Total Synthesis of Thiaplakortone A: Derivatives as Metabolically Stable Leads for the Treatment of Malaria

Rebecca H. Pouwer,* Sophie M. Deydier,† Phuc Van Le,† Brett D. Schwartz,† Nicole C. Franken,† Rohan A. Davis,† Mark J. Coster,† Susan A. Charman,§ Michael D. Edstein,§ Tina S. Skinner-Adams,† Katherine T. Andrews,† Ian D. Jenkins,† and Ronald J. Quinn‡

†Eskitis Institute for Drug Discovery, Griffith University, Brisbane, QLD 4111, Australia
‡Centre for Drug Candidate Optimisation, Monash University, 381 Royal Parade, Parkville, VIC 3052, Australia
§Australian Army Malaria Institute, Brisbane, QLD 4051, Australia

Supporting Information

ABSTRACT: Thiaplakortone A (3a), an antimalarial natural product, was prepared by an operationally simple and scalable synthesis. In our efforts to deliver a lead compound with improved potency, metabolic stability, and selectivity, the synthesis was diverted to access a series of analogues. Compounds 3a−d showed nanomolar activity against the chloroquine-sensitive (3D7) *Plasmodium falciparum* line and were more active against the chloroquine- and mefloquine-resistant (Dd2) *P. falciparum* line. All compounds are “Rule-of-S” compliant, and we show that metabolic stability can be enhanced via modification at either the primary or pyrrole nitrogen. These promising results lay the foundation for the development of this structurally unprecedented natural product.

KEYWORDS: Malaria, natural products, total synthesis

Approximately 3.3 billion people, almost half the world’s population, including the populations of Africa, Asia, Latin America, the Middle East, and South East Asia, are at risk of developing malaria. In 2010, approximately 655,000 people died due to this disease. Of these deaths, an estimated 91% occurred in sub-Saharan Africa, with children under the age of five being most at risk. The World Health Organization (WHO) recommends interventions for the prevention and treatment of malaria that include (1) the use of insecticide-treated nets; (2) drug prophylaxis in vulnerable populations; (3) rapid diagnosis; and (4) treatment with antimalarial drugs. Current drug therapy for *Plasmodium falciparum* malaria, recommended by the WHO, uses artemisinin in combination with other antimalarials. The problem of parasite resistance to artemisinin-based combination therapy (ACT) is of growing and immense concern, with the presence of artemisinin resistance having been confirmed in western Cambodia and western Thailand. In addition to the threat of parasite resistance, the poor safety profiles and undesirable side-effects associated with many of the current antimalarials are also driving the need for new small molecule therapies. New antimalarial drugs with unique mechanisms of action are urgently needed in order to combat the global problem of parasite drug resistance.

Natural products have played a key role in antimalarial drug discovery and therapy. Quinine and artemisinin, first isolated from the South American “quinine bark” (*Cinchona succirubra*) and the Chinese “sweet wormwood” (*Artemisia annua*), respectively, are well-known natural products, which have served as a structural basis for numerous antimalarial drugs, such as artesunate, artemether, chloroquine, mefloquine, and halofantrine. Many other drugs are also derived from Nature, with natural product drugs representing ~50% of small molecule approved drugs. The basis for the wide-ranging biological activity of natural products is explained by the concept of protein fold topology (PFT), where molecular recognition during biosynthesis (the biosynthetic imprint) has been shown to translate to a similar binding mode with therapeutic targets. The cavity recognition points described by PFT may be unrelated to fold and sequence similarity and define a natural product’s ability to interact with biological space.

We have developed a lead-like enhanced natural product fraction screening library, in which fractions are selected for drug-like properties including logP. Active natural products from our malaria drug discovery program include tsitsikamma-nine C (1) and makaluvamine G (2) from the marine sponge, *Zyzya sp.*, and more recently thiaplakortone A from the sponge, *Plakortis lita* (Figure 1). Thiaplakortone A (3a) contains a unique tricyclic alkaloid skeleton that is without precedent among natural products. The thiazine dioxide-fused pyrroloquinone moiety shares some structural similarity...
with the antimalarial natural products tsitsikammamine C (1) and makaluvamine G (2). Previous studies of the tsitsikammamine and makaluvamine series indicated that methylation affected antimalarial activity and metabolic stability. In comparing the structural features of 1, 2, and 3a, the right hemisphere of 3a has the intact pyrrolo moiety; however, the iminoquinone present in 1 and 2 is ring opened to the quinone with a free primary amine side chain in 3a. Tsisitsikammamine C (1) displayed potent in vitro activity (IC$_{50}$ = 13 nM [3D7], 18 nM [Dd2]), had selectivity indices of >200, and inhibited both ring and trophozoite stages of the parasite life cycle. Makaluvamine G (2) (IC$_{50}$ = 36 nM [3D7], 39 nM [Dd2]) had selectivity indices of >50 and primarily inhibited the trophozoite stage. Thiaplakortone A (3a) was found to have IC$_{50}$ values of 51 and 6.6 nM against the chloroquine-sensitive 3D7 and the chloroquine- and mefloquine-resistant Dd2 Plasmodium falciparum lines in an imaging-based growth inhibition assay, respectively.$^{22}$ These data led us to further explore the antimalarial properties of pyrroloquinone scaffolds through a diverted total synthesis of thiaplakortone A (3a).

Herein we report the first total synthesis of 3a, along with several thiaplakortone-based analogues (3b−d, 17). The in vitro antimalarial activity and mammalian cell toxicity studies for all compounds is also reported as well as metabolic profiling and in vivo studies with lead compounds in mice.

In developing a synthetic strategy toward 3a as an early lead compound for the treatment of malaria, we were mindful of expense and the need for the synthesis to be operationally simple. Furthermore, the ability to access monomethyl and dimethyl analogues ($^{3b}$−d, 17) for structure−activity relationship development was of central importance in our synthetic design.

Synthesis of the common intermediate 7 from commercially available 4-hydroxyindole (4)$^{23}$ commenced with protection of the phenol as its benzyl ether,24 followed by Vilsmeier−Haack formylation to produce aldehyde 6 (Scheme 1). This compound was subsequently subjected to Henry reaction with nitromethane, where the resulting nitroalkene was reduced by LiAlH$_4$, and the primary amine was protected as its t-butyloxycarbamate (7). Thus, 7 was synthesized in five facile synthetic steps from 4, requiring only two chromatographic separations. Selective methylation of 7 (MeI, KOH) yielded 8, thus allowing access to derivatives of the natural product in which the pyrrole nitrogen is methylated. Compound 8, which upon subsequent treatment with LiHMDS and MeI provided 9, also served as a precursor to a dimethylated analogue of 3a. To selectively access an analogue in which only the side-chain amine was alkylated, it was necessary to protect the indole nitrogen of 7. Consequently, 7 was protected as its tosylate (10), and subsequent methylation (LiHMDS, MeI) and deprotection (Mg, MeOH) gave rise to the monomethylated derivative 12. It is worth noting that 10 resembles a synthetic intermediate used in the total synthesis of tsitsikammamine A,$^{25,26}$ and it is anticipated that our synthetic strategy would equally serve to access compounds of type 1 and 2.

The tryptamine derivatives 7−9 and 12 were then subjected to a general procedure for the synthesis of the thiazine dioxide pyrroloquinones (Scheme 2). Initial hydrogenolysis of the benzyl protecting group was followed by immediate oxidation of the unstable intermediate with Fremy’s salt. The resulting

![Scheme 1](image1)

![Scheme 2](image2)

$^{a}$Reagents and conditions: (a) BnBr, K$_2$CO$_3$, acetone, reflux, 36 h; (b) POCl$_3$, DMF, rt, 1 h, then KOH, H$_2$O, reflux, 3 h; (c) CH$_3$NO$_2$, NH$_2$OAc, reflux, 1 h; (d) LiAlH$_4$, THF, reflux, 6 h; (e) Boc$_2$O, Et$_3$N, THF, rt, 16 h; (f) MeI, KOH, DMSO, rt, 19 h; (g) MeI, LiHMDS, THF, rt, 2 h; (h) TsCl, Bu$_4$NHSO$_4$, KOH, toluene, H$_2$O, rt, 24 h; (i) Mel, LiHMDS, THF, rt, 2 h; (j) Mg, MeOH, sonicate, 30 min.

$^{b}$Scheme 1

$^{c}$Scheme 2
quinones participated in a double conjugate addition/oxidation sequence upon treatment with 2-aminothanesulfinic acid to yield dihydrothiazine dioxides 13a−d as separable mixtures with their corresponding regioisomers. Dihydrothiazine dioxides 13a−d were then oxidized (O₂, KOH) and deprotected under acidic conditions to produce the natural product 3a and thiazine dioxides 3b−d as their hydrochloride salts. Compound 15, the regioisomer of 13b, was subjected to an equivalent sequence to deliver 17 (Scheme 3). From tryptamine derivatives 7−9 and 12, this synthetic sequence required only one chromatographic separation and was used to rapidly produce the thiazinedioxides on multigram scale. The NMR spectroscopic data for synthetic 3a (HCl salt) was essentially identical to that previously reported for the natural product, thiapikorone A, which was isolated as a TFA salt.22

Compounds were evaluated for in vitro antimalarial activity using the [3H]-hypoxanthine incorporation assay27,28 against the chloroquine-sensitive 3D7 and the chloroquine- and melquine-resistant Dd2 Plasmodium falciparum lines. To compare the selectivity of the compounds for malaria parasites versus normal mammalian cells, cytotoxicity tests were carried out using human neonatal foreskin fibroblast (NFF) cells. All biological data, including selectivity indices and calculated physicochemical properties, are detailed in Table 1.

The synthetic natural product 3a had an IC₅₀ of 104 nM against the 3D7 P. falciparum line and was found to be 4.3-fold more potent against the multidrug resistant P. falciparum line Dd2 (IC₅₀ = 24 nM). This interesting observation held true for analogues 3b−d, with selectivity for the P. falciparum line Dd2 ranging between 1.7- to 3.4-fold. This effect was not observed for compounds 1 and 2 and is in fact opposite to that for chloroquine, which has more than 8-fold less potency against the Dd2 P. falciparum line. For the makaluvamine series (e.g., 2), we previously reported that the presence of an N-methyl iminium moiety increased antimalarial activity by ~10− to 30-fold compared to a secondary imine skeleton, while methylation of the pyrrole nitrogen appears not to affect the bioactivity.24 With respect to the thiapikorone scaffold, our results demonstrate that alkylation of both the pyrrole and side-chain nitrogens is well tolerated, with analogues 3b−d demonstrating low nM potency in both the 3D7 (3b IC₅₀ = 128 nM, 3c IC₅₀ = 137 nM, 3d IC₅₀ = 160 nM) and Dd2 (3b IC₅₀ = 57 nM, 3c IC₅₀ = 79 nM, 3d IC₅₀ = 47 nM) P. falciparum lines. When compared with 3b, regioisomer 17 showed decreased activity against both malaria parasite lines (IC₅₀ = 416 nM [3D7], 162 nM [Dd2]), suggesting that the orientation of the thiazine dioxide moiety plays an important role in biological activity. These observations open avenues for the further manipulation of these moieties in future structure–activity relationship investigations.

Selectivity between host and parasite is of paramount concern, as quinones have been associated with toxic effects.30 All compounds were found to have greater than 9-fold selectivity for the 3D7 P. falciparum line over NFF cells, providing a reasonable therapeutic window for the development of an early lead compound. Interestingly, regioisomer 17 showed the highest levels of selectivity (32-fold in 3D7 and 81-fold in Dd2), demonstrating that minor manipulation of the core may result in an improved toxicity profile.

All compounds complied with Lipinski’s rule-of-five31 (logP < 5, hydrogen bond acceptors (HBA) < 10, hydrogen bond donors (HBD) < 5, MW < 500), and Han−Oprea’s guidelines for lead-likeness32 (logP < 4, HBA < 9, HBD < 5, MW < 460, rotatable bonds < 10). Compounds 3a−d displayed good solubility in phosphate buffered saline under both neutral and acidic conditions. These physicochemical properties support the drug-likeness of compounds 3a−d.

In vitro studies in hepatic microsomes were conducted to assess the extent of metabolic degradation (Table 2).
Compounds 3a–d were found to be stable in the microsomal matrix in the absence of cofactors, suggesting that there was no major contribution of cofactor-independent metabolism to the rate of metabolism. In the presence of NADPH in both human and mouse liver microsomes, the synthetic natural product 3a was rapidly degraded, with half-lives of 37 and 25 min, respectively. Analogues 3b and 3c, which both feature a methylated pyrrole, were minimally degraded in both species, and were found to have significantly increased half-lives (>250 min) and low predicted hepatic extraction ratios (<0.23) in comparison to 3a and 3d (Table 2). Monomethylation of the primary amine side-chain (i.e., 3d) resulted in an increased half-life of 100 min in human microsomes. These results highlight an opportunity to improve metabolic stability through a simple modification of either the primary or pyrrole nitrogen of the natural product.

The tolerability of 3a–c was assessed in healthy mice. A slight whole body tremor lasting about 1–2 h after each dose and slightly ruffled coat were observed in mice administered 3a at a daily dose of 8 mg/kg for 4 d. However, at 16 mg/kg the mice exhibited marked whole body tremors, lethargy, and ruffled coat within 2 h after the first dose. Mice administered 3b at a dose of 4 and 8 mg/kg exhibited panting and whole body tremors within 10 to 15 min of the first dose, with adverse events more noticeable with the higher dose. Compound 3c, at a dose of 8 mg/kg, led to marked physical distress in all animals, with whole body tremors, difficulty in moving about, orange urine, and swollen conjunctiva of the eyes. Overall, 3a–c were not well tolerated in mice at relatively low doses of compound. Investigations to address in vivo tolerability of this compound series are underway.

In conclusion, from 3a, an antimalarial natural product, we have developed potent antimalarial compounds with favorable physicochemical and ADME properties. We have designed and completed the rapid and operationally simple total synthesis of 3a and have diverted this synthesis to access analogues 3b–d. Methylation of the thiapikakortone scaffold was well tolerated, and analogues 3b–d showed low nM in vitro activity against drug-sensitive and drug-resistant *P. falciparum* lines. Methylation of the pyrrole nitrogen was found to dramatically increase the metabolic stability of analogues, without significant loss of antimalarial activity. These studies pave the way for the development of structurally unprecedented lead compounds for the treatment of malaria.

**ASSOCIATED CONTENT**

Supporting Information
Details regarding compound synthesis and characterization and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

Corresponding Author
*(R.J.Q.)* Tel: +61-7-3735-6000. Fax: +61-7-3735-6001. E-mail: r.quinn@griffith.edu.au.

Author Contributions
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**Notes**

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**ABBREVIATIONS**

3FT, protein fold topology; ACT, artemisinin-based combination therapy; NFF, neonatal foreskin fibroblast cells; 3D7, chloroquine-sensitive *P. falciparum* line; Dd2, chloroquine-resistant *P. falciparum* line

**REFERENCES**


(23) Purchased from AK Scientific, $200/100 g.


