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A non-cytotoxic *N*-dehydroabietylamine derivative with potent antimalarial activity

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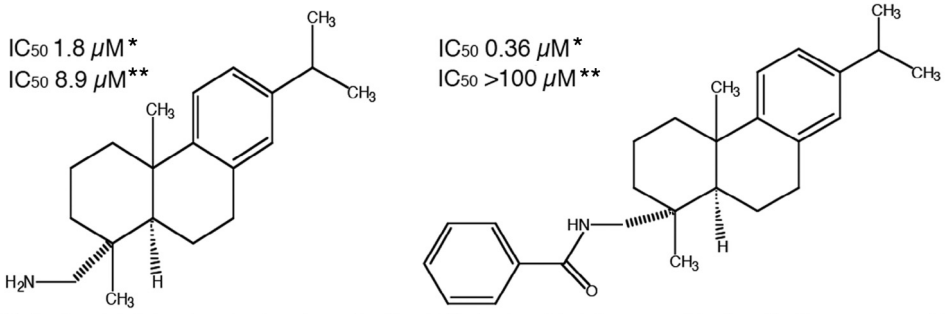
HIGHLIGHTS

- Dehydroabietylamine (DHA) has antimalarial activity, but is toxic to human cells.
- Abietic acid (AA) has neither antimalarial activity nor toxicity to human cells.
- Several DHA and AA derivatives are toxic to parasites but not to human cells.
- *N*-Dehydroabietylbezamide has potent antimalarial activity; nontoxic to human cells.

GRAPHICAL ABSTRACT



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ABSTRACT

Malaria caused by the *Plasmodium* parasites continues to be an enormous global health problem owing to wide spread drug resistance of parasites to many of the available antimalarial drugs. Therefore, development of new classes of antimalarial agents is essential to effectively treat malaria. In this study, the efficacy of naturally occurring diterpenoids, dehydroabietylamine and abietic acid, and their synthetic derivatives was assessed for antimalarial activity. Dehydroabietylamine and its *N*-trifluoroacetyl, *N*-tribromoacetyl, *N*-benzoyl, and *N*-benzyl derivatives showed excellent activity against *P. falciparum* parasites with IC_{50} values of 0.36 to 2.6 μM. Interestingly, *N*-dehydroabietylbenzamide showed potent antimalarial activity (IC_{50} 0.36), and negligible cytotoxicity (IC_{50} >100 μM) to mammalian cells; thus, this compound can be an important antimalarial drug. In contrast, abietic acid was only marginally effective, exhibiting an IC_{50} value of ~82 μM. Several carboxylic group-derivatives of abietic acid were moderately active with IC_{50} values of ~8.2 to ~13.3 μM. These results suggest that a detailed understanding of the structure–activity relationship of abietane diterpenoids might provide strategies to exploit this class of compounds for malaria treatment.

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1. Introduction

Malaria is one of the major deadly infectious diseases in many countries. Each year over 200 million clinical cases occur, resulting in more than 600,000 deaths (Snow et al., 2005; Price et al., 2007; WHO, 2013). Five species of *Plasmodium* family protozoan parasites cause malaria, but the vast majority of infections are caused by *P. falciparum* and *P. vivax*. Of these two parasites, *P. falciparum* causes fatal disease in all age groups, especially, children and pregnant women are more vulnerable. Several drugs have been effective in treating malaria and thus far this is the only available method to treat this disease. However, there is a widespread resistance of parasites to drugs such as chloroquine that have been widely used to treat malaria. In addition, currently, resistance is emerging to relatively new frontline drug, artemisinin (Dondorp et al., 2009; Murray et al., 2012). Given that parasites are likely to eventually develop resistance to newly introduced drugs and that hitherto a licensed vaccine is not available, it is critical to discover new antimalarial agents.

Natural compounds play a major role in drug discovery and have provided significant value to the pharmaceutical industry during the last 50 years (Newman and Cragg, 2012). Particularly, therapeutics for various infectious diseases, cancer, and other debilitating diseases caused by metabolic disorders have benefited from many drug classes that were initially developed based on active compounds from natural sources (Cragg et al., 2009).

The tricyclic abietane diterpenoids occur widely in plants and are used for a variety of industrial applications (Rao et al., 2008, 2012). These compounds also have medicinal values, exhibiting a wide range of pharmacological activities including anti-inflammatory, antibacterial, antifungal, and antimalarial properties (Goodson et al., 1999; He et al., 2012; Liang et al., 2013; Machumi et al., 2010; Steck, 1981; Wilkerson et al., 1991). Several abietane diterpenoids, especially those isolated from the leaves of the *Plectranthus* plant species, possess potent antimalarial activity (Van Zyl et al., 2008). However, these compounds are toxic to mammalian cells, preventing use as antimalarial agents.

Recently, dehydroabietylamine (also called leelamine), abietic acid, and their synthetic derivatives have been studied for potential anticancer activity (Huang et al., 2013; Kuzu et al., 2014; Robertson et al., 2014). Some of these compounds exhibited potent melanoma cell killing activity, while others had a negligible effect (Robertson et al., 2014). Therefore, it was interesting to determine whether these abietane diterpenoids possessed antimalarial activity, particularly those that were not cytotoxic to human cells. Thus, we assessed the antimalarial activity of the available abietane diterpenoid library of compounds for antimalarial activity. Some of these compounds effectively inhibited the growth of malaria parasites without causing cytotoxicity to human cells. Interestingly, one of the derivatives of dehydroabietylamine, *N*-dehydroabietylbenzamide, had potent antimalarial activity with negligible or no cytotoxicity to human cells. These results suggest that understanding the structure–activity relationship of abietane diterpenoids may aid development of an abietane diterpenoid compound for treating malaria.

2. Materials and methods

2.1. Parasites and culture conditions

P. falciparum parasites (3D7 strain) were cultured in RPMI 1640 medium (Gibco Life Technologies Inc., NY) supplemented with 25 mM HEPES, 29 mM sodium bicarbonate, 0.005% hypoxanthine, *p*-aminobenzoic acid (2 mg/L), gentamycin sulfate (50 mg/L) and 5% AlbuMAX II (Invitrogen, Carlsbad, CA) using fresh O-positive human red blood cells at 2% hematocrit. The Institutional Review Board of the Penn State University College of Medicine has approved the use of human blood and plasma obtained from the Hershey Medical Center Blood Bank for parasite culturing. Parasites were cultured at 37 °C under 5% O_2, 5% CO_2, and 90% N_2 (Trager and Jensen, 1976). Cultures were synchronized by the sorbitol method (Lambros and Vanderberg, 1979). Gametocytes were obtained by continuously culturing parasites for 6–8 weeks at parasitemia level >10% with weekly synchronization of cultures. At the end of this period, the majority of the parasites in cultures were gametocytes and the culture was used for assessing gametocidal activity (Aminake et al., 2011; Karl et al., 2014).

2.2. Cell lines

The human fibroblast FF2441 cell line (provided by Dr. Craig Myers, Penn State College of Medicine, Hershey) and the human melanoma UACC 903 cell line (provided by Dr. Mark Nelson, University of Arizona, Tucson) were cultured at 37 °C in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (Hyclone, Logan, UT). The cell lines were periodically monitored for genotypic characteristics and tumorigenic potential to confirm cell line identity and phenotypic behavior (Gowda et al., 2014; Kuzu et al., 2014; Robertson et al., 2014).

2.3. Antimalarial activity

Synchronous cultures of the asexual stage *P. falciparum* 3D7 parasites at the ring stage with 4–6% parasitemia were treated with 2.5, 5, 10 μM, and 20 μM of dehydroabietylamine, abietylamine, or abietic acid. At 24, 48, and 96 h, the growth and propagation of parasites were monitored by assessing Giemsa-stained thin smears of culture pellets under light microscopy. Gametocidal activity was assessed by examining under light microscopy the Giemsa-stained smears of gametocyte cultures treated with 10 and 20 μM dehydroabietylamine or abietic acid for 48 h (Sun et al., 2014).

The antimalarial activity of dehydroabietylamine, abietic acid and related synthetic derivatives was further evaluated by a SYBR Green assay (Johnson et al., 2007). The IC_{50} value, which is the effective concentration inhibiting parasite growth by 50%, of the compounds was determined using this assay. From 10 mM stock solutions of compounds in DMSO, working solutions of 400 μM were prepared, which were serially diluted with culture medium to obtain test solutions of 0.16 to 200 μM. In these solutions, the concentration of DMSO was less than 0.2%. The test solutions (100 μL each) were mixed with 100 μL of 0.4% parasitized red blood cells at the early ring stage in complete medium and were seeded into 96-well plates. After 72 h, 100 μL of lysis buffer (20 mM Tris–HCl pH 7.5, 5 mM EDTA, 0.008% saponin and 0.08% Triton X-100) containing 0.2 μL/mL of SYBR Green I (Life Technology, Eugene, OR, USA) was added. The plates were incubated in the dark at room temperature for 1 h and fluorescence intensity measured using a fluorescence plate reader at excitation and emission wavelengths of 485 nm and 535 nm, respectively. The mean IC_{50} values of three independent experiments were plotted using nonlinear regression (Sigmoidal dose response) equation by using GraphPad Prism version 4.01 (GraphPad Software, La Jolla, CA).

2.4. Cytotoxicity assay

The cytotoxic activity of compounds has previously been measured by assessing the viability of normal human fibroblast FF2441 cells and human melanoma UACC 903 cells by the MTS assay (Promega, Madison, WI) (Gowda et al., 2014; Kuzu et al., 2014; Robertson et al., 2014).