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Antimalarial activity of abietane ferruginol analogues possessing a phthalimide group

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ABSTRACT

The abietane-type diterpenoid (+)-ferruginol, a bioactive compound isolated from New Zealand's Miro tree (*Podocarpus ferruginea*), displays relevant pharmacological properties, including antimicrobial, cardioprotective, anti-oxidative, anti-plasmodial, leishmanicidal, anti-ulcerogenic, anti-inflammatory and anticancer. Herein, we demonstrate that ferruginol (**1**) and some phthalimide containing analogues **2**–**12** have potential antimalarial activity. The compounds were evaluated against malaria strains 3D7 and K1, and cytotoxicity was measured against a mammalian cell line panel. A promising lead, compound **3**, showed potent activity with an EC_{50} = 86 nM (3D7 strain), 201 nM (K1 strain) and low cytotoxicity in mammalian cells (SI > 290). Some structure–activity relationships have been identified for the antimalarial activity in these abietane analogues.

Parasitic diseases affect about 30% of the world's population.[1] Among parasitic diseases, malaria is one of the most devastating infectious diseases claiming more lives than any other parasitic infections. Malaria is endemic to more than 100 nations and remains one of the main leading causes of death in children less than five years of age worldwide.[1] A drug cocktail regimen is recommended for the treatment of malaria to prevent the development of resistance, particularly to artemisinin, one of the most effective components of this protocol. The rise of resistance to artemisinin combination therapy is an eminent threat, and resistance has already been reported in various Southeast Asian countries.[2] Hence, there is urgency in identifying new antimalarial agents, particularly new molecular scaffolds with potential novel mechanisms of actions.

Natural products have played a dominant role in the drug discovery efforts for the treatment of human diseases.[3] In fact, most antimalarial chemotherapeutic agents are based on natural products, indicating the potential of identifying new therapeutic leads from plant sources. Chemodiversity continues to be an important task in the search for antimalarial drugs.[4]

The abietane diterpenoids are widely produced by conifers belonging to the families Araucariaceae, Cupressaceae, Pinaceae, and Podocarpaceae, and also angiosperm species.[5] These natural products have a wide range of biological activities. Ferruginol (**1**) (Fig. 1) was first isolated in 1939 from New Zealand's Miro tree (*Podocarpus ferruginea*), and has been extensively studied because of intriguing chemical framework and promising biological properties.[6] Ferruginol (**1**) occurs in plants belonging to the Podocarpaceae, Cupressaceae, Lamiaceae and Verbenaceae families among others. This diterpene exhibited important bioactivities, such as antimicrobial,[7] cardioactive,[8] antioxidative,[9] antileishmanial and nematicidal,[10] and antiulcer properties.[11] In addition, ferruginol was active against prostate cancer,[12] and anti-inflammatory activity.[13] Several reports on antimalarial activity have also been described. In 2003, a report described that ferruginol (**1**) displayed significant (IC_{50} < 1 μg/mL) in vitro antiplasmodial activity against a chloroquine-resistant (K1) and -sensitive (D10) strain of *Plasmodium falciparum*, and low cytotoxicity (SI > 65) against two mammalian cell lines (CHO and HepG2).[14] In 2006, antimalarial activity for ferruginol (**1**) was determined using the D6 (chloroquine-sensitive) clone of *Plasmodium falciparum*. Promising antimalarial activity was shown by an IC_{50} of 1.95 μg/mL.[15] In 2008,

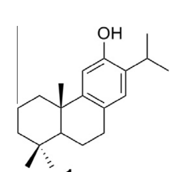

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Figure 1. Ferruginol (**1**).

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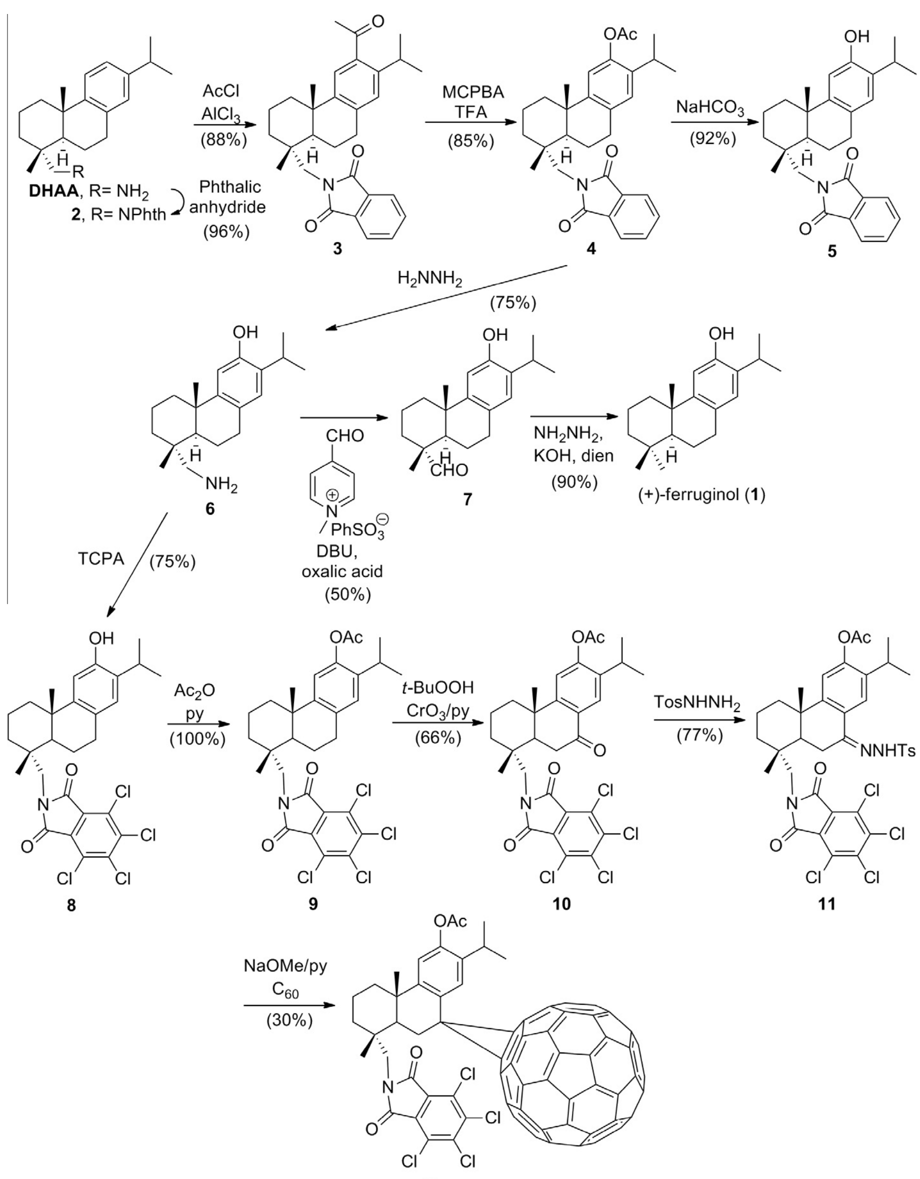
Muhammad and co-workers reported antimalarial activity against D6 (chloroquine-sensitive, IC_{50} = 4.2 μg/mL) and W2 (chloroquine-resistant, IC_{50} = 3.5 μg/mL) strains of *P. falciparum* for ferruginol (**1**).[10] Recently, an additional report confirmed significant antimalarial activity against K1 strain (chloroquine-resistant, IC_{50} = 0.9 μM), showing moderate selectivity index (SI = 15.6 (in L6 cells)).[16]

To further explore the potential of ferruginol (**1**) as antimalarial candidate, and in continuation of our research programs to discover bioactive terpenoids and new scaffolds as potential antimalarial agents,[17,18] we developed a focused compound library based on (+)-ferruginol (**1**) using a short, and efficient synthetic strategy from commercially available (+)-dehydroabietylamine (DHAA) (Scheme 1).[19] With a limited number of atoms that could be manipulated, it was decided to evaluate a series of phthalimides, which have been shown to possess antiparasitic properties.[20] A fullerene terpenoid hybrid was also envisaged as potential inhibitor of *Plasmodium falciparum* carbonic anhydrases, which has been demonstrated that also inhibits the growth of the pathogen.[21] Herein, we report the evaluation of (+)-ferruginol (**1**), and its analogues **2**–**12** against two malaria strains.

The compounds were obtained in enantiomerically pure form from commercially available (+)-dehydroabietylamine as outlined in Scheme 1.[19,22] The synthesis starts with the introduction of the phthalimide group on (+)-dehydroabietylamine to give compound **2** in 96% yield. Then, Friedel–Crafts acylation of **2** gave acetophenone **3** (88% yield), which was oxidized under Baeyer–Villiger conditions to afford acetate **4** in 85% yield (Scheme 1). Hydrolysis of the acetate in **4** gave phenol **5** in high yield, while overall deprotection of **4** afforded the amino-phenol **6** in 75% yield. Compound **6** was the intermediate of two separate approaches. Firstly, oxidative deamination of **6** gave 18-oxoferruginol (**7**) in moderate yield (50%), which was converted into ferruginol (**1**) by Wolff–Kishner reduction (90% yield). Secondly, the treatment of **6** with tetrachlorophthalic anhydride (TCPA) afforded phenol **8** in 75% yield, which was acetylated with acetic anhydride in pyridine to give acetate **9** in quantitative yield (Scheme 1). Acetate **9** was oxidized at C-7 with excess of *t*-BuOOH as oxidant and CrO_3/pyridine mixture as a catalyst in DCM, the yield of ketone **10** was 66%. Subsequently, the reaction of **10** with *p*-tosylhydrazide yielded the corresponding *p*-tosylhydrazone **11** (77% yield). The fullerene–terpenoid hybrid **12** was firstly reacted with NaOMe in anhydrous



Scheme 1. Synthesis of ferruginol analogues **2**–**12**.

Table 1
Antiplasmodial activity and cytotoxicity (EC_{50}-μM) of ferruginol (**1**) and compounds **2**–**12** on *P. falciparum* 3D7 strain, K1 strain, and HepG2, RAJI, BJ, and HEK293 cell lines

Compound	P. falciparum[a]				Cell lines[b]			
	3D7		K1		HepG2	RAJI	BJ	HEK293
	EC_{50}[c]	SI[d]	EC_{50}[c]	SI[d]	EC_{50}[c]	EC_{50}[c]	EC_{50}[c]	EC_{50}[c]
1	2.47	4.6	1.33	8.6	>25	11.49	19.57	>25
2	1.36	>18.4	15–50	n.c.	>25	>25	>25	>25
3	0.086	>290	0.201	>124	>25	>25	>25	>25
4	2.09	>12.0	15–50	n.c.	>25	>25	>25	>25
5	15–50	n.c.	4.0	>6.3	>25	>25	>25	>25
6	6.67	2.6	15–50	n.c.	>25	>25	17.43	>25
7	15–50	n.c.	15–50	n.c.	>25	>25	>25	>25
8	15–50	n.c.	15–50	n.c.	>25	>25	>25	>25
9	15–50	n.c.	15–50	n.c.	>25	>25	>25	>25
10	15–50	n.c.	15–50	n.c.	>25	>25	>25	>25
11	15–50	n.c.	15–50	n.c.	>25	>25	>25	>25
12	15–50	n.c.	15–50	n.c.	>25	>25	>25	>25
Chloroquine	0.023	n.c.	0.69	n.c	>25	>25	>25	>25
Gambogic acid	—	—	—	—	<2.0	0.24	0.21	0.21
Staurosporine	—	—	—	—	<2.0	<2.0	<2.0	<2.0

[a] 3D7 (chloroquine-sensitive) and K1 (chloroquine-resistant).
[b] BJ, normal human foreskin fibroblast; HEPG2, human liver carcinoma cell line; HEK293, human embryonic kidney cell line; RAJI, Burkitt lymphoma cell line.
[c] EC_{50} (μM): concentration corresponding to 50% growth inhibition of the parasite or cells.
[d] Selectivity index (SI): EC_{50} lower values of cytotoxic activity/EC_{50} values of antimalarial activity. N.C.: not calculated.

pyridine for 20 min at room temperature, then, a solution of C_{60} in chlorobenzene was added and the mixture was stirred for 24 h at 70 °C. This gave compound **12** in ca. 30% yield. Attempts to hydrolyze the acetate group in **12** in acidic conditions were unsuccessful.

The analogues can be divided into three groups: (a) precursors such as *N*,*N*-phthaloyldehydroabietylamine (**2**) and 12-acetyl-*N*,*N*-phthaloyldehydroabietylamine (**3**); (b) compounds possessing a hydroxy group as ferruginol (**1**) itself and 12-hydroxy-*N*,*N*-phthaloyldehydroabietylamine (**5**), 12-hydroxy-dehydroabietylamine (18-aminoferruginol, **6**), 18-oxoferruginol (**7**), and 12-hydroxy-*N*,*N*-(tetrachloro)phthaloyldehydroabietylamine (**8**); (c) compounds possessing a acetate group such as 12-acetoxy-*N*,*N*-phthaloyldehydroabietylamine (**4**), 12-acetoxy-*N*,*N*-(tetrachlorophthaloyl) dehydroabietylamine (**9**), 12-acetoxy-7-oxo-*N*,*N*-(tetrachlorophthaloyl)dehydroabietylamine (**10**), *N*,*N*-(tetrachlorophthaloyl) dehydroabietylamine-*p*-tosylhydrazone (**11**), and 12-acetoxy-7-C60-*N*,*N*-(tetrachlorophthaloyl)dehydroabietylamine adduct (**12**).

The antimalarial and cytotoxic activities of (+)-ferruginol (**1**) and its synthetic analogues **2**–**12** (Scheme 1) were evaluated against malaria strains 3D7 and K1, utilizing a previously described assay.[23] The effectiveness of the compounds on *Plasmodium falciparum* in culture were measured as the minimum drug concentration (EC_{50}) required to inhibit parasite growth by more than 50% during 72 h incubation period with test compounds.

The compounds were also evaluated against a panel of mammalian cell lines to evaluate cytotoxic properties. Only those compounds with significant selectivity index are considered as potential lead candidates. The results are shown in Table 1.

The inhibitory values against *P. falciparum* strains ranged from 0.086 to 6.67 μM, with cytotoxicity properties from 11.49 to >25 μM. However, selectivity indices are promising (from 2.6 to 290). Ferruginol (**1**) (3D7 strain, EC_{50} = 2.47 μM, SI = 4.6; K1 strain, EC_{50} = 1.33 μM, SI = 8.6) and 12-acetyl-*N*,*N*-phthaloyldehydroabietylamine (**3**) (3D7 strain, EC_{50} = 0.086 μM, SI > 290; K1 strain, EC_{50} = 0.201 μM, SI > 124) showed the highest antiplasmodial activity, followed by *N*,*N*-phthaloyldehydroabietylamine (**2**), 12-hydroxy-*N*,*N*-phthaloyldehydroabietylamine (**5**), 12-acetoxy-*N*,*N*-phthaloyldehydroabietylamine (**4**) and 12-hydroxy-dehydroabietylamine (18-aminoferruginol, **6**).

The resultant data indicates that ferruginol (**1**) and non-chlorinated phthalimide containing analogues **2**–**5** display potent antiplasmodial activity. The results indicate that the hydroxyl group at C-12 influences activity, but does not dictate complete control of outcome. The presence of an acetyl group at C-12 led to the most active compound of the series. The presence of an acetate group at C-12 led to a decrease in activity in the chloroquine-resistant strain K1, however, it interestingly increased the activity in the chloroquine-sensitive strain 3D7. When the phthalimide group is chlorinated, the analogues showed EC_{50} values between 15 and 50 μM. Also, if the amino group was left unprotected, there was a decrease in activity against the chloroquine-resistant strain K1. Gratifyingly, the dehydroabietylamine analogue **3** showed higher potency than the parent compound, with an EC_{50} of 86 nM (3D7 strain) and 201 nM (K1 strain). This compound represents a new scaffold for the development of antimalarial agents. These encouraging findings warrant further structure activity relationship and target identification studies.

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