Journal of King Saud University – Science xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Antiplasmodials soulattrolide derivatives from *Calophyllum brasiliense* and its mechanism of activity

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ARTICLE INFO

Article history:
Received 30 July 2018
Accepted 24 March 2019
Available online xxxx

1. Introduction

Parasitic diseases such as malaria, leishmaniasis, trypanosomiasis, and schistosomiasis are just one of the main causes of disease and death in the world today (WHO, 2016). In 2016, 91 countries reported a total of 216 million cases of malaria, which represents an increase to 5 million cases in relation to the previous year, with 15 countries holding 80% of the global burden of malaria. The total number of deaths worldwide reached 445.000, similar to that reported in 2015 (WHO, 2017; Chin, 2001; Newman et al., 2016), so there is an urgent need to discover new, safe and effective drugs for the prophylaxis and treatment of malaria, mainly due to the development of resistance of *Plasmodium falciparum*, the most lethal Plasmodium species to chloroquine and other antimalarial drugs. Few alternative drugs are in development and urgent measures are needed to identify new types of antimalarial agents, many of which have originated from natural products (Olumese, 2015; Leach, 2001). *Clusiaceae* is one of the families of plants in which compounds with antimalarial activity have been found, such as xanthones and derivatives of acylphloroglucinol. Since the genus *Calophyllum* is a potential source of secondary metabolites and its species are little explored in the field of malaria, their exploration is justified as a phytotherapeutic alternative in the treatment of malaria (Hay et al., 2004; Chanphen et al., 1998; Pierson et al., 2010; Guillaume et al., 2009). This paper reports the isolation of soulattrolide (**1**) from *C. brasiliense*. the preparation of eight new derivatives (**2–9**) by hemi-synthesis and the bioactivity studies thereof.

2. Materials and methods

2.1. General

NMR spectra were measured via a Varian Unity of 300 and 500 MHz for ^{1}H; 125 and 75 MHz for ^{13}C) spectrometers. The nature of the carbon signals (C, CH, CH_2, CH_3) was determined using the APT or DEPT techniques. Assignments of the signals have been carried out by two-dimensional heteronuclear correlations (COSY and HMQC/HMBC). TMS was used as an internal standard. Unless otherwise indicated, the spectra were measured in $CDCl_3$ solution. The X-ray data was measured via a Bruker CCD-SMART diffractometer. ESIMS were obtained from Q-TOF mass spectrometer (Waters, Manchester, UK) with combined electrospray and APCI ionization source with Z-spray design; the 3.5 kV capillary tension was used in the positive direction and the cone voltage was set at 20 V. UV spectra were recorded on Genesys 2 spectrophotometer and IR spectra were obtained by the use of KBr pellets in a Jasco FT/IR-6200 spectrometer, covering the region 4000–600 cm^{-1}. Silica gel 60 (Merck) (70–230 mesh) were used for column chromatography and silica gel plates used for preparative TLC.

2.2. Plant material

The aerial parts and stems were collected in the department of Antioquia in June 2009 by Felipe Cardona. Three types of samples were collected from the plant material: a sample for specimen of herbarium, samples as witnesses of the specimens collected and samples of leaves and stems to obtain the extracts. The specimen

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2019.03.005

Please cite this article as: A. M. Mesa Vanegas, S. Blair Trujillo, C. Peláez Jaramillo et al., , Journal of King Saud University – Science, https://doi.org/10.1016/j.jksus.2019.03.005

for herbarium was processed, deposited, taxonomically characterized in the herbarium Universidad de Antioquia (HUA) by Felipe Cardona, and determined as *Calophyllum brasiliense* collected in the department of Antioquia (Voucher 162,467).

2.3. Extraction and isolation

2.3.1. Isolation of soulattrolide from C. brasiliense

The air dried and powdered mixture of leaves and stems of *C. brasiliense* (0,23 kg) were extracted (3× 1L) using dichloromethane (D) at room temperature. Subsequently, the extract was filtered and concentrated in a rotary evaporator to give 47.66 g of crude extract coded as (CBE). The extract was fractionated by vacuum liquid column chromatography (VLC) over silica-gel 60 GF_{254} Merck® and eluted with organic solvents of ascending polarity, such as petroleum ether, petroleum ether/dichloromethane gradient, ethyl acetate and methanol. 50 fractions were collected and concentrated in a vacuum rotary evaporator. The fractions were unified with similar components based on the CCD analysis, developed with vanillin/sulfuric reagent and 12 fractions were obtained. The major components showed a blue coloration, indicating the presence of coumarins. Fractions 4–6 were again subjected to flash CC using silica-gel 60 GF_{254} Merck® with petroleum ether/ethyl acetate gradient. A crude white colored compound was obtained (**1**) (450 mg) which was purified by cold washing with ethyl acetate.

2.4. Ester derivatives of compound (*1*)

Triethylamine (0.3 mmol) and the corresponding acyl chloride (0.12 mmol) were successively added dropwise via syringe, under nitrogen atmosphere, to a solution of compound (**1**) (40 mg, 0.1 mmol) and DMAP (cat) in dichloromethane (8 mL) at 0 °C. The reaction mixture was allowed to warm at room temperature and then stirred at reflux temperature until the disappearance of the starting material (CCD). The crude mixture was diluted in dichloromethane (10 mL) and washed with saturated aqueous NH_4Cl (3 × 10 mL) and brine (3 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Chromatography of the residue with a mixture of hexane/ethyl acetate (9:1) gave a white powder (30 mg) which was crystallized from ethyl acetate in the form of needles. Reaction of compound (**1**) with 4-bromobenzoyl chloride, *p*-toluenesulfonyl chloride, 3,5-dinitrobenzoyl chloride, benzoyl chloride, acetyl chloride and pentafluoro benzoyl chloride gave rise to the corresponding esters **2**–**7**.

Compound (**1**) was submitted to oxidation. Thus, to a solution of compound (**1**) (44 mg, 0.1 mmol) in dichloromethane (7.6 mL) was added PCC (0 °C, 15 mmol) and celite (1.2 mmol). The reaction mixture was left stirring at room temperature for 24 h under nitrogen. The crude reaction was diluted with dichloromethane (10 mL) and washed with saturated aqueous NH_4Cl (3 × 10 mL). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure to give compound (**8**).

On the other hand, hydrogenation of compound (**1**) (50 mg, 0.1 mmol) in dichloromethane in the presence of 10% Pd/C (2 mol %), at room temperature for 24 h, filtration over celite and removal of solvent under reduce pressure afforded compound **(9)**.

2.5. Spectroscopic data of soulattrolide and derivatives

Soulattrolide (**1**): Amorphous white solid with formula $C_{25}H_{24}O_5$ (1597.6 mg), IR ν_{max} 3431.71; 2975.62, 1714.41, 1639.2; 1587.13; 1145.51 cm^{-1}. $[\alpha]_D = -33.48$ (c = 0.09, $CHCl_3$). TOF MS ES+ [M+Na]: (MeOH) *m/z* obsd 427.1529 (1,9 ppm) $[M+Na]^+$, calculated for $C_{25}H_{24}O_5Na$ 427.1521. ^{1}H-RMN-500 MHz δ ($CHCl_3$ –d1) (ppm): 7.35–7.37 (m, 3H); 7.26–7.23 (m, 2H); 6.52 (d, 1H, *J* = 10.27 Hz); 5.94 (s, 1H); 5.34 (d, 1H, *J* = 10.23 Hz); 5.02 (d, 1H, *J* = 3.42 Hz); 4.28 (dq, 1H, *J* = 10.76, 6.36 Hz); 2.88 (bs, 1H); 1.76 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz); 1.42 (d, 3H, *J* = 6.36 Hz); 1.14 (d, 3H, *J* = 7.34 Hz); 0.93 (s, 3H); 0.92 (s, 3H). ^{13}C-RMN-125 MHz δ ($CHCl_3$ –d1) (ppm): 160.6; 156.3; 153.5; 153.7; 151.1; 139.9; 127.53; 127.3 (CH × 2); 127.1 (CH × 2); 115.9; 111.6; 106.1; 106.0; 103.0; 76.9; 73.0; 61.7; 61.3; 38.22; 26.84; 26.73; 18.80; 12.49.

Compound (**2**): White crystals with formula $C_{32}H_{27}O_6Br$. IR (KBr) ν_{max} 3431, 2975, 2933, 1714, 1587, 1370, 1145, 768 cm^{-1}. $[\alpha]_D = -33.48$ (c = 0.09, $CHCl_3$). TOF MS ES$^+$ $[M+Na]^+$: (MeOH) *m/z* obsd 609.0894 (0,8 ppm) $[M+Na]^+$, calc for $C_{32}H_{27}O_6BrNa$ 609.0889. ^{1}H NMR-500-MHz, $CDCl_3$) δ 7.93 (d, 2H, *J* = 7.85) 7.55 (d, 2H, *J* = 7.88); 7.35 (m, 3H); 7.26 (m, 2H); 6.66 (d, 1H, J = 3,43); 6.50 (d, 1H, *J* = 9.78 Hz); 5.85 (s, 1H); 5.34 (d, 1H, *J* = 9.78 Hz); 4.28 (dq, 1H, *J* = 12.71, 6.36 Hz); 2.88 (bs, 1H); 1.76 (ddq, 1H, *J* = 10.76, 7.33, 3.42 Hz); 1.42 (d, 3H, *J* = 6.36 Hz); 1.14(d, 3H, *J* = 6.84 Hz); 0.93 (s, 3H); 0.92 (s,3H). ^{13}C-RMN-125 MHz (100-MHz, $CDCl_3$) 164.64; 159.50; 155.31; 154.11; 153.73; 151.78; 139.9; 131.58 (CH × 2); 131.38 (CH × 2); 129.15; 127.89; 127.54; 127.35; 127.30; 115.70; 112.536; 105.74; 103.17; 103.17; 101.92; 73.84; 64.39; 37.98; 27.07; 26.86; 18.79; 12.2.

Compound (**3**): $C_{32}H_{30}SO_7$. ^{1}H NMR (300-MHz, $CDCl_3$) δ 7.79 (d, 2H); 7. 34 (d, 2H); 7.37 (m, 3H); 7.24 (m, 2H); 6.68 (d, 1H, *J* = 9.98 Hz); 5.96 (s, 1H); 5.36 (d, 1H, *J* = 9.78 Hz); 5.23 (d, 1H, *J* = 3.49 Hz); 4.16 (dq, 1H, *J* = 10.76, 6.36 Hz); 2.22 (s, 3H); 1.96 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz); 1.44 (d, 3H, *J* = 6.36 Hz); 1.07 (d, 3H, *J* = 7.34 Hz); 0.95 (s, 3H); 0.94 (s, 3H).

Compound (**4**): $C_{32}H_{26}N_2O_{10}$ ^{1}H NMR (300-MHz, $CDCl_3$) δ 9.22 (t, 1H, *J* = 2.06 Hz); 9.18 (d, 1H, *J* = 2.07 Hz); 7.37 (m, 3H); 7.22 (m, 2H); 6.81 (d, 1H, *J* = 9.98 Hz); 6.58 (d, 1H, *J* = 3.49 Hz); 5.92 (s, 1H); 5.43 (d, 1H, *J* = 9.78 Hz); 4.26 (dq, 1H, *J* = 10.76, 6.36 Hz); 2.22 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz); 1.52 (d, 3H, *J* = 6.36 Hz); 1.17 (d, 3H, *J* = 7.34 Hz); 0.98 (s, 3H); 0.97 (s, 3H).

Compound (**5**): $C_{32}H_{28}O_6$ ^{1}H NMR (300-MHz, $CDCl_3$) δ 8.14 (m, 2H); 7.57 (m, 1H); 7.45 (m, 2H); 7.37 (m, 3H); 7.22 (m, 2H); 6.79 (d, 1H, *J* = 9.98 Hz); 6.58 (d, 1H, *J* = 3.49 Hz); 5.92 (s, 1H); 5.43 (d, 1H, *J* = 9.78 Hz); 4.26 (dq, 1H, *J* = 10.76, 6.36 Hz); 2.22 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz);1.52 (d, 3H, *J* = 6.36 Hz); 1.17(d, 3H, *J* = 7.34 Hz); 0.98 (s, 3H); 0.97 (s, 3H).

Compound (**6**): $C_{27}H_{26}O_6$ ^{1}H NMR (300-MHz, $CDCl_3$) δ 7.35 (m, 3H); 7.21 (m, 2H); 6.52 (d, 1H, *J* = 10.27 Hz); 6,45 (br, 1H); 5.92 (s, 1H); 5.34 (d, 1H, *J* = 9.78 Hz); 4.28 (dq, 1H, *J* = 10.76, 6.36 Hz); 2,18 (s, 3H); 1.88 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz); 1.42 (d, 3H, *J* = 6.36 Hz); 1.09 (d, 3H, *J* = 7.34 Hz); 0.93 (s, 3H); 0.82 (s, 3H).

Compound (**7**): $C_{32}H_{23}F_5O_6$ ^{1}H NMR (300-MHz, $CDCl_3$) δ 7.35 (m, 3H); 7.21 (m, 2H); 6,45 (br, 1H); 5.92 (s, 1H); 5.86 (d, 1H); 5.34 (d, 1H, *J* = 9.78 Hz); 4.28 (dq, 1H, *J* = 10.76, 6.36 Hz); 2,18 (s, 3H); 1.88 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz); 1.42 (d, 3H, *J* = 6.36 Hz); 1.09 (d, 3H, *J* = 7.34 Hz); 0.93 (s, 3H); 0.82 (s, 3H).

Compound (**8**): $C_{25}H_{22}O_5$ ^{1}H NMR (300-MHz, $CDCl_3$) δ 7.37 (m, 3H); 7.24 (m, 2H); 6.63 (d, 1H, *J* = 9.98 Hz); 6.03 (s, 1H); 5.41 (d, 1H, *J* = 9.78 Hz); 4.32 (dq, 1H, *J* = 10.76, 6.36 Hz); 2.22 (s, 3H); 2.56 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz); 1.54 (d, 3H, *J* = 6.36 Hz); 1.16 (d, 3H, *J* = 7.34 Hz); 0.95 (s, 3H); 0.94 (s, 3H).

Compound (9): $C_{25}H_{26}O_5$ ^{1}H NMR (300-MHz, $CDCl_3$) δ 7.41 (m, 3H); 7.32 (m, 2H); 5.92 (s, 1H); 4.28 (dq, 1H, *J* = 10.76, 6.36 Hz); 2,18 (s, 3H); 1.88 (ddq, 1H, *J* = 10.27, 6.85, 2.94 Hz); 1.42 (d, 3H, *J* = 6.36 Hz); 1.09 (d, 3H, *J* = 7.34 Hz); 0.93 (s, 3H); 0.82 (s, 3H).

2.6. Test of biological activity

2.6.1. Plasmodium falciparum culture

For the assays the culture-adapted *P. falciparum* strain NF54 (chloroquine sensitive) was used. Maintenance in continuous

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culture was according to the methodology previously described by Trager and Jensen (1976); Parasites were cultivated in suspension of 5% human A + erythrocytes in RPMI-1640 culture medium (Sigma R6504) dissolved in sterile water with 25 mM HEPES, 5.0% $NaHCO_3$, 10% fresh human A + serum (inactivated at 56 °C for 30 min); incubated in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Daily change was performed and fresh red blood cells were added twice a week.

2.6.2. Determination of antiplasmodial activity

Antiplasmodial activity was measured by the SYBR Green I® method and was adapted according to the methodology described by Smilkstein et al. (2004). Assays were performed on Falcon® 96-well flat bottom plates. A suspension of parasitized red blood cells with a hematocrit of 2.5% and a parasitaemia of 1% was prepared. Wells with compounds to test and with chloroquine as positive control were incubated at 37 °C for 48 h in an atmosphere of 5% CO_2, 5% O_2 and 90% of N_2. Subsequently, the contents of each well were transferred to Greiner Pro one dishes and the parasites were labeled with a solution of SYBR® Green I 2X in lysis buffer. The plates were incubated at room temperature for one hour in the dark and the relative fluorescence units (RFU) were read on a spectrofluorometer at an excitation wavelength of 485 nm and emission length of 538 nm. Treatments from each crude extract were prepared to a stock solution of 10 mg/mL in pure DMSO and sonicated to facilitate dissolution. From this solution, 50 μL were taken and adjusted to 1000 μL with complete RPMI-1640 medium, obtaining a final concentration of 0.5 mg/mL. The concentration of DMSO in the first dilution, which was 1%, has been shown to be non-toxic to the parasite. Seven concentrations of each extract were evaluated in a range between 100 and 1.56 μg/mL. Each concentration was evaluated in duplicate on the plate and three independent assays were performed. The CQ control was evaluated in a range between 150 and 4.7 nM and the control of Peruvian Quina extract (MeOH:H_2O; 70:30) was evaluated in the range of 0.01–10 μg/mL.

2.6.3. Determination of the inhibitory concentration

Data from three assays were analyzed to find the inhibitory concentration IC_{50} in μg/mL. Inhibitory concentrations ($IC_{50} \pm SD$) were calculated for each compound from a non-linear logistic regression model. A sigmoid concentration-response curve with slope of Hill (variable slope) was assumed. The data were analyzed and plotted using GraphPad Prism 4 for Macintosh version 4.0b which outputs the adjustment value (r) (GraphPad Software, San Diego, California, USA). To classify the antiplasmodial activity of an extract, the Malaria Group of the University of Antioquia established a consensus for the extracts evaluated: highly active <5 μg/mL, promising 6–15 μg/mL, moderate activity 16–30 μg/mL, low activity 31–50 μg/mL and non-active >50 μg/mL (Malebo et al., 2009; Muñoz et al., 2000). To classify the antiplasmodial activity of a compound, the Malaria Group estimated that a compound is promising if the IC_{50} is $\leq$10 μM.

2.6.4. Cytotoxicity testing and calculation of the selectivity index (SI)

The method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) according to Mosmann (1983) was used to evaluate the cytotoxic activity of the extracts (Mosmann, 1983) which reveals cellular damage at the mitochondrial level. U-937 cells were human histiocytic lymphoma promonocytes, which were maintained in continuous cultures in the Malaria Group Laboratory. These cells were cultured at 37 °C and 5% CO_2 in RPMI medium supplemented with 10% inactivated Fetal Bovine Serum (FBS). The media changes were performed every 48 h or according to pH changes of the medium, with centrifugation for 10 min at 1000 rpm, replaced with fresh medium. In Neubauer's chamber, U-937 cells were counted and plated in a 96-well flat bottom plate, 200,000 cells/mL in RPMI 1640 medium with 10% FBS. They were incubated at 37 °C with 5% CO_2 for 72 h in the presence of each of the seven concentrations of each extract and/or compound evaluated in a range between 100 and 1.56 μg/mL, each concentration was evaluated in duplicate in the dish and three independent assays were performed. Subsequently, mitochondrial dehydrogenase activity was measured by adding 20 μL/well of MTT to a concentration of 5 mg/mL and incubating for 3 h at 37 °C and 5% CO_2 (Moore et al., 1967). To dissolve the formed crystals, 100 μL/well of a 50% solution of isopropanol and 10% SDS were added and the absorbance read at 595 nm in an ELISA reader (Bio-Rad). Data from three independent assays were analyzed using the GraphPad Prism 5 program to find the toxic concentration in μg/mL (CC_{50}) using a non-linear logistic regression model (Reed et al., 2002). For the assay, the HepG2 cells were counted in a Neubauer chamber and seeded in a 96-well flat bottom plate 2×10^5 cells/well in 100 μL of RPMI-1640 medium with 10% fetal bovine serum. Subsequently, the cells were incubated for 30 h at 37 °C in a 5% CO_2 environment, to allow the formation of the monolayer and then add 100 μL of each of the concentrations (100–1.54 μg/mL) of the extracts and/or compounds or 100 μL of the medium in which the extracts and/or compounds were dissolved. Four wells were left, to which culture medium was added to evaluate the behavior of the cells under normal conditions (negative control). Each concentration of extracts and/or compounds and controls were evaluated in quadruplicate in 2 assays. Plates were incubated for 48 h at 37 °C. Then, 30 μL of MTT (Sigma Aldrich) was added at a concentration of 2 mg/mL and incubated again for 5 h. After incubation, 130 μL of 96% DMSO were added and the dishes were incubated for 20 min at room temperature and gently mixed to allow the MTT crystals to dissolve. The production of Formazan crystals is measured at 550 nm in an ELISA reader (Bio-Rad). The toxic effect was determined by comparing the absorbance obtained in the control with that of the treatments and thus determining whether or not there was a toxic effect. Data were analyzed with the GraphPad Prism 5 program to find CC_{50} (cytotoxic concentration inhibiting 50% growth). To classify the cytotoxicity of the extract, the Malaria Group of the University of Antioquia established a consensus for the samples evaluated: highly toxic <10 μg/mL, cytotoxic 10–40 μg/mL, moderately cytotoxic 41–100 μg/mL and not cytotoxic >100 μg/mL. In addition, the selectivity index (SI) was calculated, which indicates selectivity towards the parasite and corresponds to the relationship between cytotoxic CC_{50} activity and antiplasmodial activity IC_{50}. For the $HepG_2$ cell line, SI values above 5 were considered as promising extracts (Kaou et al., 2008).

2.6.5. Inhibition of the crystallization of β-hematin

Evaluation of the inhibition of β-hematin crystallization of compounds **1** and **2** was done by the method of Paparini et al. (2000) with some modifications (Paparini et al., 2000). A total of 100 μL of a 1.562 mM solution of hemin chloride dissolved in 0.2 M NaOH was distributed in 96-well microplates (0.4 μmol/well) with multichannel pipette together with 50 μL of different concentrations of compounds in a range: 5.0–0.078 mg/mL dissolved in dimethylsulfoxide (DMSO) and the CQ control was evaluated in a range of 3.0–0.094 mg/mL. It was added in duplicate and two replicates of the assay were performed. In the control wells, 50 μL of DMSO for wells without treatment and chloroquine control were added as reference. The formation of β-hematin was initiated by the addition of 50 μL of acetic acid 2.175 M to a final pH of 2.8 and 50 μL of sodium acetate buffer ($C_2H_3NaO_2 \cdot 3H_2O$) 0.75 M. The plate was incubated at 37 °C for 24 h and then centrifuged at 4500*g* for 1 h. The soluble fraction of the material was discarded. The rest was suspended with 200 μL of DMSO:H_2O ratio 1:1 to remove the unreacted hematin. The dishes were again centrifuged at 4500*g* for

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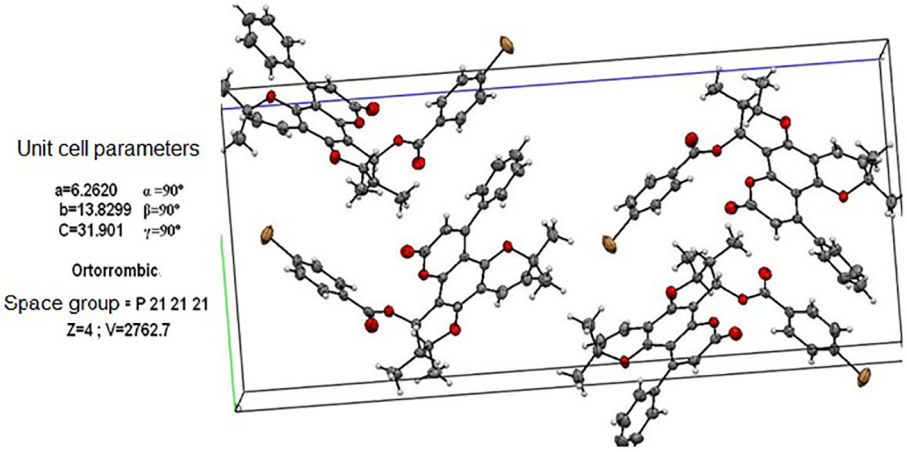

Fig. 1. X-ray characterization of soulattrolide (1).

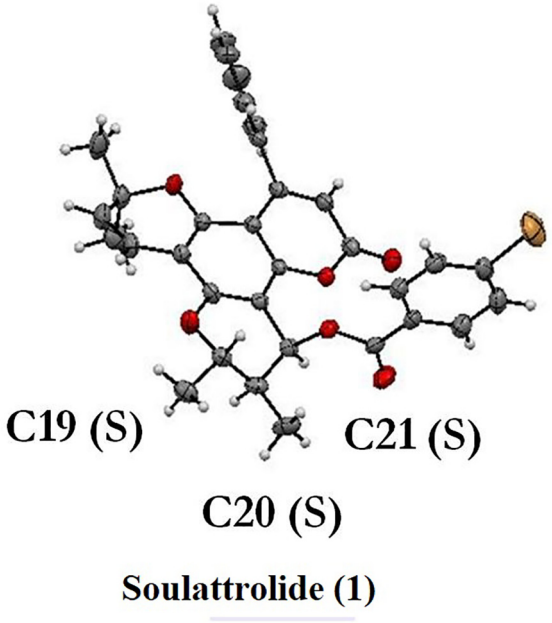

Fig. 2. ORTEP II drawing of the crystal structure for derivative (2). Major ellipses of atoms other than hydrogen are graphed with a 50% probability level. Hydrogen atoms have been drawn as spheres of arbitrary size.

15 min in triplicate and the supernatant was discarded. The precipitate was dissolved in 0.2 M NaOH for spectroscopic quantification. An aliquot of 50 μL from each well was transferred to a new dish with 150 μL of 0.2 M NaOH. A sample blank at the same concentrations dissolved in 150 μL of 0.2 M NaOH was used. Before the readings, the formation of β-hematin was determined spectrophotometrically in a Spectronic 20 UV–Vis in the range of 250–800 nm. The amount of β-hematin inhibited by each of the extracts was determined by measuring the absorbance at 595 nm using an ELISA reader (BioRad) and the % inhibition of β-hematin was calculated according to Eq. (1). Data from two assays, were analyzed with the GraphPad Prism 5 program to find the concentration of extract required to inhibit 50% of the formation of β-hematin in mg/mL (IC_{50}) by means of a non-linear logistic regression model.

$$\%Inhibition = 1 - [(Abs\ sample - Abs\ Sample\ blank)/(Abs\ Control)] \times 100 \quad (1)$$

2.6.6. Formation of complex β-hematin + compound (1, 2) by mass spectrometry ESI-MS

A Quattro LC (QhQ quadrupole-hexapolar-quadrupole) with an orthogonal mass spectrometer Z-spray-electrospray interface (Micromass, Manchester, United Kingdom) was used. The sample solutions (approximately 1×10^{-5} M) in acetonitrile: aqueous 0.5 M NH_4OH (1:1 v/v) was introduced through a fused silica capillary to the ESI source through the pump of syringe at a flow rate of 10 μL/min. The drying gas as well as the nebulization gas was nitrogen at a flow rate of 300 and 90 L/h, respectively. The temperature of the source block was set at 80 °C and the interface at 120 °C. For each ion of interest, the parameters of the instrument were optimized for the maximum ion abundance. The capillary voltage was set at 3.5 kV in positive scan mode and the cone voltage was adjusted (typically 20–40 Uc V) to control the extent of fragmentation in the source region. The extractor cone and the radio frequency lens tension were maintained at 3 V and 0.2, respectively. The chemical composition of each peak obtained in the full scan mode was assigned by comparing the experimental and theoretical isotope patterns using the program MássLynx 4.1.

3. Results and discussion

The dichloromethane extract of the air dried *C. brasiliense* was fractionated using vacuum liquid chromatography (VLC) and purified by various chromatographic techniques. The IC_{50} value of the crude extract (IC_{50} = 7.63 ± 1.84 μg/mL) and the CC_{50} (35.09 ± 0.12 μg/mL) showed moderate activity and some selectivity in the parasite *P. falciparum*, indicating that the crude extract has potential as an antiplasmodial agent. One compound (**1**) was isolated. This component called soulattrolide was isolated and structural elucidation was achieved through spectroscopic analysis and verified by direct comparison of the spectral data with values from the literature. This compound has been reported in the genus *Calophyllum* (Noldin et al., 2006). Compound **1** was obtained as a white amorphous solid. The absolute stereochemistry of the soulattrolide was confirmed by obtaining a crystalline derivative for the first time. The absolute configuration was assigned based on the X-ray test through the Analysis of the crystalline derivative with 4-bromobenzoyl chloride $C_{32}H_{27}O_6Br$, also ^1H NMR-NOE experiments were performed to determine a relative configuration by comparison of the coupling constants with other related compounds. The crystallographic data for $C_{32}H_{27}O_6Br$, were Mr = 587.44, type of orthorhombic crystal system, spatial group *P*212121, a = 6.2620 (7) Å, b = 13.8299 (16) Å, c = 31.901 (4) Å, Vcelda = 2762.7 (6) $Å^3$, Z = 4,

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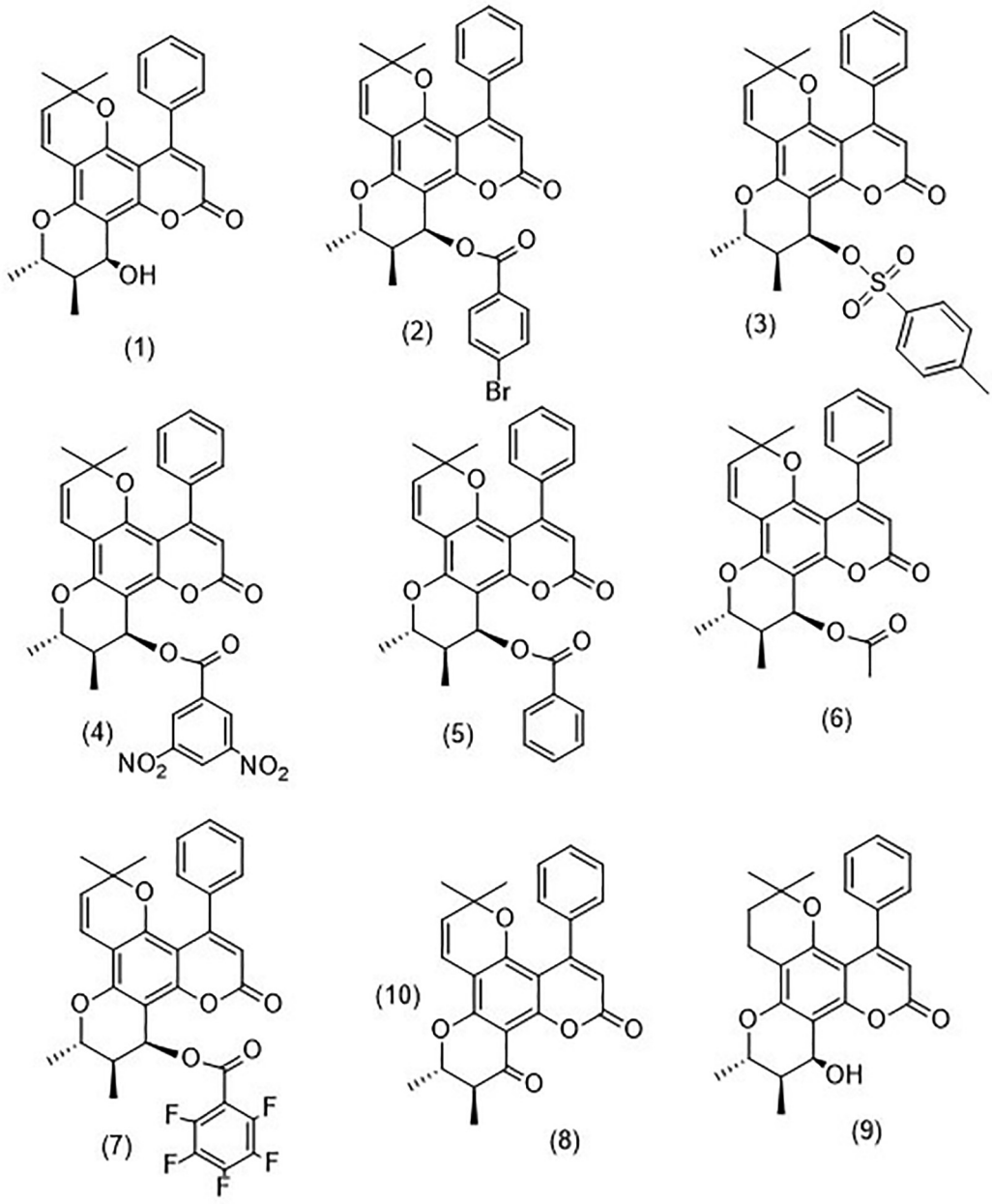

Fig. 3. Synthetic derivates of soulattrolide (1).

Table 1
In vitro antiplasmodial and cytotoxicity assays of derivates of soulattrolide (1).

Code Compound	Formula	IC $_{50}$ (μg/mL) X ± SD* *P. falciparum* NF-54***	CC_{50}(μg/mL) X ± SD HepG2 Cells	SI**
1	$C_{25}H_{24}O_5$	55.45 ± 13.89	>100	1.80
2	$C_{32}H_{27}BrO_6$	8.06 ± 0.96	4.71 ± 0.56	0.58
3	$C_{32}H_{30}SO_7$	8.71 ± 1.00	10.68 ± 0.74	1.23
4	$C_{32}H_{26}N_2O_{10}$	19.06 ± 1.55	19,16 ± 3,01	1.00
5	$C_{32}H_{28}O_6$	9.66 ± 2.53	21.84 ± 9.83	2.26
6	$C_{27}H_{26}O_6$	14.21 ± 4.95	9.16 ± 4.16	0.64
7	$C_{32}H_{23}F_5O_6$	4.39 ± 0.83	12.61 ± 2.04	2.87
8	$C_{25}H_{22}O_5$	5.13 ± 0.11	5.79 ± 1.25	1.13
9	$C_{25}H_{26}O_5$	13.89 ± 3.73	15.12 ± 0.34	1.09

* The mean ± S.D.
** SI = Selectivity index CC_{50}/IC_{50}, SI > 2 confirm efficacy and safety.
*** Control Chloroquine IC_{50} = 0.008 ± 2,78 μg/mL. The definition of the antiplasmodial activity used was: IC_{50} < 5 μg/mL – strong activity; 6–15 μg/mL – moderate activity; 16–30 μg/mL – mild activity and IC_{50} > 30 μg/mL – inactive.

Please cite this article as: A. M. Mesa Vanegas, S. Blair Trujillo, C. Peláez Jaramillo et al., , Journal of King Saud University – Science, https://doi.org/10.1016/j.jksus.2019.03.005

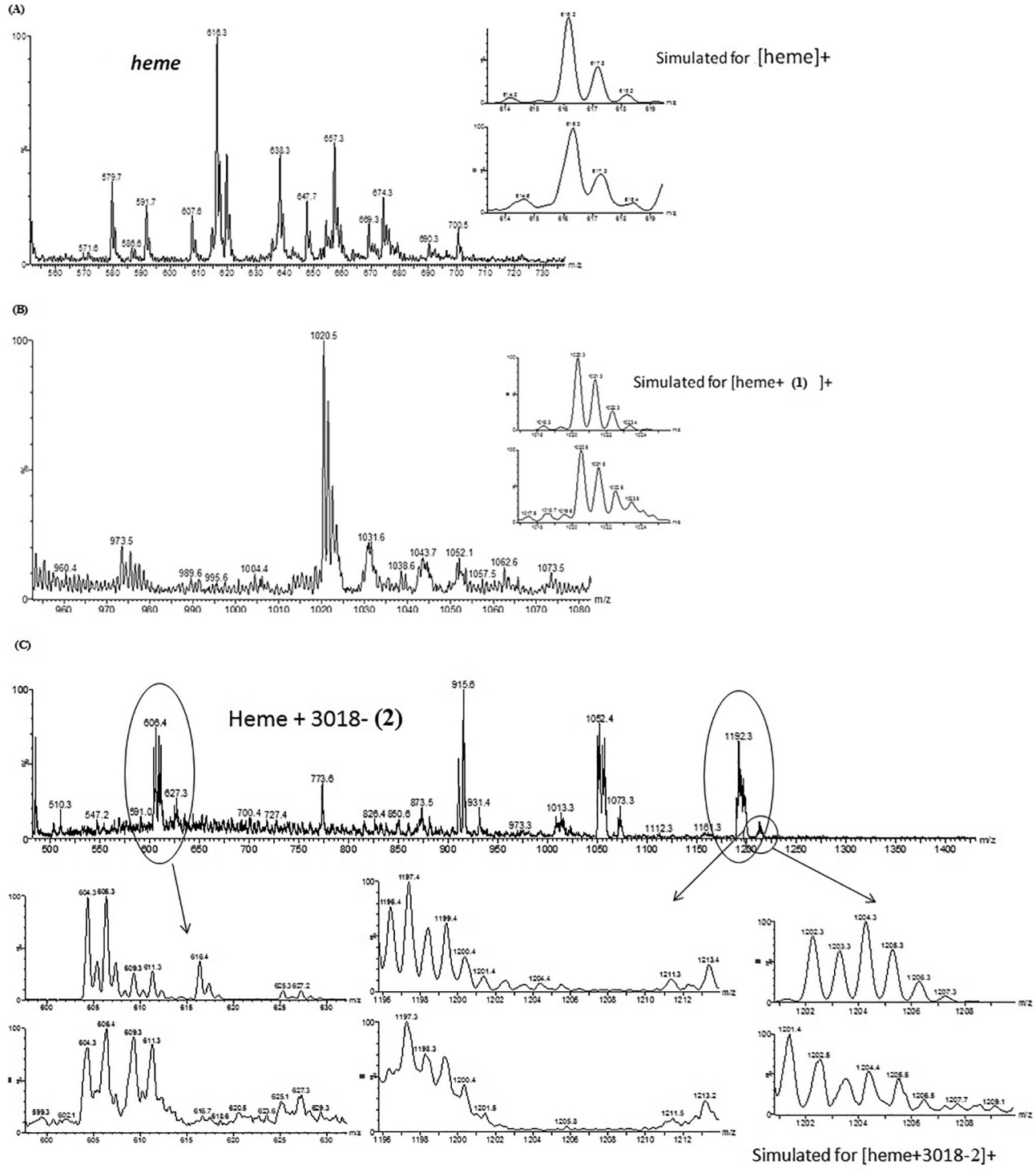

Fig. 4. Analysis by mass spectrometry of the interaction of soulattrolide (1) and compound (2) with FP. Sample solutions [heme], [heme + (1)], [Heme + (2)] (approximately $1 \times 10–5$ M) in acetonitrile: 0.5 M aqueous NH_4OH (1:1 v/v) (A) [heme]] (B) [heme + (1)] (C) [heme + (2)], followed by the positive ion recording ESI-MS, as shown. Uncertainties in masses of ±0.3 Da.

dcalc = 1.412 g cm^{-3}, μ = 1.532 mm^{-1}, (Cu, Kα, λ = 0.71073 Å), T = 516 K. The intensity data were 2θ < 30,570, 0 < *h* < 8, 0 < *k* < 19, 0 < *l* < 45 and their Friedel values were copied by means of variable-speed scans ω-2θ. The data were corrected for Lorentz and polarization factors, by absorption, and by decay of isotropic intensity. The structure was solved by direct and refined methods with the parameters of anisotropic displacement of atoms other than hydrogen (Fig. 1).

The positions of the hydrogen atoms were determined from differences of Fourier maps, but remained fixed at the positions geometrically calculated in the last refinement of the isotropic factors of assigned temperature 1.3 (Beq) of the attached atom. The complete matrix was calculated with convergent least squares (in F) refinement (max Δ/σ = 1208.0), conventional crystallographic reflections R = 0.0527 (4936) and R2w = 0.1401 (8186) for 8186 observations [I > 3σ (I)]. 460 variables and the adjustment were

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1,008. A final difference of the Fourier map was monotonous; Maximum residual densities were close to the bromine atom (0.699 and −0.845 $Å^{-3}$). This assignment of the crystalline derivative configuration (C19 (S); C20 (S); C21 (S) was confirmed by enantiopole refinement and corroborated by examination of the Friedel pairs most affected by the anomalous scattering effects (Fig. 1) In this sense, the absolute configuration of the natural compound soulattrolide is (C19 (S); C20 (R); C21 (S).) The neutral factor of atomic dispersion and the values of the anomalous dispersion terms were taken from the international society of X-ray crystallography (Fig. 2). This is the first time that this compound $C_{32}H_{27}O_6Br$ has been reported in the literature.

Nine acid derivatives of compound (**1**) were obtained for first time (Fig. 3), and were subjected to antiplasmodial and cytotoxicity assays. Biological activity of **1** was 55.45 ± 13.89 or 137.08 μM, and of the crystalline derivative of soulattrolide (**2**), which is reported for the first time**,** was 8.06 ± 0.96 or 13.73 μM. All remaining compounds (**3**–**9**) showed promising activity against the *P. falciparum* strain NF-54 (Table 1). A significant increase in the antiplasmodial activity was observed with the ester (**2**), which showed a reduction of the IC_{50} by an factor of almost 10. Fig. 4 experimentally supports the strong interaction between compound 2 and β-hematin. Molecular modeling optimized and tested the strong interaction between soulattrolide (1) and derivate (**2**) with β-hematin.

4. Conclusion

The good pharmacological activities of dichloromethane extract of *C. brasiliense* and isolated compounds and derivates, which were characterized and confirmed by obtaining a crystalline derivative, showed the importance of this study. New structural analogues were proposed with capability of inhibition of β-hematin in order to achieve a global understanding of the activity of the compounds tested and to select the best antiplasmodial compound for further studies in murine models.

5. Disclosure of funding

The authors are grateful for the financial support provided by the Colombian Ministry of Agriculture (No. 009-2007-V7552-38-07), Colciencias and the University of Antioquia.

6. Author declaration

We wish to confirm that there were no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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