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Secochiliolide ester derivatives: Preparation and evaluation of their antitrypanosomal and antimalarial efficacy

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Abstract

In the present study, a series of new esters of secochiliolide acid (SA), a diterpene isolated from *Nardophyllum bryoides,* were synthesized in good yield. All compounds were evaluated for their in vitro antiparasitic properties (on *Plasmodium falciparum* and *Trypanosoma brucei brucei*) and cytotoxicity (on WI38, normal mammalian cells). They displayed moderate antitrypanosomal activity with IC_{50} values between 2.55 and 18.14 μM, with selectivity indices >10, and low antiplasmodial effects with $IC_{50} > 29$ μM. The only exception was the *n*-hexyl ester of SA, which showed a strong and selective antiplasmodial activity ($IC_{50} = 1.99$ μM and selectivity index = 117.0). The in vivo antimalarial efficacy of this compound was then assessed according to the 4-day suppressive test of Peters in mice. An intraperitoneal treatment at 50 mg kg^{-1} day^{-1} induced a slight parasitaemia reduction by 56% which was statistically significant on day 4 post-infection and an increase in the survival time.

KEYWORDS

antimalarial efficacy, antiplasmodial activity, cytotoxicity, diterpene, *Nardophyllum bryoides*, secochiliolide acid esters

*These authors contributed equally to the work.

1 | INTRODUCTION

Neglected tropical diseases (NTDs) such as leishmaniasis, African and American trypanosomiasis, impair the lives of approximately one billion people in the developing countries of Africa, Asia and the Americas.[1] The aetiological agents of trypanosomiasis (protozoa belonging to *Trypanosoma* genus) are very closely related kinetoplastid parasites that share many biochemical characteristics and can be considered as a group in a target-based drug discovery programme.[2] Malaria, the most widespread "protozoan" infection, can also be considered as a neglected disease in environments where NTDs prevail: populations with low social visibility, low income and little political voice. These NTDs produce chronic disabilities that result in impaired physical, intellectual and cognitive child growth and decreased worker productivity.[3,4] NTDs can be controlled, prevented and/or eliminated; however, the current available treatments show limited effectiveness, with increased resistance development and/or high toxicity. Moreover, there is little commercial interest in the development of new or improved therapeutics for these poor affected populations.[1]

Nature continues to be the main source of structural diversity in molecular terms, which, in the pursuit of multidisciplinary collaborative research, can lead to the discovery of new prototype compounds, some of which may be developed into commercial drugs.[5]

Secochiliolide acid (**1**, SA) is the major secondary metabolite present in the Patagonian endemic shrub *Nardophyllum bryoides*.[6,7] This compound is present in the aerial parts of this plant and belongs to the *seco-ent*-halimane family, its spirolactone moiety is a remarkable feature, which is a fragment present in several natural trypanocidal compounds.[8,9] In a previous work, SA and some derivatives exhibited IC_{50} values against *Trypanosoma cruzi* epimastigotes similar to that of the commercial drug benznidazole (IC_{50} about 2 μg/ml - 5.8 μM). These results indicated that the carboxyl group was not essential for the bioactivity since its replacement by several other groups yielded products with comparable activity. Quite surprisingly, the *t*-butyl ester of SA, which can be metabolically converted to the former acid by hydrolysis, was inactive.[10]

As is well known, aqueous solubility and lipophilicity influence the way that a molecule crosses biological membranes and barriers.[11] Moreover, the targets of many antiparasitic drugs are inside the cellular membrane and/or the membrane itself.[12–14] In such cases, balanced lipophilic/hydrophilic properties of a drug could be a desirable feature, which would facilitate the bioactivity. In this regard, ester-based prodrugs are classical means to improve the bioavailability, by allowing passive membrane permeability of water soluble drugs due to the masking of charged groups such as carboxylic acids.[15,16] So, an homologous series of linear SA esters was synthesized, in order to evaluate the influence of the alcohol moiety chain length in the antiprotozoal activity of these compounds, especially against *Trypanosoma brucei brucei* and *Plasmodium falciparum*. A promising ester was then evaluated afterwards for in vivo antimalarial efficacy to confirm its potential as a new lead.

2 | METHODS AND MATERIALS

2.1 | Chemistry

2.1.1 | General experimental procedures

Optical rotations were determinate on a Perkin-Elmer 343 polarimeter. UV and IR spectra were obtained on a Hewlett Packard 8453 spectrophotometer and on an FT-IR Nicolet Magna 550 instrument. The ^{1}H and ^{13}C NMR spectra in $CDCl_3$ were obtained in Bruker Avance-2 (500 MHz) and AC-200 (200 MHz) spectrometers. The residual signal of $CHCl_3$ at δ 7.26 was used as reference to determinate the proton chemical shifts while ^{13}C NMR was referenced at 77.0 ppm (central peak of $CDCl_3$). Structures were verified by correlation analyses (COSY, DEPT-HSQC and HMBC), and the relative configuration was determined by gradient-enhanced NOESY experiments. The standard pulse sequences were applied to perform the 2D NMR experiments. HRESI mass spectra were recorded in a MicrOTOF QII Bruker mass spectrometer.

TLC was carried out on Merck Sílicagel $60F_{254}$ plates, read at 220 nm and revealed with 2% vanillin in concentrated H_2SO_4. Sephadex LH-20 was obtained from GE Healthcare Life Sciences. All solvents were distilled prior to use. The purity of the tested compounds was assessed by HPLC and was in all cases higher than 95%.

2.1.2 | Extraction and purification of secochiliolide acid (1) and methyl ester (2)

Compounds **1** and **2** were extracted from *Nardophyllum bryoides* as reported Siless et al.[10] A voucher specimen (HRP6865) was stored at the Herbario Regional Patagónico, Universidad Nacional de la Patagonia San Juan Bosco.

Briefly, the ethanolic extract of fresh aerial parts of *N. bryoides* was concentrated to an aqueous suspension and then partitioned between MeOH:H_2O (9:1) and cyclohexane. The polar subextract was concentrated under reduced pressure, basified with NaOH to pH 10 and then extracted with DCM. This basic aqueous fraction was then acidified with HCl and extracted with DCM. This final organic fraction was then purified by Sephadex LH-20 permeation and flash column chromatography.

2.1.3 | General procedure for the preparation of ester derivatives

The esters **3**–**9** were obtained by a Steglich esterification of **1** with linear alcohols employing $CeCl_3 \cdot 7H_2O$ as a catalyst in one step with considerably high yields.[17]

A solution of **1** (0.04 mmol), DCC (0.10 mmol) and DMAP (0.01) in 0.5 ml of THF was stirred for 30 min. Then, a solution of the corresponding alcohol (0.1 mmol) and $CeCl_3 \cdot 7H_2O$ (0.02) in 0.5 ml of THF was added dropwise. The reaction was stirred overnight. After completion, the solvent was evaporated and the crude product was dissolved in cyclohexane. The organic layer was washed with brine and taken to dryness. The resulting solid was purified by column chromatography with cyclohexane/ethyl acetate mixtures. In all cases, the yield of purified ester was higher than 58%. For NMR spectra of all derivatives see Supporting Information.

Compound 3

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, brs, H-16), 7.42 (1H, t, *J* = 1.8, H-15), 6.40 (1H, dd, *J* = 1.8, 0.6, H-14), 5.37 (1H, dd, *J* = 10.7, 6.0, H-12), 1.73 (3H, brs, H-19), 1.69 (3H, d, *J* = 2.0, H-18), 1.33 (3H, d, *J* = 6.8, H-17), 4.10 (2H, q, *J* = 7.2, -O-C$\underline{H}_2$-CH_3), 1.25 (3H, t, *J* = 7.2, -O-CH_2-C$\underline{H}_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-20), 173.6 (C-3), 144.1 (C-15), 140.2 (C-16), 128.5 (C-5), 127.8 (C-4), 124.5 (C-13), 108.6 (C-14), 70.7 (C-12), 52.2 (C-9), 45.3 (C-11), 41.6 (C-10), 36.4 (C-8), 33.0 (C-2), 29.7 (C-7), 26.2 (C-1), 21.6 (C-6), 20.9 (C-18), 20.7 (C-19), 16.1 (C-17), 60.5 (-O-$\underline{C}H_2$-CH_3), 14.3 (-O-CH_2-$\underline{C}H_3$).

Compound 4

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, s, H-16), 7.42 (1H, t, *J* = 1.6, H-15), 6.40 (1H, d, *J* = 1.2, H-14), 5.36 (1H, dd, *J* = 10.6, 5.9, H-12), 1.73 (3H, brs, H-19), 1.69 (3H, d, *J* = 1.9, H-18), 1.33 (3H, d, *J* = 6.8, H-17), 4.00 (2H, t, *J* = 6.8, -O-C$\underline{H}_2$-CH_2-CH_3), 1.64 (2H, q, *J* = 7.1, -O-CH_2-C$\underline{H}_2$-CH_3), 0.93 (3H, t, *J* = 7.5, -O-CH_2-CH_2-C$\underline{H}_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-20), 173.7 (C-3), 144.1 (C-15), 140.2 (C-16), 128.5 (C-5), 127.8 (C-4), 124.5 (C-13), 108.6 (C-14), 70.7 (C-12), 52.2 (C-9), 45.3 (C-11), 41.6 (C-10), 36.4 (C-8), 32.9 (C-2), 29.6 (C-7), 26.2 (C-1), 21.6 (C-6), 20.9 (C-18), 20.7 (C-19), 16.1 (C-17), 66.2 (-O-$\underline{C}H_2$-CH_2-CH_3), 22.2 (-O-CH_2-$\underline{C}H_2$-CH_3), 10.6 (-O-CH_2-CH_2-$\underline{C}H_3$).

Compound 5

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, brs, H-16), 7.42 (1H, t, *J* = 1.6, H-15), 6.40 (1H, d, *J* = 1.2, H-14), 5.37 (1H, dd, *J* = 10.6, 5.9, H-12), 1.73 (3H, brs, H-19), 1.69 (3H, d, *J* = 1.9, H-18), 1.33 (3H, d, *J* = 6.8, H-17), 4.04 (2H, t, *J* = 6.8, -O-C$\underline{H}_2$- CH_2-CH_2-CH_3), 0.93 (3H, t, *J* = 7.4, -O-CH_2-CH_2-CH_2-C$\underline{H}_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-20), 173.7 (C-3), 144.1 (C-15), 140.2 (C-16), 128.5 (C-5), 127.8 (C-4), 124.5 (C-13), 108.6 (C-14), 70.7 (C-12), 52.2 (C-9), 45.3 (C-11), 41.6 (C-10), 36.4 (C-8), 32.9 (C-2), 29.7 (C-7), 26.2 (C-1), 21.6 (C-6), 20.9 (C-18), 20.7 (C-19), 16.1 (C-17), 64.5 (-O-$\underline{C}H_2$-CH_2-CH_2-CH_3), 30.9 (-O-CH_2-$\underline{C}H_2$-CH_2-CH_3) 19.3 (-O-CH_2-CH_2-$\underline{C}H_2$-CH_3), 13.9 (-O-CH_2-CH_2-CH_2-$\underline{C}H_3$).

Compound 6

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, t, *J* = 0.8, H-16), 7.42 (1H, t, *J* = 1.8, H-15), 6.40 (1H, dd, *J* = 1.8, 0.8, H-14), 5.37 (1H, dd, *J* = 10.5, 5.9, H-12), 1.73 (3H, d, *J* = 1.1, H-19), 1.69 (3H, d, *J* = 2.0, H-18), 1.33 (3H, d, *J* = 6.9, H-17), 4.03 (2H, t, *J* = 6.8, -O-C$\underline{H}_2$-$(CH_2)_3$-CH_3), 0.90 (3H, t, *J* = 7.0, -O-CH_2-$(CH_2)_3$-C$\underline{H}_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-20), 173.7 (C-3), 144.1 (C-15), 140.2 (C-16), 128.5 (C-5), 127.8 (C-4), 124.5 (C-13), 108.6 (C-14), 70.7 (C-12), 52.2 (C-9), 45.3 (C-11), 41.6 (C-10), 36.4 (C-8), 32.9 (C-2), 29.7 (C-7), 26.2 (C-1), 21.6 (C-6), 20.9 (C-18), 20.7 (C-19), 16.1 (C-17), 64.8 -O-$\underline{C}H_2$-$(CH_2)_3$-CH_3), 14.2 (-O-CH_2-$(CH_2)_3$-$\underline{C}H_3$).

Compound 7

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, m, H-16), 7.42 (1H, t, *J* = 1.8, H-15), 6.40 (1H, dd, *J* = 1.9, 0.9, H-14), 5.37 (1H, dd, *J* = 10.6, 6.0, H-12), 1.73 (3H, d, *J* = 1.1, H-19), 1.69 (3H, d, *J* = 2.0, H-18), 1.33 (3H, d, *J* = 6.9, H-17), 4.03 (2H, t, *J* = 6.8, -O-C$\underline{H}_2$-$(CH_2)_4$-CH_3), 0.89 (3H, t, *J* = 6.9, -O-CH_2-$(CH_2)_4$-C$\underline{H}_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-20), 173.7 (C-3), 144.1 (C-15), 140.2 (C-16), 128.5 (C-5), 127.8 (C-4), 124.5 (C-13), 108.6 (C-14), 70.7 (C-12), 52.2 (C-9), 45.3 (C-11), 41.6 (C-10), 36.4 (C-8), 33.0 (C-2), 29.7 (C-7), 26.2 (C-1), 21.6 (C-6), 20.9 (C-18), 20.7 (C-19), 16.1 (C-17), 64.8 -O-$\underline{C}H_2$-$(CH_2)_4$-CH_3), 14.2 (-O-CH_2-$(CH_2)_4$-$\underline{C}H_3$).

Compound 8

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, brs, H-16), 7.42 (1H, t, *J* = 2.7, H-15), 6.40 (1H, d, *J* = 1.1, H-14), 5.37 (1H, dd, *J* = 10.5, 5.9, H-12), 1.73 (3H, brs, H-19), 1.69 (3H, d, *J* = 1.8, H-18), 1.33 (3H, d, *J* = 6.6, H-17), 4.03 (2H, t, *J* = 7.1, -O-C$\underline{H}_2$-$(CH_2)_5$-CH_3), 0.88 (3H, t, *J* = 6.7, -O-CH_2-$(CH_2)_5$-C$\underline{H}_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-20), 173.7 (C-3), 144.1 (C-15), 140.2 (C-16), 128.5 (C-5), 127.8 (C-4), 124.5 (C-13), 108.6 (C-14), 70.7 (C-12), 52.1 (C-9), 45.3 (C-11), 41.6 (C-10), 36.4 (C-8), 32.9 (C-2), 29.7 (C-7), 26.2 (C-1), 21.6 (C-6), 20.9 (C-18), 20.7 (C-19), 16.1 (C-17), 64.8 -O-$\underline{C}H_2$-$(CH_2)_5$-CH_3), 14.3 (-O-CH_2-$(CH_2)_5$-$\underline{C}H_3$).

Compound 9

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.47 (1H, brs, H-16), 7.42 (1H, t, *J* = 1.6, H-15), 6.40 (1H, d, *J* = 1.1, H-14), 5.37

(1H, dd, J = 10.6, 5.9, H-12), 1.73 (3H, brs, H-19), 1.69 (3H, d, J = 1.7, H-18), 1.33 (3H, d, J = 6.7, H-17), 4.03 (2H, t, J = 6.9, -O-C$\underline{H}_2$-$(CH_2)_6$-CH_3), 0.88 (3H, t, J = 6.8, -O-CH_2-$(CH_2)_6$-C$\underline{H}_3$).

^{13}C NMR (125 MHz, $CDCl_3$) δ 177.4 (C-20), 173.7 (C-3), 144.1 (C-15), 140.2 (C-16), 128.6 (C-5), 127.8 (C-4), 124.5 (C-13), 108.6 (C-14), 70.7 (C-12), 52.2 (C-9), 45.3 (C-11), 41.6 (C-10), 36.4 (C-8), 33.0 (C-2), 29.7 (C-7), 26.2 (C-1), 21.6 (C-6), 20.9 (C-18), 20.7 (C-19), 16.1 (C-17), 64.8 -O-C$\underline{H}_2$-$(CH_2)_6$-CH_3), 14.3 (-O-CH_2-$(CH_2)_6$-C$\underline{H}_3$).

2.2 | Biological evaluation

2.2.1 | Parasites, cells and media

The asexual erythrocytic stages of *Plasmodium falciparum* (3D7 strain isolated from a patient from the Nederland) were cultivated in supplemented RPMI 1640 medium (Gibco) at 37°C, under an atmosphere of 5% CO_2, 5% O_2 and 90% N_2.[18]

Trypanosoma brucei brucei (strain 427) bloodstream forms were cultured in vitro in a humidified atmosphere with 5% CO_2 in HMI9 medium supplemented with 10% heat-inactivated foetal bovine serum (Sigma-Aldrich) at 37°C.[19]

The human normal fibroblast cell line, WI38 (ATCC Number CCL-75 from LGC Standards), was cultivated in DMEM medium (Gibco, Thermo Fischer Scientific) containing 4 mM l-glutamine, 1 mM sodium pyruvate supplemented with 10% foetal bovine serum and penicillin-streptomycin (100 UI/ml–100 μg/ml).[20]

2.2.2 | Animals and parasites

Female Swiss mice (ICR (CD-1) 6–7 weeks of age, 25.9 ± 1.4 g) were obtained from Envigo laboratories (The Netherlands) and infected with the rodent parasite *Plasmodium berghei* NK173. All in vivo experiments performed in this study were approved by the Ethical Committee for animal use at the Université catholique de Louvain (2017/UCL/MD/017).

2.2.3 | In vitro test

In vitro antiparasitic and cytotoxicity tests were performed according to Bero et al.[18,20] Stock solutions of compounds were prepared in DMSO at 20 mg/ml and further diluted with medium. Compounds were tested in 96-well microtitre plates in eight serial threefold dilutions (concentration range: 100–0.05 μg/ml, maximum 0.5% DMSO shown to have no measurable effect on parasite/cell viability).

Suramin (a commercial antitrypanosomal drug, Sigma) was used as positive control with an initial concentration of 10 μg/ml. The Alamar blue fluorescence quantification was used to evaluate the parasite viability (530 and 590 nm as excitation and emission wavelengths, respectively). All tests were performed in triplicate with two wells/concentration.

As positive control for antiplasmodial activity, artemisinin (Sigma) was used with an initial concentration of 100 ng/ml. The parasitaemia and the haematocrit were 2% and 1%, respectively. Parasite viability was measured using parasite lactate dehydrogenase (pLDH) activity.[18] All compounds were tested in triplicate with two wells/concentration.

For cytotoxicity, camptothecin (Sigma) was used as positive control with an initial concentration of 25 μg/ml. The cytotoxicity was evaluated using the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (Sigma)) colorimetric method. Absorbance linked to solubilized formed formazan crystals was recorded at 570 and 620 nm. All experiments were made at least in duplicate with three wells per concentration.

The selectivity indices were calculated for each parasite according to the followed formula: IC_{50} (WI38)/IC_{50} (parasite).

2.2.4 | In vivo antimalarial activity

Based on the 4-day suppressive test of Peters recommended by OMS,[21,22] compound **7** was administrated intraperitoneally during 4 consecutive days at 50 mg kg^{-1} day^{-1} to seven mice. The treatment started 4 hr post-intraperitoneal infection by 15×10^7 parasitized erythrocytes (100 μl with parasitaemia of about 15%). In the negative control group, six mice received the vehicle (distilled water with 10% of tween 80-ethanol 7:3) via i.p., and the four positive control mice were treated with the reference drug chloroquine, at 10 mg kg^{-1} day^{-1}. Thin blood smears were made with mouse-tail blood and stained with Giemsa on days 4, 6 and 7 post-infection. At least 1,000 erythrocytes were counted under microscope to define parasitaemia, and inhibition percentages were normalized according to negative control values. One mouse, which was almost uninfected during all experiment (<5% parasitaemia), was removed from the analysis in the negative control and 86% of responder mice were taken into account for compound **7** treatment (parasitaemia between 10% and 35%).

2.2.5 | Statistical analysis

In vitro experiments were done at least in duplicate or triplicate to obtain a minimum of six repetitions for each concentration and expressed as the mean ± standard deviation. Statistical differences were determined by Kruskal–Wallis test followed by the non-parametric multiple comparison Nemenyi's test. The level of significance was set at $p < 0.05$.

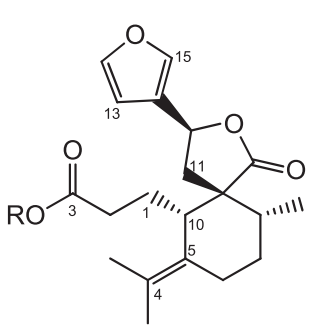
Data of in vivo experiments were analysed by GRAPHPAD PRISM 7.0 statistical software and are presented as the mean ± standard error of the mean. Comparisons of the results were made with the non-parametric Mann–Whitney test in one-tailed for parasitaemia inhibition percentage and by the log-rank test for survival curves. Statistical significance between treatments was set at $p < 0.05$ (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

3 | RESULTS

Compounds **1** and **2** were isolated from *N. bryoides* as previously reported,[10] while the new esters **3–9** were obtained by means of a Steglich esterification of **1** with linear alcohols employing $CeCl_3{\cdot}7H_27H_2O$ as catalyst in one step.[17] Compounds **3–9** are shown in Figure 1. According to the Lipinski rule of 5, lipophilicities higher than 5 and/or molecular weights higher than 500 are not advantageous and they could lead to a decrease in bioavailability.[23,24] On this basis, we synthetized a series of esters in which lipophilicity increases linearly with the length of the esterifying alcohol from 3.83 to 7.59, keeping the molecular weight always below 500 (see Table 1).

The results, compiled in Table 2, show that all esters, except **3**, displayed a higher activity against *T. brucei brucei* than the parent SA. However, no statistical differences were observed between them, due to the SA standard deviation.

Regarding the antiplasmodial activity, only compound **7** reaches the criteria to be considered as a favourable hit for further studies (IC_{50} cut off <10 μM and the SI > 10).[25] Indeed, this compound displayed a strong selective activity (IC_{50} = 1.99 μM, IS > 117.0) and it was 20 times more potent than the free acid **1**. This fact encouraged us to evaluate in vivo the effect of **7** against the rodent parasite *Plasmodium berghei*. Based on the Peters' 4-day suppressive test,[22] an intraperitoneal treatment at 50 mg kg^{-1} day^{-1} induced a slight parasitaemia reduction of 56.0 ± 8.2% which was statistically significant on day 4 post-infection compared to vehicle-treated mice. A similar level of significance was obtained for the reference group with 95.9 ± 1.2% of inhibition. On days 6 and 7, parasitaemia inhibition remained stable (32.2 ± 6.9 and 32.4 ± 4.6%, respectively). A survival increase was also observed as 50% of mice treated with compound **7** were still alive on day 11 compared to the negative control mice which all died on day 7. The in vivo antimalarial activity and the mean survival time (MST) values of the three groups are presented in Figures 2 and 3, respectively.



	R
1	H
2	CH_3
3	CH_2CH_3
4	$(CH_2)_2CH_3$
5	$(CH_2)_3CH_3$
6	$(CH_2)_4CH_3$
7	$(CH_2)_5CH_3$
8	$(CH_2)_6CH_3$
9	$(CH_2)_7CH_3$

FIGURE 1 Chemical structures of compounds **1–9**

4 | DISCUSSION

All compounds exhibited moderate to low in vitro activities against *T. brucei brucei* (IC_{50} between 2.56 and 18.14 μM, SI > 10 in all cases), with compound **8** being the most potent. In this sense, Aoyagi et al. reported an improvement of 14-fold in the activity of dihydrosalviandulin E against *T. brucei brucei* by esterification with butanoic acid.[26] Most of the compounds can be considered as non-cytotoxic on WI38 cells (IC_{50} > 70 μM).

The in vitro antiplasmodial activity of **7** was comparable to that of norcaesalpin D, a furanediterpene isolated from *Caesalpinia bonducella*.[27]

Although the length of the esterifying alcohol seems to play a critical role in the bioactivity of SA, other factors may be involved. Additional studies will be necessary to elucidate the antiparasitic mechanism of action of SA and its derivatives as well as their bioavailability.

Other halimane-type diterpenes were reported previously for their antimicrobial and antitrypanosomal activities.[10,28–33] The tricyclic methyl ester of (11R)-acetoxyhalima-5,13(E)-dien-15-oic acid displayed 90.0 ± 2.1% of growth inhibition against *T. cruzi* epimastigotes at 100 μg/ml compared to 43.8 ± 3.3% for the corresponding acid. However, this improved antitrypanosomal activity fell to 9.9 ± 2.1% at 10 μg/ml.[31]

TABLE 1 Molecular physicochemical properties of secochiliolide acid and esters

Compound	MW	Clog P[a]	Number of C-atoms
1	346.42	3.83	25
2	360.45	4.13	26
3	374.48	4.51	27
4	388.5	5.01	28
5	402.53	5.57	29
6	416.56	6.08	30
7	430.58	6.58	31
8	444.61	7.09	32
9	458.64	7.59	33

[a]Calculated from molinspiration (www.molinspiration.com).

TABLE 2 In vitro antiplasmodial, antitrypanosomal and cytotoxic activities of secochiliolide acid and esters

Compound	Cytotoxicity: IC_{50} (WI38) (μM)	Antitrypanosomal activity: IC_{50} (*Tbb*) (μM)	Selectivity index (SI) (IC_{50} WI38/IC_{50} *Tbb*)	Antiplasmodial activity: IC_{50} (*3D7*) (μM)	SI (IC_{50} WI38/IC_{50} *3D7*)
1	148.70 ± 6.06	14.59 ± 9.39	10.2	43.21 ± 4.21	3.4
2	81.29 ± 22.35	6.87 ± 3.68	11.8	78.99 ± 17.89	1.0
3	> 267.04	18.14 ± 9.89	> 14.7	65.23 ± 4.66	> 4.1
4	93.27 ± 32.42	7.26 ± 5.36	12.8	85.08 ± 8.37	1.1
5	124.05 ± 32.49	12.23 ± 8.73	10.1	87.11 ± 10.38	1.4
6	128.44 ± 15.14	4.94 ± 3.48	26.0	29.02 ± 6.65	4.4
7	> 232.25	10.83 ± 6.96	> 21.4	1.99 ± 0.18	> 117.0
8	69.29 ± 43.07	2.56 ± 2.20	27.1	35.07 ± 9.39	2.0
9	152.17 ± 49.15	9.70 ± 7.18	15.7	57.44 ± 9.05	2.6
Suramin	n.d.	0.03 ± 0.01	n.d.	n.d.	n.d.
Camptothecin	0.13 ± 0.02	n.d.	n.d.	n.d.	n.d.
Artemisinin	n.d.	n.d.	n.d.	0.013 ± 0.004	n.d.

Notes. The values are presented as the mean ± *SD* from at least 3 independent experiments. n.d., not determined.

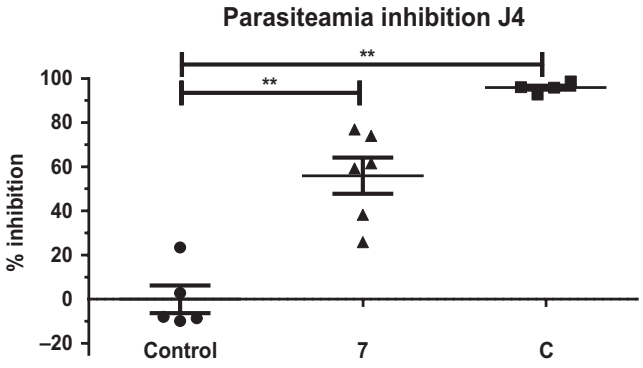


FIGURE 2 Effect of compound **7** and chloroquine on established *P. berghei* infection in mice. Control: non-treated mice. C: chloroquine-treated mice

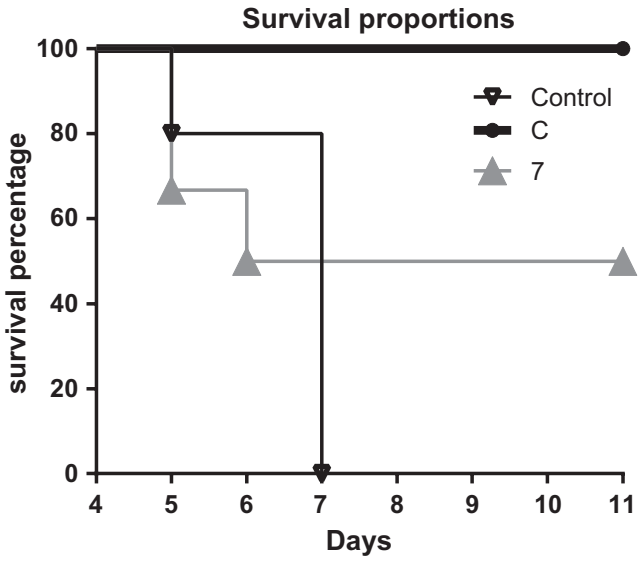


FIGURE 3 Kaplan–Meier survival analysis curves of treated mice, once daily ip for four consecutive days. Control: non-treated mice. C: chloroquine-treated mice

5 | CONCLUSIONS

This study allowed the identification of an hexyl ester of SA with promising and selective antimalarial efficacy. This compound can be obtained in good yield from a sustainable scaffold along with some other antitrypanosomal derivatives. To the best of our knowledge, this is the first report for the antimalarial activity of a *seco-ent*-halimane compound.

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CONFLICT OF INTEREST

The authors declare none.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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