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PII: S0960-894X(15)00546-6
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.05.072
Reference: BMCL 22762

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 March 2015
Revised Date: 19 May 2015
Accepted Date: 21 May 2015

Please cite this article as: Passalacqua, T.G., Dutra, L.A., de Almeida, L., Arenas Velásquez, A.M., Esteves Torres, F.A., Yamasaki, P.R., dos Santos, M.B., Regasini, L.O., Michels, P.A.M., da SilvaBolzani, V., Graminha, M.A.S., Synthesis and Evaluation of Novel Prenylated Chalcone Derivatives as Anti-leishmanial and Anti-trypanosomal Compounds, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.05.072

Synthesis and Evaluation of Novel Prenylated Chalcone Derivatives as Anti-leishmanial and Anti-trypanosomal Compounds

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Received: / Accepted: / Published:

Abstract: Chalcones form a class of compounds that belong to the flavonoid family and are widely distributed in plants. Their simple structure and the ease of preparation make chalcones attractive scaffolds for the synthesis of a large number of derivatives enabling the evaluation of the effects of different functional groups on biological activities. In this paper, we report the successful synthesis of a series of novel prenylated chalcones via Claisen – Schmidt condensation and the evaluation of their effect on the viability of the Trypanosomatidae parasites *Leishmania amazonensis, Leishmania infantum* and *Trypanosoma cruzi*.

Keywords: Prenylated chalcone; leishmanicidal activity; trypanocidal activity; *Leishmania amazonensis*; *Leishmania infantum*; *Trypanosoma cruzi;* drug discovery

Neglected Tropical Diseases (NTDs) have a higher prevalence in tropical and subtropical regions and affect more than one billion people worldwide[1]. A list of 17 NTDs[2] includes the insect vector-borne diseases leishmaniasis and Chagas´ disease. Leishmaniasis is a widespread disease caused by parasites belonging to more than 20 species of *Leishmania*, which are transmitted by phlebotomine sandflies after injection of promastigote forms into mammals during feeding. These infective stages

are phagocytized by macrophages and other types of mononuclear phagocytic cells. The internalized promastigotes transform into the tissue-stage amastigotes, which multiply by simple division and proceed to infect other macrophages[3]. The disease affects 12 million people around the world with about 1–2 million estimated new cases occurring every year[4]. About 8-10 million people are currently infected with *Trypanosoma cruzi*, the parasite that causes Chagas' disease, mostly in Latin America[5]. Chagas' disease is transmitted by triatomine insects, which release trypomastigote forms in their feces on the skin of a host. The trypomastigotes enter the host through the bite wound made by the blood-feeding insect or mucous membranes. Inside the host, the trypomastigotes invade cells near the site of entrance, where they differentiate into proliferating amastigotes and then again to trypomastigotes which are released back into the bloodstream to infect other cells. The ingested trypomastigotes transform into epimastigotes in the vector´s midgut where the parasites multiply and differentiate into metacyclic trypomastigotes[6,7].

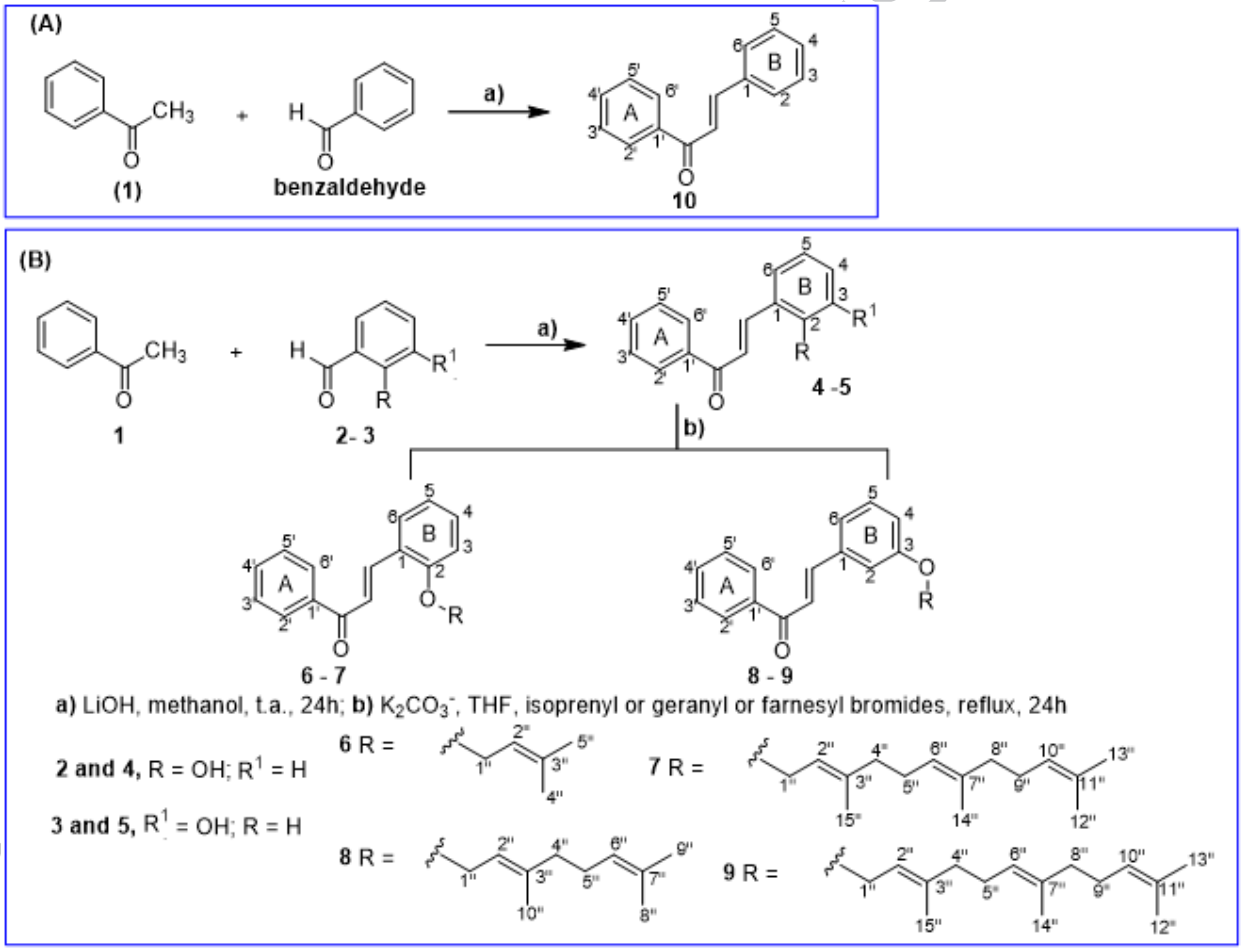
The current treatment of leishmaniasis is based on chemotherapy and includes pentavalent antimonials (sodium stibogluconate or meglumine antimoniate), the polyene amphotericin B (AmpB) (as the deoxycholate salt or its liposomal formulation), the alkylphosphocholine miltefosine and the aminoglycoside paromomycin[8]. The chemotherapy for Chagas' disease involves two drugs, nifurtimox (nitrofuran) or benznidazol (nitroimidazole), which are efficient only during the acute and initial period of the subsequent chronic phase of the disease. Unfortunately, the toxicity of all currently available drugs to treat these Trypanosomatidae-caused diseases, the inconvenience of parenteral administration, the lack of a guaranteed drug supply and the increasing incidence of treatment failure, make the development of new therapies against them urgent[9].

Chalcones are molecules consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system[10]. Their simple structure and the ease of preparation make chalcones attractive scaffolds for the synthesis of a large number of derivatives enabling the evaluation of the effect of different functional groups on biological activities[11]. Indeed, several synthetic chalcones have shown a number of biological effects such as anti-inflammatory, antibacterial, antifungal, antiviral and antiprotozoal activities[10]. Some natural prenylated chalcones such as bartericina, medicanegina and licochalcone are promising compounds for designing new drugs against neglected diseases[12,14]. Here, we present the effect of new synthetic prenylated chalcones on the viability of the Trypanosomatidae parasites *L. amazonensis, L. infantum* and *T. cruzi*.

The chalcones **4**, **5** and **10** were synthesized by Claisen-Schmidt condensation between acetophenone (**1**) and benzaldehyde derivatives (**2** and **3**)[15,16]; these compounds were used as precursors for the synthesis of four novel prenylated chalcones, **6**-**9** as shown in figure 1. For the preparation of prenylated chalcones **6** and **7,** *O*-isoprenyl and *O*-farnesyl groups were respectively added at position C-2, whereas for **8** and **9**, *O*-geranyl or *O*-farnesyl were respectively added at position C-3. Synthetic reactions in order to produce chalcones and their prenylated derivatives were followed by Nuclear Magnetic Resonance spectroscopy analysis (NMR). The ^{1}H-NMR spectra displayed a coupling constant range (*J*) for H-α and H-β of 15.0 Hz, corresponding to an (*E*)-chalcone scaffold[13]. The data obtained by NMR analyses for molecules **4** - **9** are provided as Supplementary Material, which confirmed the identity of prenylated chalcones.

The novel synthetic prenylated chalcones **6** - **9** as well as their precursor molecules, were used in screens of *in vitro* growth inhibition assays of promastigote and amastigote forms of *L. amazonensis* and *L. infantum,* as well as epimastigotes of *T. cruzi.* The cytotoxicity of all molecules for peritoneal murine macrophages was also tested, allowing the determination of the selectivity index (SI) parameter. The inhibitory activity of these compounds was evaluated at several concentrations ranging from 1.0 to 250 µM. The results expressed as the compound concentrations corresponding to 50 % of parasite growth inhibition and 50 % macrophage cytotoxicity values (EC_{50} = effective concentration; the concentration giving 50 % effect) are summarized in Table 1. Since the *O*-prenylated chalcones **6-9** are novel, no reports of the leishmanicidal activities of such compounds have been published yet (see methology in Supplementary Material).

Figure 1. General scheme for the synthesis of chalcones.



91 **Table 1.** Leishmanicidal, trypanocidal and cytotoxic activities of chalcone and its prenylated derivatives in μM.

Cpds	*L. amazonensis* (EC_{50})		*L. infantum* (EC_{50})		*T. cruzi* (EC_{50})	Swiss (EC_{50})
	Promastigotes	Amastigotes	Promastigotes	Amastigotes	Epimastigotes	Macrophages
4	333.70±23.60 (0.43)	n.d.	91.74±6.70 (1.56)	n.d.	75.89±1.88 (1.88)	143.00±0.16
5	411.60±17.70 (0.35)	n.d.	184.82±13.88 (0.96)	n.d.	68.30±0.63 (2.09)	143.00±0.63
6	4.38±0.07 (25.41)	19.93±1.34 (5.58)	4.69±0.14 (23.73)	10.41±0.99 (10.69)	21.58±1.23 (5.16)	111.30±0.34
7	6.47±0.05 (21.74)	2.90±0.19 (48.50)	10.21±0.19 (13.78)	2.24±0.86 (62.79)	69.37±4.25 (2.03)	140.65±3.62
8	8.92±0.25 (9.18)	25.81±5.05 (3.17)	7.81±0.14 (10.49)	63.89±5.89 (1.28)	26.25±3.11 (3.12)	81.94±8.11
9	9.25±0.47 (6.00)	21.89±0.47 (2.52)	5.98±0.02 (9.22)	32.55±1.89 (1.69)	23.79±1.89 (2.32)	55.14±5.60
10	13.2±1.21 (0.70)	n.d.	8.89±1.91 (1.06)	n.d.	102.64±6.11 (0.09)	9.50±0.03
Pent	10.19±0.85 (3.50)	6.25±0.58 (5.71)	67.71±8.11 (0.53)	19.77±0.52 (1.81)	n.d.	35.69±6.84
AmpB	3.22±0.03 (7.17)	4.92±0.14 (4.70)	0.92±0.01 (25.11)	2.98±0.38 (7.75)	n.d.	23.10±2.52
Benz	n.d.	n.d.	n.d.	n.d.	4.07± 0.31	n.d.

The selectivity indices (SI) are shown in parentheses

n.d. not determined

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Although chalcone (**10**) presented a mild leishmanicidal activity, it showed high cytotoxicity with SI values of 0.7 and 1.06 for *L. amazonensis* and *L. infantum*, respectively. Thus, structural changes were considered in order to obtain more effective and less cytotoxic chalcone derivatives. The intermediate molecules **4** and **5**, which present hydroxyl groups at positions C-2 and C-3, respectively, did not show antileishmanial activity and their cytotoxicity appeared to be very high. In contrast, the prenylation of **4** and **5** resulting in the derivatives **6** - **9** exhibited variable *in vitro* activity against the evaluated parasites. For both *Leishmania* species tested, *L. amazonensis* and *L. infantum*, compounds **6** - **9** were very active on the promastigote forms, with **6** and **7** being the most selective ones to the parasites (SI values of 25.41 and 21.74, respectively) (Table 1).

For the amastigote form of *Leishmania*, the clinically most relevant stage of these parasites, compound **7** was the most active and selective prenylated chalcone, for both *L. amazonensis* (EC_{50} = 2.90 µM ± 0.19; SI= 48.50) and *L. infantum* (EC_{50} = 2.24 µM ± 0.86; SI= 62.79). Compound **7** showed the most outstanding results for *L. infantum* when compared to pentamidine, with respect to both its anti-amastigote activity and its selectivity to the parasite; these values were about 9- and 35-fold higher than those of the reference drug. Regarding *L. amazonensis*, despite its low activity (only two–fold higher than pentamidine), this compound (**7**) still displayed an eight times higher selectivity index.

A reasonable explanation for the good antileishmanial activity of **6** – **9** over **4** – **5** and **10** could be the presence of prenyl moieties influencing the lipophilicity of the compounds. Indeed, there is some evidence in the literature that prenylation increases lipophilicity and, consequently, causes an improvement of the biological activity. It has been reported that prenylated flavonoids presented enhanced effects against bacteria, fungi and viruses[17]. The prenylated chalcones evaluated in this work contain *O*-isoprenyl (**6**) and *O*-farnesyl (**7**) at C-2 or *O*-farnesyl (**9**) and *O*-geranyl (**8**) at the C-3 positions; thus these prenyl moieties may result in an increased lipophilicity of the compounds that is responsible for the augmented leishmanicidal activity by facilitating the passage of the molecules through the cell-membrane barriers of the macrophages and parasites. A close relation between lipophilicity and leishmanicidal activity was also reported by Maciel-Rezende and coworkers (2013)[18], when they evaluated the leishmanicidal activity of a series of *O*-alkyl substituted benzophenones. With regard to other prenylated chalcones, Dal Picolo and coworkers showed that adunchalcone, a prenylated dihydrochalcone (possessing an isoprenyl chain), displayed good antileishmanial activity against promastigote forms of *L. amazonensis,* but lacked any effect against its intracellular amastigotes[19]. Gupta and co-workers reported that geranyl chalcones were more potent than isoprenyl chalcones against amastigote forms of *L. donovani*[20]; these findings could be attributed to a better drug delivery into host cells as a result of the size of the prenyl moiety that affects the potency of the molecules on intact cells.

In fact, for *L. infantum* and *L. amazonensis*, **7** was 4.65 and 6.87 more active than **6,** respectively. This effect should possibly be attributed to the presence of the 15 carbons prenyl chain of *O*-farnesyl in **7** compared to the 5 carbons prenyl chain of *O*-isoprenyl in **6**. The long carbon skeleton of *O*-farnesyl contributes to an increased lipophilicity, as confirmed by the calculated ClogP values (see table 2 in Supplementary Material); the ClogP of **7** was found to be 7.75 whereas for **6** it was 4.69. The partition coefficient logP is an important chemical parameter that determines the tendency of a given compound to be distributed in a biphasic system composed of two phases (octanol/water) determining its

lipophilicity / hydrophilicity[21,22,23]. All prenylated chalcones **6** – **9** presented calculated ClogP values higher than those obtained for **4**, **5** and **10**. Indeed, the anti-amastigote activity of **7** is at least five times higher than that of **6**, in agreement with its ClogP, which reinforces the hypothesis that the larger the lipophilicity, the better the antileishmanial activity.

It was also observed that the presence of the *O*-farnesyl group at position C-2 in the chalcone skeleton rendered compound **7** more active than both reference drugs tested; furthermore, considering the observed SI values, compound **7** exhibited at least eight times less cytotoxicity than the standard drug Amp B. On the other hand, the *O*-farnesyl group at position C-3 (compound **9**) resulted in a strong loss of leishmanicidal activity as well as an increased cytotoxicity profile suggesting that the position of the *O*-farnesyl group (C-2 or C-3) influences both the leishmanicidal and cytotoxicity activities. These observations suggest that the position of prenyl moieties is important for the correct interaction of the compound with its potential target molecule in the parasite, which supports the notion that the position of the prenyl groups as well as the presence or absence of other substituents in the B ring are important for the leishmanicidal activity.

For *T. cruzi*, compounds **6** (EC_{50} = 21.58 µM ± 1.23; SI= 5.16), **8** (EC_{50} = 26.25 µM ± 3.11; SI= 3.12) and **9** (EC_{50} = 23.79 µM ± 1.89; SI= 2.32) presented similar potency ranging from 21 to 26 µM, five times less active than benznidazol; moreover, their low SI values rendered these molecules unattractive for further evaluation against these parasites, at least the available epimastigote stage.

Four prenylated chalcone derivatives (**6** – **9**) were synthesized and exhibited good antipromastigote activity as well as good selectivity for both *L. amazonensis* and *L. infantum*. The most promising results were obtained for compound **7** that showed the best antiamastigote activity, the clinically most relevant parasite form. This compound showed the best relationship between the leishmanicidal effect and the cytotoxicity activity against murine macrophages, which represents a good indication for its therapeutic index for further drug development. Variation in lipophilicity of the evaluated compounds seems to be an important requisite for leishmanicidal activity. Further studies should be conducted to better understand the mechanism of action of these molecules. Thus, these series of prenylated chalcones could be further explored as potential new leishmanicidal drugs.

Acknowledgments

The authors thank Isabel Martinez for her technical support. They are also grateful to the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP: n° 2010/52327-5), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq: n° 407588/2013-2), Fundaçao Coordenaçao de Aperfeiçoamento de Pessoal de Nivel Superior (CAPES) and PROPe (Pro-Reitoria de Pesquisa da UNESP) and the FUNDUNESP for financial support.

Author Contributions:

Conceived and designed the experiments: LOR, VSB, MASG. Performed the experiments: TGP, LAD, LA, AMAV, FAET, MBS. Analyzed the data: PRY, MASG. Contributed reagents/materials/analysis tools: LOR, VSB, MASG. Wrote the paper: TGP, LAD, LA, PAMM, MASG.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at doi:

Conflicts of Interest

The authors declare no conflict of interest.

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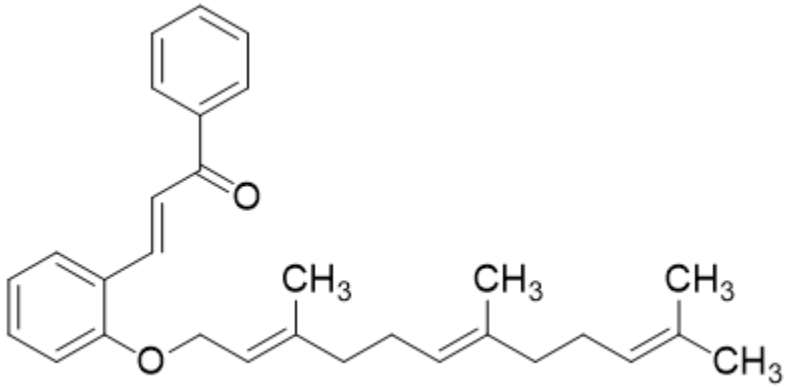
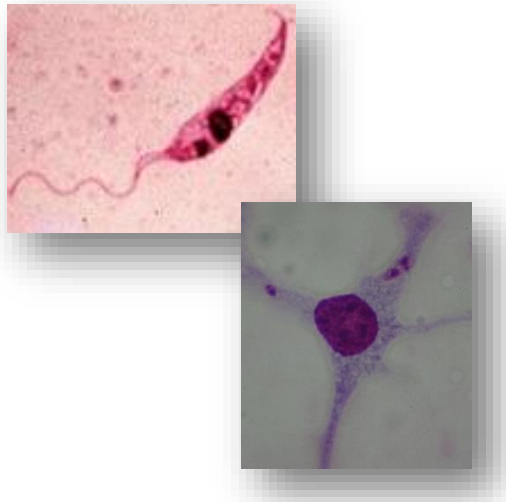
Sample Availability: Samples of the compounds 6-9 are available from the authors.



(*E*)-2-*O*-farnesyl-chalcone (**7**)



Antileishmanial activity