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# Marchantin A, a macrocyclic bisbibenzyl ether, isolated from the liverwort *Marchantia polymorpha*, inhibits protozoal growth *in vitro*

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ABSTRACT

*In vitro* anti-plasmodial activity-guided fractionation of a diethyl ether extract of the liverwort species *Marchantia polymorpha*, collected in Iceland, led to isolation of the bisbibenzyl ether, marchantin A. The structure of marchantin A (**1**) was confirmed by NMR and HREIMS. Marchantin A inhibited proliferation of the *Plasmodium falciparum* strains, NF54 ($IC_{50}$ = 3.41 μM) and K1 ($IC_{50}$ = 2.02 μM) and showed activity against other protozoan species *Trypanosoma brucei rhodesiense*, *T. cruzi* and *Leishmania donovani* with $IC_{50}$ values 2.09, 14.90 and 1.59 μM, respectively. Marchantin A was tested against three recombinant enzymes (*Pf*FabI, *Pf*FabG and *Pf*FabZ) of the *Pf*FAS-II pathway of *P. falciparum* for malaria prophylactic potential and showed moderate inhibitory activity against *Pf*FabZ ($IC_{50}$ = 18.18 μM). In addition the cytotoxic effect of marchantin A was evaluated. This is the first report describing the inhibitory effects of the liverwort metabolite marchantin A against these parasites *in vitro*.



## Introduction

Diseases caused by unicellular protozoal parasites are a major public health concern for about one billion people worldwide, particularly in tropical countries. *Trypanosoma* (African sleeping sickness and Chagas disease), *Leishmania* (leishmaniasis) and *Plasmodium* (malaria) are genera responsible for considerable medical morbidity and, in the case of malaria, high mortality (Astelbauer and Walochnik 2011; Fidalgo and Gille 2011). Despite years of effort to eradicate these diseases, the treatment options today are few, costly or with adverse effects and in addition parasite resistance against the limited number of drugs available is increasing. With the absence of functional, safe and widely available vaccines (Fidalgo and Gille 2011; Kaye and Aebischer 2011; The RTS 2011), the adequate treatment of these infections is becoming increasingly difficult. This underlines the importance and urgent need to discover new and effective drug candidates and identify novel potential chemotherapeutic targets (Astelbauer and Walochnik 2011; Pradines et al. 2002). The FAS-II pathway of *P. falciparum* has become an interesting target for malaria prophylaxis, as it is crucial for the liver stage, the first of the two infection stages in human host, of malaria parasites. Deletion or inhibition of critical FAS-II elongation enzymes such as *Pf*FabI (enoyl-ACP reductase), *Pf*FabZ (β-hydroxyacyl-ACP dehydratase) and *Pf*FabG (β-ketoacyl-ACP reductase) prevents the proceeding of the subsequent blood stage infection, thus the clinical symptoms of the disease (Min et al. 2008; Vaughan et al. 2009).

Despite a decline of natural products research in pharmaceutical industry during the past several years, a vast majority of the existing chemotherapeutic agents, particularly those used in the control of malaria, are still based on natural product scaffolds. Hence the identification of natural product leads from diverse natural sources will critically augment the search in anti-parasitic drug discovery (Bero and Quetin-Leclercq 2011; Li and Vederas 2009; Newman and Cragg 2012).

Liverworts are primitive terrestrial plants that grow worldwide. They contain cellular membrane-bound oil bodies which elaborate mainly ethereal terpenoids and lipophilic aromatics, as their major chemical constituents. Several biological activities are triggered from these secondary metabolites and various liverwort species have been used in traditional oriental medicine, generally to treat various topical disorders and bacterial infections (Asakawa 2001; Asakawa et al. 2009; Huang et al. 2010). Macrocyclic bisbibenzyls are a family of phenolic compounds belonging to the stilbenoids. They are produced exclusively in liverworts and are attracting

*Abbreviations:* VLC, vacuum liquid chromatography; MA, marchantin A; SI, selectivity index; EGCG, (−)-epigallocatechin gallate; HPLC, high performance liquid chromatography; HREIMS, high resolution electron ionisation mass spectrometry; 5-LOX, 5-lipoxygenase; COX, cyclooxygenase.

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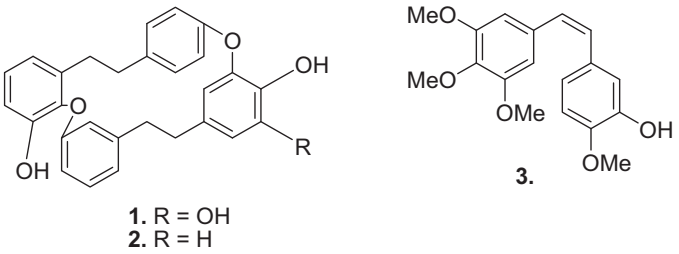


**Fig. 1.** Chemical structures of marchantin A (**1**), marchantin C (**2**) and combretastatin A-4 (**3**).

increasing attention due to their broad range of interesting pharmacological activities (Asakawa et al. 2009).

*Marchantia polymorpha* L. (Marchantiaceae) is a common thallus liverwort species known to produce a wide array of distinctive compounds and several bisbibenzyls, including marchantin A (**1**, Fig. 1). Marchantin A has been found to possess diverse biological and pharmacological activities i.e. anti-bacterial, anti-tumour and anti-leukaemia, anti-oxidant, 5-LOX-, COX- and calmodulin-inhibitory activity (Asakawa et al. 2009; Huang et al. 2010; Iwai et al. 2011; Keser and Nogradi 1995; Schwartner et al. 1995). *M. polymorpha* therefore represented an interesting candidate in a random screening for anti-plasmodial activity of Icelandic lower plants. This liverwort thrives in moist and endolithic areas of Iceland and although it is widely distributed and easily accessible (Johannsson 2002), no records of traditional uses in Iceland were found. In the present study, the *in vitro* effects of marchantin A were evaluated against four parasitic protozoa and against three different enzymes (*Pf*FabI, *Pf*FabG and *Pf*FabZ) involved in the FAS-II pathway of *P. falciparum*. The cytotoxicity against primary mammalian L6 cells was also determined and compared to the corresponding anti-parasitic activities.

## Materials and methods

### *General experimental procedures*

Analytical grade solvents for extraction and HPLC grade solvents for chromatography were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade water was obtained by a Millipore Milli-Q Academic water purification system. 1D and 2D NMR spectra using $CDCl_3$ as a solvent were recorded on a Bruker 400 spectrometer (5 mm BB-1H/D probe-head) at 25 °C, using TMS as an internal standard. HREIMS analysis was performed on an Agilent 1100 system (Supplementary data, S1). Silica gel 60H (Merck) was used for VLC and silica gel 60 F254 (Merck) was used for TLC. Further purification was performed by preparative HPLC (Dionex 3000 Ultimate; pump, UV–VIS detector).

### *Plant material*

The whole plant of *Marchantia polymorpha* L. (Marchantiaceae) was collected in July 2007 in Thverdalur in Adalvik (N66°20.32 W23°03.37), in North-Western Iceland and in September 2009 in mountain Esja close to Reykjavik (N64°12.64 W21°42.81), in South-Western Iceland. The liverwort was identified by biologist Groa Ingimundardottir at the Icelandic Institute of Natural History, and botanist Agust H. Bjarnason. Voucher speciemens (ICEL, catalogue nr. BR-44839 and BR-45237) are deposited at the Icelandic Institute of Natural History, Reykjavik, Iceland. The extracts of the plants from the two collection sites showed uniform chemical contents according to TLC and were pooled.

### *Extraction and isolation of marchantin A*

The air-dried and powdered liverwort material (300 g) was extracted with diethyl ether for one week to obtain a crude extract (7.9 g). The extract was fractionated by VLC on silica gel with an n-hexane–EtOAc gradient (100:0 to 0:100, followed by pure MeOH) to yield twenty-three 250 ml fractions. Fraction 15 (70:30, 0.5 g) was partitioned by liquid–liquid extraction using petroleum ether and 80% aqueous methanol to obtain two fractions (Mp-15-MH and Mp-15-P). The more active fraction, Mp-15-MH, was purified by preparative HPLC (C-18 column, 250 mm × 21.1 mm, 5 μm, Phenomenex Luna), eluting with MeCN–$H_2O$ (50:50), a flow rate of 10 ml/min, detection at 210 and 254 nm at room temperature. The active component ($t_R$ = 38 min) was identified as the known compound, marchantin A (**1**). Purity of marchantin A was analysed by analytical HPLC on a RP column (G.L. Sciences, Inc., Herbal medicine, C-18, 4.6 mm × 250 mm), a solvent system of MeOH:$H_2O$ (70:30), flow rate of 1 ml/min, UV-detection at 210 nm at 25 °C and injection volume 10 μl.

### *In vitro P. falciparum assays*

Anti-plasmodial activity of *M. polymorpha* extract and fractions was determined against erythrocytic stages of chloroquine-sensitive 3D7 strain of *P. falciparum* (Ziegler et al. 2002) whereas *in vitro* activity of marchantin A against erythrocytic stages of *P. falciparum* was determined by a modified [$^3$H]-hypoxanthine incorporation assay (Scala et al. 2010) using the chloroquine sensitive strain NF54 and the chloroquine- and pyrimethamine-resistant strain K1. The standard drug used as a positive control was chloroquine.

### *Anti-protozoal activity assays*

All cells were cultured on 96-well micro-titre plates and incubated at 37 °C under a 5% $CO_2$ atmosphere. The parasites tested were *Trypanosoma brucei rhodesiense* STIB 900 strain, *Trypanosoma cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene and *Leishmania donovani* strain MHOM/ET/67/L82. Bioassays were run as described by Scala et al. (2010). Experiments included untreated controls, serial compound dilutions covering a range from 90 to 0.123 μg/ml and positive control drugs melarsoprol, benznidazole and miltefosine respectively. The $IC_{50}$ values were calculated from sigmoidal inhibition dose–response curves and each $IC_{50}$ value obtained is the mean of at least two separate experiments performed in duplicate (the variation is maximum 20%).

### *Cytotoxicity against L6-cells*

The cytotoxicity assay was performed as described (Scala et al. 2010). Briefly, L-6 cells (a primary cell line derived from rat skeletal myoblasts) were incubated on 96-well micro-titre plates for 72 h with drug dilutions ranging from 90 to 0.123 μg/ml. The cell viability was assessed by resazurin staining and absorbance was read with a Spectramax Gemini XS microplate fluorometer using wavelengths 536 and 588 nm. Data were analysed using the microplate reader software Softmax Pro. Each $CC_{50}$ value obtained is the mean of at least two separate experiments performed in duplicate and the standard drug used was podophyllotoxin.

### *PfFAS-II enzyme inhibition assays*

Expression and purification of the *Pf*Fab enzymes as well as the inhibition assays were performed as described (Tasdemir et al. 2006b). The enzyme inhibition was monitored using a Perkin Elmer