https://dx.doi.org/10.21577/0103-5053.20210167

*J. Braz. Chem. Soc.*, Vol. 33, No. 5, 483-490, 2022


Article

# Natural and Semisynthetic Triterpenes from *Combretum leprosum* Mart. with Antiplasmodial Activity

***Guilherme M. Passarini,*** [*,a,b,c] ***Amália S. Ferreira,*** [b] ***Leandro S. Moreira-Dill,*** [c,d] ***Fernando B. Zanchi,*** [a,d,e] ***Aurileya G. de Jesus,*** [b] ***Valdir A. Facundo*** [c,e] ***and Carolina B. G. Teles*** [a,b,f]

[a]*Programa de Pós-Graduação em Biologia Experimental, Universidade Federal de Rondônia (PGBIOEXP), 76801-059 Porto Velho-RO, Brazil*

[b]*Plataforma de Bioensaios de Malaria e Leishmaniose, Fundação Oswaldo Cruz, Rondônia (FIOCRUZ RO), 76812-245 Porto Velho-RO, Brazil*

[c]*Laboratório de Pesquisa em Química de Produtos Naturais, UNIR, 76801-059 Porto Velho-RO, Brazil*

[d]*Laboratório de Bioinformática e Química Medicinal (FIOCRUZ RO), 76801-059 Porto Velho-RO, Brazil*

[e]*Programa de Pós-Graduação, Rede Bionorte/Rondônia, 76804-421 Porto Velho-RO, Brazil*

[f]*Instituto Nacional de Epidemiologia na Amazônia Ocidental (EPIAmO), Rua da Beira, 7671, BR 364, km 3.5, Bairro Lagoa 76812-245 Porto Velho-RO, Brazil*

Malaria is responsible for thousands of deaths each year. Currently, artemisinin combination therapy (ACT) is used as first-choice medication against the disease. However, the emergence of resistant strains prompts the search for alternative compounds. The present study aimed to investigate the antiplasmodial activities of natural triterpenes (compounds **1** and **2**), and semisynthetic derivatives **1a**, **1b**, **1c**, and **1d**. Antiplasmodial assays were carried out using the SYBR Green technique, whereas cytotoxicity was evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. Hemolytic assays were performed on human erythrocytes. An *in silico* analysis of the compounds against *Pf*ENR (*Plasmodium falciparum* 2-*trans*-enoyl-reductase) was carried out by molecular docking. Experiments with **1**, and its derivatives against *P. falciparum* showed that **1a** was very similar in terms of biological activity to compound **1** (half maximal inhibitory concentration ($IC_{50}$) ca. 4 μM), whereas **1b**, **1c**, and **1d** had reduced antiplasmodial activities ($IC_{50}$ between 8-103 μM). The selectivity indexes of **1** and **1d** for HepG2, and Vero cells were > 10. Docking results partially agreed with the *in vitro* experiments, with **1** and **1c** having the best and worst affinities with *Pf*ENR, respectively. In conclusion, the results showed that **1** and **1d** may serve as biotechnological tools in the development of antimalarial drugs.

**Keywords:** *Combretum*, triterpenes, antiplasmodial, *Plasmodium*, enoyl-reductase

## Introduction

Malaria is a disease transmitted by female mosquitos of the genus *Anopheles*, in which the etiological agents are some protozoan species of the genus *Plasmodium*. To date, five species are known to infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*.[1] This disease is responsible for an enormous social burden, especially in sub-Saharan Africa.[2] According to the World Health Organization (WHO), the estimated number of cases of the disease for 2018 was 228 million.[3] The incubation period of the parasite varies from species to species, with the average being approximately two weeks. The symptoms are nonspecific, such as fever, myalgia, malaise, headache, and vomiting. Malaria may also present in a severe form, mainly caused by *P. falciparum*, which affects mostly immunocompromised patients, and can cause extensive damage to organs such as the brain, kidneys, and lungs.[4]

*e-mail: guilhermepassarini@hotmail.com
Editor handled this article: José Walkimar M. Carneiro

Regarding therapeutics, there are currently no vaccines available for the disease, with pharmacological treatment being the only intervention to treat the disease. The artemisinin combination therapy (TCA) is the standard treatment in most cases of *P. falciparum* malaria,[5] replacing chloroquine due to the increasing appearance of resistant strains. However, with the increasing occurrence of resistance to artemisinin-based therapy, there is a need to search for new classes of drugs that can treat infections caused by future resistant strains.[6] Natural products, mainly those derived from medicinal plants, played an important role in malaria chemotherapy, in which two medicinal plants, *Cinchona officinalis*, and *Artemisia annua*, resulted in two generations of antimalarial drugs: quinine and its derivatives, and artemisinin and its derivatives.[7] Moreover, numerous medicinal plants are used in Africa by traditional populations to treat the disease, evidencing their potential for the discovery and development of antiplasmodial compounds.[8]

The above context motivates the search for compounds that can act on the parasite, reducing the impact of their resistance on malaria control and eradication programs. In this sense, natural products have been widely studied as pharmacological agents for the treatment of various diseases. Newman and Cragg[9] show, for example, that a great proportion of the Food and Drug Administration (FDA)-approved drugs from 1981 to 2014 resulted directly or indirectly from natural products, especially medicinal plants.

*Combretum leprosum*, a species popularly known as "*mofumbo*", has a restricted distribution to South America, and is found in Brazil, Bolivia, and Paraguay. Its distinctive feature is the presence of whitish or yellowish, lepidote indumentum.[10] Concerning this species, several studies have demonstrated its potential against a variety of diseases, including leishmaniasis,[11-13] cell proliferation,[14] cancer,[15] and bacterial infections.[16] Based on this context, the present study aimed to investigate the antiplasmodial and cytotoxic activities of natural triterpenes (**1** and **2**) and semisynthetic derivatives of **1**, as well as theoretically determine their binding affinities with the *P. falciparum* enzyme 2-*trans*-enoyl-ACP-reductase (*Pf*ENR) by molecular docking simulations.

## Experimental

### Obtainment of natural and semisynthetic triterpenes

Two natural triterpenes, 3β,6β,16β-trihydroxy-lup-20(29)-ene (compound **1**), and arjunolic acid (compound **2**) were previously isolated by silica gel chromatography assisted by thin-layer chromatography, from the flowers, and leaves of *C. leprosum*. The natural compounds, along with the four semisynthetic derivatives: the diacetylated triterpene (compound **1a**), diacetylated oxidated triterpene (compound **1b**), triacetylated triterpene (compound **1c**) and trioxidized triterpene (compound **1d**) were obtained from the studies of Facundo *et al.*[17,18] The compounds are presented in Figure 1.

### *In vitro* assays against *P. falciparum*

In the antimalarial activity assays, blood forms of a chloroquine-resistant *P. falciparum* (W2) clone were used. The parasites were cultured in human red blood cells under conditions established by Trager and Jensen[19] with minor modifications. Cultivation was performed in culture flasks (TPP) with 2% hematocrit diluted in Roswell Park Memorial Institute (RPMI) 1640 culture medium (Sigma-Aldrich, St. Louis, USA) supplemented with 25 mM Hepes (Sigma-Aldrich, West Chester, PA, USA), 21 mM sodium bicarbonate (Sigma-Aldrich, Waltham, USA), 11 mM

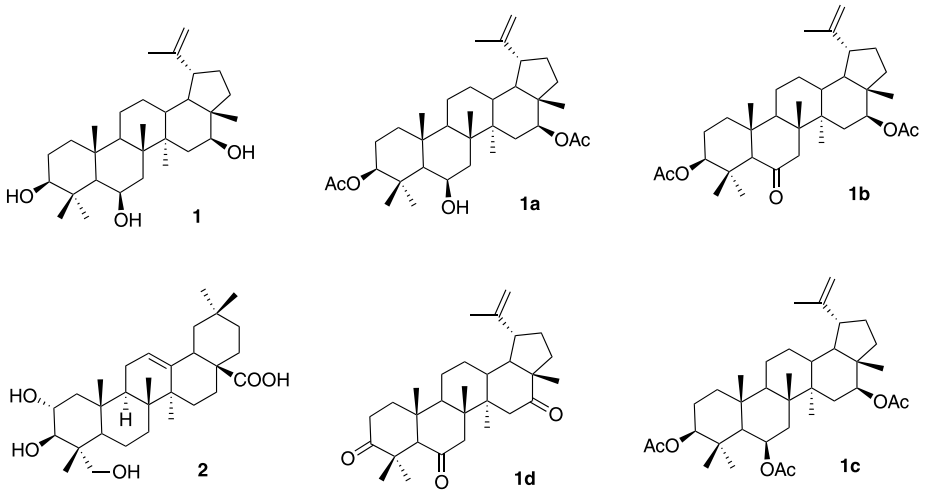


**Figure 1.** Compounds **1**, **2** and semisynthetic derivatives **1a-1d**.

glucose (Sigma-Aldrich, USA), 40 μg $mL^{-1}$ gentamycin, and 10% (v/v) albumax.[19] Cultures were maintained in desiccators at 37 ºC or in a gaseous mixture containing 5% $O_2$, 5% $CO_2$, and 90% $N_2$.

### Synchronization of *P. falciparum* culture

The predominant young trophozoite cultures that were used in the antiplasmodial assays were obtained through synchronization with sorbitol as described by Lambros and Vanderberg.[20] Hematocrit and parasitemia were adjusted to 5 and 0.5%, respectively.

### *In vitro* antiplasmodial assays

Parasite cultures were distributed in 96-well microplates by adding 180 μL *per* well of RPMI culture medium containing: 0.5% parasitemia and 2% hematocrit for the SYBR Green test. Before the addition of the parasite suspension, 20 μL of *C. leprosum* compounds to be tested were added to the test plate in triplicate and at different concentrations (100-1.56 μM). The positive control consisted of artemisinin (17.7 nM) whereas the negative control consisted of infected red blood cells without the addition of drugs. The blank consisted of uninfected red blood cells with no treatment. Plates were incubated at 37 °C for 48 h. After the incubation period, the fluorescence test was performed according to Lambros and Vanderberg.[20] After, the SYBR Green fluorimetric assay was conducted to evaluate antiplasmodial activity.[21] Plates containing the tested compounds and parasites had the supernatant discarded and for rinsing of the red cells, 100 μL of 1× phosphate buffer solution (PBS) was added *per* well and then centrifuged at 700 g for 10 min. Subsequently, the supernatant was discarded again and 100 μL of the lysis buffer (20 mM Tris, pH 7.5, 5 mM ethylenediamine tetraacetic acid (EDTA), 0.008%, m/v saponin, 0.08%, v/v Triton X-100) was added with SYBR Green I (2 μL of SYBR to each 10 mL of buffer) homogenized and transferred to the 96-well plate with 100 μL of 1× PBS in which the reading was performed. After incubation for 30 min at room temperature, the fluorescence was measured in a fluorometer with excitation of 485 nm and an emission of 590 nm in a gain of 100.

### Determination of the $IC_{50}$

The $IC_{50}$ corresponds to the concentration at which the compound causes 50% inhibition of parasite growth. We established that compounds with $IC_{50}$ values less than or equal to 25 μM were considered active, and below this threshold, inactive.[22] The $IC_{50}$ was determined by the software Origin (OriginLab Corporation, Northampton, MA, USA),[23] through non-linear regression of the concentrations tested.

### *In vitro* cytotoxicity assays

### Culture of the HepG2 and Vero cells

The adherent cell lines HepG2 and Vero were cultured as recommended.[24] They were maintained in 75 $cm^2$ culture flasks supplemented in RPMI containing 5% fetal bovine serum (FBS). Cells were maintained in 5% $CO_2$ in an incubator at 95% humidity at 37 °C and the medium of the flasks was replaced every two days.

### Preparation of the test plates

For cytotoxicity assays, cells were washed with medium without FBS, treated with 1 mL of 0.25% trypsin-EDTA (Gibco/Invitrogen) and incubated at 37 °C for 5 to 10 min. 9 mL of complete medium was added to the resulting content of the trypsinization, followed by centrifugation at 1500 rpm for 5 min at room temperature. The supernatant was discarded and the pellet was resuspended in complete medium containing 10% FBS. After counting in a Neubauer's chamber, the suspension was adjusted to $2 \times 10^4$ *per* mL and 180 μL added to each well of the microplate. The cells were incubated for 12 to 16 h in a $CO_2$ incubator at 37 °C for the adhesion process. Then, 20 μL of complete medium containing different concentrations (500-3.16 μM) of the compounds were added to the wells of the microplate. The plates were incubated for 72 h at 37 ºC, 5% $CO_2$, and 95% humidity. The negative control consisted of untreated cells, and the positive control consisted of lysis buffer. The dimethyl sulfoxide (DMSO) concentration did not exceed 1%. Due to the insufficient amount of samples, only the two natural triterpenes (**1** and **2**), and compound **1d** were tested for their cytotoxic activity.

### Preparation of the test plates

### MTT colorimetric assay

Cytotoxicity was determined by the MTT method (3-((4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium).[25] After the incubation period, 20 μL of MTT at 5 mg $mL^{-1}$ in PBS in each well, and the plates were incubated for 4 h at 37 ºC. At the end of that period, the culture medium, along with excess MTT, was discarded and then 100 μL DMSO (Sigma-Aldrich, St. Louis, USA) was added to each well. Optical reading was performed using a microplate spectrophotometer at 570 nm.

### Determination of $CC_{50}$

The cytotoxic concentration to 50% of the cells ($CC_{50}$)

was determined from non-linear regression of the tested concentrations using the software Origin, as established for antiparasitic tests.[23]

### Selectivity index

The selectivity index (SI) was calculated as the quotient between the $CC_{50}$ and the $IC_{50}$ of the respective compound. We considered that compounds whose selectivity index was greater than 10 were selective/non-toxic, whereas compounds with SI less than 10 were considered non-selective/toxic.[26]

### Hemolysis assay

The hemolytic test was performed as previously established.[27] The natural compound **1** and the semisynthetic derivatives **1a**, and **1d** were screened for their hemolytic activity for being active against *P. falciparum*. The concentrations tested were the same as those of the antiplasmodial assay. After dilution of the samples, 20 μL of them were added to 180 μL of an erythrocyte suspension to a 1% hematocrit in round-bottom 96-well microplates. A solution of saponins (0.05%) constituted the positive control (Sigma-Aldrich, St. Louis, USA) After preparation, the microplate was incubated for 30 min at a temperature of 37 °C with gentle shaking every 5 min. Subsequently, the plate was centrifuged for 10 min at 1,500 rpm and the supernatant was transferred to another flat-bottom 96-well microplate. Absorbance reading was performed at 540 nm on a microplate spectrophotometer.

### Molecular docking

The triterpenes tested *in vitro* against the W2 of *P. falciparum* were evaluated *in silico* for their binding affinity with the *P. falciparum* enzyme 2-*trans*-enoyl-ACP-reductase (*Pf*ENR). Since this enzyme is involved in the parasite's fatty acid biosynthesis FAS-II, which is not present in humans, it constitutes a promising target for screening antimalarial compounds.[28] Since there is previous evidence[29] that triterpenes might act as *Pf*ENR inhibitors, we also decided to evaluate the compound of the above-mentioned study, which presented *in silico*, and *in vitro* inhibition against the *Pf*ENR, the pentacyclic triterpene celastrol, in order to compare its results with those of the triterpenes evaluated in the present study.[29]

After the obtainment of the structures of all triterpenic analogs, the compounds were evaluated for their *in silico* affinity for 2-*trans*-enoyl-ACP-reductase through molecular docking simulations, in which the lowest ΔG (Gibbs free energy) values, resulting from various positions of the screened compounds, were evaluated. The software used for the evaluation of the *in silico* interaction was AutoDock 4.0.[30] The tridimensional structure of *Pf*ENR was retrieved from the PDB (Protein Data Bank), PDB code 3LT0.

The simulation grid was positioned at the active site with a size of 170 × 170 × 170 points and centered at 45.515, 84.230 and 39.810 Å for the coordinates x, y, and z. The Lamarckian genetic algorithm was followed with protocol consisted of 10 independent runs *per* ligand, using an initial population of 150 randomly placed individuals, with $2.5 \times 10^6$ energy evaluations, a maximum number of 27000 iterations, a mutation rate of 0.02, a crossover rate of 0.80, and an elitism value of 1. The analyses and visualizations were generated by the software USCF chimera.[31]

## Results

The antiplasmodial assays showed that the natural compound **1** was the most active for *P. falciparum* with an $IC_{50}$ = 4.4 μM. The derivatives **1a** and **1d** were also considered active, with $IC_{50}$ values of 4.56 and 8.9 μM, respectively. Compound **2**, **1b** and **1c** were considered inactive ($IC_{50}$ > 25 μM).

Concerning the comparison of the natural triterpene and its derivatives, it was observed that compound **1** obtained almost the same degree of activity as its diacetylated derivative (**1a**) at positions 3 and 16 of the molecule (both with $IC_{50}$ < 5 μM). Compound **1d** was also considered active, although it has its potency decreased (Table 1). These three compounds were assessed for their hemolytic activity in human red blood cells, in which no hemolytic activity was found (data not showed). Compounds **1b** and **1c** were considered inactive ($IC_{50}$ ≥ 25 μM). For the natural triterpene **1** and its derivative **1a**, it was possible to determine the $IC_{50}$ at a range from 13.07-0.19 μM. However, for the other triterpenes (**1b**, **1c**, and **1d**), the dose-response for the determination of the $IC_{50}$ was established with concentrations ranging from 104.62-1.56 μM.

Since the natural triterpene **1**, **1a**, and **1d** met the activity criteria of < 25 μM, they were investigated for their hemolytic potential to assess whether the antiplasmodial effects were due to hemolytic effects or direct action against the parasite. The results demonstrated (data not shown) that none of these compounds presented hemolysis for human red blood cells (RBC's) (13.07 to 0.19 μM for compound **1** and 204.62-3.19 μM for compound **2**).

### Cytotoxic assays

Cytotoxicity assays were performed concurrently with the antiplasmodial assays, with some of the compounds being tested against *P. falciparum*. Cytotoxicity tests for

**Table 1.** Antiplasmodial activity ($IC_{50}$), cytotoxicity ($CC_{50}$) and selectivity index (SI) of the two natural triterpenes and synthetic derivatives against *P. falciparum*

| Compound | $IC_{50}$ / µM | Vero ($CC_{50}$) / µM | HepG2 ($CC_{50}$) / µM | Selectivity index (SI)[a] | Selectivity index (SI)[b] |
|---|---|---|---|---|---|
| **1**[c] | 4.4 ± 2.33 | 47.22 ± 20.66 | 50.35 ± 0.32 | 10.73 | 11.44 |
| **1a**[c] | 4.56 ± 0.41 | – | – | – | – |
| **1b** | 76.25 ± 3.08 | – | – | – | – |
| **1c** | 103.38 ± 0.26 | – | – | – | – |
| **1d**[c] | 8.9 ± 2.7 | ≥ 500 | ≥ 500 | ≥ 56.18 | ≥ 56.18 |
| **2** | 52.7 ± 1.92 | 151.31 ± 10.51 | 141.53 ± 2.31 | 2.87 | 2.68 |

[a]Calculated in relation to Vero; [b]calculated in relation to HepG2; [c]compounds that were not found to be hemolytic to human RBC's. All compounds were tested in triplicates. Artemisinin was used as the positive control. Mean obtained from two independent experiments, each one with samples in triplicate. For cytotoxicity assays, all compounds were tested in triplicates. Lysis buffer was used as the positive control.

**Table 2.** Binding affinities of the triterpenes and triclosan against the *P. falciparum Pf*ENR

| Ligand | Binding energy / (kcal $mol^{-1}$) | Ki / µM | No. of H-bonds | Protein residues (H-bonds) | Ligand atoms (H-bonds) |
|---|---|---|---|---|---|
| Triclosan | –8.66 | 0.45136 | 4 | Tyr277, NAD | O |
| Celastrol | –6.58 | 9.54 | 0 | – | – |
| **1** | –6.8 | 10.38 | 1 | NAD | O |
| **1d** | –6.63 | 13.70 | 1 | NAD | O |
| **2** | –6.49 | 17.50 | 2 | NAD, Asn218 | O |
| **1b** | –3.55 | 2480 | 1 | Ala219 | O |
| **1a** | –2.7 | 10440 | 0 | – | – |
| **1c** | 3.8 | – | 1 | Arg318 | O, H |

Ki: inhibition constant; H-bonds: hydrogen bonds; Tyr: tyrosine; Asn: asparagine; Ala: alanine; NAD: nicotinamide adenine dinucleotide.

the HepG2 and Vero cell lines were performed in parallel to antiparasitic tests, with the same compounds tested against *Plasmodium falciparum*.

The cytotoxicity results showed that compounds **1**, **2**, and **1d** were inactive against the evaluated cell lines when considering the toxicity threshold of 25 µM (Table 1). **1a**, **1b**, and **1c** were not tested due to an insufficient amount of samples. Compounds **1** and **1d** presented selectivity indexes > 10 for both cell lines.

### Molecular docking simulations

The molecular docking simulations were used to predict the binding affinity of the triterpenes evaluated *in vitro*, against the enzyme *Pf*ENR (Figure 2). The results demonstrated that the natural triterpene **1** obtained the highest binding affinity amongst the triterpenes of the present study, with a ΔG of –6.8 kcal $mol^{-1}$. Celastrol obtained an estimated ΔG slightly higher than that of compound **1**, being = –6.58 kcal $mol^{-1}$. The rest of the compounds obtained lower estimated binding affinities, with **1c** obtaining a positive ΔG value. The reference inhibitor triclosan obtained the best binding affinity, with an estimated ΔG of –8.66 kcal $mol^{-1}$ (Table 2).

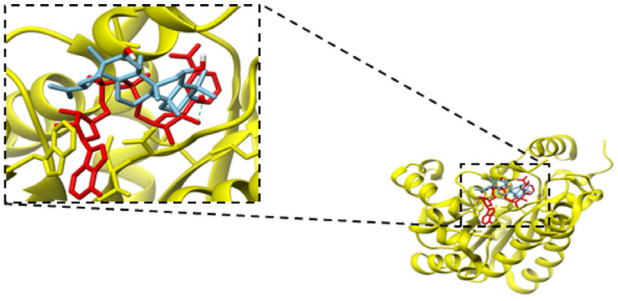

**Figure 2.** Molecular docking between *Pf*ENR (marked in yellow) and compound **1** (marked in light blue). The hydrogen bond is marked as a green traced line.

## Discussion

The literature shows that there are several antiplasmodial studies[32-35] in which natural or semi-synthetic triterpenes are active against *P. falciparum in vitro*, evidencing the importance of prospecting and conducting antiplasmodial tests with this class of substances. The biological activity of the semisynthetic derivatives of the natural triterpene against *P. falciparum* showed that the substitution of all hydroxyls by acetyl groups results in loss of activity for the molecule. Compound **1a**, which had acetyl groups replacing the hydroxyls at positions 3 and 16 but not at position 6,

maintained an $IC_{50}$ very similar to that of the natural compound **1** ($IC_{50}$ for **1** = 4.4 μM; $IC_{50}$ for **1a** = 4.56 μM). On the other hand, compound **1c**, which had two of its hydroxyls replaced by acetyl groups and one oxidized, had its activity notably decreased than those of the parental compound **1** and the diacetylated compound **1a**, with an $IC_{50}$ of 76.25 μM. Concerning compound **1d**, which had its hydroxyls oxidized in all 3 positions, its potency slightly decreased, with an $IC_{50}$ of 8.9 μM. These results suggest that, for the natural triterpene (compound **1** in the present study), the hydroxyl at position C-6 is crucial for biological activity. The presence of this hydroxyl may be crucial for interaction with the active site of the enzyme, for example, via the formation the hydrogen bonds. However, further studies are yet to be conducted to properly elucidate the mechanistic nature of this structure-activity relationship.

Natural compound **2** (arjunolic acid) was not considered active against *P. falciparum*, with an $IC_{50}$ = 52.7 μM. A previous study by da Silva *et al.*[36] agrees with our observations, with an $IC_{50}$ = 61.18 μM for arjunolic acid being reported for *P. falciparum*.

The importance of hydroxyls and other polar functional groups in the activity of lupane triterpenes for *P. falciparum* is also observed in other studies. It was found that two lupane triterpenes (messagenic acid A and messagenic acid B), both bearing carboxylates and *p*-coumarate groups in their radicals, obtained smaller $IC_{50}$'s (massagenic acid A, $IC_{50}$ = 2.42 μM, massagenic acid B, $IC_{50}$ = 6.14 μM) than lupeol, a lupane having only one hydroxyl, which was not active at 20 μg $mL^{-1}$ ($IC_{50}$ not shown).[37] Betulone and lupenone, two other lupane triterpenes, were tested against a chloroquine-resistant strain (K1) of *P. falciparum* in a hypoxanthine incorporation assay, yielding $IC_{50}$ of 3 and 4.7 μM, respectively. The difference between the two molecules was the presence of an alcohol (polar group) in a betulone radical, and the presence of a methyl (non-polar group) instead of the same lupenone radical.[38]

Some of the compounds here evaluated (**1**, **1a**, and **1d**) were previously investigated for their antileishmanial activity against *Leishmania amazonensis* promastigotes.[12] In this previous study, the authors observed that the natural compound **1** was the most active against *L. amazonensis*, agreeing with the present study. However, they found that the replacement of two acetyl groups at positions 3 and 16 rendered the compound inactive. Finally, they observed that when the compound was trioxidized (compound **1d**), it had an $IC_{50}$ value similar to compound **1**, contrasting with the present study.

Due to the hemolytic reports of triterpenes in the literature, and because the triterpenes of the present study were tested against erythrocytic stages of *P. falciparum*, a hemolytic assay was performed for the three compounds that complied with the threshold established for biological activity against *P. falciparum*: **1**, **1a**, and **1d**.[39,40] The results demonstrated that the compounds did not cause hemolysis toward human erythrocytes, in contrast to the positive control, consisting of the saponin solution. This fact is probably due to the presence of few polar groups in the structures of the triterpenes and/or absence of sugars coupled to these, a characteristic that would confer amphiphilicity to these molecules. These results also suggest that the compounds act directly against the blood trophozoites, rather than causing their death by destroying their host cells.

Parallel to the antiplasmodial assays, cytotoxicity assays were performed to assess the possible damage that these compounds might cause on human cells. In this work, tests were performed against the following cell lines: HepG2, because they are hepatocytes, cells that naturally metabolize drugs and cells where *Plasmodium* spp. replicates in a phase of its cycle in the vertebrate host;[41] and Vero cells, because they are a non-tumoral lineage that preserves characteristics of their original cells, besides being renal, playing a key role in the excretion of metabolites and being responsible for the production of EPO (erythropoietin).[42] Studies in literature usually report cytotoxic activities from triterpenes.[43] Interestingly, in our study, none of the evaluated triterpenes were classified as being cytotoxic for the two evaluated cell lines ($CC_{50}$ > 25 μM), although it does not mean they are completely devoid of toxicity since some of the $CC_{50}$'s obtained were as low as 50 μM.

In fact, a study that investigated the cytotoxic activity of **1** against a wide variety of cancer cell lines showed that this triterpene obtained a $CC_{50}$ of 13.35 μM for the HepG2 cell line by the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide)) method.[15] This study also found that the triterpene is capable of inducing apoptosis in cancer cells, increasing the population of sub-G1 cells. Its mechanism of action in cancer cells appears to be the generation of free radicals and the induction of mutations. A study by Lacouth-Silva *et al.*[44] showed that it is also cytotoxic to PBMC (peripheral blood mononuclear cells), with an estimated $CC_{50}$ of 9.4 μM after 15 h of incubation with compound **1**. Compound **2** was considered to be cytotoxic against neither the HepG2 nor the Vero cells, with $CC_{50}$ values of 141.53 and 151.31 μM, respectively. Indeed, some studies show that arjunolic acid has cytoprotective effects both *in vitro* and *in vivo*.[45,46]

Interestingly, compound **1d** has its cytotoxicity substantially decreased against HepG2 and Vero cells, with $CC_{50}$ values being ≥ 500 μM for both cell lines. Combined

with its $IC_{50}$, the selectivity index of **1d** was the highest of the evaluated compounds (≥ 56.18). Such a result encourages further analyses to investigate its antiplasmodial activity *in vivo*.

Since there is evidence that pentacyclic triterpenes may inhibit the *Pf*ENR, we screened all six compounds in a molecular docking simulation against this enzyme.[29] We also included the validated compounds celastrol and triclosan in order to compare their results with the evaluated compounds. Such a computational approach has demonstrated valuable usefulness and may direct compound selection in a drug discovery pipeline.[47] Compounds **1** and **1c** obtained the best and worst binding affinities of the six compounds screened respectively, which agree with the *in vitro* experiments, where these compounds obtained the lowest and highest $IC_{50}$ values against *P. falciparum in vitro*.

Thermodynamically, a ligand and a macromolecule represent a reaction system, where ΔG values are negative if the reaction occurs favorably and the system is stable, and positive if the reaction is unfavorable. The positive values found for **1c** (3.8 kcal $mol^{-1}$) indicate that the complex protein-ligand does not form a stable interaction and the reaction does not occur spontaneously and that therefore, this compound does not interfere with the activity of *Pf*ENR.

## Conclusions

Despite the *in silico* interaction of most triterpenes with *Pf*ENR, further *in vitro* experiments with the compounds, *Pf*ENR, and its substrate are needed to confirm whether or not they are acting via *Pf*ENR inhibition. In conclusion, the results of this study demonstrated that the natural triterpene **1**, and its derivative **1d** presented interesting antiplasmodial activity *in vitro*, and interacted *in silico* with *Pf*ENR, and thus may serve as biotechnological tools in the development of antimalarial drugs.

## Supplementary Information

Supplementary data (graphs containing the parasite growth inhibition data of the natural compound and the four derivatives) are available free of charge at http://jbcs.sbq.org.br as PDF file.

## Acknowledgments

We are grateful to EpiAmo for granting financial resources to this study and CNPq for the scholarship concession. The authors are also grateful to Malaria and Leishmaniasis Bioassays Platform - Fiocruz (Rondônia).

### Author Contributions

Guilherme M. Passarini was responsible for conceptualization, investigation, project administration, writing original draft; Amália S. Ferreira for conceptualization, investigation, methodology; Leandro S. Moreira-Dill for investigation, methodology, writing review and editing; Fernando B. Zanchi for conceptualization, investigation, software, writing review and editing; Aurileya G. de Jesus for investigation, methodology; Valdir A. Facundo for conceptualization, investigation, methodology, project administration, supervision and Carolina B. G. Teles for conceptualization, project administration, resources, supervision, visualization, writing review and editing.

## References

1. Cox, F. E.; *Parasites Vectors* **2010**, *3*, 5.
2. Rosenthal, P. J.; John, C. C.; Rabinovich, N. R.; *Am. J. Trop. Med. Hyg.* **2019**, *100*, 239.
3. World Health Organization (WHO); *World Malaria Report 2016*; WHO: Geneva, 2016, https://apps.who.int/iris/bitstream/handle/10665/252038/9789241511711-eng.pdf, accessed in November 2021.
4. Bartoloni, A.; Zammarchi, L.; *Mediterr. J. Hematol. Infect. Dis*. **2012**, *4*, e2012026.
5. Visser, B. J.; Van Vugt, M.; Grobusch, M. P.; *Expert Opin. Pharmacother*. **2014**, *15*, 2219.
6. Imwong, M.; Hien, T. T.; Thuy-Nhien, N. T.; Dondorp, A. M.; White, N. J.; *Lancet Infect. Dis*. **2017**, *17*, 1022.
7. Renslo, A. R.; *ACS Med. Chem. Lett*. **2013**, *4*, 1126.
8. Karunamoorthi, K.; Sabesan, S.; Jegajeevanram, K.; Vijayalakshmi, J.; *Vector-Borne Zoonotic Dis.* **2013**, *13*, 521.
9. Newman, D. J.; Cragg, G. M.; *J. Nat. Prod*. **2016**, *79*, 629.
10. Soares-Neto, R. L.; Cordeiro, L. S.; Loiola, M. I. B.; *Rodriguesia* **2014**, *65*, 685.
11. Barros, N. B.; Migliaccio, V.; Facundo, V. A.; Ciancaglini, P.; Stábeli, R. G.; Nicolete, R.; Silva-Jardim, I.; *Exp. Parasitol*. **2013**, *135*, 337.
12. Teles, C. B. G.; Moreira, L. S.; Silva, A. A. E.; Facundo, V. A.; Zuliani, J. P.; Stábeli, R. G.; Silva-Jardim, I.; *J. Braz. Chem. Soc*. **2011**, *22*, 936.
13. Teles, C. B. G.; Moreira-Dill, L. S.; Silva, A. A.; Facundo, V. A.; Azevedo-Júnior, W. F.; Silva, L. H. P.; Motta, M. C. M.; Stábeli, R. G.; Jardim, I. S.; *BMC Complementary Altern. Med.* **2015**, *15*, 165.
14. Horinouchi, C. D. D. S.; Mendes, D. A. G. B.; Soley, S. B.; Pietrovski, E. F.; Facundo, V. A.; Santos, A. R. S.; Cabrini, D. A.; Otuki, M. F.; *J. Ethnopharmacol.* **2013**, *145*, 311.
15. Viau, C. M.; Moura, D. J.; Facundo, V. A.; Saffi, J.; *BMC Complementary Altern. Med*. **2014**, *14*, 280.

16. Evaristo, F. F. V.; Albuquerque, M. R. J. R.; Santos, H. S.; Bandeira, P. N.; Ávila, F. N.; Silva, B. R.; *BioMed. Res. Int*. **2014**, ID 729358.
17. Facundo, V. A.; Rios, K. A.; Moreira, L. S.; Militão, J. S. L. T.; Stabeli, R. G.; Braz-Filho, R.; Silveira, R.; *Rev. Latinoam. Quim*. **2008**, *36*, 76.
18. Facundo, V. A.; Andrade, C. H. S.; Silveira, E. R.; Braz-Filho, R.; Hufford, C. D.; *Phytochemistry* **1993**, *32*, 411.
19. Trager, W.; Jensen, J. B.; *Science* **1976**, *193*, 673.
20. Lambros, C.; Vanderberg, J. P.; *J. Parasitol*. **1979**, *65*, 418.
21. Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M.; *Antimicrob. Agents Chemother.* **2004**, *48*, 1803.
22. Cos, P.; Vlietinck, A. J.; Berghe, D. V.; Maes, L.; *J. Ethnopharmacol*. **2006**, *106*, 290.
23. *Origin*, 5.0; OriginLab Corporation, Northampton, MA, USA, 2005.
24. Calvo-Calle, J. M.; Moreno, A.; Eling, W. M. C.; Nardin, E. H.; *Exp. Parasitol*. **1994**, *79*, 362.
25. Mosmann, T.; *J. Immunol. Methods* **1983**, *65*, 55.
26. Weniger, B.; Robledo, S.; Arango, G. J.; Deharo, E.; Aragón, R.; Munoz, V.; Callapa, J.; Lobstein, A.; Anton, R.; *J. Ethnopharmacol*. **2001**, *78*, 193.
27. Wang, C.; Qin, X.; Huang, B.; He, F.; Zeng, C.; *Biochem. Biophys. Res. Commun*. **2010**, *402*, 773.
28. Qidwai, T.; Khan, F.; *Chem. Biol. Drug Des*. **2012**, *80*, 155.
29. Tallorin, L.; Durrant, J. D.; Nguyen, Q. G.; McCammon, J. A.; Bukart, M. D.; *Bioorg. Med. Chem*. **2014**, *22*, 6053.
30. Trott, O.; Olson, A. J.; *J. Comput. Chem*. **2010**, *31*, 455.
31. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E.; *J. Comput. Chem*. **2004**, *25*, 1605.
32. Nogueira, C. R.; Lopes, L. M. X.; *Molecules* **2011**, *16*, 2146.
33. Isah, M. B.; Ibrahim, M. A.; Mohammed, A.; Aliyu, A. B.; Masola, B.; Coetzer, T. H. T.; *Parasitology* **2016**, *143*, 1219.
34. Ramalhete, C.; Lopes, D.; Molnár, J.; Mulhovo, S.; Rosário, V. E.; Ferreira, M. U.; *Bioorg. Med. Chem*. **2011**, *19*, 330.
35. Ramalhete, C.; Cruz, F. P.; Mulhovo, S.; Sousa, I. J.; Fernandes, M. X.; Prudêncio, M.; Ferreira, M. U.; *Bioorg. Med. Chem*. **2014**, *15*, 3887.
36. da Silva, T. B. C.; Alves, V. L.; Mendonça, L. V. H.; Conserva, L. M.; da Rocha, E. M. M.; Andrade, E. H. A.; Lemos, R. P. L.; *Pharm. Biol*. **2004**, *42*, 94.
37. Suksamrarn, A.; Tanachatchairatana, T.; Kanokmedhakul, S.; *J. Ethnopharmacol*. **2003**, *88*, 275.
38. Gachet, M. S.; Kunert, O.; Kaiser, M.; Brun, R.; Zehl, M.; Keller, W.; Munoz, R. A.; Bauer, R.; Schuehly, W.; *J. Nat. Prod*. **2011**, *74*, 559.
39. Vo, N. N. Q.; Fukushima, E. O.; Murakana, T.; *J Nat. Med*. **2016**, *71*, 50.
40. Chwalek, M.; Lalun, N.; Bobichon, H.; Plé, K.; Voutquenne-Nazabadioko, L.; *Biochim. Biophys. Acta (BBA) - Gen Subj*. **2006**, *1760*, 1418.
41. Matos, C. T.; da Silva, M. L.; Cabrita-Santos, L.; Portal, M. D.; Inês, P.; *Cell. Microbiol*. **2016**, *18*, 437.
42. Jelkmann, W.; *J. Physiol*. **2011**, *589*, 1251.
43. Patocka, J.; *J. Appl. Biomed*. **2003**, *1*, 7.
44. Lacouth-Silva, F.; Xavier, C. V.; Setúbal, S. S.; Pontes, A. S.; Nery, N. M.; Castro, O. B.; Fernandes, C. F. C.; Honda, E. R.; Zanchi, F. B.; Calderon, L. A.; Stábeli, R. G.; Soares, A. M.; Silva-Jardim, I.; Facundo, V. A.; Zuliani, J. P.; *BMC Complementary Altern. Med*. **2015**, *15*, 420.
45. Ghosh, J.; Das, J.; Manna, P.; Sil, P. C.; *Toxicol. In Vitro* **2008**, *22*, 1918.
46. Elsherbiny, N. M.; Eladl, M. A.; Al-Gayyar, M. M.; *Cytokines* **2016**, *77*, 26.
47. Baig, M. H.; Ahmad, K.; Roy, S.; Ashraf, J. M.; Adil, M.; Siddiqui, M. H.; Khan, S.; Kamal, M. A.; Provazník, I.; Choi, I.; *Curr. Pharm. Des*. **2016**, *22*, 572.

*Submitted: September 3, 2021*
*Published online: December 10, 2021*