# Evaluation of a Series of 9,10-Anthraquinones as Antiplasmodial Agents

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**LETTER ARTICLE**

# Evaluation of a Series of 9,10-Anthraquinones as Antiplasmodial Agents

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**Abstract:** ***Background***: A phytochemical study on medicinal plants used for the treatment of fever and malaria in Africa yielded metabolites with potential antiplasmodial activity, many of which are Anthraquinones (AQ). AQs have similar sub-structure as naphthoquinones and xanthones, which were previously reported as novel antiplasmodial agents.

***Objective***: The present study aimed to investigate the structural requirements of 9,10-anthraquinones with hydroxy, methoxy and methyl substituents to exert strong antiplasmodial activity and to investigate their possible mode of action.

***Methods***: Thirty-one AQs were synthesized through Friedel-Crafts reaction and assayed for antiplasmodial activity *in vitro* against *Plasmodium falciparum* (3D7). The selected compounds were tested for toxicity and probed for their mode of action against β-hematin dimerization through HRP2 and lipid catalyses. The most active compounds were subjected to a docking study using AutoDock 4.2.

***Results***: The active AQs have similar common structural characteristics. However, it is difficult to establish a structure-activity relationship as certain compounds are active despite the absence of the structural features exhibited by other active AQs. They have either *ortho*- or *meta*-arranged substituents and one free hydroxyl and/or carbonyl groups. When C-6 is substituted with a methyl group, the activity of AQs generally increased. 1,3-DihydroxyAQ (**15**) showed good antiplasmodial activity with an $IC_{50}$ value of 1.08 μM, and when C-6 was substituted with a methyl group, 1,3-dihydroxy-6-methylAQ (**24**) showed stronger antiplasmodial activity with an $IC_{50}$ value of 0.02 μM, with better selectivity index. Compounds **15** and **24** showed strong HRP2 activity and mild toxicity against hepatocyte cells. Molecular docking studies showed that the hydroxyl groups at the *ortho* (**23**) and *meta* (**24**) positions are able to form hydrogen bonds with heme, of 3.49 Å and 3.02 Å, respectively.

***Conclusion***: The activity of 1,3-dihydroxy-6-methylAQ (**24**) could be due to their inhibition against the free heme dimerization by inhibiting the HRP2 protein. It was further observed that the anthraquinone moiety of compound **24** bind in parallel to the heme ring through hydrophobic interactions, thus preventing crystallization of heme into hemozoin.

## 1. INTRODUCTION

Malaria is one of the most lethal parasitic diseases, and affects approximately 25% of the global population, with a higher concentration in tropical developing countries [1].

According to World Malaria Report 2017, there were 216 million cases of malaria infections, with 445 000 deaths reported, of which 80% of the reported deaths were young children [2]. It is estimated malaria kills a child every two minutes. For decades, malarial chemotherapy relied on several drugs, each with its own pharmacological limitations, of which parasite resistance has been the most challenging. WHO recommends Artemisinin Combination Therapy (ACT) for non-complicated falciparum malaria in sub-Saharan

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region [2]. However, due to poverty and limited access to healthcare facilities, 56-69 million children did not receive ACTs as a first-line treatment [2]. Despite the high prevalence of malarial infection's globally, only 13 out of 1300 new drugs were introduced for parasitic diseases between 1975 and 1999 [3].

One of the important chemotheraupeutic targets in combating malaria infection is its food vacuole. The *Plasmodium* parasite digests erythrocytes and releases heme [4], along with oxygen [5]. Free heme is toxic owing to its detergent-like properties and destabilizes and lyses membranes [6, 7] as well as inhibiting the activity of several enzymes, such as the cysteine proteases [7] leading to parasite death. The mechanisms of heme detoxification can be broadly classified into two types; primarily through dimerization into hemozoin and secondarily through degradation of heme by glutathione and hydrogen peroxide [8]. Histidine-Rich Protein II (HRP2) [5, 7] and lipids [8] are proposed to catalyze the reaction but there is other evidence that hemozoin formation may be spontaneous [9] and autocatalytic [7]. An oxidative mechanism has been proposed for lipid-mediated β-hematin formation, which may be mediated by the generation of some free radicals which are intermediates of heme [7]. The secondary model of heme detoxification suggests that heme which is not converted into hemozoin escapes from the food vacuole to the parasite cytosol where it is degraded by glutathione [7]. In the food vacuole, hydrogen peroxide mediates oxidation of the porphyrin ring at pH 5.2, which leads to macrocyclic ring opening and subsequent breakdown [7].

Phytochemical studies on medicinal plants used in Africa for the treatment of malaria and fever yielded many interesting metabolites with potential antiplasmodial activity. Investigation on *Morinda Lucida* Benth. [10], *Newbouldia laevis* [11], *Bulbine frutescence* [12], *Cassia siamea* [13], *Kniphofia foliosa* [14], *Stereospermum kunthianum* [15], *Tectona grandis* [16], *Pentas micrantha* [17], *Pentas longiflora* and *Pentas lanceolata* [18] yielded many active metabolites with anthraquinone moiety.

The mechanism on how anthraquinones work as antiplasmodial agents is not clearly understood; nonetheless, they are known to induce free radical formation in biological systems [19-21]. Aromatic compounds like anthraquinones often display good activities against protozoa due to bio-reduction and the formation of reactive free radicals. It is hypothesized that anthraquinones may act as anti-plasmodial agents through futile redox cycling, similar to naphthoquinones [20, 22]. They generally act as a catalytic oxidizing agent capable of undergoing cyclic one electron oxidation-reduction reactions [22]. Reactive Oxygen Species (ROS) in infected erythrocytes can induce oxidative stress causing membrane lysis and cellular damage, which consequently lead to the death of the *Plasmodium* parasite [7]. Antiplasmodial drugs that induce oxidative stress, such as primaquine and artemisinin, act by rendering the parasite more susceptible to oxygen radicals or causing enhanced production of oxygen radicals inside parasitized erythrocytes [20].

9,10-Anthraquinones have similar aromatic planar structures as xanthones except for the carbonyl group at C-

10. Anthraquinones may intervene in heme detoxification through free-radical hydroxylation and bind with free heme, consequently causing iron deprivation in parasites, similar to xanthones [22]. Ignatushchenko and co-workers proposed that carbonyl group of a hydroxyl substituted xanthone interferes with heme formation, possibly due to association with free carboxyl groups of heme polymer and π-π interactions between the respective aromatic systems [23].

There are several reports on how the substitution pattern of 9,10-anthraquinones (AQs) profoundly affects their antiplasmodial activity. Winter *et al.* suggested that the antiplasmodial activity of hydroxyanthraquinones increased as the number of hydroxyl groups increased [22]. Sittie *et al.* established that the presence of a formyl group at C-2 and a phenolic hydroxyl group at C-3 as in 2-formyl-3-hydroxyAQ exerts antiplasmodial activity [10]. In a previous report from this laboratory, it was found that, anthraquinones with a methyl group at C-2 and a phenolic hydroxyl group at C-3 showed stronger activity than 2-formyl-3-hydroxyAQ in antiplasmodial assay *in vitro* using 3D7 strain [24]. This initial observation suggested that the presence of a free hydroxyl group at C-3 is essential for strong antiplasmodial activity. 1,2-Dimethoxy-6-methylAQ **29** from *Rennellia elliptica* Korth. also displayed strong activity, with an $IC_{50}$ value of 1.10 μM. However, the hydroxylated form of this compound, 1-hydroxy-2-methoxy-6-methylAQ **26** did not show any significant activity.

In continuing to study the potential of 9,10-anthraquinones as antiplasmodial agents, a series of 9,10-anthraquinones with different substitution patterns ($R_1$, $R_2$, $R_3$, $R_4$, and $R_5$) was synthesized using a Friedel-Crafts reaction between phthalic anhydride and benzene derivatives in the presence of mixture of aluminium chloride and sodium chloride (Fig. **1**). Methylation of the hydroxyl group was accomplished through reaction of the hydroxyanthraquinones with methyl iodide and potassium carbonate. The purities of synthetic anthraquinones were determined using HPLC and the AQs with a purity of more than 95 % were assayed for antiplasmodial activity *in vitro* using a chloroquine-susceptible *Plasmodium falcipatum* (3D7) strain. The AQs were also evaluated for their toxicity, and evaluated for their potential mode of action against β-hematin formation based on HRP2 and lipid catalyses.
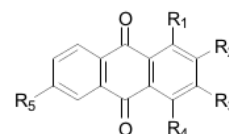
**Fig. (1).** 9,10-Anthraquinones with different substitution patterns at $R_1$, $R_2$, $R_3$, $R_4$ and $R_5$.

## 2. MATERIALS AND METHODS

### 2.1. Instrumentation

[8] $^1$H- and $^{13}$C-NMR spectra were run on a Bruker 300 Ultrashield NMR (Geneva, Switzerland) at 300 and 75.5 MHz, respectively using $CDCl_3$ or acetone-$d_6$ (Merck, Germany) as solvent. Chemical shifts are reported in ppm and δ scale with the coupling constants given in Hz. Melting points were

determined using a Hinotek X-4 (China) melting point apparatus equipped with a microscope and are uncorrected. HREIMS spectra were obtained on a Thermo Finnigan Automass Multi (Shimadzu, Japan). IR spectra were obtained using Perkin-Elmer 1600 series FTIR spectrometer (USA) using KBr pellets. The compound purity was analyzed using a Waters 600 HPLC coupled with 2414 PDA detector and Sunfire C-18 column (4.6 mm x 250 mm x 5 µM). The purification of crude synthetic products was accomplished using open column chromatography or medium pressure column chromatography using a Büchi C-40 pump regulator and cartridges packed with acid-washed silica gel.

## 2.2. Chemicals and Reagents

All chemicals for the synthesis of anthraquinones were purchased from Merck (sodium chloride) or Sigma-Aldrich (aluminum chloride anhydrous, *o*-cresol, *p*-cresol, *m*-cresol, iodomethane, potassium carbonate, 1,2-dihydroxybenzene, resorcinol, hydroxyquinone, phthalic anhydride, and 4-methyl-phthalic anhydride), unless otherwise stated. Liquid gradient grade HPLC solvents were purchased from Merck. Column chromatography was carried out using silica gel (silica gel 60, 230-400 mesh, Merck, Germany). The fractions collected were monitored using analytical TLC plates (Merck, Germany), pre-coated with silica gel 60 $F_{254}$ of 0.25 mm thickness and visualized under UV light at 245 nm and 356 nm. PTLC was carried out using pre-coated plate with PSC-Fertigplatten Kieselgel 60 $F_{254}$ (1.0 mm thickness, 20 × 20 cm) purchased from Merck. *Plasmodium falciparum* (3D7) was used for *in vitro* antiplasmodial tests. All chemicals used for the determination of antiplasmodial activity were purchased from Sigma-Aldrich, unless otherwise stated.

## 2.3. Synthesis of Anthraquinones

A series of anthraquinone derivatives was prepared as described by Singh and Geetanjali [25] with slight modification. In a dry two-necked round bottom flask was placed 45g aluminum chloride (308 mmol) and 9 g of sodium chloride (155 mmol). The mixture was heated on an oil bath until melted (external temperature, 150-170°C). Phthalic anhydride (1 g, 6.75 mmol) and hydroxyl-substituted benzene (15 mmol) were slowly introduced into the molten aluminum chloride and sodium chloride and heated with stirring for 45 min. Upon the completion of the reaction, the deep red melt was carefully poured onto 500 ml ice water. About 15 ml of concentrated hydrochloric acid was added to the mixture and it was allowed to stand overnight for product precipitation. The precipitate was collected using vacuum filtration and washed with saturated sodium bicarbonate solution to remove acidic impurities. The sample was premixed with acid-treated silica and subjected to open column chromatography or medium pressure liquid chromatography eluted with composition of *n*-hex: $CH_2Cl_2$ or *n*-hex: EtOAc to give yellow to red coloured products. The *o*-alkylation reaction was accomplished using method as described earlier [26]. In a dry round bottom flask was placed hydroxyanthraquinone (1 mmol), methyl iodide (2 mmol) and potassium carbonate (1 mmol) in 30 ml acetone. The mixture was refluxed for 8-120 h. The completion of the reaction was monitored using analytical TLC. Upon the completion of the reaction, the mixture was dried. The crude product was redissolved in dichloromethane and extracted with distilled water to remove potassium carbonate residues. The organic layer was dried over sodium sulfate anhydrous and premixed with silica prior to purification using open column chromatography or medium pressure liquid chromatography and/or preparative thin layer chromatography. Combination of *n*-hex: EtOAc and *n*-hex: $CH_2Cl_2$ were used as eluting solvents.

### 2.3.1. 1-Hydroxy-2-methyl-9,10-anthraquinone (1)

The product (300 mg, 37 %) was purified as yellowish orange solid. Mp 168-170°C; IR $\nu_{max}$ (Liq, $CH_2Cl_2$) cm$^{-1}$: 3437, 3055, 2987, 1635; MS *m/z*: 238 [M$^+$], 221; $^1$H NMR (CDCl$_3$, 300MHz): 12.90 (1H, *s*, 1-OH), 8.30 (2H, *m*, H-5, H-8), 7.80 (2H, *m*, H-6, H-7), 7.74 (1H, *d*, *J*=7.5 Hz, H-4), 7.53 (1H, *d*, *J*=7.5 Hz, H-3), 2.47 (3H, *s*, 2-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 190.0 (C=O, C-9), 182.4 (C=O, C-10), 161.1 (C-OH, C-1), 137.2, 135.0, 134.5, 134.0, 133.8, 133.3, 131.3, 127.3, 126.9, 119.2, 115.2, 16.2 (CH$_3$).

### 2.3.2. 3-Hydroxy-2-methyl-9,10-anthraquinone (2)

The compound (110 mg, 1.2 %) was purified as yellow crystals; Mp 138-140°C; IR $\nu_{max}$ (Liq, $CH_2Cl_2$) cm$^{-1}$: 3412, 3004, 2924, 1716; MS *m/z*: 238 [M$^+$], 210, 181; $^1$H NMR (Acetone-d$_6$, 300 MHz): 8.23 (2H, *m*, H-5, H-8), 8.05 (1H, *s*, H-1), 7.89 (2H, *m*, H-6, H-7), 7.67 (1H, *s*, H-4), 2.39 (3H, *s*, 2-CH$_3$); $^{13}$C NMR (Acetone-d$_6$, 75.5 MHz): 182.6 (C=O, C-10), 181.5 (C=O, C-9), 161.0 (C-OH, C-3), 134.1, 133.8, 133.7, 133.6, 132.2, 130.1, 126.6, 126.5, 111.4, 15.6 (2-CH$_3$)

### 2.3.3. 1-Hydroxy-3-methyl-9,10-anthraquinone (3)

The compound (400 mg, 49.7 %). was purified as yellowish orange solid. Mp 177-140°C; IR $\nu_{max}$ (Liq, $CH_2Cl_2$) cm$^{-1}$: 3435, 3054, 2987, 1637; MS *m/z*: 238 [M$^+$], 210, 181; $^1$H NMR (CDCl$_3$, 300 MHz): 12.61 (1H, *s*, 1-OH), 8.30 (2H, *m*, H-5, H-8), 7.81 (2H, *m*, H-6, H-7), 7.64 (1H, *s*, H-4), 7.11 (1H, *s*, H-2 ), 2.43 (3H, *s*, 3-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 188.1 (C=O, C-9), 182.7 (C=O, C-10), 162.8 (C-OH, C-1), 148.7, 134.4, 134.1, 133.6, 133.3, 133.1, 127.4, 126.8, 124.1, 120.8, 114.1, 22.3 (CH$_3$).

### 2.3.4. 1-Hydroxy-4-methyl-9,10-anthraquinone (4)

The product (900 mg; 56 %) was purified as yellowish orange solid. Mp 138-142°C; IR $\nu_{max}$ (Liq, $CH_2Cl_2$) cm$^{-1}$: 3945, 3054, 2987, 1635; MS *m/z*: 238 [M$^+$], 221, 209, 181; $^1$H NMR (CDCl$_3$, 300 MHz): 13.20 (1H, *s*, 1-OH), 8.28 (2H, *m*, H-5, H-8), 7.80 (2H, *m*, H-6, H-7), 7.50 (1H, *s*, H-3), 7.22 (1H, *s*, H-2), 2.75 (3H, *s*, 4-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 189.1 (C=O, C-9), 184.3 (C=O, C-10), 162.0 (C-OH, C-1), 141.6, 134.6, 134.6, 133.6, 134.4, 132.5, 130.0, 127.3, 126.4, 124.2, 116.5, 23.2 (CH$_3$).

### 2.3.5. 1-Methoxy-2-methyl-9,10-anthraquinone (5)

Compound **5** (40 mg, 75.6 %) was purified as yellowish brown solid. Mp 128-132°C; IR $\nu_{max}$ (Liq, $CH_2Cl_2$) cm$^{-1}$: 2987, 1635; MS *m/z*: 252 [M$^+$], 237, 223, 206; $^1$H NMR (CDCl$_3$, 300 MHz): 8.26 (2H, *m*, H-5, H-8), 7.77 (3H, *m*, H-3, H-6, H-7), 8.05 (1H, *d*, *J*=7.5 Hz, H-4), 7.61 (1H, *d*, *J*=7.5

Hz, H-3), 3.95 (3H, *s*, 1-OCH₃), 2.44 (3H, *s*, 2-CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 183.7 (C=O, C-9), 182.2 (C=O, C-10), 160.5 (C-OCH₃, C-1), 146.5, 135.4, 135.1, 134.2, 133.1, 132.5, 127.2, 126.5, 120.5, 119.2, 118.5, 56.5 (OCH₃), 22.3 (CH₃).

### 2.3.6. 1-Methoxy-3-methyl-9,10-anthraquinone (6)

Compound **6** (56 mg, 26.6 %) was obtained as yellowish brown solid. Mp 126-130°C; IR ν_max (Liq, CH₂Cl₂) cm⁻¹:2987, 1638; MS *m/z*: 252 [M⁺], 235, 223, 206; ¹H NMR (CDCl₃, 300 MHz): 8.25 (2H, *m*, H-5, H-8), 7.75 (3H, *m*, H-3, H-6, H-7), 7.42 (1H, *d*, J=8.7 Hz, H-4), 7.17 (1H, *d*, J=8.4 Hz, H-2), 4.04 (3H, *s*, 1-OCH₃), 2.50 (3H, *s*, 3-CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 183.7 (C=O, C-9), 182.2 (C=O, C-10), 160.5 (C-OCH₃, C-1), 146.5, 135.4, 135.1, 134.2, 133.1, 132.5, 127.2, 126.5, 120.5, 119.2, 118.5, 56.5 (OCH₃), 22.3 (CH₃).

### 2.3.7. 1-Methoxy-4-methyl-9,10-anthraquinone (7)

Compound **7** (180 mg, 87.2 %) was purified as golden yellow crystals. Mp 126-130°C; IR ν_max (Liq, CH₂Cl₂) cm⁻¹: 3054, 2987, 1634; MS *m/z*: 252 [M⁺], 235, 223; ¹H NMR (CDCl₃, 300 MHz): 8.12 (2H, *m*, H-5, H-8), 7.68 (2H, *m*, H-6, H-7), 7.42 (1H, *d*, J=8.7 Hz, H-4), 7.11 (1H, *d*, J=8.7 Hz, H-1), 3.99 (3H, *s*, 1-OCH₃), 2.67 (3H, *s*, 4-CH₃);¹³C NMR (CDCl₃, 75.5 MHz): 185.6 (C=O, C-9), 183.33 (C=O, C-10), 158.8 (C-OCH₃, C-1), 146.4, 139.1, 134.4, 133.6, 133.5, 133.5, 132.8, 133.1, 126.4, 126.3, 117.6, 56.8 (OCH₃), 23.1 (CH₃).

### 2.3.8. 1-Hydroxy-2,6-dimethyl-9,10-anthraquinone (8)

Compound **8** (194 mg, 25 %) was purified as a yellowish orange solid. Mp 161-165°C. IR ν_max (Liq, CH₂Cl₂) cm⁻¹; MS *m/z*: 252 [M⁺], 237, 223, 121; ¹H NMR (CDCl₃, 300MHz): 12.80 (1H, *s*, 1-OH), 7.90 (1H, *d*, J=8.1 Hz, H-8), 7.38 (1H, *d*, J=8.1 Hz, H-7), 7.79 (1H, *s*, H-5), 7.45 (1H, *d*, J=7.8 Hz, H-4), 7.30 (1H, *d*, J=7.8 Hz, H-3), 2.38 (3H, *s*, CH₃), 2.20 (3H, *s*, CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 188.3 (C=O, C-9), 181.6 (C=O, C-10), 160.6 (C-OH, C-1), 144.9, 136.7, 135.1, 134.5, 132.8, 131.1, 130.6, 127.3, 127.2, 114.9, 118.8, 21.9 (CH₃), 16.0 (CH₃).

### 2.3.9. 2-Hydroxy-3,6-dimethyl-9,10-anthraquinone (9)

The compound was purified as light yellow solid (400 mg, 4.4 %). IR ν_max (Liq, CH₂Cl₂) cm⁻¹: 3413, 3004, 2782, 1719; MS *m/z*: 252 [M⁺], 237, 223; ¹H NMR (CDCl₃, 300 MHz): 8.10 (2H, *d*, J=7.8 Hz, H-8), 7.67 (1H, *d*, J=7.8 Hz, H-7), 8.02 (1H, *s*, H-5), 7.64 (1H, *s*, H-1), 7.28 (1H, *s*, H-4),2.54 (3H, *s*, CH₃), 2.38 (3H, *s*, CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 182.3 (C=O, C-9), 181.7 (C=O, C-10), 161.0 (C-OH, C-2), 145.2, 144.6, 133.3, 133.8, 131.9, 131.4, 130.0, 126.9, 126.8, 126.1, 111.3, 20.9 (CH₃), 15.6 (CH₃).

### 2.3.10. 1-Hydroxy-3,6-dimethyl-9,10-anthraquinone (10)

Compound **10** was purified as yellowish orange solid (199.6 mg, 25.7 %). Mp 276-279°C; IR ν_max (Liq, CH₂Cl₂) cm⁻¹: 3436, 2987, 1635; MS *m/z*: 252 [M⁺], 237, 223; ¹H NMR (CDCl₃, 300 MHz): 12.60 (1H, *s*, 1-OH), 8.04 (2H, *d*, J= 7.8 Hz, H-8), 7.49 (1H, *d*, J= 7.8 Hz, H-7), 7.00 (1H, *s*,

H-2), 7.93 (1H, *s*, H-5), 7.49 (1H, *s*, H-4), 2.56 (3H, *s*, CH₃), 2.47 (3H, *s*, CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 188.1 (C=O, C-9), 187.8 (C=O, C-10), 162.6 (C-OH, C-1), 148.2, 145.6, 135.1, 134.8, 133.3, 130.8, 127.5, 126.9, 123.9, 120.6, 113.9, 22.2 (CH₃), 21.9 (CH₃).

### 2.3.11. 1-Hydroxy-4,6-dimethyl-9,10-anthraquinone (11)

The crude product was purified to give yellowish orange solid (590 mg, 37.9 %). Mp 174-176°C; IR ν_max (Liq, CH₂Cl₂) cm⁻¹: 3445, 2987, 1637; MS *m/z*: 252 [M⁺], 237, 213; ¹H NMR (CDCl₃, 300 MHz): 13.19 (1H, *s*, 1-OH), 8.15 (1H, *d*, J= 8.1 Hz, H-8), 8.04 (1H, *d*, J= 8.1 Hz, H-7), 8.05 (1H, *s*, H-5), 7.46 (1H, *d*, J=8.7 Hz, H-3), 7.19 (1H, *d*, J= 8.7 Hz, H-2), 2.73 (3H, *s*, CH₃), 2.54 (3H, *s*, CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 189.3 (C=O, C-9), 182.9 (C=O, C-10), 161.9 (C-OH, C-1), 144.6, 143.2, 141.5, 135.5, 134.4, 132.4, 130.0, 127.6, 126.6, 124.1, 116.6, 22.2 (CH₃), 21.9 (CH₃).

### 2.3.12. 1-Methoxy-2,6-dimethyl-9,10-anthraquinone (12)

Compound **12** (57.5 mg, 36.3 %) was obtained as a light yellow solid. Mp 128-132°C; IR ν_max (Liq, CH₂Cl₂) cm⁻¹: 2987, 1635; MS *m/z*: 266 [M⁺], 251, 237; ¹H NMR (CDCl₃, 300 MHz): 8.06 (1H, *d*, J= 8.1 Hz, H-8), 8.02 (1H, *s*, H-5), 7.93 (1H, *d*, J= 9 Hz, H-7), 7.21(1H, *d*, J=8.7 Hz, H-3), 7.46 (1H, *d*, J=8.7 Hz, H-4), 3.93 (3H, *s*, 1-OCH₃), 2.51 (3H, *s*, CH₃), 2.42 (3H, *s*, CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 183.3 (C=O, C-9), 182.9 (C=O, C-10), 159.0 (C-OCH₃, C-1), 145.2, 141.0, 136.4, 134.7, 133.8, 132.5, 130.5, 127.4, 126.8, 125.6, 123.4, 22.0 (CH₃), 16.7 (CH₃).

### 2.3.13. 1-Methoxy-3,6-dimethyl-9,10-anthraquinone (13)

Compound **13** (210 mg, 75.9 %) was obtained as a light yellow solid. Mp 212-215°C; IR ν_max (Liq, CH₂Cl₂) cm⁻¹: 3055, 2987, 1638; MS *m/z*: 254 [M⁺], 226, 197; ¹H NMR (CDCl₃, 300 MHz): 7.95 (2H, *d*, J= 8.1 Hz, H-8), 7.40 (1H, *d*, J = 8.1 Hz, H-7), 7.33 (1H, *s*, H-2), 7.79 (1H, *s*, H-5), 7.10 (1H, *s*, H-4), 2.59 (3H, *s*, CH₃), 2.39 (3H, *s*, CH₃); ¹³C NMR (CDCl₃, 75.5 MHz): 185.3 (C=O, C-9), 183.1 (C=O, C-10), 158.7 (C-OCH₃, C-1), 138.9, 117.5, 126.4, 143.9, 134.3, 126.5, 122.6, 132.1, 132.8, 133.4, 131.4, 134.0, 23.2 (CH₃), 21.7 (CH₃).

### 2.3.14. 1,2-Dihydroxy-9,10-anthraquinone (14)

Compound **14** was purified as red crystals (319 mg, 19.7 %). Mp 208-210°C; IR ν_max (KBr) cm⁻¹: 3413, 3004, 2924, 2782, 1716; MS *m/z*: 252 [M⁺], 240, 212; ¹H NMR (CDCl₃, 300 MHz): 12.80 (1H, *s*, 1-OH), 9.30 (1H, *s* br, 2-OH), 8.32 (1H, *m*, H-5), 8.26 (1H, *m*, H-8), 7.95 (2H, *m*, H-6, H-7), 7.79 (1H, *d*, J= 8.4 Hz, H-4), 7.32 (1H, *d*, J= 8.4 Hz, H-3).

### 2.3.15. 1,3-Dihydroxy-9,10-anthraquinone (15)

The compound was purified as bright yellow solid (705 mg, 43.5 %). Mp 262-263°C; IR ν_max (Liq, CH₂Cl₂) cm⁻¹: 3436, 3055, 2987, 1634; MS *m/z*: 240 [M⁺], 224, 212, 184; ¹H NMR (CDCl₃, 300 MHz): 12.90 (1H, *s*, 1-OH), 8.29 (1H, *m*, H-8), 8.24 (1H, *m*, H-5), 7.93 (2H, *m*, H-6, H-7), 6.69 (1H, *s*, H-2), 7.28 (1H, *s*, H-4); ¹³C NMR (CDCl₃, 75.5 MHz): 184.4 (C=O), 181.2 (C=O), 165.7 (C-OH, C-1), 164.7

(C-OH, C-3), 134.7, 133.6, 133.5, 133.2, 132.1, 127.2, 126.8, 110.4, 108.2, 107.8.

### 2.3.16. 1,4-Dihydroxy-9,10-anthraquinone (16)

The reaction mixture purified to give red crystals (550 mg, 40 %). Mp 194-198°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3436, 3054, 1630; MS $m/z$: 240 [M$^+$], 212, 183; $^1$H NMR (CDCl$_3$, 300 MHz): 12.90 (1H, $s$, 1-OH, 4-OH), 8.36 (1H, $m$, H-5, H-8), 7.85 (1H, $m$, H-6, H-7), 7.32 (1H, $s$, H-2, H-3); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 186.9 (C=O, C-9, C-10), 157.8 (C-OH, C-1, C-4), 134.5 (H-6, H-7), 133.4 (C-11, C-12), 129.4 (C-2, C-3), 127.1 (C-5, C-8), 112.8 (C-13, C-14).

### 2.3.17. 1-Hydroxy-2-methoxy-9,10-anthraquinone (17)

The compound was purified to give yellowish orange solid (37.5 mg, 16.8 %). Mp 222-224°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3337, 3055, 2987, 1634; MS $m/z$: 254 [M$^+$], 236, 255, 211; $^1$H NMR (CDCl$_3$, 300 MHz): 13.10 (1H, $s$, OH), 8.33 (2H, $m$, H-5, H-8), 7.82 (2H, $m$, H-6, H-7), 7.90 (1H, $d$, $J_o$= 8.4 Hz, H-4), 7.20 (1H, $d$, $J_o$= 8.4 Hz, H-1), 4.05 (3H, $s$, 2-OCH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 189.2 (C=O, C-9), 181.5 (C=O, C-10), 154.0 (C-OH, C-1), 152.8, 134.8, 134.1, 133.8, 133.4, 127.4, 126.9, 125.3, 121.1, 116.1, 115.8, 56.4 (OCH$_3$).

### 2.3.18. 1-Hydroxy-3-methoxy-9,10-anthraquinone (18)

Compound 18 was obtained as bright yellow solid (4 mg, 2 %). Mp 177-174°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3436, 3055, 2987, 1634; MS $m/z$: 254 [M$^+$], 255; $^1$H NMR (CDCl$_3$, 300 MHz): 12.09 (1H, $s$, 1-OH), 8.30 (2H, $m$, H-5, H-8), 7.81 (2H, $m$, H-6, H-7), 6.72 (1H, $s$, H-2), 7.39 (1H, $s$, H-4), 3.95 (3H, $s$, 3-OCH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 183.0 (C=O, C-10), 166.3 (C-OH, C-1), 165.4, 134.3, 134.1, 133.5, 127.7, 127.4, 126.8, 110.8, 107.8, 106.6, 56.1 (OCH$_3$).

### 2.3.19. 1-Hydroxy-4-methoxy-9,10-anthraquinone (19)

The compound was obtained as red solid (100 mg, 53.5 %). Mp 166-168°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3438, 3055, 2987, 1634; MS $m/z$: 254 [M$^+$], 237, 225, 211; $^1$H NMR (CDCl$_3$, 300 MHz): 13.0 (1H, $s$, 1-OH), 8.29 (2H, $m$, H-5, H-8), 7.80 (2H, $m$, H-6, H-7), 7.43 (1H, $d$, $J$=9.3 Hz, H-4), 7.34 (1H, $d$, $J$=9.3 Hz, H-3), 4.04 (3H, $s$, 4-OCH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 188.1 (C=O, C-9), 182.7 (C=O, C-10), 162.8 (C-OH, C-1), 148.7, 134.4, 134.1, 133.6, 133.3, 133.1, 127.4, 126.8, 124.1, 120.8, 114.1, 22.3 (CH$_3$).

### 2.3.20. 1,2-Dimethoxy-9,10-anthraquinone (20)

The compound was obtained as light yellow solid (118.4 mg, 52.9 %). Mp 206-210°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3055, 2987, 1637; MS $m/z$: 268 [M$^+$], 253, 239, 225; $^1$H NMR (CDCl$_3$, 300 MHz): 8.26 (2H, $m$, H-5, H-8), 7.77 (2H, $m$, H-6, H-7), 7.73 (2H, $td$, $J_o$=7.5 Hz, $J_m$=1.5 Hz, H-6, H-7), 8.18 (1H, $d$, $J_o$= 8.7 Hz, H-4), 7.29 (1H, $d$, $J_o$= 8.7 Hz, H-1), 4.02 (6H, $s$, 1,2-OCH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 182.8 (C=O, C-9), 182.4 (C=O, C-10), 159.1 (C-OCH$_3$, C-1), 149.7 (C-OCH$_3$, C-3), 135.1, 133.9, 133.6, 133.0, 127.4, 127.2, 127.0, 126.7, 125.3, 116.1, 61.3 (1-OCH$_3$), 56.3 (2-OCH$_3$).

### 2.3.21. 1,3-Dimethoxy-9,10-anthraquinone (21)

Compound 21 was recovered as bright yellow solid (31.08 mg, 14 %). Mp 150-154°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3055, 2987, 1634; MS $m/z$: 268 [M$^+$], 251, 239; $^1$H NMR (CDCl$_3$, 300MHz): 8.27 (1H, $d$, $J_o$=7.5 Hz, $J_m$= 1.2 Hz, $J_p$=0.3 Hz, H-8), 8.20 (1H, $ddd$, $J_o$=7.5 Hz, $J_m$=1.5 Hz, $J_p$=0.5 Hz, H-5), 7.73 (2H, $td$, $J_o$=7.5 Hz, $J_m$=1.5 Hz, H-6, H-7), 6.78 (1H, $s$, H-2), 7.44 (1H, $s$, H-4), 4.02 (3H, $s$, 1-OCH$_3$), 3.98 (3H, $s$, 3-OCH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 183.5 (C=O, C-9), 181.3 (C=O, C-10), 164.7 (C-OCH$_3$), 162.6 (C-OCH$_3$, C-3), 137.5, 135.1, 134.3, 132.9, 132.3, 127.2, 126.5, 116.0, 104.7, 103.3, 56.0 (3-OCH$_3$), 56.5 (1-OCH$_3$).

### 2.3.22. 1,4-Dimethoxy-9,10-anthraquinone (22)

The compound was purified as yellow crystal (62 mg, 31.5 %). Mp 171-174°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3054, 2987, 1634; MS $m/z$: 254 [M$^+$], 237, 225, 211; $^1$H NMR (CDCl$_3$, 300MHz): 8.16 (1H, $m$, H-5, H-8), 7.71 (2H, $m$, H-6, H-7), 7.35 (1H, $s$, H-2, H-3), 4.04 (6H, $s$, 1,4-OCH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 183.5 (C=O, C-9, C-10), 154.1(C-OCH$_3$, C-1, C-4), 134.2, 133.3, 126.4, 123.0, 120.2, 57.0 (1,4-OCH$_3$).

### 2.3.23. 1,2-Dihydroxy-6-methyl-9,10-anthraquinone (23)

Compound 23 was purified as a red solid (270 mg, 20 %). Mp 208-210°C; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3413, 3004, 2924, 2782, 1737; MS $m/z$: 254 [M$^+$], 239, 226; $^1$H NMR (CDCl$_3$, 300MHz): 12.90 (1H, $s$, 1-OH), 9.20 (1H, $sbr$, 2-OH), 8.15 (1H, $d$, $J$=8.1 Hz, H-8), 8.04 (1H, $d$, $J$=8.1 Hz, H-7), 8.05 (1H, $s$, H-5), 7.46 (1H, $d$, $J$=8.7 Hz, H-3), 7.19 (1H, $d$, $J$=8.7 Hz, H-2), 2.57 (3H, $s$, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 189.26 (C=O, C-9), 180.8 (C=O, C-10), 146.5, 134.8, 134.5, 130.9, 130.9, 127.2, 126.8, 121.0, 21.0 (6-CH$_3$)

### 2.3.24. 1,3-Dihydroxy-6-methyl-9,10-anthraquinone (24)

The compound was purified as a bright yellow solid (572 mg, 36.5 %). Mp 270-274°C; IR $\nu_{max}$ (KBr) cm$^{-1}$: 3436, 3056, 2987, 1633; MS $m/z$: 254 [M$^+$], 226; $^1$H NMR (CDCl$_3$, 300MHz): 12.90 (1H, $s$, 1-OH), 8.16 (1H, $d$, $J$=7.8 Hz, H-8), 7.73 (1H, $d$, $J$=7.2 Hz, H-7), 6.67 (1H, $s$, H-2), 8.01 (1H, $s$, H-5), 7.25 (1H, $s$, H-4), 2.55 (3H, $s$, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 186.6 (C=O, C-9), 182.0 (C=O, C-10), 165.0 (C-OH, C-1), 165.5 (C-OH, C-3), 107.8, 108.1, 110.0, 135.6, 131.1, 133.4, 126.7, 135.0, 145.6, 127.2, 20.9 (CH$_3$).

### 2.3.25. 1,4-Dihydroxy-6-methyl-9,10-anthraquinone (25)

The compound was recovered as red crystal (736.2 mg, 46.9 %). Mp 172-176°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3444, 3055, 2987, 1634; MS $m/z$: 254 [M$^+$], 239, 197; $^1$H NMR (CDCl$_3$, 300MHz): 12.90 (1H, $s$, 1,4-OH), 8.18 (1H, $d$, $J$= 8.1 Hz, H-8), 7.60 (1H, $d$, $J$= 8.1 Hz, H-7), 8.08 (1H, $s$, H-5), 7.27(2H, $s$, H-2, H-3), 2.55 (3H, $s$, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 187.1 (C=O, C-9), 186.8 (C=O, C-10), 157.7 (C-OH, C-1), 157.6 (C-OH, C-4), 145.9, 133.3, 131.1, 112.8, 112.8, 129.2, 129.1, 127.2, 135.3, 127.2, 22.0 (CH$_3$).

### 2.3.26. 1-Hydroxy-2-methoxy-6-methyl-9,10-anthraquinone (26)

Compound **26** was obtained as a yellow needle crystals from *Rennellia elliptica* as described previously [24]. Mps 220-221°C; UV$\lambda_{max}$ EtOH nm: 421, 278, 262, 231; UV$\lambda_{max}$EtOH/ -OH nm: 505, 314, 258; IR $\nu_{max}$(KBr) cm$^{-1}$: 3467, 1653, 1637; MS *m/z*: 268 [M$^+$], 239, 197, 169, 139, 115; $^1$H NMR (CDCl$_3$, 300 MHz): 13.20 (1H, *s*, 1-OH), 8.23 (1H, *d*, *J*=8.1 Hz, H-8), 8.12 (1H, *s*, H-5), 7.89 (1H, *d*, *J*=8.4 Hz, H-4), 7.61 (1H, *d*, *J*=8.1 Hz, H-7), 7.19 (1H, *d*, *J*=8.4 Hz, H-3), 4.04 (3H, *s*, 2-OCH$_3$), 2.56 (3H, *s*, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 189.1(C=O, C-9), 181.8 (C=O, C-10), 154.0 (C-OH, C-1), 152.7 (C-OCH$_3$, C-2), 146.2 (C-6), 134.6 (C-7), 134.0 (C-11), 131.1 (C-12), 127.8 (C-5), 127.1 (C-8), 125.5 (C-14), 121.0 (C-4), 116.1 (C-13), 115.6 (C-3), 56.4 (2-OCH$_3$), 22.0 (6-CH$_3$)

### 2.3.27. 1-Hydroxy-3-methoxy-6-methyl-9,10-anthraquinone (27)

Compound **27** was purified as a light yellow solid (53 mg, 32 %). Mp 162-166°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3436, 3055, 2987, 1633; MS *m/z*: 268 [M$^+$], 253, 237; $^1$H NMR (CDCl$_3$, 300MHz): 12.90 (1H, *s*, 1-OH), 8.13 (2H, *d*, *J*= 8.7 Hz, H-8), 7.54 (1H, *d*, *J*= 8.7 Hz, H-7), 8.03 (1H, *s*, H-5), 6.67 (1H, *s*, H-2), 7.32 (1H, *s*, H-3), 3.93 (3H, *s*, 3-OCH$_3$), 2.52 (3H, *s*, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 186.7 (C=O, C-9), 182.0 (C=O, C-10), 166.2 (C-OH, C-1), 165.3 (C-OCH$_3$, C-3), 145.3, 135.0, 133.3, 131.1, 127.7, 127.0, 110.7, 107.5, 106.6, 56.0 (3-OCH$_3$), 22.0 (CH$_3$).

### 2.3.28. 1-Hydroxy-4-methoxy-6-methyl-9,10-anthraquinone (28)

Compound **28** was purified as a red crystal (59.7 mg, 26.9 %). Mp 146-148°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3430, 3055, 2987, 1605; MS *m/z*: 268 [M$^+$], 237, 197; $^1$H NMR (CDCl$_3$, 300 MHz): 13.0 (1H, *s*, 1-OH), 8.04 (1H, *d*, *J*= 8.1, H-8), 7.48 (1H, *d*, *J*= 7.5 Hz, H-7), 7.94 (1H, *s*, H-5), 7.23 (1H, *d*, *J*=9.3 Hz, H-2), 7.33 (1H, *d*, *J*=9.3 Hz, H-2), 3.92 (3H, *s*, 4-OCH$_3$), 2.46 (3H, *s*, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 188.5 (C=O, C-9), 181.8 (C=O, C-10), 158.9 (C-OH, C-1), 157.1 (C-OCH$_3$, C-4), 144.5, 135.6, 132.0, 129.9, 115.7.

### 2.3.29. 1,2-Dimethoxy-6-methyl-9,10-anthraquinone (29)

The compound was purified as light yellow solid (480 mg, 86.5 %). Mp 193-196°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 2987, 1631; MS *m/z*: 282 [M$^+$], 253, 237; $^1$H NMR (CDCl$_3$, 300MHz): 8.17 (2H, *dd*, 8.7 Hz, 7.8 Hz, H-4, H-8), 8.06 (1H, *s*, H-5), 7.58 (1H, *d*, *J*=7.8 Hz, H-7), 7.28 (1H, *d*, *J*=8.7 Hz, H-3), 4.02 (6H, *s*, 1, 2-OCH$_3$), 2.53 (3H, *s*, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 182.7 (C=O, C-9), 182.7 (C=O, C-10), 159.1 (C-1), 149.6 (C-2), 144.6 (C-6), 134.8 (C-7), 132.9 (C-11), 132.9 (C-12), 127.5 (C-14), 127.4 (C-13), 127.1 (C-8), 126.9 (C-5), 125.2 (C-4), 115.9 (C-3), 61.3 (1-OCH$_3$), 56.3 (2-OCH$_3$), 21.8 (6-CH$_3$).

### 2.3.30. 1,3-Dimethoxy-6-methyl-9,10-anthraquinone (30)

The compound was recovered as a bright yellow crystals (78 mg, 47 %). Mp 176-180°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3055, 2987, 1637; MS *m/z*: 281 [M$^+$], 267, 253, 236; $^1$H NMR (CDCl$_3$, 300 MHz): 8.12 (1H, *d*, *J*=8.1 Hz, H-8), 7.51 (1H, *d*, *J*= 7.8 Hz, H-7), 8.06 (1H, *s*, H-5), 6.79 (1H, *d*, $J_m$=1.8 Hz, H-2), 7.47 (1H, *d*, $J_m$=1.8 Hz, H-4), 4.01 (3H, *s*, 1-OCH$_3$), 3.99 (3H, *s*, 3-OCH$_3$), 2.53 (3H, *s*, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 183.3 (C=O, C-9), 182.0(C=O, C-10), 164.7 (C-OCH$_3$, C-1), 162.5 (C-OCH$_3$, C-3), 145.5, 137.7, 135.1, 133.7, 130.2, 127.5, 126.7, 116.2, 104.6, 103.3, 56.5 (3-OCH$_3$), 56.0 (3-OCH$_3$), 22.1(CH$_3$).

### 2.3.31. 1,4-Dimethoxy-6-methyl-9,10-anthraquinone (31)

Compound **31** was purified as a bright yellow crystal (117.8 mg, 53.1 %). Mp 188-190°C; IR $\nu_{max}$ (Liq, CH$_2$Cl$_2$) cm$^{-1}$: 3055, 2988, 1634; MS *m/z*: 281 [M$^+$], 267, 253, 235; $^1$H NMR (CDCl$_3$, 300 MHz): 7.98 (2H, *d*, *J*=8.1 Hz, H-8), 7.43 (1H, *d*, *J*=7.8 Hz, H-7), 7.87 (1H, *s*, H-5), 7.27 (1H, *s*, H-2), 7.27 (1H, *s*, H-3), 3.92 (6H, *s*, 1,4-OCH$_3$), 2.42 (3H, *s*, 6-CH$_3$); $^{13}$C NMR (CDCl$_3$, 75.5 MHz): 183.6 (C=O, C-9), 183.2 (C=O, C-10), 154.0 (C-OCH$_3$, C-1, C-4), 144.2, 134.1, 132.0, 126.5, 123.0, 120.0, 57.0 (1,4-OCH$_3$), 21.8 (CH$_3$).

## 2.4. Determination of Compound Purity

The compound purity was determined using a Waters HPLC 600 coupled with a 2996 PDA detector system (Waters, USA) equipped with an autosampler (100μl). Analysis was carried out at room temperature using a Sunfire C-18 4.6 mm x 250 mm x 5 μM column (Waters, USA). A linear gradient program was performed with MeCN and H$_2$O where the ratio of MeCN was raised from 40% to 100% in 30 min at a flow rate 1 ml min$^{-1}$. Formic acid was added to both solvents at 0.1%. The samples were dissolved in 100 μL CH$_2$Cl$_2$ and 900 μL MeCN: H$_2$O (9:1, v/v) at 1 mg/ml. The peaks were monitored in the spectral window of 254-400 nm. The compound purity was expressed as percent area under the peak [27].

## 2.5. Determination of Antiplasmodial Activity

The antiplasmodial activity was determined by previously described methods [28]. The samples were dissolved in DMSO and kept at –20°C until used. The malarial parasite *P. falciparum* (3D7) clone was propagated in a 24-well culture plate in the presence of 10, 1, 0.1, 0.01, and 0.001 μg/mL concentrations of each compound. Chloroquine diphosphate was used as the positive control. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa (Merck, Germany). The antiplasmodial activity of each compound was expressed as an IC$_{50}$ value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control.

## 2.6. β-Hematin Inhibition Assay

Hemin chloride (16.3 mg) was dissolved in DMSO (1 mL). The solution was passed through a 2.0-μm-pore membrane filter to remove insoluble particles. The solution could be kept at 4°C up to one month as a stock solution [29] and diluted to 50 μM heme with 500 mM acetate buffer, pH 4.8, prior to analysis.

Chloroquine (500 µM) was used as the positive control drug. Compounds for the test were dissolved in 100% DMSO to prepare a stock concentration of 10 mg/mL and 1 mg/mL. Heme solution (110 µL, 50 µM), freshly buffered by 500 µM acetate buffer (pH 4.8), was pipetted and added into the microwell plate. Finally, lecithin (2 µg/mL) was added to each well. After incubation at 37°C for 16 h, the plate was read at 405 nm. The fraction (*f*) of heme converted to β-hematin was calculated as a previous study [30]:

$$f = (A_{control} - A_{sample})/(A_{control} - A_{min})$$

Where $A_{control}$ is the absorbance of the heme without parasite lysate or lipid extract or an antimalarial at 405 nm, while $A_{sample}$ represents the absorbance of the heme in the presence of both parasite lysate or lipid extract and plant extracts. $A_{min}$ is the absorbance of the heme with parasite lysate or lipid extract in the absence of an antimalarial at 405 nm.

Percentage of inhibition of β-hematin by plant extracts was calculated by the following equations:

$$\% \text{ Inhibition} = (1-f) \times 100 = 100 \times \frac{(A_{sample} - A_{min})}{(A_{control} - A_{min})}$$

### 2.7. HRP2 Assay

The antimalarial assay was carried out using HRP2 (HRP2 Kit Cellabs Pty. Ltd., Brookvale, New South Wales, Australia). Diluted extract solution (100 µL) and final parasite culture (100 µL) were added into the microplate. The plates were then incubated for 72 h at 37°C. They were subsequently frozen-thawed twice to obtain complete hemolysis and stored at -30°C until further processing. Each of the hemolyzed culture samples (100 µL) was transferred to the ELISA plates, which were pre-coated with monoclonal antibodies against *P. falciparum* HRP2. The plates were incubated at room temperature for 1 h in a humidified chamber. The plates were washed five times with the washing solution (200 µL of each well) and the diluted antibody conjugate (100 µL) was added to each well. After incubation for an additional 1 h in a humidified chamber, the plates were washed with washing solution (200 µL) and diluted (1:20) chromogen tetramethylbenzidine (100 µL) was added to each well. The plates were then incubated for another 15 min in the dark and the stop solution (50 µL) was added. The optical density values were measured using an ELISA microplate reader at an absorbance maximum of 450 nm. The percent Inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = (A_{control} - A_{sample})/(A_{well}) \times 100$$

### 2.8. Toxicity Study

The cytotoxicity of the samples was assessed using MTT assay [31]. Hepatocyte, Huh7it cells were treated with serial dilution of the samples in 96-well plates. The condition of the cells was observed after 46 h incubation and the toxicity was observed under microscope. The medium was removed from the 96-well plates and then MTT solution (150 µL, 10 %) was added to each well and the plates were incubated for 4 h at 37°C. MTT solution was removed from each well and

DMSO (100 µL) was added to each well prior to shaking for 30 seconds. The absorbance was measured using an ELISA microplate reader at 560 and 750 nm. The $CC_{50}$ values and selectivity indices were calculated.

### 2.9. Docking Method

The structures of the compounds were prepared using Chem3D by CambridgeSoft. The geometry and energy of the structures were optimized using the Steepest-Descent and Polak-Ribiere algorithm in HyperChem. Genetic Algorithm (GA) function in AutoDock 4.2 was employed to identify the binding modes of the active anthraquinones (**23** and **24**) responsible for the activity. Heme structure was obtained from Chemical Entities of Biological Interest database (https://www.ebi.ac.uk/chebi). The docking results were visualized using Biovia Discovery Studio visualizer version 16.1.0.15350.

## 3. RESULTS AND DISCUSSION

### 3.1. Chemistry

The selection of substituents was based on substituents that are commonly found in naturally-occurring anthraquinones which are biogenetically substituted with hydroxyl at C-1. In the previous study from this laboratory, anthraquinones with methyl substitution at C-6 exert antiplasmodial activity [24]. Thus, the present study aims to assess the contribution of methyl substituent at C-6 to antiplasmodial activity of hydroxyanthraquinones. Several compounds (**2, 5, 14, 15, 16, 14, 15, 20, 26** and **29**) were also reported from plants [10, 24, 32].

The reaction time was limited to 45 min. When the reaction time was raised up to 90 min there was no significant increase in percentage yield. Instead of adding ice-cooled water and concentrated hydrochloric acid to the cooled reaction mixture as reported by several authors [33, 34], the hot reaction mixture was slowly introduced to ice water (500 mL) followed by adding concentrated hydrochloric acid (15 mL). Upon touching ice water, the heavy fume was produced. When concentrated hydrochloric acid was added, the red to brown precipitate formed immediately. The advantage of adding hot reaction mixture to ice water is to avoid the tedious work of removing stubborn residue on the surface of the round bottom flask. In addition, the product can be conveniently recovered by vacuum filtration.

The highest percentage yield of the anthraquinone (30-50 %) was observed when phthalic anhydride was reacted with 1,4-disubstituted benzene. The percentage yield significantly dropped when 1,3-disubstituted benzenes were used with 30-40 % yield, followed by 1,2-disubstituted benzenes with less than 20 % yield. Cresols with different arrangements gave moderate yield while dihydroxybenzene gave much lower yield, except for hydroquinone. The varying reaction yield of Friedel Crafts reaction between phthalic anhydride and cresols and hydroquinones can be explained by the contradiction of resonance and inductive effects of substituents with *ortho* and *para* directors. When the contradiction effect is the lowest, higher percentage yield was obtained. In contrast, if the contradiction effects vary, hypothetically several prod-

ucts will be produced, thus giving lower percentage yield of main products. This is due to the availability of different active sites which cause the production of various intermediates.

The free hydroxyl is more susceptible to methylation due to stearic factor. Methylation of free hydroxyl took shorter time of about 5-8 h while methylation of both hydroxyls took longer time (12-120 h) due to both electronic and steric effects. The hydrogen-bonded hydroxyl at C-1 is more difficult to be methylated due to internal hydrogen bonding which creates new ring with carbonyl group at C-9.

## 3.2. Antiplasmodial Activity of 9,10-Anthraquinone Analogues

Compounds **2**, **27**, **8**, **13**, **22**, **23**, **24**, and **25** were strong inhibitors against *Plasmodium falciparum* growth *in vitro* (3D7 strain) with the IC$_{50}$ values of less than 1 µM. The active AQs have similar common structural characteristics. They have either *ortho-* or *meta-*arranged substituents and one free hydroxyl and/or carbonyl groups. However, it is difficult to establish a structure-activity relationship as certain compounds are active despite the absence of the structural features exhibited by other active AQs.

### 3.2.1. Anthraquinones with Methyl and Hydroxyl Substituents on Ring C

For AQs with methyl and hydroxyl substituents on ring C only (**1-4**), AQs **1** and **2** with *ortho-*arranged substituents showed the strongest activity, with the IC$_{50}$ values of 7.1 µM and 0.34 µM, respectively.

#### 3.2.1.1. Effect of Additional Methyl Group at C-6 on Ring A

An additional methyl substituent attached at C-6 on ring A increases the potency of the hydroxyl and methyl substituted AQs (**8-11**) as antiplasmodial agents. AQ with *ortho-*arranged substituents at C-1, C-2, and C-6 (**8**) showed the strongest activity, with an IC$_{50}$ value of 0.09 µM.

#### 3.2.1.2. Effect on Methylation of Hydroxyl Group

Methylation of the hydroxyl group at C-1 increases the activity of *ortho-*arranged AQ (**5**) with an IC$_{50}$ value of 0.08 µM (Table **1**). The activity of the *ortho-*substituted AQ with a methyl group at C-6 of on ring A (**12**) decreased when the hydroxyl substituent at C-1 was methylated. On the other hand, the *meta-*substituted AQ (**13**) showed stronger activity when the hydrogen-bonded hydroxyl at C-1 was methylated with an IC$_{50}$ value of an 0.09 µM.

### 3.2.2. Anthraquinones with two Hydroxyl Groups

For AQs with two hydroxyl substituents on ring C only, AQs with *meta-*arranged substituents showed the strongest antiplasmodial activity with an IC$_{50}$ value of 1.08 µM. However, the *meta-*arranged dihydroxyAQ were reported to be highly mutagenic [26].

#### 3.2.2.1. Effect of Additional Methyl Group at C-6 on Ring A

For both *ortho-* and *meta-*arranged dihydroxyAQ (**23** and **24**), the activity was enhanced by methyl substitution at C-6

on ring A, with IC$_{50}$ values of 0.67 and 0.02 µM, respectively.

#### 3.2.2.2. Effect on Methylation of Free Hydroxyl Group

When the free hydroxyl group (C-2 or C-3) was methylated, the antiplasmodial activity of AQs with substitution on ring C only (**17-19**) generally decreased, except for the *para-*substitutedAQ (**19**). Similarly, for AQs substituted on both rings A and C (**26-28**), methylation of free hydroxyl group decreases the antiplasmodial activity.

#### 3.2.2.3. Methyation of both Hydroxyl Groups

For AQs with substitution on ring C only (**20-22**), methylation of both free and chelated hydroxyl substituents caused the activity to decrease, except for *para-*substituted AQ (**22**). For AQs with substitution on both rings A and C (**29-31**), methylation of both hydroxyl groups decreased the activity except for the *ortho-*substituted AQ (**29**). It was observed that the presence of one free hydroxyl, especially at C-3 and one free carbonyl at C-10 contribute to the antiplasmodial activity of the anthraquinones. Ignatuschenko *et al.* also confirmed that the presence of a free carbonyl and hydroxylation at C-4 and C-5 were critical for xanthones to exert antiplasmodial activity. The docking study later affirms the contribution of hydroxyl substituents to form hydrogen bonding with free heme. This observation affirms the availability of a free hydroxyl group is critical for AQs to exert their antiplasmodial activity.

### 3.2.3. Structural Features of Anthraquinones to Exert their Antiplasmodial Activity

The most potent anthraquinone identified in this study was 1,3-dihydroxy-6-methylAQ **24**. Rubiadin (1,3-dihydroxy-2-methylAQ) from the root extract of *R. elliptica* did not show activity [24]. Both compounds possess similar substituents, but differ by the location of the methyl group. The presence of methyl group at C-6 exert its antiplasmodial activity. 1,3-Dihydroxy-6-methylAQ **24** was more potent than rufigallol [22], and gave IC$_{50}$ value three-fold higher than chloroquine. The IC$_{50}$ values for the antimalarial activity of the synthetic anthraquinones are tabulated in Table **1**.

## 3.3. β-Hematin (Lipids and HRP2) Assays and Toxicity Assays

The toxicity study was conducted using Huh7it cells in an MTT assay to determine the safety of the compounds as potential lead candidates. Compounds **1**, **2**, **5**, **8**, **15**, **19**, **22**, **23**, and **24** were selected for toxicity study since these AQs exhibited the strongest antiplasmodial activity. All compound showed the 50% cytotoxic concentration (CC$_{50}$) values of more than 3000µM, except for compounds **15**, **22**, and **24**. Compounds **15** and **24** have two hydroxyl substituents with a *meta-*arrangement and showed mild toxicity. The compounds showed high selectivity indices except for compounds **1** and **15**. Compound **15** showed moderate antiplasmodial activity, and compounds **24**, with additional methyl group at C-6 showed stronger activity with higher selectivity as compared to compound **15** (Table **2**).

**Table 1.    Antiplasmodial activity of 9,10-anthraquinone derivatives.**

| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Antiplasmodial Activity (µM) |
|---|---|---|---|---|---|---|
| **1** | OH | $CH_3$ | H | H | H | 7.1 |
| **2** | H | $CH_3$ | OH | H | H | 0.34 |
| **3** | OH | H | $CH_3$ | H | H | na |
| **4** | OH | H | H | $CH_3$ | H | 24.7 |
| **5** | $OCH_3$ | $CH_3$ | H | H | H | 0.08 |
| **6** | $OCH_3$ | H | $CH_3$ | H | H | na |
| **7** | $OCH_3$ | H | H | $CH_3$ | H | 1043 |
| **8** | OH | $CH_3$ | H | H | $CH_3$ | 0.09 |
| **9** | H | OH | $CH_3$ | H | $CH_3$ | 48.5 |
| **10** | OH | H | $CH_3$ | H | $CH_3$ | na |
| **11** | OH | H | H | $CH_3$ | $CH_3$ | 8.37 |
| **12** | $OCH_3$ | $CH_3$ | H | H | $CH_3$ | 62.3 |
| **13** | $OCH_3$ | H | $CH_3$ | H | $CH_3$ | 0.09 |
| **14** | OH | OH | H | H | H | 24.2 |
| **15** | OH | H | OH | H | H | 1.08 |
| **16** | OH | H | H | OH | H | 17.3 |
| **17** | OH | $OCH_3$ | H | H | H | na |
| **18** | OH | H | $OCH_3$ | H | H | na |
| **19** | OH | H | H | $OCH_3$ | H | 1.08 |
| **20** | $OCH_3$ | $OCH_3$ | H | H | H | na |
| **21** | $OCH_3$ | H | $OCH_3$ | H | H | 3.92 |
| **22** | $OCH_3$ | H | H | $OCH_3$ | H | 0.11 |
| **23** | OH | OH | H | H | $CH_3$ | 0.67 |
| **24** | OH | H | OH | H | $CH_3$ | 0.02 |
| **25** | OH | H | H | OH | $CH_3$ | 0.21 |
| **26** | OH | $OCH_3$ | H | H | $CH_3$ | na |
| **27** | OH | H | $OCH_3$ | H | $CH_3$ | 0.67 |
| **28** | OH | H | H | $OCH_3$ | $CH_3$ | na |
| **29** | $OCH_3$ | $OCH_3$ | H | H | $CH_3$ | 1.10 |
| **30** | $OCH_3$ | H | $OCH_3$ | H | $CH_3$ | na |
| **31** | $OCH_3$ | H | H | $OCH_3$ | $CH_3$ | na |
| **Chloroquine** | - | - | - | - | - | 6.3 nM |

The promising compounds were also assayed for ß-hematin inhibition assays. When screened using HRP2 assay at 100 µg/mL, compounds **15**, **23**, and **24** showed good activity with percent inhibition of 63.98, 56.68 and 61.34 %, respectively. Compounds **15** and **24** were further evaluated for HRP2 activity and gave $IC_{50}$ values of 1.96 µM and 0.67 µM, respectively. Compounds **2**, **23** and **24** showed moderate activity when tested against β-hematin formation assay using lipid catalysis. Other compounds did not show significant inhibition against β-hematin formation.

**Table 2.    Toxicity of selected anthraquinones and Inhibition of ß-hematin formation based on HRP2 and lipid catalysis.**

| AQ | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Antiplasmodial Activity (IC$_{50}$) µM | Toxicity (CC$_{50}$) µM | Selectivity Index | HRP2 % Inhibition at 100 µg/ml | IC$_{50}$ β-hematin (lipid) µM |
|----|-------|-------|-------|-------|-------|------|------|------|------|------|
| 1 | OH | CH$_3$ | H | H | H | 7.1 | 4201 | 592 | nt | na |
| 2 | H | CH$_3$ | OH | H | H | 0.34 | 4201 | 12,356 | nt | 129.9 ± 0.1 |
| 5 | OCH$_3$ | CH$_3$ | H | H | H | 0.08 | 3937 | 49,213 | nt | nt |
| 8 | OH | CH$_3$ | H | H | CH$_3$ | 0.09 | 3952 | 43,911 | 11.01 | na |
| 15 | OH | H | OH | H | H | 1.08 | 840 | 444 | 63.98 | na |
| 19 | OH | H | H | OCH$_3$ | H | 1.08 | 3937 | 3645 | nt | na |
| 22 | OCH$_3$ | H | H | OCH$_3$ | H | 0.11 | 1880 | 17,090 | nt | na |
| 23 | OH | OH | H | H | CH$_3$ | 0.67 | 3937 | 5876 | 56.68 | 83.1 ± 0.1 |
| 24 | OH | H | OH | H | CH$_3$ | 0.02 | 660 | 33,000 | 61.34 | 145.7 ± 0.1 |

Note: na – no activity; nt – not tested.



**23**



**24**
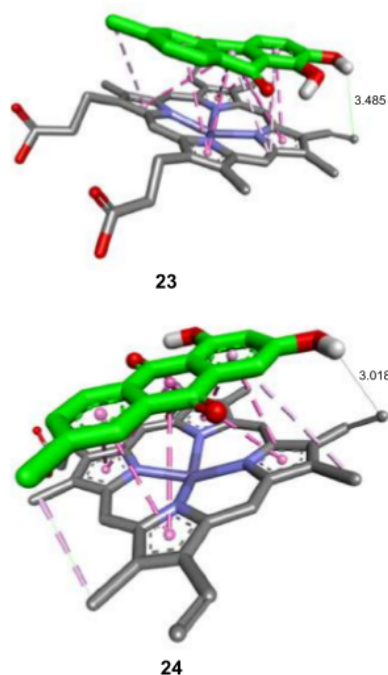
**Fig. (2).** Compounds **23** and **24** binding in parallel with heme.

### 3.4. Docking Studies

Docking was performed using AutoDock 4.2 to obtain more information on how anthraquinones **23** and **24** interact with heme. The most active AQs (**23** and **24**) are believed to form a complex with free heme, preventing the formation of hemozoin. The results gained from docking studies (Fig. **2**) showed that they are able to bind to heme and form a very stable complex having binding energies of -5.80 and -6.08 kcal/mol, respectively. The best conformation obtained for compounds **23** and **24** showed that the anthraquinone moieties were parallel to the ferriprotoporphyrin ring with

their hydroxyl groups at *ortho*- (**23**) and *meta*- (**24**) positions forming a hydrogen bond at a distance of 3.49 Å and 3.02 Å, respectively. Hydrophobic interactions were observed to be the main contributor for the binding between anthraquinone and heme. The results from the *in vitro* studies were further supported by the observation that the methyl substituent of **23** and **24** are contributing significantly to the activity. Docking studies suggest that the methyl group enhances the stability of heme-ligand complex through formation of alkyl-alkyl linkage with heme.

### CONCLUSION

The active AQs have similar common structural characteristics. They have either *ortho*- or *meta*-arranged substituents and one free hydroxyl and/or carbonyl groups. However, it is difficult to establish a structure-activity relationship as certain compounds are active despite the absence of the structural features exhibited by other active AQs. Molecular docking studies for compounds **23** and **24** showed that their hydroxyl groups formed hydrogen bonds with heme while the anthraquinone moiety of these compounds was in parallel to the ferriprotoporphyrin ring. The activity of 9,10-anthraquinones was significant with the presence of the methyl group at C-6 on ring A as supported by docking studies which suggest that the methyl group enhances the stability of heme-ligand complex through the formation of alkyl-alkyl linkage with heme. It is postulated that the activity of compound **15** and **24** could be in part due to their actions against β -hematin formation in the food vacuole by inhibiting the functions of HRP2 protein during dimerization of free heme, leading to the death of the parasite. The potential compounds are non-toxic except for compound **24** which showed mild toxicity. The finding of the present study provides an insight into the mode of action of anthraquinones as an antiplasmodial agent.

### CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## REFERENCES

[1]    Chan-Bacab, M.J.; Pena-Rodriguez, L.M. Plant natural products with antileishmanicidal activity. *Nat. Prod. Rep.;* **2001**, *18*, 674-688.

[2]    WHO, World malaria report 2017. WHO: Switzerland, **2017**.

[3]    Pink, R.; Hudson, A.; Mouries, M.-A.; Bendig, M. Opportunities and Challenges in antiparasitic drug discovery. *Nat. Rev. Drug Dis.;* **2005**, *4*, 727-740.

[4]    Egan, T.J.; Haemozoin (malaria pigment): A unique crystalline drug target. *TARGETS*, **2003**, *2*, 115-124.

[5]    Sullivan, D.J.; Theories on malarial pigment formation and quinoline action. *Int. J. Parasitol.*, **2002**, *32*, 1645-1653.

[6]    Cowman, A.F.; Foote, S.J.; Chemotherapy and drug resistance in malaria. *Int. J. Para.*,, **1990**, *20*, 503-513.

[7]    Kumar, S.; Guha, M.; Choubey, V.; Maity, P.; Bandyopadhyay, U.; Antimalarial drugs inhibiting hemozoin (β-hematin) formation: A mechanistic update. *Life Sci.*, **2007**, *80*, 813-828.

[8]    Rathore, D.; Jani, D.; Nagarkatti, R.; Kumar, S. Heme detoxification and antimalarial drugs - known mechanisms and future prospects. *Drug Dis. Today: Therap. Strateg.*, **2006**, *3*, 153-158.

[9]    Egan, T.J.; Chen, J.Y.-J.; de Villiers, K.A.; Mabotha, T.E.; Naidoo, K.J.; Ncokazi, K.K.; Langford, S.J.; McNaughton, D.; Pandiancherri, S.; Wood, B.R. Haemozoin (beta-haematin) biomineralization occurs by self-assembly near the lipid/water interface. *FEBS Lett.*, **2006**, *580*, 5105-5110.

[10]    Sittie, A.A.; Lemmich, E.; Olsen, C.E.; Hviid, L.; Kharazmi, A.; Nkrumah, F.K.; Christensen, S.B. Structure-activity studies: *In vitro* antileishmanial and antimalarial activities of anthraquinones from *Morinda lucida. Planta. Medica.*, **1999**, *65*, 259-261.

[11]    Eyong, K.O.; Folefoc, G.N.; Kuete, V.; Beng, V.P.; Krohn, K.; Hussain, H.; Nkengfack, A.E.; Saeftel, M.; Sarite, S.R.; Hoerauf, A. New bouldiaquinone A: A naphthoquinone-anthraquinone ether coupled pigment, as a potential antimicrobial and antimalarial agent from *New bouldia laevis. Phytochemistry*, **2006**, *67*, 605-609.

[12]    Abegaz, B.M.; Bezabih, M.; Msuta, T.; Brun, R.; Menche, D.; Muhlbacher, J.; Bringmann, G.; Gaboroquinones A and B and 4'-O-Demethylknipholone-4'-O-β-D-glucopyranoside, Phenylanthraquinones from the Roots of *Bulbine frutescens. J. Nat. Prod.*, **2002**, *65*, 1117-1121.

[13]    Ajaiyeoba, E.O.; Ashidi, J.S.; Okpako, L.C.; Houghton, P.J.; Wright, C.W. Antiplasmodial compounds from Cassia siamea stem bark extract. *Phytother. Res.*, **2008**, *22*, 254-255.

[14]    Abdissa, N.; Induli, M.; Akala, H.M.; Heydenreich, M.; Midiwo, J.O.; Ndakala, A.; Yenesew, A.; Knipholone cyclooxanthrone and an anthraquinone dimer with antiplasmodial activities from the roots of Kniphofia foliosa. *Phytochem. Lett.*, **2013**, *6*, 241-245.

[15]    Onegi, B.; Kraft, C.; Köhler, I.; Freund, M.; Jenett-Siems, K.; Siems, K.; Beyer, G.; Melzig, M.F.; Bienzle, U.; Eich, E. Antiplasmodial activity of naphthoquinones and one anthraquinone from Stereospermum kunthianum. *Phytochemistry*, **2002**, *60*, 39-44.

[16]    Kopa, T.K.; Tchinda, A.T.; Tala, M.F.; Zofou, D.; Jumbam, R.; Wabo, H.K.; Titanji, V.P.K.; Frédérich, M.; Tan, N.-H.; Tane, P.; Antiplasmodial anthraquinones and hemisynthetic derivatives from the leaves of Tectona grandis (Verbenaceae). *Phytochem. Lett.*, **2014**, *8*, 41-45.

[17]    Endale, M.; Ekberg, A.; Alao, J.; Akala, H.; Ndakala, A.; Sunnerhagen, P.; Erdélyi, M.; Yenesew, A.; Anthraquinones of the Roots of Pentas micrantha. *Molecules*, **2012**, *18*, 311.

[18]    Endale, M.; Alao, J.P.; Akala, H.M.; Rono, N.K.; Eyase, F.L.; Derese, S.; Ndakala, A.; Mbugua, M.; Walsh, D.S.; Sunnerhagen, P.; Erdelyi, M.; Yenesew, A. Antiplasmodial quinones from pentas longiflora and pentas lanceolata. *Planta Med.*, **2012**, *78*, 31-35.

[19]    Lai, J.-M.; Chang, J.T.; Wen, C.-L.; Hsu, S.-L. Emodin induces a reactive oxygen species-dependent and ATM-p53-Bax mediated cytotoxicity in lung cancer cells. *European J. Pharmacol.*, **2009**, *623*, 1-9.

[20]    Winter, R.W.; Cornell, K.A.; Johnson, L.L.; Ignatushchenko, M.V.; Hinrichs, D.J.; Roscoe, M.K. Potentiation of the antimalarial agents rufigallol. *Antimicrobial Agents Chemother.*, **1996**, *40*, 1408-1411.

[21]    Gutierrez, P.L. The metabolism of quinone-containing alkylating agents: Free radical production and measurement. *Front. Biossci.*, **2001**, *5*, d629-638.

[22]    Winter, R.W.; Cornell, K.A.; Johnson, L.L.; Isabelle, L.M.; Hinrichs, D.J.; Riscoe, M.K. Hydroxy-anthraquinones as antimalarial agents. *Bioorgan. Med. Chem. Lett.*, **1995**, *5*, 1927-1932.

[23]    Ignatushchenko, M.V.; Winter, R.W.; Bachinger, H.P.; Hinrichs, D.J.; Riscoe, M.K. Xanthones as antimalarial agents; studies of a possible mode of action. *FEBS Lett.*, **1997**, *409*, 67-73.

[24]    Osman, C.P.; Ismail, N.H.; Ahmad, R.; Ahmat, N.; Awang, K.; Jaafar, F.M. Anthraquinones with antiplasmodial activity from the roots of *Rennellia elliptica* Korth. (Rubiaceae). *Molecules*, **2010**, *15*, 7218-7226.

[25]    Singh, R. Geetanjali, isolation and synthesis of anthraquinones and related compounds of *Rubia cordifolia. J. Serbian Chem. Soc.*, **2005**, *70*, 937-942.

[26]    Brieger, G.; Pelletier, W.M. Oxygen alkylation in the ethyl acetoacetate synthesis. *Tetrahedron Lett.*, **1965**, *6*, 3555-3558.

[27]    Inoue, K.; Ueda, S.; Nayeshiro, H.; Inouyet, H. Quinones of *Streptocarpus dunnii. Phytochemistry*, **1983**, *22*, 737-741.

[28]    Widyawaruyanti, A.S.; Kalauni, S.K.; Awale, S.; Nindatu, M.; Zaini, N.C.; Syafruddin, D.; Asih, P.B.S.; Tezuka, Y.; Kadota, S. New prenylated flavones from *Artocarpus champeden* and their antimalarial activity *in vitro. J. Nat. Med.*, **2007**, *61*, 410-413.

[29]    Xuan, T.D.T.; Huy, N.T.; Uyen, D.T.; Sasai, M.; Shiono, T.; Harada, S.; Kamei, K. Inhibition assay of β-hematin formation initiated by lecithin for screening new antimalarial drugs. *Analyt. Biochem.*, **2006**, *349*, 292-296.

[30]    Huy, N.T.; Uyen, D.T.; Sasai, M.; Trang, D.T.X.; Shiono, T.; Harada, S.; Kamei, K. A simple and rapid colorimetric method to measure hemozoin crystal growth *in vitro. Analyt. Biochem.*, **2006**, *354*, 305-307.

[31]    Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunolog. Methods*, **1983**, *65*, 55-63.

[32]    Ismail, N.H.; Ali, A.M.; Aimi, N.; Kitajima, M.; Takayama, H.; Lajis, N.H. Anthraquinones from *Morinda elliptica. Phytochemistry*, **1997**, *45*, 1723-1725.

[33]    Ismail, N.H. Chemistry and biological activity of anthraquinones from *Morinda elliptica* (Rubiaceae). Thesis, Universiti Putra Malaysia, Serdang, **1998**.

[34]    Saha, K. The synthesis and bioactivity study of anthraquinones and the isolation of bioactive compounds from leea indica (Burm, F.) Merr. Thesis, Universiti Putra Malaysia, Serdang, **2005**.

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