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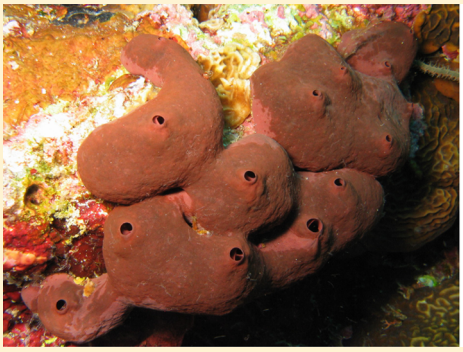
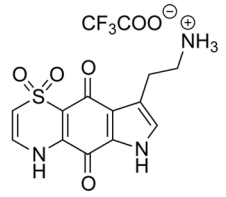
Thiaplakortones A–D: Antimalarial Thiazine Alkaloids from the Australian Marine Sponge *Plakortis lita*

Rohan A. Davis, Sandra Duffy, Sabine Fletcher, Vicky M. Avery, and Ronald J. Quinn*

Eskitis Institute, Griffith University, Nathan, Queensland 4111, Australia

Supporting Information





Thiaplakortone A

IC_{50} 6.6 nM *Plasmodium falciparum* (Dd2)
IC_{50} 51 nM *Plasmodium falciparum* (3D7)

ABSTRACT: A high-throughput screening campaign using a prefractionated natural product library and an in vitro antimalarial assay identified active fractions derived from the Australian marine sponge *Plakortis lita*. Bioassay-guided fractionation of the CH_2Cl_2/CH_3OH extract from *P. lita* resulted in the purification of four novel thiazine-derived alkaloids, thiaplakortones A–D (**1**–**4**). The chemical structures of **1**–**4** were determined following analysis of 1D/2D NMR and MS data. Comparison of the chiro-optical data for **3** and **4** with literature values of related *N*-methyltryptophan natural products was used to determine the absolute configuration for both thiaplakortones C and D as 11*S*. Compounds **1**–**4** displayed significant growth inhibition against chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) *Plasmodium falciparum* (IC_{50} values <651 nM) and only moderate cytotoxicity against HEK293 cells (IC_{50} values >3.9 μM). Thiaplakortone A (**1**) was the most active natural product, with IC_{50} values of 51 and 6.6 nM against 3D7 and Dd2 lines, respectively.

INTRODUCTION

Malaria is a significant infectious disease caused by *Plasmodium* parasites. This disease results in the deaths of 0.8–1.2 million people annually, and it is estimated that over 3.2 billion people living in Central and South America, Sub-Saharan Africa, South East Asia, the Middle East, and India and are at risk from this disease.[1] Despite recent preliminary data on a partially effective pre-erythrocytic-stage vaccine (RTS, S),[2] malaria prevention and treatment currently relies on small-molecule drugs and vector control. It is predicted that even if the RTS, S vaccine is approved for clinical use, it will be implemented as part of a multipronged approach that will also include antimalarial drugs in order to eradicate the parasite. While a number of drugs are used therapeutically to treat malaria, all have now succumbed to parasite drug resistance and subsequently display reduced efficacy.[3] Consequently, new antimalarial drugs with novel targets are urgently needed in order to combat the global problem of parasite drug resistance.

Historically, natural products and their synthetic derivatives have played a significant role in the discovery and development of antimalarial drugs.[4−6] Notable examples include artemisinin from the Chinese medicinal plant *Artemisia annua* and quinine, which was isolated from the bark of the South American tree *Cinchona succirubra*.[6] These compounds have subsequently led to the development of numerous other antimalarial drugs that are based on these natural product pharmacophores.

Natural products have an inherent understanding of biology space. Our lead discovery program is based on the druglike natural product metabolome.[7,8] Natural products and their analogues have had high impact as drugs because of the embedded biosynthetic molecular recognition that transfers to therapeutic targets as described by protein fold topology (PFT).[9] Molecular recognition during biosynthesis (the biosynthetic imprint) has been shown to carry through to a similar binding motif with therapeutic targets. PFT describes cavity recognition points unrelated to fold and sequence similarity and defines a natural product's ability to recognize biology space.[9−12] The discovery library was generated by HPLC fractionation of 18453 optimized extracts obtained from plant and marine invertebrates sourced from tropical and temperate Australia, China, and Papua New Guinea. Fractionation of each biota extract was accomplished using analytical reversed-phase C_{18} HPLC (11 fractions collected per extract) to generate the 202983 fraction library.[7] In this current study we undertook HTS[13] of this optimized screening library in

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order to identify new antimalarial lead compounds from nature. HTS data analysis identified three fractions from the sponge *Plakortis lita* (Plakinidae) that displayed significant growth inhibition against *P. falciparum* 3D7 parasites and showed no toxicity toward a human embryonic kidney cell line (HEK293). Bioassay-guided fractionation on the crude organic extract of *P. lita* resulted in the isolation of four novel thiazine-derived alkaloids, which we have named thiaplakortones A–D (**1**–**4**) (Figure 1). Herein we report the isolation and structure elucidation of **1**–**4** along with their in vitro antimalarial activity and mammalian toxicity.

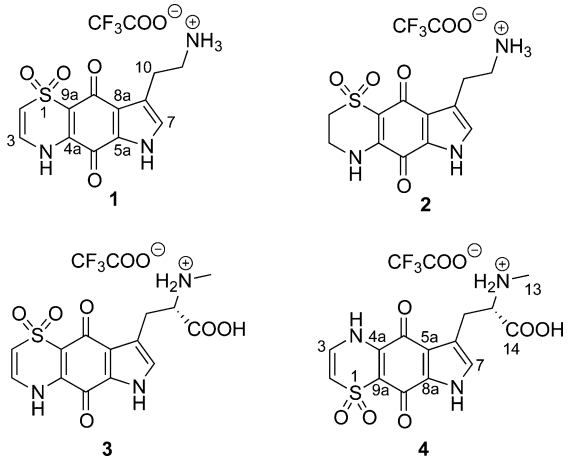

Figure 1. Structures of thiaplakortones A–D (**1**–**4**).

RESULTS AND DISCUSSION

The freeze-dried and ground sponge *P. lita* (de Laubenfels, 1954) was extracted with *n*-hexane, CH_2Cl_2, and CH_3OH. Bioassay-guided fractionation was performed on the combined CH_2Cl_2 and CH_3OH extracts using several iterations of reversed-phase C_{18} HPLC ($CH_3OH/H_2O/CF_3COOH$) and biological screening. This process yielded the antimalarial thiaplakortones A–D (**1**–**4**) as their trifluoroacetate salts.

Thiaplakortone A was isolated as a stable amorphous solid. The molecular formula of the free base of **1** was determined to be $C_{12}H_{11}N_3O_4S$ by (+)-HRESIMS of the $[M - CF_3COO^-]^+$ ion at *m*/*z* 294.0543. The ^{1}H NMR data (Table 1) of **1** showed only eight unique signals, three of which (δ_H 13.09, 11.07, and 7.84) were determined to be exchangeable, following a deuterium (D_2O) exchange NMR experiment. The remaining proton signals at δ_H 7.36, 7.06, 6.43, 3.05, and 2.97 were from protons attached to carbons resonating at δ_C 129.2, 130.2, 111.7, 38.4, and 23.4, respectively, following HSQC data analysis. The ^{13}C NMR data of **1** displayed 12 signals (Table 2), which included seven sp^2 quaternary signals that resonated between δ_C 114.1 and 178.5. Analysis of the COSY spectrum of **1** readily established three unique spin systems, which included $-CH_2CH_2NH_3^+$, –NHCH=CH–, and –NHCH= substructures (Figure 2a).

The ethylamine moiety was attached to a trisubstituted double bond on the basis of HMBC correlations (Figure 2b) from the ethyl protons at δ_H 2.97 (H-10) to carbon resonances at δ_C 123.3, 121.7, and 129.2; the signal at δ_C 129.2 was known to be associated with the –NHCH= fragment. Furthermore, the NH proton (δ_H 13.09, H-6) of this fragment showed HMBC correlations to δ_C 123.3, 121.7, 129.2, and 128.0 that

Table 1. ^{1}H NMR (600 MHz) Data for Thiaplakortones A–D (1–4) in DMSO-d_6

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]
2	6.43, d (9.0)	3.28, m	6.46, d (9.6)	6.52, d (8.4)
3	7.06, dd (9.0, 5.4)	3.81, m	7.07, dd (9.6, 6.0)	7.10, dd (8.4, 5.4)
4	11.07, d (5.4)	8.92, s	11.10, d (6.0)	11.09, d (5.4)
6	13.09, s	12.89, s	13.14, s	
7	7.36, d (2.4)	7.32, d (2.4)	7.35, d (2.4)	7.13, d (2.4)
8				13.00, s
10	2.97, t (7.8)	2.94, t (7.2)	3.24, dd (14.4, 7.8)	3.26, dd (15.0, 6.6)
			3.29, dd (14.4, 6.6)	3.32, dd (15.0, 6.0)
11	3.05, m	3.04, m	4.18, br s	4.18, br s
12	7.84, br s	7.76, br s	8.98, br s	8.80, br s
				8.93, br s
13			2.64, s	2.60, s
14			*b*	*b*

[a]Data given as δ_H, multiplicity (*J* in Hz). [b]An exchangeable proton was not observed.

Table 2. ^{13}C NMR (125 MHz) Data for Thiaplakortones A–D (1–4) in DMSO-d_6

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]
2	111.7, CH	48.4, CH_2	111.8, CH	111.8, CH
3	130.2, CH	39.0, CH_2	130.2, CH	130.2, CH
4a	139.9, C	147.3, C	140.0, C	140.5, C
5	167.8, C	167.9, C	167.8, C	174.9, C
5a	128.0, C	127.6, C	128.0, C	118.1, C
6				119.8, C
7	129.2, CH	129.6, CH	129.9, CH	126.4, CH
8	121.7, C	121.4, C	119.2, C	
8a	123.3, C	124.2, C	123.5, C	131.5, C
9	178.5, C	176.1, C	178.6, C	172.0, C
9a	114.1, C	108.6, C	114.1, C	113.2, C
10	23.4, CH_2	23.4, CH_2	24.9, CH_2	24.8, CH_2
11	38.4, CH_2	38.6, CH_2	59.6, CH	60.0, CH
13			31.3, CH_3	31.5, CH_3
14			169.6, C	169.6, C

[a]Data given as δ_C, multiplicity.

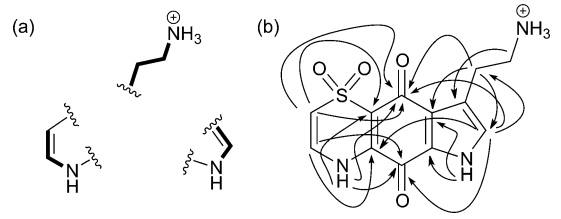

Figure 2. (a) COSY (–) identified fragments for **1**. (b) Key HMBC (→) correlations for **1**.

allowed construction of a 1,2-disubstituted 3-ethylaminopyrrolo moiety. HMBC correlations from the pyrrolo proton at δ_H 7.36 (H-7) to carbonyl signals at δ_C 167.8 (C-5) and 178.5 (C-9) suggested a 1,4-quinone pyrrolo substructure.[14] The remaining COSY-derived fragment of **1** (–NHCH=CH–) was expanded to a unsaturated 1,1-dioxo-1,4-thiazine moiety on the basis of HMBC data and the ^{13}C chemical shifts of the associated methine carbons (δ_C 130.2 (C-3) and 111.7 (C-2)), which were

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Table 3. Physicochemical and Biological Profiles of Thiaplakortones A–D (1–4)

	physicochemical params[a]				IC_{50} (nM)[b]			SI[c]	
compd	MW	*c* log *P*	HBA	HBD	3D7	Dd2	HEK293	3D7	Dd2
1	293	−2.06	6	3	51	6.6	3900	76	591
2	295	−2.21	6	3	650	92	>40000	>62	>435
3	351	−4.55	8	4	309	171	>40000	>129	>233
4	351	−4.55	8	4	279	159	>80000	>285	>500

[a]In silico calculations performed using Instant JChem software;[24] MW = molecular weight of the free base, *c* log *P* = calculated logarithm of the partition coefficient (n-octanol/H_2O), HBA = H-bond acceptors, and HBD = H-bond donors. [b]50% inhibitory concentration in vitro against *P. falciparum* drug-sensitive (3D7) and drug-resistant (Dd2) lines and a human embryonic kidney cell line (HEK293). [c]SI = (HEK293 IC_{50})/(*P. falciparum* IC_{50}).

identical with those previously reported.[15] Fusion of this moiety to the remaining unassigned quaternary carbons (δ_C 139.9 (C-4a) and 114.1 (C-9a)) was deduced by HMBC correlations from δ_H 11.07 (H-4) and 6.43 (H-2) to δ_C 114.1 and δ_H 11.07 and 7.06 (H-3) to δ_C 139.9. Furthermore, HMBC correlations from δ_H 11.07 (H-4) to the quinone carbonyl carbons established a tricyclic thiazine-fused pyrroloquinone substructure. At this stage all atoms for **1** had been accounted for, but the regiochemistry of the thiazine moiety had not been determined. Fortuitously, the HMBC data secured this assignment via long-range correlations. While several four-bond correlations to the quinone carbonyls were identified (Figure 2b), it was the correlation from both δ_H 6.43 (H-2) and 2.97 (H-10) into the carbonyl at δ_C 178.5 (C-9) that unequivocally established the regiochemistry. Thus, thiaplakortone A was assigned to structure **1**.

The free base of the minor compound, thiaplakortone B (**2**), was assigned the molecular formula $C_{12}H_{13}N_3O_4S$ on the basis of (+)-HRESIMS and NMR data (Tables 1 and 2). Comparison of the ^1H and ^{13}C NMR data of **2** with those of thiaplakortone A readily identified that these two secondary metabolites belonged to the same structure class. The only major differences observed were that **2** lacked the thiazine *Z* double-bond signals (δ_H 7.06/δ_C 130.2 and δ_H 6.43/δ_C 111.7) but contained additional upfield signals (δ_H 3.81/δ_C 39.0 and δ_H 3.28/δ_C 48.4). These data in conjunction with the MS information and literature values for related 1,1-dioxo-1,4-thiazine moieties[15−20] indicated that **2** was the 2,3-dihydro derivative of **1**. HMBC data analysis confirmed this assignment with multiple correlations identified between the thiazine-associated protons and the quinone moiety. The regiochemistry of the thiazine for **2** was determined to be identical with that of **1** on the basis of an NMR data comparison; thus, thiaplakortone B was assigned as structure **2**.

Thiaplakortone C (**3**) was isolated as an optically active amorphous solid. The molecular formula of the free base of **3** was determined to be $C_{14}H_{13}N_3O_6S$ on the basis of (+)-HRESIMS of a pseudomolecular ion $[M - CF_3COO^-]^+$ ion at *m*/*z* 352.0608. Analysis of both the 1D and 2D NMR data for **3** confirmed that the thiazine-pyrroloquinone moiety of **1** was also present in **3**; only minor differences were noted when the ^{13}C (Δ < 0.8 ppm) and ^1H (Δ < 0.06 ppm) chemical shifts were compared for the tricyclic systems of **1** and **3**. ^1H NMR and COSY data analysis for the unassigned ^1H signals for **3** established a 2-methylaminopropanoic acid side chain. HMBC data confirmed this moiety and showed that it was attached to C-8 of the pyrrolo ring. Hence, thiaplakortone C was assigned to structure **3**. In order to determine the absolute configuration of **3**, we compared the specific rotation data for thiaplakortone C with literature values of related *N*-methyltryptophan natural products, whose absolute configuration had been unequivocally determined.[21−23] The specific rotations for the hydrochloride salts of the plant metabolites (*S*)-*N*-methyltryptophan[21] and (*S*)-*N*-methyltryptophan methyl ester[22] were reported as $[\alpha]_D^{25}$ = +46° (*c* 1.554, 0.5 M HCl)[21] and $[\alpha]_D$ = +51° (*c* 0.82, CH_3OH),[22] respectively. We recorded $[\alpha]_D^{27}$ = +56° (*c* 0.025, CH_3OH) and $[\alpha]_D^{25}$ = +129° (*c* 0.007, 0.5 M HCl) for **3**; these data indicated that thiaplakortone C had the same absolute configuration as the plant natural products mentioned above, and hence **3** was assigned as 11*S*.

Thiaplakortone D (**4**) was deemed to be an isomer of **3** on the basis of NMR and MS data analysis. While the ^{13}C NMR resonances for C-7 to C-14 of **4** were essentially identical with those of **3** (Δ < 0.8 ppm), several major differences were noted in the quinone-thiazine bicyclic system. Most notably, the quinoid carbonyl signals of **3** (δ_C 167.8 and 178.6) resonated at δ_C 172.0 and 174.9 in **4**, while the pyrrolo-quinone bridging carbon signals of **3** (δ_C 128.0 and 123.5) had shifted to δ_C 131.5 and 118.1 in **4**. These data indicated that **3** and **4** were regioisomers, which was supported by literature values[15,16] and HMBC data analysis. Thus, thiaplakortone D was assigned to structure **4**. The absolute configuration of thiaplakortone D at C-11 was also assigned as *S* on the basis of a comparison of specific rotation data of **4** with literature values.[21−23]

Thiaplakortones A–D were all tested against the 3D7 (chloroquine-sensitive) and Dd2 (chloroquine-resistant) *P. falciparum* lines, and preliminary cytotoxicity data were also acquired using the human cell line HEK293. Physicochemical properties (log *P*, HBA, HBD, MW) for alkaloids **1**–**4** were calculated in silico using Instant JChem software,[24] in order to assess these molecules for lead- and drug-like properties.[25,26] Comparison of the data with Lipinski's drug-like "rule of five",[25] and Hann and Oprea's lead-like criteria,[26] identified that thiaplakortones A–D were all compliant. Details of the calculated physicochemical properties, antimalarial activity, cytotoxicity, and selectivity indices for compounds **1**–**4** are shown in Table 3.

Thiaplakortone A (**1**) showed the most promising biological profile, with IC_{50} values of 51 and 6.6 nM against the 3D7 and Dd2 lines, respectively. Furthermore, **1** displayed only moderate toxicity against HEK293 cells, which translated to selectivity indices of 76 and 591 against the 3D7 and Dd2 strains, respectively. The replacement of the unsaturated thiazine moiety of **1** with the thiazine motif in **2** resulted in 12.7- and 13.9-fold reductions in 3D7 and Dd2 antimalarial activity, respectively.

Thiaplakortones C and D both displayed similar antiparasitic activities against the 3D7 and Dd2 lines, suggesting that the regiochemistry of the thiazine is not critical for antimalarial activity. Furthermore, while the 2-methylaminopropanoic acid

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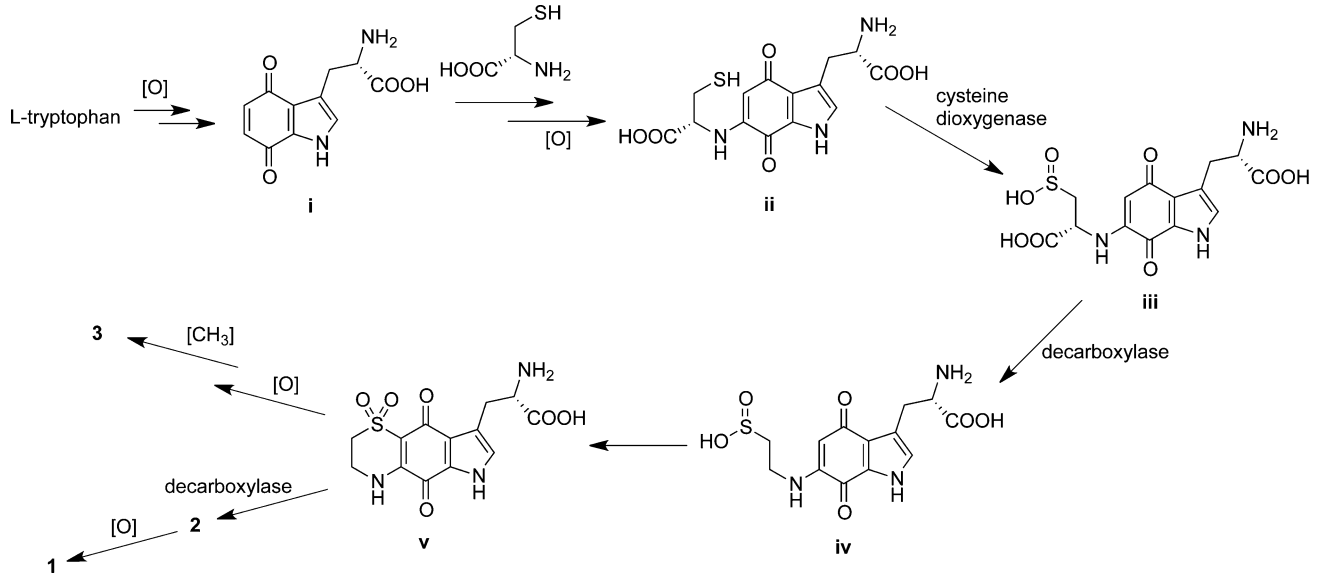

Figure 3. Proposed biogenesis of thiaplakortones A–C (**1**–**3**).

side chains of **3** and **4** reduced antimalarial activity, in comparison to the ethylamine chain of **1**, there seems to be some tolerance to variation in this side chain.

Natural products containing a thiazine-fused quinone fragment are rare.[27] A substructure search of the Dictionary of Natural Products database[27] using this bicyclic fragment identified only 14 compounds, which included thiaplidiaquinones A and B,[16] conicaquinones A and B,[19] aplidinones A–C,[17] and conithiaquinones A and B,[18] which were all isolated from the ascidian *Aplidium conicum*, adociaquinone A and 3-oxoadociaquinone A from the sponge *Adocia sp.*,[20] xestoquinolide B from the sponge *Xestospongia cf. carbonaria*,[28] and ascidiathiazones A and B from the ascidian *Aplidium sp.*[15] A variety of biological activities have been reported for these secondary metabolites, such as topoisomerase II inhibition[29] and anticancer[16,18,20,29] and anti-inflammatory properties.[15]

Although thiaplakortones A–D share some structural homology with the compounds listed above, their tricyclic alkaloid skeleton is without precedent among known natural products. We proposed that these metabolites are most likely biosynthesized from L-tryptophan, which could undergo oxygenation to form a pyrroloquinone moiety (**i**) that could react with L-cysteine to afford two potential thiazine-pyrroloquinone regioisomers,[15] only one of which (intermediate **ii**) is shown in Figure 3.

Oxidation by a dioxygenase similar to cysteine dioxygenase[30] to give the sulfinic acid derivative **iii**, followed by decarboxylation using an enzyme similar to cysteine sulfinic decarboxylase,[31] would yield the intermediate **iv**. Cyclization of **iv** to form the thiazine **v** and subsequent methylation and oxidation would yield thiaplakortone C (**3**), while decarboxylation of intermediate **v** would afford thiaplakortone B (**2**) that upon oxidation of the thiazine ring could produce **1**. The biogenesis of the thiazine-containing natural products pheomelanin and pheofungin from the coupling of cysteine with activated tyrosine and orsellinic acid, respectively, has been previously reported.[32,33]

CONCLUSIONS

The significant in vitro antimalarial activity of this novel alkaloid class combined with the low cytotoxicity is encouraging, and we believe medicinal chemistry to expand the structure–activity relationships and generate potential candidates for in vivo evaluation is warranted.

EXPERIMENTAL SECTION

General Experimental Methods. NMR spectra were recorded at 30 °C on 500 and 600 MHz NMR spectrometers. The latter spectrometer was equipped with a triple-resonance cold probe. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak for DMSO-d_6 at δ_H 2.49 and δ_C 39.5. LRESIMS were recorded on a ZQ mass spectrometer. HRESIMS were measured on a 4.7 T Fourier transform ion cyclotron resonance mass spectrometer fitted with an API source. An HPLC pump fitted with a photodiode array detector and either a C_{18}-bonded silica 5 μm 143 Å column (21.2 mm × 150 mm) or a C_{18}-bonded silica 5 μm 100 Å column (10 mm × 250 mm) were used for the semipreparative HPLC separations. End-capped C_{18}-bonded silica was used for preadsorption work, and the resulting material was packed into stainless steel guard cartridges (10 × 30 mm). An orbital shaker was used for the large-scale extraction of sponge material. All compounds were analyzed for purity by analytical HPLC using a C_{18} monolithic column (4.6 mm × 100 mm) and shown to be >95% pure. H_2O was filtered, while all other solvents used were HPLC grade. Parasite strains 3D7 and Dd2 were from the Queensland Institute of Medical Research. O+ erythrocytes were obtained from the Australian Red Cross Blood Service. Polylysine-coated plates were used for *P. falciparum* assays, and 4′,6-diamidino-2-phenylindole (DAPI), Alamar Blue, Triton-X, saponin, puromycin, artemisinin, chloroquine, and HEK293 cells were all purchased from commercial suppliers.

Sponge Material. The sponge *Plakortis lita* (family: Plakinidae) was collected by scuba diving (−42 m) at Melville Passage, Tydeman Reef, Queensland, Australia, on June 29th, 2003, and kept frozen prior to freeze-drying and extraction. A voucher sample (QMG320465) has been lodged at the Queensland Museum, Brisbane, Australia.

Extraction and Isolation. The freeze-dried and ground sponge (10 g) was sequentially extracted with *n*-hexane (250 mL), CH_2Cl_2 (250 mL × 4), and CH_3OH (250 mL × 4). All the CH_2Cl_2 and CH_3OH extracts were combined and dried down under reduced pressure to yield a dark orange solid (2.4 g). This material was divided into four equal portions (~0.6 g) and then individually preadsorbed on C_{18}-bonded silica (1 g) and packed into four guard cartridges. Each

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cartridge was attached to a C_{18}-bonded silica HPLC column (21.2 mm × 150 mm), and isocratic HPLC conditions of 90% H_2O (0.1% CF_3COOH)/10% CH_3OH (0.1% CF_3COOH) were employed for the first 10 min and then a linear gradient to CH_3OH (0.1% CF_3COOH) was run over 40 min, followed by isocratic conditions of CH_3OH (0.1% CF_3COOH) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected by time from the start of the HPLC run. This HPLC process was repeated for all four cartridges, and the corresponding fractions from each separation were combined and then assayed for antimalarial activity. Fractions 16−18 (20 mg, sample 1) and 19−26 (95 mg, sample 2) were combined on the basis on ^{1}H NMR, MS, and bioassay data analysis. Both of these samples were separately preadsorbed on C_{18}-bonded silica (1 g), and the resulting dry material was packed into guard cartridges that were subsequently attached to a C_{18}-bonded silica HPLC column (21.2 mm × 150 mm). Each sample was separately fractionated using isocratic HPLC conditions of 95% H_2O (1.0% CF_3COOH)/5% CH_3OH (1.0% CF_3COOH) for the first 10 min, followed by a 40 min linear gradient to 60% H_2O (1.0% CF_3COOH)/40% CH_3OH (1.0% CF_3COOH), and then isocratic conditions of 60% H_2O (1.0% CF_3COOH)/40% CH_3OH for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected by time from the start of the HPLC run. HPLC of sample 2 (i.e., fractions 19−26 (95 mg)) yielded the trifluoroacetate salts of thiaplakortones C (**3**, 5.4 mg, 0.054% dry wt, t_R = 29.0−29.9 min) and D (**4**, 9.3 mg, 0.093% dry wt, t_R = 30.1−31.0 min). HPLC of sample 1 (i.e., fractions 16−18 (20 mg)) yielded semipure thiaplakortones A (8.4 mg) and B (2.9 mg) in fractions 27 and 26, respectively. These semipure fractions were individually preadsorbed on C_{18}-bonded silica (1 g), and the resulting dry material was packed into individual guard cartridges that were subsequently attached to a C_{18}-bonded silica HPLC column (10 mm × 250 mm). Each sample was separately fractionated using isocratic HPLC conditions of 95% H_2O (0.1% CF_3COOH)/5% CH_3OH (0.1% CF_3COOH) for the first 10 min, followed by a 40 min linear gradient to 65% H_2O (0.1% CF_3COOH)/35% CH_3OH (0.1% CF_3COOH), and then isocratic conditions of CH_3OH (0.1% CF_3COOH) for a further 10 min, all at a flow rate of 4 mL/min. This afforded the trifluoroacetate salts of thiaplakortones A (**1**, 2.8 mg, 0.028% dry wt, t_R = 27.0−29.5 min) and B (**2**, 1.0 mg, 0.010% dry wt, t_R = 24.8−26.0 min).

*Trifluoroacetate salt of thiaplakortone A (**1**):* stable orange-brown amorphous solid; UV (CH_3OH) λ_{max} (log ε) 228 (3.49), 248 (3.47), 276 sh (3.17), 375 (3.26) nm; IR ν_{max} (KBr) 3600−3300, 1683, 1632, 1515, 1261, 1206, 1127, 1109 cm^{-1}; ^{1}H and ^{13}C NMR data (DMSO-d_6) see Tables 1 and 2; (+)-LRESIMS (relative intensity) m/z 294 (100) [M − CF_3COO^-]$^+$; (+)-HRESIMS m/z 294.0536 (calcd for $C_{12}H_{12}N_3O_4S$ [M − CF_3COO^-]$^+$ 294.0543).

*Trifluoroacetate salt of thiaplakortone B (**2**):* stable orange-brown amorphous solid; UV (CH_3OH) λ_{max} (log ε) 225 (3.83), 299 (3.61), 357 (3.33) nm; IR ν_{max} (KBr) 3600−3300, 1671, 1637, 1585, 1266, 1204, 1120, 1110 cm^{-1}; ^{1}H and ^{13}C NMR data (DMSO-d_6) see Tables 1 and 2; (+)-LRESIMS (relative intensity) m/z 296 (100) [M − CF_3COO^-]$^+$; (+)-HRESIMS m/z 296.0698 (calcd for $C_{12}H_{14}N_3O_4S$ [M − CF_3COO^-]$^+$ 296.0699).

*Trifluoroacetate salt of thiaplakortone C (**3**):* stable orange-brown amorphous solid; $[\alpha]_D^{27}$ = +56° (*c* 0.025, CH_3OH); $[\alpha]_D^{25}$ = +129° (*c* 0.007, 0.5 M HCl); UV (CH_3OH) λ_{max} (log ε) 271 (3.57), 304 sh (3.29), 373 (3.00), 474 (3.29) nm; IR ν_{max} (KBr) 3600−3300, 1670, 1657, 1630, 1411, 1266 cm^{-1}; ^{1}H and ^{13}C NMR data (DMSO-d_6) see Tables 1 and 2; (+)-LRESIMS (rel. int.) m/z 352 (100) [M − CF_3COO^-]$^+$; (+)-HRESIMS m/z 352.0608 (calcd for $C_{14}H_{14}N_3O_6S$ [M − CF_3COO^-]$^+$ 352.0598).

*Trifluoroacetate salt of thiaplakortone D (**4**):* stable orange-brown amorphous solid; $[\alpha]_D^{28}$ = +80° (*c* 0.025, CH_3OH); $[\alpha]_D^{25}$ = +143° (*c* 0.007, 0.5 M HCl); UV (CH_3OH) λ_{max} (log ε) 237 (3.67), 267 sh (3.37), 330 (3.22), 464 (2.87) nm; IR ν_{max} (KBr) 3600−3300, 1670, 1635, 1220 cm^{-1}; ^{1}H and ^{13}C NMR data (DMSO-d_6) see Tables 1 and 2; (+)-LRESIMS (relative intensity) m/z 352 (100) [M − CF_3COO^-]$^+$; (+)-HRESIMS m/z 352.0597 (calcd for $C_{14}H_{14}N_3O_6S$ [M − CF_3COO^-]$^+$ 352.0598).

In Vitro Antimalarial Image-Based Assay. Compounds were incubated in the presence of 2 or 3% parasitemia (3D7 or Dd2) and 0.3% hematocrit in a total assay volume of 50 μL, for 72 h at 37 °C and 5% CO_2, in poly-D-lysine-coated image plates. After incubation the plates were stained with DAPI in the presence of saponin and Triton X-100 and incubated for a further 5 h at room temperature in the dark before imaging on a HTS confocal imaging system. The digital images obtained were analyzed using spot detection software, where fluorescent spots which fulfilled the criteria established for a stained parasite were counted. The percent inhibition of parasite replication was calculated using 0.4% DMSO (0% inhibition) and 2 μM artemisinin (100% inhibition) control data. Artemisinin (IC_{50} = 0.0043 μM (Dd2); IC_{50} = 0.0058 μM (3D7)) and chloroquine (IC_{50} = 0.413 μM (Dd2); IC_{50} = 0.051 μM (3D7)) were used as positive controls.

In Vitro Cytotoxicity Assay. Compounds were added to 384-well black/clear tissue treated assay plates containing 3000 adherent cells/well (HEK293) in an assay volume of 45 μL. The plates were incubated for 72 h at 37 °C and 5% CO_2. After incubation the supernatant was aspirated out of the wells and 40 μL of 10% Alamar Blue added per well. Plates were incubated for a further 5−6 h and measured for fluorescence at 535 nm excitation and 590 nm emission. The percent inhibition of cell growth was calculated using 0.4% DMSO (no inhibition) and 10 μM puromycin (100% inhibition) control data. IC_{50} values were obtained by plotting percent inhibition against log dose using a graphing package and nonlinear regression with a variable slope plot. The positive control, puromycin, was shown to display an IC_{50} value of 0.382 μM toward the HEK293 cell line.

■ ASSOCIATED CONTENT

Supporting Information

Figures giving ^{1}H/^{13}C NMR spectra and tables giving NMR data for thiaplakortones A−D (**1**−**4**) and figures giving antimalarial and cytotoxicity dose response curves for thiaplakortones A−D (**1**−**4**) and the positive controls (artemisinin, chloroquine, and puromycin) used during these studies. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*R.J.Q.: tel, +61-7-3735-6009; e-mail, r.quinn@griffith.edu.au.

Notes

The authors declare no competing financial interest.

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