Published in final edited form as:

Exp Parasitol. 2015 August ; 155: 68–73. doi:10.1016/j.exppara.2015.05.002.

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

Maralinganadoddi P. Sadashiva^{a,c,*}, Raghavendra Gowda^b, Xianzhu Wu^a, Gajanan S. Inamdar^b, Omer F. Kuzu^b, Kanchugarakoppal S. Rangappa^c, Gavin P. Robertson^b, and D. Channe Gowda^{a,**}

^aDepartment of Biochemistry and Molecular Biology, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA

^bDepartment of Pharmacology, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA

^cDepartment of Chemistry, University of Mysore, Manasagangothri, Mysore, 570 006, India

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 IC₅₀ values of 0.36–2.6 μM. Interestingly, *N*-dehydroabietylbenzamide showed potent antimalarial activity (IC₅₀ 0.36), and negligible cytotoxicity (IC₅₀ >100 μM) to mammalian cells; thus, this compound can be an important antimalarial drug. In contrast, abietic acid was only marginally effective, exhibiting an IC₅₀ value of ~82 μM. Several carboxylic group-derivatives of abietic acid were moderately active with IC₅₀ 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.

Graphical abstract

*Corresponding author. mpsadashiva@gmail.com (M.P. Sadashiva). **Corresponding author. Fax: +(717) 531 7072. gowda@psu.edu (D.C. Gowda).

Keywords

Abietane diterpenoids; Dehydroabietylamine derivatives; Abietic acid derivatives; Antimalarial activity

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 anti-cancer 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% O₂, 5% CO₂, and 90% N₂ (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 IC₅₀ 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 0.16–200 µM. In these solutions, the concentration of DMSO was less than 0.2%. The test solutions (100 µL each) were mixed

with 100 μ L 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 μ L 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 μ L/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 IC₅₀ 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).

2.5. Statistical analysis

IC₅₀ values were determined by plotting the fluorescence intensity in SYBR Green assay at various inhibitor concentrations using Prism 4.0 GraphPad Software. The results shown represent mean IC₅₀ values of three independent experiments $\pm$ SEM.

3. Results and discussion

We assessed antimalarial activity of two naturally occurring abietane diterpenoids, dehydroabietylamine (**1**) and abietic acid (**3**), and various synthetic derivatives **2a–2f** and **4a–4g**, respectively. The structures of these compounds are depicted in Fig. 1. The compounds, **2a–2f** and **4a–4g**, were synthesized by derivatizing the amine and carboxylic functional groups of parent compounds **1** and **3**, respectively, and were characterized by ¹H NMR and mass spectrometry (Robertson et al., 2014). To determine whether these compounds possess antimalarial activity and, if active, inhibit parasite growth and/or invasion of erythrocytes, we initially tested the effect of dehydroabietylamine (**1**), abietic acid (**3**) and abietylamine (**4d**) on the growth and propagation of *P. falciparum* in culture. The parasite cultures were treated at the ring stage, 8–10 h post-invasion of erythrocytes, and the growth was assessed by microscopic examination of Giemsa-stained smears of culture pellets at 24, 48 and 72 h post-treatment. In untreated cultures, the initial ring stage parasites developed normally into trophozoites in 24 h and then into schizonts. By 48 h the merozoites that were released from schizont burst efficiently invaded erythrocytes, entering the second life cycle with ~22% parasitemia; at this time point, all parasites were at the late ring stage (Table 1 and Fig. 2A). By 72 h, untreated parasites progressed to the trophozoites. In contrast, treatment for 24 h with 20 μ M dehydroabietylamine completely inhibited parasite development into trophozoites as evidenced by the presence of highly condensed bodies (Table 1 and Fig. 2A). Treatment with 10 μ M dehydroabietylamine also substantially inhibited parasite growth; ~60% of the parasites developed into trophozoites during 24 h treatment. Of these, only a small number of trophozoites (~27%) matured into healthy schizonts by 48 h, producing merozoites, which invaded erythrocytes resulting in ~2.5%

parasitemia in the second life cycle. The remaining ~73% trophozoites, which accounted for 2.4% parasitemia, were still at the trophozoite and schizont stages and were unhealthy (Table 1). By 72 h, the rings barely developed into trophozoites and were unhealthy and dying. Five and 2.5 μ M dehydroabietylamine also exhibited substantial antimalarial activity; the observed growth patterns of parasites under these conditions are described in Table 1. Compared to dehydroabietylamine, abietylamine exhibited lower antimalarial activity. Upon treatment with 20 μ M abietylamine, ~60% parasites developed into trophozoites during the 24 h period. Of these, ~45% were at the trophozoite and schizont stages at 48 h. Only a small number of parasites were able to produce merozoites that could enter into the second life cycle, producing ~2.4% ring stage parasites. By 72 h, 20 μ M abietylamine completely killed the parasites (Table 1). Abietylamine also significantly inhibited parasite growth at 2.5, 5, and 10 μ M as indicated by the reduction in parasitemia after treatment for 72 h. Although during the first 24 h, 10 μ M, but not 2.5 and 5 μ M abietylamine noticeably inhibited the parasite growth. By 48 h, 5 and 10 μ M abietylamine significantly inhibited parasite growth. Even though 2.5 μ M abietylamine had no noticeable effect up to 48 h treatment, by 72 h, about 50% inhibition of parasite growth was evident (Table 1). In contrast to dehydroabietylamine and abietylamine, 2.5, 5, and 10 μ M abietic acid had no effect on parasite growth during 72 h culturing period. Parasite growth and development was comparable to those of the untreated control culture. Only at 20 μ M could abietic acid noticeably inhibit parasite growth during the second life cycle (Table 1). Thus, among the three compounds tested, dehydroabietylamine was most active, abietylamine was moderately active, and abietic acid was negligibly active.

Dehydroabietylamine (**1**) and abietic acid (**3**) were also assessed for gametocidal activity. Upon treatment for 48 h with 10 μ M dehydroabietylamine, gametocytes shrunk and condensed into non-viable, rounded condensed bodies, whereas treatment with 20 μ M dehydroabietylamine completely lysed gametocytes (Fig. 2B). On the other hand, abietic acid had no effect on gametocytes. The effect of dehydroabietylamine on different stages of gametocyte development was not assessed in this study. Nevertheless, these above data suggest that dehydroabietylamine has an excellent activity against both the asexual blood stage parasites and gametocytes, whereas abietic acid was almost inactive.

Next, the antimalarial activity of the compounds was evaluated by a SYBR Green assay (Johnson et al., 2007). Initially, the inhibitory effect of parent compounds **1** and **3** was assessed at different stages of parasite growth during the 48 h life cycle. Consistent with the results obtained by microscopic examination (see Table 1), dehydroabietylamine efficiently inhibited parasite growth in a dose dependent manner, whereas abietic acid showed negligible activity up to 20 μ M (Fig. 3). Furthermore, we analyzed inhibition by dehydroabietylamine and abietic acid derivatives at 72 h at 5, 10 and 20 μ M. All dehydroabietylamine derivatives, except **2c** and **2f**, completely inhibited parasite growth at all three concentrations (Fig. 4A). While **2c** inhibited the parasite growth in a dose dependent manner, showing complete inhibition at 20 μ M, compound **2f** was not inhibitory even at 20 μ M. In the case of abietic acid derivatives, all compounds, except **4b**, exhibited good inhibitory activity in a concentration dependent manner; **4b** showed negligible activity even at 20 μ M (Fig. 4B).

Finally, to determine the IC₅₀ values of the test compounds, antimalarial activity was evaluated at concentrations ranging from 0.08 μ M to 20 μ M. Since compounds **2f** and **4b** showed low activity even at 20 μ M, effect was tested up to 100 μ M. Data were analyzed using nonlinear regression and IC₅₀ values presented in Table 2. The dose–response curves for representative compounds among those that exhibited excellent antimalarial activity are shown in Fig. 5. Dehydroabietylamine showed excellent antimalarial activity with IC₅₀ of ~1.8 μ M, whereas abietic acid was only marginally active with an IC₅₀ of ~82 μ M (Table 2). These compounds have similar structural characteristics, each having a tricyclic carbon skeleton and three chiral carbon atoms (compounds **1** and **3** in Fig. 1). However, they differ from each other in functional groups. In dehydroabietylamine, one of the three rings is aromatic, whereas abietic acid lacks this feature. The antimalarial activity of dehydroabietylamine was substantially enhanced when amine group was derivatized to amide groups by substitution with trifluoroacetyl, tribromoacetyl, benzoyl moieties or with benzyl group. The IC₅₀ values of these compounds ranged from 0.36 μ M to ~2.3 μ M. In contrast, *N*-dehydroabietylpropionamide had only moderate activity with an IC₅₀ of ~13.3 μ M (Table 2). The presence of the bulky *N*-triphenylmethyl moiety rendered dehydroabietylamine derivative **2f** inactive (IC₅₀ ~90.6 μ M), likely due to steric hindrance. These results suggest that the activity of dehydroabietylamine and its derivatives is target specific but not owing to nonspecific toxicity. Together the structure–activity relationships of dehydroabietylamine derivatives **2a–2f** suggest that the presence of a hydrophobic *N*-substituent such as benzoyl moiety confers an effective antimalarial activity. Compared to the derivatives of dehydroabietylamine, abietic acid derivatives **4a–4g** were relatively less active (Table 2). Collectively, these results suggest that the aromatic ring of the abietane tricyclic structure is important for effective antimalarial activity.

The cytotoxic activity of these compounds against human metastatic melanoma and normal skin fibroblast cell lines was previously determined by Robertson et al. (2014) and IC₅₀ values are presented in Table 2. Several dehydroabietylamine and abietic acid derivatives, especially **2c**, **2d**, **4a**, **4c**, **4e**, **4f**, and **4g**, that possess good to excellent antimalarial activity, had low levels of mammalian cell cytotoxicity. Thus, these results support our conclusion that suitably designed abietane diterpenoid compounds may be effective antimalarial drugs.

Although abietic acid is only marginally active, the parasite growth pattern upon treatment with dehydroabietylamine and abietylamine (Table 1) suggested that active abietane diterpenoids inhibit the development of parasites from the ring stage to the trophozoite stage. This phase of the parasite life cycle corresponds to an exponential growth rate requiring efficient functioning of many metabolic pathways for rapid development. Thus, it appears that the antimalarial activity of abietane diterpenoids is due to the interference with one or more of the metabolic pathways critical for parasite survival.

In cancer cells, it has been shown that dehydroabietylamine is lysomotropic, thus accumulates in lysosomes causing disruption of lysosomal compartments (Gowda et al., 2014; Kuzu et al., 2014). Dehydroabietylamine has also been shown to interfere with intracellular cholesterol transport, affecting membrane functions and leading to cell death. Therefore, based on our observation that abietic acid is barely active, whereas dehydroabietylamine and abietylamine possess excellent antimalarial activity, it appears that

the compounds having amine basicity can accumulate in the food/digestive vacuoles (lysosome equivalent of eukaryotic cells) of parasites and disrupt digestive function, leading to parasite growth arrest and eventually death. In fact, it has been reported that, similar to chloroquine, abietane diterpenoids accumulate in the parasite's digestive vacuole and target heme to prevent its polymerization to hemozoin (Van Zyl et al., 2008). Heme is a side product of hemoglobin degradation by parasites and is a highly reactive molecule with electron donor properties. Accumulation of heme in the digestive vacuole damages the metabolic activity of parasites, leading to death (Muller, 2004; Oliaro and Goldberg, 1995). Parasites overcome the toxic effect of heme by converting it into polymeric, inert hemozoin. Therefore, optimally designed abietane diterpenoid compounds that specifically inhibit heme polymerization and kill malaria parasites may prove to be effective antimalarial drugs. Additionally, as reported previously for cancer cells (Kuzu et al., 2014), abietane diterpenoids having amine basicity may interfere with the cholesterol transport in parasites, causing membrane dysfunction and death. Therefore, based on the foregoing discussion, it appears that abietane diterpenoids target multiple functions in parasites. Understanding the precise mechanisms by which the abietane diterpenoids and synthetic derivatives cause malaria parasite death is likely to aid designing potent and specific pharmacologically viable molecules for treating malaria.

4. Conclusion

Overall, our results suggest that the structure–activity relationship of dehydroabietylamine derivatives offers a unique approach for designing compounds that have potent antimalarial activity without cytotoxicity. Among the dehydroabietylamine and abietic acid derivatives analyzed in this study, the non-cytotoxic *N*-dehydroabietylbezamide (**2d**) showed the most potent antimalarial activity. Additionally, based on the observation that dehydroabietylamine possess gametocidal activity, it is possible that the dehydroabietylamine derivatives have antigametocyte activity. Elimination of gametocyte persistence in addition to clearing asexual parasites in the blood offers an effective strategy for malaria control and elimination. Thus, our results indicate that optimally designed abietane diterpenoids might be useful as effective antimalarial drugs.

Acknowledgments

This work was supported by the Indo-US Raman Research Postdoctoral Fellowship to MPS awarded by the University Grants Commission, New Delhi, India, and by the grants AI 41139 from National Institute of Allergy and Infectious Diseases, NIH to DCG and CA127892-01A from the National Cancer Institute, NIH to GPR.

References

- Aminake MN, Schoof S, Sologub L, Leubner M, Kirschner M, Arndt HD, et al. Thiostrepton and derivatives exhibit antimalarial and gametocytocidal activity by dually targeting parasite proteasome and apicoplast. *Antimicrob Agents Chemother.* 2011; 55:1338–1348. [PubMed: 21245445]
- Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anti-cancer agents. *Chem Rev.* 2009; 109:3012–3043. [PubMed: 19422222]
- Dondorp AM, Nosten F, Yi P, Das D, Phyto AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *New Engl J Med.* 2009; 361:455–467. [PubMed: 19641202]

- Goodson B, Ehrhardt A, Ng S, Nuss J, Johnson K, Giedlin M, et al. Characterization of novel antimicrobial peptoids. *Antimicrob Agents Chemother*. 1999; 43:1429–1434. [PubMed: 10348765]
- Gowda R, Madhunapantula SV, Kuzu OF, Sharma A, Robertson GP. Targeting multiple key signaling pathways in melanoma using leelamine. *Mol Cancer Ther*. 2014; 13:1679–1689. [PubMed: 24688050]
- He Y, Zhang Y, Lu J, Lin R. Isolation and structural elucidation of abietic acid as the main adulterant in an herbal drug for the treatment of psoriasis. *J Pharm Biomed Anal*. 2012; 66:345–348. [PubMed: 22482903]
- Huang XC, Wang M, Pan YM, Yao GY, Wang HS, Tian XY, et al. Synthesis and antitumor activities of novel thiourea α -aminophosphonates from dehydroabietic acid. *Eur J Med Chem*. 2013; 69:508–520. [PubMed: 24095745]
- Johnson JD, Dennull RA, Gerena L, Lopez-Sanchez M, Roncal NE, Waters NC. Assessment and continued validation of the malaria SYBR green I-based fluorescence assay for use in malaria drug screening. *Antimicrob Agents Chemother*. 2007; 51:1926–1933. [PubMed: 17371812]
- Karl S, Laman M, Koleala T, Ibam C, Kasian B, N'Drewe N, et al. Comparison of three methods for detection of gametocytes in Melanesian children treated for uncomplicated malaria. *Malar J*. 2014; 13:319. [PubMed: 25123055]
- Kuzu OF, Gowda R, Sharma A, Robertson GP. Leelamine mediates cancer cell death through inhibition of intracellular cholesterol transport. *Mol Cancer Ther*. 2014; 13:1690–1703. [PubMed: 24688051]
- Lambros C, Vanderberg JP. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J Parasitol*. 1979; 65:418–420. [PubMed: 383936]
- Liang Y, Xie H, Wu P, Jiang Y, Wei X. Podocarpane, isopimarane, and abietane diterpenoids from *Isodon lophanthoides* var. *graciliflorus*. *Food Chem*. 2013; 136:1177–1182. [PubMed: 23194512]
- Machumi F, Samoylenko V, Yenesew A, Derese S, Midiwo JO, Wiggers FT, et al. Antimicrobial and antiparasitic abietane diterpenoids from the roots of *Clerodendrum eriophyllum*. *Nat Prod Commun*. 2010; 5:853–858. [PubMed: 20614808]
- Muller S. Redox and antioxidant systems of the malaria parasite *Plasmodium falciparum*. *Mol Microbiol*. 2004; 53:1291–1305. [PubMed: 15387810]
- Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, et al. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet*. 2012; 379:413–431. [PubMed: 22305225]
- Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*. 2012; 75:311–335. [PubMed: 22316239]
- Olliaro PL, Goldberg DE. The plasmodium digestive vacuole: metabolic headquarters and choice drug target. *Parasitol Today*. 1995; 11:294–297. [PubMed: 15275326]
- Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. *Am J Trop Med Hyg*. 2007; 77:79–87. [PubMed: 18165478]
- Rao X, Song Z, He L. Synthesis and antitumor activity of novel α -aminophosphonates from diterpenic dehydroabietylamine. *Heteroatom Chem*. 2008; 19:512–551.
- Rao X, Huang X, He L, Song J, Song Z, Shang S. Antitumor activity and structure-activity relationship of diterpenoids with a dehydroabietyl skeleton. *Comb Chem High Throughput Screen*. 2012; 15:840–844. [PubMed: 22946842]
- Robertson, GP.; Gowda, R.; Madhupantula, SV.; Omar, FK.; Gajanan, SI. Compositions and methods relating to proliferative diseases. US Patent. 8785502. 2014.
- Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature*. 2005; 434:214–217. [PubMed: 15759000]
- Steck, EA. Topical prophylaxis against schistosomiasis. US Patent. 4282253. 1981.
- Sun W, Tanaka TQ, Magle CT, Huang W, Southall N, Huang R, et al. Chemical signatures and new drug targets for gametocytocidal drug development. *Sci Rep*. 2014; 4:3743. [PubMed: 24434750]
- Trager W, Jensen JB. Human malaria parasites in continuous culture. *Science*. 1976; 193:673–675. [PubMed: 781840]

- Van Zyl R, Khan F, Edward TJ, Drews SE. Antiplasmodial activities of some abietane diterpenes from the leaves of five *Plectranthus* species. *S Afr J Sci.* 2008; 104:1–2.
- WHO. World Malaria Report. 2013. <http://www.who.int/malaria/publications/world_malaria_report_2013/wmr2013_no_profiles.pdf?ua=1>
- Wilkerson W, DeLucca I, Galbraith W, Gans K, Harris R, Jaffee B, et al. Antiinflammatory phospholipase-A2 inhibitors. *Eur J Med Chem.* 1991; 26:667–676.

HIGHLIGHTS

- Dehydroabietylamine (DHA) has antimalarial activity, but is toxic to mammalian 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.

Fig. 1.
 The structures of dehydroabietylamine 1 and abietic acid 3, and corresponding derivatives 2a–2f and 4a–4g.

Fig. 2.

Analysis of the anti-malarial activity of dehydroabietylamine and abietic acid. Cultures of the ring stage *P. falciparum* (A) and gametocytes (B) were treated with 10 μ M or 20 μ M of abietic acid or dehydroabietylamine. Untreated cultures were used as controls. The Giemsa-stained smears of culture pellets at 48 h post-treatment were examined under light microscopy. The pictures of representative microscopic fields are shown. Since both 10 and 20 μ M abietic acid had no activity, the result of 10 μ M treatment is not shown. Arrows indicate the condensed parasites in 20- μ M dehydroabietylamine-treated parasite cultures.

Fig. 3.

Assessment of the antimalarial activity of dehydroabietylamine and abietic acid by a SYBR Green assay. The ring stage *P. falciparum* was treated with the test compounds at the indicated concentrations. After 48 h, the growth of parasites was assessed by measuring the SYBR Green fluorescence intensity. The inhibitory activity was inversely proportional to fluorescent intensity. Shown are the data from a representative of at least 3 independent experiments performed in triplicate. Gray bars, abietic acid; black bars, dehydroabietylamine.

Fig. 4.

Assessment of the antimalarial activity of dehydroabietylamine and abietic acid derivatives by a SYBR Green assay. The ring stage *P. falciparum* was treated with the test compounds at the indicated concentrations. After 72 h, the growth of parasites was assessed by measuring SYBR Green fluorescence intensity. (A and B) Inhibitory activity of dehydroabietylamine and abietic acid derivatives, respectively. Shown are the data from a representative of two independent experiments performed in triplicate.

Fig. 5. Antimalarial activity of dehydroabietylamine and abietic acid derivatives. The ring stage *P. falciparum* was treated with the test compounds at the concentrations ranging from 0.08 μM to 20 μM . Compounds that showed low activity at 20 μM were tested at 40 and 100 μM . After 72 h, parasite growth was assessed by measuring SYBR Green fluorescence intensity. Results were analyzed by using nonlinear regression and dose-response data plotted. The IC_{50} values (concentrations corresponding to 50% inhibition of parasite growth) were estimated and values for all compounds are listed in Table 2. Shown are dose-response curves for representative dehydroabietylamine derivatives.

Table 1

The effect of dehydroabietylamine, abietylamine and abietic acid on the growth of intraerythrocytic *P. falciparum*.

Treatment	Percent parasitemia and parasite growth stage		
	24 h	48 h	72 h
Untreated	6 ± 0.7 (T) ^a	22 ± 1.23 (R)	20 ± 1.6% (T)
Dehydroabietylamine			
2.5 µM	6.0 ± 0.3 (T)	10.3 ± 1.3 (R)	9.1 ± 0.5 (T) ^b
5 µM	5.5 ± 0.3 (T)	6.1 ± 1.5 (T, S, R)	NVP
10 µM	3.3 ± 0.3 (T)	2.5 ± 0.2 (R) 2.4 ± 0.2 (T) ^b	NVP
20 µM	NVP ^c	NVP	NVP
Abietylamine			
2.5 µM	6.0 ± 0.6 (T)	20.4 ± 1.3 (R)	10.0 ± 0.5 (T)
5 µM	6.0 ± 0.4 (T)	16.1 ± 1.5 (R)	9.0 ± 0.4 (T) ^b
10 µM	4.3 ± 0.4 (T)	10.1 ± 0.6 (R) 0.9 ± 0.1 (S)	8.3 ± 0.1 (T) ^b
20 µM	3.7 ± 0.3 (T)	2.4 ± 0.5 (R) 1.5 ± 0.3 (T, S)	NVP ^b
Abietic acid			
2.5 µM	6.0 ± 0.2 (T)	21.6 ± 1.0 (R)	20.6 ± 1.2 (T)
5 µM	6.0 ± 0.3 (T)	22.0 ± 1.3 (R)	20.6 ± 0.4 (T)
10 µM	5.7 ± 0.3 (T)	22.3 ± 0.6 (R)	20.0 ± 0.2 (T)
20 µM	5.9 ± 0.4 (T)	20.6 ± 0.9 (R)	15.1 ± 0.3 (T)

Parasites at the ring stage (~10 h after invasion) were cultured in the presence of indicated concentrations of the test compounds. After 24, 48 and 72 h, aliquots of cultures were removed, and thin smears of parasites were prepared, stained with Giemsa, and examined under light microscopy.

^aThe letters in parenthesis refer to the parasite developmental stage: R, rings; T, trophozoites; S, schizonts.

^bUnhealthy dying parasites.

NVP, no viable parasites.

Table 2

Antimalarial and cytotoxic activities of dehydroabietylamine and abietic acid, and their derivatives.

	IC₅₀, μM $\pm$ SEM		
	3D7^a	FF 2441 cells^b	UACC 903 cells^b
1	1.8 $\pm$ 0.22	8.9 $\pm$ 0.68	1.4 $\pm$ 0.24
2a	1.5 $\pm$ 0.30	5.2 $\pm$ 0.14	1.3 $\pm$ 0.12
2b	2.6 $\pm$ 0.32	4.1 $\pm$ 0.27	1.0 $\pm$ 0.16
2c	13.3 $\pm$ 0.44	>100	89.4 $\pm$ 3.3
2d	0.36 $\pm$ 0.13	>100	>100
2e	1.3 $\pm$ 0.16	3.1 $\pm$ 0.4	6.1 $\pm$ 1.34
2f	90.6 $\pm$ 1.13	>100	>100
3	82.0 $\pm$ 1.23	>100	>100
4a	10.4 $\pm$ 0.26	>100	70.1 $\pm$ 1.82
4b	82.1 $\pm$ 0.92	>100	>100
4c	10.6 $\pm$ 0.48	>100	24.4 $\pm$ 1.32
4d	8.2 $\pm$ 0.48	8.3 $\pm$ 0.41	2.1 $\pm$ 0.25
4e	11.1 $\pm$ 0.43	>100	>100
4f	13.3 $\pm$ 0.25	>100	>100
4g	10.1 $\pm$ 0.50	>100	52.6 $\pm$ 1.46

^aThe antimalarial activity was assessed using *P. falciparum* 3D7 strains.^bThe cytotoxicity has been previously evaluated using human normal skin fibroblast FF 2441 and human metastatic melanoma UACC 903 cell lines by Robertson et al. (2014); the IC₅₀ values given here are taken from this study.