

Article

Semi-Synthesis and Evaluation of Sargahydroquinoic Acid Derivatives as Potential Antimalarial Agents

Tatenda C. Munedzimwe ¹, Robyn L. van Zyl ², Donovan C. Heslop ², Adrienne L. Edkins ³ and Denzil R. Beukes ^{4,*}

¹ Faculty of Pharmacy, Rhodes University, Grahamstown 6139, South Africa; tatendamunedzimwe@gmail.com

² Pharmacology Division, Department of Pharmacy and Pharmacology; WITS Research Institute for Malaria (WRIM); MRC Collaborating Centre for Multidisciplinary Research on Malaria, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg 2000, South Africa; robyn.vanzyl@wits.ac.za (R.L.v.Z.); donoheleslop@gmail.com (D.C.H.)

³ Biomedical Biotechnology Research Unit (BioBRU), Department of Biochemistry and Microbiology, Rhodes University, Grahamstown 6139, South Africa; a.edkins@ru.ac.za

⁴ School of Pharmacy, University of the Western Cape, Bellville 7535, South Africa

* Correspondence: dbeukes@uwc.ac.za; Tel.: +27-021-959-2352

Received: 25 February 2019; Accepted: 28 March 2019; Published: 1 April 2019

Abstract: Background: Malaria continues to present a major health problem, especially in developing countries. The development of new antimalarial drugs to counter drug resistance and ensure a steady supply of new treatment options is therefore an important area of research. Meroditerpenes have previously been shown to exhibit antiplasmodial activity against a chloroquinone sensitive strain of *Plasmodium falciparum* (D10). In this study we explored the antiplasmodial activity of several semi-synthetic analogs of sargahydroquinoic acid. **Methods:** Sargahydroquinoic acid was isolated from the marine brown alga, *Sargassum incisifolium* and converted, semi-synthetically, to several analogs. The natural products, together with their synthetic derivatives were evaluated for their activity against the FCR-3 strain of *Plasmodium falciparum* as well as MDA-MB-231 breast cancer cells. **Results:** Sarganaphthoquinoic acid and sargaquinoic acid showed the most promising antiplasmodial activity and low cytotoxicity. **Conclusions:** Synthetic modification of the natural product, sargahydroquinoic acid, resulted in the discovery of a highly selective antiplasmodial compound, sarganaphthoquinoic acid.

Keywords: sargaquinoic acid; sarganaphthoquinoic acid; antiplasmodial; malaria

1. Introduction

Despite the impressive breakthroughs in the treatment of malaria [1], it remains a life-threatening disease. Southeast Asia and sub-Saharan Africa account for the vast majority of the estimated 219 million malaria cases reported worldwide, leading to 435,000 deaths [2]. More than 90% of malaria cases and deaths occur in Africa, of which more than 70% are children under five years of age [2]. The prospect of resistance to current drugs appears inevitable and paints a bleak picture indeed [3]. Although the reasons for this dire situation are complex, there is undoubtedly a need for the continued search for and development of new antimalarial drugs. Natural products have historically offered some of the most effective antimalarial drugs [4]. In a previous study, we reported on the antiplasmodial activity of natural products isolated from the South African brown seaweed, *Sargassum incisifolium*, against a chloroquine sensitive strain of *Plasmodium falciparum* (D10) [5]. *S. incisifolium* is relatively abundant along the South African coastline and produces sargahydroquinoic acid (**1**) as the major metabolite. The accessibility of **1** thus provided an opportunity to explore the

structure activity relationships of analogs of this natural product. Herein we report on the antiplasmodial activity of semi-synthetic analogs of sargahydroquinoic acid (**1**) (Figure 1).

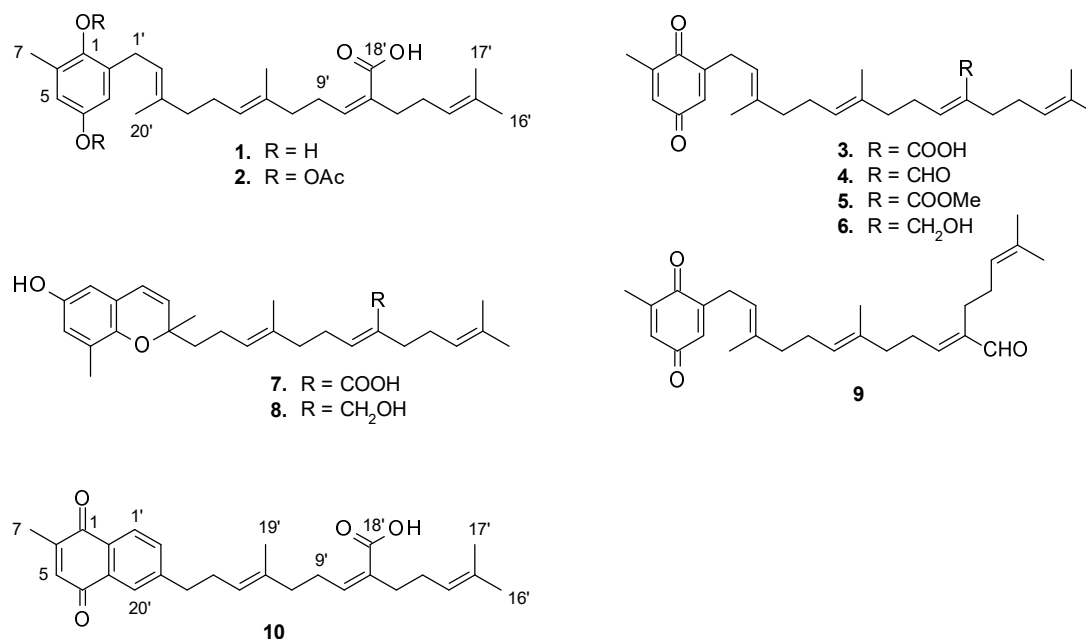


Figure 1. Natural and semi-synthetic derivatives of sargahydroquinoic acid (**1**).

2. Materials and Methods

2.1 General Experimental

All solvents were of chromatographic grade (Merck, Darmstadt, Germany) and used without further purification. Column chromatography was performed on silica gel (40–63 µm particle size) from Merck, Darmstadt, Germany. Normal Phase HPLC was carried out using a Whatman Partisil 10 semi-preparative column (Sigma-Aldrich, Schnellendorf, Germany) (10 mm × 500 mm, 10 µm), while a Phenomenex Luna C₁₈ column (Sigma-Aldrich, Schnellendorf, Germany, 10 mm × 250 mm, 10 µm) was used for reversed phase HPLC. NMR spectra were recorded on Bruker Avance 400 and 600 MHz spectrometers (Bruker Biospin, Rheinstetten, Germany) and referenced to residual undeuterated CDCl₃ solvent signals (δ_H 7.26 ppm and δ_C 77.0 ppm). UV spectra were measured on a Perkin Elmer Lambda 25 UV/Vis spectrometer (Perkin-Elmer, Norwalk, CT, USA) while FT-IR data was obtained using a Perkin Elmer Spectrum 100 FT-IR spectrometer (Perkin-Elmer, Norwalk, CT, USA). High resolution electrospray ionization mass spectroscopy (HR-ESIMS) spectra were obtained on a Waters Synapt G2 mass spectrometer (Waters Corporation, Milford, MA, USA) at 20 V.

2.2 Extraction and Isolation of Natural Products

Specimens of *Sargassum incisifolium* were collected from Port Alfred (collection code PA071b) on the south east coast of South Africa on 21 September 2007 and stored at -20 °C. The samples were authenticated by comparison with voucher specimens from previous studies [5]. Voucher specimens are stored at the School of Pharmacy, University of the Western Cape.

The following isolation protocol is representative and was repeated several times in order to generate sufficient quantities of **1** for synthetic modification. The frozen alga (38.77 g, extracted dry weight) was allowed to thaw at room temperature after which it was soaked in methanol for one hour. The methanol was removed and the alga extracted three times with MeOH-CH₂Cl₂ (1:2) at 40 °C for 30 min. Extracts were pooled and separated into aqueous and organic phases by the addition of distilled water. Concentration of the organic phase under reduced pressure gave a dark green residue (3.87 g). A portion of the organic fraction (1.09 g) was fractionated by step-gradient elution

on a silica gel column (10 g) using solvents of increasing polarity (*n*-hexane-EtOAc) to give seven fractions as follows: Fr A (H-E, 10:0, 8.6 mg), Fr B (H-E, 9:1, 27 mg), Fr C (H-E, 8:2, 132 mg), Fr D (H-E, 6:4, 218 mg), Fr E (H-E, 4:6, 65 mg), Fr F (H-E, 2:8, 9.7 mg) and Fr G (H-E, 0:10, 50 mg) followed by MeOH-EtOAc (1:1), Fr 7H (238 mg). Fraction B (19 mg) was further purified by silica gel column chromatography using a mobile phase of *n*-hexane-EtOAc (9:1) to give 1.7 mg of sargaquinal (9). Fraction C (40 mg) was purified by normal phase HPLC using *n*-hexane-EtOAc as mobile phase (8:2) to give 15 mg of sargaquinoic acid (3). Fraction D (20 mg) was purified by reversed phase HPLC using MeOH-H₂O phase (90:10) as the mobile phase to give sargahydroquinoic acid (1) (6.8 mg) and sargachromenol (7) (2.4 mg), respectively. The isolation of compounds 1, 3, 7 and 9 is summarised in Scheme S1 and their structures were confirmed by spectroscopic methods, which were in agreement with literature data (Table S1) [5]. The NMR spectra for compounds 1 (Figure S1–S2), 3 (Figure S3–S4), 7 (Figure S5–S6) and 9 (Figure S7–S8) are presented in the supplementary materials.

2.3. Sargaquinoic Acid (3) and Sarganaphthoquinoic Acid (10)

To a solution of 1 (154.0 mg, 0.36 mmol) in a mixture of CHCl₃ (8 mL) and MeOH (7 mL) was added Ag₂O (100 mg, 0.43 mmol). The reaction mixture was stirred at room temperature for 24 h, after which the resulting suspension was filtered through diatomaceous earth and concentrated under reduced pressure. The crude product was filtered through a plug of charcoal (*n*-hexane-EtOAc, 4:6) to give a yellow mixture of compounds which was separated by silica gel column chromatography (*n*-hexane-EtOAc, 7:3) to give sargaquinoic acid (3) (80 mg, 70%) and compound 10 (9.8 mg, 6%) as light yellow oils. NMR spectra for compound 10 (Figure S9–S14) can be found in the supplementary materials.

Sarganaphthoquinonoic acid (10): IR (film) $\nu_{\max}$ (cm⁻¹): 1600, 1663, 2850, 2924; ¹H and ¹³C NMR data see Table 1; HRESIMS *m/z* 419.2222 [M-H] (calcd. for C₂₇H₃₅O₃, 419.2221)

Table 1. NMR spectroscopic data for sarganaphthoquinoic acid (10) (600 and 125 MHz, CDCl₃)

Carbon Number	δ_c	Type	δ_H , mult, <i>J</i> (Hz)	COSY	HMBC
1	185.4	C	-		
2	130.2	C	-		
3	132.2	C	-		
4	185.4	C	-		
5	136.0	CH	6.81, s	H-7	
6	149.0	C	-		
7	16.4	CH ₃	2.18, s,	H-5	C-6, C-5
1'	126.7	CH	8.00, d, 7.9	H-2'	C-2, C-1
2'	133.8	CH	7.51, d, 7.9	H-4', H-20'	C-1, C-20'
3'	148.2	C	-		
4'	36.2	CH ₂	2.77, t, 7.6	H-5'	C-5', C-3'
5'	29.1	CH ₂	2.36, m	H-4', H-6'	C-4', C-6', C-7'
6'	123.2	CH	5.17, m		
7'	136.0	C	-		
8'	39.0	CH ₂	2.08, m	H-9'	C-6'
9'	28.2	CH ₂	2.57, m	H-10'	C-8', C-10'
10'	145.0	CH	5.96, t, 7.3	H-9'	C-8'
11'	130.6	C	-		
12'	27.8	CH ₂	2.26, m	H-13', H-14' (lr)	C-13', C-14'
13'	28.2	CH ₂	2.11, m	H-14'	C-15', C-11'
14'	123.4	CH	5.17, t, 7.0	H-13', H-14' (lr)	
15'	132.3	C	-		

16'	25.6	CH ₃	1.68, s	H-14' (lr)	C-15', C-14'
17'	17.7	CH ₃	1.59, s	H-14' (lr)	C-16'
18'	171.9	C	-		
19'	15.9	CH ₃	1.58, s	H-6'	C-6'
20'	125.9	CH	7.86, s	H-4'	C-4', C-4

COSY: ¹H-¹H Correlation spectroscopy; HMBC: ¹H-¹³C Heteronuclear multiple-bond correlation spectroscopy

2.4. Sargaquinoic Acid Methyl ester (5)

To a solution of **1** (122.4 mg, 0.29 mmol) dissolved in 2 mL acetone, was added K₂CO₃ (207.4 mg, 1.50 mmol) in 5 mL acetone and dimethylsulphate (250 µL, 2.63 mmol). The mixture was heated at 40 °C for 8 h followed by stirring at room temperature for 16 h. The reaction mixture was filtered, concentrated under reduced pressure and separated by silica gel column chromatography (*n*-hexane:EtOAc, 8:2) to give the methyl ester of **1**, which, upon exposure to air was completely oxidized to **5**. NMR spectra for compound **5** (Figure S15–S16) can be found in the supplementary materials.

Yellow oil, ¹H NMR (400 MHz, CDCl₃) δ 6.52 (1H, s, H-3), 6.44 (1H, s, H-5), 5.83 (1H, t, *J* = 7.0 Hz, H-10'), 5.10 (3H, m, H-2', H-6', H-14'), 3.71 (3H, s, OMe), 3.11 (2H, d, *J* = 6.8 Hz, H-1'), 2.49 (2H, m, H-9'), 2.22 (2H, m, H-12'), 2.05 (2H, m, H-4') 2.03–2.05 (6H, m, H-5', H-8', H-13'), 1.65 (6H, s, H-7, H-19'), 1.61 (3H, s, H-20'), 1.58 (3H, s, H-16'), 1.55 (3H, s, H-17'); 188.0 (C-1, C-4), 168.4 (C-18'), 148.4 (C-6), 145.8 (C-2), 142.1 (C-10'), 140.0 (C-3') 134.8 (C-7') 133.1 (C-3, C-15'), 132.1 (C-5), 131.4 (C-11'), 124.4 (C-6'), 123.5 (C-14'), 118.0 (C-2'), 51.0 (OMe), 39.8 (C-4'), 39.1 (C-8'), 34.7 (C-12'), 28.0 (C-9'), 27.8 (C-13'), 27.5 (C-1'), 26.4 (C-5'), 25.6 (C-16'), 17.6 (C-17'), 16.1 (C-7), 15.9 (C-19'); HRESIMS *m/z* 437.2710 [M-H] (calcd. for C₂₈H₃₇O₄, 437.2692).

2.5. Diacetyl Sargahydroquinoic Acid (2)

To sargahydroquinoic acid (**1**) (110.0 mg, 0.26 mmol) was added acetic anhydride (3 mL, 31.8 mmol) and pyridine (2 mL, 24.8 mmol). The reaction mixture was stirred at room temperature for 30 h. The crude product was acidified with 1 M HCl (10 mL) and extracted with EtOAc (5 mL × 3). The organic layer was collected and concentrated under reduced pressure to give a crude product which was further purified by silica gel column chromatography (*n*-hexane:EtOAc, 7:3) to give compound **2** (12.8 mg, 12%) as a yellow oil. The structure of compound **2** was confirmed by spectroscopic methods, which were in agreement with literature data [6,7]. NMR spectra for compound **2** (Figure S17–S18) can be found in the supplementary materials.

2.6. Sargaquinol (6) and Sargachromendiol (8)

To a solution of sargahydroquinoic acid (**1**) (140.7 mg, 0.33 mmol) dissolved in anhydrous THF (5 mL), was added LiAlH₄ (0.104 g, 2.74 mmol). The reaction mixture was stirred at room temperature, under a nitrogen atmosphere for 1.25 h. The reaction was quenched with a few drops of EtOAc, concentrated and partitioned between EtOAc (10 mL – 2) and H₂O (5 mL). The organic layer was concentrated under reduced pressure to give a crude product which was purified by silica gel chromatography (*n*-hexane:EtOAc, 8:2) to give sargaquinol (**6**) (12.2 mg, 30%) and the alcohol derivative of sargachromenol (**8**) (2.8 mg, 3.5%). The structure of compound **6** was confirmed by spectroscopic methods, which were in agreement with literature data (Table S1) [7]. NMR spectra for compounds **6** (Figure S19–S20) and **8** (Figure S21–S22) can be found in the supplementary materials.

Sargaquinol (**6**) yellow oil; ¹H NMR (400 MHz, CDCl₃) δ 6.54 (s, 1H) (H-3), 6.46 (s, 1H) (H-5), 5.15 (dd, *J* = 21.0, 13.2 Hz) (H-2', 6', 14'), 4.11 (s) (H-18'), 3.63 (t, *J* = 6.5 Hz) (H-10'), 3.12 (d, *J* = 7.1 Hz) (H-1'), 2.12 (s) (H-5', 9', 13'), 2.05 (s) (H-4', 8'), 1.67 (s) (H-7, 16'), 1.60 (s) (H-19', 20'), 1.57 (s) (H-17'). ¹³C NMR (100 MHz, CDCl₃) δ 188.0 (C-1), 187.97 (C-4), 148.5 (C-6), 145.9 (C-2), 139.7 (C-3'), 135.0 (C-7'), 133.1 (C-3), 131.2 (C-11'), 132.24 (C-5), 133.7 (C-15'), 124.7 (C-6'), 124.2 (C-14'), 118.1 (C-2'), 71.8 (C-18'), 62.8 (C-10'), 39.8 (C-4'), 39.5 (C-8'), 35.2 (C-12'), 27.1 (C-13'), 27.5 (C-1'), 26.2 (C-5'), 26.3 (C-9'), 25.6 (C-16'), 17.7 (C-7), 16.11 (C-17'), 16.07 (C-19'), 16.0 (C-20').

Sargachromendiol (**8**) yellow oil; ^1H NMR (400 MHz, CDCl_3) δ 6.47 (d, $J = 2.4$ Hz) (H-5), 6.32 (d, $J = 2.5$ Hz) (H-2), 6.26 (s) (H-2'), 5.98 (t, $J = 7.2$ Hz) (H-9'), 5.57 (d, $J = 9.8$ Hz) (H-3'), 5.12 (dt, $J = 19.0$, 6.4 Hz) (H-5', 14'), 2.59 (q, $J = 7.3$ Hz) (H-8'), 2.27 (t, $J = 7.4$ Hz), 2.13 (s) (H-12'), 2.09–2.04 (m) (H-4', 7'), 1.68 (s) (H-8), 1.58 (d, $J = 3.5$ Hz) (H-17', 19'), 1.36 (s) (20'); ^{13}C NMR (100 MHz, CDCl_3) δ 148.6 (C-5), 145.0 (C-10'), 144.8 (C-8), 134.7 (C-7'), 131.8 (C-15'), 130.6 (C-11'), 126.3 (C-3), 124.7 (C-6'), 124.1 (C-1'), 122.9 (C-14'), 121.3 (C-2), 117.0 (C-4), 110.3 (C-6), 77.8 (C-3'), 60.3 (C-18'), 40.7 (C-4'), 39.8 (C-8'), 35.1 (C-12'), 35.3 (C-12'), 27.0 (C-9'), 26.1 (C-13'), 25.9 (C-20'), 25.7 (C-16'), 22.6 (C-5'), 17.7 (17'), 15.9 (C-7), 15.5 (C-19').

2.7. *Z*-sargaquinal (**4**)

To a solution of sargaquinol (**6**) (37.2 mg, 0.09 mmol) dissolved in anhydrous CH_2Cl_2 (8 mL), Dess-Martin Periodinane (107 mg, 0.26 mmol) was added. The reaction mixture was stirred at room temperature for 2 h after which it was quenched with CH_2Cl_2 (10 mL) and de-ionized water (10 mL). The organic phase was separated and washed with saturated solutions of NaHCO_3 (10 mL $\times$ 3) and $\text{Na}_2\text{S}_2\text{O}_3$ (10 mL $\times$ 3), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (*n*-hexane:EtOAc, 8:2) to give compound **4** (42%, 14.9 mg), as a yellow oil. The structure of compound **4** was confirmed by spectroscopic methods, which were in agreement with literature data (Table S1) [7]. NMR spectra for compound **4** (Figure S23–S24) can be found in the supplementary materials.

2.8. Antiplasmodial Assays

All compounds were tested in triplicate against the chloroquine-resistant Gambian FCR-3 strain of *P. falciparum*. The in vitro erythrocytic stage of the parasite was maintained using the method outlined by Trager and Jensen [8]. The antimalarial activity of the compounds was determined using the tritiated hypoxanthine incorporation assay using a 0.5% parasitaemia and 1% haematocrit [9]. All assays were carried out using untreated parasites and uninfected red blood cells as controls. The concentration that inhibited 50% parasite growth (IC_{50} value) was determined from the log sigmoid dose response curve using GraphPad Prism. Quinine was used as the reference antiplasmodial agent. The selectivity index for the compounds was determined from the ratio of cytotoxicity IC_{50} to antimalarial IC_{50} .

2.9. Cytotoxicity Assay

All compounds were tested in triplicate against MDA-MB-231 breast carcinoma cells, which were purchased from the ATCC (Catalogue number HTB-26, Manassas, VA, USA). The cytotoxicity of the compounds was determined using the WST-1 assay method (Roche). The cells were treated with a range of concentrations of the test compounds or vehicle control (DMSO). Cells treated with DMSO were considered to represent 100% viability and the viability of cells at each dose was represented relative to this value. The concentration resulting in a decrease of cell viability to 50% was calculated from the linear portion of the dose response curve.

3. Results and Discussion

3.1. Isolation and Synthetic Modification of Sargahydroquinoic Acid Derivatives

Sargahydroquinoic acid (**1**) is the major component of the CH_2Cl_2 -MeOH extract of *Sargassum incisifolium* and has also been reported from several other *Sargassum* spp. [5–7]. This compound slowly converts to sargaquinoic acid (**3**) and sargachromenol (**7**) on storage of the seaweed, the extract and during purification. Specimens of *S. incisifolium* (PA071b) were collected from Port Alfred on the south eastern coast of South Africa and extracted with CH_2Cl_2 -MeOH. The crude extract was first fractionated by silica gel column chromatography, followed by normal or reversed phase HPLC to give compounds **1**, **3**, **7** and **9**. The identities of all isolated compounds were confirmed by comparison

of their NMR spectroscopic data (Table S1) to literature values [5]. The above protocol yielded sufficient quantities of **1** to perform structural modifications and biological assays.

Sargaquinoic acid (**3**) is normally isolated from fresh seaweed in relatively small quantities; however, it can be produced more efficiently by the oxidation of **1** [7,10]. Thus, treatment of **1** with Ag₂O gave **3** in moderate to good yields. Interestingly, although this conversion is facile, we consistently observed a series of unusual peaks between δ_{H} 7 and 8 in the ¹H NMR spectrum of the crude reaction product. The compound (**10**) responsible for these peaks was isolated and its structure was elucidated by NMR spectroscopy and mass spectrometry.

The HRESIMS spectrum of compound **10** showed a molecular ion peak at m/z 419.2222 [M-H] which corresponds to a molecular formula of C₂₇H₃₁O₄. Characteristic deshielded methine resonances at δ_{H} 8.00 (d, $J = 7.9$), δ 7.98 (s) and δ 7.51 (d, $J = 7.9$) were evident in its ¹H NMR spectrum. In addition, one of the aromatic singlets had shifted downfield from δ_{H} 6.46 in **3** to δ_{H} 6.81 in **10**. Data from the ¹³C NMR spectrum of compound **10** revealed no change in the number of carbon atoms when compared to the starting material (**1**). It revealed the presence of two quinone carbonyls signals at (δ_{C} 185.4 and δ 185.4) and a carboxylic acid moiety (δ_{C} 171.9). In addition, the DEPT-135 NMR spectrum indicated the loss of one methyl signal (δ_{C} 16.1, C-20') and a methylene signal (δ_{C} 27.5, C-1') when compared to **3**, together with the appearance of two additional olefinic methine signals at δ_{C} 126.7 (C-1') and 125.9 (C-20'). HMBC correlations (Figure 2) from the doublet at δ_{H} 8.00 (H-1') to carbon signals at δ_{C} 126.7 (C-1') and δ_{C} 133.8 (C-2'); the methine signal at δ_{H} 7.51 (H-2') to the carbon signal at δ_{C} 125.9 (C-20') and from δ_{H} 7.86 (H-20') to carbon signals at δ_{C} 36.2 (C-4') and δ_{C} 185.4 (C-4), allowed for the assignment of the naphthoquinone moiety. All other spectroscopic data are consistent with a polyprenyl side chain with a 6'E,10'Z-double bond geometry (as in **3**). We assigned the name sarganaphthoquinoic acid to this new compound. A related compound, chabrolonaphthoquinone, had previously been reported from the Taiwanese soft coral, *Nephthea chabrolii* [11]. The main differences between the two compounds are the methyl substituent at C-6 and the 10-double bond geometry in compound **10**.

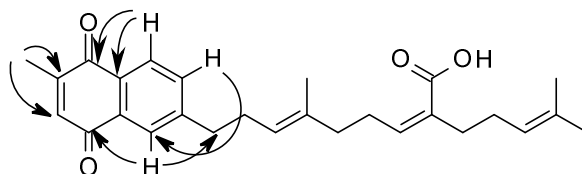
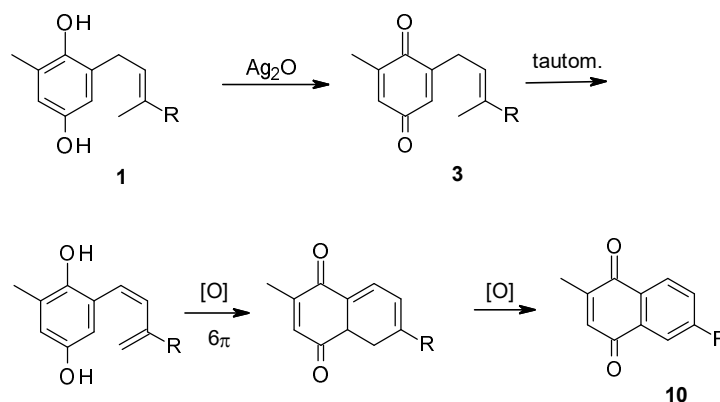


Figure 2. Key HMBC correlations for **10**.

The direct conversion of prenylated hydroquinones to naphthoquinones is uncommon and presents a novel approach to the synthesis of this important group of compounds. To the best of our knowledge there is only a single report describing the formation of a naphthoquinone as a side-product in the synthesis of chromenes from prenylated quinones [12]. Naphthoquinones are typically synthesized by Diels-Alder reactions between *p*-benzoquinones and dienes or by the prenylation of halogenated naphthoquinone moieties [13–15]. Compound **10** is proposed to form via tautomerism and oxidation of the intermediate quinone (**3**) followed by 6 π electrocyclization and further oxidation (Scheme 1).



Scheme 1. Proposed mechanism for the synthesis of sarganaphthoquinoid acid (10).

In order to establish preliminary structure-antiplasmodial activity relationships for this series of sargahydroquinoid acid derivatives, we focused our attention on modification of the carboxylic acid and quinone moieties. Acetylation of **1** with acetic anhydride/pyridine gave the diacetate (**2**), while its reduction with lithium aluminium hydride gave a mixture of sargaquinol (**6**) and sargachromendiol (**8**). The facile conversion of the hydroquinone to a mixture of the quinone and chromene on exposure to air is often seen in this series of compounds [6,7]. Spectroscopic evidence for the identity of alcohols **6** and **8** were provided by the disappearance of the ^{13}C NMR signal due to the carboxylic acid group at δ_{C} 172 ppm and the appearance of an oxymethylene carbon signal at δ_{C} 60.3 ppm in both compounds.

Mild oxidation of **6** with Dess-Martin periodinane, gave 10Z-sargaquinol (**4**). The structures of aldehydes **4** and **9** were confirmed by comparison of their spectroscopic data with literature values [5,7]. A comparison of the ^1H NMR spectra of the natural and semi-synthetic aldehydes revealed differences in chemical shifts of both proton and carbon atoms associated with the aldehyde group. The ^1H and ^{13}C NMR spectra of the semi-synthetic aldehyde (**4**) showed signals at δ_{H} 10.1 and δ_{C} 190.9 ppm compared to δ_{H} 9.55 and δ_{C} 205.4 ppm in the natural aldehyde (**9**). ^1H - ^1H NOESY correlations in both compounds confirmed the difference in the geometry of the C^{10} double bond with the semi-synthetic aldehyde (**4**) bearing a 10Z-geometry and the natural aldehyde (**9**) a 10E-geometry. The formation of 2'E,6'E,10'Z-sargaquinol (**4**) from 2'E,6'E,10'Z-sargahydroquinoid acid (**1**) has been reported in the literature [7]. However, this is the first report of its ^{13}C and 2D NMR data.

Interestingly, methylation of **1** with dimethylsulphate/potassium carbonate did not produce the dimethyl ether, but instead produced sargaquinoid acid methyl ester (**5**). This was confirmed by the appearance of an additional methyl signal at δ_{C} 51.0 and an upfield shift of the C-18' carbonyl signal from δ_{C} 172 to δ_{C} 168.4 ppm in the ^{13}C NMR spectrum of **5** (Table S1).

3.2. Biological Assays

The ten sargahydroquinoid acid derivatives were assessed for both antiplasmodial and cytotoxic activity against the chloroquine-resistant Gambian FCR-3 strain of *P. falciparum* and MDA-MB-231 breast cells, respectively (Table 2). All compounds showed moderate to good antiplasmodial activity. However, the most promising compound in this series is the naphthoquinone **10** which not only revealed good antiplasmodial activity (IC_{50} 5.4 μM), but also very low cytotoxicity (IC_{50} 2410 μM), resulting in a high selectivity index of 443. Sargaquinoid acid (**3**) also shows promising antiplasmodial activity (IC_{50} 10.8 μM), but is slightly more toxic (IC_{50} 658 μM) than **10**. It appears that the carboxylic acid in the prenyl side chain is important for activity since both aldehydes (**4** and **9**) and the alcohol (**6**) showed decreased antiplasmodial activity. The quinone/naphthoquinone scaffold is present in several antimalarial natural products and drugs [16]. It is therefore likely that the mode of action of the compounds reported here is related to this important pharmacophore [16–20].

Table 2. Bioassay results for compounds 1–10.

Compound	IC ₅₀ (μM)			Selectivity index
	D10 ¹	FCR-3	MDA-MB-231	
Sargahydroquinoidic acid (1)	15.2	38.6	70	1.8
Sargahydroquinoidic acid di-acetate (2)	-	84.3	286	3.4
Sargaquinoidic acid (3)	12.0	10.8	658	60.9
10Z-sargaquinol (4)	-	72.6	211	2.9
Sargaquinoidic acid methyl ester (5)	-	8.2	70	8.6
Sargaquinol (6)	-	93.1	99	1.1
Sargachromenol (7)	-	114.8	56	0.5
Sargachromendiol (8)	-	34.2	187	5.5
10E-sargaquinol (9)	2.0	104.4	69	0.7
Sarganaphthoquinone (10)	-	5.4	2410	443
Quinine		0.17	-	-

¹ From reference [5]

4. Conclusions

In this study we isolated the relatively abundant antiplasmodial natural product, sargahydroquinoidic acid (1) and converted it to several analogs which were evaluated for antiplasmodial and cytotoxic activity. The serendipitous formation of sarganaphthoquinoidic acid (10) gave a compound with good antiplasmodial activity while being almost non-toxic. Due to the small number of compounds no clear structure activity relationships can be established, however it appears that the presence of a quinone and carboxylic acid are important for selective activity against *P. falciparum*. Further studies are warranted to explore the mode of action of these compounds and to further improve on its antiplasmodial activity.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Scheme S1: Isolation of compounds 1, 3, 7 and 9, Table S1: Comparison of ¹³C NMR data for compounds 1, 3–9, Figure S1: ¹H NMR spectrum of sargahydroquinoidic acid (1) (400 MHz, CDCl₃), Figure S2: ¹³C NMR spectrum of sargahydroquinoidic acid (1) (100 MHz, CDCl₃), Figure S3: ¹H NMR spectrum of sargaquinoidic acid (3) (400 MHz, CDCl₃), Figure S4: ¹³C NMR spectrum of compound 3 (400 MHz, CDCl₃), Figure S5: ¹H NMR spectrum of sargachromenol (7) (400 MHz, CDCl₃), Figure S6: ¹³C NMR spectrum of sargachromenol (7) (100 MHz, CDCl₃), Figure S7: ¹H NMR spectrum of 10'E-sargaquinol (9) (400 MHz, CDCl₃), Figure S8: ¹³C NMR spectrum of 10'E-sargaquinol (9) (100 MHz, CDCl₃), Figure S9: ¹H NMR spectrum of sarganaphthoquinoidic acid (10) (400 MHz, CDCl₃), Figure S10: ¹³C NMR spectrum of sarganaphthoquinoidic acid (10) (100 MHz, CDCl₃), Figure S11: DEPT-135 NMR spectrum of sarganaphthoquinoidic acid (10) (100 MHz, CDCl₃), Figure S12: HSQC NMR spectrum of sarganaphthoquinoidic acid (10) (CDCl₃), Figure S13: COSY NMR spectrum of sarganaphthoquinoidic acid (10) (CDCl₃), Figure S14: HMBC NMR spectrum of sarganaphthoquinoidic acid (10) (CDCl₃), Figure S15: ¹H NMR spectrum of sargaquinoidic acid methyl ester (5) (400 MHz, CDCl₃), Figure S16: ¹³C NMR spectrum of sargaquinoidic acid methyl ester (5) (100 MHz), Figure S17: ¹H NMR spectrum of sargahydroquinoidic acid diacetate (2) (400 MHz, CDCl₃), Figure S18: ¹³C NMR spectrum of sargahydroquinoidic acid diacetate (2) (100 MHz, CDCl₃), Figure S19: ¹H NMR spectrum of sargaquinol (6) (400 MHz, CDCl₃), Figure S20: ¹³C NMR spectrum of sargaquinol (6) (100 MHz, CDCl₃), Figure S21: ¹H NMR spectrum of sargachromendiol (8) (400 MHz, CDCl₃), Figure S22: ¹³C NMR spectrum of sargachromendiol (8) (100 MHz, CDCl₃), Figure S23: ¹H NMR spectrum of 10'Z-sargaquinol (4) (600 MHz, CDCl₃), Figure S24: ¹³C NMR spectrum of 10'Z-sargaquinol (4) (100 MHz, CDCl₃).

Author Contributions: D.R.B. conceived and designed the work. T.C.M. isolated the natural products and synthesized the analogs, R.L.v.Z. and D.C.H. conducted the antiplasmodial assays. Cytotoxicity studies were done by A.L.E. D.R.B. and T.C.M. drafted the manuscript. All authors read and approved the final version of manuscript.

Funding: This research was funded by Rhodes University, University of the Witwatersrand and the University of the Western Cape. A.L.E. is funded by the South African Research Chairs Initiative of the Department of Science and Technology (DST) and National Research Foundation of South Africa (NRF) (Grant No 98566), and National Research Foundation CPRR (Grant No 105829).

Acknowledgments: T.C.M. acknowledges Rhodes University and the Andrew W. Mellon Foundation for a Masters scholarship.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Okombo, J.; Chibale, K. Recent updates in the discovery and development of novel antimalarial drug candidates. *Med. Chem. Commun.* **2018**, *9*, 437–453.
- World Health Organization. *World Malaria Report*; World Health Organization: Geneva, Switzerland, 2018.
- Amato, R.; Pearson, R.D.; Almagro-Garcia, J.; Amaratunga, C.; Lim, P.; Suon, S.; Sreng, S.; Drury, E.; Stalker, J.; Miotto, O.; et al. Origins of the current outbreak of multidrug-resistant malaria in southeast Asia: A retrospective genetic study. *Lancet Infect. Dis.* **2018**, *18*, 337–345.
- Fernández-Álvarez, E.; Hong, W.D.; Nixon, G.L.; O'Neill, P.M.; Calderón, F. Antimalarial chemotherapy: Natural product inspired development of preclinical and clinical candidates with diverse mechanisms of action. *J. Med. Chem.* **2016**, *59*, 5587–5603.
- Afolayan, A.F.; Bolton, J.J.; Lategan, C.A.; Smith, P.J.; Beukes, D.R. Fucoxanthin, tetraprenylated toluquinone and toluhydroquinone metabolites from *Sargassum heterophyllum* inhibit the *in vitro* growth of the malaria parasite *Plasmodium falciparum*. *Z. Naturforsch.* **2008**, *63c*, 848–852.
- Segawa, M.; Shirahama, H. New plastoquinones from the brown alga *Sargassum sagamianum* var. *yezoense*. *Chem. Lett.* **1987**, 1365–1366.
- Kusumi, T.; Ishitsuka, M.; Kinoshita, T.; Kakisawa, H.; Shibata, Y. Structures of new plastoquinones from the brown alga *Sargassum serratifolium*. *Chem. Lett.* **1979**, *8*, 277–278.
- Trager, W.; Jensen, J.B. Human malaria parasites in continuous culture. *Science* **1976**, *193*, 673–675.
- Desjardins, R.E.; Canfield, C.J.; Haynes, J.D.; Chulay, J.D. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
- Perez-Castorena, A.; Arciniegas, A.; Apan, M.T.R.; Villasenor, J.L.; de Vivar, A. Evaluation of the anti-inflammatory and antioxidant activities of the plastoquinones derivatives isolated from *Roldana barba-johannis*. *Planta Med.* **2002**, *68*, 645–647.
- Sheu, J.-H.; Su, J.-H.; Sung, P.-J.; Wang, G.-H.; Dai, H. Novel meroditerpenoid-related metabolites from the formosan soft coral *Nephthea chabroliei*. *J. Nat. Prod.* **2004**, *67*, 2048–2052.
- Chan, S.T.S.; Pullar, M.A.; Khalil, I.M.; Allouche, E.; Barker, D.; Copp, B.R. Bio-inspired dimerisation of prenylated quinones directed towards the synthesis of the meroterpenoid natural products, the scabellones. *Tetrahedron Lett.* **2015**, *56*, 1486–1488.
- Alonso, M.A.; Lopez-Alvarado, P.; Avendano, C.; Menendez, J.C. Regioselective Diels-Alder reactions of 3-vinylindoles with quinones. *Lett. Org. Chem.* **2004**, *1*, 20–22.
- de Koning, C.B.; Rousseau, A.L.; van Otterlo, W.A.L. Modern methods for the synthesis of substituted naphthalenes. *Tetrahedron* **2003**, *59*, 7–36.
- Couladouros, E.A.; Plyta, Z.F.; Papageorgiou, V.P. A general procedure for the efficient synthesis of (alkylamino)naphthoquinones. *J. Org. Chem.* **1996**, *61*, 3031–3033.
- Nixon, G.L.; Moss, D.M.; Shone, A.E.; Lalloo, D.G.; Fisher, N.; O'Neill, P.M.; Ward, S.A.; Biagini, G.A. Antimalarial pharmacology and therapeutics of atovaquone. *J. Antimicrob. Chemother.* **2013**, *68*, 977–985.
- Olliaro, P. Mode of action and mechanisms of resistance for antimalarial drugs. *Pharmacol. Ther.* **2001**, *89*, 207–219.
- Vennerstrom, J.L.; Eaton, J.W. Oxidants, oxidant drugs, and malaria. *J. Med. Chem.* **1988**, *31*, 1269–1277.
- Brandão, G.C.; Rocha Missias, F.C.; Arantes, L.M.; Soares, L.F.; Roy, K.K.; Doerksen, R.J.; Braga de Oliveira, A.; Pereira, G.R. Antimalarial naphthoquinones. Synthesis via click chemistry, *in vitro* activity, docking to PfDHOD and SAR of lapachol-based compounds. *Eur. J. Med. Chem.* **2018**, *145*, 191–205.
- Imperatore, C.; Persico, M.; Senese, M.; Aiello, A.; Casertano, M.; Luciano, P.; Basilico, N.; Parapini, S.; Paladino, A.; Fattorusso, C.; et al. Exploring the antimalarial potential of the methoxy-thiazinoquinone scaffold: Identification of a new lead candidate. *Bioorg. Chem.* **2019**, *85*, 240–252.

