *t*-butyl). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 170.1, 161.5, 157.9, 156.9, 147.9, 137.6, 133.0, 128.7, 127.9, 127.8, 110.3, 104.7, 54.2, 40.0, 30.7, 29.9, 23.5. Anal. RP-HPLC t_R = 8.55 min (method 1A, purity 95.6%). LRMS (APCI): *m*/*z* = 412.1732 [(M + H)$^+$] (anal. calcd for $C_{21}H_{26}N_5O_2S^+$: *m*/*z* = 412.1807).

(2-Chlorothiazol-4-yl)methanamine (16). To a solution of 1,3-dichloroacetone (25.0 g, 196.9 mmol) in dry acetone (100 mL) and thiourea (15.3 g, 200.8 mmol) in dry acetone (500 mL) was added dropwise and stirred at room temperature for 4 h. The reaction mixture was concentrated under vacuum, and absolute ethanol (200 mL) was added and allowed to stir at room temperature for 3 h. The reaction mixture was then filtered and washed with ethanol (2 × 50 mL). The filtrate was concentrated under vacuum, washed with dichloromethane (2 × 50 mL), and dried to afford **16** as a hydrochloric salt (26.0 g, 71%). ^{1}H NMR (400.0 MHz, [D$_6$]DMSO): δ = 9.17 (br s, 2H, NH_2), 6.97 (s, 1H, ArH), 4.68 (s, 2H, −CH_2NH$_2$). LRMS (APCI): *m*/*z* = 149.0 [(M + H)$^+$] (anal. calcd for $C_4H_6ClN_2S^+$: *m*/*z* = 149.0).

***tert*-Butyl (2-Aminothiazol-4-yl)methylcarbamate (33).** A solution of (2-chlorothiazol-4-yl)methanamine (15.0 g, 81.0 mmol) in dry methanol (250 mL) was purged with ammonia gas at −15 °C for 30 min in a sealed tube, at which time the reaction mixture was allowed to stir at room temperature for 16 h. The solution was then concentrated under vacuum to afford a yellow solid, which was used without further purification. To a suspension of 4-(aminomethyl)thiazol-2-amine (7.3 g, 56.7 mmol) and triethylamine (17.2 g, 170.1 mmol) in *N*,*N*-dimethyformamide (70 mL). *tert*-Butyloxycarbonyl anhydride (12.4 mL, 56.7 mmol) was slowly added at 0 °C. The reaction mixture was stirred at room temperature for 16 h, at which time distilled water (140 mL) was added. The mixture was extracted with ethyl acetate (4 × 200 mL). The ethyl acetate layers were combined, washed with saturated aqueous sodium hydrogen carbonate (3 × 25 mL), saturated aqueous sodium chloride (2 × 25 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a colorless solid. The remaining residue was subjected to column chromatography on silica gel using petroleum ether/ethyl acetate in a 6:4 v/v ratio as eluent to furnish **33** as a white solid (6.10 g, 47%); mp 113−115 °C. ^{1}H NMR (400.0 MHz, $CDCl_3$): δ = 6.32 (s, 1H, ArH), 5.02 (br s, 1H, NH_2), 4.18 (d, *J* = 5.6 Hz, 2H, −CH_2NH−), 1.71 (br s, 2H, NH_2), 1.45 (s, 9H, *t*-butyl). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 168.4, 155.5, 150.3, 100.5, 77.7, 40.6, 28.2. LRMS (ESI): *m*/*z* = 230.2 [(M + H)$^+$] (anal. calcd for $C_9H_{16}N_3O_2S^+$: *m*/*z* = 230.1).

General Procedure 2 for the Synthesis of 3-*tert*-Butyl-*N*-[4-(aminomethyl)thiazol-2-yl]-1-methyl-1*H*-pyrazole-5-carboxamide (18). EDCI (0.63 g, 3.27 mmol) was added in small batches to a solution of 3-(*tert*-butyl)-1-methyl-1*H*-pyrazole-5-carboxylic acid (0.44 g, 2.40 mmol) and HOBt (0.44 g, 3.27 mmol) in dry dichloromethane (10 mL). The reaction mixture was stirred for 10 min, at which time a solution of *tert*-butyl (2-aminothiazol-4-yl)methylcarbamate (0.50 g, 2.18 mmol) and *N*,*N*-diisopropylethylamine (0.56 g, 4.36 mmol) in dry dichloromethane (5 mL) was added in small portions and then the mixture was stirred for an additional 14 h at room temperature. The solution was washed with saturated aqueous sodium hydrogen carbonate

(3 × 10 mL), saturated aqueous sodium chloride (2 × 10 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give *tert*-butyl [2-(3-*tert*-butyl-1-methyl-1*H*-pyrazole-5-carboxamido)thiazol-4-yl]methylcarbamate as a colorless solid. The remaining residue was subjected to column chromatography on silica gel using hexane/ethyl acetate in a 8.5:1.5 v/v ratio as eluent (0.65 g, 76%). To a suspension of *tert*-butyl [2-(3-*tert*-butyl-1-methyl-1*H*-pyrazole-5-carboxamido)thiazol-4-yl]methylcarbamate (0.29 g, 0.73 mmol) in dichloromethane (5 mL) was added TFA (0.29 mL, 3.67 mmol). The pale-yellow solution was stirred vigorously for 12 h. The reaction mixture was concentrated under reduced pressure to give a pale-yellow solid. The resulting powder was stirred with Amberlyst A-21 resin in a dichoromethane/methanol 1:1 v/v ratio (20 mL) for 1 h. The reaction mixture was then filtered, and the solvent was removed under pressure to yield **18** as a hygroscopic powder (0.22 g, 100%); mp 235−237 °C. ^{1}H NMR (400.0 MHz, [D_6]DMSO): δ = 7.28 (s, 1H, ArH), 7.25 (s, 1H, ArH), 4.07 (s, 5H, $-CH_2NH_2$ and $-CH_3$), 1.27 (s, 9H, *t*-butyl). ^{13}C NMR (100.6 MHz, [D_6]DMSO): δ = 159.1, 158.3, 157.8, 144.1, 133.1, 111.8, 105.7, 39.1, 38.6, 31.6, 30.3. Anal. RP-HPLC t_R = 6.60 min (method 1A, purity 97.4%). LRMS (APCI): m/z = 294.18 [$(M + H)^+$] (anal. calcd for $C_{13}H_{20}N_5OS^+$: m/z = 294.14).

■ ASSOCIATED CONTENT

Supporting Information. Additional details of the characterization of selected compounds and the procedures used for the in vitro and in vivo antimalarial studies as well as metabolism and PK studies. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +27-21-6502557. Fax: +27-21-6505195. E-mail: Kelly.Chibale@uct.ac.za.

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■ ABBREVIATIONS USED

ip, intraperitoneal injection; po, oral administration; HPLC, high pressure liquid chromatography; HPMC, hydroxypropyl methyl cellulose; MSD, mean survival time; SAR, structure−activity relationship; PK, pharmacokinetics; GPCR, G protein coupled receptor; TLC, thin layer chromatography; TMS, tetramethylsilane; MMV, Medicines for Malaria Venture

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