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Structure–activity relationship studies of antiplasmodial aminomethylthiazoles

Peter Mubanga Cheuka [a], Diego Gonzalez Cabrera [a], Tanya Paquet [a], Kelly Chibale [a,b,c,*]

[a] Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa
[b] Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa
[c] South African Medical Research Council Drug Discovery and Development Research Unit, University of Cape Town, Rondebosch 7701, South Africa

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ABSTRACT

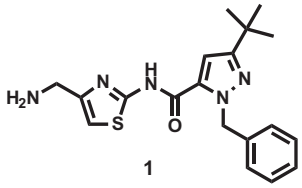
Structure–activity relationship (SAR) studies around a previously reported antimalarial aminomethylthiazole pyrazole carboxamide **1** are reported. Several analogues were synthesised and profiled for in vitro antiplasmodial activity against the drug-sensitive *Plasmodium falciparum* malaria parasite strain, NF54. Although all the reported analogues exhibited inferior in vitro antiplasmodial activity (IC_{50} = 0.125–173 μM) relative to compound **1** (IC_{50} = 0.0203 μM), one analogue, compound **5a**, retained submicromolar activity (IC_{50} = 0.125 μM).

Endemic mainly in tropical and subtropical regions of the world, malaria closely rivals human immunodeficiency virus (HIV) and tuberculosis (TB) infections in being a devastating and lead killer disease.[1] According to the World Health Organization's latest estimates released in 2013, about 207 million malaria cases and 627,000 deaths were reported in 2012 especially among children in Africa.[2] There are four species of malaria parasites of the genus *Plasmodium* implicated in human malaria infection and these include; *falciparum*, *vivax*, *malariae*, and *ovale* with *Plasmodium falciparum* being the most deadly. In addition, *Plasmodium knowlesi*, a strain responsible for infections in monkeys has been recently reported in humans.[2]

The efficacy of most marketed and commonly used antimalarial drugs has been seriously compromised due to the emergence of resistance.[3–5] Thus, there is a critical need to intensify research efforts aimed at developing effective and affordable drugs against this parasitic disease.

Aminomethylthiazoles have recently been recognised as potential antimalarial agents.[6a] Lead compound **1** (Fig. 1) was characterised by good in vitro antiplasmodial activity against drug sensitive, NF54, (IC_{50} = 0.07 μM) and multi-drug resistant, K1, (IC_{50} = 0.08 μM) strains of *P. falciparum*. In addition, compound **1** demonstrated promising in vivo efficacy in the *Plasmodium berghei* mouse model showing 99.5% reduction in parasitemia at an oral dose of 4 × 50 mg/kg. Preliminary SAR studies to establish the minimum structural requirements for in vitro antiplasmodial activity revealed that the unsubstituted aminomethyl group was critical for potent activity. Furthermore, the activity was significantly reduced in derivatives lacking the pyrazole moiety. However, in the aforementioned studies, replacements or modifications of the thiazole and pyrazole motifs, as well as other portions of compound **1** were not comprehensively studied. We, therefore, set to expand SAR studies focusing on these aspects.

In the hope of uncovering other aromatic/heteroaromatic groups that might be tolerated in place of the thiazole and pyrazole cores, analogues **5a**, **5c** and **11e–k** were synthesised. In addition, analogue **5b** was designed to investigate how tolerable an alternative position for the aminomethyl side chain on the thiazole ring is. Within the context of replacements for the pyrazole core, analogues **15a–e** and **11a** were synthesised in which substituents on the benzyl moiety were introduced or the benzyl moiety



1

Figure 1. Chemical structure of lead compound **1**.

* Corresponding author. Tel.: +27 21 6502553; fax: +27 21 6505195.
E-mail address: Kelly.Chibale@uct.ac.za (K. Chibale).

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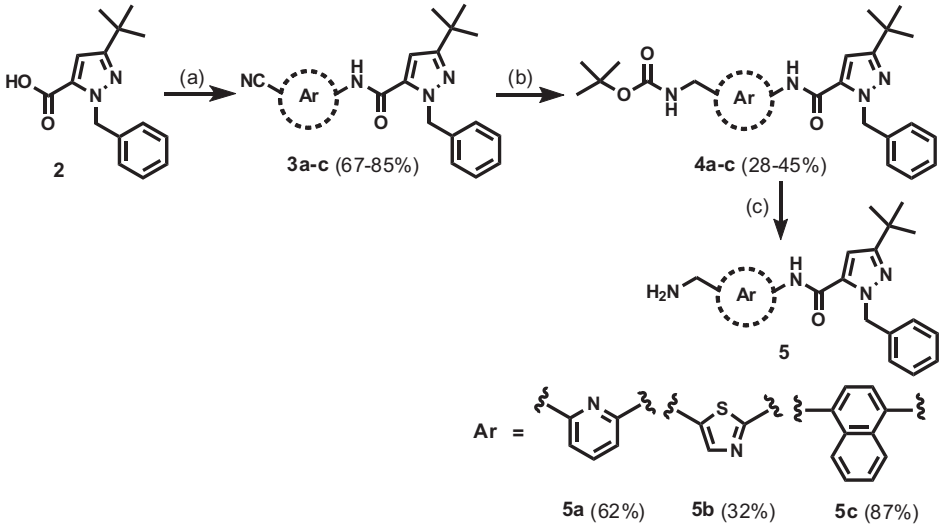
0960-894X/

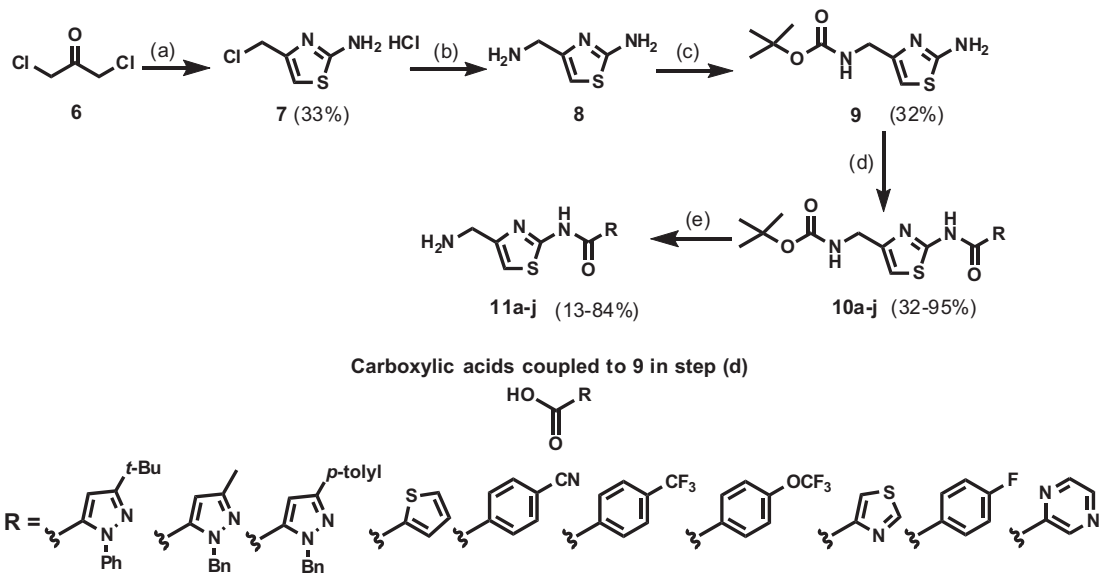
replaced with a phenyl group. Furthermore, to evaluate the importance of the *tert*-butyl group, a final set of analogues (**11b**, **11c**, and **15f**) were generated. The introduction of substituents on the aromatic portion of the benzyl group and its replacement with a phenyl group as well as replacement of the *tert*-butyl group represented new dimensions which were not explored in the reported preliminary SAR.[6a]

Analogues **5a**–**c** were synthesised following the synthetic route outlined in Scheme 1. An appropriate aromatic/heteroaromatic aminonitrile was reacted with commercially available benzyl pyrazole carboxylic acid **2**.[6b] This afforded intermediates **3a**–**c**, which were later catalytically reduced to primary amines and Boc-protected in a one pot reaction.[7] The in situ Boc-protection served to avoid undesirable dimerization which is a common side reaction in nitrile reductions.[7,8a] Deprotection of the *N*-Boc-protected precursors was accomplished using trifluoroacetic acid (TFA) followed by its removal with Amberlyst A-21 free base[8b] to afford the target compounds **5a**–**c** in relatively good yields (32–87%). A literature protocol[6] was employed to realise compound **1**. This involved the acid-amine coupling (EDCI–HOBt mediated) of the amine hydrochloride salt **7** (basified) and the benzyl pyrazole carboxylic acid **2**. Nucleophilic azide substitution of the chloro group followed by Staudinger reduction of the azide gave the free amine compound **1**.

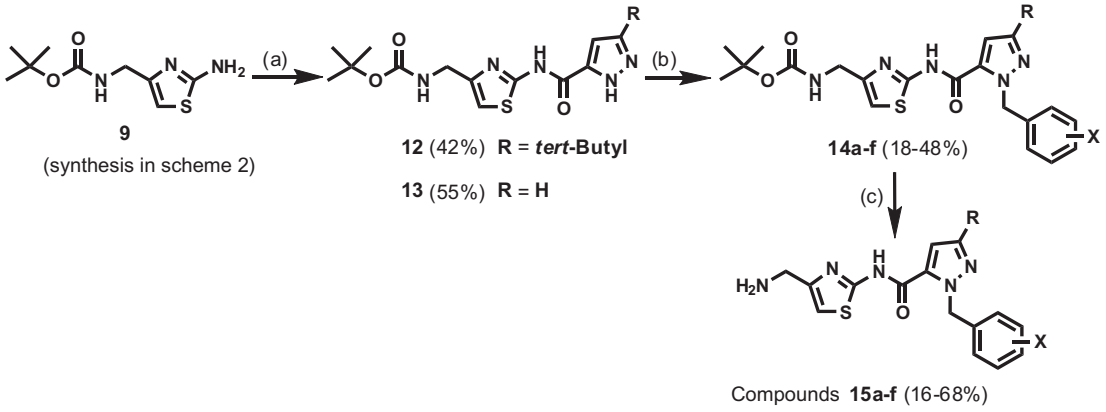
Target compounds **11a**–**j** were synthesised according to Scheme 2. Amine hydrochloride salt, **7**, was obtained in 33% yield through condensation of 1,3-dichloroacetone (**6**) with thiourea. This intermediate was further reacted with excess methanolic ammonia to afford compound **8** which was used without further purification. Intermediate **8** was regioselectively *N*-Boc-protected to afford the corresponding amino derivative **9** in 32% yield. The obtained amino intermediate, **9**, was then coupled to a variety of commercially available carboxylic acids[6b] to obtain corresponding amides **10a**–**j** in good to excellent yields (32–95%). Finally, depro-



Scheme 1. Reagents and conditions: (a) appropriate aminonitrile, EDCI, DMAP, CH_2Cl_2, rt, 4–96 h; (b) Boc_2O, $NiCl_2$, $NaBH_4$, 0 °C to rt, 3–72 h, then 1,2-ethylenediamine; (c) TFA, CH_2Cl_2, rt, 15–24 h, then Amberlyst A-21, CH_2Cl_2/CH_3OH, 1 h.

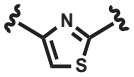
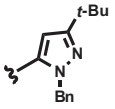
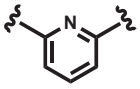
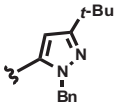
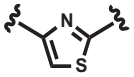
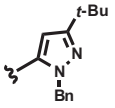
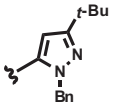
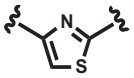
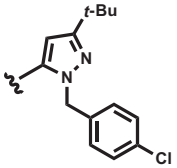
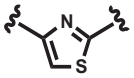
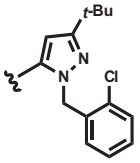
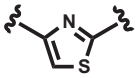
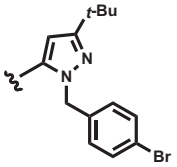


Scheme 2. Reagents and conditions: (a) $CS(NH_2)_2$, acetone, rt, 48 h; (b) methanolic ammonia (7*N*), sealed tube, rt, 72 h; (c) triethylamine, di-*tert*-butyl dicarbonate, 0 °C to rt, 20 h; (d) R = appropriate carboxylic acid, EDCI, DMAP, CH_2Cl_2, 3–48 h; (e) TFA, CH_2Cl_2, rt, 4–42 h, then Amberlyst A-21, CH_2Cl_2/CH_3OH, 1 h.



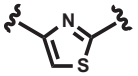
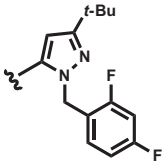
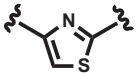
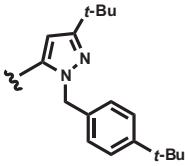
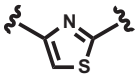
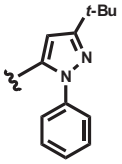
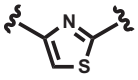
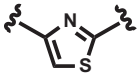
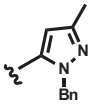
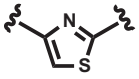
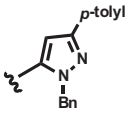
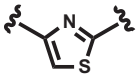
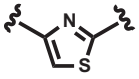
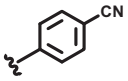
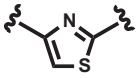
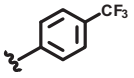
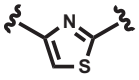
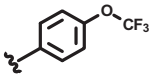
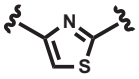
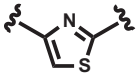
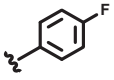
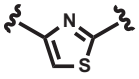
Scheme 3. Reagents and conditions: (a) appropriate pyrazole carboxylic acid, EDCI, DMAP, CH_2Cl_2 (**12**), DMF (**13**) rt, 20–48 h; (b) appropriate benzyl bromide, K_2CO_3, DMF or acetonitrile, rt, 21–48 h; (c) TFA, CH_2Cl_2, rt, 12–72 h, then Amberlyst A-21, CH_2Cl_2/CH_3OH, 1 h.

Table 1
In vitro antiplasmodial activity and aqueous solubility of compounds **1**, **5a**–**c**, **11a**–**j**, and **15a**–**f**

Compound	Ar	R	IC_{50}[a] (μM) NF54	Solubility[b] (μg/mL) pH 7.4
1			0.0203	>200
5a			0.125	40
5b			9.09	80
5c			7.59	ND*
15a			12.1	>200
15b			1.84	80
15c			12.8	>200

(*continued on next page*)

Table 1 (*continued*)

Compound	Ar	R	IC_{50}[a] (μM) NF54	Solubility[b] (μg/mL) pH 7.4
15d			4.70	ND*
15e			2.58	10
11a			67.5	160
15f			43.7	>200
11b			19.8	160
11c			2.03	40
11d			91.7	80
11e			146	20
11f			40.2	40
11g			43.9	40
11h			149	80
11i			123	80
11j			173	>200
Chloroquine	—	—	0.0106	—
Artesunate	—	—	0.00520	—

* ND = Not determined.
[a] IC_{50} values are averages of experiments independently performed in triplicate.
[b] Estimates using turbidimetric (kinetic) solubility assay.

tection of the Boc-protected amino functionalities with TFA afforded compounds **11a–j** in good to excellent yields (13–84%).

Scheme 3 was employed to access compounds **15a–f**. EDCI coupling between commercially available carboxylic acids and amine **9** afforded intermediates **12** and **13** in 42 and 55% yields, respectively, which were then N-benzylated with various benzyl bromides in presence of K_2CO_3.[9] The resulting intermediates were deprotected by treatment with TFA to give final compounds **15a–f**.

Target compounds, **1**, **5a**–**c**, **11a**–**j** and **15a**–**f**, were evaluated for in vitro antiplasmodial activity against the NF54 (drug-sensitive) strain of *P. falciparum* and for aqueous solubility at pH 7.4 (Table 1).

Analogues **5a**–**c**, resulting from the replacement of the thiazole core exhibited inferior activity to compound **1** (IC_{50} = 0.0203 μM), albeit, compound **5a** retained submicromolar activity (IC_{50} = 0.125 μM). Moreover, analogue **5a** turned out to be the most potent of all the newly synthesised compounds. Changing the position of the nitrogen and sulfur atoms, equivalent to changing the position of the aminomethyl side chain as reflected in analogue **5b**, led to a striking loss of activity (IC_{50} = 9.09 μM) indicating the importance of these positions.

Compounds **11d**–**j**, that are based on replacement of the bulky benzyl pyrazole group with simpler aromatic/heteroaromatic groups led to a significant drop in activity with IC_{50} values in the range of 40.2–173 μM. This is consistent with previous findings.[6a]

More subtle modifications such as introduction of substituents on the aromatic portion of the benzyl group as well as its replacement with a phenyl moiety as in analogues **11a** and **15a**–**e**, resulted in reduction of activity albeit less so for analogues **15a**–**e** (IC_{50} = 1.84–12.8 μM). The significant reduction in activity for analogue **11a** (IC_{50} = 67.5 μM) highlights the importance of the benzyl methylene (CH_2) linker for potent activity. Analogue **15b** bearing a chloro group on the *ortho* position of the benzyl ring was the most potent (IC_{50} = 1.84 μM) of all the analogues in this series. Interestingly, **15b** was also more potent than its *para* regioisomer **15a** (IC_{50} = 12.1 μM)—indicating that *ortho* halogenation was the most favourable. The nature of the halogen at the *para* position of the benzyl group appeared to have no significant effect on activity as analogues **15a** (IC_{50} = 12.1 μM with a *para*-chloro group) and **15c** (IC_{50} = 12.8 μM with a *para*-bromo group) were roughly equipotent.

Finally, the design of analogues **11b**, **11c**, and **15f** sought to expand the SAR with respect to replacements of the *tert*-butyl group on the pyrazole ring. For this small series of analogues, it was observed that although the activity was compromised overall in these analogues, bulky groups seemed much more tolerated than smaller ones. For instance, analogue **11c** (with a *para*-tolyl group) was more potent (IC_{50} = 2.03 μM) than the unsubstituted analogue **15f** (IC_{50} = 43.7 μM) while analogue **11b**, having a methyl group in lieu of the *tert*-butyl group had intermediate potency (IC_{50} = 19.8 μM).

To conclude, the SAR study around antimalarial aminomethylthiazole pyrazole carboxamide **1** was further expanded. It is apparent that there is a tight SAR around compound **1**. All the changes thus far made on the molecule resulted in loss of activity. Peripheral modifications such as introduction of substituents on the aromatic portion of the benzyl group as well as replacing the *tert*-butyl group of the pyrazole with other groups appeared tolerable. The benzyl group was also found to be important for activity. Replacements of the *tert*-butyl group with bulky substituents appeared much more tolerated although activity was generally reduced. Gratifyingly, replacements of the thiazole core gave rise to the pyridyl analogue, **5a** (IC_{50} = 0.125 μM), which retained potent activity. Further SAR explorations with respect to this portion of the molecule are, therefore, warranted. Lastly, replacement of the benzyl pyrazole motif with smaller aromatic and heteroaromatic groups was found to be detrimental to activity.

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Supplementary data

Supplementary data (experimental details for synthetic protocols, chemical characterisation of compounds, and biological procedures) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.09.071.

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