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Semisynthesis, cytotoxicity, antimalarial evaluation and structure-activity relationship of two series of triterpene derivatives

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ABSTRACT

In this report, we describe the semisynthesis of two series of ursolic and betulinic acid derivatives through designed by modifications at the C-3 and C-28 positions and demonstrate their antimalarial activity against chloroquine-resistant *P. falciparum* (W2 strain). Structural modifications at C-3 were more advantageous to antimalarial activity than simultaneous modifications at C-3 and C-28 positions. The ester derivative, 3β-butanoyl betulinic acid (**7b**), was the most active compound (IC_{50} = 3.4 μM) and it did not exhibit cytotoxicity against VERO nor HepG2 cells (CC_{50} > 400 μM), showing selectivity towards parasites (selectivity index > 117.47). In combination with artemisinin, compound **7b** showed an additive effect (CI = 1.14). While docking analysis showed a possible interaction of **7b** with the *Plasmodium* protease PfSUB1, with an optimum binding affinity of −7.02 kcal/mol, the rather low inhibition displayed on a *Bacillus licheniformis* subtilisin A protease activity assay (IC_{50} = 93 μM) and the observed accumulation of ring forms together with a delay of appearance of trophozoites *in vitro* suggests that the main target of 3β-butanoyl betulinic acid on *Plasmodium* may be related to other molecules and processes pertaining to the ring stage. Therefore, compound **7b** is the most promising compound for further studies on antimalarial chemotherapy. The results obtained in this study provide suitable information about scaffolds to develop novel antimalarials from natural sources.

Malaria is a devastating disease that remains a significant public health problem worldwide. There are more than 198 million cases of malaria and 584,000 deaths annually, mainly affecting children under 5 years of age.[1] Among the five protozoan species of *Plasmodium* that cause human malaria, *Plasmodium falciparum* is the most virulent, responsible, by far, for the greatest morbidity and mortality, with several hundred million cases of clinical malaria and deaths.[1,2]

The antimalarial drugs used for treatment depend on the species of malaria parasite causing the infection, where the infection was acquired, pregnancy status, and the severity of infection.[1,2] Chloroquine (CQ) was the antimalarial of choice for several decades due to its efficiency, safety, tolerance and low cost. Nevertheless, because of its irrational use, CQ treatment has been a failure, and the use of CQ to treat falciparum malaria is restricted to just a few countries.[1,3] Currently, the first-line treatment of uncomplicated malaria caused by *P. falciparum* is artemisinin combination therapy (ACT).[1] Combination therapy has been adopted to prevent drug-resistant parasites; however, the emergence and spread of drug-resistant malaria, mainly with artemisinin's efficacy declining, has been a major problem that hinders the control of malaria.[1,4,5]

The high morbidity and mortality caused by malaria, the lack of licensed vaccines, and the widespread resistance to artemisinin, together with the lack of drugs safely administered to children and pregnant women are, in general, the main issues that point to the real and critical requirement for discovering new antimalarial medications.[1,5,6] Despite the advances in malaria research and the great efforts towards developing new effective and safe drugs, the majority remain very limited in their mechanisms of action, and until now, no innovative drug is commercially available,

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proving that more studies and novel lead chemotypes are urgently needed.[5,6]

In the context of improving therapy against malaria infection, natural products could be a potential source of new classes of drugs with high activity and low toxicity, which can be further optimized by chemical modifications.[7,8] Ursolic acid (UA) and betulinic acid (BA) (Fig. 1) are pentacyclic triterpenoids extracted from several natural sources and have been reported to possess a broad and promising spectrum of biological activities, including antimicrobial, anticancer, and anti-inflammatory characteristics.[9–11]

The antimalarial activity of these compounds and their derivatives was previously assessed by our group, and they demonstrated to be promising lead compounds. The UA and BA analogues bearing an acetyl group at C-3 and piperazine moiety at C-28 were active on CQ-resistant (FcB1) and CQ-sensitive (3D7) *P. falciparum* strains.[12,13] Afterwards, two series of UA and BA derivatives possessing several ester substituents at C-3 were evaluated against CQ-sensitive (3D7) *P. falciparum*, and the derivatives with shorter side-chains were more active.[14] Two inactive BA-group compounds were selected to verify the importance of C-3 groups on cytotoxicity when C-28 is linked to piperazine. In both compounds, the piperazine moiety significantly increased the antiplasmodial activity, showing satisfactory *in vitro* selectivity.[15] However, these compounds presented some *in vivo* toxicity attributed to the piperazine moiety (unpublished data).

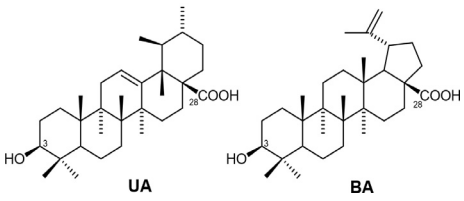
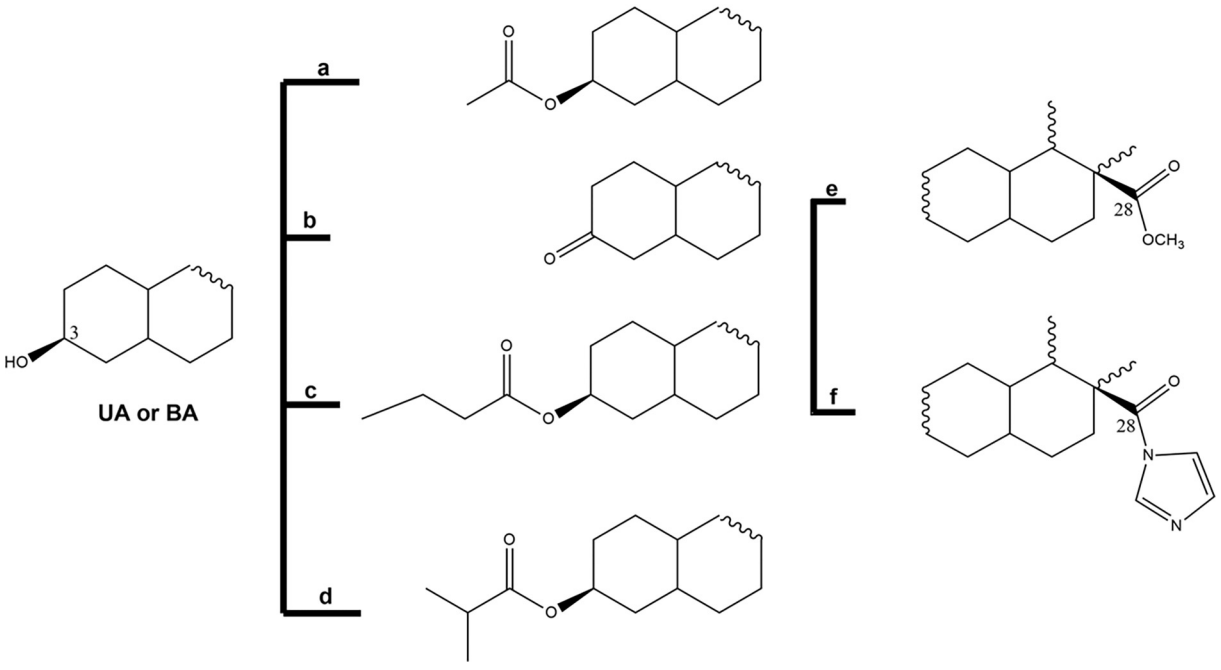


Fig. 1. Chemical structures of ursolic acid (UA) and betulinic acid (BA). (*Source:* ChemDraw Ultra® Software.)

The triterpenes UA and BA were obtained by our group from natural sources from southern Brazil, apple pomace (*Malus domestica*) and *Platanus acerifolia* bark, with good yields (3.5% and 1.5%, respectively) using simple and low-cost sources and methodologies.[13,16] A large number of structural modifications in the triterpenes are possible. Here, the semisynthesis processes were focused at the C-3 and C-28 positions, which are available sites for feasible chemical modifications in these structures. In both triterpenes, the hydroxyl group at the C-3 position was acylated using acetic (**1a**, **1b**)[13], butyric (**7a**, **7b**)[14] and isobutyric anhydrides (**10a**, **10b**).[14] Two other derivatives were obtained through the oxidation of this hydroxyl group (**4a**, **4b**),[17] totaling eight derivatives with a substitution at the C-3 position alone. Diverse natural species have antimalarial and concomitant anticancer activity. Knowing that they are unlikely to be biologically closely related because malaria involves protozoa parasite and cancer involves aberrant mammalian cells, it is equally unlikely that they share the same disease target mechanisms.[21] Due to the cytotoxic profile of methyl and imidazole groups on different cancer cells lines[18–20], starting with C-3 modified triterpene derivatives (esters or ketone groups) the C-28 carboxylic acid was replaced by methyl (**2a**, **2b**; **5a**, **5b**; **8a**, **8b**; **11a**, **11b**) and imidazole moieties (**3a**, **2b**; **6a**, **6b**; **9a**, **9b**; **12a**, **12b**). Therefore, twenty-four compounds in two groups of derivatives were successfully prepared using triterpene skeletons (Scheme 1).

P. falciparum CQ-resistant (W2 strain – IC_{50} CQ = 0.156 μM) was utilized in the *in vitro* evaluation. The parasite culture was performed as previously described by Trager and Jansen,[22] and was maintained in fresh human erythrocytes (O^+ blood) suspended at a 2% hematocrit in complete medium at 37 °C. The use of human blood was approved by the UFRGS Research Ethics Committee, under protocol number 1.242.369. An anti-*P. falciparum* assay was performed,[23] and the half-maximal drug inhibitory response (IC_{50}) was estimated by curve fivefold dilution, ranging from 100 to 3.12 μM, using software from OriginLab Corporation®. The results were compared with drug-free control wells (100% parasite viability), and artemisinin was tested in parallel as an antimalarial control. In addition, the effects of **7b** on the growth of W2



Scheme 1. Synthesis of derivatives **1a**–**12b**. Reagents and conditions: (a) dichloromethane, acetic anhydride, pyridine, rt., 24 h; (b) acetone, Jones Reagent at 0 °C, rt., 3 h; (c) dichloromethane, butyric anhydride, DMAP, rt., 24 h; (d) dichloromethane, isobutyric anhydride, DMAP, rt., 24 h (e) dichloromethane, K_2CO_3, iodomethane, N_2 atmosphere, 55 °C, 48 h; (f) dichloromethane, oxalyl chloride, N_2 atmosphere, 0 °C, 3 h – trimethylamine, 0 °C – imidazole, rt., 24 h. (*Source:* ChemDraw Ultra® Software.)