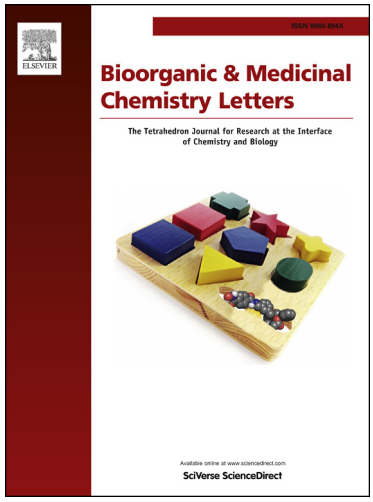

Synthesis and biological evaluation of 2-aminothiazole derivatives as antimycobacterial and antiplasmodial agents

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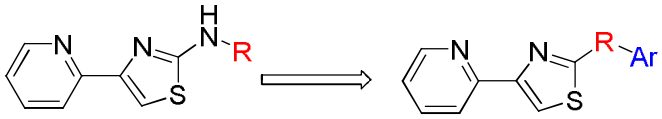

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Synthesis and biological evaluation of 2-aminothiazole derivatives as antimycobacterial and antiplasmodial agents

Faith Mjambili[a], Mathew Njoroge[a], Krupa Naran[b], Carmen De Kock[c], Peter J. Smith[c], Valerie Mizrahi[b,d] Digby Warner[b,d], and Kelly Chibale[a,d] *

[a]Department of Chemistry, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa
[b]MRC/NHLS/UCT Molecular Mycobacteriology Research Unit, Division of Medical Microbiology, Department of Clinical Laboratory Sciences, University of Cape Town, Rondebosch 7701, South Africa
[c] Division of Pharmacology, Department of Medicine, University of Cape Town, K45, OMB, Groote Schuur Hospital, Observatory 7925, South Africa
[d]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa

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ABSTRACT

A series of compounds derived from the 2-amino-4-(2-pyridyl) thiazole scaffold was synthesized and tested for *in vitro* antimycobacterial activity against the *Mycobacterium tuberculosis* H37Rv strain, antiplasmodial activity against the chloroquine sensitive NF54 *Plasmodium falciparum* strain and cytotoxicity on a mammalian cell line. Optimal antimycobacterial activity was found with compounds with a 2-pyridyl ring at position 4 of the thiazole scaffold, a substituted phenyl ring at the 2-amino position, and an amide linker between the scaffold and the substituted phenyl.The antiplasmodial activity was best with compounds that had the phenyl ring substituted with hydrophobic electron withdrawing groups.

Tuberculosis (TB) is the second leading cause of death from infectious disease globally.[1] It is an airborne disease caused by *Mycobacterium tuberculosis* (*M.tb*),[2] and was declared a global public health emergency by the World Health Organization (WHO) in 1993.[3] In 2011 the WHO estimated that TB prevalence stood at 12 million cases worldwide, with approximately 1.4 million deaths annually.[1] Although there is an existing pipeline of drugs under development that will bring new anti-tubercular agents into clinical use in the near future,[2–4] it is still not sufficient to address the need for new regimens that are effective against drug-susceptible and drug-resistant TB. This highlights the urgent need for new chemotypes with anti-mycobacterial activity.

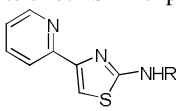
Like TB, malaria too requires new chemotherapeutic agents urgently. A parasitic disease, malaria is caused by five species of the genus *Plasmodium*; *falciparum*, *vivax, malariae, ovale* and *knowlesi* which are transmitted by the *anopheles* mosquito.[5] Malaria has a global distribution with an estimated 219 million cases and 660,000 deaths in 2010 according to WHO.[5] Although malaria can effectively be treated and cured, the rapid and widespread resistance to known

* Corresponding author. Tel.: +27216505495; fax: +27216505495; e-mail: Kelly.Chibale@uct.ac.za

antimalarial agents is the biggest challenge.[6] Recent reports of resistance to ACTs which are the current mainstay of malaria chemotherapy are worrying as there are no ready alternatives.[7] This heightens the need to search for new chemical entities with antiplasmodial activity.

Thiazole derivatives are known to exhibit a broad range of biological activities,[8] and have previously been documented to possess antimycobacterial and antiplasmodial activity.[9–12] Of interest are compounds that possess the 2-amino-4-(2-pyridyl) thiazole scaffold **1** (Figure 1) with an aryl or aryl alkyl substituent on the amino group. The scaffold **1** was identified in a high through-put screen (HTS) conducted in 2009 by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF).[13]

To the best of our knowledge, there are no Structure-Activity Relationship (SAR) studies reported on 2-amino-4-(2-pyridyl)thiazoles as either antimycobacterial or antiplasmodial agents. The objective of this work was therefore to explore the SAR of this class of compounds and evaluate them as potential antimycobacterial and/or antiplasmodial agents. Herein we describe the initial results of our SAR exploration of **1**.



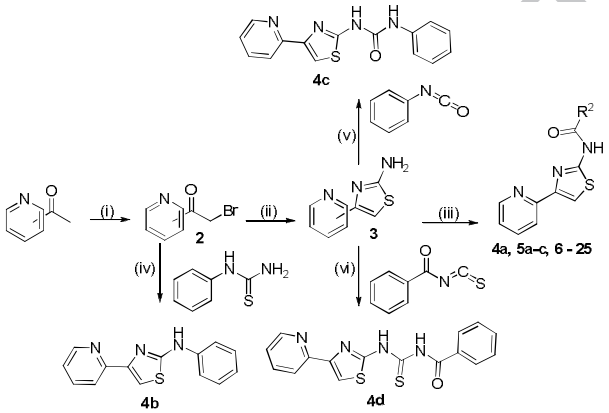
1

Figure 1. The 2-amino-4-(2-pyridyl)thiazole scaffold.

The first series of target compounds **4a**, **5a**, **6**-**25** was synthesized via a facile synthetic approach [Scheme 1, steps (i) to (iii)] starting with the bromination of commercially available 2-acetylpyridine, to yield the α brominated intermediate, **2**, which was then condensed with thiourea, to give the key intermediate **3**.

In the presence of mono substituted carboxylic acids, intermediate **3** underwent an EDCI (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)-mediated coupling to deliver the target. The HTS findings from antimycobacterial evaluation suggests that only compounds with a 2-pyridyl substituent at position 4 of the thiazole ring were active.[13] Therefore, to investigate the role of the 2-pyridyl ring, 3-pyridyl (**5b**) and 4-pyridyl (**5c**) analogues of **5a** were synthesized starting from commercially available 3-acetylpyridine and 4-acetylpyridine respectively. To investigate the role of the linker, amino (**4b**), urea (**4c**), and acylthiourea (**4d**) compounds were synthesized. Compound **4b** was obtained through the reaction of 2 and phenylthiourea [Scheme 1, step (iv)] while compounds **4c** and **4d** were obtained from the reaction of **3** with phenylisocyanate and benzoylisothiocyanate respectively [Scheme 1, step (v) and (vi)].

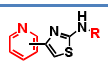
Scheme 1



[a]Reagents and conditions: (i) 48% HBr, Br_2, 65°C ,1 h, then rt, 1 h. (ii) NH_2CSNH_2, EtOH, rt, 1 h. (iii) R^2COOH, EDCI, HOBt, DCM, rt, 24 h. (iv) EtOH, 90°C, 2h, then 20°C, 1 h. (v) Toluene, 56°C, 2 h, then rt, 24 h. (vi) Acetone, 70°C, 2h, then 0°C, 1h.

All synthesized compounds were evaluated *in vitro* for their antimycobacterial activity against the drug sensitive *M.tb* H37Rv strain, antiplasmodial activity against the CQS *P.falciparum* NF54 strain, and for cytotoxicity against the Chinese Hamster Ovarian (CHO) cell line. Rifampicin, kanamycin and CQ were used as positive controls, and the results are presented in Tables 1 and 2.

Table 1. *In vitro* antimycobacterial activity and solubility of synthesized analogues.



Entry		R	*M.tb* H37Rv MIC99	Solubility (µM)	Entry		R	*M.tb* H37Rv MIC99	Solubility (µM)
3	2-		>160	>200	12	2-		5	10
4a	2-		5	80	13	2-		5	10
4b	2-		160	160	14	2-		2.5	10
4c	2-		*10	80	15	2-		2.5	20
4d	2-		*20	10	16	2-		20	10
5a	2-		1.25	10	17	2-		5	20
5b	3-		>160	40	18	2-		5	20
5c	4-		>160	40	19	2-		5	40
6	2-		5	80	20	2-		160	160
7	2-		2.5	40	21	2-		160	>200
8	2-		5	80	22	2-		10	80
9	2-		10	40	23	2-		80	>200
10	2-		10	20	24	2-		10	160
11	2-		5	40	25	2-		5	>200

The unsubstituted scaffold **1** showed no antimycobacterial activity at the highest test concentration of 160 µM. Substitution on **1** yielded varying antimycobacterial activities with compounds **20**, **21**, and **23** with a thiazole, imidazole, and 2-pyridyl ring respectively remaining inactive while compounds **22**, **24**, **25**, and **4a**, **5a**, **6**-**19** with thiophene, 3-pyridyl, 4-pyridyl and phenyl rings respectively having enhanced activity. Compounds **5a**, **16**-**19** with substituted phenyl rings had comparable activity to **4a** with an unsubstituted phenyl.

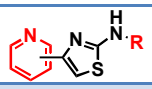
The position of the substitution on the phenyl had an influence on activity as demonstrated by the bromo-substituted compounds with activity of the *para* (**5a**) > *meta* (**7**) > *ortho* (**6**).

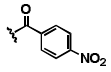
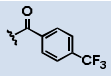
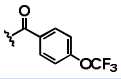
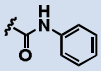
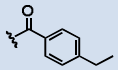
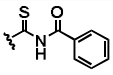
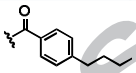
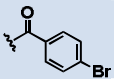
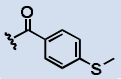
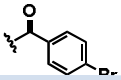
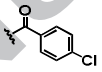
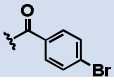
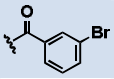
Replacing the 2-pyridyl group at position 4 of **1** (**5a**) with a 3-pyridyl (**5b**) or 4-pyridyl (**5c**) group resulted in loss of antimycobacterial activity.

In addition, compound **4a** which had an amide linker at position 2 had superior antimycobacterial activity compared to compounds **4b**, **4c**, and **4d** which had an amino, urea, and acylthiourea linkers respectively.

The unsubstituted scaffold **1** was found to have antiplasmodial activity with an IC_{50} of 47.8µM. Substitutions on **1** yielded compounds with improved antiplasmodial activity with activity of thiophene (**22**) > unsubstituted phenyl (**4a**) > 4-pyridyl (**25**) > thiazole (**20**) > imidazole (**21**) > 2-pyridyl (**23**) and 3-pyridyl(**24**). Compounds with substituted phenyls had activity ranging from 0.8µM (**11**) to 18.6µM (**13**).

Table 2. *In vitro* antiplasmodial activity and cytotoxicity of synthesized analogues



Entry		R	*P.falciparum* IC50 (μM)	CHO IC50 (μM)	SI (μM)	Entry		R	*P.falciparum* IC50 (μM)	CHO IC50 (μM)	SI (μM)
3	2-		47.8	55.0	1.2	12	2-		6.1	2.2	0.4
4a	2-		1.0	3.0	3.0	13	2-		18.6	2.0	0.1
4b	2-		3.2	1.3	0.4	14	2-		5.5	23.3	4.2
4c	2-		1.7	2.2	1.3	15	2-		6.5	6.5	1.0
4d	2-		2.7	4.3	1.6	16	2-		7.4	3.4	0.5
5a	2-		5.3	3.6	0.7	17	2-		7.6	4.5	0.6
5b	3-		15.9	89.0	5.6	18	2-		1.3	3.2	2.5
5c	4-		3.5	34.4	9.8	19	2-		1.8	3.1	1.7
6	2-		2.2	3.4	1.5	20	2-		13.8	14.3	1.0
7	2-		1.6	2.8	1.8	21	2-		35.0	1.4	0.04
8	2-		3.9	7.9	2.0	22	2-		0.9	2.8	3.1
9	2-		5.9	19.7	3.3	23	2-		>35.4	9.2	0.3
10	2-		14.8	2.1	0.1	24	2-		>35.4	0.7	0.02
11	2-		0.8	3.1	3.9	25	2-		1.9	0.0	0.005

The position of the substitution on the phenyl also had an influence on antiplasmodial activity as seen from the bromo-substituted compounds with activity of the *meta* (**7**) > *ortho* (**6**) > *para* (**5a**).

Replacing the 2-pyridyl group at position 4 of **1** (**5a**) with a 3-pyridyl (**5b**) led to a decrease in antiplasmodial activity. However, a 4-pyridyl (**5c**) group resulted in improved antiplasmodial activity.

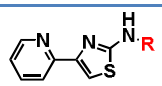
As with antimycobacterial activity, compound **4a** which had an amide linker at position 2 had better antiplasmodial activity compared to compounds **4b**, **4c**, and **4d** which had amino, urea, and acylthiourea linkers respectively.

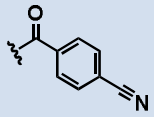
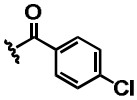
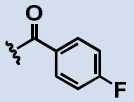
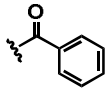
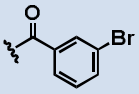
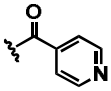
The solubility of the compounds was determined via the turbidimetric solubility method at a pH of 7.4.[14] The compounds had low to moderate solubility limits (Table 1) based on the nature of the substituents attached.

Furthermore, all compounds showed significant toxicity against the CHO cell line and thus had low selectivity indices. It is therefore possible that the antiplasmodial activity could be due to the cytotoxic nature of the compounds and may not be intrinsic.

Finally representative samples of the active compounds were selected for microsomal metabolic stability in human, rat, and mouse liver microsomes, using a single point metabolic turnover method.[15] The results are presented in Table 3.

Table 3. Microsomal Metabolic Stability



Entry	R	% remaining		
		HLM	RLM	MLM
11		24.1	64.6	1.61
18		8.40	42.1	1.33
19		14.2	72.1	3.61
4a		27.5	54.5	7.60
7		>99	>99	82.4
25		53.8	47.8	3.71

The compounds were generally unstable across all three species, with the exception of compound **7**. In conclusion preliminary SAR studies on **1** therefore suggest that the 2-pyridyl group at position 4 of the thiazole ring is essential for antimycobacterial activity as reported,[13] and the presence of a substituted phenyl group at the 2-amino position is also important for activity. In addition the linker between the groups plays a role in the antimycobacterial activity. Substitution on **1** are beneficial for antiplasmodial activity as activity is enhanced to varying degrees by substitution with different groups. However, the compounds are generally cytotoxic and metabolically unstable. Metabolite identification studies for these compounds are in progress, as part of lead optimization, and will be communicated in future work.

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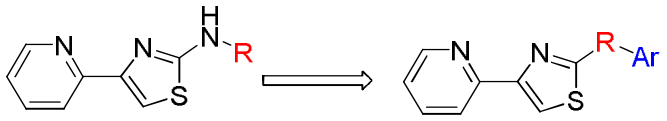
Supplementary material

Experimental procedures, characterization of final compounds and biological assays protocols.

Graphical abstract



R = amino, urea, acylthiourea, amide

Ar = phenyl, substituted phenyls, other heterocycles