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# Hairy root mediated functional derivatization of artemisinin and their bioactivity analysis

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ABSTRACT

Biotransformation of artemisinin (1) with the selected hairy root clones of three medicinally important plants, *i.e.*, *Atropa belladonna, Hyoscyamus muticus* and *Ocimum basilicum*, yielded two biotransformed products, which were identified as 3-α-hydroxy-1-deoxyartemisinin (2) and 4-hydroxy-9,10-dimethyloctahydrofuro-(3,2-i)-isochromen-11(4*H*)-one (3). Their structures were elucidated through spectroscopic analysis (NMR/MS) and X-ray crystallography. The relative transformation efficiencies of the tested hairy root clones differed concerning individual bioconversion reactions. Consequently, the HR clones of *H. muticus* and *A. belladonna* accomplished the highest conversion of (**1**) to (**2**) and (**3**) respectively, while that of *O. basilicum* imparted an intermediate response. *In-silico* and *in-vitro* bioactivity analysis of the derivatives revealed promising anti-plasmodial activity profile in tandem with notable TNF level lowering potential of compound (**2**), indicating thereby its prospective therapeutic merit in ameliorating the severity of malarial infection.



## 1. Introduction

Malaria ranks as one of the most alarming infectious parasitic diseases of the world and is imposing severe threat on approximately half of the global populations as per the existing WHO survey [1]. In spite of the tremendous worldwide research efforts to combat malaria, the global morbidity and mortality rates have not been ameliorated significantly over the last 50 years and re-emergence of malaria in many parts of the world is currently imposing fresh challenges due to rapid acquirement of drug-resistance by the parasite [2].

In general, the discovery of artemisinin, a sesquiterpene lactone containing an endoperoxide bridge and/or its analogues become increasingly popular as effective candidates of artemisinin-based combination therapy (ACT) for the treatment of drug-resistant malaria [3]. Although the ACT treatment regime showed reduction in the transmissibility of malaria by preventing gametocyte development [4], but delayed parasite clearance due to rapid emergence of artemisinin resistance [5] necessitated the discovery of new antimalarials with novel mechanism of actions [6].

Many efforts have been made to prepare simpler antimalarial molecules based on the trioxane ring of artemisinin or to produce semisynthetic and synthetic endoperoxides with greater metabolic and hydrolytic stability than artemisinin itself [7]. However, as the processes were complicated with low yields and high cost [8], efforts were diverted towards exploration of new avenues to produce more efficient artemisinin analogues or even new sesquiterpenes. Accordingly, the contemporary research findings corroborated the superior efficacies of artemisinin analogues for effective cancer chemotherapy (dihydroartemisinin) as well as in combination therapies for drug resistance malaria [3,9]. Currently

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the WHO recommended antimalarial formulations that are being used as ACT consist of several artemisinin derivatives having long half-life than artemisinin, such as artemether-lumefantrine, artesunate-mefloquine, artesunate-amodiaquine, artesunate-sulfadoxine-pyrimethamine, dihydroartemisinin-piperaquine and arteether-curcumin [10–12].

Diversified efforts have been focused towards generating novel analogues either through combinatorial biosynthesis in microbes [8] or through bioconversion of artemisinin or its analogues (artemisinic acid, α and β artemether, arteannuin B) using microbial [13–18] and plant cell/tissue culture systems [16,19–22].

Judicious exploitation of the biotransformation proficiency of hairy root cultures (HR) is gaining world-wide attention due to the practical merit of this tool in generating novel products with widened bioactivities [23]. Besides possessing characteristically distinctive and coherent growth/enzymatic profiles, long-lasting operational stability and reduced cost involvement [24], HR cultures also enjoy the added benefits of inter-clonal variations in metabolic framework through the gainful assimilation of *Ri* T-DNA mediated insertional mutagenesis. Such attributes not only broadens the range of substrate adaptability but also modulate the regio and stereo-selective reaction specificity and thereby renders this system as a potent biotransformation tool in medicinal and therapeutic chemistry [25]. Several reports document the excellent biotransformation capabilities of hairy roots which consistently catalyze reactions in a stereospecific manner, resulting in chirally pure products [23,26]. It is however pertinent to mention that HR mediated biotransformation of artemisinin is less explored and till date only two reports of its conversion to deoxy derivative are available through the use of two different plant systems, *i.e.*, *Cyanotis arachnoidea* [27] and *Rheum palmatum* L. [28], which leaves ample scope for further exploration.

The necessity for identification of novel targets through diversity-based generation of molecules has already been unanimously acknowledged which can ideally combat the rapid emergence of parasite resistance [6]. Accordingly, the structural modifications of the functional groups of artemisinin holds much promise in fighting against drug resistance malaria. Under such circumstances, the accredited uniqueness of hairy root cultures in performing regio-specific modifications [26], which are otherwise arduous to carry out by microorganisms or synthetic chemical methods, further reiterates the potential of such exploration involving hairy roots of diverse plant systems with regard to artemisinin [23].

The present study explores the competence of the preselected elite HR clones of three medicinally important plants, *i.e.*, *Atropa belladonna, Hyoscyamus muticus* and *Ocimum basilicum*, for the biotransformation of artemisinin. This communication reports the first successful hairy-root mediated biotransformation of artemisinin (**1**) to 3-α-hydroxy-1-deoxyartemisinin (**2**) and 4-hydroxy-9,10-dimethyloctahydrofuro-(3,2-i)-isochromen-11(4*H*)-one (**3**), deduced through spectroscopic analysis (NMR/MS) and X-ray crystallography. During the present course of study, the *in-silico* and *in-vitro* bioactivity analysis of the derivatives revealed encouraging activity profile of compound (**2**) with respect to antiplasmodial activity coupled with notable TNF lowering potency.

## 2. Materials and methods

### 2.1. General experimental procedures

$^{1}H$, $^{13}C$ and 2D NMR spectra were recorded using Bruker Avance 300 MHz spectrometer and the chemical shifts ($\delta$) were expressed in ppm with reference to TMS as internal standard. ESI–MS data were obtained on Shimadzu LC–MS system after dissolving compound in acetonitrile. IR spectra were measured by Spectrum BX Perkin Elmer and Spectronic® Genesys™, respectively.

### 2.2. Hairy root cultures

Two pre-selected hairy root clones of *A. belladonna* [29] and *H. muticus* [30] were used for the present study, which were maintained through sub-culturing in 1/2 strength Murashige and Skoog medium [31] supplemented with 3% sucrose (pH 5.88) and incubated with continuous shaking on a rotary shaker (80 rpm) at 25 ± 1 °C under dark condition. Additionally, one recently established hairy root clone of *O. basilicum*, having rapid growth potential and *rol* positive (both B and C) traits (data not presented), had also been utilized for the present biotransformation study following its maintenance under the aforesaid conditions.

### 2.3. Biotransformation procedures and Isolation of transformed products

The substrate (**1**), was dissolved in MeOH (40 mg/mL) and 1.0 mL of the solution was added to 50 mL of half-strength MS (3% sucrose) liquid media. These feeded media were dispensed in 2 weeks old hairy root cultures (~5.0 g FW) which were subsequently incubated at 25 ± 2 °C on rotary shaker (80 rpm) in dark. Two controls (substrate control and culture control) were also established, using MeOH instead of DMSO, following our previously reported protocol [26]. All the experiments were repeated thrice with three replicates for each category.

After co-incubation with the substrate, the cultures were harvested, the hairy root tissues were separated from the media and each were extracted with ethyl acetate in triplicates as per our earlier reported protocol [26]. The extracts were subjected to TLC analysis (silica gel 60 $F_{254}$ Plate) using the optimized solvent systems (Diethyl ether: Hexane:: 7:3) followed by the UV detection at 254 nm and after spraying with anisaldehyde solution for visualization of the transformed products.

The time course study was performed in triplicate at weekly intervals (from 7 to 21 days) and quantification of the biotransformed products in both the media and HR tissues of all the three plant systems were carried out through HPTLC following our earlier reported method [29].

Consequently, for the isolation of the biotransformed products, the ethyl acetate extracts (250 mg) were subjected to column chromatography on silica gel (20 g, 60–120 mesh, 1 × 20 cm glass) and was eluted with increasing polarity mixture of ethyl acetate-hexane. The fractions collected in 5% ethyl acetate in hexane yielded compound (**2**) (48 mg), while that collected in 8% ethyl acetate in hexane gave compound (**3**) (22 mg). The structure of the isolated biotransformed products were elucidated by 1D/2D NMR, ESI–MS and further validated through X-ray crystallography.

#### 2.3.1. 3-α-Hydroxy-1-deoxyartemisinin (*2*)

White crystalline solid; $^{1}H$ NMR ($CDCl_3$, 300 MHz) and $^{13}C$ NMR ($CDCl_3$, 75 MHz) see Table 1. ESI–MS 282.3.

#### 2.3.2. 4-Hydroxy-9,10-dimethyloctahydrofuro-(3,2-i)-isochromen-11(4H)-one (*3*)

White crystalline solid; $^{1}H$ NMR ($CDCl_3$, 300 MHz) and $^{13}C$ NMR ($CDCl_3$, 75 MHz) see Table 1. ESI–MS 240.3.

### 2.4. X-ray crystallographic data

Single crystals of (**2**) were obtained by slow evaporation from chloroform/methanol mixture. Diffraction data were collected on Bruker AXS SMART APEX diffractometer using Mo