Heliyon 8 (2022) e08939

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Coumaro-chalcones synthesized under solvent-free conditions as potential agents against malaria, leishmania and trypanosomiasis

José E. Cuellar [a], Winston Quiñones [b], Sara Robledo [c], Jesús Gil [d], Diego Durango [a,*]

[a] Universidad Nacional de Colombia-Sede Medellín, Facultad de Ciencias, Escuela de Química, Grupo de Química de los Productos Naturales y los Alimentos, Carrera 65, Número 59[a]-110, Medellín, Colombia
[b] Química Orgánica de Productos Naturales, Instituto de Química, Facultad de Ciencias Exactas y Naturales, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia
[c] PECET-Facultad de Medicina, Universidad de Antioquia, Calle 70 No. 52-21, Medellín, Colombia
[d] Universidad Nacional de Colombia-Sede Medellín, Facultad de Ciencias Agrarias, Departamento de Ingeniería Agrícola y Alimentos, Grupo de Química de los Productos Naturales y los Alimentos, Carrera 65, Número 59[a]-110, Medellín, Colombia

ARTICLE INFO

Keywords:
Coumaro-chalcones
Biological activity
Cytotoxicity
Leishmaniasis
Trypanosomiasis
Malaria

ABSTRACT

Leishmaniasis, trypanosomiasis, and malaria are a group of neglected tropical diseases present in tropical regions and they affect large numbers of people in developing countries. A series of thirteen coumaro-chalcones (**A1**-**A13**) were synthesized under solvent-free conditions and their *in vitro* anti-leishmanial, anti-plasmodial, anti-trypanosomal and cytotoxic activities were evaluated. One of these coumaro-chalcones, 3-[(2*E*)-3-(3-ethoxy-4-hydroxyphenyl)prop-2-enoyl]-2H-chromen-2-one (**A12**), is a new compound. Compounds 3-[(2*E*)-3-(3-hydroxyphenyl)prop-2-enoyl]-2H-chromen-2-one (**A5**), 3-[(2*E*)-3-(3-methoxyphenyl)prop-2-enoyl]-2H-chromen-2-one (**A2**) and 3-[(2*E*)-3-phenylprop-2-enoyl]-2H-chromen-2-one (**A1**) displayed strong inhibition against intracellular amastigotes of *Leishmania panamensis* with EC_{50} of 2.1 ± 0.1, 2.5 ± 0.2 and 3.7 ± 0.5 μM, respectively. In addition, *Plasmodium falciparum* was moderately inhibited by the coumarin-chalcone hybrids, particularly **A12** (EC_{50}: 15.0 ± 0.5 μM) and 3-[(2*E*)-3-(1,3-benzodioxol-5-yl)prop-2-enoyl]-2H-chromen-2-one (**A13**) (EC_{50}: 15.2 ± 1.1 μM). Remarkably, the coumaro-chalcone **A5** (EC_{50}: 18.7 ± 2.4 μM) exhibited an inhibition of the *Trypanosoma cruzi* intracellular amastigotes similar to the commercial drug Benznidazole (EC_{50}: 14.5 ± 0.1 μM). These results support the therapeutic potential of coumaro-chalcone hybrids.

1. Introduction

Neglected tropical diseases (NTD) are a diverse group of communicable diseases that are present in tropical conditions and affect a high number of people in developing countries. Among them are leishmaniasis, trypanosomiasis and malaria. Hematophagous vectors that ingest the pathogenic microorganisms transmit these diseases. Leishmaniasis in its different versions, cutaneous, visceral, and mucocutaneous, is caused by parasites of the genus *Leishmania* (*L. tropica*, *L. major*, *L. donovani*, *L. infantum*, *L. braziliensis*, and *L. mexicana*). It is estimated that leishmaniasis kills between 20,000 and 30,000 people in the world and almost a million new cases occur each year (World health statistics, 2018). The *Leishmania* parasites have a complex life cycle and exist in two morphological forms, as an intracellular aflagellated amastigote in the mammalian host (humans, dogs, lizards or rodents) and a motile promastigote in the insect vector (Albajar-Viñas and Jannin, 2011). Amphotericin B, pentamidine, miltefosine, and antimonials compounds are currently used as anti-leishmanial drugs.

Furthermore, American trypanosomiasis or Chagas disease is caused by the flagellated protozoan *Trypanosoma cruzi*. This parasite is transmitted mainly by the feces of hematophagous bugs (triatomines) that come into contact with human skin. It was calculated that near 7 million people worldwide were infected with Chagas disease, most of them in Latin America (Lee et al., 2013). Up to 30% of chronic patients had cardiac alterations and up to 10% have digestive, neurological or combined disorders (Junqueira, 2012). In the vertebrate host, *T. cruzi* is found in the forms of free trypomastigotes in the peripheral blood and intracellular amastigotes that are divided by binary fission, while the epimastigote is the form of division of the triatomine (Albajar-Viñas and Jannin, 2011). Chagas disease is treated with Benznidazole and Nifurtimox.

On the other hand, malaria is a parasitic disease transmitted to humans by the bite of the female Anopheles mosquito. Malaria is

* Corresponding author.
E-mail address: dldurango@unal.edu.co (D. Durango).

https://doi.org/10.1016/j.heliyon.2022.e08939
Received 28 September 2021; Received in revised form 6 December 2021; Accepted 8 February 2022

produced by a protozoon of the genus *Plasmodium*, causing the majority of the deaths *P. falciparum* and *P. vivax*. An estimated 216 million cases of malaria occurred in 2016, compared with 237 million cases in 2010, and 210 million cases in 2013 (World health statistics, 2018). Anti-malarial drugs include quinine, quinoline, mefloquine, and artemisinin.

Unfortunately, current drugs that treat leishmaniasis, Chagas disease, and malaria have limited therapeutic application due to various aspects: i.) the highest incidence of the disease occurs in marginal areas, where access to these drugs is limited; ii.) high costs; iii.) undesirable side effects, and iv.) the parasites have gradually developed resistance to the drugs used for their control. Additionally, the synthesis and purification of these compounds imply the use of heavy metals and hazardous solvents. In this regard, there has been a strategic focus to develop more environmentally friendly synthesis.

Different researches have suggested the use of substances of natural and synthetic origin of low molecular weight and easy access, like chalcones and coumarins, as chemotherapeutic candidates for these diseases. The chalcones licochalcone-A (Figure 1), crotaorixin, rhuschalcone VI, 3-methoxycitrunobin-4-methyl ether attack the parasite *L. major* (Ogungbe et al., 2014) while the 2′,4,4′-trihydroxy-3,3′-diprenylchalcone and 2′,4,4′-trihydroxy-3′,5′-diprenylchalcone are active against *L. mexicana* (De Mello et al., 2018). Furthermore, licochalcone-A, 5-prenylbutein, licoagrochalcone-A, homobutein, crotaorixin, medicagenin and xanthohumol are active against *P. falciparum* (Li et al., 1995). In addition, coumarins from *Ferula narthex* Boiss. and *Calophyllum brasiliense* are active against *L. braziliensis* (Bashir et al., 2014; Brenzan et al., 2008) while those from *Ferula szowitsiana* are active against *L. major* (Iranshahi et al., 2007). Chalepin, a compound from *Pilocarpus spicatu*, is an inhibitor of glyceraldehyde-3-phosphate dehydrogenase in *T. cruzi* cells (Pavão et al., 2002). Mammea-type isoprenylated coumarins exhibited anti-trypanosomal activity and against *P. falciparum* (Reyes-Chilpa et al., 2008).

In response to the activity shown by these two structural templates of natural origin, many authors have proposed some hybrids as promising molecules for the control of malaria, leishmaniasis, and Chagas disease (Vazquez-Rodriguez et al., 2013; Vazquez-Rodriguez et al., 2015). In the present work, a number of coumaro-chalcones were developed and their activity against parasite species that cause malaria, leishmania, and Chagas disease were evaluated.

2. Materials and methods

2.1. Materials

3-anisaldehyde (97%), 4-anisaldehyde (99+%), 3-tolualdehyde (98%), 3-nitrobenzaldehyde (99%), and 3-bromobenzaldehyde (96%) were acquired from Acros organics (NJ, USA). 2-hydroxybenzaldehyde, ethyl acetoacetate (99%), 4-hydroxybenzaldehyde, 4-nitrobenzaldehyde (98%), 4-clorobenzaldehyde (97%), 4-bromobenzaldehyde (99%), ethyl vanillin (98%), piperonal (99%), and piperidine (≥99.5%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Benzaldehyde was obtained from Merck (Hohenbrunn, Germany) and Aldrich Co. (Milwaukee, WI, USA).

2.2. Analytical equipments

Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates (0.25 mm, 60F_{254}, Merck, Darmstadt, Germany) and compounds were visualized by exposure to UV. The melting points were measured on the MEL-TEMP® fusiometer without correction. The FT-IR spectra were obtained on an IRTracer-100 FTIR spectrometer (Shimadzu, Kyoto, Japan). The ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra were performed using a Bruker AMX300 spectrometer (Bruker Bio-Spin GmbH, Rheinstetten, Germany) operating at 300 MHz

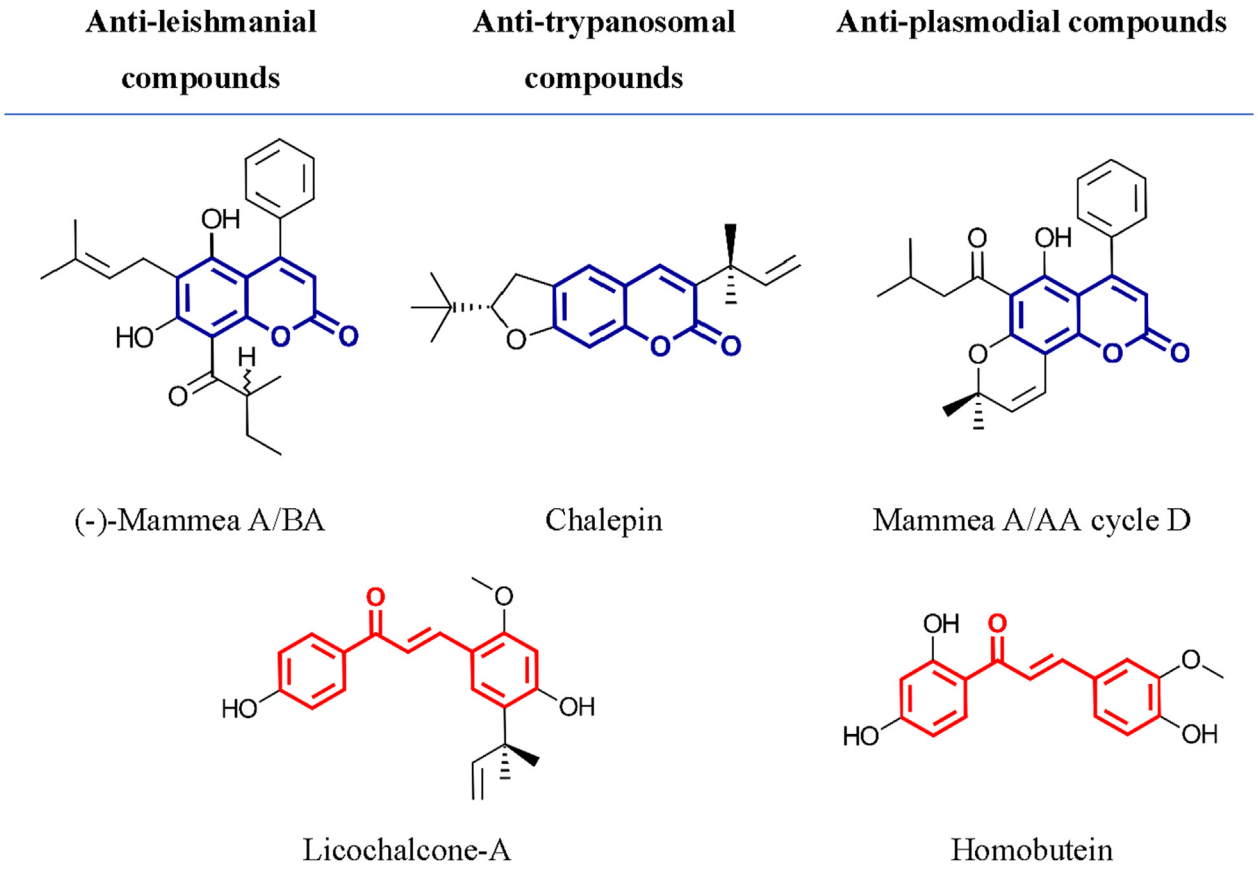


Figure 1. Structure of coumarins and chalcones with anti-leishmanial, anti-trypanosomal and anti-plasmodial activity.

for ^{1}H and 75 MHz for ^{13}C. The samples were dissolved in DMSO-d_6 or $CDCl_3$. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. Chemical shift (δ) and coupling constants (J) are expressed in ppm and hertz (Hz) units, respectively.

2.3. General experimental procedures

2.3.1. Synthesis of 3-acetyl-2H-chromen-2-one

Synthesis of 3-acetyl-2*H*-chromen-2-one was performed according to Sugino and Tanaka (2001). A mixture of 2-hydroxybenzaldehyde (20.0 g; 163.8 mmol) and ethyl acetoacetate (21.1 g; 162.2 mmol) was stirred and cooled in an ice-salt bath until the temperature become below 0 °C. Then, 1.0 mL piperidine was added dropwise and allowed to react for 1 h. With the progress of the reaction, a yellow paste was formed. Then, it was removed and placed under stirring in a 1.0 M HCl. A white precipitate was obtained, and vacuum filtered to obtain 3-acetyl-2H-chromen-2-one. M.p. 119-120 °C. IR ($\bar{\nu}$, cm^{-1}): 1740 (C=O), 1676 (C=C), 1609 (C=C), 1551 (O–C=O lactone). ^{1}H NMR ($CDCl_3$), δ: 8.55 (1H, s, H4), 7.72–7.68 (2H, m, H5, H7), 7.43–7.31 (2H, m, H6, H8), 2.77 (3H, s, Hα). ^{13}C NMR ($CDCl_3$), δ: 195.6 (C3a), 159.5 (C2), 155.4 (C8a), 147.6 (C4), 134.5 (C7), 130.3 (C5), 125.0 (C6), 124.5 (C3), 118.3 (C4a), 116.7 (C8), 30.6 (Cα).

2.3.2. Synthesis of compounds A1-A13

The mixture of 3-acetyl-2*H*-chromen-2-one (1.0 g, 5.5 mmol) and benzaldehyde derivative (5.5 mmol) was stirred and heated (below 60 °C) until obtaining a single liquid phase. Then, 0.5 mL piperidine was added dropwise and allowed to react for 30 min. After the reaction was complete, the mixture was poured into the ice/cold water. The product was placed under stirring in a 1.0 M HCl and the precipitate was vacuum filtered and recrystallized from a solution EtOH-water, 1:1.

A1. 3-[(2E)-3-phenylprop-2-enoyl]-2H-chromen-2-one: Yield: 98.9%; M.p. 162-163 °C. IR ($\bar{\nu}$, cm^{-1}): 3036 (=C–H), 1732 (C=O), 1660 (C=C), 1607 (C=C_{Ar}), 1571 (C=C_{Ar}), 1184 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.62 (1H, s, H4), 7.97 (1H, d, J = 15.9, Hβ), 7.93 (1H, d, J = 15.9, Hα), 7.73–7.71 (4H, m, H2′, H6′, H5, H7), 7.45–7.37 (5H, m, H3′, H4′, H5′, H6, H8). ^{13}C NMR ($CDCl_3$), δ: 186.6 (C3a), 159.4 (C2), 155.3 (C8a), 148.2 (C4), 145.2 (Cβ), 134.8 (C1′), 134.3 (C7), 130.9 (C5), 130.1 (C2′, C6′), 129.0 (C3′, C4′, C5′), 125.3 (C3), 125.1 (C6), 124.0 (Cα), 118.6 (C4a), 116.8 (C8).

A2. 3-[(2E)-3-(3-methoxyphenyl)prop-2-enoyl]-2H-chromen-2-one: Yield: 98.7%; M.p. 143-144 °C. IR ($\bar{\nu}$, cm^{-1}): 3053 (=C–H), 1732 (C=O), 1660 (C=C), 1607 (C=C_{Ar}), 1563 (C=C_{Ar}), 1180 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.60 (1H, s, H4), 7.97 (1H, d, J = 15.9, Hβ), 7.82 (1H, d, J = 15.9, Hα), 7.68 (2H, t, J = 7.5, H5, H7), 7.43 (2H, m, H5′, H6′), 7.30 (2H, t, J = 7.8, H6, H8), 7.20 (1H, s, H2′), 7.01 (1H, d, J = 7.8, H4′), 3.88 (3H, s, -OMe). ^{13}C NMR ($CDCl_3$), δ: 186.5 (C3a), 159.9 (C2), 159.3 (C3′), 155.2 (C8a), 148.2 (C4), 145.0 (Cβ), 136.1 (C1′), 134.3 (C7), 130.1 (C5), 129.9 (C5′), 125.2 (C3), 125.0 (C6), 124.2 (Cα), 121.7 (C6′), 118.6 (C4a), 116.9 (C2′), 116.7 (C8), 113.6 (C4′), 55.4 (-OMe).

A3. 3-[(2E)-3-(4-methoxyphenyl)prop-2-enoyl]-2H-chromen-2-one: Yield: 98.4%; M.p. 170-171 °C. IR ($\bar{\nu}$, cm^{-1}): 3050 (=C–H), 1714 (C=O), 1604 (C=C), 1553 (C=C_{Ar}), 1251 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.60 (1H, s, H4), 7.86 (2H, m, Hα, Hβ), 7.70–7.65 (4H, m, H2′, H6′, H5, H7), 7.43–7.38 (2H, m, H6, H8), 6.97–6.94 (2H, d, J = 7.5, H3′, H5′), 3.89 (3H, s, -OMe). ^{13}C NMR ($CDCl_3$), δ: 186.3 (C3a), 162.0 (C2), 159.4 (C8a), 155.2 (C4′), 147.9 (C4), 145.1 (Cβ), 134.1 (C7), 130.9 (C2′, C6′), 130.0 (C5), 127.6 (C1′), 125.5 (C3), 125.0 (C6), 121.6 (Cα), 118.6 (C4a), 116.7 (C8), 114.4 (C3′, C5′), 55.5 (-OMe).

A4. 3-[(2E)-3-(3-methylphenyl)prop-2-enoyl]-2H-chromen-2-one: Yield: 98.5%; M.p. 121-122 °C. IR ($\bar{\nu}$, cm^{-1}): 3035 (=C–H), 1726 (C=O), 1603 (C=C), 1562 (C=C_{Ar}), 1172 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.60 (1H, s, H4), 7.94 (1H, d, J = 15.6, Hβ), 7.89 (1H, d, J = 15.6, Hα), 7.72–7.67 (2H, t, J = 7.5, H5, H7), 7.52–7.49 (2H, m, H2′, H6′), 7.43–7.25 (4H, m, H4′, H5′, H6, H8), 2.42 (3H, s, -Me). ^{13}C NMR ($CDCl_3$), δ: 186.5 (C3a), 159.0 (C2), 155.2 (C8a), 148.1 (C4), 145.4 (Cβ), 138.6 (C1′), 134.7 (C7), 131.8 (C5), 130.1 (C5′), 129.4 (C2′), 128.8 (C6′), 126.4 (C4′), 125.3 (C3), 125.0 (Cα), 123.7 (C6), 118.6 (C4a), 116.7 (C8), 21.4 (-Me).

A5. 3-[(2E)-3-(3-hydroxyphenyl)prop-2-enoyl]-2H-chromen-2-one. Yield: 74.8%; M.p. 163-164 °C. IR ($\bar{\nu}$, cm^{-1}): 3330 (O–H), 3053 (=C–H), 1710 (C=O), 1598 (C=C), 1562 (C=C_{Ar}), 1226 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.66 (1H, s, H4), 7.94 (1H, d, J = 15.6, Hβ), 7.75 (1H, d, J = 15.6, Hα), 7.72 (1H, d, J = 7.5, H7), 7.64 (1H, t, J = 7.5, H5), 7.49 (1H, dd, J = 7.8, 2.1, H6′), 7.42 (1H, t, J = 2.1, H2′), 7.27 (1H, t, J = 7.5, H5′), 7.18–7.13 (2H, m, H6, H8), 6.86 (1H, d, J = 7.8, H4′), 2.50 (1H, bs, –OH). ^{13}C NMR ($CDCl_3$), δ: 187.5 (C3a), 159.0 (C2), 158.2 (C8a), 154.9 (C3′), 147.6 (C4), 144.7 (Cβ), 136.2 (C1′), 134.7 (C7), 130.9 (C5), 130.6 (C5′), 125.8 (C3), 125.4 (C6), 124.9 (Cα), 120.6 (C6′), 118.9 (C4a), 118.7 (C2′), 116.7 (C8), 115.0 (C4′).

A6. 3-[(2E)-3-(4-hydroxyphenyl)prop-2-enoyl]-2H-chromen-2-one: Yield: 82.7%; M.p. 193-194 °C. IR ($\bar{\nu}$, cm^{-1}): 3294 (O–H), 3027 (=C–H), 1696 (C=O), 1562 (C=C_{Ar}), 1171 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.48 (1H, s, H4), 7.71 (1H, dd, J = 8.1, 2.1, H7), 7.63 (1H, d, J = 15.6, Hβ), 7.62 (1H, t, J = 7.5, H5), 7.53 (1H, d, J = 15.6, Hα), 7.43 (2H, d, J = 8.4, H2′, H6′), 7.33–7.30 (2H, m, H6, H8), 6.78 (2H, d, J = 8.4, H3′, H5′), 2.07 (1H, bs, –OH). ^{13}C NMR ($CDCl_3$), δ: 186.5 (C3a), 160.8 (C2), 159.1 (C8a), 154.9 (C4′), 147.4 (C4), 145.4 (Cβ), 134.2 (C7), 130.9 (C2′, C6′), 130.2 (C5), 125.8 (C3), 125.7 (Cα), 125.1 (C6), 120.8 (C1′), 118.6 (C4a), 116.4 (C8), 116.3 (C3′, C5′).

A7. 3-[(2E)-3-(3-nitrophenyl)prop-2-enoyl]-2H-chromen-2-one: Yield: 96.9%; M.p. 250-251 °C. IR ($\bar{\nu}$, cm^{-1}): 3063 (=C–H), 1709 (C=O), 1660 (C=C), 1604 (C=C_{Ar}), 1518 (N=O), 1340 (N=O), 1176 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.68 (1H, s, H4), 8.53 (1H, s, H2′), 8.30 (1H, d, J = 7.5, H4′), 8.07 (1H, d, J = 15.9, Hβ), 8.03 (1H, d, J = 7.8, H6′), 7.89 (1H, d, J = 15.9, Hα), 7.77–7.71 (2H, m, H7, H5), 7.66 (1H, t, J = 8.1, H5), 7.48–7.31 (2H, m, H8, H6). ^{13}C NMR ($CDCl_3$), δ: 186.1 (C3a), 159.4 (C2), 155.4 (C8a), 148.9 (C4), 148.7 (C3′), 141.7 (Cβ), 136.6 (C1′), 134.7 (C7), 134.1 (C5′), 130.3 (C5), 130.0 (C2′), 126.7 (C6′), 125.2 (C3), 124.9 (C6), 124.7 (C4′), 123.4 (Cα), 118.5 (C4a), 116.9 (C8).

A8. 3-[(2E)-3-(4-nitrophenyl)prop-2-enoyl]-2H-chromen-2-one. Yield: 97.7%; M.p. 225-226 °C. IR ($\bar{\nu}$, cm^{-1}): 3098 (=C–H), 3044 (=C–H), 1722 (C=O), 1660 (C=C), 1598 (C=C), 1508 (N=O), 1333 (N=O), 1176 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.70 (1H, s, H4), 8.32 (2H, d, J = 8.7, H3′, H5′), 8.12 (1H, d, J = 15.6, Hβ), 7.88 (1H, d, J = 15.6, Hα), 7.86 (2H, d, J = 8.7, H2′, H6′), 7.73 (2H, t, J = 7.8, H5, H7), 7.49–7.41 (2H, m, H6, H8). ^{13}C NMR ($CDCl_3$), δ: 186.0 (C3a), 159.2 (C2), 155.4 (C8a), 149.1 (C4), 148.7 (C4′), 141.5 (Cβ), 141.0 (C1′), 134.8 (C7), 130.3 (C5), 129.4 (C2′, C6′), 127.7 (C6), 125.3 (Cα), 124.5 (C3), 124.2 (C3′, C5′), 118.5 (4a), 116.9 (C8).

A9. 3-[(2E)-3-(4-chlorophenyl)prop-2-enoyl]-2H-chromen-2-one: Yield: 96.7%; M.p. 270-271 °C. IR ($\bar{\nu}$, cm^{-1}): 3045 (=C–H), 1718 (C=O), 1593 (C=C), 1174 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.64 (1H, s, H4), 7.96 (1H, d, J = 15.6, Hβ), 7.82 (1H, d, J = 15.6, Hα), 7.71 (2H, t, J = 7.5, H5, H7), 7.63 (2H, d, J = 8.4, H2′, H6′), 7.46–7.31 (4H, m, H3′, H5′, H6, H8). ^{13}C NMR ($CDCl_3$), δ: 186.3 (C3a), 159.4 (C2), 155.3 (C8a), 148.4 (C4), 143.5 (Cβ), 136.8 (C4′), 134.5 (C7), 133.3 (C1′), 130.2 (C5), 130.1 (C2′, C6′), 129.3 (C3′, C5′), 125.1 (C6), 125.0 (C3), 124.4 (Cα), 118.6 (C4a), 116.8 (C8).

A10. 3-[(2E)-3-(3-bromophenyl)prop-2-enoyl]-2H-chromen-2-one: Yield: 97.5%; M.p. 200-201 °C. IR ($\bar{\nu}$, cm^{-1}): 3054 (=C–H), 1721 (C=O), 1660 (C=C), 1604 (C=C), 1553 (C=C_{Ar}), 1178 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.63 (1H, s, H4), 7.96 (1H, d, J = 15.6, Hβ), 7.83 (1H, d, J = 1.7, H2′), 7.77 (1H, d, J = 15.6, Hα), 7.73–7.68 (2H, m, H5, H7), 7.60 (1H, d, J = 7.8, H4′), 7.55 (1H, t, J = 7.8, H5′), 7.43–7.35 (2H, m, H6, H8), 7.29 (1H, t, J = 7.8, H6′). ^{13}C NMR ($CDCl_3$), δ: 186.2 (C3a), 159.4 (C2), 155.3 (C8a), 148.4 (C4), 143.1 (Cβ), 136.9 (C1′), 134.5 (C7), 133.5

(C5′), 131.5 (C2′), 130.4 (C5), 130.2 (C6′), 127.5 (C4′), 125.2 (Cα), 125.1 (C6), 125.0 (C3), 123.1 (C3′), 118.5 (C4a), 116.8 (C8).

A11. 3-[(2E)-3-(4-bromophenyl)prop-2-enoyl] -2H-chromen-2-one: Yield: 98.6%; M.p. 203-204 °C. IR ($\bar{\nu}$, cm^{-1}): 3038 (=C–H), 1722 (C=O), 1601 (C=C), 1176 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.65 (1H, s, H4), 7.99 (1H, d, J = 15.9, Hβ), 7.84 (1H, d, J = 15.9, Hα), 7.74 (2H, d, J = 7.5, H2′, 6′), 7.71 (2H, m, H5, H7), 7.43 (2H, d, J = 7.5, H3′, 5′), 7.40 (2H, m, H6, H8). ^{13}C NMR ($CDCl_3$), δ: 186.3 (C3a), 159.4 (C2), 155.3 (C8a), 148.5 (C4), 143.6 (Cβ), 134.5 (C7), 133.8 (C1′), 132.2 (C2′, C6′), 130.3 (C3′, C5′), 130.2 (C5), 125.2 (C3), 125.1 (C4′), 125.0 (C6), 124.5 (Cα), 118.6 (C4a), 116.8 (C8).

A12. 3-[(2E)-3-(3-ethoxy-4-hydroxyphenyl)prop-2-enoyl] -2H-chromen-2-one: Yield: 82.8%; M.p. 194-195 °C. IR ($\bar{\nu}$, cm^{-1}): 3446 (O–H), 1720 (C=O), 1527 (C=C), 1188 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.62 (1H, s, H4), 7.86 (1H, d, J = 15.9, Hβ), 7.84 (1H, d, J = 15.9, Hα), 7.73–7.67 (2H, m, H5, H7), 7.45–7.40 (2H, m, H6, H8), 7.31 (1H, s, H2′), 7.27–7.22 (1H, m, H6′), 7.00–6.97 (1H, d, J = 8.1, H5′), 6.11 (1H, s, –OH), 4.12 (2H, q, J = 6.9, –OCH_2-), 1.53 (3H, t, J = 6.9, -Me). ^{13}C NMR ($CDCl_3$), δ: 186.3 (C3a), 159.5 (C2), 155.2 (C8a), 148.9 (C4′), 147.9 (C4), 146.2 (C3′), 145.7 (Cβ), 134.1 (C7), 130.0 (C5), 127.5 (C1′), 125.6 (C3), 125.0 (C6), 124.5 (Cα), 121.5 (C6′), 118.7 (C4a), 116.7 (C8), 114.8 (C5′), 110.8 (C2′), 64.7 (–OCH_2–), 14.8 (-Me).

A13. 3-[(2E)-3-(1,3-benzodioxol-5-yl)prop-2-enoyl] -2H-chromen-2-one: Yield: 94.6%; M.p. 150-151 °C. IR ($\bar{\nu}$, cm^{-1}): 3428 (O–H), 3036 (=C–H), 1720 (C=O), 1591 (C=C), 1247 (C–O). ^{1}H NMR ($CDCl_3$), δ: 8.62 (1H, s, H4), 7.86–7.84 (2H, m, Hβ, Hα), 7.73–7.70 (2H, m, H5, H7), 7.46–7.40 (2H, m, H6, H8), 7.25 (1H, d, J = 1.2, H2′), 7.23 (1H, dd, J = 7.8, 1.2, H6′), 6.87 (1H, d, J = 7.8, H5′), 6.07 (2H, s, –OCH_2O-). ^{13}C NMR ($CDCl_3$), δ: 186.3 (C3a), 159.4 (C2), 155.2 (C8a), 150.3 (C3′), 148.4 (C4′), 148.0 (C4), 145.1 (Cβ), 134.2 (C7), 130.0 (C5), 129.4 (C1′), 125.9 (C6), 125.4 (C3), 125.0 (Cα), 122.1 (C2′), 118.6 (C4a), 116.7 (C8), 108.7 (C6′), 107.2 (C5′), 101.7 (–OCH_2O–).

2.3.3. Cytotoxic activity

The *in vitro* cytotoxic activity of all compounds was evaluated in mammal U-937 cells using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromine] assay as previously described (Murillo et al., 2019). The cells were adjusted in RPMI 1640 supplemented with 10% FBS and the corresponding concentration of each compound. Six series were made based on four dilutions with concentrations from 200 to 0.19 μg/mL. The amount of formazan produced by the viable cell was recorded as optical densities (O.D) obtained in a spectrofluorometer (Varioskan, Thermo) at 570 nm. The cells exposed to Amphotericin B, Benznidazole, and Chloroquine were used as positive controls (cytotoxicity), while those that were not exposed to compounds were taken as negative controls (viability). Cytotoxicity results were expressed as lethal concentration 50 (LC_{50}) corresponding to the concentration necessary to eliminate 50% of cells. The degree of toxicity was established according to the LC_{50} value using the following scale: compounds with $LC_{50} < 100$ μM were considered as potentially cytotoxic, with $100 \leq LC_{50} < 200$ μM as mildly cytotoxic, and with $LC_{50} \geq 200$ μM as noncytotoxic (Cuartas et al., 2020).

2.3.4. Anti-leishmanial activity

The anti-leishmanial activity of compounds was evaluated in intracellular amastigotes of *L. panamensis*, a species of *Leishmania* that prevails in Central America and Colombia, transferred with the gene containing the green fluorescent protein (strain MHOM/CO/87/UA140-EGF) (Pulido et al., 2012). Briefly, human U-937 cells with a density of 3×10^5 cells/mL in RPMI 1640 and 0.1 g/mL of phorbol-12-myristate-13-acetate were dispensed into a 24-well microplate and then infected with the growing stationary phase of promastigotes of *L. panamensis* in 15:1 parasites per cell ratio. The plates were incubated at 34 °C and 5% CO_2 for 3 h and then the cells were washed twice with PBS to eliminate non-internalized parasites. Fresh RPMI-1640 was added to each well (1 mL) and the dishes were again incubated. After 24 h of infection, the RPMI-1640 medium was replaced by a fresh culture medium containing each substance in four consecutive dilutions (50.0, 12.5, 3.1, and 0.8 μg/mL), and the plates were incubated at 37 °C and 5% CO_2 for 72 h. Then, the cells were removed from the bottom of the dish with 100 μL of an EDTA/trypsin solution (250 mg). The cells were centrifuged at 168 *g* for 10 min at 4 °C. The supernatant was discarded, and the cells were washed with 1 mL of cold PBS and centrifuged at 168 *g* for 10 min at 4 °C. The cells were washed twice using PBS, as a previous wash and then, for the last wash, the supernatant was discarded, and the cells were suspended in 500 μL of PBS. The cells were analyzed by flow cytometry reading at 488 nm (excitation) and 525 nm (emission) under an argon laser that counts 10,000 events (Cytomics FC 500MPL, Beckman Coulter, Pasadena, CA, USA). The infected cells were determined according to the event of green fluorescence (indicate the parasites) and the amount of parasites inside cells were registered as the median fluorescence intensity (MFI). The infected cells exposed to Amphotericin B were used as the control for anti-leishmanial activity (positive control). The non-specific fluorescence was corrected using non-infected cells. All determinations for each compound and the standard drug were made in triplicate in two experiments (Pulido et al., 2012). The effective media concentration was defined as the concentration of compound that decrease 50% of intracellular parasites. Compounds with $EC_{50} \leq 25$ μM were considered highly active (Cuartas et al., 2020).

2.3.5. Anti-trypanosomal activity

The anti-trypanosomal activity of compounds was tested in intracellular amastigotes of *T. cruzi* (Tulahuen strain transfected with β-galactosidase gene). Parasites were grown as epimastigotes in the culture at 26 °C during 10 days. Then, U-937 cells at a density of 2.5×10^4 cells/100 μL in RPMI-1640 and 0.16 μM PMA were dispensed in a 96-well microplate and then infected with *T. cruzi* epimastigotes at a 5:1 parasite per cell ratio and incubated for 24 h at 37 °C, 5% CO_2 in RPMI with 10% FBS. Non-internalized parasites were removed by two washes with PBS and then, fresh medium was added containing each of the six serial diluted concentrations of each testing compound or Benznidazole. After 72 h of incubation at 37 °C with 5% CO_2, the effect of all compounds on intracellular amastigotes viability was determined by colorimetrically measuring the β–galactosidase activity. For this, CPRG at 100 μM and nonidet P–40 at 0.1% were added to each well, and plates were incubated for 4 h at 37 °C, at biological standard conditions, protected from light. Thereafter, measurement was performed at 570 nm in a spectrophotometer (Varioskan, Thermo). The intensity of color in each experimental condition was registered as optical density (O.D.). Non–specific absorbance was corrected by subtracting the O.D. of the blank. Infected cells exposed to Benznidazole were used as controls for anti-trypanosomal activity (positive control) whereas infected cells incubated in the absence of any compound or drug were used as controls for infection (negative control). Determinations were done in triplicate with at least two independent experiments (Buckner et al., 1996). The effective media concentration was defined as the concentration of the compound that decreases 50% of intracellular parasites. Compounds with $EC_{50} \leq 25$ μM were considered highly active (Cuartas et al., 2020).

2.3.6. Anti-plasmodial activity

The activity of compounds was evaluated in asynchronous cultures of *P. falciparum* (3D7 strain), maintained in standard culture conditions (Trager and Jensen, 2005) by quantifying the parasite lactate dehydrogenase (pLDH) activity released from the cytosol of damaged cells into the supernatant (Nkhoma et al., 2007). *P. falciparum* cultures were adjusted to 0.5% parasitemia and 1% hematocrit in RPMI-1640 medium enriched with 1% Albumax II. In each well of a 96-well plate were added 100 μL of parasite suspension and parasites were exposed to 100 μL of four serial dilutions (100, 25, 3.125, and 0.78 μg/mL) of the corresponding compound. Chloroquine was used as positive anti-plasmodial drug control. Unexposed parasites were used as controls for both

growth and viability (negative control). Plates were incubated for 72 h at 37 °C with N_2 (90%), CO_2 (5%), and O_2 (5%) atmosphere. After incubation, the content of each well was harvested, and parasites were subjected to three 20-minute freeze-thaw cycles. Meanwhile, 100 μL of Malstat reagent (400 μL Triton X-100 in 80 mL deionized water, 4 g L-lactate, 1.32 g Tris buffer, and 0.022 g acetyl pyridine adenine dinucleotide in 200 mL deionized water; pH 9.0) and 25 μL of NBT/PES solution (0.16 g nitro blue tetrazolium salt and 0.08 g phenazine ethosulphate in 100 mL deionized water) were added to each well of a second dark flat-bottom 96-well plate. After five freeze-thaw cycles, cultures in each well of the first plate were re-suspended mixing by pipetting and 15 μL from each well was removed and added to the corresponding well of the plate containing Malstat and NBT/PES reagents. After an hour of incubation in the dark, color development of the pLDH reaction was monitored colorimetrically using a spectrofluorometer (Varioskan, Thermo) at 650 nm. The intensity of color in each experimental condition was registered as O.D. Non-specific absorbance was corrected by subtracting the O.D of the blank. The effective media concentration was defined as the concentration of the compound that decreases 50% of intracellular parasites. Compounds with $EC_{50} \leq 25$ μM were considered highly active (Cuartas et al., 2020).

2.3.7. Statistical analysis

All experiments were performed in triplicate. The results are expressed as the LC_{50} determined according to the viability and mortality percentages obtained for each experimental condition and calculated by the parametric method of linear regression that permits the construction of a dose-response and the calculation of the LC_{50} (Probit analysis) as described previously (Grimm, 1979).

3. Results and discussion

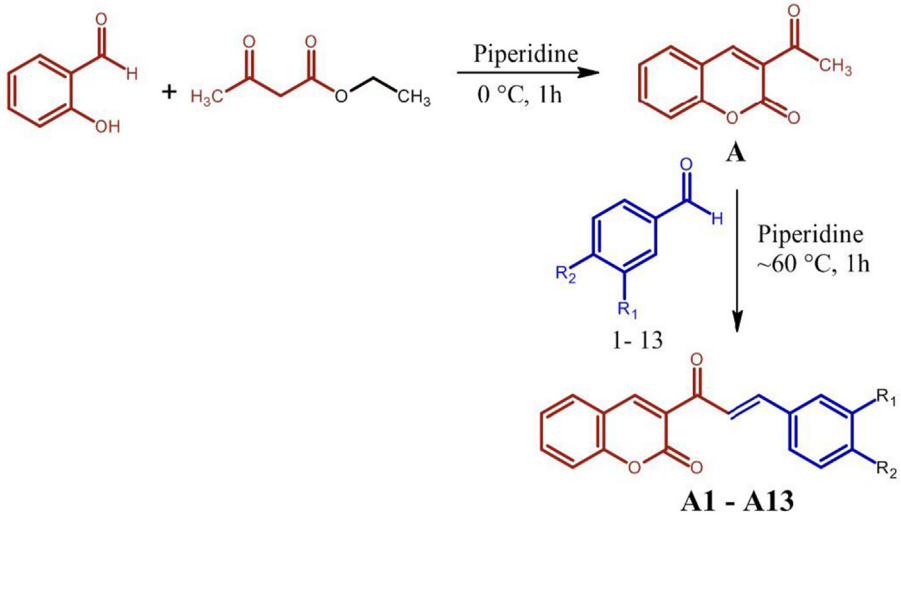
3.1. Chemistry

The coumarin-chalcone hybrids were prepared using a Claisen-Schmidt condensation from a benzaldehyde derivative and 3-acetyl-2*H*-chromen-2-one (**A**); the latter was obtained by the Knoevenagel condensation from 2-hydroxybenzaldehyde and ethyl acetoacetate using piperidine as a catalyst, cold and without solvent (Figure 2).

The methodology proposed to obtain the 3-acetyl-2*H*-chromen-2-one (**A**) has shown higher yield without using solvents (Sugino and Tanaka, 2001). In this work, the yield of Knoevenagel condensation was 99.5%. Meanwhile, the Claisen-Schmidt condensation yields ranged between 75.2 and 99.4%, being the lowest values for the reaction with the hydroxylated derivatives of benzaldehyde. Therefore, overall yields ranged from 74.8 to 98.9%, which were higher than some previous reports of synthesis of coumaro-chalcones (Vazquez-Rodriguez et al., 2015). Solvent removal in reactive systems, as was carried out in the present work, contributes to the green chemistry used in organic synthesis (Ivanković et al., 2017). The IR spectra of compounds A1 to A13 presented characteristic absorption bands at 1732-1710 (attributable to C=O), 1660 (C=C), 1600 ($C=C_{Ar}$), and 1180 cm^{-1} (C–O). Assignment of NMR signals (Figure 3) showed that the proton H4 and the carbon C4 in the coumarin skeleton were found at δ_H: 8.48–8.70 and δ_C: 149.1–147.4 ppm, respectively. The shift towards the lower field of H4 and C4 is consistent with the deshielding in the β-position of the double bond in the conjugation of the carbonyl group. The double bond in the open-chain cinnamoyl system is proved by signals of olefinic protons in the region with $\delta_{H\beta}$: 8.12–7.63 ppm and $\delta_{H\alpha}$: 7.93–7.53 ppm. These are doublets with coupling constants of J = 15.6–15.9 Hz, which indicates a *trans* double bond geometry. Interestingly, the Claisen-Schmidt condensation was selective towards the appearance of the *trans* isomer in the chalconoid system, which is in accordance with Vazquez-Rodriguez et al. (2015). The chemical shifts for H5 and H7 were found at δ 7.62–7.72 ppm (δ_{C5}: ~130 ppm, δ_{C7}: ~134 ppm) whereas those for H6 and H8 were near to δ 7.40 ppm (δ_{C6}: ~125 ppm, δ_{C7}: ~116 ppm). Carbonyl carbons of the ketone (C3a) and the ester (C2) were detected at δ 186 and 159 ppm, respectively.

3.2. Bioactivities

All synthesized compounds were tested *in vitro* against *L. panamensis* and *T. cruzi* intracellular amastigotes, and *P. falciparum* erythrocytic stages. Their cytotoxicities were tested in human macrophages (U-937



Compound	R_1	R_2
A1	H	H
A2	OMe	H
A3	H	OMe
A4	Me	H
A5	OH	H
A6	H	OH
A7	NO_2	H
A8	H	NO_2
A9	H	Cl
A10	Br	H
A11	H	Br
A12	OEt	OH
A13	OCH_2O	

Figure 2. Synthesis of coumarin-chalcone hybrids.

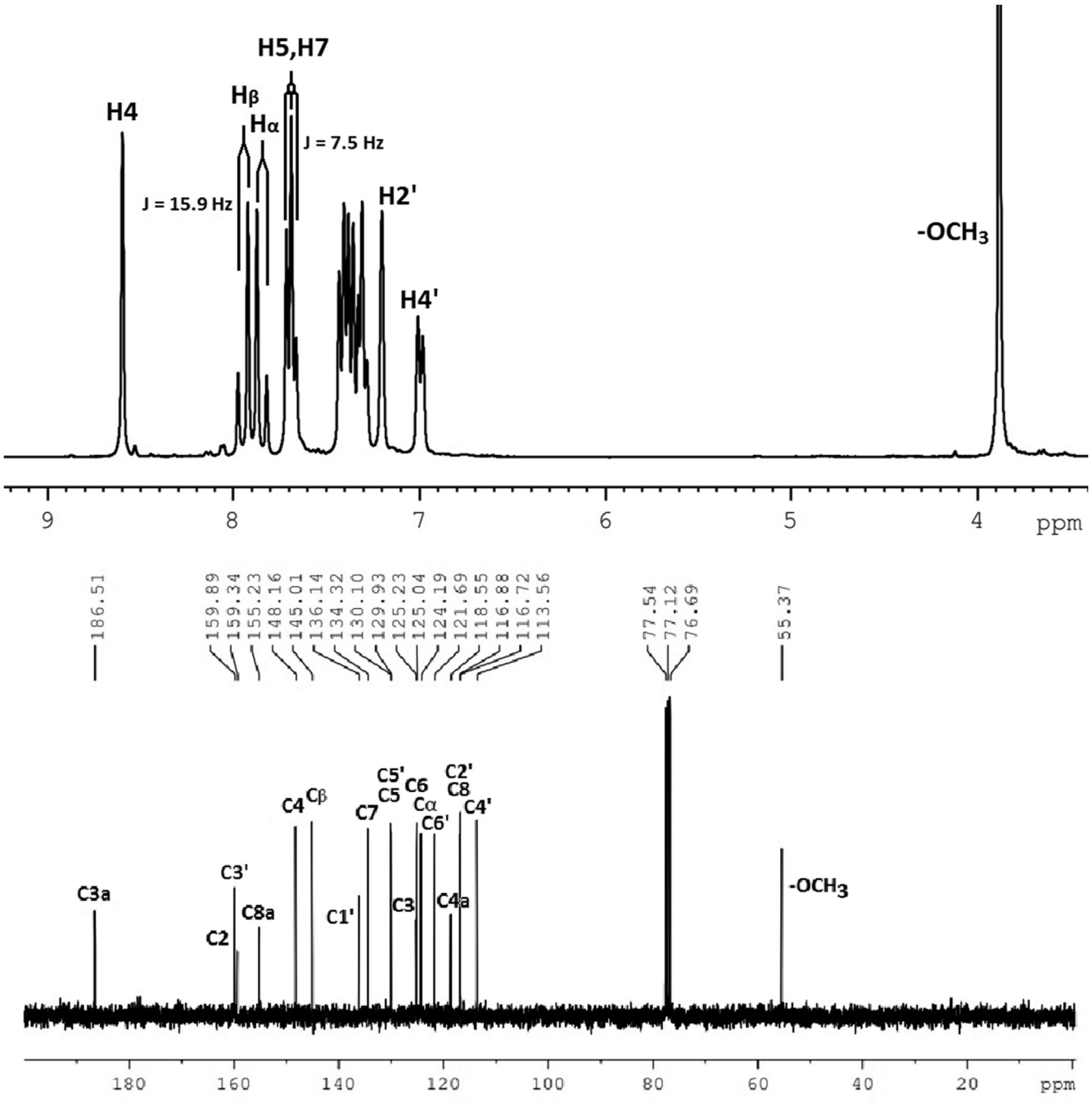


Figure 3. ^{1}H and ^{13}C NMR spectra of 3-[(2*E*)-3-(3-methoxyphenyl)prop-2-enoyl]-2H-chromen-2-one (A2).

cell line) using the MTT enzymatic micromethod. Amphotericin B was used as anti-leishmanial control with an effective media concentration (EC_{50}) of 0.30 μM; for the anti-trypanosomal activity, Benznidazole was used as control with $EC_{50} = 14.5$ μM. Finally, in order to analyze the anti-malarial activity, Chloroquine was used as a control with $EC_{50} = 3.3$ μM (Table 1). Almost all compounds were cytotoxic to U-937 cells (lethal concentration 50 (LC_{50}) < 100.0 μM), except A7 and A10, which were mildly cytotoxic and A8 was not cytotoxic (LC_{50} > 300.0 μM). Cytotoxicity (LC_{50}) of coumaro-chalcones ranged between 5.7 ± 0.1 and 601.1 ± 84.8 μM. The most cytotoxic compounds corresponded to A4 (LC_{50}: 5.7 ± 0.1 μM), A2 (LC_{50}: 5.8 ± 0.1 μM), and A5 (LC_{50}: 5.9 ± 0.2 μM). These compounds have electron-donor groups, such as -OMe, –OH, and -Me. In contrast, coumaro-chalcones with electron-withdrawing groups, such as $–NO_2$, displayed the lowest cytotoxicity (A8, LC_{50}: 601.1 ± 84.8 and A7, LC_{50}: 112.2 ± 10.6 μM). The toxicity of these two compounds (A8 and A7) was comparable, or even less, to that of Chloroquine and Benznidazole. Interestingly, the coumarin-chalcone hybrid (A1) is more cytotoxic (6.7 ± 0.1 μM) than the separated nucleus, coumarin (12.9 ± 0.5 μM) and chalcone (27.9 ± 0.5 μM).

The anti-leishmanial activity (EC_{50}) ranged between 2.1 ± 0.2 and 196.9 ± 76.9 μM. The most active compounds against intracellular *L. panamensis* amastigotes were A5 (EC_{50}: 2.1 ± 0.2 μM) and A2 (EC_{50}: 2.5 ± 0.2 μM). The lowest *in vitro* anti-leishmanial activity was exhibited by the coumaro-chalcones A8 (EC_{50}: 196.9 ± 76.9 μM) and A7 (EC_{50}: 64.7 ± 8.6 μM) having $–NO_2$ substituents. It is noteworthy that the hybrid A1 (3.7 ± 0.5 μM) displayed higher activity than the coumarin (64.4 ± 21.8 μM) and the chalcone (15.04 ± 1.44 μM). The anti-trypanosomal activity of coumaro-chalcone hybrids was found between EC_{50} values

Table 1. *In vitro* biological activity of the coumarin-chalcone hybrids.

Compound	Cytotoxicity LC_{50} (μM)	Anti-leishmanial EC_{50} (μM)	SI	Anti-trypanosomal EC_{50} (μM)	SI	Anti-plasmodial EC_{50} (μM)	SI
Coumarin	12.9 ± 0.5	64.4 ± 21.8	0.20	>7	<1.83	50.7 ± 5.7	0.25
Chalcone	27.9 ± 0.5	**15.04 ± 1.44**	1.86	99.9 ± 39.0	0.28	768.9 ± 72.0	0.04
A1	6.7 ± 0.1	**3.7 ± 0.5**	1.82	44.1 ± 4.2	0.15	**25.7 ± 3.5**	0.26
A2	5.8 ± 0.1	**2.5 ± 0.2**	2.31	**28.4 ± 5.7**	0.20	**23.3 ± 1.5**	0.25
A3	11.4 ± 2.3	>6	<1.89	326.8 ± 62.4	0.03	38.7 ± 5.6	0.29
A4	5.7 ± 0.1	**10.7 ± 5.7**	0.53	50.0 ± 5.3	0.11	35.3 ± 1.4	0.16
A5	5.9 ± 0.2	**2.1 ± 0.2**	2.85	**18.7 ± 2.4**	0.31	26.7 ± 3.6	0.22
A6	7.5 ± 0.4	**8.2 ± 4.0**	0.91	43.2 ± 6.4	0.17	**16.6 ± 0.9**	0.45
A7	112.2 ± 10.6	64.7 ± 8.6	1.73	496.5 ± 62.0	0.23	**17.9 ± 1.7**	6.28
A8	601.1 ± 84.8	196.9 ± 76.9	3.05	314.5 ± 19.9	1.91	**18.4 ± 1.0**	32.66
A9	43.4 ± 3.9	59.1 ± 12.7	0.73	52.1 ± 3.3	0.83	**18.9 ± 1.5**	2.30
A10	111.8 ± 8.2	32.8 ± 3.0	3.40	113.8 ± 8.5	0.98	**19.6 ± 1.7**	5.71
A11	32.1 ± 2.2	31.4 ± 4.2	1.02	44.4 ± 3.5	0.72	**20.9 ± 1.2**	1.54
A12	8.5 ± 1.6	**6.6 ± 1.3**	1.29	41.2 ± 1.1	0.21	**15.0 ± 0.5**	0.57
A13	38.0 ± 3.4	>20	<1.90	129.0 ± 11.0	0.30	**15.2 ± 1.1**	2.50
Amphotericin B	32.6 ± 4.0	0.3 ± 0.1	108.67	NT	NC	NT	NC
Benznidazole	>200	NT	NC	14.5 ± 0.1	>13.83	NT	NC
Chloroquine	155.2 ± 5.2	NT	NC	NT	NC	3.3 ± 0.4	46.34

Data show the lethal media concentration LC_{50} or effective media concentration EC_{50} in μM for each substance ±SD (n = 3 replies) evaluated in cell line U-937. Selectivity index (SI) = LC_{50}/EC_{50}; defined for *L. panamensis, T. cruzi y P. falciparum*. NT: not tested. NC: not calculated.

of 18.7 ± 2.4 and 496.5 ± 62.0 μM. The highest effect against *T. cruzi* was displayed by A5 (EC_{50}: 18.7 ± 2.4 μM) and A2 (28.4 ± 5.7 μM), both containing electron donor groups (-OH and –OMe) at C3'. Within the less active anti-trypanosomal compounds are A7 (496.5 ± 62.0 μM) and A8 (314.5 ± 19.9 μM), both containing the NO_2 group at C3′ and C4', respectively. Finally, the anti-plasmodial activity (EC_{50}) of coumaro-chalcones ranged between 15.0 ± 0.5 and 38.7 ± 5.6 μM. The compounds A12 (15.0 ± 0.5), A13 (15.2 ± 1.1) and A6 (16.6 ± 0.9) exhibited the highest activity; and A3 (38.7 ± 5.6), A4 (35.3 ± 1.4) and A5 (26.7 ± 3.6) have shown the lowest activity against *P. falciparum*. EC_{50} values indicate that the coumaro-chalcone A1 displayed a promising multiple biological activity; the effects of A1 against *L. panamensis* (3.7 ± 0.5 μM), *T. cruzi* (44.1 ± 4.2 μM) and *P. falciparum* (44.1 ± 4.2 μM) results improved in comparison with the coumarin (64.4 ± 21.8, >7 and 50.7 ± 5.7 μM, respectively), and the chalcone (15.04 ± 1.44, 99.9 ± 39.0 and 768.9 ± 72.0 μM, respectively).

According to literature (United Nations, 2009), substances with EC_{50} values from 0 to 50 μM are considered high-toxicity substances; from 50 to 200 μM moderately toxic, and higher than 200 μM are non-toxic substances. Agreeing with this, compounds A1-A6, A9, A11-A13 would be considered highly toxic. These compounds present in general electron-donor substituents at C3′ and/or C4' (Figure 4). Halogens at C4′ generate a great toxicity (A9, A11) while at C3' result moderately toxic (A10). A7 and A8 show the lowest cytotoxicity, both compounds have an electron-withdrawing substituent (nitro group). The above suggests strong deactivators groups decrease the toxicity of coumaro-chalcones.

Oxygenated substituents, particularly at C3′, displayed high toxicity against *L. panamensis* (A2, A5, A6, and A12); however, oxygenated substituents at C4′ presented a lower activity. Furthermore, A1 and A4 were highly active. Finally, compounds with both strong and weak deactivator groups at C3′ or C4′ exhibited a lower anti-leishmania activity. On the other hand, oxygenated compounds at C3′ position show high activity against *T. cruzi* epimastigotes. In the C4′ position, the hydroxyl group generates a better activity than other oxygenated system like alkoxy groups. It should be noted the strong anti-trypanosomal activity of A5, which has a hydroxyl group at C3'. In fact, A5 (EC_{50}: 18.7 ± 2.4 μM) exhibited an inhibition of the *T. cruzi* intracellular amastigotes similar to the commercial drug Benznidazole (EC_{50}: 14.5 ± 0.1 μM). At the end, deactivator substituents have not a considerable activity. Overall, oxygenated groups in the positions C3′ and C4' at the same time (A12 and A13) show the best activity against *P. falciparum* followed by the nitro derivatives (A7 and A8). In addition, the presence of halogens in the

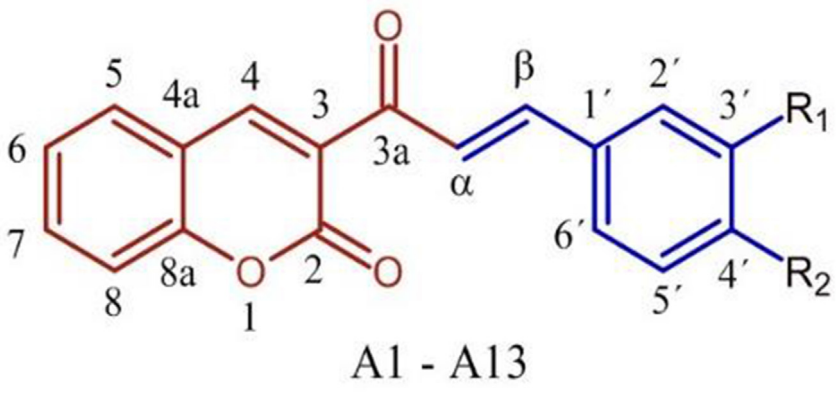


Figure 4. Structure of coumarin-chalcone hybrids.

structure generates a high toxicity. Ultimately, electron-donor groups presented a lower anti-plasmodial activity, excepting A6.

The anti-leishmanial activity of hybrids A2, A5, A8, and A10 and anti-plasmodial activity of compounds A7-A10, and A13 were higher than their cytotoxicity. Therefore, the SI values calculated for these hybrid compounds ranged from 2.31 to 3.40 in anti-leishmanial compounds, and from 2.3 to 32.66 in anti-plasmodial compounds. On the other hand, the cytotoxicity of almost all hybrid compounds, coumarin, and chalcone was higher than the anti-trypanosomal activity, with selectivity indices <1. Interestingly, compound A10 has the potential to be considered a promissory molecule for anti-leishmanial (SI = 3.10) and anti-plasmodial (SI = 5.71) drug development; while compound A8 has potential for the development of anti-malarial agents (EC_{50} = 18.4 μM and SI = 32.66).

Infections caused by *T. cruzi*, *L. panamensis* and *P. falciparum* are considered neglected diseases due to the lack of drugs to combat them. The results of the present work suggests that coumarin-chalcone hybrids with oxygenated substituents at C3′ and/or C4' can be considered useful compounds for the development of new therapeutic alternatives. However, further studies are necessary to validate the effectiveness and safety profile in the respective animal models.

4. Conclusions

Thirteen coumaro-chalcones were synthesized by coupling 3-acetyl-2H-chromen-2-one with mono and disubstituted benzaldehyde derivatives, under solvent-free conditions; Claisen-Smidt condensation was selective towards *trans* isomers in the chalconoid system, and the reaction yields ranged from 75 to 99%. Remarkably, the non-substituted hybrid (A1) exhibited better anti-protozoal activity and lowest cytotoxicity than the coumarin and the chalcone. In general, hybrids having substitution patterns with electron-donor groups were more cytotoxic to U-937 cells than the compounds with electron-withdrawing groups. Also, coumaro-chalcones with oxygenated substituents at C3′ were more active against *L. panamensis* and *T. cruzi*. In fact, the hybrid with the hydroxyl group at C3′ was almost as active as the commercial drugs Benznidazole and Amphotericin B. On the other hand, oxygenated systems at C3′ and C4' for the same molecule, as well as deactivator groups generate a good activity against *P. falciparum*. In general, coumaro-chalcone hybrids containing oxygenated substituents could be promissory for the development of new therapeutic drugs against parasitic diseases.

Declarations

Author contribution statement

Jose E. Cuellar: Performed the experiments; Wrote the paper.

Winston Quiñones and Sara Robledo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jesús Gil: Analyzed and interpreted the data; Wrote the paper.

Diego Durango: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by Universidad Nacional de Colombia-Sede Medellín, (201010027028, Hermes 50200).

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Albajar-Viñas, P., Jannin, J., 2011. The hidden chagas disease burden in Europe. Euro Surveill. 16 (38), 1–3.

Bashir, S., Alam, M., Adhikari, A., Shrestha, R.L., Yousuf, S., Ahmad, B., Iqbal Choudhary, M., 2014. New antileishmanial sesquiterpene coumarins from *Ferula narthex* Boiss. Phytochem. Lett. 9 (1), 46–50.

Brenzan, M.A., Piloto, I.C., Campana, M.V., Honda, P.A., Filho, E.R., Nakamura, C.V., 2008. Activity of extracts and coumarins from the leaves of Calophyllum brasiliense on Leishmania braziliensis. Pharm. Biol. 46 (6), 380–386.

Buckner, F.S., Verlinde, C.L., La Flamme, A.C., Van Voorhis, W.C., 1996. Efficient technique for screening drugs for activity against Trypanosoma cruzi using parasites expressing β-galactosidase. Antimicrob. Agents Chemother. 40 (11), 2592–2597.

Cuartas, V., Robledo, S.M., Vélez, I.D., Crespo, M.P., Sortino, M., Zacchino, S., Nogueras, M., Cobo, J., Upegui, Y., Pineda, T., Yepes, L., Insuasty, B., 2020. New thiazolyl-pyrazoline derivatives bearing nitrogen mustard as potential antimicrobial and antiprotozoal agents. Arch. Pharm. Chem. Life Sci. 353 (5), e1900351.

De Mello, M.V.P., Abrahim-Vieira, B. de A., Domingos, T.F.S., de Jesus, J.B., de Sousa, A.C.C., Rodrigues, C.R., de Souza, A.M.T., 2018. A comprehensive review of chalcone derivatives as antileishmanial agents. Eur. J. Med. Chem. 150, 920–929.

Grimm, H., 1979. Statistical method in biological assay. 3. In: FINNEY, D.J. (Ed.), London and High Wycombe 1978. VII, 508 S., £ 19 net. Biometrical J., 21. Charles Griffin & Co., pp. 689–690

Iranshahi, M., Arfa, P., Ramezani, M., Jaafari, M.R., Sadeghian, H., Bassarello, C., Piacente, S., Pizza, C., 2007. Sesquiterpene coumarins from Ferula szowitsiana and *in vitro* antileishmanial activity of 7-prenyloxycoumarins against promastigotes. Phytochemistry 68 (4), 554–561.

Ivanković, A., Dronjić, A., Bevanda, A.M., Talić, S., 2017. Review of 12 principles of green chemistry in practice. Int. J. Sustain. Green Energy 6 (3), 39–48.

Junqueira, L.F., 2012. Insights into the clinical and functional significance of cardiac autonomic dysfunction in Chagas disease. Rev. Soc. Bras. Med. Trop. 45 (2), 243–252.

Lee, B.Y., Bacon, K.M., Bottazzi, M.E., Hotez, P.J., 2013. Global economic burden of Chagas disease: a computational simulation model. Lancet Infect. Dis. 13 (4), 342–348.

Li, R., Kenyon, G.L., Cohen, F.E., Chen, X., Gong, B., Dominguez, J.N., Davidson, E., Kurzban, G., Miller, R.E., Nuzum, E.O., Rosenthal, P.J., McKerrow, J.H., 1995. *In vitro* antimalarial activity of chalcones and their derivatives. J. Med. Chem. 38 (26), 5031–5037.

Murillo, J.A., Gil, J.F., Upegui, Y.A., Restrepo, A.M., Robledo, S.M., Quiñones, W., Escobar, G., 2019. Antileishmanial activity and cytotoxicity of ent-beyerene diterpenoids. Bioorg. Med. Chem. 27 (1), 153–160.

Nkhoma, S., Molyneux, M., Ward, S., 2007. Molecular surveillance for drug-resistant Plasmodium falciparum malaria in Malawi. Acta Trop. 102 (2), 138–142.

Ogungbe, I.V., Erwin, W.R., Setzer, W.N., 2014. Antileishmanial phytochemical phenolics: molecular docking to potential protein targets. J. Mol. Graph. Model. 48, 105–117.

Pavão, F., Castilho, M.S., Pupo, M.T., Dias, R.L., Correa, A.G., Fernandes, J.B., da Silva, M.F., Mafezoli, J., Vieira, P.C., Oliva, G., 2002. Structure of Trypanosoma cruzi glycosomal glyceraldehyde-3-phosphate dehydrogenase complexed with chalepin, a natural product inhibitor, at 1.95 Å resolution. FEBS Lett. 520 (1-3), 13–17.

Pulido, S.A., Muñoz, D.L., Restrepo, A.M., Mesa, C.V., Alzate, J.F., Vélez, I.D., Robledo, S.M., 2012. Improvement of the green fluorescent protein reporter system in Leishmania spp. for the *in vitro* and *in vivo* screening of antileishmanial drugs. Acta Trop. 122 (1), 36–45.

Reyes-Chilpa, R., Estrada-Muñiz, E., Vega-Avila, E., Abe, F., Kinjo, J., Hernández-Ortega, S., 2008. Trypanocidal constituents in plants. 7. Mammea-type coumarins. Memorias Do Instituto Oswaldo Cruz 103 (5), 431–436.

Sugino, T., Tanaka, K., 2001. Solvent-free coumarin synthesis. Chem. Lett. 30 (2), 110–111.

Trager, W., Jensen, J.B., 2005. Human malaria parasites in continuous culture. J. Parasitol. 91 (3), 484–486.

United Nations, 2009. Part 4: Environmental Hazards, pp. 213–242.

Vazquez-Rodriguez, S., Figueroa-Guíñez, R., Matos, M.J., Santana, L., Uriarte, E., Lapier, M., maya, J.D., Olea-Azar, C., 2013. Synthesis of coumarin-chalcone hybrids and evaluation of their antioxidant and trypanocidal properties. Med. Chem Commun. 4 (6), 993–1000.

Vazquez-Rodriguez, S., Figueroa, R., Matos, M.J., Olea-Azar, C., Maya, J.D., Uriarte, E., Santana, L., Borges, F., 2015. Synthesis and trypanocidal properties of new coumarin-chalcone derivatives. Med. Chem. 5 (4), 173–177.

World health statistics, 2018. Monitoring Health for the SDGs, Sustainable Development Goals. World Health Organization, Geneva, 2018. Licence: CC BY-NC-SA 3.0 IGO.